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Livestock Production and Climate Change

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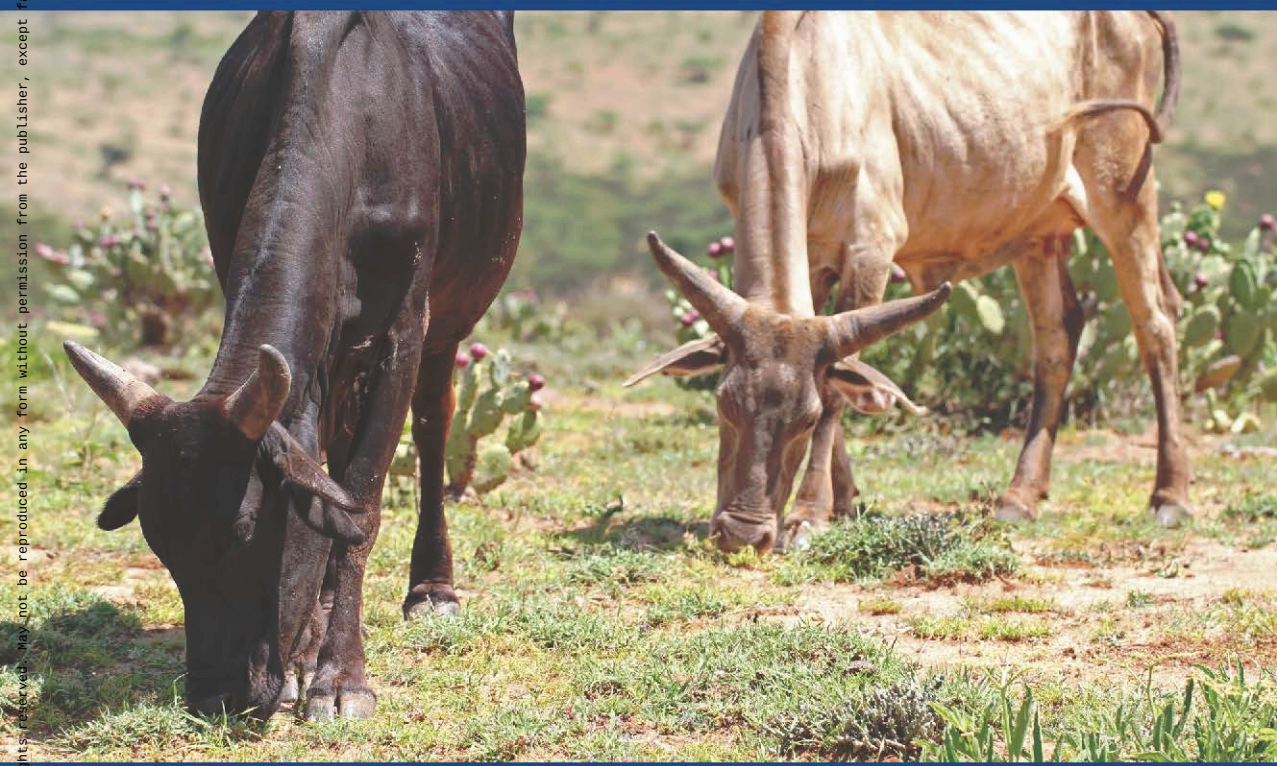

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Livestock Production and Climate Change

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Preface

In recent years, a worldwide transition in dietary ingredients has been observed where people have incorporated more livestock products in their diet. A major transition has taken place in the developing world such as India, where dietary composition has changed drastically. This has been attributed primarily to urbanization and income escalation. It is projected that by 2050, the demand for livestock food products like milk, meat and eggs will increase by 70% more than that in 1990. This growing demand will be met either by increasing livestock numbers or by enhancing their productivity. Further, the cattle and small ruminant population is likely to increase to 2.6 and 2.7 billion by 2050, respectively. The human population is also escalating at a rate of 90 million per year, and is expected to reach 9.6 billion by 2050. This increasing human population will claim first rights on the world's diminishing resources, which will therefore leave a meagre supply for livestock. Apart from shrinking water and land resources, changing climate is emerging as a major concern causing hindrance in enhancing livestock productivity.

The primary objective of this book is to raise awareness among scientists, academics, students, livestock farmers and policy makers of the twin inter-related and inter-dependent complex mechanisms of livestock rearing and climate change. Intensive livestock farming, land degradation, deforestation and greenhouse gas (GHG) emissions are the few important factors that accelerate climate change, and which in turn aggravate the adverse impacts of climate change on global livestock production. The threat of climate change on livestock will be stratified, and vulnerability will vary from one ecoregion to another. On the one hand, the prolificacy of livestock, the availability of feed and fodders, the quality of feeds, the biodiversity of animal genetic resources, livestock productivity and immunity status, as well as the emergence of new diseases and vectors, are the major issues affected by climate change. While on the other hand, livestock production is also listed in the major causes that are accountable for climate change.

Emissions of GHGs, explicitly methane and nitrous oxide, are the major concerns associated with livestock production. Enteric methane emission from livestock not only plays a considerable part in global warming and climate change but also epitomizes the substantial loss of feed energy. Livestock excrement also emits methane and nitrous oxide, albeit the magnitude of GHG emission is comparatively not as much as it is from other sources and always depends on excrement management systems, which differ from country to country. The FAO anticipate that GHG emissions from the livestock sector will double in

the next 35–40 years, in accordance with livestock numbers, and will remain a debatable issue for their large GHG contribution.

The challenges before animal researchers are multifold for achieving equilibrium between livestock numbers, production and productivity and scarce feed and fodder and other resources to satisfy the requirements of the populace across the globe. Addressing the multi-directional links of livestock production and climate change to perceive the precise impacts of one to the other is of paramount importance in a given environment for suitable acclimation approaches and to formulate ameliorative measures.

While collating this book, we have split the contents into sections: one on livestock production, one on climate change and one on enteric methane amelioration. In the first section, decisive issues such as current feed and fodder demand, the effect of climate change on feed availability and quality, projections for 2030, water requirement, etc., have been dealt in order. Given the due importance to abiotic stress, nitrogen emissions and phosphorus pollution, well-designed individual chapters are arranged in this section that suggest the appropriate corrective measures for these global glitches. As ruminants thrive mainly on fibrous feed material, particularly in the developing world, the special topics on metagenomics and proteomics are positioned in this section for the effective use of these emerging approaches in fibre degradation.

As stated earlier, livestock production and climate change are amalgamated through complex mechanisms, and one affects the other in many ways. Therefore, in the section on climate change, an attempt is made to address the interspersed link under seven chapters, focusing on the carbon footprint of producing food of animal origin, carbon sequestration, livestock diversity, animal reproduction, meat production and the role of indigenous livestock in the changing climatic scenario.

Exclusive attention is given to enteric methane emission in the final section for being the critical factor in climate change, and is deliberated under nine different chapters converging on status, thermodynamics, feeding and biological interventions to address the problem and to achieve practically viable reduction levels for minimizing the impact of global warming and saving biological energy that can be directed towards productive functions.

The reciprocity of livestock production and climate change is a global issue, which needs the collective efforts of researchers from different parts of the world. Translation research always emerges from sound basic knowledge. Therefore, attempts have been made to describe each chapter in that light, focusing on development and application. This book brings together authors from 12 countries from Asia, Africa, Europe, Australia and the USA. The selection of authors is an indication of the relevance of this subject, as they have addressed different issues in a global perspective. Each chapter is written by renowned professionals who have expertise in a given field and who have made a substantial contribution to the progressing science in that particular area.

This endeavour would not have been successful without the help of the contributory authors, who went to great pains to compile up-to-date information on these topics. The editors are indebted to all our contributors for their commendable and sincere efforts in writing the chapters and finishing the assignment within the allocated time.

We hope that the readers of this volume will find a full coverage of all the relevant topics associated with climate change in reference to livestock production, and we believe that the comprehensive, compact and up-to-date information contained in this book will empower animal scientists to cope with the climate change issue.

P.K. Malik, R. Bhatta, J. Takahashi,
R.A. Kohn and C.S. Prasad
Editors

March 2015

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1 Overview

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1.1 Livestock Sector

Worldwide livestock are an integral component of agriculture that contribute directly or indirectly to the populace by providing food, value-added products, fuel and transport, enhancing crop production and generating incomes, livelihoods, etc. In addition, livestock also diversify production and income, provide year-round employment and reduce risk. Livestock play an important role in crop production, especially in developing countries, through providing farmyard manure and draught power to cultivate around 40% of arable land. There are 1526 million cattle and buffalo and 1777 million small ruminants in the world (FAO, 2011). Worldwide, these animals are scattered under grazing (30%), rainfed mixed (38.5%), irrigated mixed (30.15%) and landless/industrial (1.15%) production systems. There are interregional differences, too, in the distribution of livestock, attributed to the agroecological features, human population density and cultural norms. Sub-Saharan Africa, Latin America and the Near East have a fairly larger land area per person engaged in agriculture, and therefore have a greater livestock proportion dependent on grasslands.

Livestock virtually support the livelihood of about 1 billion poor across the globe, of which 61% inhabit South and South-east Asia (34% in South Asia and 27% in South-east Asia). Livestock provide over half of the value of the global agricultural output, with one-third in developing countries. This share rises with income and living status, as evidenced from OECD (Organisation for Economic Co-operation and Development) countries, where livestock contribute above 50% of agricultural gross domestic product (GDP). In 2010, the export value of the livestock product in international trading was about US$180 billion, which constituted around 17% of the total agricultural product export value. Approximately 13% of total calorie consumption comes from livestock products on a global scale, while in developed countries this figure increases to ~21% of total calorie consumption. Additionally, livestock products cater to 28% of the world's total protein need, whereas in developed countries they make up 48% of the total. Due to the comparatively high cost of livestock products and low incomes, the consumption of livestock products in the developing world is still low. However, in the past few years, due to rising incomes and better living standards, a clear transition towards an animal product-based diet is becoming evident in developing countries.

1.2 Trends

The global population is expanding by 90 million per annum, and is expected to reach 9.6 billion by 2050 (UNDESA, 2013). The increase in the population of developing countries will accelerate more compared to that of the sub-Saharan and developed world. In 2050, there will be an escalation of ~70% in population growth in contrast to 1990. There is a concurrent upsurge in people's income; since 1980, an increase of

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1.5% has been recorded on a global scale, while in Asian countries the increase is around 5–7% during the same period. This hike in income is one of the major factors leading to the migration of people towards urban areas; it has been reported that 20% of the total population inhabited urban areas in 1900, which subsequently rose to 40 and >50% in 1990 and 2010, respectively. It is projected that 70% of the population will have migrated to urban areas by 2050. Higher earnings, urbanization and the preference for a better quality diet will shift the majority of people to accommodate livestock products in their meal.

The Food and Agriculture Organization (FAO, 2008) estimated the world's livestock population comprised 1.58 billion bovines (1.4 billion cattle and 0.18 billion buffalo), 1.95 billion small ruminants (1.09 billion sheep and 0.86 billion goats), 0.025 billion camels and 0.059 billion horses. It is projected that the cattle population might increase to 2.6 billion and small ruminants to 2.7 billion by 2050. This livestock population is not distributed uniformly across continents and countries; one-quarter of the total cattle population is concentrated in Brazil and India, and 56% of total world's buffalo are residing in India alone (FAO, 2008). The bovine and ovine populations have increased 13 and 22%, respectively, from the 1983 figures. Increasing the population of livestock may be one way to meet the requirement of a large human population in the coming years; however, limited feed and fodder resources, scarce water sources, land shrinkage and climate change pose a restriction on this option and a constant conflict between food–feed crops.

The overall demand for agricultural products including food, feed, fibre and biofuels is expected to increase by 1.1% $year^{-1}$ from 2005–2007 to 2050, and to meet this increased demand cereal production must increase by 940 million tonnes (Mt) to reach 3 billion tonnes (Bt), meat by 196 Mt to reach 450 Mt, and oil crops by 133 Mt to achieve the necessary figure of 282 Mt (CGIAR, 2014). The world's demand for milk, meat and eggs will increase by 30, 60 and 80%, respectively, by 2050, as compared to the demand in 1990. The aggregate demand for livestock products is projected to be 70% more in 2050 than that in 1990 (Le Gall, 2013). The growth in milk production is not as much as that seen in meat production over the past few years. However, the consumption of milk per capita per year has increased by 7 kg in the past 30 years (Alexandratos and Bruinsma, 2012). The foremost increase in milk consumption in the past few years has been seen in the developing countries, where consumption has increased by 15 kg per capita per year. To meet the requirement of the population in 2050, an increase of 1.1% per annum from a production of 664 Mt in 2005–2007 has been projected. As milk production in developed countries has already achieved a plateau and is now almost stagnant, this increase in production has hence, out of necessity, to come from the developing countries, where resources are shrinking and productivity enhancement is the only option.

To meet global demand in 2050, meat production will have to be increased at a rate of 1.3% per annum above the 258 Mt produced in 2005–2007 (Alexandratos and Bruinsma, 2012). However, there is a regional disparity projected for this growth; maximum growth in meat production is projected in South Asia (4% per annum), followed by sub-Saharan Africa (2.9%) and the Near East/North Africa (2.2%). For developed countries, the growth in meat production will remain stagnant at around 0.7% per annum. Figure 1.1 shows that meat and milk consumption in comparison to 2002 will increase by 6.4 and 0.49% by 2015 and by 14.10 and 3.47%, respectively, by 2030.

Thus, it is clear from the above discussion that the requirement for livestock products will increase dramatically in future, and the major segment of this increased demand will come from the developing world, which still has much unexplored potential and may be influential in satisfying the requirement of a large human population in 2030/2050. This increased demand shall be met in two ways, either by increasing the number of animals or through intensifying the pro-

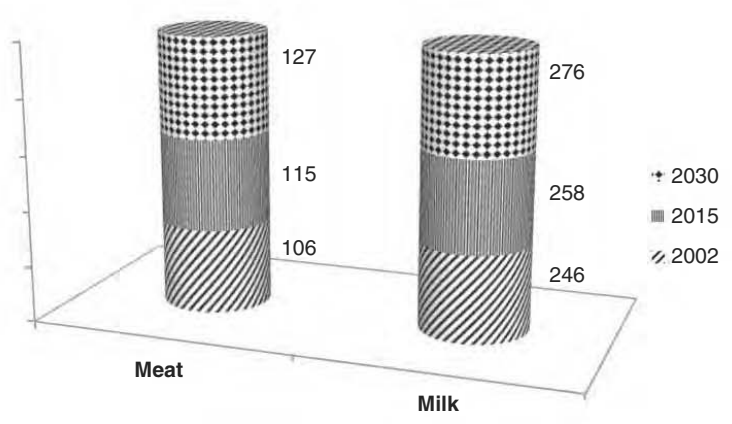

Fig. 1.1. Projections for meat and milk consumption (kilogram per capita per annum): 2015/2030. (Modified from Thornton, 2010.)

duction potential of livestock, especially in the developing world. However, this is not going to be an easy task for stakeholders, as the livestock sector is currently facing many adversities that need to be addressed urgently. Climate change is one of the major issues adversely affecting livestock production across the world. All the major issues restricting the production from this sector are discussed very briefly in the subsequent section.

1.3 Livestock and Climate Change

About one-third of the ice-free terrestrial surface area of the planet is occupied by livestock, which uses almost 15% of global agriculture water. Livestock systems have both positive and negative effects on the natural resource base, public health, social equity and economic growth (World Bank, 2009). Livestock are considered a threat to the degradation of rangelands, deforestation and biodiversity in different ecoregions, and also to supplies of nitrogen and phosphorus in water. As per one estimate, livestock are accountable for 20% of rangeland degradation and pose a threat to the biodiversity of 306 of the 825 ecoregions worldwide (Le Gall, 2013). Animal production systems and climate change are intermixed through complex mechanisms, and the threat of climate change is ubiquitous to the agriculture and livestock sector; however, the intensity of impact is stratified, depending on the agroclimatic region and country. In one way, climatic variations influence livestock production by altering the surrounding environments that govern the well-being and prolificacy of livestock; affecting the quality and quantity of crop biomass, animal health, etc. On the contrary, livestock production also has a large impact on climate change through the emission of the large quantity of greenhouse gases (GHGs) associated with livestock rearing and excreta. Climate change has both direct and indirect impacts on livestock; increase in events such as droughts, floods and cyclones, epidemic diseases, productivity losses and physiological stress are a few of the direct impacts of climate change on livestock, while indirect impacts would be on feed and fodder quality and quantity, the availability of drinking water and the interactions between the host animal and pathogens.

1.3.1 Livestock – the culprit in climate change

Carbon dioxide (CO_2) is the major GHG accountable for more than half of the greenhouse effect; however, emission from animals as such is negligible and CO_2 emits mainly from industries and fuel burning. Though methane (CH_4) is the second major

GHG, its current atmospheric concentration, i.e. ~2 ppmv (parts per million volume), is far less than the concentration of CO_2 (396.81 ppmv). CH_4 and nitrous oxide (N_2O) are the two major GHGs from animal agriculture that contribute to global warming. Livestock rearing has two components for GHG emissions; one is enteric fermentation and the other is excreta. As such, enteric fermentation contributes almost 90% of the total CH_4 emission from ruminants, and the rest comes from hindgut fermentation. The intensity of CH_4 emission from excreta depends on the management system followed; the collection and storage of dung in pits or lagoons creates the desirable anaerobic conditions and therefore leads to more CH_4 emissions from excreta; however, the emission of CH_4 from the heap system in most of developing countries, including India, is much less, as most of the heap portion is exposed to the environment and emission takes place only from the deep inner layer. To the contrary, the N_2O emission from the heap system is comparatively more, due to exposure in an open environment.

Both CH_4 and N_2O have 25 and 310 times more global warming potential, respectively, as compared to CO_2, and therefore become more important when global warming is debated. In the total CH_4 emission of 535 Tg year^{-1}, 90 Tg is derived from enteric fermentation, whereas 25 Tg comes from animal wastes. Animal production systems emit 7.1 Gt CO_2-equivalent (eq) GHGs per annum, which represents around 14.5% of human-induced GHGs. In addition, the ruminant supply chains also emit 5.7 Gt CO_2-eq year^{-1} of GHGs, of which 81, 11 and 8% are associated with cattle, buffalo and small ruminant production, respectively (Opio *et al*., 2013). In one estimate, the FAO projected that the GHG emissions from the anticipated livestock numbers may be doubled in the next 35–40 years. This shall precipitate much argument, as industrial GHG emission is anticipated to be on the decline.

Further, animal production systems are also the largest contributors of reactive nitrogen to the environment in the form of NH_4^+, NH_3, NO_3, N_2O and NO. However, most of these nitrogen losses from agriculture, except N_2O, do not affect climate change directly, but these compounds have serious environmental consequences by contributing to haze, the acidity of rain, eutrophication of surface-water bodies and damage to forests. The intensification of livestock production to satisfy requirements has led to the rigorous use of manures and fertilizers, resulting in the saturation and accumulation of phosphorus in the soil, which creates the problem of eutrophication and impairs ecosystems.

1.3.2 Impacts on livestock production

As stated earlier, both livestock and climate change are interrelated by complex mechanisms. Figure 1.2 illustrates the possible interrelated mechanisms by which livestock and climate change impact on each other. In the section above, we have seen briefly how livestock production affects climate change, and in this section, the impact of climate change on livestock production will be addressed in short, as this is deliberated in detail in subsequent chapters. Increasing demand has put pressure on the resource base, leading to more intensification and expansion of livestock production systems. Further, the cropping area in dry regions is also expanding, which forces pastoral livestock systems to relocate into still more arid lands.

Feed and fodders are the key components in livestock production, not only because these are the basic resources that fuel animal productivity but also are the key link between livestock, land and several regulating and provisioning ecosystem services such as water cycles, GHG emissions, carbon sequestration, maintenance of biodiversity and others. As illustrated earlier, livestock production and productivity has to increase substantially from the current level to meet increased demand in the future, which in turn depends on the availability and quality of feed resources; in many projections, crop yields, especially in the tropics and subtropics, are projected to fall by 10–20% by 2050, due to a combination of warming and

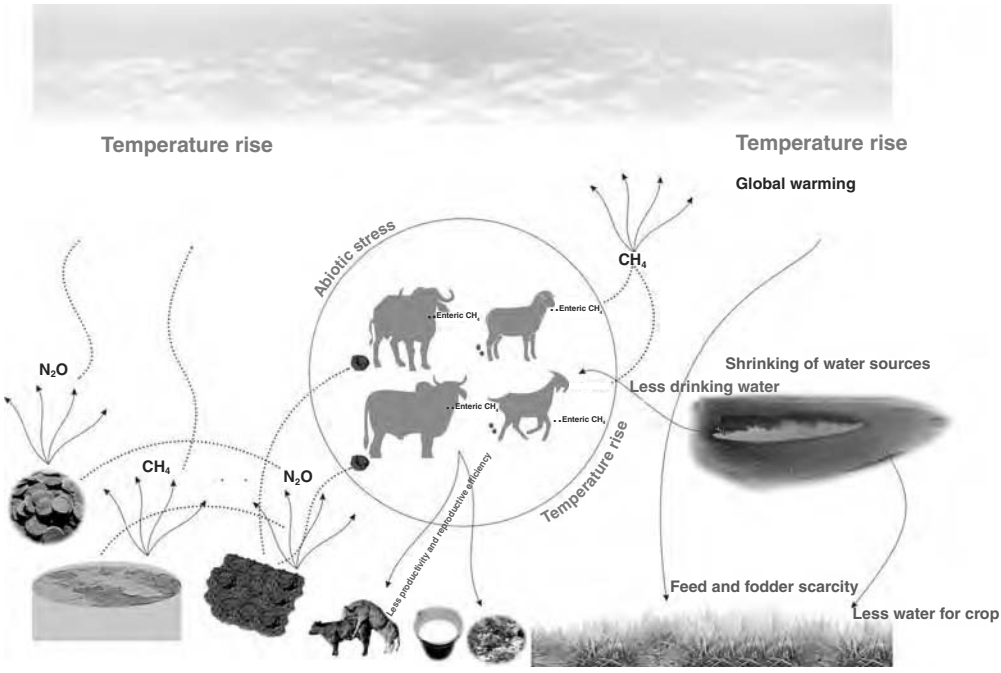

Fig. 1.2. Inter-dependence of livestock production and climate change.

drying. The impact on crop yield may be even more severe in some places.

Stover production in the intensive ruminant production systems of South Asia is now almost stagnant and needs to be complemented with some other alternative feeds to avoid an acute deficit in future. The availability of stover will vary from region to region, and a large increase is anticipated to occur in Africa, due mostly to productivity enhancement in maize, sorghum and millet. Changes in atmospheric CO_2 level and temperature will change the herbage growth pattern and pasture composition through the changing grasses to legume ratio. The greater incidences of drought may offset the yield of dry matter with the changing concentration of water-soluble carbohydrates and protein.

Ecosystems inhabited by animals are an amalgamation of biotic and abiotic factors that govern the production and productivity of the animals. Climate change is a major driving factor that exposes farm animals to abiotic stress, particularly to heat stress, which in turn adversely affects animal productivity. As one-third of cattle and most buffalo inhabit the tropical and subtropical world, heat stress through climate change will therefore have far-reaching negative consequences on livestock rearing and production in these vulnerable parts.

Climate change may also affect the future of all farm/forest animal genetic resources (AnGRs), as they may be at risk of being lost through the direct impacts of climate change mediated by increased incidences of drought/flood and the emergence of epidemic diseases. However, indirect impacts of climate change on livestock will take place through alteration of the capability to adapt to extreme climatic conditions. Climate change in terms of extreme temperatures and changes in CO_2 concentrations are likely to impose differential opportunities for several species, depending on the adaptability of a particular species in a broader range of biogeographic conditions. The impacts on favoured species may be more severe, as they will increase both in numbers

and extent and will compete for diminishing resources such as feed and water. Thus, climate change and favoured species present two of the greatest threats to biodiversity and the provision of valuable ecosystem services.

Climate change will affect reproduction in farm animals, too, through inadequate nutrition or stress conditions as the results of climate change. Nutrition and stress, in turn, will affect the entire hypothalamus–pituitary and gonadal axis in both male and female systems, and may lead to abnormal gametogenesis, folliculogenesis and ovulation, change in sexual behaviour, low conception rate, increased embryo and pregnancy loss, delayed post-partum recovery, increased calving intervals, lowered perinatal vigour and increased perinatal mortality and morbidity, etc. Climate change is likely to affect the productive and reproductive performance of animals after birth through epigenetic changes in the maternal womb.

Another issue is the availability of water for livestock drinking and crop production; temperature rise affects water availability adversely, and globally, freshwater resources are relatively scarce, amounting to only 2.5% of all water resources. In addition, the water tables in most regions of tropical countries are declining incessantly and it is projected that, by 2025, 64% of the world's population will live in water-stressed basins, compared to 38% today (Rosergrant *et al*., 2002). Increasing livestock numbers in the future to meet demand will clearly add to the additional demand for water, particularly for the production of livestock feed: 1 m^3 of water is required to produce about 0.5 kg dry animal feed in North American grasslands and about 5 kg of feed in tropical systems (Peden *et al*., 2007).

The shift from plant-based diets to more animal products will lead to the intensification of livestock production, resulting in more GHG emissions. The emission of GHGs from whole systems needs to be measured to develop strategies to minimize emissions in absolute quantity, otherwise cutting down emissions from one point may increase them from another. The carbon footprint (CF) may help to assess the GHG emissions associated with the production of food of animal origin and to identify the hotspots for reducing GHGs from whole systems. For example, the average carbon footprint (kilogram CO_2-equivalent) for producing 1 kg beef is 10 on a global scale, but there is much variation among countries, ranging from 8.5 (Germany) to 23.6 (Ireland). There is an urgent need to adopt such farming systems that ensure carbon sequestration from the whole system, comprising fossil fuel combustion, feed production, land restoration, deforestation, biomass burning, animal rearing, etc. Sustainable animal agriculture requires an understanding of crop–animal interactions and integrated natural resource management. Good agronomic practices may potentially enhance carbon sinks and ensure better ecosystem services, crop and animal productivity and less methane emissions, and may also mitigate nitrous oxide emission and ammonia volatilization.

Thus, climate change is going to have far-reaching consequences in future for dairy and meat production in vulnerable parts of the world. Therefore, the upshot of climate change is that livestock is expected to exhibit a dual role of mitigation and adaptation in order to meet the challenge of food security. This book is designed to address the multidirectional link of livestock production and climate change under the three most important segments; in other words, livestock production, climate change and enteric methane amelioration. The book covers all the recent approaches and ameliorative measures to counteract the adverse action of climate change on livestock production and to minimize the enteric methane emissions from livestock. The multidirectional link of livestock and climate change is addressed in the subsequent three segments of the book, which are indispensable to the theme.

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2 Feed Resources vis-à-vis Livestock and Fish Productivity in a Changing Climate

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Abstract

Globally, livestock contributes 40% to agricultural gross domestic product (GDP), employs more than 1 billion people and creates livelihoods for more than 1 billion poor. From a nutritional standpoint, livestock contributes about 30% of the protein in human diets globally and more than 50% in developed countries. Aquaculture accounts for nearly 50% of global seafood production and employs more than 100 million people. As outlined in the livestock revolution scenario, consumption of animal-sourced food (ASF) will increase substantially, particularly in the so-called developing countries in response to urbanization and rising incomes, offering opportunities and income for smallholder producers and even the landless, thereby providing pathways out of poverty. It is important to recognize that the increasing demand for ASF pertains to ruminants (meat and milk), monogastrics (broilers, eggs and pork) and aquatic animals such as fish. To put it differently, much more animal feed will be needed for all domestic livestock and farmed aquatic animals in the future. Competition for feed among livestock and fish species will increase, in addition to competition with human food production and biomass needs for biofuels and soil health, unless we see significant levels of intensification of ASF production, and in ways that are environmentally sustainable. Animal source food production globally already faces increasing pressure because of negative environmental implications, particularly because of greenhouse gas emissions. As livestock and aquaculture are important sources of livelihood, it is necessary to find suitable solutions to convert these industries into economically viable enterprises, while reducing the ill effects of global warming. In relation to climate change, ASFs will have to play a dual role: one of mitigation and the other of adaptation.

The most evident and important effects of climate change on livestock production will be mediated through changes in feed resources. The main pathways in which climate change can affect the availability of feed resources for livestock – land-use and -systems changes, changes in the primary productivity of crops, forages and rangelands, changes in species composition and changes in the quality of plant material

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– will be discussed in the chapter. The chapter will propose an environmentally friendly development of livestock production systems, where increased production will be met by increased efficiency of production and not through increased animal numbers. For aquaculture, the focus will be on better sourcing of feedstuffs and on-farm feed management. Feeding strategies that increase the efficiency of production by producing more from fewer livestock animals and less feed will result in reduced greenhouse gas emissions. This will be demonstrated by analysing livestock populations in India and their respective level of productivity. Thus, in India in 2005/06, the daily milk yield of cross-bred, local cows and buffalo averaged 3.61 l, resulting in a ratio of feed metabolizable energy (ME) for maintenance and production of 2.2 to 1. By increasing daily milk production in a herd model (of a mixed cross-bred, local cow, buffalo population) from 3.61 to 15 l day^{-1}, energy expended for maintenance becomes 1:1.91. As a result, the same amount of milk can be produced by fewer livestock, leading to a reduction in emissions of methane of more than 1 million tonne (Mt) $year^{-1}$.

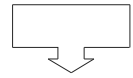
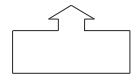
2.1 Introduction

2.1.1 Livestock: the good and the bad

Globally, livestock contributes 40% to agricultural gross domestic product (GDP), employs more than 1 billion people and creates livelihoods for more than 1 billion poor (Steinfeld *et al*., 2006). From a nutritional standpoint, livestock contributes about 30% of the protein in human diets globally, and more than 50% in developed countries. Moreover, livestock helps many farm households diversify livelihoods and reduce risks, particularly when crops fail. The relationships between livestock and the environment are complex, and appear to be viewed very differently in developed and developing country perspectives. The Food and Agriculture Organization report, *Livestock's Long Shadow*, focused on the effects of livestock on the environment (Steinfeld *et al*., 2006). The climate change impacts of livestock production (calculated in Steinfeld *et al*. (2006) at 18% of the total global greenhouse gas (GHG) emissions from human sources) have been widely highlighted, particularly those associated with rapidly expanding industrial livestock operations in Asia. Hall *et al*. (2011) estimate that 1% of GHG emissions, equivalent to 6–7% of agricultural GHG emissions, come from aquaculture. Global estimation also shows that livestock uses 30% of land, 70% of agricultural land and is an important agent of land degradation, deforestation, N and P in water supplies. Yet, in smallholder crop–livestock and agropastoral and pastoral livestock systems, livestock are one of a limited number of broad-based options to increase incomes and sustain the livelihoods of an estimated 1 billion people globally who have a limited environmental footprint. Livestock are particularly important for increasing the resilience of vulnerable poor people subject to climatic, market and disease shocks through diversifying risk and increasing assets. Given that almost all human activity is associated with GHG emissions, those from livestock and fish in these systems are relatively modest when compared to the contribution that livestock and farmed fish make to the livelihoods of this large number of people. The complex balancing act of resource use, GHG emissions and livelihoods is almost certain to get more rather than less complicated, because of the so-called livestock and blue revolutions.

As outlined in both the livestock revolution and recent fish production scenarios (Delgado *et al*., 1999; World Bank, 2013), the consumption of animal-sourced foods (ASFs) will rise in developing and emerging countries. Current and recommended future meat consumption patterns are summarized in Table 2.1. While the increasing demand for livestock products offers market opportunities and income for smallholder producers and even the landless, thereby providing pathways out of poverty (Kristjanson, 2009), livestock production globally faces increasing pressure because of negative environmental implications,

Table 2.1. Current daily meat consumption and convergent meat consumption levels recommended for 2050. (Data modified from McMichael *et al.*, 2007.)

Country/category	Current consumption	Recommended consumption
Developed countries Latin America	224 g day^{-1} 147 g day^{-1}	
		90[a] g day^{-1} or 20 g day^{-1} animal protein[b]
Developing countries Africa	47 g day^{-1} 31 g day^{-1}	

Notes: [a]A maximum of 50% of red meat; [b]equals on a yearly basis either: (i) 45 kg of fish; (ii) 60 kg of eggs; (iii) 230 kg of milk.

particularly because of GHG emissions (Steinfeld *et al.*, 2006). Besides GHGs, the high water requirement in livestock production is a major concern. As livestock is an important source of livelihood, it is necessary to find suitable solutions to convert this industry into an economically viable enterprise, while reducing the ill effects of global warming. In relation to climate change, livestock will have to play a dual role: one of mitigation and the other of adaptation.

By taking the livestock population and its current level of productivity in India, this chapter proposes a possible option that can address the issues associated with livestock–livelihood and livestock and the environment.

2.2 Climate Change on Key Livestock Systems Components Other Than Feed

2.2.1 Livestock genetics and breeding

Traditionally, the selection of animals in tropical breeds has been an adaptive one, but in recent times, market pull has stimulated a rapidly changing demand for higher production that could not be met quickly enough by breed improvement of indigenous animals. Widespread cross-breeding of animals, mostly with 'improver' breeds from temperate regions crossed with local animals, has occurred, often with poor results. Little systematic study has been conducted on matching genetic resources to different farming and market chain systems from already adapted and higher-producing tropical breeds. However, given the even greater climatic variability and stresses anticipated, this is a logical response to the adaptive challenges that will be faced. The greatest role for using the adaptive traits of indigenous animal genetic resources will be in more marginal systems in which climatic and other shocks are more common. Indigenous breeds, which have co-evolved in these systems over millennia and have adapted to the prevalent climatic and disease environments, will be essential (Baker and Rege, 1994). These systems are under substantial pressure arising from the need for increased production as well as land-use changes. Under these circumstances, ensuring continuing availability of these adapted animal breeds to meet the needs of an uncertain future is crucial.

Current animal breeding systems are not sufficient to meet this need and the improvement of breeding programmes under different livestock production and marketing contexts is a critical area for new research. The preservation of existing animal genetic diversity as a global insurance measure against unanticipated change has not been as well appreciated as has that for plants, although a recent report on the state of the world's animal genetic resources (FAO, 2007) and the accompanying Interlaken Declaration have highlighted this important issue. When conservation through use is insufficient (as is the wide-spread situation with indiscriminate cross-breeding), *ex situ*, especially *in vitro*, conservation needs to be considered as an

important component of a broad-based strategy to conserve critical adaptive genes and genetic traits.

2.2.2 Livestock (and human) health

The major impacts of climate change on livestock and human diseases have been focusing on vector-borne diseases. Increasing temperatures have supported the expansion of vector populations into cooler areas, either into higher-altitude systems (for example, malaria and tick-borne diseases in livestock) or into more temperate zones (for example, the spread of bluetongue disease in northern Europe). Changes in rainfall pattern can also influence an expansion of vectors during wetter years. This may lead to large outbreaks of disease, such as those seen in East Africa due to Rift Valley Fever virus, which is transmitted by a wide variety of biting insects. A good example is also the complexity of climate change influences with other factors associated with vector populations of tsetse flies in sub-Saharan Africa (McDermott *et al*., 2001). Helminth infections, particularly of small ruminants will be influenced greatly by changes in temperature and humidity. Climate changes could also influence disease distribution indirectly through changes in the distribution of livestock species. For example, areas becoming more arid would only be suitable for camels and small ruminants.

2.3 Feed Use and Its Projections

2.3.1 Current feed demand and use

Feed production is a key component in livestock production, not only because it is the key resource that fuels animal productivity but also because it is the key link between livestock, land and several regulating and provisioning ecosystem services such as water cycles, GHG emissions, carbon sequestration, maintenance of biodiversity, and others. The global use of feeds for livestock between 1992 and 2000 was estimated at 4.6–5.3 billion tonnes (Bt) of dry matter (DM) year^{-1} (Bouwman *et al*., 2005; Wirsenius *et al*., 2010; Herrero *et al*., 2013). Of this, grass comprises the majority of biomass consumed (2.3–2.4 Bt DM), followed by grains (0.5–1 Bt of concentrates), crop residues (0.5–1.2 Bt) and other feeds (cultivated fodders and legumes, occasional feeds, etc.). The larger ranges between grains and crop residues lie in different definitions of the feed components, with the lower bound for crop residues representing only stovers and the upper bound including some agroindustrial by-products like brans, oilseed cakes and others. The worldwide feed consumption by livestock as per animal type, system and feed type is presented in Table 2.2 and Fig. 2.1 (Herrero *et al*., 2013). Monogastrics dominate the use of grain globally and in most regions, with the exception of South Asia and the MENA region (Middle East and North Africa), where industrial monogastric production accounts for only 20–25% of production (Herrero *et al*., 2013). Meat animals, both cattle and small ruminants, consume the majority of fibrous feeds. In terms of regional differentiation, livestock in the developing world consumes most of the feed: grass (73%), crop residues (95%) and occasional feeds (90%), respectively (Herrero *et al*., 2013). This, coupled with the fast dynamics of livestock production growth in these regions, makes biomass dynamics a critical entry point in improving the sustainability of livestock enterprises in the future. Globally, mixed crop–livestock systems consume two-thirds of livestock fibrous feeds.

Herbivorous/omnivorous fish have traditionally been reared in pond systems dependent on autochthonous production (i.e. microorganisms, phyto- and zooplankton) enhanced by the application of limited quantities of on-farm crop and animal wastes that both provide a source of direct nutrition and boost autotrophic and heterotrophic production above natural levels (Brummett and Beveridge, 2015). However, through the increased use of feeds, production has been intensifying in order to generate more fish biomass per unit of land

Table 2.2. Feed consumption at the world level per animal type, system and feed type (thousand tonnes). (From Herrero *et al.*, 2013.)

		Grazing	Occasional	Stover	Grains	All feed
Cattle		1,902,557	403,187	520,441	225,987	3,052,172
	LGA	237,689	15,256	5,878	1,114	259,937
	LGH	133,285	13,914	22	733	147,953
	LGT	65,000	9,731	106	6,829	81,667
	MXA	338,742	150,439	264,856	38,677	792,714
	MXH	306,850	115,326	133,867	22,831	578,874
	MXT	296,118	27,590	76,912	108,861	509,481
	Others	408,842	35,283	24,366	30,543	499,034
	Urban	116,030	35,647	14,434	16,400	182,510
Small ruminants		359,623	155,940	51,886	59,867	627,316
	LGA	114,538	9,713	1,278	8,153	133,682
	LGH	18,021	1,450		1,726	21,196
	LGT	14,763	24,393		7,047	46,203
	MXA	97,831	40,070	33,971	17,127	188,999
	MXH	34,935	15,356	11,504	5,013	66,808
	MXT	22,293	39,604	3,038	11,277	76,212
	Other	39,166	19,596	1,327	6,180	66,269
	Urban	18,076	5,758	767	3,345	27,946
Pigs					537,129	537,129
	Smallholders				67,983	67,983
	Industrial				469,146	469,146
Poultry					476,329	476,329
	Smallholders				76,144	76,144
	Industrial				400,185	400,185
Livestock total		2, 262,180	559,127	572,327	1,299,312	4,692,946

Notes: LG = livestock grazing; MX = mixed crop–livestock system; A = arid; H = humid; T = temperate/highland.

and water use, and today only an estimated 30% of farmed fish production does not use any feeds (Tacon *et al.*, 2011). Temperature and sunlight, as well as nutrients, determine autochthonous production. Increases in temperature can be expected to affect productivity and fish growth and production up to a maximum for warm-water systems and species (e.g. catfish, tilapias and Indian major and Chinese carp) of 30°C and 25°C for common carp. Increases in rainfall may decrease autochthonous production through increased turbidity while decreases in rainfall might reduce pond volumes for production (Allison *et al.*, 2009).

Two types of feed are used: supplementary feeds, which are generally based on refractory, long-chain, carbohydrate-based crop wastes, such as rice, and wheat bran and oil cakes, sourced on-farm or locally (FAO, 2013). Such feeds, which have minimal processing and result in a moist dough or

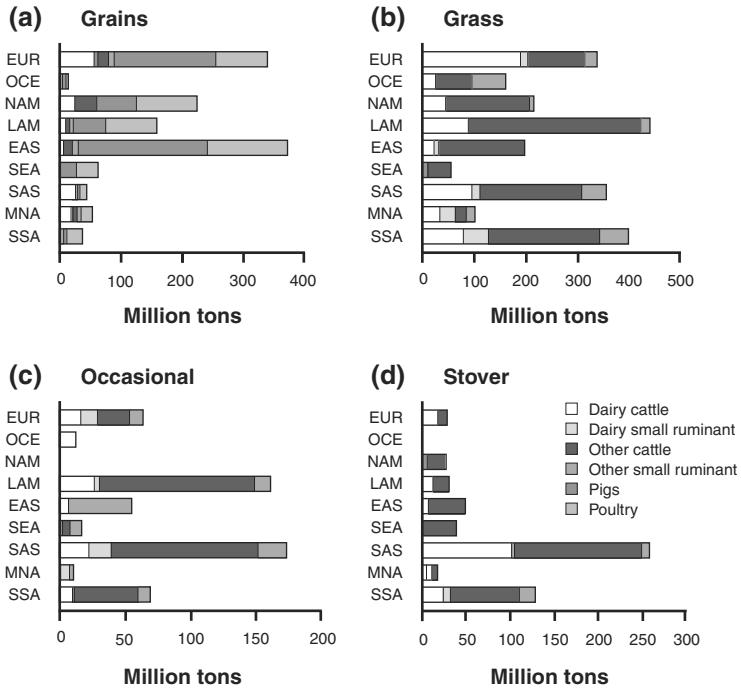


Fig. 2.1. Regional estimates of feed consumption by livestock species: (a) grains; (b) grazed grass; (c) occasional feeds; (d) stovers (million tonnes dry matter). EUR = Europe, OCE = Oceania, NAM = North America, LAM = Latin America, EAS = Eastern Asia, SEA = South East Asia, SAS = South Asia, MNA = Middle East and North Africa, SSA = Sub Saharan Africa. (From Herrero et al., 2013.)

simple moist or dried pellets, still dominate farmed fish production, especially of carp, tilapias and catfish in Asia. The impacts of climate change described above will affect crop production, and therefore quantity and quality (see following chapter). Nutritionally complete feeds, however, are becoming increasingly widely used. Such feeds were first developed for the trout and salmon industries and were based largely on fishmeal and fish oil, with crop-based feedstuffs added for energy and to bind the diet, the latter being of particular importance to maintain pellet integrity in water until consumed. For more omnivorous fish species, much less fish-based ingredients are required. Pelleted feeds are often manufactured by small, local feed mills, which often use low-quality feedstuffs and have a scant understanding of fish nutrition to produce diets that, although cheap, perform poorly. However, large multi-national companies, such as CP Foods in Asia, increasingly dominate aquaculture feed markets, bringing with them research knowledge, use of superior feedstuffs and extrusion technologies and technical support to producers. Total industrial compound aqua feed production has increased at an average rate of 11% per annum, from 7.6 million tonnes (Mt) in 1995 to 29.2 Mt in 2008 (Tacon *et al*., 2011). While extruded feeds have advantages over conventional pelleted feeds in terms of feed integrity and digestibility that translate into decreased food conversion ratios, they are more energy-intensive to produce.

The main crops used in the production of aquaculture feeds are soybean, rapeseed, maize and wheat bran (cf. livestock: maize, soybean cake, bran and wheat) (Troell *et al*., 2014, unpublished results). While there is some overlap with both demand from livestock and for human consumption,

aquaculture uses only 4% of the crop biomass used in livestock production (Troell *et al.*, 2014, unpublished results; Tacon *et al.*, 2011). While aquaculture can be expected to grow by at least 50% by 2030 (Hall *et al.*, 2011; World Bank, 2013; WRI, 2014), and while its dependency on feeds can be expected to grow by at least a similar amount, it very much depends on technology development and the markets and policy drivers as to what effects this will have on demand for feedstuffs and on the sector's impacts on climate change (WRI, 2014).

2.3.2 Projections of feed use to 2030

A number of studies have projected feed use to 2030 (Bouwman *et al.*, 2005; Wirsenius *et al.*, 2010; Havlik *et al.*, 2014; Troell *et al.*, 2014, unpublished results). Estimates of feed use by livestock to 2030 range from 6.5 to 8 billion tonnes (Bt) DM year^{-1}, depending mostly on assumptions about improvements in the quality of feed available or reductions in the demand for livestock products caused by human dietary transitions to diets with less meat (Fig. 2.2). Under business-as-usual conditions, the rate of growth of feed use to 2030 is projected to be between 2.9 and 3.3% year^{-1} (Bouwman *et al.*, 2005; Havlik *et al.*, 2014). Most of this growth is expected in tropical and subtropical areas that exhibit the highest growth rates in animal number and the highest increase in the demand for livestock products. For aquaculture, the estimates are in the order of 25 Mt of crops, growing to something like 35–45 Mt under various scenarios, within the next 30 years.

The dynamics of future feed use are dominated by large increases in grain use due to a faster increase in the consumption and production of pork and poultry, relative to ruminant products. Additionally, grassland expansion and/or intensification hold the key to future land use by ruminants (Herrero *et al.*, 2013; Havlik *et al.*, 2014). In the business-as-usual case, grasslands are

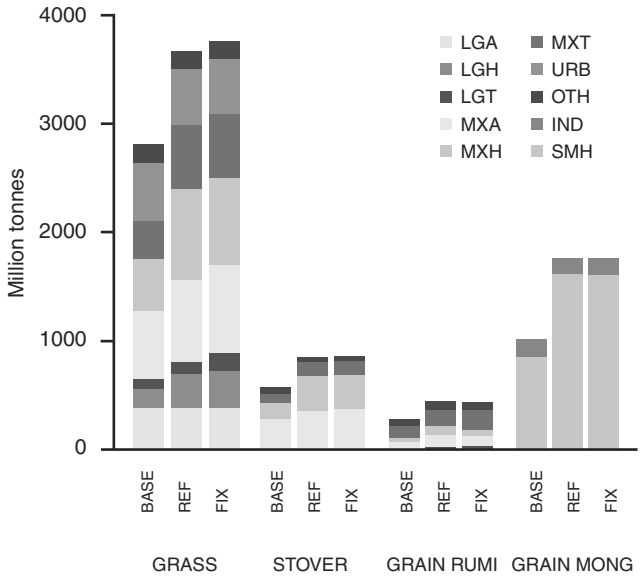


Fig. 2.2. Feed consumption in 2000 (BASE) and two contrasting scenarios (REF, FIX) to 2030. REF represents a scenario where systems transitions to more intensive mixed systems could occur to 2030, while FIX is a scenario where the proportions of production systems remain constant to 2030. LG = livestock grazing; MX = mixed crop–livestock system; A = arid; H = humid; T = temperate/highland; URB = urban; OTH = other; IND = industrial monogastrics; SMH = smallholder monogastrics; GRAIN RUMI = grain fed to ruminants; GRAIN MONG = grain fed to monogastrics. (From Havlik *et al.*, 2014.)

projected to provide an additional 0.8–1.3 Bt DM for ruminant production, while in alternative scenarios (Wirsenius *et al*., 2010; Havlik *et al*., 2014), grassland expansion contracts as systems intensify with higher-quality feeds or human diets change. Crop residues keep on playing a significant role, especially in alternative scenarios, as their relative proportions in ruminant diets increase slightly. This suggests that if these resources are also targeted for improved nutritional value, they could play an increased strategic role in using livestock as a vehicle for improving livelihoods, increased resource-use efficiencies and human nutrition in the future. The forecast for global feed availability by 2030 as per production system, animal and feed type is illustrated in Table 2.3.

2.4 Effect of Climate Change on Feed Resources and Quality

Despite the importance of livestock and fish to the poor and the magnitude of the changes that are likely to happen, the impacts of climate change on livestock

Table 2.3. Global feed projections to 2030 by livestock production system (thousand tonnes). (From Havlík *et al*., 2014.)

Row labels	Grazing	Occasional	Stover	Grains	Grand total
Cattle	2,376,674	450,973	758,563	341,388	3,927,597
LGA	177,456	9,452	2,896	518	190,323
LGH	213,086	19,998	9	1,315	234,408
LGT	68,399	5,898	77	7,308	81,682
MXA	372,966	134,724	301,582	76,858	886,129
MXH	551,769	148,338	286,885	58,140	1,045,132
MXT	468,679	61,671	128,352	150,335	809,038
Other	408,508	35,265	24,329	30,534	498,636
Urban	115,810	35,627	14,433	16,379	182,249
Small ruminants	568,116	277,486	97,459	99,039	1,042,100
LGA	173,961	28,723	1,296	12,428	216,409
LGH	49,796	4,134		3,308	57,239
LGT	14,442	46,387		10,691	71,520
MXA	162,061	77,960	60,091	30,305	330,417
MXH	83,890	53,342	30,826	18,302	186,361
MXT	26,723	41,586	3,152	14,479	85,939
Other	39,166	19,596	1,327	6,180	66,269
Urban	18,076	5,758	767	3,345	27,946
Pigs				907,391	907,391
Other				67,980	67,980
Urban				839,411	839,411
Poultry				852,073	852,073
Other				76,140	76,140
Urban				775,932	775,932
Grand total	2,944,789	728,458	856,022	2,199,890	6,729,160

Notes: LG = livestock grazing; MX = mixed crop–livestock system; A = arid; H = humid; T = temperate/highland.

systems, particularly in developing countries, is a neglected research area (Thornton *et al.*, 2009). The few existing predictions and impact assessments are qualitative, at large scale and lack comprehensiveness (Sirohi and Michaelowa, 2007; Nardone *et al.*, 2010). Most projections on climate change and its impacts focus on crop production (e.g. Sirohi and Michaelowa, 2007; Challinor and Wheeler, 2008; Thornton *et al.*, 2009). In view of increasing demand (note the preceding chapter) and the shrinking supply of livestock feed, such information gaps are worrisome.

Climate change is a significant and lasting change in the statistical distribution of weather patterns over periods ranging from decades to millions of years. It is caused by factors such as biotic processes, variations in solar radiation received by earth, plate tectonics and volcanic eruptions. Certain human activities, including crop and livestock production, have also been identified as significant causes of recent climate change (IPCC, 2007). As illustrated earlier, the impacts of climate change on livestock are multidimensional. The most evident and important effects of climate change on livestock production are mediated through changes in feed resources. Figure 2.3 illustrates a simplified flow diagram exemplifying the main pathways in which climate change can affect the availability of feed resources for livestock and links climate change and livestock feed quality and productivity. It summarizes it into: (i) impacts on biomass productivity; (ii) impacts on the composition of pasture species; and (iii) impacts on the chemical composition of feed resources (plant, e.g. Thornton *et al.*, 2009). However, information on the relative importance of these impacts is not available.

Predicted impacts are most often associated with different but interactive factors such as increase in temperature, carbon dioxide (CO_2) fertilization and land-use/land-cover changes, shortage of fresh water and greater incidences of rainfall, and change in length of growing period (LGP)

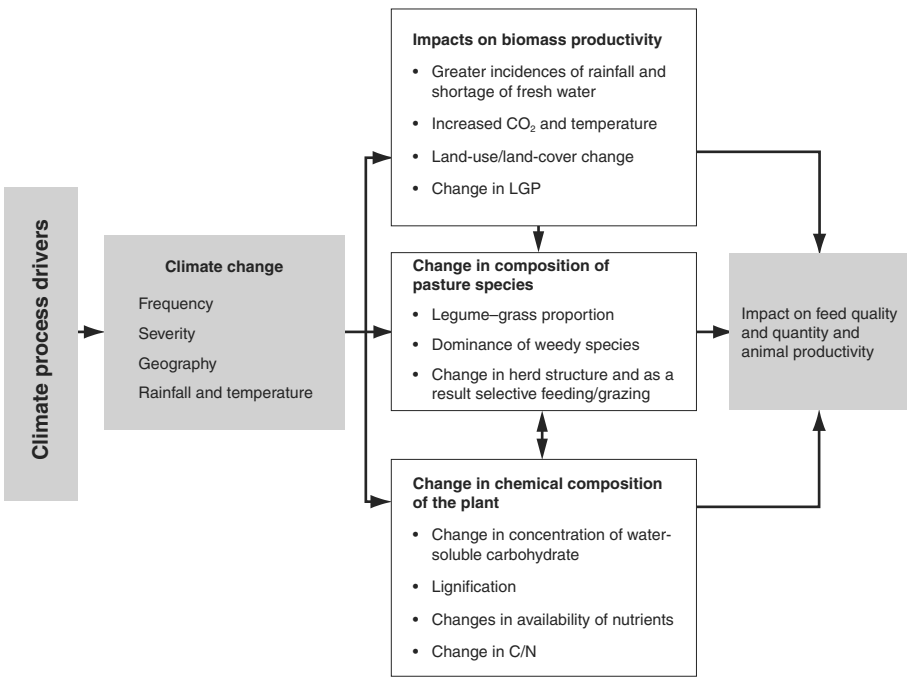


Fig. 2.3. Simplified flow diagram illustrating the main pathways in which climate change affects feed quality and productivity.

(IPCC, 2007; Sirohi and Michaelowa, 2007; Challinor and Wheeler, 2008; Thornton *et al*., 2009).

Parthasarathy and Hall (2003) suggested that 40–70% of the livestock feed sources in India, depending on the dominant ecoregions, comes from crop residues. Projections indicate that these roles will be intensified (Herrero *et al*., 2013). Pasture and grazing land is only about 3.4% (Sirohi and Michaelowa, 2007) of the total area, and thus the contribution is negligible. In view of these facts, here we try to focus only on the impacts of climate change on crop productivity, to understand its implications for livestock feed quality and productivities.

Despite acknowledged spatial variability and uncertainty on predictions, many model outputs suggest that precipitation will increase at higher latitudes and decrease in tropical and subtropical regions (IPCC, 2007). Crop yields are projected to fall in the tropics and subtropics by 10–20% by 2050 due to a combination of warming and drying, but in some places yield losses could be more severe. Future projections of climate change using global and regional climate models, run by the Indian Institute of Tropical Meteorology (IITM), with different Intergovernmental Panel on Climate Change (IPCC) emission scenarios, indicate temperature changes of about 3–5°C and an increase of about 5–10% in summer monsoon rainfall (NATCOM, 2004). It is also projected that the number of rainy days may decrease by 20–30%, which would mean that the intensity of rainfall is expected to increase. There are no comprehensive studies on the yield losses of all crops as the result of climate change. For major food–feed crops such as rice, wheat, sorghum and millet, there are fragmented studies, many concluding reduced yields, but with different magnitudes and underlying assumptions.

For example, prediction with and without CO_2 fertilization suggests different pictures. In this regard, for major food–feed crops such as millet and sorghum, losses of about 10–15% of grain yield during the second half of the 21st century are projected. Khan *et al*. (2009) suggested a strong linear decline in wheat yield with the increase in January temperature. According to these authors, for every degree increase in mean temperature, grain yield of wheat decreased by 428 kg ha^{-1}. For rice, an increase of 1°C in temperature resulted in a 5, 8, 5 and 7% decrease in grain yield in north, west, east and southern regions, respectively. An increase of 2°C in temperature resulted in a 10–16% reduction in yield in different regions, while a 4°C rise led to a 21–30% reduction. On interaction between CO_2 fertilization and increased temperature, for example at 350 ppm in north India, there was a change of –5, –12, –21, –25 and –31% in grain yield of rice with an increase of 1, 2, 3, 4 and 5°C in temperature, respectively. In the same region, and at the same temperatures but at 550 ppm, these yield changes were 12, 7, 1, –5 and –11%, respectively. Thus, in eastern and northern regions, the beneficial effect of 450, 550 and 650 ppm CO_2 was nullified by an increase of 1.2–1.7, 3.2–3.5 and 4.8–5.0°C, respectively (Challinor and Wheeler, 2008; Khan *et al*., 2009). In contrast to this nullifying effect, Thornton *et al*. (2009) discussed that such nullification of the impacts of increased temperature on productivity by CO_2 fertilization was very optimistic (for C_3 crops), and they suggested that such effects could only be partial. This argument obviously can lead to an overall decrease in grain yield and livestock feed productivity. The question is to understand what this implies for livestock feed sourcing, particularly in view of both regional- and global-scale projections illustrating a sharp increase in demand (Ramachandra *et al*., 2007; Herrero *et al*., 2013).

In a projection to 2020, Ramachandra *et al*. (2007) illustrated that crop residues as dry fodder sources would remain an important source of feed, and suggested a deficit level of about 10–11%. But the work of Ramachandra *et al*. (2007) did not take the impacts of climate change into account. Put differently, when the impacts of climate change are added to the current undersupply, the existing gaps for dry fodder will be amplified. It is important to note that the ongoing competition for uses of crop residues as a source of household energy,

soil and water management will worsen this adverse trend.

Although the pasturelands in India are increasingly shrinking, pocket-wise, they are important sources of feed. Permanent pasture and grazing land in India is about 3.4% of total agricultural land (Sirohi and Michaelowa, 2007). In addition to an expected reduction in biomass productivity, Sirohi and Michaelowa (2007) argue that additional changes of grassland in terms of composition of species (grass: legume species ratio) might be the result. The change in grassland composition could be in response to increasing temperature (resulting change in LGP) and also to a change in farmers' behaviour in adapting to certain livestock species (to capture opportunities and also to adapt to climate change), and thus selective feeding. For example, in the drylands of India in recent years, a significant increase in small ruminants has been noticed. Further controlled research as to how such a change can impact longer-term grassland composition needs to be confirmed empirically. The fact that legumes constitute important sources of crude protein (CP), such change in composition for pasture will impact the quality of livestock feed negatively.

As indicated in Roger *et al*. (2000) and Thornton *et al*. (2009), climate changes through increased CO_2 concentration will affect feed quality, particularly in terms of carbon/nitrogen (C/N). Higher C/N influences the microbial population, and thus digestibility of the feed. Also, increased temperature can result in lignification which leads to reduced digestibility and nutrient availability for livestock (Thornton *et al*., 2009).

Water scarcity has become globally significant over the period 1960–2000 or so, and is an accelerating condition for 1–2 billion people worldwide (MEA, 2005). The response of increased temperatures on water demand by livestock is well known. For *Bos indicus*, for example, water intake increases from about 3 kg kg^{-1} of DM intake at 10°C ambient temperature, to 5 kg at 30°C and to about 10 kg at 35°C (see Fig. 2.4; NRC, 1981).

However, about 100 times more water can be required for fodder production than for drinking water, resulting in low livestock water productivity. Empirical evidences across scales (both consumption and use efficiencies) vary significantly (Haileslassie *et al*., 2011). For example, in Gujarat, the heartland of the Indian white revolution, on

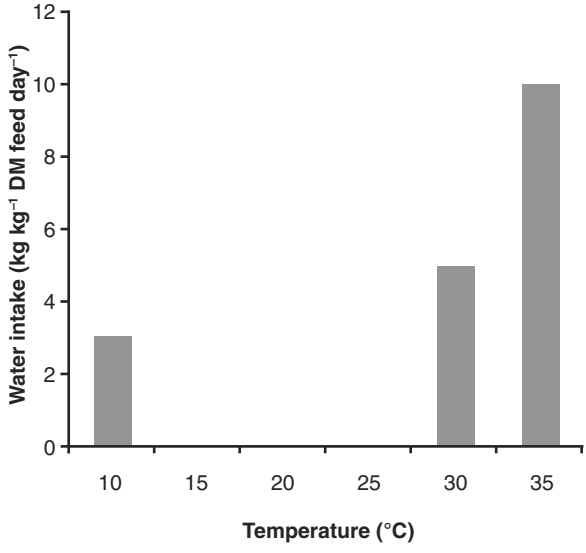


Fig. 2.4. Variation of livestock water intake as affected by temperature gradient and dry matter intake.

average 3400 l water are required for the production of 1 kg of milk (see Table 2.4).

Indirect effects on feed resources can have a significant impact on livestock productivity, carrying capacity of rangelands, buffering ability of ecosystems and their sustainability, price of stovers and grains, trade in feeds, changes in feeding options, GHG emissions and grazing management. Generally, dependency on crop residues, reduced digestibility as the result of lignification and/or change in species composition will have a negative feedback on the mitigation (e.g. increased GHG emission).

2.5 Option to Address Mitigation and Adaptation

Options to address the complex issue of feed–livestock–livelihood–climate change can be viewed from different angles. For example, one option, as is apparent from Fig. 2.5, is change in food habit from an inefficient ASF to a more efficient one. In this case, beef is a far less efficient source of calories and protein than milk and other meats (Wirsenius *et al*., 2010). But livestock uses in tropical countries are multiple and cannot be narrowed down to only energy and protein and the key issue is then how to strike a balance.

A closer look at the energy usage and productivity of animals in most tropical countries suggests that with low-producing animals, most of the feed is used for maintaining the animal and not for the production of ASF. Blümmel *et al*. (2013) used dairy production and productivity in India in 2005/06 as an example and found that only about 32% of the feed metabolizable energy was used for milk production. If daily

Table 2.4. Some water requirements and allocations in dairy production. (Data modified from Singh *et al*. (2004), who used a life cycle analysis.)

Site	Water required (l)	Produce
Gujarat	3,400	1 kg of milk
	10,000	Fodder production per animal per day
Global	900	1 kg of milk

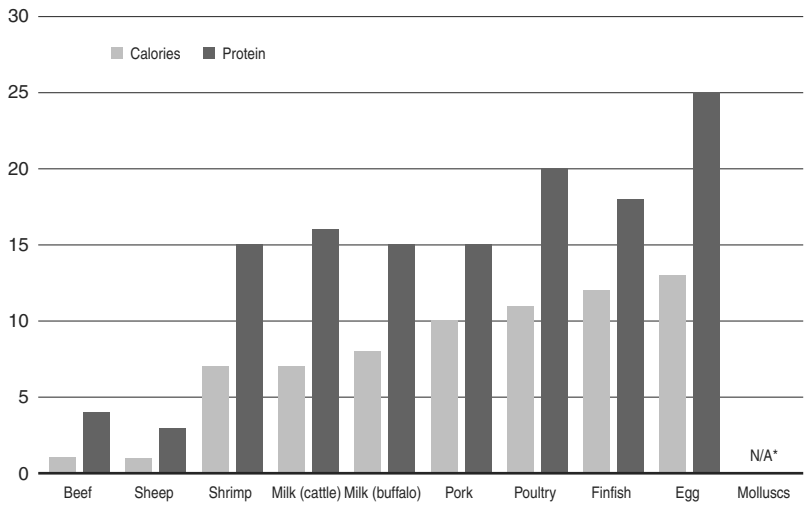


Fig. 2.5. Energy and protein production efficiencies of the different animal species (livestock species [% or "units of edible output per 100 units of feed or grass input]) Wirsenius *et al*. (2010). Note: 'edible output' refers to the calorie and protein content of bone-free carcass.

milk yield per animal would increase from the 2005/06 across-herd (buffalo, cross-bred and indigenous cattle) average of 3.61 kg to 15 kg, then the total feed metabolizable energy requirement would be reduced by over 50% (Table 2.5), resulting from fewer animals being needed to produce the same amount of milk. In other words, more than 50% less feed biomass would be required to produce the same amount of ASF.

It is highly improbable that the so-called livestock revolution can materialize without significant intensification in the production of ASF. These considerations are exemplified in Table 2.6 based on the dairy scenario in India, which in 2005 had a dairy livestock population of 69.75 million producing about 82 Mt of milk. By 2020, the demand for milk is predicted to increase to about 172 Mt. If per animal milk yield were to increase at the compound annual growth rate (AGR), average daily milk yield would be 5.2 kg and about 20 million more dairy animals would be required to meet the demand for milk. Given the already severe feed shortage and the mounting concerns about the negative environmental effects from livestock (illustrated in the preceding section), this is clearly not a viable strategy. In contrast, increasing per animal productivity as conceptualized in Table 2.5 would result in a significant reduction in animal numbers and feed requirements per unit produced (Blümmel *et al*., 2013).

The importance of per animal productivity for total feed requirement relative to ASF production can also be demonstrated for pigs, assuming a growth development from 10 to 80 kg of live weight and daily live weight gains (LWG) of 100, 200, 300, 400 and 500 g. Data were calculated according to Kirchgessner (1997), assuming that total metabolizable energy (ME) requirement equals ME for maintenance (ME) plus ME

Table 2.5. Actual across-herd average daily milk yields (3.6 kg) and scenario-dependent (6–15 kg) metabolizable feed energy requirements to support total Indian milk production of 81.8 million tonnes (Mt) in 2005 and reduction in methane production relative to milk production from fewer animals. (Data modified from Blümmel *et al*., 2013.)

Milk (kg day^{-1})	Metabolizable energy required (MJ * 10^9)			Methane production (Tg)
	Maintenance	Production	Total	CH_4 from 81.8 Mt of milk
3.6	1247.6	573.9	1821.5	2.3
6	749.9	573.9	1323.8	1.7
9	499.9	573.9	1073.8	1.4
12	374.9	573.9	948.8	1.2
15	299.9	573.9	873.9	1.1

Table 2.6. Milk demand in India in 2005/06 and in 2020 and dairy population and feed demand under across-herd yields of 3.61 kg day^{-1} in 2005/06, an estimated compounded annual growth rate in 2020 of 5.24 kg day^{-1} and needed average daily milk yield of 6.76 kg day^{-1} if the milk demand in 2020 is to be provided by the dairy livestock population of 2005/06.

	(2005/06)	2020	2020 (fixed DLP)
Milk (million tonnes)	81.8	172.0	172.0
Yield (kg day^{-1})	3.61	5.24	6.76
Dairy livestock population (DLP; millions head)	69.75	89.92*	69.76
Feed metabolizable energy requirements (MJ * 10^9)			
Maintenance	1,247.6	1,608.2	1,247.6
Production	573.9	1,075.0	1,075.0
Total	1,821.5	2,683.2	23,266.6
Feed requirements (tonnes)	247,500,000	364,570,000	315,600,000

for protein accretion (ME) plus ME for fat accretion (MEf). The following equations were used:

ME_m (kJ day^{-1}) = 719 * kg LW0.63 * 1.1. ME (2.1)

ME_p = 40.4 kJ g^{-1} protein (2.2)

ME_f = 52.7 kJ g^{-1} fat (2.3)

Protein and fat content in LWG were assumed to be 16.0% and 9.5% in LW up to 20 kg; 16.5 and 12.1% from LW 20–40 kg; 16.4 and 16.3% from LW 40–60 kg; and 15.9 and 20.9% from LW 60–80 kg, respectively (Kirchgessner, 1997).

Pigs that grow at 500 g day^{-1} would reach slaughter after 140 days, while pigs growing at 100 g daily would need 700 days. Such differences in fattening periods would obviously have severe implications for feed requirement for maintenance, and total feed requirement for the production of 70 kg of LW is much lower for faster-growing pigs (Fig. 2.6). Daily weight gains of 500 g would be approximately half of the achievable gains in highly intensified and specialized industrial state-of-the-art pig fattening enterprises, and still about 50% of the feed energy is used for maintenance purposes (Fig. 2.6). Contrarily, in dairy production, feed energy for maintenance and production would equal about 8 l day^{-1}, i.e. a moderate level of production (calculated from Table 2.4). Still, the feed input for 70 kg of live weight will decrease from about 7000 MJ ME to about 2200 MJ ME if pigs grow at a rate of 500 g day^{-1} rather than 100 g day^{-1}. In summary, if carefully planned and implemented, this approach will have both mitigation and adaptation impacts. Decreasing animal population and increasing per animal productivity will help in decreasing the total feed requirement in ruminants and monogastrics, with positive effects in terms of land and water resources and GHG emission.

The picture is less clear in aquaculture. According to recent projections in the *Fish to 2030* report (World Bank, 2013), capture fisheries production will remain fairly stable between 2010 and 2030. In contrast, global aquaculture production will maintain its

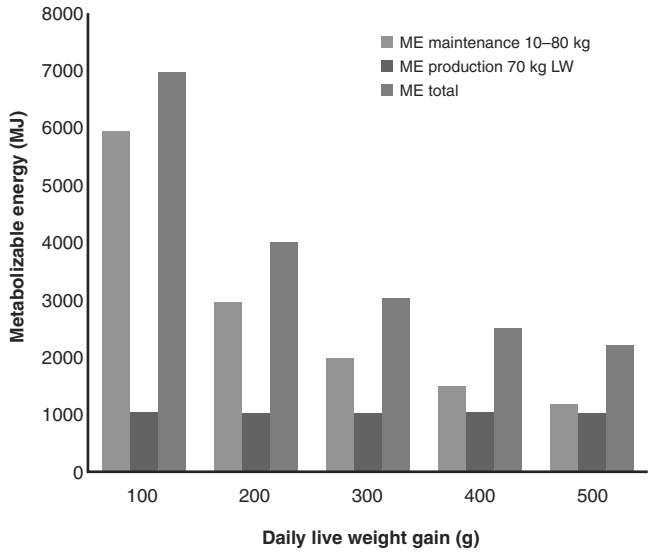


Fig. 2.6. Requirement of metabolizable feed energy (MJ) to produce 70 kg of live weight gain in pigs growing at daily rates of 100, 200, 300, 400 and 500 g.

steady rise from historical levels, reaching the point where it equals global capture production by 2030. Global fish supply, from both capture and culture, is projected to rise to 187 Mt by 2030, as compared to 147 Mt in 2010. The rapid growth of aquaculture has raised questions concerning its environmental sustainability. The *Blue Frontiers* study (Hall *et al*., 2011) compared the global and regional demands of aquaculture for a range of biophysical resources across the entire suite of species and production systems in use today. The units of analysis were the elements of a six-dimensional matrix comprising 13 species groups, 18 countries, 3 production intensities, 4 production systems, 2 habitats and 5 feed types. This gave 75 positive matrix elements that accounted for 85% of the estimated total world aquaculture production in that year. The data from the 75 species production systems reviewed showed a positive relationship between overall production levels and impact.

A comparison of environmental efficiencies across countries gave a variable picture. A look at the drivers of impact, i.e. those attributes of the production system that contributed most to environmental impact, showed that the fish production system itself contributed most to eutrophication, but impacts on climate change and acidification were dependent on the nature of the national energy supply; a factor outside the control of the local operator. The study also noted that fish convert a greater proportion of the food they eat into body mass than do livestock and therefore the environmental demands per unit biomass or protein produced are lower (Hall *et al*., 2011). A number of key conclusions and recommendations are identified in Hall *et al*. (2011) that point the way towards improved productivity for aquaculture with reduced environmental impact.

First, feed represents a significant influence on the environmental impact of aquaculture development, and reducing the dependency on fishmeal and fish oil, while requiring new innovations in technologies and management, will have spectacular payoffs both in terms of profitability, food and nutrition security and reduced environmental impact. Second, aquaculture has, from an ecological efficiency and environmental impact perspective, benefits over other forms of animal source food production for human consumption. In view of this, where resources are stretched, the relative benefits of policies for fish farming versus other forms of livestock production should be considered. Third, reductions can be made to the sector's impact on both climate change and acidification by improving energy efficiency throughout the production and value chains, and for more intensive systems shifting to alternative energy sources. Fourth, aquaculture affects climate change and climate change will affect aquaculture, and to minimize the potential for climate change, energy consumption should be minimized and new aquaculture enterprises should not be located in regions that are already high in sequestered carbon, such as mangroves, sea grass or forest areas.

2.6 Conclusion

Global and regional trends in livestock and fish feed resources need to be seen in the context of contraction (western hemispheres and Latin and Central America (LCA)) and convergence (developing and emerging countries) in the consumption of animal-sourced food and the impact of climate change. Climate change will affect livestock and fish production mainly through its effects on feed production and resourcing. A big unknown in global feed requirements resides with a decrease in ASF consumption (i.e. contraction), as recommended by health, environmental and ethical agencies for the western hemispheres and LCA; feedstuffs requirements in those regions will likely contract too. Actual feed resource demand in the developing and emerging countries will depend heavily on the degree of intensification in the sense of increasing per animal productivity. Focusing on productive animals will address both the adaptive and mitigation measures of climate change related to feeding and feed sourcing, associated natural resource usage (for

example, land and water) and GHG emission. Yet, despite the important role the sector is playing in the livelihood of smallholders in the tropical world, the livestock sector is an understudied area. For example, many studies addressing feed demand supply projections do not yet take the climate change scenario into consideration.

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3 Strategies for Alleviating Abiotic Stress in Livestock

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Abstract

The livestock sector accounts for 40% of the world's agriculture gross domestic product (GDP). It employs 1.3 billion people and creates livelihoods for 1 billion of the population living in poverty. Climate change is seen as a major threat to the survival of many species and ecosystems, and the sustainability of livestock production systems in many parts of the world. On the one hand, the current trend for the demand of livestock products is increasing, which offers market opportunities for small, marginal and landless farmers, while on the other hand, livestock production is facing the negative implications of environmental change, where abiotic stress is noteworthy. For animals, heat stress is the most stressful among all the abiotic stressors. Reducing the impact of abiotic stress on livestock requires a multidisciplinary approach with emphasis on nutrition, housing and health. Some biotechnological options may also be used to reduce abiotic stress. It is important to understand the livestock responses to environment and to analyse them carefully in order to alter nutritional and environment-related management practices. Future research needs for ameliorating abiotic stress in livestock are to identify strategies for developing and monitoring appropriate measures of heat stress; to assess the genetic components, including the genomics and proteomics of heat stress in livestock; and to develop alternative management practices for reducing abiotic stress and improving animal well-being and performance.

3.1 Introduction

Livestock systems, which occupy about 30% of the planet's ice-free terrestrial surface area, are a significant global asset, with a value of US$1.4 trillion (Steinfeld *et al*., 2006). Livestock farming is an important risk-reduction strategy for vulnerable communities, and livestock are important providers of nutrients and traction for growing crops in smallholder systems. Livestock products globally contribute 17% to calorie consumption and 33% to protein consumption (Rosegrant *et al*., 2009). The sustained improvement of incomes and rapid urbanization during 1970–2000 in parallel to population growth have prompted a higher demand for meat and other animal products, particularly in developing countries (FAO, 2009).

The World Bank has estimated that meat production should increase by 80% between 2000 and 2030 in order to meet the increased demand of livestock products. For this,

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available animal resources should be used more efficiently, while it is essential to preserve natural resources and to prevent pollution of the environment.

The livestock sector accounts for 40% of the world's agriculture gross domestic product (GDP) and creates livelihoods for 1 billion of the world's population living in poverty (FAO, 2009). Climate change is seen as a major threat to the survival of many species and ecosystems, and the sustainability of livestock production systems in many parts of the world. While the increasing demand for livestock products offers market opportunities and income for small, marginal and landless farmers, livestock production globally is facing the increasing pressure of negative environmental implications, particularly because of abiotic stresses (Sejian *et al*., 2013a).

Approximately one-third of the world's cattle population is located in arid zones, and according to IPCC predictions, the global average surface temperature may rise between 1.8 and 4°C by 2100 (IPCC, 2007). Livestock production is highly sensitive to climate change, and there is a non-linear relationship between climate change and livestock productivity (Kabubo-Mariara, 2009; Shinde and Sejian, 2013). The ecosystem that animals inhabit is a composite of biotic and abiotic factors that interact closely to determine the level of production of individual cattle. These factors or stressors, which include temperatures, diseases, pests and nutrition, are most harsh and unfavourable for production in the arid and semi-arid areas of the globe (Sejian, 2012).

Furthermore, changes in the frequency and severity of extreme climatic events will have more significant negative consequences on food production and food security. Increasing frequencies of heat stress, drought and flooding events are estimated to be more likely as a result of climate change and will have adverse effects on crop and livestock productivity (IPCC, 2007). The impacts of abiotic stress on livestock production and strategies for amelioration are subsequently discussed in this chapter.

3.2 Impact of Climate Change on Livestock Production

There is a range of thermal conditions within which animals maintain a relatively stable body temperature by altering behavioural and physiological activities. Heat stress in animals arises from their inability to dissipate sufficient heat from the body to maintain homeothermy. High ambient temperature, relative humidity and radiant energy compromise the ability of animals to dissipate heat. As a result, there is an increase in body temperature, which in turn initiates compensatory and adaptive mechanisms to re-establish homeothermy and homeostasis. These readjustments are generally referred to as adaptations and may be favourable or unfavourable to the economic interests of humans, but are essential for the survival of animals. Thus, an increase in air temperature would affect an animal's performance directly by affecting the animal's heat balance.

The potential direct and indirect impacts of climate change on livestock production have not been explored thoroughly. Changes in crop availability and quality, which have been the primary focus of previous studies, affect animal production through changes in feed supplies. The impact of climate change on feed quality and quantity is discussed in detail in Chapter 2, Section I, this volume. Changes in climate would lead directly to a reduction in milk yield and conception rates in dairy animals. Because voluntary feed intake (VFI) is the primary factor influencing the production capacity of livestock, the accurate prediction of the feed consumption of livestock under heat stress is a forerunner to assessing accurately production changes resulting from a warmer climate. The magnitude of the changes in production levels may be understood better by quantification of the potential impacts of climate change on livestock production. Projected economic losses resulting from temperature-induced reduction in production levels may justify mitigation of these temperature increases through alteration in management practices such as the

installation of shades, of sprinklers in feedlots, the evaporative cooling of barns, etc.

3.3 Impact of Abiotic Stress on Livestock Production

Factors such as temperature, solar radiation, humidity and wind, etc., have a direct impact on animals, whereas other factors such as feed intake, feed quality and quantity, digestibility, pests and diseases, which are themselves directly influenced by climate change, have indirect effects on animal production. Fundamentally, animal production is affected by climate change through:

1. Changes in livestock feed-grain availability.
2. Impacts on livestock pastures and forage crop production and quality.
3. Changes in the distribution of livestock diseases and pests.
4. Direct effects of weather on animal health, growth and reproduction (Smit *et al*., 1996).

Table 3.1 describes the direct and indirect impacts of abiotic stresses on livestock production.

3.3.1 Feed and fodder quality and availability

Change in climate is expected to have several impacts on feed and fodder crops and grazing systems in the following way: (i) changes in herbage growth brought about by changes in atmospheric carbon dioxide (CO_2) concentrations and temperature; (ii) changes in the composition of pastures, such as changes in the ratio of grasses to legumes; (iii) changes in herbage quality with changing concentrations of water-soluble carbohydrates and nitrogen at given dry matter (DM) yield; (iv) greater incidences of drought, which may offset DM yield increases; and (v) greater intensity of rainfall, which may increase nitrogen leaching in certain systems (Hopkins and Del Prado, 2007).

In terms of impacts on grasslands, sustained increase in mean temperatures results in significant changes in rangeland

Table 3.1. Direct and indirect effects of abiotic stresses on livestock production.

Growth	Milk production	Reproduction	Availability of feed and water	Distribution of livestock diseases
Reduced average daily gain	Reduction in milk quantity	Reduced intensity and duration of oestrus	Reduced pasture availability	Altered patterns of diseases in animals
Reduced body weight	Reduction in overall milk quality	Reduced LH level Reduced oestradiol	Reduced quantity of feed and fodder	Emergence of new diseases
Reduced BCS Reduced birth weight	Decrease in net energy, fat and protein	Low progesterone	Reduced micronutrient content in feed	Change in the prevalence of existing diseases
	Decreased per cent of casein and lactalbumin	Decreased quality of oocytes Increased embryonic mortality	Reduced water quantity and quality available for livestock	Changes in the distribution and abundance of disease vectors
	Decreased IgG & IgA	Reduced fertility rate		

Note: BCS = body condition scoring; LH = luteinizing hormone.

species distribution, composition and patterns, and biome distribution (Hanson *et al*., 1993). Increased temperatures lead to an increase in the lignification of plant tissues and therefore reduce the digestibility and degradation rates of plants (Minson, 1990), which can ultimately result in a below par genetic production potential of the animal. Apart from this, grasses in the tropical region are considered to be nutritionally poor, as they are C_4 plants and adopted a different photosynthetic pathway for acclimatizing themselves to the climate. C_4 plants have a higher photosynthetic rate, which in turn results in higher fibre content, low stem-to-leaf ratio and reduced digestibility and intake (Leng, 1984). Climate change will thus have the greatest impact on ruminant species (Blackburn and Mezzadra, 2006). Climate change impact on feed and fodder availability and quality is discussed at length in Chapter 2, Section I, this volume.

3.3.2 Effects on animals

A literature review by Sirohi and Michaelowa (2007) cites Hahn (1999) in giving the thermal comfort zone for temperate-region adult cattle as being in the range 5–15°C. McDowell (1972) reported that significant changes in feed intake and numerous physiological processes did not occur in the range 5–25°C. However, the thermal comfort zone is influenced by a range of factors, and is much higher in tropical breeds because of both better adaptation to heat and the lower food intake of most domestic cattle in smallholder systems. Hot weather can affect animal bioenergetics strongly, with adverse effects on the performance and well-being of livestock.

Apart from reduced feed intake, other biological mechanisms by which heat stress impacts production and reproduction include altered endocrine status, reduction in rumination and nutrient absorption, and increased maintenance requirements (Collier *et al*., 2005). This decrease in energy results in a reduction in energy balance, and partially explains why cows lose significant amounts of body weight when subjected to heat stress (reduced gut fill also contributes) (Baumgard and Rhoads, 2007). The impact of climate change on the various biological functions of animals is deliberated in the chapters of Section II, this volume.

Effects on growth

Growth, described as the increase in live body mass or cell multiplication, is controlled by both genetic and environmental factors (Marai *et al*., 2007). Sejian *et al*. (2010a) reported that elevated ambient temperature was considered to be one of the environmental factors influencing average daily gain. The reason for the effects of elevated ambient temperature on growth reduction could be due to the decrease in anabolic activity and the increase in tissue catabolism (Marai *et al*., 2007). This decrease in anabolism is essentially caused by a decrease in voluntary feed intake. The increase in tissue catabolism occurs mainly in fat deposits and/or lean body mass. In addition, the increase in tissue catabolism could be due to an increase in catecholamine and glucocorticoids after exposure to heat stress. Previous studies showed that under severe heat stress, such as trauma or shock, the systemic blood flow was redistributed (Ooue *et al*., 2007; Leon, 2008). To dissipate heat, the primary physiological autonomic responses increase blood flow to the body surface (Horowitz, 2003), resulting in early gastrointestinal ischaemia and hypoxia (Hinnebusch *et al*., 2002) and improper food assimilation, accountable for subnormal growth.

Effects on milk yield

With impounding heat stress, there is decline in physical activity and associated (direct and indirect) decline in levels of feed intake (Mader and Davis, 2004). In addition, high temperatures as well as reduced feed intake put a ceiling on dairy milk yield. In the tropics, this reduction may be between half and one-third of the actual potential of modern cow breeds (Parsons *et al*., 2001). Reduction in dry matter intake (DMI) is the major provider to decrease milk production

(Collier *et al*., 2005). When cows are affected by heat stress, there is also a reduction in rumination and nutrient absorption, with a simultaneous increase in maintenance requirement, resultant in a net deficit of the nutrient/energy available for productive traits (Collier *et al*., 2005). Studies by Rhoads *et al*. (2009) have shown that the reduction in DMI may be responsible for only a 36% decrease in milk production when cows are heat stressed, while 64% can be explained by other changes induced by heat stress. Rhoads *et al*. (2009) further demonstrated evidence of the reprioritization of post-absorptive nutrient partitioning in heat-stressed animals as the mobilization of adipose tissue is non-existent, contrary to the findings of increased cortisol, norepinephrine and epinephrine during acute heat stress (Collier *et al*., 2005) that normally stimulate lipolysis and mobilization of adipose tissues, and thus increased non-esterified fatty acids. Rather, glucose-sparing mechanisms seem to be prevented during heat stress due to metabolic adaptations (Rhoads *et al*., 2009). Hence, less availability of glucose becomes the limiting factor for milk production. During late pregnancy and the early post-partum period, a hot environment affects milk quality negatively and leads to lower colostrum net energy, fat and protein content. In addition, the analysis of protein fractions showed a reduction in the percentage of casein, lactalbumin, immunoglobulin G (IgG) and immunoglobulin A (IgA) (Nardone *et al*., 2006).

Effects on reproduction

The detrimental effects of heat stress on the reproduction processes of Holstein cattle have been well documented and include reduction in the intensity and duration of oestrus, reduction in the pulse and amplitude of luteinizing hormone, reduced oestradiol secretions, delayed ovulation, low progesterone concentration, reduced quality of oocytes, decreased blood flow to the uterus, increased uterine temperature, higher follicular persistency, changes in endometrial prostaglandin secretions, increased embryonic mortality and reduced fertility rates (Jordan, 2003). In bulls, heat stress has the followings adverse impacts: (i) hyperthermia of the scrotum; (ii) deterioration of semen quality, as evidenced by reduced semen motility, semen concentration, percentage of motile sperm and percentage of intact acrosome, increase in sperm abnormality; (iii) decreased testosterone levels; and (iv) reduced spermatogenesis (Hansen and Arechiga, 1999; Wolfenson, 2009). The effect of climate change on reproduction is described in detail in Chapter 12, Section II, this volume.

Somatic cells within the follicles (theca and granulosa cells) could be damaged by heat stress. Heat stress affects ovarian follicles and also induces a decrease in oestradiol synthesis (Sejian *et al*., 2011). It compromises oocyte growth in cows by altering progesterone, luteinizing hormone and follicle-stimulating hormone secretions during the oestrus cycle (Ronchi *et al*., 2001). Rensis and Scaramuzzi (2003) hypothesized that the dominant follicle developed in a low luteinizing hormone (LH) environment, resulting in reduced oestradiol secretion, inducing poor expression of oestrus by shortening its length and intensity. In summer, motor activity and other manifestations of oestrus are reduced (Nobel *et al*., 1997), and the incidence of anoestrus and silent ovulation are increased (Gwazdauskas *et al*., 1981). These effects are answerable for a reduction in the number of mounts, and thereby poor detection of oestrus in hot weather compared to cold weather (Pennington *et al*., 1985). Therefore, in hot climates, there is a reduction in the number of inseminations and an increase in the proportion of inseminations that do not result in pregnancy.

The plasma concentrations of insulin, IGF-I and glucose are decreased in the summer compared to the winter season (De Rensis *et al*., 2002), probably because of low dry matter intake and negative energy balance. Both IGF-I and glucose are generally stimulatory to follicular growth and implantation, and glucose is a primary fuel for the ovary (Rabiee *et al*., 1997). Glucose availability is also directly involved in

modulating LH secretion (Bucholtz *et al*., 1996), and severe hypoglycaemia inhibits pulsatile LH secretion and prevents ovulation (Jolly *et al*., 1995). This is another mechanism by which heat stress and its associated reduction in dry matter intake decrease the post-partum fertility of dairy cows. Further, the metabolic hormone, prolactin, is temperature sensitive and its levels in summer are increased (Ronchi *et al*., 2001), which can inhibit follicular development and suckling-induced prolactin secretion, leading to increased post-partum anoestrus in suckled cattle (Lupoli *et al*., 2001).

During pregnancy and prepartum, heat stress could decrease thyroid hormones and placental oestrogen levels, while increasing non-esterified fatty acid concentrations in blood; all of which can alter the growth of the udder and placenta, the nutrients delivered to the unborn calf and subsequent milk production (Collier *et al*., 1982). In males, semen quality (concentration, number of spermatozoa and motile cells/ejaculate) is lower in summer than in winter and spring (Mathevon *et al*., 1998). Heat stress reduces the libido of rams and causes sperm damage, reducing fertility, which will affect flock production. The effects of heat stress on the motility of ejaculated sperm in bulls persist for 6–8 weeks (Meyerhoeffer *et al*., 1985).

3.3.3 Distribution of livestock diseases and pests

Effects on pathogens

Higher temperatures may increase the rate of development of pathogens or parasites that spend some of their life cycle outside their animal host, which may lead to larger populations (Harvell *et al*., 2002). Climatic changes induced by global warming exert a selection pressure that will modify the biodiversity of pathogens (Lovejoy, 2008), their biomass and the epidemiology of the infections they cause. One potential consequence of significant and permanent changes to the climate is altered patterns of diseases in animals, including both the emergence of new disease syndromes and a change in the prevalence of existing diseases, including changes in the geographic range of existing vectors (Summers, 2009; Tabachnick, 2010). Therefore, animals may be exposed to entirely different parasites and diseases (Herrera *et al*., 2008), putting an even greater pressure on the production and survival of livestock breeds. Pathogens that are able to maintain and disseminate better in drought conditions would be expected to become dominant in areas where aridity would be increased under the influence of global warming. In general, it could be expected that pathogens having the lowest basic reproductive ratio, or R_0 (Morand and Guegan, 2008), would be most vulnerable to the changes induced by global warming.

Effects on the vector

There may be several impacts of climate change on disease vectors (midges, flies, ticks, mosquitoes and tsetse are all important vectors of livestock disease in the tropics). Changes in absolute rainfall or pattern and temperature regimes may affect both the distribution and abundance of disease vectors, as can changes in the frequency of extreme events (outbreaks of some mosquito-borne diseases have been linked to the El Niño–Southern Oscillation (ENSO), for example). It has also been shown that the ability of some insect vectors to become or remain infected with viruses (such as bluetongue) varies with temperature (Wittmann and Baylis, 2000). The feeding frequency of arthropod vectors may also increase with rises in temperature. As many vectors must feed twice on suitable hosts before transmission is possible (to acquire and then to transmit the infection), warmer temperatures may increase the likelihood of successful disease transmission.

Effects on the host

Baylis and Githeko (2006) observed that mammalian cellular immunity could be suppressed following heightened exposure to ultraviolet B radiation, which is an

expected outcome of stratospheric ozone depletion. So, greenhouse gas (GHG) emissions that affect ozone could have an impact on certain animal diseases, although this link has not been studied in livestock. A more important effect may be on the genetic resistance to disease. While animals often have evolved genetic resistance to diseases to which they are commonly exposed, they may be highly susceptible to new diseases. Climate change may bring substantial shifts in disease distribution, and outbreaks of severe disease could occur in previously unexposed animal populations (possibly with the breakdown of endemic stability).

Other indirect effects

Climate change may also affect the abundance and/or distribution of the competitors, predators and parasites of the vectors themselves, thus influencing the patterns of disease. It may also be that changes in ecosystems, driven by climate change and other drivers that affect land use, could give rise to new mixtures of species, thereby exposing hosts to novel pathogens and vectors, causing the emergence of new diseases (WHO, 1996).

3.4 Adaptive Mechanisms for Abiotic Stresses

3.4.1 Basic principles

The process by which animals respond to extreme climatic conditions includes: genetic or biological adaptation, phenotypic or physiological adaptation, acclimatization, acclimation and habituation. When environmental conditions change, an animal's ability to cope with (or adapt to) the new conditions is determined by its ability to maintain performance and oxidative metabolism.

An animal response to stress is influenced by a number of factors including: species, breed, previous exposure, health status, productivity level, body condition, mental state and age. Insufficient acclimatization or adaptation would determine what an animal experiences as stressful. The subsequent acclimatization or adaptation of the animal may alleviate the stress response. Animal response to stress usually results in a loss of performance (e.g. growth or reproduction) before cellular and molecular stress responses are activated. This suggests that the use of biological stress markers as an aid in selection may be limited. Figure 3.1 describes the different adaptive mechanisms in livestock for maintaining homeostasis.

3.4.2 Neuroendocrine mechanisms

Neuroendocrine responses to stress play an integral role in the maintenance of homeostasis in livestock. In general, activation of the stress circuitry inhibits functions such as growth and reproduction (Sejian, 2013; Sejian *et al*., 2013a). Substantial evidence suggests that neuroendocrine responses vary with the type of stressor and are specific and graded, rather than 'all or none'. While acute responses have important adaptive functions and are vital to coping and survival, chronic stressors elicit endocrine responses that may actually contribute to morbidity and mortality (Sejian *et al*., 2010b). Integration of these responses is possible through the network of mutual interactions that exist between the immune system, the central nervous system and the endocrine system (Sejian and Srivastava, 2010a,b). A crucial component of this network is the stress axis. Activation of the stress axis is accomplished through the release of several neurotransmitters and hormones. The stress axis, or the hypothalamo–pituitary–adrenal (HPA) axis, consists of three components: corticotrophin-releasing hormone (CRH), neurons in the hypothalamus, corticotrophs in the anterior pituitary and the adrenal cortex. A variety of molecular mediators have been implicated in the stimulation of CRH neurons, ranging from neurotransmitters, such as catecholamine to proinflammatory cytokines. CRH is an obligatory and primary stimulus for adrenocorticotropin hormone (ACTH) secretion by the pituitary gland. Subsequently, ACTH

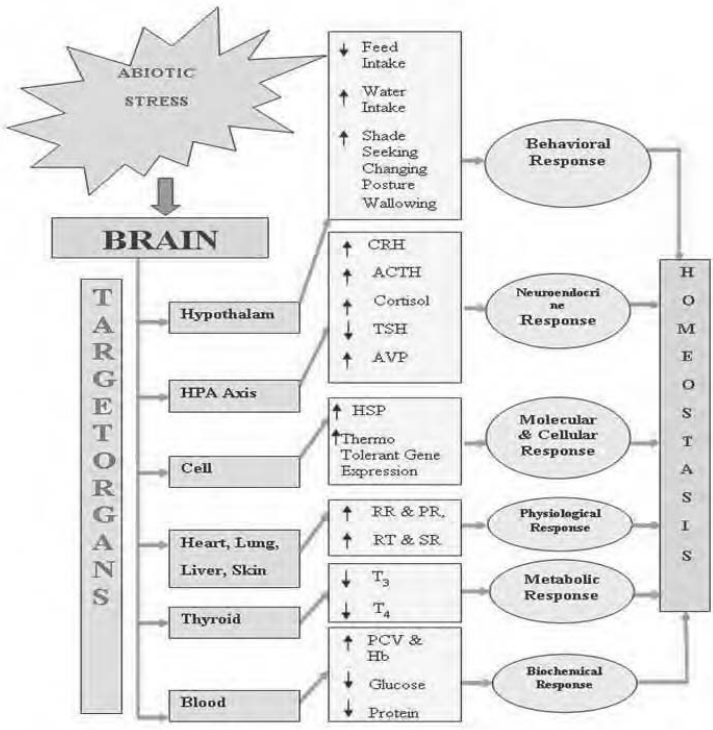

Fig. 3.1. Different livestock adaptive mechanisms to environmental stress (Sejian, 2013). HPA = hypothalamo–pituitary–adrenal; CRH = corticotropin-releasing hormone; ACTH = adrenocorticotrophic hormone; TSH = thyroid-stimulating hormone; AVP = arginine vasopressin; HSP = heat shock protein; RR = respiration rate; PR = pulse rate; RT = rectal temperature; SR = sweating rate; T_3 = tri-iodo-thyronine; T_4 = thyroxine; PCV = packed cell volume; Hb = haemoglobin.

stimulates glucocorticoid synthesis from the cells of the adrenal cortex. Glucocorticoid is the final activation product in the HPA axis and the primary effector molecule of this neuroendocrine circuit (Sejian, 2013). The secretion of ACTH, which is very crucial in this neuroendocrine response, seems to be regulated by a variety of peptides, but principally by CRH and vasopressin (VP; arginine vasopressin in most mammalian farm animal species, lysine vasopressin in pigs). CRH seems to be active mainly in the acute phase of stress, while VP is proposed to maintain HPA axis activity after repeated stimulation. In addition to the more traditional regulation of pituitary corticotrope function by hypothalamic peptides, pro-inflammatory cytokines are now recognized to play an important regulatory role in the HPA axis. Currently, interleukin-1, interleukin-6 and tumour necrosis factor are implicated strongly as stimulators of the stress axis. Cytokines are also capable of stimulating the secretion of the hormone, leptin, from adipocytes. Leptin is now recognized as an inhibitor of stress axis activity. Therefore, both leptin and glucocorticoids complete the negative feedback circuit to suppress stress axis activity and maintain homeostasis.

3.4.3 Heat shock response

Heat shock response is a rapid molecular mechanism that is transient and short acting and emerges via the production of heat shock proteins (HSPs) subsequent to exposure of the cells to sublethal stress. The heat shock response involves both heat

shock factors (HSFs) and HSPs. The heat shock response is induced by the accumulation of misfolded proteins in the cytoplasm, and is mediated by HSFs. To date, four HSFs have been identified: HSF-1, HSF-2, HSF-3 and HSF-4. HSF-1 plays a major role in heat shock response, while other members (HSF-2, HSF-4) are activated after prolonged stress or participate in normal cellular processes, embryonic development and cellular differentiation. Once activated, the HSF-1 monomer trimerizes with other HSF-1 molecules, which is essential for DNA binding. The activated complex can then enter into the nucleus and initiate transcription of HSPs.

HSFs are transcription factors regulating the expression of HSPs through interaction with specific DNA sequences in the promoter of HSP genes (heat shock elements). Heat shock elements are a stretch of DNA located in the promoter region of genes containing sequential multi-nucleotide, copies of 5′-nGAAn-3′, and are found in both HSP genes and a variety of other genes responsible for stress tolerance. The initial stimulus for HSF-1 appears to be recognition of the hydrophobic domains of denatured proteins, similar to the processes occurring during heat stress. When cells are exposed to heat stress, increased numbers of misfolded proteins are accumulated and postulated to have a competitive release of transcription factor from HSPs in the cytoplasm (Saxena and Krishnaswamy, 2012). HSPs were identified originally as proteins whose expression was increased markedly by heat shock. Several HSPs play important functions in normal cellular physiology. Induction of HSPs in mammalian cells starts within minutes after the initiation of thermal stress, with peak expression up to several hours later. HSPs possess chaperonin activity, and with this function they prevent the misaggregation of denatured proteins and assist in the proper refolding of denatured protein to native conformation. Some of the prototypical chaperonin HSPs are HSP40, HSP60, HSP70 and HSP90. HSP70, having a molecular weight ~70,000 daltons, is more abundant in cells stressed by elevated temperatures.

3.4.4 Molecular and cellular mechanisms

Understanding the cellular dynamics behind the short- and long-term adaptation of tropical food animals will be of great use in developing mollifying measures for improving productivity (Collier *et al*., 2008). HSPs are a family of approximately one dozen proteins, which are evolutionarily conserved and many of them function as molecular chaperones and critical regulators for protein folding and structural functions. Studies done on the unicellular yeast depict temporal variation in the gene expression profile when various stressors are used as treatment, and thereby many common environment-specific response genes (*CER*) have been identified constituting 18–38% of the genome. *CER* genes constitute induced expression of classical heat shock genes, osmotic stress protectants such as polyols and trehalose, protein degradation enzyme, genes involved in increased membrane permeability and ion transport, as well as compensatory expression of isozymes or allozymes and free radical scavengers like superoxide dismutase, glutathione system and cytochrome P450. *CER* also constitute genes that are repressed and associated with translation and protein synthesis to shunt energy in favour of large-scale adenosine triphosphate (ATP) requirement for chaperone function. Cells in response to stress also bring about changes in the ratio of saturated lipids to unsaturated lipids in their membrane to alter flexibility as well as transport across the membrane, which corresponds to homoviscous adaptation. Knowledge on the molecular mechanism of environmental stress is still in its infancy and may explain the biodiversity of animal genetic resources. In general, the cause of heat stress to the cell has the following consequences: (i) inhibition of deoxyribonucleic acid (DNA) synthesis, transcription, ribonucleic acid (RNA) processing and translation; (ii) inhibition of progression through the cell cycle; (iii) denaturation and aggregation of protein; (iv) increased degradation of protein through proteasomal and lysosomal pathways; (v) disruption of cytoskeletal elements (microtubules,

microfilaments and intermediate filaments); and (vi) changes in membrane permeability, leading to accumulation of intracellular sodium (Na), hydrogen (H) and calcium ions.

3.5 THI Index for Heat Stress Severity

Abiotic environmental factors having vital influence on the productivity of dairy cows are: air temperature, humidity, solar radiation and wind. Heat tolerance involves three factors: thermal environment (mainly temperature, humidity, solar radiation and wind), animal body and a suitable scale for numerical expression of the effect of the thermal environment on the animal body. Heat tolerance is normally measured on the environment or the animal. In terms of the environment, indices are normally created using factors such as temperature, humidity, wind speed and radiation load, among others.

Various indices derived from primary meteorological measures have been developed: wind-chill index (Siple and Passel, 1945); temperature–humidity index (Thom, 1959); black globe humidity index (Buffington *et al*., 1981); effective temperature for dairy cows (Yamamoto, 1983); equivalent temperature index for dairy cows (Baeta *et al*., 1987); thermal comfort index for sheep (Silva and Barbosa, 1993); heat load index for beef cattle (Gaughan *et al*., 2008); and environmental stress index (Moran *et al*., 2001). A good temperature–humidity index (THI) for measuring environmental warmth and its effect was developed for cattle (Kibler, 1964). However, sensors and indices do not adequately represent the complex physiological, behavioural and adaptive capabilities of animals. The indices must be appropriately tested for each environment and animal species. For example, a test carried out by Da Silva and co-workers (2005, unpublished results) with Holstein and Jersey dairy cows of several herds in the north-eastern region of Brazil (approximately 5° latitude) showed that the equivalent temperature index (Baeta *et al*., 1987) performed much better than other indices.

The THI, which uses dry bulb (Tdb) and wet bulb temperature (Twb), was initially developed by Thom (1959) as a heat index for human comfort, but it has remained the most common heat stress indicator used for different animal species. Various THI more adapted to cattle comfort were developed later (Bianca, 1962; Berry *et al*., 1964; NRC, 1971). Most of these indices were evaluated as potential predictors of heat stress and milk yield losses of dairy cattle using large data sets in humid and hot, tropical environments (Bohmanova *et al*., 2007; Dikmen and Hansen, 2009). The livestock weather safety index (LWSI; LCI, 1970) is a benchmark commonly used to assign heat stress levels to normal, alert, danger and emergency categories. The LWSI quantitates environmental conditions using the THI based on temperature and humidity only (Thom, 1959; NOAA, 1976). A THI between 70 and 74 is an indication to producers to be aware of the existence of potential heat stress in livestock. In the LWSI, THI values ≤74 are classified as alert, 74 < THI < 79 as danger and 79 ≤ THI < 84 as emergency. Other indices based on dry bulb temperature, wet bulb temperature, relative humidity or dew point have been developed (Buffington *et al*., 1981; Roseler *et al*., 1997).

Indices for cold stress are lesser studied than those for heat stress. A wind chill index (WCI) was developed by Siple and Passel (1945) relating ambient temperature (Ta) and wind speed (WS) to the time for freezing water, which was altered by Tew *et al*. (2002) to take biological factors into account. As indices for heat and cold stress are separate, Mader *et al*. (2010) proposed a comprehensive climate index (CCI), which incorporated adjustments for relative humidity (RH), WS and radiation (RAD) over conditions that encompassed both hot and cold environmental conditions. Although it covers a wider range of environmental conditions, index convolution confounds the analyses of variances using random regression methodology. Furthermore, solar radiation and wind speed are of minor importance for cows kept in insulated barns, implying that there is additional uncertainty if detailed information on the

management and housing conditions of farms is missing.

Although THI is still the most widespread indicator of heat stress, it does, however, have its limitations, because it is (i) an empirical representation; (ii) assumes that all animals react similarly to environmental stressors; and (iii) does not account for other environmental effects (e.g. WS and RAD) and cow-specific effects (e.g. age and breed). One problem with these indices is of not taking the cumulative effects of heat load, natural cooling, or both, into consideration. Mader *et al*. (2010) showed that animals might accumulate heat during the hottest hour of the day (with an accompanying rise in body temperature), but this heat was dissipated when the temperature fell. If this cooling process is insufficient or inefficient, the animals may enter the next hot period with an accumulated heat load (AHL; Hahn and Mader, 1997). These authors developed a THI hour model to account for intensity × duration of thermal status. St-Pierre *et al*. (2003) developed models using combinations of maximum THI, daily duration of heat stress and heat load index (HLI). Neither model accounts for air movement or solar radiation. The existing indices (THI) use the thermal situation at a point in time (intensity only). They do not take into account the effect of exposure (duration) to adverse thermal conditions. Furthermore, there is no genotype distinction, so all cattle are assumed to respond the same. As such, THI may under- or overestimate the effect of an adverse heat event, especially when night-time conditions are not considered. Night-time recovery (or a lack thereof) is an important element when assessing the heat load status of cattle (Hahn and Mader, 1997).

If night-time conditions are not considered, the heat load status of the animal may be underestimated. If the day following a heat event is cool, then underestimation is not critical. However, if the following day is hot (HLI > threshold), then cattle may enter the day with a carryover heat load and may be susceptible to heat stress at lower HLI values than expected.

Recently, technological advances have facilitated the collection of large and precise additional environmental parameters (e.g. RAD, WS and duration of exposure) and physiological parameters (e.g. respiration rate, rectal and core body temperature and sweating rate). New thermal indices (TI) incorporating environmental and physiological data in addition to cow specificities (e.g. breed and age) have been developed to overcome the various THI limitations (Gaughan *et al*., 2008; Mader *et al*., 2010). Several indices to measure heat tolerance have been developed over the years involving biological factors. The THI was adjusted for wind and solar radiation based on changes in panting scores (Mader *et al*., 2006) and on a respiration rate index using dry bulb temperature, RH, WS and solar radiation (Eigenberg *et al*., 2005). Marai *et al*. (2007) suggest the use of average relative deviations (ARD) from normal (positive or negative) in thermal, water and/or nitrogen balances of animals (or in all traits measured) due to exposure to hot climates for the detection of adaptability to a hot climate. Other indices such as tunica dartos indices (TDI; Marai *et al*., 2006) have been used during high ambient temperatures. The tunica dartos muscle extends to dissipate as much of the excess heat as possible from the testes.

Recent advances have proposed infrared thermography (IRT) as a method of measuring a larger number of animals in less time and without physical contact with the animals. Any variables that influence heat production are transmitted through blood capillaries and are dissipated in infrared waves. Stewart *et al*. (2005) stated that the effects of climatic conditions, daily and ultra-daily rhythms, feeding times, milking and rumination should be investigated when validating the method, as they interfere directly with hormone production and physiological responses. In the same study, ocular IRT was used as a stress measure in dairy cattle with a high correlation between ocular IRT temperature, cortisol and ACTH levels.

Stewart *et al*. (2007) observed that ocular IRT was related to regulation of the autonomous nervous system and therefore pain and stress (including thermal) directly interfere in this observation. According to

Knizkova *et al*. (2007), limitations involved in the method include those with images obtained in direct sunlight, exposed to wind or surfaces that are dirty. A major advantage of the method is that it does not require direct physical contact with the surface monitored, thus allowing remote reading of temperature distribution (Speakman and Ward, 1998). Stewart *et al.* (2005) recommended IRT as a non-invasive tool to study animal welfare as long as certain parameters were taken into account: the emissivity of the object, reflectance of the object and distance between the object and the camera (Knizkova *et al*., 2007).

Despite the above indices, most critical ranges cited are still those of Hahn (1985), with no consideration being taken into account of the type of animal, breed, physiological status, etc. Robertshaw (1985) showed that heat stress was a balance between metabolic heat gain and environmental exchanges (either gain or loss) through conduction, convection, radiation and evaporation (sweating or respiration). These should therefore be taken into consideration (either directly or indirectly) when evaluating heat tolerance.

3.6 Approaches for Alleviating Abiotic Stress

Adaptation options to climate change have been summarized by Kurukulasuriya and Rosenthal (2003), who define a typology of adaptation options that includes the following: (i) micro-level adaptation options, which include farm production adjustments such as diversification and intensification of crop and livestock production, land-use pattern and irrigation, and alterations in the timing of different operations; (ii) income-related responses that are potentially effective adaptation measures to climate change and include crop, livestock and flood insurance schemes, credit schemes and income diversification opportunities; (iii) institutional changes, including pricing policy adjustments such as the removal or putting in place of subsidies, the development of income stabilization options, agricultural policy including agricultural support and insurance programmes, improvements in local agricultural markets, and the promotion of interregional trade in agriculture; (iv) technological developments such as the development and promotion of new crop varieties and livestock feeds, improvements in water and soil management and improved animal health technology. In an excellent review, Beede and Collier (1986) identify three management strategies which can minimize the effects of thermal stress: (i) physical modification of the environment; (ii) genetic development of heat-tolerant breeds; and (iii) improved nutritional management. All these will be discussed below. Figure 3.2 describes the various strategies for reducing the impact of abiotic stress on livestock.

3.6.1 Genetic approach

Although all living organisms are naturally and frequently exposed to many kinds of stressors during their lifespan, it is the fittest that adapts better and survives to produce and reproduce, a process that occurs over a long period. Thermal stress has been shown to affect production indices in tropical regions (Silanikove, 2000). Nevertheless, well-adapted animals have been characterized by the maintenance or minimum loss of production during stress, high reproductive efficiency and disease resistance, as well as longevity and low mortality rates (West, 2003). The genetic approach to mitigate climate change adversity should include measures such as:

1. Identifying and strengthening of local genetic groups that are resilient to climatic stress/extremes.
2. Genetic selection for heat tolerance or bringing in types of animals that already have good heat tolerance and cross-breeding the local genetic population with heat- and disease-tolerant breeds.
3. Identifying the genes responsible for unique characteristics like disease tolerance, heat tolerance and ability to survive in low-input conditions, and using these as a

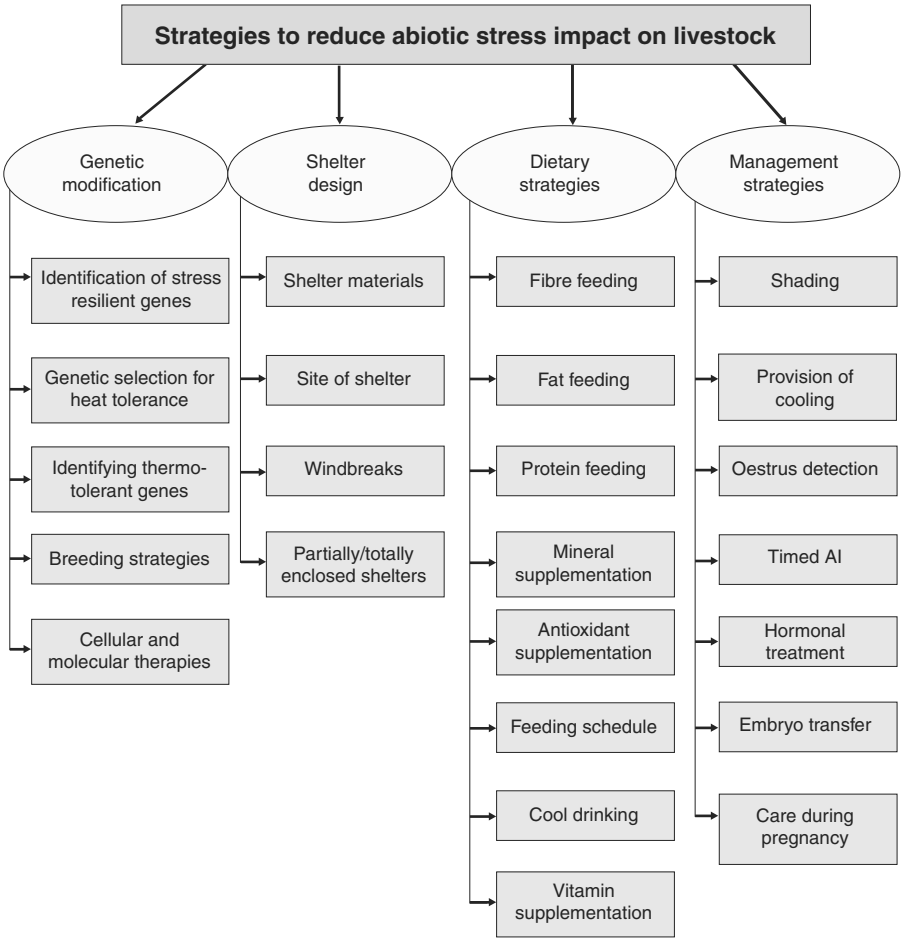

Fig. 3.2. Different strategies to reduce the impact of abiotic stress on livestock.

basis for the selection of future breeding stock will help to mitigate the adverse effects of climate stress.

Identification of stress-resilient gene pool

One strategy for reducing the magnitude of heat stress is to identify the animals that are genetically resistant to heat stress. Many local breeds have valuable adaptive traits that have developed over a long period, which include tolerance to extreme temperature, humidity, etc., tolerance/resistance to diseases and adaptation to survive and regularly produce/reproduce in low/poor management conditions and feeding regimes.

Thermotolerant strains or breeds have been developed in many livestock species (Hansen, 2011). Most of the negative effects of heat stress on animal performance are a consequence of either the physiological adaptations that homeotherms undergo to regulate body temperature or the failure to regulate body temperature (Hansen, 2011). Thus, selection for the regulation of body temperature during heat stress could result in animals that are resistant to the deleterious effects of heat stress. With novel emerging molecular techniques like

the rapid sequencing of genomes, the identification of region-specific and character-specific animals can be identified for further improvement.

Genetic selection for heat tolerance

Strictly regulated body temperature was found to promote the greatest productivity in beef cattle, and even small increases in body temperature had a negative effect on metabolic processes (Finch, 1986). The maintenance of body temperature is heritable through characteristics including sweating competence, low tissue resistance and coat structure and colour, but there is evidence that within *Bos taurus* cattle, an increased capacity for thermoregulation is accompanied by a reduction in energy metabolism (Finch, 1986). Turner (1982) reported that there was genetic variation of rectal temperature and that there was a negative correlation between rectal temperature and fertility, suggesting that selection for lower rectal temperature would improve fertility. However, the authors acknowledged that such selection had the potential to favour a lower metabolic rate or feed intake. Selection for heat tolerance without selection for an accompanied greater productivity would likely result in lower overall performance of the animal. Sweating response was found to correlate negatively with metabolic rate, suggesting difficulty in combining the desirable traits of heat adaptation and metabolic potential in cattle (Finch *et al.*, 1982).

As the genetic correlation between production and heat tolerance is approximately –0.3, the continued selection for production ignoring heat tolerance would hence result in decreasing heat tolerance. However, a combined selection for production and heat tolerance is possible because of the small correlation between the two. Further investigation in this area is necessary to exploit a genetic approach able to determine the heat tolerance of an animal while selecting for high milk yield potential (West, 2003). It may be necessary to develop strategies for the selection of dairy cattle for specific climatic conditions, enabling the improvement of genetic potential even in warm climates. Ravagnolo and Misztal (2000) proposed a model that used weather data from public weather stations to account for heat stress. Their model had two animal genetic effects, one corresponding to performance under mild conditions and the other with the rate of decline after crossing the threshold of heat stress. The application of this model in a genetic evaluation would predict rankings of animals in various environments with similar management but in different climatic conditions.

Identifying the genes responsible for tolerance

Given the complexity of the traits related to adaptation in tropical environments, the discovery of genes controlling these traits is a very difficult task. One obvious approach to identifying genes associated with acclimation to thermal stress is to utilize gene expression microarrays in models of thermal acclimation to identify changes in gene expression during acute and chronic thermal stress. Another approach would be with single gene deletions exposed to a defined thermal environment. This permits the identification of those genes that are involved in key regulatory pathways for thermal resistance and thermal sensitivity. Finally, gene knockout models in single cells will allow the better delineation of cellular metabolic machinery required for acclimatization to thermal stress. Those genes identified as key to the process of thermal acclimation will then need to be mapped to their chromosomal location, and the sequences of these genes will need to be determined in order to see if there are single nucleotide polymorphisms (SNPs) associated with changes in the coding for gene expression or protein function. Identification of SNPs associated with variation in animal resistance or sensitivity to thermal stress will permit the screening of animals for the presence or absence of desirable or undesirable alleles. However, further research is needed to quantify the genetic antagonism between adaptation and

production traits to evaluate the potential selection response.

Microarray analyses or genome-wide association-based studies identified the participating genes for cellular acclimation response and indicated that heat shock proteins were playing a major role in adaptation to thermal stress (Collier *et al*., 2008). In mammalian cells, non-lethal heat shock produces increased thermotolerance through the enhanced expression of heat shock genes. Additional genes of interest have been identified for fibroblast growth factor, solute carrier proteins, interleukins and tick resistance genes in recent studies. Genes associated with cellular metabolism and identified by microarray analysis only and not by genome-wide association studies include phosphofructo kinase, isocitrate dehydrogenase, NADH dehydrogenase, glycosyl transferase, transcription factor and mitochondrial inositol protein gene. Other genes of importance were thyroid hormone receptor, insulin-like growth factor II and annexin. Genes repressed in response to environmental stress are mostly concerned with the translation of genes for cytoplasmic ribosomal protein, DNA polymerase I, II and III, transcription, t-RNA synthetases, proteins required for processing ribosomal RNA and a subset of translation initiation factors. The identification of the variety of *CER* genes involved in stress responses suggests that these responses are aimed at the production of additional energy (ATP), maintenance of the environment, as well as the repression of protein synthesis to ensure energy conservation and minimize an unnecessary burden on the cell.

Two specific genes associated with the effects of heat stress on milk yield, the slick gene (Liu *et al*., 2011) and an allele of ATP1A1 (Olson *et al*., 2003), are associated with lower rectal temperature (RT). Olson *et al*. (2003) reported the presence of a slick hair gene in the bovine genome. This gene is dominant in mode, and cattle carrying the dominant allele of this gene have slick hair and are able to maintain body temperature at lower rates. Slick hair has a positive effect on growth and milk production under dry, tropical conditions. The phenomenon of cross-resistance where exposure to one stressor enhances resistance to other stressors has been noted by Hoffmann *et al*. (2003). This suggests that heat stress-tolerant cattle may also be tolerant to other stressors such as disease, reduced feed quality, parasites, etc. Such stress-tolerant cows may have lower culling rates and thus may stay in herds for longer. This fact has been confirmed in Drosophila, where a relationship between heat resistance and longevity has been found. However, whether or not this is valid in cattle is still unknown (Sørensen *et al*., 2003). Core body temperature during heat stress is a heritable trait in dairy cattle, with early estimates varying from 0.15 to 0.31°C (Seath, 1947) and a more recent estimate indicating a value of 0.17°C (Dikmen *et al*., 2012). Reliability of genetic estimates for rectal temperature, such as for other genetically controlled traits (Van Raden *et al*., 2009; Hayes *et al*., 2010), should be improved by genome-wide association studies (GWAS) to identify SNPs associated with the regulation of rectal temperature. Quantitative trait loci (QTL) can be identified for low heritability traits and used to improve the reliability of genetic estimates, despite the gain in reliability being less than for more heritable traits (Cole *et al*., 2011; Wiggans *et al*., 2011). In addition, GWAS can be useful for understanding the underlying biology of a trait by identifying candidate genes in physical proximity to QTL (Cole *et al*., 2011; Berry *et al*., 2012; Pfahler and Distl, 2012).

Breeding strategies

Changing the breeding animal every 2–3 years (exchange from other district herd) or artificial insemination with proven breed semen will help to enhance productivity. This may be supplemented with the supply of superior males through the formation of a nucleus herd at block level. Synchronization of the breeding period depending on the availability of feed and fodder resources results in healthy offspring and better weight gain. Local climate-resilient breeds of moderate productivity should be promoted over susceptible cross-breeds.

As the tools and techniques of breeding are changing, so too are the objectives of many breeding programmes. Although there is little evidence of direct genetic limits to selection for yield, if selection is focused too narrowly there may be undesirable associated responses (Simm *et al.*, 2004); for example, in dairy cattle, where along with genetic gain in some production traits, there is now considerable evidence of undesirable genetic changes in fertility, disease incidence and overall stress sensitivity, despite improved nutrition and general management (Hare *et al.*, 2006). Trade-offs are likely to become increasingly important between breeding for increased efficiency of resource use, knock-on impacts on fertility and other traits and environmental impacts such as methane production. Whole-system and life cycle assessment ('cradle-to-grave' analyses that assess the full range of relevant costs and benefits) will become increasingly important in disentangling these complexities.

There is considerable value in understanding better the match between livestock populations, breeds and genes with the physical, biological and economic landscape; this landscape livestock genomics approach should lead in the future to understanding the genetic basis of adaptation of the genotype to the environment (Sere *et al.*, 2008). These authors conclude that further selection for cattle lines with effective thermoregulatory control will be needed in future, although it may be difficult to combine the desirable twin traits of adaptation to high-temperature environments with high production potential. Under such challenges, balancing genotypes with production environments will become a crucial element requiring the utilization of diverse genetic resources with appropriate genetic potentials for growth, milk production, resistance to disease and prolificacy (Blackburn and Mezzadra, 2006).

Cellular and molecular therapies for abiotic stress amelioration

The tools of molecular genetics are likely to have considerable impact in the future. For example, DNA-based tests for genes or markers affecting traits that are difficult to measure currently, such as meat quality and disease resistance, will be particularly useful (Leakey, 2009). Another example is transgenic livestock for food production; these are technically feasible, although the technologies associated with livestock are at an earlier stage of development than the equivalent technologies in plants. In combination with new dissemination methods, such techniques could change livestock production dramatically. Complete genome maps for poultry and cattle now exist, and these open up the way for possible advances in evolutionary biology, animal breeding and animal models for human diseases (Lewin, 2009). Genomic selection should be able to at least double the rate of genetic gain in the dairy industry (Hayes *et al.*, 2009), as it enables selection decisions to be based on genomic breeding values, which can ultimately be calculated from genetic marker information alone rather than from pedigree and phenotypic information. Genomic selection is not without its challenges, but it is likely to revolutionize animal breeding.

Much of the effect of heat stress on the establishment and maintenance of pregnancy involves changes in ovarian function and embryonic development that together reduce the competence of the oocyte to be fertilized and the resultant embryo to develop. It is possible to manipulate the connection between hyperthermia and cellular responses to elevated temperature to improve fertility during heat stress. Three therapeutic approaches will be discussed here for doing so and what is known regarding the cellular and molecular basis for their efficacy. One of these is embryo transfer, which has been demonstrated repeatedly to reduce the magnitude of infertility associated with maternal heat stress. The second approach, manipulation of the antioxidant status of the female, has not yet been reduced to practice and has yielded equivocal results in the field. The third approach related with the selection of genes controlling cellular thermotolerance could lead to the development of lines of cattle with superior genetic resistance to heat

stress. Demonstration of the genetic differences in embryonic resistance to elevated temperature (i.e. heat shock) envisages that such genes exist, but their identity remains largely unknown (Hansen, 2013a). There are also direct effects of elevated temperature on nuclear maturation, spindle formation, cortical granule distribution, free radical formation, mitochondrial function and apoptosis (Soto and Smith, 2009; Andreu-Vazquez *et al*., 2010; Nabenishi *et al*., 2012).

Garcia-Ispierto *et al*. (2012) found that the administration of melatonin implants beginning at 220 days of gestation in cows during the summer reduced the interval to conception in the subsequent post-partum period and decreased the incidence of cows experiencing >3 breeding per conception. Melatonin has antioxidant properties in the follicle (Tamura *et al*., 2013) and had earlier been found to reduce the effects of heat stress in mice (Matsuzuka *et al*., 2005). Two antioxidants have been reported to protect embryos from heat shock in culture, anthocyanin (Sakatani *et al*., 2007) and dithiothreitol (Castroe Paula and Hansen, 2008).

Additional research into the development of practical delivery systems is warranted. It is also essential to evaluate whether the fertility-promoting effects of less known antioxidants exist in nature, because they may have different properties than the more commonly studied antioxidants. Two of these, the anthocyanins found in sweet potato (Sakatani *et al*., 2007) and epigallo catechingallate found in green tea (Roth *et al*., 2008), have been reported to protect the embryo (anthocyanins) or oocyte (epigallocatechingallate) from elevated temperature.

Genetic selection

There are also breed differences in cellular responses to elevated temperature. Nelore, Brahman and Romosinuano embryos are more resistant to the disruptive effects of elevated temperature on development than Angus or Holstein embryos. In addition, previous exposure to heat shock tended to reduce the ability of Angus blastocysts to establish pregnancy after transfer into recipients, whereas there was no effect for Nelore embryos (Silva *et al*., 2013). Breed differences in thermotolerance have also been observed in the endometrium (Malayer and Hansen, 1990) and lymphocytes (Kamwanja *et al*., 1994; Paula-Lopes *et al*., 2003).

Identification of the genes controlling cellular thermotolerance or of the genetic markers linked to those genes could lead to the selection of cattle possessing embryos with increased resistance to disruption by elevated temperature. To date, only one such genetic marker has been identified. Basiricò *et al*. (2011) studied the relationship between two SNPs in the 5′ untranslated region of the HSP70 gene and resistance of peripheral blood mononuclear cells from lactating Holsteins exposed to 43°C for 1 h *in vitro*. Both SNPs affected viability following heat shock. Moreover, the allele that was associated with increased survival also resulted in increased expression of the HSP70.1 gene. SNPs for rectal temperature during heat stress have been identified in Holsteins (Dikmen *et al*., 2013), as well as SNPs for cellular resistance to heat shock (Basiricò *et al*., 2011). Further advances in livestock genomics, including the incorporation of whole-genome sequencing into selection schemes (Hayes *et al*., 2013), should make it easier to identify and select alleles conferring thermotolerance for the whole animal and cellular level. Moreover, it will be possible to use molecular tools like transcription activator-like effector nuclease (TALENs) (Joung and Sander, 2013) to perform genome editing to change the sequence of specific genes and to introduce favourable alleles into the cattle population. Some of these alleles could represent new mutations not existing in nature that improve thermotolerance and other traits of importance in a warming world.

Marker-assisted selection through the use of high-throughput marker systems is currently being used extensively in breeding programmes to improve selection efficiency, accuracy and direct focus towards traits of great importance for adaptation (Sutton,

2009). The association between gene activity and response to abiotic stress is the major challenge involving functional genomics research and plant and animal breeding. New tools and approaches such as genetic modification, gene knockout, RNA interference, genomics, proteomics, metabolomics and metagenomics have allowed new insights in this field, and many advances in the role of genetics controlling complex traits such as those involved with response to abiotic stress.

3.6.2 Shelter design and abiotic stress

The housing of animals is a very important management aspect that can have a profound impact on livestock production, with an evident and timely result. This proves the fact that altering the microenvironment can improve the performance of animals. The major strategies providing elaborate housing involving shade, sprinklers, fans, air conditioners, etc., are capital-intensive, not very efficient and are of limited use for small and medium-size dairy farming.

Site selection

Fundamental to minimizing the effect of local weather is the selection of a site for housing. Climatic factors vary with height above the ground level at a given specific location and with terrain in a general location. Observations of the microclimate in a general location will reveal much variation in thermal conditions resulting from terrain features, differential exposure, wetlands, rivers, type and height of vegetation, human activities and other factors. Proper selection of a site to emphasize factors for enhanced heat dissipation (minimal radiation, air temperature and humidity, maximal air velocity) will have long-term protection.

Windbreaks

Grazing animals or animals giving birth will seek shelter from strong winds, especially during cold weather. Structures or trees can reduce wind speed markedly and can be beneficial to the survival of exposed animals (especially newborns). However, windbreaks have an importance much beyond these benefits, especially in the tropical and subtropical regions. First, high temperatures accompanied by dry winds may damage grass plants. Studies of the effects of wind on grass plants grown in controlled environments have shown that strong wind reduces grass growth as the result of damage to leaf surfaces, which affects the water relations of the plant (Grace, 1981); in addition, there is the more serious, indirect effect of the physical shaking of the plant. Second, while dependent on the available soil moisture, the harmful effects of high temperatures, high vapour pressure deficits and moderate to strong winds can increase the loss of water from evapotranspiration (Onyewotu *et al*., 2004).

Third, in a semi-arid region, the land is most vulnerable to wind erosion when vegetation cover is sparse and the soil is dry. Wind erosion is, in fact, one of the most important causes of desertification (Onyewotu *et al*., 2003a; Zheng *et al*., 2005). A windbreak acts as a barrier, lowering the wind speed near the ground surface, deviating and splitting the air stream. The protection achieved is determined by the configuration, height, density and thickness of the trees in a belt. The higher the windbreak, the greater will be the distance of its downwind (and upwind) protection, which involves reduction of soil erosion and soil moisture loss by evapotranspiration. The shelter effect on grassland growth has been estimated and an almost 20% increase in growth was found (WMO, 1996). If there is a depression in the immediate proximity of the trees, a maximum growth benefit can be observed at a distance of 2–5 times the height of the trees and little effect at distances greater than 15 times the height (Ruiz-Vega, 1994). However, in using trees as windbreaks, there is a trade-off between any enhanced growth of the associated grassland and the area occupied by the shelter trees, unless they have associated timber or fuel value (Onyewotu *et al*.,

2003b). The use of leguminous trees or shrubs can be a practical means to counteract the effects of wind and heat stress, as well as to improve animal diet.

Partially/ completely enclosed shelters

In temperate regions, partially enclosed shelters can reduce the thermal radiation received by animals during hot weather. Enclosed shelters are not recommended for tropical climates because of the decreased natural air velocity and sanitation. Under clear sky conditions, the average radiant heat load over a 7 h period was reduced almost 10% by the addition of a west wall to a simple shade; adding more walls helped, but to a lesser degree (Hahn *et al*., 1963). Provision of a partial west wall has been demonstrated to improve the productive performance of housed broilers in hot weather, while a partial east wall did not (Oliveira and Esmay, 1982). One can suppose that with cloud conditions, the benefit from a walled shelter should be even more pronounced, since the contribution of diffuse radiation would be reduced. There are no guidelines available for evaluating the benefits of open walled versus partially/fully enclosed shelters on animal performance, as the relative merits depend on many factors. For installations in temperate regions subject to both hot and cold weather, open front structures facing south (northern hemisphere) or north (southern hemisphere), with large doors or panels in the north (northern hemisphere) or south (southern hemisphere) wall, are an acceptable compromise. The use of fans in hot weather should be considered if natural air velocity is less than about 2 m s^{-1}.

3.6.3 Dietary strategies

Fibre feeding

There is a logical rationale of more heat production associated with acetate metabolism compared with propionate, and hence the feeding of low fibre ration during hot weather is recommended. Feeding more concentrate at the expense of fibrous ingredients increases the energy density of a diet and also reduces heat increment (Sejian *et al*., 2012). It has been proposed that a diet containing sufficient concentrates will ensure high propionate production with an adequate supply of NADPH to enable the acetate to be converted into fat, whereas on a high-roughage diet, the reverse has taken place and metabolic excess of acetate must be utilized for energy as a result of a futile cycle (Parker, 1984). In spite of negative effects, fibre quality is important all throughout the summer, as it has some buffering capacity and stimulates saliva production. Grant (1997) demonstrated that a diet having a neutral detergent fibre (NDF) value of 60% still provided sufficient fibre for the production of fat-corrected milk.

Fat

The inclusion of fat to the diet of lactating dairy cows is a common practice for increasing the energy density of the diet and potentially reduces the heat increment during hot weather. The addition of 3–5% of fat to the diet can be achieved without any adverse action on ruminal microflora (Palmquist and Jenkins, 1980). In thermoneutral conditions, cows receiving 25% of metabolizable energy (ME) from protected tallow had shown an 8–13.6% higher utilization efficiency of energy for lactation than those not receiving supplemental tallow (Kronfeld *et al*., 1980). The conversion of dietary fat to body fat is highly efficient when compared with the conversion of acetate to fatty acids (Baldwin *et al*., 1980). Heat-stressed cows do not want to oxidize body reserves for energy purposes, and hence the feeding of dietary fat (rumen inert/rumen bypass) probably remains an effective strategy of providing extra energy, especially during negative energy balance. Compared to starch and fibre, fat has a much lower heat increment in the rumen (Van Soest, 1982) and thus it can provide energy without a negative thermal side effect.

However, there are surprisingly few experiments designed specifically to

evaluate how supplemental dietary fat affects body temperature indices, or even production parameters. Reports from most of these experiments showed little or no differences in rectal temperatures (Chan *et al*., 1997; Drackely *et al*., 2003), and only the study of Wang *et al*. (2010) demonstrated a slight reduction in rectal temperature, which was at a specific time of day and not all the time. Contrarily, the findings of Moallem *et al*. (2010) indicated that cows fed on additional fat showed an increase in rectal temperatures. Wang *et al*. (2010) reported an increased respiration rate on the feeding of additional fat to cows. Overall results from a limited number of experiments varied, but little or no apparent benefit was typically observed when supplemental dietary fat was included. The reasons for the discrepancies are unclear, but could be due to the type of fats used for the supplementation (saturated versus unsaturated), the rate of inclusion, the type of protection (i.e. calcium salt versus prill), environmental factors (i.e. the severity of heat stress) or other dietary interactions. In one study (Holter *et al*., 1992), 15% whole cottonseed or 15% whole cottonseed plus 0.54 kg of calcium salt of fatty acids was added to the diet of lactating dairy cows and established that heat production declined by 6.7 and 9.7% in excess of maintenance, while total heat loss declined to the tune of 4.9 and 7.0%, respectively. Feeding unsaturated fatty acids during heat stress has illustrated benefits at different reproductive biological windows. Feeding unsaturated fatty acids to ewes has been shown to alter the lipid composition of oocytes, improving thermotolerance (Zeron *et al*., 2002). The nutritionist needs to have additional controlled experiments (besides theoretical heat calculations) in order to make intelligent ration-balancing decisions for the inclusion of supplemental fat and to arrive at the composition of fat mix to be given during heat stress.

Protein

As DMI declines, the quantity of consumed nutrients including crude protein (CP) also declines, and a negative protein balance may occur (Hassan and Roussel, 1975). During hot weather, dietary protein density is often increased to compensate for lower intake, and cows offered diets containing 20.8% CP during hot conditions had greater DMI and milk yield than those offered diets containing 14.3% CP (Hassan and Roussel, 1975). DMI usually declines with temperature increase and therefore a corresponding change in nutrient density is a must for sustaining productivity. The tendency is to increase dietary protein concentration above requirements, but there is an energetic cost associated with feeding excess protein. Excess nitrogen (N) above requirements reduces ME by 7.2 kcal g^{-1} of N (Tyrrell *et al*., 1970). When 19 and 23% CP diets were fed, milk yield was reduced by over 1.4 kg (Danfaer *et al*., 1980) and the energy cost associated with synthesizing and excreting urea accounted for the reduced milk yield (Oldham, 1984). Blood non-protein nitrogen (NPN) content was correlated positively with rectal temperature (Hassan and Roussel, 1975), suggesting reduced energy efficiency and greater heat production with excessive dietary N. Dietary protein degradability may be particularly critical under heat stress conditions. Diets with low (31.2% of CP) and high (39.2% of CP) rumen undegradable protein (RUP) fed during hot weather had no effect on DMI; however, milk yield increased by 2.4 kg day^{-1} and blood urea N declined from 17.5 to 13.3 mg 100 ml^{-1} for the diet containing higher RUP (Belibasakis *et al*., 1995). In addition, cooling the cow may affect the response of the cow to protein supplementation. When diets with a similar RUP content from high-quality (blood, fish and soybean meals) or lower-quality (maize gluten meal) proteins were fed to cows housed in shade or shade plus evaporative-cooled environments, cows fed high-quality RUP yielded 3.8 and 2.4 kg more milk in the evaporative cooled and shaded environments, respectively, than those fed on low-quality proteins (Chen *et al*., 1993). Although the interaction of protein quality by environment was not significant, the authors theorized that the greater response to high-quality protein for

cows in the cooled environment was because the amount of protein metabolized for energy was reduced and less energy was used in converting NH_3 to urea. In addition, cows in the cooled environment had higher milk yield and greater protein demand.

Minerals

During hot weather, declining DMI and high lactation demand requires increased dietary mineral concentration (West, 1999). However, alterations in mineral metabolism also affect the electrolyte status of the cow during hot weather. The primary cation in bovine sweat is potassium (K) (Jenkinson and Mabon, 1973), and sharp increases in the secretion of K through sweat occur during hot climatic conditions (Mallonee *et al*., 1985). The absorption of macrominerals including calcium (Ca), phosphorus (P) and K declined during hot temperatures (Kume *et al*., 1989). Kume *et al*. (1986) also reported that trace element requirements might increase with elevated environmental temperature. Unlike humans, bovines utilize potassium (K^+) as their primary osmotic regulator of water secretion from their sweat glands. As a consequence, K^+ requirement is increased (1.4–1.6% of DM) during the summer, and this should be adjusted through the diet (Sejian *et al*., 2013b). In addition, dietary levels of sodium (Na^+) and magnesium (Mg^{2+}) should be increased, as they compete with K^+ for intestinal absorption (West, 2002).

Water

Water intake not only is vital for milk production (milk is ~90% water) but also is essential for thermal homeostasis. In contrast to common perception, heat-stressed cows remain well hydrated (via large increases in water consumption) and actually may become hyperhydrated (Schneider *et al*., 1988). This illustrates how water availability and water/tank cleanliness become important during the summer months. Keeping water tanks clear of feed debris and algae is a simple and cheap strategy to help cows remain cool.

Glucose

Based on some of our recent data, maximizing the rumen production of glucose precursors (i.e. propionate) would be an effective strategy to maintain production. However, due to the rumen health issue, increasing grain allowance should be conducted with care. A safe and effective method of maximizing rumen propionate production is with monensin (approved for lactating dairy cattle in 2004). In addition, monensin may assist in stabilizing rumen pH during stress situations (Schelling, 1984). Proplyene glycol is typically fed in early lactation but may also be an effective method of increasing propionate production during heat stress. Ionophores are debated comprehensively in Chapter 17, Section III, this volume. Wheelock *et al*. (2010) previously demonstrated that maximizing rumen production of glucose precursors (i.e. propionate) might be an effective strategy to maintain production during heat stress. With the increasing demand for biofuels and the subsequent supply of glycerol, it will be of interest to evaluate glycerol efficacy and safety in ruminant diets during the summer season.

Vitamins

Niacin (nicotinic acid) is a potentially useful dietary supplement because it induces vasodilation, therefore transferring body heat to the periphery (Di Costanzo *et al*., 1997). Transferring body heat to the surface through peripheral or vasomotor function can perhaps alleviate some of the decrease in DMI, and thus maintain milk production. Feeding of antioxidant vitamins and minerals (vitamins A, C and E, selenium and zinc) reduces heat stress and optimizes feed intake (Pankaj *et al*., 2013).

Feeding schedule

Different feeding management strategies have been proposed to alleviate the effects of heat stress. Feeding during the cooler hours of the day or at night (night-time compensatory eating) is one technique that

has been recommended by several researchers and nutritionists. Evening-fed animals are able to cool down more quickly than morning-fed cows. However, time of feeding does not have any effect on the decline of milk production (Ominski *et al.*, 2002). Another relatively simple nutritional management strategy could be to increase the number of feedings per day (Beede and Collier, 1986). Altering feeding time and/or amount have been shown to be beneficial in reducing heat stress (Brosh *et al.*, 1998). Feeding cattle later in the day prevents the coincided occurrence of peak metabolic and environmental heat load (Brosh *et al.*, 1998). Limiting energy intake can decrease basal metabolic heat production effectively, and therefore decrease the total metabolic heat load of animals subjected to high environmental temperatures. Furthermore, energy restriction programmes have resulted in the improved efficiency of cattle maintained under a thermoneutral environment (Murphy and Loerch, 1994).

3.6.4 Management strategies

Heat stress in dairy cattle can be alleviated by different management interventions. The degree of improvement varies with the type of system provided, the climate and the production level of the cows.

Shading

In many moderate climates, shade is a cost-effective solution for reducing the radiation heat load of cows. It has been estimated that the total heat load can be reduced from 30 to 50% with a well-designed shade, and shading is one of the more easily implemented and economical options to minimize heat from solar radiation (Sejian *et al.*, 2012; Sejian, 2013). Shade changes the radiation balance of an animal but does not affect air temperature or humidity (Buffington *et al.*, 1981; Esmay, 1982). Cows in a shaded versus no shade environment had lower rectal temperatures (38.9 versus 39.4°C) and reduced respiratory rate (54 versus 82 breaths min^{-1}) and yielded 10% more milk (Roman-Ponce *et al.*, 1977). The radiant environment in shade has four constituent parts: the cold ground in the shade; the hot ground outside the shade; the lower (inner) surface of the roof; and the sky. The radiant temperature of the clear sky is, in general, much lower than that of the air, and even in a tropical location this difference may be of 25°C or even more. Thus, in areas with clear, sunny afternoons, shades should be 3–4.5 m high in order to permit maximum exposure to the relatively cool sky, which acts as an efficient radiation sink (Bond *et al.*, 1961). On the other hand, in areas with cloudy afternoons, shades of 2–2.5 m in height are better in order to limit the diffuse radiation received from the clouds by the animals beneath the shade (Hahn, 1981). In a tropical region, solar irradiance is high even during the winter, when its value is often double (1000 W m^{-2} or more) that observed in a location at 40° latitude (500 W m^{-2} or less).

As for the materials used, hay or straw shades are the most effective and cheap materials; solid shade provided by sheet metal painted white on top is next in effectiveness (Bond *et al.*, 1961). But aluminium sheets are better than a white painted surface (Bond *et al.*, 1969). Slats or other shade materials with less than total shading capabilities are considerably less effective; for example, slatted snow fencing with approximately 50% openings is only 59% as effective as new aluminium sheet (Kelly and Bond, 1958). The most effective shades are trees, as they provide protection from sunlight combined with beneficial cooling as moisture evaporates from the leaves. However, there are differences among species with respect to the protection given. Waldige (1994) observed that among *Mangifera indica*, *Caesalpinia* sp., *Pinus* sp. and *Casuarina* sp. in Brazil, the mango tree (*M. indica*) showed the best shading with the least radiant heat load; the worst type was the *Pinus*, which presented high heat loads.

Provision of cooling

Although shade reduces heat accumulation from solar radiation, there is no effect on air

temperature or relative humidity and additional cooling is necessary for lactating dairy cows in a hot, humid climate. A number of cooling options exist for lactating dairy cows, based on combinations of the principles of convection, conduction, radiation and evaporation. Air movement (fans), wetting of the cow and evaporation for cooling the air and shade to minimize the transfer of solar radiation are used to enhance heat dissipation. Fans and sprinklers offer a practical method of alleviating heat stress during the night by increasing heat loss at the animal surface through evaporative and convective means. Cooling with evaporative cooled air is effective in areas of low humidity. In more humid areas, this type of cooling is beneficial only in the daytime, when the humidity is low enough.

Correa-Calderon *et al*. (2004) investigated the effect of shade (C), spray and fan cooling (SF) and an evaporative cooling system called 'Korral Kool' (KK) on physiological responses during the summer on dairy cows in Arizona, USA. Koral Kool is a system that injects a fine mist generated at high water pressure into a stream of air. Keister *et al*. (2002) reported that the spray and fan cooling system could lower THI by two degrees compared to the outside environment. St-Pierre *et al*. (2003) proposed the functions of temperature (°C) and relative humidity (%) to quantify a decline in the temperature humidity index (ΔTHI) due to the use of different cooling systems:

$$\text{Moderate heat abatement – system of fans or forced ventilation: } \Delta THI = -11.06 + (0.257 \times T) + (0.027 \times RH) \quad (3.1)$$

$$\text{High heat abatement – combination of fans and sprinklers: } \Delta THI = -17.6 + (0.367 \times T) + (0.047 \times RH) \quad (3.2)$$

$$\text{Intense heat abatement – high-pressure evaporative cooling system: } \Delta THI = -11.7 + (0.16\ T) + (0.187 \times RH) \quad (3.3)$$

where T = temperature and RH = relative humidity. The implementation of heat-abatement facilities can enhance both pregnancy rates and milk production. Heat abatement is dependent on optimizing heat exchange via convection, conduction, radiation and evaporation. The system to be used depends on the local environment (e.g. arid to tropical) and includes the use of shades (reduction in solar radiation); sprinklers and fans under shade structures (enhances evaporative cooling from the skin surface); fans and sprinklers in the holding areas and/or exit lanes from the milking parlour; fans and sprinklers in free-stall facilities (e.g. cooling cows along the feed lines with sprinklers and fans); and evaporative cooling systems (i.e. cool the air that ultimately surrounds the cow).

A system of environmental management comprised of intermittent cooling with sprinkling and forced ventilation throughout the heat stress period in Israel improved conception rates (Berman and Wolfenson, 1992). Various cooling systems have been evaluated, and air conditioning dairy cows for 24 h day^{-1} improved fat-corrected milk (FCM) yield by 9.6% in Florida, USA (Thatcher, 1974). Work in Missouri showed that air conditioning was not an economic venture (Hahn and Osburn, 1969). Zone-cooled cows (cooled air blown over the head and neck) averaged 19% greater milk yield than the control (Roussel and Beatty, 1970), though other scientists concluded that a well-designed shade structure provided greater economic returns than the additional benefits derived from zone cooling (Canton *et al*., 1982). Large droplets from a low-pressure sprinkler system that completely wet the cow by soaking through the hair coat to the skin were more effective than a misting system (Armstrong, 1994). A combination of misters and fans was as effective as sprinklers and fans in work in Alabama, where intake and milk yield were similar for the misted cows (Lin *et al*., 1998). The fan/sprinkler system used about tenfold more water than the fan/mist system. Thus, attention to the water delivery rate through nozzle size or the use of fans and misters has proven effective in cooling cows while using substantially less water than systems evaluated in earlier research.

Evaporative cooling systems use high pressure, fine mist and large volumes of air

to evaporate moisture and cool the air surrounding the cow. Because of the evaporation, there is little wastewater to process in this type of cooling system, which is beneficial when developing a water budget for the dairy farm. Evaporative cooling systems improve the environment for lactating dairy cows in arid climates (Ryan *et al.*, 1992), and reduced air temperature results from the removal of the heat energy required to evaporate the water. Similarly, cows that were cooled using sprinklers and fans during the dry period maintained lower body temperatures and delivered calves that were 2.6 kg heavier, and cows averaged 3.5 kg more daily milk for the first 150 days of lactation than the shade-only control (Wolfenson *et al.*, 1988). Heat stress alters blood flow, potentially altering fetal development. Heat-stressed ewes delivered lambs that were 20% smaller than the control, and heat stress reduced uterine blood flow by 20–30%. The livers and brains of fetuses from heat-stressed ewes were substantially smaller than the control (Dreiling *et al.*, 1991).

Geothermal cooling

Recent interest in heat abatement has given rise to conductive cooling of lactating dairy cows. This novel approach was investigated by Bastian *et al.* (2003) and utilized waterbeds filled with chilled water as an alternative method for cooling dairy cows. However, the authors noted water accumulation as condensation on the surface of the beds, which could pose a significant health risk due to increased mastitis in lactating dairy cows. A novel approach similar to the waterbeds is currently being researched on geothermal cooling beds as heat exchangers placed 10–12 inches below sand or manure bedding in a free-stall dairy facility. The geothermal cooling technology could utilize chilled water or groundwater (depending on the temperature of water pumped from the ground) to pass through heat exchangers in order to lower the temperature of the bedding on which the cows lie. This could increase heat exchange from the cow to the cooler surface overall, lowering core body temperatures. This technology may be used in conjunction with current cooling technologies or to delay initiation of current cooling technologies, which may represent a significant reduction in energy costs and water consumption. Further studies are under way to examine the efficacy of geothermal cooling under different field conditions.

Provision of water

Water consumption is correlated positively with DMI (Murphy *et al.*, 1983), and thereby increases in DMI should improve digestion and hydration. Improving water content in the rumen tends to accelerate ruminal turnover (Silanikove, 1992), which could benefit the cow during hot weather due to a reduced digesta passage contributing to gut fill. Consumed water may also have a direct cooling effect via the reticulorumen (Beede and Collier, 1986). The benefits of improved water consumption are broad, with improvements during heat stress on cow comfort, DMI and milk yield being most apparent. Milk is approximately 90% water, and therefore water intake is vital for the production of milk and to maintain thermal homeostasis. There is a necessity for the optimization of management systems for different regions integrated with the production potential of the area. For example, in many tropical areas, the period of stress most often lasts for an extended period of the year and is coupled with diseases, parasites and low nutritional inputs. Obviously, a system under this environment needs to incorporate a management plan that not only protects animals from periods of thermal stress but also provides more stringent health care, well-being and nutritional inputs to reach the production potential of the animal in the system. Such systems involve increased investment of money to allow the maximal performance of high-producing animals.

3.7 Strategies to Improve Livestock Reproduction

One of the most important determinants in the energetic efficiency of livestock species is reproductive function. While great progress has been made in the development of pharmacological tools to regulate oestrous cyclicity in livestock, there are few tools at present for manipulating the female to increase pregnancy success (Hansen, 2013b). Key to the development of such tools is a more detailed understanding of the processes controlling the establishment and maintenance of pregnancy and of the errors in the reproductive process that limit fertility (Bisinotto and Santos, 2011).

3.7.1 Oestrus detection

One of the factors that increase the calving–conception interval of dairy cows during the hot season of the year is poor detection of oestrus. The use of tail-head paint, the heat watch system, radio-telemetric pressure transducers and pedometers can improve oestrus detection, and thus fertility. However, there are no published studies that have evaluated the effects of these aids to oestrus detection on summer infertility. Some dairy producers in Italy are turning to the use of natural breeding during summer in an attempt to overcome poor oestrus detection and improve fertility. However, the benefit of improved oestrus detection is offset by the deterioration in bull fertility caused by heat stress.

3.7.2 Timed artificial insemination

As the detection of oestrus becomes difficult in heat and humidity stress, the best strategy therefore would be to adopt oestrus synchronization and timed artificial insemination, which will improve pregnancy rates. A more detailed understanding of the processes controlling the establishment and maintenance of pregnancy and of the errors in the reproductive process that limit fertility will lead to modifications of timed artificial insemination protocols to improve the pregnancy rate (Bisinotto and Santos, 2011).

3.7.3 Hormonal treatment

The administration of either follicle-stimulating hormone (FSH) or somatotropin during one oestrous cycle in the autumn improved oocyte quality in the subsequent cycle (Roth *et al.*, 2002). Treatment with FSH increased the number of 6–9 mm follicles on the ovaries, while somatotropin increased the number of 3–5 mm follicles. In the subsequent cycle, FSH improved the proportion of aspirated oocytes that were classified as high-quality oocytes based on morphology, and also increased the cleavage rate following chemical activation. The effect of climate change on male and female reproduction is illustrated in detail in Chapter 12, Section II, this volume. Treatment with somatotropin improved oocyte morphology, but not the cleavage rate. Another study indicated that the pregnancy rate could be improved in the summer and autumn in primiparous cows by the use of gonadotropin-releasing hormone and prostaglandin F2α to generate three consecutive 9-day follicular waves, beginning at 50–60 days in milk (Friedman *et al.*, 2011). There was no benefit in multiparous cows and optimization of such treatments to improve pregnancy rates is warranted.

3.7.4 Embryo transfer

Two potential strategies for reducing the impact of heat stress on fertility have emerged from understanding how heat stress compromises oocyte function: hormonal-induced turnover of follicles and embryo transfer. The former has not yet been reduced to practice but the latter has been shown repeatedly to achieve pregnancy

rates in the summer that are equivalent to those seen with artificial insemination in cool months (Hansen, 2013). Embryo transfer is the only method presently available to improve fertility during heat stress (Hansen, 2013). When first formed, the preimplantation embryo is very susceptible to elevated temperature. Developmental competence of the zygote and two-cell embryo can be compromised by exposure to elevated temperature *in vitro* (Sakatani *et al*., 2004, 2012). Disruption of developmental competence involves reduced protein synthesis, swelling of mitochondria and cytoskeletal changes characterized by the movement of organelles towards the centre of the blastomere (Rivera *et al*., 2003). The generation of free radicals occurs in response to culture at elevated temperature, at least in embryos at day 0 and day 2 after insemination (Sakatani *et al*., 2004), and oxidative damage to macromolecules in the embryo could compromise development. Soon after the two-cell stage, the bovine embryo becomes more resistant to elevated temperature. Development of four- to eight-cell embryos can be compromised by heat shock, but to a lesser extent than for two-cell embryos (Edwards and Hansen, 1997).

Embryo transfer improves fertility during heat stress because it bypasses the loss of pregnancies caused by damage to the oocyte and preimplantation embryo (Hansen, 2013). Embryos are typically transferred into recipient females when they have reached the morula or blastocyst stages of development, typically at day 7 after ovulation. Embryos used for transfer have escaped the harmful consequences of heat stress on the oocyte and embryo, either because they were collected in the cool season, produced *in vitro* or represent the fraction of oocytes and embryos capable of continued development after heat shock. Bypassing the effects of heat stress on the oocyte and embryo, combined with the placement of a thermoresistant embryo in the uterus, means that pregnancy rates with embryo transfer during heat stress can be equivalent to those after artificial insemination during cool weather (Rodrigues *et al*., 2004). Embryo transfer can be expensive and the cost-effectiveness of the procedure depends on maintaining a high pregnancy success using a low-cost embryo (De Vries *et al*., 2011; Ribeiro *et al*., 2012).

One way to reduce the cost is to produce embryos *in vitro* using abattoir-derived oocytes. The promise represented by the use of embryos produced *in vitro* has been limited by problems with vitrification (Block *et al*., 2010; Stewart *et al*., 2011) and the reduced competence of embryos to establish pregnancy as compared to embryos produced *in vivo* (Numabe *et al*., 2000). Block and Hansen (2007) found seasonal variation in the pregnancy rate in Florida using embryos produced *in vitro*. The pregnancy rate at day 45 of gestation was 28% in the cool season and 18% in the warm season. The seasonal effect could be abolished, however, if embryos had been produced in culture in the presence of insulin-like growth factor 1 (IGF-1). In that case, pregnancy rates were 23% in the cool season and 49% in the warm season. Treatment with IGF-1 can make embryos resistant to heat shock (Jousan and Hansen, 2007).

3.7.5 Care during pregnancy

When dairy cattle are heat stressed during the last 2–3 months of pregnancy, there are clear reductions in placental function (reduced concentrations of oestrone sulfate), calf birth weight and subsequent milk production during the ensuing lactation (Collier *et al*., 1982; Moore *et al*., 1992). Indeed, cooling of dry cows during the latter stages of pregnancy is an efficient means to improve animal productivity; this is a physiologically sensitive period that often is ignored by producers. It is possible that the secretion of bovine placental lactogen has been reduced due to reduced placental function. Additional research is needed to determine if the administration of recombinant bovine placental lactogen during late pregnancy in heat-stressed cows would enhance both fetal growth and mammary development of the maternal unit. This

would potentially compensate for a potential deficiency in placental hormonal secretion induced by heat stress.

3.8 Conclusion

It is quite evident from various studies that abiotic stress factors are going to affect economic losses in livestock industries severely, and the severity is expected to be on the far end of the scale due to global warming and its ancillary effects. Livestock production and the corresponding food security can be sustained by using a broad range of scientific management practices that are region and situation specific. Right from altering the microclimate, which gives immediate effect to the genetic selection of tolerant breeds, all management practices should be executed carefully. The important constraint in developing countries where abiotic stress is going to have profound impact for ameliorative measures is the financial crunch. Maximizing the production level and the efficiency of livestock enterprises is important; however, economic considerations largely determine the level of environmental manipulation selected for livestock systems. Hence, economical alternatives for existing ameliorative measures are the need of the hour. Given the high genetic variability between and within breeds, it will be highly informative and beneficial to select for tolerance to heat stress. The major challenge, however, results from the complex nature of abiotic stress-tolerance traits and the difficulty in dissecting them into manageable genetic components feasible to modification by molecular approaches.

3.9 Future Prospects

Under the climate change scenario, different abiotic stresses will definitely impose stress on all species of livestock and will affect their production and reproduction adversely. The immediate need for livestock researchers aiming to counter abiotic stress impact on livestock production is to understand thoroughly the biology of stress response components and the measurements of animal well-being, giving researchers a basis for predicting when an animal is under stress or in distress and in need of attention. Future research needs for ameliorating abiotic stress in livestock are to identify strategies for developing and monitoring appropriate measures of heat stress; to assess the genetic components, including the genomics and proteomics, of heat stress in livestock; and to develop alternative management practices to reduce abiotic stress and improve animal well-being and performance. Substantial efforts are also needed to identify the specific genes associated with tolerance and sensitivity to different abiotic stress. Continued research evaluating genomic and proteomic approaches to improve the reproductive performance and nutritional status of abiotic-stressed animals is also warranted. Further research is also required to quantify the genetic antagonism between adaptation and production traits to evaluate the potential selection response.

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4 Nitrogen Emissions from Animal Agricultural Systems and Strategies to Protect the Environment

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Abstract

Animal production systems are among the largest contributors of reactive nitrogen to the environment. Nitrogen (N) is lost from animal agriculture through volatilization to the atmosphere (NH_3, N_2O, NO) and runoff and leaching to water resources (NH_4^+, NO_3^-, organic N). Most N losses from agriculture are in a form (NH_4^+, NH_3, NO_3^-) that does not directly affect climate change. However, these compounds have serious environmental consequences of their own, including contributing to haze, acidity of rain, eutrophication of surface water bodies and damage to forests. In addition, a significant amount of nitrous oxide (N_2O) emissions result from animal agriculture because the ammonium and nitrates from agriculture are converted to N_2O during manure storage and crop production. N_2O is a potent greenhouse gas. Although animals emit very little nitrogen directly to the air, animal excreta (urine and faeces) contains environmentally reactive nitrogen, which begins moving to the air and water from the moment it leaves the animal, unless it is incorporated into a crop or converted to molecular nitrogen (N_2). Nitrogen is lost from the barn floor or pen, storage facility and from cropland during manure application and crop growth. Additional nitrogen is lost to the environment when producing feeds for animals using nitrogen fertilizer. Strategies to control or mitigate nitrogen losses must simultaneously consider multiple forms of losses (e.g. NH_4^+, NH_3, N_2O and NO_x) in various media (e.g. air, water) for multiple processes (e.g. animal feeding, crop production, manure storage). Otherwise, management practices merely shift the losses from one form or source to another. Fortunately, process-based models assist with estimating emissions and optimizing management choices. Increased nutrient utilization efficiency of animals and crops has had the greatest impact on decreasing reactive nitrogen losses to the environment of all management strategies employed to date. Nonetheless, increased demand for animal products has continued to present environmental challenges.

4.1 Introduction

About 14.5% of all anthropogenic carbon dioxide (CO_2)-equivalent greenhouse gas (GHG) emissions are attributed to the livestock production life cycle (Gerber *et al*., 2013). Of this amount, about 44% of the GHG emissions from livestock are attributed to methane (CH_4) production from digestion by ruminants and anaerobic degradation of manure. The remaining 66% are attributed to the release of nitrous oxide (N_2O) from

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degradation of reactive nitrogen in manure and fertilizer used to grow crops for livestock. A relatively small amount of N_2O is also attributed to fuel combustion to run farm equipment and to transport and process animal products. Considering the relatively important impact of livestock production on GHG emissions, the potential to decrease GHG emissions from livestock production cannot be ignored.

According to the most recent Food and Agriculture Organization (FAO) report on GHG emissions (Gerber *et al*., 2013), the greatest potential for cuts in emissions is in low productivity livestock systems, especially in southern Asia, Latin America and Africa. Wider adoption of existing best management practices for feeding, health and animal husbandry could help decrease GHG emissions from livestock production by as much as 30% per unit of animal product. Better management of manure could also decrease emissions to a lesser extent. The principal strategy for decreasing GHG emissions is to decrease total nitrogen use in animal agriculture through improved efficiencies. This improvement has been part of an ongoing trend for the past several decades. However, as the world population grows and demands a higher-quality diet with more meat and vegetables, it will be increasingly important to improve agricultural efficiencies even faster to meet rising demands while decreasing environmental impacts.

4.2 The Fate of Nitrogen in Animal Agriculture

All living things contain nitrogen (N) in their protein and DNA, and 80% of the earth's atmosphere is molecular nitrogen gas (N_2). The triple-bonded N_2 gas in air is not chemically reactive or biologically available to most organisms until it is fixed in an energy-driven process to form reactive nitrogen such as ammonia, amines or nitrogen oxides. In nature, only a few species of bacteria and algae can reduce the N_2 in air chemically to form ammonia or amines. In addition, oxidation of N_2 can form nitrogen oxides (NO_x) at high temperatures, such as occurs from lightning. For billions of years, all life on earth depended on these natural processes as the original source of nitrogen in protein and DNA (Galloway and Cowling, 2002).

In 1913, two German scientists, Fritz Haber and Carl Bosch, invented a process to fix N chemically from the air. This process unlocked the ability to fix seemingly limitless amounts of nitrogen for agriculture, explosives and synthetic compounds. After World War II, the availability of reactive nitrogen ushered in the Green Revolution. In addition, the burning of fossil fuel in factories and automobiles accidentally produced more reactive nitrogen. Today, so much reactive nitrogen has leaked into the environment that natural ecosystems have changed substantially (Smil, 2001).

As chemically fixed N has become an inexpensive input to agriculture, we have come to consume greater quantities of animal products, fruits and vegetables that use a great deal of fertilizer per unit of N in those products. The result has been increased amounts of N going into air and water, with animal production contributing large amounts of N to air via animal manure. Although fertilizer has enabled rapid population growth and richer human diets, other aspects of modernization of agriculture have decreased nitrogen losses to the environment from what they would have been. Over the past several decades, trends in animal production have resulted in reduced output of manure N per unit of animal product. Nonetheless, increased use of N fertilizer for greater output of animal products and fruits and vegetables has resulted in an increased loss of N to the environment.

Animal agriculture is a major contributor of nitrogen (N) to air and water resources, particularly with respect to ammonia (NH_3), ammonium (NH_4^+), nitrate (NO_3^-) and, to a lesser extent, with respect to nitrous oxide (N_2O) and nitric oxide (NO). Of these, only N_2O is a major GHG; however, the other forms of reactive nitrogen can be converted to nitrous oxide in land and water systems. Worldwide,

more than half of the anthropogenic losses of reactive nitrogen to the air and more than 70% of the ammonia losses are estimated to derive from agricultural production (van Aardenne *et al*., 2001). About 50% of the anthropogenic ammonia losses to the environment derive directly from animal feedlots, manure storage or grazing systems, with additional losses occurring indirectly from cropping systems used to feed domestic animals as well as to feed humans directly. In addition, animals contribute 25% of the anthropogenic N_2O production, with an additional 25% coming from cropping systems. An overview of the flow of nitrogen in animal agriculture is presented in Fig. 4.1.

The US Environmental Protection Agency (2011) conducted an extensive life cycle analysis of the sources and sinks of reactive nitrogen into the environment for the USA. The committee estimated that about 35 Tg N $year^{-1}$ was created in the USA in 2002. Of this amount, about half was used for agriculture, including about 31% for nitrogen fixation for fertilizer and 22% fixed by legume crops. Combustion of fossil fuels in factories and transportation amounted to about 16% of the reactive N creation. About 6.4 Tg $year^{-1}$, or 18% of the total, was produced from natural causes such as biological fixation in natural landscapes and lightning.

The N in animal urine and faeces can be converted to ammonium (NH_4^+) by

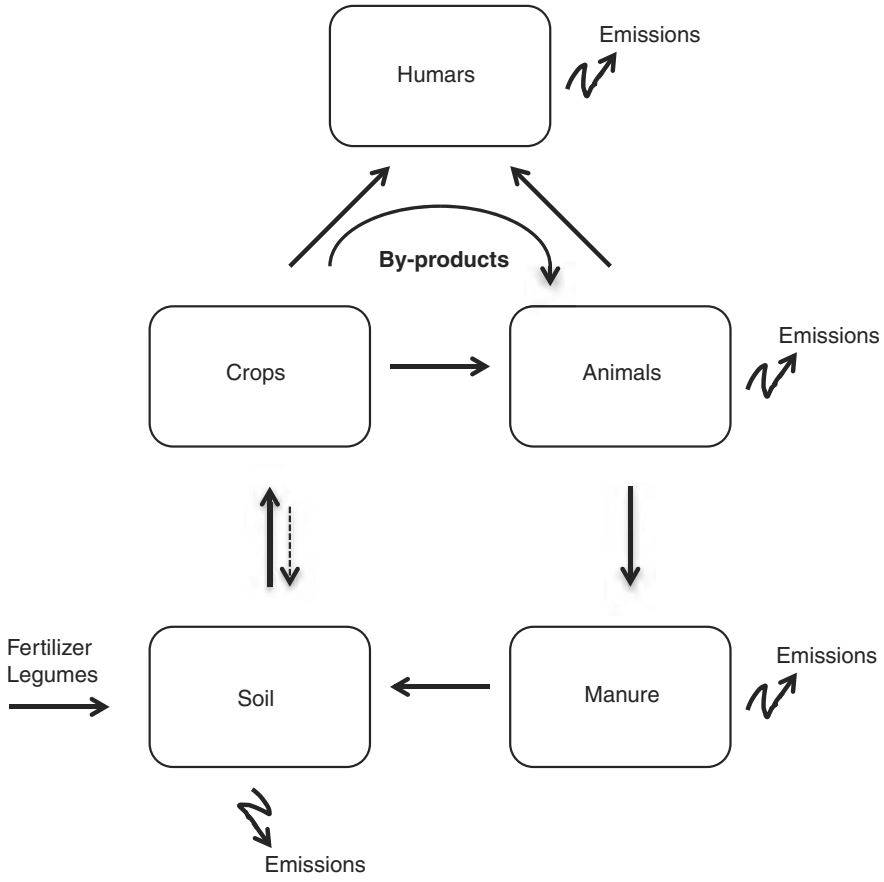

Fig. 4.1. The flow of nitrogen in animal agriculture.

hydrolysis of urea or deamination of amino acids after hydrolysis of proteins. Microorganisms and enzymes present in animals' faeces convert urea in urine to ammonium within hours of urination (Oenema *et al*., 2001). This ammonium equilibrates with ammonia (NH_3), especially at neutral pH, and the ammonia can be readily lost to air in a gaseous form from animal housing and manure storage. Ammonia emissions can occur immediately after excretion when urine N is hydrolysed, or more slowly when faecal N is decomposed and hydrolysed during manure storage and field application. Once emitted, the NH_3 can be converted back to NH_4^+ in the atmosphere, and this NH_4^+ reacts with acids (e.g. nitric acid, sulfuric acid) to form aerosols, particle matter with a diameter of less than 2.5 μm (PM 2.5). These small particles are considered a health concern for humans and a contributor to smog formation. Removal of ammonium by deposition contributes to soil and water acidity and ecosystem overfertilization or eutrophication.

Once reactive N is applied to crops or is deposited to land or water from the atmosphere, it undergoes various fates. In intensive cropping systems, almost half of the applied N is taken up for crop growth. Since manure N is not available to crops at the ideal predictable times, manure N is not used as efficiently as N from fertilizer and more N is applied to meet crop needs. Therefore, more N is also unaccounted for. The N applied that does not end up in a crop is lost to water in leachate or runoff of ammonium or nitrate, volatilized to air as NH_3 or N_2O or is denitrified back to molecular nitrogen (N_2). The various types of reactive nitrogen in soil and water are interconverted. Ammonium is converted to nitrate (NO_3^-) by aerobic microorganisms in soil in a process known as nitrification. The nitrate (NO_3^-) can be converted back to N_2 in a process called denitrification. Nitrous oxide (N_2O) is formed as a co-product of the microbial processes of nitrification and denitrification. Nitric oxide (NO) is released during nitrification in aerobic soils when manure or other fertilizer is applied.

Nitrous oxide (N_2O) is the primary nitrogenous GHG emission. Sources of nitrous oxide in the atmosphere include combustion of fossil fuels and lightning. Animals do not directly produce any nitrogenous gases, but manure storage and crop production convert ammonium and nitrate to N_2O, and reactive nitrogen that flows to natural ecosystems (e.g. forests and estuaries) can also be converted to N_2O. Therefore, animal agriculture ends up contributing significantly to the production of nitrogenous GHG.

The Intergovernmental Panel on Climate Change (IPCC) assumed that 1% of applied N fertilizer was lost from direct emissions of N_2O at the field level due to nitrification and denitrification (IPCC, 2007). This same value is applied to all forms of reactive N, including chemical fertilizer, manure and other organic forms. Studies confirm that N_2O losses from soils can exceed 1% of applied N in intensive high-yield cropping systems (Adviento-Borbe *et al*., 2006). Others have estimated that as much as 3–5% of applied nitrogen has been converted to atmospheric N_2O based on historical changes in the atmosphere over the past 50 years (Crutzen *et al*., 2008). This top-down estimate may be more accurate for the world contribution from agriculture than the IPCC approach based on field-level studies. None the less, although most N losses from animal agriculture are in the form of NH_3, NH_4^+ and NO_3^-, once these forms are applied to land as atmospheric deposition or fertilizer, a significant amount re-enters the atmosphere as N_2O.

Nitric oxide and N_2O are interconverted rapidly in the atmosphere and are referred to jointly as NO_x. Nitrous oxide diffuses from the troposphere into the stratosphere, where it can remain for hundreds of years, contributing to global warming and stratospheric ozone depletion. A molecule of nitrous oxide has a global warming potential that is 296 times that of a molecule of CO_2 (IPCC, 2001). Nitric oxide (NO) is detrimental to ozone layer depletion.

A single molecule of ammonia or nitrous oxide, once emitted to the environment, can

alter a wide array of biogeochemical processes as it is passed through various environmental reservoirs in a process known as nitrogen cascade (Galloway *et al*., 2003). A single molecule of nitric oxide can continue regenerating in the stratosphere while sequentially destroying one ozone molecule after another. Likewise, as reactive nitrogen is passed through various environmental reservoirs, a single atom can participate in a number of destructive processes before being converted back to N_2. For example, a single molecule of reactive nitrogen can contribute sequentially to decrease atmospheric visibility (increase smog), increase global warming, decrease stratospheric ozone, contribute to soil and water acidity and increase hypoxia in fresh and subsequently coastal waters.

The environmental problems caused by reactive nitrogen release into the environment are profound and ever increasing, and agriculture is the biggest source of reactive nitrogen losses to air and water (van Aardenne *et al*., 2001). Thus, it has become necessary to develop control strategies to reduce losses of reactive nitrogen to the environment.

4.3 Global Versus Local Issues

Emissions of N compounds from agriculture are important on a global scale but not on a local scale (NRC, 2003). Nitrogen emissions not only are important around the world but also it is the aggregate of these emissions throughout the world that matters more than their distribution in any specific locality. Thus, the aim should be to control emissions per unit of production (kilogram of food produced) rather than emissions per farm. This specific recommendation may directly contradict often recommended control strategies aimed at decreasing the intensity of agriculture rather than improving its efficiency. For example, governments in the USA and Europe have enacted legislation to curb livestock expansion or limit manure application rates (Berentsen and Tiessink, 2003; Sutton *et al*., 2010; Compton *et al*., 2011). It is important to emphasize the need to use nitrogen more efficiently for animal production rather than simply to use less per farm or per unit area of land. In other words, in terms of nitrogen emissions, it is not that important whether the emissions are attributed to millions of cattle owned by small farmers spread around the world or by a relatively small number of 'factory' farms in specific regions. In many cases, the larger and more industrial farm operations can afford management practices that decrease losses of reactive nitrogen from the farm, and modern practices utilize nitrogen more efficiently as a nutrient for plants and animals. On the other hand, when the concentration of animals in certain regions exceeds the true carrying capacity of the air, water and land, regional issues like eutrophication, haze or smog can become issues for those regions. Additionally, as farms become larger or more farms are concentrated in certain regions, nitrogen cannot be recycled from manure to crops as efficiently because of the cost of manure transportation to available land. Therefore, the environmentally optimal concentration of animals on a farm or in a region depends on numerous factors.

4.4 Measurements and Estimates

The National Research Council (2002) was charged with summarizing the best methods to quantify various air emissions from livestock operations. The academy found that methods to measure routinely or predict nitrogen emissions from agriculture were unreliable, expensive and too difficult to use regularly. Although predictions or measurements of any individual species of nitrogen emission (e.g. NH_3, N_2O or NO) or runoff or leachate loss (e.g. NH_4^+, NO_3) were not available for routine use, it was feasible and more accurate to estimate the sum of all nitrogen losses from farms. This sum is best estimated as the difference of all reactive nitrogen inputs (e.g. feed, fertilizer, nitrogen fixation by crops) and exports (animal products, feeds, etc.). Some management

practices increase volatilization of ammonia to the air but decrease runoff in surface water without affecting total reactive nitrogen loss. These total reactive nitrogen losses are the values of interest because, irrespective of what form of reactive nitrogen is lost to the environment, the various forms have different detrimental effects on the environment, and they are interconverted within the air, water and land system.

The US National Research Council (2002) found that protocols for measuring air concentrations, emission rates and fates of nitrogen were not sufficient to use routinely. These methods were found too expensive and inconvenient to use on the number and diversity of farms needed to provide useful data. In particular, farms with different animal commodities, using different management practices with different climate, would all need to be measured over different times of the year for each species of emission (e.g. NH_3, N_2O, NO_x). Since routine measurement is out of the question, the US Environmental Protection Agency and many governmental agencies around the world also considered estimating nitrogen emissions routinely from farms using mathematical models. The initial attempts (USEPA, 2001) to form these estimates resulted in developing emission factors for different types of animal commodities. Emission factors are coefficients that can be multiplied by the number of animal units on a farm or in a region to estimate the nitrogen emissions for that farm or region.

The NRC committee (2002) found the approach of developing emission factors to be completely inappropriate. Many different factors affect the emissions from a farm, including some that are difficult to control (e.g. climate, weather, topography, etc.) and some that are controllable (e.g. animal productivity or management practices such as tillage, manure storage, animal housing). When emission factors are used to estimate emissions, the various factors affecting emissions are not considered. As a result, the approach cannot be used to identify farms that need improvement or to provide incentives for best management practices. The conclusion would be that farms with more animals appear to emit more NH_3, N_2O or NO_x to the environment, and regulations would be directed unevenly toward the 'worst' offenders, which are simply the largest farms. We have already pointed out that it is more important to decrease emissions per unit of food produced than emissions per farm. Larger farms are not more likely to contribute to the regional or global effects of emissions than smaller farms.

The only viable approach to estimate nitrogen emissions on livestock operations is to use a process-based modelling approach (NRC, 2003). This approach involves the development and specification of mathematical models that describe the movement and conversion of various forms of nitrogen throughout the processes of agricultural enterprise. For example, urea and organic nitrogen are excreted to the barn floor, where some is converted to ammonia and is volatilized and the rest is scraped to manure storage. In storage, more urea and organic nitrogen is converted to ammonium and volatilized or converted to N_2O, NO_x or N_2. The quantity of N forms in storage, which is a function of animal intake and management, in turn affects the quantity and form of nitrogen stored. The quantity of each form stored and other factors like temperature and air exposure affect the quantity converted to other forms or volatilized. The quantity remaining when applied to crops affects the quantity of N used in crop production. This quantity affects the need for chemical fertilizer and crop growth, and how much NH_3 is further volatilized or denitrified or converted to N_2O or NO_x. Thus, the process-based modelling approach incorporates a vast amount of knowledge of chemical conversions and transport under different conditions of medium temperature and movement, and the way farm management affects conditions relevant to the chemistry. This type of model can therefore not only estimate emissions on an individual farm but also elucidate management practices to decrease the emissions. This type of model can be helpful for developing control strategies for individual farms or regions/nations.

4.5 Control Strategies

A systems approach is needed to manage N emissions from farms. This approach must integrate animal and crop production systems both on and off the farm (imported feeds and exported manure), the animal feeding operation and consider N losses to water as well as air. It is possible to reduce N emissions to air by transferring them to ground or surface water, but such solutions are not acceptable. It is also possible to decrease emissions from an animal feeding operation by exporting manure or importing crops, but the emissions will still occur, albeit on a different farm. One of the greatest opportunities to improve the efficiency of N utilization for animal production is to select crops that use N more efficiently, especially by using whole-crop legumes to fix N near crop roots rather than non-legumes that require additional N fertilizers. Of course, the selection of such crops would require the aid of an animal nutritionist to consider various options for diet formulation with different types of feeds.

Reactive N losses to the environment may occur as NH_3, N_2O or NO lost to air, as soluble nitrogen running off into surface water or as nitrate leaching into groundwater. Therefore, control strategies need to be aimed at decreasing the emissions of total reactive N from animal production systems. These strategies can include either performance standards based on process-based model estimates of N losses or technology standards to reward the use of technologies that decrease total system emissions of reactive N compounds by quantifiable amounts.

Within the animal production system, there are a number of ways to conserve nitrogen rather than let it be released to the environment in either air or water. Broad categories of improvement might include manure handling and management, crop selection and management or improved feeding and nutrition.

A mathematical model of nitrogen flows on a dairy farm (Kohn *et al*., 1997) was used to identify the critical control points for conserving nitrogen on a dairy farm system; however, the results are applicable to any animal production system. In this model, the efficiencies of N utilization (i.e. units of N used constructively per unit of N imported) were set to high and low extremes for each of these major subsystems (manure, crop, feed). For example, the efficiency of feed N utilization was calculated as the g of N in animal products (milk and meat in this case) divided by the feed N consumed by the herd, and this was allowed to vary from 16 to 24%. The gram of feed N produced per gram of N available at the root zone of crops ranged from 50 to 75%, or would be as high as 95% for forage legumes. The amount of N available to crops in soil is likely to be 25–50% of the manure N produced.

When all three efficiencies were set at lower limits, 5 units of N would be lost from the system for every 6 units of N fixed by legume crops; and 10 units of N would be lost for every 11 units applied as commercial fertilizer. Only the remaining unit would be converted to animal products. How much of the loss goes into the air and in what forms depends on the choices made regarding various management options.

For example, incorporating manure or fertilizer immediately after application may decrease ammonia volatilization considerably, but increase leaching. It is still a recommended practice because it is a means of conserving N. By improving the utilization of N by the herd, through better feeding and management programmes, these losses should be decreased by 40%. Selecting more legumes, selecting highly efficient crops and managing crops better also reduced N losses by similar levels. However, improving manure management only decreased N losses from the farm by 10–14%. Most manure N is still lost to the environment before being recycled back to the feed, even under the best of conditions. Thus, it is best not to produce it in the first place.

In the past several years, regulators and other developers of pollution control strategies have become interested in the feeding and animal management option to reduce N and phosphorus (P) losses to the environment. Nonetheless, they have been struggling with how to translate their

interest into policies to improve nutrition or feeding. Cropping systems are the other vital half of the equation; but optimizing cropping still has not received much attention. Some crops can use N more efficiently than others, but which crops are best depends on both their agronomic characteristics and how they are utilized by the animal. Ultimately, diet formulation may someday consider the environmental impact of feed selection, as it is a means to use crops that can be produced efficiently and by-products that need to be used.

Calculation of N emissions using a process-based model uses feed and production information to calculate manure output, and this estimate drives the subsequent predictions of volatile losses. Furthermore, improvements in animal nutrition that decrease manure output would be reflected immediately in the process-based model estimates. Diet formulation can affect what crops are used, and these decisions further affect the total losses of nitrogen and the forms of losses from the total animal production system. In essence, the NRC calls for an improvement in the efficiency of N utilization for animal production; animal nutrition is a key element in orchestrating this improvement.

4.6 Providing Incentives to Animal Agriculture

One of the greatest challenges for agricultural and environmental policy makers and educators is the difficulty with providing incentives to farmers to reward them for coming up with ways to reduce N losses to the environment. If we could measure or estimate these losses easily, and society paid for reduced losses, farmers and the allied industries would invent ways to profit from improved environmental management. Whole-farm N balances can be calculated where the input N from feed and fertilizer imports are balanced against N exports in meat and milk. The difference is N that is potentially lost to the environment (Berentsen and Tiessink, 2003). These balances are somewhat difficult to interpret, because every farm imports a different mix of feeds (e.g. legumes, forages, grains) and fertilizers (e.g. manure or inorganic) and exports a different mix of animal and crop products (e.g. milk, meat, compost). Since the farm has the purpose of both using low-value co-products and producing food, the environmental impact has to consider the waste disposal and off-farm production that contributes to the overall efficiency of N use. A simple farm balance potentially would show greater N lost from the system when the farm produces its own feeds because N lost from crop production is internalized to the farm. Likewise, lower efficiency of N utilization would be predicted when the farm uses organic fertilizers because the N is not made available to crops as predictably in the growing season as for inorganic N. Nonethe`less, simply importing and exporting nutrients does not make the whole agricultural system more or less efficient unless the farms specializing in either the production or use of those nutrients are more efficient themselves. Thus, the whole-farm balance idea requires some level of sophistication to work for providing incentives.

An alternative to N balance is to reward directly innovation to use less inorganic N as fertilizer. Taxes are less popular but may be more effective than incentive payments. If a high tax were to be paid for inorganic N fertilizer, the organic N would have greater value and might be used more efficiently. Legume crops might also be more cost-effective in a cropping system. The problem is that legumes are not used efficiently in diets when they are oversupplied, and much of the N in grain legumes is lost to the environment when crop residues are recycled. So even the N fertilizer tax may provide an incentive to change the system in ways that are not optimal, or again become complex. A combination of the two approaches is being used in the Netherlands (Berentsen and Tiessink, 2003). A farm balance is calculated and the predicted N losses are taxed.

4.7 Using Milk Urea Nitrogen

Because the efficiency of N utilization within the animal is so important, it would be nice to have a way to identify herds that are not efficient at using N so as to be able to troubleshoot and offer assistance. Furthermore, if N utilization efficiency could be measured or predicted efficiently on farms, one might be able to offer incentives to farmers for using practices to improve the N utilization efficiency. One technology would be to measure milk urea nitrogen (MUN) for bulk tank milk.

MUN has been shown to be an excellent predictor of nitrogen excreted into dairy cow manure (Jonker *et al*., 1998). It is high when cows are overfed protein and low when they are underfed protein. Therefore, MUN can be used to identify herds with nutritional problems, and routine analysis of MUN can help farmers fine-tune protein feeding to reduce ration cost and nitrogen excreted to manure (Jonker *et al*., 2002a). Since a portion of manure N is lost to air and water resources, these reductions in manure N translate to reductions in N lost to the environment (Kohn *et al*., 1997). We have used MUN effectively to identify herds being overfed or underfed protein and have offered assistance (Jonker *et al*., 2002a). Currently, nearly all bulk tank milk shipped in the north-eastern USA is analysed for MUN and results are sent to the farm owners.

4.8 Typical Nitrogen Efficiencies Today

Animal N utilization efficiency (N in milk and meat per N in feed) is substantially higher in industrial countries than in less industrial countries. Powell *et al*. (2013) estimated the animal N efficiency as 23.8% in Europe, North America and Oceania, 14% in Asia, 10.2% in Latin America and only 5.3% in Africa. The average efficiency (milk N/feed N) was 28.4 g 100^{-1} g (Standard Deviation (SD) = 3.9) for lactating cows on 450 commercial dairy farms in the north-eastern USA (Jonker *et al*., 2002b). Similarly, the average N utilization efficiency for lactating cows for commercial dairy farms in Wisconsin, USA, was 25.4% (Powell *et al*., 2006). Major factors contributing to differences in N efficiency are genetics, nutrition and infrastructure enabling different levels of production per cow. Figure 4.2 (reprinted with permission from Powell *et al*., 2013) shows that as milk production per cow increases, there is a non-linear increase in N utilization efficiency. The effect results from the dilution of nutritional costs to maintain an animal in the herd. When each animal produces more milk, the number of animals in the herd can be decreased. Therefore, the nutritional cost of raising replacement stock and keeping animals in the herd decreases. Once animal productivity increases enough that maintenance costs are diluted to less than half the total requirement, there is a diminishing return for continuing to increase production per animal. Therefore, less industrial countries can contribute to decreasing GHG emissions from animal agriculture by improving animal productivity, while industrial countries may need to begin looking for ways to improve the efficiency of N utilization by balancing diets more accurately or improving the efficiency of digestion and absorption.

In a study, Jonker *et al*. (2002b) investigated the source of variation among commercial dairy farms in the USA. The rolling herd average was associated positively with greater efficiency and explained 25% of the variation among farms. The level of protein feeding relative to requirements was associated negatively with efficiency and explained 71% of the variance. Interestingly, management practices associated with feeding closer to requirements, such as grouping cows by requirements, more frequent diet formulation and more frequent feed analysis, did not decrease nitrogen feeding levels. However, practices associated with higher milk production, such as using bovine somatotropin and photoperiod manipulation, were associated with improved efficiency of nitrogen utilization.

Over the past 50 years, there have been two simultaneous trends in N use for animal production. First, following World War II, the development and use of chemically fixed

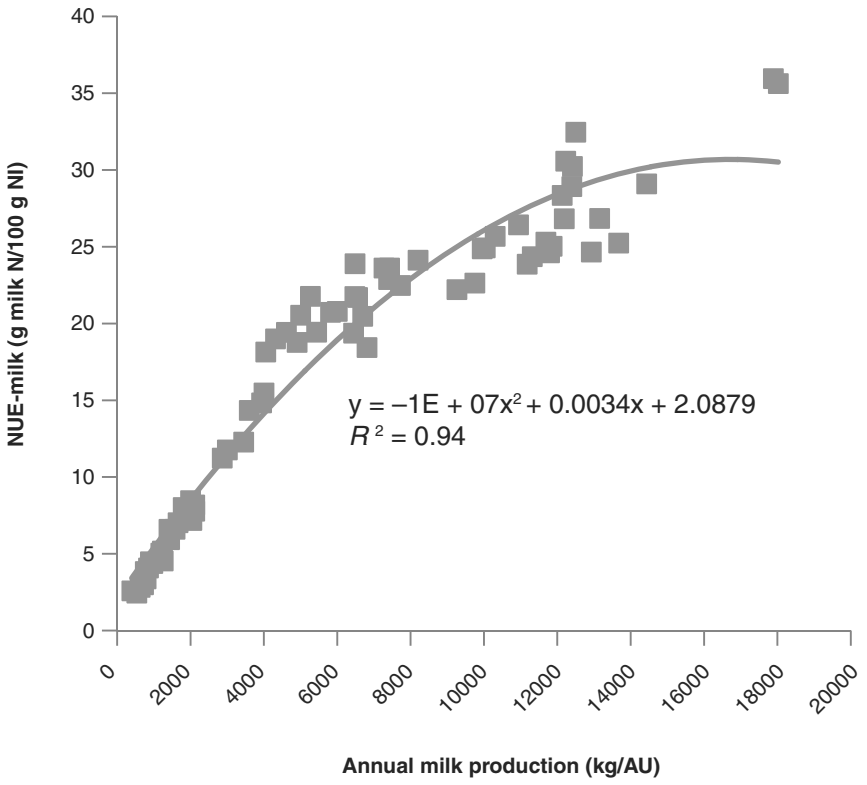

Fig. 4.2. Relationship between milk production and N utilization efficiency (N in milk per N intake) across global dairy production systems. (From Powell *et al*., 2013.)

nitrogen has increased tremendously. This means that non-legume crops have replaced the leguminous crops that were previously the source of N input to agriculture. When chemical fertilizer is applied to crops, only 25–50% of the N is taken up by the crop, while the remainder is lost to air and water and a small amount is returned to the atmosphere as harmless N_2 gas. In contrast, most N fixed by legumes ends up in harvested grains or crop residues. Thus, the increased use of N fertilizer generally represents a trend that has put a great deal more N into the environment.

The increased use of fertilizer and other aspects of agricultural intensification have made foods more available to humans around the world. As a result, we have the option to eat more meat, vegetable crops and fruits, all of which require greater N inputs per unit of N output than traditional diets of beans and rice. Today, many people eat much more protein than they actually need. In the USA, we appear to throw away about half the food N we purchase at the retail level (Smil, 2001). The human body needs about 2 kg nitrogen per person per year, but humans (collectively) create 20 kg nitrogen per person per year during food production processes. All of the reactive nitrogen is distributed to the environment, representing a biogeochemically active element that in large excess has detrimental consequences on environmental ecosystems (Galloway *et al*., 2003).

We need to reduce our dependence on N fixation if we are to reduce the losses of N to the environment. It is unlikely that

consumers will choose to eat less of the foods they like and which are good for them (e.g. animal products, vegetables and fruits). But would it impact our standard of living to decrease how much food we waste? Otherwise, we need to produce food with fewer N inputs. In this regard, there has been a positive trend for the past 50 years regarding animal production.

The availability of fertilizer N has increased our use of N in agriculture and enabled us to consume higher-quality diets. In much of Asia and Africa, the use of inorganic fertilizer has increased the loss of N to the environment per unit of food consumed. However, in the industrialized countries, N losses per unit of food consumed have remained stable since the 1970s, despite having increased the quality of the diet (Bouwman *et al*., 2005). This improvement is attributed to the increased productivity of crops and animals.

An example is provided showing improvements in dairy production efficiency over the past 50 years (Table 4.1). Production data were obtained from historical surveys conducted by the US Department of Agriculture (USDA, 2003a,b). The number of milk cows in the USA and their productivity were used in the calculations. The amount of nitrogen consumed by the average cow in 1944 and in 2001 was determined by balancing diets according to the prevailing recommendations at each time (Morrison, 1950; NRC, 2001). Excreted N was calculated (Jonker *et al*., 2002a) as the difference between N intake and N in animal products (milk and growth). The total US dairy herd peaked in 1944 with 25 million cows, although today the US produces 40% more milk with only 9 million cows. Although N excretion per cow per year has increased by about 12%, the total N excreted by all dairy cows in the USA has decreased by 60%.

4.9 Conclusion

Agricultural production in industrial countries has been decreasing GHG emissions per unit of animal protein produced. For example, nitrogen excretion by dairy cattle has decreased substantially per unit of milk protein produced. However, the trend worldwide toward consuming greater amounts of protein and more nutritious diets increases the nitrogen emissions per person (Alexandratos, 2011). As more and more people worldwide consume higher protein and more nutritious diets, the efficiency of agricultural production will need to improve even faster to overcome the dietary choices of the people who can afford them.

Table 4.1. Production and nitrogen excretion for the US dairy herd in 1944 and 2001. (Calculated from agricultural statistics and historic animal feeding recommendations: Morrison, 1950; NRC, 2001; USDA, 2003b.)

	1944	2001
Milk per cow (kg day^{-1})	7.0	27
N intake per cow (g day^{-1})	360	490
N excreted per cow (g day^{-1})	326	364
N excreted (g) per g N in milk	10	3
N in milk (g) per g N intake	0.09	0.26
Number of cows (10^6)	25	9
Milk per cow (kg year^{-1})	2073	8152
Total milk (10^9 kg year^{-1})	52	73
N excretion per cow (kg year^{-1})	119	133
Total N excretion (10^9 kg year^{-1})	3.0	1.2

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5 Nutritional Strategies for Minimizing Phosphorus Pollution from the Livestock Industry

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Abstract

Livestock manure traditionally has been considered and used as a valuable resource by farmers to improve crop production. Livestock manure is rich in nutrients (nitrogen (N) and phosphorus (P)) and thus has been land applied to enrich soils. But land application of manure nutrients in excess of crop requirements can lead to saturated soil and loss of nutrients to surface water via runoff. Environmental concerns with P from animal agriculture are significant because livestock manure has always been land applied to meet crops' N requirement, resulting in P application in excess of crops' P requirement. The problem is aggravated with the intensification of livestock production, and now animal agriculture has been identified as a primary source of water quality impairment in many regions. But intensification and continuous advancement of livestock production is required to meet the increasing demand of food supply to feed a growing global population. Therefore, management strategies are needed that will improve livestock production while supporting the environmental and social pillars of sustainability. Nutritional strategies are economically and environmentally efficient tools to reduce P excretion by livestock. This chapter discusses nutritional strategies including precision feeding, phase feeding and approaches to improve feed P availability.

5.1 Introduction

Intensification of livestock production in recent decades has resulted in challenges with appropriate manure utilization/disposal. Manure is rich in phosphorus (P) because the P utilization efficiency of livestock is less than 50%. Manure is usually land applied as fertilizer at a rate to meet crops' nitrogen (N) requirement; this results in soil P saturation. Accumulated P in soil can reach water bodies via runoff and cause eutrophication, impairing aquatic ecosystems.

If intensification of livestock and advancement of livestock production continues, perpetual impairment of water quality is expected. But continuous improvement in livestock production is required to maintain the global economy and to meet the increasing demand of food supply. Global demand for animal protein is increasing, and this trend is expected to continue as the global population is estimated to reach 9 billion by 2050. Intensification of livestock production is one of the options to maintain global food security, but a sole focus on intensification threatens the sustainability of the livestock industry by widening the gap between industry practices and societal perceptions and expectations (von Keyserlingk *et al*., 2013). Therefore, approaches are needed that increase the efficiency of animal protein production while supporting the environ-

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mental and social pillars of sustainability. Nutritional strategies to reduce nutrient excretion meet this criterion.

5.2 Livestock in P Pollution

Livestock production in the USA has evolved into an intensive production system, with the vast majority of animal products originating from animal feeding operations (AFOs, defined as farms that include confinement for 45 days or more in a year). In the span of 15 years from 1982 to 1997, the number of AFOs increased by 10% (USDA, 2001). A similar trend was observed in manure production as AFO-generated manure approximately quadrupled from 133 million tonnes (Mt) in 1997 to >500 Mt in 2003 (USEPA, 1998, 2003). The largest AFOs are defined as concentrated animal feeding operations (CAFOs). CAFOs account for a small portion of livestock farms but accounted for half of manure P produced by all livestock farms in 1997 (up from 27% in 1982; Kellogg *et al*., 2000).

Manure is a rich source of nutrients (N and P) and is used as fertilizer to enhance crop production. Spatial intensification of livestock production creates problems, as the amount of manure produced per year overwhelms the assimilative capacity of cropland. Manure has traditionally been land applied to meet crops' N requirements, but because manure N and P are in imbalance relative to crop needs, this practice leads to accumulation of soil P. A summary of US soil tests indicates that soil from areas of intensive animal agriculture had excessive P. In 1996, the majority of soil tested by several soil-testing laboratories in the north-east was categorized as high or very high in soil test P (Sharpley, 1999).

Once soil is saturated with P, it can reach surface water via runoff, increasing the risk of eutrophication, especially in fresh water (Sharpley and Tunney, 2000). A series of whole-lake experiments confirmed that P was the limiting nutrient for algal bloom in fresh water (Schindler, 1977). In the USA, an estimated 20% of agricultural impairment of water quality is from CAFOs, and more than 50% of the agricultural impairment for lakes and estuaries is by nutrients (USEPA, 1998). The source of nutrients contributing to water pollution varies regionally, and therefore a manure distribution problem may be local, regional, national or international. The semi-arid climate in the central and western regions of the USA, for instance, makes manure disposal less problematic as the risk of P runoff to surface water is less due to limited rainfall and the distant location of water bodies from farms (USEPA, 2012). The opposite scenario is observed in the Chesapeake Bay Watershed area, with its dense livestock population. In this region, manure contributes 27% of annual P load to Chesapeake Bay (Kleinman *et al*., 2012).

The P imbalance resulting from the land application of manure in excess of crop P needs is a global problem. In 62% of the global cropland area with surplus P, manure P application was in excess of crops' P use (MacDonald *et al*., 2011). The authors estimated that global recoverable manure P was 10 Mt in 2000. As is true regionally and nationally, global P surpluses result from dense livestock population in areas with inadequate cropland to assimilate manure P (Jongbloed and Lenis, 1998; Shigaki *et al*., 2006; Pathak *et al*., 2010; Wang *et al*., 2011). The association of P surpluses with intensified livestock population is not limited to one species; dairy, beef, swine and poultry production have all been identified as major contributors of P to eutrophic water bodies (Gaskin *et al*., 2001; Pote *et al*., 2003; Nelson and Mikkelsen, 2005; James *et al*., 2007; Fisher *et al*., 2009; Chebud *et al*., 2011).

At the county level, surplus manure P is more common than surplus manure N. In 1997, 155 counties in the USA had surplus manure N from CAFOs as compared to 337 counties with excess manure P (USDA, 2001). Therefore, limiting manure application to the P needs of crops (P-based nutrient management) is one way to reduce soil P accumulation and P runoff to surface water. Shifting manure application limits from N to P means increased acreage

required for manure spreading, greater cost of manure application and an increase in the number of farms that need alternative ways to dispose of manure. All of these have significant economic impacts on the livestock sector.

5.3 Assessment of Requirement and Overfeeding

Increased awareness of the problem of P accumulation on livestock farms and the impacts of P-based nutrient management regulations led to an effort to develop and implement nutritional strategies to minimize P excretion without impairing production (VandeHaar and St-Pierre, 2006). P is one of the macrominerals required for almost all living organisms with critical physiological functions including bone accretion, energy metabolism, rumen microbial growth (in ruminants), cell membrane structure, transport of fatty acids and nucleic acid structure. More than 80% of the total body P is in bone, and bone plays a role in regulating blood P by resorption (Ternouth, 1990). Therefore, bone accretion or replenishment of P in depleted bone is critical to maintain animal performance and production. As P is not synthesized in the body, P must be provided via dietary sources, but supplementation of P in excess of requirement and storage capacity results in excretion of P via faeces and urine or excreta (Knowlton *et al*., 2004). Therefore, precise assessment of dietary P requirement is critical from both nutritional and environmental perspectives.

5.3.1 Dairy cattle

The assessment of the P requirement of lactating cows has always been a matter of confusion, with variation within a feeding standard or between feeding standards used in different countries (Tamminga, 1992). The difficulty in assessing P requirements precisely is because of variation in factors that influence the P requirement, (e.g. physiological stage of production, dietary nutrient availability and the approach of calculating the requirement). In the USA, the National Research Council (NRC) uses the data from relevant animal experiments to calculate dietary P requirements. The current NRC recommended dietary P supply for lactating cows accounts for the requirement of absorbed P for milk production, maintenance, growth and reproduction (NRC, 2001). The resulting absorbed P requirement is divided by the estimated availability of feed P to calculate dietary P supply required to maintain production and performance. The requirement of total P as grams of total absorbed P and as a per cent of dietary dry matter (DM) is presented in Table 5.1. It is important to note that even though total absorbed P requirement (g day^{-1}) does not change for a particular stage of production, dietary P requirement (% of dietary DM) changes if DM intake (DMI) or feed ingredient changes.

The dietary P requirement of lactating cows for a specific age and stage of production is lower in current NRC recommendations (NRC, 2001) than it was in previous NRC recommendations (NRC, 1989), and the NRC recommended higher dietary P in 1989

Table 5.1. P requirement for Holstein lactating cows (600 kg body weight; milk yield: 40 kg day^{-1}) at different dry matter intake (DMI).[a]

DMI (kg day^{-1})	Absorbed P requirement (g day^{-1})	Dietary P requirement (per cent of dietary DM)
21.8	58	0.41
23.2	59	0.38
23.9	60	0.38
25.3	61	0.36

Note: [a]NRC (2001) predicted P requirement adapted from Knowlton *et al.* (2004).

than it did in 1978. This discrepancy was due primarily to the change in assumed P availability. Dietary P availability in ruminants is variable and difficult to measure, and thus contributes to imprecise assessment of P requirement (Park *et al*., 1999; Bravo *et al*., 2002, 2003; Kincaid *et al*., 2005; Mjoun *et al*., 2008; Wang *et al*., 2008; Martín-Tereso *et al*., 2009). This usually leads to inclusion of safety margins, and subsequently to excess dietary P.

In the 1978 and 1989 NRC recommendations, a single value was used for P availability (55 and 50%, respectively). In current NRC recommendations (2001), assumed P availability varies by type of feed (64, 70 and >75% for forages, concentrates and minerals, respectively). This attempt to account for variability in the availability of P from different sources has improved the precision of dietary P requirement calculation, but variation in P availability within a type of feed (forage or concentrate) still contributes to uncertainty.

The dietary P requirement for medium- to high-producing cows is about 0.31–0.35%; animals start showing deficiency symptoms when dietary P concentration is <0.30% of dietary DM (Valk and Šebek, 1999; Wu *et al*., 2000; NRC, 2001; Wu *et al*., 2001). In 2001, a survey involving 98 dairy farms in the Chesapeake Bay Watershed indicated that 93% of the farms were overfeeding P (Dou *et al*., 2003). The situation has improved since then (Harrison *et al*., 2012). In a nationwide 2010 survey, just 8.5% of the respondents felt that the current NRC (2001) recommendation of P for dairy cows was too low, but 40% of the respondents indicated P overfeeding, for multiple reasons.

In addition to the uncertainty about dietary P requirement, other factors contributing to the overfeeding of P to dairy cows are: the perception that high dietary P will improve reproductive performance; variation in feed P concentrations; and the inclusion of high P and relatively inexpensive by-product feed ingredients. The notion that increasing dietary P will improve reproductive performance probably originates from the studies with range or beef cattle, as mentioned by Satter *et al*. (2002) and Knowlton *et al*. (2004) in their review of animal management strategies to reduce P pollution. But in those studies, dietary P was much lower than the current recommendation (NRC, 2001).

Large variation in P concentration within a feed ingredient also contributes to P overfeeding in dairy cows. Satter *et al*. (2002) calculated within-feed ingredient variation in P concentration for the feedstuffs listed by the NRC (2001) and reported ~15% coefficient of variation. Coefficient of variation for P concentration ranged from 20–26% and 14–36%, respectively, in hay and silage samples analysed by the Northeast DHI Forage Laboratory during one 12-month period (Kertz, 1998). Coefficient of variation was lower for concentrates than for forages (3 versus 11%) in 170 samples from nine regions in the USA (Jarrett *et al*., 2011). The degree of regional variation was higher than variation by feed type (2–30% versus 3–11%). Another reason why dietary P in dairy cattle diets often exceeds requirements is the increasing popularity and inclusion of nutrient-rich, high P by-products to the dairy cattle diet. Most of these by-products are good sources of protein, but also very high in P content.

5.3.2 Beef

As in dairy, the P requirement for beef cattle is calculated using a factorial method by dividing the sum of P requirements (for maintenance, growth, pregnancy and lactation) by the absorption coefficient of P. The current beef NRC (1996) recommendation uses a fixed absorption coefficient of 0.68 to calculate dietary P requirement. As per current NRC calculations (1996), the P requirement of feedlot steers weighing between 200 and 450 kg is approximately 15–26 g day^{-1}, equivalent to 0.20–0.30% dietary P (of dietary DM) when feed intake is 10–12 kg day^{-1} (Table 5.2).

There are several reports that the NRC (1996) recommended dietary P supply for feedlot cattle is higher than necessary. This

Table 5.2. P requirement for growing and finishing Angus cattle at different body weight and average daily gain. (From NRC, 1996.)

Function	Body weight (kg)					
	200	250	300	350	400	450
	P requirement (g day^{-1})					
Maintenance	5	6	7	8	10	11
Growth (ADG[a] kg day^{-1})						
0.5	6	5	5	4	4	4
1	11	10	9	8	8	7
1.5	16	15	13	12	11	10
2	21	19	18	16	14	13
2.5	26	24	22	19	17	15

Note: [a]ADG = average daily gain.

is likely because the P requirement for gain and P availability in the 1996 NRC recommendations were calculated from data published 20–60 years ago (Ellenberger *et al*., 1950; Tillman *et al*., 1959; Martz *et al*., 1990). The P requirement of yearling steers with an average daily gain (ADG) of 1.5 kg day^{-1} was evaluated by feeding a range of dietary P from 0.14 to 0.34% of DM. These grain-fed finishing steers required 0.14% or less dietary P, about 70% of the current (NRC, 1996) recommendation (Erickson *et al*., 1999). In a similar experiment with feedlot calves, 0.16% P was found to be adequate (Erickson *et al*., 2002). Geisert *et al*. (2010) confirmed the results of Erickson *et al*. (1999) and concluded that the P requirement of finishing feedlot cattle was between 0.10 and 0.17% of dietary DM, much lower than the NRC (1996) recommendation. These results suggested that NRC (1996) overestimated the P requirement of grain-fed feedlot cattle and supplementation of P was not necessary. Similarly, Brokman *et al*. (2008) reported that P supplementation was not necessary for Holstein steers raised on pasture, given that high-quality grass was provided.

5.3.3 Swine

The assessment of P requirement for swine was originally with an empirical approach (NRC, 1979). Several studies were conducted to estimate the P requirement of pigs at different physiological stages, and until the late 1980s, the P requirement of swine was reported as total P (NRC, 1979; Combs *et al*., 1991). There has always been a discrepancy in P requirements between individual studies and NRC recommendations, largely because of the variation in P availability in different feed ingredients. The widespread adoption of exogenous phytase in swine diets has allowed reductions in dietary (and hence manure) P (Selle and Ravindran, 2008), but also contributes to the variation and uncertainty of feed P availability. The impact of phytase feeding is discussed in more detail later in this chapter. Thus, estimates of P requirements for swine continue to vary between studies or NRC standards (NRC, 1998; Hastad *et al*., 2004).

A first step toward an improved assessment system to estimate the P requirements of swine was the expression of P requirement as available or digestible P required in grams per day or per cent of diet (NRC, 1988; Ketaren *et al*., 1993; Hastad *et al*., 2004; Ruan *et al*., 2007). The data set limitations inherent to the empirical approach continued to constrain progress, because evolving production systems and changing genetics and nutrition of pigs were not accounted for. Only a limited number of studies were available to estimate empirical P requirement in growing-finishing pigs, and 60% of the studies were conducted 20 or more years ago.

For these reasons, direct assessment of P requirement from empirical results was

replaced by derivation of P requirements using nutrient-based models in the current NRC revision (NRC, 2012). In the current NRC recommendations (NRC, 2012), standardized total tract digestible (STTD) P is estimated, by refining or adding several model parameters such as whole-body P retention, endogenous P loss, marginal efficiency of using STTD-P intake and P requirement for maximal growth as a proportion of P required for maximum whole-body P retention. With this refinement, model-derived requirements of STTD-P for all stages of pigs were lower than estimated in the previous NRC recommendations (NRC, 1998; Table 5.3).

In swine, as in other species, uncertainty about P requirement and the difference in P requirements between individual pig and a group of pigs contributes to overfeeding of P. Overfeeding of P to pigs at 110–150% of the NRC (1998) recommendation for swine from the 1980s to the mid-1990s was revealed in a survey by Kornegay and Verstegen (2001). Environmental loading of P via faeces changes with production stage, as faecal P excretion (as a per cent of P intake) varies with age. The maximum contribution (up to 75% of total lifetime P excretion) is during the growing stage (Poulsen *et al*., 1999).

5.3.4 Poultry

As with dairy, beef and swine, the NRC publication is the centralized and recognized reference for nutrient requirements in poultry, but the most recent NRC recommendation for poultry (NRC, 1994) is almost 20 years old. Dietary requirements are reported as a concentration of non-phytate P (nPP). In the current NRC recommendations (NRC, 1994), dietary P recommendations for broilers, laying hens and turkeys are based on empirical research from 1952 to 1983, 1949 to 1987 and 1954 to 1986, respectively. Thus, recent studies have shown that the dietary concentration of nPP found to maintain the production and performance of broilers and laying hens is lower than that recommended by the NRC (NRC, 1994; Fig. 5.1). In contrast, although the published dietary P recommendation for turkeys (Fig. 5.1) are based on similarly old studies, they seem to match well reported results for actual P requirement of turkeys (Roberson, 2004).

In the early 1990s, there were few studies evaluating the P requirement of broilers, but most studies confirmed overestimation of P requirement for broilers by the NRC (NRC, 1994). The nPP requirement for broiler chicks (0–3 weeks of age) was 0.32 and 0.37–0.39% for optimum body weight (BW) and tibia ash, respectively (Waldroup *et al*., 2000). For the same response criteria, nPP requirement for broilers of age 3–6 weeks was 0.19 and 0.33%, respectively (Yan *et al*., 2001).

To refine P feeding (and also reduce feed costs), the broiler industry is shifting from a three-phase feeding programme (as in most studies and the current NRC recommendations) to a four-phase feeding programme. This contributes to imprecise assessment of P requirement for broilers. The fourth stage is referred to as the withdrawal phase, and P requirements are less in this stage than in the finisher stage. Dhandu and Angel (2003) reported P requirements of 0.20 and 0.16% for finisher and withdrawal phases, respectively, as compared to 0.30% for the finisher phase recommended by NRC (1994).

Table 5.3. P requirement for growing-finishing pigs. (Adopted from NRC, 2012.)

	Body weight (kg)					
	20–50		50–80		80–120	
	Year of NRC publication					
	1998	2012	1998	2012	1998	2012
STTD P,[a] per cent of diet	0.30	0.24	0.26	0.21	0.21	0.18

Note: [a]STTD P = standardized total tract digestible P.

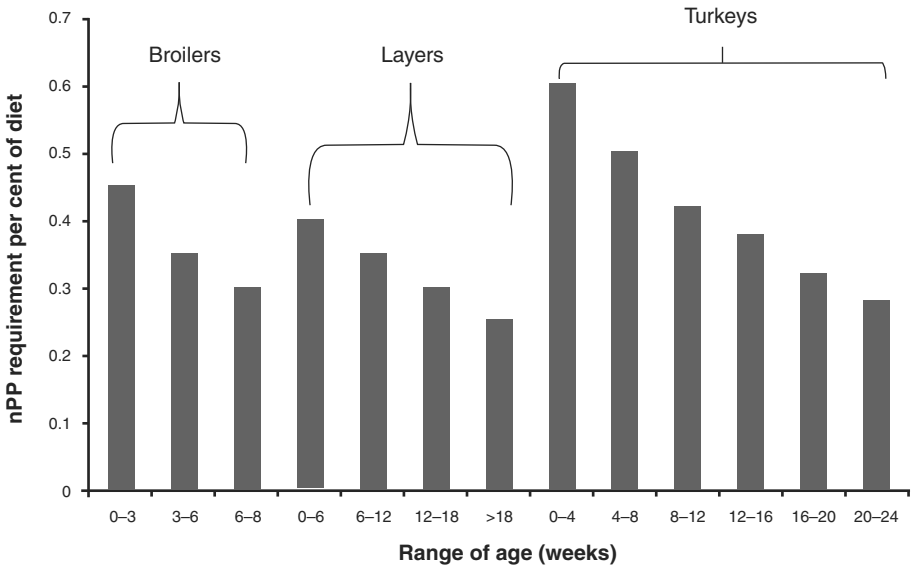

Fig. 5.1. Non-phytate phosphorus (nPP) requirements of broilers, layers and turkeys (NRC, 1994).

As with broilers, the P requirement of growing pullets is also overestimated by NRC (1994), as reported by Keshavarz (2000). Non-phytate P concentration required for optimum performance of pullets was 0.20, 0.15 and 0.10% at 0–6, 6–12 and 12–18 weeks of age, respectively, as compared to 0.40, 0.35 and 0.30% recommended by NRC (1994).

As in other species, variation in P content and bioavailability of P in feed partially explains overfeeding of P to poultry (the inclusion of a margin of safety; Huyghebaert *et al*., 1980; Waibel *et al*., 1984). For instance, the use of animal by-products as a protein source is common in the poultry industry, and P concentration varies not only between these ingredients but also within feed ingredients (coefficient of variation for P concentration in bone and meat meal from different sources: 9–17%; Waldroup, 1999). Also, advances in genetics result in continual gains in nutrient utilization and growth efficiency in all species of poultry (Havenstein *et al*., 2003). These changes justify continuing investment in research on nutrient requirements.

5.4 Nutritional Strategies for Minimizing Environmental Load of P

Nutritional strategies are more efficient than most other best management practices to reduce environmental loading of P contributed by livestock as they improve the economy of the livestock industry, reducing feed cost and reducing manure disposal costs, and thereby reducing the environmental burden of the food animal industry.

5.4.1 Ruminants: dairy and beef cattle

Remove excess dietary P

In ruminants, faecal P excretion is correlated positively with P intake (Knowlton and Herbein, 2002; Geisert *et al*., 2010; Ray *et al*., 2013). In the field, overfeeding of P to dairy and beef cattle is very common (Erickson *et al*., 1999; Dou *et al*., 2003; Arriaga *et al*., 2009). The perception that increasing dietary P will improve reproductive performance in dairy cows has persisted, but the literature data clearly indicate the opposite. For instance, no

difference in the occurrence of reproductive problems was observed in Holstein cows fed dietary P close to the NRC (2001) recommendation and in excess of its recommendation (0.37 versus 0.57% of dietary DM) (Lopez *et al*., 2004). In another experiment, decreasing dietary P from 0.49% to 0.40% or 0.31% of dietary DM did not influence the reproductive performance of lactating cows negatively during a 306-day trial, and P excretion (g day^{-1}) decreased by 23% when dietary P decreased from 0.49 to 0.40% (Wu *et al*., 2000).

Similarly, dietary P does not affect milk production, except in severe deficiency. Reducing dietary P in early lactation Holstein cows did not affect milk yield and composition (Knowlton and Herbein, 2002), and total P excretion decreased by 20 and 57% when dietary P was reduced from 0.67 to 0.51% and from 0.51 to 0.34% of dietary DM, respectively. Similarly, Odongo *et al*. (2007) did not observe any negative effect of reducing dietary P from 0.42 to 0.35% (DM basis) on the production of lactating Holstein cows, but faecal P excretion decreased. Milk production was not affected in dairy cows fed 0.31% dietary P during the first two-thirds of lactation, but in the last one-third of lactation, cows fed these very low P diets yielded less milk as compared to those fed 0.40 or 0.49% dietary P (Wu *et al*., 2000). This indicated the lowest threshold of dietary P to maintain production in dairy cows. In current feeding systems, dairy cows are fed high-concentrate diets, often with high-P feed ingredients, and thus it is nearly impossible to formulate a dairy ration with <0.33–0.35% P.

The impact of reducing overfeeding on P loading from livestock farms is dramatic. Kebreab *et al*. (2008) used a mechanistic modelling approach to simulate faecal P excretion by Ontario dairy cows. Reduction in dietary P from 0.42% to 0.35% reduced total P contribution from dairy farms by 1300 t $year^{-1}$.

As in the dairy industry, excess P can be removed from beef cattle diets without impairing production and performance. Reduction in dietary P did not affect daily gain, feed efficiency, bone ash and rib bone breaking strength when steers were fed 0.14% dietary P (per cent of dietary DM) as compared to higher dietary concentrations (0.34, 0.29. 0.24 and 0.19% of dietary DM) (Erickson *et al*., 1999). Similarly, the combined results of two experiments with finishing beef cattle suggested that dietary P could be reduced to 0.17% of dietary DM without affecting daily gain, feed efficiency, carcass quality and phalanx ash (Geisert *et al*., 2010). This will result in a greater than 40% reduction in faecal P excretion.

Improving P bioavailability

A second strategy to reduce P excretion by all species of livestock is to improve the bioavailability of feed P, allowing further reductions in dietary P. Bioavailability of P in feed ingredients depends primarily on the form of P in feed and the animal's capability to degrade organic forms of P into absorbable inorganic forms. Ruminants are blessed with the presence of microbial phytase in the rumen to degrade and utilize phytate P, the major form of P in grains (Yanke *et al*., 1998). Ruminal phytase activity can vary with type of feed and dietary phytate P concentration, and hence P bioavailability can vary even in ruminants (Godoy and Meschy, 2001). In addition, dietary phytate P degradation or P digestion varies with type of feed ingredient and feed processing (Park *et al*., 2000; Kincaid *et al*., 2005; Mjoun *et al*., 2008; Martín-Tereso *et al*., 2009). Therefore, the selection of feed ingredient is an important criterion in the effort to improve dietary P availability.

Supplementation of exogenous phytase has yielded great improvements in P availability in swine and poultry diets (see below), but the practice has less benefit in ruminant diets. Ruminal phytase activity leaves less opportunity for supplemented phytase to increase the release of inorganic P from phytate molecules. There are mixed reports about the advantage of supplementing the ruminant diet with exogenous phytase (Bravo *et al*., 2002; Kincaid *et al*., 2005; Knowlton *et al*., 2007). The results from these studies indicated that the advantage of exogenous phytase in

improving P availability might be achieved only if ruminal phytate hydrolysis by endogenous phytase was somehow limited and dietary P was at or below the P requirement of the animal (Jarrett *et al*., 2014).

5.4.2 Non-ruminants – swine and poultry

Remove excess dietary P

As in all species, eliminating P overfeeding to pigs will reduce P excretion. Thirty-six finishing boars were fed 0.15, 0.20 and 0.30% dietary available P (Varley *et al*., 2010). Digestibility of DM, ash and fibre was not influenced by dietary P, and faecal and urinary P output decreased with decreasing dietary P. Further, reductions in dietary P to below 0.20% resulted in decreased ADG, feed efficiency and bone ash. Therefore, there is no need to feed pigs over the NRC (1998) recommendation. Further research is needed to evaluate the effect of feeding P close to the current NRC (2012) recommendation on the performance and nutrient excretion by pigs.

As in cattle, faecal P excretion in poultry increases gradually as dietary P increases from deficient to the point that tibia ash content reaches maximum, and excretion increases sharply thereafter. Turkeys and broilers excreted 19–33% and 10–17% less total P when they were fed closer to their genus-specific nPP requirement (Maguire *et al*., 2004). Reducing dietary nPP concentration from 0.30 to 0.24 and to 0.15% resulted in a 19 and 30% increase in P retention by white leghorn laying hens without compromising egg production and egg quality (Panda *et al*., 2005). Therefore, reduced overfeeding is a potential strategy to reduce P excretion by poultry.

Multi-phase feeding

The concept of phase feeding is based on the use of multiple diets to match the continuously changing nutrient requirements of growing pigs (or other species) as closely as possible, and phase feeding became popular in the swine industry during the 1990s to reduce nutrient excretion by pigs. With age, the BW of growing pigs increases and the STTD-P requirement (g day^{-1}) also increases. But the needed dietary STTD-P concentration decreases due to increased feed intake with age. For example, the STTD-P requirement for pigs increases from 1.2 g day^{-1} at 5–7 kg BW to 5.95 kg day^{-1} at 100–135 kg BW, but the required dietary STTD-P concentration decreases from 0.45 to 0.21% (NRC, 2012).

Data on the effect of phase feeding on P excretion by pigs are scarce. When different feeding regimens (one-, two-, three- and four-phase feeding) were compared, the performance of pigs was not influenced, but N excretion reduced with multi-phase feeding (Lee *et al*., 2000). In that experiment, daily P excretion was numerically lower in the multi-phase feeding programme than in the one-phase feeding programme, but the effect was not statistically significant, likely due to the confounding effect of faecal DM excretion. With assumptions about BW gain and feed:gain ratio for different phases, the effect of phase-feeding strategies can be estimated (Knowlton *et al*., 2004). For instance, when one-phase feeding with constant dietary P (0.50% of diet) was compared with three-phase feeding with dietary P of 0.50, 0.45 and 0.40% of diet to achieve feed:gain of 0.42, 0.34 and 0.27 for pigs, of 20–50, 50–80 and 80–120 kg BW, P intake and subsequent excretion might be decreased by 12.5% (Knowlton *et al*., 2004).

Similar or even better improvements in the precision of P feeding can be achieved with poultry by using a four-phase feeding system. Several trials have evaluated and determined the nPP requirement of broilers in a four-phase feeding system (Angel *et al*., 2000; Ling *et al*., 2000). The replacement of average commercial usage with a four-phase feeding system can reduce dietary nPP by 5, 15 and 40% in grower, finisher and withdrawal phases, respectively (Angel *et al*., 2000).

Improving P bioavailability

The availability of P in feed is critical from a nutritional and environmental aspect, as

offering less available P than required will impair animal production and health, and available P in excess of requirement will result in increased excretion of P in a form that is most susceptible to runoff. In grains, a significant proportion of total P is present as phytate (Eeckhout and De Paepe, 1994; Ravindran *et al*., 1994; Steiner *et al*., 2007). Phytate P is not available to non-ruminants, as they lack the enzyme phytase required to degrade phytate to inorganic P. There has been a plethora of studies demonstrating the impact of improving P availability and reducing phytate P in feed ingredients commonly fed to non-ruminants.

Exogenous phytase has been used extensively in swine diets to improve dietary P bioavailability. In barrows, supplementation of 2500 and 12,500 U of *Escherichia coli* phytase kg^{-1} to a diet deficient in available P (0.15% below NRC, 1998, recommendation) improved average daily gain (ADG) and increased the breaking strength and ash weight of the metacarpal bone as compared to a no-phytase diet estimated to be adequate in available P (Veum *et al*., 2006). When compared with a P-adequate diet, supplementation of 500, 2500 and 12,500 U of *E. coli* phytase kg^{-1} diet increased P absorption and reduced P excretion by 35, 42 and 61%. The effect of phytase on production and P excretion has been observed at all phases of swine growth. The addition of phytase (500, 750 and 1000 U kg^{-1} diet) to a diet calculated to be deficient in available P to nursery, growing and finishing pigs had no effect on ADG, feed efficiency and bone ash (Brana *et al*., 2006). Rather, phytase supplementation improved apparent P digestibility (by 22–44% of P-adequate diet) and available P increased by 0.06–0.17 g U^{-1} phytase consumed. There are similar studies that reported a reduction in P excretion by pigs with phytase supplementation to a low-P diet. Supplementation of phytase at 166, 333 and 500 U kg^{-1} of low-P diet reduced P excretion by 4, 18 and 23% and 17, 19 and 22% in grower and finisher phase, respectively, and phytase supplementation at 500 U kg^{-1} diet was equivalent to 0.87–0.96 g inorganic P from dicalcium phosphate (Harper *et al*., 1997). In a similar study, phytase supplementation to a low-P diet resulted in reduced P excretion as compared to pigs fed a P-adequate diet, and phytase supplementation at 450 U kg^{-1} diet was equivalent to 0.2 g inorganic P kg^{-1} of maize–soybean meal-based finisher diet (Veum and Ellersieck, 2008).

As in pigs, the avian digestive tract lacks the phytase enzyme, so supplementation of poultry diets with exogenous phytase has been widely adopted to improve P availability. There are several reports of P excretion being reduced by 15–61% in broilers fed a diet supplemented with phytase (Simons *et al*., 1990; Żyła *et al*., 2001; Paik, 2003). Supplementation of a low nPP diet with phytase resulted in a 17–24% and 7–24% reduction in total P excretion by broilers and turkeys, respectively, as compared to P-adequate diets (Maguire *et al*., 2004). Reduction of litter P to a similar extent was reported by Leytem *et al*. (2008) and McGrath *et al*. (2010) when broiler diets were supplemented with phytase.

5.5 Novel Feed Ingredients

The development of genetically modified low-phytate grains (maize, soybeans) is another approach in improving P availability in feed for non-ruminants. Feeding low-phytate soybean increased apparent P digestibility in pigs as compared to pigs fed on regular soybeans (Powers *et al*., 2006). Excretion of total and water-soluble P decreased by 19 and 15% with low-phytate soybean meal and decreased further (by 27 and 18%) with the addition of phytase to the low-phytate soybean diet. Similarly low-phytate barley also reduced P excretion by pigs (Htoo *et al*., 2007).

The effects of phytase and low-phytate grains appear to be additive. Faecal P excretion decreased by 12 and 15% in pigs fed diets with low-phytate maize and low-phytate soybeans as compared to the pigs fed diets with regular maize and soybeans (Hill *et al*., 2009). In the same experiment, faecal P excretion decreased further in pigs fed a low-phytase diet supplemented with

phytase as compared to pigs fed a diet with regular feed and no phytase.

The low-phytate feed approach is successful in the poultry industry as well. A transgenic variety of maize developed by the US Department of Agriculture (USDA) and named 'high available phosphate corn' (HAPC) is similar to its wild variety in total P concentration, but the P is six times more available than in its wild counterpart. Replacing normal yellow dent maize with HAPC in the diet of male broilers resulted in 8–23% reductions in P excretion, irrespective of age and dietary nPP (Yan *et al*., 2000). Likewise, P excretion was 33 and 43% less in 1- to 10-day-old chicks fed low-phytate maize and barley-based diets as compared to chicks fed a wild-type grain-based diet without and with P supplementation, respectively (Jang *et al*., 2003).

The advancement of recombinant DNA technology has allowed researchers to produce transgenic plants capable of expressing microbial phytase in the endosperm of seeds, another means of providing microbial phytase to non-ruminants. The efficacy of a variety of maize expressing an *E. coli*-derived phytase gene in maintaining performance and P digestion has been evaluated (Nyannor *et al*., 2007). Diets with the maize expressing the microbial phytase gene were equivalent to microbial phytase (16,500 U kg^{-1} diet) in maintaining animal performance and in reducing faecal P excretion.

Another innovative approach of reducing P excretion in pigs is the development of phytase transgenic pigs, but this approach is expensive and controversial. Golovan *et al*. (2001) developed transgenic pigs expressing phytase and reported that true P digestibility was higher in transgenic weanling and growing-finishing pigs as compared to their non-transgenic counterparts (48 versus 88% and 52 versus 99%, respectively). This resulted in a 75 and 56% lower concentration of P in faeces of weanling and growing-finishing transgenic pigs than in non-transgenic pigs. Due to controversy about the possibility of meat from genetically engineered pigs in the food system, support for this research programme was withdrawn and the last 'Enviropigs' were slaughtered in 2012 (Schimdt, 2012).

5.6 Conclusion

Animal agriculture always has been and still is considered one of the major contributors to water quality impairment. The intensification of livestock production has yielded great benefits in terms of food supply, but has aggravated the problem of P imbalance by concentrating manure. While several management strategies may be adopted, nutritional management strategies have been identified as the most powerful and economically viable solution. Further research is needed to refine the P requirements of livestock species and to improve dietary P availability. Continued progress will improve the efficacy of nutritional strategies, allowing continued increases in animal protein with reduced environmental loading of P.

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6 Metagenomic Approaches in Harnessing Gut Microbial Diversity

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Abstract

The mechanisms involved in the digestive process of the rumen are complex, and are accomplished by a diverse and dynamic group of microbes. Microbial diversity in the rumen has been predicted to enhance the resistance of the network of metabolic pathways by increasing the number of genes encoding the pathway, enabling the ecosystem to stabilize more rapidly after change to a new equilibrium. The more resistant metabolic pathways, and the more diverse source of novel pathways, will make the microbial system more resilient. A variety of molecular methods based on direct isolation and analysis of nucleic acids, proteins and lipids from environmental samples have been discovered, and they reveal structural and functional information about microbial communities. Molecular approaches such as genetic fingerprinting, metagenomics, metaproteomics, metatranscriptomics and proteogenomics are vital for discovering and characterizing the vast diversity of microbes and understanding their interactions with biotic and abiotic environmental factors. In this chapter, efforts are made to discover the possible applications of metagenomic tools for exploring the complex microbial diversity of ruminal microbes.

6.1 Introduction

Uncultured microorganisms encompass the majority of earth's biological diversity, and about 99% of the microorganisms of different environments cannot be cultured by conventional culturing techniques. The development of culture-independent methods is a prerequisite, and is essential in understanding the genetic diversity, population structure and ecological roles of these uncultured microbes in the environment, including the rumen. Culture-independent genomic analysis, so-called 'metagenomics', of an assemblage of microorganisms has the potential to answer fundamental questions on microbial ecology. The sequencing of bacterial and archaea genomes has revolutionized the understanding of the many roles played by microorganisms. Nowadays, the complete genomes of many bacterial species, including rumen bacteria and archaea, are available in the public domain, which provides generous information on the gene(s) that possibly play an important role in different metabolic pathways. However, the perspective provided by the currently available genomes is narrow, due to biased phylogenetic distribution. In the current chapter, the different metagenomic approaches used for exploring gut microbial diversity in general, and archaeal

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diversity in particular, the limitations of metagenomic approaches and the bioinformatics tools employed for discovering microbial multiplicity are discussed.

6.1.1 Metagenomics – DNA-based technology

New genome technologies have enabled researchers to determine the DNA sequences of organisms across environments (Venter *et al*., 2004; Qin *et al*., 2010). Collective sequencing of the DNA of all organisms without the culturing and cloning of each organism is known as 'metagenomics'. A metagenome sample comprises several DNA sequences arising from all the organisms in the environment under examination. The application of metagenomics enables the study of the majority of microbes on earth to ascertain conclusive information and to classify/reclassify and to manipulate these microbes in different environments as needed. Metagenomics helps to conduct microbial surveys in specific environments such as water, soil, marshlands, the rumen and the human body (Venter *et al*., 2004; Gill *et al*., 2006; Poinar *et al*., 2006) by comprehensive study of the nucleotide sequence, structure, regulation and biological functions within the community.

The estimation of genetic diversity has become possible due to 16S rRNA gene sequencing, which provides an insight into complex microbial communities. The 16S rRNA sequences are marker genes, which exist in most microbial species but have variable regions from V1 to V9 in their sequences that allow separation into different taxonomic groups (Chakravorty *et al*., 2007). The sequencing of 16S rRNA genes is the first step in estimating the genetic diversity within a sample in many metagenomic analyses. Many computational methods have been developed for the rapid analysis of the large sets of reads obtained from targeted metagenome (16S marker genes) or 'whole' metagenome studies (Hugenholtz and Tyson, 2008). Clustering methods have also been developed to compare metagenome samples by grouping similar metagenome sequences into bins (Li and Godzik, 2006; Edgar, 2010; Hao *et al*., 2011). Other methods use classification techniques to categorize metagenome samples into different phylogenies (Huse *et al*., 2008).

6.2 Gut Metagenomics

Microorganisms represent the largest reservoir of genetic diversity on earth, outnumbering all other organisms (NRCC, 2007). Bacteria are responsible for about half of the photosynthesis on Earth, and in spite of the crucial role played by prokaryotes, familiarity with their diversity still suffers from one of the greatest gaps in the biological sciences. This remains largely unexplored (Rodriguez-Valera, 2004). There is no universally agreed estimate about their total number or diversity, or what principles govern their origin and changes. Some researchers estimate the total number of prokaryotic cells on earth as 5×10^{30}, including 10^6–10^8 individual genomes belonging to different species (Sleator *et al*., 2008). The presence of 3000–11,000 microbial genomes g^{-1} of soil (Schmeisser *et al*., 2007) makes it clear that current technologies could not support the complete sequencing of such highly diverse environments (Kowalchuk *et al*., 2007). Beyond the interspecies diversity, there is an intraspecies diversity too, which has been overlooked but which has important consequences. For example, in an easy to cultivate species, such as *Escherichia coli*, may lie a vast gene pool that is not accessible by studying one single strain. Indeed, the diversity of the genes within a bacterial species is another important facet of prokaryotic diversity (Boucher *et al*., 2001).

The biosphere contains about 10^{30} microbial genomes; at least 2–3 times more than the number of plant and animal cells combined (Whitman *et al*., 1998). The gut of livestock is home to thousands of species of microbial symbionts that play critical roles in the development and physiology of the host animal. The digestive system of

ruminants has evolved to support the retention of foregut microbiota that aid in the effective conversion of fibrous and other plant biomass into nutrients for the host animal. The fore stomach is an important and crucial part of the gastrointestinal tract, where most of the microbial degradation takes place by the microbes residing in the ruminants. However, the complete list of microbes involved, their mechanism and gene(s) accountable for the running of different metabolic pathways are not fully known, and our understanding of all these is still rudimentary due to the dependence on culture-based techniques to gather this information. But recent developments in the biological sciences make it possible to collect substantial information about these microbes without them having to be cultured. In the subsequent section, the application of metagenomic approaches for exploring the diversity of ruminal microbes in general, and archaea in particular, is deliberated.

6.2.1 Rumen microbiota

The rumen is the first chamber of the ruminant stomach, and it contains symbiotic microorganisms that break down ingested food. These microorganisms, which include representatives from all three domains of life – *Eukarya*, *Bacteria* and *Archaea* – provide nutrients, such as volatile fatty acids and bacterial protein, to the host animal. Bacteria encompass the majority of the microbial community in the rumen, and their population is usually reported as being in the range of $5.0 \times 10^6 - 6.0 \times 10^7$ μl^{-1} (Vinh *et al.*, 2011; Pilajun and Wanapat, 2012).

Variation in the microbial communities in the rumen is of great interest for the possible links to economically or environmentally important traits, such as feed conversion efficiency or methane emission levels. A key challenge here is the identification of rumen microbial profiles that are associated with, and potentially predictive of, these traits. In order to meet this challenge, whatever the methods employed for the profiling of the rumen microbial population, they should meet the following two criteria: (i) the cost of the method should be relatively low, so that large numbers of animals can be profiled for testing the associations with the above traits; and (ii) the method should be repeatable in terms of generating the microbial profile on the same dietary regimens.

Random community genomics or metagenomics where DNA is sequenced directly from environmental samples have provided a snapshot into microbial communities. Regardless of the sequencing approach, the first step in the analysis of any metagenome involves comparing the sequences to known sequence databases. Subsequent analysis includes phylogenetic comparisons, functional annotations, binning of sequences, phylogenomic profiling and metabolic reconstructions. DNA sequencing using a next-generation sequencing technique is robust, as it does not require cloning of the amplicon.

Prevotella spp. has been observed as the dominant bacteria in the rumen, suggesting that this genus plays a crucial role in the digestion and metabolism of feed nutrients (Wood *et al.*, 1998; Bekele *et al.*, 2010). *Prevotella ruminicola* and *Prevotella bryantii* ferment sugar in the rumen and present in the liquid fraction of rumen digesta. This has been validated experimentally by quantitative PCR and shotgun sequencing based taxonomic analysis. *P. bryantii* is a Gram-negative bacterium capable of utilizing soluble polysaccharides, namely xylans, justifying its higher abundance in the liquid fraction as compared to the solid fractions of rumen samples. The existence of most of the microbes in an interdependent fashion in a complex environment led the motivation for using metagenomic approaches to profile the complex structure of the microbial communities of the rumen. However, one can isolate the DNA or RNA from the community as a whole, and studies of such communities may reveal a diversity far beyond that found in conventional culture-based techniques.

6.2.2 Archaeal diversity

Bacteria and archaea are evolutionarily and biochemically distinct domains found together in various environments, and universal primer sets targeting the 16S rRNA gene can also be used to study both domains. The methyl coenzyme M gene is specific to the study of archaea, and recently universal thermosome primers have also been used by Chaban and Hill (2012) to explore the diversity of archaea. Methanogen diversity in the rumen is usually explored through the methyl coenzyme M reductase A (*mcr*A) gene (Lwin *et al*., 2012). Among the methanogens, *Methanobrevibacter* was found as prominent archaea in buffalo (Lwin *et al*., 2012); however, in cattle, a purely unknown organism filled the major space. Metagenomic studies in both cattle and buffalo revealed the presence of many unknown methanogen species that could be explored by using the latest metagenomic approaches to understand their possible role in methanogenesis.

The diversity of methanogens in the rumen of yak and cattle was investigated through 16S rRNA gene sequences by Huang *et al*. (2012), and it was found that 80.9 and 62.9% of the sequences from the respective species belonged to *Thermoplasmatales* affiliated lineage C (TALC). Sequence belonging to the *Methanobacteriales* represented the second largest clade in both libraries. Libshuff analysis indicated that the methanogen community structure of the yak was significantly different from that of cattle. A metagenomic study by Ozutsumi *et al*. (2005) provided proof of the concept of the interdependence of rumen archaea on other microbes. They reported different archaeal community composition in faunated and defaunated Holstein cattle; the high number of operational taxonomic units in the defaunated cattle suggested more diverse populations in these animals.

The archaeal community structure is also affected by the composition of the diet, as a high-roughage diet causes more methane emissions, though the total methanogen abundance may not be influenced by the proportion of roughage. Technologies to reduce methane emissions are lacking, and the development of inhibitors and vaccines that mitigate rumen-derived methane by targeting methanogens relies on the current half-complete knowledge on methanogens. Singh *et al*. (2014), in metagenomic analysis of rumen samples from Surti buffalo, reported *Methanomicrobium* spp. as the major methanogenic archaea, followed by *Methanobrevibacter* spp. and uncultured archaea. The phylogenetic analysis indicated that the methanogenic communities belong mainly to the Methanomicrobiales and Methanobacteriales orders, and the population of Methanomicrobiales, Methanobacteriales and Methanococcales was 1.94, 0.72 and 0.47% of the total archaea, respectively (Singh *et al*., 2014).

6.3 Metagenomics Approaches

Estimation of species diversity in metagenome analysis is generally based on two approaches. In the first approach, comparative or sequence similarity based methods are adopted that rely on homology to separate sequences into different taxonomic levels and classes using an annotated database (Wang *et al*., 2007; Liu *et al*., 2008). In this method, reads or contigs are aligned using global and local sequence alignment algorithms (characters) to identify regions of similarity between sequences (Huang, 1994). In the second approach, unsupervised clustering methods are used to identify groups of similar sequences within metagenome samples. The grouping of similar sequences is known as '*binning*'. Different groups in a particular sample are referred to as operational taxonomic units (OTUs), and the number of OTUs gives an approximation of species diversity in a sample (Schloss and Handelsman, 2005; Schloss *et al*., 2009; Sun *et al*., 2009). OTU-based approaches are not constrained, due to the absence of a complete coverage in taxonomic databases. Several environmental samples contain microbes that have never been laboratory cultured, and as such do not exist in genomic databases.

The OTU assignment can be used to estimate the diversity of several species, such as the Chao1 index (Chao, 1984); the Shannon diversity index (Krebs *et al*., 1999); and the abundance-based coverage estimator (ACE) index (Chao and Lee, 1992). These OTU assignments and diversity estimates facilitate the process of comparative metagenomics, i.e. comparing the genomic content of different community samples. Mothur, DOTUR and ESPRIT are the most widely used methods for OTU estimation (Hughes *et al*., 2001; Schloss and Westcott, 2011). QIIME is an open-source software package for OTU estimation, taxonomic assignment, statistical analysis and comparison of microbial communities (Caporaso *et al*., 2010), and is used primarily for analysing high-throughput 16*S* metagenomic data, generated on a variety of platforms.

The chemistry of any new sequencing technologies aims to produce longer reads, with a potential to approximate the length of the Sanger sequences. A common practice with these new technology-generated data has been to process unassembled reads, be it amplified 16*S* rRNA gene fragments or total metagenomic DNA. In the former case, only very short, 'hypervariable' regions are considered for comparisons involving millions of sequences (Gibbons *et al*., 2013). These analyses could be done with automation; the resolution of these data is very low, and information is provided at the class level without a strong linkage to the functional potential. For example, representatives of the class *Proteobacteria* are known for carrying out all types of metabolism (with the exception of methanogenesis), and representatives of this class dominate various ecosystems. Thus, the slice of a pie (or other graphic depiction) occupied by *Proteobacteria* conveys no information on what and how many metabolic functions they may be carrying out in the specific niche being addressed. This must also be true for other phyla, including the ones less represented by cultured species with known physiology.

Time-resolved metagenomics, using the above approach, elucidate that communities change over time (Caporaso *et al*., 2010), as explained by low-resolution methods such as restriction fragment length polymorphism (RFLP) and denaturing gradient gel electrophoresis (DGGE). However, these methods do not explain the function of the microbes. For example, it was concluded from the analysis of data representing various mammalian metagenomes that the communities were functionally redundant (Lozupone *et al*., 2012). The core functions of the gut microbiota may include central metabolic pathways involving carbohydrate and amino acid metabolism. Parallel sequencing provides functional insights into community functions. However, it is possible to predict this just by considering what a living system needs to survive: energy and carbon metabolism, essential to DNA and protein building.

6.3.1 Quantitative genomics

Quantitative metagenomics is used to quantify DNA molecules in a given sample as opposed to functional metagenomics, where the focus is on clone expression (Lakhdari *et al*., 2010). The sequencing of the 16*S* rRNA gene is frequently used in quantitative metagenomics. Studies of the bacterial evolution and phylogenetics provided the foundation for microbial identification. The 16*S* rRNA genes consist of a highly conserved region of variable nucleotide sequence, used for taxonomic classification. The 16*S* rRNA gene is a good marker to explore the phylogenetic composition of a given sample, to identify new species or even unknown phylogenetic groups. In quantitative metagenomics, variable regions of bacterial 16*S* rRNA genes are usually amplified by PCR and then subjected to library construction, followed by sequencing using next-generation technologies. The sequenced reads are then clustered, mapped on to a database of previously characterized sequences and used for further analyses in the studied context. Microbial 16*S* rDNA sequencing is considered the gold standard for

characterizing microbial communities, but this approach would fail to capture information about what the functions of different organisms are, knowing that organisms with identical 16S sequencing may perform different functions. A good example is the difference between various strains of *Escherichia coli* (enterohaemorrhagic – EHEC; enterotoxic – ETEC; enteroaggregative – EAEC) and related organisms such as *Shigella sonnei*, which have different clinical manifestations and different treatment modalities, yet are undistinguishable by 16S rRNA sequences (Harris and Hartley, 2003).

6.3.2 Whole-metagenome sequencing

To overcome the limitations of the 16S rRNA profiling approach, the sequencing of entire microbial genomes is now made possible by next-generation sequencing technologies. This constitutes a very attractive strategy for comprehensive metagenomics studies. The whole metagenome approach (WMS) is increasingly used, and has produced many interesting results. Sample collection from a given environment is a crucial process, since the microbial communities may be quite different between two very close locations (as in the case of rumen environments, for example), and should be determined according to research needs. DNA extraction protocols are also deciding factors and depend on the microbial composition of the sample. For example, Gram-positive bacteria, which are hard-to-lyse organisms, might be under-represented or over-represented in environmental DNA preparations, depending on the extraction protocol. Sequencing followed by mapping a selected reference gene, cataloguing and bioinformatics pre-treatment would constitute an important part of the pipeline that will ensure the biological signal is isolated, while reducing the noise caused by technical variability throughout the study. Finally, the use of the right statistical tools and data sets will be crucial in hypothesis generation and testing.

6.3.3 WMS and gene expression studies

Another important and increasingly used application of WMS is the study of gene expression. The sequencing of cDNA, which corresponds to the whole RNA in a given sample, has brought many new application possibilities. With cDNA microarrays (a gene expression measuring technology), it is possible to focus on those transcripts that have a corresponding probe on the chip, and which are usually linked to coding sequences. RNA-Seq technology allows bypassing this limitation and gives a true holistic view of the transcriptome. The RNA-Seq approach offers an unprecedented resolution with respect to the activity of a given bacteria and the functional dynamics of the genes.

However, the major disadvantage with this is the analytical challenge that underlies the complexity behind the large number of variables in the data. Other 'metaomics' approaches, such as metaproteomics or metabolomics, are still in their infancy, but just as promising. The precise biocharacterization of samples from different environments of interest is increasingly becoming routine with the help of metagenomics and other metaomics technologies, and this new science is advancing very quickly.

6.3.4 Tag-encoded FLX-amplicon pyrosequencing (TEFAP)

A comprehensive evaluation of the microbial diversity in any environment is possible. Tag-encoded FLX-amplicon pyrosequencing (TEFAP) has been utilized to evaluate bacterial, archaeal, fungal and algal as well as functional genes. Using this new bacterial tag-encoded FLX amplicon pyrosequencing (bTEFAP), it is possible to evaluate microbial diversity using a cost-effective and reproducible method that allows for sequencing the ribosomal RNA genes of microorganisms (without the need for inherent bias of culture methods). This development has ushered in a new age of microbial ecology studies (Sun *et al*., 2011).

6.4 Quantitative Metagenomics and Its Challenges

For the development of any new field, many tools and approaches need to be invented from scratch or adapted in order to answer questions on the gaps in the understanding of a subject and to gain insight on the studied topic. The field of metagenomics is no exception. This area of research is progressing very fast, and it is overwhelming to see the pace at which the whole experimental and analytical framework is being built as a consequence of international teamwork and various collaborations. The field of metagenomics is encountering great success, and is offering the possibility to explore the diversity of the microbial world at gene level. It also provides an understanding of the functions and dynamics of various habitats. Ultimately, integrated analysis of metagenomes, metatranscriptomes, metaproteomes and metabolomes will be needed to understand microbial systems biology (Sleator *et al*., 2008). Achieving such integration necessitates interdisciplinary efforts and the continuous development of appropriate bioinformatics tools to decipher the biological networks underlying molecular, functional and community structure.

The *in silico* investigation of biological networks could be effective in identifying central connected components that could bring more insight on their functionality and dynamics within the system. International projects such as metagenomics of the human intestinal tract (MetaHIT) and human microbiome project (HMP) have released data on ecosystems, along with the corresponding reference catalogues and their available functional and phylogenetic annotations. Improvement in human health by applying metagenomics studies would help in lessening the medical burdens on society and preserving active individuals who could contribute to the economy of the nation.

Sequencing technologies do not provide the whole genome of different coexisting organisms, but produce short, contiguous subsequences called sequence reads from random positions of the entire genome. The reconstruction of different microbial genomes from a mixture of sequence reads is one of the greatest challenges in metagenome study. This is referred to as the metagenome assembly problem. With high species complexity and the short length of sequencing reads obtained from current sequencing technologies, the genome reconstruction goal becomes difficult. Moreover, within a community, microbes may vary in abundance, diversity, complexity and genome length, and may have not been individually sequenced before. Likewise, the current sequencing technologies produce a large volume of sequence reads and reads that may have varying degrees of error (Hugenholtz and Tyson, 2008). Therefore, the metagenome assembly problem is complex and challenging (Charuvaka and Rangwala, 2011) and is often subject to further analysis as a collection of short reads.

Next-generation sequencing has changed the understanding of microbial ecology dramatically where large-scale and in-depth diversity studies are widely accessible. However, it has been found by Shakya *et al*. (2013) that determination of the accuracy of taxonomic and quantitative inferences and comparing the results obtained with different approaches is complicated. There has been an incongruence of experimental and computational data types. In addition, there is also a lack of knowledge of true ecological diversity.

6.5 Software Used for Analysing Microbial Diversity

6.5.1 AXIOME (automation, extension and integration of microbial ecology)

This is a highly flexible and extensible management tool for microbial ecology analysis. This software promotes reproducibility and customization in microbial research. Data analysis has become an important bottleneck of microbial ecology studies. Hence, it is imperative to develop user-friendly computational tools. AXIOME

represents an important step in this direction by automating multi-step bioinformatic analyses and customization of procedures to suit the research needs of microbial ecology. AXIOME helps to streamline and manage the analysis of small subunit (SSU) rRNA marker data in QIIME and Mothur. AXIOME also implements features that include paired-end assembler for Illumina sequences (PANDAseq), non-negative matrix factorization (NMF) and multi-response permutation procedures (MRPP). It is useful for exploring and recovering phylogenetic novelty (ssunique) and indicator species analysis. AXIOME has a companion graphical user interface (GUI). The software is designed to be extended easily to facilitate customized research workflows (Michael *et al*., 2013).

QIIME (pronounced 'chime') stands for quantitative insights into microbial ecology. QIIME is an open-source software package for the comparison and analysis of microbial communities. It is based on high-throughput amplicon sequencing data (such as SSU rRNA) generated on a variety of platforms. The software also supports the analysis of other types of data (shotgun metagenomic data). QIIME takes users from their raw sequencing output through initial analyses such as OTU picking, taxonomic assignment and the construction of phylogenetic trees from representative sequences of OTUs, and through downstream statistical analysis, visualization and production of publication-quality graphics. QIIME has been applied to the study of billions of sequences from thousands of samples.

6.5.2 DOTUR

There is copious qualitative information describing the members of the diverse microbial communities on earth. However, statistical approaches for quantifying and comparing the numbers and compositions of lineages in communities are lacking. DOTUR assigns sequences to operational taxonomic units by using either the furthest, average or nearest neighbour algorithm for each distance level. DOTUR uses the frequency at which each OTU is observed to construct rarefaction and collector's curves for various measures of richness and diversity.

6.5.3 Greengenes

This is a 16*S* rRNA gene database (http://greengenes.lbl.gov) addressing the limitations of public repositories by providing chimera screening, standard alignment and taxonomic classification by using multiple published taxonomies. It was found that there was incongruent taxonomic nomenclature among curators, even at the phylum level. Putative chimeras were identified in 3% of environmental sequences and in 0.2% of records derived from isolates. Environmental sequences were classified into 100 phylum-level lineages with archaea and bacteria.

6.5.4 MG-RAST

MG-RAST (the metagenomics RAST) server is an automated analysis platform for metagenomes. This software provides a quantitative insight into microbial populations based on sequence data. The server provides the following functions: sequence data uploading, quality control, automated annotation and analysis for prokaryotic metagenomic shotgun samples. MG-RAST was launched in 2007 and has over 12,000 registered users and 115,539 data sets. The current server version is 3.3.8.

6.6 Conclusion

Metagenomics is currently in its pioneering stages of development and is an emerging field. Many tools and technologies that are associated with the metagenomics of the rumen and other microbes are undergoing rapid evolution. The comprehensive evaluation of microbial diversity in almost any environment is being made possible using bacterial tag-encoded FLX amplicon pyrosequencing (bTEFAP), which can perform

diversity analyses of gastrointestinal microbes and can evaluate functional genes as well. In addition to paradigmatic shifts towards next-generation DNA sequencing technology, the tools of bioinformatics are also being redefined in different ways to accommodate large data volumes.

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7 Proteomics in Studying the Molecular Mechanism of Fibre Degradation

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Abstract

The degradation of plant cell walls by ruminants is of major economic importance in the developed as well as the developing world. Rumen fermentation and degradation of cell wall relies on the cooperation between the microorganisms that produce fibrolytic enzymes and the host animal, which provides an anaerobic fermentation chamber. From the 19th century, the efficiency with which the rumen microbiota degrades fibre has been the subject of extensive research. In this chapter, we will discuss various proteomic approaches such as protein fractionation (chromatography, isoelectric focusing), protein separation (two-dimensional gel electrophoresis, SDS polyacrylamide gel electrophoresis), in-gel digestion to peptides (matrix-assisted laser desorption ionization, mass spectrometry or electrospray mass spectrometry), peptide separation (two-dimensional liquid chromatography), complex protein solution digestion to peptides (electrospray ionization or MALDI-tandem MS) and proteomics of fibrolytic bacteria, which can be used to improve our knowledge of the functional framework of plant cell wall degradation in the rumen.

7.1 Introduction

Proteomics is the study of proteins, particularly their structures and functions, which participate in major cell processes, and their function, regulated by post-translational modifications such as phosphorylation, dephosphorylation, glycosylation, nitrosylation and acetylation. Proteomics involved in the molecular degradation of plant structural polysaccharides (fibre) should improve in a cost-beneficial and most effective way in order to produce biofuels and biofeeds. To understand this, we need to learn about the molecular events that control the expression of fibre-digesting enzymes, the assembly of effective degradative complexes (cellulosomes) and the signalling events required by ruminal bacteria for efficient fibre degradation.

Various proteomic approaches, such as protein fractionation (chromatography, isoelectric focusing), protein separation (two-dimensional gel electrophoresis, sodium dodecyl sulfate (SDS) polyacrylamide gel electrophoresis), in-gel digestion to peptides (matrix-assisted laser desorption ionization (MALDI)-mass spectrometry (MS) or electrospray-mass spectrometry), peptide separation (two-dimensional liquid chromatography) and complex protein solution digestion to peptides (electrospray ionization or MALDI tandem MS), can be used to obtain information on the structure of various cellulosomal and non-cellulosomal systems employed in fibre degradation. This will tell us how fibre sources have an effect on the expression of different enzymes and the kinetics of fibre degradation. A flow chart of the different proteomic approaches

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having possible application to the study of the proteomics of fibre-degrading microbes is outlined in Fig. 7.1.

7.2 Identification and Analysis of Proteins

7.2.1 Protein preparation methods

The most crucial steps in proteomics are to obtain and handle the protein sample. The dynamic range of the abundance of proteins in biological samples can be as high as 10^6 and even the best two-dimensional gels can routinely resolve no more than 1000 proteins. The ideal solution to reduce complexity and differences in abundance is to use affinity-based protein purification strategies using whole-protein complement. After obtaining the protein fraction, the method of choice for proteomic studies is one- or two-dimensional gel electrophoresis. The advantages of one-dimensional electrophoresis as a preparation method are that virtually all proteins are soluble in SDS, the range of relative molecular mass from 10,000 to 300,000 is readily covered and extremely acidic and basic proteins are easily visualized.

7.2.2 Mass spectrometric identification of proteins

MS technology has evolved as the dominant method of protein identification within the field of proteomics. MALDI (Karas and Hillenkamp, 1988) and electrospray ionization (ESI; Fenn *et al*., 1989) are the two 'soft ionization' methods used routinely to introduce analytes into mass spectrometers. MALDI relies on the co-crystallization of a peptide sample with acidified matrix on a sample plate. Sample preparation for MALDI-based MS is straightforward, and sample ionization is robust in the presence of contaminating substances such as salts and detergents. ESI requires the solubilized sample to be maintained in the liquid phase and is considerably more sensitive to contaminating substances. The major benefits of ESI include the high ionization efficiency relative to MALDI and the ability to interface the ESI-MS to high-resolution liquid-chromatographic separation apparatus.

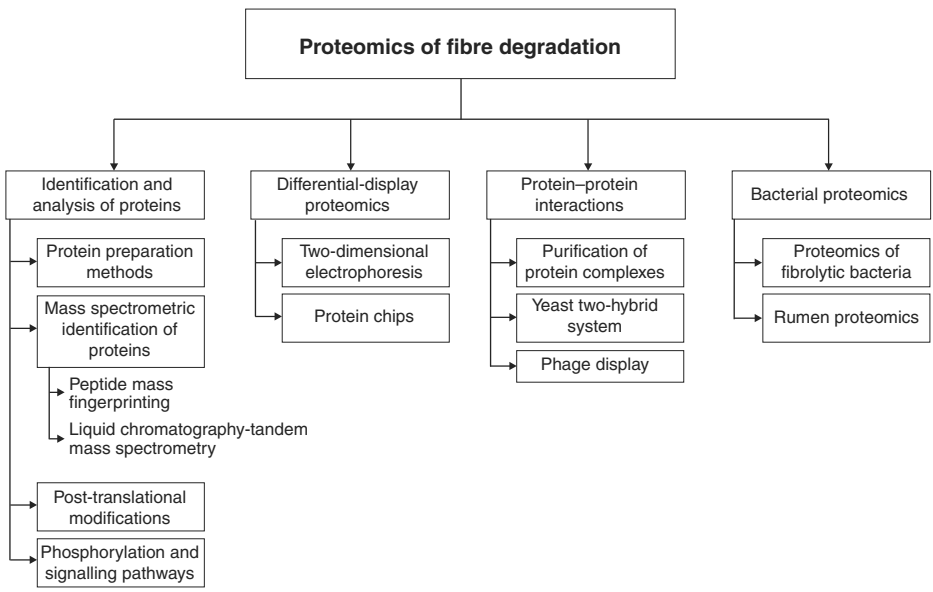

Fig. 7.1. Proteomic approaches for fibre degrading microbes.

Peptide mass fingerprinting

Peptide mass fingerprinting (PMF) is a process that involves MALDI ionization coupled to a time-of-flight (TOF) mass analyser (MALDI-TOF) and is used to measure peptide masses rapidly and identify the parent protein. The underlying principle of PMF is the comparison of experimentally derived peptide masses with theoretically calculated peptide masses generated through the *in silico* digestion of translated genomic sequences, using the expected cleavage specificities of site-specific proteolytic enzymes (Yamazaki and Tove, 1977; Yates *et al*., 1993). PMF algorithms search protein databases and return a list of search protein matches ranked according to variables including the number of matched peptide masses, the size of the individually matched peptides, the peptide mass error, the database size and the number of non-matched masses. A molecular weight search (MOWSE; Pappin *et al*., 1993) is a sophisticated scoring algorithm implemented in the PMF search algorithms Mascot (http://www.matrixscience.com; Perkins *et al*., 1999) and Protein Prospector (http://prospector.ucsf.edu/prospector/mshome.htm; Clauser *et al*., 1999). ProFound is a commonly used PMF search algorithm (http://prowl.rockefeller.edu; Zhang and Chait, 2000), which uses the Bayesian theory to rank search results according to their probability of occurrence. An evaluation of the commonly used PMF algorithms concluded that Mascot and ProFound were superior to Protein Prospector (Chamrad *et al*., 2004).

There is always a risk of obtaining false positive protein identification with the use of PMF, as measured peptide masses can randomly match to peptides from a sequence database due to the necessity to set a maximum allowable mass tolerance. The probabilistic approach mentioned above also necessitates the analysis of peptides derived from single protein species wherever possible, and that is why two-dimensional electrophoresis (2-DE) is often coupled with MALDI-TOF. When several proteins are identified as being present in a single gel plug, manual analysis of the individual identifications is necessary. MALDI ionization is biased towards arginine carboxy-terminating peptides and typically results in only 30–45% coverage of the complete protein sequence (Resing and Ahn, 2004). 2-DE is also biased against hydrophobic proteins, basic proteins and proteins below 15 kDa. The alternative approach to the 2-DE MALDI-TOF is to maintain protein or peptide mixtures in the liquid phase, separate the components using high-pressure liquid chromatography (HPLC) and analyse the elute using tandem mass spectrometry (MS/MS).

Liquid chromatography-tandem mass spectrometry

Liquid chromatography-tandem mass spectrometry (LC-MS/MS) is used to obtain peptide sequence data that are difficult and sometimes impossible to generate by MALDI-TOF. The comparison of tandem mass spectrometry (MS/MS) spectral data with predicted peptide sequences by peptide fragmentation fingerprinting (PFF) can be used to identify proteins with high confidence, sometimes when as few as two peptides are matched to the parent protein. The hydrophobic (Wolff *et al*., 2008), basic and low molecular weight proteins (Kuntumalla *et al*., 2009), which are difficult to detect using a 2-DE MALDI-TOF approach, can be identified easily by LC-MS/MS.

LC-MS/MS-based quantitative proteomic analyses typically use either isotopic protein labelling prior to chromatographic separation, such as isobaric tagging for relative and absolute quantitation (iTRAQ; Ross *et al*., 2004) and stable isotope labelling with amino acids in cell culture (SILAC; Ong *et al*., 2002), or a label-free approach that uses data acquired during separation and analysis to derive an estimation of relative protein abundances (Old *et al*., 2005). A benefit of the latter approach is the elimination of potential bias induced by variable protein labelling or detection of labelled sample. Label-free quantitation utilizes measurement of variables such as mass spectral peak intensities (Chelius and Bondarenko, 2002)

and spectral counting (Zhang *et al*., 2006). The peak intensities of peptide ions correlate with protein abundances, while the spectral counting methods measure protein abundance by comparing the number of MS/MS spectra assigned to each protein, based on the assumption that the number of observed peptides correlates with protein abundance (Rappsilber *et al*., 2002). LC-MS/MS can be used to identify a protein species within complex mixtures without prior subcellular fractionation (Wang *et al*., 2004; Francis *et al*., 2005).

7.2.3 Post-translational modifications

Proteomics studies provide a unique ability to analyse the post-translational modifications of proteins. Phosphorylation, glycosylation, acetylation and sulfation, as well as many other modifications, are extremely important for protein function, as they can determine turnover, localization, activity and stability. Mass spectrometry is the proteomic method of choice to determine protein modifications, and it is more difficult than the meagre determination of protein identity. Furthermore, these modifications are not generally apparent from genomic sequence or mRNA expression data. Progress is being made in this field, especially in the case of phosphorylation. Phosphorylation events can be studied by generic strategies, because phosphopeptides are 80 Da heavier than their unmodified counterparts, give rise to a specific fragment (PO^{3-}, mass 79) and bind to metal resins. This is recognized by specific antibodies, and the phosphate groups can be removed by phosphatases (Nuwaysir and Stults, 1993; Betts *et al*., 1997; Zhang *et al*., 1998; Cortez *et al*., 1999; Neubauer and Mann, 1999).

7.2.4 Phosphorylation and signalling pathways

The receptor-mediated signalling pathways result in serine/threonine or tyrosine phosphorylation of a large set of substrates. To identify these substrates, the lysates from non-stimulated and stimulated cells can be prepared and resolved by two-dimensional gels. The proteins of interest can be detected by ^{32}P labelling or by western blotting with antibodies that recognize only the activated state of molecules (such as phosphotyrosine- or phosphoserine-specific antibodies). These spots can then be identified by MS, as demonstrated recently (Soskic *et al*., 1999). A better alternative, however, is first to enrich the substrates by using anti-phosphotyrosine antibodies in an immunopreciptation step, followed by mass spectrometric identification. Several known and new components were recently reported in one such study on the epidermal growth factor (EGF)-receptor pathway (Pandey *et al*., 2000).

7.3 Differential-Display Proteomics

7.3.1 Two-dimensional electrophoresis

Two-dimensional electrophoresis (2-DE) is used to separate complex protein mixtures into single protein species using each protein's unrelated properties of isoelectric point (p*I*) and molecular weight (MW). 2-DE can resolve hundreds to thousands of proteins on a single polyacrylamide gel, and can rapidly expose differences in protein abundance, identify protein isoforms and elucidate the presence of post-translational modifications (PTMs) such as phosphorylation and glycosylation. The development of immobilized pH gradient (IPG) strips has had a major impact on the utility of the 2-DE technique (Bjellqvist *et al*., 1982). Wide- and medium-range IPG strips such as IPGs 3–10, 4–9 or 4–7 are excellent for the analysis of simple proteomes or when an overview of more complex proteomes is required, while narrow-range, overlapping IPG strips enhance the resolving power of the first dimension focusing. Using this approach, Cho *et al*. (2003) resolved 1237 individual protein species between p*I* 3.5 and 5.5 when analysing a cytosolic protein sample of the halophilic bacterium, *Halobacterium*

salinarum. When total protein extract of *Saccharomyces cerevisiae* was analysed using a series of IPG strips covering as little as one pH unit per IPG strip, 2286 individual protein spots were visualized, compared with 755 when using a standard single pH 3–10 gradient IPG strip (Wildgruber *et al*., 2000).

7.3.2 Protein chips

Nowadays, the protein chip approach is used widely, in which a variety of 'bait' proteins such as antibodies can be immobilized in an array format on to specially treated surfaces. The surface is then probed with the sample of interest and only the proteins that bind to the relevant antibodies remain bound to the chip (Lueking *et al*., 1999). Protein chip can also be probed with fluorescently labelled proteins from two different cell states. Cell lysates are labelled by different fluorophores and mixed such that the colour acts as a readout of the change in abundance of the protein bound to the antibody. This version depends on reasonably specific and well-characterized antibodies and a number of technical problems would still need to be overcome. In some other modifications, peptides–protein fragments or proteins may also be immobilized on to chips and samples applied on to the chip followed by detection of binding.

7.3.3 Protein–protein interactions

Protein–protein interaction plays a major role in biological functions and can potentially be exploited for therapeutic purposes. The creation of a protein–protein interaction map of the cell would be of immense value to understand the biology of the cell.

Purification of protein complexes

Protein–protein interaction can be studied easily with the help of proteomics (Lamond and Mann, 1997; Neubaer *et al*., 1997; Blackstock and Weir, 1999; Link *et al*., 1999). To study protein–protein interactions, it is better to purify the entire multi-protein complex by affinity-based methods. This can be achieved in a variety of ways, such as by using glutathione *S*-transferase (GST) fusion proteins, antibodies, peptides, DNA, RNA or a small molecule binding specifically to a cellular target. One of the generic ways of identifying the interaction partners of a new protein is to tag it with an epitope. This protein can then be overexpressed in cells and together with its interaction partners, immunoprecipitated by an antibody against the epitope. This requires only the full-length complementary DNA clone of the gene, and no time is spent in generating a precipitating antibody against the gene of interest. Because full-length cDNAs may soon be available for most human genes (Strausberg *et al*., 1999), large-scale interaction studies will become possible. Making fusion proteins such as GST fusions is another generic way to obtain interaction partners. The multi-protein complex associates with the 'bait', which is immobilized on a solid support. After washing away the proteins that interact non-specifically, the protein complex is eluted, separated by gel electrophoresis and analysed by mass spectrometry. Thus, in a single experiment, the components of an entire multi-protein complex can be identified. Its protein components were then displayed by two-dimensional gel electrophoresis. Nineteen new factors were obtained from a single, two-dimensional gel (mostly in expressed sequence tag (EST) databases), and several of them were cloned and analysed further. Co-localization using immunofluorescence of the new protein with other members of the complex served to establish that they were bona fide members of the complex. Several of the new factors identified from this study were cloned and GST fusion proteins generated. Using this strategy, one of the proteins, designated S14, precipitated a subset of the spliceosome proteins, which indicated a function of this protein. Many protein complexes have now been characterized using the strategy outlined above. Some of these complexes include the yeast Arp2/3

complex (Winter *et al*., 1997), proteins found in the yeast nuclear-pore complex (Rout *et al*., 2000) and proteins bound to the chaperonin, GroEL (Houry *et al*., 1999).

Once members of a multi-protein complex have been identified by mass spectrometry, their function is studied by pertinent assays. At this stage, proteomics can be used in an iterative fashion to define either direct interaction partners of a new protein in the complex and/or to connect to other complexes in the cell (Shevchenko and Mann, 1999).

Yeast two-hybrid system

The yeast two-hybrid system has emerged as a powerful tool to study protein–protein interactions (Fields and Song, 1989). It is a genetic method based on the modular structure of transcription factors wherein close proximity of the DNA-binding domain to the activation domain induces increased transcription of a set of genes. The yeast hybrid system uses open reading frames (ORFs) fused to the DNA binding or activation domain of GAL4, such that increased transcription of a reporter gene results when the proteins encoded by two ORFs interact in the nucleus of the yeast cell. One of the main consequences of this is ORF identification based on the detection of positive interaction. For these reasons, it is a simple and generic method, amenable to high-throughput screening of protein–protein interactions.

On a large scale, this strategy has been used in two formats. In the array method, yeast clones containing ORFs as fusions to DNA or activation domains are arrayed on to a grid and the ORFs are tested (as reciprocal fusions) and screened against the entire grid to identify interacting clones. In the library screening method, one set of ORFs are first pooled to generate a library and then the reciprocal ORF fusions are mated with the library one by one or several at a time.

A recently described modification of the yeast two-hybrid method, termed 'reverse' two hybrid, can be used to identify the compounds and peptides that disrupt protein–protein interactions (Vidal and Endoh, 1999). This can lead to the development of drugs that have activities *in vivo* as opposed to drug screens that are conventionally done *in vitro*.

Phage display

Phage display is a method where bacteriophage particles are made to express either a peptide or protein of interest fused to a capsid or coat protein. It can be used to screen for peptide epitopes, peptide ligands, enzyme substrates or single-chain antibody fragments. Although combinatorial peptide libraries are generally used in most phage display-based studies, more large-scale protein interaction studies can now be performed if the products of cDNA libraries are displayed on phage particles. Any target protein can then be immobilized to capture phage particles displaying interacting proteins. This method is similar to the yeast two-hybrid system and can be performed simply with high throughput. Depending on the particular class of proteins being studied (such as cytoplasmic versus cell surface proteins), this method might be superior or inferior to the two-hybrid system, because of the interactions that take place in solution rather than in the nucleus of the yeast cell. Furthermore, this method is applicable in principle to transcription factors, which are not amenable to the yeast two-hybrid system. Methods have recently been optimized to display cDNA libraries on phages to isolate signalling molecules in the EGF-receptor signalling pathway, as well as to identify antigens that react with certain antibodies (Hufton *et al*., 1999; Zozulya *et al*., 1999).

7.4 Bacterial Proteomics

Proteomic technologies have been used to examine the proteomes of many industrially and medically important bacteria, cultured under a variety of defined conditions. The model Gram-negative and Gram-positive organisms, *Escherichia coli* and *Bacillus subtilis*, have naturally been the focus of extensive proteomic examination, with

other important *Bacillus* species such as *Bacillus anthracis* and *Bacillus licheniformis* (Ohlmeier *et al*., 2000; Deutscher and Saier, 2005; Tam *et al*., 2006; Voigt *et al*., 2006; Gilois *et al*., 2007; Hecker *et al*., 2008; Chitlaru and Shafferman, 2009). Several groups have demonstrated the utility of a combined gel-based and gel-free proteomic approach in achieving improved proteome coverage of *Bacillus* species and other Gram-positive bacteria (Schmidt *et al*., 2004; Wolff *et al*., 2006; Hahne *et al*., 2008; Jungblut *et al*., 2010). Proteomic technologies have been utilized in the analysis of several important human gastrointestinal bacteria, including *Fusobacterium varium* and *Lactobacillus plantarum*, as well as the polysaccharilytic *Bifidobacterium longum* (Cohen *et al*., 2006; Yuan *et al*., 2006; Potrykus *et al*., 2008). In particular, analyses of the cytosolic, exported and cell envelope proteins of *B. longum* and the adaptation of cells to low pH environments has revealed important insights into the glycosyl hydrolases produced by this bacterium (Yuan *et al*., 2006; Sanchez *et al*., 2007, 2008; Ruiz *et al*., 2009). An LC-MS/MS label-free quantitative proteomics approach was used recently to examine the cytosolic proteomes of *B. longum* strains differing in their heat shock resistance (Guillaume *et al*., 2009).

7.4.1 Proteomics of fibrolytic bacteria

Proteomics approaches have been used with success to investigate the fibrolytic enzyme systems of a number of polysaccharide-degrading bacteria. Murashima and co-workers identified several cellulosomal enzymes expressed by the cellulolytic soil bacterium, *Clostridium cellulosolvens* (Murashima *et al*., 2002), and subsequently demonstrated through 2-DE/MALDI-TOF that the cellulosome composition and relative enzyme abundance were both regulated by the growth substrate (Han *et al*., 2004, 2005). Similarly, the cellulosome composition of *Clostridium cellulolyticum* and the effect of polymeric substrates on the organization of extracellular multi-enzyme complexes in *Paenibacillus curdlanolyticus* have recently been determined (Blouzard *et al*., 2007; Waeonukul *et al*., 2008). The effect of growth substrates on the relative abundance of fibrolytic enzymes has also been examined for a diverse range of 38 bacteria, including *Bacillus* sp. K1, *Cellulomonas flavigena* and the marine bacterium, *Saccharophagus degradans* (Chu *et al*., 2000; Sanchez-Herrera *et al*., 2007; Sato *et al*., 2007).

7.4.2 Rumen proteomics

In spite of the variety of gut bacteria having been the subject of intensive proteomic efforts, and the importance of the rumen microflora, the application of proteomic technologies for studying the rumen fibrolytic bacteria has been very limited so far. Devillard *et al*. (2004) used 2-DE and MALDI-TOF MS to investigate the mechanisms underlying the attachment of *Ruminococcus albus* 8 to cellulosic substrates. Later, the group used a similar approach to demonstrate the increased abundance of the molecular chaperone, GroEL, within the cell membrane-associated proteome when cells were exposed to linoleic and conjugated linoleic acids (Devillard *et al*., 2006). Following the publication of the completed genome sequence of the Gram-negative, non-fibrolytic, succinic acid-producing rumen bacterium, *Mannheimia succinici-producens* (Hong *et al*., 2004), the proteome of this bacterium has been the subject of recent attention. 2-DE reference maps of the whole cellular, membrane and secreted proteins allowed the identification of more than 200 proteins and revealed important insights into growth phase-dependent physiological alterations (Lee *et al*., 2006). This information was subsequently used to engineer a mutant strain that improved succinic acid-producing capability (Lee and Lee, 2010). There are currently no reports of proteomics being applied to the analysis of the polysaccharide-degrading enzyme systems of fibrolytic rumen bacteria.

7.5 Conclusion

The study of gene function on a large scale can be executed directly at the protein level with the help of proteomics. The mass spectrometric study of proteins separated by 2-D gel electrophoresis is leading to a resurgence in biochemical approaches to protein function. Protein characterization will continue to improve in throughput, sensitivity and completeness, and will help in understanding the physiology of proteins. Post-translational modifications cannot currently be studied at high throughput, but certain categories such as acetylation, methylation and phosphorylation are beginning to be amenable to generic approaches. Mass spectrometry-based methods that use affinity purification followed by only one-dimensional electrophoresis will continue to gain in importance. In future, proteomics will provide a wealth of protein–protein interaction data, which will probably be its most important and immediate impact on biological science. Because proteins are one step closer to function than are genes, these studies frequently lead directly to biological discoveries or hypotheses. The ready availability of many human genes as full-length clones is itself an extremely important extension of the genome projects that will make several proteomic strategies possible. Assays to determine protein function using purified proteins will be automated and performed in miniaturized grid formats in parallel for thousands of proteins. Advances in genomics will directly fuel large-scale protein assays that use genetics as a readout, such as the two-hybrid screen. The resulting proteomic study of the rumen microflora will be instrumental in developing new approaches to improving feed digestibility, reducing animal waste and presenting a positive image of animal agriculture.

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8 Perspective on Livestock-Generated GHGs and Climate

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Abstract

The greenhouse gases (GHGs) attributed to agriculture and animal agriculture are methane (CH_4) and nitrous oxide (N_2O). The relative absorptivity of the infrared radiation of carbon dioxide (CO_2) is about 21-fold and 310-fold higher than for each molecule of CH_4 or N_2O, respectively. As the absorptivity in both gases is not saturated like CO_2, the contribution of CH_4 and N_2O to the greenhouse effect have therefore been prospectively increasing linearly, because atmospheric increases in the concentration of both gases correlate closely with human activities, and the world population is currently expanding to more than 7 billion. Rumen CH_4 production emitted to the atmosphere can be accounted as the biggest anthropogenic source. The abatement mechanism of rumen CH_4 emission may be divided into direct and indirect suppression of methanogens in the rumen. The most significant strategy to mitigate rumen CH_4 emission in an indirect manner is to promote alternative metabolic pathways to dispose of the reducing power, competing with methanogenesis for H_2 uptake. In an attempt to identify natural manipulators with the efficacy to mitigate rumen CH_4 emission, efficient prebiotics and probiotics have been developed in various institutions instead of ionophores in respect to food safety. The relatively lower molecular weight compounds produced by *Lactobacillus plantarum* have recently revealed the ability to suppress rumen methanogenesis. Some tropical and subtropical legume trees fed to cattle and buffalo as a protein source, due to their high protein content, are rich in secondary metabolites such as saponin and tannin, which is a type of polyphenol, etc. Some of them have been reported to abate rumen CH_4 production remarkably well *in vitro* or *in vivo*. In some cases, however, experimental results are in agreement but may not match. Meta-analysis might be useful to integrate statistically the experimental results from independent studies for comprehensive understanding in the next stage of research.

Although the inventory assessment of N_2O is still ongoing, animal manures, including those from monogastrics, have been listed as a significant contributor. The incomprehensible fates of organic and inorganic nitrogen compounds contained in the aerobic composting of animal manures may confuse an accurate evaluation of the actual emission of N_2O from animal manures. Of late, there has been an urgent need for renewable energy production using anaerobic digesters to capture biogas (CH_4) from animal effluent. Most manure nitrogen during fermentation has been converted to an inorganic form in the digested slurry.

8.1 Introduction

Mitigation of the six anthropogenic GHGs, such as CO_2, CH_4, N_2O and Fluorinated gases, i.e. sulfur hexafluoride (SF_6),

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hydrofluorocarbons (HFCs) and perfluorocarbons (PFCs), has been established as a legally binding commitment of the Kyoto Protocol (IPCC, 1996). Figure 8.1 shows the emission rate of CO_2-equivalent (eq) GHGs in the highest-ranking countries (WRI, 2011). The emission rate of GHGs closely correlates with economic development, exemplified by GDP.

The important GHGs attributed to animal agriculture are CH_4 and N_2O. The rumen fermentation of ruminants and the anaerobic fermentation of agricultural organic wastes including animal manures are major contributors of CH_4 emission as anthropogenic sources (Moss, 1993). To abate GHGs, the development of methods to mitigate enteric CH_4 is the most significant issue in world ruminant livestock production (Van Nevel and Demeyer, 1996). The most significant indirect strategy to mitigate rumen CH_4 emission is to promote alternative metabolic pathways to dispose of the reducing power, competing with methanogenesis for H_2 uptake. In an attempt to identify natural manipulators with the efficacy to mitigate rumen CH_4 emission, efficient prebiotics and probiotics have been developed in various institutions instead of ionophores in respect to food safety. Some tropical and subtropical legume trees fed to cattle and buffalo as a protein source, due to their high protein content, are rich in secondary metabolites such as saponin and tannin, which is a type of polyphenol, etc. Some of them have been reported to abate remarkably rumen CH_4 production *in vitro* or *in vivo*. In some cases, however, the experimental results are in agreement and may not match. Meta-analysis might be useful to integrate statistically the experimental results from independent studies for comprehensive understanding in the next stage of research (Jayanegara *et al.*, 2012, 2013).

The gradual increase of atmospheric N_2O since the 19th century is related closely to the sudden expansion of the human and animal population following the innovation of the Haber–Bosch process. Severe environmental pollution was caused at the same time, although the reactive nitrogen withdrawn from the atmosphere as stable paired nitrogen brought about prosperous food production. Although the inventory

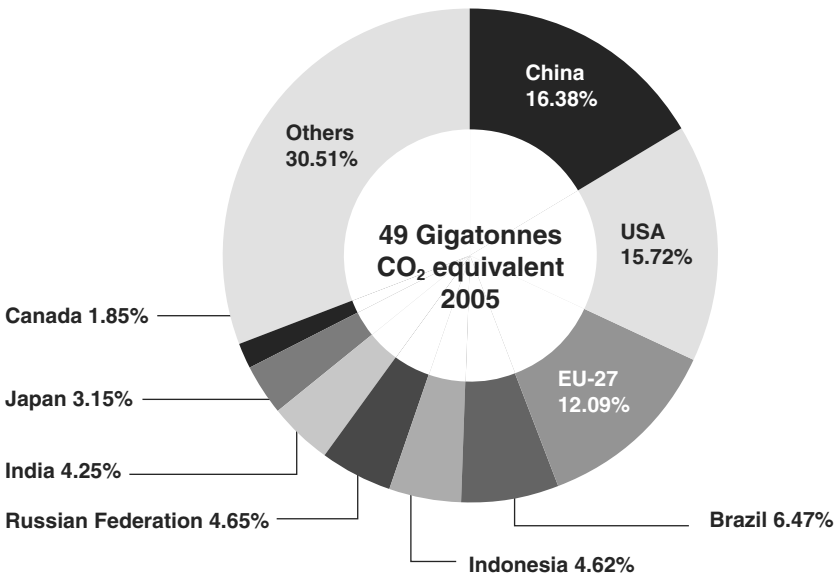

Fig. 8.1. Nation wise greenhouse gas emissions (WRI, 2011).

assessment of N_2O is still ongoing, animal manures including those from monogastrics have been listed as significant contributors. The incomprehensible fates of organic and inorganic nitrogen compounds contained in the aerobic composting of animal manures may confuse accurate evaluation of the actual emission of N_2O from animal manures. There has been an urgent need for renewable energy production using anaerobic digesters to capture biogas (CH_4) from animal effluent. Most manure nitrogen during fermentation has been converted to inorganic form in the digested slurry. This chapter proposes the use of an advanced biogas plant equipped with an ammonia stripping system, or the enzymatic reduction of ammonia to paired nitrogen from animal wastes, in order to mitigate N_2O emission from animal manure, as it is the second highest agricultural GHG that contributes to climate change.

To secure food production preventing environmental catalysis by global warming, the sustainable development of animal agriculture should be sought as an alternative strategy, not only in industrialized but also in developing countries.

8.2 Global GHGs Emission Trends

Figure 8.2 shows the global trends in GHG emissions during 1970–2004 and their contribution to global warming. These GHGs account for about 97% of the direct climate forcing by the increase in long-lived GHGs since 1750. The increase in CO_2 originates largely from the combustion of fossil fuel due to the development of the ore industry in advanced nations after the industrial revolution. The CH_4 and N_2O attributed to animal agriculture still continue to increase. CO_2 contributes more than half of the greenhouse effect of global warming, and CH_4 is the next largest GHG to CO_2. The concentration of atmospheric CH_4 is only 2 ppmv or less, far less than the 396.81 ppmv of carbon dioxide (Mauna Loa Observatory: NOAA-ESRL in December 2013). However, the greenhouse effect of CH_4 and N_2O is 21 times and 310 times, respectively, more than that of carbon dioxide for each molecule. The total CO_2-eq concentration of all long-lived GHGs is currently estimated to be about 455 ppmv CO_2-eq (IPCC, 2007). As CH_4 is removed after reacting with radical OH of the troposphere, its longevity (12–17

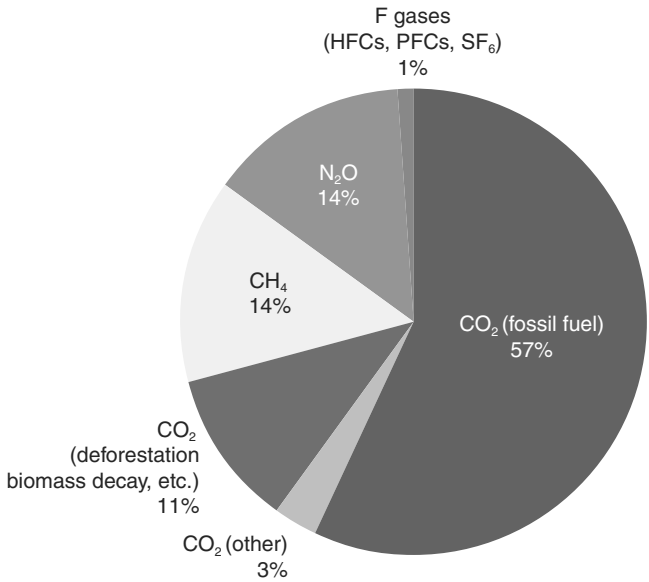

Fig. 8.2. The contribution of greenhouse gases to global warming. (From IPCC, 2007.)

years) in the atmosphere is less than 50–200 years, compared to CO_2. The concentration of atmospheric CH_4 at present is twice its value before the Industrial Revolution.

The annual rise in the concentration of CH_4 has shown a rapid increase of 1.0–1.3% in the past decade compared with the 0.5% increase of CO_2. Although atmospheric CO_2 has a large absorption region at around 16 μm wavelength in solar radiation, infrared absorptivity is already presumed to be almost saturated by water vapour, of which the wavelength region for far-infrared absorption is overlapped by CO_2. For CH_4 and N_2O, however, the relative absorptivity of far-infrared radiation is large, and their absorptivity is assumed not to be saturated like that of CO_2, because their absorption wavelengths of far-infrared are shorter than those of CO_2. The absorptivity of far-infrared radiation is assumed to rise proportionally with the rise in temperature. Therefore, even a small increase in CH_4 and NO_2 concentration in the atmosphere will exert an extreme and strong effect on global warming.

It is possible to divide the sources of CH_4 roughly into natural and anthropogenic sources. The annual emission of CH_4 in the atmosphere amounts to 535 Tg (Tg = million metric tonnes (Mt); IPCC, 1995), of which 85 Tg derives from the rumen fermentation of ruminant animals and 25 Tg derives from animal wastes, and thus ruminants contribute 20% of the total CH_4 emissions. This source of CH_4 emission is as large as the marsh (wetland) contribution of ~115 Tg. Sixty per cent or more of CH_4 production comes directly from human-related activities. The expansion of rice paddy (60 Tg) and livestock is almost proportional to the expansion of the human population. This may be the main cause of the increase in CH_4 in the atmosphere. CH_4 produced in rumen fermentation is emitted into the atmosphere by the eructation process of animals. The ruminant species is a large group that contains the *Bos* subfamily, classified into five genera and 14 species, wild kinds of giraffe, deer families, etc. However, the target for controlling CH_4 emissions from ruminants includes domesticated milking and beef cattle. The amount of CH_4 emitted by eructation from a lactating cow is presumed to be more than 200–400 l day^{-1}, and around 1.49 billion domestic cattle are being raised on earth as per the latest FAO estimate (http://faostat.fao.org/). So, almost two drums of pure CH_4 are being emitted into the atmosphere by one milking cow in a day.

To abate GHGs, the development of mitigation strategies for enteric CH_4 is the most significant issue in livestock production (Van Nevel and Demeyer, 1996). Currently, artificial nitrogen fixation accounts for 1.6×10^8 t, whereas natural nitrogen fixation by nitrogenase contributes 1.8×10^8 t. Severe environmental pollution was caused at the same time, although the reactive nitrogen withdrawn from the atmosphere as stable paired nitrogen brought about prosperous food production. The flow of nitrogen in animal agriculture, measurement estimates and strategies to protect the environment, etc., are debated at length in Chapter 4, Section I, this volume. To secure food production and prevent environmental catastrophe by global warming, the sustainable development of animal agriculture should be sought as an alternative, not only in industrialized but also in developing countries. Inventories of emitters and their abatements should be assessed accurately in both GHGs. The key element of this recycling must be low input for sustainable animal agriculture in developing countries. Carbon and nitrogen recycling of agricultural biomass as a renewable energy and nitrogen resource may contribute to mitigate CH_4 and N_2O emission (Takahashi *et al*., 2003, 2004; Takahashi, 2007; Takahashi and Uemura, 2009).

8.3 Mitigation of Enteric CH_4 Emission

In the rumen, metabolic H_2 is produced during the anaerobic fermentation of carbohydrates. This H_2 can be used during the synthesis of volatile fatty acids, and excess H_2 from nicotinamide adenine dinucleotide (NADH) is eliminated primarily

through methanogenesis, a process executed by methanogenic archaea, which are natural occupants of the rumen ecosystem (Baker, 1999). The stoichiometric balance of volatile fatty acid (VFA), CO_2 and CH_4 indicates that acetate and butyrate promotes CH_4 production, whereas propionate formation consumes H_2, and thereby reduces CH_4 production (Wolin and Miller, 1988). Therefore, a strategy for the mitigation of ruminal CH_4 emission requires that alternative metabolic pathways compete with methanogenesis for H_2 uptake, ensuring the disposal of reducing power from the rumen. One of the alternative ways for safe H_2 disposal from the rumen is reductive acetogenesis, which not only ensures the safe deployment of H_2 from the rumen but also leads to an energetic gain to the host animal. The potential of reductive acetogenesis as an alternate mechanism for methanogenesis and the competitiveness of rumen acetogens are discussed elsewhere in this volume (Chapter 19, Section III, this volume). Nitrate reduction in the rumen is another passage for removing H_2 from the methanogens, due to relatively higher affinity than methanogen hydrogenotrophs. The administration of nitrate (NO_3^-) remarkably suppressed ruminal methanogenesis in the studies conducted by Takahashi and Young (1991, 1992).

Figure 8.3 shows that the formation of toxic nitrite, a reduced metabolite of nitrate reduction, is successfully prevented by L-cysteine (Takahashi *et al*., 1989, 1998, 2000, 2002; Takahashi and Young, 1991, 1992). In these studies, the researchers mitigated ruminal CH_4 emission effectively with simultaneous administration of nitrate and L-cysteine without nitrate intoxication (Takahashi, 2001). Furthermore, genetically modified *Escherichia coli* was developed in an attempt to promote the nitrite reduction abating ruminal methanogenesis (Sar *et al*., 2004a, 2005a,b,c; Fig. 8.4). The mitigating effects of two kinds of *E. coli* strain, wild-type *E. coli* W3110 and *E. coli* nir-Ptac, which has enhanced nitrite (NO_2^-) reduction activity, on CH_4 emission and NO_3 toxicity has been confirmed in *in vitro* and *in vivo* trials. Wild-type *E. coli* W3110 is known to have a certain NO_2^- reductase activity, in which NO_2^- reductase, consisting of two kinds of subunits encoded by nir*BD* operon, is usually involved in NO_3^- respiration and is induced under oxygen-limited conditions (Gennis and Stewart, 1996). The *E. coli* strain

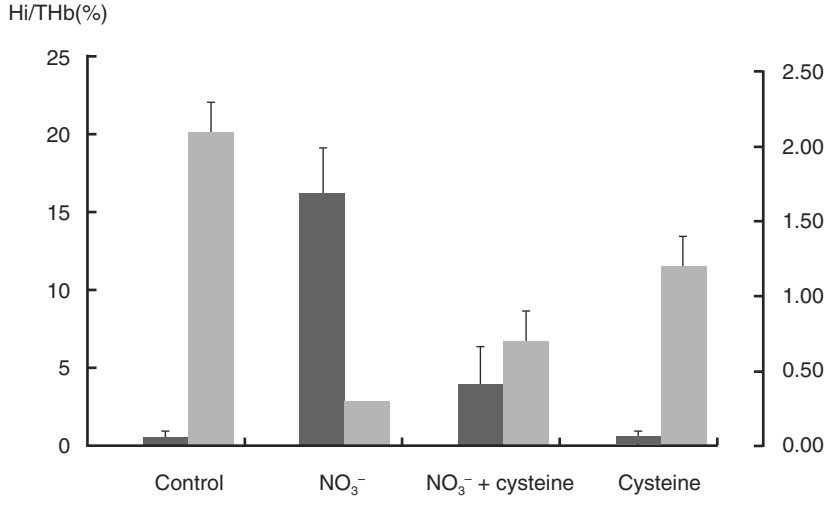

Fig. 8.3. The suppressing effect of nitrate (1.3 f $NaNO_3$ kg^{-1} $W^{0.75}$) on CH_4 emission and the prophylactic effect of L-cysteine (0.21 g S kg^{-1} $W^{0.75}$) on nitrate induced methaemoglobinaemia in sheep.

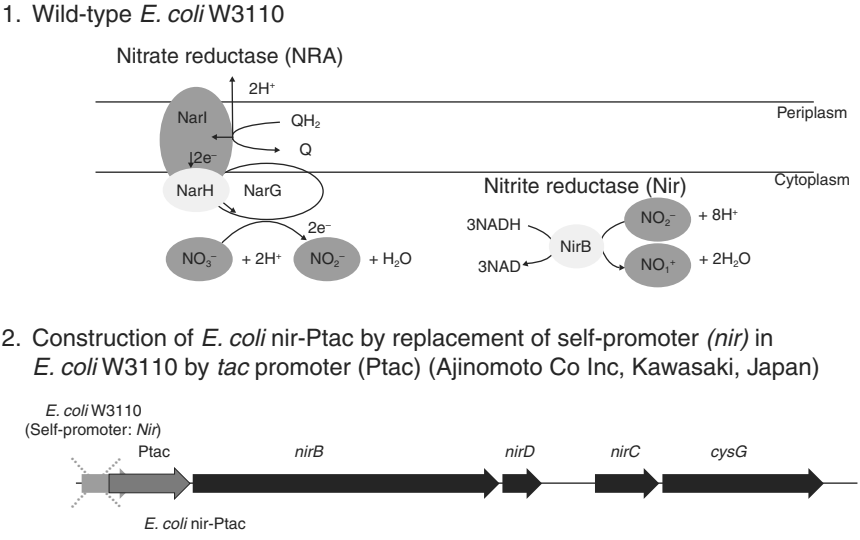

Fig. 8.4. Wild-type *Escherichia coli* W3110 and the construction of *E. coli* nir-Ptac.

nir-Ptac with higher NO_2^- reductase activity, in which the *nirBD* in *E. coli* W3110 is expressed constitutively by replacing its promoter with a *tac* promoter, has been constructed (Ajinomoto Co Inc, Tokyo, Japan). The NO_2^- reductase activity of the *E. coli* strain nir-Ptac was found to be approximately two times higher than its wild counterpart, *E. coli* W3110. *E. coli* nir-Ptac inhibited *in vitro* rumen NO_2^- accumulation and CH_4 production more than wild-type *E. coli* W3110 in NO_2^--containing cultures, and feeding of anaerobic cultures (Sar *et al*., 2005c). Rumen methanogenesis was reduced by the inoculation of *E. coli* nir-Ptac or *E. coli* W3110 (Sar *et al*., 2005a). However, rumen and plasma NO_2^- accumulation and erythrocyte methaemoglobin production in sheep were not affected by the inoculation of *E. coli* nir-Ptac.

Rumen manipulation with ionophores such as monensin has been reported to abate rumen methanogenesis (Mwenya *et al*., 2005). Ionophores in respect of CH_4 abatement are debated comprehensively in Chapter 17, Section III, this volume. There is an increasing interest in exploiting prebiotics and probiotics as natural feed additives and alternatives to antibiotics, due to mounting concerns about the incidences of bacterial resistance and environmental pollution (Mwenya *et al*., 2006). Nisin and extract of *Yucca schidigera* and *Quillaja saponaria* comprising saponin have been categorized as 'generally recognized as safe' (GRAS) for human consumption by the US Food and Drug Administration. Nisin produced by *Lactococcus lactis* subsp. *lactis* showed a mitigating effect on ruminal CH_4 emission (Mwenya *et al*., 2004a; Santoso *et al*., 2004b; Sar *et al*., 2006). Saponins are the natural detergents found in a variety of plants. Yucca saponins have a steroidal nucleus, whereas quillaja saponins are triterpenoid in structure (Fig. 8.5). Supplementation of commercial-grade saponin, saponin-rich plants or extracts decreased ruminal protozoa and methanogenesis accompanied by narrowing ruminal acetate/propionate (A/P) ratio *in vitro* and *in vivo* (Wallace *et al*., 1994; Takahashi *et al*., 2000; Wang *et al*., 2000; Mwenya *et al*., 2004a; Santoso *et al*., 2004a; Pen *et al*., 2006; Malik and Singhal, 2008; Malik *et al*., 2010). However, the studies of Pen *et al*. (2007, 2008) showed no effect of *Q. saponaria* on ruminal methanogenesis and A/P ratio, although it suppressed protozoal numbers.

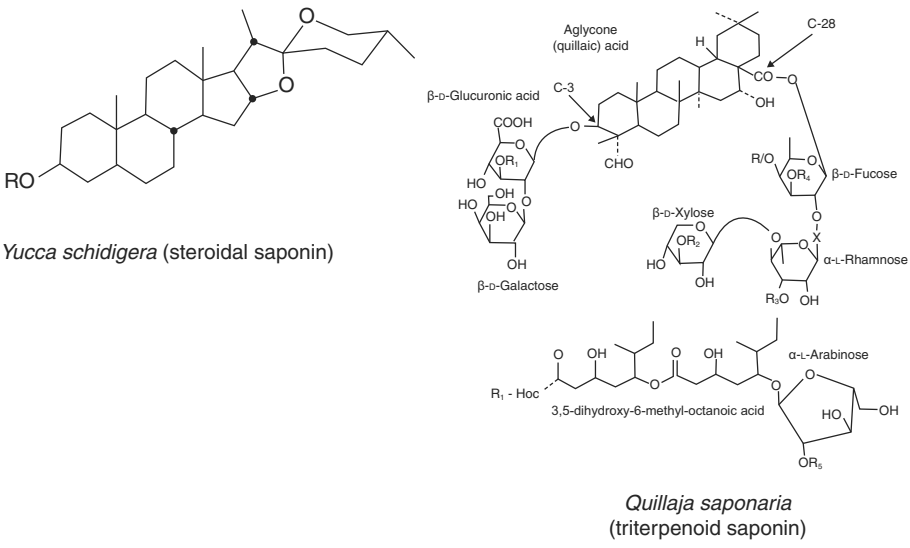

Fig. 8.5. Chemical structure of *Yucca schidigera* and *Quillaja saponaria*.

Galacto-oligosaccharides (GOS) are non-digestible carbohydrates in non-ruminants and have a long history of research as a prebiotics food ingredient (Fig. 8.6). GOS are resistant to gastrointestinal enzymes, but are utilized selectively by *Bifidobacteria* (Sako *et al*., 1999). In the rumen, *Bifidobacteria* and *Lactobacillus* species utilize fructose, galactose, glucose and starch as substrates to produce lactate and acetate. Lactate is an intermediate compound of the acrylate pathway during propionate production in the rumen. The intensity of propionate production competes indirectly with rumen methanogens for the available hydrogen. As *Bifidobacteria* and *Lactobacillus* species in the rumen can utilize GOS and produce more lactate, ruminal methanogenesis is suppressed by β1-4 galacto-oligosaccharides with or without direct-fed microbe yeasts and lactic acid bacteria (Gamo *et al*., 2001; Sar *et al*., 2002, 2004b,c; Takahashi *et al*., 2002, 2003; Mwenya *et al*., 2004b,c,d, 2005; Santoso, 2004a). However, the efficacy of β1-4 galacto-oligosaccharides with probiotics on different diets and animal species remains to be elucidated.

There has been a growing interest in utilizing antimicrobial substances for selectively targeting rumen methanogens to reduce CH_4 emissions (Nollet *et al*., 1998; Less *et al*., 2002; Asa *et al*., 2010). Figure 8.7 shows the effect of protease-resistant antimicrobial substances (PRA) produced by

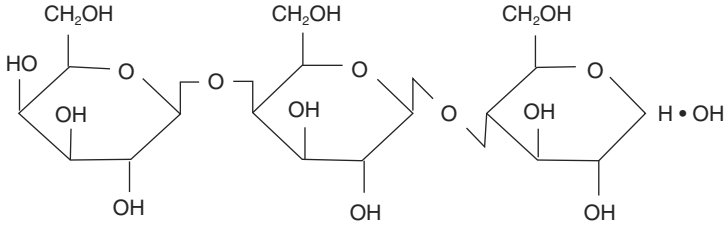

Fig. 8.6. Chemical structure of β1-4 galacto-oligosaccharides.

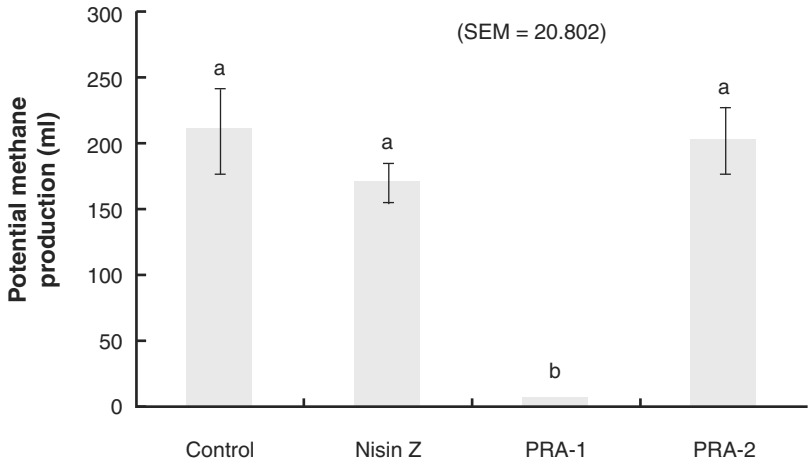

Fig. 8.7. Effect of PRA on potential CH_4 production. Vertical bars represent standard deviation ($n = 4$). Means with different letters differ significantly ($p < 0.01$).

Lactobacillus plantarum and *Leuconostoc citreum* on rumen methanogenesis in *in vitro* continuous CH_4 quantification systems (Asa *et al*., 2010). Four different strains of lactic acid bacteria, Control: *Lactococcus lactis* ATCC19435 (non-antibacterial substances), Nisin-Z: *L. lactis* NCIMB702054, PRA-1: *L. plantarum* TUA1490L and PRA-2: *L. citreum* JCM9698, were cultured individually in GYEKP medium. PRA-1 remarkably decreased cumulative CH_4 production. For PRA-2, there was no effect on CH_4 and CO_2 production and fermentation characteristics in mixed rumen cultures. The results suggested that PRA-1 reduced the number of methanogens or inhibited utilization of hydrogen in rumen fermentation.

The supernatant of *L. plantarum* TUA1490L (LP) reduced *in vitro* CH_4 production, but the non-proteinaceous antimicrobial substance (PRA-1) was not identified, which subsequently has been shown as hydrogen peroxide (Takahashi, personal communication). The antimicrobial effect of H_2O_2 has been attributed to its strong oxidizing effect on bacterial cells and to the destruction of the molecular structure of cell proteins (Ito *et al*., 2003; Zalán *et al*., 2005). However, there is no information available on the effect of H_2O_2 on rumen fermentation. Thus, it was necessary to assess if methanogenesis was more sensitive to H_2O_2 in the LP supernatant than primary and secondary rumen fermentation processes that produce volatile fatty acids (VFA). The effect of the supernatant of *L. plantarum* TUA1490L on *in vitro* rumen CH_4 output was investigated minutely with *in vitro* gas quantification system (Takahashi and Kawabe, 2011) installed auto infrared CH_4 and CO_2 analysers (O'Brien *et al*., 2013). In consequence, H_2O_2 was detected in the supernatant and CH_4 output was reduced by 72%. However, the supernatant had an adverse effect on total VFA concentration.

8.4 Innovative Reuse of the Digested Slurry to Mitigate N_2O

The increased emissions of CH_4 and N_2O from decomposing unmanaged and bio-based industrial wastes along with the expansion of human activities contribute to climate change. The biogas plant produces biogas including combustible CH_4 as a renewable energy using unused resources like animal manures, and can provide fuel, heat and electricity (Takahashi *et al*., 2004; Umetsu *et al*., 2005, 2006; Komiyama *et al*., 2006) and minimize the impact on the environment by reducing the amount of

pollutants discharged. The biogas system and its application have been expanded in APEC (Asia-Pacific Economic Cooperation) as a mitigation strategy with an economic incentive (Takahashi, 2009).

The conventional biogas system based on the anaerobic fermentation of organic wastes, however, is not specifically for nitrogen recycling but rather is more specifically for carbon recycling. Therefore, isometric fertilization of the digested slurry after anaerobic fermentation may not be the solution to the current issue of the abatement of excess nitrogen, although N_2O is almost completely suppressed during anaerobic fermentation (Takahashi, unpublished results). It causes not only CH_4 emission, but also nitrate leaching and N_2O emission from soil (Takahashi, 2006). The introduction of ammonia stripping from the digested slurry of a thermophilic biogas plant might be a solution to reduce the total nitrogen content of the slurry as a liquid fertilizer containing suitable nitrogen, and eventually could contribute to the mitigation of N_2O emission as a new concept of the biogas system (Fig. 8.8). Furthermore, the stripped ammonia can be put to practical use as a low-input and renewable nitrogen resource without energy supply from outside, because an abundant amount of organic wastes exists in developing countries and the energy required for ammonia stripping can be supplied from a biogas plant attached to the ammonia-stripping apparatus. The following three options have been examined for future nitrogen recycling:

1. Production of high-quality feed from cellulose biomass in agricultural waste with ammonia-stripping process from digested slurry of biogas plant (Takahashi, 2007).
2. Saccharification of soft cellulose biomass to create bioethanol and hydrogen using ammonolysis by stripped ammonia from effluent and hydrolysis of rumen bacteria.

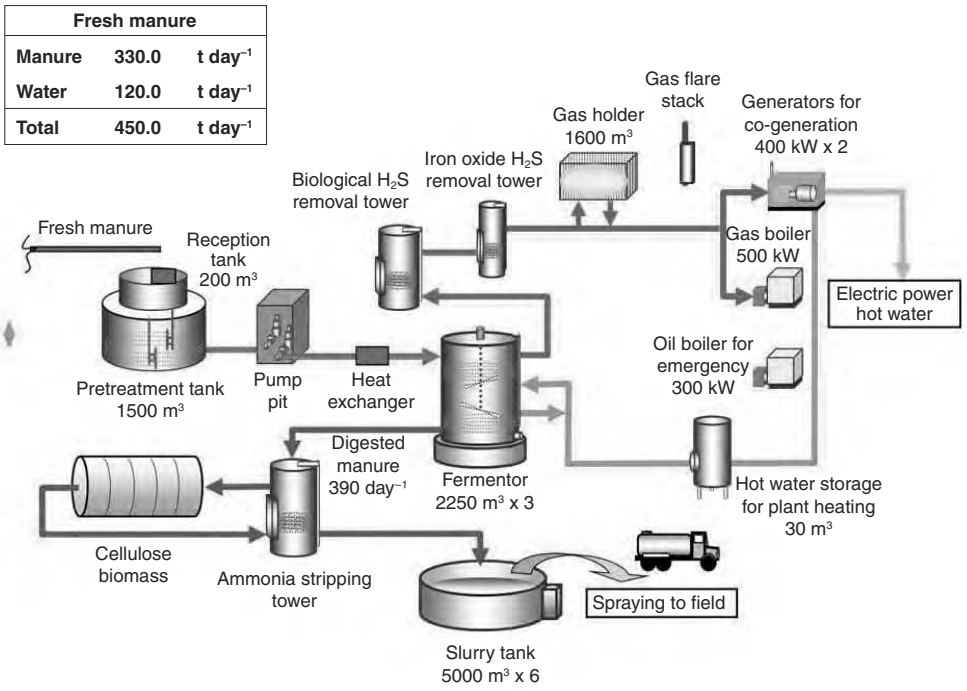

Fig. 8.8. New advanced biogas system.

3. Ammonia fuel cell with ammonia stripping from digested slurry (Takahashi and Uemura, 2009).

8.5 Ammonia Removal with Heterotrophic Nitrification

Improper management of livestock wastewater will cause eutrophication in the hydrosphere due to nitrate and N_2O emission in the atmosphere attributed to an excess amount of ammonia nitrogen (also see Chapter 4, Section I, this volume). It is a common issue in Asian developing countries where a gradual increase in population and urbanization has been progressing along with economic development. So far, most biotechnological approaches to ammonia removal from livestock wastewater have been implemented by aerobic nitrification and anaerobic denitrification using autotrophs. However, the autotrophic bacteria are presumably unsuitable for livestock wastewater treatment because of the high strength of the ammonium and organic matters. Furthermore, the long retention for autotrophic nitrification has been designated due to the slow proliferation rate of the bacteria. In an attempt to seek the ability of heterotrophic bacteria to remove ammonia, *Alcaligenes faecalis* strain No 4 was isolated from the sewage sludge (Joo *et al*., 2006). As an alternative for mitigating N_2O and nitrate emission derived from animal agriculture (Fig. 8.9), heterotrophic nitrification and the aerobically denitrifying effect of *A. faecalis* strain No 4 on an excess amount of ammonia nitrogen was evaluated in wastewater collected from a piggery in a suburb of Shanghai, China, according to the procedure reported by Joo *et al*. (2006). Possible removal of ammonia from piggery wastewater using *A. faecalis* strain No 4 was confirmed with proper controlling of the C/N ratio and pH.

8.6 Conclusion

The major GHGs attributed to animal agriculture are CH_4 and N_2O. Approximately 18–20% of atmospheric CH_4 is derived from enteric fermentation, and organic effluent comes from the animal agriculture sector. Methanogen archaea in the rumen reduce CO_2 into CH_4 using metabolic H_2. Some prophylactics such as probiotics, prebiotics and natural compounds including nitrate are used as alternatives to replace ionophores for rumen CH_4 mitigation. These prophylactics manipulate rumen microbial

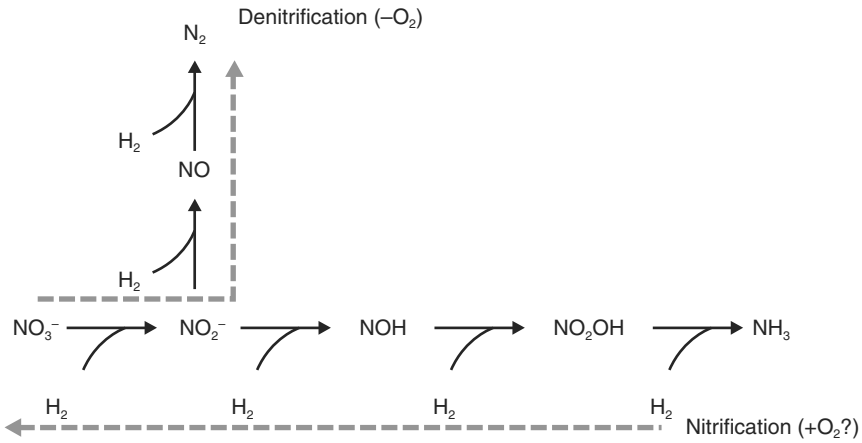

Fig. 8.9. Ammonia removal with heterotrophic nitrification and aerobic denitrification abilities of *Alcaligenes faecalis*.

fermentation by enhancing propionate production via the acrylate pathway. Ammonia derived from animal manures and chemical fertilizers also contribute to nitrate pollution in the hydrosphere and N_2O pollution in the atmosphere. Nitrogen pollution might be mitigated by recycling using ammonia-stripping technology combined with thermophilic anaerobic fermentation. Biotechnological approaches using highly exploited profitable organisms for co-existence and co-prosperity in sustainable animal agriculture may be an alternative way for developed and developing countries.

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9 Carbon Footprints of Food of Animal Origin

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Abstract

Animal production contributes substantially to global greenhouse gas emissions (about 14.5%). So-called carbon footprints (CFs) consider the greenhouse gas potential of climate-relevant gases (e.g. $CO_2 \times 1$; $CH_4 \times 23$; $N_2O \times 296$), which is given in carbon dioxide (CO_2)-equivalent g^{-1} or kg^{-1} of product or unit of edible protein. CFs may help to assess the greenhouse gas emissions associated with the production of food of animal origin such as milk, meat, eggs or fish, and they may contribute to sensitizing producers and consumers to a more resource-efficient and environmentally friendly production and consumption of food of animal origin and to avoiding food wastage. The highest CFs per unit edible protein are calculated for products of growing ruminants (beef and lamb), followed by milk, pork, eggs and poultry meat, with the lowest values. Discrepancies in the results of various studies are explained mainly by different system boundaries, allocation methods and computation of emissions, especially with regard to land-use changes, enteric methane (CH_4) and nitrous oxide (N_2O) emissions. A more standardized approach for CF calculations would be a very useful tool to compare CFs between production systems, regions and countries, and as an indicator for food labelling. The production of food of animal origin is a very complex process, and a selective consideration, i.e. focusing on single factors, does not provide an assessment that reflects the complexity of the subject.

9.1 Introduction

A growing population and higher need for feed and food are associated with a growing demand for limited natural resources, and elevated emissions with greenhouse gas (GHG) potential, such as carbon dioxide (CO_2), methane (CH_4), nitrous oxide (N_2O) and other substances (e.g. nitrogen (N), phosphorus (P), trace elements, etc.), characterize the situation all over the world (IPCC, 2006; Steinfeld *et al*., 2006; FAO, 2009, 2010; Godfray *et al*., 2010). The shift from plant-based diets to more animal products (Delgado, 2003; Tilman *et al*., 2011; Kastner *et al*., 2012), based on higher income (Keyzer *et al*., 2005), requires higher yield or more land for providing adequate feed to livestock (Gerbens-Leenes and Nonhebel, 2002; Naylor *et al*., 2005; Wirsenius *et al*., 2010). Therefore, some experts propose a redefinition of agricultural yield and agriculture in general, from tonnes to people nourished per hectare (Kastner *et al*., 2012; Cassidy *et al*., 2013), and ask more and more for sustainable animal agriculture (Kebreab, 2013).

The balance between planet (global resources and emissions), people (population all over the world) and profit (money making), in the so-called 3P concept (Boonen *et al*., 2012), is an important prerequisite for

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sustainable life and development on earth. Profit should not be the single objective of production. We need to find a balance between a careful and sustainable use of limited resources such as arable land, water, fuel and some minerals on the one hand (Fedoroff *et al*., 2010; Giovannucci *et al*., 2012) and low emissions with local and global consequences for later generations (Foley *et al*., 2011) on the other hand.

Attention has been paid to so-called carbon footprints (CFs), but these have also been modified or called ecological footprints (EFs), eco-balances (EBs), life cycle assessments (LCAs) or life cycle impact assessments (LCIAs). In all cases, the term means a summarized parameter for all gaseous emissions with GHG potential to sensitize producers and consumers (Upham *et al*., 2010; Young *et al*., 2010), for an efficient use of fossil carbon sources and to reduce GHG emissions per product (de Alvarenga *et al*., 2012). CFs or LCAs are used as a tool for estimating the environmental effects caused by products or processes. Furthermore, CFs may also contribute to assessing the resource and feed efficiency between various regions and production systems. Recently, some experts have written about problems with LCAs (Ciroth *et al*., 2004; Reap *et al*., 2008; Morais and Delurue-Matos, 2010; Caffrey and Veal, 2013), and new developments such as more comprehensive life cycle sustainability analysis (LCSA; Guinee *et al*., 2011), but a unified solution to the subject is still lacking. Therefore, CFs are calculated and interpreted in this chapter in the common way.

Agriculture, and especially animal husbandry, are considered as important GHG sources because of the high GHG potential of some emissions associated with animal production (e.g. CH_4 and N_2O), which are estimated at 7.1 Gt CO_2-equivalent (eq) per annum, representing 14.5% of human-induced GHGs (see Gerber *et al*., 2013). Table 9.1 summarizes the present production of food of animal origin, expected growing rates and emissions for animal groups. Globally, ruminant supply chains are estimated to emit 5.7 Gt CO_2-eq $year^{-1}$, of which 81%, 11% and 8% are associated with cattle, buffalo and small ruminant production, respectively (Opio *et al*., 2013).

Based on these, the objective of the present contribution is to analyse the sources and amounts of gases associated with the production of food of animal origin and to deduce parameters (so-called carbon footprints) to assess the level of emissions from animal production.

9.2 Fundamentals to Calculate Carbon Footprints

Carbon footprints are defined as the total amount of GHG emissions (under consideration of their GHG potential) associated with a product along its supply chain (see Fig. 9.1).

CFs sometimes include emissions from consumption, end-of-life recovery and disposal. Usually, CFs are expressed in kilograms or tonnes of carbon dioxide

Table 9.1. Present production, growing rates and emissions for some groups of food of animal origin.

Animal groups	Ruminants	Pigs	Poultry
Production	Milk: 864	CW:[a] 110	Eggs: 58
(million tonnes)	Meat: 81		CW: 72
Growth rate	Milk: 1.1	1.3	Eggs: 1.6
(% $year^{-1}$; until 2030/50)	Meat: 1.3		Meat: 2.4
Emissions	Milk: 2.0	0.7	Eggs: 0.2
(Gt;[b] CO_2-eq $year^{-1}$)	Meat: 3.7		Meat: 0.4

Notes: [a]Carcass weight; [b]gigatonnes calculated from the data of Gerber *et al*. (2013), MacLeod *et al*. (2013) and Opio *et al*. (2013).

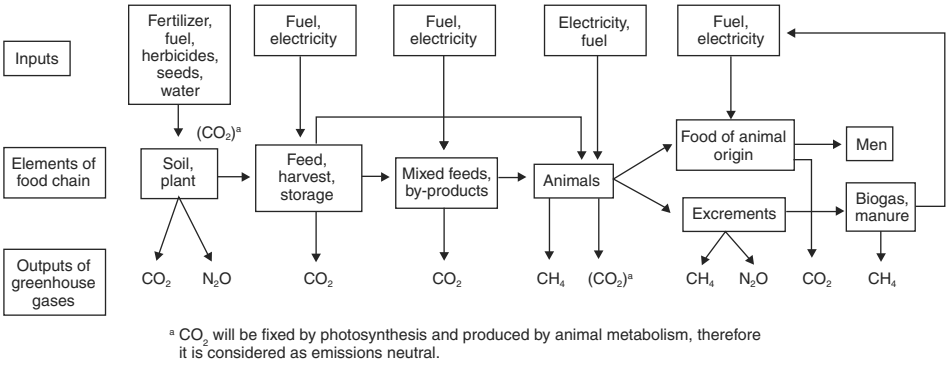

Fig. 9.1. Substantial elements of the food/supply chain to produce food of animal origin as well as selected inputs of resources and outputs of greenhouse gases (base for system boundaries).

equivalents (CO_2-eq) per unit of product (Opio *et al*., 2013). Studies and publications on CFs have increased dramatically during the past few years, demonstrating that the interest for more resource-efficient and cleaner production is enhanced. Agriculture, and especially animal husbandry, is considered as an important GHG source because of the high greenhouse potential of the emissions (e.g. $CO_2 \times 1$; $CH_4 \times 23$ and $N_2O \times 296$; IPCC, 2006). CFs consider the GHG potential of climate-relevant gases and are given in CO_2-eq g^{-1} or kg^{-1} product. Various authors have calculated such CFs for agriculture in general, but also for separate segments.

The public interest in CFs is discussed in the context of global warming and possible climate changes (IPCC, 2006). The chapter presents the most important factors in agricultural primary production along the whole food chain, i.e. soil, plant production (harvesting, conservation), industrial feed production, livestock keeping (including excrement management) and possible other influencing factors such as feed and food processing, transport and trade (Fig. 9.1) resulting in climate-related emissions. The consequences of land-use change (LUC, i.e. change of forest into cropland or pasture) for CF calculations should also be considered, but in some cases the values are not known or not considered in calculations (e.g. import of feeds).

A number of factors (e.g. plant yield, animal species and performances, type of production) cannot be ignored when taking into account the GHG potential of the various gases (see above) to derive CFs and for the comparison of values along the food chain (see Fig. 9.1).

9.2.1 Carbon dioxide (CO_2)

The direct CO_2 emission from animals can be considered as emission neutral. The CO_2 will be fixed by the photosynthesis of plants and excreted by the animals as a result of animal metabolism (see Fig. 9.1). But nevertheless, the CO_2 emission must be viewed along the whole food chain and include burning of fossil carbon during feed production (Fig. 9.1) and land-use changes. In general, non-carbon dioxide GHGs such as CH_4 and N_2O come directly from animals or from animal manure practices.

9.2.2 Methane (CH_4)

CH_4 is emitted from the enteric fermentation in the digestive tract of animals, mainly in the rumen, but also during manure management (see Fig. 9.1). Details of enteric CH_4 emission are described in many papers and prediction equations are given (Flachowsky *et al*., 2011; Hristov *et al*.,

2013a,b; also see Section III, this volume). The CH_4 emissions from manure management are generally not directly associated with animals, but the losses can be substantially high (Hristov *et al*., 2013a,c; Montes *et al*., 2013), especially if the excreta are stored under anaerobic conditions.

9.2.3 Nitrous oxide (N_2O)

Animals do not excrete N_2O directly, but it can be formed in manure depending on the storage conditions and the following land application (Flachowsky and Lebzien, 2007; Hristov *et al*., 2013a,c; Montes *et al*., 2013). These microbial processes depend on the temperature, moisture content and oxidation status of the environment. Further details are described in Chapter 4, Section I, this volume.

9.3 Calculation of CFs of Food of Animal Origin

Over the past 15 years, beginning with one or two studies being published per year from 1998 to 2000, the number of studies published has increased, with about 20 studies being published in 2011 (Avadi and Fréon, 2013). The studies dealt with calculations of the CFs of nearly all types of food of animal origin (see summaries by Williams *et al*., 2006; Leip *et al*., 2010; Gruenberg *et al*., 2010; Flachowsky, 2011; Flachowsky *et al*., 2011; Lesschen *et al*., 2011; Gerber *et al*., 2013; MacLeod *et al*., 2013; Opio *et al*., 2013).

The results of CF calculations for foods of animal origin depend on many influencing factors, such as the end points of animal production. The advantages and weaknesses of end points (outputs) of various forms of animal production are summarized in Table 9.2. All end points are characterized by some advantages and disadvantages. From nutritional and scientific points of view, edible protein seems to be the most favourable measurement, but in the case of meat production, its measurement is not easy and requires some analytical work.

For practical reasons, carcass weight or weight gain (warm or cold) would be the most important end point to measure the yield of slaughtered animals, because this weight is measurable in the slaughter house (Peters *et al*., 2010) and can be used for further calculations. Ways to calculate CFs and examples for various foods of animal origin are shown and discussed in the following sections.

9.3.1 Milk

Table 9.3 demonstrates some important emission sources and steps to calculate emissions per cow per year and per kilogram of milk. The values per cow or per kilogram

Table 9.2. Advantages and disadvantages of various outputs/end points of animal yields.

Animal yields	Advantages	Disadvantages
Milk, eggs	Easily measurable, almost completely edible	Variation in protein, fat and energy yield, analysis may be useful
Body weight gain	Easily measurable	High portion of non-edible fractions in the gains
Carcass weight	Easily measurable	Still contains fractions that are not edible (e.g. bones)
Meat, edible fraction	Completely edible	Categorization and separation not easy
Edible protein	Most important objective of animal production; comparison of various ways and sources to produce protein of animal origin	Categorization of various fractions as edible and difficulties in measuring; additional analytical work; variation in N-protein content

Table 9.3. Calculation of emissions per cow per year (body weight: 650 kg; milk yield: 8000 kg and 1 calf year^{-1}).

Emissions (kg cow^{-1} year^{-1})			
Sources of emission	CO_2	CH_4	N_2O
Fertilizer	210	5.5	1.1
Feed	83	–	1.2
Transport, treatment	43	–	
Rumen fermentation		119	
Fermentation of excrement management		19	0.9
Emissions from soil[a]		–1	1.8
Total	336	143	5
CO_2-equivalents of emission (kg cow^{-1})	336	3290	1500
CO_2-equivalents (kg cow^{-1} year^{-1})	5200		
CO_2-equivalents (g kg^{-1} milk)[b]	650		
(Per cent of total emissions)	6	65	29

Notes: [a]No land-use change; [b]without calf and heifer. (Daemmgen and Haenel, 2008.)

of milk depend on the levels of emissions and on the milk yield. The calculation shows that in this case about two-thirds of emissions come from CH_4.

Table 9.4 shows the exemplary CF for milk, taking various boundaries into consideration. A clear definition and understanding of these system boundaries are important prerequisites for comprehending the calculations and making the results easily comparable (Casey and Holden, 2006a,b; Matthews *et al*., 2008). Furthermore, a clear definition of milk (e.g. energy, protein or fat-corrected milk; see Table 9.4) is also necessary to compare the calculations of various authors. Scientists working in this field should agree on the system boundaries and the GHG factors of the climate-relevant gases.

The large range in the CFs of milk in comparing the results of various authors and depending on the many influencing factors is shown in Tables 9.5 and 9.6. The CF for milk calculated by various authors ranges between 0.4 and 1.5 kg CO_2-eq kg^{-1} in Europe, North America and Oceania, and taking into consideration the different world regions, between 1.3 (Europe and North America) and >10 kg CO_2-eq kg^{-1} in sub-Saharan Africa or the highlands of Peru (FAO, 2010; Bartl *et al*., 2011; see Tables 9.5 and 9.6). Most authors considered only the emissions during production, but sometimes LUC as well as processing, transport and trade were also included in the calculations. In their recent publication, Opio *et al*. (2013) mentioned ranges from 1.6 to 9.0 kg CO_2-eq kg^{-1} fat- and protein-corrected milk (FPCM)

Table 9.4. Model calculation to demonstrate the effects of various system boundaries of CFs of milk (g CO_2-eq kg^{-1} of milk; 30 kg milk cow^{-1} day^{-1}; diet on DM-base: 60% roughage, 40% concentrate; 4% milk fat, 3.4% protein; 305-day lactation; 60-day dry period, 3-year lactation; 30 months calf and heifer period).

System	System boundaries	CF (g CO_2-eq kg^{-1} milk)
1	Animal-caused emissions (including CH_4 during lactation period)	280
2	1+ Emissions of feed production (without LUC)	430
3	2 + Dry period of cows	500
4	3 + Heifer period	730
5	4 + Animal housing and milking	760
6	5 + Excrement management	820
7	6 + Processing, transportation and trade of milk	1100

(Flachowsky *et al*., 2011.)

Table 9.5. Examples of CFs (kg CO_2-eq kg^{-1} milk) depending on the type of production.

Country	Type of production/farming		Reference
	Conventional	Organic	
Germany	0.83	0.84	Woitowicz (2007)
	0.85	0.78	Hirschfeld *et al.* (2008)
	0.94	0.88	Fritsche and Eberle (2007)
	0.98	0.92	GEMIS (2009)
	1.30	1.30	Haas *et al.* (2001)
Sweden	0.90	0.94	Cederberg and Flysjö (2004)
	0.99	0.94	Cederberg and Mattsson (2000)
The Netherlands	0.97	1.13	Van der Zijpp (2001)
	1.40	1.50	Thomassen *et al.* (2007)
UK	1.06	1.23	Williams *et al.* (2006)
	1.20	1.30	PAS (2011)
	1.6 (1.0–3.2)	1.3 (0.9–2.4)	Plassmann and Edwards-Jones (2009)
Austria	1.20	1.00	Lindenthal *et al.* (2010)

Table 9.6. CFs (kg CO_2-eq kg^{-1} milk) in different countries without considering production type.

Country	CF kg CO_2-eq kg^{-1} milk	Reference
Germany	0.40 (40 kg milk)	Flachowsky (2008)
	0.65	Daemmgen and Haenel (2008)
	0.98 (10,000 kg milk $year^{-1}$) 1.35 (6000 kg milk $year^{-1}$)	Zehetmeier *et al.* (2011)
Model calculation	0.55 (20 kg milk day^{-1}) 1.00 (10 kg milk day^{-1})	Flachowsky (2008)
New Zealand	0.65–0.75	Basset-Mens *et al.* (2009)
USA	0.86	Ledgard *et al.* (2004)
	1.09	Phetteplace *et al.* (2001)
	1.35	Peters *et al.* (2010)
Sweden	1.00	Cederberg *et al.* (2009)
Canada	1.00	Verge *et al.* (2007)
UK	1.06	Williams *et al.* (2006)
EU	1.3 (1.0–2.3; EU-27)	Lesschen *et al.* (2011)
Ireland	1.3–1.5	Casey and Holden (2005)
Norway	1.5–1.6 (combined milk/meat; expanded boundaries)	Roer *et al.* (2013)
Peru; Coast Highlands	3.2 13.8	Bartl *et al.* (2011)
Global	2.4 (1.3–7.5; global)	FAO (2010)
Literature review	0.8–1.4 (on farm) 0.9–1.8 (on-farm + post-farm emissions)	Sevenster and de Jong (2008)
Global – cow Global – buffalo Global – small ruminants	2.8 3.4 6.5	Opio *et al.* (2013)

for regional emission intensity. Generally, milk production in low productive systems has higher emissions per kilogram FPCM than in high production systems (Gerber *et al*., 2011). CH_4 and N_2O emissions per cow increase, but they decrease per kilogram of milk with increasing productivity, while CO_2 increases because of a higher feeding of concentrates, but on a much lower scale.

9.3.2 Slaughtered animals

It is much more difficult to measure the yield from the animal body after the slaughtering and processing of the animal (see Table 9.2). Calculation of CFs may be based on various outputs. For practical reasons, carcass weight or weight gain (warm or cold) would be the most important end point to measure the yield of slaughtered animals. Based on the values derived from Table 9.7, the CFs are calculated for the various end points taking into consideration the differences in feeding and GHG emissions, and are shown in Table 9.8.

The co-products of feed and the food industry (Makkar, 2012) can reduce the CF of food of animal origin (Elferink *et al*., 2008), because of their lower environmental costs (Bockisch *et al*., 2000). Under farm conditions, only the GHG balance per kilogram of body weight gain can be calculated. Normally, the GHG emissions for the whole beef system include also the emissions of the cows, calves and heifers needed to produce beef. They are much higher than in the dairy cow–growing/fattening bulls for beef system. The GHG emissions to produce pork and poultry meat should also include the emissions of parents (sows and laying animals).

The term 'meat' is generally used, but what is actually meant by the term is not

Table 9.7. Model calculations of CFs for beef (150–550 kg body weight)[a] depending on feeding[b,c], weight gain, CH_4 and N emissions.

						Carbon footprints (kg CO_2-eq kg^{-1})			
Gain[d]	Feed intake[e]	Concentrate[f]	CH_4 emission[g]	N excretion[h]	N_2O synthesis[i]	Weight gain	Empty carcass weight gain	Edible fraction gain	Edible protein
500 (pasture)	6.5	0	26	110	2	11.5	23.0	28.0	110
1000 (indoor, grass silage)	7.0	15	24	130	1	5.5	11.0	13.8	55
1500 (indoor, maize silage)	7.5	30	22	150	0.5	3.5	7.0	9.0	35

Notes: [a]Production of calf up to 150 kg BW is not considered; [b]CO_2 output: 120 g kg^{-1} roughage DM; 220 g kg^{-1} concentrate DM; [c]feed sources may have a strong influence on CFs. Units for [d, e, f, g, h] and [i] are measured in g day^{-1}; kg DM $animal^{-1}$ day^{-1}; per cent of DMI; g kg^{-1} DM; g day^{-1}; and per cent of N excretion, respectively. (Flachowsky, 2008.)

Table 9.8. Model calculation to show various end points for growing/fattening bulls (150–550 kg body weight).

Weight gain (g day^{-1})	Weight gain without content of intestinal tract (g day^{-1})	Carcass weight (warm; per cent of weight gain)	Carcass weight gain (warm; g day^{-1})	Meat gain (per cent of weight gain)	Meat gain (g day^{-1})	Edible fraction gain[a] (g day^{-1})	Edible protein (g day^{-1}; 19% protein in edible fraction)
500	438	50	250	40	200	250	48
1000	900	53	530	44	440	490	93
1500	1385	56	840	48	720	770	146

Note: [a]Meat plus other edible tissues; calculation based on data by Flachowsky (2002).

clearly described: real meat or meat plus bones? Peters *et al*. (2010) introduced the term 'hot standard carcass weight' (HSCW) as the weight at the exit gate of the meat processing plant. It varies between 50 and 62% of the live weight of slaughtered cattle, but it may vary between 50% in the case of sheep and up to 80% for fattening turkeys (Flachowsky, 2002; Williams *et al*., 2006; Peters *et al*., 2010; Table 9.8).

Therefore, it is really difficult to find an adequate CF for the meat or edible products from slaughtered animals. Various authors used different bases to calculate the CF of products from slaughtered animals. Williams *et al*. (2006) estimated the killing out percentages for beef and poultry as 55 and 70%, respectively, and 72, 75 and 77% for pigs with body weights of 76, 87 and 109 kg, respectively. Lesschen *et al*. (2011) used fixed values to calculate the carcass fraction from the final body weight of animals (e.g. 58% for beef, 75% for pork and 71% for poultry). Most authors used a fixed fraction of 0.9 for all animal species for converting carcass weight to edible meat. One reason for the high range of CFs for food from slaughtered animals is understandable, as shown for beef in Table 9.9.

Table 9.9. Examples by various authors of CFs (kg CO_2-eq kg^{-1} carcass weight gain) of beef cattle depending on type of production.

	Type of production/farming		
Country	Conventional	Organic	Reference
	8.5	29.0 (beef cow)	Reitmeyr (1995)
Germany	8.7/10.1	10.2	Woitowicz (2007)
	13.3	11.4	Fritsche and Eberle (2007)
	15.2	17.5	Schlich and Fleissner, 2005
Australia	9.9 (grain finished)	12.0 (grass finished)	Peters *et al*. (2010)
UK	15.8	18.2	Williams *et al*. (2006)
Ireland	23.6	20.2	Casey and Holden (2006)
Global	10 (intensive – dairy beef)	32–40 (suckler beef)	PAS (2011)
	24.5	20.9	Subak (1999)
Without differentiation between conventional and organic			
Germany	5.6 (6000 kg milk $year^{-1}$)–14.6 (10,000 kg milk $year^{-1}$, see Table 9.6)		Zehetmeier *et al*. (2011)
	7.0–23.0		Flachowsky (2008)
	8.4 (fattening from dairy cows) 16.8 (fattening from beef cows)		Hirschfeld *et al*. (2008)
Canada	5.9–10.4		Verge *et al*. (2007)
Sweden	10.1		Cederberg and Stadig (2003)
Ireland	13.0 (11.3–15.6)		Casey and Holden (2006a,b)
EU	16.0–19.9; 27.3 (suckler herd)		Nguyen *et al*. (2010)
Norway	17.7–18.4 combined milk/meat; expanded boundaries		Roer *et al*. (2013)
Japan	19.6		Ogino *et al*. (2004)
	36.4 (beef cows – fattening bulls; 40% meat yield)		Ogino *et al*. (2007)
Global	15.6 (fattening from dairy cows) 20.2 (fattening from beef cows)		FAO (2010)
Beef	46.2		Opio *et al*. (2013)
Buffalo	53.4		Opio *et al*. (2013)
Small ruminants	23.8		Opio *et al*. (2013)
Literature review; five studies	32		Tan *et al*. (2014)

9.3.3 Beef

The ruminant sector contributes to about 29% of the global meat production, but 5.7 Gt CO_2-eq, representing about 80% of the global livestock emissions per year, should come from all ruminants (see Table 9.1; Opio *et al*., 2013). The large range in CFs, comparing the results of various authors and depending on the many influencing factors, is shown for beef in Table 9.9. The values are much higher than those for milk (compare Tables 9.5 and 9.6 with 9.9), and are influenced by body weight gain, feed production with or without LUC and the feeding and keeping of the animals, as well as the system boundaries. The calculation base for the output of growing animals is more difficult (see Tables 9.7 and 9.8) to calculate than that for milk or eggs (see Table 9.2). Depending on the calculation base, the authors found a high variation in the CFs of beef. The highest values are given for beef cows (Table 9.9). In general, all the results indicate (Peters *et al*., 2010; Flachowsky *et al*., 2011, O'Mara, 2011; Opio *et al*., 2013) that policies which are targeted at improvements in productivity and efficiency of resource use will result in a lower GHG emission or lower CFs per unit of product. In the case of beef production, about 15% of total emissions are associated with the expansion of grassland into forest (Opio *et al*., 2013).

9.3.4 Pork

The pig sector contributes 37% to global meat production; it will grow by 32% from 2005 to 2030 (MacLeod *et al*., 2013). Only 0.7 Gt CO_2-eq per annum, representing about 10% of the livestock sector's emissions, comes from pigs (see Table 9.1; MacLeod *et al*., 2013).

The main emission sources from global pig supply chains are feed production (60%) and excrement management (27%). The remaining 13% arises from post-farm processing, transport, enteric fermentation and indirect energy use in pig production (MacLeod *et al*., 2013). Thirteen per cent of the total emissions arise from LUC driven by the increasing demand for feed crops (e.g. forest into soybean area).

MacLeod *et al*. (2013) compared the results of 14 studies with pigs from Europe, North America and Australia and found a range in emissions between 2.01 and 6.36 kg CO_2-eq kg^{-1} carcass weight. Later, the same authors adjusted all studies to the same scope according to FAO rules and calculated values between 3.34 and 6.37 without LUC and values between 4.71 and 9.85 kg CO_2-eq kg^{-1} carcass weight with LUC. Tan *et al*. (2014) analysed three case studies from Australia and Canada and found similar results (4.5 kg CO_2-eq kg^{-1} pork).

All the values mentioned above are much lower than the data from beef (Table 9.9). The main reasons for this are the enteric CH_4 production in ruminants and the low growth intensity of beef cattle (mostly <0.5% weight gain day^{-1} of body weight) compared with pigs (mostly >1% weight gain day^{-1} of body weight; see also Table 9.11).

9.3.5 Poultry

Chicken meat accounts for about 24% of global meat production. The global demand for chicken meat and chicken eggs is forecasted to grow by 61 and 39%, respectively, during the period 2005–2030. Chickens are estimated to emit 0.6 Gt CO_2-eq $year^{-1}$, representing about 8% of the livestock sector's emissions (also see Table 9.1; MacLeod *et al*., 2013).

In the case of chicken meat on the global scale, 78% of emissions come from feed production and only small amounts directly from farm energy use (8%), processing and transport of meat (7%) and excrement management (6%; MacLeod *et al*., 2013). In the case of eggs, feed production contributes to 69% of emissions, direct on-farm energy uses 4% and post-farm processing and transport 6%; the rest (20%) is manure storage and processing.

Some authors did not consider emissions from LUC, where it occurred. In such cases, about 21% of poultry meat emissions and

13% of egg emissions came from LUC (from forest into soybean; MacLeod *et al*., 2013). These authors compared the emissions of 18 studies with broilers from Europe, North America, Brazil and Australia and found a range between 1.30 and 5.53 kg CO_2-eq kg^{-1} carcass weight. Later, the same authors adjusted all studies to the same scope according to FAO rules and calculated values between 1.89 and 5.00 without LUC and values between 1.93 and 7.71 kg CO_2-eq kg^{-1} carcass weight with LUC. Tan *et al*. (2014) calculated 2.9 kg CO_2-eq kg^{-1} meat in three case studies on chicken from Brazil and Finland. The most important influencing factors of the CFs of broilers are the feed amounts needed per weight gain (feed conversion rate) and feed transport (Thevenot *et al*., 2013). LUC should not be neglected.

In the case of eggs, feed production contributes to 69% and excrement management to about 20% of the emissions (Table 9.1; MacLeod *et al*., 2013). Pelletier *et al*. (2013) came to a similar assessment after analysis of egg production in the Midwestern USA. The composition of eggs is well defined, but may differ between various sources and depend on the animal breed, feeding of animals and other influencing factors (Table 9.10). The yield can be measured as weight (kilogram, etc.) or on the basis of standardized products (e.g. standardized protein, fat, dry matter or energy). Therefore, analysis of egg composition (protein, fat) may contribute to a more specific measuring of animal yield including energy yield. Eggs may be used entirely as food (except for small amounts of eggshells; see Table 9.11).

In conclusion, growing intensity, laying performance, feed conversion rate (FCR), healthy animals and low animal losses are the key determinants of the emission intensity per kilogram of food of animal origin from non-ruminants (pork, broiler meat and eggs).

9.3.6 Aquaculture

Aquaculture is a strong, upcoming way to produce food protein of animal origin. Recently, some authors have tried to determine the CF of various forms of aquaculture. Mungkung *et al*. (2013) carried out a case study of combined aquaculture systems for carp and tilapia. The studied system included fingerling production in hatcheries, fish rearing in cages and transport of feed, as well as that of harvested fish to markets.

Avadi and Fréon (2013) reviewed 16 LCA studies applied to fisheries and considered in the comparison the following aspects: scope and system boundaries; functional unit allocation strategies for co-products; conventional and fishery-specific impact categories; fuel use; impact assessment methods; level of detail of inventories; normalization of results and sensitivity analysis. Fishery-specific impact categories and fuel use in fishing operations were identified as the main contributors to environmental impact. Nijdam *et al*. (2012) analysed 18 and 11 studies for seafood from fisheries and agriculture, respectively. The authors summarized the CF as being between 1–86 kg CO_2-eq for seafood from fisheries and 3–15 kg CO_2-eq for seafood from aquaculture, respectively. These authors, as well as Avadi and Fréon (2013), defined the need for standardization of fisheries LCA research for further studies on the sustainability of seafood and fisheries-based agrifood.

9.3.7 Edible protein

The production of protein of animal origin is one of the most important goals of animal husbandry (De Vries and de Boer, 2010; Lesschen *et al*., 2011; Flachowsky and Kamphues, 2012; Nijdam *et al*., 2012). On the other hand, the efficiency and the emissions of food of animal origin can also be compared on the basis of edible protein. The N or protein (N × 6.25) content of various foods of animal origin may vary from the values used for the calculations in Table 9.11. These data agree with the values used by Lesschen *et al*. (2011) and they do not disagree substantially with the values from human food tables (Table 9.10).

Table 9.10. Protein content of some edible animal products, by various authors (g kg^{-1} edible product).

	References					
Product	Flachowsky (2002)	GfE (1995; 1999; 2001; 2006)	Souci *et al.* (2008)	Andersen (2011)	Lesschen *et al.* (2011)[a]	Nijdam *et al.* (2012)
Cow milk	34	34	33.3 (30.8–37.0)	34	34.4	35
Beef	190	170–200	220[b] (206–227)	206–212	206	200
Pork	150	157 (129–178)	220[b] (195–240)	183–216	156	200
Poultry	200	n.d.	199	182–242	206	200
Eggs	120	121 (110–124)	125	125	119	130

Notes: [a]N content × 6.25; [b]muscles only; n.d. = no data.

Nijdam *et al*. (2012) used, to calculate CFs for seafood from fisheries, 160–200 g and, for seafood from agriculture, 170–200 g protein kg^{-1} food.

Taking into consideration various influencing factors such as animal yields, feeding, edible fractions and protein content in the edible fractions, the yield of edible protein per day and per kilogram of body weight of animals is given in Table 9.11. Feeding may influence the CFs of food of animal origin. In the case of ruminants, higher amounts of concentrate are required with higher animal yields. The proportion of co-products used in animal nutrition has not only nutritional implications (Makkar, 2012) but also affects the results of calculations on land use (Vandehaar, 1998).

Table 9.11. Influence of animal species, categories and performances on yield of edible protein.

Protein source[a]	Performance (day^{-1})	DMI (kg day^{-1})	R:C ratio (per cent DM basis)	Edible fraction[b]	Protein in edible fraction[c]	Edible protein[d]	Edible protein[e]
Dairy cow (650 kg)	10 kg milk	12	90/10			323	0.5
	20 kg milk	16	75/25	95	34	646	1.0
	40 kg milk	25	50/50			1292	2.0
Dairy goat (60 kg)	2 kg milk	2	80/20	95	36	68	1.1
	5 kg milk	2.5	50/50			170	2.8
Beef cattle (350 kg)	500 g[f]	6.5	95/5			48	0.14
	1000 g[f]	7.0	85/15	50	190	95	0.27
	1500 g[f]	7.5	70/30			143	0.41
Growing/fattening pig (80 kg)	500 g[f]	1.8	20/80			45	0.56
	700 g[f]	2	10/90	60	150	63	0.8
	1000 g[f]	2.2	0/100			81	1.0
Broiler (1.5kg)	40g[f]	0.07	10/90	60	200	4.8	3.2
	60g[f]	0.08	0/100			7.2	4.8
Laying hen (1.8kg)	50%[f]	0.10	20/80			3.4	1.9
	70%[g]	0.11	10/90	95	120	4.8	2.7
	90%[g]	0.12	0/100			6.2	3.4

Notes: [a] Body weight is given in brackets; [b]represents the fraction in per cent of product or body mass; [c]represents the edible fraction in g kg^{-1} fresh matter; [d]edible protein in g day^{-1}; and [e]represents the edible protein in g kg^{-1}; [f]daily weight gain; [g]laying performance BW. (Flachowsky, 2002.)

There are large differences in animal protein yield per animal per day or per kilogram of body weight and day depending on animal species and categories, as well as their performances and the fractions considered as edible (see Table 9.11).

Table 9.11 shows the highest protein yields per kilogram of body weight for growing broilers as well as for laying and lactating animals and the lowest values for growing/fattening ruminants. Based on those values, emissions per kilogram of edible protein are given in Table 9.12. Higher portions of edible fractions or higher protein content as shown in Tables 9.8 and 9.12 may increase protein yield and reduce the CF per product. At high levels of performance, there are remarkable differences in CO_2 emissions due to human consumption of 1 g of protein from food of animal origin (eggs and meat from poultry < pork < milk < beef; see Table 9.12).

Nijdam *et al*. (2012) analysed 52 LCA studies (Table 9.13) and summarized the CF per kilogram of product and per kilogram of edible protein of animal origin. The results indicate that large differences exist between the studies and the products. The outcomes for milk, pork, poultry meat and eggs show much more homogeneity than those for beef, mutton, lamb and seafood. This is largely because of the very wide variety in production systems of the last food groups. Meat from non-ruminants has a lower CF than that from ruminants, because CH_4 is the main contributor to the CF of ruminants. Because of too low values for feed production and processing (see Tables 9.3 and 9.4), most values shown in Table 9.12 are considerably lower than the data given in Table 9.13.

Apart from protein, food of animal origin also contains other main nutrients (fat and lactose in the case of milk; fat in the case of meat and eggs), which contribute to human nutrition and may replace energy of plant origin in human food. But, at this point, it has to be emphasized that this protein intake is accompanied, willingly or not, by an energy intake from the protein itself. Therefore, exclusive attribution of the CO_2 burden to the protein fraction ('edible protein') should be avoided. There are different alternatives to prevent this fact from being neglected. In a

Table 9.12. Influence of animal species, categories and performances on emissions (per kilogram of edible protein; calculations based on the data from Tables 9.10 and 9.11).

				Emissions in kg kg^{-1} protein			
Protein source (BW)	Performance (day^{-1})	N excretion (per cent of intake)	CH_4 emission (g day^{-1})[c]	P	N	CH_4[c]	CO_2-eq
Dairy cow	10 kg milk	75	310	0.10	0.65	1.0	30
(650 kg)	20 kg milk	70	380	0.06	0.44	0.6	16
	40 kg milk	65	520	0.04	0.24	0.4	12
Dairy goat	2 kg milk	75	50	0.08	0.5	0.8	20
(60 kg)	5 kg milk	65	60	0.04	0.2	0.4	10
Beef cattle	500 g[a]	90	170	0.30	2.3	3.5	110
(350 kg)	1000 g[a]	84	175	0.18	1.3	1.7	55
	1500 g[a]	80	180	0.14	1.0	1.2	35
Growing/	500 g[a]	85	5	0.20	1.0	0.12	16
fattening pig	700 g[a]	80	5	0.12	0.7	0.08	12
(80 kg)	900 g[a]	75	5	0.09	0.55	0.05	10
Broilers	40 g[a]	70	Traces	0.04	0.35	0.01	4
(1.5 kg)	60 g[a]	60		0.03	0.25	0.01	3
Laying hen	50%[b]	80	Traces	0.12	0.6	0.03	7
(1.8 kg)	70%[b]	65		0.07	0.4	0.02	5
	90%[b]	55		0.05	0.3	0.02	3

Notes: [a]Daily weight gain; [b]laying performance; [c]CH_4 emission depending on composition of diet.

Table 9.13. Carbon footprints of protein of food of animal origin (Nijdam *et al*., 2012).

Protein source (studies)	kg CO_2-eq kg^{-1} product	kg CO_2-eq kg^{-1} protein
Cow milk (n = 14)	1–2	28–43
Beef, intensive system (n = 11)	9–42	45–210
Meadow, suckler herds (n = 8)	23–52	114–250
Extensive pastoral systems (n = 4)	12–129	58–643
Mutton and lamb (n = 5)	10–150	51–750
Pork (n = 11)	4–11	20–55
Poultry meat (n = 5)	2–6	10–30
Eggs (n = 5)	2–6	15–42
Seafood from fisheries (n = 18)	1–86	4–540
Seafood from aquaculture (n = 11)	3–15	4–75

first simple method, the CO_2 emissions due to 1 kg of edible protein could be used as the CO_2 burden of consumed energy (for example: 1 kg of the edible protein of eggs corresponds to about 8 kg of eggs and corresponds to 51.6 MJ energy; this combined intake is related to a certain amount of CO_2-eq). 'Nutritional allocation', described below, may distribute CO_2 emissions to different functions of the food.

9.4 Allocation as Influencing Factor on CFs

The results of LCAs may be substantially influenced by system boundaries (see Table 9.4). Apart from the factors mentioned above, the allocation of animal products (e.g. Cederberg and Stadig, 2003; Thomassen *et al*., 2007; Gruenberg *et al*., 2010; Zehetmeier *et al*., 2011; Avadi and Fréon, 2013; Roer *et al*., 2013) may be used whenever the systems under study generate more than one saleable output (e.g. milk and meat). Such studies also influence the results of LCAs. Mass-based and economic-based allocations were applied in the case of saleable products (Peters *et al*., 2010). For example, Zehetmeier *et al*. (2011) calculated a CF of 1.35 and 0.98 kg CO_2-eq kg^{-1} milk of cows producing 6000 or 10,000 kg milk $year^{-1}$. In the case of lower milk yield, beef was produced by calves of dairy cows with a CF of 5.58 kg; in the case of higher milk yields, beef cows were needed to produce sufficient beef and the CF increased to 14.62 kg CO_2-eq kg^{-1} beef (Table 9.14). Taking the economic aspects (prices for milk and beef; economic allocation) into consideration, the CF of milk decreased and that of beef increased (see Table 9.14). Allocation could also be used as a 'nutritional allocation' (as described for 'economic allocation' above); this means, that the CO_2 emissions are attributed to the different functions of the food (e.g. source of protein/source of energy/source of further essential nutrients).

Furthermore, animal products are not only used as food or as protein/amino acids and energy sources; they also offer some other important side products such as skins or hides, fishmeal or meat and bonemeal, or the animals are used as draught animals. A kind of combined 'nutritional/further purposes allocation' may contribute to a more scientific assessment of CFs for nutrient and energy supply, as well as further uses. Taking into consideration all the aspects mentioned above, it is extremely difficult to compare the results of the LCAs from various authors. This variability has caused confusion between scientists, among policy makers and for the public. A methodical agreement generated by internationally recognized scientific panels with expertise across a range of disciplines and clear science-based orientation (Herrero *et al*., 2011; PAS, 2011) seems to be urgently necessary.

Table 9.14. Influence of increased milk yield on greenhouse gas emissions, taking into consideration various allocation methods.

Without allocation of GHG emissions in dairy husbandry			
	Dairy cattle including rearing heifers	Dairy cattle including rearing heifers	Dairy cattle including rearing heifers
Milk yield (kg cow^{-1} year^{-1})	6,000	8,000	10,000
GHG emissions (kg CO_2-eq kg^{-1} milk)	1.35	1.13	0.98
Origin of beef	Culled dairy cows, fattening of all male and female calves, except calves for rearing	Culled dairy cows, fattening of all male and female calves, except calves for rearing; beef cows	Culled dairy cows, fattening of all male and female calves, except calves for rearing; beef cows
GHG emissions (kg CO_2-eq kg^{-1} beef)	5.58	9.53	14.62
With economic allocation of GHG emissions in dairy husbandry			
	Dairy cattle including rearing heifers	Dairy cattle including rearing heifers	Dairy cattle including rearing heifers
Milk yield (kg cow^{-1} year^{-1})	6,000	8,000	10,000
GHG emissions (kg CO_2-eq kg^{-1} milk)	1.06	0.93	0.89
Origin of beef	Culled dairy cows, fattening of all male and female calves, except calves for rearing	Culled dairy cows, fattening of all male and female calves, except calves for rearing; beef cows	Culled dairy cows, fattening of all male and female calves, except calves for rearing; beef cows
GHG emissions (kg CO_2-eq kg^{-1} beef)	10.75	13.13	16.24

(Zehetmeier *et al*., 2011)

9.5 Reduction Potentials

Animal health, low animal losses, long periods of productive life of reproductive animals such as dairy cows and sows, a reduction in the number of unproductive or low-yielding animals and feeding of animals according to species/categories, as well as performance avoiding excess and deficiencies, are general potentials for lower greenhouse emissions. Some recent reviews by Hristov *et al*. (2013a,b,c), Montes *et al*. (2013) and Opio *et al*. (2013) analysed and summarized animal feeding and management mitigation options. In an excellent review, Hristov *et al*. (2013a) summarized the mitigation opportunities for non-CO_2 GHGs, which included the following:

- feed additives and feeding strategies
- manure-handling strategies
- animal management strategies
- reproductive management strategies
- interactions among non-CO_2 GHGs.

The authors assessed the relative effectiveness, the input required to achieve the desired effects and the applicability to regions. Improvement of feed conversion

rate (FCR) is one of the most efficient ways to reduce emissions per kilogram of animal product and to decrease the CF of ruminants (Opio *et al*., 2013), non-ruminants (Pelletier *et al*., 2013) and fisheries (Mungkung *et al*., 2013; Wilfart *et al*., 2013). Special attention should be paid to non-CO_2-emissions.

9.5.1 N_2O

There are a number of animal and management practices that are feasible and can effectively reduce N_2O emissions from manure storage and/or land application (Montes *et al*., 2013). Optimizing the animal diet to improve N use efficiency and balancing N input with production level are important steps in reducing N_2O emissions from manure (Flachowsky and Lebzien, 2006; also see Chapter 4, Section I, this volume, for details). Due to the complex interaction between nutrition, production, animal health and economic performance, diet modification to reduce N inputs should be done carefully to prevent reduced fibre digestibility and to maintain animal productivity (Montes *et al*., 2013).

9.5.2 CH_4

Some possibilities for the reduction of CH_4 emissions, which include increasing forage digestibility, digestible forage intake, dietary lipids, high-concentrate feeding or the application of various feed additives, are mentioned in Table 9.15. Long lists of substances, i.e. nitrates and nitrooxy compounds (e.g. Haisan *et al*., 2014; Martinez-Fernandes *et al*., 2014; Reynolds *et al*., 2014), tannins and other phytogenic substances, direct-fed microbials such as yeast-based products, fumaric acid and further H_2 binders, vaccines against rumen archaea, etc., that have a certain potential to reduce enteric CH_4 production are discussed with regards to their applicability and limitations in Section III, this volume. Flachowsky and Lebzien (2012) proposed a five-stage programme to evaluate the effects of such additives, taking phytogenic substances into special consideration:

1. Botanical characterization of the plant(s) and their composition.
2. Analytical characterization of the active phytogenic substance(s).

Table 9.15. Feed-based approaches to reduce enteric CH_4 emission, importance at the farm level and research need.

Measurements	Significance (especially for Europe) at farm level	Research need
More concentrate, less fibre in the diet	Limited, because of high amount in many diets	~
Forages with high digestibility, low fibre content	Consideration in practical feeding	⇑
Fats and fatty acids in the diet	Limited, because of some side effects	(⇑)
Feed additives		
Halogen compounds	Banned in the EU	~
Ionophores (e.g. monensin)	Banned in the EU	⇑
Addition of H-binder, such as fumaric acid, acrylic acid, etc.	Presently no significance	⇑⇑
Addition of phytogenic substances or plants containing such substances (e.g. tannins, saponins)	Presently no significance	⇑⇑
Further additives, such as yeasts, enzymes, etc.	Presently no significance	⇑⇑

Note: ⇑ ⇑ = high need; ⇑ = need; ~ = not so important.

3. *In vitro* studies to test the effects of substances on rumen fermentation and methanogenesis (i.e. screening).
4. *In vivo* studies (e.g. feed intake, rumen fermentation, CH_4 emissions).
5. Long-term feeding studies with target animal species/categories (e.g. animal health and performance, quality and safety of food of animal origin, environmental impact, adaptation of microbes, etc.).

Enhanced animal productivity and feed efficiency with metabolic modifiers, such as growth hormones and ionophoric antibiotics (Hristov *et al*., 2013b), would reduce GHG emissions, but the applicability of these mitigation practices is limited to the regions where the use of these substances is not permitted. Ranga Niroshan Appuhamy *et al*. (2013) analysed the CH_4 reduction potential of the ionophoric substance, monensin, via meta-analysis. Data from 22 controlled feeding studies were used. The CH_4 mitigation effects of monensin were small (12 or 14 g day^{-1} in dairy cows and beef cattle) when adjusted for the monensin dose (see Chapter 17, Section III, this volume). Improved genetics and animal health care as well as animal management, in combination with better reproduction and feeding (higher digestibility and quality of forages) and reduction of the breeding overhead (i.e. animals kept to maintain the herd and old animals without lactation) may contribute to reducing emissions, especially CH_4, and the CF (Niemann *et al*., 2011; Hristov *et al*., 2013a; Opio *et al*., 2013). Gerber *et al*. (2013) estimated that reducing the gap between livestock operations that generate high emissions versus those that put out low emissions per unit of product could cut emissions by about 30%.

In summary, the reduction of the CF in ruminant production per product should focus on a lowering of CH_4 emissions from enteric fermentation and an increase of low production levels as well a reduction of ineffective animal numbers (Flachowsky and Brade, 2007; Gerber *et al*., 2013; Hristov *et al*., 2013a,c). In the future, results of plant (Flachowsky *et al*., 2012) and animal breeding (Niemann *et al*., 2011; Forabosco *et al*., 2013; and debated at length in Chapter 18, Section III, this volume) may also contribute substantially to lower GHG emissions.

9.6 Conclusion

Global emissions from livestock are estimated as 7.1 Gt CO_2-eq $year^{-1}$, which represents about 14.5% of human-induced GHG emissions. Beef and milk cattle, pigs for meat, and poultry meat and eggs contribute to 41, 20, 9 and 8%, respectively, of the total emissions. Feed production and processing and enteric fermentation from ruminants represent 45 and 39%, respectively, of the total emissions. Carbon footprints may help to assess the GHG emissions associated with the production of food of animal origin. They may contribute to sensitizing producers and consumers to a more resource-efficient and environmentally friendly production and consumption of food of animal origin and to avoid food wastage (FAO, 2013). Clear areas with high mitigation potential are the following:

- Improving feed production, especially fertilization (N, manure), management and reduced LUC.
- Improving feed supply, feeding practices and digestibility of diets.
- Improving animal yields through genetics, animal health, feeding practices and animal management (including excrement management), and in consequence, reduction of the number of low-yielding animals.

A more standardized approach for CF calculations would be a very useful tool to provide an indicator for food labelling to compare the CF between production systems, regions and countries, as well as to assess resource efficiency, especially in non-ruminants. The high portion of CH_4 in the CF of food from ruminants does not allow the use of that CF to compare it with food from non-ruminants in order to draw conclusions concerning feed efficiency.

Therefore, some authors (Reap *et al*., 2008; Laurent *et al*., 2012; Owsianiak *et al*.,

2013) analysed the limitations of the CF as an indicator of environmental sustainability and recommended significant efforts in more dynamic modelling to ameliorate the problems of spatial variation and local environmental uniqueness. Furthermore, methodical problems must be solved and more diverse researchers should be involved in such studies in order to improve the database (Caffrey and Veal, 2013).

In summary, the production of food of animal origin is a very complex process and selective consideration, i.e. focusing on single factors, does not provide an assessment that reflects the complexity of the subject.

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10 Carbon Sequestration and Animal-Agriculture: Relevance and Strategies to Cope with Climate Change

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Abstract

Carbon sequestration is an important pathway to stabilize the environment with minimum effects of climate change. Farming systems provide a non-compensated service to society by removing atmospheric carbon generated from fossil fuel combustion, feed production, land restoration, deforestation, biomass burning and drainage of wetlands. The resultant increase in the global emissions of carbon is calculated at 270 Gt, and increasing at the rate of 4 billion tonnes $year^{-1}$. Strategies to maximize carbon sequestration through enhanced farming practices, particularly in crop–animal systems, are thus an important priority to reduce global warming. These pathways also respond to agricultural productivity in the multifaceted, less favoured rainfed environments. Sustainable animal-agriculture requires an understanding of crop–animal interactions and integrated natural resource management (NRM), demonstrated in the development of underestimated silvo-pastoral systems (tree crops and ruminants). It has been reported that mitigation can potentially sequester carbon by 0.70–3.04 t carbon dioxide (CO_2)-equivalent (eq) ha^{-1} $year^{-1}$, reduce methane (CH_4) emission by 0.02 t CO_2-eq ha^{-1} $year^{-1}$ and reduce nitrous oxide (N_2O) emissions by 0.02–2.30 t CO_2-eq ha^{-1} $year^{-1}$. Good agronomic practices potentially enhance carbon sinks and soil organic matter through leguminous trees (e.g. *Leucaena*), integrated nutrient management, regulation of grazing pressure and use of animal manure. These interventions significantly increase ecosystem services, crop and animal productivity, reduce CH_4 emissions and mitigate N_2O emissions and ammonia volatilization. Research and development (R&D) efforts on the characterization of forages and research on heat stress and economic animal productivity are urgently needed. Multinational interdisciplinary R&D, investment to reduce the effects of climate change, enhancement of the value of C sinks and food security are high priorities. These issues are rarely and inadequately researched in South-east Asia, West and East Africa, Latin America and the Caribbean and merit collective action.

10.1 Introduction

Agriculture involves the science, art and business of cultivating soil, growing plants and raising animals for producing food, feeds, fibre and a whole range of other services. Together with forestry and fisheries, it provides the primary source of food and nutritional security for the welfare of people. Beyond food production, the

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subject is a complex multidimensional and multifaceted sector, which concerns the efficient use of natural resources, productivity enhancement and the safeguarding of ecosystems in a manner that can sustain the needs and improvement of human livelihoods in the future (Devendra, 2010). Presently, the sector is grappling with two defining issues of global concern: the onset of climate change and the inadequacies of food production systems. Climate change is likely to induce significant changes in the agricultural landscape, stresses on natural resources and the livelihoods of the poor and the landless.

Agriculture is a major sector in Asia and contributes 25–43% to the gross domestic product (FAO, 1996, 1997). Much of this contribution is made by fertile, irrigated areas, which are presently overused, and yields are plateauing. On the other hand, the rainfed areas of lower importance are currently underutilized, despite having good potential and merit for future development. About 43–88% of the human population depends on agriculture for their livelihoods, of which 12–93% live in rainfed areas and use 26–84% of the arable land. Around 5–41% of agricultural output comes from these areas. Due to low productivity, the share of total crop and livestock outputs from rainfed areas is much lower than the share of the total area under irrigation.

About 2.6 billion farmers produce the majority of food, products and services in agriculture throughout the world on small farms with limited land (<2 ha). IFAD (2009) has reported that climate change is expected to put 49 million additional people at risk of hunger by 2020, rising to 132 million by 2050. Of great concern about the anticipated effects of climate change is that livelihoods will be affected more severely in the developing world. This is already evidenced in several countries, the most recent being the trauma of massive typhoons in the Philippines, with large losses of human lives, farms and property, severe food shortages, disease outbreaks and induced poverty.

Livestock contribute 10–45% to the agricultural gross domestic product (GDP) in the developing world, and could be higher if the value of draught power is included in the calculation. It is one of the fastest growing subsectors in agriculture (World Bank, 2009) and plays an important multifunctional and socio-economic role. It is estimated that 70–90% of ruminant livestock is found in rainfed mixed farms. In India, for example, the rainfed ecosystem occupies 68% of the total cultivated area and supports 40% of the human and 65% of the livestock population. This ecosystem produces 44% of the food requirements and will continue to play a critical role in Indian agriculture (Singh *et al*., 2004). The importance of rainfed areas is reflected in the creation of a rainfed network, the main objective of which is to seek improved understanding of the appropriate policy and programmes for livestock (Köhler-Rollefson and Kishore, 2010).

The Asian region is also home to small farm systems, where 87% of the world's (total 470 million) small farms are located (Nagayets, 2005). Many of these small farms are models for efficient integrated natural resource management (NRM), but the small farmers continue to experience deprivation, poverty, hunger and vulnerability (Devendra, 2010). These small farms generally have higher yields than large farms on a per hectare basis (Cornia, 1985), attributed to low labour and production costs. This inverse relationship weakens as agriculture becomes more capital-intensive, as is shown to prevail in India (Hanumanth Rao, 1994). The resilience of this relationship will depend to a large extent on the use of improved technologies and policy support.

Concerning animal agriculture, there is increased emphasis and justification for improved production systems to accelerate the output of foods of animal origin in most of the countries in South-east Asia. This is linked directly to the fact that the current output of meat and milk from ruminants is relatively low, and the levels of self-sufficiency in these products exacerbated further by increasing imports at high cost. Increased costs can trigger higher commodity prices, which can be associated with strong global demand.

This increased requirement is associated with several demand-driven factors and includes inadequate animal protein supplies and rising incomes, which encourages people to diversify their diet to a variety of meat, eggs and dairy products. Equally awesome is the inadequacy of animal protein supplies to meet current and projected future human requirements and escalating costs. Improved animal production and productivity enhancement is therefore urgent in direct response to the need for more animal protein. Major opportunities and challenges need to be addressed thoroughly to the greatest extent possible (Devendra, 2007a, 2010).

Agriculture drives economic growth during transformation. The dramatic benefits of the green revolution's use of capital inputs like irrigation and fertilizer have already demonstrated this fact. Agriculture is also a powerful means of reducing poverty through increasing labour productivity and creating employment and opportunities for rural–urban growth. However, it is the most important user of environmental natural resources, including water, forests and soil nutrients. Well-managed agriculture and a stable environment are therefore important to provide the environmental services that are conducive to good human health.

This chapter focuses on the role and importance of carbon sequestration in the context of variable biophysical features, agroecological zones (AEZs), ecosystems and land-use systems. AEZs have great diversity for food production in crop–animal small farm systems, the poverty complex and the livelihoods of the poor. These issues are discussed in depth, with particular reference to land use in Asia, ways to increase carbon sequestration, mitigation and adaptation in response to harmful climate changes, effects and reduced greenhouse gases (GHGs) on food production systems, and the opportunities for R&D. Discussion also touches on the urgency related to the multifunctional capacity and potential of animals to contribute to food security and stable livelihoods.

10.2 Biophysical Environment

The biophysical environment is extremely diverse, with variable soil quality, crop growth and different types of livestock. Features of the biophysical environment vary between and within individual regions. Rainfall and temperature are the two key causative factors for this variation. These variable biophysical features, to a large extent, also determine the level of productivity. Revitalizing agriculture will require more innovative ways to be resource-efficient to promote the development of sustainable agriculture.

10.3 Land-use Systems

The justification for targeting rainfed areas for food production is urgent, and is linked to the following:

- human-induced climate change, with an anticipated harsher climate, will cause a slide into extreme poverty and a fight for survival
- need for the efficient use of available natural resources and to define the objectives of production clearly in terms of potential outputs and profitability
- understanding of the significance and implications of soil–crop–animal interactions
- ensuring that the resulting benefits are consistent with productivity enhancement, environmental integrity and sustainable development of rainfed areas.

These issues altogether emphasize the need of the efficient use of arable and other lands. Table 10.1 indicates the extent of rainfed agriculture and its importance in the different AEZs of the Asia-Pacific region (ADB, 1989). Rainfed areas contribute 38.8, 16.9 and 26.3% of the total in arid/semi-arid, sub-humid and humid AEZs in Asia. The size of the human population dependent on rainfed agriculture is also of particular importance (Table 10.1).

Table 10.1. Distribution of land types by region. (From CGIAR/TAC, 2000.)

	Land type (per cent of total land)				
Region	Favoured	Marginal	Sparsely populated arid lands	Forest and woodlands	Rural population living in favoured lands (%)
Asia	16.6	30.0	18.5	34.6	37.0
Latin America and the Caribbean	9.6	20.3	8.1	61.9	34.0
Sub-Saharan Africa	8.5	23.1	24.6	43.7	27.0
Near East and North America	7.8	22.6	65.8	3.9	24.0
Total	10.7	24.0	25.9	39.4	35.0

10.4 Agro-ecological Zones (AEZs)

10.4.1 Definition

Rainfed areas refer to all the lands outside of the irrigated, more favoured or high/low potential areas. They have been referred to variously as fragile, marginal, dry, waste, problem and threatened, range, less favoured, low potential lands, forests and woodlands, and include a reference to lowlands and uplands. Of these terms, less favoured areas (LFAs), low or high potential, is used quite widely and has been adopted in this chapter.

10.4.2 Features

Biophysical characteristics include lands that are variable, with low agricultural potential, low rainfall, poor soils and steep slopes.

These are the areas that have been bypassed during the green revolution.

- The poorest of the poor are found here due to the disparity with richer farmers, who have benefited from the green revolution.
- Poverty, low agricultural productivity and natural resource degradation are very common. Poverty in India has been shown to respond more to rural agricultural growth than to urban growth. A 1% increase in agricultural productivity will reduce poverty by 0.37% and can take 26 million people out of poverty (ESCAP, 2008).
- Diversification and reducing risks are key features of these areas.
- These features together, and inadequate R&D in the past, overture the major opportunities for increasing the contribution from these areas.

Rainfall dictates the value of rainfed areas. When the rains fail, severe calamities and potential disasters are explosive, with several resultant implications:

- more droughts and climate instability
- failure of crop production and reduced grazing lands and feed availability
- millions of households and people with their animals are forced into semi-nomadism and nomadism
- poor people are marginalized further into extreme poverty, starvation and vulnerability, and damage to the environment is inevitable.

10.4.3 Farming systems in rainfed areas

Key features of the farming systems are:

- involves the semi-arid/arid, subhumid and humid AEZs
- average growing period, i.e. LGP, is 120–160 days
- long dry seasons are common, with occasional droughts
- land is of low quality and has greater production risks

- usually farmed by very poor small farmers and the landless
- rainfed agriculture is essentially subsistence farming
- poverty and nutritional and food insecurity are very common
- mixed farming of annual and perennial crops (millets, sorghum, oilseeds, cotton, rice and wheat) is the norm
- crop failures occur more commonly in semi-arid/arid areas
- crop cultivation is dependent to a large extent on the return of manure from rearing animals.

Using the classifications of the Technical Advisory Committee (TAC) (1994), the rainfed AEZs of relevance are as follows:

- rainfed temperate and tropical highlands – mainly the Hindu Kush/Himalayan region
- rainfed humid/subhumid tropical systems – mainly countries in Indochina, South-east and East Asia, the Pacific Islands and parts of South Asia to include Bangladesh and Sri Lanka
- rainfed arid/semi-arid tropical systems – mainly countries in South Asia, excluding Nepal and Bangladesh.

Rainfed arid/semi-arid and humid/subhumid tropical systems are priority AEZs. Most of the humid and subhumid lowlands are found in South-east Asia, while the lowlands of South Asia are semi-arid and arid. Within the AEZs, two broad rainfed areas, the lowlands and rainfed uplands, are recognized. The lowlands have larger, high potential areas of arable and permanent cropland, which accounts for the greater crop production in these areas.

South Asia is characterized by dry climates in which total rainfall and its distribution can limit crop growth. Arid/semi-arid (warm arid and semi-arid tropics consolidated with summer rainfall) and subhumid climates predominate. The LGP for the arid and semi-arid zones varies from 0–74 days to 75–179 days, respectively. Annual rainfall in the semi-arid zone ranges from about 500 to 1000 mm and < 500 mm in the arid zone. In contrast, in South-east Asia, humid (warm humid tropics consolidated with summer rainfall) and subhumid (warm subhumid tropics consolidated with summer rainfall) predominate. Humid zones are characterized by an LGP of 180–270 days and a rainfall regime ranging from 1000 to 1500 mm annually.

Shifting agriculture is common. Rural poverty is more acute in these areas. The average annual rainfall of these AEZs is between 1500 and 2300 mm. Rice-based cropping systems are common, but also include other annual crops and tree crops. Both ruminants and non-ruminants are reared, with the overriding major constraint of 5–7 months of dry period and potential droughts.

10.4.4 Distribution and types of livestock

These rainfed farms are diverse with relatively large individual animal populations. These are widely distributed across small farms, which are the reservoirs of a large proportion of the main animal species. It is estimated that 70–90% of the ruminant livestock are found in the rainfed mixed farms. Native pigs and chickens are also very common and contribute significantly to food security. Table 10.2 gives an idea of the diversity of the available species and their wide distribution.

Animals form an important economic and ecological niche, especially in rainfed small farms, and their ownership is related to their numerous multifunctional contributions in which women and children are involved with their management (Devendra, 1983; Chantalakhana, 1990). These include:

- diversification in the use of production resources and reduction of socio-economic risks
- promotion of linkages between system components (land, crops and water)
- generation of value-added products (e.g. meat, milk, eggs and skins)
- income generation, investment, insurance and economic security
- supply of draught power for crop cultivation, transportation and haulage operations

Table 10.2. Distribution of domestic animals by ecosystem and sub-regions in Asia. (From Devendra, 1996.)

	Agroecosystems and animal species								
	Lowland irrigated			Lowland/upland rainfed			Semi-arid and arid		
Subregion	Buffalo/ Cattle	Goat/ Sheep	Pig/ Poultry/ Duck	Buffalo/ Cattle	Goat/ Sheep	Pig/ Poultry/ Duck	Buffalo/ Cattle	Goat/ Sheep	Pig/ Poultry/ Duck
China	***	*	***	**	***	***	*	***	*
Hindu Kush	***	*	**	**	***	*	*	–	–
South Asia	***	*	**	**	***	**	*	***	–
Mekong countries	***	*	***	**	**	***	**	*	–
South-east Asia	***	*	***	**	**	***	*	**	*

Notes: * = low concentration; ** = medium concentration; *** = high concentration.

- contribution to soil fertility through nutrient cycling (dung and urine)
- contribution to sustainable agriculture and environmental protection
- prestige, social and recreational values
- development of stable farm households.

10.5 Carbon Sequestration

10.5.1 Definition

Carbon sequestration is defined as a process of increasing the carbon content of a reservoir rather than the atmosphere.

10.5.2 Relevance

Carbon is sequestered from the atmosphere by growing plants, trees and pastures, but in differential ways. Carbon is sequestered from the atmosphere by trees, shrubs and pastures and is stored in extensive root systems. The amount stored is influenced by several factors and includes biophysical factors, notably rainfall and temperature, type of plant or tree, density of plant and tree growth, soil fertility status and type of farming system. Farming systems thus provide a non-compensated service to society, removing atmospheric carbon generated by fossil fuel combustion, feed production, land restoration, deforestation, biomass burning, animal production conversion and drainage of wetlands. Whereas plants store tonnes of carbon in the aerial parts, pastures by comparison have deep and extensive root systems and the carbon sequestered is influenced by soil type, pasture management, agronomic practices, type and quantity of fertilizer use, soil fertility status, soil microorganisms, presence of animals and soil erosion.

Carbon concentration in the atmosphere is estimated at 4 billion tonnes (Bt), transferred primarily from fossil fuel and biotic and soil pools. The increase is linked to two problems. First, the loss of carbon from terrestrial pools reduces ecosystem services and the goods that these systems provide. Second, an increase in atmospheric CO_2 accentuates global warming pools, with shifts in the frequency and intensity of extreme events including droughts: an obvious solution to this is to transfer the atmospheric carbon dioxide (CO_2) into potential sinks such as agroforestry or silvopastoral systems – a process that is called carbon sequestration (Lal, 2009). In plant-based systems, sequestration is a natural process whereby CO_2 is photo-synthesized into organic compounds and stored in plant products or soil organic matter substances. Lal (2009) also reported that the natural rate of photosynthesis in the global biosphere sequestrated about 120 Bt of carbon year^{-1}. Fossil fuel combustion emits around 8 Bt carbon annually, and deforestation and land-use conversion emit

an additional 1.6 and 2 Bt carbon year^{-1}, respectively.

The increase in global emissions of carbon is estimated at around 270 Gt, which is determined to be growing at a rate of 4 Bt year^{-1} (Lal, 2009). With increasing attention and concerns about ways to increase carbon storage, producers and farmers will be paid on the basis of the net amount of carbon sequestered from the atmosphere. This service is paid in tandem with the carbon that is sequestered in perpetuity, as well as the possible risks in farming activities. Arrangements have been created to seek the best ways to pay farmers for these services and include the World Bank's Global Environment Facility. In Nicaragua, Costa Rica and Columbia, US$5–10 was paid for 1 t of carbon sequestered.

The potential exists for the accumulation of higher levels of C sequestration in both temperate and tropical environments. In the Chiang Mai region of northern Thailand, researchers under the Royal Thai Organic Project have recorded a 5% increase in soil organic matter over 8 years, equal to 187.2 t of CO_2 ha^{-1} or 23.4 t of CO_2 ha^{-1} year^{-1}. If this were applied globally, it would sequester 114 Gt CO_2 year^{-1}, more than double the world's current GHG emission (Royal Thai Organic Project, Chiang Mai, Thailand, personal communication).

10.5.3 Role and importance

There are two observations that need to be highlighted concerning the importance of carbon sequestration. First, much of the information available refers to tree crops, mainly coconuts, cocoa, oil palm, rubber, cashew, teak and citrus. Of these, oil palm has received most attention because of the economic importance of palm oil. Among these, the oil palm is a particularly important 'golden crop' and Asia has about 84% of the total world land area under oil palm of about 10.6 million ha (Mha). The largest land areas under oil palm of 8.4 Mha are found in Malaysia and Indonesia, where these two together own over 79% of the world planted area and produce about 87% of the total world output of palm oil, followed by much smaller areas being found in Thailand, the Philippines, India and Papua New Guinea.

In Malaysia, about 86% of the total agricultural area of 6.9 Mha in 2010 was under tree crops. Oil palm alone occupies 63.4%, of which about 49% is found in Sabah and Sarawak. The integration model with oil palm offers extension of the principles involved with other tree crops like coconuts in the Philippines, Sri Lanka and South Asia, rubber in Indonesia and citrus in Thailand. Elsewhere, oil palm, cocoa and coconuts are found in West Africa. Currently, Côte d'Ivoire is the largest producer of cocoa, and the supply is increasing. Oil palm is growing in importance in Columbia, Costa Rica and Nicaragua.

The second observation is that most of the R&D efforts have been focused singularly on very discipline-oriented crop production; unfortunately to the exclusion of the potentially valuable livestock sector. The opportunity is lost to study the implications of fewer negative and more positive crop–animal–soil interactions, increased productivity and income generation in the context of the development of environmentally sustainable integrated production systems.

10.5.4 Small versus large farms

It is emphasized that there is an extreme paucity of knowledge on carbon sequestration on both small and large farms. However, the word 'plantations' is the term used for large farms, and hence the reference to tea and forest plantations (Parrotta, 1992). Woody plants like oil palm and rubber compared to annual crops sequester carbon more efficiently, which can be equal to a lowland rainforest.

10.6 Enhancing Carbon Sequestration

Figure 10.1 illustrates the development pathways in the process of economic transformation. Agriculture-induced growth has resulted in clear benefits, notably

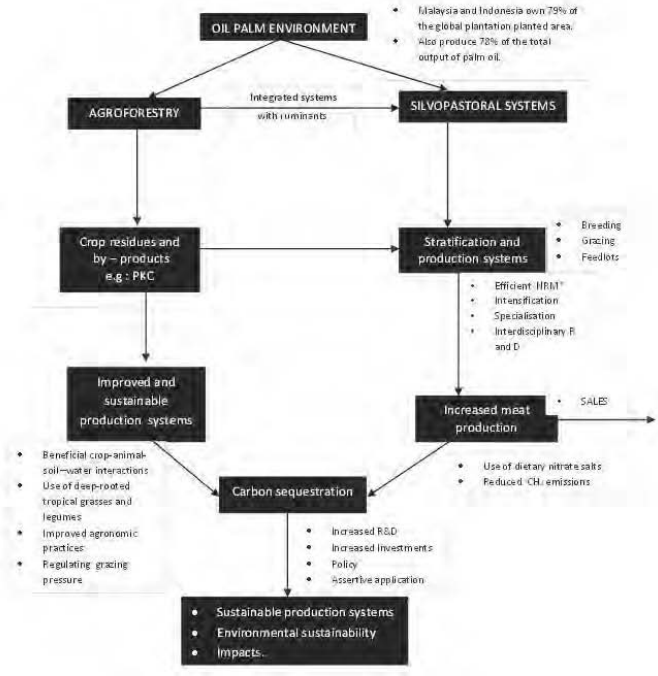

Fig. 10.1. Agroforestry and silvopastoral systems' potential and carbon sequestration. NRM = natural resource management.

commodity exports, import substitution and export manufacturing. In Malaysia, the development of agriculture has involved a combination of the diversification of agriculture and the finding that tree crops, notably oil palm, rubber and cocoa, are well suited to the local environment, and has led to a rapid shift and expansion to tree crop-based agriculture. Although an agriculture-led economic transformation has been achieved, agriculture *per se* has been on the decline in most countries. The overriding issue, and one that is of grave concern, is the overarching effects of climate change on agriculture.

10.6.1 Improved agronomic practices

Carbon sequestration is an important pathway to stabilize the environment with minimum effects on climate change. Carbon is sequestered from the atmosphere by trees, shrubs and pastures and is stored in the aerial parts. Trees, shrubs, pastures and expanding land areas under oil palm provide good opportunities for carbon sequestration through the widespread use of grasses and tree legumes, and improved forage management practices, resulting in decreased carbon atmospheric emissions and global warming. Table 10.3 from Pretty *et al*. (2002) indicates the range of practices one can use to reduce GHG emissions.

10.6.2 Cultivation and use of mixed legume–grasses forage systems

There are several mechanisms to increase carbon sinks with the cultivation of tree legumes for grazing and other production systems. Pretty *et al*. (2002) calculated that in mixed farming systems, 0.32 t carbon ha^{-1} $year^{-1}$ or 8.03 Mt carbon $year^{-1}$ was sequestered. The practical implication of this is that agronomic practices need to enhance these carbon sinks through enrichment of

Table 10.3. Approaches to increasing carbon storage and greenhouse gas emissions. (From Pretty *et al.*, 2002.)

Increase carbon sinks in soil organic matter and aboveground biomass
• Replace inversion ploughing with conservation and zero-tillage systems
• Adopt rotations with cover crops and green manure to increase biomass additions to the soil
• Adopt agroforestry in cropping systems to increase aboveground biomass
• Minimize summer fallowing and periods with no ground covers to maintain soil organic matter stocks
• Use oil conservation measures to avoid soil erosion and soil organic matter
• Apply composts and manures to increase soil organic matter stocks, including crop residue recycling
• Improve pasture/rangelands through grazing, vegetation and management to reduce degradation and increase soil organic matter
• Cultivate perennial grasses (60–80% biomass below ground)
• Restore and protect agricultural wetlands
• Convert marginal agricultural land to woodland to increase standing biomass of carbon
Reduce use of direct and indirect energy to avoid GHG emissions
• Conserve fuel and reduce machinery use to avoid fuel consumption
• Adopt grass-based grazing systems to reduce methane (CH_4) emissions from ruminant livestock
• Use composting to reduce manure CH_4 emissions
• Substitute biofuels for fossil fuels
• Increase N fertilizer use efficiency (as manufacture of N fertilizer is highly energy intensive)
• Use integrated pest management to reduce pesticide use (avoid indirect energy consumption)
Increase biomass-based renewable energy production
• Cultivate annual crops for biofuel production such as ethanol from maize and sugarcane
• Cultivate annual and perennial crops, such as grasses and coppiced trees, for combustion and electricity generation, with crops replanted each cycle for continued energy production
• Use biogas digesters to produce CH_4, substituting for fossil fuel sources
• Use improved cook stoves to increase efficiency of biomass fuels

soil organic matter and the forage biomass under oil palm. Reducing land-use changes is an effective way to enhance mitigation (FAO, 2013). The FAO (2013) study also showed improvements in animal herd efficiency in Brazil. It has been estimated that reducing grazing land and associated land-use changes, reduced emissions up to 25 %.

Similarly in Indonesia, the development of the three-strata forage system is another success story. The three-strata forage system (TSFS) in Bali, Indonesia, is a traditional people-centred smallholder system for dry areas (8 months dry and 1000 mm annual rainfall), involving grasses and herbaceous–shrub legumes and forage trees in strata one, two, and three, respectively. It is a sustainable system and meets the technical, biological, economic and sociological needs of smallholder farming families. TSFS aims to enhance year-round feeding and increase productivity in integrated systems involving food cropping (cassava, groundnuts and beans) and ruminant production (cattle and goats). Nitis *et al*. (1990) reported that the additive effects of the integration and crop–animal–soil interactions on applying TSFS resulted in many improvements: increased forage production, higher stocking rates, total weight (375 kg ha^{-1} compared to 122 kg ha^{-1}), 57% less soil erosion, 64% self-sufficiency in household fuelwood requirements, 31% more farm income and economically stable farm households. The concept and technology originally applied to 32 farmers was subsequently extended to another 144 farm households. The technology has now been institutionalized and officially promoted in Indonesia.

10.6.3 Greenhouse gas emissions

Livestock production is responsible for 18% of GHG emissions. It accounts for 9% of anthropogenic carbon emissions. The strategy obviously is to keep these emissions to a minimum. Strategies to reduce GHGs

have largely focused on methanogen inhibitors and substrate levels, rather than on feed quantity and quality. Recent studies have overcome the problem with the use of nitrate salts to replace the fermentable nitrogen requirements of ruminants. Methane (CH_4) production has consistently been shown to be reduced in feeding trials with goats (Leng, 2008; Trinh *et al*., 2009), sheep (van Zijderveld *et al*., 2010) and dairy cows (van Zijderveld *et al*., 2011).

10.6.4 Microbial ecology and reduced CH_4 emission

Parallel to feeding trials, which have the objective of consistently reducing CH_4 production, there have been concerted efforts at the rumen level to investigate alternatives for potential reduction in CH_4 emission.

The subject has recently been examined in depth and up to date by Emeritus Professor Leng (2014) in an authoritative and excellent paper titled 'Interactions between microbial consortia in biofilms: a paradigm shift in rumen microbial ecology and enteric methane mitigation'. The basic premise is that most research approaches have not considered the rumen ecology under different feeding conditions and the ecological changes that occur when perturbed by the processes aimed at inhibiting/mitigating methanogenesis. Professor Leng emphasizes two key factors that are important in CH_4 mitigation approaches:

- Adjust the diet of ruminants based on local resources to overcome deficiencies and to optimize feed conversion efficiency for optimum production such that there is a greater predictable potential to reduce actual enteric CH_4 production per unit of meat, milk or fibre.
- Manage other CH_4 mitigation approaches without jeopardizing this efficiency.

10.6.5 Silvopastoral systems and carbon sequestration

Silvopastoral systems are underestimated and also underutilized throughout the developing countries, and especially where tree plantations are abundant, such as with oil palm in Indonesia, Malaysia and Colombia. While the term 'agroforestry' is more widely recognized, 'silvopastoral' tends to be neglected or marginalized, probably because of the link with animals. The system differs from agroforestry as:

- *Agroforestry* refers to the use of various types of multifunctional trees that are incorporated into farming systems. They are usually integrated with annual crops, often in the more fertile areas outside of irrigated zones.
- *Silvopastoral systems* refer to an integrated system involving agroforestry options and notably perennial trees (e.g. coconuts, oil palm and rubber) and animals, usually in rainfed areas.

Integration involves the system components or natural resources, namely crops, animals, land and water, but integrated systems refer to approaches that link the components to economic, social and ecological perspectives. The process is holistic, interactive and multidisciplinary, and promotes participatory activities between farmers, researchers and extension personnel and efficiency in NRM. The integration of various crops and animals enables synergistic interactions and value additions that have a greater total contribution than the sum of their individual effects.

Characteristic features of silvopastoral systems and advantages

These include *inter alia:*

- Diversified and integrated use of production resources, mainly crops, animals, land and water. Integrated use promotes the use of existing resources, e.g. forage biomass under the trees, by-product feeds and reduced cost of production.
- With oil palm, the range of feeds available enables the development of *in situ* feeding and production systems.
- Use of both ruminants and non-ruminants.

- Animals and crops play multi-purpose roles.
- The process is holistic, interactive and multidisciplinary, and promotes NRM.
- Crop–animal–soil interactions are varied and have socio-economic and ecological implications.
- Low inputs used and indigenous and traditional systems.
- Associated with demonstrable sustainability and sustainable production systems.

Associated with the above, various forage biomass and the inclusion of tree legumes, providing an opportunity for *in situ* feeding and also the presence of shade, the oil palm presents excellent opportunities for carbon sequestration. This is further justified by the large area under this crop in Indonesia and Malaysia and, above all, the considerable economic benefits from promoting the system.

10.7 Strategies to Cope with Climate Change

The strategies for coping with climate change involve a combination of mitigation and adaptation. Both have to be addressed simultaneously. Ways and means, to the extent possible, have to be found to reduce CO_2 emissions, and therefore global warming. Adaptation requires accelerating the process of adaptation that will be required to cope especially with increased temperature. Both crops and animals will have to be more heat tolerant without compromising productivity. Together with greater emphasis on dryland agriculture, cropping systems and patterns will need to be geared to more suitable plants like sorghum and pigeon pea. As of now, the answer to many strategies remains largely unknown, which underlines the need for vigorous R&D on numerous issues.

10.7.1 Mitigation

Discussions on the mitigation aspects draw attention to the excellent piece of information reported in the Asian Development Bank's report (ADB, 2009). The principal strategy in biological terms relates to the GHG emissions from the agricultural sector and includes the following:

- reducing fertilizer-related emissions
- reducing CH_4 emissions from rice paddies
- reducing emissions from land-use change
- sequestering carbon in agroecosystems
- producing fossil fuel substitutes.

The keys are the type of technology being applied, type of practice, relative mitigation potential, challenges, opportunities and co-benefits and contribution to sustainable development. The types of practices identified include cropland management, rice management, agroforestry, set-aside, land-use change, grassland management, peatland management, restoration of degraded lands, bioenergy, livestock management, feeding practices, etc.

The highlights of the ADB (2009) study are reflected in the following:

- The study estimates the existing range of the economic mitigation potential of agricultural practices in South-east Asia.
- South-east Asia has a higher technical mitigation potential to reduce GHG emissions from agriculture than any other region.
- South-east Asia's vast area of croplands, could, through cropland management, be an important channel to sequester carbon in soils.
- As a major world rice producer, South-east Asia can contribute to a reduction of CH_4 emissions while ensuring food security.
- Other potential mitigation options exist with perennial tree crops; good examples include coconuts, cocoa, oil palm and rubber, all of which involve several thousands of hectares.

Concerning the latter group, the integration of ruminant animals in silvopastoral systems presents an important opportunity to sequester carbon, improve soil management and fertility, and enable feeding systems for animals, with good possibilities of reduced CH_4 from enteric fermentation, and the

introduction of improved grasses and forage legumes that can have a nutritional and ecological impact (Devendra, 2009).

By comparison, in South Asia more variable AEZs are common, typified in most of northern India and Pakistan by semi-arid to arid conditions, and by southern India and Bangladesh being similar to humid South-east Asia. The former AEZs are characterized by temperatures even higher than those in the humid AEZs, and lower rainfall and shorter LGP. Table 10.4 summarizes the main issues, challenges and opportunities that are of major concern in South Asia, together with many common practices that have been identified for South-east Asia. These include droughts, dryland agriculture, rangeland management, animal production and landlessness. Climate change impacts seriously on the agricultural sector, as well as on poor people and livelihood systems in South Asia. In India, agroforestry systems have recently been identified as an important resource conservation tool to maintain soil health in the more fragile north-east states (Saha *et al*., 2010).

10.7.2 Adaptation

Asian farmers have a long history of adapting to the changes and effects of the biophysical environment. Adaptation has entailed in practice the use of risk-minimizing strategies and the adoption of innovative low-input practices that can adapt to environmental change. They have done this through deep understanding of farming systems and experience, and more particularly the use of traditional knowledge.

The ADB (2009) report also reviewed the adaptation options and practices in the agricultural sector for South-east Asia. While readers are encouraged to study the report in full, for present purposes, a summary of the adaptive options in the agriculture sector is as follows:

- adjustment of cropping calendar and pattern
- changes in management and farming practices
- use of heat-resistant varieties
- development of water-efficient crops
- diversified farming, intercropping, crop rotation and food–feed systems
- utilization of southern oscillation index (SOI) designing cropping strategy
- implementation of index-based insurance
- development of early warning systems
- improvement of irrigation efficiency.

To these, the following can be added:

- plant breeding for increased drought and flood tolerance and disease resistance
- application of new technologies for water harvesting, conservation and recycling
- development of food–feed systems
- nutrient management and soil fertility
- integration of animals with annual and tree crop systems
- sustainable intensification of improved crop–animal systems
- appropriate economic incentives, subsidies, pricing and taxes
- linking production to post-production systems and the international food supply chain.

Many of the adaptation options identified for South-east Asia by the ADB (2009) will also be similar to the options for South Asia. Strategies for crop production, cropping patterns and the cropping calendar, for example, will be broadly similar, except the types of crops to grow and the choice of animals and breeds to fit in with the particular AEZ. In general, both the types grown and the animals reared in South Asia will need to be more heat tolerant than required for South-east Asia.

10.8 High Priority for R&D

Much of the foregoing discussions emphasize the considerable challenges for R&D. Agricultural research can go a long way to reduce the risks in agriculture. With the science of climate change, the fact is that there is a great paucity of information in understanding the effects of the biophysical factors of temperature and rainfall on natural resources and ecology, and needs to start at the grass roots level. A recent review

Table 10.4. Mitigation options in agriculture in South Asia.

Issue and practice	Challenges	Opportunities	Co-benefits and contribution to sustainable development
Droughts	• Minimize risks to farming systems • Coping with heat stress • Adapting to heat stress • High mortality in animals • Resilience of livelihood systems • Improved storage and conservation of seeds and crops	• Risk-minimizing strategies • Sustainable dryland agriculture • Heat-tolerant technologies • Reduced animal mortality • Enhancing nutritional and food security • Improved management and use	• Increased adaptation • Ecosystem resilience • Reduced vulnerability • Increased self-reliance • Stable households • Sustained agricultural production
Dryland agriculture • Heat-tolerant crops and animals • Improved water harvesting and conservation • Agronomy and feeding regimes • Nutrient management • Soil fertility and water balance	• Use of indigenous knowledge and traditional systems • Improved rainfed agriculture • Alleviation of hunger and poverty • Improved livelihoods • Improved health and resilience • Improved agricultural water management	• Major opportunities in R&D • Expanded use of rainfed areas • Increased food production • Improved R&D • Empowerment • Improved land management and agronomic practice	• Sustainable production system • Improved understanding of the landless • Environmental integrity • Increased nutritional and food security • Cooperative development • Ensuring sustained crop production
Animal production • Species and breeds • Adaptation • Feed resources • Heat stress • Soil nutrient management	• Heat tolerance • Optimum productivity • Totality of availability and potential value • Priorities and efficiency of use • Heat tolerance and adaptation • Increased carbon sequestration	• Identification of more adaptable breeds • Distinctive adaptation traits • Integration with farming systems • Production and conservation • Development of integrated ruminants–tree crop systems (south India and Sri Lanka) • Improved use of forage and legume varieties and in food–feed systems	• Increased productivity • Increased sustainability • Increased food and nutritional security • Development of year-round feeding systems • Stable households • Sustainable production systems
Landless • Nomadism • Transhumance • Livelihoods • Animal ownership	• Rationale • Way of life • Migratory patterns • Contribution by animals	• Improved understanding • Traditional systems • Security • Extent of contribution to poverty alleviation	• Environmental protection • Survival • Increased ownership of animals
Rangeland management • Grazing systems	• Overstocking • Control of management	• Effective use of browse • Improved fodder production • Increased meat production	• Prevention of environmental damage • Improved livelihoods

on the subject suggests that research will require a rethinking of research structures, and further suggests that governance issues will be a central consideration (Lahsen *et al*., 2010). The challenges and opportunities for R&D are numerous, and the overriding issues make this very complex. No single discipline can resolve the problem and that is why the call is for intensive interdisciplinary efforts that focus at the system level, along with prioritization of the research agenda. The range of issues is large, very complex and interrelated, and varies from country to country, with more specific and individual

needs. Current problems in agriculture and food production systems require more than technical solutions and practical application. To be thorough, they require an R&D agenda that integrates many disciplines, but notably biological constraints, demographics, socio-economic issues, systems perspectives, resource allocation, value chains and trade and marketing considerations at the national, regional and global levels. Finally, there is also the issue of ensuring the successful delivery and adoption of the improved technology through community-based joint efforts between farmers, researchers, extension personnel and municipal officials (Devendra, 2014).

10.9 Policy Framework

The implementation of R&D activities for coping with the effects of climate change in agriculture will also need realistic policy elements to ensure the success of a pragmatic agenda. The policy requirements that are appropriate for agriculture are reflected in the following:

- affirmation of the official policy to address waning agriculture, its revitalization and integrated NRM to cope with climate change
- priority for enhancing nutrition and food security, and increased self-reliance
- priority for concerted R&D of rainfed agriculture and small farm systems to include carbon sequestration, mitigation and adaptation strategies
- priority for the development of rainfed less favoured or marginal lands
- improved water efficiency for cropping systems and land-use systems
- priority for pro-poor community-based activities that can adapt to climate change
- promotion of ways and means to enhance carbon sequestration and reduce emissions of GHGs, for example, the development of sustainable integrated tree crops–ruminant systems
- building R&D capacity and application of systems perspectives to deal with climate change
- substantial increase in investments in agriculture to promote greater engagement and productivity
- promote public–private sector partnerships to address agricultural development in the context of climate change.

In addition to the above, it is also pertinent to draw attention to the policy recommendations made by ADB (2009) for the mitigation and adaptation of agriculture and land-use sectors in South-east Asia.

10.9.1 Mitigation

- Improve land-use systems, temperature-tolerant crops and animal practices and farm management.
- Promote emissions reduction through a combination of market-based programmes, regulatory measures, voluntary agreements and international programmes.

10.9.2 Adaptation

- Strengthening adaptive capacity by providing public goods and services such as better climate information, R&D on heat-resistant crop variety and other techniques, early warning systems and efficient irrigation systems, and explore innovative risk-sharing instruments such as index-based insurance schemes.
- Implement aggressive public–private partnerships for reforestation and afforestation.

10.10 Conclusion

The scale and nature of the effects of climate change is uncertain, but there is general agreement that these are imminent and will make all regions warmer, with declining soil moisture corresponding with high temperatures and evapotranspiration, which can favour severe drought and flooding, especially in semi-arid and arid AEZs. Therefore, a reorientation of R&D strategy is

necessary to improve inadequate food production, enhance food and nutrition security, combat spiralling food costs and crises, and promote sustainable development across the ecological landscape and the livelihood systems of the poor. The priorities for R&D should include *inter alia* pro-poor community-based activities that can adapt to climate change and the use of sustainable yield-inducing technologies, along with the promotion of ways and means to enhance carbon sequestration and reduce emissions of GHGs. Trait-based breeding and conservation of animal genetic resources with their inherent adaptation traits is a prerequisite to mitigate the adversity of climate change. Also, strengthening R&D capacity with transdisciplinary systems perspectives and human resources to deal with the problems of climate change, and promote increased investments in agriculture to drive increased agricultural development, is the need of the time.

The revitalization of agriculture, its development and transformation to vibrant and sustainable agricultural systems to produce more food for humans is at the crossroads of uncertainty and anticipation. With climate change, the potential effects are even more uncertain, and the agricultural landscape hangs in a balance. Telescoping time to address R&D and adapting to the impact of any inevitable consequences is our collective challenge to ensure harmony with the environment in the near future. Problem identification, priority setting, adaptation and innovation are key considerations and vision must lead the way.

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11 Climate Change: Impacts on Livestock Diversity in Tropical Countries

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Abstract

The effect of changing climate will not only be confined to limited production, and the productivity of agricultural commodities, but will also have far-reaching consequences on dairy, meat, wool and other animal products. The impact of climate change on the livestock sector as a whole will be felt more in tropical countries compared to temperate countries, largely because of the structure of production system and economics. The resultant pressure, both direct and indirect, is likely to result in further dilution of livestock diversity, which would specially affect the nutritional security and livelihood of small and marginal farmers. The challenge is to sustain genetic diversity and productivity by different adaptation strategies like production adjustment, breeding strategies, alteration of management systems, developing appropriate policies, scientific intervention and capacity building of livestock owners. In light of concerns over the impacts of climate change and climate variability, this chapter provides an overview of the opportunities for adaptation and mitigation strategies in tropical climatic conditions.

11.1 Introduction

Climate change as evidenced in tropical areas is expected to cause strong negative impacts, where most of the developing countries are concentrated. The latest report of IPCC (2013) indicated sharp changes in annual rainfall in the tropical and subtropical world as temperatures rise and oceans become warmer. Climate change in these regions is projected to cause an increase in extreme weather events, such as droughts, heatwaves, storms, desertification, cyclones, flash floods, etc. Long-term changes in climate may significantly affect the future of all animal genetic resources (AnGRs), including those on farms or in forests.

In most tropical areas, livestock are the key assets of underprivileged people for providing multiple economy, nutritional confidence, social security and economic insurance during emergencies. The effect of changing climate will not only be confined to the limited production and productivity of agricultural commodities, but will also have far-reaching consequences on dairy, meat, wool and other animal products. The AnGRs of the tropics are at risk of being lost through the direct impacts of climate change, arbitrated via increased incidences of drought and flood and the emergence of epidemic diseases, whereas the indirect impacts are through change of adaptation capability of animals to extreme climatic conditions. There arises health implications too related to biodiversity loss, and subsequently many of the expected or emerging health risks.

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The challenge is to sustain genetic diversity and productivity by different adaptation strategies like production adjustment, breeding strategies, alteration of management systems, developing appropriate policies, scientific intervention and capacity building of livestock owners. The identification and optimum utilization of different adaptable traits of local AnGRs need to be included in breeding programmes for sustainable growth of the livestock sector, as well as to maintain genetic diversity. Proper *ex situ* conservation strategies, especially *in vitro* conservation, need to be considered as an important component of a broad-based strategy to conserve critical adaptive genes and genetic traits.

The chapter will cover some of the likely impacts of climate change on livestock and livestock system variability, and will discuss some of the resultant priority livestock development issues, including breeding strategies for climate-resilient animal husbandry to sustain livestock biodiversity in the tropics.

11.2 Animal Diversity

Diversity in general refers to the array of differences among some set of entities. Biological diversity thus refers to variety within the living world – the biosphere. The term 'biodiversity' is commonly used to describe the number, variety and variability of living organisms. Diversity can be measured only if some quantitative value can be ascribed to it and these values compared. To do this, biodiversity is divided into its constituent elements, i.e. genes, species and ecosystems, which correspond to three fundamental and hierarchically related levels of biological organization. The most common usage of the word 'biodiversity' is as a synonym of species diversity or species richness. This is perhaps because the living world is most widely considered in terms of species. Thus, discussion of global biodiversity is typically presented in terms of global numbers of species in different taxonomic groups. Estimates for the total number of species currently existing on earth vary from 5 million to nearly 100 million. One estimate suggests there might be around 12.5 million; of these, only an estimated 1.7 million have been described to date. In terms of species number alone, life on earth appears to consist essentially of insects and micro-organisms.

Genetic diversity represents the heritable variation within and between populations of organisms. Populations may be entire species or a specific collection of individuals within a species such as a breed, strain, line, herd/flock, etc. Diversity ultimately resides in the variations in the sequence of the four base pairs that, as components of nucleic acids, constitute the genetic code. New genetic variation arises in individuals by mutations of gene and chromosome. During sexual reproduction, by recombination of the chromosome, the variation is spread through the population.

Genetic diversity, the pool of genetic variation in an interbreeding population, is acted upon by selection, be it natural or artificial. Differential survival results in changes of the frequency of genes within a population, and this constitutes population evolution. Thus, genetic variation enables both natural evolutionary change and artificial selective breeding to occur.

Livestock populations developed in different ecological or geographical areas have become genetically distinct as a result of genetic drift, shift and differential selection pressures, provided they have also been isolated reproductively from other populations developed under different conditions. Thus, the indigenous livestock from different regions of the world should probably be assumed a priority to represent different 'breeds' that have different adaptive characteristics or possess unique physiological characteristics.

11.3 Adaptability of AnGRs

The speciation and development of geographically isolated breeds in the tropics has taken place with the consideration of harsh

climatic variation present in the region since time immemorial. For example, zebu cattle are uniquely suited to hot and humid climates because of their smooth coat, primary hair follicles, improved sweat and sebaceous glands, and better ability to lose moisture by evaporation than *Bos taurus* cattle (Turner, 1980). Variation in adaptability between *Bos indicus* and *B. taurus* cattle may be due to their origin in distinct climates in which *B. indicus* might have acquired thermotolerant genes (Hansen, 2004). Among temperate breeds, Jersey dairy cows are more resistant to heat stress than Holstein cows under tropical climatic conditions (Sharma *et al*., 1983). Typically, goats are regarded as the best-adapted species to harsh environments (Silanikove, 2000b). In addition, sheep and goat are more thermotolerant than cattle (Silanikove, 2000a,b; Khalifa *et al*., 2005), which have a high metabolic rate and a poorly developed water retention mechanism (Bernabucci *et al*., 2010).

Similarly, resistance/tolerance to numerous infectious diseases and parasitic infestations like trypanosomiasis, dermatophilosis, or foot rot, tick-borne diseases and internal parasites afford them a distinct advantage, which will be of increased importance in changing climatic conditions. Efficient conversion of low-grade feed resources to high-quality animal proteins and enhanced immunity to diseases is evidence of adaptation by these animals living in the tropics (Naskar *et al*., 2012).

Some of the commercially successful and thriving indigenous breeds of livestock well adapted to an often harsh environment include the Sahiwal cattle of India (Ogilvie, 1947), the Kenana cows of Sudan (Alim, 1960) and Nandi cattle in Kenya (Wilson, 2009). Some prized tropical beef breeds are the Caracu from Criollo, Indubrasil or Nellore (originally from the Ongole cattle of India) in Brazil (Cardellino, 2000), Brahman cattle (originally from India) in the USA, the Boran in Ethiopia and Kenya (Haile-Mariam *et al*., 1998; Homann *et al*., 2005) and Mashona and Tuli cattle in Botswana and Zimbabwe (Moyo, 1990; Homann *et al*., 2005). Similarly, Haryana and Kankrej cattle in India, and Afrikander cattle in South Africa (Wilson, 2009), are renowned for draught or pack purpose. Among the sheep breeds, the Chios and Awassi breeds of the Near East for milk purpose, the Sudan Desert for the dual purpose of milk and meat (Wilson, 2009), the Awassi breed of the Near East as multi-purpose (Amin and Peters, 2006; Wilson, 2009) and the Chios breed of the Near East and eastern Mediterranean region (Hatzimimaogiou *et al*., 1990) are front-runners in productivity. The Boer goat of South Africa (SASBA, 2004), the Jamunapari, Beetal and Black Bengal goats of India and the Damascus goat in Cyprus (a native of Syria and Lebanon; Mavrogenis *et al*., 2006; Wilson, 2009) are widely used for profitable goat farming under tropical climatic conditions.

Genetic studies also suggest the presence of a few major genes influencing better adaptability in harsh climates, like the presence of: the trypanosome resistance gene in the N'dama cattle of Africa; the slick hair gene in Senepol and Criollo cattle breeds (Spanish origin) in Central and South America for the development of hairs to resist heat; the *Nramp1* gene for resistance/susceptibility to *Brucella abortus* (Adams and Templeton, 1998; Barthel *et al*., 2001), *Salmonella* and Paratuberculosis infection in cattle (Pinedo *et al*., 2009) and buffalo (Ganguly *et al*., 2008); and the halothane gene in pigs which causes malignant hyperthermia (heat shock) when pigs are exposed to environmental stressors.

The widespread use of Brahman cattle in cross-breeding programmes in the USA (Ames and Ray, 1983), the import, development and re-export of African Boran and Tuli cattle breeds (Rege and Gibson, 2003), the resilience of Sahiwal breeds in India and Kenana, Butana and N'dama cattle breeds are a few good examples that illustrate the importance of adaptability to local environmental conditions. There are many Indian breeds of cattle (*B. indicus*) that perform equally well in a hot climate, as they have been selected unintentionally for their ability to survive in unfavourable environments. The Australian Milking Zebu (AMZ), a cross of Sahiwal and Red Sindhi bulls with

Jersey cows, was developed during the 1960s, with milk production over 2100 kg lactation^{-1} and 4.5% fat (Stephens, 2006). This breed has remarkable heat tolerance and tick resistance. Similarly, the Australian Friesian Sahiwal (AFS), whose development is of recent origin, has better performance and fertility than the AMZ (Stephens, 2006). The development of Senepol cattle (lesser known as Nelthrop) in the Caribbean Islands combined N'dama traits of heat tolerance with the higher milk production, good meat quality and docility of Red Polls (Wilson, 2009). Santa Gertrudis, a composite breed of 3/8 zebu and 5/8 Shorthorn developed in Texas, USA, is an excellent beef breed that has found wide acceptability in tropical and subtropical countries also. The Droughtmaster, a composite of mainly Brahman and Shorthorn developed in Queensland, Australia, produces quality beef with good reproductive performances. It is also credited for docility, heat and drought tolerance and tick resistance. The Jamaica Hope, developed through the crossing of native cows with Jersey bulls, and subsequently Sahiwal bulls of India, had mixed success. The Droper sheep, derived from British Dorset Horn and Persian Blackhead in South Africa, is one of the few examples of sustained success in small ruminants.

11.4 Impact of Climate Change on Diversity

Climate changes in terms of extreme temperatures and changes in carbon dioxide concentrations are likely to impose differential opportunities for several species, depending on adaptability to changes to a broader range of biogeographic conditions and environmental controls. The impacts of those favoured species may be more severe as they increase both in numbers and extent, and as they compete for diminishing resources such as water, feed, etc. Changed air and water temperatures may also facilitate movement of species along previously inaccessible pathways of spread, both natural and man-made. Individually, climate change and favoured species present two of the greatest threats to biodiversity and the provision of valuable ecosystem services. The estimated damage from invasive species worldwide totals more than US$1.4 trillion per annum – 5% of the global economy, with impacts across a wide range of sectors including agriculture, forestry, aquaculture, transportation, trade, power generation and recreation (Pimentel *et al*., 2001). Based on regional climate models, it is predicted that the temperatures in the Indian subcontinent will rise between 3.5 and 5.5°C by 2100, and on the Tibetan Plateau by 2.5°C by 2050 and 5°C by 2100 (Rupa Kumar *et al*., 2006).

11.4.1 Direct impact

A particular set of ecological and climatic conditions is necessary for the survival of a species. A shift in the environmental variables, such as air and water temperature and water availability, will have implications for species, particularly if variables shift outside the range of the species' bioclimatic envelope for survival. The array of anticipated climatic and biogeographic changes has significant implications for both native and non-native species. This may prompt species to migrate to new areas where conditions may be a better match, or simply to go into decline if such movements are not biologically or physically possible. Relationships with symbiotic hosts, the presence/absence of predators and other ecological dynamics will also play a significant role in regulating population sizes.

11.4.2 Indirect and secondary impact

Changing climate may also facilitate biological invasions without necessarily being the direct source of their introduction. Particular areas of concern include the role of events and their impacts on ecosystems, as well as ongoing shifts in species composition and trophic chains responding to climate change. These phenomena may also increase ecosystem vulnerability to the

establishment and spread of a particular species (Campbell *et al*., 2009).

Changes in soil composition, hydrological cycles and glacial extent may all provide favourable ground for few invasive species. Finally, the range of human responses to climate change, both intentional and unintentional, will influence the impact of invasive species.

11.4.3 Species composition and ecosystem function

The broad categories of climate change impacts on species composition and ecosystems are gradually becoming better defined; however, the full implications of these types of changes are still largely unknown and could be unique to each case.

Observed areas of impact include changes in the geographic range of species, their phenology, as well as photosynthetic rates, carbon uptake and productivity (SCBD, 2009). All these events will affect interactions between species, and more broadly, community composition, trophic webs and corresponding ecosystem functions. For example, earlier flowering dates for plants may not coincide with the emergence of symbiotic pollinators. Similarly, from a management perspective, the efficacy of species used for biological control may vary depending on the changes in the development, morphology and reproduction of the targeted species (Ziska, 2005). Altered feeding behaviour and reproduction rates from insects and mammals due to warmer temperatures and longer seasons may impact plant reproduction. These individual interactions may have compounded effects on broader ecosystem services such as groundwater retention and filtering, pollination, disease suppression and carbon sequestration.

11.5 Impact of Climate Change on Livestock

Climate change has become an important area of concern for tropical countries to ensure food and nutritional security for a growing population. The impacts of climate change are global, but tropical countries are more vulnerable in view of the high population depending on agriculture.

Animal agriculture, the rearing of animals for food, clothing and draught power, is a major contributor to climate change, responsible for 18% of greenhouse gas emissions (Steinfeld *et al*., 2006). Climatic changes will have a negative impact on all animals. The climate debate may lead to a greater increase in intensive production practices at the expense of medium- and long-term environmental and animal welfare friendly extensive production methods. Harming the health and well-being of animals directly compromises the societal, economical, physiological and cultural aspects of humans. Climate change, particularly global warming, may affect strongly the production performances of farm animals and impact worldwide on livestock production (Nienaber and Hahn, 2007). The impacts of climate change are not equally distributed and are affecting more or less all regions. Since farm and livestock production and productivity are closely associated with climate, it is obvious that any change in climate would render rural areas more vulnerable.

Sharma and Rai (2012) described a number of changes at low, medium and high altitudes, primarily in response to reduced pasturelands, warming and decrease in precipitation. Pastoralists at high altitudes would move around less and become sedentary. The productivity of several crops may decrease, while crop diversification may increase. While documenting changes in the quality of milk from cattle, Senthil Kumar (2012) found the amount of sour milk being gathered by milk cooperatives had increased: sourness, presumably, was being caused by the warm weather.

Climatic effects on animal health and production involve complex interaction between climate and animal factors. Climate elements include the direct effect of air temperature, humidity, wind velocity, solar radiation and other factors. Heat is the major constraint in tropical and subtropical

climatic conditions, and thermal stress is a major factor negatively affecting the production and reproduction of livestock species. Heat stress causes a chain reaction of physiological, behavioural and anatomical alteration, leading to reduction in growth and productive and reproductive functions. All these are debated at length in Chapter 3, Section I, this volume. In addition, there is a decrease in activity, increase in respiration and body temperature, increased peripheral blood flow and alterations in endocrine functions. The impact of climate change on reproduction and endocrine functions is described in Chapter 12, Section II, this volume. Animal production is affected by climate change in four ways (Smit *et al*., 1996):

- through changes in livestock feed–grain availability and price (see Chapter 2, Section I, this volume)
- impacts on livestock pastures and forage crop production and quality
- changes in the distribution of livestock diseases and pests
- changes in physiological parameters.

Heat stress will have long-term effects on milk production, body growth rate, physiology and birth rates. Increased summer temperature leads to depressed and low feed intake, reduction in the body weight of animals and lower milk production. Hence, the negative effects of this global change are not limited to crops and agricultural production only, but will have far-reaching consequences for livestock and related sectors also. A thermal environment is a major factor that can affect milk production in dairy cows negatively, especially in animals of high genetic merit. Johnson *et al*. (1962) showed a linear reduction of dry matter intake (DMI) and milk yield when the temperature humidity index (THI) exceeded 70. The reductions were –0.23 and –0.26 kg day^{-1} per unit of THI increase for DMI and milk yield, respectively. The increase in milk yield increases the sensitivity of cattle to thermal stress and reduces the 'threshold temperature' at which milk losses occur (Berman and Kofinas, 2004). This is because metabolic heat production increases with the increasing production level of a cow (Kadzere *et al*., 2002).

Heat stress has a detrimental effect on reproductive efficiency. Since the 'bull is half of the herd', it is imperative that the impact of climate change on the fertility of males, which has wide implications on the reproduction and production parameters of the cows, be taken into consideration (also see Chapter 12, Section II, this volume). Climate change may impact the fertility of the male animals in ways such as delayed puberty due to non-availability of balanced feeding during summer and depressed immune status during summer season. Expression of heat shock proteins due to harsh tropical climate condition is considered to be a major hurdle limiting germplasm quality and fertility (Ali *et al*., 2013). In boars, scrotal and testicular temperature rises when the ambient temperature increases, due to their low capacity for compensatory sweating (Stone, 1982). The effect of elevated temperature includes a decrease in the total sperm count and ejaculate volume and an increase in abnormal sperm cells, which leads to poor fertility (Suriyasomboon *et al*., 2004). Further, the negative effects of heat stress persisted for 4–6 weeks after the heat stress was removed.

High ambient temperature leading to heat stress has been associated with seasonal infertility in both tropical as well as temperate countries (Tummaruk *et al*., 2000). In sows, temperatures exceeding 27°C delay or prevent the occurrence of oestrus, reduce the conception rate and increase early embryonic death. Conception rate was found to be 90%, 85% and 78% in sows reared under an environment temperature of 26–27°C, 30°C and 33°C, respectively (Serres, 1992). Heat stress has been reported to reduce implantation and embryonic survival by 30–40% (Curtis, 1981). Omtvedt *et al*. (1971) found in their study that there was a greater reduction in the number of viable embryos among gilts exposed to elevated temperatures during day 8–16 post-breeding than day 0–8, indicating that the time of implantation would be the most sensitive stage of pregnancy to stress.

Heat stress reduces the reproductive performance of laying hens by interrupting egg production. This may be caused not only by a reduction in feed intake but also by a disruption of hormonal balance responsible for ovulation and a decrease in the responsiveness of granulosa cells to luteinizing hormone (Donoghue and Doyel, 1989; Novero *et al.*, 1991). Significant reduction of body weight and feed consumption occur in heat-stressed hens. Egg production, egg weight, shell weight and shell thickness are considerably compromised by heat exposure (Mashaly *et al.*, 2004).

11.5.1 Biodiversity loss due to climate change

Climate change has had a considerable effect on livestock by being a major driver of the processes of speciation. Climate change is a natural event that has occurred throughout history. However, with the recent increased emission of CO_2 in the earth's atmosphere, abrupt climate change has occurred. It has been hypothesized that greenhouse gas emissions due to anthropogenic causes has significantly influenced global climate since about 8000 years before now (Van Hoof *et al.*, 2006).

Species respond to climate changes by migration, adaptation, or if neither of those occur, by death. These migrations can sometimes follow an animal's preferred temperature, elevation, soil, etc., as said species move due to climate change. Adaptation can be either genetic or physiological, and death can occur in a local population only (extirpation) or as an entire species, otherwise known as extinction.

Climate change is projected to affect individual organisms, populations, species distributions and ecosystem composition and function both directly (e.g. increased temperatures and changes in precipitation) and indirectly, through the climate changing intensity and frequency of disturbances such as wildfires and severe storms (IPCC, 2002). Average temperature changes itself does not provide simple predictions about ecological consequences. Average temperatures have changed more in high latitudes than in the tropics, but tropical species are likely more sensitive to temperature changes than temperate species (IPCC, 2007).

Every organism has a unique set of preferences or requirements, called 'niche', and biodiversity has been tied to the diversity of animals' niches. These can include or be affected by temperature, aridity, resource availability, habitat requirements, soil characteristics, competitors and pollinators. Since the factors that compose a niche can be so complex and interconnected, the niches of many animals are bound to be affected by climate change (Parmesan and Yohe, 2003). Animals are adapted to specific temperature ranges. As temperatures increase, there is a high probability that animals will shift their habitat to higher altitude, particularly in mountains, where the temperature is comparatively cooler.

A changing global climate threatens species and ecosystems, as the distribution of species (biogeography) is largely determined by climate. Climate change may simply shift these distributions but, for a number of reasons, plants and animals may not be able to adjust. The pace of climate change will be more rapid than most plants are able to migrate. The presence of roads, cities and other barriers associated with human presence may provide no opportunity for distributional shifts. The climate that characterizes the present-day Yellowstone Park (USA) may shift several hundred miles northward. For these reasons, some species and ecosystems are likely to be eliminated by climate change. Agricultural production likely will show regional variation in gains and losses, depending on crop and climate. As a consequence of these multiple forces, many scientists fear that by the end of the next century, perhaps 25% of existing species will be lost (Wilson, 1992).

11.5.2 Genetic and environmental interaction

It has important implications in the development of breeding programmes if selection is undertaken in good production environments (feeding, health, housing or climate). We need to know beforehand if the genetic improvement achieved is to be exploited in a poor production environment.

The selection of breeding stock should be undertaken in environments that are similar to where their offspring are expected to be raised. More often, failures in realizing the full genetic potential of exotic temperate breeds when they are exported to more stressful tropical environments or production systems is due to failure of the farmers and technical staff to recognize fully the importance of Genotype × Environment (G × E), the most common of which is the continued use of high-producing North American Holstein–Friesian bull semen to produce daughters in tropical farmers' herds, where husbandry is generally inadequate. Many such examples exist in ill-designed (rather too sophisticated, given the existing infrastructure) exotic breed-based livestock development programmes in the tropics.

The increase in species richness or biodiversity that occurs from the poles to the tropics, often referred to as the latitudinal diversity gradient, is one of the most widely recognized patterns in ecology. In general, localities at lower latitudes generally have more species than localities at higher latitudes (Adams and Hadly, 2012). Tropical areas play a prominent role in the understanding of the distribution of biodiversity, as their rates of habitat degradation and biodiversity loss are exceptionally high (Gaston, 2000). There are two climate-related hypotheses that suggest the biological diversity in relation to the latitudinal diversity gradient.

Climate harshness hypothesis

This hypothesis suggests that the latitudinal diversity gradient may exist because fewer species can physiologically tolerate conditions at higher latitudes than at low latitudes, as higher latitudes are often colder and drier than tropical latitudes.

Climate stability hypothesis

Climate stability is suggested to be the reason for the latitudinal diversity gradient. The mechanism for this hypothesis is that while a fluctuating environment may increase the extinction rate or preclude specialization, a constant environment can allow species to specialize on predictable resources, allowing them to have narrower niches and facilitating speciation. The fact that temperate regions are more variable, both seasonally and over geological timescales, suggests that temperate regions are thus expected to have less species diversity than the tropics.

There are many exceptions to the assumption that climate stability means higher species diversity. For example, low species diversity is known to occur often in stable environments such as tropical mountaintops. Additionally, many habitats with high species diversity do experience seasonal climates, including many tropical regions that have highly seasonal rainfall (Brown and Lomolino, 1998).

11.5.3 Livestock system biodiversity

Climate can affect livestock both directly and indirectly (Adams *et al*., 1999; IPCC, 2001). Direct effects from air temperature, humidity, wind speed and other climate factors influence animal performance such as growth, milk production, wool production and reproduction. Climate can also affect the quantity and quality of feedstuffs such as pasture, forage and grain, and the severity and distribution of livestock diseases and parasites. The effect of climate on crops can also affect the desirability of livestock. Livestock net revenues, the number of livestock per farm and earnings per livestock are all highly sensitive to climate (Niggol and Mendelsohn, 2008). Lack of water and increased frequency of drought in certain parts will also lead to a loss of resources. The

impact of climate change is expected to heighten the vulnerability of livestock systems and reinforce existing factors that are affecting livestock production systems, such as rapid population and economic growth, rising demand for food (including livestock) and products and conflict over scarce resources (land tenure, water, biofuels etc.). For rural communities, losing livestock assets could trigger a collapse into chronic poverty and have a lasting effect on livelihoods.

The impacts of climate change also depend on rainfall, which generally affects crop and grassland productivity, and thereby livestock net income (Niggol and Mendelsohn, 2008). There are three plausible explanations: first, farmers shift to crops as rainfall increases; second, grassland shifts to forests as rain increases, reducing the quality and quantity of natural grazing for most animals; and third, increasing precipitation intensifies the incidence of certain animal diseases.

Climatic factors, such as high ambient temperature, high relative humidity (RH), high solar radiation and low wind speed, can induce a heat stress response in heat-susceptible animals. The heat load may, for at least part of the year, induce physiological (Ramana *et al*., 2013) and behavioural changes (Pankaj *et al*., 2013) that contribute to a decrease in production and reproduction, and could impair immune function (Finocchiaro *et al*., 2005). Biologically, animals are able to minimize the adverse effects of a high heat load by invoking physiological mechanisms, such as increased respiration rate and sweating rate, changes in endocrine function and a reduced metabolic rate (Sevi *et al*., 2001; Ramana *et al*., 2013; also see Chapter 3, Section I, this volume, for details). When the physiological mechanisms fail to alleviate the effect of heat load, the body temperature may increase to a point at which animal well-being is compromised. The loss in body weight during hot conditions is essentially a result of reduced DMI and an increase in maintenance requirement caused by the increased physiological functions (Marai *et al*., 2007).

Under heat stress, a number of physiological and behavioural responses vary in intensity and duration in relation to the animal's genetic make-up and environmental factors (Pankaj *et al*., 2013a). Climatic, environmental, nutritional, physical, social or physiological stressors are likely to reduce the welfare and performance of animals (Freeman, 1987). Adaptation to heat stress requires the physiological integration of many organs and systems, specifically the endocrine, cardiorespiratory and immune systems (Altan *et al*., 2003). Heat stress reduces libido, fertility and embryonic survival in animals. The primary effect of environmental stress in neonates includes the increasing disease incidence associated with reduced immunoglobulin content in plasma. Heat stress in late gestation reduces fetal growth and alters the endocrine status of the dam. The carry-over effects of heat stress during late gestation on post-partum lactation and reproduction are also detectable (Collier *et al*., 1982). Thermal stress lowers the feed intake of animals, which in turn reduces their productivity and reproductive performance (Kimothi and Ghosh, 2005). High ambient temperature can affect the structure and physiology of cells adversely, causing impaired transcription, RNA processing, translation, oxidative metabolism, membrane structure and function (Iwagami, 1996).

Rise in temperature during summers affect reproductive functions and milk production negatively in buffaloes (Upadhyay *et al*., 2007). The incidence of silent heat or poor expression will be more common at high temperatures during the summer season and beyond, particularly in buffaloes that have limited access to water for either drinking and/or wallowing. Buffaloes at high temperatures may also fail to conceive due to silent heat or poor expression of heat, loss of conception, causing long dry periods and intercalving intervals (Roy, 1969), ultimately affecting milk production.

Most of the economic traits in livestock species are under the control of many genes (at many loci). Such traits are combined

expressions of many different physiological systems, each contributing to the metric value additively or through interaction with other physiological mechanisms. If we take milk production as an example, the observable value is the overall expression of several 'macro functions' such as appetite, feed intake, digestion efficiency, efficiency of utilization of body reserves, udder function and volume, health status, ability to handle other environmental stresses, etc. In addition, behind each macro function, there are chains of enzymatic, hormonal and other biochemical reactions regulated by gene products. Thus, the number of genes involved in one trait is usually or likely to be very large. For such complex quantitative traits, the different genotypes cannot be distinguished on the basis of the phenotype (production record, measurement or appearance) of the individual. An important complicating factor is that environmental effects modify the expression of such characters and therefore contribute to the phenotypic variation among individuals. For example, the milk production from an individual is influenced by factors such as quality and quantity of feed, housing, effect of disease, etc. To the extent that environmental conditions are affected by climatic conditions, season becomes an important factor influencing animal performance (Rege and Okeyo, 2006).

In the tropics, both the quality and quantity of feeds and disease and parasite burdens can fluctuate considerably between seasons in response to differences in rainfall, temperature, humidity, etc. These have important implications for housing and overall animal management, and for herd/flock structures. In turn, management (housing, feeding, health care, etc.) considerably influences the expression of quantitative traits.

To handle the complexity of these traits, quantitative genetic theory provides us with powerful tools for analysing quantitative variation to enable us to use the results in practical animal breeding. There are several analytical methods available. All of these are based on the fact that, no matter how complex the underlying causal mechanisms are for any trait, the expressed phenotype (P) can be attributed to two main sources, the genetic (G) and the environmental (E) components. In complex models, these components are divided into subcomponents, and interactions among components are also included. While for a single trait in one environment, quantitative estimates of the causal components (G and E), which are usually expressed in terms of variances, provide a good indication of the contribution of the environment relative to the total phenotype. The situation is a bit more complicated for multiple traits and for one trait being evaluated in multiple environments. For quantitative estimates of multiple traits and one trait being evaluated in multiple environments, the concept of genotype by environment interaction ($G \times E$) is of utmost importance. Where $G \times E$ exists, the breed or genotype with the best performance in a given trait in one environment may not give their best performance in another environment, or the extent of superiority will differ between environments. Such differences provide a framework for quantitative analysis. An instructive approach to the analysis of $G \times E$ is to treat records of the same trait taken in different environments as representing different traits and to estimate genetic correlations between these traits. The existence of $G \times E$ will be indicated if the genetic correlation is low.

11.5.4 Health risks brought by climate change

Heat stress in lactating animal results in dramatic reduction in roughage intake, gut motility and rumination, which in turn contribute to decreased volatile fatty acid production and may contribute to alteration in the acetate to propionate ratio. Rumen pH also declines during thermal stress (Collier *et al.*, 1982). Electrolyte concentrations, in particular Na^+ and K^+, are reduced in the rumen fluid of heat-stressed cattle. The decrease in Na^+ and K^+ are related to an increase in loss of urinary Na^+ and loss of skin K^+, as well as a decline in plasma

aldosterone and an increase in plasma prolactin (Collier *et al.*, 1982). Thermal stress alters dietary protein utilization and body protein metabolism (Ames *et al.*, 1980), and ultimately the body immune system is also affected.

In some areas, climate change could also generate new transmission models. Livestock productivity is reported as being severely affected by vector-borne livestock diseases that are known to be climate sensitive (Ford and Katondo, 1977). The direct effects of climate change could translate into the increased spread of existing vector-borne diseases and macroparasites, accompanied by the emergence and circulation of new diseases (also see Chapter 3, Section I, this volume).

Many species that are already vulnerable are likely to become extinct. Species with limited climatic ranges and/or with limited geographical opportunities (e.g. mountaintop species, species on islands) and species with restricted habitat requirements and/or small populations are typically the most vulnerable. Changes in the frequency, intensity, extent and locations of climatically and non-climatically induced disturbances will affect the way and rate of replacement of existing ecosystems by new plant and animal assemblages. All the species in an ecosystem are unlikely to migrate at the same rates; long-lived species will persist longer in their original habitats, leading to new plant and animal assemblages. Many ecosystems will be dominated by opportunistic 'weedy' species, i.e. species well adapted to dispersal and rapid establishment, especially if the frequency and intensity of disturbance is high.

Alteration of micro–macro environments due to climate change may offer differential opportunity for various pathogens/pests/parasites to grow at varying rates. The incidences of various diseases may also be altered due to a change in virulence level by genetic shift and drift. There may be an emergence of new diseases due to the altered combination of genetic material of pathogens. The severity of losses by disease may also be altered due to altered susceptibility as well as change in virulence level. There is an indirect effect to health due to changed socio-economic factors in the climate change scenario, which may result in some of the species/breeds becoming more vulnerable/susceptible than others. This also affects biodiversity naturally.

11.6 Mitigation/Adaptation Strategies

Adaptation activities must include projects specifically designed to preserve ecosystems and the biological diversity on which we rely (Pankaj and Ramana, 2013). Sharma and Rai (2012) emphasize that traditional ecological knowledge, agrobiodiversity, multiple land uses and diversification of livelihoods allow local communities to cope with changes, as each of them play an important role in true adaptation to climate change.

11.6.1 Breeding strategies

To exploit the potential of indigenous local germplasm efficiently and to be sustainable, simple strategies with long-term vision are required. An important consideration for a breeding programme to be successful is that it shall somehow be flexible and responsive to variable factors. Designing a breeding programme is more than just increased productivity. It should be integrated with infrastructure, community development, improved livelihood and biodiversity, agricultural development policy, environment, production system and market. Similarly, required integration of all activities, adequate training of personnel and incentives to run the breeding programme successfully are often ignored. Across species, the successful use of temperate breeds for 'upgrading' has been mostly successful in peri-urban areas, in some highlands and in maritime climates. There is no better way of conserving a breed for future use than keeping it viable commercially or culturally through a demand-driven and efficient long-term breeding programme.

Changing the breeding animal every 2–3 years (male animal exchange from another

district herd) or artificial insemination with proven breed semen will not only help in enhancing productivity (Pankaj *et al*., 2013) but also in maintaining heterogeneity. This may be supplemented with the supply of superior males through the formation of a nucleus herd at village level. Synchronization of the breeding period depending on the availability of feed and fodder resources results in healthy offspring and better weight gain. Concentrate mixture supplementation to breeding animals should be initiated at least 1 month before the breeding season and continued until weaning. Local climate resilient breeds of moderate productivity should be promoted over susceptible cross-breds.

In developing countries with small herd sizes, large fluctuations in rearing conditions and management between herds, and over time within a herd, lack of systematic livestock identification, inadequate recording of livestock performances and pedigrees, constraints related to the subsistence nature of livestock rearing, the accuracy of selection will be much lower, resulting in even lower rates of genetic gain. However, locally adapted breeds are likely to be highly variable, and the highest performing animals of such breeds shall have great productive potential. Therefore, the screening of livestock populations previously not subjected to systematic selection is likely to give quicker results to provide a high genetic merit foundation stock for nucleus flocks.

Cross-breeding with a more productive breed can yield faster improvement. However, an appropriate improver breed has to be available that will adapt to the conditions where it is to be introduced. If it is a completely new introduction, then it is better to conduct a trial and monitor the performance of crosses for a few years before undertaking a large-scale cross-breeding programme. There are many other considerations such as ensuring the supply of good-quality animals of the improver breed, the livestock keeper's acceptance of the improver germplasm, a distribution strategy for breeding males or semen, and a strategy for maintenance of the local breed, so that it is not displaced completely by the crosses. Financial consideration is also important, because it could be expensive to import the improver breed.

Improvements in the components of livestock production other than genetics are more effective and successful in improving incomes and livelihoods if they are accompanied by genetic upgradation. One important consideration for breeding in the changed climate is whether to match the genotypes with the environment, or the other way round. There are two main approaches: one is modifying the environment, making it less harsh, and the other is to select stock that is most adaptable to the prevailing conditions. In most traditional livestock production systems of developing countries, it has been experienced that the existing levels of management, nutrition and veterinary care are not sufficient to support the production potential of improved breeds. Further, the extent to which variables like climate, disease, pasture and associated nutrition are modifiable will determine the approach. Cost–benefit analysis and the application of both avenues as per requirement should be the norm. For this purpose, a thorough understanding of the genetic constitution of the animal population and how it interacts with the environment is required. Since not many determinants of the environment can be changed in a low-input tropical production system, the identification of a production system and finding the best match for it will be the most appropriate choice Prima facie, utilization of the best locally available and adapted genotypes along with improvements in environment to the extent feasible and economical, is going to be the choice. For this, the most productive and adapted animals for each environment need to be identified for breeding purposes.

It is debatable whether to select animals for important traits, for example production or reproduction, along with traits adaptive to harsh environments. It is evident that physiological adaptability is expressed on performance; hence, the question arises whether the selection of an animal on the basis of performance in a given environment (stressors) alone will be

sufficient or not. Favourable correlation suggests that if we place major importance on performance traits (e.g. reproduction, growth, etc.) in stressful environments, adaptability traits will not be compromised and will lead to the selection of the most favourable animals. Addition of adaptive traits as such might not be that rewarding, due to the following reasons: first, estimates of the heritability of adaptability traits in most of the population are not available; and second, since genetic correlation between adaptive and productive traits is normally not very high, genetic progress for individual traits may slow down with the inclusion of additional traits. Regarding designing a sustainable breeding programme, it must be set in relation to the resources available and the stage of development in the action area concerned. It also needs to be integrated with farmer participation. The design may vary depending on the breed, the production system, or other circumstances.

The breeding objective at the micro level will be determined by the relative importance assigned to different traits in the given production environment, while at the macro level, it will be determined by the agricultural development policy, market, production system and output required from the system in the locality, region or the country over the longer term. While the long-term goals determine the breeding objectives and relative importance of each trait, the short-term benefits for farmers must be considered in order to confirm farmer participation. It might be difficult to value precisely in economic terms the change in all the desired traits; fundamental traits must always be considered in the selection programme, for example through independent culling or other appropriate methods, if the indexing method does not work. Special care must be taken to give due importance to fitness and adaptive traits.

The choice of breeding method, i.e. pure breeding or cross-breeding, is the most important decision to be undertaken during the design of the breeding programme. The important factors that will determine the breeding programme are the performance level and potential of genetic improvement in indigenous breeds through selection, the availability of alternate breeds for cross-breeding, along with their performance and adaptability, heterosis for major traits, the availability and feasible expansion of infrastructures to maintain two or more pure breeds for a long-term cross-breeding programme, cost–benefit analysis of cross-breeding over within-breed selection, or whether a synthetic breed would be a better alternative to a pure-bred or cross-bred.

11.6.2 Conservation strategies

Conservation of biodiversity among AnGRs will bring species-specific understanding within their boundaries, especially those which are rare or endangered or that are characteristic of the region, ecosystem, or location. Active consideration for management of conservation biodiversity in a changing climatic scenario will help us to regain the gene pool of AnGRs.

In situ conservation strategies

Conservation of AnGRs in the local climate or the climate as near to its native breeding tract is referred to as *in situ* conservation. The strategy for *in situ* conservation includes:

- proper surveying and monitoring of AnGRs in the native tract of the animals
- development of an organized herd/farm in the farmer's field
- development of an organized breeding farm by a government organization
- formation of a breeders' society
- implementation of a herd registration scheme.

Ex situ conservation strategies

This is the conservation strategy for maintaining the animal gene pool away from its breeding tract. *Ex situ* conservation strategies should be developed for the endangered or near-to-extinct group of animals by following two methods:

1. *In vivo* conservation strategy refers to maintaining fewer numbers of live animals away from their breeding tract. However, this is mostly followed in the case of wild animal conservation.
2. *In vitro* conservation is a type of *ex situ* conservation strategy wherein the genetic material of animals is kept in a frozen condition for a longer period. Long-term preservation of a gene pool in the form of frozen semen and cryopreserved oocyte or embryo is the major strategy of *in vitro* conservation. The development of a DNA data bank or a c-DNA library of different AnGRs will also help to conserve the vital gene pool.

11.6.3 Production management strategies

The number of animals being raised for human consumption also poses a threat to the earth's biodiversity. Livestock account for about 20% of the total terrestrial animal biomass, and the land area they now occupy was once habitat for wildlife. Of the 825 terrestrial ecoregions, 306 have been identified by the Worldwide Fund for Nature as under 'current threat', while 23 of Conservation International's 35 'global hotspots for biodiversity' are characterized by serious levels of habitat loss. In the following section, the means of maintaining biodiversity through production management strategies are discussed.

Agroforestry systems have the potential to sequester carbon and can reduce soil erosion, moderate climate extremes on crops, improve water quality and provide alternative income and fuel to local people (see also the preceding chapter on carbon sequestration). Agroforestry incorporates trees and shrubs into agricultural lands, to achieve conservation and economic goals while keeping the land in agricultural production. Agroforestry can increase biodiversity greatly, especially in landscapes dominated by annual crops or lands that have been degraded. Plantings can be used to link the forest fragments and other critical habitat functionally as part of a broad landscape management strategy.

Improved management of grasslands (e.g. grazing management, protected grasslands, grassland productivity improvements, etc.) can enhance carbon storage in soils and vegetation, while conserving biodiversity. Productivity, and thus the potential for carbon sequestration of such lands, is restricted mainly by the availability of water, nitrogen and other nutrients, and the unsuitability of some native species to high-intensity grazing by livestock.

Farmers have evolved farming systems and strategies that are compatible with specific resource features of ecosystems over millennia. Common property resources (CPRs), mainly community lands under forests and grasslands, have been central to these strategies. Farmer's access to and control over CPRs, which are often exceptionally rich in natural biodiversity, makes them resource rich and ensures the survival and sustainability of their systems and life. Uncultivated CPR lands provide a natural subsidy to farmlands via the agency of livestock. They provide fodder to livestock, due to which no proportion of cultivated area has to be earmarked for fodder cultivation. Almost all fuelwood is extracted from CPR areas, and in the absence of CPR, livestock dung could be used as a fuel for cooking purposes. Ecological niches full of exceptionally high-value biodiversity, including those in CPRs, and genetic resources in farmlands could be the basis of ecosystem sustainability.

The biophysical resource base of land, soil, water sources, forest ecosystems and agrobiodiversity are basic to the sustainability of food production and livelihoods in rural areas. The forest:cropland ratio must be as wide as possible in order to provide a sound ecological balance and a natural subsidy to farmland, and must be at least five times larger than the cropland area (Singh, 2004). Forest ecosystems infuse vitality into the whole food production system by maintaining strong organic linkages among livestock, cropland and humans. Living soil, one of the basic capitals of sustainable agriculture, is the product of forest ecosystems.

Continuous biomass flow from the forest (bedding material in animal sheds and fodder being converted into manure, for example) enriches the soil ecosystem, a process that supports biodiversity cultivation and manifests into crop production. Forests play a crucial role in nature's hydrological cycle, and thus water sources in the mountains are also largely a product of the forests. The flow of water and moisture circulation within a farming system is vital for the sustenance of all forms of life. The availability of water in the system also ensures a higher level of food production.

Forest areas in the mountains are also a source of high-quality food. These uncultivated areas, in fact, have the potential to provide more quality food per unit area on a sustained basis than cultivated land. People in the mountains have been obtaining food (edible fruits, flowers, buds, seeds, vegetables, mushrooms, honey, etc.) from the forests for centuries. To manage uncultivated areas for food, CPRs should be in our focus. CPRs are a richer source of food than cultivated land, and obtaining food from uncultivated land/CPRs is dependent on a more stable system than that of the more vulnerable cultivated land.

Farmers rear all types of animals including draught, carry pack, milch, wool animals, meat animals, poultry, etc., that are suitable for farming systems. They achieve functional diversity through combining complementary plant and animal species in synergetic interactions, injecting sustainability into agroecosystems. Livestock form the core of the livelihood systems of livestock-dependent marginal communities. Livestock is farmers' best companion by serving them as manure providers, exploiters of wastes, sources of power, forms of investments, and in many more ways. They form the cultural identity of people. By transferring nutrients from forest ecosystem to farmlands and maintaining the cyclic flow pattern in the farming system, they contribute to the ecological integrity of the system. A high degree of biodiversity creates barriers against any natural calamity and pest epidemics. A CPR centred farming system is inevitably a sustainable farming system (Singh, 2004).

The introduction of nitrogen-fixing legumes and high-productivity grasses or additions of fertilizer can increase biomass production and soil carbon pools, but can decrease biodiversity. The introduction of exotic nitrogen fixers poses the risk of them becoming invasive. Irrespective of whether a grazing land is intensively managed or strictly protected, carbon accumulation can be enhanced through improved practices, especially if native species are properly managed to enhance the biodiversity associated with the system. Examples of activities that promote the mitigation of or adaptation to climate change include:

- maintaining and restoring native ecosystems
- protecting and enhancing ecosystem services
- managing habitats for endangered species
- creating refuges and buffer zones
- establishing networks of terrestrial, freshwater and marine protected areas that take into account projected changes in climate.

11.6.4 Feeding management strategy

Feeding management practices also offer opportunities for alteration in trying to minimize the effects of heat stress. Some of the key considerations in this area are:

- Fresh, palatable, high-quality feed should be in the feed bunk at all times to provide the maximum opportunity for feed consumption. If the feed in the bunk is warm, musty or spoiled, it needs to be removed and discarded.
- Feed should be provided within a reachable distance of the animal, so that animal can enjoy feeding.
- Uniform mixing and regular delivery by feed staff should be assured.
- Feed should be distributed in such a manner so that all the animals reach the feeding area.

- Avoid wastage of feed. The addition of water or molasses will help the feed to stick together better and thus reduce wastage.
- It may be useful to shift feeding times to match animal behaviour. Usually, animals tend to change meal patterns and eat more feed during the cooler times of the day.

11.6.5 Health management strategies

There is a need to develop a comprehensive plan (e.g. health, disaster reduction) to deal with the migration of disease due to climate change. Apart from this, there should be positive animal welfare contingency plans to control zoonoses caused by climate change. Prophylactic vaccinations should be used as a control measure in appropriate regions where disease is endemic. There is a need to improve biosecurity at animal production sites, and the transportation of live animals should be restricted.

Increased prevalence of endemic diseases due to malnutrition is becoming a potential threat to the profitability and diversity of livestock farming during summer by lowering production and income. Hence, preventive vaccination against endemic diseases, especially Peste des Petits Ruminants (PPR), Foot and Mouth Disease (FMD), Anthrax, Haemorrhagic Septicaemia (HS) and Black Quarter (BQ), should be given to susceptible animals. An increase in humidity along with temperature would result in a higher internal worm burden; hence, de-worming should be planned accordingly. Continuous rains may lead to more vector populations and outbreaks of diseases like bluetongue, and this needs proper hygiene and sanitation measures in sheds and the surroundings. Small ruminants have every chance of intoxication due to the consumption of toxic plants, which are green during summer/famine, and also feeding on spoiled/fungal feed ingredients. These require proper care of animals during famine conditions. In case of severe heat stroke, oral rehydration therapy should be advocated, along with feeding gruel mixed with molasses and salt. Mapping animal disease outbreaks through advanced technology like GIS and providing updated information related to small ruminant management and health aspects through mass communication systems in villages will facilitate the knowledge empowerment of stakeholders and containment of animal diseases in rural areas.

11.6.6 Capacity building of livestock owners

The adoption of a multi-pronged approach for capacity building of farmers focusing on the supply, production and marketing chain will make the best utilization of the livestock sector, with efficient resource use. The following agenda should be considered for proper capacity building among livestock keepers:

- Assessment of existing laws and regulations influencing animal genetic resources and their implementation in conjunction with environmental sustainability.
- Increasing awareness and acceptability of the animal husbandry sector to larger audience beyond traditional rearers.
- Involvement of government, public and private sector organizations, including the banking sector.
- Establishment of educational institutions of veterinary science and livestock management.
- Increasing and upgrading the knowledge and skills of the stakeholders.
- Strengthening the existing stakeholder associations and establish the institutions that are missing.

11.6.7 Development of appropriate policy

Initially, there is a need to monitor climate change through meteorological changes, which induce biological changes, ending with sociocultural changes. Based on this, there is a need to develop climate scenarios at the regional scale. Locally available coping and adaptive capacity and resources need to

be estimated, and vulnerability at the district or village level needs to be assessed, which will be helpful in designing interventions. These developed interventions need to be implemented and evaluated on a pilot basis. After phasing and testing all of these steps, policy adjustments for the region can be advocated.

11.7 Conclusion and Future Direction

The impact of climate change on the livestock sector as a whole will be felt more in tropical countries compared to temperate countries, largely because of the structure of production systems and economics. Fundamentally, climate change or variability will influence the production (growth, meat and milk yield and quality, egg yield, weight and quality, wool production and quality, etc.) and reproductive performance, metabolic and health status and immune response owing to individual acclimation and acclimatization of animals to a specific range of environmental variables. The resultant pressure, both direct and indirect, is likely to result in further dilution of livestock diversity, which would especially affect the nutritional security and livelihood of small and marginal farmers. Arrest of the dilution of livestock diversity will depend on many interlinked factors, including conservation. The success of conservation measures may only be realized in actual scale when it is worked out in association with the social and economic viability of the breed or species of livestock concerned.

An important prerequisite for the consideration of conservation measures is characterization of the livestock under threat to its fullest term. While formulating conservation measures, whether the design is in synchronization with natural selection should also be looked at. It is widely known that livestock of tropical countries are to a large extent under-characterized. Linking under-characterized livestock and its production system to the mainstream will require substantial research. While the medium- and long-term objectives of maintaining livestock diversity will depend largely on government and institutional players through appropriate policies and support, the short- and to some extent medium-term objective may need to be addressed by the livestock rearers, their societies and different non-governmental organizations. The formation of livestock breed societies may be a key determinant of conservation success. The practice of selective breeding in a livestock breed (under threat) for the production environment(s) under which it is to perform will be important. Otherwise, G × E interaction is required to be involved in the evaluation of genetic merit. The management of pasture and range, along with the necessary policy support for its renewal and sanctity, will be tremendously important for a conservation scheme. The animal husbandry sector will, on the whole, require more budgetary support beyond veterinary care than at present, to keep it relevant in the tropics. The knowledge gap about the livestock breeds that need to be conserved, their required number for conservation and mode of conservation at national and regional level while maintaining their economic viability and resource use efficiency, will be the major research challenges for the scientific community.

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12 Climate Change: Effects on Animal Reproduction

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Abstract

The amounts of greenhouse gases in the atmosphere have been increased as a result of human activity, causing rise in climatic temperature. In recent times, climate has been changing faster than ever; as a result, plants and animals are exposed to more adverse conditions and are finding it difficult to adjust in temperate and tropical regions. The existence of some animals and plants is threatened. Threat to existence is due mostly to low or no reproduction. Photoperiodic action is mediated through the hypothalamus; however, nutrition and stress affect the entire hypothalamus–pituitary and gonadal axis of both male and female systems. The effects are: aberrant gametogenesis, folliculogenesis and ovulation, reduced male and female sexual behaviour, low conception rates, increased embryo and pregnancy loss, delayed post-partum recovery, increased calving intervals, lowered perinatal vigour and increased perinatal mortality and morbidity, etc. These losses are difficult to recognize and diagnose, and the consequence is expensive maintenance of animals with reduced reproductive efficiency. Low productive–reproductive performance of animals after birth is related to the epigenetic changes in the maternal womb due to nutritional deficiency and exposure to stressors. Therefore, animal farming activities are facing, and will continue to face, a tough challenge until some steps are taken to counteract these anticipated damages. Slow adoptive animals would be endangered, as they will face more problems with successful reproduction. It needs a coordinated effort for faster identification of allelic variation of important genes and transfer of adoptive variability to existing animal population by cross-breeding or any other suitable breeding policy. This chapter describes in detail the climatic factors, the mechanisms of their effect and the way forward to counter these effects.

12.1 Introduction

Human activity has increased the amount of greenhouse gases in the atmosphere and the climate is changing faster than ever. The world's temperature is set to rise as global warming traps the sun's heat in our atmosphere due to the presence of greenhouse gases, namely methane, nitrous oxide, halocarbon, carbon dioxide, etc., and water vapour. The predicted consequences are melting of ice caps, rise in sea level and sinking of low-lying countries. It is said that the world's climate is repeating its 4.5 billion years of history when it was hot. It is predicted that we could experience an average global temperature rise of 3.5°C within the next 100 years. It is unlikely that it would lead to a major catastrophe for plants, animals, humans, weather and habitats. But with this kind of rapid change,

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plants and animals would need to adapt to new conditions quickly or otherwise would face possible extinction if they do not evolve new strategies for survival and reproduction. Survival and reproduction depend on how well an individual is adapted to local climate patterns. The climate change can disrupt the match between organisms and their local environment, reducing survival and reproduction and causing subsequent impacts on populations' or species' distribution across geographic regions. Climate change may benefit some species and cause extinction for others. Cumulatively, it will alter biological communities and the functioning of ecosystems. The earth is already experiencing sufficient climate change to affect biological systems; well-documented changes in plant and animal populations are related to recent climate change.

Migratory birds adapt quickly to changing climate by moving from one place to another. But most species of tropical birds are not migratory and stay in one small area year-round; they will therefore have to face great challenges ahead. Birds living at high altitude will also face challenges as they migrate higher where breathable oxygen is low, which might not suit their physiology. Even if they survive, they would struggle for food and with their metabolism, thus affecting reproduction. For other ground-dwelling and domesticated animals, the obstacles might be even greater, as they do not migrate. Threats to reproduction could lead to the extinction of a whole species; therefore, it is of paramount importance to assess the impact of climate change on reproduction, as it is of great concern to wild aquatic birds or farm animal species. It is said that reproduction is a luxury of the body system, meaning that if everything goes well, reproduction will occur. There are reports suggesting that climate change contributed to the defects in cheetah (*Acinonyx jubatus*) sperm. It has been observed that many cheetahs (*A. jubatus*) have abnormal coils in the sperm tail and low sperm counts, as well as extremely low testosterone levels (The Guardian, 2012). This would contribute to the infertility problem, might be one of the reasons for the reduced big cat population in the world. Climate changes might affect reproduction directly by affecting the function of male or female organs or by blocking the hormone-mediated cellular functions of the hyopthalamo–pituitary–gonadal (HPG) axis, mostly through heat stress and nutrient deficiency. The general deterioration of health due to climate change would also lead to compromise in immunity, so disease susceptibility will increase, which would further affect reproduction. Changing climate would lead to changes in seasonality that ultimately will affect the seasonal breeding pattern in animals. There might be an increase in the duration of seasonal anoestrous cases if no stress ameliorative measures are taken.

12.2 Changing Climate Scenario

Prediction of the effect of global warming through computer simulation-based modelling (Huang *et al*., 2013) indicated that there would be increased rainfall in the current high-rain regions; warm regions would get more rain at a specific time point but have reduced average annual rainfall; there will be increased mean annual temperature and increased atmospheric carbon dioxide concentrations. The frequency of extreme events such as flood and drought will increase/change the distribution of pests and weeds. All of these singly or in combination would impact agricultural and forage production, on which animals are dependent for nutrition and survival. The availability of animal feeds and fodders will be affected directly due to their being in short supply as a result of natural calamities and crop failure. In addition, the quality of feed and fodder will decline, with varying levels of energy and protein due to crop stress. Inclusive information on current feed demand, regional estimates of feed use, climate change effects on feed resources quality and projections for 2030 is given in Chapter 2, Section I, this volume.

Animals in tropical countries will be exposed to heat stress during hot summer due to high temperature humidity index (THI; THI = t_{db} + 0.36 t_{dp} + 41.5), where t_{db} is dry-bulb temperature in °C; t_{dp} represents the dew point temperature in °C. A THI score above 70 is considered stressful for *Bos taurus* cattle, and climate change in tropical countries is expected to expose livestock to a THI of 70–80 with increased duration. For example, in the northern plains of India, days with THI >80 (severe heat stress) occur predominantly from May to August (Upadhyay *et al*., 2012). At present, the total number of days with >80 THI is about 40 in a 1-year period. The maximum temperature rise during May–August for the time slice 2079–2099 is projected at between 3.07 and 4.42°C, with an unlikely change in precipitation (Ruosteenoja *et al*., 2003). This will lead to a rise in the number of uncomfortable days for livestock from 40 days (10.9%) to 104 days (160% increase) in the northern plains of India. In heat stress, the body directs the flow of blood from the core to the periphery, which facilitates heat loss by evaporation and sweating. Thus, reproductive organs and the brain, which control reproductive functions, are expected to receive less blood supply, leading to energy, protein and micronutrient deficiency in these organs. Also, there will be a lack of appetite during the compensatory adjustment of reducing metabolic heat, which again would lead to nutritional deficiency. In the climate change scenario, a negative energy balance is certain to occur in both wild and domestic animals. It is therefore pertinent to understand where energy is prioritized in the case of a negative energy balance.

12.2.1 Energy partitioning in negative energy balance

Animals have to adjust their energetic priorities due to the deficiency of oxidizable metabolic fuel (glucose), so that the body functions essential for living are maintained. The most prioritized activities are basic cell and organ functions, flow of blood circulation, neural activities and homeostasis. The medium prioritized activities are thermoregulation, physical activities, production and maintaining body growth. As reproduction and the storage functions become secondary, being the least prioritized activity of the body, reproduction would, therefore, be affected in such cases (Fig 12.1).

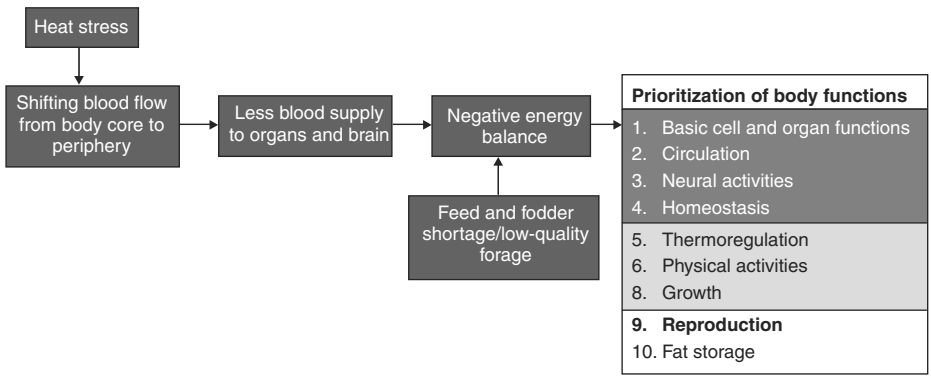

Fig. 12.1. Negative energy balance caused by heat stress and feed and fodder scarcity due to climate change leading to prioritization of body functions. The body functions are classed here in three orders of priority (highest, medium and lowest) and kept in three differently shaded boxes. The dark shade indicates the highest priority and the lightest shade indicates the lowest priority body functions. Reproduction falls in the lowest priority body function.

12.3 Climatic Factors and Regulatory Mechanisms of Reproduction

The entire process of reproduction, namely gamete development, production, fertilization, hatching, maternal signalling, implantation, maintenance of gestation and successful live birth at delivery, happens only after attainment of puberty and sexual maturity. The production of sperm is regulated by the HPG axis. The hypothalamic gonadotropin-releasing hormone (GnRH) causes release of gonadotrophins luteinizing hormone (LH) and follicle stimulating hormone (FSH) from the pituitary gland. The LH acts on the Leydig cells to synthesize testosterone, whereas FSH converts testosterone to oestrogen in Sertoli cells. Sertoli cells also secrete inhibin, which controls FSH secretion by negative feedback control (Fig 12.2). Sperm cells are formed with the help of Sertoli cells under the influence of testosterone and the direct effect of oestrogen on the germ cells at the terminal differentiation step. The role of the male in reproduction is restricted only to ensuring the production of the sperm (male gamete) and depositing it into the female tract at the right time of the cycle coinciding with ovulation. The rest of the reproductive process happens in the female reproductive tract, where control is more composite.

In the female, the oocytes (gametes) are stored at the arrested meotic prophase stage (diplotene of prophase) in resting follicles called primordial follicles. The primordial follicles develop from preantral to antral (cavity) and then to the pre-ovulatory stage with the help of local and gonadotropic hormone support. Preantral

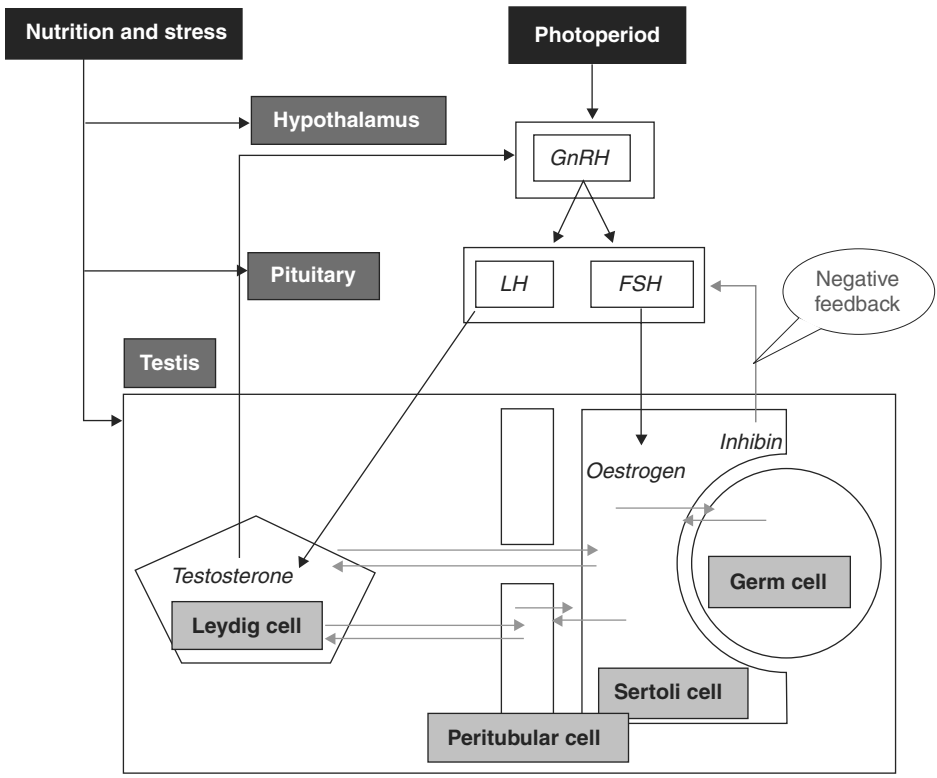

Fig. 12.2. Male gamete production under the influence of climatic factors.

follicle development occurs at any point of time in life, whereas antral follicle development and ovulation occur only after puberty, when the gonadotropin LH and FSH secretions are regular. The antral follicles have theca cells on which LH acts to synthesize testosterone from cholesterol and granulosa cells, which converts testosterone to oestrogen by FSH action. Granulosa cells also synthesize inhibin for negative control on FSH secretion from the pituitary. The oestrogen then reaches a threshold, acts on the hypothalamus to release a huge amount of LH, called a surge release. This only acts on preovulatory follicles (>10 mm diameter in cattle and buffalo), causing the release of oocytes by the process called 'ovulation' (Fig. 12.3).

Male and female gametes are transported to the site of fertilization (the amulla–isthmus junction of the oviduct) through the female reproductive tract. After ovulation, the oocytes are picked up by the infundibulum and transported through the ampulla of the oviduct, whereas the sperms, when deposited, are transported through the entire uterus and then through the isthmus of the oviduct to the site of fertilization. After fertilization, the developing embryos are transported to the uterus through the isthmus. Contraction and relaxation of the oviduct muscle and the movement of epithelial cell cilia under the influence of prostaglandin (PG) E_2 and $F_{2\alpha}$ control the transport of the gametes and fertilized embryo to the uterus (Wijayagunawardane *et al.*, 2001). The uterus takes care of the remaining part of gestation, starting from hatching, early embryonic development, establishment of communication with the conceptus, growth and development of the whole conceptus and finally delivery at term. The male and female gonads produce the steroid hormones essential for the maintenance of other reproductive tract functions.

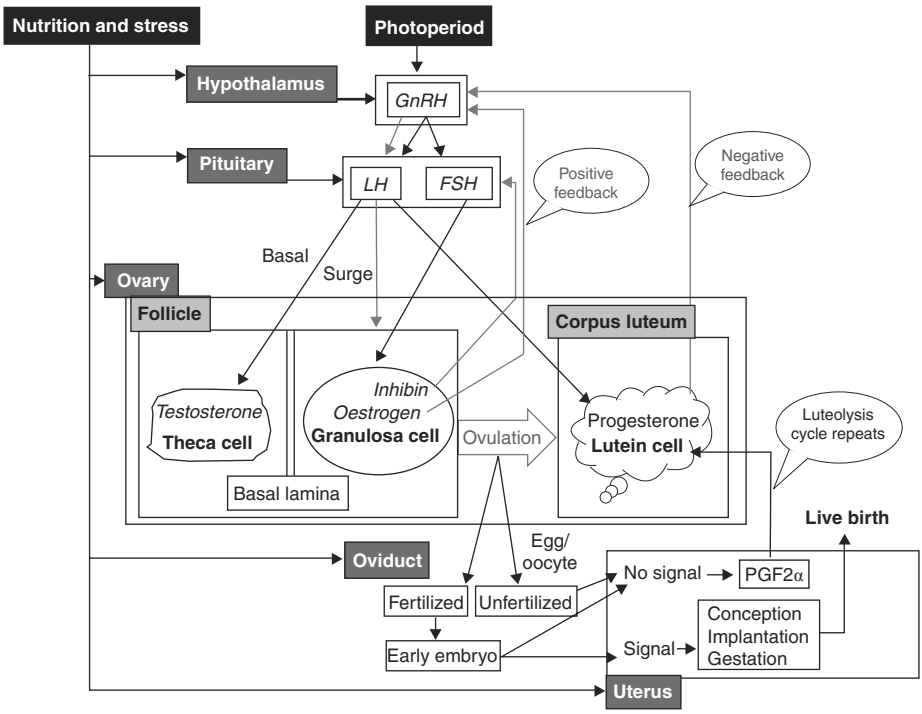

Fig. 12.3. Female reproduction under the influence of climatic factors.

The intervening climatic factors such as nutrition, stress and photoperiod affect the entire process of reproduction negatively. These factors adversely affect gamete production in both males and females. They also alter the events of pre-puberty, post-puberty, gamete transport, fertilization, early embryonic development, embryo signalling, implantation, gestation, delivery and post-partum recovery. The photoperiod is important in a temperate-zone climate, and exerts its effect through the hypothalamus, whereas nutrition and stresses are important in a tropical-zone climate, where they act in all the organs of the HPG axis. The intervention points of the photoperiod, nutrition and stress in the entire axis are shown in Fig. 12.2 for males and Fig. 12.3 for females. The anticipated effects of climate change on reproduction would be delayed attainment of puberty, defect in gametogenesis, aberrant expression of sexual behaviour, fertilization failure, early embryonic loss, failure to signal mother, failure of implantation, intrauterine growth retardation of conceptus, premature termination of pregnancy, problem in delivery and delayed post-partum recovery. The effects of climate change on the reproductive performance of animals in temperate and tropical regions are presented in Fig. 12.4. All of these reproductive problems are known to occur in animals; however, the incidence will increase as a result of climate change, culminating in low reproductive efficiency of animals. Hypothalamic

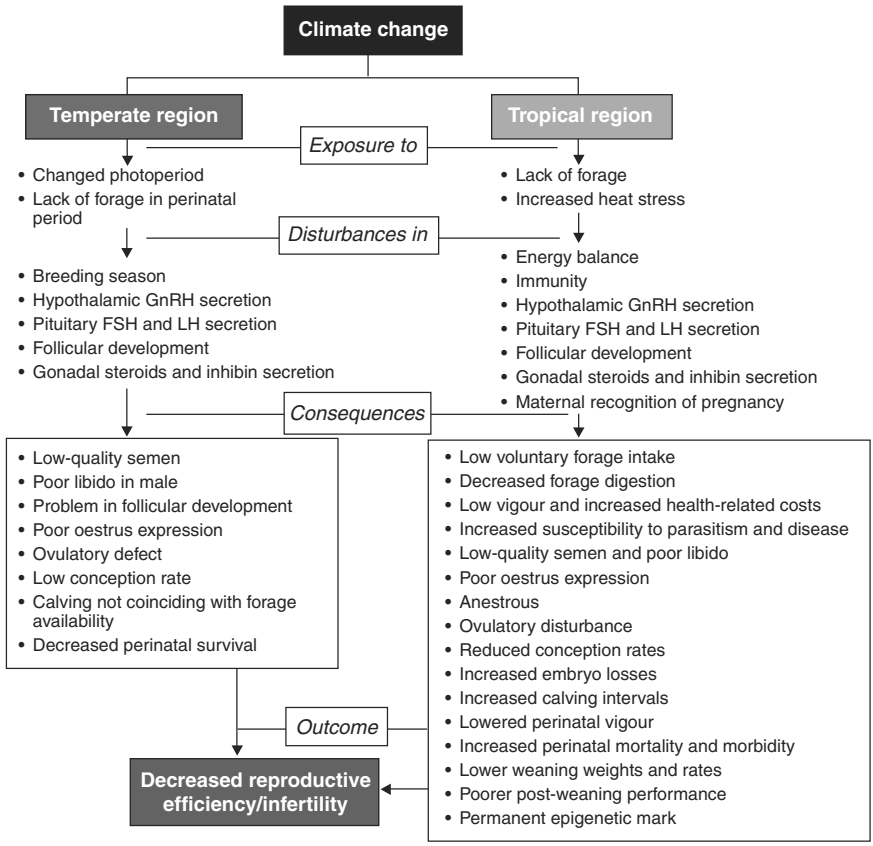

Fig. 12.4. Effects of climate change on animal reproduction in two main climatic regions.

gonadotropin-releasing hormone (GnRH) is the key to controlling the release of pituitary gonadotropins (LH and FSH). These two hormones act on the gonads, ensuring gametogenesis and steroid synthesis, essential for reproduction and maintenance of reproductive tract functions. The following section enumerates how GnRH release is influenced by climatic factors.

12.3.1 Climatic factors on GnRH release

GnRH is released under the direct influences and feedback control of the hypothalamo–pituitary–adrenal (HPA) and HPG axes, and is controlled by several other factors, which influence both HPA and HPG axes. Activation of the HPA axis would lead to the release of corticosteroids under the influence of the corticotrophin-releasing hormone (CRH). The neurons that release CRH directly inhibit GnRH neuron activity. Any activities or conditions such as undernutrition due to deficient feeding, excessive energy expenditure, diabetes mellitus and heat stress limit the availability of oxidizable metabolic fuels (glucose) in circulation and can inhibit the release of GnRH, either indirectly through leptin and hind-brain fuel detectors (Wade and Jones, 2004) or directly through action on the GnRH neuron (Zhang *et al*., 2007). The indirect signals are carried to the GnRH neuron via the area postrema and the mediobasal hypothalamus. POMC (pro-opiomellanocortin), neuro peptide-Y (NPY) and catecholamine (CA) neurons are located in this region. These neurons project directly on to the GnRH neuron and modulate GnRH neuron activity through μ-opioid and NPY-5 receptors, reducing GnRH pulse frequency (Zheng *et al*., 2005). The hind-brain fuel detector may also inhibit GnRH secretion indirectly via CRH neuron stimulation (Wade and Jones, 2004). The direct effect of glucose is exerted on the GnRH neuron of the organum vasculosum of the lamina terminalis area, where there is a leaky blood–brain barrier due to the anatomical location. The GnRH neurons have classical glucose responsive mechanism because of the presence of glucokinase enzyme in the cell. The K_{ATP} channel-mediated GnRH neuron firing activity increases with a high glucose level and decreases with a low glucose level (Zhang *et al*., 2007). The GnRH neurons are responsive to multiple neural and humoral signals originating from different metabolic states and physiological conditions, indicating that overall control of GnRH secretion is complex. The signals of energy imbalance might come to the brain from liver, pancreas, stomach, duodenum and adipose tissues through vagus nerves or by hormones such as leptin, insulin, insulin-like growth factor 1 and ghrelin. These hormones could act directly on the neural circuits controlling the GnRH neuron or they could act by modulating the availability of metabolic fuel. Similarly, the other neuropeptides, galanin, orexin, urocortins and endogenous opioids of the fore brain also can regulate GnRH secretion (Bronson, 2009). Variation of the photoperiod is sensed through the olfactory bulb, and this ability is acquired by linking the retina to the GnRH-secreting neurons with melatonin hormone. The energy balance, singly or in combination with the photoperiod can control the release of GnRH, and thus control seasonal reproduction. The heat stress-related environmental temperature rise would also affect GnRH secretion by direct influences on the HPA and HPG axes. These effects would be reflected in the circulatory levels of gonadotropin (FSH and LH), inhibin and testosterone in the male and progesterone and oestrogen in the female.

12.4 Impact on Gonadotropin and Steroid Hormone

The impact of nutritional deficiency and heat stress on males and females is described separately to enable a better understanding on the individual effects of climatic factors.

12.4.1 Male

Low nutrition will have a serious impact on calfhood before 25 weeks of age, as it would

result in suppressed LH release, causing delayed puberty and reduced testicular development. This defect could not be corrected, even with a high nutritional regimen in later age (Barth *et al*., 2008). Under the low plane of nutrition, GnRH release is affected by direct or indirect action on the neuron. As a result, the LH and testosterone levels in circulation will be less. Studies of bulls and boars have shown that heat stress initially causes a decline in circulatory testosterone level lasting for about 2 weeks; however, it returns to normal in the case of continuing heat stress (Rhynes and Ewing, 1973; Wettemann and Desjardins, 1979). It has been shown in bulls that the LH level drops in serum after 6 days of exposure, but it remains unaffected in short-term exposure (Minton *et al*., 1981). No systemic data are available on the circulatory FSH and inhibin level in heat-stressed males of farm animal species. However, Steinberger (1991) reported that heat stress caused a 60% decrease in inhibin production in male rats, and as a result, the FSH level in circulation increased, which probably triggered Sertoli cells to protect them by eliminating cell-surface FSH receptors accountable for oestrogen deficiency and effect on spermatogenesis. The major disruption of male reproduction due to heat stress related to climate change and deprivation of nutrition would happen in the spermatogenic cell lineage mediated via testosterone and oestrogen deficiency.

12.4.2 Female

Nutritional deficiency affects steroid synthesis at the gonadal level both directly and indirectly via gonadotropin action. Restricted energy intake results in the suppression of LH release in pre-pubertal heifers, ending in delayed puberty and sexual maturity (Day *et al*., 1986). In post-pubertal animals, malnutrition affects the synthesis of progesterone by corpus luteum (CL) (Apgar *et al*., 1975), without affecting lifespan (Imakawa *et al*., 1983). However, energy deficiency does not affect oestrogen synthesis by follicular cells (Staigmiller *et al*., 1979). Feed-restricted cycling cattle showed high LH in circulation, and that might be due to the decreased negative feedback of less progesterone from CL. In more severe nutritional deficiency, cows enter into anoestrus, probably due to acquired hypersensitivity to oestrogen, so do not respond to the progestin–oestrogen treatment for oestrus induction (Imakawa *et al*., 1986). Feed-restricted cattle show greater inhibition of LH secretion in response to oestrogen implant. However, if animals are able to cycle normally and show the regular oestrus symptom, the pre-ovulatory LH surges are not affected for ovulation. The effect of nutrition on normal follicular development and ovulation is explained better in a cold-exposed domestic mice experiment. These mice do not ovulate in winter; however, when they are allowed to consume more feed to compensate the increased thermoregulatory demand, follicles develop and ovulate, and the animals reproduce even in low temperature (Manning and Bronson, 1990). In post-partum animals, feed restriction would delay the recovery period, as it delays follicular development, oestradiol release and ovulation (Henricks *et al*., 1986). Post-partum cows do not respond to the GnRH challenge in low nutrition.

Heat stress reduces steroid synthesis capacity in granulosa and theca cells of more than 0.5–1 mm diameter follicles in cattle (Roth *et al*., 2001a). It probably affects steroid synthesis by altering endocytosis, and selective membrane receptors mediated cholesterol uptake by the granulosa and theca cells (Argov *et al*., 2005). In heat stress, follicular responsiveness to gonadotropin action becomes reduced, as observed in goats (Kanai *et al*., 1995). In dominant follicles, elevated temperature reduces gonadotropin (LH/FSH) induced androstenedione and oestradiol production, and increases the progesterone production *in vitro* in bovine (Bridges *et al*., 2005). Such changes are indicative of premature differentiation/leutinization of dominant follicles, which might confer fertility disorder in animals during the summer season. Heat stress reduces LH (Wise *et al*.,

1988) and inhibin (Roth *et al*., 2000), but increases FSH in circulation (Roth *et al*., 2000). Such cows are found to have an increased number of small (2–<4 mm diameter) and medium-sized (4–<8 mm diameter) follicles on the ovary as a result of increased recruitment of antral follicles in the growing pool (Roth *et al*., 2000). High ambient temperature shortens the duration of oestrus, and symptom of oestrus expression in buffalo, along with lower circulating progesterone, oestradiol, LH and FSH during the summer than during the cooler period in India (Rao and Pandey, 1982, 1983; Madan and Prakash, 2007). A long duration of summer anoestrus in buffalo is attributed to the combined effects of low nutrition and high environmental temperature (Kaur and Arora, 1984). In severe heat stress, female animals turn infertile for a temporary period, termed as summer infertility. In Spain, where heat stress is frequent in the summer season, the proportion of inseminated dairy cows becoming pregnant during the warm months of the year was 22.1 versus 43.1% inseminated in the cool season (Lopez-Gatius, 2003). The magnitude of summer infertility is much lower in non-lactating heifers or cows producing low amounts of milk than in those producing more milk. This is because the high yielders are prone to develop negative energy balance as they direct their energy into milk production as a priority. In the climate change scenario, one would expect intensification of these activities in tropical countries.

12.5 Impact on Oocyte Function

Follicles developed during a heat stress period would not yield good-quality oocyte. One study in Israel had shown that resumption of fertility of lactating dairy cows could be hastened by removing the follicles formed in the summer season (Roth *et al*., 2001b). It is interesting to note that in normal conditions, the ovarian temperature is 1–1.5°C cooler than the rectal temperature, and the large antral follicles are a further 1–1.5°C cooler than the surrounding stroma (Grondahl *et al*., 1996; Hunter *et al*., 2000). No information is available on ovarian temperature variation during heat stress, so it is not known whether follicles are exposed to elevated temperature even in hyperthermia due to thermoregulation failure. Nevertheless, induction of heat stress during ovulation and oocyte maturation in mice (Baumgartner and Chrisman, 1988) and cattle (Putney *et al*., 1989) may or may not affect fertilizing ability, but the resultant embryos develop slowly or abnormally. There are several reports to suggest that elevated temperature during the pre-ovulatory period disrupts the process of oocyte maturation. The direct damage to the oocyte due to heat stress seems to be related to reactive oxygen species (ROS), as the adverse effects are reduced by the administration of antioxidants in mice (Roth *et al*., 2008). Heat stress generated ROS damage to oocytes through induced apoptosis, proved by inhibition of the apoptotic effect by caspase inhibitors, sphingosine-1-phosphate (Roth and Hansen, 2004) and tetrahydrobiopterin (BH4) peptide (Soto and Smith, 2009).

12.6 Impairment of Testicular Function

Testes in most animals are maintained at lower than body temperature by a separate thermoregulatory mechanism. This is controlled by the involvement of the pampiniform plexus (an arteriovenous plexus) of the spermatic cord, the tunica dartos muscle of the scrotum (regulates the scrotal surface area) and the cremaster muscle (controls the position of the scrotum relative to the body). Rise in testicular temperature leads to reduction in sperm output, decreased sperm motility and increased proportion of morphologically abnormal spermatozoa in the ejaculate. Spermatid and spermatocyte are the most susceptible to damage, followed by B-type spermatogonial cells (Setchell, 1998). The damage to the cells is due to oxidative stress-related apoptosis and DNA strand break (Paul *et al*., 2009). Testicular heating in

cattle decreases the proportion of progressively motile and live spermatozoa and increases the incidence of defective heads (Barth and Oko, 1989). A considerable animal-to-animal variation exists in the proportion of sperm defect in bulls. In testes, all stages of germ cells are very sensitive to damage by heat shock, in addition to the effect caused indirectly by impairment of Sertoli and Leydig cell functions. However, the degree of damage depends on the extent and duration of temperature exposure (Waites and Setchell, 1990). In rams, spermatocytes in meiotic prophase are killed by heat, whereas spermatozoa that are more mature usually have metabolic and structural abnormalities (Setchell *et al*., 1971). Spermatogonial (stem) cells have a remarkable capacity to repair even DNA damage very quickly, thus they tend to bring the changes to normalcy once heat stress is withdrawn. The interval from cessation of heating to restoration of normal spermatozoa in the ejaculate corresponds to the interval from the beginning of differentiation to ejaculation (Waites and Setchell, 1990). Spermatogenesis in bulls takes about 61 days; alteration in semen quality after heat exposure occurs in 2 weeks, and normalcy returns within 8 weeks once heat shock is withdrawn (Meyerhoeffer *et al*., 1985). The most worrying point is that sperm morphology returns to normal but fertilization rates decrease, with an increased incidence of embryonic death in rabbits (Burfening and Ulberg, 1968). As an immediate effect, elevated scrotal temperature affects fertility without change in semen quality, as the damaged spermatozoa enter the ejaculate much later. The ambient temperature of 40°C and relative humidity of 35–45% for as little as 12 h reduce semen quality in Holstein bulls (Skinner and Louw, 1966). *Bos taurus* is found more susceptible to heat stress than *Bos indicus*; thus, a decrease in semen quality is found less severe in the latter species (Skinner and Louw, 1966). Also, there is evidence that cross-bred (*B. indicus* × *B. taurus*) bulls recover more rapidly from heat shock than pure-bred *B. taurus* bulls when exposed to high ambient temperatures (Johnston *et al*., 1963). However, hyperthermia up to 40°C in the female tract does not affect the sperm's fertilizing ability and competence to develop blastocyst (Hendricks *et al*., 2009). Hendricks and Hansen (2009) showed that ejaculated bull and stallion spermatozoa did not undergo apoptosis, even though they were cultured at the physiological hyperthermia temperature. There have been reports that X- and Y-bearing spermatozoa behave differently to thermal stress. Perez-Crespo *et al*. (2008) showed that when female mice were bred with males exposed to scrotal heat treatment, more female pups were produced, indicating the effect on the skewing of sex ratio. In contrast, the *in vitro* fertilization experiment revealed that incubation of sperm at 40°C for 4 h compared to 38.5°C tended to reduce the proportion of female embryos (Hendricks *et al*., 2009).

12.7 Impairment of Oviduct and Uterine Environment

Climate change related heat stress and nutritional deficiency alter the oviduct and uterine environment, as well as the growth, and secretory activity of the conceptus. Heat stress and nutritional deficiency probably have more effect in the preimplantation period, when embryos are susceptible to damage, leading to failure of maternal recognition of pregnancy during implantation and mid-gestation, when angiogenesis is more extensive. This results in the loss of conception or compromised development of the fetus and placenta, leading to either in-between termination or continuance with the deficiency until term. In heat-stressed Holstein cows, increased prostaglandin E_2 (PGE_2) production in the ampullary oviduct is reported due to increased expressions of PGE synthases and *HSP90AA1*genes. Periods that activate cytosolic PGE synthase and upsets the normal PG synthesis, thereby reduces oviductal smooth muscle motility, which in turn decrease gamete/embryo transport through the oviduct (Kobayashi *et al*., 2013). Alteration of the uterine environment in heat-stressed cows between

days 8–17 of pregnancy has been reported by Geisert *et al*. (1988). They observed increased rectal temperature, reduced conceptus wet weight, increased protein and calcium content in the uterine horn ipsilateral to the CL, along with enhanced synthesis and secretion of trophoblastic protein in heat-stressed cows. Exposure of pregnant ewes to heat stress causes reduction in fetal and placental weights, and concentrations of placental hormones in blood (Wallace *et al*., 2005). There are reports suggesting that heat stress reduces the concentration of circulating progesterone. This is probably due to impaired CL function as an effect of the dominant follicles from which it is developed (Wolfenson *et al*., 2002). As a result, low progesterone would again alter the uterine environment. Thus, the effect of elevated temperature, or nutritional deficiency on embryonic survival, depends on both direct and indirect effect on the maternal system. However, embryos develop their own resistance and survival mechanism to counteract the threat.

12.7.1 Acquisition of heat-stress tolerance by conceptus

In lactating cows, Ealy *et al*. (1993) found that in cows exposed to heat stress at day 1 after oestrus/artificial insemination (one- to two-cell stage embryo), the proportion of blastocyst development was reduced, as compared to those stressed at days 3 (8–16 cells), 5 (morula) and 7 (blastocysts), meaning that *in vivo* embryos in a later stage of development are relatively more resistant to heat shock. Similar acquisition of thermal resistance is reported in sheep (Dutta, 1964) and pig (Tompkins *et al*., 1967). *In vitro* embryo culturing in cattle also showed the same trend of reducing the adverse effects of heat shock during the advanced stage of blastocyst development (Edwards and Hansen, 1997). However, mouse embryos remain sensitive to heat shock, even if they are exposed to heat shock in the late developmental stages (Arechiga and Hansen, 1998). The direct effect on the embryo is due to the generation of ROS in the oviduct and embryos, and to reduced glutathion content (Ozawa *et al*., 2002). There is a gender difference in terms of susceptibility, as female mice embryos are found to be less susceptible than their male counterparts due to less ROS production (Perez-Crespo *et al*., 2008). The increased resistance of the developing embryo is acquired due to the reduced level of ROS production and the increased availability of intracellular antioxidant glutathione (Lim *et al*., 1996). The acquisition of thermotolerance also involves capability of heat shock protein 70 (HSP70) synthesis, which in turn stabilizes intracellular proteins and organelles. HSP70 synthesis is noted as early as at the two-cell stage in cattle (Edwards and Hansen, 1996) and mice (Christians *et al*., 1997).

Maternal undernutrition reduces placental and fetal growth in both domestic animals and humans as an adaptation mechanism of survival under nutritional threat. In addition to energy, protein and micronutrient supply are most important during the peri-implantation period (early gestation) and during the period of rapid placental development (mid-gestation). The grazing animals are more susceptible to nutritional deficiencies. It has been observed that unsupplemented pregnant grazing ewes lose body weight and compromise their health, fetal growth and lactation performance (Thomas and Kott, 1995). In pigs, a disproportionate supply of nutrients along the uterine horn results in 15–20% low-birth-weight piglets (<1.1 kg), whose postnatal survival and growth performance are severely reduced (Wu *et al*., 2004). Ozanne and Hales (1999) showed that in rats, 50% protein deficiency during pregnancy would result in altered fetal, and possibly newborn, growth (quantitatively and qualitatively) due to maternal diet-dependent changes in maternal and placental hormones. These changes selectively alter the relative growth rates of the different developing organs of the fetus according to priorities, sex and the metabolic setting of various tissues (namely, liver, muscle and adipose tissue) postnatally, so that the offspring survive under

poor nutrition. This would result in the generation of 'thrifty phenotype' as a general feature of vertebrate adaptability to enhance the survival of species in nutritionally unfavourable conditions; however, this becomes detrimental when adult nutrition is abundant or overabundant (Ozanne and Hales, 1999). Climate change-related nutritional deficiency and increasing heat stress would increase the incidence of intrauterine growth retardation (IUGR) of the conceptus. Maternal undernutrition due either to forage deficiency or increased heat stresses would impair nitric oxide (NO) and polyamine synthesis in the placenta. These two substances help DNA and protein synthesis essential in regulating placental growth, blood vessel relaxation and angiogenesis from pre-existing blood vessels by cell proliferation and differentiation. Deficiency of NO and polyamines would impair placental development, utero-placental blood flow and placental angiogenesis, resulting in reduced transfer of nutrients and O_2 to the fetus and restricted fetal growth. Evidence in support of this view includes: (i) IUGR is observed in NO synthase inhibitor-treated rats or eNOS-knockout mice (Hefler *et al*., 2001); (ii) the inhibition of polyamine synthesis prevents mouse embryogenesis, reduces placental size and impairs fetal growth (Ishida *et al*., 2002); (iii) IUGR in humans is associated with impaired whole-body NO synthesis (Hata *et al*., 1998), with decreases in arginine transport, eNOS activity and NO synthesis in umbilical vein endothelial cells (Casanello and Sobrevia, 2002); and finally (iv) maternal arginine deficiency causes IUGR, increases fetal resorption and death, and increases perinatal mortality in rats, the effect of which is reversed by dietary arginine supplementation (Vosatka *et al*., 1998).

12.7.2 Alteration of epigenetics and impairment of conceptus development

Maternal hyperthermia is considered to be one of the causes of fetal teratologies during pregnancy and might be due to epigenetic phenomenon. Similarly, the energy, protein and micronutrient deficiency during the critical period of gestation might leave a permanent memory called an epigenetic mark throughout life. An epigenetic mark is the stable alteration of gene expression due to covalent modifications of DNA and core histone proteins by environmental factors such as maternal nutrition or heat stress. The epigenetic state of the fetal genome and imprinting gene expression is carried forward to subsequent developmental stages. In epigenetic phenomenon, DNA is methylated at the 5′-positions of cytosine (C) within the CpG islands in the promoter region of a gene in the genome. Modifications of the chromatin structures may alter gene expression, due to the modification of histone proteins by deacetylation or methylation. Acetylation of histone helps gene expression, whereas deacetylation reverses the process. Methylation of DNA is catalysed by DNA methyltransferases, where *S*-adenosylmethionine (SAM) acts as a methyl donor (Jaenisch and Bird, 2003). SAM is synthesized from methionine and ATP by methionine adenosyltransferase, depending on the presence of serine, glycine and B vitamins (i.e. folate, B12 and B6). The entire process of DNA and histone modification may be altered by the overall availability of amino acids and micro-nutrients. Evidence to support this view is: (i) a marked reduction in genomic DNA methylation and aberrant expression of H19 allele (a normally silent paternally imprinted gene) in mouse embryos cultured in amino acid deficiency (Doherty *et al*., 2000); (ii) hypomethylation of the *p53* gene in post-natal kidney (Pham *et al*., 2003) and global DNA hypomethylation with increased histone acetylation in post-natal liver (MacLennan *et al*., 2004) in rat uteroplacental insufficiency; (iii) increased CpG methylation at the A^{vy} locus of agouti mice, in maternal supplementation of methyl donors and cofactors (i.e. folic acid, vitamin B12, choline and betaine), and the methylation patterns are retained into adulthood (Waterland and Jirtle, 2004).

12.7.3 Conception and pregnancy outcome

Reproductive performance decreases in high-producing dairy cows, especially when animals are under severe negative energy balance (Diskin *et al*., 2006). Increased incidences of early pregnancy loss occur if animals are exposed to heat stress before and immediately after the breeding season. Hansen (2007) opined that this effect might be due to compromised oocyte quality and development of the early embryo in cattle. Conception rates are affected only when the animals are not adapted to the changing climatic condition. The rise in minimum daily temperature is critical for conception. The first 21 days in the spring and summer breeding time are critical for animals of a temperate climate. The conception rate of *B. taurus* cattle will decrease by 4.6% for each unit increase in THI (Hahn, 1995). Amundson *et al*. (2006) reported that THI 72.9 was critical, as animals adapted up to this temperature; however, the pregnancy rate reduced in pasture-bred beef cattle (*B. taurus*) during a 60-day spring–summer period when the average daily mean temperature and THI equalled or exceeded 16.7°C and 72.9, respectively. According to these researchers, each unit change in THI above 70 would cause 3.2% decreases and each degree centigrade temperature rise from 23.5°C would cause 3.5% decreases in the conception rate of *B. taurus* cattle. Peripartum heat stress has an effect on post-partum recovery in large animals.

12.8 Impairment of Male and Female Sexual Behaviours

Male sexual behaviours, namely, copulation, aggression, scent marking and ultrasonic vocalizations, are controlled by testosterone hormone, which acts through androgen receptors (AR) and oestrogen receptors (ER) located in specific brain areas. In the hypothalamus, the medial preoptic area (MPOA) and the amygdala (AMY) are important in the neural control of male sexual behaviour (Hull and Dominguez, 2007). Whereas in females, sexual behaviour is mediated through the oestrogen receptor (ER) present mainly in the ventromedial nucleus of the hypothalamus (VMH). Undernutrition inhibits male and female copulatory behaviour, due partly to low steroid and partly to decreased neural responsiveness to the steroid by reduction in hormone receptors in those areas. The experimental evidence indicates that metabolic fuel deprivation can inhibit both LH secretion and oestrous behaviour in females without activating the HPA axis. The mechanism of reduced sexual behaviour due to heat stress in females is yet to be elucidated fully; however, available evidence from humans and other species indicates that the pattern may be oxytocin mediated and moderated by sex hormones and endogenous opoid peptides. In heat stress, sexual activity of males is reduced by direct influences on the HPA and HPG axes mediated through AR (Cunningham *et al*., 2012).

12.9 Changes in Seasonal Breeding Pattern and Post-weaning Survivability in Temperate-zone Animals

Climate change has altered the seasonal breeding pattern in many plants, insects, amphibians and birds (Parmesan, 2007). Over a 7-year period, a 4°C increase of ambient temperature was observed in west Greenland, which resulted in a mismatch between the caribou migration to their calving grounds and the availability of vegetation. As a result, a fourfold decrease in the production of weaned young was observed (Post and Forchhammer, 2008). The yellow-bellied marmots (*Marmota falviventris*) of the Rocky Mountains (USA) are emerging 38 days earlier than they did 28 years ago, when snow cover still remained, resulting in non-availability of forage for grazing (Inouye *et al*., 2000). Variation in the rates of pregnancy and litter size in these marmots could be explained by the change in timing of hibernation, time of snow melt

and availability of feed (Van Vuren and Armitage, 1991). Among farm animal species, the individual variation in photo-responsiveness has only been documented in Pelibuey sheep of Mexico, the Caribbean and northern South American regions. They show considerable individual variation in the onset of oestrus when housed outside at 19°N (Arroyo *et al*., 2007). Therefore, these animals of mid- to high latitude will be hard hit by climate change. Time will tell as to whether they will be able to survive by adapting to the new condition of steadily increasing temperature and changes in rainfall pattern or become extinct.

12.10 Conclusion and Future Perspectives

Climate changes could impact the economic viability of livestock production systems due to loss of production and reproduction. The surrounding environment directly affects the mechanisms and rates of heat gain or loss of animals; therefore, lack of prior conditioning to weather events would result in catastrophic losses in the livestock industry. There are conflicting opinions on whether climate change will have more of an effect on the reproduction of domestic than of wild mammalian species. The common perception is that some ameliorative control can be taken for domestic animals, whereas it is not possible to exert control on wild animals. Even minimum deficiency in their food would affect their health and reproduction. Wild animals have a tendency to migrate to a favourable climate as a natural instinct; however, by doing so they are likely to be exposed to a different photoperiodic regimen or climatic condition where they might have less availability of food/forage. The likely effects of climate change on reproduction in tropical countries are due to nutrition and/or heat stress, and in temperate countries are due to change in the photoperiod. The consequences of climate change are reduced conception rates, increased embryo losses, increased calving intervals, low voluntary forage intake, decreased forage digestion, increased susceptibility to parasitism and disease, lowered perinatal vigour and increased perinatal mortality and morbidity, lower weaning weights and rates, poorer post-weaning performance and increased health-related costs depending on the extent, the stage of occurrence and the magnitude of the deficiency or stress. These losses are difficult to recognize and diagnose. Animals acquire adaptive mechanisms; however, some acquire them very quickly and others slowly. Slowly adapting animals could be endangered as they will have more problems with reproducing successfully. There exists allelic variation in the genes controlling body temperature regulation and cellular resistance to heat shock, indicating genetic adaptation in many species. In cattle, distinct breed differences in thermo-regulatory ability are observed (Pereira *et al*., 2008). Zebu cattle have superior thermo-regulatory ability due to their lower metabolic rate, reduced resistance to heat flow from the body core to the periphery. They also posses special properties in hair coat (Hansen, 2004). In pig, three different genetic lines have shown a different magnitude of reduction in sperm output/concentration in the summer season (Flowers, 2008). Similarly, the adverse effect of heat shock on preimplantation embryo development is found less in breeds such as Brahman, Romosinuano and Nelore, which have evolved in a hot climate, than in the breeds of a cooler climate, namely, Angus and Holstein (Barros *et al*., 2006). In addition, the fertility of Holstein cows in the summer season is found to increase if they are served with the semen of the Gyr breed (*B. indicus*) as compared to Holstein semen (Pegorer *et al*., 2007). Therefore, current animal breeding policy should target conferring genetic resistance to the next generation so that they are better prepared for survival in the climate change scenario. Recent research interest has focused on elucidating the role of kisspeptin, a protein product of the *KISS1* gene, in overriding the detrimental effect of mild food restriction on reproduction. Kisspeptin depolarizes gonadotropin-releasing hormone neurons through the activation of canonical transient

receptor potential (TRPC)-like cationic channels to control GnRH release (Zhang *et al*., 2008). More and more research needs to be directed to find similar genes and their functions in nutritionally compromised animals. The epigenetic mechanism should remain an active area of research. Since it has yet to be explained whether CpG methylation of the important genes (namely, NO synthase: responsible for NO synthesis; guanosine triphosphate (GTP) cyclo-hydrolase I: rate-limiting enzyme for BH_4 synthesis; and ornithine decarboxylase: which forms polyamines from ornithine) is important, or the alteration of histone proteins at chromatin level in the uterus, placenta, fetal and post-natal tissues such as the vascular bed, adipose tissue, liver, kidney, skeletal muscle, or pancreas play the crucial role. Certainly, different domestic and wild animal species are going to face different kinds of challenges. The management of animals to ameliorate stressors and nutritional deficiency will be most crucial for increasing reproductive efficiency in the climate change scenario. A cooler animal shed with facilities for showers would greatly help animals coping with inclement hot and humid weather conditions. Similarly, arginine nutrition, as proposed by Wu *et al*. (2013), might improve conception rates and the development of the conceptus, ensuring the supply of nutrients by improved blood circulation and dilation of blood vessels in the uterus and placenta. The beneficial effect of this amino acid supplement has been proven in pig, sheep and mice; however, more research would help to confirm this hypothesis.

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13 Climate Change: Impact of Meat Production

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Abstract

Between 1961 and 2009, the world recorded a continued increase in the demand for meat, driven by the fast growth in population, economic improvement, changes in eating habits and rapid urbanization. This has resulted in improved livestock production that is projected to continue even into the future. However, raising animals for food has been identified as a major contributor to climate change. As more meat is produced to satisfy the increasing demand, it is important to understand its effect on climate change, which continues to be a threat to food security. Livestock production contributes 14.5% of the total greenhouse gases (GHGs) that originate directly from the animal in the form of enteric emissions (39%), or indirectly from activities in the meat production value chain like animal feed production and processing (45%), manure decomposition (10%) and slaughter, processing and transportation of animal products (6%). The amount of GHGs emitted in meat production depends on the type of feed and the capability of the animals to digest and utilize feeds, thus minimizing the amount of waste excreted. The production of meat is a very inefficient system where animal proteins require 11 times more fossil fuel compared to plant protein. The efficiency of meat production reduces in the order of fish, poultry, pork and beef. Efficient meat production systems also cut down on the emissions associated with the production of massive feeds. Meat production systems involving ruminant animals and feeding fibrous or poor-quality feeds contribute significantly to enteric emission and manure production, and the inefficiency of feed utilization and meat from these systems has a high GHG effect.

13.1 Introduction

One of the major challenges in the world is to increase meat production for the projected world population of 9 billion by 2050. Efforts have been made to achieve this through improved livestock production systems, a step towards realizing food security, which requires massive amounts of land, food, energy and water. Globally, raising animals for food requires about 30% of the earth's land mass (Steinfeld *et al*., 2006). Forests have been destroyed to open up grazing land or grow crops that are used to feed animals. Even in intensive farms where animals are raised on small pieces of land, they require extensive land to produce feeds. It should not be underestimated that huge amounts of energy also go into this system to grow, process and transport feeds and slaughter, process, refrigerate and transport meat. Considering all these, it is expensive to produce animal products, which are estimated to consume 11 times more energy to produce one calorie of animal protein as compared to the

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production of one calorie of plant protein (Pimentel and Pimentel, 2003). Apart from the meat that is produced, there is massive waste production from animals and meat processing plants that require treatment before disposal. In countries where there are no laws governing the disposal of these wastes, they are sprayed in crop fields or left to decompose in huge heaps. All these activities in the meat production value chain have direct or indirect effects on the environment.

Climate change continues to be a threat to food security, especially in the developing world, where productivity is heavily dependent on climate. A lot of research has been directed at mitigating factors to curb this change, but it is a complex scenario attributed to many factors. The Fourth Assessment Report of the Intergovernmental Panel on Climate Change (IPCC, 2007) attributes the increase in global temperatures to human activities such as fossil fuel burning and land-use changes. Animal production has been identified as a major contributor to global warming. As more meat is produced to satisfy increasing demand, it is important to access its impact on climate in order to bring into play appropriate corrective measures.

13.2 Climate Change

13.2.1 Global climate change

Global warming has been noted over time, and this has become a big concern in the world (IPCC, 2007). The earth receives radiations from the sun that are partly absorbed and radiated back to space, thereby maintaining a mean temperature of about 14°C. Observed changes in climate are brought about by an imbalance in the radiative properties of the earth and the atmosphere. Some of these changes are due to interference in the movement of radiations, referred to as radiative forcing. The composition and properties of the atmosphere contribute to the retention of heat from the sun in the earth-atmosphere system. More important are the changes in atmospheric levels of greenhouse gases (GHGs), aerosols and particles in the atmosphere that reabsorb radiations that are redirected back into the earth and atmosphere. The presence of these gases retains more heat on the earth and its atmosphere, giving rise to the 'greenhouse effect', or global warming. In contrast, the reflection of radiations into space by volcanic sulfate aerosols and particles originating from burning give rise to a cooling effect. Emission or removal of GHGs emanates from activities like fossil fuel burning, decomposition of biomass and land use (agricultural production).

Changes in the absorptive and reflective properties of the earth surface can also lead to an imbalance in global net energy. This can be brought about by land-use activities mainly involving agricultural production, a major driver of deforestation, which opens up land for grazing or growing crops for humans and animals.

13.2.2 Greenhouse gas emissions

The observed change in global climate in the past 50 years has been attributed mainly to GHG emissions in the atmosphere (IPCC, 2007). The most abundant gases in the atmosphere, namely nitrogen (N_2) and oxygen (O_2), have no GHG effect. Carbon dioxide (CO_2), methane (CH_4), water vapour, nitrous oxide (N_2O), chlorocarbons and atmospheric aerosols have been identified as the anthropogenic gases in the atmosphere responsible for climate change (Wang *et al*., 1976; Twomey, 1977; IPCC, 2007). They differ in their global warming potential (GWP), which is tagged on their radiative properties and stability or retention period in the atmosphere, thereby contributing to long-term radiative forcing. Because of the variable greenhouse potency, every GHG is usually expressed as an amount of CO_2 with the same global warming potential. According to the IPCC, the greenhouse impact of CH_4 and N_2O are 72 and 289 times stronger, respectively, than CO_2 over a 20-year time horizon. The effects due to aerosols and particles are short-lived,

because they do not stay in the atmosphere for long.

The concentrations of GHG in the atmosphere recorded over time show that levels of CO_2 increased up to 367 in 1999 (IPCC, 2001) from a pre-industrial level of 280 ppm (Indermühle *et al*., 1999). Most of this production has been associated primarily with anthropogenic activities in terms of increased burning of fuel (66.7%) and agricultural activities (33.3%). The review by IPCC (2007) also notes an increase in the CH_4 and N_2O that have also been associated with anthropogenic activities. The global production from different sources of these gases is summarized in Table 13.1.

In the IPCC (2007) report, global GHGs arise from various sources, i.e. energy supply (26%), industry (19%), deforestation (17%), agriculture (14%), transport (13%), residential and commercial buildings (8%) and waste and wastewater (3%). In the agricultural sector, the emissions originate from the management of agricultural soils, livestock and crop production, and biomass burning.

13.3 Meat Production and Climate Change

All food production systems have environmental impacts, and livestock production has been singled out as a major cause of climate change (Steinfeld *et al*., 2006). The effect of livestock on global warming is potentially huge, as it is associated primarily with CH_4 emissions. It is estimated that the production levels of meat contribute between 14 and 22% of the 36 billion tonnes (Bt) of CO_2-equivalent (eq) GHGs the world produces every year. In an FAO report (Gerber *et al*., 2013), it is estimated that livestock contributes 14.5% of the GHGs. Apart from direct emission of GHGs, growth of livestock for food is an extremely inefficient process that requires a lot of energy to produce, process and transport the enormous amount of feeds required to raise the animals. The rapid growth in the sector due to increased demand for meat has opened up forests to grow feeds required by animals. As the numbers of meat animals increase, poor husbandry practices are inevitably resulting in overgrazing, where land is left open and exposed to degradation (Fig 13.1). All these activities contribute either directly or indirectly to climate change.

13.3.1 Meat production and consumption trends

In an FAO report, it was reported that the world's livestock sector was growing faster than other agricultural subsectors (Steinfeld *et al*., 2006). The global contribution of animal products to the total energy consumed in the period 2005–2007 was 17%, with many variations (Gill, 2013). Meat consumption increased from 47 million tonnes (Mt) in 1950 to 260 Mt in 2005, more than doubling the consumption per person from 17 to 40 kg. The report estimates an increased world consumption of 460 Mt in 2050 to meet the requirement of 9 billion people. A strong relationship exists between increased per capita income and consumption of livestock products at

Table 13.1. Global production of GHG from different sources. (From IPCC, 2007.)

GHG	Sources	Emissions (%)
CO_2	Fossil fuels	57
	Land use (deforestation, decay of biomass)	17
	Other	3
CH_4	Agricultural activities, waste management and energy use	14
N_2O	Agricultural activities such as fertilizer use	8
F-gas HFCs, PFCs, and SF_6	Industrial processes, refrigeration and the use of a variety of consumer products	1

Fig. 13.1. Land degradation in overgrazed rangelands.

lower income levels, which stagnates and even becomes negative at high income levels. This is referred to as the 'food transition' process (Guyomard *et al*., 2013). Although there has been growth in both the world population and meat supply, more growth has been recorded in the latter leading to increased meat consumption that stands at 115 g day^{-1} (42 kg $year^{-1}$; FAOSTAT, 2014).

Over the years, there has been growth in the world meat supply, with a shift in meat production and consumption towards poultry and fish from beef and pork (Fig 13.2) because of increased concern about cholesterol. Production and consumption of animal products in developed countries is either decreasing or has stagnated as compared to developing countries. This is attributed to the growing awareness of the negative health effects of high meat consumption. However, the consumption per head is still high in developed countries.

In developing countries, the growing taste for animal products has been associated with rising incomes (Delgado *et al*., 1999). Thus, with more people crossing the poverty line, the production and consumption of meat is expected to continue rising. Another area with an expected increased growth is pastoral communities, where animal production serves as a source of income, credit and insurance, and efforts towards food security cannot ignore growth in this sector. Thus, meat production will continue to be a major activity for subsistence in these areas.

13.3.2 Inefficiencies in meat production

The process of meat production involves storage of energy in the form of either protein or fat within the body. The relative growth in these two forms is dependent on many factors that include variations in

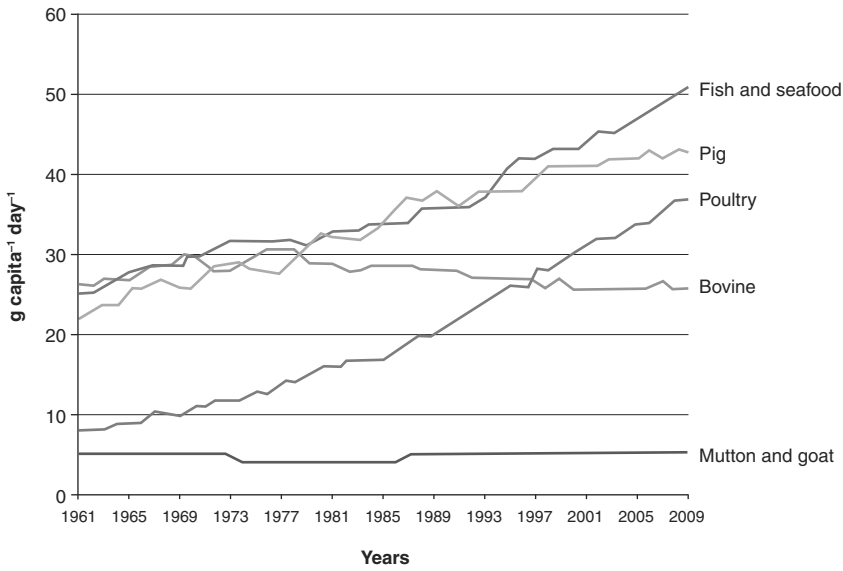

Fig. 13.2. Meat supply by types from 1961 to 2009. (From FAOSTAT, 2014.)

species, breed, age of animal, type of feed and level of feeding (Vermorel and Bickel, 1980). In the production of meat, there is the direct cost of converting feeds into meat and the indirect cost of maintaining the animal. Efficiency of retention of this energy depends on factors affecting its partition, ranging from digestion, absorption and utilization of nutrients. It also depends on the level of feeding/production, which makes the nutrients that are used in maintenance negligible in comparison to the amount converted to meat. As the food is processed in the body, part of the feed energy may be lost directly as heat arising from failure to capture this energy in the form of adenosine triphosphate (ATP) during nutrient metabolism in the synthesis of proteins and fats. The efficiency of metabolizable energy (ME) utilization is lower for protein deposition (45%) than for lipid (75%), due mainly to different biochemical pathways and high protein turnover (Edmunds *et al*., 1980). This has contributed to high efficiency of meat production in older animals, females and breeds that tend to deposit relatively more lipids in their growth. Similarly, animals fed a high-concentrate ration favour more deposition of fat and exhibit high efficiency of energy gain compared to those on low-quality ration. The carbon that is not retained in body tissues also leaves in the form of gases like CH_4 and CO_2.

Emission of heat and gases into the environment varies with the meat production system. Animals fed roughage diets lose most of the feed energy to the environment in the form of heat, waste and emission of gases, resulting in low feed conversion efficiency (FCE) (Table 13.2). This means that intensification of meat production has a low greenhouse effect. In contrast, ruminant production systems have lower efficiencies of utilizing ME compared to non-ruminant animals (McDonald *et al.,* 1999). Thus, ruminant production systems based on low-quality roughages, the case in most tropical developing countries, are characterized by low efficiencies, with more impact on climate change.

The production of meat is a very inefficient process that varies with the system involved. It was found to be more climate friendly to produce protein from vegetable sources than from animal sources (Carlsson-Kanyama and González, 2009). Pimentel and Pimentel (2003) noted that

Table 13.2. Efficiency of utilization of metabolizable energy for growth and fattening. (From McDonald *et al.*, 1999.)

Animal	Type of feed	Efficiency
Ruminants		
Growth (k_g)	Maize (grain)	0.62
Growth (k_g)	Barley (grain)	0.60
Growth (k_g)	Soybean meal	0.48
Growth (k_g)	Dried ryegrass	0.34
Growth (k_g)	Dried grass	0.31
Growth (k_g)	Wheat straw	0.24
Pigs		
Protein deposition (k_p)	Amino acids	0.45–0.55
Fat deposition (k_f)	Normal diet	0.74
Growth (k_g)	Normal diet	0.71

the fossil energy required to produce animal protein was about 11 times higher than that required to produce plant protein. The ability of animals to convert grain into protein varies widely; it takes roughly 7 kg of grain to produce 1 kg beef. For pork, the figure is close to 4 kg for kg^{-1} gain, for poultry it is just over 2 and for herbivorous species of farmed fish it is <2. Pimentel and Pimentel (2003) estimated that the energy required to produce 1 kcal protein depended on the product in the following order: beef (40 kcal), pork (14 kcal), milk (14 kcal) and broiler (4 kcal). Thus, the efficiency of meat production reduces in the order: fish > poultry > pork > beef. The inefficiency associated with meat production requires more feeds, which in turn need massive land and fossil energy to produce, process and transport, and so it is less environmentally friendly. It also contributes to increased enteric emission and manure production.

13.3.3 GHG emissions from meat production

It is estimated that about 35% of global GHG emissions comes from agricultural activities and land use, with most of it originating from livestock production, i.e. ~80% (Steinfeld *et al.*, 2006; McMichael *et al.*, 2007). In the most recent review, livestock is estimated to emit 7.1 Gt of CO_2-eq $year^{-1}$, representing 14.5% of all human-induced emissions (Gerber *et al.*, 2013). There are three main GHGs: CO_2, accounting for only 9% of agricultural emissions, CH_4 (35–45%), while 45–55% comes from N_2O (IPCC, 2007; McMichael *et al.*, 2007). CH_4 and N_2O make a large contribution to global warming because of their high GWP of 25 and 296, respectively, using a 100-year period (IPCC, 2007).

Emissions in the meat value chain originate from animal feed production and processing (45%), enteric emission (39%), manure decomposition (10%) and slaughter, processing and transportation of animal products (6%). In feed production, it arises from deforestation activities for expanding grazing land and feeds production, soil carbon loss in grazing lands, the energy used in growing feed crops and in processing and transportation of feeds and the N_2O released from the use of nitrogenous fertilizers. The amount emitted from this sector is dependent on the type of meat produced and the type of feeds used, which play a key role in determining the efficiency of meat production.

Fermentation of feeds in rumen is the main source of CH_4 from animals. The fermentation patterns in the rumen are quite variable in response to the type of ration offered to the animal. Acetate and butyrate production pathways in the rumen promote CH_4 production, while propionate formation can be considered as a competitive pathway for hydrogen use in rumen (Moss *et al.*, 2000). Highly fibrous rations cause higher CH_4 emissions, which are quite common under the range/extensive systems,

while feeding more concentrate in the intensive ruminant systems results in lower CH_4 emission (Herrero *et al*, 2013). The dietary approaches for reducing enteric CH_4 emission are debated elsewhere in the book (see Section III, this volume, for details). Non-ruminant species such as pigs and poultry do also produce CH_4, but in much lower quantity by comparison. At the maintenance level of feeding, CH_4 accounts for a loss of 7–9% of the gross energy of feeds, which decreases to 6–7% at a high level of feeding (McDonald *et al*., 1999). This loss of energy from feeds contributes to the inefficiency of feed energy utilization in ruminant production systems. Thus, apart from the direct effect of CH_4 emission on global warming, the accompanying inefficiency of feed utilization associated with CH_4 production requires large quantities of feeds to produce meat. CH_4 also originates from manure during storage and processing due to the anaerobic decomposition of organic material when stored in deep lagoons. Nitrogen emits from manure in the form of NH_3 and N_2O (see Chapter 4, Section I, this volume).

CO_2 emissions in the meat value chain originate from the expansion of feed crops and pasture into natural habitats, which causes the oxidation of carbon in soil and vegetation. CO_2 also originates from the production of nitrogen fertilizers and fossil fuel and energy use on the farm in the production of feeds. In the FAO report (Gerber *e al*., 2013), the total GHG emissions from meat production systems vary with the types of meat produced (Table 13.3). Weber and Matthews (2008) estimated that the production of red meat generated 150% more GHG than chicken or fish. In another study (de Vries and de Boer, 2010), the GHG emissions (kg CO_2-eq kg^{-1} product) in life cycle assessments for developed countries were estimated as: 14–32 (beef), 3.9–10 (pork), 3.7–10 (chicken), 3.9–4.9 (eggs) and 0.84–1.4 (milk). Comprehensive information on GHG emission for producing different animal products in developed and developing countries is given in Chapter 9, Section II, this volume, on carbon footprints.

Greenhouse gas emissions are driven by animal productivity, quality of feed and feed scarcity (Herrero *et al*., 2013). Thus, the intensive systems and breeds with high feed conversion efficiency tend to be more environmentally friendly than the animals belonging to low feed conversion capacity. According to FAO, the GHG emissions from intensive production systems are lower than those from extensive systems. However, the bulk of global meat is produced from extensive systems, where there are more animals, and specifically more ruminants. The production of these gases can be reduced drastically through intensification of livestock production.

13.4 Meat Production Systems and Climate Change

Meat is produced from many species of animals, but more commonly from cattle,

Table 13.3. Global production of greenhouse gases per unit product. (From Gerber *et al*., 2013.)

	Emission (kg CO_2-eq kg^{-1} product)		
Source	Meat	Milk	Egg
Layers	6.9	–	3.7
Broiler	5.3	–	–
Pig	6.1	–	–
Sheep	23.4	8.4	–
Goats	23.8	6.5	–
Buffalo	53.4	3.4	–
Dairy cattle	18.2	2.6	–
Beef cattle	67.6	–	–

sheep, goats, camels, poultry and pigs. The animals have different digestive systems that are adapted to handle different feeds. These differences have given rise to meat production systems in which the efficiency of meat production and the accompanying emissions in the environment depends on animal species and diet.

13.4.1 Meat production systems

In a broader sense, meat production can be categorized in two main systems: extensive systems and intensive systems. Production from the different systems is summarized in Table 13.4.

Extensive livestock production is an old traditional system of meat production where animals are raised free range, characterized by inadequate and poor-quality feeds, which results in a slow growth rate (Auriol, 1978). In addition, the animals raised are of low growth rate in order to cope with the poor quality feeds. All these factors add to a very inefficient system, giving rise to consumption of large quantities of biomass, high emission of GHGs and manure in the environment. Although the systems are inefficient per se, they are more efficient in producing food per unit area of dryland than other forms of agricultural land use under the same conditions (Kratli *et al.*, 2013). Due to scarcity of land, many countries are putting more emphasis on the logistics of utilizing the arid and semi-arid regions where crops cannot be grown. In a country like Kenya, the development plan targets increased utilization of arid and semi-arid areas (ASALs) that are only suitable for livestock farming (GOK, 2007). Overgrazing is a common phenomenon in this system, especially in the dry season, which increases the evapotranspiration level and consequently promotes climate warming (Du *et al.*, 2004). Thus, the ASALs have great potential for meat production that will continue affecting climate change.

In extensive systems, animals take longer before reaching slaughter and may spend about 85% of the feed on maintenance. This reduces to about 63% with intensification of the system. In developed countries, more animals are raised under this system, and more so in concentrated animal feeding operations (CAFOs), or factory farms. Meat production from this system accounts for 80% of the growth in livestock production, 72% of poultry production, 43% of egg production and 55% of pork production in the world (Steinfeld *et al.*, 2006). Intensive meat production is associated with increased efficiency of production due to higher daily live weight gain, better feed conversion and the shorter period involved. Even in ruminant systems, intensification produces less CH_4 than the systems based exclusively on rangeland (Capper *et al.*, 2009; Capper, 2011). These systems are more environmentally friendly in terms of emissions and require less feeds to produce meat. However, these systems rely on the production of high-quality feeds elsewhere, which uses a lot of fossil fuel and energy. Large quantities of manure from these systems also pose a major challenge in terms of disposal. They produce N_2O and CH_4 gases that have high global warming potential.

Sometimes, the feeding method is wasteful due to poor management (Fig. 13.3). In

Table 13.4. Livestock production (Mt) from different systems (2001–2003 average). (From Steinfeld *et al.*, 2006.)

	Livestock production system			
Product	Grazing	Rainfed mixed	Irrigated mixed	Industrial/landless
Beef and mutton	18.4	33.3	16.9	4.0
Pork	0.8	12.5	29.1	52.8
Poultry meat	1.2	8.0	11.7	52.8
Milk	0.5	5.6	17.1	35.7
Eggs	71.5	319.2	203.7	–

Fig. 13.3. Poor methods of feeding, leading to feed contamination and wastage.

some areas, there are no proper feeding structures; animals are fed from the ground, where feeds get mixed with urine and faeces. Most of this feed is rejected, leading to excess wastage that ends up in composite pits, where it decomposes and gives rise to GHGs.

13.4.2 Fossil energy use in meat production systems

Feed is a major component in the meat production industry. It accounts for about 45% of the total emissions from the livestock sector. Production of these feeds requires production and storage of manure, production of nitrogen fertilizers and use of manure and fertilizers, which contribute to the production of CH_4 and N_2O. The amount of N_2O produced in this sector accounts for about 50% of the emissions from feeds. Fertilizers and pesticides are sometimes made from fossil fuels. More fuel is also used directly in the production and transportation of feeds to the farms.

As the demand for meat increases, its production requires more land to produce feeds, and in some countries it has been a major cause of deforestation. The contribution of animal feed production to climate change depends on the quantities required, which vary with the demand of meat. Thus, the projected increase in meat consumption will be accompanied with more production of feeds. On the other hand, the high inefficiency in the utilization of feed for meat production (see Section 13.3.2) also contributes to the large quantities of feeds required. Inefficient meat-producing systems like beef, especially where animals are raised on poor-quality feeds like rangeland grasses, require more fertilizers to produce feeds, and thereby contribute

more to global warming. This type of system is found in the drier rangelands and in developing countries where meat is derived mainly from ruminant animals. Intensive production systems improve the efficiencies of meat production, thus cutting down on the energy required to produce large amounts of feeds. Globally, it is estimated that feed crop production uses about one-third of all arable land in the world (Steinfeld *et al*., 2006).

Meat is a perishable product that is produced in rural areas and needs to be transported to urban areas. To extend its shelf life, it must be processed and refrigerated, which again requires a lot of fossil fuels.

13.5 Deforestation and Land Degradation

As developed countries are running short of land for crop expansion, the increasing demand for meat is forcibly extending intensive agriculture into the tropical rainforests, opening up more land. Much of livestock's contributions to global warming come from deforestation, as trees are cut down to make space for pasture or farmland to grow animal feed. About one-quarter of animal feed emissions are related to land-use change (Gerber *et al*., 2013). Livestock takes up a lot of space, and nearly one-third of the earth's entire landmass is used under livestock production. This has led to deforestation that increases atmospheric CO_2 by either burning or decomposition. Meat production requires a lot of feeds, and its improvement has resulted in the loss of forests. In Latin America, FAO estimates that some 70% of former forest cover has been converted to grazing. The resulting animal feeding pressure is transferred to the few drought-tolerant plants found in the area, which are cut to feed the animals (Fig 13.4).

Fig. 13.4. Feed shortage contributing to deforestation.

Livestock farming, the only source of livelihood in arid and semi-arid lands, is much limited by unreliable weather. Low productivity in this environment and over-reliance on animals for subsistence results in a type of farming that puts more emphasis on numbers. Thus, most of the time throughout the year there are limited feeds for the animals, which results in overgrazing and land degradation (see Fig. 13.1). Overgrazing causes loss of plants that may require a long period to regenerate, thus leaving the soil exposed to external forces. It also causes soil compaction and erosion, decreased soil fertility and water infiltration, and a loss in organic matter content. It is estimated that about 20% of the global pastureland has been degraded by grazing animals. Oldeman *et al*. (1991) estimated that, since 1945, degradation has been in the region of 15%; while Dregne *et al*. (1991) argued that 73% of the world's rangeland was either moderately or severely degraded. Meat production from rangelands requires proper management to reduce its effect on climate change, especially considering the fact that grazing occupies 26% of the earth's ice-free terrestrial surface.

13.6 Conclusion

Raising animals for meat contributes significantly to GHG production. High emissions are associated with inefficient meat production, mainly from ruminant animals, poor-quality feeds and animals with low production potential. As global meat production is expected to grow with the increasing population, it will either open up more forest land or push production to arid or semi-arid areas, where crop farming is not practical without irrigation. In the latter case, ruminant animals are most suited, but have a big climate change effect. This is expected to be made worse with overgrazing, which is common in these areas with feed scarcity. This effect can be reduced by intensifying meat production.

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14 Indigenous Livestock Resources in a Changing Climate: Indian Perspective

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Abstract

Biological diversity, the variability of life on earth, exists in the form of different species and breeds within the animal kingdom. This diversity is created in the process of molecular/biochemical/metabolic reactions, and acts as a critical measure of adaptation in changing climatic conditions. Indigenous breeds have adapted to climatic variations since time immemorial, and hence have acquired unique traits that make them suitable in given agroclimatic zones; for example, the Indian cattle breeds, Tharparkar and Sahiwal, are heat and tick resistant. Similar cases have also been observed worldwide in Asia, Africa, Europe, Latin America, North America and the south-west Pacific region, having a total of 1144, 1300, 345, 104 and 108 breeds of major livestock species, respectively. Native breeds, namely N'Dama cattle, Red Massai sheep, etc., have developed trypanosomiasis resistance and gastrointestinal nematode tolerance by continuous natural selection. The overwhelming majority of indigenous livestock around the world are bred locally and kept by small-scale livestock keepers; hence, there is a need to promote local indigenous livestock species, as they represent a genetic resource that is relatively resilient to climate variability.

14.1 Introduction

Biological diversity is the variability of life on earth. The most obvious aspect of biodiversity is genetic, in the form of different breeds and species. This genetic diversity or variability is due to molecular diversity in the process of molecular or biochemical metabolic reactions. Diversity in the gene provides the basis of molecular variability and phenotypic variation between breeds and species. Genetic diversity has resulted due to the process of evolution over thousands of years, during wild and domesticated stages, and to the efforts made by humans to meet market demand. Existing biodiversity is critically important to help livestock keepers to adapt to a changing climate.

Animal husbandry has always been an integral part of Indian civilization, and the country possesses a rich biodiversity of animal genetic resources that are spread over diverse agroclimatic regions. These resources have been developed by our ancestors over several generations and have acquired special attributes like remarkable adaptability to the environment and management conditions, and genetic resistance to most tropical diseases, besides survival on poor quality feed and fodder. In spite of the multiple threats of changing

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climate, replacement, degradation and extinction, this biodiversity of farm animals comprises 30 well-described breeds of cattle, 10 of buffalo, 20 of goats, 40 of sheep, 8 of camel, 6 of horses and 20 of poultry, besides a large number of variants of native ponies, pig, donkey, yak and mithun (Sahai and Vijh, 2000). This megadiversity is not accidental, nor is it purely natural; rather, it is the outcome of thousands of years of deliberate selection and planned exposure to a range of natural climatic conditions.

Climatic changes have a negative impact on all animals, as they are essentially dependent on the environment, and any fluctuations in weather and climate can affect them. Climate change will not only impact the health and welfare of animals but also the more than 1 billion people who depend on them.

14.2 Climate Versus Animal Genetic Resources (AnGR) in India

14.2.1 Cattle

There are 30 well-defined breeds of cattle that have evolved through the serious efforts of the breeders. Breed characteristics, however, are influenced by climatic factors, and the selection and management processes through which these have evolved. As per the Livestock Census of India 2007, the cattle population in India is 199.08 million.

The zebu cattle of India are classified into five types, based on their colour and phenotypes: (i) the large white cattle of the north; (ii) the very distinct Mysore type of the south, with the characteristic formation of the head and horns; (iii) the highly peculiar cattle of Kathiawar and the western part of India; (iv) the small black, red or dun cattle from the north to the south and the east to the west of India, mainly in hilly tracts and forests; and (v) the broad-face, lyre-horned, grey-white cattle of western India. Based on utility pattern, the indigenous cattle breeds have been further classified as:

- Milch breeds: Gir, Sahiwal, Red Sindhi and Tharparkar.
- Draught breeds: Nagori, Bachaur, Kenkatha, Malvi, Kherigarh, Hallikar, Amritmahal, Khillari, Bargur, Kangayam, Ponwar, Siri, Gaolao, Krishna Valley and Alambadi.
- Dual-purpose breeds: Nimari, Dangi, Hariana, Mewati (Kosi), Rathi, Ongole, Kankrej, Deoni, Red-Kandhari, Punganur and Umblachery.

The cattle breeds that have evolved and the climatic conditions of the various agro-climatic regions of the country are described below.

High-altitude region

The climate in the high-altitude and alpine regions above the timberline supports only small cattle. Typical small hill cattle are predominant in this zone. There is a vast diversity in the morphometric and performance levels of hill cattle, but they remain largely ignored as far as classification into breeds. The major reason for this is that they produce a very small quantity of milk, mainly for domestic consumption, and do not form part of the dairy industry. In the foothills, cattle breeds of medium-sized stature are available where the bullocks perform mild work on small fields on hilly terrain, while the females produce some milk for domestic consumption. Kherigarh and Ponwar cattle breeds are found in the Himalayan foothills in Uttar Pradesh and Uttarakhand. Siri cattle are found in the hills of Sikkim and Darjeeling in the north-eastern parts. These breeds of cattle have evolved as per the climatic conditions of the foothills and perform reasonably well in these areas.

Dry north-western plains

The dry north-western plains include the entire Rajasthan, Gujarat, parts of Punjab, Haryana and the adjoining regions of western Uttar Pradesh. The climate of this

region is dry. The fragile ecosystem and frequent drought affect agriculture crop failures, and the milk obtained from cattle supports the human food chain. The breeds of these areas have been selected and further developed for milk production so that they consistently provide milk for humans. Excellent milch-type cattle breeds, namely Sahiwal, Tharparkar and Gir, and Rathi, Hariana and Kankrej dual-purpose breeds have been developed. These cattle breeds have been producing a reasonably fair quantity of milk subsisting on the poor-quality feed and fodder resources available under the local agroclimatic conditions. Tharparkar is one of the important heat-tolerant and tick-resistant cattle breeds of this region (Fig. 14.1).

The Indo-Gangetic plains

The Indo-Gangetic plains comprise of part of the doabs of the Ganges, Yamuna, and other perennial rivers. Farmers of this type of agroecosystem have developed dual-purpose cattle breeds for milk production and draught power. The Hariana, Gangatiri and Mewati cattle in the northern parts, the Kankrej in Gujarat, the Ongole, Kangayam, Krishna Valley and Deoni in the southern plateau and the Nimari and Dangi in the central parts are excellent dual-purpose cattle breeds.

Central parts and Deccan plateau

The central parts and the Deccan plateau, where the soil is hard, require powerful bullocks for farm operation under hot and humid climatic conditions. Here, specialized draught cattle breeds with massive build has been produced. These are the Kenkatha and Malvi in central India, the Gaolao and Khillari in Maharashtra, the Hallikar, Amritmahal and Krishna Valley in Karnataka and the Kangayam in Tamil

Fig. 14.1. Tharparkar: a heat-tolerant breed of cattle.

Nadu. Females of these breeds provide comparatively less milk, to support the male calves for raising them to powerful bullocks and also for human consumption.

Eastern and coastal regions

Cattle breeds in the eastern and coastal regions, because of the climatic conditions (high humidity and soft agricultural land), are of small stature and poor body conformation, and are not well recognized as distinct breeds. However, a few breeds, like the Bachaur in Bihar, the Umblachery in Tamil Nadu, the Vechure in Kerala and the Punganur in Andhra Pradesh, are found in these regions.

14.2.2 Buffalo

Buffalo are prominent and well-recognized dairy animals in India, as well as in many other countries in the world. India is the richest source of buffalo germplasm in the form of the Murrah and Bhadawari, with a population of 105.34 million (Livestock Census of India, 2007).

The highest genetic improvement in water buffalo for milk, work and meat has taken place in the Indian subcontinent (mainly India and Pakistan), and they are being reared in different climatic conditions. Buffalo contribute around 54% of India's and 12% of the world's total milk production (ICAR, 2002). The buffalo is a multi-purpose animal, providing milk with a high fat content, meat, draught power and transport. It also provides employment and generates income, mainly for marginal and landless rural masses, and is a part of the cultural fabric of society. On the basis of habitats, climatic conditions and genetic constitution, buffalo can be classified into riverine and swamp buffalo.

Riverine buffalo

These buffalo have 50 chromosomes (Kumar *et al*., 2007). They are mostly dairy breeds and prefer the clear water of rivers, irrigation canals and ponds to wallow.

Swamp buffalo

These buffalo have 48 chromosomes (Bhattacharya *et al*., 2007). Breeds in this species are Assamese and Manipuri; however, recent studies have confirmed that only the Manipuri breed is a true swamp buffalo having 48 chromosomes, while some of the Assamese buffalo have 48 chromosomes, a few have 49, but the majority possesses 50 chromosomes typically like riverine buffalo.

There are 72 breeds of buffalo in the world, out of which India has ten well-defined riverine buffalo breeds, namely Bhadawari, Jaffarabadi, Marathwada, Mehsana, Murrah, Nagpuri, Nili Ravi, Pandharpuri, Surti and Toda. Besides these, there are also lesser-known breeds. Of the 15 known population groups, also known as 'lesser-known breeds', Orissa has seven (namely Kujang, Chilka, Jerangi, Paralakhemundi, Manda, Kalahandi and Sambhalpuri). The other eight defined populations are Tarai (Uttar Pradesh and Uttarakhand), South Kanara (Karnataka), Kuttanad (Kerala), Godavari (Andhra Pradesh), Sikkimese (Sikkim), Swamp (Assam and Manipur), Banni (Gujarat) and Gojri (Himachal Pradesh).

Domestic buffalo (*Bubalus bubalis*) differ from agroclimatic region to region in temperament, physical characteristics and milk yield. Most of the defined dairy breeds of Indian buffalo have been developed in north-west India based on productivity and physical appearance. The Murrah and Nili Ravi are known for high milk production. They are distributed mainly in Haryana, the western part of Uttar Pradesh and Punjab. However, the Murrah has a much wider distribution than the Nili Ravi. The Bhadawari breed, known to have the highest fat percentage (8–13%), has very limited distribution, mainly in the erstwhile Bhadawar region covering parts of Uttar Pradesh and Madhya Pradesh. Gujarat, in the western part of India, has Jaffarabadi in Saurashtra, Mehsana in central Gujarat and Surti in southern Gujarat.

Maharashtra, relatively dry parts of the southern plateau, has some good germplasm

of longhorn buffalo breeds, namely Nagpuri, Pandharpuri and Marathwada. In the south, the Toda is more or less a hill feral buffalo breed.

Buffalo breeds can be classified according to their size, body weight and morphological characteristics, as below:

- Large buffalo: Murrah, Nili Ravi, Jaffarabadi and Banni.
- Medium-sized buffalo: Mehsana, Nagpuri, Bhadawari, Sambalpuri and Tarai.
- Small buffalo: Surti, Manda, Kalahandi, Jerangi, Assamese and Manipuri.

Buffalo breeds are also classified according to ecogeographical distribution, as below:

- North-western India: Murrah, Nili-Ravi, Banni, Mehsana, Surti and Jaffarabadi.
- Central India: Bhadawari, Tarai, Nagpuri and Pandharpuri.
- South India: Toda, Kuttanad and South Canara.
- East India: Manda, Jerangi, Kalahandi, Sambalpuri, Paralakhemundi and Chilka.
- North-eastern India: Assamese and Manipuri.

These breeds of buffalo have been developed over time in various parts of the country having different climatic conditions and are performing very well in their areas. For example, the Bhadawari breed of buffalo found in the Chambal areas of Uttar Pradesh and Madhya Pradesh is well adapted for those areas and produces milk with a very high fat content (up to 13%). Similarly, the Toda breed found in the Nilgiri hills in South India is the backbone of the people of the Toda community in this region.

14.2.3 Sheep

Sheep have been bred by humans to suit different agricultural and climatic conditions. They are reared for a variety of purposes, and can be maintained under diverse environmental conditions. Utilizing uncultivable wastelands and weeds from fields, they contribute to the sustenance of humans by providing food and material for clothing. There is no substitute for wool yet as a durable, warm and health-promoting raw product for clothing. As per the Livestock Census of India (2007), the sheep population in India is 71.56 million.

Indigenous sheep genetic resources are endowed with many desirable attributes like disease resistance and better tolerance to varying climatic conditions (high temperature and humidity). Indigenous breeds are more efficient in feed conversion. They generally perform better than exotic breeds under low-input conditions and climatic stresses, especially during times of drought. Different breeds have been developed in response to the economic requirements of humans and agroecological conditions. These breeds have generally been named after their place of origin. A few breeds have been bred from the base populations by crossing native and exotic breeds. The 40 breeds of Indian sheep found in the different agroecological regions of India are as follows:

- Northern temperate: Bhakarwal, Changthangi, Gaddi, Gurez, Karnah, Kashmir Merino, Poonchi and Rampur Bushair.
- North-western arid and semi-arid: Chokla, Hissardale, Jaisalmeri, Jalauni, Kheri, Magra, Malpura, Marwari, Muzaffarnagri, Nali, Pattanwadi, Pugal, Sonadi and Munjal.
- Southern peninsular region: Bellary, Coimbatore, Daccani, Hassan, Kanguri, Kilakarsal, Madras Red, Mandya, Mecheri, Nellore, Nilgiri, Rammand White, Tiruchy Black and Vembur.
- Eastern region: Balangir, Bonpala, Chottanagpuri, Ganjam, Garole and Tibetan.

The sheep breeds that have evolved and the climatic conditions of the various agroclimatic regions of the country are described below.

Northern temperate region

The northern temperate region comprises the states of Jammu and Kashmir, Himachal Pradesh and Uttarakhand. This region is characterized by steep gorgeous hills rising from the plains. The tops of the hills and the valley bottoms provide a source of grazing at

different times during the year. In winter, flock owners bring back their sheep to the lower plains and move to higher elevations when required under certain conditions of grazing and rainfall during the summer months. There are nine distinct breeds in the cold climatic conditions of Jammu and Kashmir, Himachal Pradesh and Uttarakhand. In Kashmir, there are three distinct breeds, namely Poonchi, Karnah and Kashmir Valley. The Poonchi and Karnah breeds yield a heavier and softer fleece. The Gurej produces fine carpet wool and is probably the heaviest sheep breed of the Himalayan region. The Gaddi and Bhakarwal, the two important breeds of the Kashmir, Kangra and Kullu valleys originated in the lower Himalayan foothills. These breeds, when kept at higher altitudes, develop a fine undercoat. The Rampur Bushair, another important breed, originates in the Mahsu district of Himachal Pradesh and is known to produce fine, carpet-type wool.

North-western arid and semi-arid region

The states of Rajasthan, Punjab and Haryana and parts of Uttar Pradesh, Gujarat, Madhya Pradesh and Chhattisgarh come under the north-western arid and semi-arid regions. These drought-prone areas of the semi-arid ecosystem have peculiar environmental conditions that affect life forms, including sheep. The major sheep production systems in this zone are of the migratory type. However, migration may be longer or shorter, depending on the availability of grazing and water resources, and the social status of farmers within each area.

Sheep breeds of this climatic zone are primarily of coarse and long wool types. The Lohi, Chokla, Magra, Malpura, Marwari, Sonadi, Pugal, Jaisalmeri and Nali breeds from Rajasthan, and the Patanwadi from Gujarat are the principal breeds of this area. The Lohi sheep are small in number in Punjab and Haryana. The Munjal breed, considered to be an admixture of the Nali and Lohi breeds, is found around the Hisar, Rohtak and Karnal districts of Haryana and in the Amritsar and Ferozepur districts of Punjab. In addition to the above local breeds, the Hisardale breed has evolved at the Government Livestock Farm, Hisar, by crossing Chokla ewes with Merino rams. The cross is nearly 7/8 Merino × Chokla. It is confined mainly to government sheep farms. Rams, however, have been used for upgrading local flocks in selected rural areas.

Southern peninsular region

This region has varying climatic conditions. The drier areas of the southern region, extending from the Vindhyachal Mountains to the Nilgiris, have a large sheep population, with a greater diversity than in the northern Indian plains. This region covers the states of Maharashtra, Andhra Pradesh, Karnataka and Tamil Nadu. The region is characterized by dry lands with little grazing resources. Often, high ambient temperature and humidity affect the animals. Basically, there are sheep of two main types in this area, namely those producing mixtures of coarse, coloured wool and those producing no wool at all. Hairy sheep are raised primarily for mutton production; their skin is useful for making leather garments. Woolly sheep are found in Maharashtra, the adjacent areas of Andhra Pradesh and Karnataka, and some areas of Tamil Nadu. The Deccani breed, commonly found in Maharashtra and parts of Andhra Pradesh and Karnataka state are medium to large in size and produce hairy and coloured fleece intermixed with kemps. They are hardy and well adapted to poor pastoral conditions. Deccani sheep are predominantly black or coloured. A unique mutton-type sheep breed, Mandya, is found in the Mandya district of Karnataka.

In the more humid and warm climate of the coastal part of southern India, large numbers of sheep of a unique phenotype are found. They are more akin to goats in appearance than to woolly sheep. The breeds of this type are Nellore in Andhra Pradesh, and Yalag or Tenguri or Kenguri in Karnataka. Mecheri, Kilakarsal, Vembur, Coimbatore, Remand White and the Madras Red are other important sheep breeds of Tamil Nadu.

Eastern region

The climatic conditions of this region are partly cold and humid. The eastern region comprises the states of Bihar, Jharkhand, West Bengal, Orissa, Assam, Sikkim and the north-eastern states. This region does not have many sheep breeds, although in certain isolated places sheep are reared on a small scale. The Chotanagpuri breed is prevalent in the Chotanagpur hilly terrain and plateau region of Jharkhand.

The Shahabadi breed is found in the plains of Bihar, besides the adjoining districts of Burdwan and Bankura in West Bengal. Orissa has the Ganjam and Bolangir breeds of sheep. The Bolangir is a woolly breed, whereas the Ganjam is a hairy breed. Garole sheep are small in size and are found in the Suderban areas of West Bengal. They are known for high fecundity. The breed possesses the *Fec+B* gene, exploited globally for improving fecundity. Such small sheep are also found in the coastal region of Orissa.

14.2.4 Goat

There are 20 well-defined breeds distributed throughout the country, ranging from the high-altitude, cold, arid region in the Himalayas to the hot, humid and coastal regions. Many of these breeds have been characterized for accurate phenotypic description and evaluation in terms of their population, population trends, flock size and structure, ecology, feed resources, management practices, conformation and morphometric performance characteristics using modern biological and statistical methods. The goat population in India is 140.54 million, as per the Livestock Census of India 2007. The majority of the Indian goat breeds are highly prolific, especially the Bengal type. The goat breeds found in the extreme cold climate (Changthangi and Chegu) produce pashmina fibres of finest quality. Breeds like Jamunapari, Barbari and Beetal have been utilized as improver breeds for milk and meat within India and in South Asian countries.

Most goat breeds have evolved through natural selection for adaptation to the agroecological conditions. Goat breeds in India have been classified according to (i) the agroclimatic zones, (ii) their body size and (iii) their production functions. Various breeds of goat found in the different agroclimatic conditions of the country are as follows.

Temperate Himalayan

This region comprises the states with cold climatic conditions, namely Jammu and Kashmir, Himachal Pradesh and Uttarakhand. The breeds found in this area are Gaddi, Changthangi and Chegu.

North-western region

The north-western region comprises the states of Delhi, Punjab, Haryana, Uttar Pradesh, Rajasthan, Gujarat, Madhya Pradesh and Chhattishgarh. Various breeds found in this region are Jamunapari, Marwari, Zalawadi, Beetal, Kutchi, Sirohi, Barbari, Mehsana, Surti, Jhakrana and Gohilwadi.

Southern region

The states of Maharashtra, Andhra Pradesh and Tamil Nadu come under the southern region. Sangamneri, Osmanabadi, Kanai Adu and Malabari breeds are found in this region.

Eastern region

Bihar, Jharkhand, Orissa, West Bengal and Assam are part of the eastern region. The breeds found in this region are Ganjam and Bengal.

14.2.5 Camel

Both the *Camelus dromedarius* (single-humped or dromedary) and *Camelus bacterianus* (double-humped or bactrian) species of camel are found in India. The dromedary camel is an important domestic livestock in the hot, arid and semi-arid north-western region of India. The primary

utility of camels has been carting for short distances (30–40 km day^{-1}) and agriculture operations. They have been used as a baggage animal for the transport of fodder, water, fuelwood and other materials when other forms of transport are not available, especially in deserts. The camel is well adapted to the most rigorous environment of arid and semi-arid lands. It provides milk, meat, traction power, mobility and quality fibres and hides. The camel produces a large quantity of milk with an excellent nutritional and therapeutic value. Camel meat is valued for its low fat content; fat is stored mainly in the distinct hump. The camel population in India is 0.52 million as per the Livestock Census of 2007, and is declining (0.63 million in the 2003 census).

Indian camels have been classified into two classes, plain camel and hill camel. There are only four distinct breeds of plain camel, namely the Bikaneri, Jaisalmeri, Mewari and Kachchhi. These camel breeds are well suited for the hot and dry climatic conditions of Rajasthan and other adjoining states.

A small population of Bactrian (double-humped) camel exists in the Nubra valley of Ladakh (Fig. 14.2). This type of camel is called the ship of the cold desert, where the temperature goes as low as –30°C. Indian double-humped camels have a well-developed muscular body with a soft, fine, long hair coat or woolly undercoat.

14.2.6 Horse

For centuries, the horse has been a most faithful servant during demanding and often gruelling conditions. In war, in sport, in agriculture and even as part of police and security forces, horses continue to play a vital role all over the world. The horse population in India is 0.61 million as per the Livestock Census of India, 2007, and is declining (0.75 million in the 2003 census). India has six indigenous breeds of horse, developed through the immigration of genes mainly from Arabian countries, namely the Kathiawari and Marwari, and from

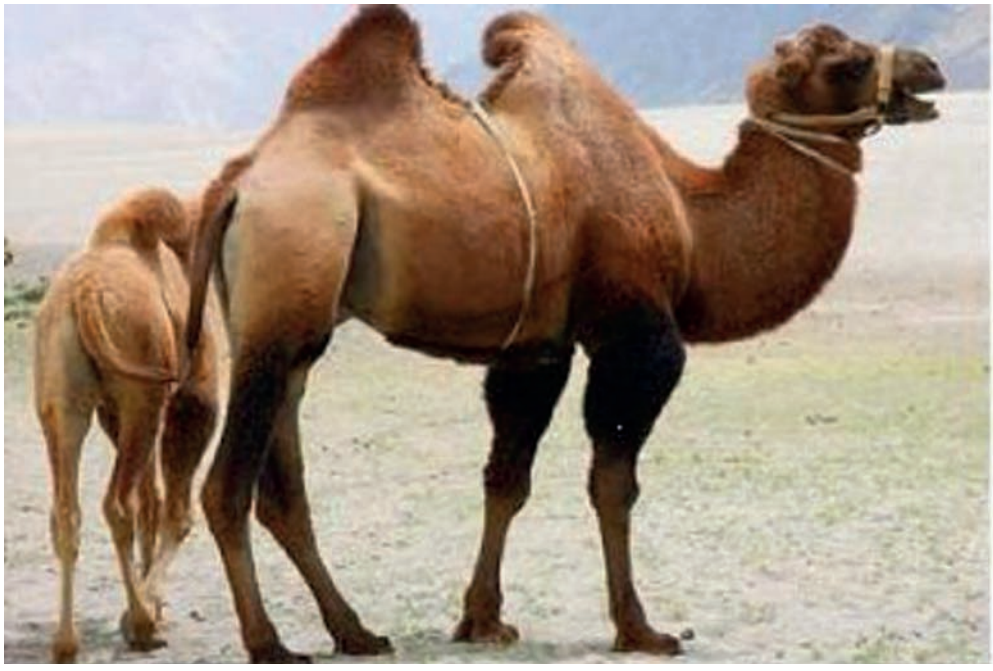

Fig. 14.2. Double-humped camel.

Mongolia, namely the Manipuri, Bhutia, Spiti and Zanskari. All the indigenous horse breeds show declining trends. Zanskari horses are available in the cold desert (Leh and Laddakh) area of Jammu and Kashmir. The horses are known for their ability to work, run adequately and carry loads at high altitude. Only a few hundred Zanskari horses exist at present in the Zanskar and other valleys of Laddakh.

14.2.7 Yak

The yak is a multi-purpose, domesticated animal of high-altitude, cold mountain regions. Yaks provide wool and leather for clothing, shoes, blankets, bags, implements, rugs and tents; bones for carving; meat and milk for fresh food, dried food, processed butter and cheese for consumption, sale and ceremonial offerings; transport for trade and agricultural production; financial assets and security for investments, accidents and family ceremonies; and manure for cooking, heating and nutrient recycling. Movement of the yak through diverse herding regimes and trading patterns has maintained economic and social networks among the tribal communities keeping yaks in India and across the borders. These have also contributed to regional stability and mutual understanding. The yak is also responsible for maintaining agrobiodiversity in the world's most fragile ecosystem by utilizing low-growing grasses, refurbishing it by seed dispersal and by providing manure. The yak is often termed as a 'ship of the snow'. It is one of the most important animals of Ladakh and the other Himalayan mountains adjoining the Tibetan border. Yaks are also found in isolated pockets of the cold climatic regions of Himachal Pradesh, Sikkim, Arunachal Pradesh and the Garhwal Hills of Uttarakhand. The yak population in India is 0.08 million, as per the Livestock Census of India, 2007.

In India, no specific breeds of yak have been classified, but the wide variability in phenotypes and colour pattern in different yak herds presents ample scope for the classification of yak into breeds. The yak population in India is very small and spread over isolated hill pockets. Due to an unpredictable environment and other natural calamities, these small yak populations are more vulnerable than any other species of farm animals. In India, yaks are found in the northern and western Himalayan states in regions that experience heavy snowfall and where the temperature may drop below –50°C in a few places. These small populations of yaks are distributed in the Himalayan states, namely the Ladakh region of Jammu and Kashmir, the Kinnaur, Spiti and Chamba districts in Himachal Pradesh and a small population in the Garhwal Hills of Uttarakhand State (Gupta *et al*., 1996). In the north-eastern region, they are found in western and eastern Sikkim, and the Twang and West Kameng districts of Arunachal Pradesh. These populations have been geographically isolated from each other since the distant past, and no interbreeding has taken place. Thus, Indian yaks can be described as of four types based on their geographic distribution. This has also led to considerable variability in their phenotypes.

14.2.8 Mithun

Mithun (*Bos frontalis*), the domesticated, free-range bovine species, is an important component of the livestock production system of the north-eastern hilly region of India. This unique bovine species is believed to have been domesticated more than 8000 years ago. The mithun population in India is 0.26 million, as per the Livestock Census of 2007, and is declining (0.28 million in 2003 census). The mithun is reared primarily as a meat animal and is highly preferred among the tribal people of the north-eastern region of India. The mithun is also used as a ceremonial animal, and plays an important role in the economic, social and cultural life of the tribal people of the north-east. Besides, it has now been established that superior-quality milk and hide can be obtained from mithun (Gupta *et al*., 1999).

There are two distinct breeds of mithun, namely Nagami and Arunachali, in the north-

eastern region of India. These are distinguishable on the basis of their body conformation, shape of forehead, coat colour, hair pattern, body weight, etc. Based on the available information on different phenotypes including coat colour pattern, horn pattern, body size and conformation, the mithun population of Arunachal Pradesh can be classified into three groups, that is Aka, Nishi and Adi. These animals also differ markedly according to their sacrificial requirements. There is an urgent need to classify them into definite breeds or strains by using standard breed descriptors. The National Research Centre on Mithun was established by ICAR in 1988 in the state of Nagaland to conserve, propagate and improve this species for future use. The mithun are well suited to the climatic conditions of the north-eastern hilly region of India, where cattle face difficulties in their survival.

14.2.9 Pigs

Pigs are the most efficient domestic animals in converting feedstuffs and household and agricultural wastes into edible meat. They grow fast and have high prolificacy. As per the Livestock Census of India, 2007, the pig population in India is 11.13 million. Indigenous pigs produce the cheapest meat among all carcass-producing animals that can be afforded by the poorer sections of society.

Pigs are normally fed with kitchen waste and homemade feed and other available vegetable/crop by-products, such as banana stems, tubers, brewer's grain and other ingredients, including a salt and mineral mixture. Some degree of selection has been made and the indigenous pig population has been classified into distinct breeds/groups such as the Ankamali from the Ankamali Block of the Ernakulum district of Kerala; the Ghori in parts of West Bengal and Assam; the Mali and Dome in Tripura, Manipur and Nagaland; and the Ghoongroo in the Darjeeling and Siliguri districts of West Bengal. Indigenous pig breeds, though given nomenclature at the local level, are yet to be characterized.

Indigenous small pigs (known as desi) are present throughout India. There is a wide variation in its phenotypes, colour pattern, litter size and growth rate under different agroecosystems. The pig populations/breeds available in different parts of the country are performing reasonably well in the climatic conditions of the area.

14.2.10 Poultry

Indigenous poultry breeds are hardy but poor in growth and productivity. They are reared as part of Indian farmers' tradition. They are kept in the backyards of houses under a zero-input system. Poultry rearing is associated with the culture of the area, and they are kept for meat, egg or game purposes. A wide range of variations is found among these breeds in relation to body weight, plumage and skin colour, feathering and comb type. These breeds have acquired considerable adaptability to local climatic conditions and resistance to tropical diseases. They are best suited to contribute to the economic benefits in their respective home tracts. The traditional system of poultry keeping, although losing its importance from day to day under the impact of modernization and industrialization, is still prevalent in rural and tribal areas of the country.

As per the 2007 Livestock Census of India, the poultry population in India is 648.88 million. There are 20 indigenous breeds of chicken in India. Some native breeds have better meat quality, while some others resemble Leghorn in size and shape, but are poor layers. These native breeds are broody and are the best mothers for hatching. They are good foragers and have comparatively better resistance to poultry diseases.

The Kadaknath is a dual-purpose breed with poor mothering ability, and is found in the Jhabua and Dhar districts in western Madhya Pradesh. It is also known as 'Kalamasi' because of its black flesh (Fig. 14.3). The Aseel breed is native of Andhra Pradesh, particularly coastal Andhra Pradesh. These birds are also found in other

Fig. 14.3. Kadaknath poultry, known for its black flesh.

countries, being kept by people who love cockfighting. It is a game bird, well known for its pugnacity, high stamina, majestic gait and dogged fighting qualities. It is the largest of the native breeds, and measures 28 inches from back to toe. The Denki is a native of Andhra Pradesh, having a glossy and lustrous plumage, with a compressed single comb. The Kalahasthi also is a native of Andhra Pradesh. These birds are smaller in size. The cocks have long necks and legs. They are good fighters. These birds are comparatively resistant to some of the common diseases. The Ghagus breed is a native of Andhra Pradesh and Karnataka. These birds also are small in size and have a single comb. The Nicobari is a native of the Andaman and Nicobar Islands and is known locally as 'Takniet hyum', which means short-legged chicken. The birds produce around 130–140 eggs year^{-1} of a relatively small size (40–45 g). The naked neck is found in the Andaman and Nicobar Islands and the north-eastern states. The homozygous naked neck birds have no feathers on their neck. These birds can dissipate heat because of the lack of feathers in the neck. The home tract of the Frizzle fowl breed is the north-eastern states and Andaman and Nicobar. These birds have curled feathers because of the presence of a dominant frizzle (*F*) gene and have better heat tolerance. The home tract of the Ankleshwar breed is Gujarat.

The Busra is found in Gujarat and Maharastra. These birds are small to medium in size. The home tract of this breed can be found in all parts of the country. The birds are small in size, dual-purpose, attractive foragers and excellent sitters. They can tolerate high temperature very well. The home tract of the Brown Desi breed is Uttar Pradesh. These birds are light to deep brown, layer-type, single-combed bird. The Doathigir breed is found in Assam. It is a fairly heavy breed, with good juvenile

growth. The Haringhata Black is from West Bengal. It is a small-bodied, black bird with typical layer conformation. The home tract of the Kashmiri Faverolla breed is Kashmir. The birds are small in size, with a small comb and wattles. The feathered comb is the peculiarity of this breed. The native tract of the Miri is the Dhemaji, Lakhimpur and Sivasagar districts of Assam. These birds are small in size and black in colour and are reared mostly by Miri tribes. The Punjab Brown is a native of Punjab and Haryana. It is a meat-type bird having brown plumage with a yellow beak, legs and feet. Titri birds are small, with speckled black and white feathers and yellow beak and legs. The Tellichery breed is from Kerala. It is a small bird having black skin. Plumage colour varies from black to grey, and sometimes with various combinations of colour. The meat is said to have medicinal value. The Indian breeds of poultry available in different parts of the country are very hardy and well adapted in the climatic conditions of their breeding tract.

14.3 Indigenous Livestock Resources of Asia and Other Continents

The words 'indigenous breeds' mean the locally available breeds, for a particular country or continent (Kohler-Rollefson, 2005). Looking at the worldwide perspective, there is huge diversity in locally available breeds among different continents. Starting with the world's biggest and most populous continent, Asia, it is very rich in terms of biodiversity, with a number of major indigenous livestock breeds including 239 cattle, 88 buffalo, 265 sheep, 182 goats, 141 horses and 229 pigs, making a total of 1144 breeds. Such huge diversity is expressed due to the diverse climatic conditions and geographical features ranging from arctic and subarctic in Siberia to tropical in southern India and South-east Asia (FAO, 2007b).

Africa is the second largest continent on the earth, with a comparatively lesser number of livestock breeds locally available. There are 154 cattle breeds, 2 buffalo breeds, 109 sheep breeds, 86 goat breeds, 36 horse breeds and 49 pig breeds, making 436 altogether as locally available breeds of livestock (FAO, 2007b). Africa has 44 indigenous breeds of camel, which is the highest in the world. This is due to the fact that most of the northern half of the continent is primarily desert.

Europe, the world's second smallest continent by land area, has a tremendous livestock breed diversity, with a total of 1300 breeds, which includes 277 breeds of cattle, 11 of buffalo, 458 of sheep, 170 of goat, 165 of pig and 269 of horse. A large portion of the region's land is suitable for agriculture, especially in the north, where the moist, cool climate is conducive to the growth of rich pastures that can support a high density of livestock (FAO, 2007b).

Latin America and the Caribbean, comprising South America, the Caribbean and Middle America, are relatively low in breed diversity, with a total of 345 breeds, 129 of which cattle, 11 of buffalo, 26 of goat, 47 of sheep, 67 of pig and 65 of horse. Most of the breeds have been domesticated during exploration and colonization (FAO, 2007b). North America has a total 104 breeds of major livestock species, which include 29 of cattle, 3 of goat, 31 of sheep, 18 of pig and 23 of horse (FAO, 2007b). The south-west Pacific region around Australia has a total 108 breeds of major livestock species that include 26 of cattle, 2 of buffalo, 11 of goat, 35 of sheep, 12 of pig and 22 of horse (FAO, 2007b).

As far as the major species of cattle, buffalo, sheep, goat, horse and pig are concerned, it can be seen clearly from the data above that diversification acts as a critical measure of adaptation in changing climatic conditions. The preponderance of such diversified livestock breeds throughout the continents, as well as the world, indicates the ongoing adaptation process towards changing climate over periods of time. The advantage of locally adapted breeds over the 'exotic' breeds can be very well envisaged in the following examples. The East African shorthorn zebu cattle is moderately resistant to brucellosis; the N'Dama and Guadalupe Creole cattle of Africa are highly resistant to

dermatophilosis; N'Dama cattle are resistant to trypanosomiasis; cattle belonging to the *Bos indicus* group, i.e. Indian native cattle, are tolerant to haemoparasites and ticks, and Indian Sahiwal cattle are highly resistant to tick attacks. Among small ruminants, the Red Maasai sheep of Eastern Africa are found to be tolerant of gastrointestinal nematodes; similarly, breeds of sheep like the St Croix of the US Virgin Islands, the Garole sheep of India, the West African Dwarf sheep, the Barbados Blackbelly sheep of the Caribbean Islands and the Indonesian thin-tailed sheep are found to be more or less tolerant to gastrointestinal nematodes and fluke infestation. The rare Wensleydale sheep of the UK is highly resistant to scrapie infection, and the West African Dwarf goats are found to be resistant to gastrointestinal nematodes and trypanosomosis. Looking into other economically important livestock animals, the Pantaneiro horses of Brazil are resistant to infectious equine anaemia; the local indigenous pigs of the Democratic Republic of the Congo, Angola, Sudan and Mozambique are moderately to highly resistant to African swine fever (Kohler-Rollefson, 2005).

14.4 Indigenous Versus Cross-bred/ Exotic Animals in Changing Climate

It is generally found that the production of cross-bred animals is higher than that of native animals when only the production traits are analysed between cross-breeds and native animals, due to heterosis in F_1. However, when the criteria for adaptability are also observed, native breeds excel in performance. As a simple example, the Indian dairy sector has been the most researched and policy emphasized sector, even pre-independence. If we observe the long-term analysis of the milk production data of India, it indicates that India's annual milk production has increased by approximately 5.8 times during 1950/51 to 2005/06. As per the Indian Livestock Census, this increase in milk production is credited largely to the significant increase of the native dairy buffalo population, which is a major source of milk in the country (Chhabra *et al*., 2009), though there is also a considerable share of exotic/cross-bred cattle. The cross-bred animals are reared mostly on fully intensive systems, with management practices aimed at reducing climatic variations; however, the buffalo population is reared under semi-intensive or extensive systems, with the least inputs and negligible control over climatic changes. This shows that with constant selection, the production performance of native livestock can be improved to the level desired, even with rapid global climatic changes. Thus, looking from the perspective of climate change, it can be concluded without a doubt that the native Indian dairy buffalo has performed well under changing climatic conditions over a period of time.

The reproductive performance of the Mashona, Nkone, Tuli and Afrikaner indigenous cattle of Africa was found to be superior to their crosses (Moyoa *et al*., 1996). Scholtz and Theunissen (2010) have also advocated the use of indigenous cattle in a terminal cross-breeding programme to improve beef production in sub-Saharan Africa to increase the feedlot performance in F_1 and reduce calving difficulties.

14.5 Breeding of Locally Adapted Livestock Species

The majority of indigenous livestock around the world are bred locally and kept by small-scale livestock keepers and pastoralists, especially in developing nations. These breeds may be less productive than their high-yielding exotic relatives, but they are supremely adapted to the harsh environments where they dwell and can produce under conditions where other breeds cannot survive. Indigenous breeds are drought tolerant, as well as disease and heat resistant. In the 12,000 years since livestock were first domesticated, more than 7000 breeds have been developed, many of which have adapted to a specific habitat and been shaped, often over centuries, by the cultural preferences of

a particular community (FAO, 2007a). Examples of local adaptation are the N'Dama cattle and the West African dwarf goats, both of which have been bred in the tsetse fly infested zones (subhumid and humid zones) of West and Central Africa, where trypanosomiasis is prevalent (Bosso, 2006). These breeds have a proven ability to survive, reproduce and remain productive without recourse to drugs. Djallonke sheep and goats in Central Africa have demonstrated similar resistance to tsetse flies. The raising of these indigenous, trypano-tolerant livestock is one approach to control disease and reduce the risk of inducing drug resistance in trypanosome strains. It has also been reported that trypano-tolerant cattle, especially the N'Dama breed, show superior heat tolerance than zebu cattle. Moreover, they metabolize water with greater economy, making them better adapted to the hot and water-stressed regions of Africa, conferring obvious advantages in the face of climate change.

Presently, more emphasis is given to production, reproduction, growth and morphological traits in the selection of animals. Inclusion of climatic/disease-resistance traits in selection is a relatively new concept and has received great attention from researchers. The differential environmental adaptability of breeds is the new attraction due to global climatic changes. Indigenous breeds are said to be comparatively heat tolerant; however, this lacks scientific evidence. This needs to be characterized and understood at the genetic level. Climate change projections suggest that further selection for breeds with effective thermoregulatory control may be needed. Breeding for climate change adaptation or mitigation will not be fundamentally different from existing breeding programmes; however, the phenotypes relevant to adaptation have to be identified. Breeding indices should also include traits associated with thermal tolerance, low-quality feed and disease resistance, and give more consideration to genotype–environment interactions (G × E) to identify the animals most adapted to specific conditions.

14.6 Conclusion

The world possesses a rich biodiversity of animal genetic resources spread over diverse agroclimatic regions. Various breeds/species of livestock have been developed over thousands of years, able to survive and withstand climatic conditions, geographical limitations and locally available feed and fodder resources. These adverse climatic conditions have reduced the productivity of livestock to a great extent, and therefore climate change is one of the important factors affecting the livestock sector. Hence, research efforts need to be undertaken to improve the productivity of the animals that are being maintained in very adverse climatic conditions.

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15 Enteric Methane Emission: Status, Mitigation and Future Challenges – An Indian Perspective

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Abstract

Atmospheric CH_4 is increasing at a rate of 1% per annum. To stabilize this greenhouse gas in the atmosphere, global CH_4 production needs to be reduced by 10–20%. Ruminants fed on low-quality feed/fodder produce over 75% of the CH_4 generated by ruminants worldwide. Strategic supplementation to improve digestive efficiency in these animals could halve this CH_4 production per unit of feed consumed. Supplementation to improve the efficiency of feed utilization coupled with increased product output may thus reduce CH_4 production per unit of milk or meat by a factor of 4–6. The dietary/nutritional strategy that improves productivity with no potential negative effects on livestock health and production is cost-effective and has a better chance of being adopted. Other strategies (biotechnologies, additives) are promising, but the diversity and plasticity of the functions of the rumen bacterial and methanogenic communities may be the limiting factor for their successful application. In addition, the environmental impacts of strategies should also be taken into consideration. A global vision of production systems that considers all greenhouse gas emissions from the animal up to the farm scale, as well as grassland use, is essential. Further, the sustainability of CH_4-suppressing strategies is an important issue. An effort is made in this chapter to address enteric CH_4 emissions and their current status. An overview on the possible ameliorative strategies has also been given.

15.1 Introduction

In February 2007, the IPCC of the United Nations released a report that said global warming was 'very likely' – meaning that there was an at least 90% certainty that it was caused by human activity (IPCC, 2007). That document forecasts that the average temperature will rise by 1.8–4°C by 2100, and in turn sea levels will also creep up by 17.8–58.4 cm. In addition, a rise of 9.9–19.8 cm is likely to occur if polar sheets continue to melt. The IPCC also reported that snow cover since the late 1960s had decreased by about 10%. The lakes and rivers in the northern hemisphere are frozen over about 2 weeks less each year than they were in the late 1960s. Mountain glaciers in non-polar regions have also been in 'noticeable retreat' in the 20th century, and the average global sea level has risen between 0.1 and 0.2 m since 1900. In simple words, the world is

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getting warmer and the temperature is rising faster than ever. The IPCC predicts more floods, intense storms, heatwaves and droughts. Its study forecasts a rise of 1.4–5.8°C in the global mean surface temperature over the next 100 years, where developing countries like India are most vulnerable.

15.2 Consequences of Global Warming

Even given the moderate 0.8°C increase in temperature to date, the impacts are already felt in many parts of the world, including India. For example, the summer extent of the ice mass in the Arctic Ocean has already shrunk by about half since the 1970s (Stroeve *et al*., 2007). Since the ice is also thinning dramatically at the same time, the volume of ice declines even more rapidly (Kwok *et al*., 2009). If warming should escalate unabated to 4°C or more, it might fundamentally change the earth system, along with all its ecological resources and services. Global temperature differences on such a scale would correspond roughly with the difference between temperature at the peak of the last Ice Age 20,000 years ago and temperature today. In a nutshell, global warming has the following consequences:

- The sea level rises due to the thermal expansion of seawater and the influx of meltwater into the oceans, and the warmer it becomes, the faster the sea level rises (Rahmstorf, 2007). Since 1880, the global sea level has risen by around 20 cm. It could, however, rise by 50–150 cm by 2100 (Rahmstorf, 2007).
- Already, an increase in extreme weather events such as heatwaves, droughts, extreme rainfall, floods and tropical storms has been observed in many regions (IPCC, 2007). A further rise of extreme weather events in the wake of additional warming is probable, depending on the type of event.
- Global warming in excess of 2°C threatens to accelerate the loss of genetic species and ecosystem diversity, since many regions in the world will very rapidly enter climatic conditions not experienced for several million years. According to the IPCC (2007), this would place such an intolerable strain on nature's adaptive and regenerative capacity as to risk the irreversible loss of 20–30% of animal and plant species and associated genetic resources. Ecosystems such as mangrove forests and coral reefs would suffer irreversible damage or destruction. Biodiversity loss would result in loss of ecosystem resources and services, for example the availability of clean drinking water and genetic resources, which are also crucial functions for society's efforts to adapt to climate change.

15.3 Greenhouse Gases

The IPCC included six gases, i.e. carbon dioxide (CO_2), methane (CH_4), nitrous oxide (N_2O), hydrofluorocarbons (HFCs), perfluorocarbons (PFCs) and sulfur hexafluoride (SF_6), which have high global warming potential (i.e. the CO_2 equivalence of a particular gas, when integrated over a time horizon of 100 years, is referred to as its global warming potential (GWP)). Three gases, namely CO_2, CH_4 and N_2O, occur naturally in the atmosphere in small quantities and are being added continuously, and therefore their concentration is increasing due to human activities. The other three gases normally do not exist in the atmosphere, but are added, due to industrial activities, in small quantities that are sufficient to increase global warming substantially, as these gases have comparatively high GWP. The percentage of the relative contribution of greenhouse gases (GHGs) is represented in Fig. 15.1.

GHGs allow solar radiation to pass through the earth's atmosphere. But after the earth absorbs part of that radiation, it reflects the rest back; that is where the problem lies. Particles of GHGs absorb the radiation that warm up the atmosphere. The increasing levels of GHG are causing too much energy to be trapped – the so-called 'greenhouse effect' (IPCC, 2001). Globally, agriculture, and in particular enteric

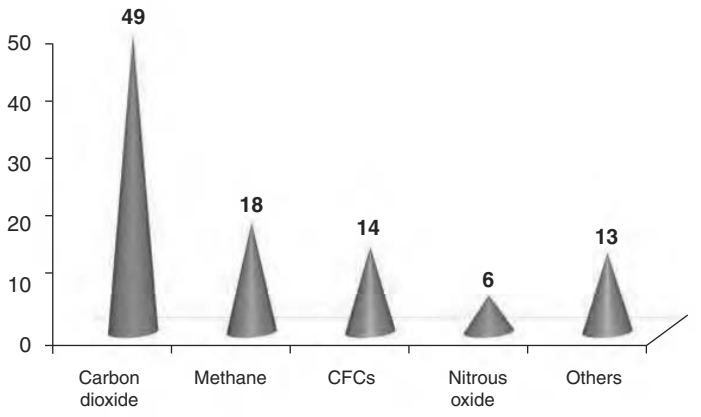

Fig. 15.1. Relative contribution (%) of greenhouse gases to atmospheric warming. (From WRI, 1990.)

fermentation in ruminants (predominantly cattle and small ruminants), produces between 21 and 25% of the total anthropogenic emission of CH_4 (Fig. 15.2).

The GHG emissions from a country also depend on the food habit of the populace, as depicted in Table 15.1. Maximum GHGs (kg CO_2-equivalent (eq) kg^{-1} product) are produced from beef, followed by pork, poultry, milk and wheat. A relative comparison for the consumption of plant and animal based product is given in Table 15.1. Therefore, communities consuming either vegetarian foods or pork and poultry based non-vegetarian foods are responsible for lower global warming than the communities that consume beef or other similar meat.

CH_4 is an important component of the increasing gases in the atmosphere, and is the one most associated with animal agriculture (Bhatta *et al*., 2005, 2006a). The accumulation of CH_4 has increased dramatically since the 18th century (see Fig. 15.3), due to intense industrialization. Prior

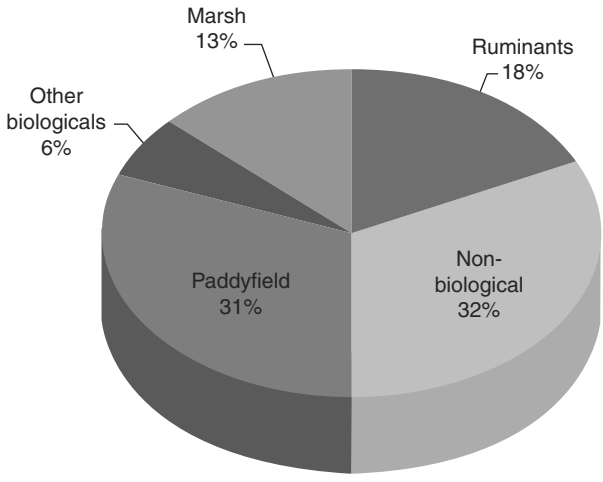

Fig. 15.2. Relative contribution of biological resources to the global production of CH_4 (Tg $year^{-1}$). (From Bolle *et al*., 1986.)

Table 15.1. Greenhouse gas emissions for a meal. (Based on Garnett, 2009; Lesschen *et al.*, 2011; Kamra, 2014.)

Type of food	GHG emission (kg CO_2-eq kg^{-1})
Wheat	0.8
Milk	1.3
Poultry	1.6
Pork	2.5
Beef	22.6–30.0

to this, the rise in temperature and composition of the atmosphere had changed little, but now appears to be in an exponential period (Fig. 15.3). CH_4 is accumulating at a faster rate, and apparently is responsible for a small proportion of the depletion of the protective ozone layer.

15.4 Livestock Production and Enteric CH_4 Emission

Livestock production plays four important roles in the release of gases into the atmosphere (Leng, 1991): (i) directly by anaerobic digestion in rumen and indirectly contributes to global warming by (ii) anaerobic decomposition of a part of the dung (iii) CO_2 production from fossil fuels associated with production and marketing infrastructure and inputs such as motorized transport, fertilizers, herbicides and insecticides and (iv) deforestation. Ruminants in natural production systems are inefficient, and increase in general production depends on an expansion of numbers. There is a growing appreciation that efficiency per animal can be improved manifold with simple technology inputs, which would have an impact on all four aspects of the contributions to global warming discussed above. The most important approach to be discussed in relation to the amelioration of GHG production by ruminants is to increase the efficiency of animal production from the resources available and to develop the capacity to produce more from fewer animals. It is critical to note that it is the rates of CH_4 production per unit product over a lifetime that identify the hotspots for major reductions in CH_4 emissions. Species wise, as well as region wise, estimates of enteric CH_4 emissions are shown in Table 15.2, to enable easy comparison.

Recently, Key and Tallard (2012) concluded that Asia Pacific contributed the most (32.74% of the total) to worldwide

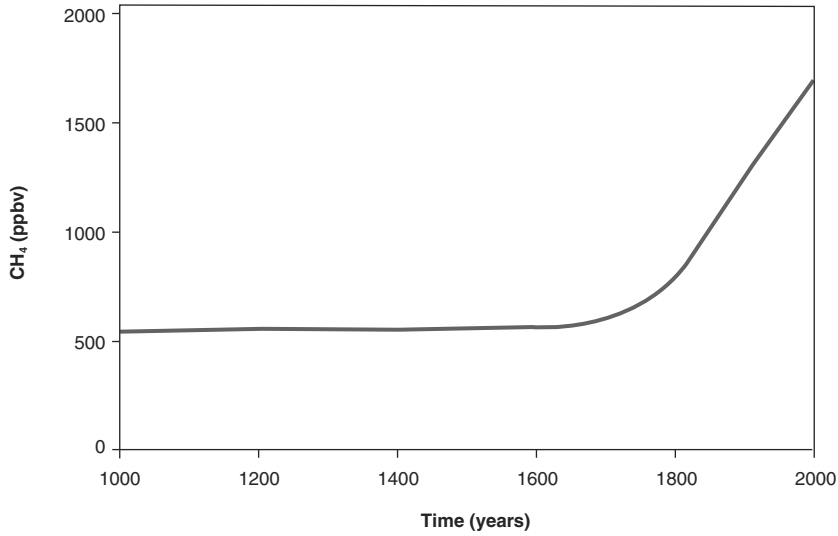

Fig. 15.3. Trends in atmospheric CH_4 accumulation. (From Khalil and Rasmussen, 1986.)

Table 15.2. Estimates of enteric CH_4 emissions from animals.

Animal type	Region	World population ($\times 10^6$)	CH_4 (kg head^{-1} year^{-1})	Total CH_4 (Tg)[b]
Cattle	Developed countries	573	55	31.8
	Developing countries[a]	653	35	22.8
Buffalo		142	50	6.2
Sheep	Developed countries	400	8	3.2
	Developing + Australia	738	5	2.4
Goats		476	5	2.4
Camels		17	58	1.0
Pigs	Developed countries	329	1.5	0.5
	Developing countries	445	1.0	0.4
Horses		64	18	1.2
Mules and asses		54	10	0.5
Humans		4670	0.05	0.3
Wild ruminants		100–500	1–50	2–6
Total				76–80

Notes: [a]Includes Brazil and Argentina (adapted from Crutzen *et al.*, 1986); [b]total estimate for emissions from domestic animals has an uncertainty factor of ± 15%.

enteric CH_4 emissions, followed by Latin America (23.47%), Europe (13.94%), Africa (13.54%) and North America (11.47%). They also projected the per cent change in total CH_4 emissions (Mt CO_2-eq) from livestock during 2008–2013 and documented that the maximum change during the past 5 years took place in the Asia Pacific region (14.9%), followed by Africa (12.6%). Among countries, Brazil ranked first in terms of total livestock CH_4 emissions, and contributed 13.10% to the total. India, China, the USA and the European Union (27) occupied the second, third, fourth and fifth positions, respectively, in the list of top livestock CH_4-emitting countries (Key and Tallard, 2012). Therefore, these countries may be the hotspots for livestock CH_4 emission reductions. Enteric CH_4 reduction, particularly from Asian and African countries, is most urgent and imperative due to the high emissions per unit of product as a result of feeding highly fibrous feed material to livestock.

15.4.1 Enteric methanogenesis

The major ruminal microorganisms are accountable for hydrolysing the proteins, starch and plant cell wall polymers into amino acids and sugars. These simple products are then fermented to volatile fatty acids (VFAs), hydrogen (H_2) and CO_2. Major fatty acids like acetate, propionate and butyrate are then absorbed and utilized by the host animal (Fig. 15.4). The major producers for H_2 are those microorganisms that produce acetic acid in the fermentation pathway. Acetate and butyrate production from fermentation results in more CH_4 production, while propionate formation serves as a competitive pathway for H_2 use in the rumen. CH_4 accounts for a significant energy loss to the ruminant animal, amounting to about 8% of gross energy at the maintenance level of intake and falling to about 6% as the level of intake rises. The excreta of animals, if not managed properly, also contribute significantly to GHGs (Johnson and Johnson, 1995).

15.4.2 Indian scenario

Many agencies have made attempts to estimate enteric CH_4 emission from Indian livestock; estimates from such agencies are compiled in Table 15.3. The estimated emissions figure for enteric CH_4 revealed much variation (Table 15.3), depending on the methodology/approach used for

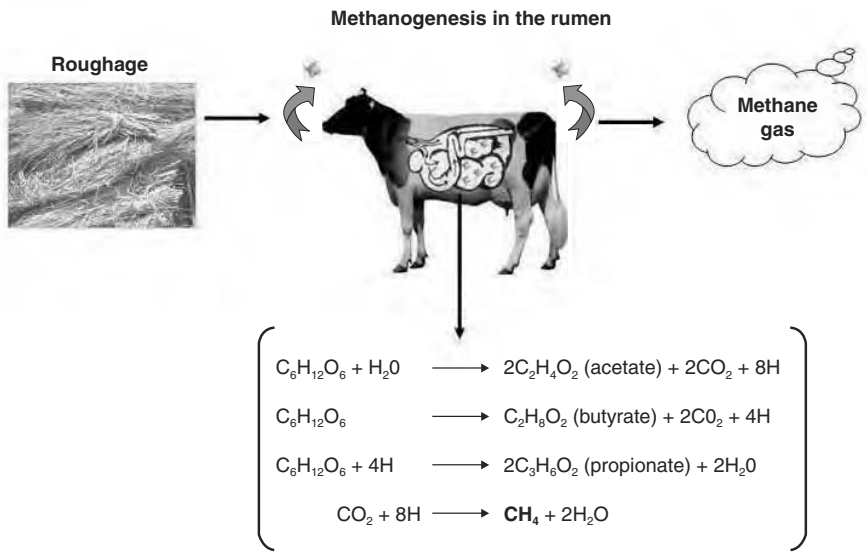

Fig. 15.4. Schematic representation of methanogenesis in the rumen.

calculation purposes. The Department of Animal Husbandry, Dairying and Fisheries, the Ministry of Agriculture, Government of India (2012) provided a figure of 13.27 Tg $year^{-1}$ for enteric CH_4 emissions from Indian livestock. To this, cattle and buffalo contribute 6.73 and 6.56 Tg $year^{-1}$, respectively, and were responsible for ~91% of the total emission from the country. Among cattle, indigenous cattle emit more enteric CH_4 than cross-bred cattle, attributed to their large population (Table 15.4) and poor feeding practices. Other livestock species like goats, sheep, yak, mithun, horses, donkey, mules, pigs, etc., contributed the rest (Table 15.4). Based on livestock and milk production data for 1994, Singhal *et al*. (2005) reported that lactating cattle and buffalo collectively emitted 53.6 g of CH_4 kg^{-1} of milk. A comparison of the emissions from different livestock species kept on different feeding regimens is illustrated in Table 15.5.

Slow growth, low milk yield and poor reproductive performance results in poor feed conversion and large CH_4 output relative to the product. CH_4 output relative to the product of ruminants depends on two factors: the efficiency of fermentation in the rumen and the efficiency of conversion of nutrients into product (e.g. milk, beef, draught).

15.5 Challenges

There may be direct or indirect effects of climate change caused by enteric CH_4 emission from livestock. The quantity and quality of the feed supplied to the animal is a major factor. In addition, the direct relationship between the feeding management of the animal, its thermal environment and the seasonal availability of forage may also have implications for livestock production systems.

15.5.1 Direct effects

The response of livestock in adjusting to changing environmental temperature emphasizes the key difference between ruminant and non-ruminant species in their comfort zones. Since ruminants have a wide comfort zone and a high degree of thermal tolerance, it is likely that climate change resulting in an increase of a few degrees is

Table 15.3. Estimates of enteric CH_4 emissions from Indian livestock.

Authors	CH_4 emission Tg year^{-1}	Base year	Approach
Swami and Bhattacharya (2006)	9.0	1997	CLRI estimates
	13.2	1997	IPCC default values (Tier 1)
	11.2	1997	NPL
	7.9	1997	ALGAS project
Singh *et al*. (2012)	9.1	2003	Tier 2
Patra (2012)	11.17	2003	Tier 2
	11.89	2007	Tier 2
Kamra (2014)	13.3	2012	Tier 1
Government of India	13.27	2012	Tier 1

Notes: CLRI = Central Leather Research Institute; IPCC = Intergovernmental Panel on Climate Change; NPL = National Physical Laboratory; ALGAS = Asia Least-cost Greenhouse Gas Abatement Strategy.

Table 15.4. Enteric CH_4 emission from different livestock species in India. (From Kamra, 2014.)

		Enteric CH_4 emissions		CH_4	CH_4
Category	Number × 1000	kg head^{-1} year^{-1}	Tg head^{-1} year^{-1}	emission Tg year^{-1}	emissions (%)
Cattle – Cross-bred	43,266	46	1.990	2.163	14.87
Indigenous	1,64,935	25	4.123	4.562	31.36
Buffalo	1,09,316	55	6.012	6.559	45.09
Yak	71	55	0.004	0.005	0.03
Mithun	264	55	0.015	0.016	0.11
Sheep	72,676	05	0.363	0.378	2.60
Goat	1,41,155	05	0.706	0.737	5.06
Horse/pony	611	18	0.011	0.012	0.08
Mule	168	10	0.002	0.003	0.04
Donkey	458	10	0.005	0.006	0.04
Camel	517	46	0.024	0.025	0.17
Pig	11,905	01	0.012	0.083	0.57
Total	5,45,342		13.267	14.549	100.00

Note: IPCC default emission factors were used for calculations.

Table 15.5. Enteric CH_4 emission (kg^{-1} milk) on feeding different diets.

Category of animal	Diet fed	Milk yield kg^{-1} head^{-1} day^{-1}	CH_4 g kg^{-1} milk	Reference
Holstein dairy cows, mid-lactation, Australia	Concentrate + lucerne hay + algal meal	22.6–23.5	22.1–24.9	Moate *et al*. (2013)
Cross-bred cattle	Concentrate + roughage crop residues	5.16	25.1	Patra (2012) (data based on 2007 Indian livestock census)
Indigenous cattle	Concentrate + roughage crop residues	1.92	51.6	
Buffalo	Concentrate + roughage crop residues	3.99	30.4	
Goat	Grazing + supplemental feeding	0.3	50.8	

not going to have a major effect on their performance. The regions currently characterized by high rainfall and low temperatures may become more favourable for livestock production. On the other hand, high summer temperature may present the dairy cow and the buffalo with thermal stress that results in reduced feed intake and performance. Improved management systems such as the provision of shade and water should be adequate to counter this. However, non-ruminant species have a very narrow comfort zone. This is one of the reasons why pig and poultry enterprises have often been based on intensive housed systems in developed and developing countries such as India. Higher environmental temperatures in winter may lead to a saving of heating costs for poultry buildings in layers and in extra covering for small ruminants. However, in the summer months, the existing housing systems may not be able to cope with the increased thermal load. This may lead to an increased requirement for costly cooler systems, especially in the arid and semi-arid regions of India (Rowlinson, 2008).

15.5.2 Indirect effects

Climate change can be expected to have several impacts on feed crops and grazing systems, such as:

- change in herbage growth brought about by change in atmospheric CO_2 concentration and temperature
- changes in the composition of pastures, such as changes in the ratio of grasses to legumes
- changes in herbage quality, with changing concentrations of water-soluble carbohydrates and N at given dry matter (DM) yields
- greater incidences of drought, which may offset any DM yield increases
- greater intensity of rainfall, which may increase N leaching in certain systems (Hopkins and Del Prado, 2007).

Non-ruminant livestock will continue to receive diets consisting of cereals and cakes and therefore are likely to suffer more than ruminant animals as the latter have forage as a major component in the diet. In the case of extensive systems like those in sheep and goats, this may make up the entire diet, whereas in more intensive dairy cattle or goat rearing systems, the forage will be balanced by a more concentrated supplement. It is the source, quality and quantity of the forage component of a ruminant's diet that is likely to be affected by climate change (see Chapter 2, Section I, this volume for details). The result may be either advantageous or deleterious effects on the existing forage species. With respect to our existing forage species, low environmental temperature is one of the major limitations to higher DM production. Thus, any increase in temperature might be expected to have benefits on early-season growth. If the mean rainfall declines, this would lead to soil moisture deficits, which in turn would require more expenditure on irrigation, unless reductions in DM yield are accepted. For existing species, the stage of maturity at which the crop is cut is a major determinant of quality, and in any altered climatic scenario the interplay between increasing quantity and declining quality would continue to be of major importance, although the alterations in climate may be favourable to conservation and reduce losses during haymaking. In many hill and upland areas that are currently characterized by low temperatures and waterlogged soils, climate change may be expected to lead to more favourable conditions and result in a shift towards more productive species, with accompanying implications in animal production. The other major possibility is that climate change will lead to a shift in the forage species grown in a country. For example, elevated temperature may lead to an increase in the acreage of maize grown. This might be expected to result in improvements in both the quantity and quality of forage for ruminant livestock, and which could lead towards the forage component of rations fed to dairy cattle elsewhere being improved. In semi-arid regions of India, such as Rajasthan, palatable plant species

might be replaced by non-palatable species, which would result in a lower quantity of edible biomass for sheep on extensive rearing systems.

15.6 Mitigation Strategies

Increased understanding and improved quantification of CH_4 production in the rumen has implications not only for global environmental protection but also for efficient animal production. However, there appears to be uncertainty in the estimation of CH_4 emissions from livestock due to the limited availability of data to document variability at the farm level and also due to the significant impact of diet on enteric CH_4 production. CH_4 mitigation strategies require a robust prediction of emissions from the rumen. There are many methods available that would be suitable for measuring the CH_4 produced from the various stages of animal production. For the development of an accurate inventory, or to implement mitigation strategies, it is important that there is confidence in the accuracy of the CH_4 measurement technology. CH_4 emissions from livestock have been measured as part of the studies on ruminal fermentation, energy balance, evaluation of feed additives and, most recently, to characterize and reduce the contribution of ruminants to the global CH_4 burden. Livestock CH_4 emissions have been measured using respiration calorimetry systems such as whole-body chambers, head boxes, ventilated hoods and face masks (Johnson and Johnson, 1995). Data obtained from these techniques have been the foundation of the prediction equations used to generate mathematical models and countrywide and global inventories (Benchaar *et al*., 1998; Mills *et al*., 2001). To develop strategies to mitigate CH_4 emissions, the precise quantification of CH_4 emissions from ruminants under a wide range of circumstances is very essential. However, several factors need to be considered in order to select the most appropriate technique, such as the cost, level of accuracy required and the scale and design of the experiments to be undertaken. The selection of any technique depends on accuracy, as each one has its advantages and disadvantages.

15.6.1 Challenges and opportunities for CH_4 mitigation

Management of the H_2 production in the rumen is the most challenging factor to be considered when developing strategies to control ruminant CH_4 emissions (Joblin, 1999). It should therefore be possible to reduce CH_4 production by inhibiting H_2-liberating reactions or by promoting alternative H_2-using reactions or routes for the disposing of H_2 during fermentation (Fig. 15.5; Boadi *et al*., 2004).

There are many reports on *in vivo* CH_4 mitigation strategies, including the use of chemicals against methanogens, defaunation, plant secondary metabolites, electron acceptors, etc., but each one of the techniques has some advantages and disadvantages of using them in the diet of ruminants. These have been summarized in Table 15.6. All the approaches that have the potential for enteric CH_4 mitigation are debated in detail elsewhere in subsequent chapters of this book.

15.6.2 Nutritional strategies

Johnson *et al*. (2000) reported that fermentation of cell wall carbohydrates produced more CH_4 than fermentation of soluble sugars. This is a consequence of decreased rates of ruminal fermentation and passage out of the rumen that favour a higher Acetate:Propionate (A:P) ratio. On the contrary, high grain diets fed at high intake levels are associated with high rates of ruminal digestion and passage that favour higher propionic acid production. For example, when brewer's grain was added to a Timothy hay (60 parts) and maize (25) based diet, the CH_4 output in goats was reduced by 10% (Bhatta *et al*., 2005, 2007, 2008). However, although an increased grain component in ruminant rations reduces CH_4 production, the challenge is the importance of ruminants in converting fibrous feeds

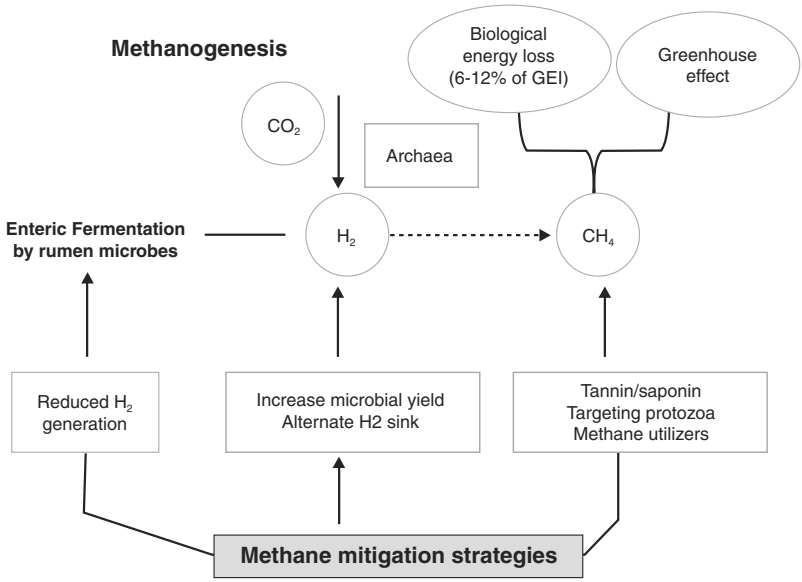

Fig. 15.5. Schematic presentation of CH_4 mitigation strategies in ruminants.

unsuitable for human consumption to high-quality protein.

Feeding of oils and fats

Oil cakes are better feed ingredients that can be included in the ration of livestock to increase energy and protein content. Some of the components of oils have antimicrobial and antifibrolytic activity, and therefore free oils cannot be fed beyond a certain level. Beauchemin and McGinn (2006) reported that adding canola oil at the rate of 4.6% of DM intake inhibited CH_4 emission by 32% and decreased CH_4 emissions as a per cent of gross energy (GE) intake by 21%, but this decrease in CH_4 emission was attributed primarily to reduced feed intake and lower total tract digestibility of feed, especially the fibre component.

Diet effect on CH_4 production

Methanogenesis decreases by improving the quality of feed, either by increasing the digestibility of lignocellulosic feeds by some chemical means (Moss *et al*., 2000) or microbiological treatment or by replacing these feeds with good-quality concentrate feeds (Bhatta *et al*., 2008). The inhibition of methanogenesis is accompanied by increased propionate levels and decreased acetate to propionate ratios and better feed conversion efficiency. Livestock grazing poor-quality pasture will produce more CH_4 per unit of product (Kirschgessner *et al*., 1995). The treatment of paddy straw with urea resulted in a significant improvement in the digestibility of nutrients and a significant reduction in methanogenesis. Even different roughage sources like paddy straw, sugarcane bagasse and wheat straw caused different levels of CH_4 production. Paddy straw supported more CH_4 production than the other two roughage sources (Chatterjee *et al*., 2005). Therefore, proper selection of the source of roughage in ruminant rations may help to reduce methanogenesis to some extent.

Stage of forage growth

McAllister *et al*. (1996) and Moss *et al*. (2000) noted that CH_4 production in ruminants tended to increase with the maturity of the forage, and the CH_4 yield

Table 15.6. Possible technologies available for practical application in CH_4 mitigation.

Technique	Strategies advantages/limitations	Potential for use in field conditions	Reference
Reducing the number of low/or non-producing livestock	Due to high number of low-producing or non-producing ruminants, CH_4 emission kg^{-1} livestock product is high. However, removal of such livestock is not possible due to the ban on cow slaughter in many states of India. But it is possible by reducing their number strategically over a long period	Slow	Bhatta *et al*. (2012c)
Use of low CH_4-producing diets (cereals and oil cakes)	It is clearly established that feeding concentrates produces less CH_4 than roughage; economic feasibility is a question mark	Low	Bhatta *et al*. (2008)
Improving the quality of feed offered to ruminants	Increased digestibility of roughage reduces CH_4 emission and improves ruminant productivity	High	Das and Singh (1999), Sahoo *et al*. (2000), Haque *et al*. (2001), Bhatta *et al*. (2008)
Removal of protozoa from rumen defaunation	Removal of ciliate protozoa from the rumen results in 20–30% lower CH_4 production. However, it is practically impossible to maintain protozoa-free ruminants	Moderate	Bhatta *et al*. (2011, 2012c)
Reductive acetogenesis in the rumen	Acetogens in the rumen compete with methanogens for hydrogen in the rumen, but their affinity is low as compared to methanogens	Low	Van Nevel and Demeyer (1996) Lopez *et al*. (1999)
Plants containing tannins, saponins and essential oils	There are a good number of reports on the efficacy of plant secondary metabolites (PSMs) on CH_4 reduction	High	Patra *et al*. (2006), Kamra *et al*. (2008), Bhatta *et al*. (2009, 2012c, 2013a,b,c)
Ionophores	Effective in reducing CH_4 by 30–35%, but is banned in many countries	Low	Nagaraja *et al*. (1997)
Inorganic compounds as terminal electron acceptor	Nitrate can be a good CH_4 inhibitor, but use of nitrate-reducing bacteria as a probiotic is a must to check nitrate poisoning	Good	Sakthivel (2011), Sakthivel *et al*. (2012)
Vaccine against methanogens/ciliate protozoa	Still a potential area	High	Wright *et al*. (2004)

from ruminal fermentation of legume forages was generally lower than the yield from grass forages.

Feeding frequency of the ration

It was observed that low frequency of feeding increased propionate, reduced acetic acid production and lowered CH_4 production in dairy cows. This was attributed to the lowering of methanogens as a result of high fluctuations in ruminal pH, since low frequency of feeding increases diurnal fluctuations in ruminal pH that can be inhibitory to methanogens. On the other hand, more frequent feeding was shown to increase the A:P ratio (Sutton *et al*., 1986).

Use of complete feed blocks/total mixed ration

Feeding complete feed blocks comprising 70% roughage and 30% concentrate ingredients could be an alternative approach as it ensures nutrient balancing, leading to better productivity and lowering CH_4 production by 10%. Similar effects were observed when animals were provided total mixed ration blended with roughage and concentrate feeds. This approach could be best suited to Indian conditions, as it helps in better utilization of unconventional feeds, reduces wastage and helps in lowering CH_4 production (Bhatta *et al*., 2008).

15.6.3 Manipulation of rumen fermentation

Feeding of ionophores

Mathison *et al*. (1998) described ionophores as highly lipophilic substances that were able to shield and delocalize the charge of ions and facilitate their movement across membranes. Their feeding has been associated with the selective reduction of gram-positive ruminococci and the proliferation of gram-negative bacteria with the concurrent shift in fermentation from acetate to propionate and decrease in CH_4 production (Newbold *et al*., 1997).

Defaunation

Defaunation, which is the partial/total elimination of protozoa from the rumen by dietary or chemical agents, has been shown to reduce ruminal CH_4 production by about 20–50%, depending on the diet composition (Itabashi *et al*., 1994). Protozoa in the rumen are associated with a high proportion of H_2 production, and are closely associated with methanogens by providing a habitat for up to 20% of rumen methanogens (Newbold *et al*., 1997). The reduced ruminal methanogenesis observed with defaunation can be attributed to factors such as a shift of digestion from the rumen to the hindgut (Van Nevel and Demeyer, 1996) or the loss of methanogens associated with protozoa during defaunation (Hegarty and Gerdes, 1998). However, this process could lead to reduced digestibility of poor-quality roughages, not very suitable where agricultural crop residues are the major roughage source, such as in India.

Feeding of plants containing tannins and saponins

Tannins constitute one of the important secondary metabolites that have antimicrobial activity and are widely present in different groups of plants. More attention has been paid to condensed tannins in comparison to hydrolysable tannins, perhaps due to lower risk of toxicity. Bhatta *et al*. (2006b,c, 2009, 2010) confirmed that tannins suppressed methanogenesis directly through their antimethanogenic property and indirectly through antiprotozoal activities. Samples containing both condensed tannin (CT) plus hydrolysable (HT) were more potent in reducing methanogenesis than those containing HT only. This was further confirmed through respiration chamber studies in goats (Bhatta *et al*., 2010, 2012c).

Microbial activity in the rumen may be affected by the use of saponins. The microbial population increased with low saponin supply but decreased when doses became excessive (Wallace *et al*., 2002). Protozoal counts in the rumen fluid decreased with higher saponin doses, as with sarsaponin from *Yucca schidigera* and quillaja saponin (Makkar and Becker, 1996), and with saponin-rich plants or fruit pulp (Kamra, 2006).

At the National Institute of Animal Nutrition and Physiology, Bangalore, India, under the Outreach programme on CH_4, various tree leaves and medicinal and aromatic plants have been screened to assess their efficacy as CH_4 suppressants. Promising results were obtained *in vitro* with some of the leaves tested. The data on the protozoa count indicated that the CH_4 suppression recorded with plant tannins was not due primarily to their defaunation property (Bhatta *et al*., 2012a,b).

Addition of propionate enhancers

As a result of growing awareness of the threat of microbial resistance to antibiotics, there is an increasing interest in alternatives to antibiotics as growth promoters (Moss *et al*., 2000). Dicarboxylic acids such as fumeric and malic acids have been studied *in vitro* as feed additives in ruminant diets to reduce CH_4 emissions. Reducing fumeric acid may provide an alternate electron sink for H_2, since H_2 ions are needed in this reaction.

Vaccination against methanogens

Researchers at CSIRO, Australia, have vaccinated sheep with a number of experimental vaccine preparations against methanogens, so that the animals produce antibodies against the residing methanogens. CH_4 production was reduced by between 11 and 23% in vaccinated sheep, with no long- or short-term adverse effects. Researchers anticipate that commercial vaccines will allow a 3% gain in animal productivity and a 20% reduction in CH_4 production. These vaccines were based on cultivable methanogens. However, the work of Whitford *et al*. (2001) showed that most ruminal methanogens had not yet been cultivated.

Alternate H_2 sink-reductive acetogenesis

In the gut of termites and rodents, acetogens convert excess H_2 to acetic acid, which is then utilized by the host (Joblin, 1999). However, in the rumen the number of acetogens is few and cannot compete effectively with methanogens for H_2, because they have a lower affinity for H_2 than methanogens (Nollet *et al*., 1998). Increasing the populations of acetogens through exogenous inoculations into the rumen could be useful for competing against methanogens (Joblin, 1999).

15.7 Conclusion

Mitigation of rumen CH_4 emission is essential and can be achieved effectively by strategies that improve the efficiency of animal production. The dietary/nutritional strategy that improves productivity with no potential negative effects on livestock health and production, and that is cost-effective, is a challenge for animal nutritionists. The approach to mitigation also depends on the production systems and agronomic practices in the region. Generation of a database on CH_4 production under different production systems adopting a common protocol would be useful in drawing mitigation strategies. Possible strategies to mitigate the emission of CH_4 from livestock are: replacing non-productive or low productive livestock with high productive livestock, enhancing the degradability of poor-quality roughages (Bhatta *et al*., 2005, 2008), use of alternate hydrogen sinks, feed additives like ionophores, plant secondary metabolites (Bhatta *et al*., 2006b, 2009) and chemicals, and the use of biotechnology techniques. Other strategies could be ration balancing and strategic supplementation of feeds.

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16 Thermodynamic and Kinetic Control of Methane Emissions from Ruminants

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Abstract

CH_4 emissions occur directly from animal digestion (enteric) and from animal waste that is stored under anaerobic conditions. In both regards, CH_4 emissions depends on kinetic and thermodynamic factors. With kinetic control, the profile of products formed depends on the relative rates of the different competing reactions. In turn, the rates of reactions depend on substrate concentrations and enzyme activities, and these enzyme activities depend on microbial growth or enzyme synthesis. With thermodynamic control, which pathway branches are available and the direction of metabolite flow depends on the concentrations of reactants and products. Biologists have focused on controlling the kinetic elements of fermentation such as enzyme function, microbial activity, gene expression or provision of substrates. However, fermentation is often controlled by thermodynamics. In chemistry, thermodynamics is quantified using Gibbs energy calculations. Whether or not a reaction can proceed spontaneously in the forward direction is represented by the change in Gibbs energy (ΔG), which can be calculated based on the ratio of products and reactants in the system. Using this calculation, a strongly negative ΔG indicates that a reaction could proceed strongly in the forward direction without the addition of energy to the system. A strongly positive value of ΔG indicates the reaction cannot proceed in the forward direction without the addition of energy to the system, and it may even run in the reverse direction. The analysis of thermodynamics in different situations in the rumen or manure storage facility can identify when CH_4 may be controlled kinetically by affecting the rates of reactions, or thermodynamically by affecting substrate or product concentrations.

16.1 Introduction

Livestock production worldwide generates about 7.1 Gt of carbon dioxide (CO_2)-equivalent (eq) greenhouse gas (GHG) emissions year^{-1}, or about 14.5% of all anthropogenic GHG emissions (Gerber *et al*., 2013). Methane (CH_4) emissions from livestock represent 44% of this total (Gerber *et al*., 2013). The roughly 3 billion cattle and sheep in the world convert 3–10% of their dietary gross energy to CH_4 gas (Johnson and Johnson, 1995). In addition, when animal manure is stored under anaerobic conditions, it releases CH_4 and nitrous oxide gas to the environment as it degrades. On a molar basis, CH_4 is as much as 50 times more potent as a GHG than carbon dioxide (Moss, 1993). Thus, ruminants are among the greatest contributors to global climate change of all anthropogenic sources,

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exerting effects similar to the airline industry or ground transportation (Steinfeld *et al*., 2006). Not only are these CH_4 emissions destructive to the environment, but wasting significant energy resources by converting them to emitted CH_4 gas is economically inefficient and wastes both food and energy resources needed by a growing and more demanding world human population. With such significant impacts, it is essential to find ways to decrease CH_4 emissions from livestock.

Unfortunately, despite the importance to the environment, food and energy security, there are few options to decrease CH_4 emissions reliably from cattle (Casmiglia *et al*., 2007; Buddle *et al*., 2011). Some types of ration and feed ingredients have been shown to affect CH_4 emissions. For example, CH_4 emissions per unit of fermentable carbohydrate increase as the ratio of forage to grain in the ration increases, and the use of ionophores shifts fermentation toward propionate and away from CH_4 (for details, see Chapter 17, Section III, this volume). Additionally, some feed ingredients like high-tannin feeds (also see Chapter 20, Section III, this volume) or oils decrease CH_4 production *in vitro*. However, one persistent problem is that fermentation systems often adapt to our manipulations and find a way to resume making CH_4 in the presence of the inhibitors. It seems to be the nature of fermentation to make CH_4 gas. One of the objectives of the chapter is to describe the underlying physics of fermentation that causes CH_4 gas to be produced.

Microbial anaerobic digestion and fermentation are important in nature, agriculture and industry. Understanding how fermentation is regulated could help improve waste disposal, fuel production and abatement of GHGs, as well as animal agriculture. Fermentation of simple sugars can result in many different products including lactate, acetate, ethanol and CH_4, among others. In order to understand why CH_4 is produced, we need to understand how fermentation processes are controlled. We need to understand why different fermentation systems convert the same simple sugars to so many different end products. Ultimately, this knowledge may help us optimize fermentations for various purposes.

It is clear that the microbial species present in a system determine the fermentation products produced by possessing different enzymes that catalyse reactions of different fermentation pathways. It is also well known that physico-chemical characteristics of the system like temperature, pH, osmolarity and passage rates determine which microorganisms can survive and grow and which ones cannot (Jay, 1996). Examples are abundant: acetate-utilizing methanogens grow in anaerobic digesters, but they grow too slowly to thrive in the rumen, where passage rates are faster (Russell and Wallace, 1997). Fermented foods like cheese, yogurt or sauerkraut only allow growth of microbial species that are adapted to low pH (Jay, 1996). Microorganisms also differ in the range of temperatures at which they can grow (Jay, 1996). However, the question remains as to why microorganisms that thrive in certain environments have evolved to catabolize sugars by a particular pathway or pathways instead of others.

Apart from the physico-chemical properties of the system, the polymer from which sugars are released can affect the pattern of fermentation products. For example, both cellulose and starch are hydrolysed to glucose in the rumen, which is catabolized through glycolysis to pyruvate (Russell and Wallace, 1997). When glucose is released from cellulose digestion, pyruvate is preferentially metabolized to acetate, along with CO_2 and H_2. The gases are converted to CH_4. When glucose results from starch digestion, more propionate is produced from pyruvate with decreased production of H_2. The decreased H_2 concentration decreases CH_4 production. Thus, CH_4 emissions are affected by diet (for more details on how dietary factors affect enteric CH_4 emission, see Chapter 22, Section III, this volume). The question is why microorganisms that digest cellulose preferentially metabolize glucose to acetate and CH_4, while starch-digesting microorganisms produce more propionate and less CH_4. Both cellulose and starch are

broken down to glucose and pyruvate through a common pathway. Starch digestion occurs faster than cellulose and results in lower pH, but even when pH was held constant, maize digestion resulted in a lower acetate to propionate ratio than hay (Russell, 1998).

Most fermentation systems are limited by the rates at which substrates can be obtained and products released. In industrial food and alcohol fermentations that occur in closed systems, where substrate is not added and products are not removed, thermodynamic equilibrium is eventually reached. But even open systems like the rumen have been shown to be near equilibrium. Using this knowledge, we demonstrated that the way glucose was utilized in the rumen was controlled in part by the equilibrium among end products (Ungerfeld and Kohn, 2006). A similar mechanism of control may be common to fermentation and other biological systems. The chapter will show how the pattern of fermentation products in the rumen changes, while still remaining near thermodynamic equilibrium, when the rumen environment is modified by feeding different diets or antimicrobials. Results suggest ways to improve our understanding of how fermentation is regulated in nature to yield different desirable or undesirable end products, and strategies to decrease CH_4 emissions are suggested.

The implications of the present chapter extend beyond the rumen ecosystem. In addition to decreasing CH_4 emissions to the atmosphere and decreasing loss of dietary energy from ruminant production, the understanding also applies to the way CH_4 is produced in manure storage or fuel production, as well as other agricultural systems like rice paddies and natural ecosystems like bogs.

16.2 Fermentation Balance

CH_4 synthesis cannot be understood or controlled without understanding all of the processes that produce substrates or remove products from the system. The amount of CH_4 produced in the rumen is a function of volatile fatty acid (VFA) production, as depicted in Fig.16.1. The principal catabolic reaction in the rumen providing energy to drive fermentation is the conversion of glucose to acetate

$$C_6H_{12}O_6 + 2H_2O \rightarrow 2CH_3COOH + 2CO_2 + 4H_2.$$

Another major reaction producing reducing equivalents is the conversion of glucose to butyrate

$$C_6H_{12}O_6 \rightarrow CH_3CH_2\,CH_2COOH + 2CO_2 + 2H_2.$$

The hydrogen does not accumulate because it is used readily in various pathways including formation of CH_4 and propionate

$CO_2 + 4H_2 \rightarrow CH_4 + 2H_2O$ (methane formation)

$C_6H_{12}O_6 + 2H_2 \rightarrow 2CH_3CH_2COOH + 2H_2O$ (propionate formation).

Wolin (1960) calculated the fermentation balance in the rumen to show how profiles of VFAs and gases (CH_4, CO_2) are linked. A different example will be calculated here. In order to conserve the mass of each element, and particularly to balance H_2, each reaction must be scaled appropriately. For example, if the relative molar concentrations of acetate, propionate and butyrate on a high-grain diet are 5, 2 and 1, the CO_2 and H_2 production that must accompany the VFA production can be quantified as the sum from each scaled reaction

5 ($C_6H_{12}O_6 + 2H_2O \rightarrow 2CH_3COOH + 2CO_2 + 4H_2$)

2 ($C_6H_{12}O_6 + 2H_2 \rightarrow 2CH_3CH_2COOH + 2H_2O$)

1 ($C_6H_{12}O_6 \rightarrow CH_3CH_2\,CH_2COOH + 2CO_2 + 2H_2$)

$8\,C_6H_{12}O_6 + 6H_2O \rightarrow 10CH_3COOH + 4CH_3CH_2COOH + CH_3CH_2\,CH_2COOH + 12CO_2 + 18H_2$.

Thus, 4.5 moles of CH_4 could be formed from the remaining CO_2 and H_2

$$4.5CO_2 + 18H_2 \rightarrow 4.5CH_4 + 9H_2O.$$

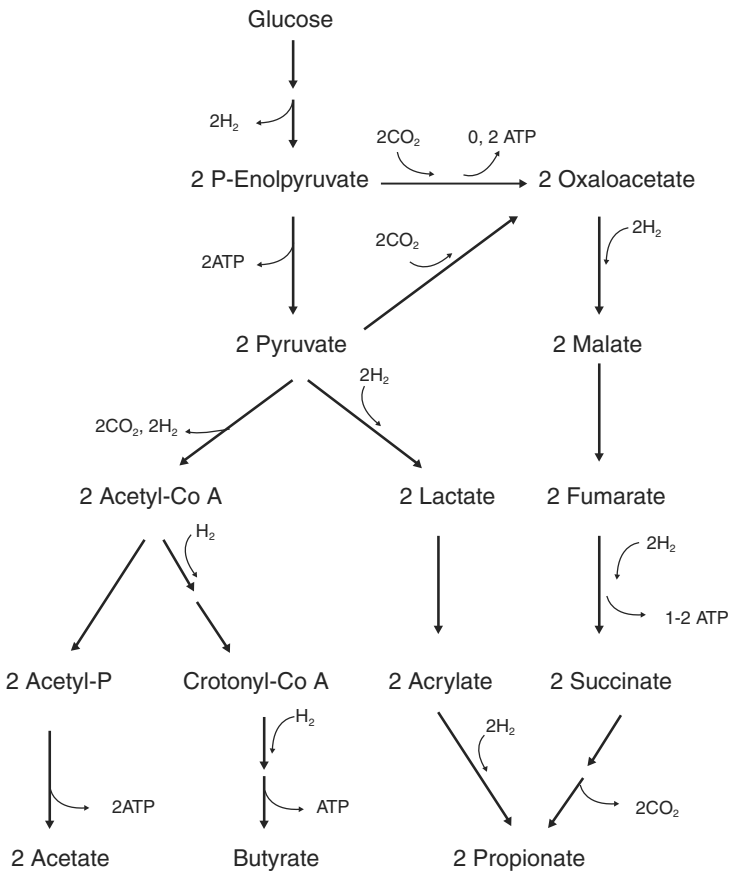

Fig. 16.1. Pathways for producing volatile fatty acids in the rumen.

The final balance is

$8C_6H_{12}O_6 \rightarrow 10CH_3COOH + 4CH_3CH_2COOH + CH_3CH_2\,CH_2COOH + 3H_2O + 7.5CO_2 + 4.5CH_4$.

The net reaction can be verified by ensuring that each element is balanced on both sides of the arrow: 48C, 96H and 48O. The result is an approximation excluding substrates and products that have minor effects. Additional sinks for H_2 that may decrease the CH_4 to CO_2 ratio include: microbial protein, which tends to contain more reducing equivalents than glucose, swallowing of air and transport of O_2 across the rumen wall, and sulfate (SO_4) and nitrate (NO_3) in the ration.

Thus, when the acetate to propionate ratio is 2.5 to 1, the estimated CH_4 to CO_2 is 4.5 to 7.5 or 0.60 to 1. The CH_4 per glucose molecule fermented is 4.5 per 8 or 0.56. For a high-fibre diet, the relative profile of VFA may be 8, 2, and 1 for acetate, propionate and butyrate

$8\,(C_6H_{12}O_6 + 2H_2O \rightarrow 2CH_3COOH + 2CO_2 + 4H_2)$

$2\,(C_6H_{12}O_6 + 2H_2 \rightarrow 2CH_3CH_2COOH + 2H_2O)$

$1\,(C_6H_{12}O_6 \rightarrow CH_3CH_2\,CH_2COOH + 2CO_2 + 2H_2)$

$11C_6H_{12}O_6 + 12H_2O \rightarrow 16CH_3COOH + 4CH_3CH_2COOH + CH_3CH_2\,CH_2COOH + 18CO_2 + 30H_2$.

Thus, 7.5 moles of CH_4 could be formed from the remaining CO_2 and H_2

$$7.5CO_2 + 30H_2 \rightarrow 7.5CH_4 + 15H_2O.$$

The final balance is:

$$11C_6H_{12}O_6 \rightarrow 16CH_3COOH + 4CH_3CH_2COOH + CH_3CH_2CH_2COOH + 3H_2O + 10.5CO_2 + 7.5CH_4.$$

Thus, when the acetate to propionate ratio is 4 to 1, the estimated CH_4 to CO_2 is 7.5 to 10.5 or 0.71. The CH_4 per glucose molecule fermented is 7.5 per 11 or 0.68. Results for both scenarios are summarized in Box 16.1.

The increase in CH_4 for the high-forage compared to the high-grain diet was about 17%. Observed differences in CH_4 between high-forage and high-grain diets are typically much higher than predicted from the fermentation balance calculated here. CH_4 emissions from cattle fed high-grain diets are typically about 3% of gross energy intake compared to at least twice that amount on high-forage diets (Johnson and Johnson, 1995). For a more specific example, acetate to propionate ratios for beef steers on forage versus 90% grain diets were 4.5 and 2, but methanogenesis was more than twice as high on the forage diet (Lana *et al*., 1998). An experiment conducted by Christophersen *et al*. (2008) in sheep for comparing high-fibre and high-grain diets showed the change in acetate to propionate ratio from 3.5 to 1.5, with a 21% increase in CH_4. Therefore, at times, there may be more to the change in CH_4 production due to diet than can be explained by the stoichiometry of the major products alone.

High-grain diets have higher H_2 partial pressures than high-forage diets. This environment thermodynamically favours methanogenesis, but methanogenesis is apparently inhibited. Russell (1998) showed that at least part of the inhibition of methanogenesis could be attributed to lower pH or accumulation of VFA on the high-concentrate diet. Therefore, the reducing equivalents are used to produce propionate instead of acetate. However, as the fermentation balance shows, the response in the acetate to propionate ratio is not as high as would be expected; so much of the additional reducing equivalents must be used in another way. Therefore, the acetate to propionate ratio cannot be the only factor to consider. Some of the other uses of H_2 include: elongation of fatty acids, saturation of fatty acids, microbial growth, and even reductive acetogenesis (i.e. acetate synthesis from CO_2 and H_2). Only the latter is nearly limitless in the ability to capture CO_2 and H_2 (reductive acetogenesis is deliberated in Chapter 19, Section III, this volume).

Complete oxidation of glucose to CO_2 and H_2O yields 2.8 MJ mol^{-1} (calculated as change in enthalpy, or $-\Delta H$ of the reaction), and complete oxidation of a mole of CH_4 yields 0.81 MJ mol^{-1}. Therefore, using the fermentation balance above, the simulated high-grain diet would release 16.2% of a fermented glucose molecule's energy as CH_4, and the high-forage diet would release 19.7%. These estimates are about twice as high as typically observed. For example, 4–10% of the digestible energy was lost as CH_4 across a range of diets (Johnson and Johnson, 1995). However, fermentation balance only considers the glucose converted to VFAs or gases and not glucose incorporated into microbial protein or lipids. Microbial matter may incorporate more reducing equivalents than the original glucose. Nonetheless, the fact that

Box 16.1

Simulation	Acetate/propionate	CH_4/CO_2	CH_4/glucose
High grain	2.5:1	0.60	0.56
High forage	4:1	0.71	0.68

considerably less CH_4 is produced than predicted by fermentation balance suggests the existence of alternative pathways that decrease CH_4 or increase VFAs or both.

The fermentation balance studies explain why shifts in the VFA profile are associated with changes in CH_4 production, and why it is necessary to study multiple pathways together. However, fermentation balance studies do not explain why we get the profiles of the products that are observed rather than some other stoichiometrically balanced combination. For example, there are known microbes that convert glucose to 3 moles of acetate with no CO_2 or H_2. Why not just use those microbes instead of producing CO_2 and CH_4? We do not choose the microbes that thrive in the rumen, the microbes that survive are the best fit, and an aspect of fitness is that they carry out a role to capture Gibbs energy faster or to a greater extent than their competitors. Understanding why we consistently see similar profiles of VFAs and CH_4 under similar conditions requires an understanding of the fundamental physics of the rumen.

16.3 Kinetics and Thermodynamics

Underlying all of the processes that occur in the rumen that contribute to CH_4 formation are the principles of kinetics and thermodynamics. All chemical reactions are controlled kinetically or thermodynamically (Chang, 1981). With kinetic control, the rates of reactions depend on substrate concentrations or enzyme activities, and these enzyme activities in turn may depend on microbial growth or enzyme synthesis. The profile of products depends on the relative rates of the different competing reactions. With thermodynamic control, which pathway branches are available depends on the second law of thermodynamics. This law governs whether or not a reaction can proceed spontaneously in the forward direction based on the concentrations of reactants and products.

Biologists have focused on controlling kinetic elements of fermentation such as enzyme function, microbial activity, gene expression or provision of substrates. However, fermentation is often controlled by thermodynamics (Kohn and Boston, 2000). For example, in a mixed-culture anaerobic digester, as soon as a glucose molecule is released by digestion of cellulose, there are several microbes that can transport it into their cells and metabolize it to any number of products. The amount of energy any particular organism can obtain depends on the concentration of all the products of the reaction relative to all the reactants. Since the free glucose concentration is very low due to competition among microorganisms in the fermentation, and the products of fermentation are removed slowly, only very efficient microbes can use the small amount of glucose at all, and they can only use it when concentrations of the products they produce are low. Therefore, when their products start to build up, they can no longer obtain energy by converting the reactant to a product, and they leave the glucose behind for another microbe that produces a different product. In this way, a consistent ratio of products is produced.

In chemistry, whether or not a reaction can proceed spontaneously in the forward direction is represented by the change in Gibbs energy (ΔG), which can be calculated based on the ratio of products and reactants in the system (Chang, 1981). Using this calculation, a strongly negative ΔG indicates that the reaction could proceed strongly in the forward direction without the addition of energy to the system. A strongly positive value of ΔG indicates the reaction cannot proceed in the forward direction without the addition of energy to the system, and it may even run in the reverse direction. If one calculates the ΔG values between many of the products in the rumen of a cow, assuming typical metabolite concentrations, one finds that they are usually very near to 0 (Kohn and Boston, 2000; Ungerfeld and Kohn, 2006). This means that the products made are a function of thermodynamics. Additional products cannot be made unless the reactant concentrations increase or the product concentrations decrease. If one product increases the substrates to produce that product increase slightly, so more of a

different kind of product can be produced. Mathematical models were developed incorporating this knowledge by solving multiple simultaneous equations using thermodynamic data to predict the concentrations of products that would result.

16.3.1 Thermodynamics of anaerobic digestion and rumen fermentation

Kohn and Boston (2000) and Ungerfeld and Kohn (2006) calculated that the key reactions producing and utilizing hydrogen were close to equilibrium in the rumen based on thermodynamic (ΔG) data. The same was found to be true in anaerobic digesters (Hoh and Cord-Ruwisch, 1997). These reactions are shown below:

Methanogenesis: $CO_2 + 4H_2 \leftrightarrow CH_4 + 2H_2O$

Volatile fatty acid interconversion:
CH_3CH_2COOH (propionate) + $2H_2O \leftrightarrow$
CH_3COOH (acetate) + $3H_2 + CO_2$

$CH_3CH_2CH_2COOH$ (butyrate) + $2H_2O \leftrightarrow$
$2CH_3COOH$ (acetate) + $2H_2$

Acetate degradation: $CH_3COOH + 2H_2O \leftrightarrow$
$2CO_2 + 4H_2$.

Of course, there are many additional reactions that can be considered simultaneously, but these major reactions can be used to demonstrate how thermodynamic analysis can be used with multiple simultaneous equations to understand fermentation. When using thermodynamic analysis to determine whether or not a single reaction is feasible, some insight is required to know why a reaction might or might not proceed. But when using multiple equations at once, the same calculations can show which pathway branches might be taken or why fermentation might shift from one set of pathways to another. To understand methanogenesis, it is necessary to understand all of the major reactions in the pathway for CH_4 production as well as other uses of the same metabolites. Simultaneous equations are needed to understand which pathways are favoured.

First, the ΔG calculation shows the feasibility of converting CO_2 and $4H_2$ to CH_4 and $2H_2O$. If the total gas pressure in the rumen approximates 1 atm, and CO_2 comprises 70% of that pressure, the pCO_2 would be 0.7 atm. Multiplied by the solubility constant (Segel, 1976) for this ionic strength, temperature and pressure (0.0229 mol atm^{-1}), this pCO_2 would provide 0.016 mol l^{-1} of CO_2 (aq) at equilibrium. The H_2 concentration can be calculated using the Nerst equation from the reducing potential and pH (Segel, 1976)

$$\Delta E = \Delta E^\circ + R\,T / (n\mathrm{F}) \text{ times } \ln([H^+] / [H_2]_g),$$

where ΔE is the reducing potential in volts measured using a hydrogen electrode, ΔE° is the change in reducing potential for the reaction under standard conditions, which is equal to zero for the H^+ to H_2 half reaction, R is the gas constant (8.3145 J $°K^{-1}$ mol^{-1}), T is the temperature in °K, n is the number of moles reduced (2) and F is Faraday's constant (9.6487×10^4 C mol^{-1}), which converts ΔE from ΔG. The solution for $[H_2]_g$ in this case yields the concentrations under equilibrium conditions for the H_2 half reaction. Typical ruminal conditions are E = –0.315 V and pH = 6.5 (Barry *et al*., 1977). Thus, $[H_2]_g$ would be 1.6×10^{-3} atm. For this example, let us assume that the partial pressure of CH_4 is 0.3 atm. Multiplied by its solubility constant (2×10^{-5}; Fogg and Gerrard, 1985) yields a concentration of 6×10^{-6} mol l^{-1}. The molecular weight of water is 18 g mol^{-1} with 1000 g l^{-1}. Therefore, the molarity of pure water is 55.6 mol l^{-1}. Assuming 10% dry matter of the ruminal solvent yields a molarity of approximately 50 mol l^{-1} in rumen liquid, typical ruminal conditions allow for at least 0.05 mol l^{-1} of acetate. Thus, the concentrations of all products and reactants for a particular set of ruminal conditions have been defined.

Table 16.1 shows key thermodynamic data under standard conditions for these reactants and products, as well as some other important ruminal metabolites. These values represent the free energy of formation (ΔG_f°) and enthalpy of formation (ΔH_f°) of the metabolites from the elements (e.g. H_2,

Table 16.1. Standard free energy of formation and enthalpy of formation in kJ mol^{-1} of key rumen metabolites at 298.15°K and 1 atm.

Metabolite	$\Delta G°_f$	$\Delta H°_f$
D-Glucose (aq) ($C_6H_{12}O_6$)	–916.97	–1263.78
Acetate (aq)	–376.89	–485.6
Propionate (aq)	–373.82	–511.70
Butyrate (aq)	–372.04	–535.55
Lactate (aq)	–516.72	–686.64
Methane (aq)	–50.79	–74.85
Carbon dioxide (aq)	–386.23	–412.92
Water (l)	–237.19	–285.84
Hydrogen (g)	0.0	0.0

Notes: Data are *not* adjusted to pH 7 and are from Chang (1977), except for propionate (CRC, 1991). Standard conditions are 1 M concentration of each soluble reactant and product, 1 atm of all gases and 298.15°K.

O_2, graphite). Free energy under standard conditions and concentrations, $\Delta G°$, can be determined from these tabular values for each reaction of interest (Chang, 1981)

$$\Delta G° = \Delta G°_f \text{ of products} - \Delta G°_f \text{ reactants.}$$

Adjustment to each $\Delta G_f°$ for temperature can be made using a transformation of the vant Hoff equation (Chang, 1981) and enthalpy of formation, $\Delta H_f°$, where T_1 and T_2 are the initial and final temperatures, respectively, and $\Delta G°T_1$ and $\Delta G°T_2$ are the respective standard free energy values

$$\Delta G°T_2 = T_2 / T_1 [\Delta G°T_1 - \Delta H°(T_2 - T_1) / T_2].$$

Table 16.2 shows the resulting standard change in free energy calculated for several reactions important to ruminal metabolism under standard conditions and adjusted for 311°K.

Now it is possible to determine the ΔG of methanogenesis under the ruminal conditions that were just described. For methanogeneis,

$$\Delta G = \Delta G° + RT \ln \{[CH_4]_{aq} [H_2O]^2 / ([CO_2]_{aq} [H_2]_g^{\,4})\}$$

$$\Delta G = -134.9 + 0.008314 \times 311 \ln \{(6 \times 10^{-6}) (50^2) / [(0.016) (1.6 \times 10^{-3})^4]\}$$

$$\Delta G = -68.5 \text{ kJ mol}^{-1}.$$

The ΔG for this reaction is negative, so the reaction is feasible. Now consider the ΔG for the use of H_2 for acetate production under the same ruminal conditions

$$\Delta G = \Delta G° + RT \ln \{[C_3H_3O_2^-] [H^+][H_2O]^2 / ([CO_2]^2_{aq} [H_2]^4)$$

$$\Delta G = -72.2 + 0.008314 \times 311 \ln \{0.050 (1 \times 10^{-6.5}) 50^2 / [(0.016)^2(1.6 \times 10^{-3})^4]\}$$

$$\Delta G = -10.4.$$

The ΔG is negative, so the reactions are feasible under these conditions. However, the production of Adenosine Triphosphate (ATP) was not considered for these reactions. Generally, about 1 ATP is produced per CH_4 molecule synthesized from gases. An ATP releases about 44 kJ mol^{-1}, so under these typical conditions CH_4 can be produced at about 65% efficiency (44/68.5). Reductive acetogenesis allows the microbes to produce a fraction of an ATP (e.g. 0.2 ATP) at very high efficiency. For example, if the ΔG is –10.4, the efficiency is nearly 100% at producing 0.23 ATP.

Both of these reactions, when considering ATP production, are very close to thermodynamic equilibrium. The methanogens could grow much faster under typical ruminal conditions since they can obtain 1 mole of ATP per mole of CH_4 produced, but the reductive acetogens can only make 0.2

Table 16.2. Key reactions in the rumen: standard change in enthalpy in kJ mol^{-1} ($\Delta H°$), standard change in free energy at 298°K in kJ mol^{-1} ($\Delta G°_{298}$) and standard change in free energy at 311°K in kJ mol^{-1} ($\Delta G°_{311}$).

Reaction	Formula	$\Delta H°$	$\Delta G°_{298}$	$\Delta G°_{311}$
Glucose to acetate	$C_6H_{12}O_6 + 2H_2O \rightarrow 2C_2H_3O_2 + 2H^+ + 4H_2(g) + 2CO_2(aq)$	38.4	–134.9	–142.4
Glucose to propionate	$C_6H_{12}O_6 + 2H_2 \rightarrow 2C_3H_5O_2 + 2H^+ + 2H_2O$	–331.3	–305.0	–303.9
Glucose to butyrate	$C_6H_{12}O_6 \rightarrow C_4H_7O_2 + H^+ + 2H_2(g) + 2CO_2(aq)$	–97.6	–227.5	–233.1
Glucose to lactate	$C_6H_{12}O_6 \rightarrow 2C_3H_5O_3 + 2H^+$	–109.5	–116.5	–116.8
Lactate to propionate	$C_3O_3H_5 + H_2 \rightarrow C_3H_5O_2 + H_2O$	–110.9	–94.3	–93.6
Methanogenesis	$CO_2(aq) + 4H_2 \rightarrow CH_4(aq) + 2H_2O$	–233.6	–138.9	–134.9
Acetogenesis	$2CO_2 + 4H_2 \rightarrow C_2H_3O_2 + H^+ + 2H_2O$	–231.4	–78.8	–72.2
ATP generation	$ADP + Pi + H^+ \rightarrow ATP + H_20$	24.3	–9.0	–10.4

Notes: Data are calculated from values in Table 16.1, except for ATP, which is from Rekharsky (1986). Data are *not* adjusted to pH 7.
Adenosine Triphosphate (ATP) and Adenosine Diphosphate (ADP) wherein energy is stored in the ATP form of the molecule.

ATP per mole of acetate produced. Methanogens have H_2 thresholds 10–100 times lower than reductive aceotogens (Cord-Ruwisch *et al*., 1988; Fievez *et al*., 1999). Thus, methanogenesis would outcompete reductive acetogenesis in the rumen by keeping H_2 pressure under the H_2 threshold for acetogenesis. If methanogens effectively deplete the rumen of free H_2, the recovery of acetogens after a meal must be much slower. Further calculations also show that interconversion of acetate with propionate or butyrate is also near thermodynamic equilibrium. Thus, under typical ruminal conditions, there is very little that can be done to shift fermentation away from CH_4 and toward other VFAs other than to shift conditions away from typical ruminal conditions.

We first considered that the ΔG values so close to 0 indicated that it would not be effective to add reductive acetogens to the rumen because enzymes or microbes could only have kinetic effects; they can make the system approach thermodynamic equilibrium faster, but cannot change equilibrium (Ungerfeld and Kohn, 2006). Since the system is already at thermodynamic equilibrium, speeding up the rate of movement toward equilibrium is futile. Therefore, adding microbes or enzymes would not produce observable effects, and the literature (Nollet *et al*., 1997; Le Van *et al*., 1998; Fievez *et al*., 1999; Lopez *et al*., 1999) confirms this observation.

On further reflection, the calculations have additional meaning. While it is true that under typical conditions the rumen is near its thermodynamic equilibrium and there is no effect of adding microbial activity, the fact that the ruminal environment is typically close to thermodynamic equilibrium means that the microbial activity in the rumen has already brought about those conditions. For example, when more H_2 or CO_2 are produced, they are used to make CH_4 or acetate. If more acetate is generated from cellulose or starch, more H_2 and CO_2 must be generated too, because as the acetate concentration increases, the pressures of H_2 or CO_2 and propionate must also increase to maintain equilibrium.

Theoretically, when the ΔG for a reaction is close to zero, there is little energy available to be captured for microbial growth (Crabtree and Nicholson, 1988). For example, the ΔG for CH_4 production is very close to zero in anaerobic digesters, so methanogens would need to grow slowly, if at all. Empirically, previous research did not find a clear relationship between methanogen numbers and CH_4 production (Machmüller *et al*., 2003); or in other words, CH_4 production per methanogen was highly

variable. Under very low H_2 availability (which would result in ΔG close to 0), CH_4 formation becomes decoupled from growth (Nölling and Reeve, 1997). Expression of genes encoding methanogenic enzymes can change depending on H_2 availability (Reeve *et al*., 1997). These empirical results under conditions with little available H_2 confirm theoretical predictions for slow methanogen growth when ΔG for CH_4 production is close to 0.

Janssen (2010) proposed a descriptive model to explain CH_4 formed in the rumen based on molecular hydrogen (H_2) currency. This qualitative model described the effects of methanogen growth rates under different H_2 concentrations, pH and CH_4 inhibitors, and suggested what conditions would enable methanogens to grow fast enough not to wash out of the rumen at different passage rates.

Likewise, the growth of reductive acetogens would also be much slower when the pressures of H_2 and CO_2 decreased. Since much more energy can be captured by the methanogens, their growth rates are greater than for the acetogens. Yet, under typical ruminal conditions, the concentrations of acetate relative to H_2 and CO_2 pressure can still be explained on the basis of thermodynamics. If the acetate concentration was lower relative to H_2 and CO_2, it would be more thermodynamically favourable (more negative ΔG) to convert H_2 and CO_2 to acetate, and the reductive acetogens would grow faster.

Thermodynamics suggests that it may be difficult to control fermentation in a way that will decrease CH_4 production. The CH_4 is produced because it is a low-energy state for the system. While thermodynamics can explain why certain ratios of CH_4 to acetate or acetate to propionate are established, it is far more difficult to explain why these ratios ever change. Given the role of thermodynamics, how can we decrease CH_4 production? One opportunity may be to control the kinetics of the fermentation, or to slow the rate that some pathways within the fermentation system approach equilibrium. For example, immediately after a meal, the size of the population of microbes in the rumen may limit the rate of digestion and the formation of some products. With plenty of substrate available, certain microbes may grow more quickly than others because they can capture more energy because the ratio of their products to reactants is greater. The faster these types of microbes grow, the more they are able to carry out their reactions.

16.4 Effect of Gas Composition on CH_4 Production

One way to study the thermodynamics of fermentation is to perturb a natural system and see how or if the system returns to equilibrium. Changing gas composition is one of the most direct ways to perturb the fermentation.

With this in mind, we (Kohn, 2008) hypothesized that vacuum pressure or purging with N_2 would shift the reaction: $CO_2 + 4H_2 \leftrightarrow CH_4 + H_2O$, to the left. There are five moles of gas on the left side of the equation compared to one mole of gas on the right side. Considering only the reaction for methanogenesis, vacuum pressure or purging with N_2 should increase H_2 production and decrease CH_4 production. Furthermore, based on conventional wisdom, one would expect CO_2 and CH_4 production to be correlated with VFA production. It was thought that VFAs and gases were produced in combination from carbohydrate in accordance with the fermentation balance discussed, and therefore VFA production should be correlated with production of CO_2 and CH_4. The results from incubating hay with ruminal fluid and buffer under vacuum, N_2 or CO_2 headspace partly fit expectations. The H_2 production increased immediately, to return to the original H_2 partial pressure. When the total pressure was lower, H_2 comprised a greater percentage of the total gas. When N_2 replaced CO_2, H_2 comprised a greater percentage of the fermentation gas (CO_2, CH_4 and H_2). When CO_2 or N_2 were perfused through the system, a similar partial pressure of H_2 was maintained, so more H_2 was produced and released to maintain the partial pressure.

However, other than the effects on H_2, results were inconsistent. The CH_4 did not consistently decrease as a result of vacuum or N_2 perfusion, and in several experiments, CH_4 production increased with low CO_2 pressure. Application of vacuum or purging with N_2 increased the ratio of CH_4 production to VFA production, which contradicted the model wherein gas production was coincident with VFA production (i.e. the expected fermentation balance). These results prompted us to consider a reaction thought not to be of much consequence in the rumen: acetate formation or degradation: $2CO_2 + 4H_2 \leftrightarrow CH_3COOH + 2H_2O$. Conventional wisdom is that acetate-degrading organisms grow too slowly to survive washout from the rumen, and therefore acetate is not degraded. Previous studies showed that acetate production from CO_2 and H_2 was barely feasible thermodynamically (Kohn and Boston, 2000; Ungerfeld and Kohn, 2006). Therefore, little consideration was given to the pathway. However, decreasing gas pressures would shift this reaction to the left. When considering that both CH_4 production and acetate production from CO_2 and H_2 could be in equilibrium, it becomes apparent that CO_2 pressure could decrease CH_4 and increase VFA concentration. The following equations result from the assumption that these two reactions are near equilibrium

$$K_{eq16.1} = \{[CH_4][H_2O]^2\} / \{[CO_2][H_2]^4\} \quad (16.1)$$

$$K_{eq16.2} = \{[CH_3COOH][H_2O]^2\} / \{[CO_2]^2 [H_2]^4\}. \quad (16.2)$$

Dividing Eqn 16.1 by Eqn 16.2 shows:

$$K_{eq16.1} / K_{eq16.2} = \{[CH_4][CO_2]\} / \{[CH_3COOH]\}.$$

Combining the two equilibrium constants yields a new constant. Now it is clear that vacuum pressure or nitrogen perfusion could decrease the equilibrium concentration of acetate. In contrast, increasing pressure could increase acetate concentration. Thus, short-term effects might include increases in CH_4 formation until the VFA concentration approaches a new equilibrium. Changing gas composition could alter the VFA concentrations over the long term. Immediately after perturbing the system, gas composition would affect VFA production only until it returns to a new equilibrium. Once the fermentation adjusts to the new gas composition with new VFA concentrations, the gas composition may not further affect VFA production rates. Vacuum pressure or N_2 purging could increase the ratio of CH_4 produced per VFA concentration, as VFAs may be degraded or just not produced while CH_4 is produced.

Results in the literature using anaerobic digester fluid generally confirm that CO_2 pressure in the headspace decreases VFA concentration and increases CH_4 compared to N_2 pressure. Finney and Evans (1975) and Finney (1978) hypothesized that the rate-limiting step in the biological production of CH_4 in anaerobic digesters was the removal of gases, and they demonstrated increased degradation of acetate to CH_4 from vigorous mixing and application of slight vacuum pressure. Finney and Evans (1975) discussed the thermodynamics of molecular gas transfer in the system, but not the chemical thermodynamics. Hashimoto (1982) also found digesters with continuous mixing, and pressures of 0.96 atm had up to 5% greater CH_4 production rate compared to digesters at 1 atm.

Hansson (1979) reported that CO_2 pressure inhibited CH_4 production from glucose and increased acetate concentration compared to N_2 or CH_4 pressure. Higher CO_2 pressure increased sensitivity to heat for a mixed thermophilic culture, but a culture grown under N_2 atmosphere was able to produce CH_4 from glucose at 80°C (Hansson, 1982). Hansson and Molin (1981a) reported that CH_4 production from acetate was maximized when the CO_2 pressure was about 0.3 bar, but it became substantially decreased as the CO_2 pressure increased to 1 bar. Decreasing CO_2 in the headspace increased the rate of degradation of acetate, propionate and butyrate to CH_4 and CO_2 (Hansson and Molin, 1981b). Propionate

degradation rate increased from 60 to 200 mg l^{-1} day^{-1} as the CO_2 pressure decreased from 1 to 0.2 atm (Hansson and Molin, 1981b). Kasli *et al*. (1990) reported that H_2 supplementation initially stimulated methanogenesis and acetogenesis in decaying refuse, but later inhibited them. A gas headspace of CO_2 strongly inhibited methanogenesis and partially inhibited acetogenesis compared with N_2. A gas mixture of CO_2–CH_4 (40:60) also inhibited methanogenesis, but not acetogenesis. In contrast, a study with rumen fluid showed the opposite effects. Patra and Yu (2013) found greater CH_4 production when CO_2 was the headspace gas instead of N_2 or a mixture of N_2, CO_2 and H_2. They did not find changes in VFA concentrations from the treatments.

Intuitively, if a reactant or product concentration is added or removed, one would expect the rate of the reaction to increase or decrease until a new equilibrium concentration is reached. For example, if CO_2 is a co-product of acetate degradation to CH_4, decreasing CO_2 pressure should increase the degradation rate of acetate. Thermodynamics pertains to concentrations, not necessarily rates. So, the thermodynamics suggest that decreasing the partial pressure of CO_2 might increase acetate degradation and CH_4 formation until the acetate *concentration* decreases to a new equilibrium. However, the *rates* of VFA or CH_4 production may not be affected by thermodynamics over the long term. This distinction may explain why CO_2 effects on rates of CH_4 formation are inconsistent. Since chemical thermodynamics was not the driving theory for these previous investigations, treatments did not change the concentrations of products and reactants in concert, and the final concentration was not the target response variable. Beyond the thermodynamics of the single pathway, it is also necessary to integrate the results from multiple pathways.

Still unresolved is the fact that increasing CH_4 in the headspace does not affect acetate or CH_4 production rates. Based on the thermodynamics, one would expect a higher CH_4 concentration to decrease subsequent CH_4 production or to decrease acetate degradation.

16.5 Differences Between the Rumen and Anaerobic Digesters

The difference in size between anaerobic digesters and the cow's rumen might drive differences in thermodynamics. The rate of removal of produced gases from each system depends on mixing and diffusion across the medium. The larger size of the anaerobic digester would slow the removal of the gases, causing them to become more concentrated in fermentation broth. Furthermore, hydrostatic pressure in the bottom of the digester would also increase gas concentrations in the digester.

A feed like grass is converted rapidly to VFAs in the cow's rumen. About half of the cellulosic biomass can be degraded in about 24 h, and 6–10% of the digestible energy is converted to CH_4. The same feed is converted nearly entirely to CH_4 and CO_2 in an anaerobic digester. The material is digested over several weeks or months. The same feed could also be preserved, with some of the fermentable sugars converted to lactic acid if the feed is stored to produce silage. What causes the same feed to be converted to different products when stored in anaerobic conditions?

An anaerobic digester to produce CH_4 differs from silage in that the gases in the digester are allowed to escape, keeping the total pressure in the digester at about 1 atm at the surface; however, the gases in the silo are trapped. The degradation of acetate to CO_2 and H_2 would not be thermodynamically favourable in the silo, but it is favourable in the digester. The CO_2 and H_2 are converted to CH_4 in the anaerobic digester and the CH_4 is removed. The silage pH decreases make further acid production impossible and the feed is preserved. In the digester, the CH_4 continues to be produced and removed until the feed is degraded. Another difference between the digester and the silo is the concentration of feedstock relative to moisture. The digester usually has much more water to dilute the acids to further

favour the digestion of biomass. However, compost has a similar level of moisture as silage, but compost is degraded because H_2 chemically reduces the O_2 that enters. The O_2 allows for a much lower H_2 concentration, even at lower moisture content, making it thermodynamically favourable to degrade the acetate and other acids. Thus, the compost pH is neutral and the biomass can be digested.

The digestion in the cow's rumen is the fastest microbial biomass digestion on earth, but it results in acetate as a final product. The difference between the rumen and an anaerobic digester includes: (i) the removal of VFA by absorption from the gut; (ii) the recycling of sodium and CO_2 via the bicarbonate buffer system of the ruminant; and (iii) the smaller size of the ruminant compared to the anaerobic digester. Because the VFAs are continually removed from the rumen, the concentration of VFA is decreased, which makes VFA degradation less thermodynamically favourable. The conventional wisdom is that organisms that degrade VFAs grow too slowly and wash out of the rumen, but this is not true. The investigators isolated several species of microorganisms from the rumen that degrade or synthesize VFAs to or from CO_2 and H_2. The ΔG for acetate synthesis or degradation from CO_2 and H_2 is near 0 in the rumen. Therefore, this reaction is near equilibrium, making it controlled by thermodynamics. The removal of VFAs from the rumen would shift the reaction away from degrading the VFAs.

The recycling of CO_2 into the rumen via bicarbonate buffers might be thought to also shift the fermentation toward sparing VFAs compared with anaerobic digesters. Sodium bicarbonate ($NaHCO_3$) is secreted directly into the rumen and into saliva that flows into the rumen. The salt form dissociates to form a sodium ion and a bicarbonate ion, and the bicarbonate ion is in equilibrium with the CO_2 in the gas phase: $HCO_3^- + H^+ \leftrightarrow H_2CO_3 \leftrightarrow H_2O + CO_2$. The recycling of bicarbonate to remove CO_2 therefore uses protons or provides cation (Na^+) to neutralize pH. However, it also produces CO_2 gas, which dilutes the CH_4 and H_2.

In the rumen, the total gas pressure is the sum of the partial pressure of CH_4 and CO_2, and because the ruminant eructates (burps) when the pressure exceeds an atmosphere, the total gas pressure is approximately 1 atm. The bicarbonate system therefore increases CO_2 partial pressure and decreases CH_4 partial pressure. However, the ratio of CH_4/CH_3COOH decreases as the partial pressure of CO_2 increases. In other words, there should be a decreased tendency for the system to degrade acetate to ultimately produce CH_4. Adding $NaHCO_3$ or other bicarbonate salts to the rumen could decrease CH_4 production. On the other hand, both VFA concentration and $NaHCO_3$ feeding or recycling could have opposite effects, as mediated by their effects on ruminal pH. The higher VFA concentration may be associated with lower pH, and the $NaHCO_3$ may be associated with higher pH. Methanogens are known to be inhibited by low pH (Russell, 1998), and therefore direct inhibition of methanogenesis could shift fermentation toward propionate.

16.6 Strategies for Controlling Enteric CH_4 Emissions

16.6.1 Higher production per animal unit

CH_4 emissions from animal agriculture are a global concern, as they affect climate change, but they have minimal environmental impact at the farm or regional level. Microorganisms in the rumen produce CH_4, because doing so enables them to capture Gibbs energy to survive. One of the most effective ways to decrease CH_4 emissions from animal agriculture is to increase animal productivity, so fewer animals are needed for the same level of production of animal product. This strategy is likely to be the most important (Gerber *et al*., 2013).

16.6.2 Forage:concentrate ratio

It has long been known that feeding ruminants a diet with higher energy concentration decreases CH_4 emissions per

unit of energy consumed. For example, beef cattle fed high-grain diets produced about half as much CH_4 as a fraction of gross energy intake as cattle consuming forages (Johnson and Johnson, 1995). As shown in the discussion on fermentation balance, the lower acetate to propionate ratio observed on high-grain diets is at least partially linked stoichiometrically to decreased CH_4 emissions. However, fermentation balance studies show that the change in the acetate to propionate ratio accounts for less than half of the effect of high-grain diets on decreasing CH_4 emissions. The other half may relate to other hydrogen sinks that are activated on high-grain diets. The mechanism for the decrease in CH_4 emissions on high-concentrate diets is believed to involve a decrease in pH, which selectively inhibits methanogens (Russell, 1998). This leaves more H_2 available to be converted to propionate and other compounds. Thermodynamics provides an explanation for why the observed profiles of VFA occur, and it may provide a theoretical basis for why acetate to propionate ratios decrease on high-grain diets. On high-grain diets, inhibition of methanogenesis by low pH or higher acidity (Russell, 1998) would result in higher H_2 pressures (which are observed). Therefore, propionate synthesis would be favoured thermodynamically because it uses H_2, and acetate synthesis would be disfavoured because it produces H_2. Ungerfeld and Kohn (2006) showed that ΔG for interconversion of acetate and propionate remained close to 0 for both high-grain and high-forage rations. The higher H_2 pressure on high-grain diets would cause the shift toward propionate.

Calculations presented earlier in this chapter suggest that acetate may be made from CO_2 and H_2 in the rumen. This could explain why the fermentation balances for high-forage diets and especially for high-grain diets overpredict CH_4 production compared to observations. In reality, some of the CH_4 and CO_2 might be accounted for as additional VFAs instead. As the H_2 pressure increases on high-grain diets, calculations of the ΔG for acetogenesis from CO_2 and H_2 become more negative. This suggests that more acetate could be synthesized from CO_2 and H_2 until the reactants and products approach equilibrium. Alternatively, some glucose may be converted to 3 acetate without production of CO_2 or H_2.

When high-grain diets cause low pH, which inhibits methanogens, the increase in H_2 pressure could increase acetate concentration as well as propionate concentration. Therefore, it might seem that the acetate to propionate ratio would not change. However, the high-grain diets would have higher VFA concentrations, and these higher total concentrations would shift the fermentation equilibrium toward a lower acetate to propionate ratio. Assuming the VFA concentrations are in equilibrium with CO_2 and H_2, the equilibrium approached would be:

$$K_{eq} = [CH_3COOH][H_2O] / [CO_2][H_2]^4$$

$$K_{eq} = [CH_3CH_2COOH] / [CH_3COOH][CO_2][H_2]^2.$$

If the acetate concentration is higher on a high-grain diet, the fastest adjustment toward equilibrium would be for the H_2 concentration to increase. For example, equilibrium could be re-established at double the acetate concentration if the $[H_2]^4$ doubles or H_2 concentration increases by 1.4. But if the concentration of acetate doubles, and the concentration of H_2 increases 1.4-fold, the equilibrium concentration of propionate would increase fourfold (two times 1.4^2) and the ratio of acetate to propionate would decrease by half. At the same time, the CH_4 partial pressure would also double. However, CH_4 production would not increase in proportion to its concentration, because the shift toward propionate would decrease CO_2 production. In addition, the ΔG for methanogenesis becomes more negative relative to ΔG for acetogenesis under low pH conditions.

In other words, part of the effect of the increasing propionate concentration due to high-grain diets could result from the immediate increase in propionate relative to

acetate due to the higher H_2 pressure. However, the higher H_2 pressure could also cause an increase in acetate concentration, but if it did, the propionate concentration would increase even more than the acetate and the acetate to propionate ratio would still decrease. The calculation of the ΔG for each reaction (i.e. methanogenesis, reductive acetogenesis, VFA interconversion, etc.) on high-grain and high-forage rations shows that methanogenesis is the primary pathway that is directly inhibited, because the ΔG for that reaction decreases the most.

16.6.3 Ionophores

For comprehensive information on the use of ionophores in relation to enteric CH_4 emission, the deliberations made in the next chapter would also be supportive in understanding. Kohn and Boston (2000) described the behaviour of a theoretical model on the use of ionophores. Ionophores result in decreased CH_4, increased propionate and higher pH. They permit ions to penetrate gram-negative bacteria such as acetate producers (Russell and Strobel, 1989). This effect increases the cost of acetate production by causing the gram-negative organisms to expend ATP to repair internal ion concentrations. The cost would directly decrease the threshold ΔG (make it more negative) for acetate production and decrease the acetate concentration. The glucose spared from this change could be used to produce propionate instead, and this shift would decrease H_2 available for CH_4 production. Thus, the mechanism is quite different from the mechanism causing a decrease in the acetate to propionate ratio on high-grain diets. In this case, the pH is increased and the H_2 pressure is decreased. Thus, we would expect the ΔG for acetogenesis to decrease the most, because this is the pathway that is directly inhibited.

16.6.4 Feed additives

A number of feed additives decrease CH_4 emissions *in vitro*, including oils, saponins and tannins (Woodward and Reed, 1997; Machmüller, 2006; Casmiglia *et al*., 2007; Tatsouka *et al*., 2008; Waghorn, 2008; see Chapters 8, 20 and 22, this volume, for more information on these additives). These tend to inhibit methanogens directly. However, the rumen microbial population often appears to adjust to the additives, and finds a way to work around the direct inhibition of methanogens. Direct inhibition of methanogens results in higher H_2 pressure, which increases use of other sinks for reducing equivalent. It would be advantageous to determine ΔG for methanogenesis and other relevant reactions (e.g. reductive acetogenesis, VFA interconversion) in the rumen or *in vitro*, to evaluate which reactions are inhibited most. The most inhibited reactions will have the most negative ΔG. The same thermodynamic calculations were used to show which pathways were directly inhibited when feeding high-grain diets or using ionophores.

16.7 Temporary Benefits

Perhaps the best we can do is to cause temporary decreases repeatedly in CH_4 production. The kinetic and thermodynamic analysis presented in this chapter suggests that CH_4 production occurs in the rumen and in piles of manure, because releasing CH_4 allows the microorganisms in the system to capture Gibbs energy. It may be possible to inhibit CH_4 production directly using microbial or enzyme inhibitors, but doing so would make more Gibbs energy available for CH_4 production. This change in chemistry would select for alternative microorganisms that can make CH_4, and once the population adapts, CH_4 may be produced again. Often, feeding trials allow 3–5 weeks for adaptation to new diets before collecting measurements, and by the time measurements are made, the CH_4 production has resumed to the original rate. One strategy for managing CH_4 emissions from ruminants might be to decrease CH_4 emissions temporarily, and then change the diet again once the microbial population adapts. In other words, the decreased

emissions would occur immediately after the dietary change. A dynamic model (Offner and Sauvant, 2006) incorporating thermodynamics might be used to predict when these temporary effects might occur relative to feeding.

Ultimately, a mathematical model to estimate enteric CH_4 production may include calculation of ΔG for each major reaction contributing to reactants or products for methanogenesis. These reactions would include at least carbohydrate degradation to VFA, CO_2 and H_2, and methanogenesis from CO_2 and H_2. Under different conditions, the ΔG for any given reaction may change. For instance, if the passage rate is higher, organisms carrying out a specific reaction may need to grow faster. For example, H_2 concentration may be higher, as methanogens would have a higher threshold for growth at the higher rate (Janssen , 2010). Also, the threshold H_2 pressure for reductive acetogens would increase.

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17 Ionophores: A Tool for Improving Ruminant Production and Reducing Environmental Impact

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Abstract

Ruminal fermentation is an inherently inefficient process converting up to 12% of dietary carbon and energy into end products (e.g. CH_4) that are largely unusable by the animal. Ruminant nutritionists seek to modify fermentation, specifically by increasing ruminal propionic acid yield, reducing methanogenesis and decreasing ruminal proteolysis and deamination of dietary proteins in order to improve production efficiency. To date, a variety of methods have been investigated in an effort to meet these objectives. Carboxylic polyether compounds, 'ionophores', are an effective means of decreasing enteric CH_4 emissions when included in ruminant diets. Although ruminant nutritionists have historically focused on feeding ionophores to increase efficiency and profitability, recent attention has focused on the ability of ionophores to impact global greenhouse gas production. This chapter examines the use of ionophores in cattle diets for the mitigation of enteric CH_4 production. Issues like ionophore resistance and the impact of ionophore feeding on human health are also addressed.

17.1 Introduction

Ruminant livestock production receives negative attention as a direct result of methane (CH_4) production and its contribution to the greenhouse gas effect and global warming. Livestock currently contribute up to 18% (O'Mara, 2011) of global greenhouse gas emissions in the form of CH_4. Ruminant livestock represent one of the few natural sources of CH_4 emissions that can be manipulated (Shibata and Terada, 2010), and as such have been identified as an avenue to reduce global CH_4 emissions (Guan *et al*., 2006). As an added advantage, reduction in CH_4 is associated with improved animal production efficiency. Enteric CH_4 production is associated with 2–15% loss of gross energy intake for the ruminant animal (Johnson and Johnson, 1995; Van Nevel and Demeyer, 1996; Moss *et al*., 2000). CH_4 emission and the associated loss of dietary energy give rise to nutritional and environmental concerns within the livestock industry.

Native populations of microorganisms in the rumen endow ruminant animals with unique feed utilization capabilities (Schelling, 1984). The symbiotic relationship

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between ruminant animals and their ruminal microbial populations allows them to utilize fibrous/cellulosic materials effectively (Hungate, 1966). Ruminal bacteria, fungi and protozoa ferment carbohydrates and proteins primarily to produce volatile fatty acids (VFAs), H_2 and CO_2. Methanogenic archaea reduce CO_2 to CH_4 by using H_2 and/or formate to provide energy for growth and maintenance. Methanogenic archaea play an important role in rumen function and ruminant production efficiency because they possess a high affinity uptake system for scavenging these substrates from the rumen environment. Complete H_2 removal leads to an increased rate of fermentation by other ruminal bacteria by eliminating the inhibitory effect of H_2 on microbial fermentation, creating a more favourable environment for VFA (primarily acetate) formation. However, ruminal fermentation is an inherently inefficient process converting up to 12% of dietary carbon and energy into CH_4 and heat, end products that are largely unusable by the animal (Blaxter, 1962). Although CH_4 production represents a significant energy loss, recent investigations have focused primarily on the environmental impact and potential contribution to climate change.

Ruminant nutritionists seek to modify fermentation, specifically increasing ruminal propionic acid yield, reducing methanogenesis and decreasing ruminal proteolysis and deamination of dietary proteins in order to improve production efficiency (Bergen and Bates, 1984). Augmented production efficiency results from decreased livestock contribution to global CH_4 production. To date, a variety of methods have been investigated in an effort to meet these objectives.

Compounds that are capable of modifying fermentation to achieve improved production efficiency are currently used in ruminant livestock production. One particular class of these compounds, carboxylic polyether ionophore antibiotics (commonly known as ionophores), are an effective means of decreasing enteric CH_4 emissions when included in ruminant diets. Although ruminant nutritionists historically have focused on feeding ionophores to increase efficiency and profitability, recent attention has focused on the ability of ionophores to impact global greenhouse gas production. This chapter examines ionophore use in cattle diets for the mitigation of enteric CH_4 production.

17.2 Effects of Ionophores at the Animal Level

Originally used to control internal parasites in poultry (Bergen and Bates, 1984), ionophores have been fed to ruminants to improve production efficiency, resulting in: (i) a reduction in methanogenesis; (ii) increased propionate production; (iii) decreased protein degradation (Russell and Strobel, 1989); and (iv) decreased lactic acid production (Dennis *et al*., 1981).

Use of ionophores substantially reduces CH_4 emissions from ruminant animals in the short term. Because of their beneficial effect on ruminant feed efficiency, their use is not only economically justifiable on the farm scale, but reduced CH_4 emissions further provide global incentive for ionophore usage. Although findings regarding long-term impact conflict, ionophores generally decrease CH_4 production per unit product (meat or milk; Tedeschi *et al*., 2003), therefore reducing CH_4 emissions to the atmosphere from ruminant livestock.

17.2.1 CH_4 production

Ionophores such as monensin, lasalocid, laidlomycin and salinomycin reduce methanogenesis by inhibiting hydrogen-producing bacteria, a precursor of CH_4 (Van Nevel and Demeyer, 1977). Methanogens from the group *Archaea* use their high affinity for H_2 to remove much of the hydrogen from the rumen ecosystem. Reducing H_2 production leads to a reduction in the efficiency and extent of CH_4 formation by methanogens. Studies reporting CH_4 reductions from ionophore supplementation in cattle vary both in magnitude and length

of response (Sauer *et al*., 1998; Guan *et al*., 2006; Odongo *et al*., 2006). Ionophore response is dependent on diet composition and type and amount of ionophore administered. Additionally, different methods utilized to quantify CH_4 production may explain some of the differences in findings (Johnson and Johnson, 1995). In a comprehensive review, Van Nevel and Demeyer (1996) summarized *in vitro* studies in which the CH_4 inhibition caused by ionophore inclusion ranged from 0 to 76%. *In vivo* studies have demonstrated a reduction in CH_4 production of up to 31% (Joyner *et al*., 1979; Delfino *et al*., 1988; O'Kelly and Spiers, 1992). O'Kelly and Spiers (1992) reported a 10.5% reduction in daily feed intake due to monensin feeding, and suggested this was associated with a 25.6% reduction in CH_4 production. Delfino *et al*. (1988), however, reported only a 5% decrease in CH_4 production resulting from ionophore inclusion. On average, Van Nevel and Demeyer (1996) indicated that short-term ionophore inclusion caused an 18% decrease in *in vivo* methanogenesis.

Although it is evident that ionophore inclusion decreases CH_4 emissions from livestock, the long-term effects on CH_4 mitigation are inconsistent. Monensin reduced CH_4 production by 7% and sustained this reduction for 6 months in lactating dairy cows (Odongo *et al*., 2006). However, Rumpler *et al*. (1986) reported CH_4 production returned to control values by day 12 of monensin supplementation. Additionally, in a study by Guan *et al*. (2006) on CH_4 production, cattle were returned to the baseline level of emission by the third week of supplementation when fed on a high-forage diet and by the sixth week of supplementation when fed on a high-concentrate diet. While observations conflict, there is a general indication that ionophore-based inhibition of methanogenesis does not persist in the long term.

17.2.2 Volatile fatty acid

When ruminal CH_4 production is decreased, reducing equivalents must be disposed of via alternative electron sinks, such as propionate (Russell and Houlihan, 2003). Propionate is the most reduced VFA and is glucogenic to the animal, meaning it is utilized most efficiently by the animal (Yokoyama and Johnson, 1988). Molar proportions of ruminal propionic acid increased from 31.9 to 41.0 and 43.5% for 100 and 500 mg monensin supplementation, respectively, and maintained this increase throughout the 148-day experiment. In a meta-analysis of studies by Ellis *et al*. (2012), monensin inclusion consistently resulted in a decreased acetate:propionate ratio, which appeared to be related to decreased CH_4 production per unit feed (Ellis *et al*., 2012). Decreased acetate:propionate ratio is often associated with reduced CH_4 production, because reducing equivalents derived from the fermentation process must be disposed of in an alternative electron sink, in this case propionate production (Yan *et al*., 2010; Ellis *et al*., 2012). Therefore, ionophore feeding results in both increased propionate and reduced CH_4 production, leading to increased energy retention by the animal from feed.

17.2.3 Protein degradation

'Protein sparing' describes the beneficial effect of ionophores on ruminal amino acid degradation and the resultant decrease in ammonia production (Russell and Strobel, 1989). Ruminal bacteria that utilize amino acids as their sole carbon and energy source are known as 'obligate amino acid fermenting' or 'hyper-ammonia producing bacteria', which are characterized by high specific activities of ammonia production. The first obligate amino acid fermenting ruminal species isolated (*Peptostreptococcus anaerobius*, *Clostridium aminophilum* and *Clostridium stricklandii*) are capable of deaminating over 25% of the protein in feeds (Krause and Russell, 1996). Because these gram-positive obligate amino acid fermenting bacteria are ionophore sensitive, populations can be reduced tenfold by ionophore inclusion (Krause and Russell, 1996). Reduction of obligate amino acid

fermenting bacteria results in increased nitrogen retention, improved feed efficiency (Potter *et al*., 1976) and decreased ammonia nitrogen excretion in urine, which represents an environmental pollutant.

17.2.4 Effect on lactic bacteria

When ruminant animals are fed rations containing large amounts of starch, ruminal pH decreases. Increased lactate production is associated with lowered pH, and because lactate is a stronger acid than the typical VFAs, ruminal acidosis therefore occurs. Ruminal acidosis has been associated with reduced feed intake, lowered feed efficiency (Callaway *et al*., 2003), ulceration, founder, and in severe cases, death (Russell and Strobel, 1989). Most lactate-producing rumen bacteria are inhibited by ionophores (Dennis *et al*., 1981). The resulting decreased lactic acid production reduces the occurrence of ruminal acidosis and improves efficiency of production.

17.3 Mechanism of Ionophore Action

Ionophores carry ions across membranes, killing some bacterial species. Targeted bacterial death changes rumen microbial populations, potentially leading to reduced CH_4 emission and consequently improved production efficiency (Schelling, 1984). Ionophores have moderate molecular weights, typically 200–2000 (Pressman, 1976), and are capable of interacting with metal ions, serving as ion carriers across biological membranes (Ovchinnikov, 1979). Ionophores have a hydrophobic exterior, while the interior is hydrophilic, resulting in the ability to bind with cations (Russell and Strobel, 1989). Ionophores form lipid-soluble complexes with polar cations, K^+, Na^+, Ca^{2+}, Mg^{2+}, and biogenic amines (Pressman, 1976). Some ionophores act as uniporters, binding only one cation, while others act as antiporters, transporting more than one cation simultaneously (Russell and Strobel, 1989). Ionophores have differing affinities for the cations; monensin's affinity for Na^+ is ten times greater than for K^+, allowing it primarily to mediate Na^+–H^+ exchange due to the large Na^+ and pH gradients maintained by rumen bacteria (Pressman, 1976). Lasalocid, however, has a higher affinity for K^+. Ionophore modes of action are explained by their affinity for cations, the presence and degree of ion gradients and interference with normal ion flux through interaction of the ionophore with biological membranes and gradients. Ionophores shield and delocalize the charge of ions and facilitate their movement across membranes due to their hydrophobicity (Russell and Strobel, 1989), disrupting crucial ion gradients (Pressman, 1976). Mobile carriers such as monensin function within the membrane and are selective for specific ions; transport cycles across biological membranes can reach thousands of cycles a second (Pressman, 1976). Other ionophores, referred to as pore formers, have far less specificity and span the cell membrane (Russell and Strobel, 1989). Although pore formers are capable of translocating ions at a faster rate than mobile carriers, they have not been used as ruminal feed additives (Russell and Strobel, 1989).

Improvements in animal performance efficiency resulting from ionophore inclusion as discussed above are a secondary effect caused by the disruption of normal bacterial membrane physiology (Bergen and Bates, 1984). Bacterial cell membranes are relatively impermeable to ions; accordingly, ionic gradients are used to facilitate nutrient uptake at minimal adenosine triphosphate (ATP) cost (Rosen, 1986). Rumen bacteria maintain high intracellular K^+ and low intracellular Na^+ concentrations. Conversely, the ruminal environment is characterized by high Na^+ and relatively low K^+ concentrations. Consequently, ruminal bacteria utilize K^+ and Na^+ gradients for nutrient uptake. Ruminal pH is mildly acidic (ranging from 5.5 to 6.8 under most conditions), while intracellular pH is near neutral, creating an inwardly directed proton (or pH) gradient.

Monensin is the most extensively investigated ionophore used in ruminant rations, making it the most complete model

of collective ionophore modes of action. Monensin is a metal/proton antiporter that exchanges H^+ for either Na^+ or K^+ (Russell and Strobel, 1989; Fig. 17.1). Antiporter activity mediates the exchange of intracellular K^+ ions for extracellular protons, and extracellular Na^+ ions for intracellular H^+ (Callaway *et al*., 2003). Potassium is the predominant intracellular cation of microorganisms; however, the extracellular K^+ concentration is typically four to fivefold lower than Na^+ (the predominant extracellular cation, 90–150 m*M*; Durand and Kawashimi, 1980), causing the K^+ gradient to be greater than the Na^+ gradient. Because monensin is an antiporter, K^+ is expelled and an accumulation of protons occurs inside the bacteria on monensin inclusion. In an effort to maintain ionic equilibrium and/or pH neutrality within the cell, it activates a reversible F_1F_0 ATPase to pump protons from the cell. Hydrogen ions are exported by facilitated diffusion via an exchange of Na^+ or by active transport involving ATP (Grooms, 2010). Active transport results in an energy expenditure, decreasing ATP pools and resulting in reduced growth and eventually death of the cell.

Ionophores are generally most effective against gram-positive bacteria, because the cell membrane is surrounded by a porous peptidoglycan layer, which allows small molecules such as ionophores to pass through and dissolve into the membrane. Conversely, gram-negative bacteria are surrounded by a lipopolysaccharide layer, outer membrane and periplasmic space (Callaway *et al*., 2003), resulting in a relatively impermeable structure. Although ionophores are capable of binding to both gram-positive and gram-negative bacteria, CH_4 mitigation has been related to effects on gram-positive species (Bergen and Bates, 1984). It has been suggested that ionophore inclusion provides a competitive advantage to gram-negative bacteria by inhibiting ionophore-sensitive gram-positive species (Chen and Wolin, 1979; Newbold *et al*., 1993).

Rather than directly inhibiting methanogens, ionophores inhibit the bacteria

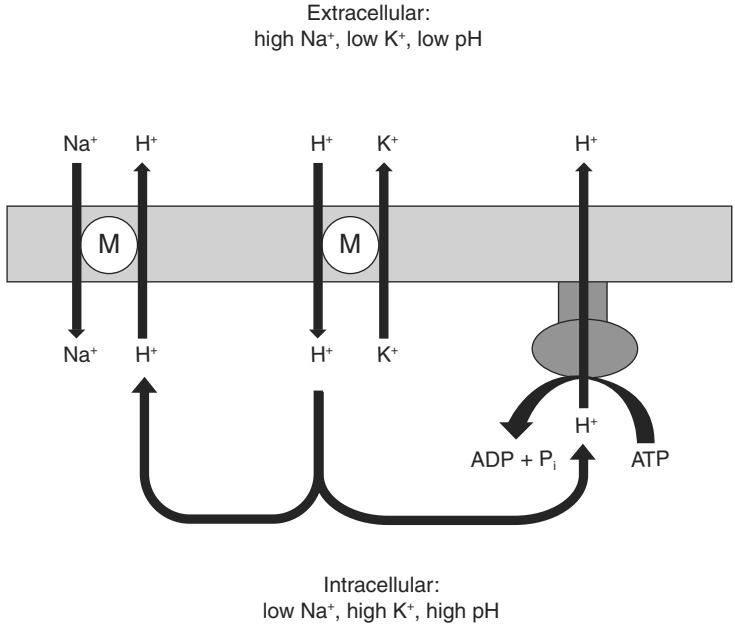

Fig. 17.1. Mechanism of monensin (M). (From Callaway *et al*., 2003.)

responsible for cross-feeding nutrients (H_2) to methanogenic archaea (Van Nevel and Demeyer, 1977; Dellinger and Ferry, 1984). Gram-positive bacteria generally produce acetate, butyrate, hydrogen and ammonia (end products of ruminal fermentation). Inhibition of these species by ionophores results in fewer hydrogen-, ammonia- and lactate-producing bacteria. The rate of ruminal CH_4 production is correlated with dissolved H_2 concentration; hence, a reduction in H_2 level results in reduced methanogenesis (Czerkawski *et al*., 1972).

Many ruminal acetate-producing bacteria are sensitive to ionophores, and it has been reported that a decrease in acetate production will further reduce CH_4 production because of its connection to the disposal of reducing equivalents via methanogenesis (Hegarty, 1999). Additionally, some acetogenic bacteria produce formate (which can be used as a substrate by methanogens), which further limits substrate availability to the methanogen population (Van Nevel and Demeyer, 1977).

17.4 Mechanisms of Ionophore Resistance and Potential Impact on Human Health

The effectiveness of many antibiotics has been reduced because bacteria can freely transfer genes encoding for resistance factors between species (Salyers and Shoemaker, 2006; Salyers *et al*., 2007). Although humans are not prescribed ionophores for treatment of bacterial infection, the concern exists that ionophores pose a threat to public health in regard to antibiotic resistance. It is important when examining antimicrobial resistance in a bacterial species to determine if resistance results from the selection of intrinsic resistance or the emergence of novel resistant populations (Callaway *et al*., 2003). Some ruminal microorganisms are intrinsically resistant (insensitive) to ionophores, even when ionophores are not fed; other species are sensitive to ionophores, but can become or acquire a reduced sensitivity (resistance). Bergen and Bates (1984) suggested that ionophore resistance was related to the membrane-bound enzyme, fumarate reductase, a proton-translocating enzyme, because ruminal bacteria that produce succinate and propionate were resistant and they hypothesized that this enzyme might counteract ionophore-dependent ion flux. Morehead and Dawson (1992) noted the appearance of more fumarate reductase activity in monensin-resistant *Prevotella ruminicola* strains than in monensin-sensitive strains. This hypothesis was contradicted by Chen and Wolin (1979), who found *Ruminococcus flavefaciens*, a fumarate reductase-containing species that produced large amounts of succinate, to be highly sensitive to monensin. Sensitivity of ruminal microorganisms to ionophores has been suggested to be relatively stable because the pattern of resistance results from differences in cell membrane structures (Russell and Strobel, 1989). Dawson and Boling (1983) monitored monensin sensitivity in calves. They found nearly 60% of bacterial isolates to be monensin sensitive prior to treatment, with only a marginal increase as a result of subsequent monensin administration.

Although gram-positive bacterial species are generally ionophore sensitive, some gram-positive bacteria do adapt following repeated exposure to the ionophore (Dennis *et al*., 1981; Dawson and Boling, 1983; Rumpler *et al*., 1986; Newbold *et al*., 1988; Callaway *et al*., 1999). Callaway *et al*. (1999) found some gram-positive bacteria to be sensitive to monensin initially, but following repeated exposure, these bacteria were able to increase their resistance significantly. The resistance appeared to result from an increase in extracellular polysaccharides (glycocalyx). Extracellular polysaccharides may play a key role in ionophore resistance of ruminal bacteria (Russell and Houlihan, 2003), because they prevent binding of the ionophore to the cell wall (Rychlik and Russell, 2002). On withdrawal of the ionophore, resistant bacteria did not persist in the population, indicating the changes were a result of the selection of naturally resistant subpopulations as opposed to the

emergence of novel/acquired traits (Callaway *et al*., 2003).

Ionophore resistance of bacterial populations has been monitored by measuring the amount of ionophores needed to catalyse K^+ depletion from bacterial cells due to the K^+ efflux from ionophore sensitive cells (Lana and Russell, 1996; Callaway *et al*., 1999). Rapid K^+ depletion occurred when mixed ruminal bacteria were taken from cattle treated with ionophores while consuming hay (Lana and Russell, 1996). Resistance of the mixed ruminal bacterial population was significantly greater within 3 days of the initiation of daily ionophore supplementation (Lana and Russell, 1996).

There is little rationale for common mechanisms of resistance between traditional antibiotics and ionophores (other than glycocalyx formation), because ionophores utilize a different mode of action than most therapeutic antibiotics (Russell and Houlihan, 2003). Due to the intrinsic physiological mechanisms of ionophore resistance, there is little evidence that ionophore resistance can be spread from one bacterium to another, as with therapeutic antibiotics. After many years (>35 years in the USA) of widespread use, highly ionophore-resistant isolates have rarely been isolated (Aarestrup, 1995; Aarestrup *et al*., 1998), and ionophores continue to improve the efficiency of ruminal fermentation. Russell and Houlihan (2003) concluded, based on collective observations, that the use of ionophores in animal diets is not likely to impact significantly the transfer of antibiotic resistance from animals to humans.

17.5 Ionophore Rotation Programmes

Ionophore rotation programmes have been suggested as a method to alleviate the long-term loss in the effectiveness of utilizing a single ionophore and maximizing the reduction of CH_4 production. Ionophore rotation schemes, however, have often produced inconsistent results. The potential benefits of rotation have been hypothesized as being due to reduced adaptation to a specific ionophore (Morris *et al*., 1990), altered site and extent of digestion (Galyean and Hubbert, 1989), and the direct impact of ionophores on host tissue metabolism (Armstrong and Spears, 1988). Johnson *et al*. (1988) found a daily rotation of lasalocid and monensin plus tylosin (an antimicrobial) improved daily gain and feed efficiency in feedlot cattle compared with feeding lasalocid or monensin plus tylosin continuously. Branine *et al*. (1989) also suggested that feeding a daily rotation of monensin and lasalosid enhanced feed efficiency and gain more than continuously feeding either ionophore alone. However, Morris *et al*. (1990) found no differences in the effect of daily rotation of lasalocid and monensin plus tylosin, concluding that a daily rotation scheme was too frequent to overcome any possible adaptation to ionophores. Guan *et al*. (2006) investigated a rotation of monensin and lasalocid every 2 weeks, but found that it did not extend the duration of reduced enteric CH_4 emission. Although ionophore rotation programmes do improve feed efficiency, the extent and mode of action of the synergistic benefit of ionophore rotation remains unclear.

17.6 Conclusion

Agriculture has come under intense scrutiny in recent years due to increased concern over greenhouse gas emissions. Of the total CH_4 production, 70% comes from anthropogenic sources, of which two-thirds are related to agriculture (Moss *et al*., 2000). Because ruminant livestock produce CH_4 via gastrointestinal fermentation, they have been targeted as a CH_4 source that potentially can be reduced. CH_4 emissions from ruminant animals represent not only a significant contribution to atmospheric concentrations but also a significant loss of energy to the animal and expense to the producer. For these reasons, a number of strategies are being utilized in an effort to reduce CH_4 emissions in cattle. Ionophore use in ruminant diets generally reduces CH_4 production significantly, thereby reducing the contribution of ruminants to global CH_4

emissions. CH_4 mitigation improves feed efficiency without changing the amount of meat or milk produced. Tedeschi *et al*. (2003) suggests that because of their potential to improve feed conversion and CH_4 production per kilogram of meat or milk, ionophores are effective, even in the case of acquired resistance. Use of ionophores can therefore be associated with a more environmentally friendly cattle production system.

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18 Residual Feed Intake and Breeding Approaches for Enteric Methane Mitigation

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Abstract

The expanding world human population will require greater food production within the constraints of increasing societal pressure to minimize the resulting impact on the environment. Breeding goals in the past have achieved substantial gains in environmental load per unit product produced, despite no explicit inclusion of environmental load (and in most instances, even feed efficiency) in these goals. Heritability of feed intake-related traits in cattle is moderate to high, implying that relatively high accuracy of selection can be achieved with relatively low information content per animal; however, the genetic variation in feed intake independent of animal performance is expectedly less than other performance traits. Nonetheless, exploitable genetic variation does exist and, if properly utilized, could augment further gains in feed efficiency. Genetic parameters for enteric methane (CH_4) emissions in cattle are rare. No estimate of the genetic variation in enteric CH_4 emissions independent of animal performance exists; it is the parameters for this trait that depict the scope for genetic improvement. The approach to the inclusion of feed intake or CH_4 emissions in cattle breeding goals is not clear, nor is the cost benefit of such an endeavour, especially given the cost of procuring the necessary phenotypic data.

18.1 Introduction

The world human population is expanding and the demand for food in 2050 is expected to be approximately 70% greater than the demand in 2010 (FAO, 2009). Demand for meat and other livestock products is highly elastic to income (Delgado *et al*., 1999), and therefore as population affluence improves, the demand for livestock products will increase further. Also, the global human population is ageing, and older people typically consume larger quantities of animal-derived protein than children (Steinfeld *et al*., 2006). The expected 70% increase in food demand requires an annual increase in food production of 1.3% per annum. This increase in food demand can only be met by increased efficiency of food production, both animal and crop derived. Moreover, competition for land from other industries (e.g. biofuels) implies this increased animal and crop production must be achieved from an ever-declining land base. Although feed efficiency, as currently defined, is not synonymous with production efficiency, it undoubtedly will be a major contributor to increasing production from

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an ever-decreasing, food-producing land base. The global production of red meat is expected to increase from 229 million tonnes (Mt) in 1999–2001 to 465 Mt in 2050, while milk production is expected to increase from 580 Mt globally to 1043 Mt over the same period (Steinfeld *et al.*, 2006). This increased production must, however, be achieved in an environmentally responsible and sustainable manner.

There is considerable commentary nowadays on climate change and its implications, as well as possible mitigation strategies. Animal agriculture generates greenhouse gas (GHG) emissions as methane (CH_4) from enteric fermentation and manure, nitrous oxide (N_2O) from the widespread use of nitrogenous fertilizers and animal manure, and carbon dioxide (CO_2) from the fossil fuels for energy usage plus land-use change. CH_4, however, is not only an environmental hazard but is also associated with a loss of carbon from the rumen, and therefore an unproductive use of energy (Johnson and Johnson, 1995). There is wide variation in the documented calculations of animal agriculture contributions to GHGs (Herrero *et al.*, 2011). O'Mara (2011) stated that animal agriculture was responsible for 8.0–10.8% of global GHG emissions based on calculations from the Intergovernmental Panel on Climate Change (IPCC), but if complete life cycle analysis (i.e. accounting for the production of inputs to animal agriculture as well as change in land use such as deforestation) is undertaken, this figure can be up to 18%. Cattle are the largest contributors to global GHGs (O'Mara, 2011).

Thirty-seven industrialized countries plus the EU have signed up to the UN Kyoto Protocol and its target of reducing GHGs between 2008 and 2012 by 5%, on average, from 1990 levels. The EU countries have further committed to reduce, from the 1990 level, emissions by 20% by 2020 (UNFCCC, 2011). Although most discussion is on the possible impact of food production on climate change, few have considered the converse, which is the impact of possible climate change on food production. Climate change is expected to result in rising global temperature, changes in patterns of precipitation, and more extreme weather events. Therefore, the animal of the future, as well as being efficient, will have to be resilient to these perturbations.

Agricultural contributions to GHG and food security are, nonetheless, not mutually exclusive. The growing food demand from an ever-decreasing land base can only be met by increased system efficiencies. Feed efficiency, both gross feed efficiency and net feed efficiency, as well as environmental efficiency, as part of the entire production system, will contribute to achieving the goal. Moreover, the conflict between food for direct human consumption versus feeds for the production of animal products for human food consumption is of increasing concern (Hume *et al.*, 2011). Galloway *et al.* (2007) stated that, globally, the conversion rate of feed to meat was 20:1 in ruminants and 3.8:1 in non-ruminants; however, after adjustment for feed not directly edible by humans (e.g. grass, crop residues), this ratio changed to 3:1 and 3.4:1, respectively. Therefore, animal efficiency in ruminants is particularly important. Grasslands cover over one-third of the ice-free land (Ellis and Ramankutty, 2008; Wang and Fang, 2009), which is approximately twice the arable cropland. Despite this, most research on feed efficiency and enteric CH_4 emissions is undertaken using high-concentrate diets.

This chapter reviews the state-of-the-art on breeding for improved efficiency of production and reduced enteric CH_4 emissions of modern day dairy and beef cattle production systems.

18.2 Enteric CH_4 Production

In ruminants, CH_4 is a natural by-product of anaerobic respiration, produced predominantly in the rumen (90%) and to a small extent in the large intestine (Ellis *et al.*, 2012). The contribution of CH_4 released by flatulence is only 1%, while eructation and air from the lungs accounts for the remainder of total CH_4 produced by ruminants. The major factors that determine CH_4 production include the amount of feed consumed by the ruminant, as well as the

digestion of that feed. As more feed is ingested, more CH_4 is produced, but the ratio of CH_4 per kilogram of dry matter intake decreases with increasing feed intake (Jentsch *et al*., 2007).

The conversion of feed material to CH_4 in the rumen involves the integrated activities of several different microbial species, the final step being carried out by methanogenic archaea. CH_4 production serves as the principal electron sink within the rumen. The formation of acetate and butyrate, largely as the result of the fermentation of structural carbohydrate, ultimately results in the production of CH_4. On the other hand, propionate, largely produced from the fermentation of non-structural carbohydrates, serves as a competitive pathway for electron use in the rumen, and is accompanied by a decrease in overall CH_4 production. Differences between animals are being identified as high or low efficient feed utilizers, or high or low CH_4 emitters. Due to the diurnal rhythm of feed intake, individual CH_4 production may vary by 100% during the day (Jentsch *et al*., 2007; Garnsworthy *et al*., 2012). The major portion of CH_4 emissions cannot simply be explained by feed intake, but rather is determined by dietary nutrient composition. The quantity and quality of digestible nutrients, especially the carbohydrate fraction, determines CH_4 production; for example, a greater portion of starch reduces, while greater fibre content elevates CH_4 production (see the details in Chapter 22, Section III, this volume). Feeding supplements capable of utilizing H_2, such as unsaturated fatty acids (Martin *et al*., 2008) or nitrate (Hulshof *et al*., 2012), but also ionophores, antibiotics and immunization (Buddle *et al*., 2011), may help to reduce ruminal CH_4 production without any repercussion for feed intake and milk production: this is discussed elsewhere in the book.

18.3 Sector Efficiency and Environmental Implications

It must first be recognized that CH_4 emissions per unit product produced in cattle has reduced in the past decades from improvements in animal performance, with no direct cognizance of animal CH_4 emissions in either breeding or management strategies. The CO_2-equivalent (eq) kg^{-1} milk produced in the US dairy sector has reduced by 37% between 1944 and 2007 (Capper *et al*., 2009). Similar achievements have been observed in the US beef sector, with a 16% reduction in CO_2-eq kg^{-1} beef produced over the shorter period of 1977–2007 (Capper, 2011). Therefore, the contribution of performance gains and efficiency (through, for example, genetics) to improved environmental footprint of the agri-food sector cannot be ignored.

One of the most commonly cited examples of the contribution of breeding to achieving improvements in production efficiency is the comparison of a 1957 random-bred broiler strain with the 2001 commercial-broiler strain fed diets representative of each period (Havenstein *et al*., 2003). The more recent strain required only one-third of the duration to reach market weight, eating only one-third the quantity of feed when compared to the 1957 strain; 85–90% of this gain was attributed to genetic selection (Havenstein *et al*., 2003). Such rapid gains were achievable in broilers because of, among other things, access to data on feed intake; the generation interval in poultry (Dekkers and Chakraborty, 2001) is considerably lower than in cattle (McParland *et al*., 2007). Similar results have been observed in pigs, with an improvement in feed efficiency in the Dutch Landrace breed from 3.5 kg in the 1930s to 2.8 kg in the 1990s (Merks, 2000). The implications of such improvements in production efficiency on the resulting environmental footprint are expected to be large; these reductions in environmental footprint were generally achieved without any direct cognizance of environmental traits in the breeding programmes.

In beef production systems, between 60 and 75% of the total dietary energy in the cowherd is used for maintenance (Ferrell and Jenkins, 1985; Montaño-Bermudez *et al*., 1990). The cowherd accounts for 65–85% of the feed used in the entire beef production system (Montaño-Bermudez *et al*., 1990).

Feed costs account for 43–67% of the total costs across a range of dairy production systems in southern Australia (Ho *et al.*, 2005). Similarly, feed costs account for approximately 80% of the overall costs in Irish dairy production systems (Shalloo *et al.*, 2004). The large contribution of the mature animal herd to the overall costs of production of the entire sector is primarily because of the reproductive rate in cattle, which is considerably lower than in species like poultry and pigs. Therefore, reducing the cost of maintaining the mature cow herd, all else being equal, will contribute to increased production efficiency. This obviously has implications for also reducing enteric CH_4 emissions because of the known association between feed intake and daily CH_4 emissions in cattle (Fitzsimons *et al.*, 2013).

A common misconception is that daily feed efficiency and the overall efficiency of the system are equivalent. It is the efficiency of the production system in its entirety that governs overall performance and the resulting environmental footprint. Although some producers may only be concerned with particular subsystems (e.g. cow and calf, feedlot, etc.) within the entire system, animal breeders must generally concern themselves with the entire (national or global) production sector. The exceptions may include systems where the parents of subsequent generations are generated by specialist breeders (e.g. producers of F_1 pigs).

Herd feed conversion efficiency (FCE) in the beef sector may be defined as:

$$\text{Herd FCE} = \frac{n_{Off} \cdot VALUE_{Off} + n_{Cow} \cdot VALUE_{Cull}}{n_{Cow} \cdot DMI_{Cow} + n_{Replace} \cdot DMI_{Replace} + n_{Off} \cdot DMI_{Off}},$$

where $VALUE_{Off}$ and $VALUE_{Cull}$ is the value of the offspring and culls, respectively; DMI_{Cow}, $DMI_{Replace}$ and DMI_{Off} is the total feed intake of the cow, replacements and other offspring, respectively; and n_{Cow}, $n_{Replace}$ and n_{Off} is the total number of cows, replacements and other offspring, respectively. This clearly shows that factors other than feed intake, such as fertility (i.e. weaning rate) and cow replacement rate and death rate, can also affect herd efficiency. Similarly, the growth rate of the animal, or in other words the ability of the animal to achieve its target slaughter weight rapidly, thereby remaining on the farm for a shorter duration, will also impact herd FCE (and environmental footprint). The denominator of the equation may be modified to reflect herd environmental efficiency as:

$$\text{Herd ENVIRONMENTAL EFFICIENCY} = \frac{n_{Off} \cdot VALUE_{Off} + n_{Cow} \cdot VALUE_{Cull}}{n_{Cow} \cdot ENV_{Cow} + n_{Replace} \cdot ENV_{Replace} + n_{Off} \cdot ENV_{Off}},$$

where ENV_{Cow}, $ENV_{Replace}$ and ENV_{Off} are the total environmental footprint of the cow, replacements and other offspring, respectively. Total environmental footprint includes all contributing factors including emissions and nitrogen loss. Moreover, the denominator environmental terms may be broken down into different diet types (e.g. grazed grass, ensiled forage, concentrates, etc.) and the associated complete life cycle analysis environmental cost of each component included.

The FCE of a dairy herd may be defined as:

$$\text{Herd FCE} = \frac{\text{Milk value} + \text{beef value}}{n_{Cow} \cdot DMI_{Cow} + n_{Replace} \cdot DMI_{Replace} + n_{Off} \cdot DMI_{Off}},$$

where milk value is the total value of the milk produced, taking cognizance of the tiered milk pricing system on milk composition (if implemented); beef value is the farm revenue for beef (i.e. cull cows and surplus animals); DMI_{Cow}, $DMI_{Replace}$ and DMI_{Off} is the total feed intake of the cow, replacements and other offspring (i.e. for beef), respectively; and n_{Cow}, $n_{Replace}$ and n_{Off} is the total number of cows, replacements and other offspring (i.e. for beef), respectively. The feed intake variables in the denominator of the definition of dairy herd feed efficiency may be replaced by the appropriate phenotypes for environmental load, to define a dairy herd environmental efficiency index.

Therefore, in dairy and beef production systems, reproductive efficiency and cow survival are important contributors to the overall system efficiency, and therefore also the environmental load including enteric CH_4 emissions. Garnsworthy (2004) documented, using modelling, that if dairy cow fertility in the UK national herd could be restored to 1995 levels from the 2003 levels, then herd CH_4 emissions could be reduced by 10–11%, while ammonia emissions could be reduced by 9% under a milk quota environment; the respective reductions were 21–24% and 17% if ideal fertility levels were achieved. A reduction of 4–5% in herd CH_4 emissions is expected in the UK if fertility levels are restored to 1995 levels from 2003 levels where no milk quota existed (Garnsworthy, 2004). These improvements were due primarily to a reduced number of non-producing replacement animals and, to a lesser extent, greater milk yield (i.e. in beef would result in greater calf growth rate) when fertility was improved. No cognizance was taken here of the impact of the replacement rate on genetic gain.

18.4 Phenotypes for Breeding

18.4.1 Residual feed intake and feed efficiency traits

Table 18.1 summarizes the plethora of definitions of (feed) efficiency that exist (Berry and Pryce, 2014). FCE was the traditional measure of feed efficiency in

Table 18.1. Definitions of (feed) efficiency in growing and lactating/mature animals; analogous definitions of feed efficiency in growing and mature animals are on the same row. (From Berry and Pryce, 2014.)

Growing animals	Lactating/mature animals
$FCE=\frac{ADG}{FI}$	$FCE=\frac{ECM}{FI};FCE_{Adj}=\frac{ECM+b_{1_5}\Delta WT^{+}}{FI-b_{1_6}\Delta WT^{-}}$
$PEG=\frac{ADG}{FI\text{-}FI_{Maintainence}}$	$PEMP=\frac{ECM}{FI-b_1WT^{0.75}}$
$RGR=\frac{100\cdot(Log_eWT_{END}\text{-}Log_eWT_{START})}{Days\ on\ test}$	
$KR=\frac{ADG}{WT^{0.75}}$	$KR=\frac{ECM}{WT^{0.75}};KR=\frac{ECM+b_{1_5}\Delta WT^{+}}{WT^{0.75}}$
$RFI=FI-(b_1WT^{0.75}+b_2ADG+b_3(\Delta)FAT+b_4WT^{0.75}\cdot FAT+b_5ADG\cdot\Delta FAT)$	$FtW=\frac{FI}{WT^{0.75}}$ $RFI=FI-(Parity\cdot\sum_{i=1}^{n}DIM^n+b_1WT^{0.75}+b_2ECM+b_3(\Delta)BCS+b_4\Delta W+b_5WT^{0.75}\cdot BCS+b_6\Delta WT\cdot\Delta BCS)$
$RG=ADG-(b_1WT^{0.75}+b_2FI+b_3(\Delta)FAT+b_4WT^{0.75}\cdot FAT)$	$RSP=ECM-(Parity\cdot\sum_{i=1}^{n}DIM^n+b_1WT^{0.75}+b_2FI+b_3(\Delta)BCS+b_4\Delta WT+b_5WT^{0.75}\cdot BCS+b_6\Delta WT\cdot\Delta BCS)$
$RIG=RG-RFI$	$RISP=RSP-RFI$

Notes: ADG = average daily gain; BCS = body condition score; DIM = days in milk; ECM = energy-corrected milk; FAT = fat depth; FCE = feed conversion efficiency; FI = feed intake; FtW = feed to weight ratio; KR = Kleiber ratio; PEG = partial efficiency of gain; PEMP = partial efficiency of milk production; RFI = residual feed intake; RG = residual gain; RGR = relative growth rate; RIG = residual intake and gain; RISP = residual intake and solids production; RSP = residual solids production; WT = live weight; ΔWT^{+} = live weight gain; ΔWT^{-} = live weight loss.

growing animals, but has now been replaced by residual feed intake (RFI) in animal breeding research studies in ruminants in particular (Koch *et al.*, 1963). Feed conversion efficiency in lactating animals is still, however, the most commonly used measure, probably because of its ease of calculation and explanation. The numerator used to calculate FCE must somehow account for the differential in the energy cost of producing milk fat, protein and lactose, and using some measure of total milk energy (Tyrrell and Reid, 1965) is one option to achieve this. Nonetheless, FCE in mature animals has serious shortcomings, especially during periods of body tissue anabolism or catabolism, such as in the immediate post-partum period (Roche *et al.*, 2007). All else being equal, females mobilizing body tissue have greater energy available for production and other bodily functions, but considerable body tissue mobilization can result in compromised health and fertility (Roche *et al.*, 2009). Moreover, the lost body tissue will generally have to be replaced in late lactation or during the dry period; therefore, any definition of efficiency in mature animals must be based on measurements over a long period. Berry and Pryce (2014) suggested an alternative definition of FCE, which they termed FCE_{adj}; this new trait included body tissue gain (e.g. growth) in the numerator and body tissue mobilization in the denominator (Table 18.1). The coefficients applied to both parameters could be derived from nutritional tables or estimated from the data (i.e. coefficients from an RFI equation).

Residual feed intake is increasing in popularity as a proxy for feed efficiency in growing cattle (Berry and Crowley, 2013), sheep (Knott *et al.*, 2008) and pigs (Gilbert *et al.*, 2007). Residual feed intake is defined as the difference between energy intake and demand and is usually estimated as the residuals from a least squares regression model regressing feed intake on the various energy sinks. An extensive discussion on RFI is outlined in the review of Berry and Crowley (2013). Figure 18.1 gives an example of a two-dimensional plane predicting, from the average of the population, the expected feed intake for each combination of individual animal metabolic live weight and average daily gain; the RFI model in this instance includes just metabolic live weight and average daily gain as the energy sinks and is typical of most RFI models fitted (Berry and Crowley, 2013). Animals above the plane (i.e. dots) eat more than predicted based on their performance (i.e. positive RFI), and are therefore deemed

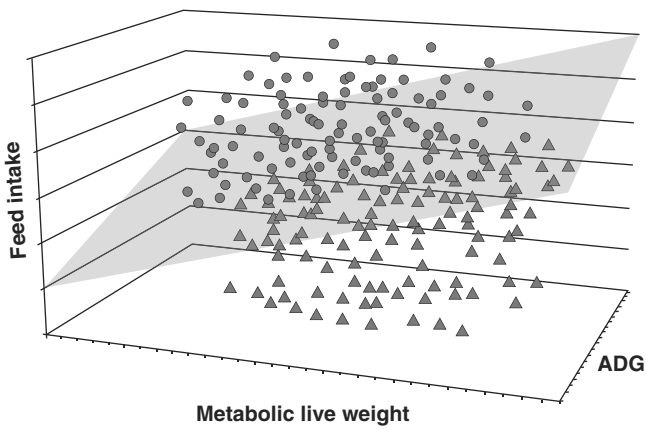

Fig. 18.1. Two-dimensional plane of expected feed intake based on metabolic live weight and average daily gain (ADG).

to be inefficient. Animals below the plane (i.e. triangles) eat less than predicted based on their performance (i.e. negative RFI), and are therefore considered to be efficient relative to the average population. The population variability in RFI reduces as the complexity and completeness of the RFI statistical model increases. However, as the complexity of the statistical model increases, the contribution of measurement error and errors due to an inaccurate model to the residual term also increases. Savietto *et al*. (2014) outlined some of the deficiencies of the commonly used RFI models, including the lack of measures of body fat and protein mass (change). Savietto *et al*. (2014) also documented inter-animal variation in the regression coefficients on the energy sink. This suggests heritable variation in individual animal energy conversion efficiencies; Savietto *et al*. (2014) did, however, caution that such variation could also be attributable to inter-animal variation in correlated contributors to differences in feed intake that were not included in the statistical model.

One of the most important points, which is often ignored, is that feed efficiency as currently defined relates only to feed efficiency per day over a given period. Berry and Crowley (2012), using a relatively crude example, showed that greater feed efficiency per day achieved using RFI did not necessarily equate to the most optimal approach to improve feed efficiency over a given period of an animal's life relative to selection on other traits. This is because RFI is independent of growth rate, and although the animal may eat less on average per day, animals that grow faster may eat less over an entire period to grow a predefined amount. However, this example only considered the finishing production system and did not consider the entire production system, as well as the impact on long-term genetic gain for either trait. A similar conclusion that RFI may not be the most appropriate index to select more efficient animals was also documented in turkeys (Willems *et al*., 2013).

In dairying, RFI and residual solids production (RSP), as defined by Coleman *et al*. (2010) and others (for RFI; Lopez-Villalobos *et al*., 2008; Prendiville *et al*., 2011; Vallimont *et al*., 2011), does not differentiate between the energy used for different functions, despite clear differences in the economic importance for the different energy sinks. For example, if the regression coefficient from regressing dry matter intake (DMI) on metabolic live weight in the multiple regression used by Coleman *et al*. (2010) in their definition of RFI (multiple regression equation also included other energy sinks) was 0.20, then, all else being equal (e.g. energy-corrected milk is identical), a cow weighing 500 kg (i.e. metabolic live weight 106 kg) eating 15.0 kg DM will have the same RFI as a cow weighing 600 kg (i.e. metabolic live weight 121 kg) and eating 18.1 kg DM. Although the feed efficiency, defined by RFI, of both animals was identical, the production efficiency of the latter animal was obviously inferior. Therefore, in order to select for production efficiency, traits other than feed efficiency must be taken into consideration.

18.4.2 CH_4 emissions traits

CH_4 production (MP; g CH_4 day^{-1}) is an obvious CH_4 emissions trait, but many alternative definitions of CH_4 emissions exist. There are three levels in which a CH_4 trait can be defined (Pickering *et al*., 2014, unpublished results); first, the farm system level, which uses information on the number of animals present within a system boundary, with a related estimate of CH_4 emissions per head, calculated, for example, from the IPCC (2006) tier 2 calculations. These calculations have embedded within them a number of assumptions about the factors that affect CH_4 emission per head, such as feed intake, feed quality and CH_4 yield. Second, the animal production level that uses information about productivity per head (i.e. milk yield or kilogram carcass weight) from individual animals to derive CH_4 intensity (MI; g CH_4 kg^{-1} product). Finally, at the animal level, individual CH_4 emissions and feed intake measurements to enable genetic progress on CH_4 yield (MY; g

CH_4 kg^{-1} DMI). Currently, there is little consistency in the use of these CH_4 emission traits. Discussion is ongoing to reach a consensus on which traits and terms to use in order to be able to compare methods to measure CH_4 and to share data so as to undertake joint analyses. This initiative is challenged by the fact that a number of the spot CH_4 sample methods developed in Europe, where cows are indoors (Garnsworthy *et al.*, 2012; Lassen *et al.*, 2012) are not very suitable to use in, for example, Australia and New Zealand, where pasture-based production systems predominate, and therefore portable accumulation chambers have been used to make large-scale recordings in sheep (Pinares-Patiño *et al.*, 2011).

The main advantage of most of the ratio traits (i.e. CH_4 per unit of product (MI) or per unit of intake (MY)) is their ease of calculation (once the appropriate performance measures are available) and interpretation, as well as the ability to compare statistics easily across populations. The main disadvantages, however, of the ratio traits are: (i) an increase in the error variance as a proportion of the total variance in the statistical analysis can result; (ii) strong correlations exist between the ratio trait and its component traits; and (iii) no distinction is made between the CH_4 used for separate functions. The latter is particularly true for lactating dairy cows where live weight is of little or no economic value. The expected responses to selection on ratio traits are also difficult to determine (Gunsett, 1984), due to the poor statistical properties of ratio traits owing to the antagonism between the desirable response in the numerator and the denominator and the unknown relative selection pressure on each. A disproportionate amount of selection pressure will be exerted on the trait in the ratio with the greater genetic variance (Sutherland, 1965); for CH_4 intensity, this is likely to be growth rate (growing cattle) or milk yield (lactating cows), since it generally has a higher coefficient of genetic variation. This correlation structure with the ratio traits also implies that genetic selection for growth or milk yield will also improve the ratio traits, despite no difference in total daily CH_4 emissions per animal. Figure 18.2 describes the expected genetic correlation between an output trait (i.e. milk yield in dairy or growth

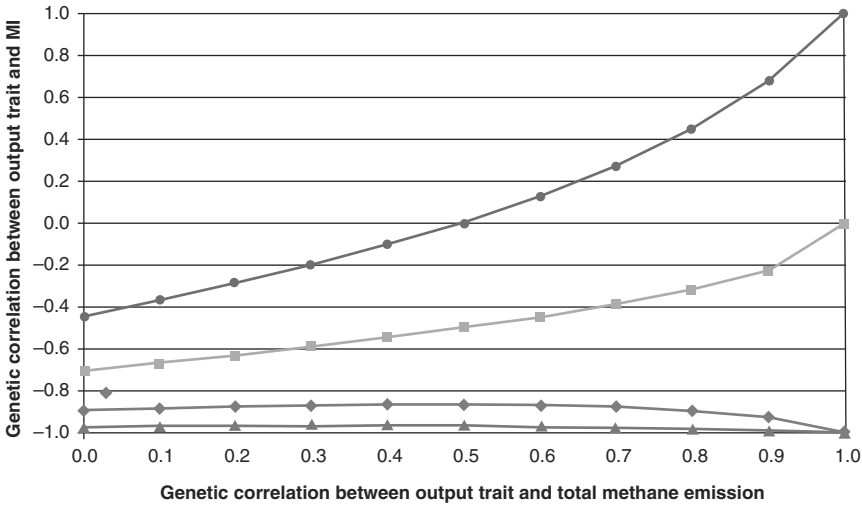

Fig. 18.2. Expected genetic correlation between output trait and CH_4 intensity (MI), as the correlation between the output trait and total CH_4 emissions varies from 0 to 1.0. The ratio of the genetic variation of the output trait relative to total CH_4 emissions was 4 (▲), 2 (♦), 1(■) and 0.5 (●).

rate in beef) and CH_4 intensity, where the ratio of variation in the output trait to total CH_4 emissions differs but also the genetic correlation between the output trait and total CH_4 emission varies.

Berry (2013) proposed an alternative definition of CH_4 emissions using a statistical approach analogous to the definition of RFI. He proposed the trait, residual CH_4 production (RMP), which he defined as the residuals from a least squares regression model regressing total daily CH_4 emissions on various energy sinks (e.g. live weight, growth rate, milk production) and possibly feed intake. The variation and genetic parameters for this trait provide information on the potential for genetic selection for reduced daily emissions per animal without impacting animal performance. If feed intake were also included in the regression model, then this trait would also depict the potential benefit of measuring daily CH_4 emission over any above knowledge on animal performance and feed intake. To our knowledge, genetic parameters for such a trait have not been quantified.

18.5 Genetic Parameters for Feed Efficiency and CH_4 related Traits

18.5.1 Feed efficiency traits

Heritability estimates for feed intake and efficiency (Berry and Crowley, 2013) in growing animals are summarized in Fig. 18.3. Pooled heritability estimates from up to 45 different studies or populations of growing cattle varied from 0.23 (FCR) to 0.40 (feed intake) and were similar to those observed for other performance traits like average daily gain (0.31), live weight (0.39) and feed intake (0.40). Moreover, considerable variation in heritability estimates existed across populations. This is not unexpected, given the diversity in breeds and feeding systems contributing to the meta-analysis.

Pooled heritability estimates from the scientific literature for feed intake and efficiency traits in mature animals (Berry and Crowley, 2013) are given in Fig. 18.4; heritability estimates in cows were considerably lower than those reported in growing animals. The lower heritability

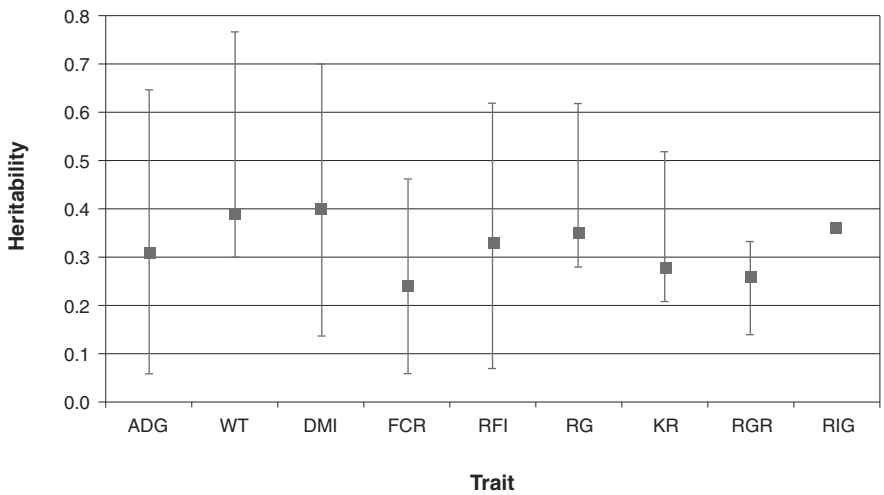

Fig. 18.3. Least square mean heritability estimate and the minimum and maximum heritability estimates for average daily gain (ADG), live weight (WT), dry matter intake (DMI), feed conversion rate (FCR), residual feed intake (RFI), residual gain (RG), Kleiber ratio (KR), relative growth rate (RGR) and residual intake and gain (RIG) across 45 studies on growing animals. (From Berry and Crowley, 2013.)

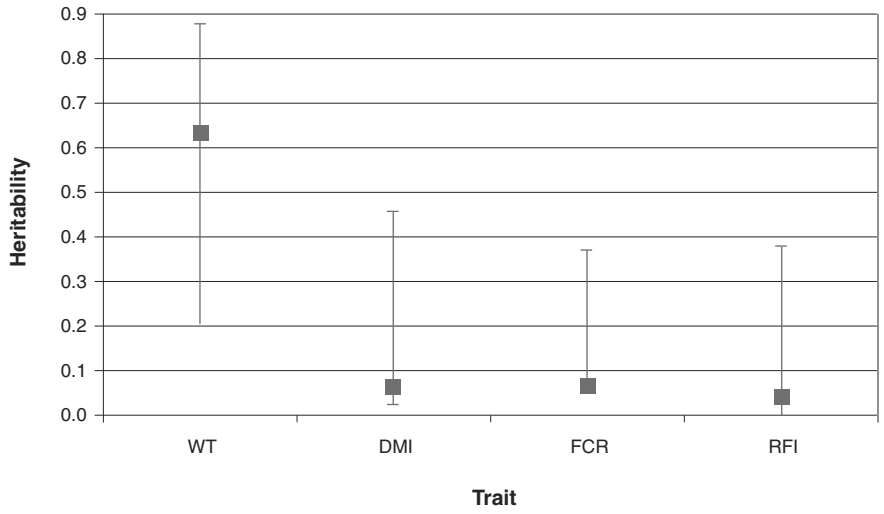

Fig. 18.4. Least square mean heritability estimate and the minimum and maximum heritability estimates for live weight (WT), dry matter intake (DMI), feed conversion rate (FCR) and residual feed intake (RFI) across 11 studies on mature (lactating) animals. (From Berry and Crowley, 2013.)

estimates for mature animals is likely attributable to increased contribution of random noise to the residual variance attributable to potential errors in the collection of the data (e.g. estimated grass feed intake at pasture as well as the influence of gut fill on live weight measures) and an incomplete or inappropriate statistical model (i.e. not properly accounting for body tissue mobilization patterns).

The phenotypic and genetic correlations between feed efficiency traits, although generally strong, were all less than unity (Berry and Crowley, 2013), implying that they were measuring different characteristics of animal feed efficiency. The genetic parameters and areas of further research are discussed in detail elsewhere in the chapter.

18.5.2 CH_4 emissions

Genetic parameters for total CH_4 production and CH_4 yield (CH_4 emissions divided by daily feed intake) measured in respiration chambers at fixed levels of feed intake have been reported for sheep (Pinares-Patiño *et al*., 2013) and cattle (Donoghue *et al*., 2013). The heritability for total CH_4 production in sheep and cattle was 0.29 and 0.40, respectively. Heritability estimates in sheep and beef for CH_4 emissions per kilogram of feed intake was 0.13 and 0.19, respectively. The coefficient of phenotypic variation for both CH_4 traits in sheep was 0.10–0.13, suggesting genetic variation does indeed exist.

Heritability estimates for measured CH_4 emissions in dairy cows do not exist. A heritability of the ratio between CH_4 and CO_2 of 0.21 was documented by Lassen and Løvendahl (2013) from 683 commercial dairy cows. De Haas *et al*. (2011) reported a heritability of 0.35 for predicted CH_4 emissions in Dutch cows; predicted CH_4 emissions were derived from feed intake and maintenance and therefore was likely to be heritable, given the heritability of DMI and live weight (Berry and Crowley, 2013). Milk fatty acid composition has also been suggested as a means of predicting enteric CH_4 output in lactating dairy cattle because of the common biochemical pathways among CH_4, acetate and butyrate in the rumen. A stoichiometric relationship between CH_4 and ruminal acetate, propionate and butyrate was proposed by Demeyer and Van Nevel (1975). The short-

chain fatty acids formed in the rumen in particular act as precursors for the *de novo* synthesis of milk fatty acids in the mammary tissue. CH_4 predictive equations from milk fatty acid composition in dairy cows have been developed in several studies (Chilliard *et al*., 2009; Dijkstra *et al*., 2011; Mohammed *et al*., 2011). Kandel *et al*. (2013) reported heritability estimates of 0.34–0.37 for predicted CH_4 emissions in dairy cows based on milk fatty acid composition.

Berry (2013) cautioned about the inferences of heritability estimates from CH_4 emissions per unit feed intake (or any CH_4 phenotype that includes known heritable traits in the numerator or denominator). Berry (2013) simulated individual animal daily CH_4 emissions for a data set of growing bulls used previously in the estimation of variance components of feed efficiency by Crowley *et al*. (2010). CH_4 yield was defined as daily CH_4 emissions divided by daily feed intake. Berry (2013) reported an expected heritability of the simulated daily CH_4 emissions of 0 but a heritability of 0.19 for CH_4 yield. The existence of a significant heritability of CH_4 yield was an artefact, not of heritable variation in CH_4 emissions but because the heritability of feed intake was 0.49 (Crowley *et al*., 2010). A similar conclusion could be hypothesized for daily CH_4 emissions, as it is likely to include genetic variation in feed intake and also performance traits such as milk production or growth rate. Therefore, of particular interest is the heritability and genetic variation in residual CH_4 production (described earlier).

18.5.3 Genetic correlations – feed efficiency and enteric CH_4

A favourable association between feed efficiency and CH_4 production is expected, given that CH_4 production represents a source of energy loss (Johnson and Johnson, 1995), and therefore inefficiency. In direct contrast, however, the likely improved digestive ability of more efficient animals could result in greater CH_4 emissions per unit feed intake.

No literature exists on the genetic correlation between CH_4-related traits and feed efficiency. Most studies that attempted to evaluate if a genetic association existed between feed efficiency and CH_4 emissions used controlled experiments of animals divergent from RFI (Hegarty *et al*., 2007). RFI is strongly correlated with feed intake (Berry and Crowley, 2012) and, as evidenced by the lack of differences in CH_4 per unit intake between animals divergent from RFI (Hegarty *et al*., 2007), the cause and effect of the observed association between RFI and CH_4 emissions needs to be elucidated. Using the correlation of 0.44 and 0.38 between daily CH_4 production and both RFI and DMI, respectively, as well as the mean correlation of 0.72 between RFI and DMI from the meta-analysis of Berry and Crowley (2012), 26% of the phenotypic variation in daily CH_4 production could be explained by RFI after accounting for differences in feed intake. However, this needs further investigation. Nonetheless, advocating selection on RFI, which is highly correlated to feed intake (Berry and Crowley, 2013), should be undertaken with caution, since reducing feed intake, even if production is held constant, may reduce CH_4 emissions per day, but unless appropriately addressed within the breeding goal, may result in greater negative energy balance and body tissue mobilization in lactating cows (McParland *et al*., 2014). More severe and prolonged negative energy balance in early lactation is known to have unfavourable consequences for dairy cow health and fertility (Roche *et al*., 2009), thereby negating or even increasing CH_4 emissions within the herd or entire sector.

18.6 Breeding Programmes

For a trait to be included in a breeding goal, the following three criteria should be fulfilled:

1. It should be economically, socially or environmentally important.
2. It should exhibit exploitable additive genetic variation.

3. It should be measurable in relatively large populations (ideally at a low cost) or be genetically correlated with (a) heritable measurable trait(s).

The criterion of having to be routinely measurable in a large population of animals has been somewhat relaxed with the development of genomic selection (Meuwissen *et al.*, 2001). The importance of feed efficiency and CH_4 emissions to feed a growing demand for animal-derived human edible protein without an associated increase in environmental load has already been discussed at length. Although CH_4 emissions currently do not have any direct economic value in most countries, breeding is a long-term strategy and therefore must take cognizance of the potential economic incentives or penalties that may be enforced in the future (Wall *et al.*, 2010). Albeit based on a few studies only, heritable genetic variation in total CH_4 emissions has been documented (Donoghue *et al.*, 2013; Pinares-Patiño *et al.*, 2013), although the extent of the genetic variation that is independent of performance traits, and thus amenable for selection without compromising performance, is not clear. The remaining issues to consider prior to recommending the inclusion of direct measures of CH_4 emissions in a breeding strategy include options for the routine generation of estimated breeding values for CH_4 emissions and what emission phenotype to include in the breeding goal.

18.6.1 Generation of breeding values

An estimated breeding value (EBV) is an estimate of the genetic merit of an animal for a given trait or series of traits based on an evaluation of all available data on the performance of the animal, and close relatives, for a trait (Berry *et al.*, 2011). Although genomic selection is now routinely used in the estimation of breeding values of dairy (Hayes *et al.*, 2009; Spelman *et al.*, 2013) and beef (Saatchi *et al.*, 2012) cattle, actual phenotypes for the trait of interest (i.e. feed intake or CH_4 emissions) are still required to: (i) estimate the association between each of the DNA markers and the phenotype; and (ii) estimate the component of the EBV or total genetic merit (i.e. including non-additive genetic variation) not captured by the DNA markers. Hence, the incorporation of genomic information into genetic evaluations does not forgo the necessity to collect phenotypic information on traits of interest. Genomic selection algorithms have, however, the potential to generate (low to moderate) accurate EBVs for animals who have no phenotypic information. The accuracy of the genomic predictions is a function of, among others, the number of animals with both genotypic and phenotypic information for the trait under investigation and the relationships between those animals and the candidate animals.

Unlike most performance traits (e.g. growth rate, milk yield), the measurement of individual feed intake and animal CH_4 emissions currently requires specialized equipment and facilities; moreover, accurate measurement of individual animal intake and CH_4 emissions generally disrupts normal management practices. Hence, there is considerable interest in low-cost, rapid measures of individual animal feed intake and CH_4 emissions that can be implemented under normal herd environments without compromising accuracy of measurement. Approaches to the measurement or prediction of feed intake and efficiency have already been discussed at length and are not considered further here.

Many of the approaches being investigated to measure or predict CH_4 emissions focus on obtaining samples of expired air from animals over a short period. The benefit of collecting phenotypic data on CH_4 emissions is dependent on the cost of procuring the data versus the marginal benefit of increased accuracy of selection over and above the use of other routinely recorded information; how this increased accuracy alters genetic gain and its associated economic benefits is also a key driver. For CH_4 emissions, a 'public good' monetary value on reducing CH_4 emissions may have to be considered (Wall *et al.*, 2010; van Middelaar *et al.*, 2014).

CH_4 emissions exhibit extreme diurnal variation, and emissions are also influenced by diet selection. Under identical conditions (i.e. same sheep, feed and level of feeding, animal handling and 24 h respiration chamber measurement), the repeatability of CH_4 yield on consecutive days was 0.89, but the repeatability of CH_4 yield 2 weeks and 1 year later was 0.26 and 0.24, respectively (Pinares-Patiño *et al*., 2013). No such data exist for cattle except repeatability estimates of daily CH_4 emissions across days (0.49; Vlaming *et al*., 2008). Moreover, no information exists on the genetic correlations between repeated measures over time, which may in fact be greater than the reported repeatability estimates and is arguably more important for use in genetic evaluations. The lack of such information is simply an artefact of the cost of procuring large data sets from which to generate precise estimates of the necessary genetic (co)variance components.

For measurement of CH_4 emissions on individual animals, the methodology must provide a consistently reliable measure of the true CH_4 emission of the individual for the period of measurement and be suitable for the production system in which the generated EBVs will be used. The period of measurement of CH_4 and the number of measurement periods should be sufficient to rank sires reliably for EBVs for the trait of interest. Respiratory chambers have traditionally been advocated as the gold standard of measurement. Isolating animals in respiratory chambers, however, can impact feed intake and both feeding pattern and diet selection (Hegarty, 2004); this may be particularly disadvantageous if the resulting EBVs are to be used in, for example, grazing animals. These shortcomings may result in a poorer prediction of actual CH_4 emissions under normal conditions compared to less precise measures of actual CH_4 emissions generated under more realistic conditions. Moreover, as previously alluded to, short-term measures of CH_4 emissions may not be a very good indicator of long-term CH_4 emissions of an animal, thereby necessitating repeated measures of the same animal over time. Given the limited number of respiratory chambers generally available, this implies CH_4 emissions on a limited number of animals, which may not be sufficient to generate accurate (genomically enhanced) EBVs.

Other options to measure individual CH_4 emissions include portable accumulation chambers (PACs), the Greenfeed® Emissions Monitor (GEM) and SF_6 (Table 18.2). Methods where recovery is less than 100% might be useful if they show consistent recovery; these include sniffers, which permit losses of CH_4 between animal and sensor. Genetic evaluations are based on contemporary comparison, and therefore systematic under- or overestimation across individuals within a contemporary group should not bias genetic evaluations. Ratio methods (e.g. CH_4/CO_2) may also be useful if the ratio is able to be equated to the CH_4 production rate.

The repeatability of CH_4 using respiratory chambers (RCs) (Pinares-Patiño *et al*., 2013) suggests that at least two measurements

Table 18.2. Comparison of methods for measuring CH_4 traits against practical criteria likely to influence implementation of measurement for genetic evaluation.

Method	Robust	Intrusive	Cost	Throughput
Respiration chamber	Yes	Yes	High	Low
Short-term accumulation chamber	Yes	Yes, but easily managed with grazing animals	Low	High
Greenfeed®	?	Moderately, requires modified grazing pattern	High	Moderate
SF_6	?	Yes for sampling, less so for grazing	High	Moderate
CH_4/CO_2	Moderate	No, if implemented in AMS	High	High

Note: AMS = automatic milking system.

with a minimum of 2 weeks apart are required to obtain reliable estimates of CH_4 emissions in RCs. The repeatability of measures in PACs is slightly less than in RCs. Limited data exist, however, to estimate repeatability of CH_4 emissions reliably using the SF_6 and GEMs, but it is anticipated that it will not be better than in RCs.

Mid-infrared (MIR) spectroscopy of individual milk samples has recently been reported to be able to predict, with some degree of accuracy, CH_4 (Dehareng *et al.*, 2012), feed intake (McParland *et al.*, 2012) and RFI (McParland *et al.*, 2014). MIR spectroscopy is the study of the interaction between matter and electromagnetic waves in the 900 cm^{-1} to 5000 cm^{-1} region. Because milk MIR is the routine method employed globally to determine the fat, protein and lactose composition concentration in milk, prediction equations for feed intake/efficiency or CH_4 emissions can be implemented rapidly at negligible marginal cost for both day-to-day herd management, but also within breeding programmes. Several studies have documented associations between milk fatty acids and CH_4 emissions (Chilliard *et al.*, 2009; Dijkstra *et al.*, 2011; Mohammed *et al.*, 2011); MIR analysis of milk has clearly been shown to be able to predict some milk fatty acids with a high degree of accuracy (Soyeurt *et al.*, 2011), thereby providing a biological justification for the predictive ability of CH_4 from milk MIR. As well as the contribution of CH_4 production to differences in RFI and its prediction from milk MIR, McParland *et al.* (2014) speculated that the milk MIR may be detecting inter-animal variation in protein turnover rate, which is likely to contribute to differences in RFI (Richardson and Herd, 2004). The use of milk MIR, however, is limited to lactating dairy cows.

18.6.2 Breeding goal trait

Strong similarities exist between the ongoing discussions on which phenotype to include in a breeding goal to reflect best the feed intake complex and what phenotype to use to reflect CH_4 emissions. For feed intake/efficiency, some advocate the inclusion of RFI directly in the breeding goal, while others suggest the inclusion of feed intake itself concomitant with the inclusion of the energy sinks also in the breeding goal (Berry and Crowley, 2013). Kennedy *et al.* (1993) showed that both scenarios, if undertaken correctly, were actually mathematically equivalent. Berry and Pryce (2014) summarized the advantages and disadvantages of including either RFI or feed intake in a breeding goal (Table 18.3); similar points may be made for whether or not to include total CH_4 emissions or CH_4 emissions independent of performance in the breeding goal. Using the mathematical equations reported by Kennedy *et al.* (1993), it can also be clearly shown that including either total CH_4 yield or total CH_4 yield independent of the performance traits is the mathematical equivalent, assuming that the performance traits are also included in the breeding goals of both scenarios. Including a ratio trait such as CH_4 per unit of product produced or CH_4 per kilogram of feed intake is not advocated, for reasons already alluded to.

One of the main advantages of providing EBVs for total CH_4 emissions is that they are easy to explain and the concept is likely to be readily acceptable by producers (and scientists). The main disadvantages, however, of just presenting EBVs for total CH_4 emissions is that it is not easy to determine whether the animal is CH_4 efficient or inefficient. For example, an animal with a positive EBV for total CH_4 emissions may actually be more CH_4 efficient than an animal with a negative EBV for total CH_4 emissions if the former animal is producing proportionally more.

The main disadvantage of presenting just EBVs for CH_4 emission adjusted for energy sinks (and feed intake) is that such a trait can be a difficult concept to understand and explain. Furthermore, the accuracy of the EBV for CH_4 independent of other traits could be low (at least in the short term) because EBVs for CH_4 emissions are likely to be genomic based or from a predictor trait (e.g. milk MIR) where the maximum accuracy

Table 18.3. Reasons in favour and against including dry matter intake (DMI) or residual feed intake (RFI) in a breeding goal.

DMI in the breeding goal	
For	Against
Easy to explain and understand	Cannot identify efficient animals easily
Economic value is relatively easy to calculate	May be misunderstood (positive EBV may be efficient)
Amenable to customized indices	Correlated with performance
Economic value of other components reflect reality in the marketplace (e.g. fat:protein price ratio)	Independent culling levels may be harmful to overall gain
Good predictors available	Misinterpreted that negative EBV might imply poorer performing animals
Higher 'reliability' through selection index theory	
May be less susceptible to genotype by environment interactions (G × E)	
RFI in the breeding goal	
For	Against
Economic value is relatively easy to calculate	Difficult to explain technically
Can slot into current breeding goals 'easily'	Low reliability (currently)
(Theoretically) uncorrelated with performance	Possibly more susceptible to G × E
Relatively simple message (if not caught up in details)	Selection index within a selection index
Could materialize in faster genetic gain for efficiency	Sensible to select on something we do not understand?
	(Never stopped us before!)
	Mixed messages from 'pros' and 'cons' camps
	RFI in lactating animals (as currently defined) is not ideal
	EBVs may change as the RFI model changes
	Possibly correlated with fertility (so is DMI!)

Note: EBV = estimated breeding value.

achievable will be the genetic correlation between the predictor trait and CH_4 emissions; because of the low reliability, EBVs may fluctuate wildly.

18.7 Conclusion

The advantages of implementing a breeding strategy for improved feed efficiency and reduced enteric environmental load are that genetic change is cumulative and permanent. The main disadvantage of breeding strategies in animals with low reproductive rates (e.g. cattle) is that progress can be slow. However, developments in reproductive technologies and genomic technologies to increase the accuracy of selection can achieve rapid genetic gain. Although breeding strategies to date have improved gross feed efficiency and CH_4 intensity dramatically, measurement of actual feed intake or CH_4 emissions will help exploit the net efficiency component of both.

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19 Acetogenesis as an Alternative to Methanogenesis in the Rumen

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Abstract

Bacteria capable of producing acetate from H_2 and CO_2 using the acetyl-CoA pathway ($4H_2 + 2CO_2 \rightarrow CH_3COOH + 2H_2O$) are known as acetogens. They have been found in a variety of anaerobic ecosystems, including sediments, wastewater treatment systems, soils and animal gut systems. In recent years, acetogens have received attention as a hydrogenotrophic population that may play a role in reducing methane (CH_4) emissions from ruminant animals. During ruminal fermentation, methanogenic archaea reduce CO_2 ($4H_2 + CO_2 \rightarrow CH_4 + 2H_2O$) or methylated compounds to CH_4. Ruminal methanogenesis represents a loss of 2–12% of gross energy of ingested feed and contributes significantly to global greenhouse gas emissions. If methanogenesis could be suppressed in the rumen, acetogenesis may serve as an effective alternative hydrogen sink, resulting in an energy gain for the ruminant through the production of acetate. Acetogenesis is the dominating hydrogenotrophic pathway in other gut systems such as those of humans, pigs, termites and potentially some native Australian marsupials. The latter are of particular interest as they exhibit foregut fermentation analogous to that of ruminants, though resulting in significantly less CH_4 production, suggesting the functioning of alternative hydrogen sinks to methanogenesis. Recent molecular investigations of the bovine and ovine rumen and the tammar wallaby forestomach revealed the presence of a diversity of functional gene sequences associated with reductive acetogenesis that demonstrate only low similarity to sequences from presently isolated authentic rumen acetogens. Giving the natural rumen acetogen population a competitive advantage over methanogens may be a strategy for establishing reductive acetogenesis rather than methanogenesis as the major hydrogen sink in the rumen. Methanogen inhibitors may aid this process, allowing hydrogen in the rumen to accumulate to levels where it can be utilized by acetogens. Previous researchers have found that reductive acetogenesis was enhanced in the presence of methanogen inhibitors in ruminal incubations. Also, acetogen enhancers may provide acetogens with an advantage over methanogens in the rumen. Continued study into the factors affecting ruminal acetogens and their interactions with methanogens is certainly needed.

19.1 Methanogenesis

Methane (CH_4) is a potent greenhouse gas, implicated in global warming (Moss *et al*.,

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2000). Of the 600 Tg of CH_4 released into the atmosphere each year, 55–70% is anthropogenic (Thorpe, 2009), and of this, enteric fermentation in ruminant livestock is the largest contributor, responsible for approximately 20–25% of anthropogenic CH_4 emissions globally (Thorpe, 2009). During enteric fermentation of carbohydrates, hydrogen is produced. Methanogenic archaea scavenge this hydrogen and use it for reducing carbon dioxide (CO_2) ($4H_2 + CO_2 \rightarrow CH_4 + 2H_2O$) or methylated compounds to CH_4 (Thauer *et al*., 1993), which is a waste product for the host and is expelled to the atmosphere by eructation. The partial pressure of hydrogen in the rumen needs to remain low for healthy functioning of the rumen (Joblin, 1999); therefore, methanogens occupy an essential niche in the rumen. However, in addition to the environmental problem of CH_4 emissions from livestock, ruminal methanogenesis is also a loss of potential energy to the host, as 2–12% of ingested energy is lost as CH_4 (Johnson and Johnson, 1995). For decades, researchers have attempted to improve the efficiency of ruminal fermentation and redirect the energy that would otherwise have been lost to the atmosphere as CH_4 into a form useful for the ruminant animal (Nagaraja *et al*., 1997).

19.2 Reductive Acetogenesis

Reductive acetogenesis via the acetyl-CoA pathway ($4H_2 + 2CO_2 \rightarrow CH_3COOH + 2H_2O$) is a hydrogenotrophic process that could serve as an alternative hydrogen sink to methanogenesis in the rumen and at the same time result in an energy gain for ruminant animals through the production of acetate, a short-chain fatty acid readily absorbed across the rumen wall (Morvan *et al*., 1994; Chaucheyras *et al*., 1995; Morvan *et al*., 1996a,b; Boccazzi, 1997; Nollet *et al*., 1997; Russell and Wallace, 1997; Joblin, 1999; Olsson *et al*., 2006). The anaerobic bacteria, capable of using the acetyl-CoA pathway to produce acetate as the sole or primary reduced end product, are known as homoacetogens (Drake, 1994) and they will be referred to simply as acetogens throughout this chapter.

19.3 The Acetyl-CoA Pathway

The acetogens are a phylogenetically diverse group of bacteria united by their ability to use the acetyl-CoA pathway for the reduction of CO_2 to acetyl-CoA, for the conservation of energy and assimilation of CO_2 into cell carbon (Drake, 1994; Drake *et al*., 2008). The acetyl-CoA pathway was resolved only relatively recently (mid-1980s) and the pathway is often referred to as the Wood/Ljungdahl pathway after the first researchers to elucidate the unique biochemistry and enzymology simplified in Fig. 19.1 (Ljungdahl, 1986; Drake *et al*., 2008; Ragsdale, 2008).

The two 'branches' of the acetyl-CoA pathway (Fig. 19.1) reflect the flow of CO_2 through to either the methyl or the carbonyl group of acetyl-CoA during acetogenesis. The methyl branch of the pathway is folate dependent, and variations of it can be found in a wide range of organisms, including in methanogens as a methanopterin-dependent process (Ragsdale, 1997). CO_2 that enters the methyl branch of the acetyl-CoA pathway is reduced to formate, which then condenses with tetrahydrofolate to form formyltetrahydrofolate. Formyltetrahydrofolate then undergoes transformation to methylenetetrahydrofolate and finally to methyltetrahydrofolate (see Fig. 19.1; Ragsdale, 2008). CO_2 that enters the carbonyl branch of the acetyl-CoA pathway is reduced to CO, which in turns condenses with the methyl group to produce acetyl-CoA. The carbonyl branch of the acetyl-CoA pathway and the enzymes catalysing it are unique and are found in acetogens, sulfate reducers and methanogens that can utilize the acetyl-CoA pathway for acetate production, the assimilation of cell carbon or acetate oxidation (Ragsdale, 1997; Drake *et al*., 2008; Ragsdale, 2008).

Acetogenesis via the acetyl-CoA pathway yields no net adenosine triphosphate (ATP) by substrate-level phosphorylation (one ATP

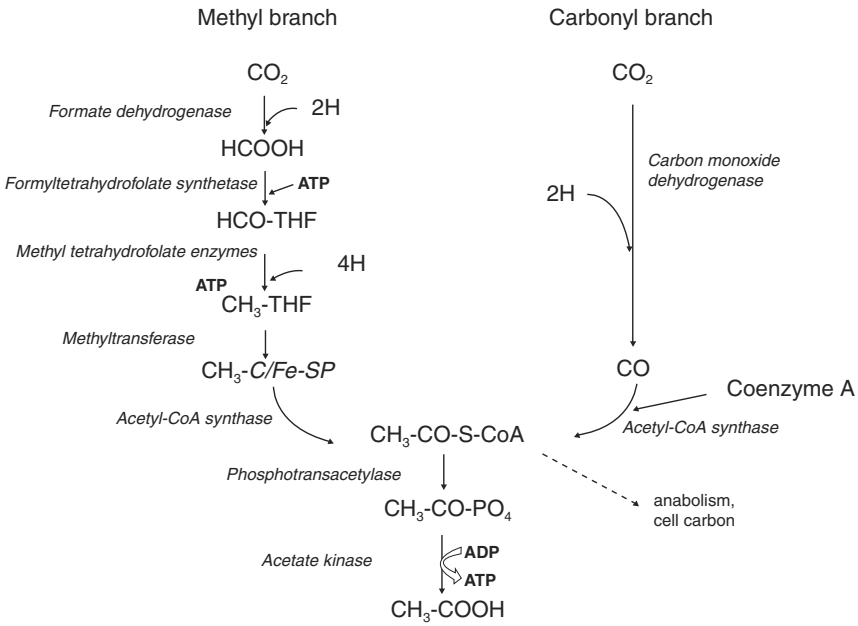

Fig. 19.1. Simplified diagram of the acetyl-CoA pathway of reductive acetogenesis. (From Drake, 1994). THF = tetrahydrofolate. Enzymes are given in italics beside the various reactions. C/Fe-SP = corrinoid iron sulfur protein. Methyl tetrahydrofolate enzymes include: methylene tetrahydrofolate cyclohydrolase, methylene tetrahydrofolate dehydrogenase and methylene tetrahydrofolate reductase.

is consumed in the formation of formyltetrahydrofolate and one ATP is formed in the acetate kinase reaction; Fig 19.1), and as such, acetogens growing autotrophically are dependent on other mechanisms for energy conservation (Müller, 2003). In acetogens, this mechanism of ion gradient-driven energy conservation is linked to the acetyl-CoA pathway, and it involves either protons or sodium ions (Müller, 2003; Drake *et al*., 2008). Proton-dependent acetogens possess membrane-bound electron transport systems such as cytochromes, menaquinones and oxido-reductases that transport protons out of the cell and the subsequent proton gradient drives ATP synthesis via proton-dependent ATPases. Sodium-dependent acetogens lack membrane-bound electron transport systems and translocate sodium out of the cell, probably during the methyl-transferase reaction of acetogenesis (refer to Fig. 19.1), with the subsequent sodium gradient driving ATP synthesis via sodium-dependent ATPase (Müller, 2003; Drake *et al*., 2008).

19.4 Phylogeny and Diversity of Isolated Acetogens

To date, 22 genera of acetogens have been identified. Some are monophyletic, containing only acetogens, for example the genus *Moorella*, while others group closely related acetogens and non-acetogens, such as the genus *Clostridium* (Drake *et al*., 2008). Acetogens exist in a wide range of habitats and environmental conditions including soils, guts, plant roots, sediments and faeces, with psycrophilic, mesophilic and thermophilic isolates all reported. Acetogens have been isolated from various rumen ecosystems including beef cattle (Leedle and Greening, 1988; Jiang *et al*., 1995; Boccazzi and Patterson, 2011), dairy cattle (Jiang

et al., 1995; Olsson *et al*., 2006; Boccazzi and Patterson, 2011), calves (Bryant *et al*., 1958), young lambs (Morvan *et al*., 1994; Rieu-Lesme *et al*., 1996, 1998; Fonty *et al*., 2007), the forestomach of deer (Rieu-Lesme *et al*., 1995), llama and bison (Rieu-Lesme *et al*., 1996) and the forestomach of native Australian macropods, which is analogous to the rumen (Ouwerkerk *et al*., 2009). It is likely that acetogens are normal flora of all ruminants (Joblin, 1999). Rumen acetogens have been classified as belonging to the genera *Acetitomaculum*, *Blautia*, *Clostridium* and *Eubacterium*, though acetogens that are yet to be formally identified have also been isolated (Table 19.1).

19.5 Molecular Tools for Investigation of Rumen Acetogens

In most environments, only a small proportion of the total microbial population can be cultured (Raskin *et al*., 1994); therefore, molecular techniques are the preferred tools for investigating complex microbial communities. The rumen environment is quite typical in this regard (Hess *et al*., 2011), and it is likely that there are a range of acetogenic bacteria in the rumen that are presently uncultivable. Additionally, cultivation techniques for acetogens can be particularly difficult, as some organisms initially isolated as reductive acetogens appear to lose their hydrogenotrophic capacity with prolonged laboratory maintenance (Küsel *et al*., 2000; Fonty *et al*., 2007). The use of molecular techniques, such as those targeting nucleic acids, proteins or cell components for example, is therefore critical in investigations of ruminal acetogens.

As the acetogens are phylogenetically diverse, traditional 16S rRNA gene-based molecular tools are not useful for detecting all acetogens or for identifying new acetogens in environmental samples. A functional gene-based molecular approach is ideal, and at present there are two available functional gene-based assays for acetogen diversity analysis. The first targets the formyltetrahydrofolate synthetase gene (*fhs*) (Leaphart and Lovell, 2001) and it has been used in rumen investigations (Matsui *et al*., 2008; Gagen *et al*., 2010, 2012; Henderson *et al*., 2010; Mitsumori *et al*., 2013); however, it is compromised by lack of specificity due to the presence of the enzyme

Table 19.1. Acetogens isolated from ruminants.

Isolate	Source	Reference
Acetitomaculum ruminis	Steer rumen	Greening and Leedle (1989)
Blautia coccoides (8F)	Methanogen-free lambs	Fonty *et al*. (2007)
Blautia producta	Calf rumen	Bryant *et al*. (1958)
Blautia schinkii	Young lamb	Rieu-Lesme *et al*. (1996)
Clostridium difficile-like	Newborn lambs	Rieu-Lesme *et al*. (1998)
Clostridium symbiosum[a]	Methanogen-free lambs	Fonty *et al*. (2007)
Enterococcus gallinarum[a]	Methanogen-free lambs	Fonty *et al*. (2007)
Escherichia coli[a]	Methanogen-free lambs	Fonty *et al*. (2007)
Eubacterium limosum	Sheep rumen	Genthner and Bryant (1987)
Propionibacter australiense[a]	Methanogen-free lambs	Fonty *et al*. (2007)
ser 8 and ser 5 Cluster XIV Clostridia	Young lamb	Morvan *et al*. (1994); Fonty *et al*. (2007)
Unidentified	Dairy or beef cattle	Boccazzi and Patterson (2011)
Unidentified	Deer	Rieu-Lesme *et al*. (1995)
Unidentified	Cow rumen	Joblin (1999)
Unidentified	Sheep rumen	Joblin (1999)
Unidentified coccoid spore-forming bacteria	Lambs, llamas and bison	Rieu-Lesme *et al*. (1996)

Note: [a]Isolated as reductive acetogens but not retaining acetogenic capacity after subculture (Fonty *et al*., 2007).

formyltetrahydrofolate synthetase (FTHFS) in other biochemical pathways (Drake *et al*., 2008; Pierce *et al*., 2008). To improve on this approach, Henderson *et al*. (2010) developed a homoacetogen similarity (HS) score based on key residues within FTHFS sequences of authentic acetogens that can be applied to help classify FTHFS sequences from acetogens and non-acetogens. The HS score method has value for distinguishing FTHFS sequences from known acetogens (HS >90%) and known non-acetogens (HS <60%), though is limited for determining the origin of sequences with intermediate HS scores (60–90%).

The second available functional gene-based molecular tool for the acetogens targets the acetyl-CoA synthase gene (*acsB*; Gagen *et al*., 2010). Acetyl-CoA synthase (ACS) is one of the four enzymes unique to the acetyl-CoA pathway (the others being carbon monoxide dehydrogenase, a corrinoid iron–sulfur protein and a methyltransferase) (Ragsdale, 1991; Fig. 19.1); therefore, this approach is more specific than FTHFS-based analyses. Combined approaches using both *fhs*- and *acsB*-based molecular tools have been used to investigate and compare the acetogen diversity in the bovine and ovine rumen, the tammar wallaby forestomach and the developing rumen of young lambs (Gagen *et al*., 2010, 2012). Collectively, *fhs*- and *acsB*-based analyses of various rumen systems, i.e. from beef cattle, dairy cattle, sheep and deer located in different parts of world (Australia, New Zealand, Japan, France) and feeding grain, silage or pasture (Matsui *et al*., 2008; Gagen *et al*., 2010, 2012; Henderson *et al*., 2010; Mitsumori *et al*., 2013), revealed a large diversity of putative acetogens that are not closely affiliated with any presently isolated acetogens (see Figs 19.2 and 19.3 for a selection of FTHFS and ACS sequences recovered from rumen ecosystems in relation to those from isolates). Based on these molecular analyses, the majority of putative rumen acetogens affiliate broadly with the *Clostridiaceae* and *Lachnospiraceae* (Gagen *et al*., 2010, 2012). It is essential that cultivation attempts to isolate and characterize members within this diverse group are undertaken in order to gain a complete picture of the natural rumen acetogen population. Traditional cultivation approaches that select for acetogens capable of strictly autotrophic growth are probably not ideal for many members of this diverse group of as yet uncultured acetogens. Future acetogen cultivation approaches may need to accommodate a wider metabolic potential; for example, alternative electron acceptors and/or stimulatory substances that acetogens can use during mixotrophic growth (discussed below).

19.6 Physiology and Metabolic Potential of Acetogens

Acetogens display diverse metabolic potential and can use a wide range of electron donors and electron acceptors in addition to H_2 and CO_2. Growth on some of these substrates can result in hydrogen production, rather than consumption (Mackie and Bryant, 1994). Electron acceptors that acetogens can use include fumarate, nitrate, thiosulfate, dimethylsulfoxide, acetaldehyde, aromatic aldehydes and aromatic acrylate groups. Alternative electron donors include formate, carbon monoxide, alcohols, aldehydes, carboxylic acids, glycolate, hexoses, pentoses, betaine and substituent groups of aromatic compounds (Drake *et al*., 2006, 2008). As a result, acetogens can form a large variety of reduced end products other than acetate, depending on the available substrates (Drake *et al*., 2008). Also, although use of the acetyl-CoA pathway is a unifying feature of all acetogens, acetogenesis is not necessarily the preferred biochemical pathway and some acetogens favour alternative terminal electron accepting processes. For example, when nitrate is available, *Moorella thermoacetica* uses nitrate as an electron acceptor rather than CO_2, and nitrate dissimilation to nitrite and ammonium is preferred over acetogenesis (Seifritz *et al*., 1993; Fröstl *et al*., 1996). Other acetogens can harness the acetyl-CoA pathway to consume hydrogen and CO_2 simultaneously with growth on organic substrates, a characteristic known as mixotrophy (Braun and Gottschalk, 1981;

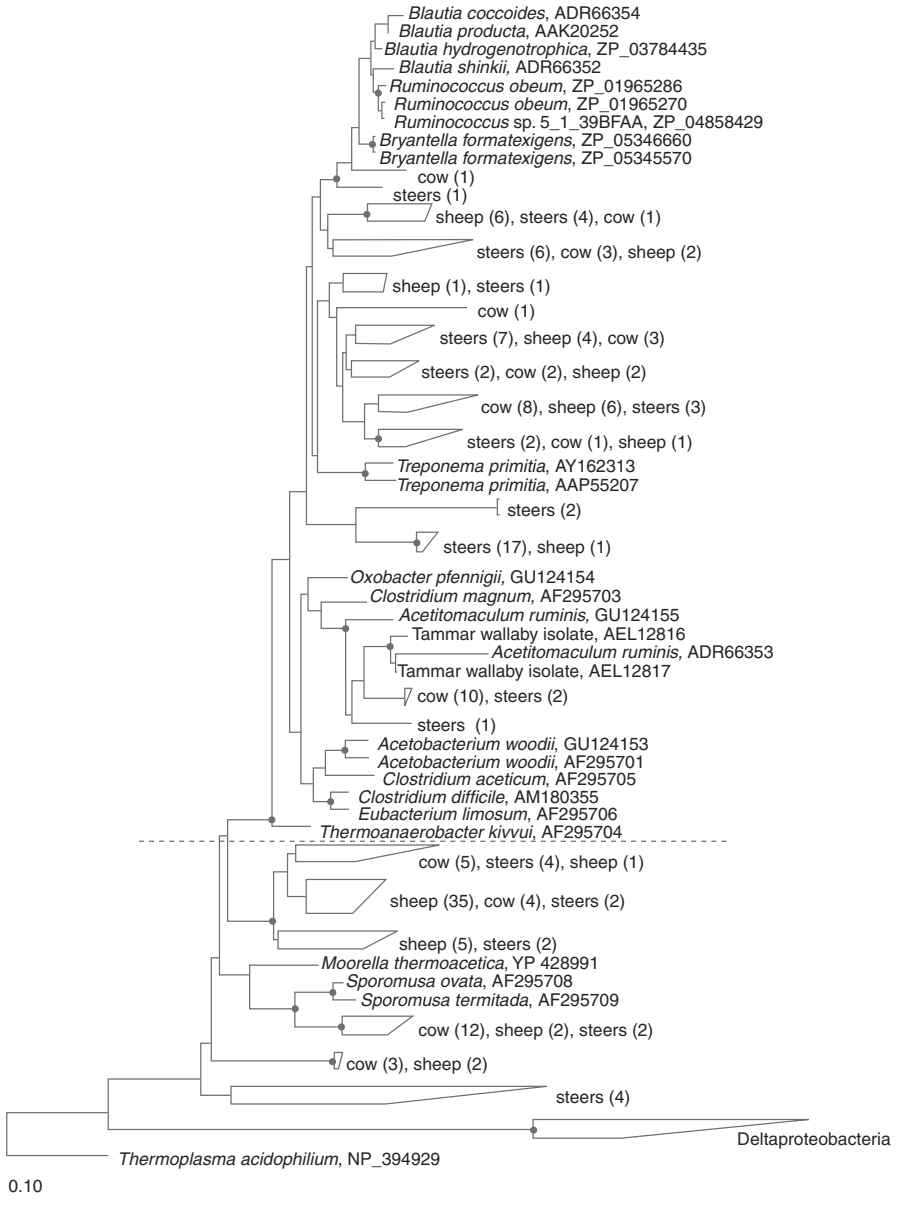

Fig. 19.2. Maximum likelihood tree of deduced FTHFS amino acid sequences from selected rumen studies. Tree was constructed using RaXML (Stamatakis, 2006; Stamatakis *et al*., 2008) at the CIPRES Science Gateway (Miller *et al*., 2010) with the Jones Taylor Thornton (Jones *et al*., 1992) model of amino acid substitutions and a gamma rate of substitution, 25 discrete rate categories and bootstrap analysis performed for the best tree topology with 100 re-samplings. Rumen sequences are from five pasture-fed Brahman steers (Gagen *et al*., 2010), a Holstein cow (Matsui *et al*., 2008) and two Texel sheep (Gagen *et al*., 2012), with sequences from each study indicated by the term 'steers', 'cow' and 'sheep', respectively. The number of sequences in closed groups is indicated in brackets. Genbank accession numbers for reference sequences are shown after species name. Bootstrap values of ≥75% are indicated by circles at branch nodes. The dashed line represents an approximate separation between FTHFS sequences from authentic acetogens and those from non-acetogens, though the distinction is not strict. The scale bar indicates 10% sequence divergence.

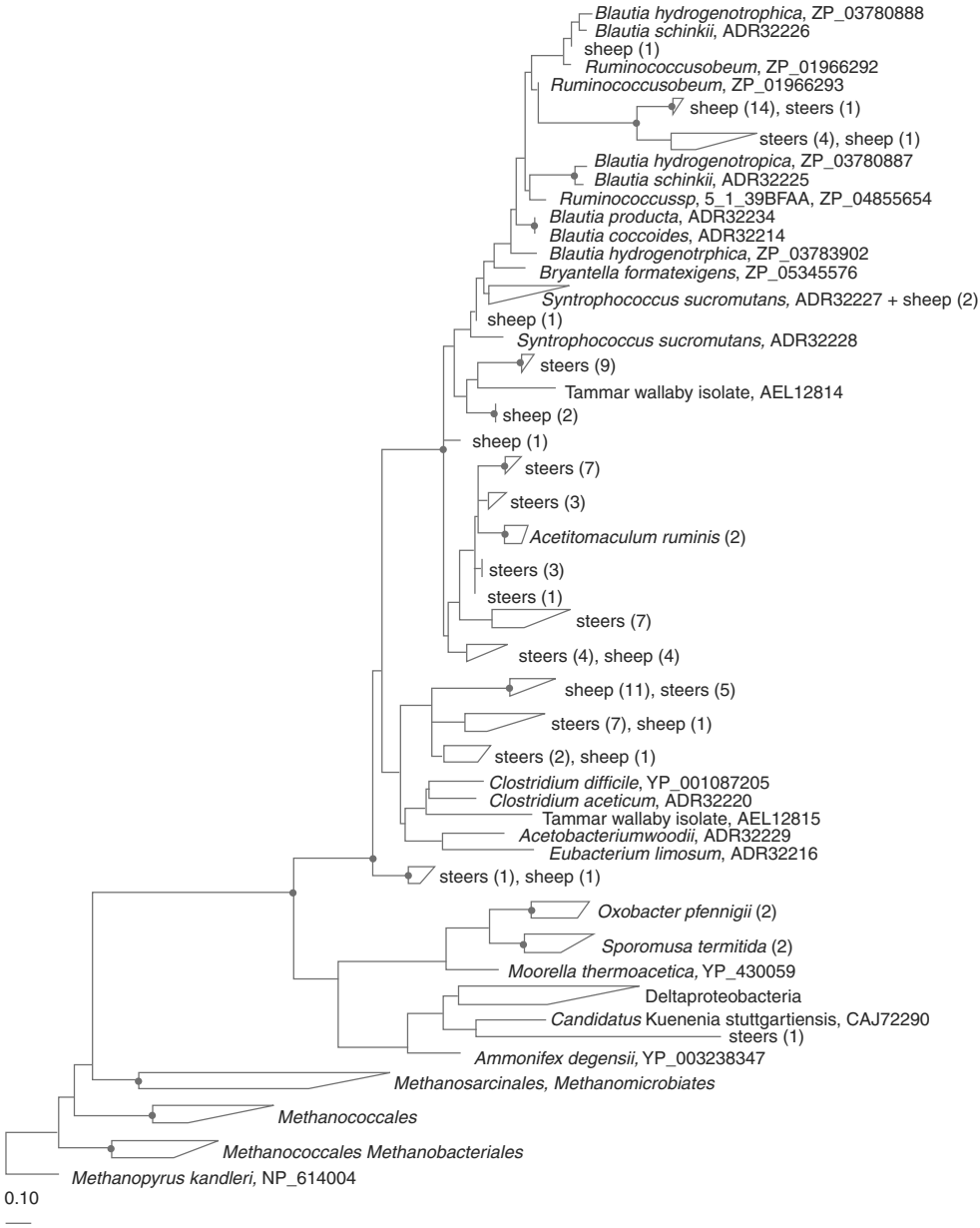

Fig. 19.3. Maximum likelihood tree of deduced ACS amino acid sequences from selected rumen studies. Tree was constructed as outlined for FTHFS sequences (Fig. 19.2). Rumen sequences are from five pasture-fed Brahman steers (Gagen *et al*., 2010) and two Texel sheep (Gagen *et al*., 2012), indicated by the terms 'steers' and 'sheep', respectively. The number of sequences in closed groups is indicated in brackets. Genbank accession numbers for reference sequences are shown after species name. Bootstrap values of ≥75% are indicated by circles at branch nodes. The scale bar indicates 10% sequence divergence.

Breznak and Blum, 1991; Breznak, 1994). Mixotrophy influences the competitiveness of acetogens over methanogens in some ecosystems (Breznak and Blum, 1991) and has been suggested as an important trait required for any acetogen that will be used in strategies for reducing ruminal methanogenesis (Morvan and Fonty, 1996; Joblin, 1999). However, to date, only one of the few isolated rumen acetogens is reported to be capable of mixotrophy (Morvan and Fonty, 1996), and in some cases hydrogenotrophy is even repressed by the presence of carbohydrates (Pinder and Patterson, 2012). It is likely that mixotrophic rumen acetogens exist, but are yet to be isolated since current culturing strategies employ predominantly autotrophic conditions.

19.7 Thermodynamic Factors Controlling Reductive Acetogenesis in the Rumen

In the typical rumen, reductive acetogenesis is not a major hydrogenotrophic pathway and methanogenesis is the primary hydrogen sink (Breznak and Switzer, 1986; Breznak and Kane, 1990). This may be because methanogenesis is energetically more favourable than reductive acetogenesis (Breznak and Kane, 1990). Under typical rumen conditions, the Gibbs free energy change (ΔG) for reductive acetogenesis is –10.2 kJ mol^{-1}, while for methanogenesis from the same substrates ΔG is –68.3 kJ mol^{-1} (Ungerfeld and Kohn, 2006). For reactions with hydrogen as a substrate, there is an inverse relationship between ΔG and the minimum partial pressure of hydrogen required for the reaction to occur. That is, the less energetically favourable a hydrogen-consuming reaction is, the greater partial pressure of hydrogen is required before it will proceed (Ellis *et al*., 2008). The minimum partial pressure of hydrogen required for reductive acetogenesis is higher than that required for methanogenesis and sulfate reduction, and as a result methanogens and sulfate reducers have a competitive advantage over reductive acetogens for hydrogen use in the rumen. Sulfur levels in the rumen are limiting, which prevents sulfate reducers dominating over methanogens (Ellis *et al*., 2008). Methanogens are therefore the major hydrogen sink in the rumen naturally and they consume hydrogen rapidly, never allowing it to accumulate to the levels at which most acetogens can utilize it (Nollet *et al*., 1997; Ellis *et al*., 2008).

None of the ruminal acetogens identified so far (Table 19.1) are obligate hydrogenotrophs (Nollet *et al*., 1997; Joblin, 1999), and it is possible that acetogens in the rumen simply use alternative substrates, potentially contributing to hydrogen production rather than hydrogen consumption via autotrophic growth (Mackie and Bryant, 1994; Pinder and Patterson, 2012). However, Joblin (1999) reports that ruminal acetogens with the ability to utilize hydrogen at low concentrations had been isolated and found to be able to dominate over methanogens (*Methanobrevibacter* sp.) and reduce CH_4 formation *in vitro*. Also, *in vivo* acetogens dominate in the rumen of very young animals but are replaced with methanogens as the rumen develops fully, indicating that there is competition for hydrogen between these two groups (Morvan *et al*., 1994). In some other gut systems, reductive acetogenesis rather than methanogenesis is the principal pathway for the disposal of hydrogen generated during digestion (Breznak and Kane, 1990; Mackie and Bryant, 1994), suggesting that thermodynamic control is not the only factor regulating methanogen–acetogen interactions. A better understanding is needed of the activity of rumen acetogens *in vivo*, as well as methanogen–acetogen interactions in the rumen naturally and during rumen development, if reductive acetogenesis is to serve as a successful alternative to methanogenesis in the rumen, as it appears to in some other ecosystems.

19.8 Enhancing Reductive Acetogenesis in the Rumen

Giving the natural rumen acetogen population a competitive advantage over

methanogens may be a strategy for establishing reductive acetogenesis rather than methanogenesis as the major hydrogen sink in the rumen. Methanogen inhibitors may aid this process, allowing hydrogen in the rumen to accumulate to levels where it can be utilized by acetogens (Ungerfeld and Kohn, 2006). Le Van *et al*. (1998) and Nollet *et al*. (1997) found that reductive acetogenesis was enhanced in the presence of the methanogen inhibitor, bromoethansulfonate, in ruminal incubations. Mitsumori *et al*. (2013) also reported a change in acetogen diversity *in vivo* in Holstein steers fed bromochloromethane (BCM), suggesting that acetogens in the rumen were able to adapt in response to the inhibition of methanogenesis. Additionally, lambs removed from their mothers within 2 days of birth and raised in isolation produced significantly less CH_4 than conventionally raised lambs and appeared to utilize more metabolic hydrogen via reductive acetogenesis (Faichney *et al*., 1999).

Another strategy may involve acetogen enhancers to provide acetogens with an advantage over methanogens in the rumen. Chaucheyras *et al*. (1995) found that the yeast, *Saccharomyces cerevisiae*, stimulated an isolated ruminal acetogen to use hydrogen *in vitro*, even in the presence of a methanogen. Alternatively, acetogens with the ability to grow mixotrophically, which is energetically more favourable than methanogenesis per mole of hydrogen consumed (Breznak and Blum, 1991), may be able to compete with methanogens.

19.9 Reductive Acetogenesis in Other Gut Environments

Gastrointestinal environments where reductive acetogenesis dominates over methanogenesis may be a useful source of acetogens with potential to compete successfully with methanogens in the rumen. In the gut systems of some humans, pigs, termites, rats and cockroaches, reductive acetogenesis is the principal pathway for the disposal of hydrogen generated during digestion, and CH_4 production is limited (Breznak and Kane, 1990; Mackie and Bryant, 1994). There may be gradients of hydrogen in these ecosystems where acetogens dominate or host factors that influence the presence of acetogens and methanogens in these guts (Ungerfeld and Kohn, 2006). Potentially, the ability to grow mixotrophically gives natural acetogens a competitive advantage in these ecosystems (Breznak and Blum, 1991; Breznak, 1994; Ungerfeld and Kohn, 2006). Of particular interest is the gut system of some native Australian macropods such as kangaroos and wallabies. These marsupials exhibit foregut fermentation analogous to that of the rumen; however, they appear to emit minimal amounts of CH_4 compared to ruminants (Kempton *et al*., 1976; Engelhardt *et al*., 1978; Dellow *et al*., 1988). The mechanisms behind this are poorly understood and could be physiological, such as body temperature, retention time of feed in the gut or host regulation of microorganisms in the gut (von Engelhardt *et al*., 1978). However, potentially, acetogenesis acts in concert with methanogenesis in these animals. Acetogens have been isolated from eastern grey (*Macropus giganteus*) and red (*Macropus rufus*) kangaroos (Ouwerkerk *et al*., 2009), as well as from the forestomach of the tammar wallaby (Gagen *et al*., 2014), and all isolates are potent hydrogenotrophs. The recently isolated tammar wallaby acetogen also demonstrates mixotrophic capabilities, as well as the ability to grow and consume hydrogen when in co-culture with a methanogen with hydrogen available at high partial pressures (e.g. >5 mM hydrogen; Gagen *et al*., 2014). Furthermore, when grown in co-culture with a methanogen, the tammar wallaby acetogen has been found to recycle hydrogen generated from fermentative growth rather than release it for methanogenesis. Isolates like these, with favourable metabolic characteristics, may be a contributing factor to lower CH_4 emissions in other gut ecosystems and could potentially be useful in strategies for reducing CH_4 emissions from ruminants and redirecting the otherwise lost energy into acetate.

19.10 Conclusion

Naturally, methanogenesis is the primary hydrogen sink in the rumen. If methanogens could be suppressed with inhibitors and/or acetogens enhanced with stimulants or through mixotrophic growth, acetogenesis could serve as an alternative hydrogenotrophic pathway in the rumen. In addition, an active population of acetogens growing under mixotrophic conditions would result in less hydrogen being produced from fermentation of available carbohydrates. Further study is needed into the diversity of acetogens in the rumen and molecular approaches using functional gene-based (*acsB*, *fhs*) molecular tools are appropriate for this purpose. Cultivation attempts must also continue in order to understand fully the function of acetogens as well as methanogen–acetogen interactions in the rumen and analogous gut ecosystems.

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20 Immunization and Tannins in Livestock Enteric Methane Amelioration

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Abstract

The complexity of the rumen microbial ecosystem supports the efficient conversion of various carbohydrates to volatile fatty acids for fulfilling host energy requirement via stepwise disposal of hydrogen (H_2) through the reduction of carbon dioxide (CO_2) to methane (CH_4). Although, this mechanism is indispensable for rumen homeostasis, CH_4 production in ruminants has attracted a great deal of attention due to its contribution to the greenhouse gas effect and global warming. Various strategies have therefore been considered for its mitigation. Rumen methanogen targeting vaccination is a promising means of reducing CH_4 emissions by decreasing the number or activity of rumen methanogens. However, trials of this strategy have provided inconsistent results, and need for further consideration of the composition, function and microbial interactions within the ecosystem. Alternatively, to establish a more efficient way for the mitigation of CH_4 emission, systematic intervention in rumen microbial populations by a combination of vaccination and other chemical means may also be feasible. Although some of the CH_4 abatement strategies have shown efficacy *in vivo*, more research is needed to make any of these approaches applicable to animal production systems. This chapter provides the background to the diversity and plasticity of functions of the rumen bacterial and methanogenic communities, as well as some of the CH_4 abatement options that aim to manipulate the rumen community, including immunization and other concepts.

20.1 Rumen Bacterial and Archaeal Community

Ruminant animals harbour a complex microbial community consisting of a diverse array of bacteria, archaea, protozoa and fungi in the rumen (Stewart *et al*., 1988). These different microbes interact with one another and play an important role in the digestion of fibrous plant material, anaerobically fermenting them into end products, which are in turn used as an energy source by the host. This nutritional change is a unique step in the energy metabolism of ruminants. Among ruminal microbes, bacteria decompose the feed into short-chain (C1–C5) fatty acids, amino acids, hydrogen, carbohydrates, etc. The archaeal component of the ecosystem, which is thought to be represented exclusively by methanogens, is responsible for the removal of hydrogen (St-Pierre and Wright, 2013).

Ruminal bacteria and archaea are obligate anaerobes. For many years, descriptions of bacterial diversity in the rumen were based mainly on the use of anaerobic culture techniques. More than 200 bacterial species have been isolated from the rumen, and many of these have been characterized

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both phylogenetically and physiologically (Dehority, 2003). Although culture-dependent studies have improved our understanding of rumen microbiology, there are still methodological obstacles to the precise and rapid monitoring of the entire ruminal microbial community. For example, many of the rumen microbes have yet to be cultured. There is renewed interest in this question due to the development of various molecular techniques, especially those based on the 16S rRNA genes (Uyeno *et al*., 2004). These technologies provide not only a phylogenetic framework of the microbiota but also insights into the impact of host and environmental factors on the microbiota community structure and dynamics. Finally, the extreme bacterial molecular diversity uncovered in such investigations reflects the complex metabolic network in which rumen bacteria are involved. We succeeded in monitoring the community members predominating in ruminal ecosystems using a probe set coupled with an RNA-based quantitative detection method (Uyeno *et al*., 2007). This enabled quantitative detection of 11 groups, including those that have not yet been applied to rumen samples by any of the molecular quantification methods. We then determined representative bacteria and archaea inhabiting the rumen of Holstein heifers. The results were generally consistent with previous observations of the quantitative detection of the target groups, *Bacteroides/Prevotella* (Krause *et al*., 2000), *Ruminococcus flavefaciens* and *Ruminococcus albus* (Krause *et al*., 1999, 2001) and genus *Fibrobacter* (Stahl *et al*., 1988; Ziemer *et al*., 2000). The coverage with the probe set was 71–78% of the total bacterial 16S rRNA. Kong *et al*. (2010) also investigated the composition and distribution of bacterial populations associated with liquid and solid rumen contents from ruminal-cannulated Holstein dairy cows fed grass hay or barley silage diets with or without flaxseed using another RNA-based quantitative method (fluorescence *in situ* hybridization (FISH)). The results of these two studies are summarized in Table 20.1. *Bacteroidetes* and *Firmicutes* were abundant in the rumen fractions in these studies. In Kong's study, the classes *Deltaproteobacteria* and *Gammaproteobacteria* were found to be major constituents of the rumen microbial community. Fibrolytic species including *Fibrobacter succinogenes* and *Ruminococcus* spp. and archaeal methanogens accounted for greater proportions of the microbiota in our study compared to the other study ((Kong *et al*., 2010). Overall, minor differences in the results of these two studies were probably due to the age and stages of the experimental animals, as well as the feeding conditions. However, both analyses showed a certain unknown proportion that none of the probes could determine. This implies difficulty in producing a complete description of the whole community by these methods, due mainly to the genetically diverse structure of the bacteria in rumen ecosystems. Methanogens are present (0.1–0.6%) in normal rumen samples as determined by FISH, which is close to or within the range (0.3–3.3%) estimated in our and other studies (Lin *et al*., 1997; Sharp *et al*., 1998). Archaea have been shown to already occupy an ecological niche in the rumen of young ruminants no more than 8 weeks of age (unpublished data).

Cultivation-based analysis has identified *Methanobrevibacter*, *Methanomicrobium* and *Methanobacterium* as predominant methanogens genera in the rumen (Stewart *et al*., 1997). Due to the fastidious growth requirements of rumen methanogens, the information is very limited in both quantity and quality to determine the true activity and potential of these microbes compared to other rumen bacteria. Tajima *et al*. (2001) succeeded in recovering a wide range of the rumen archaeal molecular diversity, as well as several 16S rRNA sequences that did not cluster with known methanogens, using two different sets of archaeal primers that had not been used previously in culture-based microbiological studies. Studies revealed the presence of five methanogen species namely, *Methanobacterium formicicum*, *Methanobacterium ruminantium*,

Table 20.1. Rumen microbial community profiles determined by RNA-based methods.

	Uyeno *et al*. (2007)	Kong *et al*. (2010)
Sample	Holstein heifers	Dairy cows
Method	RNaseH cleavage method[a]	FISH
Population profile		
High (>10%)	*Bacteroides/Prevotella* *Clostridium coccoides– Eubacterium rectale* group	*Bacteroides/Prevotella* *C. coccoides –E. rectale* group *Deltaproteobacteria*
Moderate (>3%)	*Archaea* *Fibrobacter*	*Gammaproteobacteria*
Low (≤3%)	*Selenomonas* *Ruminococcus flavefaciens* *Ruminococcus bromii* *Ruminococcus albus*	*Archaea* *Spirochaetaceae* *Fibrobacter* *Ruminococcus* spp.
Coverage rate[b]	71–78%	37–92%

Notes: [a]See Uyeno *et al*. (2004) for details of the method; [b]the numbers represent the sum of percentages of the individually defined microbial groups in a rumen sample.

Methanosarcina barkeri, *Methanosarcina mazei* and *Methanosarcina mobile* (Stewart *et al*., 1988; Kumar *et al*., 2009). Rumen protozoa are also involved in methanogenesis because of their ecto/endosymbiotic association with methanogenic archaea that utilize the hydrogen produced during fermentation (McAllister and Newbold, 2008; Kumar *et al*., 2009). The protozoa-associated population consists mainly of the family *Methanobacteriaceae* and accounts for about 90% of all rumen methanogens, while the remainder consists of free-living organisms represented by the *Methanomicrobiales* (Sharp *et al*., 1998). Similarly, 54% of the total methanogens in the sheep rumen is *Methanomicrobium mobile* (Yanagita *et al*., 2000). The molecular diversity of rumen methanogens in sheep in Australia was investigated using individual 16S rRNA gene libraries prepared from the rumen contents (Wright *et al*., 2004). A total of 733 clones were examined, and the analysis revealed 65 phylotypes with sequences similar to those of cultivated methanogens belonging to the order *Methanobacteriales*, and genus *Methanobrevibacter*. The use of molecular techniques has already revealed the enormous methanogen diversity and putative novel species in the rumen (Yanagita *et al*., 2000; Tajima *et al*., 2001).

The ruminal microbial community is viewed as an ecosystem that can be perturbed; for example, by altering diet (Russell and Rychlik, 2001; Tajima *et al*., 2001). The effects of diet on diversity and number of a wide range of bacterial species in the rumen are well known (Whitford *et al*., 1998; Tajima *et al*., 1999; Yanagita *et al*., 2000; Kocherginskaya *et al*., 2001). In addition, cattle with higher feed efficiencies were reported to produce 20–30% less methane (Eckard *et al*., 2010; Buddle *et al*., 2011). Methane (CH_4) production is influenced by feed intake and the digestibility of dry matter. The probable association between the 'methanogenic biome' and feed efficiency in cattle has been studied (Wright *et al*., 2004; Guan *et al*., 2008). Limited genera and/or species comprising the methanogenic ecology may play an important role in contributing to the difference in CH_4 emission between animals with different feed efficiencies. An application of high-resolution detection techniques is warranted to learn more about the linkage between the microbial ecology of methanogens and feed efficiency in cattle, especially the energy-harvesting mechanism.

20.2 Enteric CH_4 Emission and Its Mitigation

The ability of the rumen microbial system to convert carbohydrates of plant origin

efficiently to fermentable sugars relies on the effective disposal of hydrogen through methanogenesis. For several decades, the importance of methanogen archaea in rumen function has been studied intensively with regard to CH_4 emission and its suppression (Dumitru *et al*., 2003; Eun *et al*., 2004; Mohammed *et al*., 2004; Sar *et al*., 2004). More than 95% of the CH_4 emitted by ruminants is produced in the rumen, and is associated with 2–15% of gross energy loss from ruminants, which contributes to 13–19% of global greenhouse gas (Patra, 2012). This shows the role of these organisms in global warming contributed from domesticated livestock. CH_4 reduction strategies should improve ruminant production efficiency and mitigate global warming.

In ruminants, CH_4 production principally depends on dry matter (DM) intake (Shibata and Terada, 2010). For carbohydrate digestion in the rumen anaerobic oxidation is required in the disposal of hydrogen (Russell and Wilson, 1996; Wright and Klieve, 2011). The principal requirement for minimizing CH_4 emission from the rumen is to divert/rechannel the hydrogen away from methanogens. An alternative metabolic pathway for rumen microbes to dispose of hydrogen is higher propionate production from succinate. An inverse relationship has been described between CH_4 and propionate production in the rumen (Shibata and Terada, 2010), which reflects the competitiveness of both processes for the net consumption of reducing power. In respect of H_2 production/utilization, rumen bacteria are classified into three groups (Bryant, 1979): (i) bacteria that produce propionate, butyrate, ethanol and/or lactate; (ii) bacteria that produce acetate and hydrogen; and (iii) methanogenic microorganisms (i.e. hydrogen users; Fig. 20.1). The first type represents those bacteria that

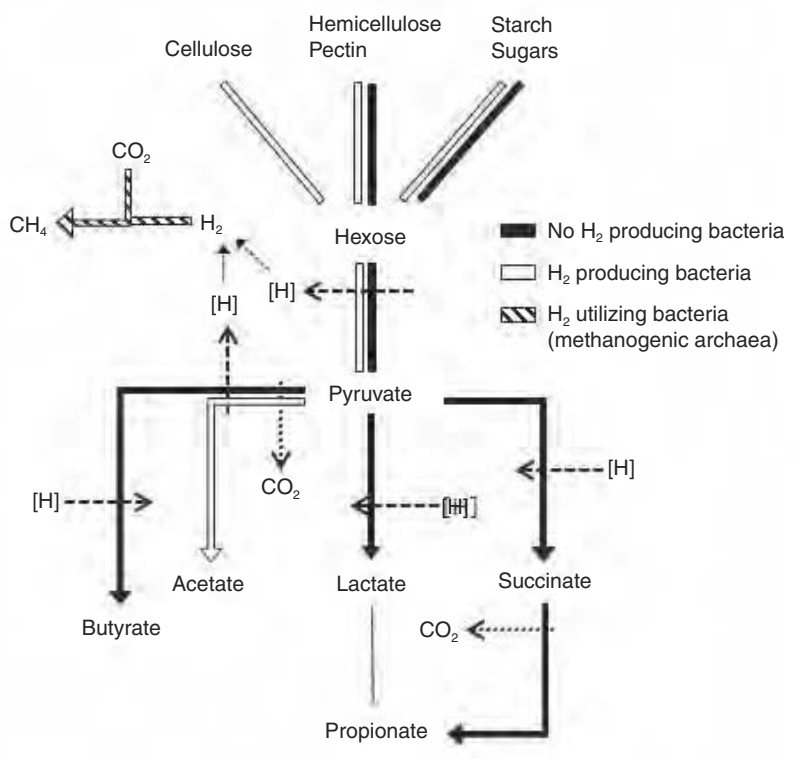

Fig. 20.1. A schematic diagram of carbohydrate fermentation and predominant ruminal bacteria categorized as per their need for H_2 production/utilization.

theoretically undergo complete fermentation without any requirement of hydrogen disposal (i.e. not reliant on CH_4 formation), and the second group shows a clear dependence on the third group for efficient fermentation.

The level of feed intake, forage processing, type of carbohydrate and addition of lipids and ionophores are a few of the important factors associated with CH_4 production and lead a change in the pattern of rumen fermentation. A number of mitigation strategies for decreasing enteric CH_4 emissions from ruminants have been considered. Various compounds and feed supplements are found effective in reducing CH_4 emissions directly or indirectly. Biological approaches such as the immunization of host animals either active or passive, bacteriophages, reductive acetogenesis, etc., directly target the methanogens (Lee *et al*., 2002; Malik *et al*., 2012; Mitsumori *et al*., 2012). Other approaches, such as defaunation, dietary manipulation through various plant extracts or organic acids and promotion of acetogenic populations, seek to lower the supply of metabolic hydrogen to methanogens (Malik *et al*., 2012).

20.2.1 Immunological approaches

Immunological approaches to alter rumen microbial populations (methanogens, other bacteria and protozoa) have been investigated as an alternative to feeding ionophores or direct-fed microbial agents. The concept of passive immunization was first introduced to counteract rumen acidosis. Providing oral doses of an avian-derived polyclonal antibody against lactic acid-producing bacteria was effective in decreasing ruminal lactate concentration and target bacteria, and therefore in preventing the onset of acidosis in cattle and sheep fed high-grain diets (Shu *et al*., 1999; Gill *et al*., 2000). DiLorenzo *et al*. (2006) determined that feeding a polyclonal antibody preparation (PAP) made from hen eggs immunized against *Streptococcus bovis* and was successful in improving the rumen environment (decreasing ruminal counts of target bacteria and increasing pH). They subsequently evaluated the effects of feeding PAP on performance, carcass characteristics and ruminal fermentation variables of feedlot steers during a 2-year study (DiLorenzo *et al*., 2008). PAP against *S. bovis* was effective in enhancing the gain:feed (G:F) ratio of steers fed high-grain diets. The mechanisms to enhance the G:F ratio are unknown, but may be related to changes in ruminal counts of target bacteria and the associated effects on ruminal fermentation products. The use of passive immunization strategies with avian antibodies appears to be effective in preventing the deleterious effects associated with these bacteria, and has the potential to enhance animal production. This technology could be utilized to target other microorganisms such as methanogenic bacteria.

On the other hand, vaccination (i.e. a major active immunization concept) against rumen methanogens can reduce CH_4 emissions by decreasing the number or activity of methanogens in the rumen (Wright and Klieve, 2011). Information is scant on the interaction of archaea and the immune system, because these organisms are rarely involved in causing disease in humans. However, there is some precedent for immunological control of rumen microbes through the vaccination of animals by spawning substantial salivary antibodies. The average volume of saliva produced is between two to three times the volume of the rumen (Kay, 1960; Bailey, 1961). Methanogens contain immunogenic fraction and vaccinating with methanogens directed the generation of antibodies against a variety of structures in rodents (Canway de Macario *et al*., 1982) and provided a clue to the biological control of methanogens through active immunization. Methanogens have the properties, such as unusual lipids, to elicit impact on the immune response. Methanogen antigens are not immunologically inert, but are recognized as a foreign body by the mammalian immune system. A preliminary study of vaccination targeting approximately 20% of the methanogen population reduced CH_4 production (kg^{-1} DMI) by 7.7%, although

the results were not repeatable with subsequent vaccine preparations (Wright *et al*., 2004). The same research group also developed a vaccine based on five methanogen strains that was administered in three vaccinations to sheep. Although the vaccine targeted 52% of the methanogens present in the rumen of the sheep, the CH_4 output unpredictably increased by 18% with vaccination. When the study was repeated with a mixture of five methanogens, vaccination failed to demonstrate any CH_4 abatement (Williams *et al*., 2009). Currently, producing effective vaccines to reduce CH_4 emissions in ruminants based on crude whole-cell preparations does not seem easy to achieve. This may be because the diversity and plasticity of the functions of the rumen bacterial and methanogenic communities may hamper their successful application. The vaccines may not target the methanogens capable of producing most of the CH_4. As a result, the killing of some rumen methanogens may also allow for other CH_4-producing microbes to take their place. Another consideration when using vaccines against methanogens is that the rumen methanogen population can vary as per diet and geographical location of the host, making a single-targeted approach difficult (Wright *et al*., 2004).

Wedlock *et al*. (2010) also conducted an *in vivo* experiment for controlling CH_4 emission through immunization. Four subcellular fractions, namely cytoplasmic, two cell wall preparations and cell wall-derived proteins were prepared from a *Methanobrevibacter ruminantium* strain. Twenty 10-month-old sheep were divided into five groups and vaccinated repeatedly with each of the fractions or with whole cells. Vaccination with the antigenic fractions induced strong antibody responses in serum. Antigens from methanogens are immunogenic in ruminants, and antisera from sheep vaccinated with the fractions of methanogens induced cell agglutination and decreasing growth of methanogens and production of CH_4 in an *in vitro* assay, demonstrating the feasibility of a semi-active immunization to mitigate CH_4 emission.

Rumen protozoa are also involved in methanogenesis because of their ecto- and endosymbiotic relationship with methanogenic archaea. Defaunation (removing protozoa) is a method to lower the supply of metabolic hydrogen to methanogens. Williams *et al*. (2008) determined changes in rumen protozoal numbers in Merino sheep with two rumen protozoa vaccine formulations containing either whole fixed *Entodinium* or mixed rumen protozoa cells, with the aim of decreasing the number and/or activity of protozoa in the rumen. It was expected that the change in protozoa numbers would be indicated by a decrease in rumen ammonia-nitrogen (N) concentration and increased wool growth. Vaccination with protozoal formulations resulted in the presence of specific immunoglobulin G (IgG) in rumen saliva, but the titer was low. Rumen protozoa were not decreased by the vaccination. The lack of change in rumen ammonia concentration and wool growth was accompanied by the vaccination. As the rumen is not an immunologically active organ, antibody via the saliva may be inefficiently delivered to the rumen. The majority of rumen salivary IgG is transferred from serum, but efficiency is low (*c*.1–2% of the concentration of total immunoglobulin in serum). The generated antibodies do bind to and reduce protozoa numbers, but insufficient amounts of specific IgG in the saliva consequently fail to reducing the numbers of protozoa in the rumen. Even animals can be defaunated successfully; protozoa coming from other faunated subjects are able to re-establish themselves in the rumen. To date, applications including immunization approaches for the selective suppression of protozoa have been promising in reducing CH_4 production but have not yet been applied in practice.

The situation is similar with the development of a methanogen vaccine. The vaccine could be improved if specific antigens against methanogens are and isolate, this will require improved understanding of the actions of antibodies in the rumen fluid and of the relationships between the levels of antibodies and the number of

methanogens in the rumen. That is, killing of some methanogens in the rumen may be possible, but it will open the way to the activation of other methanogens. While a highly specific vaccine can be made to target specific strains of methanogens, a more broad-spectrum approach is preferred for success in the rumen. A flow chart depicting the steps in controlling enteric CH_4 emission from ruminants through active immunization (vaccination) is given in Fig. 20.2.

20.2.2 Tannins in CH_4 abatement

Direct ruminal interventions aim to manipulate the steps where there appears to be a possibility to mitigate CH_4 emission in ruminants. Several efforts have focused on decreasing enteric CH_4 emission through the addition of lipids, plant compounds, monensin and other organic compounds, or by otherwise controlling diet composition (Odongo *et al*., 2007; Yang *et al*., 2009; Eckard *et al*., 2010; Hook *et al*., 2010; Liu *et al*., 2011; Mitsumori *et al*., 2012). Recently, there has been increasing interest in the use of plants and plant extracts to mitigate enteric ruminal CH_4 emissions (Hook *et al*., 2010; Martin *et al*., 2010). The use of plant secondary metabolites (PSMs) in many parts of the tropics to reduce livestock CH_4 emission, and thereby improve animal performance, is increasing (Jayanegara *et al*., 2010). The PSMs are well recognized as antimicrobial agents that act against bacteria, protozoa and fungi (i.e. do not necessarily target archaea) as a substitute for chemical feed additives. Tannins and saponins constitute the major classes of PSMs that are currently under investigation in a number of laboratories (Guo *et al*., 2008; Kong *et al*., 2010; Bodas *et al*., 2012). They are widely distributed in nutritionally important forage trees, shrubs, legumes,

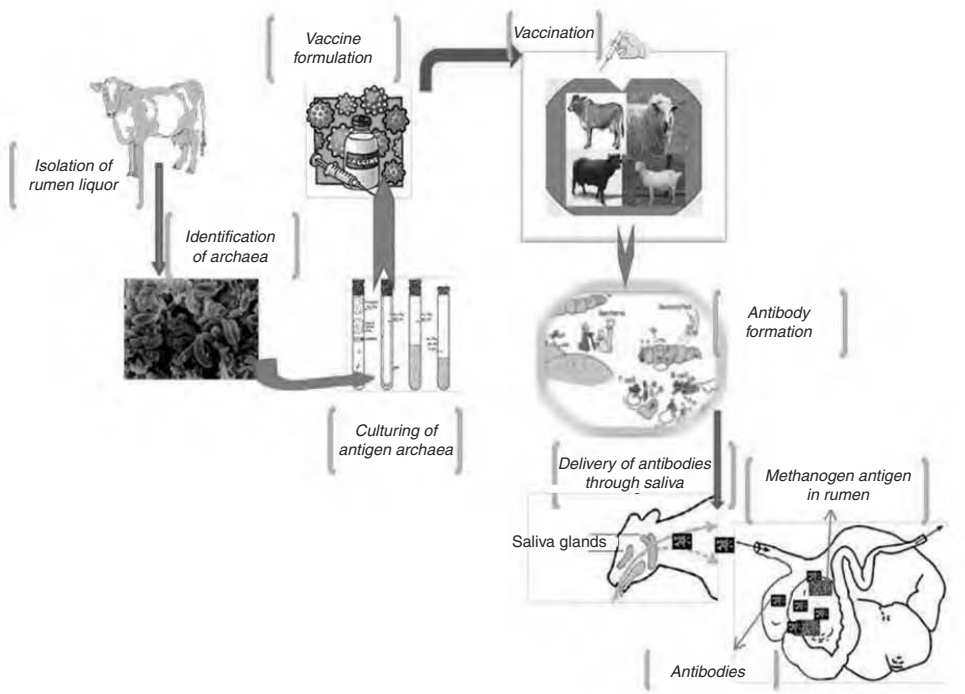

Fig. 20.2. Flow chart for the active immunization of animals against methanogens. (From Malik *et al*., 2013.)

cereals and grains. The antimicrobial actions and effects on rumen fermentation of these compounds depend on their nature, activity and concentration in a plant or plant product. The beneficial effects of tannins (hydrolysable tannins and condensed tannins) and saponins (Malik and Singhal, 2008b; Malik *et al*., 2010) have been observed in *in vitro* and *in vivo* studies.

Tannins are polyphenol substances of diverse molecular weights and variable complexity that can form complexes mainly with proteins, due to the presence of a large number of phenolic hydroxyl groups. Based on their structure and chemical properties, tannins are classified into two groups: hydrolysable tannins (HTs), which consist of a central sugar to which a number of phenolic carboxylic acids are bound by esters of gallic acid (gallotannin) or ellagic acid (ellagitannins); another group of tannins, condensed tannins (CTs) or proanthocyanidins, represents the large group of natural polyphenols widely distributed in the plant kingdom (Ayres *et al*., 1997). HTs are widely distributed in oak and acacia species, especially in the browse, with a level of up to 200 g kg^{-1} on a DM basis (Reed, 1995). They are large molecular weight compounds (500–20,000 Da), which are composed of chains of flavan-3-ol unit. They can also be toxic, especially when large quantities are given to ruminants with insufficient time for microbial adaptation. However, once animals are adapted to diets containing HTs, acceptable levels of production can be achieved with appropriate feed management. CTs are complex polymers derived by condensation of flavan-3-ol (catechin) or flavan-3,4-diol (epigallocatechin or delpinidin) subunits linked through interflavan bonds (Aerts *et al*., 1999). Differences in the degree of polymerization produce a large variety of chemical structures, which contributes to variation in the biological properties of CTs (Patra and Saxena, 2009). Chestnut tannins are the most common HTs extracted from temperate plants; and quebracho and mimosa are CTs extracted from tropical plants, which sometimes have levels of 170 g CT kg^{-1} DM (Puchala *et al*., 2005).

When ruminants are fed tannin-rich forage, CH_4 production from ruminal fermentation decreases by up to 50% (Patra and Saxena, 2011; Goel and Makkar, 2012). The antimethanogenic activities of HTs or CTs have been demonstrated extensively in several *in vitro* and *in vivo* studies (Hess *et al*., 2004, 2011; Malik and Singhal, 2008a; Buddle *et al*., 2011). However, it has not been determined which type of tannin is more effective in suppressing CH_4 production. Different types of tannins show differences in affinity for bacterial and plant proteins (Deaville *et al*., 2010). It should be noted that not all types of tannins produce beneficial nutritional and environmental responses (Goel and Makkar, 2012). CTs from various plant species show different magnitudes of effect on rumen fermentation (Tiemann *et al*., 2008), which could be related to their different chemical structures and molecular weights (Patra, 2012). Generally, tannins with low molecular weight have greater inhibitory effects on rumen microbes, because of their higher protein-precipitating capacities, than high molecular weight polymeric tannins. On the other hand, Huang *et al*. (2011) showed that CT fractions with the highest molecular weight had the greatest inhibitory effect on CH_4 production and lowered CH_4 production by 62% compared to the control. Tavendale *et al*. (2005) reported that the polymeric CT fraction from big trefoil completely inhibited CH_4 production. Although a direct antimethanogenic activity of CT has been suggested, mechanisms for the inhibition of methanogenesis by CTs are largely hypothetical.

Feeding with tannin sometimes results in decreasing ammonia concentration, exhibit the efficient use of volatile fatty acid (VFA) for microbial protein synthesis. Multiple phenolic hydroxyl groups of tannins can react with proteins, forming tannin–protein complexes, thus preventing degradation by proteases and binding proteins at ruminal pH. Thereafter, they can allow protein release at the abomasum level. The effects of CTs on ruminal N metabolism are well documented (Beauchemin *et al*., 2003; Waghorn, 2008). Tannins commonly make a shift in N excretion from urine to

faeces during nutrition conversion in the host. Sometimes, CH_4 production is reduced with CT supplementation, and there are no negative impacts of feeding CT on VFA concentration or neutral detergent fibre (NDF) digestibility (Misselbrook *et al*., 2005; Deaville *et al*., 2010).

Therefore, CT contents of diet from low to moderate (20–40 g kg^{-1}) level may have beneficial effects on protein metabolism and further improvement in animal performance, such as body weight, milk yield and reproduction, because of increased small intestinal absorption of amino acids (Aerts *et al*., 1999). Accordingly, the effects of tannins on ruminant productivity depend on the quality (e.g. degradability and composition of essential amino acids) and quantity of dietary protein, requirements of amino acids and status of other nutrients (Deaville *et al.,* 2010). It should be noted that some tannins are known to produce excessive escape of crude protein (CP) from the rumen (Krause *et al.,* 2001; Kariuki and Norton, 2008).

20.3 Impact of PSMs on Rumen Microbial Ecology and Host Nutrition

Tannins are also regarded as antimicrobial compounds that exert inhibitory effects on bacteria through complex formation (Smith and Mackie, 2004). Inhibition of archaeal growth is due mainly to the bacteriostatic and bactericidal effects of CTs. The formation of complexes with bacterial cell wall membrane components causes morphological changes and secretion of extracellular enzymes. Either interaction is likely to inhibit the transport of nutrients into the cell and/or retard the growth of the organism. Tannin-induced membrane disruption, direct action on microbial metabolism, deprivation of substrates for microbial growth and chelation of cation by tannins, all reduce its availability to microbes. The modes of action of tannins have been examined because different types of CTs with varied biological activities influence CH_4 emission in different manners (Beauchemin *et al*., 2003). Tannins lower CH_4 production probably by directly inhibiting the activities of methanogenic archaea and/or reducing fibre digestion in the rumen.

The effects of tannins on ruminal bacteria were reported to be dependent on the species of microorganism and the type or source of tannin. Tannins could reduce fibre digestion by complexing with lignocellulose and preventing microbial digestion and/or by directly inhibiting cellulolytic microorganisms due to anti-nutritional effects. Within PSMs, mimosa tannins showed a marked inhibitory effect on microbial fibre degradation in the rumen (Deaville *et al*., 2010). Carulla *et al*. (2005) suggested that inhibition of CH_4 emission by CTs is primarily attributed to the reduction in cellulolytic and/or total bacteria numbers that take part in nutrient digestion, and as a result, decreased rumen hydrogen production. Thus, on feeding of tannins from quebracho or mimosa, sumach or chestnut, ruminal acetate concentration decreased (Beauchemin *et al*., 2007; Castro-Montoya *et al*., 2011). Synchronized increase in the molar proportion of propionate with less hydrogen production from enteric fermentation also affects CH_4 production negatively. Rumen digestion of readily fermentable carbohydrate and hemicellulose is also reduced, albeit compensated by increased post-ruminal digestion.

The effects of PSM on ruminal fermentation are desirable if they lead to an increase or do not alter the VFA concentration and decrease both ammonical N and CH_4 production. Many studies have shown that forages containing CTs reduce CH_4 emissions in ruminants (Hess *et al*., 2004; Animut *et al*., 2008), but in most cases the reduction in CH_4 production was accompanied by negative effects on digestibility (McAllister and Newbold, 2008). Presumably, tannins at a level of higher than 5–6% of DM are generally regarded as an anti-nutritional factor for livestock, showing reduced voluntary feed intake, crude protein and fibre digestibility and growth of ruminants (Makkar, 2003). Voluntary feed intake and the performance of animals may depend on the type of CT present in the

forage. High dietary CT concentrations also depress voluntary feed intake, probably because of interaction between diets and the chemical characteristics of CT (and other secondary metabolites), with a subsequent reduction of the palatability of diets. The concentration range of tannins that show *in vivo* anti-methanogenic effects without decreasing organic matter digestibility in animals has yet to be determined.

CTs mainly decrease CH_4 through the reduction in fibre digestion (indirect effect), while HTs appear to act through the inhibition of growth and/or activity of methanogens and/or hydrogen-producing microbes (direct effect). However, there have been very few studies investigating the effect of tannins on rumen archaea (Tavendale *et al*., 2005). Bhatta *et al*. (2009) evaluated the effects of six commercially available natural sources of tannins (three sources of HT and three sources of CT in different combinations) on total archaea using mixed cultures. They found that CT reduced CH_4 production by 5.5% and suppressed the population of methanogenic archaea by 12.0%. The total archaeal population was lower with the combination of HT and CT than with HT alone. The different modes of action of two kinds of tannins may explain why the effects of HT + CT on total gas and CH_4 production were greater than those of HT alone. Mohammed *et al*. (2011) evaluated the effects of dry maize distillers' grain (DDG) and CT from *Acacia mearnsii* on rumen methanogens using PCR denaturing gradient gel electrophoresis and quantitative real time (qRT)-PCR. Their findings indicated that inclusion of DDG or a mixture of DDG and CT altered methanogenic diversity without altering the total copy numbers of methanogenic 16S rRNA gene in beef cattle, and they suggested that the total methanogen population in the ruminal digesta was similar among diets, similar to the findings of Hook *et al*. (2009) and Kong *et al*. (2010). Since the symbiosis of protozoa with methanogenic bacteria in the rumen is well established, a reduction in methanogens would probably affect the protozoal population. Total protozoa decreased when CTs were added (Buddle *et al*., 2011). Reduction in rumen protozoal counts may decrease archaeal counts as well, which may indirectly impact on CH_4 emission (Cieslak *et al*., 2012).

20.4 Conclusion

For many years, researchers have tried to manipulate the numbers and/or activities of rumen methanogens to improve the efficiency of ruminant production. Although ruminal interventions are sometimes achieved with unintended consequences, for instance complete blockage of eructation could result in an unhealthy build-up of hydrogen in the animals. Immunization approaches have been shown to modulate rumen fermentation favourably, but consistent beneficial effects of rumen modulation and animal performance have not been observed. This indicates that the essential features have yet to be determined. Abatement strategies are often limited by the diet fed, the management conditions and the physiological state and use of the animals, as well as government regulations, resulting in difficulties in applying an optimum approach to the problem of enteric CH_4 mitigation. All of the present strategies appear to be promising, either singly or in combination, but more research is needed to validate these approaches.

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21 Phage Therapy in Livestock Methane Amelioration

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Abstract

Viruses of prokaryotes (phages) are obligate microbial pathogens that can, in the lytic phase of development, infect and lyse their respective bacterial or archaeal hosts. As such, these viruses can reduce the population density of their hosts rapidly, and have been viewed as possible agents of biological control (phage therapy). Phage therapy is becoming increasingly important as a means of eradicating or controlling microbial populations as the use of antibiotics and chemical treatments becomes both less effective and less publicly acceptable. Phage therapy has therefore been raised as a potential strategy to reduce methane (CH_4) emissions from ruminants, providing an innovative biological approach, harnessing the potent, yet targeted, biocidal attributes of these naturally occurring microbial predators.

21.1 Introduction

Enteric methane (CH_4) produced by ruminant livestock is a major concern as the agricultural sector seeks to reduce their contribution to global greenhouse gas emissions. The use of phage-based therapies to control populations of the organisms responsible for the majority of ruminant enteric CH_4 emissions, methanogenic archaea (methanogens), was first suggested in the late 1990s (Klieve and Hegarty, 1999). Initial studies to investigate the viruses infecting rumen methanogens were hampered by a lack of fundamental knowledge surrounding both the archaeal viruses to be potentially utilized in any phage therapy approaches and the biology and species diversity of the host methanogens. Extensive international research efforts have since led to a greater understanding of the diversity of rumen methanogens and the identification of the factors influencing the relative composition of rumen methanogen populations, such as diet and ruminant host species (Hook *et al*., 2010, King *et al*., 2011; St-Pierre and Wright, 2013). An increasing number of methanogenic archaeal isolates have also been introduced into microbial culture collections, and our understanding of rumen methanogen biology and the specialist technical requirements for their cultivation has considerably increased. Advances in high-throughput sequencing technologies have also greatly facilitated the genome sequencing of rumen methanogen isolates (Attwood *et al.,* 2011; Leahy *et al*., 2013a). They have also enabled the diversity of the rumen microbial ecosystem to be characterized to an unprecedented extent, encompassing not only the most abundant, well-recognized genera of bacteria, but also incorporating previously uncultured, less understood microbial populations including

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the methanogenic archaea (Hess *et al*., 2011; Kang *et al*., 2013). As well as the microbial populations of the rumen, high-throughput sequencing technologies have also enabled the genetic characterization of the abundant, highly diverse phage populations known to be endemic to the rumen microbial ecosystem (Berg Miller *et al*., 2012; Ross *et al*., 2013).

The continued acquisition and accumulation of this knowledge is vital for the development of any strategy to reduce ruminant enteric CH_4 emissions, particularly those focused on the reduction of the naturally occurring rumen methanogen populations. This knowledge is also important for the development of any phage-based therapies, as these approaches require a thorough understanding of three major factors: first, the target organism or organisms; second, the phages or phage-encoded products to be employed to reduce or eradicate the target organisms; and third, a thorough understanding of the microbial ecosystem and physical environment into which the phage-based therapy is to be introduced. This chapter will therefore encompass research developments in the field of rumen methanogenic archaea; the archaeal viruses associated with methanogens; briefly describe the naturally occurring phage populations of the rumen; and describe alternative phage-based approaches for the control of rumen methanogen populations and subsequent enteric CH_4 amelioration.

21.2 Methanogenic Archaea in the Rumen: Potential Phage Hosts

The rumen of herbivores contains a microbial ecosystem that evolves from birth and following weaning, to digest plant material often high in fermentable sugars and complex carbohydrates. The microbial population of the rumen has evolved primarily to ferment this otherwise indigestible feedstuff and provide the animal with volatile fatty acids and microbial protein (Hungate, 1966). Interactions between the various members of the microbial community are known to occur, and include predation and cross-feeding on fermentation products (Wolin *et al*., 1997). Within the rumen, therefore, there is effectively a secondary fermentation whereby products released by the primary microbial fermentation of feedstuffs and microbial protein are recycled and further utilized as substrates for the growth of other microbes. Methanogen populations are actively involved in this secondary fermentation, utilizing the hydrogen and other methyl compounds, for example methanol, formate and acetate, to reduce carbon dioxide (CO_2) to CH_4 (Bonin and Boone, 2006; McAllister and Newbold, 2008).

Rumen methanogen populations were initially described as methanogenic bacteria rather than archaea, but were generally considered to represent an important, metabolically active proportion of the total microbial population (Smith and Hungate, 1958; Wolin, 1981). Several methanogens were isolated from the rumen and investigations into their antigenic properties and substrate utilization requirements gave initial insights into the extent of rumen methanogen physiological diversity (Balch *et al*., 1979; Lovley *et al*., 1984; Miller *et al*., 1986). Advances in sequence-based molecular methods have subsequently revealed a wide range of genetically diverse methanogenic archaea that are metabolically active within the rumen. Studies utilizing the 16S rRNA gene as a phylogenetic marker for methanogen populations (Raskin *et al*, 1994; Tymensen and McAllister, 2012) have shown that methane-producing archaeal populations become established soon after birth (Skillman *et al*., 2004). While methanogen species classified within the order Methanobacteriales, such as *Methanobrevibacter smithii* and *Methanosphaera stadmanae* predominate in non-ruminant intestinal tracts (Liu and Whitman, 2008), in the rumen of domesticated sheep and cattle, a wide range of CO_2-reducing methanogens establish and dominate (St-Pierre and Wright, 2013). These methanogens include species of the genus *Methanobrevibacter*, such as *Methanobrevibacter ruminantium*, *M. millerae* and

M. thaurei, and species classified within the genera *Methanobacterium* and *Methanomicrobium* (Miller *et al*., 1986; Janssen and Kirs, 2008). Under certain dietary conditions, for example high-forage diets with increased fibrous content (Wright *et al*., 2006; Popova *et al*., 2011), other genera of methanogenic archaea, classified within the order Methanomicrobiales, such as *Methanosarcina* and *Methanosphaera* may increase in relative abundance. Novel methanogens phylogenetically classified with the largely uncultured Thermoplasmatales-affiliated lineage C (TALC), also described in the literature as Rumen Cluster C (RCC) and rice cluster C Thermoplasmatales (Kemnitz *et al*., 2005; Paul *et al*., 2012), can also utilize methyl compounds as alternative substrates for growth, and subsequently rise to significant intra-ruminal concentrations (Poulsen *et al*., 2013).

In addition to using substrates present within the liquid or rumen fluid fraction, methanogenic archaea may also form mutualistic associations with the protozoa living in the rumen (Krumholz *et al*., 1983; Tymensen *et al*., 2012), and it has been suggested that the physical, endo- and ecto-associations of these microbes with protozoa improve the efficiency of interspecies hydrogen or formate transfer (Vogels and Stumm, 1980; Whitman *et al*., 2006). The reduction of certain protozoa in the rumen that occurs following, for example, a change in diet from forage to high starch content grain diets (Mosoni *et al*., 2011; Ozutsumi *et al*., 2012) may alter rumen methanogen community diversity, but does not totally remove these organisms from the rumen, rather causing a shift in the archaeal community, reducing populations of TALC and increasing the relative abundance of *Methanobrevibacter* spp. described as free living, or not associated with protozoa (Tymensen *et al*., 2012).

No archaeal isolates classified within the phylum Crenarchaeota have been isolated from the rumen. Studies utilizing 16S rRNA gene analysis techniques to characterize rumen archaeal communities have rarely encountered sequences representing operational taxonomic units (OTUs) with homology to Crenarchaeota (Shin *et al*., 2004; Janssen and Kirs, 2008). Crenarchaeal OTUs detected in very low concentrations within bovine rumen fluid (Shin *et al*., 2004) have not been observed in recent studies that have utilized high-throughput sequencing to improve the corresponding community diversity coverage (Carberry *et al*., 2014) or studies of the rumen metatranscriptome focusing on total rumen RNA (Poulsen *et al*., 2013). Further studies taking into consideration the choice of primers employed for community analysis so as not to preclude or bias the detection of different archaeal genera (Tymensen and McAllister, 2012) will be required to confirm or refute the presence of these novel archaea.

The complexity of the rumen methanogen population must be considered and taken into account during the development of any new approaches for the reduction of ruminant enteric CH_4 and may preclude the use of CH_4 mitigation strategies targeting only a single methanogen species or strain. In this regard, phage therapy approaches for the control of rumen methanogen populations will also need to target a broad spectrum of methanogenic archaeal genera.

21.3 Archaeal Viruses

The naming of viruses infecting organisms within the domain archaea has been undertaken using archaeal host strains a source of conjecture, with many researchers preferring the use of the term 'archaeal virus' (Abedon and Murray, 2013) instead of archaeaphage or more simply, phage. For the purposes of this chapter within the context of phage therapy development, archaeal viruses will be referred to as phages.

The majority of research investigating phages infecting archaea has been sourced from extreme environments and classified within the phylum Crenarchaeota (Pina *et al*., 2011; Krupovic *et al*., 2012; Prangishvili, 2013). Perhaps as a consequence of the selective pressures and challenges imposed by the extreme environmental conditions and the relatively unique cellular attributes of their Crenarchaeal hosts, these phages

may exhibit considerable evolutionary divergence. As a result, these phages sometimes differ in their morphology, to such an extent that they are phylogenetically classified in viral families typified by their novel morphologies: for example, the bottle-shaped Ampullaviridae, the two-tailed Bicaudaviridae, the lemon- or spindle-shaped Fuselloviridae and the droplet-shaped Guttaviridae (Krupovic *et al*., 2012; Ackermann and Prangishvili, 2012).

The second major archaeal phylum, the Euryarchaeota, incorporates organisms found in a wide range of habitats such as the high hydrostatic pressure and extreme temperatures surrounding deep-sea hydrothermal vents (Roussel *et al*., 2011), high salt conditions of hypersaline lakes (Porter *et al*., 2007) and anaerobic, yet more mesophilic and pH neutral conditions such as those found in the rumen of herbivores (St-Pierre and Wright, 2013). This phylum therefore includes a phenotypically diverse range of archaea, including methanogens, halophiles, thermophilic and extremely acidophilic archaea, and the hyperthermophilic orders Thermococcales and Archaeoglobales. Phages infecting some Euryarchaea may also have unusual morphologies similar to those found infecting Crenarchaea, such as spindle-shaped virions resembling lemons. Examples include the phage TPV1 infecting the thermophilic euryarchaeota, *Thermococcus prieurii*, which also clusters into rosette-like aggregates (Gorlas *et al*., 2012), and the lemon-shaped phages His 1 and His 2 classified within the novel virus family Salterprovirus (Bath and Dyall-Smith, 1998, 2011) infecting the halophilic archaean, *Haloarcula hospanica*. In addition, the phage HRPV-1, infecting a halophilic isolate from a solar saltern of the genus *Halorubrum*, has a pleomorphic morphology and a single-stranded DNA genome (Pietila *et al*., 2009), in distinct contrast to the usual dsDNA genomes of the Caudovirales. Interestingly, electron microscopy surveys of the types of phage morphologies present in more extreme environments dominated by euryarchaeota, such as hypersaline lake environments (Porter *et al*., 2007; Sime-Ngando *et al*., 2011) and the more moderate conditions of freshwater, anoxic sediments (Borrel *et al*., 2012), have encountered a diverse range of viral morphotypes, with the spindle-shaped particles being the more commonly observed (Pietila *et al*., 2013). Although the genus *Salterprovirus* and some unclassified spindle-shaped phage isolates have yet to be assigned comprehensive taxonomy, these spindle-shaped archaeal phages are generally classified into two viral families, the Bicaudaviridae and the Fuselloviridae, on the basis of genetics and the number and length of the particle fibres or tail-like structures (Pina *et al*., 2011; King *et al*., 2012).

In contrast to the relatively unusual phages found to infect the Crenarchaea, the majority of phages infecting the Euryachaeota tend to possess double-stranded DNA genomes and exhibit the tailed (or head-tail) particle morphology characteristic of the more ubiquitous viral order, the Caudovirales (Clokie *et al*., 2011; Pietilä *et al*., 2014). The majority of characterized phages infecting Euryarchaeota have been isolated using halophilic host strains of the genera *Halorubrum*, *Haloarcula*, *Haloferax*, *Halobacterium*, *Natrialba* and *Natrinema*. Within the context of the extreme, hypersaline environments, phages are the most predominant microbial predators and have therefore been a focus of research (Bettarel *et al*., 2011; Oren, 2013). Over 60 haloarchaeal viruses have been described (Porter and Dyall-Smith, 2006; Pina *et al*., 2011; Atanasova *et al*., 2012), and several have been morphologically and genetically characterized. For example, the haloarchaeal viruses, φCH1, φH, HF2 and BJ1, all belong to the Myoviridae or Siphoviridae families within the order Caudovirales (Pietilä *et al*., 2014), with phages of the family Myoviridae being isolated more frequently in a relatively large-scale isolation study utilizing haloarchaeal hosts (Atanasova *et al*., 2012). It has been suggested, however, that the isolation of tailed phages of the extreme halophiles does not correspond to the variety of viral morphotypes observed in microscopy surveys of hypersaline waters, where tailed

phages do not always predominate (Ackermann and Prangishvili, 2012).

The number of published reports describing phages infecting methanogenic euryarchaeal hosts is considerably less than those infecting the halophilic euryarchaea. For the purposes of this review, with an emphasis on the development of phage therapy approaches, lytic phages, i.e. those which attach to their host, replicate then lyse and release progeny phage particles (Ackermann and DuBow, 1987), are described separately to those that infect then form a stable, dormant state, sometimes referred to as a chronic infection. This latter state is formed by the phage integrating their genome into the host chromosomal DNA, either at a specific site or at several sites, with some phages integrating at or near palindromic structures, such as transfer RNA genes (Campbell, 1992; Bobay *et al.*, 2013), or persisting in the cytoplasm as a plasmid (Ackermann and DuBow, 1987). Phages with this ability are described as lysogenic or temperate phages, are ubiquitous to microbial ecosystems and have also been found to be associated with Crenarchaea isolated from extreme environments and Euryarchaea (Prangishvili and Garrett, 2005; Pina *et al.*, 2011).

To date, very few lytic phages infecting methanogens within the Euryarchaeaota have been reported in the literature. Archaeal isolates of the genera *Methanothermobacter* and *Methanobrevibacter* have been used successfully as host species for the isolation of lytic phages (Table 21.1). The most characterized phage is ψM1, infecting the thermophilic methanogen, *Methanothermobacter marburgensis* (DSM 2133), formally known as *Methanobacterium thermoautotrophicum* Marburg. Phage ψM1 is a virulent, double-stranded DNA phage with a polyhedral head of 55 nm diameter and a tail of 210 nm in length (Meile *et al.*, 1989). The *Methanobacterium* phage ψM2 (Pfister *et al.*, 1998) is highly related to *Methanobacterium* phage ψM1, being a deletion mutant of ψM1, which occurred spontaneously during repeated phage subculture (Jordan *et al.*, 1989; Pfister *et al.*, 1998). While the complete genome sequence is available for the phages ψM1 and ψM2, to date there have been no published studies describing the other reported lytic methanogen phages in any further detail. In addition, although the isolation of these phages was reported in the literature, the phages were not preserved or deposited in publicly available international culture

Table 21.1. Phage isolates infecting methanogenic archaea and taxonomic classification reported in the literature.

Methanogen host	Virus name	Virus classification	Origin	Reference
Methanothermobacter thermoautotrophicus (*Methanobacterium thermoformicicum*) 5 isolates; Z-245 (DSM 3720), FTF (DSM 3012), FF1, FF3, CSM3 and DSM 1035[a]	ΦF1	Caudovirales, Myoviridae	Anaerobic sludge bed reactor (55°C)	Nölling *et al.* (1993)
Methanothermobacter thermoautotrophicus isolate FF3	ΦF3	Caudovirales, Siphoviridae	Anaerobic sludge bed reactor (55°C)	Nölling *et al.* (1993)
Methanothermobacter marburgensis	ψM1/ψM2	Caudovirales, Siphoviridae	Experimental anaerobic digester (55–60°C)	Jordan *et al.* (1989); Meile *et al.* (1989)
Methanobrevibacter smithii	PG	Caudovirales	Rumen	Baresi and Bertani (1984)

Note: [a] DSM 1035 is the type strain currently classified as *Methanothermobacter thermoautotrophicus* Delta H. (From Wasserfallen *et al.*, 2000.)

collections, limiting the potential for further genetic and biological characterization and any subsequent utilization of these phages in the development of phage-based therapies.

The majority of more recently acquired information regarding phages infecting methanogenic archaea has arisen from studies of fully sequenced methanogen genomes (Krupovic *et al*., 2010; Leahy *et al*., 2013b). Individual archaea may play as host to integrated prophages, and sometimes archaeal genomes can contain fragments of several integrated prophages (Krupovic *et al*., 2011). Once a prophage becomes fragmented, it may lose the ability to produce intact, viable phage particles, or may be completely unable to emerge from the lysogenic state. These defective phage-related sequences may still be of interest, as they can still be long fragments of sequence, encompassing multiple open reading frames encoding for complete phage proteins. Intact prophages, however, may be able to emerge from the lysogenic state, in a process referred to as phage induction, and enter the lytic cycle of phage production, culminating in the lysis of the host cell and release of progeny phage particles (Ackermann and DuBow, 1987).

The occurrence of lysogenic phage infection of an archaeal methanogen was first noted in studies of the *Methanococcus voltae* PS type strain (ATCC 33273, DSM 1537), sourced from salt marsh estuarine sediments (Balch *et al*., 1979; Bertani, 1999). Further studies investigating gene transfer mechanisms of this organism indicated that tailed, phage-like particles could be detected (Eiserling *et al*., 1999); however, following genetic analysis, only a relatively small, 4.4-kb genome fragment could be purified and the observed particles were attributed to the activity of a defective, chromosomally integrated prophage. Similarly, early studies of the *M. voltae* strain A3, which is closely related to the *M. voltae* PS-like group (Wood *et al*., 1989), reported the isolation of ovoid, phage-like particles. These *Methanococcus* strains have been revisited in more recent genome-sequencing studies, and the presence of prophage elements has been confirmed for both the *M. voltae* PS type strain and the *M. voltae* strain A3 (Krupovic *et al*., 2010). Similarly, prophages have been described for methanogen isolates classified within the orders Methanococcales, Methanobacteriales and Methanosarcinales (detail is given in Table 21.2.) and at least four of these prophages, including Hlac-Pro1, Mace-Pro1, Mjan-Pro1 and Mmar C6-E2, are genetically related to tailed phages of the order Caudovirales (Krupovic *et al*., 2010).

While genome sequences are continually being published for archaeal methanogens sourced from environments such as sewage sludge, river and estuarine sediments and deep-sea hydrothermal vents, there has recently been an increasing focus on the sequencing of mesophilic, gut-associated methanogens. Several archaeal isolates from the order Methanobacteriales including *M. smithii* sourced from the human gut and the *M. ruminantium* type strain M1 (ATCC35063) from the rumen have been found to contain chromosomally integrated prophages (Attwood *et al*., 2008; Hansen *et al*., 2011).

Human gut-associated methanogen prophages were first reported by Knox and Harris (1986) following growth observations of the 6-mercaptopurine-resistant mutant of *M. smithii* strain PS, which showed spontaneous lysis in the late-exponential growth phase. Electron microscopy demonstrated the presence of phage particles with a hexagonal head and flexible tail, representative of Siphoviridae morphology. This phage was then termed PMS1 and found to have a 35 kb DNA genome, and further genome sequencing of this organism confirmed the presence of an integrated prophage (Hansen *et al*., 2011).

A more recent study of the human gut-associated *M. smithii* pan genome (Hansen *et al*., 2011) found that 7 out of the 20 sequenced *M. smithii* strains contained prophage elements. Sequence reads of these prophages could then be aligned with the prophage element found within the *M. smithii* PS type strain (ATCC35061), indicating sequence homology between the identified prophage elements, although

Table 21.2. Examples of archaeal methanogen prophage elements reported in the literature.

Methanogen host	Prophage name	Host origin	Reference
Methanobrevibacter ruminantium M1 type strain (ATCC 35063)	φmru	Bovine rumen	Attwood *et al.* (2008); Leahy *et al.* (2010)
Methanobrevibacter smithii PS type strain (ATCC 35061)	Msmi-Pro1[a]	Sewage digester	Knox and Harris (1986); Krupovic *et al.* (2010); Hansen *et al.* (2011)
Methanobrevibacter smithii; 7 isolates; METSMITS96C, METSMIT145A, METSMIT145B, METSMIT146A, METSMIT146B, METSMIT146C, METSMIT146D	nd[b]	Human faeces	Hansen *et al.* (2011)
Methanobrevibacter sp. JH1	nd[b]	Bovine rumen	Leahy *et al.* (2013a); Lee *et al.* (2013)
Methanothermobacter wolfeii DSM 2970	ψM100	Sewage sludge and river sediment	Stettler *et al.* (1995); Luo *et al.* (2001)
Methanococcus maripaludis C6	MmarC6-E2	Salt marsh sediment	Wood *et al.* (1985); Krupovic *et al.* (2010)
Methanococcus voltae A3	Mvo1-Pro1	Salt marsh estuarine sediments	Wood *et al.* (1989)
Methanococcus voltae PS	VTA	Salt marsh estuarine sediments	Wood *et al.* (1989); Eiserling *et al.* (1999)
Methanosarcina acetivorans C2A	Mace-Pro1	Marine sediment	Krupovic *et al.* (2010)
Methanocaldococcus vulcanius M7	Mvul-Pro1	Deep-sea hydrothermal vent chimney	Krupovic *et al.* (2010)
Methanocaldococcus jannaschii DSM 2661	Mjan-Pro1	Deep-sea hydrothermal vent	Krupovic *et al.* (2010)
Methanocaldococcus fervens AG86	Mfer-Pro1	Deep-sea hydrothermal vent	Krupovic *et al.* (2010)

Notes: [a]Prophage initially reported as being a lytic phage; [b]nd = prophage name not designated.

none of the identified prophages appeared to be completely homologous to the PS prophage. When these prophages were mapped to 20.84 kb of the *M. smithii* PS type strain prophage in order to identify regions of prophage sequence variation, the genes encoding for the phage's tail protein (Msm1684), a putative protein (Msm1691) related to the previously described, predicted pseudomurein endoisopeptidase gene, *Pei*W (Luo *et al.*, 2002), and several hypothetical proteins (Msm1674 and Msm1688; Hansen *et al.*, 2011), were identified. This extent of genetic homology also suggests that these prophages are derived from tailed phages of the viral order Caudovirales.

The most characterized rumen-derived methanogen prophage is the prophage φmru, infecting the type strain *M. ruminantium* M1 ATCC35063, DSM 1093 (Smith and Hungate, 1958). This prophage spans an approximately 40 kb region and incorporates genes for phage DNA integration, DNA replication and packaging, enzymes for host cell lysis and capsid and tail proteins (Attwood *et al.*, 2008; Leahy *et al.*, 2010), suggesting that this prophage originates from a lysogenic Caudovirales phage. As genome sequencing of microbes becomes more commonplace, facilitated by international sequencing efforts (Leahy *et al.*, 2013b), it is anticipated that more rumen-derived methanogen genome sequences will be annotated and analysed to reveal more novel, methanogen-associated prophage elements.

21.4 Naturally Occurring Rumen Phage Populations

While viruses of prokaryotes are ubiquitous in the environment and the gastrointestinal tracts of all animals (Weinbauer, 2004), particularly dense and diverse phage populations occur in the rumen of herbivores. The first reported isolation of phages from the bovine rumen was made in the mid-1960s (Adams *et al*., 1966). Soon afterwards, it was suggested that rather than being transient viruses ingested into the rumen with plant feed material, phages were common inhabitants of the rumen (Hoogenraad *et al*., 1967), actively using the rumen microbes as hosts to proliferate. It is now accepted that phages are endemic to the rumen, occurring in dense populations (Klieve and Swain, 1993), and have been studied at both the individual and ecosystem level (Letarov and Kulikov, 2009; Gilbert and Klieve, 2014). Compared to other rumen microbial inhabitants, however, relatively little is known about the biological properties or genetic make-up of the majority of phages naturally endemic to the rumen.

The presence of phages in the rumen capable of infecting rumen methanogen populations has not been confirmed by studies based on a cultivation and phage isolation approach (Gilbert *et al*., 2010b). Investigations of the rumen viral metagenome utilizing high-throughput sequencing methodology to study the DNA phage fraction of rumen fluid (Berg Miller *et al*., 2012; Ross *et al*., 2013), however, have detected the presence of phage sequences with homology to known archaeal phages. For example, an updated analysis (Fig. 21.1.) of the viral metagenome data sets obtained for three Holstein cows at various stages of lactation (Berg Miller *et al*., 2012) shows the presence of several archaeal phage-related genes. In addition, sequences homologous to previously studied *Methanobrevibacter* prophages can be found in rumen metagenomic data sets. Whether these sequences have arisen directly from phage particles actively replicating with the methanogen populations of the rumen has yet to be verified through either a culture-independent approach, such as assembly of a complete methanogen phage genome from a rumen viral metagenomic sequence data set or studies utilizing a cultivation-dependent phage isolation approach.

Analysis of archaeal genome sequences and metagenomic data sets can also provide information regarding the phage–host interactions that may occur (Sorek *et al*., 2013). A relatively recent approach being used to investigate these interactions involves the identification of clustered, regularly interspaced, short pallindromic repeats (CRISPR) and CRISPR-associated genes (*Cas* genes). CRISPR have been found within both bacterial and archaeal genomes, with archaeal genomes tending to contain a relatively high proportion (Barrangou *et al*., 2007). CRISPR consist of sequence repeats followed by spacer regions, with the spacer regions incorporating short segments of sequence homologous to the invading DNA. This invading DNA is thought most often to originate from infecting phages, plasmids and other transposable elements (Sorek *et al*., 2008; Barrangou, 2013). Biologically, CRISPRs provide prokaryotes with a heritable mechanism of phage resistance (Fineran and Charpentier, 2012); they can also be used to reveal genetic information of past phage infections. A CRISPR-based approach has been utilized to investigate the phage–host interactions occurring in several microbial ecosystems such as the human gut (Minot *et al*., 2013), saliva (Pride *et al*., 2012) and aquatic ecosystems (Heidelberg *et al*., 2009). This approach has also been used to examine virus–host interaction occurring in extreme environments rich in archaeal species such as hyperthermophilic, hydrothermal vent and hypersaline microbial communities, and is becoming increasingly important in determining the extent to which phages may modulate archaeal populations in the environment (Garrett *et al*., 2010; Anderson *et al*., 2011; Emerson *et al*., 2013).

In the context of rumen methanogens, CRISPR sequences have been found to occur in the complete genome sequences of rumen methanogen isolates, for example *M.*

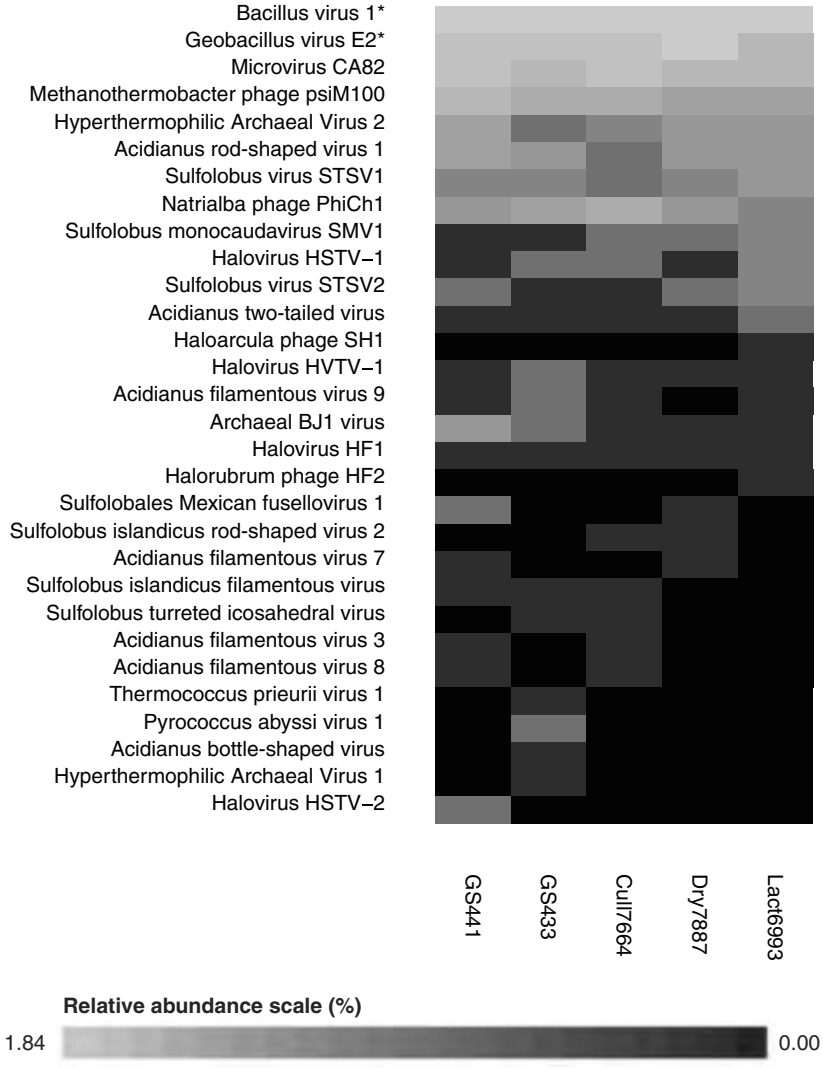

Fig. 21.1. Relative abundance of archaeal phage sequences in rumen viral metagenome data sets. Sequences related to archaeal viruses were detected within three published rumen viral metagenomes (Cull 7664; Dry 7887; Lact 6993) generated from Holstein cows at various stages of lactation (Berg Miller *et al*., 2012), deposited in MGRAST (Meyer *et al*., 2008), and two rumen viral metagenomes from *Bos indicus* cross steers maintained on a forage diet (Gilbert *et al*., unpublished results). Virus reads were identified following Blastx analysis (10^{-5} e-value threshold) using the National Center for Biotechnology Information (NCBI) complete viral genomes protein sequence database (13 March 2014 release) and relative abundance determined as a percentage of total read counts (Metavir, Roux *et al*., 2011). Reads homologous to any of the 64 archaeal virus genomes listed on the European Molecular Biology Laboratory-European Bioinformatics Institute (EMBL-EBI). Archaeal virus database were identified within the sequence data sets and presented as a heatmap of relative abundance (RStudio: integrated development environment for R Version 3.0.3).
* The phages *Bacillus* virus 1 and *Geobacillus* virus E2 were classified as archaeal viruses in the EMBL-EBI Archaeal virus database and were both isolated from an extreme, deep-sea thermophilic environment.

ruminantium M1-type strain (Leahy *et al*., 2010) and *Methanobrevibacter* sp. AbM4 (Leahy *et al*., 2013a). CRISPR sequences have also been noted to occur in rumen phage metagenomic data (Berg Miller *et al*., 2012), although this approach has not been directly applied to the comprehensive investigation of rumen methanogen phage–host interactions. The effect of naturally occurring populations of phage on rumen methanogen populations therefore remains relatively unknown and may provide an avenue for future investigations.

21.5 Prospects for Using Phage Therapy to Control Rumen Methanogenesis

As the agricultural production sector shifts away from the use of antibiotics, phage research is becoming increasingly focused on the development of phage therapy-based approaches for the biological control of bacterial populations. To date, phage therapy approaches developed specifically for the control of rumen bacteria have only been investigated for the amylolytic bacterium, *Streptococcus bovis*, (Klieve *et al*., 1999; Tarakanov, 2006) implicated in the disease syndrome affecting cattle on high-grain diets, lactic acidosis (Nagaraja and Titgemeyer, 2007). Research into these therapies focused on utilizing formulations of intact, lytic phages of the order Caudovirales, classified morphologically on the basis of a long tail, within the family Siphoviridae or the shorter-tailed Podoviridae family. Host ranges tended to be narrow, with individual phages able to infect only one or two strains of *S. bovis* (Klieve *et al*., 1999). The phage designated as F4 was reported on examination to have a wider host range and able to infect five out of ten *S. bovis* strains (Styriak *et al*., 1994). The development of phage therapies for the control of *S. bovis*, however, often did not progress beyond the initial stages of *in vitro* testing. While on the administration of a mixture of *S. bovis* phages resulting in an increase in milk fat content was reported by a Russian study (Tarakanov, 1994), no phage therapies for modulating rumen bacterial populations have been subsequently developed on a commercial scale, or tested for authorized for use in mainstream animal production systems.

In recent years, however, phage therapies have been developed successfully for the control of pathogens and biofilms (human clinical) in the plant, poultry, aquaculture and ruminant livestock industries (Donlan, 2009; Monk *et al*., 2010). These therapies employ either a cocktail of lytic phages, which specifically infect the bacterial (or archaeal) species of interest (Callaway *et al*., 2008), or utilize preparations of phage-encoded proteins (O'Flaherty *et al*., 2009).

Phage therapies based on lytic phages have the advantage over conventional antibiotics and chemical antimicrobials, as phages (i) are naturally occurring biological agents that do not contribute to the development of therapeutic antibiotic resistance in the environment; (ii) can be applied without the negative health side effects associated with some antibiotics; (iii) can be administered directly to living tissue without causing harm; and (iv) can attach, self-propagate and penetrate microbial biofilms that may otherwise be unaffected by chemical antimicrobials (Abedon, 2012; Chan *et al*., 2013). Phages chosen for use in phage therapy also tend to have a very narrow spectrum of activity directed against a target organism, are free of toxin and transducing virulence factor genes, replicate only through the lytic cycle of phage reproduction and are incapable of forming a lysogenic association with their host (Chan *et al*., 2013). In order for phages to encompass all of these desirable properties, they must be extensively genetically characterized and tested for biological efficacy, lysis efficiency and persistence, prior to any inclusion in a phage therapy formulation.

To date, phage therapies developed for ruminant livestock have focused on controlling organisms such as *S. bovis* in the rumen (Iverson and Millis, 1977; Tarakanov, 1994), *Staphylococcus aureus* causing bovine mastitis (Dias *et al*., 2013) and *Escherichia coli* strains such as the enterohaemorrhagic serotype O157:H7, which can be shed in

faeces and progress usually through the food chain to become a zoonotic pathogen (Niu *et al*., 2012).

While all of these therapies have progressed from *in vitro* isolation studies to *in vivo* testing in animals, further developments in phage therapies targeting *S. bovis* have not been reported in recent years and have not been progressed to large-scale production and testing. The emergence of drug-resistant strains of *S. aureus* in the dairy industry has prompted interest in the development of phage-based alternatives to chemical antibiotic mastitis treatments, with both intact phage- and enzyme-based projects being explored (Han *et al*., 2013; Mishra *et al*., 2013). Similarly, using lytic phages for the control of *E. coli* O157:H7 has been shown to be an effective treatment for reducing both the faecal shedding of this organism (Haiqing *et al*., 2006; Callaway *et al*., 2008; Rozema *et al*., 2009; Raya *et al*., 2011) and for use as biosanitizers for food contact surfaces and hides (Coffey *et al*., 2011; Sillankorva *et al*., 2012). Interestingly, it has also been suggested that the endemic, naturally occurring phage populations of ruminants may play a role in modulating enteric *E. coli* populations (Raya *et al*., 2011; Kropinski *et al*., 2012).

Initial research projects into the development of phage-based strategies to control rumen methanogen populations has been largely hampered by a lack of knowledge of both phages and hosts to be employed in the development and testing of these strategies. As detailed previously in this chapter, there is a lack of phages known to infect rumen methanogens, or even phages infecting related, mesophilic archaeal methanogens, which could be potentially employed as controls in phage isolation methodologies, such as plaque assays. In addition, there are only a few rumen archaeal isolates deposited in established, publicly available microbial culture collections. Rumen methanogen isolates that encompass the diversity of species found in the rumen are essential to further phage research, as they are required as potential phage hosts and to facilitate phage isolation studies. The development of new methodologies for the cultivation of rumen methanogens under anaerobic conditions, within layers of rumen fluid-based agar media (Gilbert *et al*., 2010a), has facilitated the development of techniques for phage isolation; however, factors such as: (i) the lack of host strains; (ii) the slow-growing nature of many of the available methanogen host strains; and (iii) the absence of phages available to be utilized as positive controls in plaque assays has impeded the progress of phage isolation studies.

As CRISPR/Cas systems provide a heritable mechanism of phage resistance, their formation by rumen methanogens may pose an additional complication for the development of archaeaphage-based therapies; however, factors such as the time frame required for the development of this mechanism of resistance, particularly in an environment such as the rumen, are currently unknown. The relatively high prevalence of CRISPR in archaeal genomes (Barrangou *et al*., 2007) does, however, further emphasize the importance of incorporating multiple phages into any therapies based on the use of intact, viable lytic phages.

As an alternative to using preparations of intact phage particles to target specific microbes, phage-encoded enzymes may be employed as alternatives to conventional antimicrobials (Fenton *et al*., 2010; Shen *et al*., 2012). With this approach, sometimes described as enzybiotics (Shen *et al*., 2012), the types of phage-encoded enzymes that may be utilized include those that in nature have evolved specifically to degrade the host cell wall and, for example, enable progeny phage particles to be released following phage replication within the infected host cell. Phage enzymes targeting the integrity of cell walls include amidase, muramidase and endopeptidase enzymes (Oliveira *et al.,* 2013). This enzyme-based approach may also employ phage proteins that have a structural role forming the coat tail structures or spikes, or have a functional enzymatic role (Ackermann and DuBow, 1987). Tailed phages, for example, may have a lysozyme located at the tail tip, and the spikes of some capsule-specific phages

demonstrate endoglycosidase activity allowing these structures, even when used in isolation of complete phage particles to bind to cell surfaces (Paul *et al*., 2011; Andres *et al*., 2012). This approach has moved beyond the initial *in vitro* developmental stages, with several preparations of phage enzymes that have been shown to be potent antimicrobials progressing to the stage of being tested in animal models, targeting bacteria such as *S. aureus*, *Streptococcus pneumoniae* and *Bacillus anthracis* (O'Flaherty *et al*., 2009; Shen *et al*., 2012).

Lytic enzymes encoded by the methanogen phages ψM2 infecting *M. marburgensis* (Pfister *et al*., 1998) and ψM100, the prophage of *Methanothermobacter wolfeii* (Luo *et al*., 2001), have been relatively well characterized. These enzymes have also been classified as pseudomurein endoisopeptidases (*Pei*W and *Pei*P) and have been over-expressed and purified in order to ascertain their activity (Luo *et al*., 2002). They have not been employed in any phage therapy approaches to reduce either the *Methanothermobacter* hosts or related methanogens, but have been utilized *in vitro* to increase the permeability of methanogen cell walls and enable the hybridization of oligonucleotide probes used in the molecular microbiology technique, fluorescence *in situ* hybridization (FISH; Nakamura *et al*., 2006; Kubota *et al*., 2008). Examples of phage-encoded enzymes with lytic activity have also been identified in annotated rumen methanogen prophage sequences (Attwood *et al*., 2008), and the endoisopeptidase enzyme, *Pei*R, encoded by the *M. ruminantium* M1 prophage φmru has been shown to lyse host cells in pure cultures (Leahy *et al*., 2013b). These phage-encoded enzymes therefore represent a powerful new avenue for the development of phage-based therapies to reduce and control methanogen populations.

21.6 Conclusion and Future Directions

The future of rumen phage research can be anticipated to expand rapidly, taking advantage of the technological advances in high-throughput sequencing in order to characterize individual rumen phages genetically and benefit from their unique enzymatic and structural properties and biological attributes such as host specificity and lytic potential. Adoption of new sequence-based technologies will also enable researchers to elucidate the roles of phages in maintaining rumen microbial population balance and genetic transfer, determining the overall contribution in rumen bacterial lysis and nutrition more comprehensively.

New phage-based therapies for the control of rumen methanogen populations and subsequent CH_4 amelioration could potentially be developed using a two-pronged approach of: (i) phage therapy with intact lytic phages that specifically target a broad spectrum of methanogen genera; and (ii) phage therapy based on phage-encoded products, for example formulation of lytic enzymes, tails. Both of these approaches would need to be developed with the premise of targeting a range of methanogen genera in order to address the natural complexity and diversity of the rumen methanogen community.

This could be achieved by utilizing a phage preparation or cocktail, incorporating a number of intact lytic phages that are either capable of infecting a range of methanogen species or have a narrow host range, infecting a single methanogen species. The phage cocktail would then be able to target and cause infection across the diversity of methanogen species found in the rumen. Continuing to sequence the genomes of different methanogen species found in the rumen will also benefit the discovery of new phage-encoded products greatly, which could potentially be blended into a broad-spectrum formulation targeting various rumen methanogen populations.

In addition, phage-based therapies could be used to reduce methanogens in the rumen per se, or they may be utilized to establish alternative microbial populations that will out-compete methanogens for hydrogen, such as reductive acetogens. Such an approach could be implemented at an early stage of ruminant development, for example

at weaning, and would potentially overcome any problems that may result from the overaccumulation of hydrogen within the rumen.

In conclusion, the approach of using phage therapy to reduce rumen methanogen populations is still in the relatively early stages of development. Improvements in the methodologies for the cultivation of rumen methanogens and the application of new technologies for the sequencing of methanogen genomes will facilitate progress greatly. In this way, phage therapy represents a novel biological approach for reducing ruminant enteric CH_4 emissions, building on the naturally occurring biocidal attributes of these unique microbial predators.

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22 Feed-based Approaches in Enteric Methane Amelioration

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Abstract

Mitigation of methane (CH_4) emissions from ruminants is necessary not only from the global warming point of view but also for saving dietary energy. Livestock being the significant contributors to the anthropogenic CH_4 pool have remained the prime target of global research for the past two decades, in order to find suitable, sustainable and economical possibilities of reducing enteric CH_4 emission. The adoption of a particular strategy by the stakeholders depends on the input cost, economic status, toxicity to host/inhabiting microbes, mitigation potential and persistency in long run. Among all the available options, feed-based intervention seems remarkable, and can be tried anywhere by making little alterations to the available feed resources and prevailing feeding practices. This chapter deliberates the pros and cons of various nutritional interventions, along with their future prospects to reduce enteric CH_4 emission. Issues like necessity of methanogenesis in the rumen, the feasibility of reducing livestock numbers and cutting down emissions, and the expected reimbursements that arise from this practically feasible reduction, are well debated in the chapter.

22.1 Introduction

Global warming is a key issue affecting the environment and agriculture and livestock production throughout the world. Agriculture as such is accountable for about 25–32% of greenhouse gas (GHG) emissions (ILRI, 2011). Crop production emits 14% of emissions, while livestock are accountable for almost 11–18% of emissions (FAO, 2006; Westhoek *et al*., 2011). Goodland and Anhang (2009) opined that the livestock sector, including its by-products, produced 51% (32,564 million tonnes (Mt) of carbon dioxide (CO_2)-equivalent (eq) year^{-1}) of worldwide GHG emissions; far more than the estimate of the FAO (2006), which reported 7516 Mt CO_2-eq or 18% of GHG emissions from livestock. Carbon dioxide provides most GHG (55–60%), followed by methane (CH_4; 15–20%). GHG emissions from livestock include 30% enteric CH_4, 30% nitrous oxide from manure and 40% carbon dioxide associated with feed production and grazing land (FAO, 2006). Apart from this, livestock production also produces a substantial amount of nitrogen in various forms, namely ammonia and nitrates, which in turn cause the loss of terrestrial and aquatic (including marine)

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biodiversity. By and large, around 30% of total terrestrial biodiversity loss may be attributed to livestock production, and the land conversion that is ascribed to livestock activities.

CH_4 is a potent GHG that contributes to global warming, and its concentration has increased enormously to 1782 ppb (155% increment) compared to the pre-industrial concentration (IPCC, 2007; Malik *et al*., 2012). CH_4 production from ruminants not only is important from the point of global warming but also is due to the significant loss of dietary energy in the form of CH_4, which otherwise would have been utilized by the host animal for productive functions (Takahashi, 2001; Malik *et al*., 2012). According to the FAO (2006), 37% (103 Mt) of anthropogenic CH_4 comes from livestock, including enteric fermentation and manure management, which is equivalent to 2369 Mt of CO_2 considering the global warming potential (GWP) of 23 over a 100-year period (IPCC, 2007); but when the GWP of CH_4 is 72 over a 20 year period, it is equivalent to 7416 Mt of CO_2 (Goodland and Anhang, 2009).

Livestock, being significant contributors to the anthropogenic CH_4 pool, have remained a prime target for worldwide research to find the most suitable, sustainable and economical ways of reducing enteric CH_4 emission over the past 20–25 years. Approaches pertaining to feeding intervention and biological control have been investigated and tested for the vested purpose of CH_4 reduction. These approaches have promulgated mixed and variable responses of failure and success, due to various reasons. The appositeness and prospects of feed-based approaches will be debated in subsequent sections; while other relevant approaches having good prospects for significant enteric CH_4 reduction in the future are discussed elsewhere in the book.

22.2 Rumen Methanogenesis: An Obligation or Wasteful Process?

Ruminants are excellent convertors of fibrous crop residues and lignified materials into animal food used by humans for their nutritional need. All this conversion of fibrous materials ensues in the rumen, where divergent microbes act on the feed material and degrade it into various end products such as volatile fatty acids (VFAs), microbial protein, etc. Gases like CO_2, H_2, N_2, traces of H_2S and O_2 are also produced in the rumen as a result of enteric fermentation. The accumulation of these gases in the rumen system may cause the complete cessation of fermentation, and the ultimate death of animal if the safe disposal of these fermentative gases is not ensured. That is why nature has blessed these animals with a safe disposal mechanism from the complex rumen system through burping, or eructation.

In enteric fermentation, hydrogen is a central metabolite, where its partial pressure is an important determinant for rumen methanogenesis. The balance between hydrogen ion (H^+) and dissolved hydrogen gas (H_2) determines the redox potential in the rumen, and therefore the possible extent of the oxidation of feedstuffs (Hegarty and Gerdes, 1998). Hydrogen gas (H_2), reduced cofactors (such as reduced form of nicotinamide adenine dinucleotide (NADH) and nicotinamide adenine dinucleotide phosphate (NADPH)) and free protons are the three key states of hydrogen for CH_4 synthesis in the rumen and are due to the central regulator of rumen fermentation, also referred to as the 'currency of fermentation' (Hegarty and Gerdes, 1998). The concentration of dissolved hydrogen in bulk rumen fluid at 39°C varies between 90 to 250 μM (IUPAC, 1981). Pelchen and Peters (1998) reported that 100 l of hydrogen was produced daily in sheep rumen, which is enough to generate 25 l CH_4, considering that 4 mol of hydrogen are required mol^{-1} of CH_4 produced.

In general, methanogenesis is the main route of hydrogen removal from the rumen (Beauchemin *et al*., 2008). It is impossible to reduce enteric CH_4 emission, without ensuring the safe disposal of H_2 through other routes, i.e. alternative sinks to keep the rumen functional or animal alive. Theoretically, propionogenesis and reductive

acetogenesis seem promising alternative sinks for methanogenesis in the rumen, for receiving H_2 and converting it to beneficial products rather than to CH_4 (Molano *et al.*, 2008). Other sinks for H_2 disposal may also be generated in the rumen through nitrate, sulfate and/or oil, particularly unsaturated fatty acid supplementation. However, the activation of these alternative sinks in the rumen has some limitations, such as feeding animals on fibrous diet that restricts the propionate production in rumen; thermodynamically favouritism for methanogenesis, toxicity as well as the cost of supplemental materials are among the limiting factors for H_2 clearance through these alternative routes in rumen. Major H_2 removal sinks in the rumen are discussed comprehensively in Chapter 16, Section III, this volume.

Due to the low H_2 threshold level of methanogens, they are thought to outcompete acetogens, and therefore utilize most of the available H_2 for CH_4 production (Martin *et al.*, 2010). In the case of CH_4 suppression, the rumen acetogens should be capable of utilizing H_2 at a low level. Acetogens often outweigh methanogens in the gut of young lambs, humans, wood-digesting termites, rodents and pigs; hence, reductive acetogenesis is the major hydrogenotrophic pathway in these microbial ecosystems. *Peptostreptococcus productus*, a sewage sludge acetogenic isolate, has been shown to outcompete methanogens in a simulated gastrointestinal fermenter (Nollet *et al.*, 1997). A wide range of the H_2 threshold has been reported as from 340 to 8060 ppm for acetogens, while the range of rumen acetogens in kangaroo and cattle varies from 342 to 4200 ppm (Rieu-Lesme *et al.*, 1996). If the factors answerable for augmentation of the reductive function of acetogens are identified from non-rumen ecosystems, a clue can be provided for intensifying reductive acetogenesis coinciding with enteric CH_4 reduction.

Methanogenesis due to the low H_2 threshold level of archaea is the only prominent hydrogenotrophic sink for large H_2 clearance from the rumen system; therefore, this pathway is an obligatory but wasteful mechanism, leading to a substantial loss of feed energy. Complete inhibition of methanogenesis is neither possible nor practical, due to the highly dynamic and diverse archaeal community. Our efforts should focus on targeting the prominent methanogens, which have a low hydrogen threshold level and usually do not allow the acetogens to utilize hydrogen. At the same time, efforts should also be made to identify and promote acetogens that appear competent to methanogens in H_2 utilizing capacity.

22.3 Dwindling Livestock Numbers

Livestock are the major determinant of GHGs from agricultural systems, as pronounced elsewhere in the book. Worldwide, livestock numbers are always fluctuating, and the degree of fluctuation depends on profitability, market place response and the sustainability of other alternatives. There are approximately 3.6 billion head of ruminants, which is about half the global human population (Foley, 2014). The United Nations predicts that the world population will exceed 9 billion by the middle of the century and has called for a 100% increase in world food production by 2050. This doubled food requirement must come from virtually the same land area as today and approximately 70% of this additional food must come from the use of existing and new agricultural technologies. Globally, more than 1 billion people keep livestock, 60% of rural households do so, and it is a major income source for the poor (von Braun, 2010). Livestock are a significant global asset, with a value of at least US$1.4 trillion (Thornton, 2010), and employ at least 1.3 billion people globally and directly support the livelihoods of 600 million poor smallholder farmers in the developing world (Thornton *et al.*, 2006). Livestock products contribute 17% of the energy and 33% of protein consumption by humans on a global scale (Rosegrant *et al.*, 2009).

It is clear that livestock are an integral part of the agricultural system; livestock play a pivotal role in the survival of millions

and will remain a principal and crucial fragment of the food chain, in spite of the massive GHG emissions from the sector. In one study, Pelletier and Tyedmers (2010) projected that the global emissions of direct generated greenhouse gases from livestock will increase to nearly 40% above the 2000 level by 2050. Researchers from both the developed and developing world are unremittingly examining the possibilities and practical applicability of various nutritional and biological interventions for reducing enteric CH_4 emission from ruminants. It is implicit that reducing livestock numbers will be the simplest way to reduce CH_4 emission (USDA, 2009). A significant reduction in livestock numbers will undoubtedly reduce GHG emissions in a short time frame and in an effective way. Greater reduction in livestock CH_4 emission may be achieved by reducing the numbers of ruminants, particularly of low or non-productive animals. This will not only help in reducing the global warming phenomenon but will also provide extra feed energy for bridging the gap between availability and demand, which is otherwise used by these non-performing animals for maintenance purposes. Goodland and Anhang (2009) recommended that a 25% reduction in livestock numbers would eliminate worldwide enteric CH_4 emission by a significant extent, and this could be made possible to selective locations.

Reducing livestock numbers, particularly of low productivity, looks a very realistic option as such, but most of the developing countries possess large numbers of such low producing animals as they are very well suited to and can withstand harsh climatic conditions, poor quality feeds, feed scarcity and poor management practices, whereas most of the quality exotic germplasm would fail to tolerate, produce and survive. The dependency of landless and marginal poor farmers on such low-producing animals for their survival restricts the killing of these livestock for the sake of stabilizing global warming. In addition, the slaughtering of a cow or the killing of other animals is religious taboo in a country like India, which holds 18% of the world's livestock. In 2014, Denmark signed a regulation, where the ritual slaughter of kosher animals (kosher animals are those that have split hooves and that chew the cud; for example, cows, sheep, goats and deer) is being made difficult by the imposition of a ban on such customs. Under these circumstances, in those countries where farmers are heavily dependent on their livestock for generating income, the imposition of regulations aimed at reducing livestock numbers would not be well received by the farmers. Thus, the killing of low-productive animals to reduce CH_4 emissions from livestock seems impractical in many developing countries, where livestock have a strong bond with the stakeholders. Researchers therefore have to focus on other alternatives that can make a substantial difference to CH_4 emission and that have universal and ethical acceptance. One such universally and widely accepted option, nutritional intervention, is discussed in subsequent sections of the chapter.

22.4 Mitigation Strategies

Practical strategies to reduce agricultural GHG emissions are urgently sought, particularly for ruminant enteric CH_4 (Pinares-Patiño *et al*., 2013). The most successful strategies will be those that lead to a profitable increase in animal productivity, as well as reducing enteric CH_4 emission. A brief overview of the opportunities for controlling CH_4 emission from livestock is presented in Fig. 22.1. It is evident from this figure that the opportunities for mitigating enteric CH_4 emission revolve around H_2 production or its utilization. Any strategy that restricts enteric H_2 production, obstructs its utilization or redirects it away from the methanogenic archaea will certainly control emissions. CH_4 is emitted from livestock by two mechanisms; the major mechanism is enteric fermentation, while the other is excreta storage or its handling. Of the total emission, 87% is produced in the rumen during enteric fermentation, which is later burped out into the atmosphere (Murray *et al*., 1976), while the remaining 13% comes from hindgut fermentation (Moss *et al*., 2000).

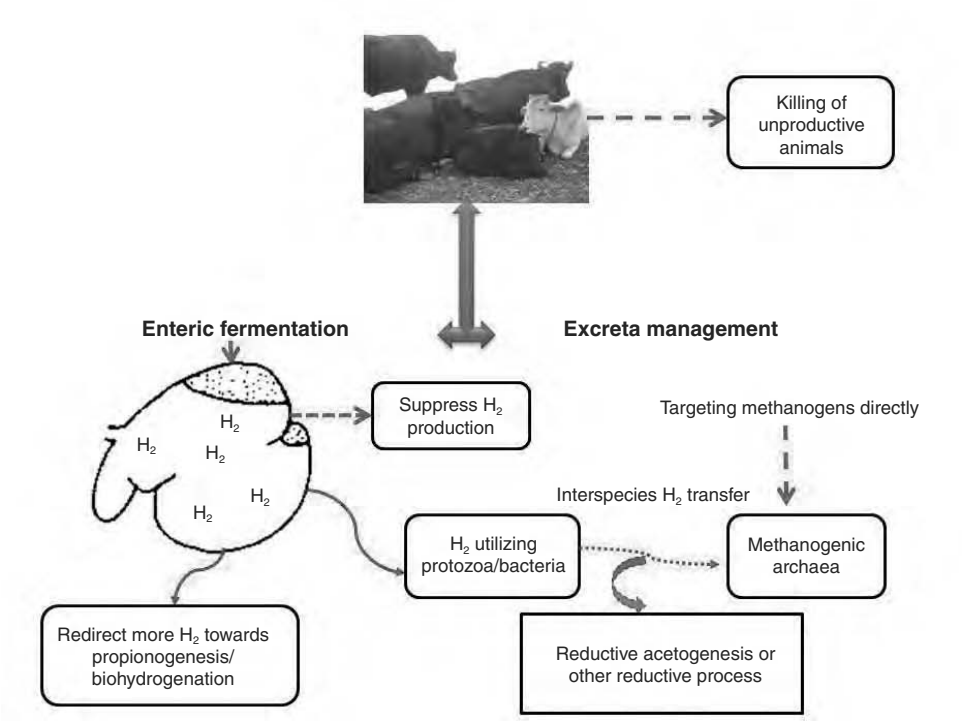

Fig. 22.1. Strategic options for controlling livestock CH_4.

Researchers are trying to explore the best possible options for the eradication of enteric CH_4 emission through feeding and nutritional interventions, improved management practices and biological control of rumen archaea (Malik *et al.*, 2012). The espousal of a particular strategy for CH_4 reduction by stakeholders depends on the cost of inputs, the economic status of the livestock keepers, the toxicity to the host/inhabiting microbes, persistency in the long run, etc. (Malik *et al.*, 2012). One of the best available options for livestock CH_4 reduction is to cut down the numbers of livestock by killing/culling the low or unproductive animals, as described in the section above. Biological control of enteric CH_4 emission or rumen archaea appears to have good prospects through promoting reductive acetogenesis, active and passive immunization of host animals against inhabiting methanogens, archaeaphage therapy, protozoa removal, etc., and is argued elsewhere in the book. Due to the major CH_4 emission through eructation, nutritional and management strategies in particular will be addressed in this chapter.

Among all the options available, interventions pertaining to feeding and feed management seem to be most favourable and can be tried anywhere in the world by making slight adjustments to feed resources and feeding practices. Improved animal productivity and dietary manipulation are two such strategies that have shown potential for reduced emissions and at present appear to be the most viable options (Clemens and Ahlgrimm, 2001). Composition of diet, forage type, processing, feeding frequency, nature of concentrate, fermented starch, intake level, etc., can make remarkable changes in enteric CH_4

emission. In the subsequent section, the prospects of feed-based approaches are debated in detail.

22.4.1 Diet composition

Composition of diet influences CH_4 production in ruminants, as digestion in the rumen is dependent on the activity of microorganisms, which need energy, nitrogen and minerals (Moss, 1994). Therefore, the quality of diet affects the activity of rumen microbes and CH_4 production in the rumen. Enteric CH_4 emission is highly reliant on diet composition and tends to decrease with high protein content, while the reverse occurs with increasing fibre content (Johnson and Johnson, 1995; Kurihara *et al*., 1997). When dairy cows were fed on high roughage, CH_4 production (per kilogram of dry matter intake (DMI)) was 35% higher than when they were fed on high-concentrate feed (Kurihara *et al*., 1997), since the amount of digested cellulose contributed to CH_4 production more than the amount of other carbohydrate components (Moe and Tyrrell, 1979). CH_4 production on high-concentrate feed is lower than that on high roughage at near maintenance (Lovett *et al*., 2003).

The major effect of diet composition on CH_4 production in ruminants is due to a shift in fermentation pattern through alterations in the acetate:propionate ratio, and thereby the proportion of dietary energy lost as CH_4. Major constituents of the diet, namely sugars, starch, fibre, protein and lipid, appear to have varying impacts on CH_4 emission. Kirchgessner *et al*. (1995) concluded, from a regression analysis, that on average crude fibre provides about 60%, nitrogen free extract 30%, crude protein 10% and ether extract a minor proportion of the total CH_4 emission from dairy cows. However, variations within and between the major classes of nutrients can cause major shifts in CH_4 emission. Forage species, forage processing, proportion of forage in the diet, fibre level and grain sources are a few important factors that affect diet composition, and so enteric CH_4 emission.

22.4.2 Fodder quality and type

Fodder type and quality are well known for affecting the activity of rumen microbes, and thus the CH_4 generation from the rumen. Kurihara *et al*. (1995) showed that CH_4 production in cows fed on Italian ryegrass hay was lower than that from cows given maize silage. Fodders that are highly digestible stay in the rumen for a short time only, due to the high passage rate, while fodders of lower digestibility stay in the foregut comparatively longer and consequently lead to more CH_4 emission. CH_4 emission from animals feeding on leguminous forages is reported to be lower than from those feeding on grasses, because legumes promote higher intake and production from the animals (Ramirez-Restrepo and Barry, 2005). The desirable reduction of 15–21% in enteric CH_4 emission may be achieved by the feeding of more digestible feeds like legumes (Benchaar *et al*., 2001).

Legumes like lucerne or red clover tend to decrease CH_4 losses due to the condensed tannins (Ramirez-Restropo and Barry, 2005) or saponins (Malik *et al*., 2009, 2010) usually present in these fodders. Not only the fodder type but also the form of feeding also has an impact on CH_4 emission. There is evidence that fresh grass results in lower CH_4 losses than grass silage. The saponin content in legumes varies in the range of 30–50 g kg^{-1} DM (Fenwick and Oakenfull, 1983). Hess *et al*. (2003) found that the inclusion of *Sapindus saponaria* in a roughage diet at a level of 14% decreased the protozoal population by 54% and CH_4 emission by 20%. Feeding of leguminous fodder, however, does not have any direct impact on enteric CH_4 emission, and an indirect reduction is achieved through the shift in fermentation towards more propionogenesis, high digestibility, passage rate (Beauchemin *et al*., 2008), protozoa removal action, inhibition of H_2-producing bacteria, etc.

22.4.3 Maturity stage

CH_4 emission from animals is highly correlated with neutral detergent fibre intake and digestibility. In an interesting study, Kasuya and Takahashi (2010) compared CH_4 emission in dry cows fed with first-cut Timothy silage, second-cut Timothy silage, second-cut Italian silage, third-cut Italian silage, or second-cut red clover silage as their sole feed. They reported emissions in the range of 258.2–396.5 l day^{-1} in dry cows; emissions from red clover silage were lower than those from grass silage. On comparing three different cuts of berseem fodder (*Trifolium alexandrinum*), Malik (2007) reported the highest CH_4 production from third cut berseem fodder compared with first and second cuts in an *in vitro* study. The same trend was also observed for lucerne fodder, where the first- and second-cut fodder produced less CH_4 than the third cut; however, lucerne (*Medicago sativa*) fodder produced less CH_4 than berseem fodder, irrespective of the cuts at 15, 30 and 45% levels of supplementation to a wheat straw-based diet in *in vitro* studies (Malik, 2007). The fibre content of forage crops tends to increase with maturity, which has inverse relations with protein and saponin content. Thus, the feeding of matured fodder to animals leads to high CH_4 emission due to low digestibility and slow passage rate. Wims *et al.* (2010) reported a 14% reduction in CH_4 emissions from dairy cows grazing on more digestible swards as compared to those grazing on less digestible swards, while Boadi *et al.* (2002) reported a 44% reduction in CH_4 production per unit of DMI from steers grazed on early-season pastures rather than late-season pastures.

The impact of forage quality on enteric CH_4 emissions has been inconsistent in the literature. CH_4 production tended to increase when mature dried forages were fed (Sundstol, 1981). Contrarily, Chung *et al.* (2013) demonstrated that heifers fed at maintenance produced more CH_4 when offered freshly cut legumes at early maturity compared with late maturity. Mc Geough *et al.* (2010) evaluated four maturity stages of maize silage in finishing beef cattle and concluded that maize harvest maturity did not affect the beef cattles' performance but reduced CH_4 output relative to DMI and carcass gain. It is presumed that highly digestible forages promote improvements in animal performance, which reduces the emissions per unit of product (milk or meat), and fodder harvested at early maturity is more digestible than that harvested at late maturity.

22.4.4 High-grain/concentrate feeding

Concentrates are usually lower in cell wall components than forages and normally ferment faster than forages due to the presence of soluble carbohydrates, i.e. starch and sugars. It is well known that an increase in the proportion of concentrate in the diet decreases CH_4 emission (Martin *et al.*, 2010). This fermentation phenomenon of concentrates is quite different from structural carbohydrates and gives rise to elevated levels of propionic acid, at the cost of acetate. Johnson and Johnson (1995) showed that digested cell walls normally led to higher losses than non-cell wall components. Within non-cell wall components, soluble sugars are more methanogenic than starch (Johnson and Johnson, 1995).

When forage quality is poor, grain supplementation is generally recommended for improving efficiency and reducing CH_4 loss in ruminants, as evidenced from the study of Boadi *et al.* (2002), where they found that grain supplementation for pastured yearling steers increased DM intake and gain but there was no benefit relative to enteric emissions. That study clearly established that forage quality was the major factor affecting enteric emissions from animals. Feeding high-grain diets to cattle unequivocally lowers the formation of CH_4 in the rumen. With high grain diets, the energy loss in the form of methane is reduced to about 3% from 6.5% (Beauchemin and McGinn, 2005). CH_4 production can be lowered by almost 40% when a forage-rich diet is replaced with high concentrate (Veen, 2000). High-starch diets generally result in a decline in the protozoal population; a close

negative relationship between CH_4 emission and protozoa numbers exists (Morgavi *et al*., 2010). Sauvant and Giger-Reverdin (2007), in a meta-analysis, showed a curvilinear relationship between CH_4 production and concentrate proportion and reported low CH_4 emission when the concentrate comprised more than 70% of the diet. High-concentrate diets result in low pH (Doreau *et al*., 2011), reduction in protozoa numbers and reduced fibrolytic activity (Martin *et al*., 2010), which collectively decrease enteric methanogenesis. Feeding of large amounts of concentrates may sometimes be associated with higher risk of lameness (Manson and Leaver, 1988). Increasing the proportion of concentrates is limited by a required minimum level of physical structure in the diet and the balance between energy intake and requirements in low-producing animals. Feeding of high-concentrate diet is sometimes not practical, due to direct competition with humans, and escalating prices is also putting a major constraint in using grain or other concentrates for animal feeding.

22.4.5 Type of carbohydrates fermented

The VFA profile, and thereby CH_4 yield, is affected by the type of carbohydrates fermented in the rumen (Bannink *et al*., 2006). On a concentrate-based diet, fermentation of sugars and starch showed 25 and 15% lesser CH_4 yields, respectively, as compared to a roughage diet (Bannink and Dijkstra, 2005). As the starch content in a diet increases, rumen pH decreases, making the environment more hostile for methanogens to survive. It is therefore expected that less CH_4 should be produced per unit of starch than per unit of cell wall carbohydrate digested. Considerable variation in starch content exists among grains, as Gozho and Mutsvangwa (2008) reported the starch content of barley, maize, wheat and oat in the tune of 19.2, 21.8, 22.4 and 15.2%, respectively. For a drop in CH_4 production, the diet of ruminants needs to have starch-based grain rather than high-fibre by-product feeds (i.e. screenings, millrun). Starch-containing grains lower the formation of CH_4 in the rumen by forming more propionate and less acetate. Feeding high-grain diets also causes the rumen environment to become more acidic, which inhibits the growth of rumen methanogens.

The extent to which high-grain diets lower CH_4 emissions depends on the source of the grain. For example, greater reductions can be achieved with maize than barley (Beauchemin and McGinn, 2005). McGinn *et al*. (2009) observed 23.9% less CH_4 on the feeding of maize distillers' dried grains as compared to the feeding of barley grain in growing beef cattle. However, adding maize distillers' dried grains to cattle diets reduced CH_4 emissions, but the emissions of nitrous oxide and ammonia increased, which should also be taken into account when evaluating the environmental impact of feeding maize distillers' dried grains (Todd *et al*., 2006). Feeding of starchy crop waste, which is readily available in South-east Asia, may be an effective way of reducing CH_4 emissions. Shioya *et al*. (2002) examined the effects of supplementing low-quality hay with sweet potato and reported a reduction of 42% (260 versus 146 l day^{-1}). Starchy sweet potato waste feeding may stimulate a higher rate of rumen fermentation and suppression of fibrolytic activity in cellulolytics (Hino and Hamano, 1993). At higher intakes, cell wall carbohydrates are more methanogenic than soluble carbohydrates (Moe and Tyrell, 1979); therefore, anything that ensures a wider soluble/cell wall carbohydrate ratio will decrease CH_4 formation.

More degradable starch sources on the one hand lead to added fermentation in the rumen, but on the other hand may also lower ruminal fibre digestion. Increased use of grains in ruminant diets reduces enteric CH_4 emissions, but there is concern that increased grain production may also increase the use of fossil fuels for fertilizer, machinery and transport, resulting in more GHG emissions (Beauchamin and McGinn, 2005). Grain feeding ignores the importance of ruminants in converting fibrous feeds to high-quality protein sources. Furthermore, high-grain diets can affect cow health negatively, due to acidosis. With escalating

grain prices and competition, the scope of using more grains, especially in the developing world, seems impractical under the present scenario.

22.4.6 Intake, feeding frequency and processing

The level of feed intake, feeding frequency and processing of feed materials are a few vital principles that decide the amount of CH_4 generated during enteric fermentation. Intake level is governed by the animal itself, depending on the quality of diet. Feeding frequency and feed processing are at the discretion of livestock keepers, however, feed and fodders availability; quality, stock strength at farm, stakeholder's economic status, purpose of livestock farming etc. are few of the important criteria to decide the feeding frequency and processing method.

It is expected that at a higher level of intake, the loss of dietary energy in the form of CH_4 will be less than that at low intake. The percentage of gross energy lost as CH_4 on average decreases by 1.6% with a per unit increase in intake level (Johnson *et al.*, 1993). At higher intake, the CH_4 emission per unit of intake is decreased by 12–30%; however, the total emission of CH_4 as such will be more. Generally, at high intake, the passage rate of digesta through the gastrointestinal tract increases; thus, the extent of substrate access to microbes decreases, which in turn reduces the rate, or extent, of ruminal fermentation, and consequently CH_4 emission (Owens and Goetsch, 1986). The relationship between CH_4 emission and DM intake is positive and varies with each individual animal (Lassey *et al.*, 1997). The differences in DM intake per se accounted for about 14% of the variation in CH_4 emission.

Efficiency of livestock for CH_4 emission is related closely to the maintenance requirements of the animal; the higher the intake above maintenance, or the higher the level of production, the lower the CH_4 emission per unit of product, and thus the higher the productive efficiency. This suggests that feeding of animals above the maintenance level is advantageous for efficient animal production and less so for CH_4 emission. However, CH_4 production does not necessarily depend on feed intake, as high fractional CH_4 losses can occur when highly available carbohydrates are fed at limited intakes, and the reverse can happen when a high intake of a highly digestible diet takes place (Johnson and Johnson, 1995). Changes in DM intake not only affect the amount of substrate available for microbial degradation but also change fermentation conditions and the size of the microbial population. For example, the fate of ingested starch alters with changes in the amount of DM ingested; increased intake levels lead to a proportionally higher amount of starch digestion in the small intestine rather than fermentation in the rumen.

Feeding frequency also affects CH_4 emission associated with propionate production (Sutton *et al.*, 1986). Some of the earlier studies revealed the effect of feeding frequency from the perspective of optimization of carbohydrate fermentation in the rumen. Mathers and Walters (1982) concluded that even with frequent feeding, there was considerable deviation in the rate of carbohydrate fermentation from steady state in sheep fed every 2 h. CH_4 production increased rapidly within 30 min of feeding, and then decreased until the next 2-h cycle (Mathers and Walters, 1982). In the 1980s, a series of trials were conducted in Germany, where it was established that frequent feeding did not improve dietary energy use but rather increased CH_4 emission when concentrate was fed more often and separately from forage (Röhrmoser *et al.*, 1983). Crompton *et al.* (2010) also did not find any effect of feeding frequency on CH_4 production in dairy cows.

Various feed processing methods like grinding and pelleting reduced CH_4 emission from the rumen markedly, due to change in rumen pH, fermentation shift and increased fractional outflow of particulate matter. CH_4 losses are higher for coarsely chopped fodder than for finely ground, pelleted diets (Hironaka *et al.*, 1996). Johnson and Johnson (1995) pointed out that CH_4 loss per unit of diet could be reduced by 20–40%

by processing of forage (ground or pelleted) at high intake, due to high passage rate. In developing countries, enhancing productivity by improving feed quality is very important, as the productivity of animals in these countries is low, and therefore more animals and extensive time are required to acquire enough animal products in order to meet demand. On comparing various dietary manipulation means for reducing CH_4 production, Moss (1994) observed that chemical treatments such as sodium hydroxide or ammonia and protein supplementation in low-quality feeds were the best options, while others proposed the use of urea molasses mineral block (Srivastava and Garg, 2002) for reducing CH_4 emission from animals thriving on poor-quality feeds such as straw in developing countries.

22.4.7 Use of fat and oil

Supplementation of fats and oils conquers enteric CH_4 production through redirecting hydrogen towards a biohydrogenation process (alternative H_2 sink), triggering a toxic effect on ruminal microorganisms, particularly on H_2-producing microbes and methanogens, suppressing rumen protozoa liable for interspecies H_2 transfer to archaea and depressing enteric digestion. The addition of unsaturated fatty acids to the rumen decreases CH_4 emission; however, the extent of suppression is dosage dependent and influenced by the type of fatty acid. The extent of CH_4 reduction through lipid supplementation depends mainly on the quantity, degree of unsaturation and the chain length (Johnson and Johnson, 2002). It appears that the effect of the degree of unsaturation is relatively small, and is due mainly to digestion depression (Johnson and Johnson, 1995). Certain oils, such as coconut oil, seem to reduce CH_4, possibly by suppressing protozoa (Dohme *et al*., 1999).

Johnson and Johnson (1995) stated that the amount of H_2 used in the biohydrogenation process of unsaturated fatty acids was insignificant to lead to a substantial reduction in CH_4 emission, and considerable effects of lipid supplementation were likely to occur only when basal digestion was inhibited. However, Dong *et al*. (1997) opined that it was not necessary for CH_4 reduction to be always accompanied with depressed digestion. For enteric CH_4 reduction, medium-chain fatty acids are more effective than long-chain fatty acids (Johnson and Johnson, 2002). Dietary fat supplements, especially those containing unsaturated fatty acids, can reduce enteric CH_4 emissions, but oil production for animal feed consumption is usually associated with increased net GHG emissions (Beauchemin *et al*., 2008, 2009; Grainger *et al*., 2010). Eicosahexanoic acid (EPA or C20:5) and docosahexanoic acid (DHA or C22:6) are the major bioactive unsaturated fatty acids found in fish oil and marine algae (Givens *et al*., 2000) and decrease CH_4 production by up to 80% in i*n vitro* studies (Fievez *et al*., 2003).

22.4.8 Organic acids

It is stated that the addition of carbohydrate degradation intermediates (organic acids) stimulates the production of propionic acid (Kolver *et al*., 2004) and reduces CH_4 losses (Castillo *et al*., 2004) through serving as H_2 scavangers in the rumen (Krause *et al*., 2002). Sodium acrylate and sodium fumarate were found most promising in decreasing CH_4 production on comparing 15 potential precursors of propionate, and showed a reduction of between 8 and 17% in a short-term batch cultures study (Newbold *et al*., 2005). Newbold and associates (2005) further reported that free acids rather than salts were more effective in reducing CH_4. Using free acid as a means for CH_4 reduction may decrease rumen pH, which in turn poses possible negative effects on fibre degradation. Fumarate addition decreased CH_4 production by 28% in an *in vitro* study without any negative effect on DM degradation, whereas malate was not found effective in reducing CH_4 production, or was found marginally effective (Carro and Ranilla, 2003). Some anaerobic bacteria synthesize propionate from fumarate or

malate using a reverse citric acid cycle. Malate must be converted to fumarate, which in turn reduces to succinate, a process that requires H_2, and thereafter, succinate is decarboxylated to form propionate. It was concluded from the literature that the effect of organic acid supplementation on CH_4 production was inconsistent, as McGinn *et al*. (2004) and Beauchemin and McGinn (2006) did not find any measurable effect of fumaric addition on CH_4 production. Further, malate and fumarate are expensive chemicals so it is doubtful that the amount required could be used as a feed additive, and the concentrations of these organic acids vary naturally in plants.

22.4.9 Ionophores

Ionophores, the 'gold standard' feed additives due to their highly lipophilic nature and capacity to alter the ion exchange gradient across the bacterial membrane, cause an energy-spilling cycle in bacteria to maintain the ion gradient (Russell and Strobel, 1989). Outer membrane impermeability of the gram-negative bacteria provides a protective barrier, and is likely cause for the selectivity of ionophores. Ionophores may differ slightly in their mode of action but mostly end with a selective decrease of gram-positive bacteria in the rumen (Coe *et al*., 1999). As gram-negative bacteria are mostly propionate and succinate producers (Nagaraja *et al*., 1997), ionophores in ruminant diets therefore often narrow down the acetate to propionate ratio. Ionophores have been chosen strategically for the mitigation of enteric CH_4 emission from ruminants (Guan *et al*., 2006).

Monensin is the ionophore mostly used in animal production for the purpose of enteric CH_4 mitigation; however, many other ionophores, such as lasalocid, salinomycin, narasin and lysocellin, are also used as CH_4 inhibitors. Ionophore (monensin) possibly reduces CH_4 through the inhibition of protozoa, which transfer H_2 to methanogens (Guan *et al*., 2006), or by altering the VFA production pattern. The role of ionophores in animal production and enteric CH_4 mitigation is discussed comprehensively in Chapter 17, Section III, this volume.

Monensin supplementation decreased CH_4 production in cows by 25% (O'Kelly and Spiers, 1992), with a 10.5% lateral reduction in daily feed intake. Barman *et al*. (2001) also reported a reduction in CH_4 emission in buffalo calves following dietary supplementation of monensin. Supplementation in both faunated and unfaunated goats also reduced CH_4 production, though the degree of reduction in unfaunated animals was less than that in faunated animals (Itabashi *et al*., 1984). Guan *et al*. (2006) systematically evaluated the effects of short- and long-term feeding, as well as rotation of two ionophores (monensin and lasalocid) on enteric CH_4 emissions in Angus steers. They observed that the supplementation of ionophores decreased enteric CH_4 emissions by 30% for the first 2 weeks and by 27% for the first 4 weeks. Further, they reported that rotation of ionophores did not exhibit a greater decrease and did not have a longer period of depressed enteric CH_4 emissions. Long-term supplementation of ionophores shows erratic results in the inhibition of methanogenesis, and pre-supplementation values usually restored after 2 weeks. The use of antimicrobials including monensin is also banned in some European countries, which is again instigating limitation of the use of ionophores for enteric CH_4 mitigation.

22.4.10 Use of bioactive phytochemicals

Bioactive phytoextracts have traditionally being used for their medicinal value for years, but their potential for reducing CH_4 emission has only been explored recently (Patra and Saxena, 2009; Malik *et al*., 2012). The use of bioactive photochemicals, or plant secondary metabolites like tannin and saponin, and essential oils as an anti-methanogenic agent (Wallace *et al*., 2002) in the diet is generally considered safe. Low cost and easy availability also make them a favourable agent for CH_4 reduction. The European Union has sponsored the projects like 'RUMENUP' and 'REPLACE' to identify

the wide range of plant materials that could modify rumen fermentation, useful in reducing CH_4 emission, and could replace antibiotics in the diet of monogastrics.

The antimethanogenic activity of tannin can be attributed to both condensed and hydrolysable tannins. Forage legumes such as *Lotus corniculatus* and *Lotus uliginosus* encompass condensed tannins (CTs) in their leaves. A considerable variation exists between and within the varieties of *L. corniculatus* and *L. uliginosus* (Marley *et al*., 2006) for tannin content. Tannins suppress methanogenesis directly by acting on rumen methanogens and indirectly through lowering feed degradation, and thereby H_2 production and protozoa removal. Supplementation of *Phaseolus calcaratus* hay (PCH) was found beneficial in swamp buffalo fed rice straw as basal roughage; it resulted in reduced protozoal population and CH_4 emission (Chanthakhoun *et al*., 2011). Legumes containing condensed tannin are able to lower CH_4 by 12–16% kg^{-1} DM intake (Beauchemin *et al*., 2008; Grainger *et al*., 2009). McAllister and Newbold (2008) reported that extracts from plants such as rhubarb and garlic could also decrease CH_4 emissions. Most of the trials where tannin was used as a source for CH_4 suppression were conducted *in vitro*, and only a few studies have been undertaken *in vivo*, which showed highly inconsistent effect on methanogenesis. The efficacy of these compounds remains disputed, and it is generally argued that the suppression in methanogenesis is achieved through the reduction in feed fermentability rather than having a direct impact on rumen archaea. Efforts should made for exploring the novel feed resources that contain measurable quantity of tannin; dose optimization for different livestock species/breeds for substantial methane reduction without adversely affecting feed fermentation.

Saponin, naturally found in numerous plants, has antiprotozoal, and so CH_4, reduction properties. Saponin probably does this through the selective inhibition/enhancement of ruminal bacteria, the removal of protozoa, reducing feed fermentability and H_2 production, a shift in the VFA production pattern, etc. The literature has revealed that saponin from different sources can inhibit CH_4 production both *in vitro* and *in vivo*; however, inhibition from all sources is not equally effective (Rowlinson *et al*., 2008). Recently, Sirohi *et al*. (2014) emphasized that plant secondary metabolites (PSMs) at low concentrations could be used to manipulate rumen fermentation favourably. *In vitro* studies (Holtshausen *et al*., 2009) where different phytosources of saponin were used revealed a considerable variation in CH_4 reduction, ranging from 2.2 to 64%, depending on the source (Sirohi *et al*., 2014). They also compiled the results from *in vivo* studies, where a reduction of 8–15% in CH_4 emission was apparent. The reduction in CH_4 production on the inclusion of saponin sources is possibly due to inhibitory action on protozoa, which are accountable for interspecies H_2 transfer to the associated methanogens (Holtshausen *et al*., 2009).

In a series of experiments, Li and Powers (2012) did not find any measurable difference in CH_4 emission in steers fed on a *Quillaja saponaria* (QS) or *Yucca schidigera* (YS) saponin-based diet (at 1.5% of DM). A 31% reduction was apparent on the inclusion of tea saponin, accompanied by a 27% reduction in DMI. Results from *in vivo* studies are inconsistent (Patra and Saxena, 2009) and dietary saponin supplementation usually fails to reduce CH_4 emissions, except when fed at high concentrations that inhibit performance (Li and Powers, 2012). One of the reasons for the failure of a long-term effect or inconsistent results may be the microbial adaptation or degradation of saponin in the rumen (Teferedegne *et al*., 1999). Further research is required for exploring the optimum sources of saponin, feeding methods and optimizing dose rates for different livestock to ameliorate CH_4 emission without affecting feed fermentation.

Essential oils are volatile components, well known for their antimicrobial properties and recently for methanogen inhibition (Benchaar and Greathead, 2011). *In vitro*

studies have indicated that CH_4 production can be decreased in a dose-dependent manner on using essential oils derived from thyme, oregano, cinnamon, garlic, horse radish, rhubarb and frangula (Benchaar and Greathead, 2011). In these studies, inhibition was apparent at high doses (>300 mg l^{-1} of culture fluid) and in many cases was associated with decreased feed fermentation and volatile fatty acid concentrations. The blended application of thymol, guajacol and limonene at 110 mg day^{-1} in sheep did not provide the desired results (Newbold *et al*., 2004). Beauchemin and McGinn, 2006 also found no measurable effect of essential oils (1 g day^{-1}) on CH_4 emissions in beef cattle. Like other secondary metabolites, results from *in vivo* studies where essential oil is used as the mitigating agent are erratic, perhaps due to the adaptation of rumen microbes and ruminal degradation of bioactive compound.

22.4.11 Novel phyto-sources

Competition for conventional feed materials, especially for grains, between ruminants, non-ruminants and humans has led to a scarcity of feed for livestock. The escalating cost of conventional feed ingredients also exacerbates the situation and increases the cost of animal production (Akinmutimi, 2004). Due to the circumstances of short availability of feedstuffs, especially in the dry season, farmers are searching for alternative feed ingredients that not only substitute the usually expensive conventional ingredients (Ojebiyi *et al*., 2008) but also ensure contemporaneous reduction in enteric CH_4 emission.

Algae (marine and freshwater) have been used in human nutrition, cosmetics and pharmaceuticals for long time, but their use as a feed supplement for livestock has only recently been explored (Baptiste *et al*., 2013). Algae are one of carbon dioxide's severest natural adversaries. *Ascophyllum nodosum* has specifically received attention for its application in ruminant diets and effects on GHG production (Wang *et al*., 2008). Microalgae are a good source of unsaturated fatty acids that have potential for CH_4 mitigation (Pacheco, 2011). A project focusing on proof of concept for the development of algae-based functional foods to reduce enteric CH_4 emission is coordinated under the National Livestock Methane Programme (NLMP) of Meat and Livestock Australia, where researchers are testing a range of algae for antimethanogenic activity and are looking for lines of algae that may be tested in future. Preliminary data from *in vitro* studies indicate that the freshwater algae, *Spirulina*, does not affect CH_4 emissions.

A patent for red macroalgae species, which have shown powerful CH_4-reducing properties, has been filed by the CSIRO and James Cook University, Australia, working together under the NLMP (Tomkins, 2014). Trials at the James Cook University disclosed that cattle fed an algae-based diet emitted 20–40% less CH_4. A decrease in DMI with increasing milk yield was reported in dairy cows supplemented with microalgae-based commercial DHA (Boeckaert *et al*., 2008). Due to less cellulose and high starch, algae are more digestible than many terrestrial plants. However, CH_4 emissions from the feeding of algae or algae products have not been measured exclusively and only a few reports are available. In a study of Holstein dairy cows, Moate *et al*. (2013) found no reduction in CH_4 emission due to algal meal supplementation to a forage-based diet. Marine and freshwater algae, namely *Caulerpa lentil lifera*, *Caulerpa taxifolia*, *Cladophora patentiramea*, *Ulva* sp. 3, *Ulva ohnoi*, *Derbesia tenuissima* and *Oedogonium* sp., were evaluated for their inclusion in ruminant diets and the subsequent effect on GHG emission. The results from the studies indicated that *Cystoseira trinodis* had a significant effect in promoting CH_4 reduction (Baptiste *et al*., 2013). However, other algal species showed a lesser mitigation effect.

Another novel source of phyto origin is cashew nut shell liquid (CNSL), which has shown a promising effect on CH_4 production

in recent years. CNSL is rich in the antibacterial phenolic compound, 'anacardic acid'; the principal factor selectively affects the rumen microbial composition and shifts the fermentation pattern, with contemporaneous CH_4 decrease. This promising new active compound from cashew nuts has reduced CH_4 emissions in dairy cows by 20% (Shinkai *et al*., 2010). In a recent study, Shinkai *et al*. (2012) observed a remarkable decrease in CH_4 emission and stimulation of propionate production on the feeding of CNSL pellets to non-lactating Holstein cows. On CNSL feeding, the relative numbers of methyl coenzyme M reductase subunit A and its expression decreased as compared to control group (Shinkai *et al*., 2012).

In a series of batch culture system experiments, Watanabe *et al*. (2010) tested the CH_4 inhibition efficacy of raw versus heated CNSL, the optimum dosage and the susceptibility of bacteria to CNSL. They found raw CNSL more effective than heated CNSL for inhibition of CH_4 production in a dose-dependent manner; maximum inhibition was at 200 μg ml^{-1} supplementation. In addition, raw CNSL also prevented the growth of hydrogen-, formate- and butyrate-producing rumen bacteria, but not the growth of bacteria involved in propionate production (Watanabe *et al*., 2010). Akin to other phyto-derived materials, there is a need to conduct more *in vitro* and *in vivo* trials to test the efficacy of CNSL and to formulate diets for the purpose of CH_4 mitigation.

22.5 Ration Balancing

The majority of the livestock in developing countries are scattered randomly and are reared by marginal and landless farmers. Livestock, particularly ruminants, are exclusively being fed a fibrous diet, with negligible or zero concentrate, leading to more enteric CH_4 emission. On the other hand, livestock in the developed world are fed on entirely different feeding regimens comprising concentrates and succulent pasture fodder. The situation in the developing world is aggravated by the availability of limited feeding options, due to shrinking land holdings, limited land allocation under fodder production, non-scientific feeding, lack of awareness, and of course a flourishing human population that always claims first rights to natural resources. Due to the lack of awareness among livestock keepers in the developing world, they hardly know about enteric CH_4 emissions from livestock and consequently feed an imbalanced diet, which promotes more enteric CH_4 emissions.

One of the best ways of mitigating enteric CH_4 emission from livestock is to utilize locally available feed resources judiciously, in such a way that not only ensures less CH_4 emission but also sustains/increases productivity. This can be done through the development of a minimum CH_4 model by country-specific scientific agencies, by taking all locally available feed resources into consideration. One such least-cost minimum CH_4 model has been developed by Moreas *et al*. (2014) to formulate diets that minimize CH_4 emission while supplying the nutrients required to maintain milk production. However, achieving both goals is conflicting, as minimizing diet costs may result in increased CH_4 emissions, and vice versa. This is where scientific minds and inputs become involved, to arrive at a balance between the two goals (Moraes *et al*., 2012). The National Dairy Development Board (NDDB) of India recently launched a ration-balancing programme for small dairy farmers in different agroclimatic regions of the country, and in one study, Garg *et al*. (2012) found a 13.3% reduction in CH_4 emissions on feeding buffalo balanced rations prepared from locally available feed resources. Further, they reported a 15–20% reduction in CH_4 emission from cattle/buffalo kept in Gujarat, Maharashtra, Andhra Pradesh and Uttar Pradesh states and fed on a balanced ration. The findings clearly point out that balancing rations with locally available feed resources on the farmer's doorstep may be one viable and practical option to ameliorate CH_4 emission from livestock.

22.6 Genetic Upgrading – Forage and Animal

Improving the nutritive value of feed offered to animals is one way of reducing enteric CH_4 emissions from livestock, as the options for substituting one feed with another are very limited under the present scenario of feed shortages. Another approach for achieving this indispensable goal is the genetic improvement of forage quality through the latest and conventional breeding preferences. For plant breeders, genetic improvement in the quality and quantity of cereal and other crops directly used for human consumption is a top priority. They rarely consider the genetic improvement of fodder crops on their research radar, which is why the magnitude of the compositional changes in forage crops is very low all over the world. Through conventional breeding, genetic changes in composition can be made to affect the ratio of soluble to cell wall carbohydrates in biomass, and thus CH_4 emission. Benchaar *et al*. (2001) predicted that a reduction of 10–40% could be achieved by the breeding of forage crops. In many studies, it has been shown that the grazing of animals on high-quality pastures can lessen CH_4 emission by up to 50%, due to low fibre content. A potential decrease in enteric CH_4 emission through increased polyunsaturated fatty acid content of the forage crop may be another aim of the breeders. Genetic variation in polyunsaturated fatty acid content is minute (Dewhurst *et al*., 2001) as compared to variations due to growing season (Misselbrook *et al*., 2013). Therefore, the genetic improvement of local forage crops should be promoted to bring compositional changes towards low fibre content and improve the ratio of soluble to cell wall carbohydrates.

Low CH_4 emissions by animals may be a potential criterion for their selection in future, along with other productive traits. Presently, researchers are looking at whether livestock can be bred for low CH_4 emission without compromising the production level. The technologies for the implementation of selective breeding programmes are well established and provide a low-cost option for control. Nonetheless, within animal production there is currently little or no concerted research effort on long-term breeding strategies to mitigate GHG emissions from ruminants are explored (Misselbrook *et al*., 2013).

The production potential of animals is ultimately associated with feed conversion efficiency (FCE); animals with high FCE will eat less, with consequently less enteric CH_4 emission. A 40–45% reduction in CH_4 emissions is possible through the selection of individual animals by combining the CH_4 production potential with low residual feed intake (RFI; Misselbrook *et al*., 2013). It seems difficult to select animals directly on the basis of a low CH_4 emissions trait, as there is no selection index for low CH_4 heritability. In addition, measuring CH_4 emission directly from animals under field conditions is also practically difficult. However, the selection of animals for low CH_4 emission can be made by looking at traits that are correlated with high FCE. Hegarty *et al*. (2007) reported that feed conversion efficient animals produced 25% less CH_4 as compared to those that converted feed into product less efficiently. The question of the persistency of CH_4 emission differences over a long period has also yet to be resolved. Pinares-Patiño *et al*. (2003), however, reported a consistency in CH_4 emission between low and high emitters for up to 4 months. A difference of 8% in CH_4 emission (g kg^{-1} DMI) was reported in sheep after one generation of selection, as reported by Pinares-Patiño *et al*. (2013).

In one study, it was found that the progeny from low CH_4 emitting bulls produced 24% (29 versus 38 g) less CH_4 than that from the control group (NSW-DPI, 2010). For identifying and quantifying the association between CH_4 emissions and production traits, the trait must be heritable, with a reasonable amount of genetic variation (Pickering *et al*., 2013). Recent research has indicated that the CH_4 emission trait is heritable (Donoghue *et al*, 2013; Pinares-Patiño *et al*., 2013), repeatable and has shown sizeable genetic variation between animals, even after correcting the

emission for feed intake (Pinares-Patiño *et al*., 2013).

Factors like trait inheritance, the dispersal of the efficient animals in the population and robustness affect the selection of animals for low CH_4 emission on the basis of FCE. Further, divergence between the expected and actual CH_4 emissions due to environmental variation and the prediction of CH_4 emission from high- and low-efficiency animals on different feeding regimes are a few deterrents that need to be resolved on the basis of instant and focused research. Further, the selection of genetically superior bulls on the basis of a low CH_4 emission trait can affect the other desirable traits badly.

22.7 Paybacks from Enteric CH_4 Amelioration

Many scientific agencies have concluded that the average global temperature is 2°C/3.5ºF higher than pre-industrial threshold levels; however, keeping the temperature rise below threshold does not guarantee the avoidance of significant adverse impacts of climate change (Romm, 2009). But, if the temperature increases further, it will certainly have much more severe, widespread and irreversible impacts that could trigger large-scale catastrophic events. To avoid a temperature rise above this level, the atmospheric concentration of CO_2 should be stabilized at between the 400- and 450-ppm level. The IPCC (2007) summarized that an emissions reduction scenario would result in an estimated reduction in cumulative global GDP of about 3% by 2030, which is equivalent to a 0.12% global annual GDP reduction.

Annual GHG emissions grew by 1 Gt CO_2-eq at 2.2% year^{-1} from 2000 to 2010 as compared to 0.4 Gt CO_2-eq at 1.3% year^{-1} from 1970 to 2000, despite the numbers of emergent mitigation policies (IPCC, 2014). The total anthropogenic GHG emissions were the highest in human history from 2000 to 2010, and reached 49 Gt CO_2-eq year^{-1} in 2010 (IPCC, 2014). Among the GHGs, CO_2 remains the major anthropogenic GHG, accounting for 76% of anthropogenic emissions, while CH_4 is next, adding 16% (7.8 Gt CO_2-eq year^{-1}) to the anthropogenic pool of GHGs (IPCC, 2014). It is stated that livestock is the major source of anthropogenic CH_4 emissions (~90 Tg year^{-1}) worldwide. The reduction in enteric CH_4 emission is not only obligatory from a global warming point of view but is also required for improving the productive efficiency of animals, especially under the scenario of a feeds and fodders deficit that prevails in most developing countries. Reducing enteric CH_4 emissions will decrease the GHGs in the atmosphere and will also improve the efficiency of converting plant material into milk and meat (Beauchemin *et al*., 2008). Climate change is a collective action problem at the global scale, because most GHGs accumulate over time and mix globally, and emissions by any agent (e.g. individual, community, company, country) affect the others; hence, the effective mitigation of GHGs requires cooperation between continents, areas and communities (IPCC, 2014).

Discussing the advantages that emerge from reducing the emission of all anthropogenic GHGs is outside the limit of this chapter; the predictable reimbursements that arise from the mitigation of enteric CH_4 emission from livestock is discussed here in a hypothetical example (see Table 22.1). All the calculations used in Table 22.1 are theoretical and are based on certain assumptions. As most of the researchers/agencies have suggested that an enteric CH_4 reduction of up to 20% is practical from the unending fermentation point, the example furnished below therefore suggests the probable paybacks arising from a 20% reduction globally as well as locally (i.e. India). Table 22.1 depicts that a 20% reduction in enteric CH_4 emission can cut 450 Tg CO_2-eq GHGs year^{-1} from the anthropogenic pool, which seems a meagre quantity (~1%) when looking at the total emission of 49 Gt CO_2-eq year^{-1}. But the benefit that arises from this 20% reduction is substantial when looking at the additional milk production of ~210 million t on a global scale, or 23 million t in India alone, without

Table 22.1. Expected benefits from 20% reduction in enteric CH_4 emission – world and India.

Attributes	Values
World	
Enteric CH_4 emissions	90 Tg
Desirable reduction 20%	18 Tg
CO_2-eq	450 Tg
CH_4 emission after attaining the reduction level	72 Tg
Additional feeding (adult cattle)[a]	~60 million
Extra milk[b]	~210 MMt
India	
Enteric CH_4 emissions	10 Tg
Desirable reduction 20%	2 Tg
CO_2eq	50 Tg
Additional feeding (adult cattle)[c]	~7 million
Extra milk	~23 MMt

Notes: [a]maintenance requirement of 121 Kcal/kg$^{0.75}$ is taken into consideration for calculation purposes; [b]a requirement of 1.144 mcal for 1 kg milk was taken into consideration for calculation purposes; [c]400 kg body weight was used for calculation purposes for the adult cattle.
MMt = Million metric tonnes

any extra inputs and just by saving feed energy that is otherwise lost as CH_4. With the same feeding inputs, the energy requirement to maintain an additional 60 million adult cattle can be fulfilled through this saved energy, while in India, the saved energy is adequate to fulfil the maintenance requirement of an additional 7 million adult cattle. The extra milk production and feeding of additional adult cattle from a 20% CH_4 reduction looks very promising, especially in the developing world, where an acute shortage of feeds and fodders is a general phenomenon.

22.8 Conclusion

Livestock are significant global assets, with a value of approximately US$1.4 trillion, employing at least 1.3 billion people and directly supporting the livelihoods of 600 million poor smallholder farmers in the developing world. Livestock products provide 17% of the energy and 33% of the protein consumption on a global scale. Despite all these virtues from the livestock sector, the world's 3.6 billion ruminants emit 90 Tg CH_4 year^{-1}, because of rumen methanogenesis, an obligatory pathway for the safe disposal of H_2 from the rumen. This enteric CH_4 emission bequeaths almost 16% of the anthropogenic GHGs. Recently Pelletier and Tyedmers (2010) have projected that livestock production would emit nearly 40% more GHG by 2050. Therefore, livestock, being the significant contributors to the anthropogenic GHG pool, have remained the prime research priority for the past three decades, in order to find a suitable, effective and economic way of extenuating enteric CH_4 emission.

Reducing livestock numbers through killing/culling of non-/low-productive animals is the simplest way to reduce enteric CH_4 emission. This measure will undoubtedly cut emissions from livestock in a short time, but controlling emissions this way does not seem practical or realistic, as most livestock in the developing countries are, of course, low producing, but very well suited to harsh climatic conditions and can thrive and produce milk where exotic high-producing breeds usually fail to do so. Hence,

researchers have focused on other options, including feed-based interventions and biological control of rumen methanogens for the vested interest of CH_4 reduction from livestock. As CH_4 production in the rumen revolves around H_2 production and its clearance mechanism, therefore, any strategy that lays down a restriction on enteric H_2 production, or redirects it towards a desirable mechanism away from archaea, is helpful in controlling enteric CH_4 emission.

Among all the options, feed-based interventions are the best tool to tackle enteric CH_4 emission without any hardship to feed fermentability. Feeding of succulent green fodder and starchy concentrates (grain) is a good option for improving animal performance by cutting down emissions; however, there is direct competition between ruminants, non-ruminants and humans for these grains. The escalating prices of grain and other concentrates also pose a restriction on their use in large animal feeding. The use of a grain-based diet in developing countries does not seem practical, due to the economic status of the stakeholders and the low productivity of the livestock. The use of green and quality fodder for reducing emissions is undoubtedly practical and feasible, but again limited land availability under permanent fodder production and the seasonal rainfall are the major constraints in a regular supply of succulent fodder throughout the year.

In recent years, plant secondary metabolites have shown very promising results, but most studies have been conducted *in vitro*, and only a very few studies have been done in the animal system, which showed inconsistent results. On using secondary metabolites for CH_4 mitigation, many studies also recorded a decrease in feed fermentability. Therefore, prior to a recommendation for large scale use in animal diet, *in vivo* studies should be undertaken. Further, phyto secondary metabolite dosage also needs to be optimized for different categories of livestock. New phyto-sources possessing these secondary metabolites should also be explored for their use in animal diets.

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23 Methanotrophs in Enteric Methane Mitigation

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Abstract

In recent years, greater concern for environmental health, especially the mitigation of greenhouse gases, has been debated on various platforms. Methane (CH_4) is a potent greenhouse gas (GHG) and is produced globally by both biotic and anthropogenic activities. The most important recognized sources of CH_4 are natural wetlands (21%); fossil fuel related to natural gas, coal mines and the coal industry (16%); enteric fermentation (16%); paddy (11%); biomass burning (7%); landfills (7%); and animal waste (5%). Enteric fermentation in ruminants represents a major source of anthropogenic CH_4. Several CH_4 lowering strategies are being attempted by animal scientists across the globe to enhance livestock production vis-à-vis lower CH_4 production. Each strategy that is being attempted has its advantages as well as limitations. So it is imperative to look for a strategy that is viable and environmentally friendly too. CH_4-oxidizing bacteria, obligate and facultative, as well as anaerobic CH_4-oxidizing archaea are known to play a fundamental role in the carbon cycle by metabolizing CH_4 before it is released into the atmosphere. Therefore, CH_4 mitigation by employing methanotrophic microorganisms may be a viable and novel approach in controlling enteric CH_4 emissions in ruminants. The prospects of methanotrophs for eradicating enteric CH_4 emission are debated elaborately in the following chapter.

23.1 Introduction

Methane (CH_4) is a potent greenhouse gas (GHG), 23 times more potent than carbon dioxide, and is produced by both natural and anthropogenic sources. The major natural and anthropogenic sources of CH_4 include natural wetlands, paddy fields, raising of livestock (ruminants), termites, lakes and oceans, landfills, oil recovery operations and methane hydrates (Dalton, 2005). The most important sink for dissipating CH_4 is the lower atmosphere, where it is oxidized into carbon dioxide and water. But soils are also a significant sink, capturing approximately 10% of CH_4 emissions. Natural processes in soil and chemical reactions in the atmosphere help in removing CH_4 from the atmosphere. Methane's lifetime in the atmosphere is much shorter than carbon dioxide, but CH_4 is more efficient in trapping radiation than carbon dioxide (CO_2). CH_4 gas has a strong absorbance of infrared radiations, which are not able to escape from the earth's atmosphere, leading to global warming. The release of CH_4 to the atmosphere results in an increase in global warming and causes changes in the chemical composition of the atmosphere (Sonnemann and Grygalashvyly, 2014). It has been predicted that increased levels of CH_4 in the atmosphere will decrease

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hydroxyl (OH) radical concentration, and thus increase the lifetime of CH_4 in the atmosphere (Lelieveld *et al*., 1993) leading to decrease in the tropospheric ozone concentration.

As far as CH_4 production from ruminants is concerned, it has its origin from microbial fermentation in the rumen, and to a lesser extent from the lower digestive tract, referred to as enteric CH_4 emissions. CH_4 emissions from enteric fermentation results in feed energy loss (2–12%), and the extent of energy loss depends on the type of diet given to the animal (Johnson and Johnson, 1995). To enhance the utilization of this energy, which is otherwise a loss, several strategies have been attempted for lowering CH_4 emission. In recent years, CH_4 mitigation research has gained impetus, because of the greenhouse effects backed by CH_4 release in the atmosphere. Production of food of animal origin, i.e. milk, meat and eggs, has increased several fold globally, and this expansion has been witnessed more in the countries showing rapid economic development (Steinfeld *et al*., 2006). With an increasing global human population, the demand of livestock products is bound to increase manyfold, with a concomitant increase in livestock numbers. The increase in the ruminant population will, in turn, add to enteric CH_4 emission to the atmosphere. Therefore, suitable CH_4 mitigation strategies need to be explored to lower enteric CH_4 emission vis-à-vis increased livestock production.

Ruminant CH_4 mitigation strategies that are researched intensely and reported in the literature include the use of CH_4 inhibitors (bromochloromethane (BMC), 2-bromoethane sulfonate, chloroform and cyclodextrin), which have reduced CH_4 production by up to 50% *in vivo* (Lila *et al*., 2004; Lalu *et al*., 2009; Knight *et al*., 2011; Mitsumori *et al*., 2011), and electron receptors, i.e. fumarate, nitrates, sulfates and nitroethane (Gutierrez-Banuelos *et al*., 2007; Brown *et al*., 2011), reported to have reduced CH_4 production by up to 50% (Sar *et al*., 2004; Nolan *et al*., 2010; van Zijderveld *et al*., 2010, 2011a,b; Hulshof *et al*., 2012). Commonly used ionophores (monensin, for example) have been the most studied and are routinely used in meat (beef) production in developed countries. Ionophores in regard to CH_4 mitigation and livestock production are very well discussed in Chapter 17, Section III, this volume. The drawback in the use of some of the above-mentioned chemicals is that they are toxic to animals, cause microbial resistance, show ineffectiveness when used long term, and hence are banned in many developed countries.

Other widely explored CH_4 suppressants from natural sources are plant secondary metabolites, which are inherent and aid in the defence mechanism against insects and pests. This class includes a multiplicity of plant secondary compounds, specifically tannins, saponins and essential oils and their active ingredients. Tannins and saponins have been widely studied in many species of livestock and have shown the most mitigating potential. Leaves of jatropha (*Jatropha curcas* L.), khejri (*Prosopis cineraria*), pala (*Ziziphus nummularia*), ardu (*Ailanthus excelsa*) and neem (*Azadirachata indica*), rich in plant secondary metabolites, lowered CH_4 production to a significant extent when incubated with different levels of *Cenchrus ciliaris in vitro* (Sahoo and Soren, 2011). Use of essential oils and their active ingredients in enteric CH_4 mitigation has been reported, mostly from *in vitro* experiments (Bodas *et al*., 2008; Calsamiglia *et al*., 2008; Benchaar *et al*., 2009; Soren *et al*., 2010, 2011). The inclusion of spice seed residual material, namely coriander (*Coriandrum sativum* L.), fenugreek (*Trigonella foenum-graecum* L.), sowa (*Anethum sowa* Roxb.), fennel (*Foeniculum vulgare* Mill.) and ajowin (*Trachyspermum ammi* L.) at a graded level in sewan (*Lasiurus sindicus*) and *C. ciliaris* grass-based diets lowered CH_4 production *in vitro*, and this reduction was attributed possibly to the presence of essential oils (Soren *et al*., 2010, 2011; Soren and Sahoo, 2011). Very few *in vivo* experiments for testing the efficacy of plant secondary metabolites and essential oils have been piloted (Tekippe *et al*., 2011; Soren, 2012, personal communication; Hristov *et al*.,

2013). The uses of exogenous enzymes (xylanase, β-glucosidase, etc.) in the diet of ruminants are reported to increase feed efficiency and lower enteric CH_4 emission. Defaunation (removal of protozoa) from the rumen is often linked with an increased microbial protein supply and improvement in animal productivity (Patra, 2012). Many methanogens remain attached to the exterior surface of rumen ciliate protozoa as endosymbionts; thus, they are responsible for up to 37% of rumen methanogenesis (Tokura *et al*., 1997).

All the accessible CH_4 mitigation strategies to date have their own advantages, as well as disadvantages. Therefore, a suitable strategy needs to be explored that may prove to be animal and environment friendly and sustainable, and have a long-lasting impact. Among the novel strategies that are currently being explored, the use of microorganisms that are able to oxidize CH_4 is one of them. Some bacterial species have been reported to have a methane-oxidizing capability. These bacteria may be obligate, or facultative, anaerobes. Therefore, CH_4 mitigation by employing methanotroph microorganisms that are present in the rumen may be a viable and novel approach in controlling enteric CH_4 emissions in ruminants. A concerted effort has been made in this chapter to highlight different approaches that are currently being employed for lowering enteric CH_4, a novel approach using methane-oxidizing bacteria, or methanotrophs, and their taxonomy, physiology and marker genes for studying their diversity, etc.

23.2 Enteric CH_4 Emission

The digestive system of ruminant animals are unique, since they possess a four-chambered stomach. The largest part of the stomach, called the rumen, serves as a fermentation vessel, wherein poor-quality fibrous feeds (unusable plant materials) are broken down into nutrients required by the host animal. In the process, the same helpful digestive system, however, generates CH_4 as a wasteful by-product, which is a potent GHG that contributes immensely to global warming and climate change. The fermentation of carbohydrates in an anaerobic environment (the rumen) results in the production of hydrogen. Methanogenic archaea residing in the rumen utilize this excess hydrogen and dispose of it through the reduction of CO_2 into CH_4. Therefore, methanogenesis often uses the hydrogen and carbon dioxide that are produced as by-products in carbohydrate fermentation to volatile fatty acids. As methanogens remove excess hydrogen from the ruminal environment (the terminal step of carbohydrate fermentation), this therefore helps the other microorganisms involved in fermentation to function optimally and sustain the complete oxidation of substrates (Sharp *et al*., 1998). If hydrogen is not removed from the rumen environment, then it can affect the metabolism of the other rumen microorganisms.

About 89% of CH_4 in ruminants is produced from the rumen and exhaled through the mouth and nose (Murray *et al*., 1976); however, non-ruminants produce comparatively negligible enteric CH_4 from the lower gastrointestinal tract. In general, about 8–12% of dietary energy is lost in the form of CH_4 in ruminants (Blaxter, 1967). CH_4 production in ruminants depends on so many factors, such as the quality and quantity of feed, the production status of the animal and the quality and digestibility of the pasture. Ruminants can thrive on relatively low-quality forages and crop residues. Therefore, reduced intake coupled with low digestibility of these feed resources contributes substantially to lower productivity, with the emission of a sizeable quantity of CH_4. All these factors are debated critically in Chapter 22, Section III, this volume.

The rumen is a complex and diverse system and mostly harbours obligate anaerobic microbes, including methanogens archaea. Methanogens harbouring in the rumen belong to a separate domain archaea, and are also found in a wide range of anaerobic environments (Liu and Whitman, 2008). Most rumen methanogens derive their energy for growth through a series of

biochemical reductions of CO_2 with H_2, and some methanogens use acetate and methyl group containing compounds to produce CH_4. By scavenging hydrogen gas, methanogens play a key ecological role in keeping the partial pressure of hydrogen low so that fermentation can proceed efficiently. Thermodynamics and the kinetic control of methanogenesis and other metabolically important pathways have been well argued previously (also see Chapter 16, Section III, this volume). An illustration of the various activities involved in CH_4 production (methanogenesis) and the possible whereabouts for interventions are depicted in Fig. 23.1.

Methanogens differ from bacteria as they lack peptidoglycan in their cell wall: this is replaced with pseudomurein in *Methanobrevibacter* and *Methanobacterium*, heteropolysaccharide in *Methanosarcina* and protein in *Methanomicrobium* (Hook *et al*., 2010). All methanogens have coenzyme F_{420}, which is a cofactor necessary for enzymes such as hydrogenase and formate dehydrogenase. Another characteristic of methanogens is coenzyme M, which is either produced by methanogens such as *Methanobacterium* or is required from an external source, as in the case of *Methanobrevibacter ruminantium* (Rouvière and Wolfe, 1988). Coenzyme M, or 2-mercaptoethanesulfonic acid, is methylated to produce CH_4 (Hobson and Stewart, 1997). Although about 70 methanogenic species belonging to 21 genera have been identified from anaerobic environments, and a range of different methanogens coexist in the rumen (Jarvis *et al*., 2000), to date only seven ruminal species have been isolated and purified. These are *Methanobacterium formicicum*, *Methanobacterium bryantii*, *M. ruminantium*, *Methanobrevibacter millerae*, *Methanobrevibacter olleyae*, *Methanomicrobium mobile* and *Methanoculleus olentangyi*. The population density of methanogens in the rumen is influenced by diet, and more precisely, the fibre content of the diet (Kirchgessner *et al*., 1995).

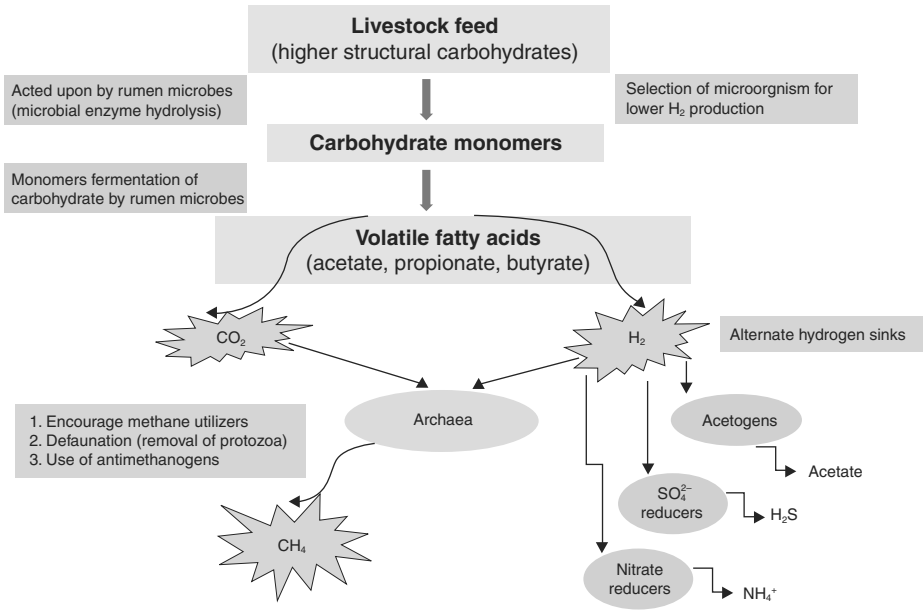

Fig. 23.1. Schematic outline of steps involved in CH_4 formation in the rumen and probable intervention sites for lowering CH_4 emissions.

23.3 Mitigation Strategies

As CH_4 is formed as a by-product of enteric fermentation in the reticulorumen, there is, hence, every possibility that other microorganisms may also regulate and alter CH_4 production (Morgavi *et al*., 2010). Existing CH_4-alleviating strategies encompass increasing feed intake, feeding of concentrates, feeding of high-quality forages, secondary metabolites (tannins, saponins, essential oils), dietary oils (coconut, linseed, palm, soy) and ionophores. Recent research has focused on the potential of novel feed ingredients (probiotics, acetogens, bacteriocins, archaeal viruses, organic acids and plant extracts), vaccination of the host animal against some methanogenic bacteria and the selection of cows with inherently lower losses of CH_4 (Boadi *et al*., 2004). The different mitigation strategies stated above have been dealt with in detail elsewhere in the book.

23.4 Methanotrophs

Methane-oxidizing bacteria, or methanotrophs, are bacteria that are able to metabolize CH_4 (Mancinelli, 1995) and do not require oxygen for their growth. These organisms are generally found in soils like swamps, rice fields, etc., where the concentration of CH_4 is high. Wetlands are by far the biggest source of CH_4 production (34%), followed by coastal water (25%); ruminants contribute to about 14% of CH_4 (Rodhe and Svensson, 1995). CH_4 is oxidized by methanotrophs under either aerobic or anaerobic condition. Under aerobic conditions, methanotrophs oxidize methane to formaldehyde, which is then incorporated into organic compounds via the serine pathway or the ribulose monophosphate (RuMP) pathway. On the other hand, anaerobic methanotrophs oxidize CH_4 under anaerobic conditions. Anaerobic oxidation of methane (AoM) is a microbial process that occurs mostly in anoxic marine and freshwater sediments. During AoM, CH_4 is oxidized with different terminal electron acceptors such as sulfate, nitrate, nitrite and metals.

Aerobic methanotrophic bacteria are divided into two taxonomic groups, type I and II, depending on their cell morphology, metabolism and phylogeny. Type I methanotrophs can survive in environments with limited CH_4, and type II methanotrophs require high levels of CH_4 (Hanson *et al*., 1991) for their survival. Type I methanotrophs belong to the class Gammaproteobacteria, while type II methanotrophs belong to the class Alphaproteobacteria. Type I include the genera *Methylobacter*, *Methylomicrobium*, *Methylomonas*, *Methylocaldum*, *Methylosphaera*, *Methylothermus*, *Methylosarcina*, *Methylohalobius*, *Methylosoma* and *Methylococcus*. Type II methanotrophs include the genera *Methylocystis*, *Methylosinus*, *Methylocella* and *Methylocapsa* (McDonald *et al*., 2008). Other than the above two groups of methanotrophs, there are some reports of methanotrophic strains that can use multi-carbon compounds for their growth (Whittenbury *et al*., 1970). These methanotrophs were reported to show enhanced growth on culture media containing methane, along with malate, acetate or succinate. Such findings suggested the existence of facultative methanotrophic strains that could utilize both the multicarbon compounds as well as CH_4 as a sole growth substrate (Semrau *et al*., 2011). Some of the facultative methanotrophs like *Methylocella silvestris*, *Methylocella palustris*, *Methylocella tundrae*, *Methylocapsa aurea*, *Methylocystis* strain H2s and *Methylocystis* strain SB2 have been studied extensively for their ability to utilize wide substrates other than CH_4 (Dedysh *et al*., 2000, 2004, 2005; Dunfield *et al*., 2003, 2010; Belova *et al*., 2011; Im *et al*., 2011).

There are scant reports on methanotrophs in curtailing enteric CH_4 in different ruminant species. Although methanotrophs (*Proteobacteria*) have been isolated from a wide range of environments, including the rumen, there has been little investigation on their physiology and molecular evidence of their role in methanotrophy in the rumen (Mitsumori *et al*., 2002). To date, there is little evidence to suggest that methanotrophy is important in the rumen, and Kajikawa *et al*. (2003) has reported that methanotrophy

accounts for only 0.2–0.5% of rumen fluid. More recently, Jha *et al*. (2013) has explored methanotrophs harbouring in the rumen of buffalo by amplifying the partial sequence of 16S rRNA gene from the genomic DNA in the rumen contents by using type I and type II methanotroph-specific primer sets. The sequence of type I methanotrophs had 83% similarity with *Methylophaga thiooxydans*, 81% with *Methylobacter tundripaludum* SV96 and 77% with *Methylococcus capsulatus* strain in the study of Jha *et al*. (2013). On the other hand, the sequence of type II methanotrophs showed 83% sequence similarity with *Methylocystis* sp. ATCC 49242, *Methylosinus trichosporium* OB3b, *Methylocella silvestris* BL2 and *Methylobacterium nodulans* ORS.

23.4.1 Taxonomy and physiology

Methanotrophic bacteria are ecologically important, because they form a vital link in the global carbon cycle, act as nitrogen fixers and ammonia oxidizers, degrade a wide group of organic contaminants and have biotechnological potential for single-cell protein production and novel enzyme functions. Therefore, more insight into their taxonomical features and physiology will help provide us with a better understanding. As far as methanotrophs are concerned, they are physiologically and phylogenetically unique.

The isolation of the first methanotrophs dates back to 1906 (Söhngen, 1906), but it was not until 1970 that Whittenbury *et al*. isolated and characterized over 100 new methane-utilizing bacteria (Whittenbury *et al*., 1970). This formed the basis for the current classification of these bacteria. Initially, Whittenbury and co-workers classified methane-utilizing bacteria into five groups, based on morphological differences, types of resting stages formed, the fine structures of intracytoplasmic membranes and some physiological characteristics (Whittenbury *et al*., 1970; Whittenbury, 1981; Whittenbury and Dalton, 1981; Whittenbury and Krieg, 1984). The genera proposed by them initially were *Methylomonas*, *Methylobacter*, *Methylococcus*, *Methylocystis* and *Methylosinus*, similar to those currently accepted, except for the addition of one new genus, *Methylomicrobium* (Bowman *et al*., 1993, 1995).

Methane-oxidizing bacteria are classed into types I, II and X, depending on the guanine and cytosine content of their DNA, intracellular membrane arrangement, carbon assimilation pathway and phospholipid fatty acid (PLFA) composition (Bull *et al*., 2000). The major dissimilarity between the two types of methanotrophs is the pathway through which formaldehyde is incorporated into cell biomass. Type I methanotrophs assimilate biomass via the ribulose monophosphate (RuMP) pathway, while type II methanotrophs use the serine pathway for an identical process. Hanson and co-workers classified methanotrophs into three distinct types: type I methanotrophs include the genera *Methylomonas* and *Methylobacter*, while type II include the genera *Methylosinus* and *Methylocystis* (Hanson and Hanson, 1996); type X methanotrophs were included to accommodate methanotrophs related to *M. capsulatus*, which utilize ribulose monophosphate (RuMP) as the primary pathway for formaldehyde assimilation, similar to type I methanotrophs. Type X methanotrophs are distinguished from type I methanotrophs, because they also possess low levels of enzymes of the serine pathway ribulose biphosphate carboxylase, an enzyme that is required in the Calvin–Benson cycle (Whittenbury, 1981; Whittenbury and Dalton, 1981; Whittenbury and Krieg, 1984). The main characteristic features of type I, type II and type X methanotrophs are summarized in Table 23.1.

From phylogenetic studies based on 16S rRNA gene sequences using MEGA4, Tamura *et al*. (2007) reported that type I and II methanotrophs resemble γ- and α-proteobacteria, respectively. From their studies, they report that type I methanotrophs include genera *Methylobacter*, *Methylomicrobium*, *Methylomonas*, *Methylocaldum*, *Methylococcus*, *Methylosoma*,

Table 23.1. Characteristics of different types of methanotrophs. (Modified from Hanson and Hanson, 1996.)

Characteristics	Type I	Type II	Type X
Cell morphology	Short rods, occurs singly, some cocci or ellipsoids	Crescent-shaped rods, rods, pear-shaped cells, sometimes occur in rosettes	Cocci, often found as pairs
Growth at 45°C	×	×	✓
Membrane arrangement			
Bundles of vesicular discs	✓	×	✓
Paired membranes aligned to periphery of cells	×	✓	×
Nitrogen fixation	×	✓	✓
Exospores	×	×/✓	×
Cysts	×/✓	×/✓	×/✓
Primary pathway for utilizing oxidized product	RuMP	Serine path	RuMP, ribulose 1, 5 biphosphate carboxylase or sometimes Serine path
Major PLFAs	14:0, 16:1ω7c, 16:1ω5t,	18:1 ω8c	16:0, 1:1ω7c
Proteobacterial subdivision	ϒ	α	ϒ
Phylogenetic signature probe(s) available	✓	✓	×

Notes: PLFAs = phospholipid fatty acids; RuMP = ribulose monophosphate.

Methylosarcina, *Methylothermus*, *Crenothrix*, *Clonothrix* and *Methylosphaera*, and type II methanotrophs include genera *Methylocapsa*, *Methylocella*, *Methylosinus* and *Methylocystis*. Lately, the same workers have isolated acidophilic methanotrophs that are not classified into either α- or γ-proteobacteria, but are phylogenetically located under Verrucomicrobia phylum. Even though these bacteria are not fully characterized, they have striking dissimilarities to other methanotrophic bacteria (Dunfield, 2007; Hou *et al*., 2008).

Methanotrophs metabolize methane uniquely, and it is imperative to understand the factors that are involved in this metabolism and the ecology of methane-oxidizing bacteria. Therefore, an insight into the physiological aspect of methanotrophs will throw more light on to the processes involved. The first step involved in bacterial methane oxidation is mediated by methane monooxygenase (MMO), which occurs in two forms: a membrane-bound or particulate (pMMO) and a cytoplasmic or soluble (sMMO) monooxygenase. One of the oxygen atoms is reduced to form H_2O, and the other is incorporated into CH_4 to form CH_3OH. All the identified methanotrophs, except the *Methylocella* species, possess pMMO that consists of three membrane-associated polypeptides encoded by *pmoC*, *pmoA* and *pmoB* (McDonald and Murrell, 1997b; Murrell *et al*., 2000; Pacheco-Oliver *et al*., 2002). In addition to pMMO, most type II (*Methylosinus*, *Methylocystis*) and some type I methanotrophs (*Methylomonas*, *Methylomicrobium*), as well as *M. capsulatus* (type X), possess sMMO. The sMMO utilizes reduced nicotinamide adenine dinucleotide ($NADH+H^+$) as an electron donor and remains soluble after centrifugation of cell extracts at 15,000× g for 75 min (Dalton, 1992; Lipscomb, 1994).

In the second step, oxidation of formaldehyde takes place, which results in the formation of carbon dioxide via format. There are multiple enzyme systems for the oxidation of formaldehyde to formate in methylotrophs (Anthony, 1991; Dijkhuizen

et al., 1992). These include NAD(P)-linked aldehyde dehydrogenases that may or may not require reduced glutathione or other cofactors, and dye-linked dehydrogenases (Dijkhuizen *et al*., 1992). Formate is oxidized to carbon dioxide by NAD-dependent formate dehydrogenase in most methanotrophs (Anthony, 1991; Dijkhuizen *et al*., 1992). A cyclic pathway for the oxidation of formaldehyde to carbon dioxide has been known to exist in methylotrophs that possess the RuMP pathway for formaldehyde assimilation (Anthony, 1991). In this pathway, formaldehyde and ribulose-5-phosphate react to form hexulose-6-phosphate, which is isomerized to fructose-6-phosphate, and this substrate is converted to glucose-6-phosphate, which is in turn oxidized to 6-phosphogluconate. The 6-phosphogluconate is then oxidized to produce carbon dioxide and ribulose-6-phosphate to complete the cyclic pathway for formaldehyde oxidation. The electron acceptors for the two oxidations in the cycle are NAD1 or NADP1. The majority of the obligate methanotrophs make use of the linear pathway for formaldehyde oxidation, while many non-methane-utilizing methylotrophs employ the cyclic pathway as the major route (Anthony, 1991; Dijkhuizen *et al*., 1992).

The α-proteobacterial methanotrophs (type II methanotrophs) utilize the serine cycle for formaldehyde assimilation (see Fig. 23.2). In this cycle, methane is converted to formate, which is converted to methylene tetrahydrofolate (H_4F) via a standard H_4F pathway. Methylene H_4F condenses with glycine to generate serine. The remainder of the cycle is involved in the regeneration of the glycine acceptor. The equation for generating one phosphoglycerate molecule is as follows:

$$1.5\ CO_2 + 1.5\ HCHO + 1.5\ NADH + 1.5\ ATP + 0.67\ FAD \Longrightarrow 3\text{-phosphoglycerate} + 1.5\ NAD + 1.5\ ADP + 0.5\ Pi + 0.67\ FADH$$

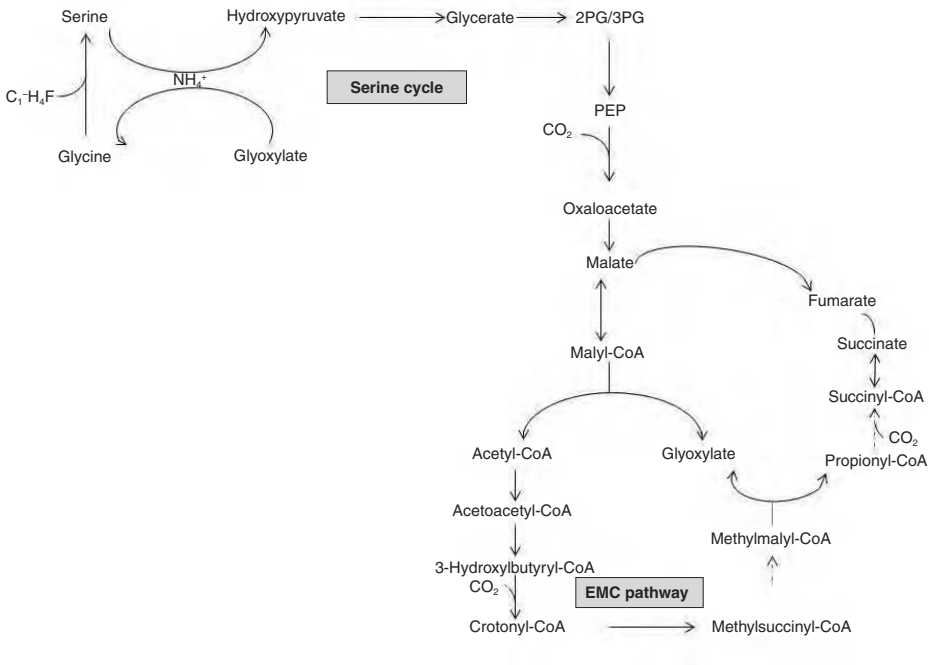

Fig. 23.2. Serine cycle for assimilation of formaldehyde in type II methanotrophs.

The γ-proteobacterial methanotrophs (type I and type X) use the ribulose monophosphate cycle (see Fig. 23.3). Formaldehyde is condensed with ribulose monophosphate to form a hexulose phosphate, which is further converted to fructose-6-phosphate. Two routes are likely to convert fructose-6-phosphate to pyruvate, the Entner–Douderoff (EDD) pathway and the Embden-Meyerhof-Parnas (EMP) pathway (glycolysis) in type I and type X methanotrophs. Although enzyme activities suggested that the EDD pathway was the course that occurred in methanotrophs, it has recently been shown by ^{13}C-labelling in a γ-proteobacterial *Methylomicrobium* strain that the dominant pathway for generating pyruvate is the EMP pathway (Kalyuzhnaya *et al*., 2013). In the latter case, the reaction for generating a 3PGA is:

3 HCHO + NAD + 0.67 ADP + 5/3 Pi ==> PGA + NADH + 0.67 ATP

The methanotrophs belonging to Verrucomicrobia phyllum utilize the classic Calvin–Benson–Bassham cycle and oxidize methane to CO_2 for energy by incorporating CO_2 into cell material by this cycle (Khadem *et al*., 2011). Even though some methanotrophs like *M. capsulatus* have genes for all the three assimilatory pathways, evidence implies that only the RuMP pathway plays a major role in carbon assimilation (Stanley and Dalton, 1982).

23.5 Marker Genes for Methanotrophs

There is an increased interest to explore the diversity of methanotrophs, as they play a vital role in neutralizing environmental CH_4. Aerobic methanotrophs have been studied extensively for quite some time. As far as anaerobic methanotrophs are concerned, fewer reports are available. For diversity studies, 16S rRNA, 18S rRNA or internal transcribed spacer (ITS) regions are targeted by primers, since these genes are present in all organisms, they have distinct regions for taxonomic classification that are not subject to horizontal transfer and have sequence databases available to researchers. The 16S rRNA gene is a commonly used phylogenetic marker for studying the microbial ecology and diversity in the environment. The sequencing of functional genes that are unique to the physiology of the studied group of microorganisms has generally been done.

The enzyme unique to methanotrophs is methane monooxygenase. The *pmoA* and *mmoX* genes encode a subunit of the pMMO and the sMMO, respectively, and these subunits are the most frequent targets of researchers studying methanotrophic diversity (Dumont and Murrell, 2005; McDonald *et al*., 2008). As the pMMO is present in nearly all methanotrophs, the current *pmoA* sequence database available is larger than the *mmoX* sequence database.

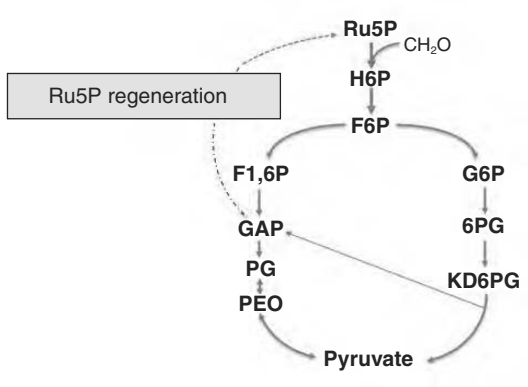

Fig. 23.3. Ribulose monophosphate cycle in type I and type X methanotrophs.

The pMMO gene cluster in type I and type II methanotrophs includes three open reading frames (ORFs) arranged as pmoCAB, with a putative transcriptional start upstream of the *pmoC* gene (Semrau *et al*., 1995; Stolyar *et al*., 1999; Gilbert *et al*., 2000). Two identical copies of pmoCAB have been found in these organisms. Conversely, several type II methanotrophs are known to have a supplementary but different copy of *pmoA*, which is referred as *pmoA-2* (Dunfield *et al*., 2002; Tchawa Yimga *et al*., 2003). The enzyme from the strains belonging to *Methylosinus*, *Methylocystis* and *Methylococcus* has been studied meticulously, and the nucleotide sequence of the sMMO gene cluster *mmoX*, *mmoY*, *mmoB*, *mmoZ*, *mmoC* and *mmoD* appears to be highly conserved in these strains. The *pmoA* gene encoding a 26 kDa subunit that harbours the active site of the pMMO and *mmoX* gene coding for a subunit of the sMMO hydroxylase component can be used as appropriate gene markers for the presence of the enzymes in various methanotrophs.

Methanol dehydrogenase (MDH), the second enzyme involved in methane oxidation, is present in all gram-negative methylotrophs including methane and methanol utilizers, and *mxaF* is an appropriate indicator gene for their occurrence in the natural environment (McDonald and Murrell, 1997a). All the proteobacterial subdivision, namely type I, type II and type X, methanotrophs possess distinct patterns of phospholipid ester-linked fatty acids (PLFAs) that differentiate them from each other. The type I methanotrophs contain mainly 14C and 16C PLFAs, whereas the type II contain mainly 18C PLFAs. This difference can be practical for distinguishing methane consumption by either type I or type II species using isotope labelling. In addition, methanotrophs also possess signature fatty acids that are not found in any other known microorganism (type I: C16:1ω8c and C16:1ω5t versus type II: C18:1ω8c) and are therefore especially valuable biomarkers (Bodelier *et al*., 2009).

23.6 Methanotrophs in Enteric CH_4 Mitigation

Methanotrophs are ubiquitous in the environment and play a major role in the global carbon cycle and scavenging of environmental CH_4. Thus, they play a significant role in the mitigation of GHGs and global warming. Methanotrophy in the rumen has been little studied and has been reported to account for <0.5% of rumen CH_4 production *in vitro* (Kajikawa *et al*., 2003). Methanotrophs are also capable of using CH_4, and therefore prevent its release into the atmosphere. Understanding the pathways involved in the metabolism of these compounds may provide a new insight into the biological control of enteric CH_4 emission. The work of Kajikawa *et al*. (2003) has demonstrated that oxidation of CH_4 in the rumen is anaerobic and is associated with sulfate reduction. Mitsumori and co-workers (2002) identified bacterial clones closely associated with *Nitrosomonas* spp. from a clone library constructed from samples of bacterial communities attached to the rumen epithelium. The members of the genus *Nitrosomonas* are ammonia-oxidizing bacteria, and they have the capability to oxidize CH_4 under some conditions (Hyman and Wood, 1983; Jiang and Bakken, 1999). There is always a presence of ammonia near the rumen wall because the excess urea coming from the blood of the ruminant is acted upon by the ureolytic bacteria attached to the rumen wall (Cheng and Wallace, 1979). Therefore, there is a possibility that *Nitrosomonas* spp. in the rumen wall may also be involved in CH_4 oxidation.

The metabolic pathways involved in enteric methane oxidation by methanotrophs have not been elucidated to date. The ultimate end product of methane oxidation is CO_2, which does not supply any energy, and therefore the nitrogen and energy contained in the biomass of methanotrophs would be negligible. Consequently, in the rumen environment, the presence of microorganisms capable of utilizing excess CO_2 can aid its removal. Some bacterial

species such as *Prevotella*, *Fibrobacter*, *Ruminococcus*, *Lachnospira* and *Succinomonas* require CO_2 for their growth (Dehority, 1971). Capnophilic bacteria (bacteria that can utilize CO_2) also use H_2 to produce organic acids, mainly succinic acid, as the final products, but the influence of this metabolic conversion on H_2 balance is unknown. A rumen bacterium, *Mannheimia succiniciproducens*, whose genome has been sequenced, produces succinic acid as the main metabolic product and consumes both CO_2 and H_2 in the process (Hong *et al*., 2004). This, and other similar bacteria, may have an effect on the net H_2 and CH_4 balance if their numbers and activity are sufficient in the rumen.

Though methanotrophy is very low in the rumen, there are some studies that have suggested that some members of bacteria belonging to the phylum Verrucomicrobia (Romero-Perez *et al*., 2011; Godoy-Vitorino *et al*., 2012) may be involved in the oxidation of methane. Although their role in the rumen is not well understood, some members of the Verrucomicrobia have been found to oxidize CH_4 as the sole source of carbon and energy in non-rumen environments (Hou *et al*., 2008). There is no concrete proof as to whether or not Verrucomicrobia are capable of this function in the rumen. Furthermore, Klieve *et al*. (2012) recognized clones related to CH_4-oxidizing archaea in the rumen of cows. Methane-oxidizing archaea have been ascribed to play an important role in aquatic ecosystems (Hallam *et al*., 2003; Knittel *et al*., 2005). But it is not clear how far the methane-oxidizing archaea will be able to withstand rumen conditions that have a nutrient-rich environment and a high turnover rate, and this needs to be assessed.

More recently, Jha *et al*. (2013) has reported the presence of methanotrophs from the rumen of buffalo by amplifying the partial sequence of 16S rRNA gene from the genomic DNA in the rumen contents by using type I and type II methanotroph-specific primer sets. In the study, they observed both type I and type II methanotrophs. The sequence of type I methanotrophs in their study had 83% similarity with *M. thiooxydans*, 81% with *M. tundripaludum* SV96 and 77% with *M. capsulatus* strain. On the other hand, the sequence of type II methanotrophs showed 83% sequence similarity with *Methylocystis* sp. ATCC 49242, *M. trichosporium* OB3b, *M. silvestris* BL2 and *M. nodulans* ORS. Novel methanotrophs from buffalo rumen were identified in the molecular study of Jha *et al*. (2013).

Work related to enteric CH_4 mitigation by methanotrophs is being carried out by Australian researchers wherein methanotrophs in natural ecosystems are being studied extensively and their role in ruminant CH_4 mitigation is being explored. Efforts have been made to fabricate fermentation apparatus to simulate the rumen environment to see if the methanotrophs available are effective at reducing CH_4 emissions in a rumen-like environment, and also to monitor the population dynamics of both methanotrophs and methanogens using specifically designed real-time polymerase chain reaction assays (Finn *et al*., 2012).

23.7 Conclusion

Anthropogenic activities contribute immensely to the production of CH_4 and its subsequent release into the atmosphere. Enteric CH_4 production is inherent in ruminants, and several mitigation strategies exist. Each has its own benefit and limitation. Methane-oxidizing bacteria, or methanotrophs, play an important role in neutralizing global CH_4 release to the atmosphere by their unique ability to utilize methane for their requirements. Few reports have revealed the presence of methanotrophs in the rumen and therefore exploration of the diversity of and associations with methanogens will help us to understand their role in CH_4 scavenging. Therefore, CH_4 mitigation by employing methanotrophic microorganisms may be a viable and novel approach.

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24 Summary

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Abstract

This chapter summarizes the full content of this book where contributors have addressed various aspects of livestock production vis-à-vis climate change. Improving livestock production and productivity is the need of the hour to accomplish the ever increasing demand of the populace. Climate change appears to be a major constraint in this endeavour as it severely affects livestock directly or indirectly. Further, livestock itself is accountable for the climatic variations and negative environmental complications of climate change by dispensing large quantities of greenhouse gases into the atmosphere. The implications of climate change on livestock, the involvement of livestock in climate change, mitigation approaches and adaptation strategies for minimizing the adverse impact and reducing enteric methane (CH_4) emissions debated in the various chapters of this book are summarized here.

24.1 Livestock Production

Livestock is an integral component of agriculture that directly or indirectly serves society by providing food, value-added products, fuel and transport, improving crop production and generating incomes, livelihoods, etc. By 2050, the worldwide bovine and ovine population is projected to be 2.6 billion and 2.7 billion, respectively. This distribution of livestock is not uniform across continents; one-quarter of the cattle population will be dispersed in Brazil and India, whereas 56% of the world's buffalo will be concentrated in India alone. Since 1983, the bovine and ovine population has increased by 13 and 22%, respectively. The overall demand for animal food products is also increasing steadily, and by 2050 it is expected to increase by 30, 60 and 80% for milk, meat and eggs, respectively. However, the aggregate demand for livestock products is projected to be 70% higher than it was in 1990. Thus, it is clear from the above figures that the requirement for livestock products will increase considerably in times to come, and this sector will play a pivotal role in satisfying the hunger of the populace. As livestock production has already peaked and currently almost stagnated, this increasing demand must, hence, come primarily from the developing world, which still has much unexplored potential.

Livestock production is facing multi-dimensional challenges across the globe; however, its intensity varies from one region to another, depending on resource availability and management practices. Changing climate is now emerging as a primary concern for livestock production and poses the greatest threat to diversity and survival. It is also creating a barrier to productivity enhancement, which is a prerequisite for meeting the nutrient requirement of humans in the future. Animal production systems and climate

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change are inter-mixed through complex mechanisms. The threat of climate change to the livestock sector is ubiquitous. However, the intensity of the impact is stratified and depends on many factors. Both livestock production and climate change are interconnected in such a way that alteration in one exerts a significant impact on the other. Climatic variations influence livestock production by altering the surrounding environments that govern the well-being and prolificacy of the livestock, affecting crop biomass quality and quantity, animal health, etc. On the contrary, livestock production also has large impact on climate change through the emission of large quantities of greenhouse gases (GHGs) associated with livestock rearing and excreta. Enteric methane (CH_4) emission from the livestock sector is a major element that contributes to global warming and depletion of the ozone layer. Enteric CH_4 emission from livestock is now widely debated on all national and international platforms, not only because of their global warming and ozone depletion potential but also due to the consequential loss of biological energy in the form of CH_4. Although the extent of energy loss varies in accordance with diet composition, it is still meaningful, as worldwide, a large livestock population can be fed and more production can be achieved with the biological energy saved from enteric CH_4 reduction.

The impact of climate change on animal comfort, productivity, availability and quality of feed resources, livestock biodiversity and reproduction is debated in different chapters of this book and summarized in subsequent sections. In addition, the contribution of livestock production to climate change has delineated issues such as the carbon footprint of producing animal food products, the impact of meat production and enteric CH_4 and nitrous oxide (N_2O) emissions. Other forms of nitrogen emissions and phosphorus pollution instigated by livestock are also addressed. Mitigation/adaptation strategies for minimizing the impact of abiotic stress and reducing enteric CH_4 emission are addressed in detail. The role of indigenous livestock and carbon sequestration through silvopastoral farming, which are very pertinent in the current climatic changing scenario, are discussed. Ruminal fibre degradation is not completely understood. The emerging technologies that may prove helpful in understanding its mechanism and microbial profile are discussed in Chapters 5 and 6.

Feed production is a key component in livestock production, not because it is the key resource that fuels animal productivity but it is also the main link between livestock, land and several regulating and provisioning ecosystem services. More animal feed is required for all domestic livestock and farmed aquatic animals to attain the extra production needed to fulfil the increasing demand of the population in the future. Competition for feed among livestock and fish will increase, in addition to competition with human food production and biomass needs for biofuels. The most evident and important effects of climate change on livestock production will be mediated through changes in feed resources. The main pathways in which climate change can affect the availability of feed resources for livestock include land use and systems changes, changes in the primary productivity of crops, forages and rangelands, changes in species composition and changes in the quality of plant material – are summarized in Chapter 2. This chapter also addresses the impacts of climate change on biomass productivity, the composition of pasture species and change in the chemical composition of feed resources. Worldwide current feed demand and its use, regional estimates of feed consumption and projections about the availability of feed resources by 2030 are also made in the chapter. It explains the impacts of climate change on livestock genetics and breeding, health; mitigation and adaptation options in context of feed–livestock–livelihood–climate change are also addressed in chapter 2.

Meeting the demand for animal products in future will be very difficult under the negative implications of environmental change, where abiotic stress is noteworthy.

Heat is the most stressful among all the abiotic stressors for livestock. An animal's ability to adapt to climatic variations is determined by its ability to maintain performance and oxidative metabolism. Given the high genetic variability between and within breeds, it is beneficial to select heat-tolerant breeds. The major challenge here is the complex nature of abiotic stress tolerance traits and the difficulty in dissecting them into manageable genetic components. Chapter 3 illustrates the different adaptive mechanisms in livestock for maintaining homeostasis and also explains the different approaches for alleviation. The authors have highlighted that a deep understanding of the biology of stress response components is a prerequisite for predicting the intensity of stress and for finding appropriate measures to counteract the stress impacts on livestock.

The aggregate of nitrogen (N) emissions throughout the world is more important than their distribution in any specific locality. Animal production systems are among the largest contributors of reactive nitrogen to the environment, where nitrogen is lost from animal agriculture through volatilization to the atmosphere, runoff and leaching. Most nitrogen losses from animal agriculture are in a form that does not affect climate change directly. The exception is N_2O emissions that come from excrement storage and crop production and which affect climate change directly due to its high global warming potential. However, these compounds have serious environmental consequences and contribute to haze, acidity of rain, eutrophication of surface water bodies and damage to forests. In Chapter 4, all forms of nitrogen emissions are discussed at length, along with suitable strategies to control or mitigate these losses so that the losses may not shift from one form or source to another.

Chapter 5 represents the role of livestock in phosphorus pollution. Livestock manure has traditionally been considered a valuable resource of nutrients to enrich soils. But land application of manure in excess of crop requirements saturates the soil and loss of nutrients to surface water via runoff occurs. Environmental concerns with phosphorus from animal agriculture are identified as a primary source of water-quality impairment and eutrophication. Intensive livestock production aggravates the environmental consequences of phosphorus pollution. In this chapter, nutritional strategies are recommended for improving the bio-availability of phosphorus in livestock and minimizing the environmental load of phosphorus to avoid excess feeding. Chapters on metagenomics and proteomics are also accommodated for understanding the complex rumen microbial population and unravelling the less understood mechanism of fibre degradation. The latest techniques and approaches for deciphering the diversity of microbes and mechanism of function are elaborated.

24.2 Climate Change

The recent shift from plant to more animal product-based diets is evidenced, due primarily to the migration of large numbers of people to urban areas and higher income. To produce more animal products to satisfy the projected requirement in 2030/2050, more land and biomass yield is required. This would increase GHG emissions from the animal agriculture chain. The balance between planet (global resources and emissions), people and profit in the so-called 3P concept is an important prerequisite for sustainable life and development on earth. We should ensure the balance and sustainable use of limited resources such as arable land, water, fuel and some minerals for low emissions of GHGs from the animal agriculture chain. In Chapter 9, special attention is given for addressing carbon footprints (CFs). The emphasis is to sensitize producers and consumers for an efficient use of fossil carbon sources and to reduce GHG emissions per unit of product. Carbon footprints are defined as the total amount of GHG emissions associated with a product along its supply chain. CFs or LCAs (life cycle assessments) are tools for estimating the environmental effects caused by products or processes. They also help to

assess resource and feed efficiency between various regions and production systems. The author of Chapter 9 sheds light on the criteria for calculating the CFs of milk and meat. The chapter summarized very well the advantages and weaknesses of outputs from various animal productions and also established the effect of various system boundaries and types of production on the CFs of milk and other food of animal origin. Areas that have high mitigation potential are also suggested in the chapter, to minimize the carbon footprints of producing more animal food products.

Carbon concentration in the atmosphere is estimated at around 4 billion metric tonnes (Bt), which primarily transfer from fossil fuel and biotic and soil pools. The increase in the global emissions of carbon is estimated at around 270 Gt, which is linked to two major problems: first, the loss of carbon from terrestrial pools reduces the ecosystem services and goods provided by these systems; and second, an GHG emission in the atmosphere accentuates global warming pools. An obvious solution for this is to transfer the atmospheric CO_2 into potential sinks through carbon sequestration. 'Carbon sequestration' is defined as a process of increasing the carbon content of a reservoir rather than of the atmosphere. Carbon is sequestered from the atmosphere by growing plants, trees and pastures, and is stored in extensive root systems. The amount of carbon sequestered is influenced by several factors such as rainfall, temperature, type of plant or tree, plant density, growth, soil fertility status and type of farming system, etc. Farming systems thus provide a non-compensated service to society by removing atmospheric carbon. The author of Chapter 10 has discussed the relevance of carbon sequestration in the context of variable biophysical features, agroecological ecosystems and land-use systems. He has also focused on ways to increase carbon sequestration, mitigation of greenhouse gases and adaptation. The author opines that agroforestry and silvopastoral-based farming systems are influential ways of increasing carbon sequestration from the atmosphere. Special emphasis has been given to the opportunities for R&D activities to sequester more carbon from the atmosphere.

Livestock population developed in different ecological or geographical areas is genetically distinct due to genetic drift, shift and differential selection pressures. The indigenous livestock of various agro-ecological regions representing different breeds is assumed to have diverse adaptive or physiological characteristics. The speciation and development of geographically isolated breeds in the tropics has taken place with consideration of harsh climatic variation since time immemorial. For example, zebu cattle are uniquely suited to a hot and humid climate because of their smooth coat, primary hair follicles, improved sweat and sebaceous glands and better ability to lose moisture by evaporation than *Bos taurus* cattle. The impact of climate change will not only be confined to the production and productivity of an agricultural commodity but will also have far-reaching consequences on dairy, meat, wool and other animal products. The animal genetic resources (AnGR) of the tropics are at risk of being lost through the impact of climate change. This is directly arbitrated via increased incidences of drought, flood and the emergence of epidemic diseases, whereas indirect impacts are through changing of the adaptation capability of animals to extreme climatic conditions. Climate change is likely to impose differential opportunities for various species/breeds, depending on their adaptability in a broader range of biogeographical conditions. The impact on favoured species will be more severe. Climate change will also facilitate the movement of species along previously inaccessible pathways of spread, both natural and human-made. Individually, climate change and favoured species present two of the greatest threats to biodiversity and the provision of valuable ecosystem services.

Each organism has a unique set of preferences or requirements called niche, and biodiversity has been tied to the diversity of animals' niches. These can include, or be affected by, temperature,

aridity, resource availability, habitat requirements, soil characteristics, competitors and pollinators. Since the factors that compose a niche can be so complex and interconnected, the niches of many animals are bound to be affected by climate change. The authors of Chapter 11 have deliberated different adaptation strategies that deal with production adjustment, breeding, alteration of management system, development of appropriate policies, etc., for sustaining the diversity of livestock and their ecosystems. They emphasize that the identification and optimum utilization of different adaptable traits of local animal genetic resources need to be included in breeding programmes for sustainable growth of the livestock sector and to maintain genetic diversity. Proper *ex situ* conservation strategies, especially *in vitro* conservation, need to be considered as an important component of a broad-based strategy to conserve critical adaptive genes and genetic traits.

Livestock reproduction is another economically important trait that is affected badly by the changing climate. In Chapter 12, the effect of climate change on male and female animal reproduction is debated. Threats of climate change on reproduction may lead to the extinction of species/breeds. Therefore, assessing the true impacts and making precise projections about climate change and reproduction interactions becomes very important to prevent the extinction of a species/breed through impairment of reproduction. Environment directly affects the regulatory mechanism and rate of heat gain/loss of animals. Lack of prior conditioning to persistent adverse climatic conditions may result in catastrophic losses to the livestock industry worldwide. It is generally perceived that the impact of climate change on wild mammals would be more severe than on domestic mammals, due to the lack of ameliorative measures to minimize adverse impacts. Poor nutrition and heat stress are likely to affect the reproduction of livestock in tropical countries; while in the temperate world, it will be mediated through change in photoperiod. The consequences of climate change are reduced conception rates, increased embryo losses, increased calving intervals, lowered perinatal vigour and increased perinatal mortality and morbidity, lower weaning weights and rates, poorer post-weaning performance and increased health-related costs depending on the extent, stage of occurrence and the magnitude of stress. All these issues for both male and female animals are argued systematically in this chapter. Epigenetic alteration in relation to impairment of reproduction is also addressed by the authors.

In the past few years, on many platforms, the issue of meat production in relation to climate change has been questioned, as meat production from animals requires 11 times more fossil fuel than producing plant protein. Keeping the importance of this topic in view, chapter 13 in the book addresses the inefficiencies of meat production systems and their influence on climate change. Rapid urbanization, improved income levels and changing eating habits have led to the transition of food from plant-based proteins to animal protein. Total meat consumption has increased, as evidenced, from 47 million tonnes (Mt) in the 1950s to 260 Mt in 2005, more than double. Likewise, per capita consumption has also increased from 17 to 40 kg year^{-1}. Meat consumption is expected to increase further and is projected to be 460 Mt by 2050. This increased quantity can be met by raising the number of animals, which will further amplify the contribution of the livestock sector to GHG emissions. The author has taken note of different meat production systems, fossil energy consumption, deforestation and land degradation, while arguing the impact of meat production on climate change.

Chapter 14 describes the importance of indigenous livestock resources in the changing climatic scenario. Indigenous breeds of livestock have been adapting to climatic variations for a long time, and hence have acquired unique traits that make them suitable in given agroclimatic zones. For example, Indian cattle breeds, Tharparkar and Sahiwal, are heat and tick resistant. Similar cases are also observed in Asia,

Africa, Europe, Latin America and North America and the south-west Pacific region, possessing 1144, 1300, 345, 104 and 108 breeds of major livestock species, respectively. The mega diversity of livestock in India is neither accidental nor purely natural; rather, it is the outcome of thousands of years of deliberate selection and planned exposure to a range of natural climatic conditions. The production potential of cross-bred animals is generally more as compared to the native local breeds, but when criteria for adaptability and local feed and other resources are taken into account, the performance of indigenous breeds is always better in a given harsh local environment. Long-term analysis of milk production data from 1950/51 to 2005/06 revealed a 5.8 times increase in production where native Indian dairy buffalo contributed the most. Nevertheless, there is also a considerable share from exotic/cross-bred cattle. The cross-bred animals are reared mostly on fully intensive systems. They require good management practices to control climatic variations. Buffalo are reared primarily under semi-intensive or extensive systems and need the least inputs and negligible control over climatic conditions. The indigenous breeds may be less productive than their high-yielding exotic counterparts, but they are well adapted to the harsh environments and also produce under these conditions where other breeds cannot survive. The performance of indigenous livestock can be improved to a desirable level through a constant selection process.

24.3 Enteric CH_4 Amelioration

Chapters 15–23 in Section III deal with enteric CH_4 emissions, their status, thermodynamic and kinetic control, and mitigation strategies for feasible reduction in enteric CH_4. In this section, feed-based interventions, ionophores, plant secondary metabolites, etc., are debated at length. Emerging approaches such as reductive acetogenesis, residual feed intake and breeding interventions, immunization, archaeaphage therapy and methanotrophs, which seem to hold promise for substantial enteric CH_4 reduction, are also pondered upon.

CH_4 is an important component of the greenhouse gases in the atmosphere, and is the major gas associated with animal agriculture. The atmospheric concentration of CH_4 has increased significantly since the 18th century. This is primarily accountable for depletion of the protective ozone layer, along with other greenhouse gases. Around 90 Tg of enteric CH_4 per annum is produced by livestock. Enteric CH_4 emission across the globe is not uniform, and varies considerably from one region to another, depending on livestock population, species, available feed and fodder resources, etc. Recently, it was concluded that Asia-Pacific contributed the most (32.7%), while North America (11.5%) contributed the minimum to the total enteric CH_4 emissions from livestock. During the past 5 years, the maximum change (%) in livestock CH_4 emission was seen in the Asia-Pacific region (14.9%). Among countries, Brazil tops the list in livestock CH_4 emissions, while India, China, the USA and European Union (27) are next in sequence. Chapter 15 illustrates the enteric CH_4 emissions status on a global scale in general and in India in particular. The authors also discuss the challenges and opportunities for enteric CH_4 mitigation, along with the weakness and potential of different interventions.

Rumen methanogenesis is stated as an obligatory but wasteful mechanism for animals, as enteric emission represents a loss of biological energy in addition to its contribution to global warming. CH_4 synthesis cannot be understood or controlled without understanding the processes that produce substrates or remove products from the system. Underlying all the hydrogenotrophic processes that occur in the rumen are controlled kinetically or thermodynamically. In Chapter 16, the author explains the kinetics and thermodynamic control of enteric CH_4 emission. He elaborates why the disposal of H_2 through methanogenesis is thermodynamically most favourable among the

reducing pathways. This chapter also illuminates how the pattern of fermentation products in the rumen changes, while remaining near thermodynamic equilibrium, when the rumen ecosystem is modified by different interventions.

Ruminant nutritionists have historically focused on feeding ionophores to increase the efficiency and profitability of livestock. But nowadays these carboxylic polyether compounds are better known for their enteric CH_4 abatement. The extent of CH_4 inhibition with ionophores ranged from 0 to 76% in *in vitro* studies and up to 31% in animal studies. The use of ionophores reduces CH_4 emissions from ruminants substantially in the short term, and it is now evident that enteric CH_4 mitigation from livestock is inconsistent in the long term. The authors of Chapter 17 explore the view that animals return to their baseline level of emissions within 3–6 weeks, depending on diet composition. The authors suggest adopting a rotation policy for the use of different ionophores to minimize the adaptation of rumen denizen microbes and to achieve CH_4 mitigation in the long term, too. Ionophores also have a beneficial effect on ruminant feed efficiency. Therefore, their use is not only economically justifiable on the farm scale but also reduced CH_4 emissions provide a global incentive for ionophore usage.

Residual feed intake (RFI) is gaining popularity as a proxy for feed efficiency in growing cattle, sheep and pigs. RFI is defined as the difference between energy intake and demand, and is usually estimated as the residuals from a least squares regression model regressing feed intake on the various energy sinks. Chapter 18 illustrates a two-dimensional RFI model considering metabolic live weight and average daily gain as typical in most RFI models. As an alternative to RFI, they propose the trait, residual CH_4 production (RMP); residuals from a least squares regression model regressing total daily CH_4 emissions on various energy sinks and possibly feed intake. The genetic parameters for feed efficiency and CH_4-related traits are also discussed in detail, along with heritability estimates for the CH_4 trait compiled from recent studies carried out all over the globe. The genetic variation in feed intake independent of animal performance is expectedly less than other performance traits; but still, exploitable genetic variation does exist. Genetic parameters for enteric CH_4 emissions in cattle are rare, and no estimate of the genetic variation in enteric CH_4 emissions independent of animal performance exists.

In Chapter 19, the possibilities of reductive acetogenesis as an alternative hydrogenotrophic pathway to rumen methanogenesis is elaborated. This approach ensures the safe disposal of H_2 away from methanogenesis, in addition to energetic gain for the host animal. The authors stress the thermodynamic factors that control reductive acetogenesis in the rumen and how this process can be augmented as a competitive pathway of methanogenesis. Methanogen inhibition, or letting H_2 accumulate up to a desirable level where reductive acetogens become more active for utilizing this fermentation currency, can be potent strategies for augmenting reductive acetogenesis. The writers also elaborate that reductive acetogenesis is a prominent hydrogenotrophic pathway in other gut environments such as in kangaroos and wallabies. These marsupials exhibit foregut fermentation analogous to that of the rumen; however, they appear to emit minimal amounts of CH_4, ascribed to potential reductive acetogenesis that utilizes most of the H_2 in these animals.

Many strategies have been developed for enteric CH_4 mitigation. The limited applicability of a few successful strategies such as inadequacy of concentrate or succulent green fodder has forced researchers to focus on some alternative options where minimum inputs are required to ameliorate CH_4 in natural way. Recently, researchers have established that enteric CH_4 emissions may be controlled by immunizing the host animals against their own methanogens. Biological approaches such as immunization of host animals, through either active or passive means, offer good prospects for the future. These immunization approaches directly target the rumen methanogens that are accountable for rumen CH_4 production.

Methanogen antigens are not immunologically inert, but are recognized as a foreign body by the mammalian immune system. Properties such as the presence of unusual lipids that elicit an immune response offer the opportunity to control these rumen archaea through a vaccination approach. Although research on this aspect is still in its infancy, very limited work has been done by Australian researchers, with variable results. To counteract rumen acidosis, passive immunization has been introduced for the first time, where polyclonal antibody derived from egg was given to target lactic acid-producing bacteria. This may be applied to control rumen archaea, and thereby enteric CH_4, through avian-derived, ready-made antibodies produced in response to methanogen vaccination. In Chapter 20, the author has elucidated the prospects of both active and passive immunization approaches for the vested interest of enteric CH_4 mitigation. He also discusses the attainability of plant secondary metabolites (PSM), particularly of tannin in animal diet, to eradicate CH_4 emission substantially.

The concept of using biological agents in regulating enteric CH_4 emission from livestock strengthened when Australian researchers perceived for the first time that bacteriophages, the so-called 'archaeaphages', might be an innovative way to reduce enteric methanogenesis through targeting rumen archaea, and so the methanogenesis. Phage therapy is becoming increasingly important as a means of eradicating or controlling microbial populations as the use of antibiotics and chemical treatments becomes both less effective and less publicly acceptable. The first isolation of phages from the bovine rumen was reported way back in the 1960s, and since then there have been only sporadic reports on the rumen bacteriophages/archaeaphages. Very little is known about the biological properties of the genetic make-up of the rumen phages. It is now accepted that phages are endemic to the rumen, occurring in dense populations. Technological and scientific advancements have made it possible to understand the greater diversity of rumen methanogens, and the interaction of archaeaphages, which are prerequisites for contemplating the use of phages as a bioagent to control archaea. The authors emphasize that the development of any phage-based therapies requires a thorough understanding of three major factors: first, the target organism(s); second, the phages or phage-encoded products to be employed to reduce or eradicate the target organisms; and third, a thorough understanding of the microbial ecosystem and physical environment into which the phage-based therapy is to be introduced. The authors of Chapter 21 delineate the prospects of using phage therapy for the control of rumen methanogenesis. They also provide a list and taxonomic details of phage isolates known to infect the methanogenic archaea.

Any strategy that ensures a profitable increase in animal productivity as well as reduces enteric CH_4 emission will be easily adoptable by stakeholders. Opportunities for mitigating enteric CH_4 emissions revolve around H_2 production or its utilization; therefore, a strategy that restricts fermentative H_2 production, or obstructs/redirects its utilization away from methanogenic archaea, certainly has the potential to be useful. Further, the espousal of a strategy for CH_4 reduction by stakeholders depends on the cost of the inputs, the economic status of the livestock keepers, the toxicity to host/inhabiting microbes and persistency in the long run. Feed-based interventions appear ideal, and can be tried anywhere in the world by making slight alterations to available feed resources. The effect of feed-based approaches on enteric CH_4 emission is discussed in Chapter 22 under the headings such as diet composition, fodder type and quality, maturity stages, high-grain feeding, types of carbohydrates fermented in the rumen, intake, feeding frequency, feed processing, fat and oil supplementation, organic acids, bioactive phytochemicals, novel phyto-sources, ration balancing and genetic upgrading of forage. All these interventions are debated, with their advantages and limitations. The authors illustrate a hypothetical example for ease of

understanding the reimbursement that may arise from a 20% reduction in enteric CH_4 emission, taking into account both India and the rest of the world.

Few bacterial species reported to have CH_4-oxidizing capability can be employed in rumen CH_4 mitigation. The use of methanotroph microbes may be a viable and novel approach in controlling enteric CH_4 emissions in ruminants. Reports on the use of methanotrophs for enteric CH_4 abatement are scant, and little is known about their physiology and methanotrophy in the rumen. But there are some studies suggesting that selective members of the bacterial phylum, Verrucomicrobia, may be involved in the oxidation of CH_4. The authors of Chapter 23 summarize the physiology and taxonomy of methanotrophs, along with discussing the future prospects of using them for enteric CH_4 abatement.

24.4 Conclusion

There is a need to establish a set of adaptation and mitigation strategies that could be taken up by stakeholders. Identifying the hotspots for enteric CH_4 reduction and mitigation through new innovative technologies in a natural way should be the priority of livestock researchers in order to save not only the planet but also the biological energy needed to feed extra heads with the same quantity of locally available feed resources.

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