Methane emissions and mitigation technologies in cattle, sheep and red deer

A thesis presented in partial fulfilment of the requirements for the degree of

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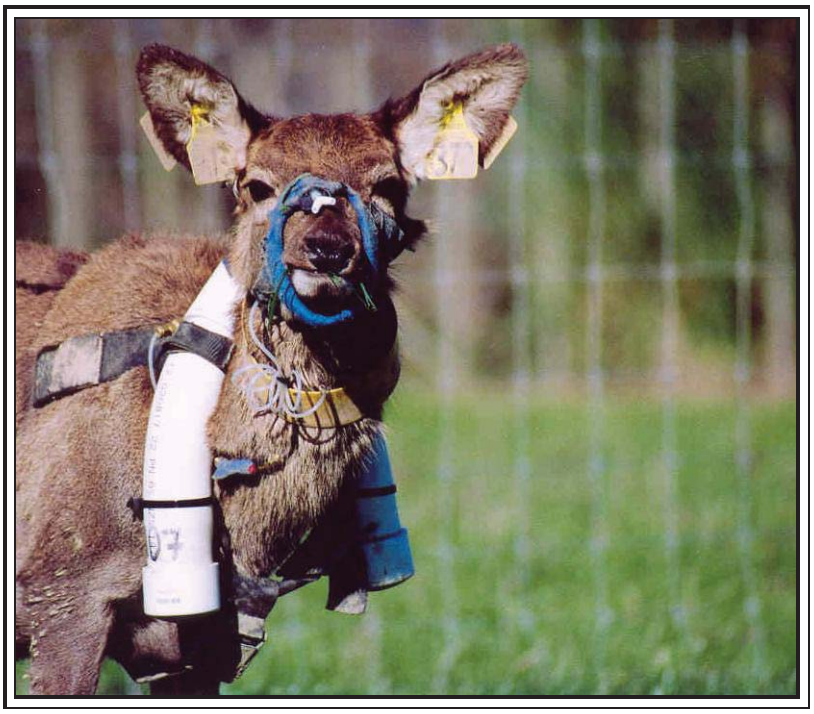

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ABSTRACT

Enteric fermentation of ingested feed by ruminant livestock is an important source of methane (CH_4), a potent greenhouse gas (GHG). Ruminant CH_4 emissions contribute approximately 31% of New Zealand's total GHG inventory; therefore reducing CH_4 emissions from ruminant livestock is a national priority.

The aims of this research were to investigate the effectiveness of potential mitigation technologies on the CH_4 emissions in sheep. This included the supplementation of monensin and coconut oil, individually or in combination, and the feeding of chicory as an alternative forage to perennial ryegrass-based pasture (pasture). The influence of ruminant age (grazing red deer) and ruminant species (housed cattle, sheep and red deer) on CH_4 yield were also explored.

This research showed that the supplementation of monensin to sheep may provide reductions in CH_4 yield (g CH_4/kg dry matter intake, DMI) of up to 30%, but this was not consistent between experiments. Sheep fed chicory yielded less CH_4 (17%) compared with sheep fed pasture, which was suggested to be due to faster degradation rates of chicory, leading to the increased outflow rate of digesta from the rumen; this theory needs to be tested. Neither, the supplementation of coconut oil or the combination of mitigation technologies resulted in a significant reduction in CH_4 yield. Nevertheless, as the power to detect a significant difference between treatments was reduced, due to the high variability of estimated CH_4 production, it is recommended that the effects of combined mitigation technologies be retested.

Methane yield was influenced by deer age, but only at 4.5 months of age as CH_4 yields of deer aged 6.5 to 11.5 months did not differ and may be an artefact of the method used to estimate DMI. Mean differences of CH_4 yield (up to 32%) between ruminant species was found when animals were offered the same diet and constant feeding levels; cattle > sheep > deer. This study indicates that the use of a single ruminant species to model potential CH_4 mitigation technologies may not represent all target populations due to differences of age or species found in this study. Research is required to confirm if differences between ruminant species persist when animals are fed fresh forages and to determine if responses to potential mitigation technologies are similar with age or between ruminant species.

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Literature review

1.1 INTRODUCTION

The earth's climate is becoming warmer, with global surface temperatures estimated to have increased by 0.74°C for the 100 year period from 1906 – 2005 (Solomon *et al.,* 2007). The accelerated warming of the earth's climate is attributed to the accumulation of greenhouse gases (GHG) from anthropogenic sources. As a result, it is anticipated that there will be an increase in the number and intensity of extreme weather events (Solomon *et al.,* 2007). The United Nations Framework Convention on Climate Change (UNFCCC) has defined climate change as "a change in climate which is attributed directly or indirectly to human activity that alters the composition of the global atmosphere and which is in addition to natural climate variability" (UNFCCC, 2008).

Greenhouse gases are important in creating the earth's climate as they, along with clouds, act as an insulation barrier and radiate solar energy reflected by land and ocean back towards the earth's surface. This subsequently traps energy and warms the earth's surface and is termed the greenhouse effect (Le Treut *et al.,* 2007). The main GHGs produced from human activity that have intensified the greenhouse effect, enhancing global warming and climate change are carbon dioxide (CO_2), methane (CH_4), nitrous oxide (N_2O) hydrofluorocarbons, perfluorocarbons, and sulphur hexafluoride (SF_6), (Le Treut *et al.,* 2007).

Methane has a global warming potential estimated to be 21 times that of CO_2 (weight basis, Solomon *et al.,* 2007). However, CH_4 has a shorter atmospheric residence time, of approximately 12 years, compared with CO_2, which is around 100 years (Solomon *et al.,* 2007). Consequently, reducing CH_4 emissions will have positive effects on the climate within a shorter time period than reductions in CO_2 emissions.

Agriculture is considered to be responsible for 10 to 12% (5.1 to 6.1 GT CO_2 equivalents; $CO_{2\text{-e.}}$) of total anthropogenic GHG emissions, with the principal sources being CH_4 (3.3 GT $CO_{2\text{-eq}}$) and N_2O (2.8 GT $CO_{2\text{-eq}}$; Smith *et al*, 2007). Enteric CH_4 produced from ruminant livestock is responsible for 15 to 33% of total anthropogenic CH_4 emissions (Smith *et al.,* 2007; Solomon *et al.,* 2007).

By 2030, CH_4 from agriculture is predicted to increase by 60%; in direct proportion to livestock numbers, unless international efforts are made to reduce emissions (Smith *et al.,* 2007). New Zealand is unique amongst developed nations as approximately 48% of GHG come from agriculture, with an increase of 12.7% in total agriculture GHG emissions between 1990 and 2007 (Anon, 2009). Methane emissions arising from ruminant enteric fermentation are accountable for 31% of New Zealand's total GHG and 64% of all agriculture GHG emissions, which have increased by 6.9% between 1990 and 2007 (Anon, 2009). This large contribution of enteric CH_4 emissions to New Zealand's total GHG emissions means that the implementation of mitigation strategies to reduce emissions from livestock is a priority (Anon, 2009).

The aim of this literature review is to briefly describe the process of methanogenesis in the ruminant animal and discuss the current knowledge of factors that influence the production of CH_4 and its mitigation, with an emphasis on grazing ruminants. The review will describe the New Zealand Greenhouse Gas Inventory for enteric CH_4 emissions (Section 1.2), followed by the process of CH_4 formation (methanogenesis) in the gastro-intestinal tract of ruminants and its measurement with reference to the two dominant methods (indirect calorimetry and the SF_6 technique; Section 1.3). Factors affecting CH_4 emissions from ruminants (Section 1.4) will be considered, and lastly, current and potential CH_4 mitigation technologies will be discussed, with particular emphasis on studies relevant to grazing ruminants fed fresh forages (Section 1.5).

1.2 NEW ZEALAND'S ENTERIC METHANE INVENTORY

Under the requirements of the Kyoto Protocol, New Zealand is required to develop and produce accurate inventories of all GHG emissions. Enteric CH_4 emissions reported by the New Zealand Greenhouse Gas Inventory (1990 to 2007 (Anon, 2009)) are calculated using the "Tier 2" method. However, this is expected to be moving towards a "Tier 3" method in the future (Clark Pers. Comm., 2009).

As discussed by Clark (2002), the International Panel on Climate Change (IPCC) has developed guidelines for three types of inventory methodologies, “Tier 1”, “Tier 2” and “Tier 3”. In essence, Tier 1 inventories are simplistic and are used for emissions from minor sources, whilst Tier 2 and Tier 3 inventories are more complex. These latter inventories are recommended for major sources of GHG and are appropriate for calculating New Zealand’s enteric CH_4 emissions. Other countries using the Tier 2 method to calculate enteric CH_4 emissions include Australia, Ireland and Holland (Clark Pers. Comm., 2009). Under the Tier 2 method (Figure 1.1), ruminants are divided into livestock type, i.e. sheep, deer, beef and dairy cattle, and then further subdivided into respective livestock classes, such as breeding hinds, weaners, and stags. Within each class of livestock, population changes within a year are accounted for by monthly time-step intervals, i.e. seasonal births and slaughter, animals changing from one class to the next (i.e. young animals entering the breeding herd), and deaths from natural causes.

Total animal numbers are based upon data collected by Statistics New Zealand and supplemented with data from the Ministry of Agriculture and Forestry (MAF) (Anon, 2009). Total CH_4 emissions for each livestock class are based on the population of each livestock class and its estimated feed intake based on average live weight and liveweight change, calving/lambing percentage, milk production and dietary chemical composition/quality. The total feed consumed for each species is then multiplied by the CH_4 energy loss as percentage of gross energy intake (CH_4 as a % GEI) for each species to determine the total CH_4 emissions. At present, the CH_4 estimates used for cattle and sheep are based on the SF_6 marker dilution technique.

The 2007 New Zealand Greenhouse Gas Inventory (Anon, 2009) recognises that CH_4 emissions from immature sheep (< 1 year of age) are lower than mature sheep (> 1 year of age), whilst immature cattle and deer are not recognised to have lower CH_4 as a % GEI compared with mature animals. Research measuring CH_4 (as a % GEI) does not include all livestock species and classes. For example, the CH_4 emission factor for deer is an average of mature sheep and dairy cattle, that from dairy cattle is applied to non-dairy

cattle, and there are no data available for goats and some classes of sheep (Anon, 2009). The accuracy of the livestock population's dynamics may also present another area for concern. Statistics for estimating livestock populations are sourced from Statistics New Zealand and are not consistent with industry statistics or sufficiently detailed to provide accurate estimates of the dynamics of each livestock class presented in the GHG inventory calculations (Anon, 2009).

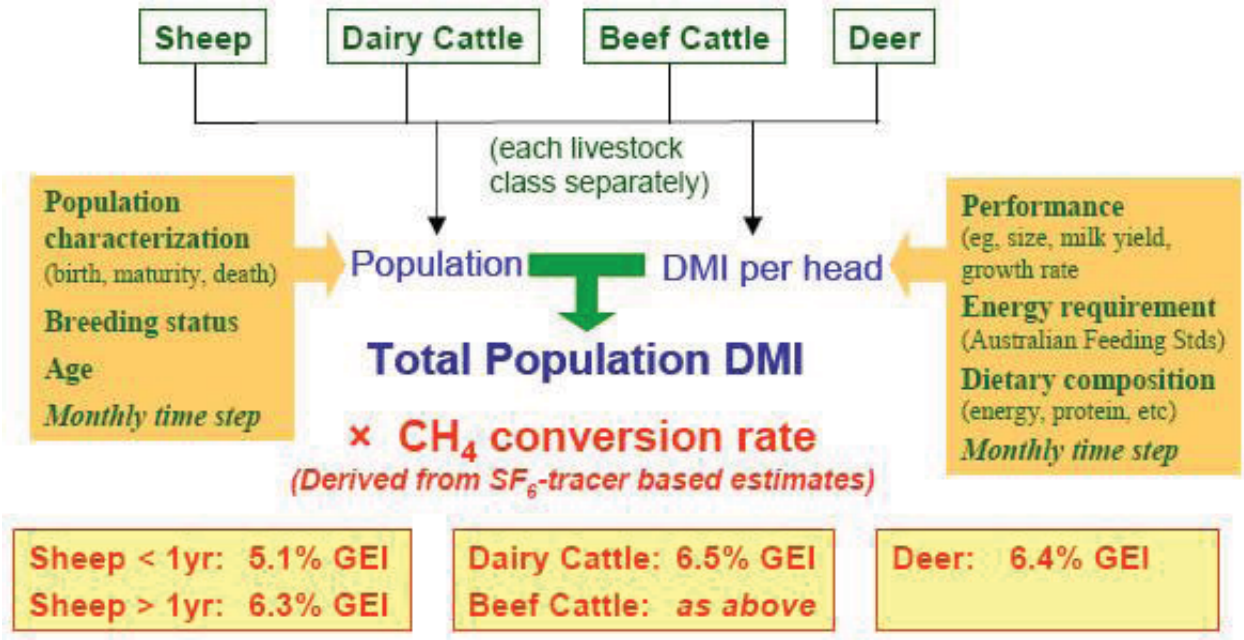

Figure 1.1: Schematic diagram of the model used to calculate New Zealand's ruminant enteric methane (CH_4) emissions. (DMI, dry matter intake; GEI, gross energy intake) (Anon, 2007).

1.3 RUMINANT ENTERIC METHANE PRODUCTION

1.3.1 Sites of methanogenesis and excretion

Ruminants, like all mammals, do not secrete the digestive enzymes essential for digesting plant structural carbohydrates (cellulose and hemicellulose). Forage digestion, especially of structural carbohydrates, arises from a symbiotic association between the host ruminant and the rumen microflora (bacteria, archaea, protozoa and fungi) (Akin, 1993). The majority of digestion takes place

in the rumen-reticulum (rumen) where up to 65%, 82% and 91% of organic matter, cellulose and hemicelluloses, respectively, in fresh forage diets are digested by sheep (Ulyatt & Egan, 1979; Waghorn *et al.,* 2007). Microbial digestion also occurs in the hindgut (large intestine and caecum), but its contribution to digestion is smaller (5 to 15%; Waghorn *et al.,* 2007).

The rumen functions as a large anaerobic fermentation vat which provides optimal conditions for microflora responsible for the fermentation of feed (Morgavi *et al.*, 2010). Ingested feed is retained in the rumen where it is extensively digested by microbial enzymes to produce end products such as volatile fatty acids (VFAs), as summarised in Figure 1.2, and these are utilised by the host animal as an energy source. The major VFAs are acetate, butyrate and propionate. The former two can be used in lipogenesis, whilst propionate is the main precursor of glucose and glycogen (Waghorn *et al.,* 2007). Other end products of microbial fermentation that are not used by the animal as a source of energy are CO_2 and hydrogen (H_2), which methanogenic archaea utilise to form CH_4 (termed methanogenesis; Section 1.3.2). This removal of H_2 maintains a low partial pressure of H_2 in the rumen and allows for optimal microbial fermentation of feed (Janssen, 2010).

The dominant site of methanogenesis occurs in the rumen with an estimated 87 to 92% (Murray *et al.,* 1976; Torrent & Johnson, 1994) of total CH_4 produced. The remaining 8 to 13% (Murray *et al.,* 1976; Torrent & Johnson, 1994) of CH_4 is produced in the hindgut (caecum and large intestine). Murray *et al.* (1976) reported that the major route of CH_4 excretion is eructation via the mouth (98% of total CH_4), with the remaining 2% excreted in the flatus. This is due to 89% of the CH_4 produced in the lower intestine being absorbed into the blood and expired through the lungs (Murray *et al.,* 1976). Nevertheless, the partitioning of CH_4 between eructation, breath and flatus has not been well studied.

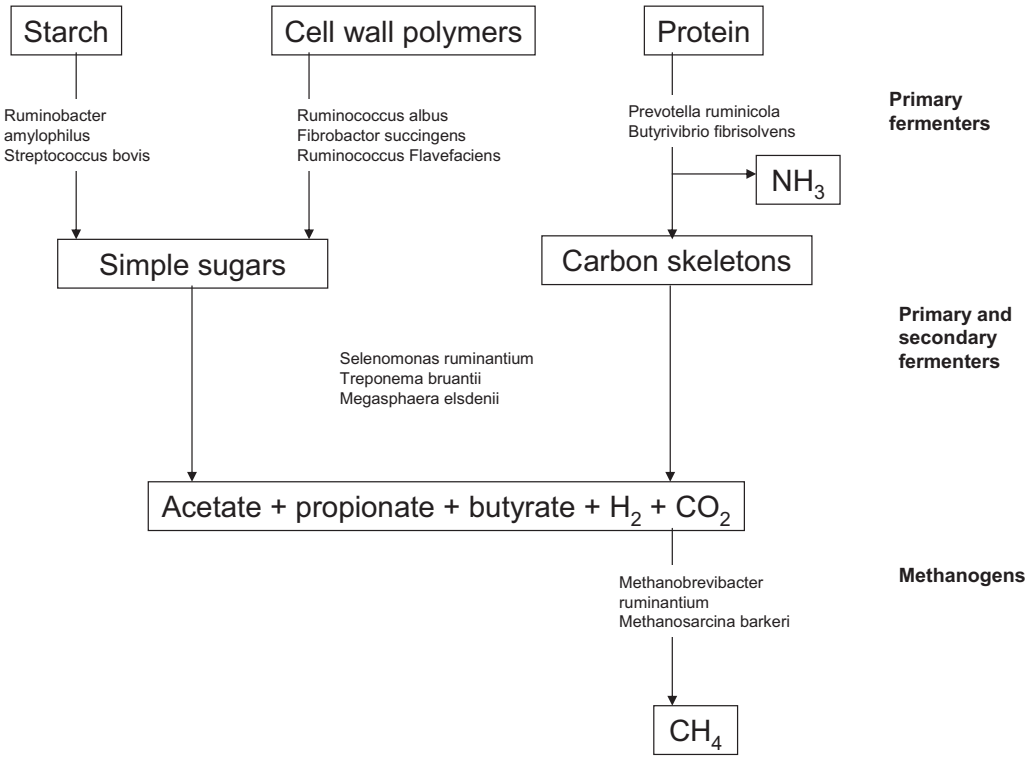

Figure 1.2: Microbial fermentation in the rumen. Microbes digest feed to simple monomers, which in turn are utilised by both primary and secondary fermenters. Methanogens prevent the accumulation of hydrogen (H_2) by reducing carbon dioxide (CO_2) to methane (CH_4). (NH_3, ammonia) (McAllister *et al.*, 1996).

1.3.2 Methanogens

Methane is produced by methanogenic archaea (McAllister *et al*., 1996; Janssen & Kris, 2008), whose only means of acquiring energy is by the formation of CH_4 (Mathison *et al.,* 1998). Members of the archaea domain are found in a wide range of environments; however, those isolated from the rumen are strictly anaerobic (Janssen & Kris, 2008).

The identification and quantification of methanogens residing within the gastrointestinal tract of ruminants is difficult and imprecise when using traditional culture methods (Jarvis *et al.,* 2000; Janssen & Kris, 2008). Although 113 methanogenic species from a wide a range of environments have been identified and classified, only seven species have been isolated and cultured from the rumen (Janssen & Kris 2008). Advances in molecular techniques have improved the precision of methanogenic detection from the rumen, and as a consequence, identification of methanogens has increased (Nicholson *et al.,* 2007; Janssen & Kris, 2008). Janssen and Kris (2008) reported that 92.3% of

the rumen archaea are placed within 3 genus levels which are *Methanobrevibacter* (61.6%), *Methanomicrobium* (14.9%), and a yet uncultured group known as 'Cluster C' (15.8%).

Between 9 to 25% of methanogens in the rumen are reported to be associated with protozoa (Newbold *et al.*, 1996; Takahashi, 2001) and this relationship has been shown to be responsible for up to 37% of the CH_4 produced in the rumen (Hegarty, 1999; Kumar *et al.,* 2009). McAllister & Newbold (2008) proposed that protozoa are responsible for up to 50% of the fibrolytic activity in the rumen, and are an important source of H_2 (Takahashi, 2001). The partial H_2 pressure in the rumen appears to affect the colonisation of protozoa by methanogens, as after a feeding event, the number of methanogens associated with protozoa is decreased, but increased under low concentrations of H_2 (Takahashi, 2001). In the absence of protozoa, rumen CH_4 emissions were reduced by 13% in *in vivo and in vitro* experiments (Hegarty, 1999), but this decreased to 10.5% when only *in vivo* experiments were considered (Morgavi *et al.,* 2010). The reduction of CH_4 production due to the elimination of protozoa is diet dependent, with the effect being greatest when animals are fed concentrate diets compared with forage diets (Hegarty 1999; Morgavi *et al.,* 2010).

1.3.3 Methanogenesis

Enteric CH_4 from ruminants is produced when feed is degraded by microorganisms within the digestive tract and results in the production of VFAs, ammonia (NH_3), CO_2 and H_2. Although CO_2 and H_2 are the main substrates used for methanogenesis, formate, acetate, methanol and mono-, di- and tri-methylamine can also be utilised as CH_4 sources (Wolin *et al.,* 1997). The products from digestion result in different amounts of H_2 formation and so the stoichiometry of CH_4 formation varies (Janssen, 2010). The formation of CH_4 uses CO_2 as the source of carbon and H_2 as the electron acceptor and is the dominant pathway of methanogenesis in the rumen (Morgavi *et al.,* 2010). The utilisation of CO_2 denotes methanogens as the terminal user of carbon within the fermentation process, while providing an important sink for H_2.

The breakdown of plant materials to provide energy for microbial metabolism is through a series of catabolic reactions, named the Embden-Meyerhof-Parnas pathway (McAllister & Newbold, 2008). Energy associated with the phosphorylation of ADP to ATP can be used to drive biochemical reactions. The conversion of ADP to ATP requires the reduction of a cofactor such as NAD^+ (nicotinamide adenine dinucleotide) to NADH. To enable the continuation of the fermentation process, NADH must be re-oxidised to NAD^+. In the anaerobic rumen environment, the transfer of electrons and protons, i.e. H_2, must be to acceptors other than oxygen (Demeyer & Van Nevel, 1974; Russell & Wallace, 1997). In the presence of methanogens, this is normally CO_2, which is reduced to CH_4 (McAllister & Newbold, 2008; Figure 1.3). This process is termed 'interspecies H_2 transfer' (Attwood & McSweeney, 2008; McAllister & Newbold, 2008) and the removal of H_2 is considered to lead to nutritionally more favourable patterns of microbial fermentation compared with when H_2 is not removed (Janssen & Kris, 2008).

The build-up of H_2 within the rumen activates inhibitory feedback pathways, which slow the rate of microbial fermentation (Figure 1.3; McAllister *et al.*, 1996). Therefore, although methanogens do not directly contribute to the digestion of fibre, the rate of plant fibre digestion is enhanced. The presence of methanogens effectively decreases the build-up of H_2 and reduced cofactors (e.g. NADH) via interspecies H_2 transfer (McAllister *et al*., 1996; Russell *&* Wallace, 1997). This is based on the belief that the only major sink for excess H_2 in the rumen is CH_4, with the assumption that H_2 is not emitted from the rumen as a gas (Czerkawski, 1986; Van Soest, 1994). The evidence derived for this relationship, and the pathways of interspecies H_2 transfer has largely been based on *in vitro* studies and is not well established *in vivo*.

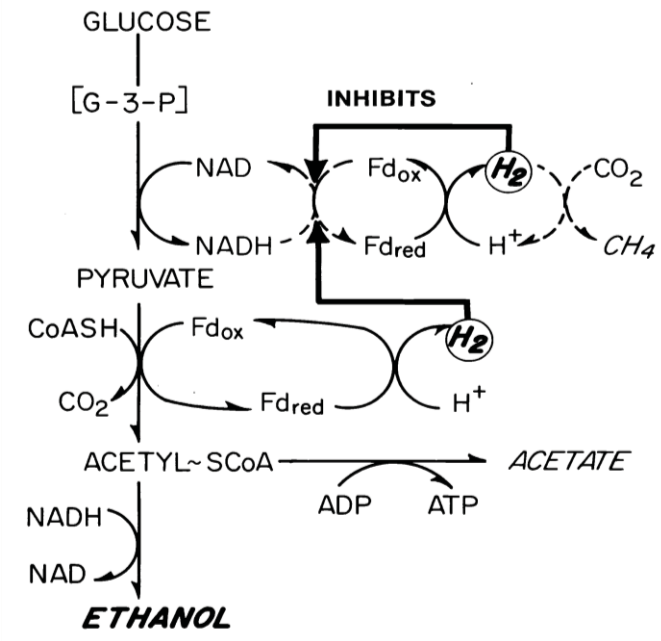

Figure 1.3: Hexose fermentation by *Ruminococcus albus* in the absence and presence of methanogens. In mono-culture, *R. albus* degrades glucose to acetate, ethanol, hydrogen (H_2), and carbon dioxide (CO_2). Hydrogen inhibits NAD^+ (nicotinamide adenine dinucleotide) formation from NADH. In co-culture with methanogens, NADH is used to reduce protons to H_2 and the final products are acetate and methane (CH_4) (Fd = ferredoxin) (Miller, 1995).

1.3.4 Methods to measure methane emissions

Methods to measure CH_4 emissions from ruminant livestock have been reviewed extensively in the literature, e.g. O'Hara *et al.* (2003), Vlaming (2008) and Pinares-Patiño and Clark (2008), and therefore, a focus on the advantages and disadvantages of two dominant methods used to measure CH_4 emissions from ruminants will be examined.

1.3.4.1 Indirect calorimetry

Measurements of ruminant CH_4 emissions have been undertaken using indirect calorimetry or respiration chambers techniques that calculated energy expenditure by the consumption of oxygen and expiration of CO_2. In contrast, direct calorimetry determines the energy expenditure of an animal by directly measuring heat production (Pinares-Patiño & Clark, 2008). Respiration chamber measurements have recently become the preferred technique to

measure CH_4 emissions from ruminants in New Zealand, and are considered to be the 'gold standard' against which other methods are compared (Pinares-Patiño & Clark, 2008; Vlaming, 2008). This technique can either be closed or open circuit, with the open circuit systems being the most common (Pinares-Patiño & Clark, 2008). Open circuit-systems involve the continuous flow of air through a chamber in which an animal is housed. The difference between CH_4 in air entering and exiting the chamber, once adjusted for flow rate, allows the determination of CH_4 emissions. In contrast, closed circuit chambers do not continuously pump air through the chamber. The air is circulated within the chamber and around and, air quality (humidity and temperature) and CO_2 concentration is maintained by air conditioning and CO_2 absorbents. Thus, the closed circuit chamber must not have any air leakage to ensure the accurate measurement of gas emissions. A distinct disadvantage of the closed circuit respiration technique is that despite the conditioning of the air gaseous emissions can only be measured for very short periods, as animals are at risk of running out of oxygen. A sample of air for gas analysis is taken at the start and end of the measurement period and therefore does not give a continuous measurement of gases whilst the animal is in the chamber.

Because the total production of CH_4 or other gases are measured and air quality is maintained indirect calorimetry is accurate and can be maintained for long periods (days). However, the disadvantage of this method is that it is labour intensive and expensive relative to the other indirect CH_4 measurement techniques. However, due to the precision of measurements from respiration chambers, fewer animals per treatment group are required to ensure adequate statistical power. There is also concern that CH_4 emissions are not representative of those from grazing animals and animal behaviour differences when animals are confined and fed conserved or freshly-cut forages. Partial enclosures, such as the use of masks or hoods, can also measure CH_4 emissions and the sample principles as those used with open circuit respiration chambers apply. The main disadvantage is that gas measurements are only made from the breath of the animal (Suzuki *et al.,* 2007). Furthermore, measurements using masks is limited as animals unable to eat or drink.

1.3.4.2 Sulphur hexafluoride tracer technique

The SF_6 technique was first developed by Johnson *et al.* (1994) and involves the placement of a permeation tube charged with SF_6 liquid into the rumen of the animal. Sulphur hexafluoride is released from the permeation tube as a gas via the Teflon® membrane. The rate of SF_6 gas release is determined by gravimetric weighing for at least two months prior to placement into the rumen, whilst permeation tubes are kept at 39°C (Lassey *et al.,* 1997; 2001). Breath samples from the animal contain both SF_6 and CH_4 gases, and are collected continuously over a 24 hour period via equipment mounted on the animal's head. Samples are then stored in an evacuated plastic yoke worn by the animal (as illustrated later in Chapters 2.3.3 and 4.3.5) and later analysed via gas chromatography (Lassey *et al.,* 1997; 2001). Total CH_4 production (Q_{CH4}; g/day) for each animal in a 24 hour period is calculated from the mixing ratio (µmol/mol) of SF_6 to CH_4 gas concentrations in the breath sample and the background concentrations of CH_4 and SF_6 (C^b_{CH4} and C^b_{SF6}). These concentrations are expressed in relation to SF_6 gas (µmol/day (Q_{SF6})) release from the permeation tubes per day where MW is the molecular weight of the gases (Equation 1; Lassey *et al.,* 1997; Grainger *et al.,* 2008).

$$Q_{CH4} = ((C_{CH4}-C^b_{CH4})/(C_{SF6} - C^b_{SF6})) \times Q_{SF6} \times (MW_{CH4} / MW_{SF6}) \qquad \text{Equation 1}$$

Relative to indirect calorimetry, the SF_6 method is less expensive as chambers are not required and measurements can be made from large numbers of grazing animals, which is important for the New Zealand pastoral system. However, this latter advantage is offset by the difficulty in determining the feed intake of grazing animals. The lack of accuracy in estimating DMI from grazing animals could mean that any small decrease in intake, due to the intensive handling of animals or to the wearing of breath collection equipment, will not detected, thereby diminishing the precision of calculated CH_4 yield (CH_4 expressed per unit of DMI).

The accuracy of the SF_6 technique to estimate CH_4 emissions is reliant on several assumptions, which are: that the SF_6 gas simulates the emissions of

CH_4 and is uniformly mixed; dilution rates of SF_6 and CH_4 gases are identical; SF_6 is inert; the release rate of gas from the permeation tube follows a constant linear pattern; and there are no interactions between rumen contents and SF_6 gas (Johnson *et al*., 1994; Ulyatt *et al*., 1999; Boadi *et al*., 2002; Vlaming *et al*., 2007; Pinares-Patiño & Clark, 2008). In addition, it is critical that the release rate of SF_6 from the permeation tubes in the rumen is accurately determined.

1.4 FACTORS EFFECTING ENTERIC METHANE PRODUCTION AND YIELD

In the context of this thesis, CH_4 emissions from ruminant enteric fermentation, are described in three ways; either as CH_4 production (total CH_4 produced per day; g/day); CH_4 yield (total production per unit of dry matter intake; g/kg DMI; or CH_4 as a % of GEI (CH_4 energy as a percentage of gross energy intake).

1.4.1 Feed Intake

Sheep and cattle fed conserved forage or grain-based diets increase CH_4 production as intake increases, but decrease CH_4 yield, in response to increasing DMI (Blaxter & Clapperton, 1965; Johnson *et al.,* 1994; Lassey *et al.,* 1997; Mbanzamihigo *et al.,* 2002; Yan *et al.,* 2009). The effect of decreasing CH_4 yield is possibly driven by changes in rumen physiology i.e. rumen kinetics and rumen fill, that occur in response any increase in DMI (Weston, 1996).

The relationship between CH_4 production or yield and intake has been established primarily with conserved forages and appears to be highly variable. Attempts to consistently describe this relationship across a range of diets have been largely unsuccessful (Johnson & Johnson, 1995). Because differences in diet structure and proportions (i.e. forage vs. grain) can impact on the relationship between CH_4 production or yield and intake (Johnson *et al.,* 1993).

Sheep and cattle fed fresh forages appear to have a similar relationship between CH_4 yield and DMI, as discussed above with concentrate-based diets. Methane yields from lambs fed fresh perennial ryegrass-based pasture at four differing levels of intake (0.8, 1.2, 1.6 and 2.0 times maintenance energy

requirements) were found to decrease linearly (R^2 = 0.66) when measured in respiration chambers, and DMI was shown to account for 69% of the variation (Knight *et al.,* 2008a). Muetzel *et al.* (2009) fed ewes fed fresh pasture at various intake levels and reported CH_4 yield to decrease by 5.3 g/kg DMI for every increase of DMI as a multiple of ME requirements for maintenance. In agreement with this, a review of New Zealand data on CH_4 emissions from sheep and cattle fed indoors on fresh ryegrass-based pasture found that DMI explained 51% and 81% of CH_4 production, when measured using either the SF_6 technique or respiration chambers, respectively (Hammond *et al.,* 2009). Increasing DMI reduced CH_4 yield by 3.9 g CH_4/kg DMI (SF_6 technique) and 3.2 g CH_4/kg DMI (respiration chambers) for each multiple of maintenance in sheep (Hammond *et al.,* 2009).

However, as reported with conserved and concentrate diets (Blaxter & Clapperton, 1965), the relationship between the DMI of fresh forages on CH_4 yield varies markedly. Molano and Clark (2008) compared five differing levels of intake from sheep fed fresh pasture and found that CH_4 production, when measured using the SF_6 technique, increased with intake, but CH_4 yield was not affected. This suggests that the relationship between CH_4 yield and DMI is variable and not consistent between experiments. A better understanding of the relationships between DMI, CH_4 yield, diet and digestive physiology are needed before CH_4 emissions can be accurately described using DMI.

1.4.2 Chemical composition of the diet

1.4.2.1 Quantification and prediction of relationship between dietary chemical composition and CH_4 yield

Attempts to mathematically describe the relationship between dietary chemical composition and CH_4 yield have been undertaken in a number of studies with animals fed conserved forage and grain diets (e.g. Blaxter and Clapperton (1965), Holter and Young (1992) and Ellis *et al.* (2009)). However, these equations have not explained variations in CH_4 yield due to diet alone, and cannot predicted CH_4 yield from ruminants fed fresh forages (Waghorn & Woodward, 2006).

Similar attempts by Waghorn and Woodward (2006) and Hammond *et al.* (2009) were made to describe the variation in CH_4 yield from sheep and cattle across a range of experiments on the basis of dietary chemical composition and failed to find any significant relationships. The latter study only included animals fed fresh perennial ryegrass (*Lolium perenne*), widely differing in chemical composition, and with measured intakes. Hammond *et al.* (2009) found that dietary chemical composition only explained 2% (SF_6 technique) to 20% (respiration chambers) of the variation of CH_4 yield and suggested that the remaining variance could be due to DMI and animal factors, such as digestive physiology.

1.4.2.2 Carbohydrates

The dietary proportions of cellulose, hemicellulose and soluble carbohydrates influence microbial fermentation pathways and consequently, production of CH_4 (Moe & Tyrrell, 1979; Holter & Young, 1992; Moss *et al.,* 2000). The digestion of structural carbohydrates is predicted to increase the production of CH_4 compared with soluble carbohydrates as pathways producing acetate and butyrate are favoured, resulting in the production of H_2 (Janssen, 2010).

In contrast, soluble carbohydrates have a greater rate of fermentation than structural carbohydrates (Moe & Tyrrell, 1979) and promote propionate production as an end-product of fermentation. The formation of propionate does not result in a release of H_2 and therefore is associated with lower CH_4 formation (Moss *et al.,* 2000; Jansen, 2010). Furthermore, the rapid fermentation of readily fermentable carbohydrates, particularly starch, is associated with lower rumen pH and renders the rumen environment less favourable for methanogens, which prefer a pH of 6.0 to 6.4 (Johnson & Johnson, 1995; Lee *et al.*, 2000; Moss *et al.,* 2000; Jarvis *et al.,* 2000).

1.4.2.3 Non-carbohydrate components

The effect of dietary concentrations of nutrients other than carbohydrates on CH_4 yield has not been well researched, and has been principally investigated with conserved/mixed ration diets. Digestible crude protein and dietary lipids were found to be negatively related to CH_4 as a % of GEI (Moe & Tyrrell, 1979;

Holter & Young, 1992). The inclusion of crude protein and fat in equations relating diet composition to CH_4 yield has improved prediction of CH_4 yield (Ellis *et al.,* 2009; Hammond *et al.,* 2009). Dietary chemical components that may provide an alternative H_2 sink, i.e. nitrate and sulphate, have been shown to reduce CH_4 emissions. For example, the CH_4 yield of sheep fed a basal diet of corn-silage was reduced by 32% and 14% (from 18.3 to 12.6 or 15.8 g/kg DMI respectively) when supplemented with nitrate or sulphate (2.6% of DM; van Zijderveld *et al.,* 2010).

1.4.2.4 Secondary plant compounds

Recognition of the potential impact of plant secondary compounds on CH_4 yield is steadily growing, with current research focused on saponins and condensed tannins (Beauchemin *et al.,* 2008). The majority of these studies, reviewed by Beauchemin *et al.* (2008) were conducted *in vitro* and there are limited studies investigating the effect of these compounds *in vivo.* Other secondary compounds found in fresh forage herbs such as sesquiterpene lactones have not been evaluated as a potential mitigating factor for CH_4 yield. Waghorn *et al*. (2002) and Woodward *et al.* (2002; 2004) showed that forages containing condensed tannins such as sulla (*Hedysarum coronarium*) and lotus major (*Lotus pedunculatus*) fed to sheep and cattle reduced CH_4 yields by up to 50% compared with animals fed pasture. These authors proposed that 13% to 16% of this reduction was due to the presence of condensed tannin, which were inactivatd by supplementation with polyeththylene glycol as opposed to other differences in dietary chemical composition. Similar effects on CH_4 yield were reported ryegrass-based pasture was supplemented with *Acacia mearusii* (Black wattle tree containing approximately 0.615 g condensed tannin (CT)/g DM). Reductions in CH_4 yield were up to 12% from sheep (25g CT/day; Carulla *et al.,* 2005) and 14 – 29% in lactating dairy cows (163g CT/day or 326 g CT/day, respectively; Grainger *et al.,* 2009).

Not all sources of condensed tannins appear successful in lowering CH_4 yield, no reduction in cattle supplemented with CT extract from quebracho trees (*Schinopsis quebracho-colorado*; red quebracho) was observed (Beauchemin *et al.,* 2007). Explanations for the lack of uniformity with different condensed

tannin sources are thought to be due to the reactivity of condensed tannins from different plant species (Ramíez-Restrepo & Barry, 2005; Hoste *et al.,* 2006). Hence, there may also be a difference in activity between condensed tannins in forage cells and extracted condensed tannins added to diets, which is yet to be explored.

1.4.3 Apparent nutrient digestibility

Blaxter and Clapperton (1965) reported increased CH_4 yields reported for sheep and cattle with increasing apparent digestibility feeds (fed at maintenance energy requirements including roughages, pelleted or milled feeds, and mixed roughage/grain; Figure 1.4). This suggests that as the apparent energy digestibility of the diet increases, so does the proportion of feed fermented by the microbial population in the rumen, resulting in an increase in CH_4 production (Moss *et al.*, 2000).

The impact of apparent digestibility on CH_4 emissions appears to interact with DMI (Figure 1.4). Methane yield decreased when sheep are fed amounts greater than maintenance energy requirements, but only for the higher quality diets; in comparison, the CH_4 emissions from diets of low digestibility/quality were less affected by the level of intake (Figure 1.4; Blaxter and Clapperton, 1965). This implied that diets with higher apparent digestibilities, have a greater rate of particle breakdown and passage from the rumen, thereby reducing the microbial fermentation of the diet. Methane emissions did not change with increasing DMI when low quality diets were fed. However, the studies of Blaxter and Clapperton (1965), do not take into account the effect of diet presentation, i.e. particle size of the feed. Therefore, this relationship maybe confounded by changes in the physical structure of the diets.

In contrast to experiments conducted with conserved forages, sheep fed fresh ryegrass-based pasture at either 62.5% or 73.5% apparent DM digestibility had no effect on CH_4 yield (Molano & Clark, 2008). Similarly, Pinares-Patiño *et al.* (2003a) fed cattle timothy (*Phleum pratense*) pasture at four stages of maturity and found no relationship between either organic matter digestibility or neutral detergent fibre digestibility and CH_4 yield.

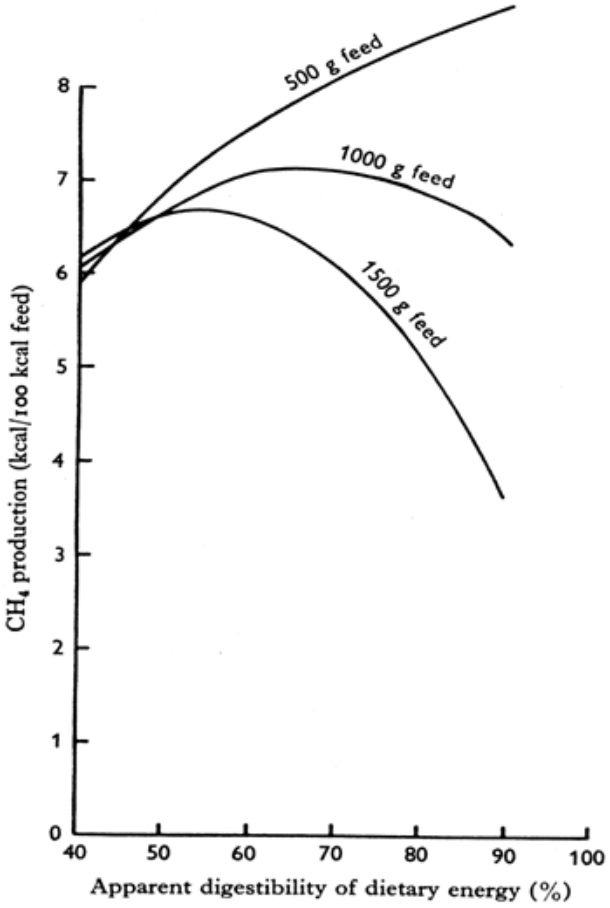

Figure 1.4: Estimated methane (CH_4) production, expressed as kcal/100 kcal feed, of sheep when fed diets of differing apparent digestibilites of energy and at three different levels of energy maintenance requirements (Blaxter & Clapperton, 1965).

1.4.4 Rate of digesta passage and particle degradation

Current evidence indicates that the residence time of digesta in the rumen influences the rate of CH_4 production and yield. The rate of digesta passage appears to be linked with feed intake (Section 1.4.1), apparent digestibility (Section 1.4.3), and feeding frequency (Okine *et al.,* 1989; Pinares-Patiño *et al*., 2003b; Boadi *et al.,* 2004). By placing weights in the rumen of steers to simulate rumen fill without increasing intake, Okine *et al.* (1989) found a 63% increase in the fractional outflow rate (FOR) of particulate matter, coinciding with a 29% decrease in CH_4 production and yield, while apparent digestibility remained unchanged.

Similarly, the FOR of the particulate fraction explained 57% of the variation in CH_4 yield between sheep and was negatively related to CH_4 yield, rumen fill and apparent digestibility of cellulose (Pinares-Patiño *et al*., 2003b). The variability in passage rates between animals and its apparent impact on CH_4 yield, was proposed as a candidate for genetic selection for low CH_4 emitting ruminants by Boadi *et al.* (2004), which could potentially reduce CH_4 emissions and improve animal production.

The rate of material leaving the rumen is influenced by two components; particle breakdown by chewing and ruminating, and rate of passage through the rumen-omasal orifice (Ulyatt *et al*, 1984). For particles to have a greater probability of exiting the rumen, they must be reduced to a critical particle size, approximately 1 mm for sheep and 2 mm for cattle (Ulyatt *et al.,* 1984), The selective retention of digesta in the rumen effectively exposes feed particles to prolonged degradation and microbial attack (McDonald, 1995). Therefore, the physical structure of the diet may impact on the rate of fermentation and consequently CH_4 yield. There is a lack of available data on the effect of the diet particle size and physical structure on microbial fermentation and CH_4 yield, although its effect on digestibility has been documented. Uden (1988) found that the apparent DM digestibility (67.1 vs. 72.8%, respectively), degradation rate (0.052 vs. 0.094 per hour, respectively), and the retention of solids from the total digestive tract (16 vs. 21 hours, respectively) were reduced in cows fed ground and pelleted hay compared with chopped hay.

1.4.5 Ruminant maturity/age

The digestive tract of ruminants is reported to reach maturity by eight weeks of age for sheep (Wardorp & Coombe, 1961) and cattle (Godfrey, 1961a), with key cellulolytic bacteria and methanogenic archaea present in the rumen as early as 3 weeks of age (Anderson *et al.,* 1987; Fonty *et al.,* 1987; Skillman *et al.,* 2004). However, the age that defines maturity varies, with Godfrey (1961a) reporting that the rumen and omasum of bovine calves were still growing at 17 weeks of age. The digestive tract of deer is different from that of sheep and cattle, as it appears to continue to develop up to approximately 1 year of age,

with the greatest growth rates occurring between 9 and 12 weeks (Hammond, 2008).

Determining the age of digestive tract maturity is important, as its function in immature ruminants appears to differ from their mature counterparts. Bovine calves (14 weeks of age) were reported to have shorter mean digesta retention times compared with calves at 20 to 38 weeks of age (2 hours; Leibhoiz, 1991) when fed roughage diets and housed indoors. A functional difference between immature and mature animals may indicate potential differences in CH_4 yield with animal age.

Reports from the literature where CH_4 was measured from immature ruminants do not appear to give consistent results. The mean CH_4 yield determined using the SF_6 technique from sheep less than one year of age have been reported to be lower (16.8 g CH_4 /kg DMI) than that from adult sheep (20.9 g CH_4/kg DMI) (Lassey *et al.,* 1997; Ulyatt *et al.,* 2005; Anon, 2009). Measurements, using the SF_6 technique, from beef cattle aged 6 to 24 months of age (Molano *et al.*, 2006), were also lower (19.5 g CH_4 / kg DMI) than those from mature dairy cows (21.6 g CH_4/kg DMI) (Anon, 2009). Conversely, Graham (1980) measured CH_4 emissions using closed-circuit respiration chambers and found that age did not affect CH_4 yield when measured from sheep at 2, 4 and 10 months of age and concurrently from adult sheep. In agreement, a meta-analysis by Pelchen and Peters (1998) found no significant difference of CH_4 as a % GEI between growing sheep (age not defined) and adult sheep (7.23 vs. 7.22 CH_4 as a % GEI, respectively) when methane was measured using respiration chambers and fed a broad range of diets. Similarly, a recent comparison of young (3.7 months of age) and mature cattle (6.8 years of age) fed ensiled ryegrass chaff reported no influence of age on CH_4 yields, measured using respiration chambers (Ramirez-Restrepo *et al.,* 2009).

The SF_6 measurements of CH_4 yield from young and mature sheep and cattle in these studies may be compromised, as DMI was not measured directly, rather estimated using energy regression equations (Molano *et al.,* 2006), or calculated from apparent DM digestibility and total faecal output (Lassey *et al.,* 1997; Ulyatt *et al.,* 2005). The lack of accuracy in estimating DMI from grazing

animals could mean that any small decrease in intake, e.g. due to wearing breath collection equipment or intensive handling, will not be detected and may create errors in the estimate of CH_4 yield. Therefore, further studies are needed to confirm if CH_4 yields from immature animals differ from their mature counterparts and if this is consistent across all ruminant species. No studies have yet been conducted to measure the CH_4 emissions from immature farmed deer or goats, especially whilst grazing or fed fresh forages.

1.4.6 Farmed ruminant species

The total daily CH_4 production differs between ruminant species, for example sheep produce 25 to 55 L/day and cattle produce 150 to 240 L/day (Czerkawski, 1986; Holter & Young, 1992; McAllister *et al.,* 1996), which reflects differences in animal size and the amount of DM consumed per day. As reported in the New Zealand Greenhouse Gas Inventory (Anon, 2009), CH_4 yield, did not differ between the dominant ruminant species, cattle, sheep and deer, farmed in New Zealand.

However, studies comparing the digestive physiology, i.e. intake, apparent digestibility and passage rate, of cattle and sheep (Aerts *et al.,* 1984/85; Pearson *et al.,* 2006) and sheep and red deer (*Cervus elaphus;* Milne *et al.,* 1978; Fennessy *et al.,* 1980; Domingue *et al.,* 1991) fed the same diet, have identified differences in digestive physiology between these species. Thus CH_4 emissions from different ruminant species could also differ. For example, When fed lucerne chaff, the apparent DM digestibilities in deer (56% DMI) and sheep (55% DMI) were similar, but the fractional outflow rates (FOR) of the solid (sheep, 3.31 vs. deer, 3.12%/hour) and liquid (sheep, 10.4 vs. 16.1%/hour) fractions from the rumen were found to differ (Domingue *et al.,* 1991).

Furthermore, although the apparent DM digestibility of the diet remained constant between summer and winter when fed to sheep (54 vs. 56% DMI) and deer (57 vs. 55% DMI), in deer the FOR of solids decreased (2.77 vs. 3.47 %/hour) whilst that of liquids increased (15.8 vs. 16.3 %/hour) from summer to winter. There was no difference in solid and liquid FOR from the rumen between summer and winter for sheep (Domingue *et al.,* 1991).

Although the above studies did not measure CH_4 emissions, DMI (Section 1.4.4), apparent digestibility (Section 1.4.3) and rate of passage (Section 1.4.4) have been shown to affect CH_4 yield within a ruminant species. The study of Domingue *et al.* (1991) reported that the passage rate of digesta through the digestive tract of deer changes between summer and winter and this could indicate CH_4 emissions may also be influenced by season. However, unlike sheep, seasonal effects on deer had an associated effect on DMI, which increased in summer (62.5 g/kg $LW^{0.75}$) compared with winter (46.7 g/kg $LW^{0.75}$). Therefore, it is unclear if the changes in rate of digesta passage between summer and winter in deer are due to differences of DMI or a seasonal change in digestive tract function.

Evidence for differences between ruminant species in CH_4 as a % GEI (respiration chambers) was presented by Galbraith *et al*. (1998). When fed lucerne pellets, CH_4 was greater from bison (*Bison bison*; 6.6 CH_4 as a % GEI) than wapiti (*Cervus elaphus*; 5.2 CH_4 as a % GEI), and greater from wapiti than white-tailed deer (*Odocoileus virginianus*; 3.3 CH_4 as a % GEI). Similarly, CH_4 from red deer was lower than that from sambar deer (*Cervus unicolor*) when fed a pelleted concentrate diet at either maintenance (4.8 vs. 6.5 CH_4 as a % GEI, respectively) or twice maintenance energy requirements (4.7 vs. 6.8 CH_4 as a % GEI, respectively; Semiadi *et al.,* 1998).

In contrast, CH_4 (as a % GEI) from sheep (8.0%) and cattle (7.6%), across a number of experiments, did not differ significantly when fed diets consisting of low quality dried grass and rolled oats at different feeding levels (Blaxter & Wainman, 1961). Except for the study of Semiadi *et al.* (1998), animals were fed *ad libitum*. This implies that differences between reported CH_4 emissions may not be due to fundamental differences in digestive physiology between ruminant species, but instead due to differences of digestive physiology caused by differing levels of DMI. Further research is required to address whether there are fundamental differences between the dominant species of farmed ruminants in New Zealand in CH_4 yields, when fed the same diet at the same feeding level and within the same season.

1.5 METHANE MITIGATION

The Kyoto Protocol requires that total GHG gases emitted from participating countries (i.e. New Zealand) be reduced to the baseline levels of 1990. New Zealand's CH_4 emissions from enteric fermentation are derived by estimating the total population's DMI for each livestock class. This is done by taking into account population characteristics, animal performance, and dietary composition, based on monthly time steps, and multiplying this by a CH_4 conversion rate (CH_4 as a % GEI) specific to each class of livestock (Section 1.2). This implies that by altering either the DMI or the CH_4 conversion rate for a given population group, total CH_4 emissions could be reduced. As the conversion factor is expressed as CH_4 as a % GEI, potential mitigation technologies are aimed at lowering CH_4 per unit of feed intake (i.e. CH_4 yield or CH_4 as a % GEI).

An alternative approach is to express CH_4 per unit of animal product. However, this approach can fail to recognise the potential increases in DMI needed to maintain higher levels of production, and could result in an overall increase in total CH_4 emissions.

Extensive and detailed reviews exploring possible options for CH_4 mitigation have been previously undertaken (Baker, 1999; Hegarty, 1999; Lee *et al.*, 2000; Moss *et al.*, 2000; Pinares-Patiño, 2000; Boadi *et al.,* 2004; Waghorn & Woodward, 2006; Beauchemin *et al.,* 2008; McAllister & Newbold, 2008) and potential technologies for CH_4 mitigation are summarised in Table 1.1. Some of these are described in more detail in Section 1.5.2. This section aims to explore some of the options that may be appropriate for mitigating CH_4 emissions from grazing ruminants.

Table 1.1: Options for reducing methane emissions, in total, per unit of feed intake or per unit of product from ruminants fed fresh forages (Waghorn & Woodward, 2006).

Technique	Application	Limitations	Consequences[a]	Potential uptake
Short term				
Maintain forage quality	Medium-high fertility grazing	No limitations; require skilled management	Improved animal performance, must limit excess fertilizer use	High
Feed legumes/herb, high-quality grasses	All situations depending on species	Costs of establishment and maintenance lower yields could lower profitability	Improved animal performance but more agronomic care needed	Moderate
Incorporate condensed tannins into diet	Widespread, especially with lotuses, sainfoin	Lower yield and persistence except in low fertility	Very good animal performance, 13-17% reduction in methane and lower N_20 emissions	Moderate
Specific lipids	Currently limited to dairy unless expressed in forage plants	Cost effectiveness	May affect product flavour	High with incentive[b]
Balance rations to meet animals needs	Systems involving supplementary feeding	Requires nutritional knowledge and advice	Improved performance from high producers. Could lessen N_20 emissions by lowering N intake	Moderate
Select high-producing animals	Normal practice	High producers require good feeding and management	Lower stock numbers, increased profitability	High
Optimal farm management	Widespread but requires good skills	Depends on commodity process; need consultant advice	Potential for high profitability	Moderate
Medium Term				
Selection of low methane producing animals	Widespread if trait is heritable	But may be diet dependent	Unlikely to have detrimental consequences	High with incentive

Table 1.1(continued): Options for reducing methane emissions, in total, per unit of feed intake or per unit of product from ruminants fed forages (Waghorn & Woodward, 2006)

Technique	Application	Limitations	Consequences[a]	Potential uptake
Use of ionophores	Widespread if viable	Current data show inconsistent responses, variable persistence with forage diets. Market acceptance	If viable, an added benefit is protection form bloat and possible improved feed conversion	Low to medium
Probiotics	Dairy, unless available as slow release	Minimal evidence of efficacy *in vivo*	Unknown	Unknown
Halogenated compounds	Could be widespread if in slow-release form	Need approval and verification of persistence	Consumer avoidance of products	High with incentive
Acetogens	Dairy cows	Require dairy administration	Responses not defined; excess acetate will not benefit ruminants fed forage	Low unless incentive
Defaunation	Moderate, depending on diet	Current technology risky, a vaccine would help	Beneficial for animals fed poor forages	Moderate if safe
High-efficiency animals	widespread	Require selection of animals with efficient nutrient utilisation	Selection may be feed specific	Moderate
Long term				
Vaccines - methanogens	Widespread	Good opportunities hampered by lack of funding	Potential for improved animal performance	High
Vaccines - protozoa	Moderate	Probably minimal	OK when poor feed is available	Moderate
Specific methanogen inhibitors (HMG-S-CoA[c] and Phage)	Widespread	Depends on specific inhibition of methanogens	Improved performance in intakes maintained	High with incentive

[a] Consquences refer to animal or environment; a net reduction in CH_4 kg^{-1} feed or product is implied.

[b] If performance is not enhanced an incentive may be required to use these materials.

[c] HMG-CoA, hyroxymethyl glutaryl-S-CoA

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1.5.1 Targets for CH_4 mitigation management

Targets for CH_4 mitigation include the management of the microbial population, the type of diet fed to ruminants, and animal management including whole farm techniques and animal selection. To avoid repetition the effect of dietary management on CH_4 yield has been addressed elsewhere (Section 1.4.2 and 1.5.2.1).

1.5.1.1 Microbial management

Management of the microbial populations within the digestive tract, particularly the rumen, is fundamental for reducing CH_4 emissions from ruminants. Mitigation technologies aimed at nutrition or animal management will interact and alter microbial populations, which consequently can have a flow-on effect on methanogenesis.

Sites for targeting microbial intervention were highlighted by Joblin (1999), as shown in Figure 1.5. These include: alterations to the microbes involved in digestion to change H_2 availability; provisions of alternative sinks for H_2; and the reduction of methanogen populations in the rumen. If total methanogen populations are to be reduced, consideration must be given to the fate of H_2. Methanogens have an important role in maintaining the low H_2 partial pressure in the rumen so that fermentation pathways can proceed (Section 1.3) (McAllister *et al.*, 1996; Janssen & Kirs, 2008; Newbold & McAllister, 2008). Therefore, any microbial intervention strategies must either provide alternative H_2 sinks or decrease CH_4 without decreasing fibre digestion.

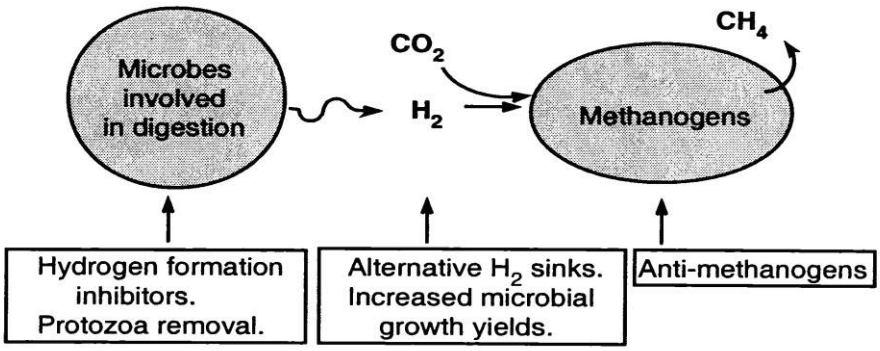


Figure 1.5: Possible sites of microbial-intervention for lowering ruminant methane (Joblin, 1999).

1.5.1.2 Animal management

Improvements in farm production efficiency and animal production can reduce total CH_4 emissions, as fewer animals would need to be farmed for the same amount of product produced. However, the reductions in livestock numbers need to be greater than increases in DMI per animal required to maintain the increased rate of productivity. A successful example of this is the New Zealand sheep industry, which has been able to decrease total CH_4 emissions by 14.7% from 1990 to 2001 (Anon, 2003). This was achieved by decreasing animal numbers, whilst maintaining total production, as lambing percentages from 1990 to 1999 have increased by 12%, and lamb and sheep slaughter weights have increased by 12 and 11%, respectively (Clark, 2002). Within the dairy sector improvements have also been made with an increase in milk solid production per cow of 14%, from 1994 to 2004 (Clark *et al.,* 2007). However, as a sector, enteric CH_4 emissions have increased by 70% (3519.8 Gg $CO_{2\text{-e}}$) from the 1990 levels of 5011.4 Gg $CO_{2\text{-e}}$, as the total number of dairy cattle have increased 1.9 million (from 3.4 to 5.3) from 1990 to 2007 (Anon, 2009).

Increased animal production and intake will decrease CH_4 per unit of animal product or per kg of DMI; however, as DMI increases, so too will total CH_4 emissions (Howden & Reyenga, 1999). Therefore, ways to increase production efficiency without affecting DMI need to be investigated (Howden & Reyenga,

1999). This can be done by exploiting animal variation in CH_4 emissions, with genetic or animal selection; selecting for animals efficient at converting feed into product; or digesta parameters that relate to decreased CH_4 yields, such as apparent digestibility and digesta kinetics (Howden & Reyenga, 1999; Boadi *et al.,* 2004; Waghorn & Woodward, 2006). However, there is the possibility that improving animal efficiency per unit of intake could encourage farmers to increase the total number of animals farmed, and so increase total GHG emissions.

1.5.1.3 Combining management strategies

To date, there has been mixed success in the development of CH_4 mitigation technologies. This is due the difficulty of identifying technologies that consistently reduce CH_4 yield and can be applied to grazing production systems. It is unlikely that a single technology will suit all ruminant livestock production systems and in practice, a combination of strategies may be employed. As highlighted by Beauchemin *et al.* (2008), the effect of combining two or more technologies in a single experiment is not known, despite the potential for additive effects. Furthermore, many farm systems in New Zealand are multi-species enterprises and it is not known if responses to mitigation technologies or their application would be the same across all ruminant species.

In the development of any successful CH_4 mitigation technology there needs to be consideration of the economic, animal, social, and environmental costs, as well as the impact on farm production systems. Technology uptake should also be considered, as it is unlikely to occur without any production benefits or economic incentives (Waghorn & Woodward, 2006; Beauchemin *et al*., 2008).

1.5.2 Potential mitigation technologies

1.5.2.1 Diet

Forage quality

Maintaining high quality forages is advantageous, as they tend to have higher apparent digestibility (Section 1.4.2) and nutritive value (Beauchemin *et al.,*

2008), which means that animals are able to maintain high DMI (Section 1.4.1). Whether forage quality has an effect on CH_4 yield is under debate, with recent work by Molano and Clark (2008) finding no difference in CH_4 yield from sheep fed reproductive and vegetative pasture. This implies that pasture management and its effect on CH_4 yield needs to be better understood and may only offer limited potential in mitigating CH_4 emissions. However, should diet quality prove to be an effective mitigation strategy, its incorporation into pastoral farming systems would be relatively straightforward (Beauchemin *et al.,* 2008; Waghorn & Woodward, 2006), with forage management skills of the farmer being the main concern.

Alternative fresh forages to ryegrass pasture

The dominant forage fed to ruminants in New Zealand is perennial ryegrass based pasture (Ramírez-Restrepo & Barry, 2005). Research conducted in New Zealand has showed that feeding fresh herb and legume forages can reduce CH_4 emissions from anywhere between 6.4 to 52% (Table 1.2), compared with ruminants fed fresh ryegrass diets. The feeding of legumes and herbs has been shown to increase animal performance in terms of liveweight gain (g/day). For example, the liveweight gain of lambs and deer has been shown to increase by 14% to 87% when fed red clover or chicory compared with pasture (Barry *et al.,* 1998). The use of forage legumes and herbs will require specific forage management, may only be suitable to selected livestock classes and limited to particular regions of New Zealand.

The mechanism by which CH_4 is reduced by feeding legumes and herbs (Table 1.2) is not well understood. It is thought that the lower fibre and greater soluble carbohydrate concentrations of legumes compared with grasses contribute to their greater apparent digestibility (Sections 1.4.2-1.4.3; Waghorn *et al.,* 2002); faster degradation rate, and an increased passage rate (1.4.3) within the rumen, all of which could contribute to lower CH_4 emissions

The rate of digesta passage through the rumen has been shown to affect CH_4 emissions, (Section 1.4.4) but studies comparing the digesta kinetics of

legumes and herbs with perennial ryegrass have not measured CH_4 yield. Kusmartono *et al,* (1996; 1997) found that the rumen FOR of the particulate phase of digesta of red deer fed chicory was greater (4.08 %/hour) than pasture (2.78 %/hour), which was attributed to the rapid breakdown of chicory (Kusmartono *et al.,* 1996). Similarly, a comprehensive screening of different forages using *in vitro* and *in sacco* techniques by Burke *et al.* (2000) found large differences in fractional degradation rate between pasture (0.114 %/hour), white clover (0.195 %/hour), sulla (0.121 %/hour) and chicory (0.260 %/hour).

Plant secondary compounds are present in some legumes and herbs and have been found to lower CH_4 yield by 13 to 16% (Section 1.4.2.4). However, not all plant secondary compounds appear effective at reducing CH_4 yield and this needs to be better understood. Further research is needed to elucidate the impact of dietary chemical composition, potential degradation rates and digesta kinetics of fresh forages on ruminant CH_4 emissions.

Table 1.2: Methane (CH_4) production (g CH_4/day), and yield (expressed in terms of dry matter intake (g CH_4/kg DMI) or digestible dry matter intake (g CH_4 per kg DDMI)), from cattle, sheep and red deer fed a range of fresh forage diets indoors.

Species	Diet	DMI kg/day	DM digestibility	CH_4 g/day	CH_4 g/kg DMI		CH_4/kg DDMI		Reference
						% Diff.[3]		% Diff.	
Sheep	*Trial 1*								Waghorn *et al.*, 2002[4]
	Pasture	1.1	74.0	28.7	25.7	-	34.7	-	
	Lucerne	1.5	71.3	30.2	20.6	19.8	29.0	16.4	
	Sulla	1.5	72.8	26.3	17.5	31.9	24.1	30.5	
	Sulla/lucerne	1.7	71.1	31.8	19.0	26.0	26.7	23.1	
	Trial 2								
	Chicory	1.1	79.3	18.1	16.2	37.0	20.4	41.2	
	Red clover	1.8	75.6	31.2	17.7	31.1	23.4	32.6	
	Sulla	1.2	63.2	20.5	17.5	31.9	27.7	20.2	
	Chicory/sulla	1.4	71.1	23.2	16.9	34.2	23.8	31.4	
	Chicory/red clover	1.4	76.5	26.8	19.7	23.3	25.6	26.2	
	Lotus	0.9	70.0	10.8	11.5	55.3	16.4	51.9	
	Lotus + PEG	0.9	76.9	12.9	13.8	46.3	17.3	50.1	
	Dried lucerne	0.9	58.0	14.1	15.7	38.9	27.1	21.9	
	Pasture	0.8	na[1]	16.0	20.1	-	20.3	-	Woodward *et al.*, 2001[4]
	Lucerne	0.7	na	13.5	18.6	7.5	19.0	6.4	
	Lotus	0.7	na	10.6	14.5	27.9	14.5	28.6	
Red Deer	Pasture	1.5	79.8	41.8	25.6	-	33.8		Swainson, 2004
	Plantain	1.7	69.5	39.5	25.0	2.3[ns]	33.0	2.4[ns]	
Dairy cattle	Pasture	10.2	67.1[2]	344.4	35.1	-	50.3	-	Woodward *et al.*, 2001
	Lotus	14.1	68.6[2]	376.7	26.9	23.4***	38.9	22.6[4]	
	Pasture	10.7	na	260.0	24.6	-	na	na	Woodward *et al.*, 2002
	Sulla	13.1	na	253.9	19.5	20.7*	na	na	

[1]na; no data available.

[2]Dry matter apparent digestibility determined *in vitro*.

[3] Percentage difference compared with pasture. Statistical differences between pasture and other forages within the same study denoted as; * P < 0.05; ** P < 0.01; *** P < 0.001; ns not-significant .

[4] Statistical differences between forages not presented in the original report

1.5.2.2 Oils

The addition of lipids with anti-microbial activity to the diet may offer a means of increasing the energy density of the diet and improving animal production, whilst lowering CH_4 emissions (Beauchemin *et al.,* 2008). Refined oils, such as coconut oil, contain high concentrations of medium-chain fatty acids (MCFA), particularly lauric and myristic fatty acids (Machmüller *et al*., 2001). Reductions in CH_4 yield attributed to coconut oil are reported to be mediated by these MCFA (Dohme *et al*., 2001) as they have anti-microbial properties and are toxic to methanogens. Reductions in both protozoa and methanogen numbers in response to MCFA have been reported by Machmüller (2006). Sheep fed a mixed hay and grain diet and supplemented with coconut oil at a rate of 3.5% or 7% of DMI were reported to have reduced CH_4 yields of 28 and 78%, respectively (Machmüller & Kreuzer, 1999; Table 1.3). However, the supplementation of coconut oil as a potential CH_4 mitigation agent has not been tested when animals are fed a fresh forage diet.

Table 1.3: Summary of sheep supplemented with coconut oil and its effect on methane (CH_4) production (CH_4p, g/day) and CH_4 yield (CH_4y, g/kg DMI).

Diet	Dose rate %DM	Total ether extract % DM	DMI kg/day	CH_4p	CH_4y	Reference
Hay (63%)-concentrate	0	6.8	0.992	18.6	18.8	Machmüller *et al.,* 2001
	5.9	6.1	0.992	17.2	17.3	
Hay (62%)-concentrate	0	1.8	0.685	29.5[a]	22.1[a]	Machmüller & Kreuzer,1999
Hay (71%)-concentrate	3.5	4.4	0.599	21.2[b]	17.3[b]	
Hay (46%)-concentrate	7	7.0	0.694	7.4[c]	8.0[c]	
TMR (35% concentrate)	0	3.1	0.943	15.5	16.4	Machmüller *et al.,* 2000
	2.5	5.4	0.908	11.5	12.7	

[abc] Differing letters within column and group denoted a significant difference between means

Oils containing high concentrations of long-chain fatty acids (LCFA), such as oilseeds and animal fats, have reduced CH_4 emissions (Beauchemin *et al.,* 2008), and are reported to have a greater effect compared with MCFA (Machmüller, 2006). This is partially attributed to reduced fibre degradation and decreased DMI when animals are supplemented with LCFA (Beauchemin *et al.,* 2008). Long chain fatty acids reduce CH_4 production by consuming H_2 as they undergo hydrogenation in the rumen, which differs from the proposed anti-microbial mechanism of MCFA (Machmüller, 2006).

The reports detailing the ability of lipids to reduce CH_4 yields of ruminants fed fresh forages are few and conflicting. The combination of sunflower and linseed oil (LCFA), supplemented to sheep over a range of infusion concentrations (0, 1.2, 2.5, 3.8, and 5% DMI), and fed a ryegrass diet pasture had no impact on CH_4 yields (Cosgrove *et al.,* 2008). However, the statistical power of this study was low, and CH_4 production was determined using the SF_6 technique. In contrast, CH_4 yield decreased (18.5 to 13.5 g CH_4/kg DMI) when lactating cows fed fresh ryegrass were supplemented with differing proportions of fish and sunflower oil at 500 g/day (or 3.7% DM) (Woodward *et al.,* 2006). The ability to supplement oils to animals fed forages is limited because these forages can contain up to 4% of the total lipids as a percentage of DM (Cosgrove *et al.,* 2008) and the maximum allowable total lipid content of ruminant diets is approximately 9% DM. The logistics of supplementing oil to grazing ruminants could limit its use on farm, but it may be suitable for animals receiving daily supplements in addition to pasture, such as high performing dairy cows.

The success and degree of CH_4 mitigation appears to be dependent on the level of oil supplementation (Figure 1.6). Beauchemin *et al.* (2008) reported that over a range of experiments, including different lipid types and diets, CH_4 yield was reduced by 5.6% for every addition of lipid as a percentage of DMI. It is not clear how diet and the individual fatty acids impact on CH_4 yield and if the 5.6% reduction of CH_4 yield for every percentage increase of lipid supplementation (% DM) is the same across all lipid types. There are also likely to be interactions between the diet and the presentation of the supplementary lipid. For example,

Martin *et al.* (2008) also found a reduction in CH_4 yield (22.0 to 10.5 g CH_4/kg organic matter intake (OMI)), when cows were fed a mixed ration diet supplemented with linseed oil. The presentation of the linseed oil affected CH_4 yield, with refined oil having a greater impact on CH_4 than crude linseed or extruded linseed (10.5 vs. 19.8 vs. 16.3 g CH_4/kg OMI, respectively) (Martin *et al.,* 2008).

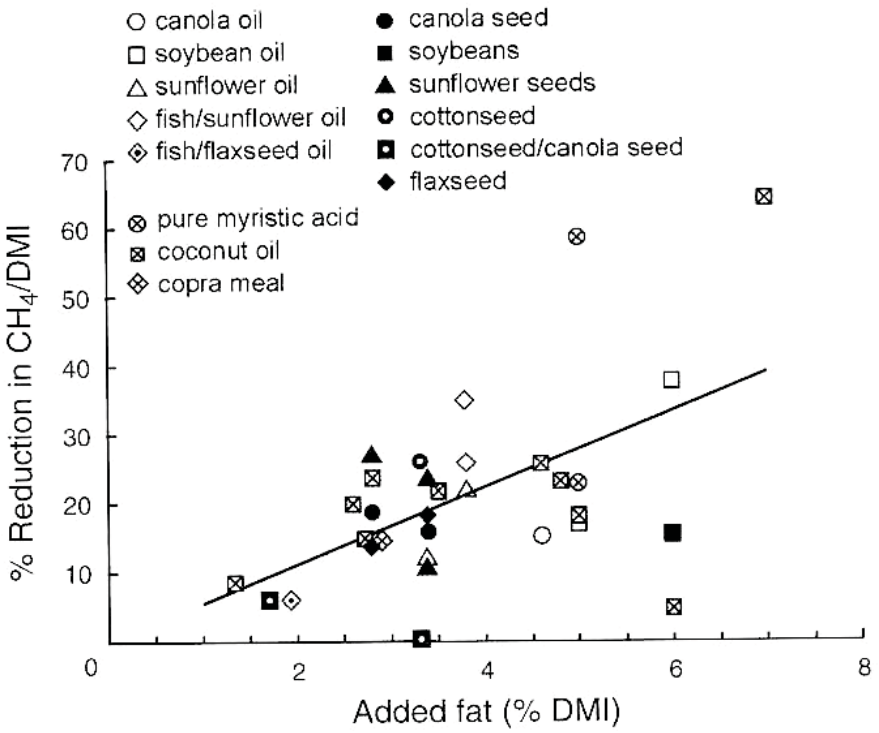


Figure 1.6: Summary of literature results for 33 treatment means showing the effect of fat from various sources on the percentage reductions in methane (CH_4) (g/kg dry matter intake, DMI) relative to the control diet. The solid line represents the regression accounting for the effect of study; y = 5.562 (s.e. = 0.590) x percentage added fat; (r^2 = 0.67; P = 0.004) (Beauchemin *et al.,* 2008).

1.5.2.3 Monensin

Monensin is an ionophore that has anti-microbial properties. It is widely used for the control of bloat in cattle, and has production benefits for dairy cows in terms of milk production and feed conversion efficiency (McGuffey *et al.,* 2001;

Ruiz *et al.,* 2001; Ipharraguerre & Clark, 2003). Monensin affects gram-positive microbes, such as protozoa, which are associated with a high proportion of H_2 production and can indirectly account for up to 20% of methanogenesis in the rumen (Guan *et al.,* 2006).

The effectiveness of monensin as a CH_4 mitigation agent is widely debated. Extensive reviews by Van Nevel and Demeyer (1996) and Beauchemin *et al.* (2008) have reported that CH_4 yields were reduced by 4 to 31% in cattle and sheep fed a wide range of diets.

Studies measuring the CH_4 yield from dairy cows fed fresh perennial ryegrass diets and supplemented with monensin have given conflicting results. Van Vugt *et al.* (2005) reported a 9% reduction in CH_4 yield from dairy cows fed pasture and maize silage supplemented with monensin at a rate of 29.6 mg/kg DMI, whereas, when dairy cows were fed fresh ryegrass diets, lower monensin doses, of 11 and 13 mg/kg DM, had no effect on CH_4 (Grainger *et al.,* 2008; Waghorn *et al.,* 2008; Table 1.4). The persistence of reduced CH_4 yield through monensin supplementation is also questionable. Guan *et al.* (2006) reported that the 30% reduction in CH_4 yield from beef cattle supplemented with monensin disappeared after four weeks. Therefore, in addition to dose rate and possible dietary influences, the persistence of reduced CH_4 emissions need to be further elucidated, if monensin is to be used as a mitigation technology.

Table 1.4: Effect of monensin supplementation on methane (CH_4) production (g/day) and yield (g/kg dry matter intake, DMI) (Beauchemin *et al.,* 2008).

Animals	Diet	Dose rate		Days after dose	CH_4 emissions				Reference
		(mg/day)	(mg/kg DMI)		Control (g/day)	Monensin (g/day)	Control (g/kg DMI)	Monensin (g/kg DMI)	
Controlled release capsules									
Dairy cows	Ryegrass pasture	166	11	30-90	328	313^{ns}	19.2	20.0^{ns}	Waghorn *et al.,* 2008
Dairy cows	Ryegrass pasture	320	29.6	11	179^{a}	158^{b}	16.9^{a}	15.3^{b}	Van Vugt *et al.,* 2005
Non-lactating dairy cows	Ryegrass pasture	320	35.2	72	246^{a}	223^{b}	25.5	24.8^{ns}	Van Vugt *et al.,* 2005
Dairy cows	Ryegrass + white clover	320	17.5	23	330^{a}	309^{b}	17.5	16.9^{ns}	Van Vugt *et al.,* 2005
Dairy cows	Ryegrass + maize silage	320	18.1	58	350	356^{ns}	19.2	20.5^{ns}	Van Vugt *et al.,* 2005
Dairy cows	Ryegrass + grain	240	13	25,85	341	365^{ns}	-	-	Grainger *et al.,* 2008
Dairy cows	Ryegrass + grain	240	13	83	376	386^{ns}	-	-	Grainger *et al.,* 2008
Dairy cows	Ryegrass + grain	240	13	75	309	306^{ns}	16.7	17.0^{ns}	Grainger *et al.,* 2008
Added to the ration									
Dairy cows	Grain + forage	385	24	8–28	572^{a}	517^{b}	38.6^{a}	35.7^{b}	Sauer *et al.,* 1998
		385	24	8–28*	599	598^{ns}	34.9	33.7^{ns}	Sauer *et al.,* 1998
Dairy cows	Grain + forage	473	24	**	458.7^{a}	258.6^{b}	23.3^{a}	22.4^{b}	Odongo *et al.,* 2007
Beef cattle	High forage	246	33	19	166.2^{a}	159.6^{b}	22.6^{a}	20.7^{b}	McGinn *et al.,* 2004
Beef cattle	High grain	271	33	Weekly for 16 weeks		-27% for 2 weeks, 0 by week 6			Guan *et al.,* 2006
Beef cattle	High forage	240	33	Weekly for 16 weeks		-30% for 4 weeks, 0 by week			Guan *et al.,* 2006

* Second CH_4 measurement

** CH_4 measured monthly for six months

[ab] Within rows and the CH_4 variable, values followed by the same letter are not significantly different at P = 0.05.

[ns] No effect of monensin on CH_4 emissions reported.

1.6 CONCLUSIONS

Methane emissions from ruminant livestock contribute to global GHG emissions, which if unmanaged, are expected to increase up to 61% by 2030 (Smith *et al.,* 2007). Due to the short half-life of CH_4 (12 years) in the atmosphere, any benefits of reducing CH_4 emissions will be realised within a relatively short-time period compared with CO_2 (half-life of 100 years). New Zealand is unique because a high proportion of its GHG arise from agriculture and CH_4 emissions from New Zealand ruminant livestock have increased by 6.9% since 1990 (Anon, 2009). Therefore, developing technologies to mitigate CH_4 emissions from ruminant livestock within New Zealand is of high priority.

Methane emissions reported in the New Zealand Greenhouse Gas Inventory have been measured from a limited number of ruminant species and livestock classes. Furthermore, generalisations have been made with CH_4 emissions from deer based on the average emissions from adult sheep and dairy cattle.

Animal factors, such as intake, apparent digestibility, and digesta kinetics, have been shown to affect CH_4 yield from ruminants; however, their impact is not well understood and may vary with diet type. A better understanding of the interactions between diet and digestive physiology on CH_4 yield is needed, if mitigation strategies are to be indentified and applied to animals fed fresh forage diets.

In addition to dietary effects, comparisons between ruminant species have shown differences in digestive physiology and CH_4 emissions. However, the differences observed in CH_4 yield between species may be an artefact of the effect of intake. It is not known if CH_4 yields differ between ruminant species when animals are fed the same amount of feed relative to maintenance energy requirements. If differences between ruminant species in CH_4 yield exist, this needs to be considered in further development of CH_4 mitigation technologies.

The chemical composition of conserved forage or grain-based diets fed to sheep and cattle have been shown to influence methanogenesis. However, the

relationship between diet chemical composition and CH_4 yield is inconclusive for fresh forage diets, and may not have as large an impact as previously envisaged. Nevertheless, the feeding of some herbs and legumes has been shown to reduce CH_4 yields, and are of greater feeding value compared with ryegrass diets.

Further research is required to investigate the potential of herbs and legumes to reduce CH_4 emissions and to determine the underlying mechanisms. If these are better understood, they could provide important information for the selection and development of new forages for reducing CH_4 emissions, while also improving animal productivity.

Supplementary monensin and/or coconut oil have been suggested as CH_4 mitigation technologies for ruminants fed conserved forage or grain-based diets. Coconut oil has been shown to reduce CH_4 yields by 28 to 78%; however, as yet this has not been tested in ruminants fed fresh forage diets. The success of lipid supplementation to reduce CH_4 yield appears to be related to amount of lipid supplemented. With an upper limit of about 9% total lipid in a ruminant diet, the amount of lipid given to animals fed fresh forages (containing approximately 3 to 5% lipid) is limited.

Monensin supplementation has achieved mixed results, with approximately 9% reduction in CH_4 with cattle fed fresh pasture, and a range from 4 to 31% across all diet types. Supplementation with monensin has not been tested in sheep fed fresh forages and this needs to be undertaken before the role of monensin as a mitigation technology can be determined for New Zealand.

The three principal areas for CH_4 mitigation are animal, microbial and nutritional management. However, interactions between these three target areas make it difficult to predict the effectiveness of potential mitigation technologies. Thus, a better understanding of these factors and their interactions are needed before successful mitigation technologies can be developed for on-farm use.

The effect of combining CH_4 mitigation technologies has not yet been investigated. It is not known if a combination of mitigation technologies will have

an additive, neutral or negative impact on CH_4 yield. This is important as it is unlikely that a single technology will be sufficient to mitigate CH_4 emissions in all farm system types and several technologies may be needed.

1.6.1 Aims of this thesis

1. To quantify the influence of red deer age on CH_4 production and yield by measuring CH_4 emissions at two monthly intervals from weaning until slaughter at one year of age.

2. Investigate the use of monensin as a mitigation technology by determining its effect on CH_4 emissions from ewes during early lactation.

3. Determine the effect of combining mitigation technologies, such as the supplementation of monensin and/or coconut oil, on the CH_4 emissions from sheep fed fresh perennial ryegrass-based pasture or chicory.

4. Compare the CH_4 yields from three farmed ruminant species in New Zealand (cattle, sheep and red deer), apparent digestibility (sheep and deer), and rumen fermentation parameters (cattle, sheep and deer) when fed at the same level of maintenance energy requirements in summer and winter.

CHAPTER 2

Methane emissions from red deer (*Cervus elaphus*) stags post-weaning until one year of age grazing perennial ryegrass (*Lolium perenne*) based pasture

Chapter published in-parts in:

Swainson N.M.; Hoskin S.O.; Clark H.; Lopez-Villalobos N. 2007: The effect of age on methane emissions from young, weaned red deer (*Cervus elaphus*) stags grazing perennial-ryegrass (*Lolium perenne*)-based pasture. *New Zealand Journal of Agricultural Research* **50:** *(3),* 407- 416.

Swainson N.M.; Hoskin S.O.; Clark H; Lopez-Villalobos N. 2007: Effect of age on methane emissions of red deer stags from weaning until one year of age grazing perennial ryegrass-based pasture. *Journal of Animal and Feed Sciences Special Issue. Sept:* 16-21. *(*The VII International Symposium on the Nutrition of Herbivores, Beijing China, 16-21 September 2007).

Swainson N.M.; Hoskin S.O.; Clark H.; Lopez-Villalobos N. 2007: Effect of age on methane emissions of red deer stags from weaning until one year of age grazing perennial ryegrass-based pasture. *Proceedings of the New Zealand Society of Animal Production*, **67:** 276-279.

Swainson N.M.; Hoskin S.O; Clark H.; Lopez-Villalobos N. 2007: Effect of age on methane emissions of red deer stags from weaning until one year of age grazing perennial ryegrass-based pasture. *Recent Advances in Animal Nutrition Conference*, Australia, 9-11 July 2007, University of New England, Armidale.

Swainson, N.M; Hoskin S.O.; Clark H. 2005: Methane production by farmed red deer. *Proceedings of a Deer Course for Veterinarians, Deer Branch NZVA Deer Course for Veterinarians No. 22*, Te Anau, New Zealand: 78 -81.

Swainson N.M.: Hoskin S.O.; Clark H.; Lopez-Villalobos N. 2007: Effect of age on methane emissions of red deer stags from weaning until one year of age grazing perennial ryegrass-based pasture. *Greenhouse Gas and Animal Agriculture Conference, Christchurch, November 2007* (Abstract + Poster).

2.1 ABSTRACT

Sheep less than one year of age have been shown to produce approximately 20% less methane (CH_4) per unit of feed intake, referred to as CH_4 yield (g CH_4/kg dry matter intake, DMI), than adult sheep. If CH_4 yield changes with age in all farmed ruminants, this has implications for The New Zealand Greenhouse Gas Inventory. The primary aim of this study was to determine if the CH_4 yields from immature farmed deer grazing pasture increased with age. This study also included a comparison of two methods to determine the rumen-release rate of sulphur hexafluoride (SF_6) gas and the impact this had on estimating CH_4 production (g CH_4/day) using the SF_6 technique. Release rates of SF_6 gas from the rumen-permeation tubes were determined by calibration either pre-deployment-only (referred to as uncorrected SF_6 release rates), or by pre-deployment with adjustment for SF_6 release rates of permeation tubes recovered at the end of the experiment (referred to as corrected SF_6 release rates).

The CH_4 emissions of 20 red deer stags grazing permanent perennial ryegrass-based pasture were determined four times post-weaning, at 4.5, 6.5, 9.0 and 11.5 months of age. Methane yield, calculated using corrected SF_6 release rates, were found to be lower at 4.5 months of age (14.9 g CH_4/kg DMI) than at either 6.5, 9.0 or 11.5 months of age (17.0, 17.4 and 17.7 g CH_4/kg DMI; $P < 0.05$), when DMI was calculated from estimated energy requirements, based on live weight and liveweight gain.

Corrected SF_6 release rates (1.110 mg/day) from retrieved permeation tubes ($n = 13$) were 0.208 mg/day lower at the experimental midpoint than uncorrected SF_6 release rates (1.318 mg/day; $P < 0.05$). As a consequence, the estimated CH_4 production of deer was reduced by 15% when calculated using corrected SF_6 release rates compared with uncorrected SF_6 release rates (33.1 vs. 39.1 g CH_4/day, respectively; $P < 0.001$).

The average (corrected) CH_4 yield (16.8 g CH_4/kg DMI) of immature deer aged 4.5 to 11.5 months of age appears to be appears to be less than that previously

measured in mature deer (22.5 g CH_4/DMI) (Swainson, 2004). However, this needs to be confirmed with measurements from immature deer alongside adult deer consuming the same diet, with accurate measures of DMI. The results from this study imply that the release rate of SF_6 obtained prior to the placement of the permeation tube in the rumen differs from the long-term average SF_6 release rate while the permeation tube resides in the rumen. Therefore using uncorrected SF_6 release rates may give misleading overestimates of CH_4 production.

2.2 INTRODUCTION

Enteric methane (CH_4) emissions from ruminant livestock arise from the enteric fermentation of ingested feed and are a dominant source of non-carbon dioxide greenhouse gas (GHG), representing approximately 31% of New Zealand's total GHG emissions (Anon, 2009). Contributors to ruminant CH_4 emissions, as of 2007 were; dairy cattle 36.6%, non-dairy cattle, 22.6%; sheep, 37.6%; and deer, 2.8% (Anon, 2009). Although total enteric CH_4 emissions from sheep have decreased (22.1%) since 1990, the baseline year of the Kyoto Protocol, CH_4 emissions from all other livestock classes have increased. This has led to an overall increase of 6.9% across the agricultural sector (Anon, 2009). The main cause of increasing CH_4 emissions from ruminant livestock appears to be the combination of increasing livestock numbers and changes in the level of animal performance (Anon, 2009).

The CH_4 yields of adult dairy cattle, sheep and deer, (i.e. animals greater than one year of age) are currently reported in the New Zealand GHG inventory to be 21.6, 20.9, and 21.3 g CH_4/kg DMI, respectively. The CH_4 yield used for deer is an average of adult dairy cattle and sheep. Currently, within the New Zealand GHG inventory CH_4 yields of immature sheep, less than one year of age, (16.8 g CH_4/kg DMI) are recognised as being lower than adult sheep (Anon, 2009). This is based on reports by Lassey *et al.* (1997) and Ulyatt *et al.* (2005) who estimated CH_4 yields using the sulphur hexafluoride (SF_6) technique. Molano *et*

al. (2006) also reported that the mean CH_4 yield of grazing beef cattle aged 6 to 26 months of age was lower (19.5 g CH_4/kg DMI) than that previously estimated for adult cattle. These studies supported the hypothesis that immature ruminants yield less CH_4 than adult animals. Nevertheless, the studies of Molano *et al* (2006), Lassey *et al.* (1997) and Ulyatt *et al.* (2005) did not simultaneously estimate CH_4 emissions from adult and immature animals and did not measure DMI. The only direct comparison between adult and immature ruminants using the SF_6 technique, reported that the average CH_4 yields of lambs (21.9 g CH_4/kg DMI), aged 13, 17, 25 and 35 weeks of age, was up to 8% lower compared with adult sheep (23.8 g CH_4/kg DMI), but the difference was only statistically significant when lambs were 35 weeks of age (17.9 vs. 21.9 g CH_4/kg DMI) (Knight *et al.,* 2008b).

Estimates of CH_4 emissions from immature deer are lacking in the literature, especially when grazing fresh forages. As at June 2005, 38% of the New Zealand deer herd were less than one year of age (Anon, 2006). Consequently, if immature deer produce less CH_4 than adult deer, total CH_4 emissions in the New Zealand GHG inventory for deer may be overestimated.

The SF_6 technique, which has been used extensively to calculate ruminant CH_4 emissions in New Zealand, is a tracer technique that enables the estimation of daily CH_4 production by determining the ratio of the tracer gas (SF_6) to CH_4 gas collected (Lassey *et al.,*1997). The SF_6 technique involves the insertion of a permeation tube, filled with SF_6, into the rumen of the experimental animal. The SF_6 is released as a gas from the permeation tube and acts as a tracer for the CH_4 produced and expelled from animal. Breath samples from individual animals are collected for 24-hour periods for 4 to 5 consecutive days (Lassey *et al.,* 1997).

As the SF_6 gas is a tracer for CH_4, the technique is reliant on the accurate determination of the SF_6 gas released from the permeation tube (referred to as release rate). The release rate of the SF_6 gas is established by the routine weekly weighing of the permeation tube for at least two months, whilst maintained at an air temperature of approximately 39ºC. Only those permeation

tubes with highly linear (R^2 > 0.997) release rates of SF_6 are selected for experimental use. Release rates of SF_6 are established in air and it is unclear what influence the rumen environment has on SF_6 release rate. If the rumen environment changes the release rate of SF_6 (Vlaming, 2008), the accuracy of the calculated CH_4 emissions will also be affected. Lassey *et al.* (2001) compared the release rates of 17 tubes sourced from the same batch. Tubes in the rumen were calculated to have a lower SF_6 release rate than in air, thus resulting in a 15% overestimated in CH_4 production. Further research is needed to confirm that this basis is consistent and if it applies to deer.

The aims of this study were to:

- Measure CH_4 emissions from growing red deer stags, aged four months to one year of age, whilst grazing conventional perennial ryegrass-based pasture.

- Compare CH_4 production in deer using either uncorrected or corrected SF_6 release rates.

2.3 MATERIALS AND METHODS

2.3.1 Experimental design and animals

An experiment to measure the CH_4 production from 20 growing red deer (*Cervus elaphus)* stags was conducted at Massey University's Deer Research Unit, (Palmerston North, New Zealand) from March to October 2004. Methane production was estimated for 5 consecutive days from the same animals at 4.5, 6.5, 9.0 and 11.5 months of age, with measurement periods starting on the 29 March, 24 May, 16 August and 18 October, respectively.

Twenty-five red deer stags were weaned on the 26 February at approximately 3.5 months of age, weighing an average 44.1 ± 6.2 (SD) kg live weight. The age of the deer was based on the mean calving date for that year, as the exact age of each individual animal was not known. From weaning until the first CH_4 measurement period, animals underwent a training period for 3 weeks to become accustomed to normal handling procedures in the yards and the CH_4

measuring equipment. A final selection of 20 deer, based on behaviour, was made 7 days prior to the first CH_4 measurement. From weaning and throughout the experiment, deer were weighed every 2 weeks, at 10am.

During the experiment, four animals were removed; two due to uncertainty regarding the release rates of the SF_6 gas from their permeation tubes, and two to recover apparently normally functioning permeation tubes to re-establish the release rate of SF_6 gas from the permeation tubes halfway through the experiment. From these animals, three were euthanized to recover their permeation tubes.

2.3.2 Diet and dry matter intake

Animals grazed pasture that contained perennial ryegrass (*Lolium perenne* cv Nui) and white clover (*Trifolium repens* cv Huia), consisting of 87% and approximately 2 % of the total available dietary DM, respectively. The remaining dietary DM consisted of weeds (0.8% DM) and dead material (11% DM). For one week prior to, and during each 5 day measurement period, deer grazed the same three paddocks, with an average paddock area of 0.44 hectares. Daily DM allowance was set at 7 kg DM of 'edible' pasture per animal, allowing *ad libitum* intake.

Edible pasture was defined as the total pre-grazing pasture mass (kg DM/paddock) less the proportion of dead material and weeds. Total DM pre-grazing pasture mass was measured by randomly cutting 8 quadrats (0.15 m^2) herbage samples to ground level the day before the animals entered the paddock. Pasture was then washed and dried (24 h at 100 ^oC) to determine available DM (kg DM/ha). This was then multiplied by the paddock size to determine the pre-grazing herbage mass per paddock. The edible proportion of the pasture was determined from eight additional pasture samples, cut from the outside edge of the quadrat (approximately 40 x 10 cm). These samples were then pooled within paddock and a sub-sample taken (~200 grams), which was dissected into 4 components, i.e. grass, clover, dead and weed. Each

component was then washed and dried (24 h at 100 ^{o}C) and the proportions determined from the DM sum of all the components (Adu *et al.*, 1998).

Animals grazed pasture throughout the experiment with the exception of winter (1 June to 31 August) when pasture supply was limited. Therefore deer received ensiled pasture baleage as a supplement (approximately 0.6 kg DM per deer per day) to maintain *ad libitum* intakes. For the ten days prior to and during the measurement period in August, when deer were 9.0 months of age, animals were fed *ad-libitum* pasture-only.

Dry matter intake was estimated by the calculating the energy requirements for maintenance and liveweight gain for each deer. The mean liveweight gain for the group of deer within each season was determined from linear regression equations of live weight against time. To account for changes in deer metabolism due to day-length, the energy requirement for maintenance was adjusted according to each season (Equations 1 to 3; Fennessy *et al.*, 1981; Suttie *et al.*, 1987).

Autumn (20 March – 23 May)

$MR = 0.74 \text{ MJ ME / BW kg}^{0.75} \text{ / day}$ Equation 1

(MR, Maintenance requirements; BW, body weight)

Winter (24 May–31 August)

$MR = 0.85 \text{ MJ ME / kg}^{0.75} \text{ / day}$ Equation 2

Spring (1 September – 9 December)

$MR = 0.68 \text{ MJ ME / kg}^{0.75} \text{ / day}$ Equation 3

The energy requirement for liveweight gain was assumed to be 37 MJ ME/kg liveweight gain (Fennessy *et al.*, 1981; Suttie *et al.*, 1987). The total metabolisable energy (ME) required per day by each individual deer was then divided by the estimated dietary ME concentration (MJ ME/kg DM), to calculate DMI.

The ME concentration of the diet in each CH_4 measurement period was determined by daily forage sampling for five days. Samples were collected by hand plucking pasture to grazing height, focussing on the areas that animals were most often observed to graze, until approximately 500 g fresh weight was collected (Semiadi *et al.,* 1993). Once collected samples were washed and stored frozen at –20 ^{o}C until required for chemical composition analysis.

2.3.3 Methane measurements

Daily CH_4 emissions of deer were calculated using the SF_6 tracer technique. This technique was developed by Johnson *et al.* (1994) and applied for use in New Zealand (Lassey *et al.,* 1997; Ulyatt *et al.*, 1999). The SF_6 technique utilises the dilution of SF_6 in gases expired or eructed from the mouth and nose of an animal to calculate the emission of CH_4. The method assumes firstly, that the SF_6 gas is inert and as such there are no detrimental effects or interactions with substances in the animal or experimental environment. Secondly, the method assumes that the emission of SF_6 exactly simulates that of CH_4 and therefore the rate of dilution of the two gases are identical. This assumes that gases are eructated from the digestive tract by force and therefore dilution by turbulence is more important than dilution by molecular diffusion and that more than 95% of the gases produced by enteric fermentation are expelled through mouth and nose (Murray *et al.,* 1976). Thirdly, it is assumed that the release of the SF_6 gas from the permeation tube is at a constant and known rate.

Breath samples from the deer were continuously collected from around the mouth and nose for 24-hours periods over five consecutive days. To enable breath sampling, each deer wore a modified halter and a pre-evacuated polyvinyl chloride (PVC) yoke that was attached to a harness (Plate 2.1a & b). The halter had tubing attached to it, with one end resting above the nose and the other end connected to the yoke via a QuickConnect® valve. The flow of air into the yoke was controlled by a short length of small-diameter metal capillary tubing, situated on the halter. Yokes were changed each morning at approximately 8.30 am and the pressure was checked. Yokes were considered to have a successful breath sample when the pressure in the yoke was between

0.4 and 0.8 of atmospheric pressure. In addition, two background samples to determine ambient air concentrations of both SF_6 and CH_4 were collected daily.

For each yoke a gas sample was extracted and analysed by gas chromatography (Hewlett Packard 5890 Series II) using flame ionisation and electron capture detectors to determine the concentration of CH_4 and SF_6 gases, respectively (Lassey *et al.,* 1997). Accuracy and consistency of gas analysis was based on a calibration curve of three standard gas mixtures (New Zealand Institute of Water and Atmospheric Research), where concentrations of SF_6 and CH_4 ranged from 15 – 1000ppt and 2 – 200ppm, respectively. All three standards were run at the start and end of each day of sample analysis and the mid-range gas standard was run after every tenth experimental sample, to account for any drift in the detection of SF_6 or CH_4.

The SF_6 permeation tubes were provided by New Zealand Institute of Water and Atmospheric Research. This is a brass tube around 30 mm in length that has a Swagelok® nut fitted on one end. The escape of SF_6 from the permeation tube is restricted by a Teflon® membrane. A stainless steel frit and nylon washer were placed either side of the Teflon® membrane to protect it from the internal pressure and distortion during the tightening of the Swagelok® nut. The permeation tubes were charged at liquid nitrogen temperature with 0.8 to 0.9 grams (sheep) SF_6 liquid.

The permeation tubes used in this experiment were all from the same batch and were filled with SF_6 gas on the 5th February 2004. After charging with SF_6 the permeation tubes were maintained at a 39°C dry air environment and individually weighed weekly for 8 weeks prior to placement in the animal. A linear regression was fitted to the weights of each tube to determine the SF_6 release rate for each tube. Only those tubes that had a linear regression fit of R^2 > 0.998 were used. The mean release rate of SF_6 for all 20 deer at the start of the experiment was 1.24 ± 0.356 (SD) mg/day. Seven days prior to the first CH_4 measurement, a permeation tube was inserted into the rumen of each deer via the mouth using an applicator gun.

Total CH_4 production (Q_{CH4}; g/day) for each animal in a 24 hour period is calculated from the mixing ratio (µmol/mol) of SF_6 to CH_4 gas concentrations in the breath sample and the background concentrations of CH_4 and SF_6 (C^b_{CH4} and C^b_{SF6}). These concentrations are expressed in relation to SF_6 gas (µmol/day (Q_{SF6})) release from the permeation tubes per day where MW is the molecular weight of the gases (Equation 4). Methane production and yield were calculated from the mean CH_4 production rate across the five-day collection period.

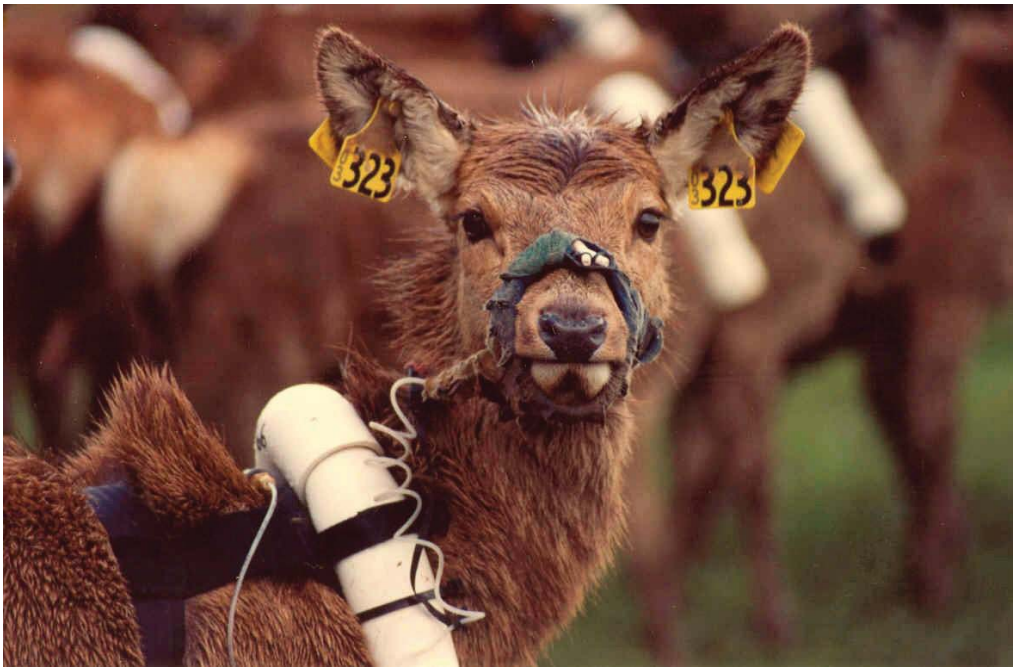
$$Q_{CH4} = ((C_{CH4}-C^b_{CH4})/(C_{SF6} - C^b_{SF6})) \times Q_{SF6} \times (MW_{CH4} / MW_{SF6}) \quad \text{Equation 4}$$

2.3.3.1 SF_6 release rate correction

Permeation tubes were recovered from 13 deer after slaughter. Ten tubes were recovered shortly after the final CH_4 measurement (October), and three after the second CH_4 measurement (May). The three permeation tubes recovered in May included two from deer whose permeation tubes appeared to be emitting SF_6 gas, and one from an animal with a permeation tube that did not appear to be emitting SF_6 gas. This latter permeation tube appeared to be emitting SF_6 gas when monitored in the laboratory after retrieval. Release rates of SF_6 gas from the recovered permeation tubes were re-determined. This involved cleaning the tube, with water, and reweighing each tube weekly for at least 4 weeks. After retrieval from an animal, the regression of the SF_6 release rates based on emissions rates before and after placement in the rumen was no longer linear and therefore a curvilinear linear regression was applied to determine the 'new' release rate of SF_6 during the measurement periods (Lassey *et al.,* 2001). Methane emissions calculated using the corrected SF_6 release rate or the uncorrected release rates were compared. Seven permeation tubes were unable to be retrieved as these deer were retained for future experiments. For these tubes a mean correction factor based on the curvilinear regression curves was applied (Lassey *et al.,* 2001) for each measurement period.

Plate 2.1 (a) & (b): Methane (CH_4) collection equipment, halter and yoke, used to collect breath samples from red deer during CH_4 measurements, when deer are standing (a) and grazing (b).

(a)



(b)

2.3.4 Chemical analysis and forage sampling

All pasture samples were oven dried at 60 ^{o}C for 48 h, ground to pass through a 1 mm sieve mill, and then analysed for chemical composition by near-infrared spectroscopy (NIRS, FeedTEC, AgResearch Ltd, Palmerston North, New Zealand; Corson *et al.*, 1999).

2.3.5 Statistical analysis

All statistical analyses were performed with SAS (Statistical Analysis System, 2003, version 9.1; SAS Institute Inc., Cary, NC, USA) using the MIXED procedure. Significance was declared at $P \leq 0.05$ and a trend reported if $0.05 < P \leq 0.10$.

Methane production, CH_4 yield and DMI were analysed with a mixed linear model that included the fixed effect of age. The chemical composition of the pasture samples were analysed with a similar model, but the fixed effect of age was replaced by time. A compound symmetry error structure (Kuehl, 2000) was determined as the most appropriate residual covariance structure for repeated measures over time within animals. Botanical composition of the pasture over time was analysed with a mixed linear model after data was transformed by arcsin square root transformation (Bromiley & Thacker, 2002), as the data did not appear normally distributed.

2.4 RESULTS

2.4.1 Dietary chemical and botanical composition

The chemical composition of the diet is shown in Table 2.1. The concentrations of, ash, acid detergent fibre (ADF), neutral detergent fibre (NDF), lignin, predicted organic matter digestibility (OMD) and ME content changed ($P < 0.05$) between CH_4 measurement periods. The concentration of ash increased from the first measurement period (March) to the last measurement period (October).

Both NDF and ADF concentrations were highest in August, when deer where 9.0 months of age. Organic matter digestibility and pasture ME concentration was greatest in May and October. In contrast, the dietary concentrations of lignin did not change significantly between measurement periods, but starch and soluble sugars, and lipids tended to differ between measurement periods.

The botanical composition of the pasture offered during each measurement period is shown in Table 2.2. The proportion of weed (0.002 to 0.02) and clover (0.01 to 0.03) remained relatively minor and did not change ($P > 0.1$) over time. In contrast, the dead material and ryegrass components of the pasture changed ($P < 0.05$) with time, with a higher proportion of ryegrass and a lower proportion of dead material in October compared with March, May and August.

Table 2.1: Chemical composition (g/kg dry matter, DM) of pasture offered during the five day methane measurement periods when deer were 4.5, 6.5, 9.0 and 11.5 months of age.

	March[6]	May	August	October	Average	P-Value
Age (months)	**4.5**	**6.5**	**9.0**	**11.5**	**SEM**	
Ash	10.3[a]	10.4[a]	10.5[a]	11.1[b]	0.17	0.010
ADF[1]	193[ab]	174[a]	202[b]	187[ab]	6.0	0.002
NDF[2]	376[ab]	344[a]	390[b]	381[ab]	10.1	0.034
Lignin	17	10	14	11	2.5	0.051
SSS[3]	121	124	114	109	7.6	0.491
Crude protein	247	266	251	288	11.3	0.130
Lipid	40	41	42	46	1.9	0.094
OMD[4] (%)	82.0[a]	84.7[b]	82.9[a]	85.0[b]	0.53	0.002
ME [5]	11.9a	12.5[b]	12.2[a]	12.6[b]	0.10	0.001

[1]Acid detergent fibre.

[2]Neutral detergent fibre.

[3]Starch and soluble sugars.

[4]Organic matter digestibility.

[5]Metabolisable energy (MJ/kg DM).

[6] n = 4 for each measurement period.

[abc] Differing letters within rows denote a significant difference of the means ($P < 0.05$).

Table 2.2: Botanical composition (clover, dead, ryegrass and weeds) expressed as a proportion of the total sample DM of the pasture during the 5 day methane measurement periods when deer were 4.5, 6.5, 9.0 and 11.5 months of age.

	March	May	August	October	SEM	P-value
Age (months)	**4.5**	**6.5**	**9.0**	**11.5**		
Clover	0.02	0.02	0.01	0.00	0.010	0.407
Dead	0.15[a]	0.16[a]	0.09[b]	0.04[b]	0.005	0.005
Ryegrass	0.82[a]	0.81[a]	0.89[ab]	0.94[b]	0.027	0.021
Weed	0.00	0.00	0.01	0.02	0.017	0.177

[ab] Means with differing letters denotes a significant difference between means within rows ($P < 0.05$).

2.4.2 Live weight and liveweight gain

The mean live weight of the deer at the start of each measurement period and overall liveweight gain is shown in Table 2.3. Age/season had a significant ($P < 0.001$) effect on liveweight gain with lower growth rates in winter, at 9.0 months of age (69 g/day) compared with early- autumn (160 g/day) and late autumn (169 g/day) and spring and (266 g/day) months of age. Animals grew at a greater ($P = 0.001$) rate in spring at 11.5 months of age than in autumn at 4.5 or 6.5 months of age and there was no difference ($P > 0.1$) between the rate of growth between deer at 4.5 and 6.5 months of age.

2.4.3 Dry matter intake

The mean DMI from estimated from energy requirements and dietary ME concentration (Table 2.3) increased significantly ($P < 0.001$) as deer aged and grew. An exception to this was at 9.0 months of age (1.85 kg DM/day) when the DMI of deer tended to be similar ($P = 0.07$) to that at 6.5 months of age (1.93 kg DM/day). Despite a greater live weight (67.1 kg versus 58.3 kg), the 9-month old deer had lower liveweight gains (69 vs. 169 g/day at 6.5 months of age), due to reduced day-length in winter.

Table 2.3: Live weight (kg), liveweight gain (g/day) and calculated dry matter (DM) intake (DMI; kg/day) of red deer stags at 4.5, 6.5, 9.0 and 11.5 months of age when grazing perennial ryegrass pasture.

	March		May		August		October		
Age (months)	**4.5**	**SEM**	**6.5**	**SEM**	**9.0**	**SEM**	**11.5**	**SEM**	**P-value**
Live weight (kg)	48.3[a]	1.72	58.3[b]	1.72	67.1[c]	1.87	83.5[d]	1.87	0.001
Liveweight gain (g/day)[1]	160[a]	13.5	169[a]	13.5	69[c]	14.7	266[d]	14.7	0.001
DMI_2 (kg/day)	1.66[a]	0.030	1.93[b]	0.033	1.85[b]	0.036	2.27[c]	0.028	0.001
DMI g/kg of metabolic live weight	90[a]	0.6	92[b]	0.6	81[c]	0.6	82[c]	0.6	0.001

[1] Determined by linear regression of deer liveweight within each season. Age relating to seasons are; early-autumn, 4.5 months of age (n = 20); late-autumn, 6.5 months of age (n = 18); winter, 9 months of age (n = 16); and spring, 11.5 months of age (n = 16).

[2]DMI calculated from energy requirements for maintenance and growth (Fennessy *et al.*, 1981; Suttie *et al.*, 1987).

[abc] Differing letters within rows denotes a significant difference between means ($P < 0.05$).

2.4.4 Methane production and yield

2.4.4.1 SF_6 release rates

The release rates of SF_6 were re-determined from permeation tubes recovered after slaughter from a sub-set of the deer (n = 13) to investigate the effect of permeation tube age/residence time on the release rate and calculated CH_4 production. Each measurement period mid-point was related to permeation tube age. Permeation tube age was defined as the number of days since the tube was charged with SF_6. The post slaughter corrected SF_6 release rates were used to adjust the uncorrected SF_6 release rates and calculate a new corrected SF_6 release rate, whilst the permeation tube resided in the rumen. The corrected SF_6 release rates at the mid-point (1.110 mg/day, permeation tube age 115 days) and end (1.021 mg/day, permeation tube age 258 days) of the experiment were lower than the uncorrected release rates determined before the permeation tubes were placed into the rumen (1.318 mg/day, permeation tube age 46 days; $P < 0.05$) (Table 2.4 and Appendix Table 2.8).

The mean CH_4 production, from the deer (n = 13), calculated using the corrected SF_6 release rates were 6 g CH_4/day lower than when uncorrected SF_6 release rates were used (i.e. corrected, 33.1 vs. uncorrected, 39.1 g CH_4/day; $P < 0.001$) (Table 2.5 and Appendix Table 2.9). The difference between CH_4 production calculated using corrected or uncorrected release rates increased the longer the permeation tube resided within the rumen. During the first three measurement periods (deer ages 4.5, 6.5, and 9.0 months) CH_4 production calculated using corrected release rates (25.6, 34.1, and 33.2 g CH_4/day, respectively) were not significantly lower than when uncorrected release rates were used (28.2, 39.0, and 39.1 g CH_4/day, respectively) . However, the percentage difference of CH_4 production calculated using corrected or uncorrected release rated increased from 9% to 15.3%. The CH_4 production calculated from corrected release rates (39.6 g CH_4/day) when deer where 11.5 months of age was significantly lower (20.5%; $P < 0.03$) than CH_4 production calculated using uncorrected release rates (49.8 g CH_4/day). There was a

tendency towards an interaction (P = 0.097) between deer age and SF_6 release rate (corrected or uncorrected).

Table 2.4: Uncorrected (P_{uncorr}) and corrected (P_{corr}) release rates from permeation tubes (n = 13) during methane measurement periods when deer were 4.5, 6.5, 9.0 and 11.5 months of age.

Deer age	Permeation tube age	Mean	Range	SD[1]	CV[2]
	P_{uncorr}[3], days 15-46 (February – March)	1.318[a]	0.827-1.858	0.306	23.2
4.5	P_{corr}[4], day 55 (March)	1.148[ab]	0.767-1.682	0.250	21.8
6.5	P_{corr}[4], day 111 (May)	1.111[b]	0.729-1.599	0.253	21.2
	P_{corr}[4], day 115 (exp. midpoint)	1.110[b]	0.758-1.593	0.230	20.7
9.0	P_{corr}[4], day 195 (August)	1.061[b]	0.746-1.473	0.211	19.9
11.5	P_{corr}[4], day 258 (October)	1.021[b]	0.737-1.380	0.202	19.8
	P-value	0.0486			

[1]Standard deviation

[2]Coefficient of variation (= SD/Mean) (%)

[3]P_{uncorr} is the SF_6 release rate determined prior to insertion into the rumen (uncorrected) (mg/day) based on linear regression based on weights measured over 31 days

[4]P_{corr} is the corrected SF_6 release rate (mg/day) based on the midpoint of each methane measurement period and the experimental midpoint. This is calculated from the slope of each quadratic regression fit (Lassey *et al.,* 2001).

[ab] Differing letters within the same column denote significant differences between means (P < 0.05).

Table 2.5: Methane (CH_4) production (g CH_4/day) of red deer stags calculated using uncorrected and corrected SF_6 release rates when deer were 4.5, 6.5, 9.0 and 11.5 months of age.

Deer age (month)	Uncorrected (A)	Corrected (B)	SEM	CH_4 g/day P-value	n	% difference[#]
Overall mean	39.1[a]	33.1[b]	0.77	0.001	13	15.3
4.5 (March[*])	28.2[1a]	25.6[1a]	1.04	0.086	13	9.4
6.5 (May)	39.0[2a]	34.1[2a]	2.36	0.153	11	12.6
9.0 (August)	39.1[2a]	33.2[2a]	2.44	0.102	11	15.3
11.5 (October)	49.8[3a]	39.6[3b]	2.98	0.026	11	20.5
Age P-value	0.0001	0.0001		0.097		

[*]CH_4 measurement period when CH_4 was measured from deer. Deer age (months) was 4.5, 6.5, 9.0 and 11.5 months of age and corresponded to permeation tube age of 55, 111, 195, and 258 days.

[ab] Denotes significant differences between means within the same row (P < 0.05).

[123] Denotes significant differences between means within the same column (P < 0.05).

[#] Percentage (%) difference = ((A-B)/A) x 100

2.4.4.2 Methane production and yield

Methane emissions reported in this section and presented in Table 2.6 are calculated using corrected SF_6 release rates[1] and include estimates of CH_4 from all 20 deer. The mean CH_4 production of deer was found to increase ($P < 0.001$) as the deer aged and grew; 4.5 < 6.5 = 9.0 < 11.5 months of age (24.6, 32.8, 32.3, and 40.2 g CH_4/day, respectively). The CH_4 yield of the deer was lower ($P < 0.001$) at 4.5 months of age (14.9 g CH_4/kg DMI) compared with 6.5 , 9.0 and 11.5 months of age (17.0, 17.4, and 17.7 g CH_4/kg DMI, respectively), which did not differ significantly ($P > 0.1$).

Table 2.6: Dry matter (DM) intake (DMI; kg/day) and methane (CH_4) production (g CH_4/day) and yield (g CH_4/kg DMI) based on corrected SF_6 reelase rates of 20 red deer stags, at 4.5, 6.5, 9.0 and 11.5 months of age, grazing perennial ryegrass pasture.

	Age (months)[2]								
	4.5	**SEM**	**6.5**	**SEM**	**9.0**	**SEM**	**11.5**	**SEM**	**P-value**
DMI [1]	1.66[a]	0.030	1.93[b]	0.033	1.85[b]	0.036	2.27[c]	0.028	0.001
CH_4 production	24.6[a]	1.12	32.8[b]	1.62	32.3[b]	1.79	40.1[c]	1.68	0.001
CH_4 yield	14.9[a]	0.58	17.0[b]	0.72	17.4[b]	0.85	17.7[b]	0.75	0.001

[1]DMI based on calculations of energy requirements for maintenance and growth

[2] Deer age months, 4.5 (March) n = 20; 6.5 (May) n = 18; 9. (August) n = 16; 11.5 (October) n = 16

[abc] Differing letters within rows denotes a significant difference between means ($P < 0.05$)

[1]Corrected release rates of SF_6 as determine from permeation retrieval or for those deer whose permeation tubes were not retrieved release rates of SF_6 was corrected based on the mean correction factor calculated from retrieved tubes.

2.5 DISCUSSION

2.5.1 Methane yield

The total CH_4 production of immature red deer increased with age and accompanying increases in DMI and live weight. Methane yield was shown to be influenced by age, due to deer at 4.5 months of age (14.9 g CH_4/kg DMI) having a lower CH_4 yield compared with deer aged 6.5, 9.0 and 11.5 months of age (17.0, 17.4 and 17.7 g CH_4/kg DMI, respectively). Nevertheless, the effect of age on CH_4 yield in this study cannot be separated from the method used to calculate DMI, as any errors in estimating the DMI of the deer while grazing would have influenced the findings presented here.

The average CH_4 yield of immature deer (16.8 g CH_4/kg DMI) reported in this study are lower than previous values obtained from adult red deer hinds grazing pasture (37.8 g CH_4/kg DMI) and adult deer fed pasture and housed indoors (22.5 g CH_4/kg DMI) (Swainson, 2004). The total number of studies that have measured CH_4 yield from red deer using the SF_6 technique is limited to those mentioned above. Therefore, it is impossible to quantify if the CH_4 yields of deer presented in this study are statistically different to those of adult deer.

The current finding that the CH_4 yields of deer appeared to be influenced by age agrees with previous findings with sheep less than one year of age (18.9 g CH_4/kg DMI, Lassey *et al.,* 1997; 14.8 g CH_4/kg DMI, Ulyatt *et al.*, 2005) and and beef cattle six to 24 months of age (mean 19.5 g CH_4/kg DMI, Molano *et al.,* 2006). These values are lower than CH_4 yields reported in adult sheep (20.9 g CH_4/kg DMI) and cattle (21.6 g CH_4/kg DMI) (Anon, 2009), as shown in Table 2.7. Nevertheless, in these studies DMI was not measured directly, but instead DMI was estimated and CH_4 production from adult animals was not concurrently measured.

The only direct comparison of immature sheep (13, 17, 25 and 35 weeks of age) and adult sheep (ewes 3 to 4 years of age), all fed pasture indoors at 1.5 times maintenance, was reported by Knight *et al.* (2008b). In this study, the CH_4

yields of the lambs were only significantly lower than those of ewes, when lambs were 35 weeks of age.

Measurements of CH_4 conducted with sheep and cattle using respiration chambers have not identified any difference in CH_4 yield between young and mature animals (Graham, 1980; Pelchen & Peters, 1998; Ramírez-Restrepo *et al.,* 2009). For example, Graham (1980) measured CH_4 emissions, expressed as proportion of dietary energy intake from immature sheep, at 2, 4, and 10 months of age, and adult sheep fed a conserved forage/grain-based diet and found that age did not affect CH_4 emissions. In agreement with this, Ramírez-Restrepo *et al.* (2009) found no difference between the CH_4 yield of immature dairy heifers (3.7 to 10 months of age, mean 24.5 g CH_4/kg DMI) and mature dairy cows (6.8 years of age, 24.0 g CH_4/kg DMI) fed an ensiled ryegrass diet, using either respiration chambers or the SF_6 technique (Table 2.7).

Table 2.7. Dry matter intake (DMI kg/day) and methane (CH_4) yields (CH_4y, g CH_4/kg DMI) from young and old sheep, cattle and deer.

Animal	Diet	Method CH4	Age (months)/DMI/ CH4 yield						Reference
Sheep	Pasture*	SF_6	Age	8					
			DMI†	1.214	1.264	1.334			
			CH4y	17.7	19.0	20.1			Lassey et al., 1997
Sheep	Pasture	SF_6	Age	8	9	7	>24		
			DMI†	1.389	1.704	1.206	1.685‡		
			CH4y	13.8	12.9	17.8	21.1		Ulyatt et al., 2005
Sheep	Pasture	SF_6	Age	3.1	4.1	6.3	8.8		
			DMI§ (ewes, mature)	0.98a	0.86b	0.89b	1.01a		
			DMI§ (lambs)	0.51d	0.52d	0.69c	0.78bc		
			CH_4y (ewes)	22.7bc	25.1a	25.4a	21.9bc		
			CH_4y (lambs)	20.9cd	23.3ab	25.4a	17.9de		Knight et al., 2008b
Cattle	Pasture	SF_6	Age	6	11	13	15	23	26
			DMI††	3.6	5.4	6.2	10.7	10.2	12.0
			CH_4y	26.1	18.3	18.3	17.9	17.3	19.0	Molano et al., 2006
Cattle	Chaffage**	SF_6	Age	3.7	7.1	9.1	10.1		
			DMI§ (cows > 6.8 years)	5.60	6.62	6.64	5.72		
			DMI§ (calves)	1.64	2.17	2.32	3.09		
			CH4y (cows > 6.8 years)	21.7	21.7	26.9	27.3		
			CH_4y (calves)	23.8	28.1	23.9	25.3		
		Chambers		8.4	10.1	13.3	14.5		
			DMI§ (cows > 6.8 years)	6.15	5.65	5.05	6.08		
			DMI§ (calves)	2.09	3.07	2.92	3.76		
			CH4y (cows > 6.8 years)	24.3	24.8	20.71	24.8		
			CH4y (calves)	23.3	24.8	23.72	23.2		Ramírez-Resptrepo et al., 2009
Deer	Pasture	SF_6	Age	4.5	6.5	9.0	11.5		
			DMI††	1.66a	1.93b	1.85b	2.27c		
			CH_4y	14.9a	17.0b	17.4b	17.7b		Chapter 2

*Pasture consisting of predominantly perennial ryegrass (*Lolium perenne*) and white clover (*Trifolium repens*)

** Ensiled ryegrass chaffage.

†DMI calculated by total faecal collections and *in vitro* estimates of dry matter digestibility.

††DMI calculated by estimating energy requirements for animal age, live weight and liveweight gain.

‡DMI estimated using the n-alkane technique

§DMI measured directly from animals housed indoors

Changes in the chemical composition of pasture make it impossible to isolate the effect of animal age on CH_4 yields presented in this study (deer) and others (Lassey *et al.,* 1997; Ulyatt *et al.,* 2005; Molano *et al.,* 2006; Knight *et al.,* 2008b) with sheep and cattle. Nevertheless, based on an analysis of the New Zealand Enteric CH_4 database, no significant relationship has been found between CH_4 yield (sheep and cattle) and the chemical composition of fresh forage diets (Waghorn & Woodward, 2006; Hammond *et al.,* 2009). In addition, changes in pasture dry matter digestibility (vegetative state: 75.3% versus reproductive state: 62.5%) had no impact upon the CH_4 yields of sheep (22.9 vs. 23.7 g CH_4/kg DMI; Molano & Clark, 2008). The minor differences in pasture chemical composition between seasons reported in this study are therefore unlikely to have a large impact on the CH_4 yield from deer.

Although, the chemical composition of fresh pasture does not appear to have a clearly defined impact on CH_4 yield, it may contribute towards some of the variance of CH_4 yield in immature animals. Knight *et al.* (2008b) reported that diet quality, particularly the percentage of NDF in the pasture (ranging from 46.4 to 55.5% DM), had a greater effect on the variation in CH_4 yield from lambs (41.9%, with a range of 17.9 to 25.4 g CH_4/kg DMI) compared with ewes (16.0%, with a range of 21.9 to 25.4 g CH_4/kg DMI) when fed the same diet. This suggests that dietary components may have a greater influence on CH_4 yield in immature ruminants compared with adults, but needs to be confirmed with further research.

The reasons for reduced CH_4 yield in immature ruminants compared with older ruminants are not clear, and could be attributed to either rumen microbial populations, animal factors or an artefact of the method used to calculate DMI. Microbial populations in the rumen are established at an early age with cellulolytic bacteria (Anderson *et al.,* 1987; Fonty *et al.,* 1987) and methanogens are present within the first week of life (Anderson *et al.,* 1987; Skillman *et al.,* 2004). Within 3 weeks of birth, flock-reared lambs appeared to have cellulolytic and methanogenic bacteria population densities similar to those found in the mature ruminant (Fonty *et al.,* 1987). Joyce and Rattray (1970) reported that the

rumen fluid inocula taken from lambs under 3 weeks of age resulted in lower *in vitro* digestibilities of hay and grass, compared with rumen inocula taken from adult sheep, but there was no difference when rumen fluid was taken from lambs aged 3 weeks or older. As the deer in this study were 18 weeks (4.5 months) of age or greater, it is unlikely the microbial population could be responsible for the lower CH_4 yield of deer at 4.5 months of age.

No clear evidence from the literature is available to suggest whether animal factors are responsible for reduced CH_4 yield in immature ruminants. By 8 weeks of age, the digestive tract of lambs is considered to function similarly to that of a mature sheep (Wardorp & Coombe, 1961). The rumen function of bovine calves, with unlimited access to roughage, reached functional maturity by 8 weeks of age, with volatile fatty acid (VFA) concentrations stable from 5 weeks of age onwards (Godfrey, 1961b). Nevertheless, examination of the development of the bovine calf digestive tract showed that the reticulo-rumen and the omasum was still growing by 17 weeks of age, although the abomasum was developed by 5 weeks of age (Godfrey, 1961a). In addition, Hammond *et al.* (2008) reported that the digestive tract of deer continued to develop beyond 3 months of age, with development continuing up to 1 year of age, with the greatest growth of the rumen occurring between 8 and 12 weeks of age.

Methane yield has been inversely related to the fractional outflow rate of the particulate phase of digesta and the buffering capacity of the rumen (Okine *et al.,* 1989; Pinares-Patiño *et al.,* 2003b). Therefore, a difference in digesta passage rate between immature and mature ruminants could explain the difference in CH_4 yield with age. Although there is some published evidence to support this theory, reports of differences in digesta passage rate between immature and mature ruminants are not consistent. For example, bovine calves (14 weeks of age) were reported to have shorter mean digesta retention times compared with calves at 20 to 38 weeks of age (2 hours; Leibhoiz, 1991) when fed roughage diets and housed indoors. Nevertheless, Weston & Margan (1979) reported no differences in the digesta kinetics of lambs aged 15, 24 and 40 weeks of age when fed a dried clover diet.

Changes in the site of digestion could explain the apparently lower CH_4 yield of young animals. Methane appears to be produced at a lower rate per unit of feed fermented in the hindgut compared with the rumen (Torrent & Johnson, 1994); however this has not been clearly demonstrated with either changes in diet and ruminant age. For lambs fed milk and pasture up until 8 weeks of age and fed pasture or a pelleted TMR diet thereafter and receiving milk, the percentage of total volatile fatty acids (VFAs) originating from the rumen was 32, 87 and 92% at 1, 11 and 21 weeks of age, respectively, with the remaining VFAs of caecal and large intestinal origin (Oh *et al.,* 1972). Krehbiel et al. (2000) found that the apparent rumen OMD in yearling cattle fed a high-grain diet was greater compared with that of calves (age not stated); however, post-ruminal disappearance of organic matter was greater in calves than in yearlings. There appears to be no information in the literature addressing the changes in the site of digestion or digestion efficiency with age in deer. As deer were at least 14 weeks of age in this study, it is unlikely that a shift in the site of digestion, as the digestive tract develops, would have resulted in a large enough change of CH_4 yield to be detected using the SF_6 technique.

The significantly lower CH_4 yield of deer at 4.5 months of age, compared with deer at 6.5, 9.0 and 11.5 months of age, may simply be an artefact of the method used to estimate intake. The intake of grazing animals cannot be measured directly and therefore must be estimated or calculated. In this study this was achieved by calculating each deer's energy requirement based on metabolic body weight and liveweight gain. The equations used to calculate the energy requirements for maintenance and growth were based on those of Fennessy *et al.*, (1981) and Suttie *et al.* (1987) and were developed in New Zealand from deer fed indoors or outdoors in pens. Any inaccuracies in determining either live weight or liveweight gain would have a significant influence on the calculated CH_4 yield.

Liveweight gain is difficult to accurately and consistently determine for short time periods. Any variation on the digestive tract fill or accumulation of mud and water on the animal will influence measurements of live weight. To overcome

this liveweight gain was determined the by linear regression of live weight against time. However, any short-term reductions of feed intake, and hence liveweight gain, arising from deer wearing the breath collection equipment would not be detected. As a consequence estimated DMI could have been overestimated. As CH_4 yield is equally reliant on the accurate measurement of both CH_4 production and DMI, the the lower CH_4 yield of deer at 4.5 months of age could be due to an overestimation of DMI resulting in an underestimation of CH_4 yield.

The nutrition and biology of temperate deer species (e.g. red deer), unlike domesticated sheep and cattle, are strongly influenced by day length, as an adaptation to the temperate environment where these species of deer evolved. Phenotypic expressions of adaptation to temperate environments are evident in strong seasonal patterns of liveweight gain, voluntary feed intake, digestive physiology and reproduction (Fennessy *et al.*, 1981; Suttie *et al.*, 1987; Barry *et al.,* 1991; Domingue *et al.,* 1991). The equations of Fennessy *et al.* (1981) and Suttie *et al.* (1987) attempt to account for these changes in seasonality, by having a different multiplying factor for metabolic live weight for calculating maintenance energy requirements in each season (see Equations 1-3 in Section 2.3.2). These equations are the only available estimates of deer metabolic energy requirements, based upon experimental measurements.

The patterns of deer live weight from weaning until the termination of the experiment, as presented in Table 2.3, followed the typical patterns reported in the literature, in that liveweight gain is slowest during winter and greatest during spring (Fennessy *et al.*, 1981; Suttie *et al.*, 1987; Barry *et al.,* 1991). The change in deer live weight was (69 g/day) in winter and (266 g/day) in spring, with the liveweight gain being intermediate for summer and autumn (160 and 169 g/day).

2.5.2 SF_6 release rate

The 15% difference in CH_4 production, calculated using uncorrected release rates of SF_6 gas, compared with corrected release rates from recovered tubes,

agree with the findings of Lassey *et al.* (2001) and Pinares-Patino *et al.* (2008). In addition, this study demonstrated that that the size of the error increased from 9.4% at a tube age of 55 days (approximately 16 days after tube insertion into the animal) to 20.5% at a permeation tube age of 258 days (approximately 219 days after tube insertion into the animal). Unfortunately, at the time this experiment was conducted, there were no facilities to measure CH_4 and SF_6 production from deer using respiration chambers. Without a total collection of SF_6 gas from the deer, there is some uncertainty regarding the accuracy of either method of predicting SF_6 release rate whilst the permeation tube is in the rumen.

The 22.5% difference between uncorrected and corrected SF_6 release rates (1.310 vs. 1.021 mg/day at 258 days of permeation tube age, respectively) reported in this study was similar to the 22.2% difference reported for sheep by Pinares-Patiño *et al.* (2008), after the permeation tube had resided in the rumen for 250 days. In the study of Pinares-Patiño *et al.* (2008), total CH_4 production was measured using respiration chambers, which enabled the calculation of SF_6 release rates whilst in the rumen. The SF_6 release rate calculated from chamber concentrations of SF_6 was closest to that of corrected SF_6 release rates. Methane production from chamber measurements (18.3 g CH_4/day) was not significantly different from estimates using corrected SF_6 release rates (19.5 g CH_4/day), but were significantly lower than uncorrected SF_6 release rate estimates (27.0 g CH_4/day) (Pinares-Patiño *et al*., 2008). This indicates that corrected release rates are more representative of the SF_6 release rate in the rumen and that these values are not influenced by ruminant species.

The coefficient of variation in SF_6 release rate for 13 permeation tubes did not increase from pre-insertion till recovery at slaughter, and ranged from 19.8% to 23.2%. However, the standard error of the mean for CH_4 production did increase from 1.04 g CH_4/day at 55 day of permeation tube age to 2.98 g CH_4/day at 258 days (Table 2.5), suggesting the estimated CH_4 production became more variable as the permeation tubes aged. This indicates that the increased variability of CH_4 production was due to permeation tube age and/or

residence time in the rumen. This increase in variability indicates that the statistical power to detect a significant difference between two means is reduced with time.

A retrospective power analysis of CH_4 production using the means and standard deviations of CH_4 production based on uncorrected release rates of SF_6 showed that the statistical power to detect a difference of 20% at a significant level of 5% was 98% at 55 days of permeation tube age, but this power was reduced to 55% at 258 days. In contrast, retrospective power analysis, calculated using corrected SF_6 release rates, indicated that the power to detect a 20% difference between two means at 55 days of permeation age was 86% and was only reduced to 83% by 258 days. This indicates that if release rates of SF_6 gas are uncorrected, the experimental design needs to take into account the diminishing statistical power to detect differences between treatment means for CH_4 production as permeation tubes age.

When using SF_6 release rates determined pre-insertion into the rumen, it is assumed that this rate remains constant whilst in the rumen; however, evidence from this study and others (Ulyatt *et al.,* 1999; Lassey *et al.,* 2001; Vlaming, 2008) strongly suggests that this is not the case. The reasons for this change are not known. Possible reasons for the change in the rate of SF_6 gas release from the permeation tubes following deployment include: the co-freezing of impurities in the SF_6 gas; interactions between the SF_6 gas and the Teflon® membrane; and/or physical distortion of the Teflon® membrane (Lassey *et al.*, 2001). Recovered permeation tubes are often encrusted with microbial deposits and digesta over and around the Teflon® membrane, which penetrates around the lock-nut holding the Teflon® membrane in place (Ulyatt *et al.,* 1999). The degree of microbial encrustation is difficult to accurately quantify, but could influence the surface area and interaction of the Teflon® membrane. It is not clear if this alone could account for changes in SF_6 gas release rate after recovery from the rumen.

In addition, the determination of SF_6 rate in dry air may not be representative of the release rate of SF_6 gas in liquids. Based on short-term monitoring of SF_6

gas release rates, rather than long-term serial weighing in dry air, Vlaming (2008) showed that the release rate of SF_6 gas from permeation tubes differed when incubated in either air (3.51 mg SF_6/day), water (2.55 mg SF_6/day) or rumen fluid (2.34 mg SF_6/day). The release rates of SF_6 from tubes incubated in either water and rumen fluid were lower than in air by 27 and 33% respectively ($P < 0.01$). There was no difference between the SF_6 release rate of tubes incubated in either rumen fluid or water ($P < 0.05$). This shows that the current method of determining SF_6 release rates from permeation tubes needs to be reassessed.

2.5.3 Conclusion

Methane production and yield from deer in the present study was lower at 4.5 months of age. However, it was not possible to attribute this to an effect of age, changes in the dietary chemical composition or method used to estimate the DMI of deer. Therefore, to validate these results, measures of deer CH_4 production need to be undertaken when DMI can be accurately measured, and ideally to use respiration chambers, with concurrent measures of CH_4 from mature and immature deer.

The comparison of CH_4 production, calculated using either uncorrected or corrected SF_6 release rates, resulted in a difference of up to 20% and a mean difference of 15%. This implies that using SF_6 release rates determined prior to insertion-only may overestimate CH_4 production when tubes reside in the rumen for an extended period of time, e.g. longer than 1 month. Therefore, it is recommended that when SF_6 permeation tubes remain in the rumen for extended periods of time, they should be recovered at the end of the experiment, in order to determine a corrected SF_6 release rate.

2.6 APPENDIX

Table 2.8: Uncorrected release rates of sulphur hexafluoride (SF_6) pre-deployment and corrected release rates when recovered from red deer stags at slaughter (n = 13). The uncorrected release rates of SF_6 gas are determined from the linear regression of a permeation tube's weights over time and then extrapolated to estimate release rates *in situ*, but corrected values are based on a curvilinear linear regression.

		Un-corrected*	Corrected SF_6 release rate for each measurement period* (mg/d)				Corrected mean**
Deer No	Perm Tube	SF_6 release rate (mg/d)	March	May	Aug	October	SF_6 release rate (mg/d)
302	407	1.350	1.1178	1.0884	1.0443	1.0112	1.0864
303	548	0.827	0.9296	0.9463	0.9713	0.9902	0.9474
305	579	1.578	1.1879	1.1360	1.0581	0.9996	1.1323
310	561	1.334	0.9895	0.9461	0.8810	0.8322	0.9431
311[1]	385	1.340	1.1504	1.0808	0.9763	0.8978	1.0759
312	411	1.360	1.1321	1.0911	1.0297	0.9836	1.0883
317	416	0.997	0.8335	0.8130	0.7822	0.7590	0.8115
318	454	0.835	0.7671	0.7288	0.7464	0.7371	0.7582
319[1]	550	1.646	1.4970	1.4150	1.2906	1.1915	1.4088
330[1]	584	1.858	1.6817	1.5985	1.4738	1.3802	1.5927
333	415	1.440	1.1321	1.0911	1.0297	0.9836	1.0883
334	479	1.450	1.2361	1.2133	1.1791	1.1535	1.2117
335	455	1.114	1.2650	1.2900	1.3275	1.3556	1.2918
Average		1.3176	1.1477	1.1165	1.0608	1.0212	1.1105

*Based on the linear midpoint of SF_6 release rates per measurement period.

**Based on the overall linear midpoint of the whole experiment when SF_6 release rates were corrected for the rates of SF_6 release when tubes where recovered from animals.

[1]Permeation tubes recovered deer; deer were euthanased after the May methane measurement.

Table 2.9: Methane (CH_4) production (grams per day) averaged over a 5 days of collection of red deer stags using uncorrected and corrected release rates of sulphur hexafluoride (SF_6).

Deer No.	Perm. Tube No.	Permeation Rate[1] (mg/d)	March (4.5 months of age)		May (6.0 months of age)		August (9.5 months of age)		October (11.5 months of age)	
			Uncorrected	Corrected	Uncorrected	Corrected	Uncorrected	Corrected	Uncorrected	Corrected
302	407	1.350	25.1	20.5	35.7	28.8	34.9	27.0	38.1	28.6
303	548	0.827	27.0	30.4	30.3	34.7	32.1	37.7	34.9	41.8
305	579	1.578	24.6	18.5	31.3	22.5	35.4	23.8	55.7	35.3
310	561	1.334	30.0	22.2	55.3	39.2	43.4	28.7	58.8	36.7
311	385	1.340	28.7	24.6	45.6	36.8	n.d.	n.d.	n.d.	n.d.
312	411	1.360	28.7	23.1	34.8	27.9	33.7	25.5	54.7	39.6
317	416	0.997	28.4	23.8	30.7	25.1	44.4	34.8	71.0	54.1
318	454	0.835	26.4	24.3	50.2	45.6	36.3	32.4	43.7	38.5
319	550	1.646	32.3	29.4	49.7	42.8	n.d.	n.d.	n.d.	n.d.
330	584	1.858	33.1	29.0	n.d.*	n.d.	n.d.	n.d.	n.d.	n.d.
333	415	1.440	33.1	26.1	42.9	32.5	53.8	38.5	53.0	36.2
334	479	1.450	21.9	27.1	29.0	35.2	48.3	48.7	53.0	42.1
335	455	1.114	28.6	32.5	33.1	38.3	28.9	34.4	35.5	43.2
Average			28.23	25.50	39.06	34.12	39.12	33.15	49.84	39.61
SD			3.36	4.07	9.20	7.04	7.96	7.45	11.61	6.62

*n.d. no data available

[1]Permeation tube release rate shown is the initial rate determined pre-deployment

CHAPTER 3

The effect of monensin on the methane emissions of twin-bearing ewes in early lactation

3.1 ABSTRACT

The aim of this experiment was to evaluate the ability of monensin monosodium salt (monensin) to mitigate the methane (CH_4) emissions of twin-bearing ewes in early lactation and evaluate if monensin supplementation could provide production or nutritional benefits to ewes and their lambs.

Ewes were fed ensiled lucerne (*Medicago sativa*) chaff and 20 twin-bearing ewes were selected post-lambing. During the post-lambing periods, in order to induce moderate nutritional stress, ewes were fed the same diet, but the quantity of the feed offered was limited to 80% of the theoretical total energy requirement of each ewe. Due to a greater than anticipated weight loss, feed offered was increased to 90% of estimated feed requirements at 4 weeks post-lambing. Methane emissions were estimated using the sulphur hexafluoride (SF_6) technique. Monensin supplementation to ewes commenced 2 days post-lambing at a rate of 21 mg/day.

Monensin was found to have no effect on either CH_4 yield (g CH_4/kg dry matter intake; DMI) (monensin 19.2 vs. control, 20.6 g CH_4/kg DMI), apparent digestibility, total rumen and blood metabolites. The average milk production of ewes supplemented with monensin was 41% greater than control ewes (P = 0.042). This was due to a difference in milk production (70%) at 3 weeks post-lambing (P = 0.012), which had disappeared by 6 weeks post-lambing. However, this may be attributed to ewe health rather than an effect of monensin, as milk production was not clearly related to lamb live weight.

Based on the results from this study monensin is not recommended as a technology to reduce CH_4 yields from forage fed-sheep. Monensin in this study offered limited benefits in terms of sheep productivity. The effects of monensin as a supplement to improve the production efficiency and nutritional status of forage-fed ewes during early lactation need to be tested further.

3.2 INTRODUCTION

The New Zealand sheep flock of approximately 38.5 million sheep (Anon, 2009) contributes approximately 37.6% of the national total enteric methane (CH_4) emissions (Anon, 2009). Total enteric CH_4 emissions from sheep in New Zealand have decreased by 22.1%, from 1990 to 2007 (Anon, 2009), due to a 19.4 million reduction in population and improved animal performance, rather than through the implementation of CH_4 mitigation technologies (Anon, 2009). This suggests that further reductions in CH_4 emissions from the sheep sector are achievable by employing appropriate mitigation technologies.

Methane mitigation technologies for the New Zealand agricultural sector must heed the year-round reliance on pastoral grazing, and be beneficial for animal production to ensure technology uptake by producers. This is because the financial savings gained from mitigating CH_4 emissions, i.e. carbon credits, must be greater than the financial cost of the mitigation technologies. Technologies already used within pastoral grazing production systems, for reasons other than CH_4 mitigation, should be tested to determine their potential to mitigate CH_4 emissions. An example is monensin, an ionophore antibiotic that is widely used for the control of bloat, improving feed conversion efficiency and the nutritional status of dairy cows during early lactation (Clark, 2005; Waghorn *et al.,* 2008). Monensin is not currently used within New Zealand's commercial sheep flock (Clark, 2005). An advantage of monensin for pastoral grazing is that it can be formulated in rumen-controlled release capsules (e.g. Captec) (Beauchemin *et al.,* 2008), and therefore could be adopted in current New Zealand pastoral farming practices.

The potential effectiveness of monensin as a CH_4 mitigation technology is widely debated. A recent review by Beauchemin *et al.* (2008) reported that CH_4 yields from dairy cows fed ryegrass pasture, whilst receiving monensin in the form of rumen-controlled release capsules, varied from an increase of 4% to a decrease of 10%. Both *in vitro* and *in vivo* studies suggested that the impact of monensin on CH_4 production (g CH_4/day) and yield (g CH_4/kg DMI) was

influenced by diet and dose rate (Potter *et al.,* 1976; Richardson *et al.,* 1976; Van Nevel & Demeyer, 1996).

Improvements in the utilisation of energy and protein in response to the supplementation with monensin can improve the health and nutrition of dairy cows, prior to calving and during early lactation. Monensin supplementation has been shown to elevate blood glucose concentration, reduce the mobilisation of body fat reserves (indicated by lowered blood concentrations of non-esterified fatty acids (NEFA) and β-hydroxybutyrate (BOH)), reduce ketosis, and improve milk production and milk production efficiency (McGuffey *et al.,* 2001; Ruiz *et al.,* 2001; Ipharraguerre & Clark, 2003). Sheep fed pasture in spring and autumn have also been found to have increased rumen fluid propionate concentration (19%) and decreased acetate concentration (2.5%) in response to monensin; however, blood glucose concentration was not affected (Maas *et al.,* 2001). This suggests that monensin supplementation may improve the production efficiency and nutritional status of high producing ewes during early lactation.

The recommended pasture sward height needed to meet the nutritional requirements of twin- and triplet-bearing ewes during mid- and late-pregnancy is 4 cm or 1200 kg DM/ha (Morris & Kenyon, 2004; Corner, 2007). However, within some regions of New Zealand, pasture growth in winter may be insufficient to meet the nutritional requirements of ewes during mid- to late-pregnancy and possibly early lactation (Mathews *et al.,* 2000). Morris and Kenyon (2004) showed that under-nutrition of ewes could lead to retarded foetal growth resulting in lowered birth weights and possibly increased mortality rates of lambs. The improved propionate production with monensin supplementation in non-pregnant/lactating sheep (Mathews *et al.,* 2000), suggests it may provide important nutritional benefits.

A decision was made to restrict the feed availability to lactating ewes to achieve intakes similar to those of grazing animals. Indoor feeding can result in higher intakes compared with grazing when feed is offered *ad libitum*. Stocking rates of grazing animals ensure high competition to maintain low residual dry matter of the pasture sward and therefore pasture quality. Nevertheless, this can be at

the expense of animal productivity (Hoogendoorn *et al.*, 1986; Waghorn *et al.,* 2007).

If monensin is shown to reduce CH_4 yields, it could be a useful tool for the mitigation of CH_4 emissions from New Zealand's extensively-based sheep industry, whilst providing nutritional benefits to ewes during periods of nutritional stress. The aims of this study were to;

- Investigate the effect of monensin supplementation of twin-bearing ewes in early lactation on CH_4 production and yield.

- Determine if monensin supplementation of twin-bearing ewes could provide production benefits, i.e. milk production and live weight, and/or nutritional benefits, i.e. apparent digestibility, nitrogen utilisation and concentration of blood metabolites during early lactation.

3.3 MATERIALS AND METHODS

3.3.1 Experimental design

An experiment to measure CH_4 emissions and diet apparent digestibility in twin-bearing ewes during early lactation was conducted at AgResearch, Grasslands campus, Palmerston North, New Zealand, from the 29th August to the 16th November 2005. The experiment was approved by the AgResearch Ltd, Grasslands, Animal Ethics Committee. The experiment consisted of three measurement periods that included: 1) pre-lambing, (12 September 2005, a covariate measurement); 2) 3 weeks post-lambing; and 3) 6 weeks post-lambing, as shown in Table 3.1.

As ewes lambed, they were allocated to either monensin or control groups (not receiving monensin). The allocation was balanced for successful pre-lambing CH_4 measurements, sire of lambs and the number of lambs that each ewe had. Treatment groups were balanced for the number of sets of twin-lambs, as not all ewes were able to rear both twin lambs, through apparently malfunctioning udders due to previous injury or the death of one twin.

The pre-lambing measurement period consisted of four days of 24 hour CH_4 measurements with corresponding measurements of DMI. Both the 3 and 6 week post-lambing measurement periods consisted of 2 days adaptation to housing in metabolism cages and 4 days of consecutive CH_4 measurements. This coincided with 8 days of apparent digestibility, urine production and DMI measurements, and the measurement of milk production and sampling of rumen fluid at the end of each measurement period.

There was a 9-day range in lambing dates, with a mean lambing date of the 23 September 2005. The timing of measurements during the post-lambing period were based on each ewe's lambing date and each lamb's birth date.

Table 3.1: Timetable of main activities during the experiment.

Day of experiment	Activity
1	Permeation tubes administered and all housed indoors
4 – 79	Blood sampling on Monday and Thursdays
14 – 19	CH_4[1] measurement
19	Rumen sampling – all ewes
21 – 30	Lambing
Days post-lambing	
1	All animals weighed
2	Start of monensin supplementation to ewes in monensin group and placebo to ewes in the control group
7	Ewes and lambs weighed
14	Ewes and lambs weighed
22	Lambs weighed
28	Ewes weighed
29	Lambs weighed
21 – 29	Apparent digestibility and CH_4 measurement one
29	Rumen fluid sampling (sub-group) and milk production sampling
35	Ewes weighed
36	Lambs weighed
42 – 50	Apparent digestibility and CH_4 measurement two
50	Rumen fluid sampling (sub-group) and milk production sampling

[1]Methane (CH4)

3.3.2 Animals and housing

Initially, 30 Romney mixed-aged ewes (60.1 ± 6.5 (S.D.) kg live weight) in late gestation with twins, were selected. The sires of the lambs were either Romney (n = 20) or South Suffolk (n = 10). Of the original 30 ewes, 20 were selected for measurements 3 and 6 week post-lambing. Ewe selection was based on lambing date, successful birth of live twin lambs, and adaptation to indoor housing. Three ewes did not lamb within the required two-week time period and therefore were excluded.

Ewes were weighed weekly pre-lambing. Post-lambing, ewes and lambs were weighed every 7 days when possible. Animals were always weighed prior to the morning meal.

Prior to lambing, including the pre-lambing CH_4 measurement, ewes were housed in individual pens, which consisted of raised plastic mat flooring and were approximately 2 by 3 m^2 in size. After lambing, ewes were housed individually with their lambs in the same pens. During the 3 and 6 week post-lambing measurement periods ewes were housed with their lambs in metabolism cages. To ensure there was enough room for both the ewe and the lambs, two standard metabolism cages were tied together. The partitioning wall was removed at 3 weeks post-lambing measurement period.

However, at 6 weeks post-lambing, to prevent the lambs consuming the ewes' feed, lambs were separated from the ewe during the day. Lambs were allowed access to their mother twice a day to suckle. When separated from the ewes, lambs had unlimited access to feed and water. This arrangement of the metabolism cages was tested prior to the commencement of the experiment with non-experimental animals. Approximately 1 week after lambing, all ewes were treated with a broad-spectrum anthelmintic oral drench (Arrest, Ancare New Zealand Limited) at a rate of 1 ml per 5 kg live weight.

At 4 weeks post-lambing, a number of ewes were found to have sores on their udders, which may have arisen from excessive suckling by the lambs, possibly due to boredom. To alleviate this, ewes were treated daily with an iodine-based udder cream. Lambs were removed during the day for 6 to 8 hours and group-housed in three large pens with unlimited access to feed and water.

3.3.3 Diet and feeding

Prior to lambing, ewes were fed *ad libitum* a diet consisting of ensiled lucerne (*Medicago sativa*) chaff with added molasses 'FibrePro', (Fibre Fresh Feeds, Reporoa, New Zealand). However, ewes initially took longer to adapt to the diet than anticipated, based on previous experience. Thus, ewes were supplemented with freshly-cut pasture for two weeks. All animals had access to a mineral/salt block (Summit multi mineral salt block, Dominion Salt NZ Ltd, Mount Maunganui, New Zealand) and unlimited access to water. Lambs were offered ensiled lucerne chaff from 3 weeks of age.

The individual feed intakes of all ewes were monitored daily. Sheep were fed twice daily at 8.30 am and 4 pm, with approximately half of their daily allocation offered at each meal. Post-lambing, ewes were fed FibrePro, at 0.8 times their total energy requirements for maintenance and lactation. The aim was to induce moderate nutritional stress. Energy requirements were based on the UK ruminant feeding standards (Anon, 1984) and the energy requirement for milk production was estimated using milk production data reported by Peterson *et al.* (2005; 2006). Due to a greater than anticipated initial weight loss, feed offered was increased to 90% of estimated feed requirements at four weeks post-lambing. The manufacturer's specifications stated that the ensiled lucerne contained a metabolisable energy (ME) concentration of 13 MJ ME/ kg dry matter (DM). To allow for a margin of safety for the purposes of this experiment, feed allocation was based on the feed having an energy content of 12 MJ ME/kg DM.

Samples of feed offered were collected twice weekly between measurement periods and daily during measurement periods to establish the DM concentration of the feed as determined by oven drying samples (100 ˚C; 24 hrs) in triplicate. Daily feed samples (approximately 200 g) were also taken during the measurement periods, stored frozen at –20 °C and later pooled per measurement period for chemical analysis. Feed refusals for individual animals were determined daily, both between and during measurement periods. Between measurement periods, the feed refusals for each treatment group were pooled before DM was determined in triplicate. During the measurement periods a sample (approximately 200 g) of the feed refused for each animal was collected daily, chilled, and pooled at the end of the measurement period. The DM of the feed refused was determined from the pooled sample. Additional daily feed refusal samples were also pooled per animal, per measurement period, and stored frozen (–20 ˚C) for later processing and chemical analysis.

3.3.4 Apparent digestibility and urine production

Feed offered, feed refused, urine and faeces were collected daily for seven days during the 3 and 6 week post-lambing measurement periods in order to determine apparent digestibility of DM, organic matter (OM), nitrogen (N) and

gross energy (GE) and DMI. Samples (200 g) of feed offered were taken daily, pooled per period and stored at –20 °C. Feed refused consisted of feed refused in the feed bins ('refusals') and feed spilt in or around the metabolism cages ('sweepings'). These were collected separately, weighed, and the DM content determined (100˚C; 24 hrs). Feed refused from the feed bins was sub-sampled and frozen (–20 °C) to be later pooled per period and per animal for chemical analysis. Faeces were collected daily, mixed by hand, sub-sampled and frozen (–20 °C). Later, faeces were pooled per animal per measurement period, homogenised and sub-sampled in triplicate (approximately 200 g) for DM content determination. An additional sample of approximately 200 g was taken and stored frozen (–20 °C) for later chemical analysis.

Total urine volume (kg) was measured daily and a 2% sub-sample taken and pooled per animal per measurement period and frozen (–20 °C) for later analysis. Urine was collected in buckets and sufficient hydrochloric acid (HCl) (50% diluted) was added to lower the pH to 3 or below.

3.3.5 Monensin treatment

Monensin was administered to ewes in the treatment group at a rate of 21 mg/day (Kim Agnew, Elanco Animal Health, a division of Eli Lilly and Company, Greenfield, Indiana, USA, personal communication). Sheep received rumensin oral drench for cattle (60 mg/ml of monensin sodium (undiluted), Elanco Animal Health, a division of Eli Lilly and Company, Greenfield, Indiana, USA), diluted at a rate of 1 ml oral drench to 19 ml water, thus receiving 3.5 ml of solution prior to each meal. A total of 1 litre of the monensin solution was made up at a time. The control group was given an equivalent volume of a placebo (water) treatment via an oral drench gun prior to each meal event. Placebo or monensin drenching commenced two days after lambing, and continued thereafter twice daily until the end of the experiment (50 days).

3.3.6 Methane measurements

Methane emissions were determined during the pre-lambing measurement period and in the first 4 days of apparent digestibility measurements at 3 and 6 weeks post-lambing. Methane production was calculated using the sulphur

hexafluoride (SF_6) technique as described in detail in Chapter 2. The release rate of SF_6 gas from the permeation tubes (0.71 ± 0.193 (SD) mg/day) was determined, prior to insertion, by serial weighing of the permeation tubes when incubated at 39 °C for eight weeks. The release rates were not corrected for post-deployment (Chapter 2) as the permeation tubes were unable to be retrieved at the end of the CH_4 measurements.

3.3.7 Blood sampling

Blood concentrations of glucose, non-esterified fatty acids (NEFA), β-hydroxybutyrate (BOH) and plasma urea were determined twice weekly from the ewes. Blood samples were collected using one vacutainer with no additives (10 ml) and one heparinised tube (5 ml), for blood glucose by jugular venipuncture from alternative jugular veins (to reduce the impact of repetitive blood samples). Samples were collected four hours after the morning feeding event (08:30 hours) (Marie *et al.,* 2001).

3.3.8 Milk production

Milk production was estimated at the end of each of the digestibility periods, using methods described by Cardellino and Benson (2002). Ewes were machine-milked and hand stripped, and lambs removed from the ewes (Table 3.1). After 3 hours, ewes were milked a second time, the milk weighed, sub-sampled, pooled and then frozen at –20 °C. To aid in the letdown of milk, oxytocin (1 to 1.5 ml at 10 ui/ml Vetpharm, Auckland, New Zealand) was administered inter-muscularly into the hind leg of ewes at each milking. Milk production was multiplied by eight to estimate milk yields over a 24 hr period. For chemical analysis milk was sub-sampled from each ewe (approximately 10%) which was then pooled, so that each pool contained milk from five ewes within each treatment group at 3 and 6 weeks post-lambing, resulting in a total of 8 pooled samples.

3.3.9 Rumen fluid sampling

Rumen fluid was sampled by oral-gastric tubing five hours after the morning meal event, with a total of 16 ml required per animal per sample. The pre-

lambing collection of rumen fluid occurred at the end of the CH_4 measurements from all ewes. Post-lambing rumen fluid samples were collected from a sub-group of ewes (n = 5) within each treatment group at the end of the apparent digestibility measurements, 3 and 6 weeks post-lambing.

Rumen fluid pH was determined for each animal immediately after the collection of the sample using a pH meter (PHM210, Radiometer, Copenhagen). The rumen fluid was analysed for volatile fatty acid (VFA) samples (5.0 ml) requiring the addition (1.0 ml) of a protein precipitant. Samples were then frozen and later centrifuged at 3000 rpm for 15 minutes. The supernatant was transferred to pre-labelled tubes and frozen. Rumen fluid for ammonia (NH_3) concentration determination (1.0 ml) was transferred to a microcentrifuge tube containing 15 ul of concentrated hydrochloric acid, and the samples mixed thoroughly. Samples were then frozen and later centrifuged (15 minutes at 1400 rpm) with a microcentrifuge. After centrifuging, the supernatant was transferred to another microcentrifuge tube and re-frozen for later analysis.

3.3.10 Laboratory analysis

All feed, faecal, urine, rumen fluid and milk samples were analysed by the Nutrition Laboratory, Massey University, Palmerston North. Samples of feed offered, refused and faeces were analysed for GE (bomb calorimetry), organic matter (OM: Furnace 5500 C, AOAC 942.05), nitrogen (N; Leco, total combustion method, AOAC 968.06), crude protein (CP) concentrations were calculated by multiplying N by 6.25 and *in-vitro* DM digestibility (DMD) (feed offered only, Roughan & Holland, 1977). However, the chemical composition of the feed refused from the pre-lambing measurement period was not analysed. Urine and milk samples were analysed for GE and nitrogen (N) concentrations. Rumen fluid NH_3 concentrations were measured by enzymatic determination and rumen fluid VFA concentrations were determined by gas chromatography (Wronkowska *et al.,* 2006).

Blood samples were analysed for concentrations of glucose, non-esterified fatty acids (NEFA), β-hydroxybutyrate (BOH) and plasma urea (New Zealand Veterinary Pathology Laboratory, Palmerston North, New Zealand).

3.3.11 Statistical analysis

Data were statistically analysed using the PROC MIXED model with repeated measures (SAS, version 9.1, 2007). Lamb birth weight and pre-lambing measurements of ewe live weight, DMI, CH_4 production and yield, and rumen VFA and NH_3 concentrations were used as covariates in the analysis of the subsequent data, which are presented as covariate adjusted means. The means of DMI presented along with apparent DM digestibility means (Table 3.2) were not covariate adjusted. The main effects of treatment, time and their interactions were compared, and significance was declared at $P \leq 0.05$ and a trend reported if $0.05 < P \leq 0.10$.

3.4 RESULTS

3.4.1 Dietary chemical composition

Despite being sourced from the same batch of FibrePro, dietary chemical composition was not consistent between the three measurement periods, as shown in Table 3.2. The CP (257 g/kg DM) and GE (20.0 MJ/kg DM) concentration of the diet fed pre-lambing was greater ($P < 0.05$) than the mean concentrations of the diet fed post-lambing (227 g/kg DM and 19.5 MJ/kg DM, respectively). The concentration of DM (380 g/kg DM) and OM (890 g/kg DM) were lower ($P < 0.05$) in the pre-lambing diet, compared with the post-lambing diets (mean, 443 g/kg DM and 908 g/kg DM, respectively). There was no difference ($P > 0.1$) in the chemical composition of the diet offered between the 3 and 6 week post-lambing measurement periods. At 3 weeks post-lambing, feed refusals had a greater concentration of DM (462 vs. 401 g/kg DM) ($P < 0.001$) and OM (920 vs. 401 g/kg DM) ($P = 0.005$) and a lower concentration of CP (176 vs. 221 g/kg DM) ($P = 0.003$) and GE (18.9 vs. 19.4 MJ/kg DM) ($P = 0.001$) compared with feed refused at 6 weeks post-lambing.

Table 3.2: Chemical composition (g/kg dry matter, DM) of the FibrePro diet (ensiled lucerne, *Medicago sativa,* chaff with added molasses; n = 2) to and refused by sheep and the *in vitro* digestibilities (% DM) and organic matter (% DM) of feed offered to sheep during the three measurement periods; pre-lambing, 3 weeks and 6 weeks post-lambing.

	Pre-lambing	Post-lambing		SEM	P-values
		3 weeks	6 weeks		
Feed offered					
Dry matter	380[a]	447[b]	437[b]	10.8	0.041
Organic matter	890[a]	910[b]	906[b]	2.3	0.009
Crude protein	257[a]	224[b]	230[b]	5.0	0.023
Gross energy (MJ/kg DM)	20.0[a]	19.5[b]	19.5[b]	0.10	0.044
Dry matter digestibility (%)[#]	nd*	58.6	60.7		
Organic matter digestibility (%)[#]	nd	57.8	62.0		
Metabolisable energy (MJ/kg DM)	nd	8.7	8.7	0.04	0.879
Feed refused		*(n = 5)*	*(n = 10)*		
Dry matter	380[a]	462[b]	401[a]	7.9	0.001
Organic matter	nd	920[a]	907[b]	2.7	0.005
Crude protein	nd	176[a]	221[b]	8.6	0.003
Gross energy (MJ/kg DM)	nd	18.9[a]	19.4[b]	0.09	0.001

* nd not determined

[#] n = 1

[ab] Denotes a significant difference between means within the same row ($P < 0.05$)

3.4.2 Live weight and liveweight gain

The change in average ewe live weight over the course of the experiment is shown in Figure 3.1. The first measurement of live weight was used as a covariate in the analysis of subsequent ewe live weights ($P < 0.001$). Overall, monensin supplementation had no effect on ewe live weight (overall mean monensin, 50.9 vs. control, 50.6 ± 1.15 kg) ($P = 0.69$). Post-lambing ewes lost weight with the greatest weight loss between day one (0.1 week, 48.3 kg LW) and 1 week (43.8 kg LW) post-lambing, when ewes lost 10% ($P = 0.001$) of their live weight. By 1 week post-lambing ewe live weight loss appeared to have stabilised. There was no significant interaction between treatment and time for ewe live weight ($P = 0.486$).

The pattern of live weight change in lambs successfully reared as twins until the end of the study, is shown in Figure 3.2. The mean (± SEM) birth weights of lambs born in the monensin group (4.16 ± 0.14 kg) were greater ($P < 0.002$) than those lambs born to control ewes (3.30 ± 0.17 kg). However, monensin had no effect on lamb birth weight as supplementation did not commence until after lambing. Lamb birth weight, when used as a covariate, had a significant ($P < 0.001$) effect on lamb live weight, but not for liveweight gain ($P = 0.968$). At 5 weeks of age lambs born to ewes treated with monensin (7.7 ± 0.17 kg) were significantly heavier (9%, $P = 0.016$) than those born to control ewes (7.1 ± 0.17 kg), but no difference of live weight was found prior to 5 weeks of age (Figure 3.2 and Appendix 3.7, Table 3.9).

The mean (± SEM) liveweight gain of lambs over the period from birth until 5 weeks of age was 18% greater for lambs from ewes treated with monensin (111 ± 5.2 g/day) compared with lambs from the control ewes (91 ± 7.2 g/day) ($P = 0.034$). There was no statistically significant interaction between liveweight gain and time ($P = 0.406$). The weekly liveweight gain of lambs with time is shown in Appendix 3.7, Table 3.9.

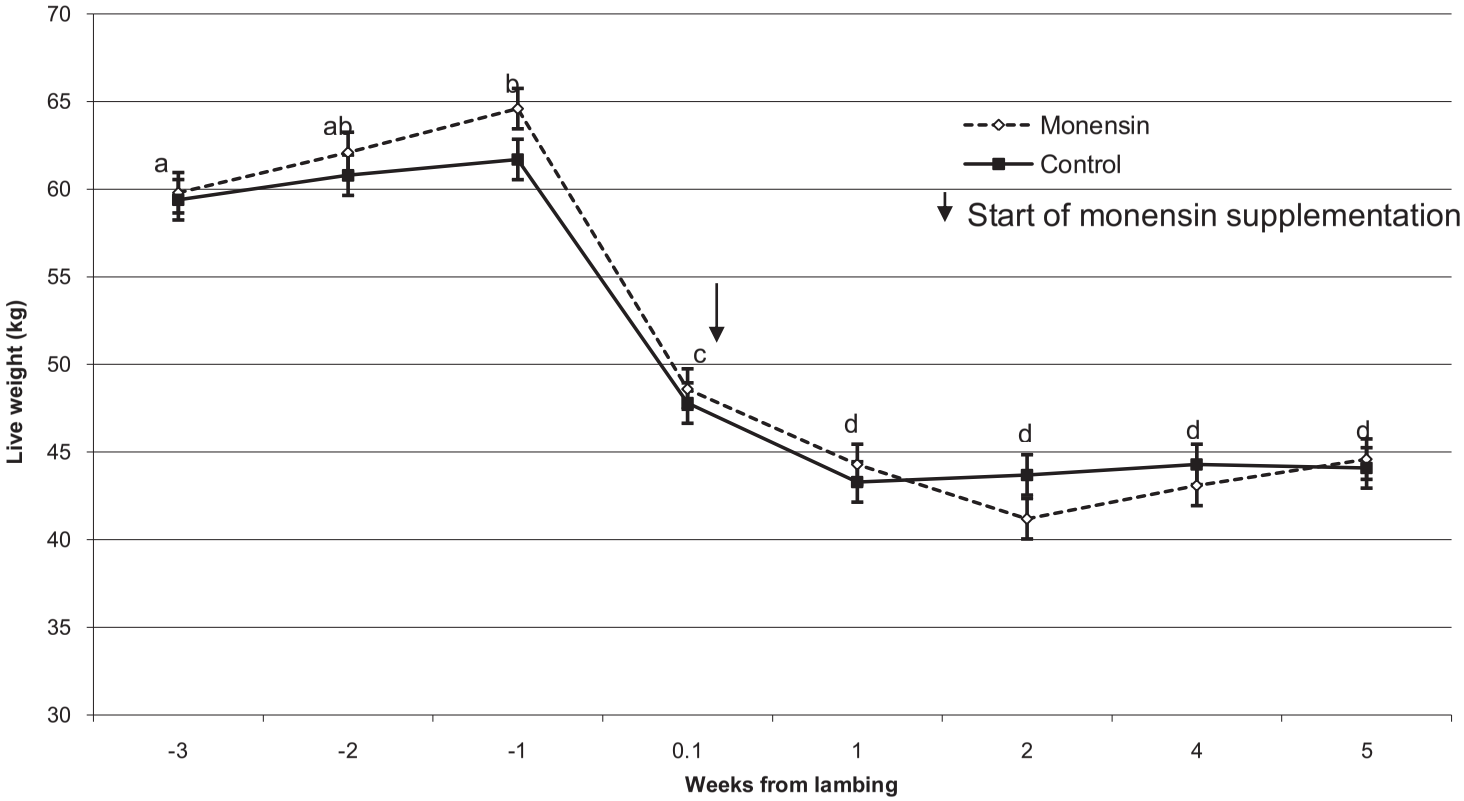


Figure 3.1: Live weight (kg, covariate adjusted means) of ewes pre- and post-lambing either receiving monensin (21 mg/day, monensin; n =10) or a placebo (control; n = 10). Monensin and placebo drenching commenced two days after lambing. [abcd] Differing letters denote a difference between each time point in the overall mean live weights ($P < 0.05$).

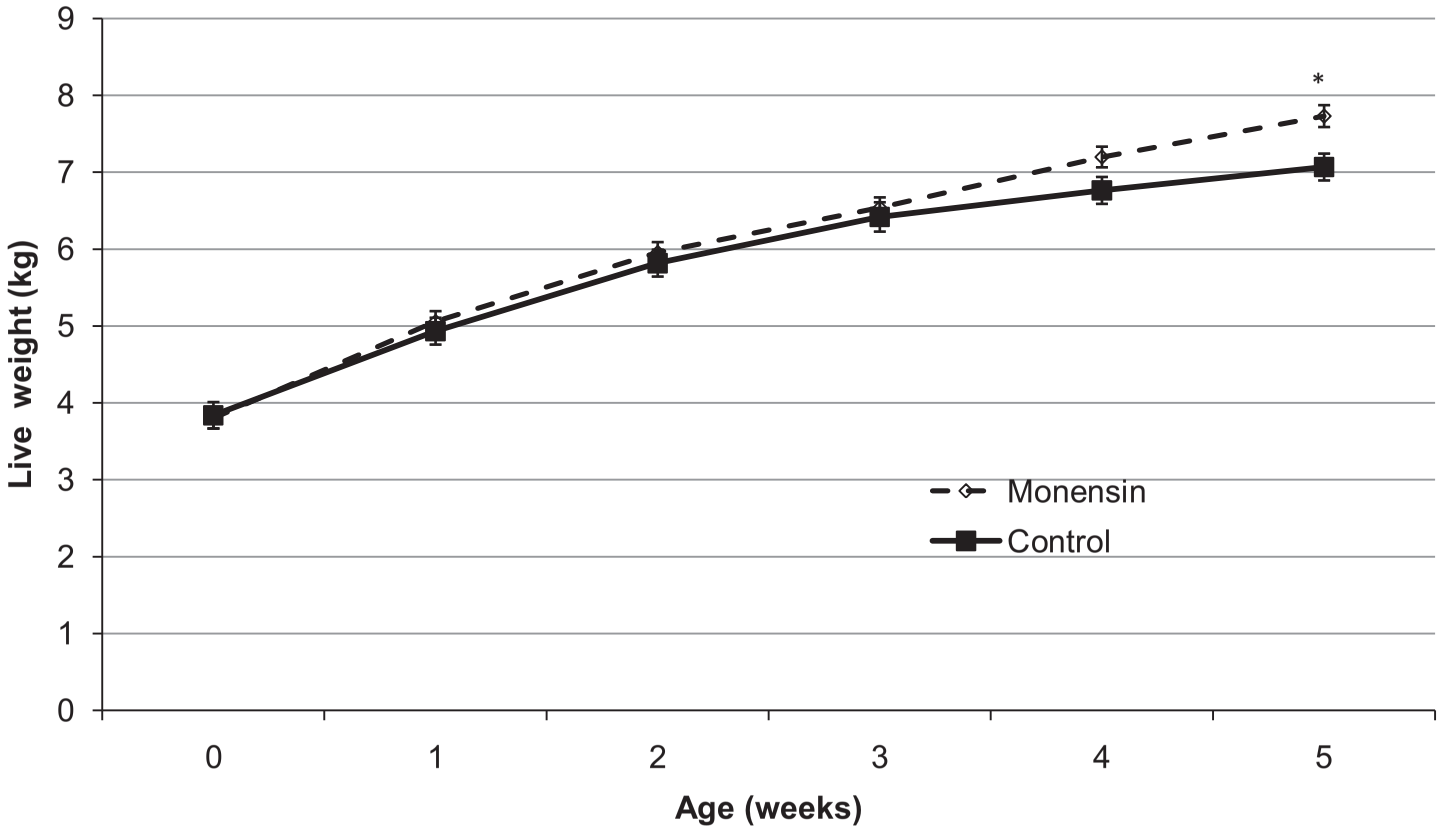


Figure 3.2: Live weight (kg, covariate adjusted means) of twin lambs from birth until 36 days of age, with birth weight used as a covariate (P = 0.001). Lambs were from ewes treated with monensin at 21 mg/day (monensin, n =20) or from ewes receiving a placebo (control, n = 12). Monensin and placebo drenching of ewes commenced two days after lambing. * Denotes a significant difference between means at the same point in time (P < 0.05).

3.4.3 Milk production

The milk production of ewes that successfully reared both lambs for the duration of the experiment and had fully functioning udders (n = 15; monensin n = 8 and control n =7) was used in the analysis of the data. The mean (± SEM) milk production of ewes receiving monensin (1297 ± 121.1 g/day) was 41% greater ($P < 0.042$) than the control group (919 ± 129.5 g/day). This was largely caused by a 70% difference in milk production at 3 weeks post-lambing (monensin, 1665 ± 171.3 g/day; control, 981 ± 183.1 g/day; $P = 0.0114$), which had disappeared by 6 weeks post-lambing (monensin, 930 ± 171.3 g/day; control, 856 ± 183.1 g/day). Ewe milk production was greater ($P = 0.023$) at 3 weeks post-lambing (1323 ± 125.4 g/day) compared with 6 weeks post-lambing (893 ± 125.4 g/day). There tended to be an interaction ($P = 0.098$) between treatment and time for milk production.

Despite the difference in calculated milk yield, there was found to be no statistically significant difference in the mean (±SEM) DM percentage of milk from ewes treated with monensin compared with control ewes at 3 weeks (13.9 vs. 14.0 ± 1.83 % DM) or 6 weeks, (13.0 ± 1.83 vs. 10 ± 1.83 % DM; $P = 0.4$).

3.4.4 Feed intake

The DMI of ewes during the pre-lambing measurement period, prior to monensin supplementation, did not differ ($P = 0.815$) between treatment groups (1.3 ± 0.04 (SEM) kg DM/day). Pre-lambing DMI had a significant ($P < 0.001$) effect when it was used as a covariate in the analysis of DMI post-lambing. The mean DMI of ewes supplemented with monensin (1.34 ± 0.032 (SEM) kg/day) was lower ($P = 0.005$) than the control group (1.41 ± 0.032 (SEM) kg/day). No significant interaction between time and treatment was found ($P = 0.237$) for DMI. Results for feed intake calculated per kg of ewe metabolic live weight, OM intake (OMI) and digestible OMI, mirrored that of DMI (Table 3.3).

3.4.5 Apparent digestibility

The *in vivo* apparent DM and OM digestibility (DMD and OMD, respectively) of the ensiled lucerne diet are shown in Table 3.3. Monensin supplementation did

not affect the DMD (P = 0.938) or OMD (P = 0.835) of the diet. At 3 weeks post-lambing, the apparent DMD (50.0%) and OMD (48.6%) was 10% lower than (P < 0.001) the DMD (59.3%) and OMD (61.1%) determined at 6 weeks post-lambing. In addition, the DMD at 3 weeks post-lambing was less than the DMD determined *in vitro* (58.6%). However, at 6 weeks post-lambing the *in vitro* DMD (60.7%) and OMD (62.0%) (Table 3.2) were similar to those determined *in vivo*. As the *in vitro* DMD did not change from 3 to 6 weeks post-lambing, it indicates that a change in the sheep's ability to digest the diet may have been responsible for the increase of DMD and OMD from 3 to 6 weeks post-lambing. There was no significant (P > 0.1) interaction between time and treatment for *in vivo* DMD or OMD.

Table 3.3: Daily dry matter (DM) intake (DMI), digestible DMI, organic matter (OM) intake (OMI) and *in vivo* apparent digestibilities of DM and OM at 3 and 6 weeks post-lambing in twin-bearing ewes either with (monensin, n = 10) or without (control, n = 10) monensin supplementation and fed FibrePro (ensiled lucerne, *Medicago sativa,* chaff with added molasses).

	Post lambing						P-values		
	3 weeks			6 weeks					
	Control	Monensin	SEM	Control	Monensin	SEM	Time	Treat	Time X treat[1]
DMI (kg/day)	1.51	1.40	0.030	1.29	1.22	0.035	0.001	0.004	0.237
DMI (g/LW $^{0.75}$)	85	75	1.9	77	72	1.93	0.003	0.001	0.147
Faecal DM loss (kg/day)	0.77	0.69	0.017	0.52	0.49	0.017	0.001	0.002	0.145
Digestible DMI (kg/day)	0.74	0.69	0.025	0.78	0.76	0.025	0.186	0.110	0.939
DM digestibility (% DMI)	48.8	51.1	1.7	60.3	58.2	1.5	0.001	0.938	0.163
OMI (kg/day)	1.37	1.25	0.057	1.17	1.11	0.028	0.004	0.002	0.296
Faecal OM loss (kg/day)	0.71	0.64	0.016	0.46	0.43	0.016	0.001	0.002	0.127
Digestible OM intake (kg/day)	0.72	0.68	0.023	0.79	0.77	0.023	0.022	0.080	0.703
OM digestibility (% OMI)	48.0	49.2	1.32	60.7	61.5	1.30	0.001	0.835	0.215

[1]Interaction between time and treatment

3.4.6 Methane emissions

The estimations of CH_4 production and yield were only partially successful due to the lower than expected concentrations of SF_6 gas in the collected breath samples from some of the sheep, (normal range being 82 – 823 ppt, Annex A). This resulted in only 55%, 45% and 50% of the total breath samples collected in the pre-lambing, and 3 and 6 week post-lambing measurement periods, respectively, being included in the final analysis of the data (Table 3.4). The criteria for data inclusion were based on the decision tree that is presented in Annex A.

Neither CH_4 production ($P = 0.765$), yield ($P = 0.986$) or CH_4 as a % GEI ($P = 0.986$) differed between the groups of ewes pre-lambing; the means (± SEM) were 25.8 ± 1.93 g CH_4/day, 19.2 ± 1.08 g CH_4/kg DMI and 5.3 ± 0.30 CH_4 as a % GEI, respectively. These pre-lambing measurements of CH_4 production and yield were subsequently used as covariates in the analyses of CH_4 data obtained from the post-lambing measurement periods (CH_4 production, $P = 0.008$; CH_4 yield, $P = 0.041$; CH_4 as a % GEI; $P = 0.041$).

No effect of monensin supplementation on CH_4 production, CH_4 yield, or CH_4 as a % GEI was found at either 3 or 6 weeks post-lambing ($P > 0.1$) (Table 3.5). The overall mean (± SEM) CH_4 production across both treatment groups decreased by 27% from 3 weeks (32.8 ± 2.65 g CH_4/day) to 6 weeks post-lambing (23.8 ± 2.33 g CH_4/day; $P = 0.001$). Similarly, CH_4 yield (21.9 vs. 18.0 g CH_4/kg DMI, $P = 0.026$) and CH_4 as a % GEI (6.3 vs. 5.1 CH_4 as a % GEI; $P = 0.026$) decreased from 3 to 6 weeks post-lambing. No significant interactions between time and treatment for total CH_4 production, CH_4 yield or CH_4 as a % GEI were found.

Table 3.4: The planned number of animals to be used within each treatment group (control, and monensin), versus the actual number of animals used in the final analysis of methane emissions (pre-lambing and post-lambing).

	Treatment		Included data points	
	Control	**Monensin**	**Total**	**%[5]**
Planned No. of animals[3]	10	10	20	
Total available data points[3]	44	44	88	
Treatment measurement period number of animals (data points) included				
Pre-lambing[4]	6[1] (23)[2]	9 (25)	48	55
Post-lambing:				
3 weeks	7 (19)	6 (21)	40	45
6 weeks	9 (27)	6 (17)	44	50

[1] Denotes the number of animals.

[2] Denotes the number of data points.

[3] Per measurement period

[4]Includes only those animals that were selected for measurements post-lambing. Animals received no monensin supplementation at this time.

[5] Percentage difference = (total included data points/total available data points) x 100

Table 3.5: Methane emissions (covariate adjusted means ± SEM) of twin-bearing ewes fed FibrePro (ensiled lucerne, *Medicago sativa,* chaff with added molasses) , with (monensin) and without (control) monensin supplementation at 3 and 6 weeks post-lambing.

	Post lambing[2]								
	3 weeks			6 weeks			P-values		
	Control	Monensin	SEM	Control	Monensin	SEM	Time	Treatment	T x T[1]
CH_4 (g/day)	35.9	29.7	2.65	24.5	23.0	2.33	0.001	0.136	0.354
CH_4 (g/kg DMI)	22.8	20.9	1.77	18.4	17.5	1.55	0.026	0.406	0.768
CH_4 (g/kg DDMI)	42.6	39.2	3.10	31.8	30.1	2.89	0.001	0.392	0.768
CH_4 (g/kg DOMI)	48.9	43.5	3.38	34.7	32.9	3.15	0.001	0.282	0.581
CH_4 energy (% GEI)	6.5	6.0	0.50	5.2	5.0	0.44	0.026	0.406	0.768

[1]Time by treatment interaction

[2]n per group per measurement period is presented in Table 3.4

3.4.7 Energy and nitrogen balance

The overall daily intake of N was lower in the monensin ewes than the control ewes (48 g vs. 51 N/day respectively; P = 0.014); as was faecal N loss (16.9 vs 15.6 g/day respectively; P = 0.004). There was no difference between treatment groups in either urinary N excretion (control, 22.8 g/day; monensin 20.9 g/day) or retained N (control, 4.9 vs. monensin, 6.4 g/day; Table 3.6).

At 3 weeks post-lambing, ewes supplemented with monensin had lower (P = 0.088) milk N per day (g/day) compared with the control group (4.2 vs. 5.1, respectively). This difference in milk N had disappeared by 6 weeks post-lambing (monensin, 8.0 vs. control 7.3). No significant interactions between treatment and time were found for daily N intake, faecal N loss, urine N loss, or N retained (P >0.1).

Monensin supplementation did not result in any significant difference in GEI, faecal GE loss, apparent energy digestibility, urine GE loss or GE lost as CH_4 (P > 0.1) (Table 3.7). The total GE of milk from ewes supplemented with monensin (6.1 MJ/day) at 3 weeks post-lambing was greater (P = 0.001) than the control group (3.7 MJ/day), but this difference had disappeared (P = 0.7) by 6 weeks post-lambing (control, 2.7 vs. 2.6 MJ/day).

Table 3.6: Nitrogen flows (mean ± SEM, g/day) in ewes supplemented with monensin and fed FibrePro (ensiled lucerne, *Medicago sativa,* chaff with added molasses) at 3 and 6 weeks post-lambing.

	Post-lambing								
	3 weeks			6 weeks			P-values		
	Control[2]	Monensin[2]	SEM	Control	Monensin	SEM	Time	Treat	Time X Treat[1]
N intake	55	51	0.8	47	45	0.13	0.001	0.014	0.633
Faecal N loss	20	19	0.4	13	12	0.5	0.001	0.004	0.332
Urine N loss	25	24	3.0	21	18	0.9	0.051	0.378	0.687
Milk N	5.1[a]	4.2[a]	0.36	7.3[b]	8.0[b]	0.41	0.001	0.848	0.038
N retention [*]	*4.5*	*4.4*	*2.39*	*5.2*	*8.4*	*2.65*	*0.348*	*0.534*	*0.523*

[*] N retention = N DM intake – (faeces N + urine N + milk N)

[1]Interaction between time and treatment (treat)

[2] Monensin, n = 10; control, n = 10

[ab] Letters denote a significant differences between means within the same row ($P < 0.05$), based on a significant interaction between time and treatment

Table 3.7: Energy flows (mean ± SEM, MJ/day) in ewes supplemented with monensin and fed FibrePro (ensiled lucerne, *Medicago sativa,* chaff with added molasses) at 3 and 6 weeks post-lambing.

	Post-lambing[4]						P-values		
	3 weeks			6 weeks					
	Control	Monensin	SEM	Control	Monensin	SEM	Time	Treat	Time X Treat[3]
GE intake	29.5	26.7	0.70	25.0	23.7	0.71	0.001	0.004	0.281
Faecal energy loss	14.2	13.9	0.37	9.7	9.9	0.37	0.001	0.887	0.505
Digestible energy (%)	50.6	50.7	1.73	59.7	58.1	1.72	0.001	0.669	0.621
Urine energy loss	1.06	1.07	0.046	1.06	1.03	0.046	0.716	0.813	0.505
CH_4 energy loss	1.67	1.45	0.12	1.24	1.22	0.13	0.006	0.319	0.398
Milk energy	3.7[a]	6.1[b]	0.23	2.7[c]	2.6[c]	0.21	0.001	0.001	0.001
Energy retained [1]	*8.3[ab]*	*4.5[a]*	*0.92*	*9.4[b]*	*9.0[b]*	*0.79*	*0.011*	*0.761*	*0.032*
Calculated dietary ME (MJ ME/kg DM)[2]	7.8	7.6	0.46	9.6	9.8	0.45	0.001	0.998	0.604

[1] Energy retained = GE intake – (Faecal energy loss + urine energy loss + CH_4 energy loss + milk energy loss)

[2]Calculated dietary ME (MJ/kg DM) = Energy retained / DMI (Table 3.2)

[3]Interaction between time and treatment

[4]Monensin, n = 10; control, n = 10

[abc] Letters denote a significant differences between means within the same row ($P < 0.05$), based on a significant interaction between time and treatment

3.4.8 Blood metabolites

No effect (P > 0.1) of monensin supplementation on blood glucose, BOH, NEFA or plasma urea concentrations was detected as shown in Figures 3.3 to 3.6. The blood concentrations of glucose, BOH and NEFA changed after lambing (P < 0.05), but there was no significant (P > 0.1) interaction between time and treatment for any of the blood metabolites measured.

3.4.9 Rumen fermentation

There was no significant effect (P > 0.1) of monensin treatment on rumen fermentation, in terms of ammonia, total rumen fluid VFA concentration and molar proportions of individual VFAs (Table 3.8). The ammonia concentration (mmol/100 ml) in the rumen fluid did not change (P > 0.1) from 3 weeks (18.4) to 6 weeks (16.1) post-lambing. In contrast, the total VFA concentration in the rumen fluid doubled (P = 0.001) from 3 to 6 weeks post-lambing (3.68 vs. 6.83 mmol/100 ml, respectively). The molar proportions of individual VFAs, excluding acetate, changed significantly from 3 to 6 weeks post-lambing (P < 0.05). The molar proportion of propionic acid decreased by 26% (0.244 vs. 0.180; P = 0.0023), while the proportion of iso-butyric (0.014 vs. 0.025; P = 0.001), n-butyric (0.027 vs. 0.065; P = 0.001) increased. There was no significant interaction between treatment and time for ammonia, total VFA concentration or molar proportions of individual VFA.

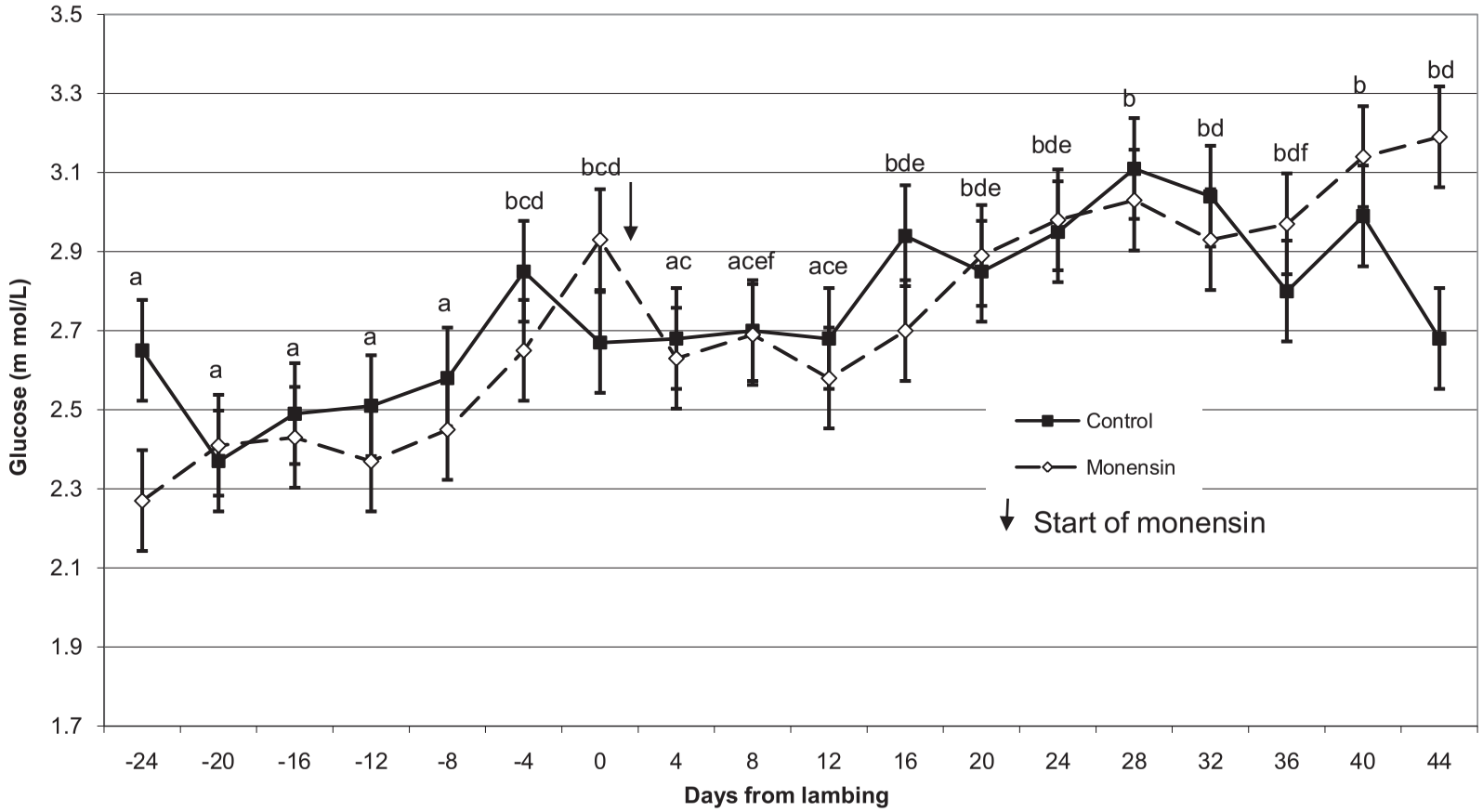


Figure 3.3: Pre- and post-lambing concentrations (m mol/L) of blood glucose in twin-bearing ewes with (monensin, n = 10) and without (control, n = 10) monensin supplementation, which commenced two days post-lambing. Lambing is denoted as day 0. Error bars represent the standard error of the mean. Differing letters denote a significant difference ($P < 0.05$) between days in the average blood glucose concentrations across both treatment groups.

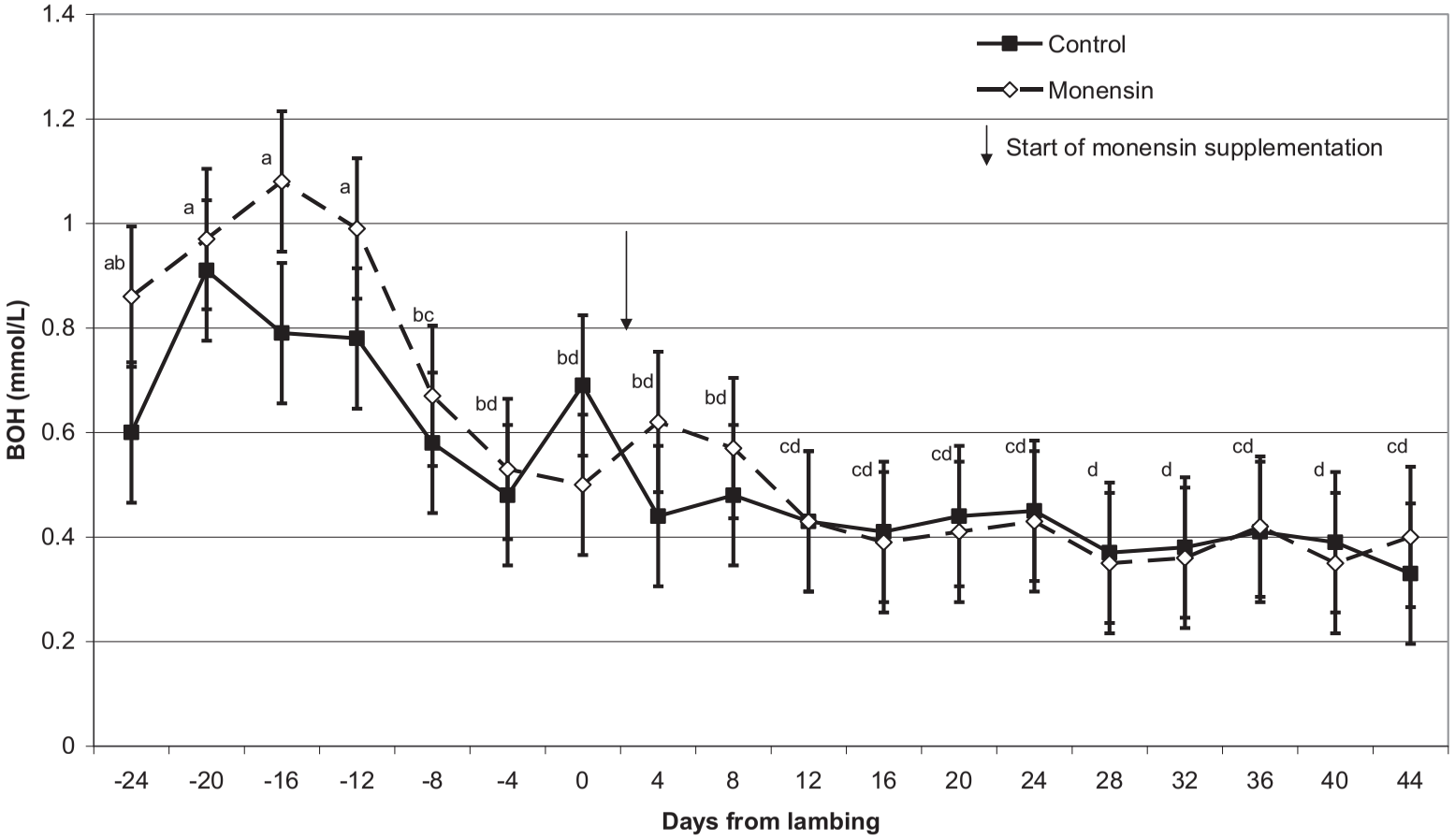


Figure 3.4: Pre- and post-lambing concentrations (m mol/L) of blood β-hydroxybutyrate (BOH) in twin-bearing ewes, with (monensin, n = 10) and without (control, n = 10) monensin supplementation, which commenced two days post-lambing. Lambing is denoted as day 0. Error bars represent the standard error of the mean. Differing letters denote a significant difference ($P < 0.05$) between days in the average blood BOH concentrations across both treatment groups.

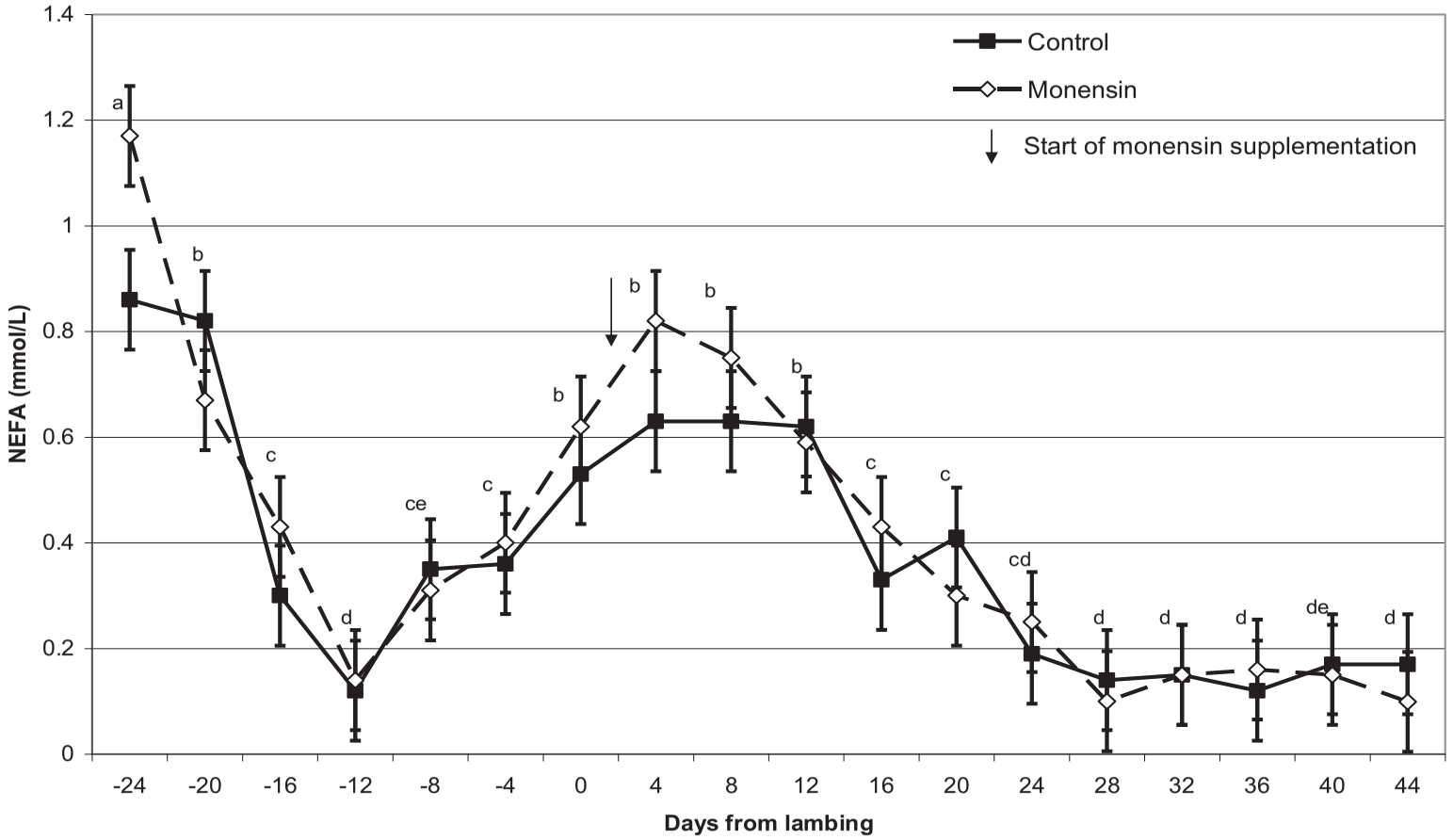


Figure 3.5: Pre- and post-lambing concentrations (m mol/L) of blood non-esterified fatty acids (NEFA) in twin-bearing ewes, (monensin, n = 10) and without (control, n = 10) monensin supplementation, which commenced two days post-lambing. Lambing is denoted as day 0. Error bars represent the standard error of the mean. Differing letters denote a significant difference ($P < 0.05$) between days in the average blood NEFA concentrations across both treatment groups.

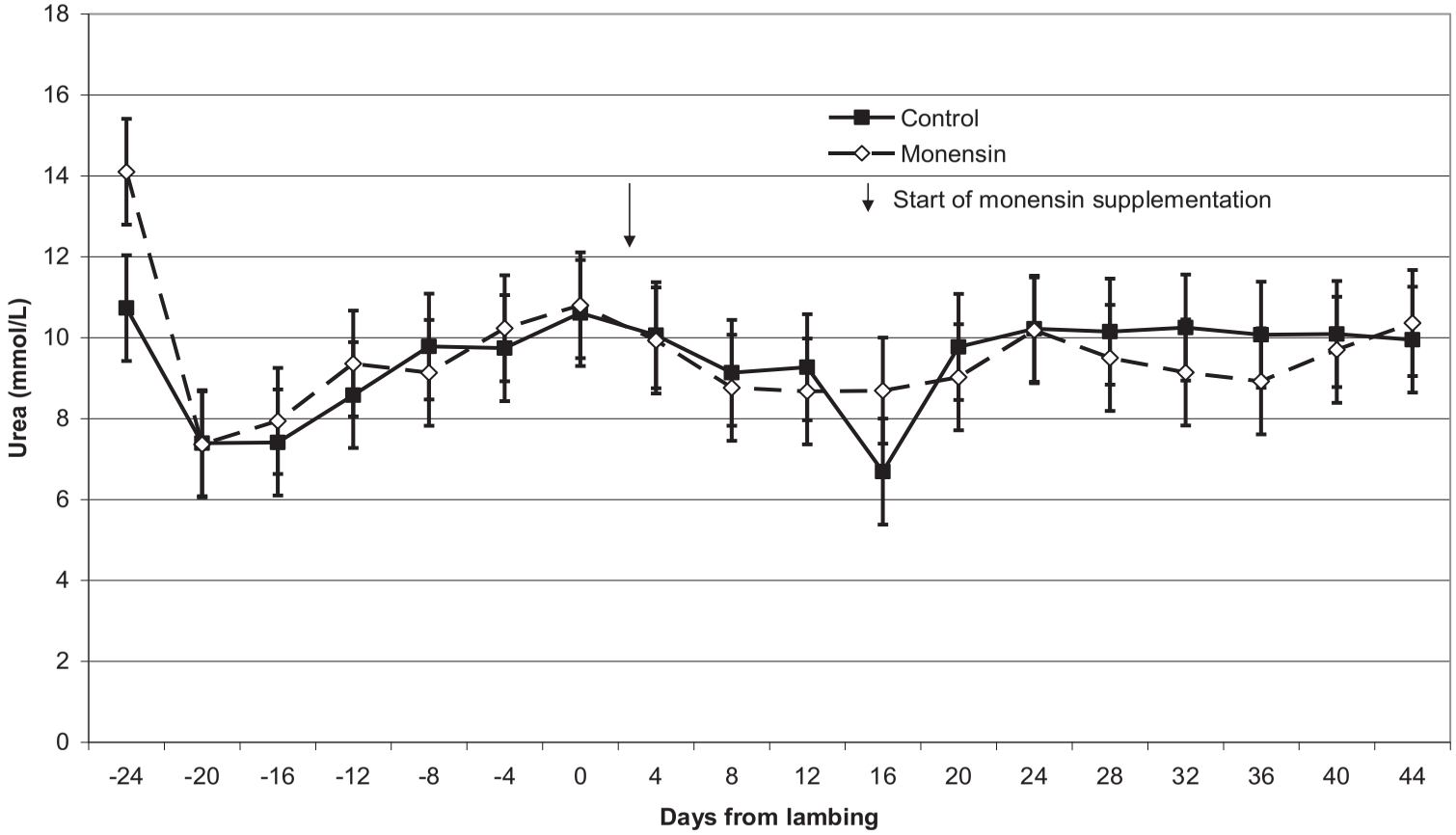


Figure 3.6: Pre- and post-lambing concentrations (m mol/L) of blood urea in twin-bearing ewes, with (monensin, n = 10) and without (control, n = 10) monensin supplementation, which commenced two days post-lambing. Lambing is denoted as day 0. Error bars represent the standard error of the mean.

Table 3.8: Mean (covariate adjusted ± SEM) total volatile fatty acid (VFA), ammonia concentrations (m mol/100 ml), and individual VFA as a proportion of total VFA concentration in the rumen fluid of twin-bearing ewes fed FibrePro (ensiled lucerne, *Medicago sativa,* chaff with added molasses) , with (monensin) and without (control) monensin supplementation at 3 and 6 weeks post-lambing.

	Post-lambing[3]								
	3 weeks			6 weeks			P-values		
	Control	Monensin	SEM	Control	Monensin	SEM	Time	Treat[1]	Time X Treat[2]
Ammonia (m mol/100 ml)	18.8	17.9	1.19	18.8	13.3	2.67	0.285	0.148	0.281
Total VFA (m mol/100 ml)	3.53	3.84	0.623	7.03	6.63	0.623	0.001	0.942	0.575
VFA as proportion of total VFA									
Acetic	0.703	0.690	0.0142	0.691	0.688	0.0141	0.649	0.565	0.730
Propionic	0.234	0.256	0.0177	0.174	0.186	0.0178	0.002	0.347	0.790
Iso-Butyric	0.017	0.011	0.0025	0.026	0.024	0.0024	0.001	0.237	0.261
n-Butyric	0.027	0.027	0.0076	0.073	0.057	0.0073	0.001	0.366	0.306
Iso-Valeric	0.012	0.008	0.0032	0.017	0.016	0.0032	0.061	0.487	0.600
n-Valeric	0.010	0.012	0.0020	0.024	0.024	0.0020	0.001	0.603	0.543

[1]Treatment

[2]Interaction between time and treatment

[3]Monensin n = 5; control n = 5

3.5 DISCUSSION

This study found that monensin supplementation of ewes during early lactation did not significantly reduce CH_4 production or yield. These findings are consistent with the reports of Waghorn *et al.* (2008) and Grainger *et al.* (2008), who also found no significant reduction of CH_4 yield in lactating dairy cows fed pasture and receiving monensin at a similar rate to ewes in this study. The study presented here also found that *in vivo* apparent digestibility, VFA as a proportion of total rumen fluid VFA concentration, and blood concentrations of metabolites, i.e. glucose, NEFA, BOH and urea, of ewes were unaffected by monensin supplementation. However, monensin supplemented ewes had greater milk production at 3 weeks post-lambing 1665 g/day compared with control ewes (981 g/day), but there was no significant difference at 6 weeks post-lambing. The final mean live weight at 5 weeks of age of twin-lambs born to ewes supplemented with monensin was greater than for twin-lambs born to control (unsupplemented) ewes (7.74 vs, 7.08 kg, respectively). Nevertheless, from this study, it is difficult to determine with confidence that this is a direct effect of monensin supplementation.

3.5.1 Methane emissions

In this study, the CH_4 yield from ewes during early lactation i.e. 3 and 6 weeks post-lambing was not significantly reduced by the supplementation of monensin. This lack of a significant difference between treatment groups in this study could have been due to low statistical power, as a consequence of the increased variability of calculated CH_4 production, arising from low and variable concentrations of SF_6 gas in the collected breath samples from some of the ewes. As a result, 45%, 55% and 50% of the total number of breath samples collected in the pre-lambing, and 3 and 6 week post-lambing measurement periods, respectively, were excluded in the final analysis of the data.

A retrospective power analysis found that at 3 weeks post-lambing, the power to detect a 20% difference in CH_4 yield between group means was 19%, and at 6 weeks post-lambing the power was 22%. It was also estimated that to achieve an 80% probability of detecting a significant difference ($P < 0.05$) between the

treatment and control groups, a difference of 59% would been required. Therefore it was unlikely that any difference in CH_4 yield between the monensin and control sheep would reach statistical significance.

The dose rate of monensin has been shown to affect liveweight gain in cattle fed conserved forage-grain-based diets (Raun *et al.,* 1976; Potter *et al*., 1976), and therefore it is likely that CH_4 yield may be similarly affected (Richardson *et al.,* 1976; Beauchemin *et al.,* 2008). The dose rate of monensin in the studies where monensin was not found to reduce CH_4 yield (Waghorn *et al.,* 2008; Grainger *et al.,* 2008), were 10.8 mg/kg DMI and 12 to 14.5 mg/kg DMI, respectively, which are similar to the dose rate used in this study (14 mg/kg DMI).

In comparison, CH_4 yield, measured using the SF_6 technique, was reduced by 9% (from 16.9 to 15.3 g CH_4/kg DMI), when dairy cows fed pasture were supplemented with monensin at a rate of 29.6 mg/kg DMI (Van Vugt *et al.,* 2005). However in other measurements, reported in the same study, monensin did not influence the CH_4 yield of cows, when supplemented at rates of 35.5, 17.5 and 18.1mg/kg DMI. This agrees with the study of Grainger *et al.* (2010), in which monensin (23 mg/kg DMI) did not reduce CH_4 yield in dairy cows fed fresh pasture and grain (22.7 vs. 22.2 g CH_4/kg DMI), as measured using respiration chambers. Therefore the hypothesis that the ability of monensin supplementation to reduce CH_4 yield is dependent on dose rate does not appear to be supported when animals are fed forages.

The efficacy of monensin supplementation may be influenced by the basal diet the animals are fed (Beauchemin *et al.,* 2008). Guan *et al.* (2006) compared a low-concentrate diet (86% silage plus 13% canola meal) and a high-concentrate diet (31% silage plus 68% barely grain), and found that CH_4 yield was reduced by similar amounts for each diet (27% and 30% respectively) when monensin was supplemented at a rate of 33 mg/kg DMI. The FibrePro diet (ensiled lucerne chaff with added molasses) used in this study was initially assumed to be of high nutritive value, with the product description specifying a value of 13 MJ ME/kg DM. The ME, determined by in vitro analysis and calculated from the in vivo results, found that the ensiled lucerne chaff had a mean ME of 8.7 MJ

ME/kg DM. In addition, the mean apparent DMD of the diet was 50% at 3 weeks post-lambing and 59% at 6 weeks post-lambing. These values are lower than the DMD obtained when FibrePro (sourced from a different batch) was fed to sheep (67%; Chapter 5) and could have influenced the ability of monensin to reduce CH_4 yield.

Johnson & Johnson (1995) and Guan *et al.* (2006) noted that in both forage- and grain-fed cattle and sheep, CH_4 yield returned to normal 4 to 6 weeks after the initiation of monensin supplementation. Guan *et al.* (2006) also identified that protozoa counts were reduced in response to monensin supplementation, but returned to pre-treatment levels after 4 or 6 weeks of monensin supplementation, when cows where fed the low and high-concentrate diets, respectively.

The return of protozoa to levels observed pre-treatment suggests a development of resistance to monensin. Nevertheless, the effect of monensin on reducing DMI, bloat and improving production efficiency appeared to be persistent when examined across a large number of studies (Duffield *et al*., 2008a,b,c). This suggests that the adaptation of CH_4 production to monensin is not always a direct effect of monensin resistance. From the few microbes tested by Chen & Wolin (1979), monensin was not found to impact directly upon methanogens. Therefore, a possible explanation for this is that there is a change in the dynamics of the methanogen community, and/or, with the reduction of protozoal number and activity, another group of microbes provides the hydrogen to enable methanogenesis, giving the appearance of monensin resistance.

3.5.2 Diet and animal production

The apparent DM digestibility of the diet fed to ewes at 3 (control, 48.8 vs. monensin, 51.1) or 6 (control, 60.3 vs. monensin, 61.5) weeks post-lambing was not influenced by monensin supplementation. This agrees with Maas *et al.* (2001) who compared the DMD of spring and autumn pasture fed to rumen fistulated sheep. Monensin was also shown to have no effect on the apparent digestibility of a diet containing whole crop barley silage (75%) and steam rolled

barely (19%) fed to Holstein steers (monensin, 63.8% vs. control, 62.0%) (McGinn *et al.,* 2004). However, the review of McGuffey *et al.* (2001) reported that the supplementation of monensin could improve apparent DM digestibility, albeit by only 2%, which would be difficult to detect within a single animal experiment.

As a means of evaluating ewe performance, the live weight and liveweight gain of the lambs was monitored. At birth, the lambs from the monensin group were heavier than the lambs from the control group (4.1 vs. 3.8 kg), but this was not an effect of monensin, as monensin supplementation did not begin until post-lambing. When birth weights were taken into account, there was no difference in live weight between the two groups until 5 weeks of age. This suggests that the monensin supplementation to ewes may improve growth efficiency in twin-lambs. However, it is not clear if this is a direct effect of improved milk production.

The mean milk production of ewes supplemented with monensin in this study was 41% greater than the control ewes and was found to be driven by the 70% difference between groups at 3 weeks post-lambing. It was also found that the significant difference in milk production between treatment groups detected 3 weeks post-lambing had disappeared by 6 weeks post-lambing. The increase in milk production due to monensin supplementation, as reported in this study, is greater than those reported for lactating dairy cattle.

Ipharraguerre and Clark (2003) reviewed 27 published reports, of which 18 reported no effect of monensin on milk production, whilst 14 reported an increase. The increases in milk production ranged from 2.8 (11.2%) to 0.4 (2.6%) with a mean of 1.5 kg per day (7%), with a wide range of diets at monensin supplementation rates ranging from 80 to 350 mg per cow per day. For cows fed diets predominately based on pasture, there was a mean increase in milk yield of 0.81 kg per day (Ipharraguerre & Clark, 2003). A meta-analysis of data from 36 published papers conducted by Duffield *et al.* (2008b) also showed that monensin increased milk production in cows by 0.7 kg per day, coinciding with a 0.3 kg (2%) decrease in DMI. From this study, cows fed high-forage diets typically had a greater response to monensin (1.5 kg/day) than

grain-fed animals (0.7 kg/day) (Duffield *et al.,* 2008b). The small differences in milk production identified by Ipharraguerre and Clark (2003) and Duffield *et al.* (2008b) would be very difficult to statistically detect in single experiments (Duffield *et al.,* 2008b).

In contrast, recent studies by Waghorn *et al.* (2008) and Grainger *et al.* (2008) found no significant difference in milk production between lactating dairy cows treated with monensin and cows receiving no monensin. Cows in these studies were supplemented with monensin at a similar rate to the sheep in this study, as discussed earlier. Van Vugt *et al.* (2005) and Wilson *et al.* (1993) both found no effect of monensin on the milk production of dairy cows when fed pasture and treated with rumen-controlled release capsules. Given the results from these reported studies, it is difficult to confidently conclude that the 70% increase in milk production of sheep at 3 weeks post-lambing was directly due to monensin supplementation.

The apparent increase of milk production at 3 weeks post-lambing in this study was not supported by any statistically significant differences in blood glucose concentrations, molar proportions of rumen VFA and lamb live weight or liveweight gain. It is suggested that the large difference in estimated milk production could be an artefact of the method used to determine milk production or ewe health. As some of the ewes were found to have developed sores on their udders, and whilst they were under close veterinary supervision, it is not known if udder health impacted on the collection of milk from these ewes and if ewes were experiencing sub-clinical mastitis.

At 6 weeks post-lambing, the estimated milk production did not statistically differ between the monensin and control groups (930 vs. 856 g/day respectively). Furthermore, no statistical difference in milk solids was detected between the monensin and control group (13.0 vs. 10.0 % DM per day). However, as noted by Duffield *et al.* (2008b), differences in milk production resulting from the supplementation of monensin are not often statistically detectable with a single experiment. In the study presented here, the milk production and total milk solids from the monensin groups were numerically greater than from the control group, and if this were a real effect, it may help explain the greater rate of

liveweight gain of lambs at 5 weeks of age. However, more research is needed to confirm this.

3.5.3 Blood metabolites

In the study reported here, no difference in blood metabolites concentrations i.e BOH, NEFA and blood glucose was found between the monensin and control group. This indicates that monensin did not improve the nutritional status of the ewes in this study. In support of this conclusion, propionic acid concentrations in the rumen fluid of sheep supplemented with monensin did not differ from the control group. These findings are similar to those reported by Maas *et al.* (2001) where the blood glucose, BOH, and urea concentrations in non-lactating sheep fed pasture in autumn and spring were unaffected by monensin supplementation. Nevertheless, blood glucose concentrations may not be a good indicator of glucose production, especially during early lactation, due to the rapid uptake of glucose by the body tissue and its regulation (homeostasis) (Ipharraguerre & Clark, 2003).

The findings presented here are in contrast to studies with dairy cattle, where the supplementation of monensin is reported to result in significant decreases of BOH from 6 to 51% (average 23%). However, NEFA was largely unaffected by monensin supplementation when compared across a range of diets (Ipharraguerre & Clark, 2003). In agreement with Ipharraguerre and Clark (2003), Duffield *et al.* (2008a) also demonstrated that monensin was able to reduce blood concentrations of BOH and NEFA by 13 and 7% respectively, when using data from 114 experiments. The largest increase of blood BOH and NEFA concentrations was found in dairy cows fed pasture, which may be an indication of the greater potential of pasture-fed dairy cows to be energy deficient, particularly during early lactation (Duffield *et al.* 2008a). The impact of monensin on blood metabolites is likely to be a result of an increased propionate supply to the liver (Duffield *et al.,* 2008a). However, no effect on rumen propionate concentrations was detected between the monensin and control groups in this study. The ewes were under significant nutritional stress and in poor body condition. Under these circumstances ketones (i.e. BOH) concentrations in the blood may not be elevated, as the animals were unlikely to

be able to mobilise significant quantities of body fat. Therefore, the condition of the animals used in this study could have impacted on the experimental findings.

3.5.4 Conclusion

This study did not find a reduction in methane emissions following monensin supplementation of lactating ewes. Combined with the inability to directly attribute improvements of ewe and lamb productivity to monensin supplementation, monensin is not recommended as a technology to reduce enteric CH_4 emissions in lactating ewes. The poor quality of the diet fed to sheep in this study is not representative of the diet of grazing sheep in New Zealand. It is recommended that the use of monensin in lactating ewes as a means to improve the nutritional status needs to be re-evaluated under conditions that better represent pastorally farmed sheep in New Zealand.

3.6 Appendix

Table 3.9: Adjusted live weight (kg) and liveweight gain (g/day) (mean ± SEM g/day) of twin-lambs from ewes with (monensin) and without (control) monensin supplementation and fed FibrePro (Fibre Fresh Feeds, Reporoa, New Zealand).

	Days of age	Treatment[1]			P-value
		Control[2]	Monensin[2]	SEM	(treatment)
Live weight	0	3.30	4.16	0.155	0.892
	7	4.94	5.08	0.164	0.571
	14	5.83	5.97	0.164	0.546
	22	5.97	6.43	0.172	0.615
	29	6.77	7.21	0.164	0.064
	36	7.08[a]	7.74[b]	0.168	0.007
Liveweight gain	7	156	180	12.6	0.192
	14	126	128	12.6	0.937
	22	72	73	13.2	0.947
	29	57	95	13.2	0.052
	36	43[a]	81[b]	13.0	0.048

[1] Birth weight used as a covariate of live weight (P = 0.0001) and liveweight gain (P = 0.9684). All means except for birth live weight are covariate adjusted means. The interaction between treatment and time was not significant for live weight (P = 0.283) or liveweight gain (P = 0.406)

[2] Monensin n = 20; Control n = 12

[ab] Letters denote a significant differences between means within the same row (P < 0.05).

CHAPTER 4

The effect of monensin and/or coconut oil supplementation on methane emissions of sheep fed either perennial ryegrass-based pasture or chicory

Chapter published in-part in:

Swainson N.M.: Hoskin S.O.; Clark H.; Brookes I.M. 2007: The effect of coconut oil and monensin on methane emissions from sheep fed either fresh perennial ryegrass pasture or chicory. *Greenhouse Gas and Animal Agriculture Conference, Christchurch, November 2007* (Abstract + Poster).

4.1 ABSTRACT

The aim of this experiment was to evaluate the effect of potential methane (CH_4) mitigation technologies, singly or in combination, on the CH_4 yield of sheep. The CH_4 production of 40 Romney wether hoggets, approximately 10 months of age and individually housed in metabolism cages, was estimated using the sulphur hexafluoride (SF_6) technique. The experiment consisted of three measurements periods 2 weeks apart, comprised of a covariate period and two treatment periods. Sheep received an experimental diet of either perennial ryegrass (*Lolium perenne*)-based pasture or chicory (*Cichorium intybus*) at 1.5 times estimated maintenance energy requirements, plus mitigation agents (monensin and/or coconut oil). There were four main treatment groups; no agent (n = 8), monensin (n = 8), coconut oil (n = 12) and coconut oil plus monensin (n = 12).

Sheep fed chicory produced (g/day) 33% less ($P < 0.001$) CH_4 (g/day) and yielded (g/kg dry matter intake, DMI) 19% less ($P < 0.001$) than sheep fed perennial ryegrass-based pasture (17.7 v 26.5 g CH_4/day and 24.9 v 30.7 g CH_4/kg DMI for chicory and perennial ryegrass respectively). The CH_4 yield of sheep supplemented with monensin (26.0 g CH_4/kg DMI) and monensin plus coconut oil (22.8 g CH_4/kg DMI) were 30% lower ($P < 0.05$) than the control group (32.8 g CH_4/kg DMI). There was no significant difference in CH_4 yield of sheep supplemented with coconut oil (29.6 g CH_4/kg DMI) compared with the control group.

This study indicates that reductions in CH_4 yield can be achieved by feeding chicory and this has the potential to be an important tool to reduce CH_4 emissions from grazing ruminants. However, the mechanisms responsible for the reduction of CH_4 yield in sheep fed chicory need to be elucidated. Monensin also reduced CH_4 yield; however, before it can be used with confidence, dose response trials need to be conducted with animals fed fresh forages. The combination of potential CH_4 mitigation technologies did not result in additional reductions in CH_4 yield that were statistically significant.

4.2 INTRODUCTION

Methane (CH_4) produced from the enteric fermentation of feed consumed by farmed ruminants has been identified as a contributor to anthropogenic greenhouse gases (GHG) and therefore climate change (Smith *et al.,* 2007). Technologies to reduce enteric CH_4 emissions are needed to mitigate the impact of agriculture on climate change. Currently, no single technology is able to consistently mitigate CH_4 emissions beyond a 10% reduction in CH_4 yield (Grainger, Pers. Comm. 2011). To provide reductions of enteric CH_4 yields that exceed the 10% threshold, a combination of mitigation technologies may be needed to be used at any one time. Investigations into methods to reduce enteric CH_4 yields have focused on the addition of one technology at a time. The effect of combining more than one mitigation technology at the same time on CH_4 yield is unknown.

Mitigation technologies that could be used to reduce CH_4 yield include the manipulation of the diet, for example the addition of grain, or the introduction of dietary supplements (mitigation technologies), such as monensin or coconut oil (Beauchemin *et al.,* 2008). Livestock production systems that rely on ruminants grazing forages place severe constraints on the type of CH_4 mitigation technologies that can be used. The dietary manipulation of grazing ruminants requires either the addition of dietary supplements, or changes in plant species grown in the forage sward. Mitigation technologies for use in grazing systems require intra-animal (application or delivery) technology, such as rumen slow-release devices or long-acting injections. This is particularly important for sheep, non-dairy cattle and deer production systems where animals are more extensively farmed and are not yarded or fed supplement on a daily basis.

New Zealand's pastures consist predominantly of perennial ryegrass (87% dry matter, DM, *Lolium perenne*) and white clover (13% DM, *Trifolium repens*; Ramírez-Restrepo & Barry, 2005). Alternatives to pasture that may be suitable for CH_4 mitigation include forages such as legumes and herbs. Waghorn *et al.* (2002) tested a range of legumes and herbs and found that the CH_4 yield of sheep could be reduced by 20 to 55% (20.6 to 13.8 g CH_4/kg DMI) compared with pasture (25.7 g CH_4/kg DMI).

Chicory (*Cichorium intybus*), a temperate forage herb, has reduced the CH_4 yields of sheep by 37%, from 25.7 to 16.2 g CH_4/kg DMI, compared with pasture (Waghorn *et al.,* 2002). Chicory has a superior feeding value compared with pasture, and can result in a mean increase in liveweight gain of 41% in red deer, from 182 to 256 g/day, and 68% in lambs, from 136 to 228 g/day (Barry, 1998). Therefore, chicory could be suitable as an alternative forage for mitigating CH_4 yield, whilst also being able to provide production benefits to grazing ruminants.

Compounds such as monensin and coconut oil have been reported to reduce CH_4 yield (Boadi *et al.,* 2004; Beauchemin *et al.,* 2008). Monensin, an ionophore antibiotic (Beauchemin *et al.,* 2008) used for the control of bloat in cattle, is also reported to have production benefits in terms of milk production and feed conversion efficiency for dairy cows (McGuffey *et al.,* 2001; Ruiz *et al.,* 2001; Ipharraguerre & Clark, 2003). Nevertheless, the efficacy of monensin as a CH_4 mitigation agent is widely debated. Beauchemin *et al.* (2008), identified six studies in which monensin significantly reduced the CH_4 yields by 4 to 30% in cattle fed primarily fed conserved forage and grain based diets. In another seven studies, no influence of monensin on CH_4 yield was observed.

Studies investigating the effectiveness of monensin on reducing CH_4 emissons when animals are fed fresh pasture have reported mixed results. Van Vugt *et al.* (2005) reported a 9% reduction, from 16.9 to 15.3 g CH_4/kg DMI, in CH_4 yield from dairy cows fed pasture and maize silage when supplemented with monensin at a rate of 29.6 mg/kg DMI. However, recent reports by Grainger *et al*. (2008) and Waghorn *et al.* (2008) found that monensin was not effective at reducing CH_4 yield in dairy cattle fed pasture. The cattle in these trials and sheep, as reported in Chapter 3, received lower dose rates of monensin per kg DMI (ranging from 11 to 14 mg/kg DM) compared with the dose rate used by Van Vugt *et al.* (2005).

The addition of lipids to the diet has been identified to have the potential to lower CH_4 yield by up to 40%, with realistic reductions of CH_4 yield ranging from 10 to 25% in commercial production systems (Beauchemin *et al.,* 2008). Beauchemin *et al.* (2008) also reviewed 17 studies and found for every

additional 1% of lipid in the DMI, CH_4 yield decreased by 5.6%. However, considerable variation in the reduction of CH_4 yield was found according to the type of fat used, with coconut oil achieving the highest reductions in CH_4 yield. Reductions in CH_4 yield by coconut oil are reported to be mediated by its high concentrations of medium-chain fatty acids (MCFA), particularly lauric (C12) and myristic (C14) fatty acids (Dohme *et al*., 2001; Machmüller *et al*., 2001). Sheep fed a hay/grain diet and supplemented with coconut oil at a rate of 3.5 or 7.0% of DMI were reported to have reduced CH_4 yields by 22 and 64%, from 22.1 to 17.3 and 8.0 g CH_4/kg DMI, respectively (Machmüller & Kreuzer, 1999). Coconut oil as a potential mitigation technology has not been tested in animals fed fresh forage diets.

Monensin and coconut oil appear to have different modes of action on the rumen microbial population to potentially reduce CH_4. Monensin affects the ion exchange of gram-positive microbes with simple single cell membranes, and protozoa within the rumen. Protozoa are associated with a high proportion of hydrogen (H_2) production and are suggested to indirectly account for up to 37% of methanogenesis in the rumen (Hegarty, 1999; Guan *et al.,* 2006). In contrast, coconut oil appears to act directly on methanogens, reducing population numbers and their metabolic activity (Machmüller, 2006). The effect of combining two potential mitigation technologies, such as coconut oil and monensin, is unknown. Furthermore, it is not known if any reduction in CH_4 yield will be dependent on the type of forage fed.

The aim of this study was to;

- Investigate the effect of diet (perennial ryegrass and chicory) and mitigation technologies (monensin and coconut oil), singly or in combination on the CH_4 yield of sheep.

4.3 MATERIALS AND METHODS

4.3.1 Experimental design

An experiment to investigate the effect of coconut oil and monensin, singly or in combination, and to compare the CH_4 yield of sheep fed chicory or pasture was

conducted at AgResearch, Aorangi, October to November 2006. The experiment was approved by the AgResearch Ltd, Grasslands, Animal Ethics Committee. The experiment consisted of three, 4-day measurement periods: (1) covariate pre-treatment period (from 4 October 2006); (2) treatment period 1 from 24 October 2006, and (3) treatment period 2 from 12 November 2006. All measurement periods were separated by 2-week intervals (Table 4.1).

Table 4.1: Timetable of activities during the experiment.

Day of experiment	Activity
1	Permeation tubes administered and all sheep grazing pasture
4 – 12	Sheep fed pasture at 1.5 x maintenance energy requirements
4 – 8	Sheep housed individually in metabolism cages
8 –12	Covariate CH_4[1] and DMI[2] measurements
12	Rumen fluid sampling (pH, ammonia and volatile fatty acids)
12 – 51	Commencement of treatment diets; chicory or pasture
12 – 26	Sheep housed in pens
15 – 52	Commencement of monensin and coconut oil supplementation
26 – 32	Treatment measurement one. Sheep housed in metabolism cages, CH_4 and DMI measured.
32	Rumen fluid sampling (pH, ammonia and volatile fatty acids)
33 – 47	Sheep housed in pens
47 – 52	Treatment measurement two. Sheep housed in metabolism cages, CH_4 and DMI measured.
52	Rumen fluid sampling (pH, ammonia and volatile fatty acids)
	Sheep returned to grazing pasture

[1] CH_4, methane

[2] DMI, dry matter intake

The pre-treatment measurement period was undertaken prior to the administration of coconut oil or monensin, and while all animals were fed pasture. During the treatment period, there were four main treatment groups: control, no agent (n = 8); monensin (n = 8); coconut oil (n = 12); and coconut oil plus monensin (n = 12). Within each main treatment group, equal numbers of sheep were fed either pasture or chicory.

4.3.2 Animals and treatments

Forty wether Romney hoggets (40.6 ± 2.69 (SD) kg live weight), approximately 10 months of age, were randomly assigned to treatment groups and diet. All groups were balanced for live weight, and prior to the experiment, all sheep

were grazed on perennial ryegrass-based pasture. During the three measurement periods, sheep were housed individually in sheep metabolism cages to enable the accurate measurement of individual daily DMI. Sheep were placed in metabolism cages 4 days prior to the covariate CH_4 measurement period and 2 days prior to the commencement of each of the following CH_4 measurement periods. Between measurement periods, sheep were housed in four large pens (10 sheep per pen) with two pens of sheep fed chicory and two pens of sheep fed pasture. Treatment groups were randomly distributed throughout the pens, within their respective diets. Raceways were constructed between pens to facilitate the twice daily administration of coconut oil and monensin. Animals were weighed at the start and end of each measurement period.

The supplementation of monensin and coconut oil commenced 11 days prior to the start of the first treatment measurement period and was administered to sheep prior to each meal event. Monensin was given orally as two 4 ml doses of diluted Rumensin™ oral drench for cattle (60 mg/ml monensin sodium, undiluted). This provided 15 mg/day/sheep of monensin, (Kim Agnew, Pers. Comm.; Elanco Animal Health, a division of Eli Lilly and company, Greenfield, Indiana, USA).

Coconut oil supplementation was set at 3% of dry matter (DM) allowance, on an individual animal basis, during CH_4 measurements and on a treatment group basis between measurement periods. This dose rate was to ensure that the maximum daily total lipid intake (coconut oil plus lipid in the forage) did not exceed 8% of DMI. Sheep received half of their daily dosage of oil (approximately 12 mls of oil depending on DM allowance) prior to each meal. The coconut oil was melted slowly in a microwave oven and placed in a hot water bath to prevent it from re-solidifying. The oil was given orally to sheep using a 20 ml syringe with a 5 cm flexible tube attached to the outlet point. The combination treatment group of monensin plus coconut oil received two separate drenches for each treatment.

4.3.3 Diets and feeding

During the pre-treatment period, all sheep were fed a tetraploid perennial ryegrass-based pasture (pasture; *Lolium perenne* cv. Wrightson seeds Quartet, tetraploid perennial ryegrass (82% DM) and white clover (*Trifolium repens* cv. Grasslands Kopu II; 13% DM), both sown in 2005. After the covariate measurement period, sheep were offered their assigned treatment diet of either chicory (*Cichorium intybus* cv. Wrightson seeds Choice (91%), sown in spring 2005) or pasture (as described above). This allowed 14 days of dietary adaptation prior to the first treatment measurement. Sheep were offered the same diet throughout both treatment measurement periods.

Feeding allowances were based on 1.5 x maintenance energy requirements, as per the Australian Feeding Standards (CSIRO, 2007). All diets were assumed to have a metabolic energy (ME) concentration of 12 MJ ME/kg DM, based on pre-trial forage analysis by near infrared spectroscopy (FeedTECH, AgResearch, Palmerston North, New Zealand). During the CH_4 measurement periods, sheep were fed on an individual live weight basis and when sheep were housed in pens, they were fed according to the group mean live weight. The energy concentration of the coconut oil was not taken into account when considering feed allowances.

The daily feed allowance was split into two meal events at 8.30 am (40%) and 4 pm (60%). Forages were cut in the morning using a sickle bar mower, starting at approximately 9.30 am every day during CH_4 measurements and every second day while the sheep were in pens. Forages were stored at 1 to 4 °C for a maximum of 48 hours.

During the measurement periods, samples of the feed offered were collected daily. Dry matter concentration (% DM) was determined by oven-drying triplicate samples at 100 ˚C for 24 hrs. Samples of feed offered were also collected for botanical and chemical analysis (approximately 500 g); samples for chemical analysis were stored frozen (–20 ˚C) and later pooled per forage within each measurement period. Samples for the determination of botanical composition were refrigerated and pooled at the end of each measurement period; a sub-

sample (approximately 200 g, fresh weight) of the pooled forage sample was taken and the DM proportions of leaf, reproductive stem, weed, clover and dead material determined (Chapter 2). Each sheep's feed refusal was collected daily and weighed. Feed refusals consisted of feed spilt under the metabolism cages, as there were no feed refusals left in the feed bins. Refusals for each forage were then pooled, homogenised, sub-sampled and the DM concentration determined by oven drying triplicate samples (24 h, 100 °C). Daily DMI was determined as DM of feed offered less DM of feed refused. Between measurement periods, samples of feed offered were taken three times per week and the DM concentration determined. There were no feed refusals when sheep were housed in pens.

4.3.4 Methane measurements

Methane production was calculated using the sulphur hexafluoride (SF_6) technique developed by Johnson *et al*. (1994; Chapter 2). A permeation tube charged with SF_6 gas was inserted into the rumen 8 days prior to the covariate measurement period. The release rate (mg/day) of SF_6 gas from the permeation tubes (1.19 ± 0.810 (SD) mg/day) was determined prior to insertion by the serial weighing of permeation tubes when incubated at 39 °C for eight weeks. The release rates were not corrected for post-deployment (Chapter 2) as the permeation tubes were unable to be retrieved at the end of the experiment. Allocation of permeation tubes to individual sheep was balanced for SF_6 release rate across treatment groups. Using equipment mounted on the head (Plate 4.1), breath samples for each individual animal were collected consecutively for four days per measurement period. Within each measurement period, breath samples were continuously sampled for 24 hours and each day's collected breath samples was then analysed. In addition, ambient air (background) samples were also collected, simultaneously to the animal samples, from two yokes at opposite sides of the building.

Total CH_4 production per day was calculated from the ratio of the concentration of SF_6 to CH_4 in the breath sample. The ratio was adjusted to the rate of SF_6 gas release from the permeation tube and for background levels of SF_6 and CH_4.

Plate 4.1: A sheep wearing the breath collection apparatus, while in a metabolism cage.

4.3.5 Rumen fluid sampling for pH, volatile fatty acids, and ammonia

Rumen fluid was collected by stomach tubing sheep on the day after they were returned to the pens at the end of each measurement period. The collection of rumen fluid occurred five hours after the morning meal and a minimum of 16 ml was collected per animal.

The pH of the rumen fluid was measured immediately after collection using a pH meter (PHM210, Radiometer, Copenhagen). Samples for volatile fatty acid (VFA) analysis (5.0 ml) were treated with 1.0 ml of a protein precipitate, and were then frozen (-20 ˚C). These were later thawed and centrifuged at 3000 rpm for 15 minutes and the supernatant was transferred to pre-labelled tubes and re-frozen. Rumen fluid for ammonia (NH_3) analysis (1.0 ml) was transferred to a 1 ml centrifuge tube containing 15 μl of concentrated hydrochloric acid and mixed thoroughly. Samples were initially frozen and later thawed and centrifuged (15 minutes at 1400 rpm) with a micro-centrifuge. After centrifuging the supernatant was transferred to another micro-centrifuge tube and re-frozen for later analysis.

4.3.6 Laboratory analysis

All feed offered, rumen fluid samples and coconut oil were analysed by wet chemistry at the Nutrition Laboratory, Massey University, Palmerston North. Samples of feed offered were prepared for analysis by freeze-drying and grinding the samples until fine enough to pass through a 1 mm sieve (Wiley Mill). Samples were analysed for gross energy (GE: Bomb calorimetry), organic matter (OM: Furnace 550ºC, AOAC 942.05), crude protein (CP: Leco, total combustion method. AOAC 968.06), neutral detergent fibre, acid detergent fibre and lignin (NDF/ADF/Lignin Tecator Fibretec System, Robertson & van Soest, 1981), hot water-soluble carbohydrates (HWSC: Nelson's determination of reducing sugars), pectin (Blumenkrantz Method for Uronic Acid (Pectin) Determination; Blumenkrantz & Asboe-Hansen, 1973), lipid (Soxtec extraction. AOAC 991.36) and *in vitro* DM and OM digestibility (DMD and OMD, respectively; Roughan & Holland, 1977). Rumen fluid NH_3 concentrations were measured by enzymatic determination and rumen fluid VFA concentrations were determined by gas chromatography (Wronkowska *et al.,* 2006).Coconut oil was analysed to determine the fatty acid profile, using fatty acid methyl esters analysis via gas chromatography (GC) separation (Sukhija & Palmquist, 1988).

4.3.7 Statistical analysis

The data were statistically analysed using the PROC MIXED model, with repeated measures (SAS, version 9.1, 2007). Pre-treatment measurements of DMI, CH_4 production, CH_4 yield, rumen pH, and VFA and NH_4 concentrations were used as covariates in the analysis of the data collected during the treatment periods. The main effects of forage, treatment (i.e. coconut oil and monensin), time and their interactions were compared. Where proportions and not absolute values are reported, e.g. VFA as a proportion of total VFA, the data were transformed by an arcsin square root transformation (Bromiley & Thacker 2002). Significance was declared at $P \leq 0.05$ and a trend reported if $0.05 < P \leq 0.10$.

4.4 RESULTS

4.4.1 Dietary chemical and botanical composition

The chemical composition and *in vitro* digestibility of the two forages are summarised in Table 4.2 and presented in full in Appendix 4.7, Table 4.12. Chicory had a lower DM concentration (P = 0.001) and lower cellulose (P = 0.004), hemicellulose (P = 0.001), and GE (P = 0.006) concentrations than pasture, but higher lignin (P = 0.003) and pectin (P = 0.001) concentrations. The *in vitro* DMD and OMD were 10% greater for chicory (DMD, 78.8 and OMD, 82.4%) compared with pasture (DMD, 68.1 and OMD, 69.8%). Chicory (12.4 MJ ME/kg DM) contained a higher ME concentration than pasture (10.8 MJ ME/kg DM; P = 0.03). Chicory tended to have lower OM (857 vs. 895 g/kg DM) (P = 0.083) and lipid (31 vs. 47 g/kg DM; P = 0.053) concentrations compared with pasture. The ratio of readily fermentable carbohydrate to structural carbohydrate tended to be greater (P = 0.063) for chicory (1.45) than pasture (0.40), but the CP and HWSC concentrations did not differ between forages (P > 0.1). The GE concentration of the coconut oil was 37.8 MJ/kg DM and the fatty acid profile of the oil is shown in Table 4.3.

Botanical composition analysis showed that the chicory diet had a low proportion of reproductive stems (0.08 (SD)), but the pasture diet had minimal reproductive material present (0.01 ± 0.002 (SD)). The proportion of weeds present in the chicory diet (0.03 ± 0.021 (SD)) was greater than that in the pasture diet (0.00 ± 0.62 (SD)). The proportion of clover in the chicory diet (0.01 ± 0.12 (SD)) was less than pasture diet (0.13 ± 0.105 (SD)). Both diets had a similar proportion of leaf (chicory 0.83 ± 0.132 (SD); pasture 0.82 ± 0.107 (SD)) and dead material (chicory, 0.05 ± 0.027 (SD); pasture, 0.05 ± 0.189 (SD)).

Table 4.2: Dietary chemical composition (± SEM, g/kg dry matter, DM), gross energy, *in vitro* DM digestibility (%) and metabolisable energy concentration of the pasture and chicory offered to sheep during treatment measurement periods.

	Forage[1]			P-value
	Chicory	Pasture	SEM	
Dry matter (%)[2]	9.8[a]	16.5[b]	0.98	0.001
Organic matter	857	895	8.2	0.083
Lignin	59[a]	15[b]	1.7	0.003
Crude protein	149	153	15.6	0.860
Lipid	31	47	0.1	0.053
Hot water soluble carbohydrate (a)	174	155	25.0	0.635
Pectin (a)	84[a]	15[b]	1.80	0.001
Hemicellulose (b)	60[a]	189[b]	3.2	0.001
Cellulose (b)	132[a]	230[b]	4.2	0.004
Ratio RFC:SC (a/b)[3]	1.45	0.40	0.177	0.063
Gross energy (MJ/kg DM)	17.1[a]	18.1[b]	0.06	0.006
Dry matter digestibility (%)	79.8[a]	68.1[b]	0.19	0.001
Organic matter digestibility (%)	82.4[a]	69.8[b]	0.52	0.006
Metabolisable energy (MJ ME/kg DM)	12.4[a]	10.8[b]	0.10	0.030

[1] n = 2 per forage, except for dry matter

[2] n = 8 per forage

[3] Readily fermentable carbohydrates: structural carbohydrate

[ab] Differing letters denote a significant difference ($P < 0.05$) between means within the same row

Table 4.3: Fatty acid (FA) profile (expressed as either g/100g or percentage of total FA) of the fatty acids detected in the coconut oil administered to sheep.

Fatty acid	g/100g	%FA/total FA
C 8:0 Caprylic	8.98	8.59
C 10:0 Undecanoic	6.28	6.01
C 12:0 Lauric	52.19	49.92
C 14:0 Myristic	18.77	17.96
C 16:0 Palmitic	7.83	7.49
C 18:0 Stearic	2.82	2.69
C 18:1-cis9 Oleic	6.68	6.39
C 20:0 Arachidic	1.00	0.96

4.4.2 Dry matter intake

The mean DMI was similar between all groups DMI (0.80 ± 0.011 (SEM) kg DM/day; $P < 0.1$) throughout the covariate measurement period, when all animals were fed pasture. The measured DMI from the covariate measurement period was significant ($P = 0.001$) when applied as a covariate in the analysis of subsequent measurement data.

Averaged over both treatment measurement periods, sheep fed chicory had lower ($P < 0.001$) DMI (0.70 vs. 0.88 kg/day), metabolisable energy intake (MEI, 7.0 vs. 8.2 MJ/kg DMI), feeding level (defined as multiples of ME for maintenance energy requirement) (1.45 vs. 1.58) and GE intake (GEI) compared with those fed pasture. No difference in DMI, feeding level or MEI ($P > 0.7$) was found between treatment groups. In contrast, the GEI of the sheep receiving coconut oil was significantly greater than the control or monensin-only treatment groups ($P < 0.05$; Table 4.4). The mean DMI (0.74 vs. 0.83 ± 0.012), MEI (7.2 vs. 8.0 ± 0.11 MJ/kg DM), feeding level (1.33 vs. 1.70 ± 0.022) and GEI (13.2 vs. 14.7 ± 0.21 MJ/kg DM), increased ($P = 0.001$) from treatment period 1 to 2 by 6%; however, no interaction ($P > 0.1$) between time and treatment or forage was found.

Table 4.4: Intake of forage dry matter (DM), feeding level (multiples of ME energy requirements for maintenance), metabolisable energy (ME) and gross energy (GE) of sheep fed either chicory or pasture and treated with monensin and/or coconut oil. Values presented are the mean (± SEM) of the two treatment periods.

		Treatments[5]							P - value		
		Control	Monensin	Coconut oil	Monensin + Coconut oil	SEM	Forage	SEM	Forage	Treat[3]	F x T[4]
DM intake (kg/day)	Chicory	0.67	0.69	0.71	0.71	0.022	0.70[a]	0.012	0.001	0.732	0.662
	Pasture	0.89	0.88	0.90	0.87	0.025	0.88[b]	0.012			
DM intake (g / $LW^{0.75}$)	Chicory	40.6	42.3	41.2	41.1	1.33	41.4[a]	0.11	0.001	0.649	0.696
	Pasture	53.7	52.9	52.8	51.0	1.39	52.6[b]	0.12			
ME intake (MJ/kg DM)[1]	Chicory	6.8	6.9	7.2	7.2	0.22	7.0[a]	0.11	0.001	0.724	0.629
	Pasture	8.3	8.2	8.3	8.1	0.24	8.2[b]	0.12			
Feeding level[1]	Chicory	1.43	1.46	1.45	1.45	0.043	1.45	0.022	0.001	0.954	0.871
	Pasture	1.60	1.59	1.59	1.55	0.044	1.58	0.022			
Total GEI [2]	Chicory	11.5	11.7	13.1	13.1	0.42	12.4[a]	0.21	0.001	0.003	0.662
	Pasture	16.1	16.1	17.2	16.6	0.44	16.5[b]	0.22			

[1] Does not include the energy content of coconut oil supplemented to sheep

[2] GEI includes the gross energy content of coconut oil (37.8 MJ/kg) supplemented to sheep.

[3] Treatment

[4] Interaction between forage and treatment

[5] Chicory n = 20; pasture n = 20; control n = 8; monensin, n = 8; coconut oil n = 12; coconut oil plus monensin n = 12

[a,b,] Denotes a significant difference ($P < 0.05$) between means within the same column

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Table 4.5: Planned number of animals versus the actual number of animals used and the number of data points included in the final analysis of methane (CH_4) production (g CH_4/day) and yield (g CH_4/kg DMI) within each measurement period.

			Treatments				Included data points	
		Forage	Control	Monensin	Coconut oil	Monensin + Coconut oil	Total	%[3]
Planned No. of animals	Chicory	20	4	4	6	6		
	Pasture	20	4	4	6	6		
	Total	40	8	8	12	12		
Total available data points[4]		160	32	32	48	48		
Treatment measurement period number of animals and data included								
Covariate	Chicory	17[1] (63)[2,4]	4 (16)	4 (15)	3 (10)	6 (22)	63	79
	Pasture	19 (64)	4 (10)	4 (15)	6 (22)	5 (16)	64	80
	Total	36 (127)	8 (28)	8 (30)	9 (32)	11 (38)	127	79
Treatment one	Chicory	15 (52)	3 (10)	2 (7)	5 (17)	5 (18)	52	65
	Pasture	18 (63)	3 (11)	3 (11)	6 (18)	6 (14)	63	21
	Total	33 (115)	6 (21)	5 (18)	11 (35)	11 (32)	115	72
Treatment two	Chicory	16 (59)	3 (12)	4 (15)	4 (15)	5 (17)	59	74
	Pasture	18 (72)	4 (16)	3 (12)	5 (20)	6 (24)	72	90
	Total	34 (131)	7 (28)	7 (27)	9 (35)	11 (41)	131	82

[1] Denotes the number of animals used.

[2] Denotes the number of data points available from all animals used.

[3] Percentage difference = (total included data points/total available data points) x 100.

[4]Each data point is approximately a 24 hour period of breath collection.

4.4.3 Methane emissions

The estimation of CH_4 production was not as successful as anticipated. This was due to the low and/or variable concentrations of SF_6 in some of the collected breath samples. Apparently normal concentrations of CH_4 were collected from the breath samples; this therefore suggests a problem with the release of the SF_6 from the permeation tubes or the rumen. Because of the low and variable concentrations of SF_6, data were only included in the final analysis if they met defined criteria (Annex A). Consequently, the final number of animals in each forage and treatment group was reduced, as shown in Table 4.5.

Methane production calculated from all sheep across all treatments increased ($P < 0.005$) by 20% from treatment measurement 1 (20.1 ± 1.03 (SEM) g/day) to treatment measurement 2 (24.10 ± 1.03 (SEM) g/day). In addition, CH_4 yield (26.2 vs. 29.4 ± 1.35 (SEM) g CH_4/kg DMI, $P = 0.081$) tended to increase between treatment periods 1 and 2. Methane production, CH_4 yield and CH_4 as a % GEI differed significantly with forage and treatment (Table 4.6), but no significant interaction between forage, treatment and/or treatment measurement period was found. Therefore values reported and presented in Table 4.6 are the mean of both treatment measurement periods.

Sheep fed chicory (24.9 g CH_4/kg DMI) had lower CH_4 yields than those fed pasture (30.7 g CH_4/kg DMI), which was similar to CH_4 was expressed as a % GEI (7.8 vs. 9.1, respectively; $P = 0.03$). When CH_4 was expressed per kg of digestible DMI (DDMI) sheep fed chicory (31.2 g CH_4/kg DDMI) compared with sheep fed pasture (45.4 g CH_4/kg DDMI) had lower CH_4 emissions ($P < 0.001$). This indicates that difference in DMD between chicory and pasture is unlikely to be entirely responsible for the lowered CH_4 yield of chicory fed sheep.

When averaged across both forages, the CH_4 yield of sheep receiving monensin (26.0 g CH_4/kg DMI) or the combination of monensin plus coconut oil (22.8 g CH_4/kg DMI) was lower than the control group (32.8 g CH_4/kg DMI; $P < 0.05$). The supplementation of coconut oil (29.6 g CH_4/kg DMI) had no effect on CH_4 yield. However, sheep supplemented with monensin had similar a CH_4 yield to both the coconut oil and monesin plus coconut oil groups, whilst the monensin plus coconut oil group had significantly lower CH_4 yield compared with the coconut oil group. Methane as a % GEI of mirrored the results for CH_4 yield.

Table 4.6: Methane (CH_4) production (CH_4 g/day), CH_4 yield (CH_4 g/kg dry matter intake, DMI), CH_4 per kg digestible DMI (CH_4 g/kg DDMI) and CH_4 energy expressed as a percentage of gross energy intake (GEI) (CH_4 as a % GEI) for sheep fed chicory or pasture and supplemented with monensin and/or coconut oil. Values presented are the mean (± SEM) of the two treatment periods.

		Treatment							P - value		
		Control	Monensin	Coconut oil	Monensin + Coconut Oil	SEM	Forage	SEM	Forage	Treat. ‡	F x T†
CH_4 g/day	Chicory	20.0	15.7	20.0	15.0	1.64	17.7[1]	1.08	0.001	0.001	0.324
	Pasture	32.1	25.0	29.0	19.9	1.78	26.5[2]	1.03			
	Mean	26.1[a]	20.3[b]	24.5[a]	17.5[b]	1.23					
CH_4g/kg DMI*	Chicory	29.5	22.9	26.5	20.5	2.15	24.9[1]	1.42	0.003	0.002	0.979
	Pasture	36.1	29.0	32.6	25.1	2.36	30.7[2]	1.35			
	Mean	32.8[a]	26.0[bc]	29.6[ab]	22.8[c]	1.60					
CH_4 g/kg DDMI#*	Chicory	37.2	29.3	32.6	25.7	2.99	31.2[1]	1.96	0.001	0.001	0.864
	Pasture	53.9	42.6	48.1	36.8	3.22	45.4[2]	1.87			
	Mean	45.6[a]	36.0[b]	40.3[ab]	31.2[c]	2.22					
CH_4 as % GEI^*	Chicory	9.5	7.4	8.2	6.1	0.64	7.8[1]	0.40	0.028	0.001	0.991
	Pasture	11.0	8.7	9.4	7.2	0.70	9.1[2]	0.41			
	Mean	10.3[a]	8.1[bc]	8.8[ab]	6.6[c]	0.48					

[1,2] Means with differing numbers denote a significant difference ($P < 0.05$) between means within the same column.

[abc] Means with differing letters, within the mean row denote a significant difference ($P < 0.05$) between means.

[#] Apparent dry matter digestibility's sourced from Table 4.9.

[^] CH_4 as % GEI includes the gross energy content of coconut oil supplemented to sheep.

* Covariate significant ($P < 0.05$) in the analysis of the data.

[‡] Treatment.

[†] Interaction between forage and treatment

4.4.4 Rumen fermentation

Total VFA production and molar percentage of VFAs

The total VFA concentration or the molar percentage of individual VFAs did not have an effect when used as a covariate in the analysis of VFA data from sheep during the treatment measurement periods and was removed from the statistical model. Total VFA production (Table 4.7) was not affected by either forage fed ($P > 0.8$) or treatment ($P > 0.7$).

The rumen fluid from sheep fed chicory had a greater ($P < 0.001$) molar percentage of propionic acid (23.8%) compared with pasture (20.2%) fed sheep. In contrast the percentages of acetic (69.1 vs. 71.2%) and n-butyric (5.1 vs. 5.9%) acids were lower in the sheep fed chicory compared with the pasture fed group.

The percentage of propionic acid in the rumen fluid of sheep treated with monensin and monensin plus coconut oil was up to 17% greater ($P < 0.05$) than the control or coconut oil-only groups. No difference between monensin-only and coconut oil plus monensin ($P = 0.603$) or control and coconut oil-only ($P = 0.2712$) were found. Similarly, no significant ($P = 0.107$) interaction between forage and treatment was found for the percentage of propionic acid in the rumen fluid of sheep.

The molar percentage of acetic acid from sheep treated with monensin (68.7%) or monensin plus coconut oil (68.8%) groups was less ($P = 0.005$) than the control (71.1%) or coconut oil (71.9%) groups. No statistically significant difference in acetic acid between the control and coconut oil, or the monensin and monensin plus coconut oil groups was found ($P > 0.1$). A significant interaction between forage and treatment for acetic acid ($P = 0.05$) was found. This is due to the lower molar percentage ($P < 0.001$) of acetic acid in the rumen fluid of sheep fed chicory and treated with monensin plus coconut oil (66.5%), compared with sheep fed pasture and receiving the same treatment (71.0%).

Rumen fluid pH and ammonia concentration

Rumen fluid pH and ammonia concentration are shown in Table 4.8. Sheep fed chicory (6.4) had a lower rumen fluid pH than sheep fed pasture (6.7; $P < 0.001$). There was a tendency for rumen pH to differ between treatments ($P = 0.068$), which was driven by the higher rumen pH in the control group (6.7), compared with the monensin (6.5), coconut oil (6.5) or coconut plus monensin group (6.6).

The ammonia concentration in the rumen fluid of sheep fed chicory (8.1 m mol/L) was 36% lower than the pasture fed sheep (12.7 m mol/L; $P < 0.001$) pasture. However, the supplementation of either coconut oil or monensin singly or in combination had no effect on rumen fluid ammonia concentration.

Table 4.7: Rumen fluid concentrations of total volatile fatty acid (VFA, m mol/100 ml) and individual VFA as a percentage of total VFA concentration of sheep fed chicory (n = 20) or pasture (n = 20) and treated with monensin (n = 8) and/or coconut oil (n = 12 coconut oil-only; n = 12 coconut oil plus monensin). Values presented are the mean (± SEM) of the two treatment periods.

		Treatment							P- value		
		Control	Monensin	Coconut oil	Monensin + Coconut Oil	SEM	Forage	SEM	Forage	Treatment	F x T[†]
Total VFA (m mol/100 ml)	Chicory	8.81	9.05	9.17	8.76	0.557	8.95	0.280	0.846	0.676	0.926
	Pasture	8.74	8.50	9.43	8.80	0.577	8.87	0.280			
	Mean	8.78	8.78	9.30	8.78	0.397					
VFAs molar percentage											
Acetic	Chicory	70.2^{ab1}	68.6^{a1}	70.1^{b1}	66.5^{c1}	0.77	69.1^{1}	0.39	0.001	0.001	0.050
	Pasture	72.0^{a1}	68.8^{a1}	72.8^{a1}	71.0^{a2}	0.82	71.2^{2}	0.41			
	Mean	71.1^{a}	68.7^{b}	71.9^{a}	68.8^{b}	0.56					
Propionic	Chicory	23.0	24.2	22.0	26.7	0.86	23.8^{1}	0.43	0.001	0.001	0.107
	Pasture	19.3	22.2	18.5	20.6	0.90	20.2^{2}	0.45			
	Mean	21.1^{a}	23.1^{b}	20.2^{a}	23.7^{b}	0.63					
n-butyric	Chicory	4.9	5.4	5.2	4.7	0.39	5.1^{1}	0.20	0.004	0.267	0.951
	Pasture	6.0	6.0	6.1	5.5	0.41	5.9^{2}	0.21			
	Mean	5.5	5.7	5.7	5.1	0.29					
Iso-butyric	Chicory	0.9	0.9	0.9	1.0	0.06	0.9^{1}	0.03	0.001	0.362	0.220
	Pasture	1.1	1.2	1.0	1.1	0.06	0.11^{2}	0.03			
	Mean	1.0	1.1	0.1	1.1	0.04					
Iso-valeric	Chicory	0.5	0.5	0.5	0.6	0.07	0.6^{1}	0.03	0.001	0.394	0.459
	Pasture	0.9	1.0	0.8	0.8	0.07	0.9^{2}	0.03			
	Mean	0.7	0.8	0.7	0.7	0.05					
n-valeric	Chicory	0.5	0.5	0.5	0.6	0.07	0.6	0.04	0.001	0.847	0.568
	Pasture	0.7	0.80	0.7	0.7	0.08	0.7	0.04			
	Mean	0.6	0.65	0.6	0.7	0.05					

[12] Denote significant differences (P < 0.05) between means with in a column, comparisons made between chicory and pasture

[abc] Denote significant differences (P < 0.05) between means within rows

[†] Interaction between forage and treatment

Table 4.8: Rumen fluid pH and ammonia (NH_3) concentrations (m mol/L) from sheep fed fresh chicory (n = 20) or pasture (n = 20) and treated with monensin (n = 8) and/or coconut oil (n = 12 coconut oil; n = 12 coconut oil plus monensin). Values presented are the mean (± SEM) of the two treatment periods.

		Treatment							P - value		
		Control	Monensin	Coconut oil	Monensin + Coconut Oil	SEM	Forage	SEM	Forage	Treatment	F x T†
pH*	Chicory	6.6	6.4	6.3	6.5	0.07	6.4[1]	0.04	0.001	0.068	0.446
	Pasture	6.7	6.7	6.7	6.8	0.08	6.7[2]	0.04			
	Mean	6.7	6.5	6.5	6.6	0.05					
NH_3	Chicory	8.5	6.8	9.5	7.9	1.07	8.1[1]	0.53	0.001	0.171	0.075
	Pasture	15.1	13.4	11.4	10.9	1.11	12.7[2]	0.55			
	Mean	11.8	10.1	10.4	9.4	0.77					

* Covariate significant (P < 0.001) in the analysis of the data

[12] Denotes significant differences (P < 0.05) between means with in a column, comparisons made between chicory and pasture

† Interaction between forage and treatment

4.5 DISCUSSION

The evaluation of mitigation technologies in this experiment found that chicory, as a fresh forage alternative to pasture, and monensin supplementation, when fed in conjunction with either fresh chicory or pasture, reduced the CH_4 emissions of sheep. The combination of monensin plus coconut oil, when supplemented to sheep fed fresh forages, did not result in a cumulative reduction in CH_4 yield that was significantly greater than for monensin supplementation alone. Nevertheless the performance of the SF_6 technique in this study was poor, owing to the intermittent collection of SF_6 in the breath samples of animals, which resulted in the development of a set of criteria to screen the data, (the decision tree in Annex A). Therefore, the performance of the SF_6 technique needs to be considered in the interpretation of the results from this study.

4.5.1 Chicory

Sheep fed chicory in this study had 17% lower CH_4 yields compared with pasture (chicory, 29.5 vs. pasture, 36.1 g CH_4/kg DMI). This is consistent with, although smaller than, previous findings of Waghorn *et al.* (2002) who found a 37% reduction of CH_4 yield when sheep were fed chicory, compared with those fed pasture (16.2 vs. 25.7 g CH_4/kg DMI). The reasons for a reduction in CH_4 yield in response to chicory are not known. Factors influencing CH_4 yield include DMD, passage rate, and the chemical composition of the diet (Moss *et al.,* 2000; Boadi *et al.,* 2004); differences between chicory and pasture in these factors may help to explain the lower CH_4 yield from chicory fed sheep.

Models that have been developed in an attempt to describe and/or predict CH_4 yield (Blaxter & Clapperton, 1965; Holter & Young, 1992; Ellis *et al.,* 2009; Yan *et al.,* 2009) and relationships derived from these models generally indicate that an increase in DMD, which coincides with a decrease in the proportion of dietary fibre, relates to a decrease in CH_4 yield. Nevertheless, these models describe and predict CH_4 yield with mixed success, and many of these models are based on data from ruminants fed TMR type diets. Blaxter and Clapperton (1965) reported that the CH_4 emissions of ruminants decreased in response to

increasing diet apparent digestibility when fed above maintenance energy requirements. The *in vitro* DMD of chicory in the current study was 12% greater than pasture (68.1 vs. 79.8%). This difference is somewhat larger than the 7% in previous *in vivo* reports with sheep (79.3 vs. 74.0%, Waghorn *et al.,* 2002) and red deer (75.2 vs. 68.5%, Hoskin *et al.,* 1995; 78.5 vs. 72.7%, Kusmartono *et al.,* 1997). Chicory in this study had a greater concentration of pectin, a higher ratio of readily fermentable carbohydrates to structural carbohydrates and lower cellulose and hemicellulose concentrations compared with pasture, which agrees with previous reports (Hoskin *et al.,* 1995; Barry, 1998).

From models developed to predict CH_4 yield (Blaxter & Clapperton, 1965; Holter & Young, 1992; Ellis *et al.,* 2009; Yan *et al.,* 2009), the greater DMD and concentration of readily fermentable carbohydrates supports the finding that sheep fed chicory yielded less CH_4 than pasture-fed sheep. Nevertheless, the analysis of the New Zealand CH_4 database of sheep and cattle fed fresh forage diets found no significant relationship between DMD or chemical composition and CH_4 yield (Waghorn & Woodward, 2006; Hammond *et al.,* 2009). Therefore, it is difficult to speculate what contribution the differences in diet DMD and/or chemical composition between chicory and pasture would have made to the differences in CH_4 yield reported in this study and further research is required to elucidate this.

The above models are predominantly based upon conserved forage diets that are high in structural fibre, and the proportion of readily fermentable carbohydrates is often in the form of starch sourced from grains. In contrast, the readily fermentable carbohydrates in fresh forage diets contain very low amounts of starch and normally consist of differing types of fructans (Chalmers *et al.,* 2005). The effect of differing types of readily fermentable carbohydrates on the rate of fermentation and CH_4 yield is not understood.

The wet chemistry analysis of chicory only accounted for 860g of the chemical components per kilogram of DM. This implies that a proportion of the chemical components of chicory were not identified and the impact of this proportion on CH_4 yield is unaccounted for. In addition, the chicory fed to sheep in this study had a greater ash concentration than pasture. Therefore the reduced CH_4 yield

of sheep fed chicory could simply be a result of these animals consuming less fermentable material compared with pasture fed animals.

The 31% reduction in CH_4 when expressed per kg digestible DMI, from sheep fed chicory compared with pasture, suggests that differences in apparent DMD between chicory and pasture cannot fully explain the reported difference in CH_4 yield. This implies that there may be a modification of the microbial fermentation pathways affecting methanogenesis in sheep fed chicory. This is supported by 18% greater propionic acid (23.8 vs. 20.2% molar % of total VFA) and 3% lower acetic acid (69.1 vs. 71.2% molar % of total VFA) concentrations found in the rumen fluid of sheep fed chicory compared with pasture. Moss *et al.* (2000) reported that a greater production of propionic acid is associated with a reduction in methanogenesis. However, VFA concentrations from rumen fluid have been shown to have no clear relationship with CH_4 yield (Robinson *et al.,* 2010).

Pinares-Patiño *et al.* (2003b) and Okine *et al*. (1989) demonstrated that CH_4 yield is inversely related to the rumen fractional outflow rate (FOR) of the particulate phase of the digesta. The passage rates of chicory or pasture through the digestive tract were not determined in this study. Nevertheless, previous studies with red deer reported particulate FOR of chicory (4.08%/hour) from the rumen to be twice that of pasture (2.78%/hour; Kusmartono *et al.,* 1996; 1997), which was attributed to the rapid breakdown of chicory in the rumen (Kusmartono *et al.,* 1996). Sun *et al.* (2007) identified that the rapid degradation of chicory, compared with pasture and white clover, was due to a unique cell wall structure, which is rich in pectin (Sun *et al.,* 2007). Both the rapid breakdown and increased fractional outflow of chicory in the rumen compared with pasture could limit the amount of time feed particles are exposed to microbial fermentation, thus contributing to a reduction in CH_4 yield.

In this study, it was intended that all sheep were offered the treatment diets at the same feeding level (1.5), but the actual feeding level of animals fed chicory (1.45) was lower than those fed pasture (1.58). The difference in feeding level reflected a mean difference of 0.18 kg DM/day between chicory and pasture fed sheep (0.70 vs. 0.88 kg DM/day), which could have impacted upon CH_4 yield.

The reduction of CH_4 yield and reported superior nutrient value of chicory for grazing ruminants (Barry, 1998) suggests that chicory as a CH_4 mitigation tool that could be successfully implemented into grazing systems, with appropriate climate, topography and soil types for growing chicory. In this study, chicory was fed as pure culture forage, but farm practices often result in multiple forage species on offer to animals at the same time. The proportion of chicory required in the diet to achieve significant reductions in CH_4 yield is not known and needs further investigation if chicory is to be proven as a useful on-farm mitigation tool.

Additionally, the testing of diets and their relative effects on the ruminant animal and associated digestive processes, including CH_4 yield, are mostly conducted over a relatively short period of time. There have been no long term investigations to ascertain if adaptation to the diet may occur and if differences in CH_4 yield changes over time.

4.5.2 Potential mitigation agents

4.5.2.1 Monensin

In this study, the CH_4 yield of sheep supplemented with monensin was 22% less compared with sheep in the untreated control group (20.3 vs. 26.1 g/kg DMI). Coinciding with the decrease in CH_4 yield, a 9% increase in propionate (23.1 vs. 21.1 % of total VFA) and a 4% decrease in acetate (68.7 vs. 71.1 % of total VFA) concentration was observed in the rumen fluid of sheep supplemented with monensin compared with sheep in the control group. This indicates that the amount of H_2 available for methanogenesis was reduced when sheep were supplemented with monensin. However, if the assumption that VFA concentrations reflect total VFA production then the increase in propionate acid combined with the decrease in acetate acid concentration cannot account for the reduction in H_2 required to lower CH_4 by 22% as shown in this study.

Van Nevel and Demeyer (1996) and Beauchemin *et al.* (2008; Table 4.9) reviewed *in vivo* studies examining the effect of monensin on CH_4 emissions and strongly suggested that the degree of CH_4 mitigation is dependent on both the diet and the dose rate. Therefore, the reduced CH_4 yield could be due to the

higher dose rate of monensin of 21 mg/kg DMI, compared with no reduction in CH_4 yield at monensin supplementation rates of 10.8 mg /kg DMI (Waghorn *et al.,* 2008), 12.0 - 14.5 mg/kg DMI with pasture-fed dairy cattle (Grainger *et al.,* 2008) and 14 mg/kg DMI with ewes fed ensiled lucerne (Chapter 3).

Nevertheless, the response of dose rate on CH_4 yield when animals are fed fresh forages is not consistent. In the study of Van Vugt *et al.* (2005), monensin was given to dairy cows fed pasture and maize silage at rate of 29.6 mg/kg DMI, yet CH_4 yield was reduced by 9%, from 16.9 to 15.3 g CH_4/kg DMI. A recent study by Grainger *et al.* (2010) showed that there was no effect of monensin on CH_4 yield in dairy cows fed pasture and barley grain, when monensin was supplemented at a rate of 471 mg/day or 22.5 mg/kg DMI. Therefore the dose rate of monensin when animals are fed fresh forage diets does not appear to explain the inconsistent effectiveness of monensin to reduce methanogenesis.

Monensin is proposed to indirectly reduce methanogenesis by targeting gram-positive bacteria and protozoa (Chow *et al.,* 1994). No direct impact of monensin on the methanogen population has been observed, as there does not appear to be a direct action on the archaea community (Van Nevel & Demeyer, 1996). By reducing the protozoa population size, it is hypothesised that there is both a reduction in the total amount of available H_2 for methanogenesis and an elimination of a symbiotic host for some methanogens, thereby reducing the total number of methanogens in the rumen. In reviews by both Hegarty (1999) and Morgavi *et al.* (2010), it was estimated, from both *in vivo* and *in vitro* data, that the elimination of protozoa from the rumen across all diets would result in a reduction of methane production by 13 and 10.3%, respectively.

Nevertheless, Hegarty (1999) reported that defaunation of ruminants had little effect on methanogensis, when animals were fed forage diets. This is most likely because when animals are fed forage diets, protozoa are not the only significant source of H_2 for methanogenesis. Instead a large proportion of the hydrogen could arise from production of acetic acid. In addition, the protozoa biomass in forage-fed ruminants is reduced compared with grain-fed ruminants (Jouany & Ushida, 1999). Therefore, a reduction in protozoa in forage-fed

animals may not have the same impact as it would in grain-fed animals. For example, Brossard *et al.* (2003) showed that the protozoa community, one hour before feeding lucerne hay to sheep, averaged 106 x 10^3 per ml of rumen fluid, which increased significantly to 340 x 10^3 per ml of rumen fluid when the same sheep were fed a diet consisting of 60% ground wheat and 40% lucerne hay.

In a comparison of the effect of diet type when monensin was supplemented to steers (33 mg/kg DMI), Guan *et al*. (2006) compared a low-concentrate diet (86% silage) and a high-concentrate diet (31% silage) and found that CH_4 yield was reduced by 27% and 30%, respectively. However, the reduction of CH_4 yield did not differ significantly between the two diets. No direct comparison of this type has been made between a fresh forage diet and a conserved-forage or grain-based diet. This suggests the need for CH_4 measurements in both dose-response and longevity trials with monensin-supplemented animals fed fresh forage diets.

Table 4.9: Effect of monensin on methane (CH_4) production and yield. Adapted from Beauchemin *et al.* (2008).

Animals	Diet	Dose rate		Days after dose	CH_4 emissions				Reference
		(mg/day)	(mg/kg DMI)		Control (g/day)	Monensin (g/day)	Control (g/kg DMI)	Monensin (g/kg DMI)	
				Controlled release capsules					
Dairy cows	Ryegrass pasture	166	11	30-90	328	313^{ns}	19.2	20.0^{ns}	Waghorn *et al.*, 2008
Dairy cows	Ryegrass pasture	320	29.6	11	179^{a}	158^{b}	16.9^{a}	15.3^{b}	Van Vugt *et al.*, 2005
Non-lactating dairy cows	Ryegrass pasture	320	35.2	72	246^{a}	223^{b}	25.5	24.8^{ns}	Van Vugt *et al.*, 2005
Dairy cows	Ryegrass + white clover	320	17.5	23	330^{a}	309^{b}	17.5	16.9^{ns}	Van Vugt *et al.*, 2005
Dairy cows	Ryegrass + maize silage	320	18.1	58	350	356^{ns}	19.2	20.5^{ns}	Van Vugt *et al.*, 2005
Dairy cows	Ryegrass + grain	240	13	25,85	341	365^{ns}	-	-	Grainger *et al.*, 2008
Dairy cows	Ryegrass + grain	240	13	83	376	386^{ns}	-	-	Grainger *et al.*, 2008
Dairy cows	Ryegrass + grain	240	13	75	309	306^{ns}	16.7	17.0^{ns}	Grainger *et al.*, 2008
				Added to the ration					
Dairy cows	Grain + forage	385	24	8–28	572^{a}	517^{b}	38.6^{a}	35.7^{b}	Sauer *et al.*, 1998
	Grain + forage	385	24	8–28*	599	598^{ns}	34.9	33.7^{ns}	Sauer *et al.*, 1998
Dairy cows	Grain + forage	473	24	**	458.7^{a}	258.6^{b}	23.3^{a}	22.4^{b}	Odongo *et al.*, 2007
Dairy cows	Ryegrass + grain	471	23.6	35	429	435	22.0	21.6	Grainger *et al.*, 2010
	Ryegrass + grain	471	22.5	70	466	470	22.5	23.7	Grainger *et al.*, 2010
Beef cattle	High forage	246	33	19	166.2^{a}	159.6^{b}	22.6^{a}	20.7^{b}	McGinn *et al.*, 2004
Beef cattle	High grain	271	33	Weekly for 16 weeks		-27% for 2 weeks, 0 by week 6			Guan *et al.*, 2006
Beef cattle	High forage	240	33	Weekly for 16 weeks		-30% for 4 weeks, 0 by week			Guan *et al.*, 2006
				Daily oral drenching					
Lactating ewes	Ensiled lucerne chaff	21	14	12 – 33	30.2	26.4^{ns}	20.6	19.2	Chapter 3
Hoggets	Pasture or chicory	15	21	11 – 46	26.1^{a}	20.3^{b}	32.8^{a}	26.0^{b}	Chapter 4

* Second CH_4 measurement

** CH_4 measured monthly for six months

^{ab} Within rows and the CH_4 variable, values followed by the same letter are not significantly different at $P > 0.05$.

^{ns} No effect of monensin on CH_4 emissions reported.

4.5.2.2 Coconut oil

In this study when feeding fresh forages, there was no suppression of CH_4 yield sheep when supplemented with coconut oil. This is in contrast to literature reviews of Beauchemin *et al.* (2008) and Machmüller (2006) reporting CH_4 yield reductions of 8 to 64% (Table 4.10) when sheep were fed a conserved forage and grain diet, supplemented with coconut oil. The effectiveness of coconut oil in reducing CH_4 yield can be influenced by large variety of factors, with the most predominate appearing to be dose rate and diet (Machmüller, 2006). Both of these factors in the present study differed from those in the studies reviewed by Machmüller (2006).

Machmüller & Kreuzer (1999) and Beauchemin *et al.,* (2008) concluded that the CH_4 mitigation response to added dietary fat was dependent on the amount of lipid supplementation. When sheep were fed hay and concentrates and supplemented with coconut oil at 0% (control), 3.5% or 7% of DM, daily CH_4 yield decreased from 22.1 to 17.3 and 8.0 g CH_4/kg DMI, respectively (Machmüller & Kreuzer, 1999). In the present study, coconut oil supplementation was 3% DM offered and may have been insufficient to reduce CH_4 production or yield. The total lipid concentration of a ruminant's diet appears to be 9%, above which intake and apparent digestibility are negatively affected (Beauchemin *et al.,* 2008; Cosgrove *et al.,* 2008). As both chicory and pasture contain approximately 3 to 5% total lipids, additional supplementation of lipids above the rate of 3 to 4% DMI may not be practical without risking animal health.

The use of coconut oil to reduce CH_4 yield when animals are fed fresh forages have not been previously tested *in vivo.* From research conducted with grain-based diets, the effectiveness of coconut oil and/or MCFA appears to be influenced by the composition of the dietary carbohydrate fraction, as defined by the amount of NDF (Machmüller, 2006). Machmüller (2006) reviewed studies where sheep were supplemented with coconut oil and reported that in sheep fed a hay-based diet (46% DM NDF) CH_4 yield was unaffected (Machmüller *et al*., 2001), but a reduction in CH_4 yield of up to 70% was reported when the diet

(grain and silage-based diet) contained less than 31% DM NDF (Machmüller & Kreuzer, 1999). It is difficult to determine from the current study if dietary chemical composition of the forages fed may have impacted on the effectiveness of coconut oil to reduce CH_4 yield of sheep. Despite a difference in NDF concentration between the forage diets (chicory, 25% DM NDF; pasture, 43% DM NDF), there was no significant difference in CH_4 yield.

Table 4.10: Summary of in vivo experiments of sheep supplemented with coconut oil and its effect on methane (CH_4) production (CH_4p, g/day) and CH_4 yield (CH_4y, g/kg DMI).

Diet	Dose rate %DM	Total ether extract % DM	DMI kg/day	NDF g/kg DM	CH_4p	CH_4y	% difference[3] of CH_4y	Reference
Hay (63%)-concentrate[1]	0	6.8	0.992	453	18.6	18.8	-	Machmüller et al., 2001
Hay (63%)-concentrate[1]	5.9	6.1	0.992	461	17.2	17.3	8.0	
Hay (62%)- concentrate[1]	0	1.8	0.685	418	29.5[a]	22.1[a]	-	Machmüller & Kreuzer,1997
Hay (71%)- concentrate[1]	3.5	4.4	0.599	446	21.2[b]	17.3[b]	21.7	
Hay (46%)- concentrate[1]	7	7.0	0.694	348	7.4[c]	8.0[c]	63.8	
TMR (35% concentrate)[1]	0	3.1	0.943	367	15.5	16.4	-	Machmüller et al., 2000
	2.5	5.4	0.908	373	11.5	12.7	22.5	
Chicory[2]	0	4.7	0.67	251	20.0	29.5	-	Chapter 4
	3	nd	0.71	251	20.0	26.5	10.1	
Pasture[2]	0	3.1	0.89	434	32.1	36.1	-	
	3	nd	0.90	434	29.0	32.6	9.6	

[1] CH_4 measured using open circuit respiration chambers

[2] CH_4 calculated by SF_6 technique

[3] Percentage difference of CH_4 yield between control sheep and those treated with coconut oil

[abc] Differing letters within column and group denoted a significant difference between means

4.5.2.3 Combining potential mitigation agents and technologies

In this study, the combination of monensin and chicory initially appeared additive. Relative to pasture, chicory reduced sheep CH_4 yields by 17%, which was reduced by a further 8% when sheep fed chicory were supplemented with monensin. However, this reduction was not statistically significant. In addition, the CH_4 yield of sheep fed both pasture and chicory were reduced by up to 22% when animals were supplemented with monensin. This reduction was increased to 30%, when an additional supplement of coconut oil was given, but this was not statistically significant. However, the monensin group was small in number, and there was greater than expected variation within and between animals in the study, due to low and highly variable concentrations of SF_6 in some of the collected breath samples. A retrospective power analysis based on CH_4 yield, using the mean standard deviation from the treatment groups (10.97 g CH_4/kg DMI) and a sample size of seven animals (seven being the number of animals identified to be needed in the pre-experimental power analysis, Chapter 6, 6.2.3), found that there was a 3% probability that a significant ($P < 0.05$) difference of 20% between two means would be detected. A difference of 64% between the two means would have been required to have an 80% probability of detecting a significant difference ($P < 0.05$). This implies that no definite conclusion can be made on the efficacy of combining the mitigation technologies.

Results from this study support the need for further research to investigate the potential impact of combining potential mitigation technologies. It is recommended that further investigations be conducted using either a greater number of animals and/or a different technique to measure CH_4 production that has greater accuracy than SF_6 .

4.5.3 SF_6 technique

The CH_4 yield values presented in this study for chicory (29.5 g CH_4/kg DMI) and pasture (36.1 g CH_4/kg DMI) are greater than those previously reported for sheep when fed these forages (chicory: 16.2 g/kg DMI (Waghorn *et al.,* 2002) v Pasture: 23.4 g/kg DMI (Hammond *et al.,* 2009)). Although CH_4 as a

percentage of GEI (chicory, 9.5 and pasture 11.0 CH_4 as a % GEI) are within the biological range reported in the literature of 2 to 12% (Johnson & Ward, 1996), these high CH_4 yields, combined with the inconsistent detection of SF_6 gas from the breath samples, raises concerns regarding robustness of the data and the SF_6 technique.

The mean concentration of CH_4 in the breath samples collected from sheep in this study (44.9 ppm) was lower than the mean of experiments in the New Zealand database (72.7 ppm) (Table 4.11). In addition, the coefficient of variance (CV) of CH_4 from this study (65.7%) was greater than that from the New Zealand database (29.1%), indicating that the collection of breath samples was not as consistent as in previous studies. It is assumed that SF_6 behaves in a similar manner to CH_4. The much greater CV of SF_6 compared with CH_4 suggests that the SF_6 gas did not behave in a similar manner to CH_4 (99.8 vs. 65.7%). The CV of the ratio of SF_6:CH_4: from this study (111.4%) is greater than that from the New Zealand database (79.7%).

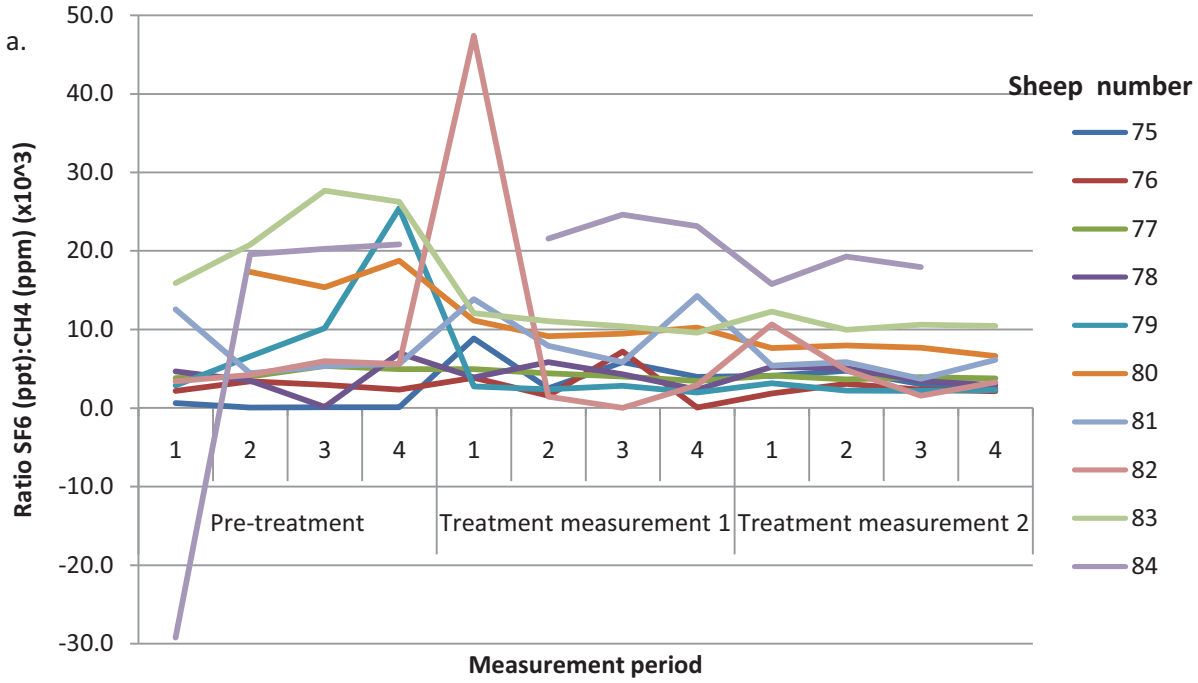
Both the concentration of SF_6 and ratio of SF_6:CH_4 are influenced by the SF_6 release rate. Therefore, some of the variability of SF_6 concentrations and ratio of SF_6:CH_4 could be accounted for by the variability of the SF_6 release rates from the permeation tubes. Compared with the New Zealand database, the CV of the SF_6 release rate determined from permeation tubes used in this study pre-deployment, was slightly greater (51.3 vs. 68.2%). The high CV of SF_6 and the ratio CH_4:SF_6 is unlikely to be accounted for by the CV of SF_6 release rate alone. In addition, when examining the ratio of SF_6:CH_4 gas concentration between days, no discernable pattern was apparent and the ratio of SF_6:CH_4 appeared unstable within animals (Figure 4.1). Thus, it is difficult to formulate any hypotheses for the inconsistent concentrations of SF_6 in the breath samples of sheep in this study.

Table 4.11: A comparison of SF_6 and CH_4 gas variance from the breath samples collected from sheep.

	SF_6 (ppt)	**CH_4 (ppm)**	**Ratio CH_4:SF_6 ($x10^3$)**	**SF_6 release rate**
New Zealand Methane database (n = 3542)				
Mean	382.9	72.7	7.29	1.129
Standard deviation	127.5	21.2	5.81	0.5791
Coefficient of variation (%)[2]	33.3	29.1	79.1	51.3
This study[1]				
Mean	268.3	44.9	7.0	1.187
Standard deviation	268.7	29.5	7.8	0.8096
Coefficient of variation (%)[2]	99.8	65.7	111.4	68.2

[1] All data samples that were analysed for SF_6 and CH_4 are included. Concentrations of gases are corrected for background concentrations of SF_6 and CH_4.

[2]Coefficient of variation = (standard deviation/mean)*100.



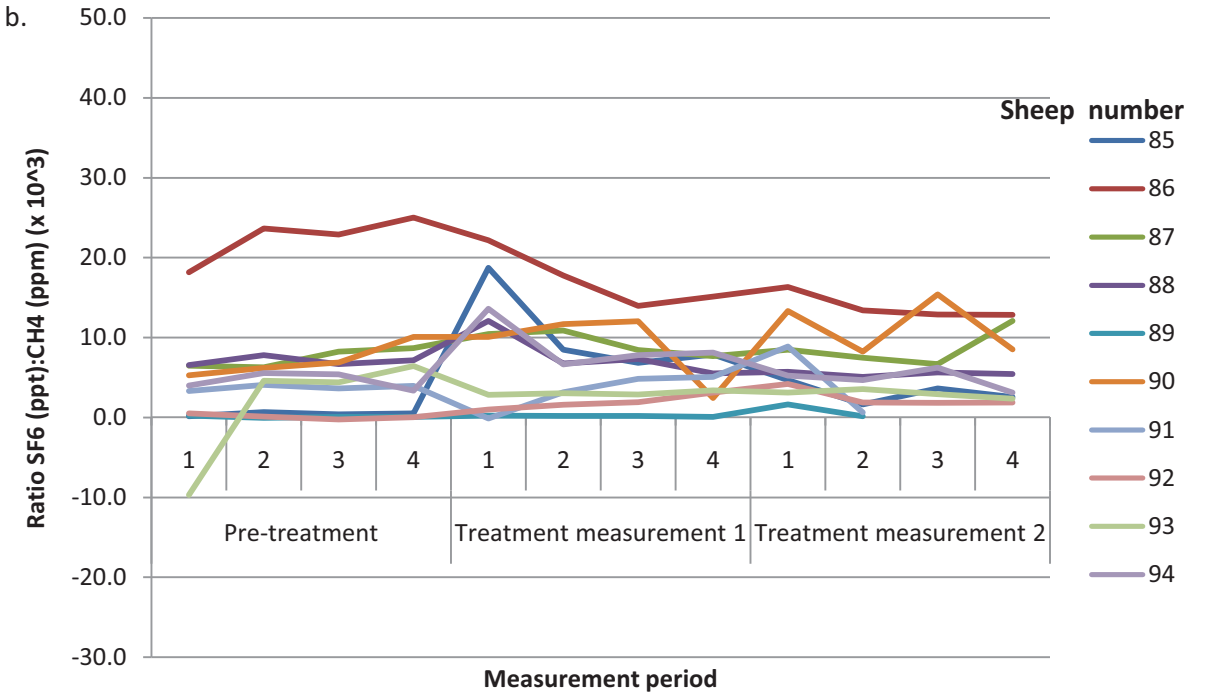
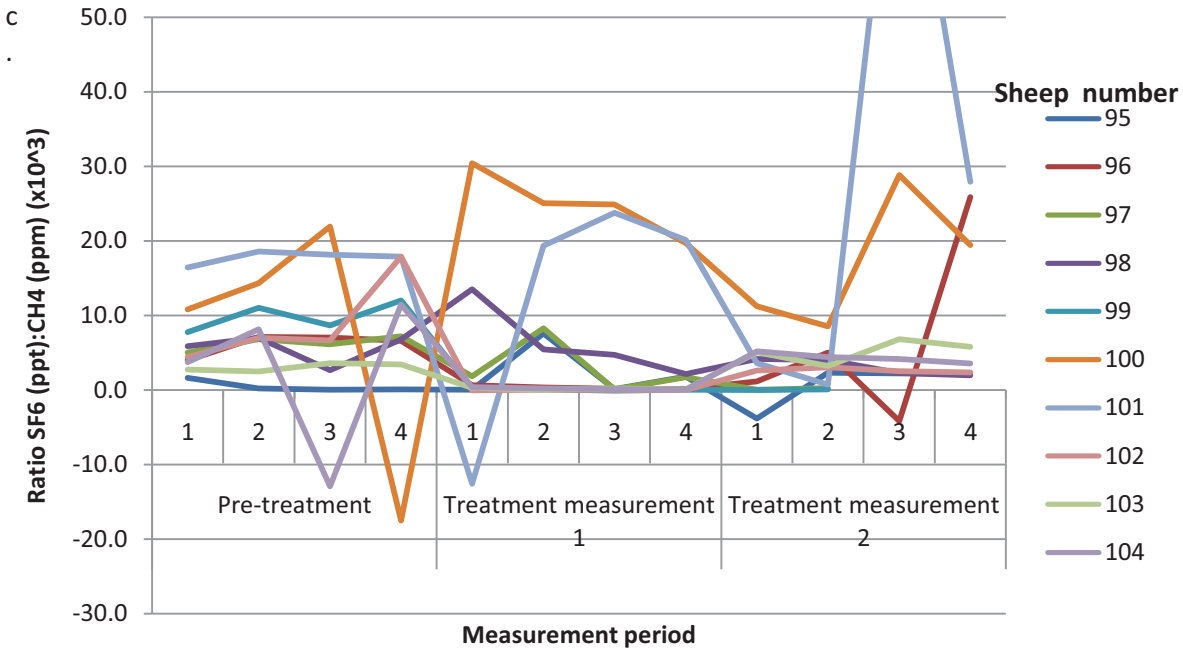


Figure 4.1a.b: Ratio of concentration of sulphur hexafluoride (SF_6) and methane (CH_4) gases in the breath samples of individual sheep within each measurement period, after correction for ambient concentrations of SF_6 and CH_4. Each graph shows a sub-group (n = 10) of animals that is not related to treatments or diets fed.

c.



d.

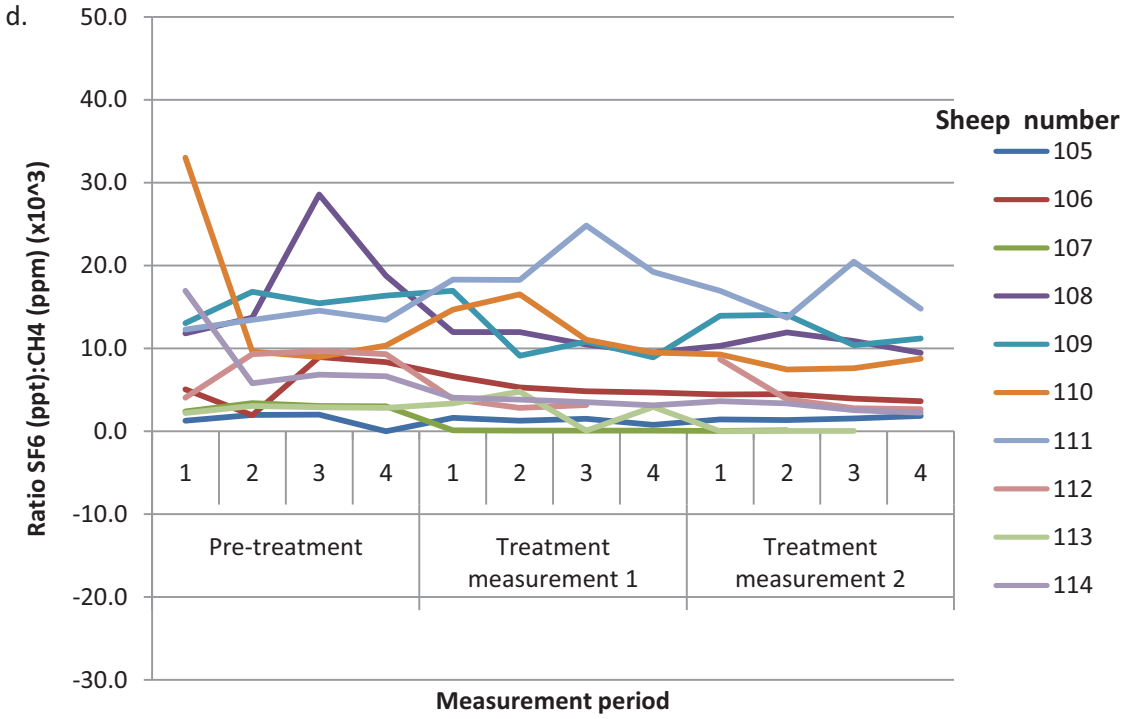


Figure 4.1c.d: Ratio of concentration of sulphur hexafluoride (SF_6) and methane (CH_4) gases in the breath samples of individual sheep within each measurement period, after correction for ambient concentrations of SF_6 and CH_4. Each graph shows a sub-group (n = 10) of animals that is not related to treatments or diets fed.

4.5.4 Conclusion

Chicory, as a fresh forage alternative to pasture, significantly reduced CH_4 yields in sheep and has the potential to be an important CH_4 mitigation tool in pastoral systems. However, the processes responsible for the reduction of CH_4 yield in sheep fed chicory need to be to be elucidated. Monensin also appeared to be beneficial in reducing the CH_4 yield of sheep; but before monensin can be used with confidence, there a need for a dose-response trial to be conducted with animals fed fresh forages. The combination of potential mitigation technologies did not result in significant reductions of CH_4 yield. Nevertheless, there is a lack of clarity in interpreting these results, due a greater than expected variation within and between sheep in this study. This suggests further research is needed to confirm the effect of combining CH_4 mitigation technologies.

4.6 APPENDIX

Table 4.12: Chemical composition (g/kg DM), *in vitro* digestibility (%) and metabolic energy (ME; MJ ME/kg DM) concentration of chicory and pasture offered to sheep during each measurement period.

	Methane measurements period				
	Covariate	Measurement one		Measurement two	
	Pasture	Chicory	Pasture	Chicory	Pasture
Organic matter	901	867	889	847	901
Lignin	13	61	13	57	16
Crude protein	204	138	173	160	134
Lipid	42	32	43	30	51
Hot water soluble carbohydrate (a)	133	206	139	143	170
Pectin (a)	21	87	14	82	15
Hemicellulose (b)	170	56	187	64	191
Cellulose (b)	215	127	230	138	230
Ratio RFC:SC (a/b)[1]	0.40	1.60	0.37	1.11	0.44
Gross energy (MJ/kg DM)	18.5	17.2	18.2	17.0	18.1
Dry matter digestibility (%)	70.0	79.8	68.4	79.8	67.8
Organic matter digestibility (%)	71.8	82.6	69.8	82.2	69.8
Metabolic energy (MJ ME/kg DM)	10.5	11.8	10.2	13.0	11.4

[1] Readily fermentable carbohydrates (hot water soluble carbohydrates plus pectin) : structural carbohydrate (hemicelluloses plus cellulose).

CHAPTER 5

Comparative methane emissions in cattle, sheep and red deer and apparent digestibility in sheep and red deer fed ensiled lucerne chaff (*Medicago sativa*, FibrePro®) during summer and winter

Chapter published in-parts in:

Swainson N.M.; Hoskin S.O.; Clark H.; Pinares-Patiño C.S.; Brookes I.M. 2008: Comparative methane emissions from cattle, red deer and sheep. *Proceedings of the New Zealand Society of Animal Production* **68:** 59-62.

Swainson N.M.; Hoskin S.O.; Clark H.; Pinares-Patiño C.S.; Brookes I.M. 2008: Comparative methane emissions from cattle, red deer and sheep. *Proceedings of a Deer Course for Veterinarians, Deer Branch NZVA Deer Course for Veterinarians No. 22*, Te Anau, New Zealand.

Swainson N.M.; Hoskin S.O.; Clark H.; Pinares-Patiño C.S.; Brookes I.M. 2007: Comparative methane production and yields from adult sheep, cattle and red deer. *Greenhouse Gas and Animal Agriculture Conference, Christchurch, November 2007.* (Abstract + Poster).

5.1 ABSTRACT

Methane (CH_4) yield, expressed per unit of dry matter intake (DMI) (g CH_4/kg DMI) from the three predominant ruminant species farmed in New Zealand (cattle, sheep and deer) are considered to be similar in the New Zealand Greenhouse Gas Inventory (Anon, 2009). However, the CH_4 yield reported for deer (21.3 g CH_4/kg DMI) is an average of values used for cattle (21.6 g CH_4/kg DMI) and sheep (20.9 g CH_4/kg DMI). Furthermore, these CH_4 yields are based on measurements conducted with animals fed a variety of diets in different seasons. No measurements of CH_4 yield have been previously conducted with cattle, sheep and deer when fed same diet within the same season.

Methane production (g CH_4/day) and yield in non-lactating dairy cows (cattle), sheep and red deer (deer), and apparent digestibilities in sheep and deer, were determined in summer and winter. In each season, CH_4 production (g/day) and apparent digestibility were measured concurrently over 4 and 8 days, respectively, following a 10-day acclimatisation period. All animals were individually housed in metabolism cages and fed ensiled lucerne chaff with added molasses (FibrePro) at a rate of 1.17 times estimated maintenance energy requirements. Methane measurements were made using the sulphur hexafluoride (SF_6) tracer technique.

When averaged across summer and winter, the total mean daily CH_4 production (cattle, 145.6 g CH_4/day > deer, 31.4 g CH_4/day > sheep, 18.7 g CH_4/day; $P < 0.0001$) and yield (cattle, 21.4 g CH_4/kg DMI > sheep, 18.7 g CH_4/kg DMI > deer, 16.1 g CH_4/kg DMI; $P < 0.001$) differed between species. A significant interaction between species and season was found ($P = 0.002$), which was driven by the higher CH_4 emissions (up to 27%) of cattle in winter compared with summer (23.9 vs. 18.8 g CH_4/kg DMI). No significant differences in CH_4 production when expressed per kilogram of digestible DMI (DDMI; g CH_4/kg DDMI) or digestible organic matter intake (DOMI; g CH_4/kg DOMI) between sheep and deer were found. This suggests the amount of CH_4 produced during the process of digestion was similar for deer and sheep; however, the mean apparent DM digestibility (DMD) of the diet by deer (60.1%) was lower ($P < 0.05$) than sheep (66.9%).

This study shows that CH_4 yield can differ between ruminant species when measured when fed the same diet. Species differences in CH_4 yield were attributed to differences in diet DMD between sheep and deer, but relationships between DMD and CH_4 yield in cattle were not determined.

5.2 INTRODUCTION

Factors that influence methane (CH_4) yield (g/kg dry matter intake, DMI) can be broadly split into three categories; these are animal factors, nutrition, and microbial. When fed the same diet differences of CH_4 yield between animals within a species have been proposed to be due to the inherent animal variability of apparent DM digestibility (DMD) and digestive kinetics that occurs between individual animals (Pinares-Patiño *et al.,* 2003b). Possible reasons for differences in CH_4 yield between ruminant species could be due to differences in DMI and/or digestive physiology, such as DMD, passage rate (Milne *et al.,* 1978; Fennessy *et al.,* 1980; Aerts *et al.,* 1984/85; Domingue *et al.,* 1991; Pearson *et al.,* 2006), microbial populations, or the site of digestion (Hoffman, 1993).

Blaxter and Wainman (1961) found no difference in the mean CH_4 production (respiration chambers, expressed as CH_4 kcal/100 kcal of ingested energy) between sheep (7.55 kcal/100 kcal) and cattle (7.37 kcal/100 kcal) when comparing 24 sheep and 21 cattle experiments conducted in series and across differing feeding levels. Conversely, Galbraith *et al.* (1998) reported that bison (*Bison bison*) produced more CH_4 as a percentage of gross energy intake (GEI; CH_4 as a % of GEI) than either wapiti (*Cervus elaphus*) or white-tailed deer (*Odocoileus virginianus*), fed lucerne pellets *ad libitum* (6.6 vs. 5.2 vs. 3.3 CH_4 as a % GEI, respectively) and CH_4 as a % GEI was greater from wapiti deer than white-tailed deer. Additionally, in a comparison of energy expenditure between red deer (*Cervus elaphus*) and sambar deer (*Cervus unicolor*) fed a pelleted concentrate diet, the proportion of GEI lost as CH_4 was 26% less from red deer compared with sambar deer when fed at maintenance energy requirements (4.8 and 6.8 as a % GEI, respectively; Semiadi *et al.,* 1998). This difference increased to 45% between deer species when the feeding level was

increased to twice maintenance energy requirements (red deer 4.7 vs. samber deer 6.8 CH_4 as a % GEI).

Independent of CH_4 measurements, animal factors (e.g. DMD and passage rate) have been demonstrated to change significantly in temperate species of deer between summer and winter (Milne *et al.,* 1978; Domingue *et al.,* 1991). Domingue *et al.* (1991) compared the digestive physiology of sheep and deer in summer and winter, when fed lucerne hay offered *ad libitum* deer ate more in summer than winter (62.5 vs. 46.7 g/kg $LW^{0.75}$), while the DMI of sheep (52.2 vs. 54.8 g/kg $LW^{0.75}$) did not change. The greater DMI of deer in summer compared with winter was associated with a 51% greater fill (289 vs. 191 g/kg $LW^{0.75}$) and a decrease in the fractional outflow rate (FOR) of particles (2.77 vs. 3.47 %/hour, as measured by lignin) in summer compared with winter (Domingue *et al.,* 1991). Despite this evidence for animal factors changing between summer and winter, it is not known if CH_4 yield would also be influenced.

Fresh forage diets can have a large impact on methanogenesis as demonstrated by Waghorn *et al.* (2002) and Woodward *et al.* (2001; 2002) when CH_4 was calculated using the SF_6 technique. Althrough no direct relationship between diet chemical compostion and CH_4 yield has been determined (Waghorn & Woodward, 2006; Hammond *et al.,* 2009), the feeding of a diet that did not change between summer and winter was an important requirement of this study. The reasoning behind this was to lessen any confounding effects between season or species and diet.

The aims of this experiment were to;

- Compare the CH_4 yields of adult cattle, sheep and red deer in summer and winter, whilst housed under the same conditions with diet and feeding level the same.

- Measure the DMD (sheep and deer), and rumen fermentation parameters (cattle, sheep and deer) between cattle, sheep and deer, during summer and winter, when fed the same diet and feeding level.

5.3 MATERIALS AND METHODS

5.3.1 Experimental design

An experiment to measure CH_4 emissions in mature non-lactating dairy cows (cattle; n = 11, 9 fistulated), wether sheep (n = 11, 4 fistulated) and castrated red deer (deer; n = 11, 4 fistulated) and DMD from a sub-group of deer (n = 6, 4 rumen fistulated) and sheep (n = 6, 4 rumen fistulated) was conducted at AgResearch Grasslands (sheep and cattle) and Massey University (deer), Palmerston North, New Zealand. The experiment was conducted in summer (January) and winter (June) of 2007. No DMD measurements were conducted with cattle, as facilities were not available for total faecal collections. Each measurement period consisted of 18 days, which comprised 10 days of adaptation to diet and housing, followed by four days of CH_4 measurements. The CH_4 measurements coincided with eight days of apparent digestibility measurements and rumen fluid sampling. All animals were individually housed in metabolism cages suited to each species, allowing for daily individual DMI to be measured.

5.3.2 Animals

Friesian-Jersey cross dairy cows, Romney sheep (wethers), and castrated red deer stags were used in this study. The mean (± standard deviation, SD) live weight of each species at the start of each measurement period was; summer - cattle 573 ± 29.3 kg, sheep 61 ± 16.7 kg and deer 158 ± 21.6 kg; winter - cattle 542 ± 29.7 kg, sheep 55 ± 12.7 kg and deer 144 ± 25.0 kg. The mean (± SD) age of the animals at the commencement of the summer experiment was approximately; cattle 6 ± 0 years; sheep 2.6 ± 1.48 years; and deer 5.6 ± 4.41 years. The same animals, except for two deer, were used in both the summer and winter measurement periods. Cattle were given access to a feed-pad for four hours each day, to allow for movement to prevent the onset of lameness. No animal health remedies were used during the experimental measurement periods.

5.3.3 Diets and feeding

Animals were fed ensiled lucerne (*Medicago sativa*) chaff with added molasses 'FibrePro' (FibrePro®, Fibre Fresh Feeds, Reporoa, New Zealand) twice daily,

with 40% and 60% of total daily feed allowance fed at 8 am and 4 pm, respectively. All feed was sourced from the same batch to ensure consistency of diet between species and across seasons. Animals were fed on an individual live weight basis. The level of feeding was intended to be 1.2 times the estimated energy requirements for maintenance (Nicol & Brookes, 2007), based on an estimated metabolisable energy (ME) of 11 MJ ME/kg dry matter (DM) and a DM of 40%. The actual feeding level offered was 1.17 times maintenance energy requirements, based on the DM measured during the experiment and calculated ME of 9.52 MJ/kg DM as determined by *in vitro* analysis (Section 5.3.8). All animals had unlimited access to water.

Samples of the feed offered to the animals were collected daily during each measurement period to determine the DM of the feed (approximately 200 g in triplicate), as established by oven drying (100 °C for 24 hours). An additional sample of feed offered (approximately 200 g) was taken, stored frozen (–20 °C) and later pooled within each measurement period for chemical analysis. Feed refusals for individual animals were measured daily throughout the entire experiment. During the ten-day adaptation period, feed refusals were pooled per ruminant species, sub-sampled, and dried in triplicate to determine DM. During the CH_4 and apparent digestibility measurements, each individual animal's feed refusal was sub-sampled and DM determined.

5.3.4 Apparent digestibility and urine measurements

Apparent digestibility was determined for sheep and deer only, using a sub-group of animals undergoing CH_4 measurements. Measurements were made on five sheep in the summer and six in the winter, four of which were rumen-fistulated. Data from only four sheep in summer and five sheep in winter were used, due to an animal not eating in each of these periods. Five deer in summer and six in winter, of which four were rumen-fistulated, were used to determine apparent digestibilities. Originally, five rumen-fistulated deer were intended to be used for the apparent digestibility measurements. However, one fistulated deer was euthanased halfway through the summer experiment due to unforeseen health complications. This deer was replaced by a non-fistulated animal in winter.

During the DMD measurements, the feed offered, feed refused, faeces and urine from all animals undergoing DMD measurements were collected daily for 7 days from the start of week three of each measurement period. Samples (200 g) of feed offered were taken daily for later chemical analysis, which were pooled per period and stored frozen at –20°C. Each animal's feed refusal was collected daily, weighed and a sample (approximately 200 g) frozen (–20 ºC) for chemical analysis. To reduce analytical costs, feed refusal samples were combined into two pools per species; pool one consisted of animals that had feed refusals above 10% of daily feed offered and pool two had feed refusals from animals that refused less than 10% of daily feed offered. Faeces were collected daily, homogenised, sub-sampled and frozen (–20 °C). Later, total faecal samples were pooled per animal within each measurement period, mixed and sub-sampled. Triplicate faecal samples (approximately 200 g) were taken for DM determination and a sample (approximately 200 g) was taken for chemical analysis.

Total urine volume was measured daily from each animal and 2% of the total urine volume was sub-sampled, pooled per animal within each measurement period and frozen (–20 °C) for later chemical analysis.

5.3.5 Methane emissions

Methane measurements were made using the SF_6 technique (Ulyatt *et al.,* 1999; Chapter 2), and the collection equipment was modified to fit each species. Permeation tubes manufactured for cattle were used for the cattle and deer, with a mean (± SD) SF_6 release rate of 2.937 ± 0.1298 mg/day and 3.107 ± 0.2336 mg/day, respectively. Smaller 'sheep' permeation tubes were used for the sheep, which had a mean (± SD) release rate of 1.153 ± 0.6541 mg/day.

Some deer had been used previously for CH_4 measurements and still retained existing SF_6 permeation tubes. These tubes were two to three years old and were considered to be expired. To ensure that any residual SF_6 gas released from the tubes was taken into account, breath samples were taken before the new tubes were inserted. The SF_6 gas concentration from the breath samples above background levels were added to the background levels measured during the actual CH_4 measurement periods in summer and winter.

Permeation tubes in the fistulated animals were retrieved after each measurement period, but were not retrieved from the non-fistulated animals. The release rates of SF_6 from the permeation tubes while in the rumen of the animals were corrected based on the gravimetric weighing of the permeation tubes after recovery from the animal, based on the methods described by Lassey *et al.* (2001) and Chapter 2. Fistulated animals had the same permeation tube throughout the entire experiment. To facilitate the retrieval of permeation tubes from the fistulated sheep and deer, permeation tubes were placed in nylon mesh bags with a nylon string attached to the rumen cannula. The permeation tubes in the rumen of the fistulated cattle were retrieved by hand.

5.3.6 Rumen fermentation

Rumen fluid samples were taken from the fistulated animals (5 cattle, 4 sheep and 4 deer) on day 17 of each experimental period, 3 days after the final CH_4 sampling. Fluid samples (minimum 16 mls) were taken 2, 4, 6 and 8 hours after the morning meal event, by squeezing the rumen contents through at least four layers of cheese-cloth. The remaining rumen contents were returned to the rumen.

Rumen pH was determined for each animal immediately after collection of the sample using a pH meter (PHM210, Radiometer, Copenhagen). Rumen fluid samples for volatile fatty acids (VFAs) analysis (5.0 ml) were added to 1.0 ml of protein precipitant, frozen and later centrifuged at 3000 rpm for 15 minutes. The supernatant was transferred to a pre-labelled tube and frozen (–20 °C).

Samples for ammonia (NH_3) concentrations consisted of 1.0 ml of rumen fluid transferred to a microcentrifuge tube, mixed thoroughly with 15 µl of concentrated HCl and then frozen at –20 °C. Preparation for NH_3 analysis initially involved defrosting samples and centrifuging for 15 minutes at 1400 rpm. The supernatant was then transferred to another microcentrifuge tube and re-frozen for later analysis. Samples for both VFA and NH_3 analysis were pooled within animal and each measurement period.

5.3.7 Laboratory analysis

Samples of feed offered and feed refused were analysed by wet chemistry, according to the methods described in Chapter 4, for gross energy (GE), organic matter (OM), acid and neutral detergent fibre (ADF and NDF), lignin, hot water-soluble carbohydrates (HWSC), pectin and *in vitro* digestibility (feed offered only). The faeces of sheep and deer were analysed for GE, OM, ADF and NDF, and the urine of both sheep and deer were analysed for GE. Faecal and dietary concentrations of cellulose were calculated by subtracting the concentration of lignin from ADF concentrations and hemicellose was calculated by subtracting the concentration of ADF from NDF. Rumen fluid samples were analysed for VFA and NH_3 as described in Chapter 4.

5.3.8 Statistical analysis

The statistical analysis of the data was undertaken using SAS (version 9.1, 2007), using the PROC MIXED model, with repeated measures. Main effects of species and season and their interactions were compared for DMI, CH_4 production and yield, DMD, nitrogen and energy balance, and parameters of rumen fermentation. Where proportions, rather than absolute values are reported, e.g. VFA as a proportion of total VFA, the data was transformed by an arcsin square root transformation (Bromiley & Thacker, 2002). Significance was declared at $P \leq 0.05$ and a trend reported if $0.05 < P \leq 0.10$.

5.4 RESULTS

5.4.1 Dietary chemical composition

The chemical composition of the diet is shown in Table 5.1. There was no significant difference in the chemical composition of the diet fed to cattle, sheep and deer between summer and winter. One pooled sample of the feed offered was analysed for *in vitro* digestibility. The *in vitro* analysis found that the dry matter digestibility of the diet was 63.5% and the calculated ME of the diet of the diet was 9.5 MJ ME/kg DM.

Table 5.1: Chemical composition (± SEM g/kg DM), ratio of readily fermentable carbohydrate to structural carbohydrate (RFC:SC) and gross energy (GE, MJ/kg DM), of the FibrePro diet (ensiled lucerne, *Medicago sativa,* chaff with added molasses) offered to cattle, sheep and red deer in summer and winter.

	Season			
	Summer[1]	Winter[1]	SEM	P-value
Dry matter (%)[2]	44.9	45.8	0.37	0.145
Organic matter	901	899	0.4	0.094
Acid detergent fibre	337	340	1.3	0.300
Neutral detergent fibre	451	452	2.6	0.808
Hemicellulose (b)	114	113	1.2	0.493
Cellulose (b)	254	257	1.6	0.312
Lignin	8.3	8.2	0.08	0.689
HWSC[3] (a)	40	40	0.9	0.919
Pectin (a)	48	49	0.7	0.585
Ratio RFC:SC (a:b)	0.24	0.24	0.006	0.908
Crude protein	21	21	1.9	0.466
GE (MJ/kg DM)	19.6	19.5	0.04	0.303

[1] Samples for chemical analysis by wet chemistry n = 2 per season

[2] Samples for the determination of dietary dry matter n = 8 per season

[3]Hot water soluble carbohydrate

5.4.2 Dry matter intake

Despite all species being offered the same feeding allowance of 1.17 times maintenance energy requirements the feeding level consumed by each ruminant species differed (Table 5.2). This was due to sheep and deer refusing a proportion of their daily feed allowance. Cattle did not have any feed refusals in either summer or winter and therefore the feeding level of cattle did not change (1.16 vs. 1.17; P > 0.1). The mean feeding level consumed by cattle (1.17), across summer and winter was greater than that of either sheep (1.09)

or deer (1.00; P < 0.001). The feeding level of sheep differed between summer and winter (1.01 vs 1.17; P = 0.001) and there was a trend for feeding level of deer to be greater in winter (1.02) than in summer (0.98; P = 0.075). A significant interaction between season and species (P = 0.001) was found for feeding level (Table 5.2), which appears to be driven by the lower feeding level of sheep in summer.

Table 5.2: Daily dry matter intake (DMI), gross energy intake (GEI; MJ/day) and feeding level (as a multiple of maintenance energy requirements) of cattle, sheep and red deer fed FibrePro (ensiled lucerne, *Medicago sativa,* chaff with added molasses) in summer and winter.

		Species				P-values		
	Season[1]	Cattle	Sheep	Deer	SEM	Species	Season	Sp x S[3]
DMI (kg/day)	S	6.96	0.98	2.04	0.050	0.001	0.013	0.093
	W	6.83	1.00	1.86	0.047			
DMI (g/$LW^{0.75}$)	S	60	45	46	1.2	0.001	0.068	0.070
	W	61	50	46	1.2			
GEI	S	136.3	19.1	39.9	0.98	0.001	0.013	0.094
	W	133.8	19.5	36.3	0.93			
Feeding level[2]	S	1.16[a]	1.01[b]	0.98[b]	0.016	0.001	0.001	0.001
	W	1.17[a]	1.17[a]	1.02[b]	0.016			

[1]S, summer and W, winter.

[2]Based on the calculated ME from the *in vitro* analysis of the diet (9.52 MJ ME/kg DM, Table 5.1).

[3] Interaction between species and season (Sp x S).

[abcd] Differing letters denotes a significant difference (P < 0.05) between means within rows and columns, based on a significant (P < 0.05) interaction between species and season

5.4.3 Apparent digestibility

The DMD of the FibrePro diet fed to sheep and deer is shown in Table 5.3. In summer, sheep digested ADF (51.6%) and NDF (51.5%) to a greater extent than deer (45.5% and 45.3%, respectively). Additionally, sheep tended to digest the DM (69.4%) and OM (70.2%) to a greater extent than deer (58.0 and 60.3%, respectively; P < 0.1). In winter, only DM (64.4%) and hemicellulose (50.6%) were digested to a greater extent by sheep compared with deer (62.2 and 46.8%, respectively). No significant interaction between season and species was found for any of the dietary components tested for apparent digestibility (P > 0.1), except for N (P < 0.001). This was driven by deer having a lower N digestibility in summer (58.0%) compared with winter (71.8%; P < 0.001) or sheep in summer (62.8%) and winter (65.0%; P = 0.02). There was no significant difference of N digestibility in sheep between summer and winter.

Table 5.3: Dry matter intake (DMI; kg/day) and apparent digestibility (% DMI) of dietary chemical constituents of the FibrePro diet (ensiled lucerne, *Medicago sativa,* chaff with added molasses) fed to sheep and deer in summer and winter.

		Species				P-value
	Season[1]	**Sheep**	**SEM**	**Deer**	**SEM**	**Species**
DMI	Summer	0.90[a]	0.189	2.00[b]	0.189	0.0061
	Winter	1.10[a]	0.127	2.05[b]	0.127	0.0007
Apparent digestibility						
Dry matter	Summer	69.4	3.74	58.0	4.18	0.082
	Winter	64.4[a]	0.58	62.2[b]	0.58	0.022
Organic matter	Summer	70.2	3.45	60.3	3.85	0.096
	Winter	64.4	0.73	64.0	0.73	0.708
Acid detergent fibre	Summer	51.6[a]	1.31	45.5[b]	1.47	0.017
	Winter	51.8	1.85	50.8	1.85	0.713
Neutral detergent fibre	Summer	51.5[a]	1.55	45.3[b]	1.55	0.029
	Winter	51.4	1.39	49.0	1.39	0.257
Cellulose	Summer	61.0[a]	1.06	56.3[b]	1.06	0.019
	Winter	60.8	1.61	58.4	1.61	0.323
Hemicellulose	Summer	51.8[a]	1.19	42.5[b]	1.33	0.001
	Winter	50.6[a]	1.16	46.8[b]	1.16	0.050
Nitrogen[2]	Summer	62.8[a]	0.53	58.0[b]	0.53	0.007
	Winter	65.0[a]	1.41	71.8[c]	1.41	0.009

[1] Summer, sheep n = 4 and deer n = 4; Winter sheep n = 5 and deer n = 6

[2] A significant interaction between species and season ($P < 0.001$), therefore differing letters indicate a significant ($P < 0.05$) difference between rows and columns.

[abc] Differing letters indicate a significant difference ($P < 0.05$) between means within rows.

5.4.4 Methane emissions

The concentrations of SF_6 gas in the breath samples collected from cattle, but not sheep and deer, in winter were very low. Only two animals had concentrations of SF_6 within the expected range for cattle (40 to 277 ppt, Annex A). Thus, data from the first attempt to determine CH_4 production in winter were not used. The position of the permeation tubes in the rumen of the fistulated cattle (n = 9) were checked after the last day of breath sampling and breath collections were repeated 2 days later. All sheep and cattle data were screen according to the methods decribed in Annex A. No such criteria for deer were defined because too few measurements of CH_4 emissions have been made with deer using 'cow' permeation tubes. Nevertheless, the data from two deer within each measurement period were not included in the final results. This was because one deer continuously destroyed the halters and associated tubing to collect the breath samples and a deer in summer and another deer in winter ate very little. The final number of animals and breath samples used for the analysis of the data are shown in Table 5.4.

Table 5.4: Planned versus the actual number of animals and maximum planned versus actual number of data points used in the final analysis of methane (CH_4) emissions in summer and winter.

		Species			Included data points[4]	
	Season	**Cattle**	**Sheep**	**Deer**	**Total**	**%[3]**
Planned number of animals	Summer	11	11	11		
	Winter	11	11	11		
Total available data points[4]		88	88	88		
Actual number of animals and data points included						
	Summer	11[1] (41)[2,4]	10 (37)	9 (34)	112	85
	Winter	7 (22)	10 (39)	9 (33)	94	71

[1] Number of animals

[2] Number of data points

[3] Percentage difference = (total included data points/total available data points) x 100

[4] Each data point is approximately a 24 hour period of breath collection

The production and yield of CH_4 from cattle, sheep and deer are shown in Table 5.5. The mean CH_4 yield of each species, when averaged over summer and winter, was greatest from cattle (21.4 ± 0.69 g CH_4/kg DMI) and least from deer

(16.1 ± 0.69 g CH_4/kg DMI, P < 0.001). The mean CH_4 yield of sheep (18.7 ± 0.59 CH_4/kg DMI) was intermediate between both cattle and deer, and was significantly different from both species (P < 0.05). A significant interaction between season and ruminant species was found (P = 0.001). The CH_4 yield of cattle in summer was less than in winter (18.8 vs. 23.9 g CH_4/kg DMI; P < 0.001). In contrast the CH_4 yields from both sheep (19.3 vs. 18.1 g CH_4/kg DMI) and deer (16.8 vs. 15.4 g CH_4/kg DMI) between summer and winter did not differ (P > 0.1).

The exclusion of the cattle CH_4 yield data from the overall analysis of CH_4 removed the interaction between species and season and confirmed that the mean CH_4 yield of sheep (18.7 ± 0.59 CH_4/kg DMI) was 14% greater than deer (16.1 ± 0.69 g CH_4/kg DMI; P = 0.001). Furthermore, there was a tendency for the overall mean CH_4 yield of both sheep and deer to be greater in summer (18.1 g CH_4/kg DMI) than winter (16.8 g CH_4/kg DMI; P = 0.082). The differences between species, seasons and the interaction between species and season for CH_4 as a % GEI and CH_4 per unit of maintenance energy requirements (feeding level) were the same as that for CH_4 yield.

Methane yield, expressed per kilogram of either digestible DMI (DDMI; g CH_4/kg DDMI) did not differ between sheep (28.0) and deer (26.9) when the mean DMD determined for each species was used (Table 5.3). A significant (P < 0.05) interaction between species and season was evident for both CH_4 yield expressed per kg of digestible DMI and digestible OM intake (OMI; Table 5.5). In both instances, this was driven by the lower CH_4 yield of deer in winter, which itself was a consequence of a lower CH_4 production, combined with a higher digestible OM for deer in winter.

5.4.5 Energy balance

The calculated dietary energy intakes and losses of dietary energy are shown in Table 5.6. The percentage of gross energy lost as faecal energy was lower in sheep (37.8%) than deer (39.6%; P < 0.001). This contributed towards the mean percentage of GEI as DE being significantly greater in sheep (65.4%) than deer (61.2%; P < 0.001). No significant effect of season was detected for

digestibility, but a significant interaction between species and season was found ($P = 0.036$). The percentage of GEI as DE was similar in summer and winter in sheep (66.7% vs. 64.0%), but values from sheep were higher than deer in summer (59.6%), but not winter (62.7%). Nevertheless sheep (64.0%) and deer (62.7%) in winter had a similar percentage of GEI as digestible energy.

Gross energy intake lost as urine was greater in sheep (5.2%) than deer (3.4%; $P < 0.001$) and was greater in summer (4.7%) than winter (3.9%; $P < 0.05$). However the interaction between season and species for GEI lost as urine energy (Table 5.6) was not significant ($P < 0.062$).

The GE lost as CH_4, as presented here, was calculated for animals that also underwent dietary digestibility measurements. When the GE of CH_4 was expressed as a percentage of GEI no significant difference between sheep (4.77) and deer (4.77; $P > 0.1$) was found, despite small differences in the digestibility of energy by sheep (65.3%) and deer (61.2%; $P < 0.01$).

Table 5.5: The calculated methane (CH_4) production, CH_4 yield, CH_4 per of feeding level, CH_4 per kg of digestible dry matter intake (DDMI) or digestible organic matter intake (DOMI) and CH_4 as a percentage of gross energy intake (CH_4 as a % GEI) from cattle, sheep and red deer fed FibrePro (ensiled lucerne, *Medicago sativa,* chaff with added molasses) in summer and winter.

		Species						P-values		
	Season	**Cattle**	**SEM**	**Sheep**	**SEM**	**Deer**	**SEM**	**Species**	**Season**	**Sp x S[1]**
CH_4 production (g /day)	Summer	130.8[a]	3.92	18.7[be]	2.81	33.6[c]	4.24	0.001	0.020	0.001
	Winter	160.4[d]	5.34	18.5[be]	4.02	29.2[ce]	4.24			
CH_4 yield (g/kg DMI[3])	Summer	18.8[ab]	0.82	19.3[a]	0.82	16.8[bd]	0.89	0.001	0.246	0.001
	Winter	23.9[c]	1.12	18.1[ab]	0.84	15.4[d]	0.89			
CH_4 (g/feeding level)	Summer	18.9[ab]	0.87	19.2[a]	0.85	16.5[bd]	1.01	0.001	0.237	0.001
	Winter	24.2[c]	1.18	18.2[ab]	0.90	15.0[d]	0.93			
CH_4 (g/kg DDMI)	Summer	nd[2]		27.8[a]	1.08	29.0[a]	1.17	0.308	0.094	0.039
	Winter	nd		28.2[a]	1.17	24.7[b]	1.17			
CH_4 (g/kg DOMI)	Summer	nd		27.5[a]	1.06	27.9[a]	1.15	0.105	0.144	0.048
	Winter	nd		28.1[a]	1.09	24.0[b]	1.15			
CH_4 as a % GEI	Summer	5.32[a]	0.232	5.47[a]	0.232	4.82[ac]	0.255	0.001	0.240	0.001
	Winter	6.85[b]	0.324	5.14[a]	0.238	4.36[c]	0.251			

[1] Interaction between species (Sp) and season (S)

[2] Not determined

[3]Dry matter intake

[abcde] Indicates a significant difference ($P < 0.05$) between means within both rows and columns, based on a significant ($P < 0.5$) interaction of species by season.

Table 5.6: Calculated dietary energy intake and dietary energy losses as a percentage of gross energy intake (GEI) from sheep and red deer fed FibrePro (ensiled lucerne, *Medicago sativa,* chaff with added molasses) chaff in summer and winter.

		Species[1]			P - values		
	Season	**Sheep**	**Deer**	**SEM**	**Species**	**Season**	**S x S[4]**
Gross energy intake (GEI)	Summer	21.9	40.8	1.10	0.001	0.826	0.845
	Winter	21.4	40.8	0.97			
Faecal energy as a % of GEI	Summer	33.7[a]	41.0[b]	1.26	0.001	0.756	0.036
	Winter	35.9[ac]	38.1[bc]	1.11			
Digestible energy (DE) intake	Summer	14.6	24.3	0.90	0.001	0.946	0.308
	Winter	13.7	25.3	0.80			
Digestible energy % of GEI[2] (DEI/GEI %)	Summer	66.7[a]	59.6[b]	1.26	0.001	0.757	0.036
	Winter	64.0[ac]	62.7[bc]	1.11			
Urine energy as a % of GEI	Summer	5.3	4.2	0.44	0.001	0.043	0.062
	Winter	5.2	2.6	0.37			
Methane as a % of GEI[5]	Summer	4.74	4.93	0.260	0.984	0.568	0.432
	Winter	4.80	4.60	0.228			
Metabolisable energy intake (MEI)	Summer	12.4	20.8	1.18	0.001	0.669	0.605
	Winter	11.6	22.8	1.00			
Metabolisable energy as a % of GEI [3]	Summer	55.0	52.0	1.83	0.283	0.311	0.497
	Winter	55.6	54.6	1.55			
Metabolisable energy diet (MEI MJ/ kg DMI)	Summer	10.8	10.4	0.36	0.668	0.419	0.470
	Winter	10.8	10.9	0.31			

[1] Summer sheep n = 5, deer n = 6; Winter, sheep n = 4, deer n = 4

[2] Digestible energy divided by gross energy intake

[3] Metabolisable energy intake (MEI) divided by gross energy intake

[4] Sp x S interaction between species and season.

[5] Methane production only from animals who also underwent apparent digestibility measurements and assuming 1g CH_4 = 0.55 MJ GE

[abc] Indicates a significant difference ($P < 0.05$) between means within both rows and columns, based on a significant interaction between species and season

5.4.6 Rumen fermentation

The concentration of total VFA and NH_3 in the sampled rumen fluid are shown in Table 5.7. The total concentration of VFA did not differ between season or ruminant species ($P > 0.1$).

The molar percentage of individual VFA did not differ with season; however some did differ with ruminant species as shown in Table 5.7. The molar percentage of acetate in summer (cattle, 70.4; sheep, 71.7 and deer 73.8%) and winter (cattle, 69.5; sheep, 71.2 and deer, 74.4%) tended to differ ($P = 0.06$) between species and between season. This was a consequence of deer having a greater ($P < 0.05$) percentage of acetic acid compared with cattle in both summer and winter. The percentage of n-butyric acid in winter was greater in cattle (9.1%) compared with deer (6.5%; $P = 0.012$), but not compared with sheep (8.9%; $P = 0.734$). Sheep had a greater percentage of n-butyric acid in their rumen fluid compared with deer ($P = 0.026$).

The ratio of acetic acid to propionic acid tended to differ between species in summer ($P = 0.084$). Deer (5.1) had a higher ratio of acetic acid to propionic acid than cattle (4.6; $P = 0.034$) and sheep (4.7; $P = 0.102$), which were similar ($P = 0.586$). In winter, there were no significant differences in the ratio of acetic acid to propionic acid between ruminant species; cattle, 4.5; sheep, 4.9; and deer, 5.4 ($P = 0.135$).

Overall, the NH_3 concentrations in the rumen fluid from cattle, sheep and deer were found to differ with species in both summer ($P = 0.053$) and winter ($P = 0.034$). No effect of season was found ($P > 0.1$) for rumen fluid NH_3 concentrations (Table 5.7). Closer examination reveals that in summer, the rumen fluid ammonia concentration of cattle (12.4 mmol/L) and sheep (13.5 mmol/L) were not different ($P = 0.390$). Nevertheless, deer (15.9 mmol/L) had a greater rumen fluid ammonia concentration compared with cattle ($P = 0.019$), but not sheep ($P = 0.102$). In winter, the ranking of ammonia concentrations changed, with sheep (16.8 mmol/L) having greater ammonia concentration than deer (11.1 mmol/L; $P = 0.011$), but not

cattle (14.1 mmol/L; P = 0.120). The ammonia concentrations of the rumen fluid from sheep and cattle were similar (P = 0.116).

The pH of the rumen fluid differed with the time of sampling in summer (P = 0.001), but not winter (P = 0.702; Table 5.8). In summer the mean rumen fluid pH of all three ruminant species, 8 hours (6.98) after the morning meal was greater than at 2 (6.72), 4 (6.72) or 6 (6.84; P < 0.05) hours post-feeding, which did not differ (P > 0.1). Deer in winter had a higher rumen pH (7.04) compared with both cattle (6.84) and sheep (6.79; P = 0.018 and P = 0.007, respectively). No significant difference in rumen fluid pH was found between ruminant species in summer (cattle, 6.76; sheep, 6.81; and deer, 6.88; P = 0.117).

Table 5.7: Rumen fluid concentrations of ammonia and total volatile fatty acids (VFA) (m mol/L), the molar percentage of individual VFAs of cattle, sheep and red deer fed FibrePro (ensiled lucerne, *Medicago sativa,* chaff with added molasses) in summer and winter.

			Species[1]			P-values
	Season	**Cattle**	**Sheep**	**Deer**	**SEM**	**Species**
NH_3	Summer	12.4	13.5	15.9	1.04	0.053
	Winter	14.1[ab]	16.8[b]	11.1[a]	1.06	0.034
Total VFA	Summer	77.6	84.8	74.8	4.28	0.298
	Winter	84.5	91.6	77.2	6.21	0.348
Ratio acetic: propionic acid	Summer	4.6	4.7	5.1	0.20	0.084
	Winter	4.5	4.9	5.4	0.21	0.135
Molar percentage of VFA's (%)						
Acetic	Summer	70.4	71.7	73.8	0.90	0.064
	Winter	69.5	71.2	74.4	1.23	0.064
Propionic	Summer	15.3	15.2	14.6	0.31	0.296
	Winter	15.4	14.8	13.8	0.58	0.233
n-butyric	Summer	8.8	8.1	6.4	0.77	0.118
	Winter	9.1[a]	8.9[a]	6.5[b]	0.57	0.029
Iso-butyric	Summer	1.4	1.4	1.5	0.07	0.351
	Winter	1.5	1.3	1.4	0.10	0.628
Iso-valeric	Summer	1.9	1.6	1.8	0.11	0.324
	Winter	2.3[a]	1.6[b]	1.8[ab]	0.16	0.042
n- valeric	Summer	2.3	2.0	2.0	0.10	0.121
	Winter	2.2	2.1	2.0	0.15	0.572

[1] Summer, cattle n = 5, sheep = 4 and deer = 3; Winter, cattle n = 5, sheep = 4 and deer = 4.

[ab] Denote significant differences ($P < 0.05$) between means within rows

Table 5.8: Rumen fluid pH (mean ± SEM), taken 2, 4, 6 and 8 hours after the morning feeding event, in cattle, sheep and deer during summer and winter when fed FibrePro (ensiled lucerne, *Medicago sativa,* chaff with added molasses).

		Time after morning feeding (hours)							P-value	
Season	**Species**[1]	**2**	**4**	**6**	**8**	**SEM**	**Average**	**SEM**	**Species**	**Time**
Summer	Cattle	6.68	6.69	6.78	6.90	0.044	6.76	0.053	0.117	0.001
	Sheep	6.69	6.66	6.85	7.06	0.079	6.81	0.039		
	Deer	6.79	6.83	6.90	6.99	0.079	6.88	0.039		
	Average	6.72[a]	6.72[a]	6.84[a]	6.98[b]	0.044				
Winter	Cattle	6.84	6.79	6.78	6.94	0.107	6.84[*]	0.053	0.015	0.702
	Sheep	6.67	6.87	6.81	6.81	0.120	6.79[*]	0.060		
	Deer	6.96	7.13	7.16	6.90	0.124	7.04[†]	0.062		
	Average	6.83	6.93	6.92	6.88	0.068				

[1] Summer, cattle n = 5, sheep n = 4, deer n = 4; Winter cattle n = 5, sheep n = 4, deer n = 3

[ab] Denotes significant differences ($P < 0.05$) between means within rows.

[*†] Denotes significant differences ($P < 0.05$) of means within columns.

5.5 DISCUSSION

5.5.1 Methane emission differences between ruminant species

This study was the first to simultaneously determine the CH_4 yield of domestic cattle, sheep and deer whilst fed the same diet at a similar feeding level. The principal finding was that CH_4 yield was not affected by season, except for cattle. Differences of CH_4 yield between ruminant species were found. The highest CH_4 yields were measured from cattle (21.4 g CH_4/kg DMI) which were greater than sheep (18.7 g CH_4/kg DMI) and deer (16.1 g CH_4/kg DMI). The CH_4 yield measured from deer was lower than that from sheep. Nevertheless, the greater CH_4 yield of cattle and the difference of cattle CH_4 yield between summer (18.8 g CH_4/kg DMI) and winter (23.9 g CH_4/kg DMI) cannot be separated from problems arising from the performance of the SF_6 technique.

Previous studies have also reported a difference in CH_4 emissions between ruminant species. However, those studies were predominantly based on undomesticated ruminant species, and there are none that have measured CH_4 emissions from domestic sheep and deer or cattle and deer, when fed the same diet and level of feeding. Galbraith *et al.* (1998) reported that when fed lucerne pellets, bison produced 27% more CH_4 as a percentage of GEI than wapiti deer and 50% more than white-tailed deer (6.6, 5.2 and 3.3, CH_4 as a % of GEI, respectively). Animals in the study of Galbraith *et al.* (1998) were fed *ad libitum* and CH_4 was measured by respiration chambers. Despite *ad libitum* feeding, DMI did differ not significantly between species (bison, 77.7 g/live weight (LW) kg $^{0.75}$; wapiti deer, 89.7 g/LW kg $^{0.75}$; and white-tailed deer, 92.9 g/LW kg $^{0.75}$), and are unlikely to have contributed to differences of measured CH_4. Semiadi *et al.* (1998) also reported differences of CH_4 (respiration chambers; as a % of GEI) between species of deer fed a pelleted diet. At maintenance energy intakes, red deer produced 26% less CH_4 compared with sambar deer (4.9 vs. 6.1 CH_4 as a % GEI), and this difference increased to 46% when deer were fed twice maintenance energy requirements (4.7 vs. 6.8 CH_4 as a % of GEI). This indicates that the magnitude of difference in CH_4 yield or CH_4 as a % of GEI between ruminant species can be influenced by feeding level.

Nevertheless, in contrast to the above studies, Blaxter and Wainman (1961) using respiration chambers showed that across 24 sheep and 21 cattle serial experiments, sheep and cattle did not differ significantly in CH_4 as a % GEI (8.0% and 7.6%, respectively), when fed diets consisting of low quality dried grass and rolled oats over a range of feeding levels.

5.5.2 Digestive physiology and CH_4 emissions

The causes of the observed differences in CH_4 yield between ruminant species in this study (24.4, 18.7 and 16.1 g CH_4/kg DMI for cattle, sheep and deer, respectively) and others are not clear (Galbraith *et al.,* 1998; Semiadi *et al.,* 1998). Aspects of digestive physiology, such as intake, apparent digestibility, and digesta passage rate, within the same species, have been previously shown to impact on CH_4 yield (Okine *et al.,* 1989; Pinares-Patiño *et al.,* 2003b; Yan *et al.,* 2009). Despite reported differences in digestive physiology between ruminant species, it is accepted within the New Zealand Greenhouse Gas Inventory that cattle, sheep and deer emit similar amounts of CH_4 yield (Anon, 2009).

It was intended that all three species in this study would consume a similar level of feed (1.2 times maintenance energy requirements). Despite efforts to standardise the feeding level offered to all three species, the DMI of cattle was greater than both deer and sheep, when expressed per kilogram of metabolic live weight or feeding level, which was a consequence of some deer and sheep refusing a proportion of their offered feed. Dry matter intake within a ruminant species has been shown to influence CH_4 yield; increasing DMI is associated with increasing CH_4 production and decreasing CH_4 yield (Blaxter & Clapperton, 1965; Lassey *et al*., 1997; Mbanzamihigo *et al.,* 2002; Yan *et al.*, 2009). This relationship possibly occurs because of compensatory changes in rumen clearance due to changes in DMI (Weston, 1996), thus the exposure feed particles to microbial fermentation is reduced, thereby reducing CH_4 yield. Nevertheless, DMI is not the only factor influencing CH_4 yield and if CH_4 emissions rigidly followed this pattern, the CH_4 yield of cattle in this study would have been lower than that of both sheep and deer, which was not the case.

Moss *et al.* (2000) postulated that ruminant CH_4 emissions may be characterised by the microbial fermentation patterns that lead to differences in the percentages of VFAs in the rumen fluid. This was based on the idea that the production of propionate utilises hydrogen (H_2) and results in less H_2 available for methanogens, whereas in contrast, the production of acetate releases H_2 making it available for methanogenesis. It also assumes that the production of VFA is represented by the percentage of VFA in the rumen fluid calculated from VFA concentrations. However the theory of Moss *et al.* (2000) does not explain the species differences in CH_4 yield found in this study, because VFA proportions were similar Nevertheless, Robinson *et al.* (2010) found that VFA concentrations were a poor predictor of daily CH_4 production and explained only 26% of the total variation in CH_4 production. This suggests that VFA concentrations are not an ideal indicator to characterise the fermentation patterns of rumen microbes.

Furthermore, Jeyanathan *et al.* (2011) sampled rumen contents from cattle, sheep and deer in this and other studies to compare the methanogen species present in the rumen of cattle, sheep and deer fed a range of diets including chicory (*Cichorium intybus*), fresh pasture (*Lolium perenne*) and ensiled lucerne chaff. They used PCR-DGGE (Polymerase Chain Reaction - Denaturant Gradient Gel Electrophoresis) molecular techniques, but there were no differences in methanogen species detected between ruminant species and/or diets fed. Given no difference in microbial populations, this implies that the production and transfer of H_2 during the fermentation process to methanogen species should be similar between ruminant species. However, PCR-DGGE does not quantify the activity of the methanogen populations. The review by Morgavi *et al.* (2010) identified that the relationship between the number of methanogens and the production of CH_4 is not clear.

In this study, despite differences in CH_4 yield per unit of DMI, no significant difference in CH_4 when expressed as g CH_4/kg digestible DMI were found between sheep and deer (28.0 and 26.7 g CH_4/kg DDMI, respectively). In addition, sheep digested the ensiled lucerne chaff to a greater extent (up to 11% more DMD and OMD) than deer. Therefore, it is proposed that the greater

digestion of the diet by sheep compared with deer had the greatest impact on species differences of CH_4 yield.

Differences in DMD between sheep and deer have been reported previously, but the direction of difference in DMD between sheep and deer are conflicting. Milne *et al.* (1978) found that deer were less efficient at digesting dried grass pellets compared with sheep, similar to the findings in the current study. In contrast, Fennessy *et al.* (1980) reported that deer had greater apparent DMD when fed pelleted hay and mature meadow hay (10% and 6%, respectively), compared with sheep. Domingue *et al.* (1991) also found the apparent DMD, OMD and digestion of fibre by deer fed chaffed lucerne hay was up to 4% greater than by sheep. Due to the conflict in the direction of DMD difference between sheep and deer it is important to compare the relationship between CH_4 yield, DMD and ruminant species when measurements are conducted during the same experiment.

Generalised relationships, mainly developed with animals fed conserved forages and grain-based diets, have shown 1) an increase in CH_4 yield associated with decreasing DMD in animals fed at maintenance, and 2) a decrease in CH_4 yield associated with increasing DMD for animals fed above maintenance (Blaxter & Clapperton, 1965). However, Moss *et al.* (2000) and Waghorn and Woodward (2006) suggest that these relationships are more variable in animals fed fresh forages compared with animals fed conserved diets. This current study and those of Moe and Tyrrell (1979), Blaxter and Clapperton (1965), and Pinares-Patiño *et al.* (2003b) are based upon conserved forages. Therefore the finding of an animal species difference in CH_4 yield that appears to be linked to DMD needs to be confirmed and explored further, especially with fresh forages. Recent studies by Hammond *et al.* (2009), Molano and Clark (2008), and Waghorn and Woodward (2006) found no significant relationships between CH_4 yield and DMD (measured *in vivo and in vitro*) of fresh forage-based diets, within the same ruminant species.

The rate of digesta passage through the digestive tract, measured as either mean retention time (MRT) or fractional outflow rate (FOR), particularly from the rumen, has been linked to CH_4 emissions within - but not between - species

(Okine *et al.,* 1989; Pinares-Patiño *et al.,* 2003b). The influence of passage rate is suggested to be associated with the relatively slow growth of methanogens in comparison with other rumen bacteria (Miller & Wolin, 2001). Faster passage rates may modify the rumen environment, making it less favourable for methanogens and/or methanogenesis or by reducing the length of time feed particles are exposed to microbial attack. The passage rate of digesta was not determined in the study presented here and in order to fully explore the possible explanations for the difference in CH_4 yield between ruminant species, factors such as digesta passage rate should be investigated in future studies of this type. This is particularly important as previous experiments have reported differences in digesta passage rate between cattle and sheep of up to 15% (MRT, Pearson *et al.,* 2006) or sheep and deer ranging from 6 to 20% (FOR, Milne *et al.,* 1978; Domingue *et al.,* 1991).

5.5.3 Seasonality

The digestive physiology of deer species from temperate regions, which include red deer and wapiti, are strongly linked to day length as an adaptation to the climate where these deer species evolved. A consequence of this adaption is that the feeding behaviours, digestion, growth and reproductive cycles of these deer species are linked to day-length and therefore seasons, which is in contrast to other domesticated ruminant species (Barry *et al.,* 1991). Milne *et al.* (1978) and Domingue *et al.* (1991) observed that voluntary DMI, digesta passage rate and rumen volume were influenced by season in deer, but not sheep, when animals were fed *ad libitum*. Both passage rate and rumen volume have been implicated in CH_4 yield (Pinares-Patiño *et al.,* 2003b; Okine *et al.,* 1989); therefore, it was hypothesised that CH_4 yield would also be influenced by season. Nevertheless, this study did not find a significant difference of CH_4 yield between seasons. The seasonal difference of CH_4 yield from cattle (summer, 18.8 vs. winter 23.9 g CH4/kg DMI) may have been associated with SF_6 release rates, as indicated by the high variability of CH_4 production between and within-animals in winter.

It is likely that the driver behind the changes in digestive passage rate and rumen volume is appetite, driven by endocrine responses to changing day-

length (Barry *et al.,* 1991). In contrast to the studies of Milne *et al.* (1978) and Domingue *et al.* (1991), the DMI in this study was constant between summer and winter. The constant feeding level offered to animals may have suppressed any seasonal changes of passage rate and rumen volume that have been previously associated with an increase of DMI in summer (Domingue *et al.,* 1991), therefore explaining the consistency of CH_4 yield between summer and winter from deer.

5.5.4 Variability in CH_4 production measurements

In the present study, the estimation of both CH_4 production and yield was highly variable in cattle. This appeared to be, in part, due to the very low and variable concentrations of SF_6 in the collected breath samples. These problems were less evident in sheep and deer. Possible reasons for the low concentrations of SF_6 gas in the breath samples specifically from cattle are unclear, and were not perceived to be due to the breath collection equipment. Implications of the highly variable CH_4 yields in cattle in this study are that the estimated CH_4 yield, particularly in winter, may be overestimated and that differences between the CH_4 yield of cattle and other ruminant species in this study are exaggerated. Caution is therefore advised in extrapolating these findings beyond the animals used in this study, especially in regards to the findings from cattle, until further comparisons of CH_4 yield between ruminant species have been confirmed, preferably using respiration chambers.

5.5.5 Conclusion

This study has shown that there are differences in CH_4 emissions between ruminant species, including differences in CH_4 yield and CH_4 as a % of GEI. However, further research is required to confirm this, particularly for cattle, and to elucidate the reasons for these differences. Future investigations should include measurements of rumen microbial ecology, digesta passage rate, DMD, and CH_4 measurements using respiration chambers rather than the SF_6 technique. It is also recommended that further research is conducted to determine if animal species differences in CH_4 yield persist at different feeding levels and diets, especially fresh forages.

CHAPTER 6

General discussion

6.1 INTRODUCTION

The aims of this research were to investigate the effects of diet (chicory vs. pasture, Chapter 4) and mitigation technologies (monensin, Chapters 3 and 4, and/or coconut oil, Chapter 4) on methane (CH_4) emissions in sheep. In addition, the influence of ruminant age (red deer 4.5 to 11.5 months of age, Chapter 2) and ruminant species (cattle, sheep and red deer, Chapter 5) on CH_4 yield (g CH_4/kg dry matter intake, DMI) was explored.

6.2 DISCUSSION

6.2.1 The effect of potential mitigation technologies for reducing on methane emissions

6.2.1.1 Chicory

Feeding chicory for 6 weeks reduced the CH_4 yield of sheep by 17%, from 30.7 to 24.9 g CH_4/kg DMI, compared with pasture when fed at 1.2 times maintenance energy requirements. This suggests that the type of forage species fed can influence sheep CH_4 yield. Waghorn *et al.* (2002) also reported a reduction in CH_4 yield of 37% when sheep were fed chicory compared with pasture (16.2 vs. 25.7 g CH_4/kg DMI, respectively). The greater reduction of CH_4 yield reported by Waghorn *et al.* (2002) could be because the DMI of sheep was unrestricted. Fresh forages other than chicory, have been demonstrated to reduce CH_4 yields from ruminants, as the feeding of lucerne, sulla, red clover and lotus reduced CH_4 yields by 16% to 52% compared with sheep and cattle fed pasture, when CH_4 was estimated using the sulphur hexafluoride (SF_6) technique (Waghorn *et al.,* 2002; Woodward *et al.,* 2001; 2002).

The reasons for the reduced CH_4 yield in sheep fed chicory compared with pasture are not clear, but may be attributed to differences between forages in chemical composition and digestibility (Hoskin *et al.,* 1995; Kusmartono *et al.,* 1996; 1997; Burke *et al.,* 2000). The rapid degradation rate of chicory while in

the rumen is attributed to the unique cell wall structure of chicory, where the structural components of the leaf are rich in pectin (Sun *et al.,* 2007). Because chicory breaks down at a faster rate compared with pasture, it spends less time in the rumen. This could reduce the time available for microbial fermentation, resulting in less CH_4 produced per kg of DM consumed. Particle breakdown and digesta passage rate were not measured in this study, and research is needed to investigate if the rate of particle breakdown affects CH_4 yield.

Differences in dietary chemical composition between chicory and pasture may have contributed towards the lower CH_4 yields in sheep consuming chicory. However, no significant relationship between the chemical composition of fresh forages and CH_4 yield has been identified (Waghorn & Woodward, 2006; Hammond *et al.,* 2009). This suggests that physical characteristics of chicory rather than the dietary chemical composition reported in these studies may be contributing towards the reduction in CH_4 yield. However, the role of secondary plant compounds, such as condensed tannins, has not been extensively investigated and should be explored to elucidate the mechanisms for reduced CH_4 yields in ruminants when fed chicory or other legumes and herbs.

In this study, and those reported by Waghorn *et al.* (2002) and Woodward *et al.* (2001; 2002), legumes and herbs were mainly fed as monoculture forages. Nevertheless, in practice, farm systems often result in a variety of forage species being offered to animals at any one time to maximise both herbage yield and animal productivity. The optimal proportion of chicory, or other herbs and legumes, required in the diet to achieve significant reductions of CH_4 yield is unknown and needs investigation.

6.2.1.2 Monensin

From the experimental results reported in this thesis it appeared that monensin was able to reduce CH_4 yields in sheep, but this effect was not consistent between the two studies. Monensin supplementation resulted in a mean CH_4 yield reduction of 30% when sheep were fed fresh pasture or chicory (32.8 vs. 26.0 g CH_4/kg DMI, respectively), whereas no effect of monensin supplementation was reported for ewes fed ensiled lucerne chaff during early lactation. The reasons for the inconsistent effect of monensin supplementation on CH_4 yield between the experiments are not clear.

Experimental differences between the studies include differences in sheep age and physiological status, diet type and quality, and dose rate of monensin (mg/kg DMI). The different rates of monensin supplementation (21 vs. 14 mg/kg DMI) could have contributed towards the inconsistent responses in CH_4 yield. As shown in Table 6.1, dose rates of less than 20 mg monensin/kg DMI fail to promote a reduction in CH_4 yield for most diets. Beauchemin *et al.* (2008) suggested that the higher dose rates of monensin (24 – 35 mg/kg DMI) required to reduce CH_4 yields were similar to those needed to improve feed efficiency in both beef and dairy cattle. Nevertheless, monensin dose rates above 20 mg/kg DMI do not guarantee a reduction in CH_4 yield, especially when animals are consuming pasture (Grainger *et al.,* 2010).

The persistence of reduced CH_4 emissions due to monensin appears questionable. Guan *et al.* (2006) showed that monensin decreased CH_4 yields (31%) and protozoa counts, and increased propionate production in steers; however, after 6 weeks of monensin supplementation, these parameters were not statistically different from the control animals. In contrast to this, Rogers *et al*. (1997) found no evidence of adaptation to the long-term supplementation of monensin. This study found a 50% reduction in protozoa counts and an elevated molar proportion of propionate (up to 29%), which remained until monensin supplementation was stopped at 96 days. Unfortunately, no measurements of CH_4 emissions were made. In addition, a meta-analysis of the

effect of monensin on animal production, metabolism and animal health did not indicate any adaptation to monensin (Duffield *et al.,* 2008a, b, c).

The inconsistent ability of monensin supplementation to reduce CH_4 yields from animals fed forage diets may be due to its mode of action. Monensin does not appear to directly influence the methanogen community, but does alter the activity of gram-positive bacteria and protozoa (Chow *et al.,* 1994; Guan *et al.,* 2006). By reducing the activity of protozoa, it is anticipated that the amount of hydrogen (H_2) available for methanogenesis is reduced (Guan *et al.,* 2006). Compared with concentrate diets, the ruminal concentration of protozoa is lower than with forage diets (Brossard *et al.,* 2003). Therefore the H_2 produced from the protozoal community of forage-fed animals may not be a key source of H_2 for methanogenesis and could explain the inconsistent effect of monensin supplementation on CH_4 yield.

Table 6.1: Effect of monensin on methane (CH_4) production (g/day) and yield (g/kg dry matter intake; DMI). Adapted from Beauchemin *et al.* (2008).

Animals	Diet	Dose rate		Days after dose	CH_4 emissions				Reference
		(mg/day)	(mg/kg DMI)		Control (g/day)	Monensin (g/day)	Control (g/kg DMI)	Monensin (g/kg DMI)	
				Controlled release capsules					
Dairy cows	Ryegrass pasture	166	11	30-90	328	313[ns]	19.2	20.0[ns]	Waghorn *et al.*, 2008
Dairy cows	Ryegrass pasture	320	29.6	11	179[a]	158[b]	16.9[a]	15.3[b]	Van Vugt *et al.*, 2005
Non-lactating dairy cows	Ryegrass pasture	320	35.2	72	246[a]	223[b]	25.5	24.8[ns]	Van Vugt *et al.*, 2005
Dairy cows	Ryegrass + white clover	320	17.5	23	330[a]	309[b]	17.5	16.9[ns]	Van Vugt *et al.*, 2005
Dairy cows	Ryegrass + maize silage	320	18.1	58	350	356[ns]	19.2	20.5[ns]	Van Vugt *et al.*, 2005
Dairy cows	Ryegrass + grain	240	13	25,85	341	365[ns]	-	-	Grainger *et al.*, 2008
Dairy cows	Ryegrass + grain	240	13	83	376	386[ns]	-	-	Grainger *et al.*, 2008
Dairy cows	Ryegrass + grain	240	13	75	309	306[ns]	16.7	17.0[ns]	Grainger *et al.*, 2008
				Added to the ration					
Dairy cows	Grain + forage	385	24	8–28	572[a]	517[b]	38.6[a]	35.7[b]	Sauer *et al.*, 1998
	Grain + forage	385	24	8–28*	599	598[ns]	34.9	33.7[ns]	Sauer *et al.*, 1998
Dairy cows	Grain + forage	473	24	**	458.7[a]	258.6[b]	23.3[a]	22.4[b]	Odongo *et al.*, 2007
Dairy cows	Ryegrass + grain	471	23.6	35	429	435	22.0	21.6	Grainger *et al.*, 2010
	Ryegrass + grain	471	22.5	70	466	470	22.5	23.7	Grainger *et al.*, 2010
Beef cattle	High forage	246	33	19	166.2[a]	159.6[b]	22.6[a]	20.7[b]	McGinn *et al.*, 2004
Beef cattle	High grain	271	33	Weekly for 16 weeks		-27% for 2 weeks, 0 by week 6			Guan *et al.*, 2006
Beef cattle	High forage	240	33	Weekly for 16 weeks		-30% for 4 weeks, 0 by week			Guan *et al.*, 2006
				Daily oral drenching					
Lactating ewes	Ensiled lucerne chaff	21	14	12 – 33	30.2	26.4[ns]	20.6	19.2	Chapter 3
Hoggets	Pasture or chicory	15	21	11 – 46	26.1[a]	20.3[b]	32.8[a]	26.0[b]	Chapter 4

* Second CH_4 measurement

** CH_4 measured monthly for six months

[ab] Within rows and the CH_4 variable, values followed by the same letter are not significantly different at $P > 0.05$.

[ns] No effect of monensin on CH_4 emissions reported.

6.2.1.3 Coconut oil

The supplementation of coconut oil (3% DMI) had no influence on CH_4 emissions in sheep fed fresh pasture or chicory (Chapter 4). This is in contrast to research reviewed by Machmüller (2006) where coconut oil reduced CH_4 yields by 28 to 78% in animals fed different concentrate-based diets. Nevertheless, Machmüller (2006) identified that the suppression of CH_4 emissions in sheep was greatest at the higher levels of coconut oil supplementation (7% DMI). Because of the natural lipid content of fresh forages, approximately 3 to 5% DM, the level of oil supplementation may be limited compared with concentrate-based diets. The tolerance of total lipid content in the diet of ruminants is 8 to 9% DMI. Above this dietary lipid level, both DMI and apparent digestibility are negatively affected (Beauchemin *et al.,* 2008; Cosgrove *et al.,* 2008).

The supplementation of lipids, other than coconut oil, to ruminants has been shown to reduce CH_4 yields (Beauchemin *et al.,* 2008), but data are less numerous and less consistent with fresh forage diets. Woodward *et al.* (2006) supplemented mixtures of fish and sunflower oil at a rate of 3.7% DMI to lactating dairy cows and reduced CH_4 yields by 21 to 34% after supplementation for 10 days. In contrast, Cosgrove *et al.* (2008) reported that when sheep were fed a diet of pure perennial ryegrass pasture and supplemented with a blend of linseed and sunflower oils at four different levels (1.2%, 2.5%, 3.7%, 5.0% and 6.0% of DMI) there was no difference in CH_4 yield compared with the unsupplemented control group. However, the experiment may have lacked the statistical power (n = 4 per treatment) to detect significant differences in CH_4 yield using the SF_6 technique.

6.2.1.4 Combining potential mitigation technologies

The combination of more than one mitigation technology may offer a means of achieving greater reductions of CH_4 yields, compared with using a single mitigation technology. The combination of coconut oil and/or monensin supplemented to sheep fed chicory or pasture was investigated (Chapter 4) and

did not show a significant cumulative reduction in CH_4 yield. Nevertheless, the results from that study are compromised by the poor performance of the SF_6 technique to estimate CH_4 production. Therefore the experiment did not have enough statistical power to detect 20% difference between groups (Section 4.5.2.3 and Section 6.2.3).

Although not statistically significant, the combination of monensin and chicory produced numerically cumulative reductions of CH_4 yield. Chicory reduced sheep CH_4 yields by 17%, which were reduced by a further 8% when sheep were also supplemented with monensin. The mean reduction of CH_4 yield when sheep were supplemented with monensin was 22% and increased to 30% when sheep were also supplemented with coconut oil, although again this was not statistically significant. No definitive conclusion can be made on the efficacy of combining mitigation technologies on CH_4 yield. The possible array of complex interactions between animal, microbial populations and nutrition needs to be better understood to realise the full potential of combining CH_4 mitigation technologies. Further research is needed to confirm this hypothesis, with either a greater number of animals and/or a more precise method to determine CH_4 production, i.e. respiration chambers.

6.2.1.5 Economic benefit of methane mitigation technologies

Potential CH_4 mitigation technologies need to be feasible under New Zealand pastoral conditions. Grazing systems place constraints on the type of CH_4 mitigation technologies able to be used and accepted by both the public (consumers) and producers. In developing mitigation technologies, the cost effectiveness of the technology must also be considered. To be economically viable, the cost of any CH_4 mitigation technology must be less than the savings made from reducing those emissions, and financial gains must be realised from increased animal production.

The potential savings that could be realised in carbon credits (cost per tonne of CO_2) with reductions in CH_4 emissions are shown in Table 6.2 for cattle, sheep and deer. For each ruminant species, the total cost of enteric CH_4 as CO_2-equivalents ($CO_{2\text{-e}}$) is calculated from the total tonnes of $CO_{2\text{-e}}$ produced per

year per animal and multiplied by the value of a tonne of CO_2 'carbon credit'. From the total cost of each carbon credit, the amount of money saved by reducing enteric CH_4 CO_2-equivalent emissions, ranging from 5% to 35%, is calculated. As shown in Table 6.2, any savings gained by reducing enteric CH_4 emissions without any added benefit in animal productivity are unlikely to provide enough financial incentive for farmers to use these technologies until there is a significant increase in the cost of carbon. For example, if a mitigation technology costs $3 /sheep/year and reduces CH_4 emissions by 15% then the savings made by reducing CH_4 will not cover the cost of the mitigation agent, until the cost of each carbon credit is greater than $60. However, if that technology also increases animal productivity, with a net gain of $2 /sheep/year, then the cost of carbon only needs to be $30 /tonne/year to break-even.

Table 6.2: Scenario analysis of the predicted total cost ($) of enteric methane ($CH_4$) for cattle, sheep and deer and the estimated savings ($) made by mitigation total CH_4 emissions per animal per year.

			Cattle[1]			
% reduction in CH_4 emissions	Value ($) per carbon credit (cost per tonne of carbon)					
	10	20	30	40	50	60
5	0.81	1.62	2.43	3.24	4.05	4.86
10	1.62	3.24	4.86	6.49	8.11	9.73
15	2.43	4.86	7.30	9.73	12.16	14.59
20	3.24	6.49	9.73	12.97	16.21	19.45
25	4.05	8.11	12.16	16.21	20.27	24.32
30	4.86	9.73	14.60	19.45	24.32	29.18
35	5.67	11.35	17.02	22.70	28.37	34.05
Cost/cattle	*16.21*[4]	*32.42*	*48.64*	*64.85*	*81.06*	*97.27*
			Sheep[2]			
% reduction in CH_4 emissions	Value ($) per carbon credit (cost per tonne of carbon)					
	10	20	30	40	50	60
5	0.14	0.23	0.34	0.46	0.57	0.69
10	0.23	0.46	0.69	0.91	1.14	1.37
15	0.34	0.69	1.03	1.37	1.71	2.06
20	0.46	0.91	1.37	1.83	2.29	2.74
25	0.57	1.14	1.71	2.29	2.86	3.43
30	0.69	1.37	2.06	2.74	3.43	4.11
35	0.80	1.60	2.40	3.20	4.00	4.80
Cost/sheep	*2.28*[4]	*4.57*	*6.86*	*9.14*	*11.42*	*13.71*
			Deer[3]			
% reduction in CH_4 emissions	Value ($) per carbon credit (cost per tonne of carbon)					
	10	20	30	40	50	60
5	0.23	0.47	0.70	0.94	1.17	1.41
10	0.47	0.94	1.41	1.87	2.34	2.81
15	0.70	1.41	2.11	2.81	3.51	4.22
20	0.94	1.87	2.81	3.75	4.68	5.62
25	1.17	2.34	3.51	4.68	5.85	7.03
30	1.41	2.81	4.22	5.62	7.03	8.43
35	1.64	3.29	4.92	6.56	8.20	9.83
Cost/Deer	*4.68*[4]	*9.37*	*14.05*	*18.73*	*23.42*	*29.20*

[1] Cattle assumes an effective CH_4 emission of 77.2 kg CH_4/head/year (Anon, 2009)

[2] Sheep assumes an effective CH_4 emission of 10.9 kg CH_4/head/year (Anon, 2009)

[3]Deer assumes an effective CH_4 emission of 22.3 kg CH_4/head/year (Anon, 2009)

[4] Estimated total cost per animal for each price /tonne of carbon. The total carbon dioxide equivalent produced per animal is calculated from CH_4/head per year x global warming potential of CH_4 (21, weight basis)

6.2.2 Influence of age and ruminant species on methane emissions

6.2.2.1 Age

The CH_4 yield calculated for weaner deer was influenced by age, as a consequence of the lower CH_4 yields calculated for deer at 4.5 months of age (14.9 g CH_4/kg DMI), compared with those of deer aged from 6.5, 9.0 to 11.5 months (17.0, 17.4 and 17.7 g CH_4/kg DMI; Chapter 2). The mean CH_4 yield of young deer in this study (16.8 g CH_4/kg DMI) was lower than previous measurements of CH_4 yield from adult deer (22.5 g/kg DMI; Swainson, 2004) using the SF_6 technique. However, as the total number of measurements from adult deer fed pasture is small and highly variable, the values of CH_4 yield reported here maybe within the normal range of adult deer.

The indirect method of determining DMI and the quality of the diet consumed by grazing deer in this study make it impossible to isolate these effects from any influence of deer age on CH_4 yield. The accuracy of determining DMI and CH_4 production are equally important for the calculation of CH_4 yield. This study presented here (Chapter 2) and others from the literature (Lassey *et al.,* 1997; Ulyatt *et al.,* 2005; Molano *et al.,* 2006) did not directly measure DMI, thus compromising the precision of calculated CH_4 yield. The DMI of the immature deer was estimated by the calculation of each animal's energy requirement for maintenance and production, i.e. liveweight gain. As a result, any small reductions of actual DMI, due to handling stress or the wearing of the breath collection equipment, could remain unnoticed over a 5-day measurement period. These undetected reductions of actual DMI could have resulted in an underestimation of CH_4 yield, explaining the supposedly lower CH_4 yields of deer at 4.5 months compared with deer at 6.5 to 11.5 months of age.

Knight *et al.* (2008b) reported that the CH_4 yields (g CH_4/kg DMI) of lambs were numerically lower than those of adult ewes at 13 (lambs, 20.9; ewes 22.1) and 17 (lambs, 23.3; ewes, 25.1) weeks of age, and significantly lower at 35 weeks of age (lambs, 17.9; ewes 21.9). These authors identified that diet quality had an important influence on the CH_4 yield of lambs, but was confounded with the

effect of age. No effect of diet quality on CH_4 yield was observed in mature ewes. These results suggest that diet quality may have a greater influence on apparent digestibility, passage rate of digesta and methanogenesis in immature ruminants compared with their mature counterparts, but this has not been tested under controlled conditions.

In contrast to Knight *et al.* (2008b, sheep) and this study (deer, Chapter 2), Ramírez-Restrepo *et al.* (2009) reported no difference of CH_4 yield between young (3.7 months of age) and adult cattle (6.8 years of age), when CH_4 emissions were measured by respiration chambers and animals were fed a uniform diet of ensiled ryegrass silage. This could imply that cattle are different to sheep and deer, and/or the apparent influence of age in both sheep and deer may be a function of the variability in the SF_6 technique used to measure CH_4 emissions. Therefore, the influence of animal age in both deer and sheep needs to be confirmed with measurements using respiration chambers.

If younger ruminants yield less CH_4 than their adult counterparts, this has implications for CH_4 mitigation, whilst interacting with drivers of production efficiency, particularly in meat production systems. Production systems may yield less CH_4 if animals reach slaughter weights at a younger age. Therefore, CH_4 mitigation may become an additional driver to increase growth efficiency of immature ruminants. However, changes in management would be needed, taking into consideration all GHG emissions from the production system. For example, the supplementation of grain to immature grazing ruminants increases growth rates, but comes at an additional cost associated with grain production and transport (Clark *et al.,* 2007).

6.2.2.2 Ruminant species

The study comparing the CH_4 yield of cattle, sheep and deer (Chapter 5), was the first to compare CH_4 emissions using the SF_6 technique, within the same season and fed the same diet at a similar feeding level (multiple of metabolisable energy (ME) required for maintenance). The CH_4 yields of cattle (21.4 g CH_4/kg DMI) were greater than sheep (18.7 g CH_4/kg DMI), which were greater than deer (16.1 g CH_4/kg DMI). The finding that ruminant species can

produce differing amounts of CH_4 is supported by the studies of Galbraith *et al.* (1993; bison, wapiti and white-tailed deer) and Semiadi *et al.* (1998; red and sambar deer). Nevertheless in this study, the calculation of CH_4 was problematic due to the variable collection of SF_6 in the breath samples from cattle. Therefore the cattle CH_4 yields may be misleading and these differences between ruminant species cannot be extrapolated to farmed ruminants.

The differences in diet apparent DM digestibility (DMD) between sheep (69.4% DMI) and deer (58.0% DMI) may have contributed towards the difference of CH_4 yield between these two animal species (DMD was not measured in cattle). Therefore, the differing ability of sheep and deer to digest the diet may contribute to the greater CH_4 yield of sheep compared with deer. In addition, no difference in CH_4 production per kg digestible DMI was observed between sheep and deer. Diet DMD differences between sheep and deer have been previously reported (Domingue *et al.,* 1991; Milne *et al.,* 1978; Fennessy *et al.,* 1980, but the direction of DMD difference between sheep and deer was not consistent. Therefore it is recommended that measurements of DMD, and digesta passage rate be conducted, in conjunction with measurements of CH_4 production, to help explain any differences or similarities between ruminant species in terms of CH_4 yield.

No differences between summer and winter were found in CH_4 yield or DMD in sheep and deer, in this study. This is despite Domingue *et al.* (1991) and Milne *et al.* (1978) reporting that rumen fill and turnover of digesta differs between summer and winter in deer, but not in sheep. However, the animals were fed *ad libitum* and deer (summer, 62.5 vs. winter, 46.7 g DM/kg $LW^{0.75}$) had the greatest change in DMI, compared with sheep (summer, 52.2 vs. winter, 54.8 g DM/kg $LW^{0.75}$). Therefore, the changes in deer digestive physiology in response to season are confounded with differences of DMI between summer and winter. This makes the link between digestive physiology, CH_4 yield and ruminant species tenuous, highlighting the need for future research at similar feeding levels to explore these mechanisms.

6.2.3 Retrospective power analysis and the SF_6 technique

6.2.3.1 Retrospective power analysis

Power analysis at the start of each experiment (Chapters 2 – 5) was used to determine the number of animals required in each treatment group. The analysis used standard deviations (SD) of CH_4 yield for each ruminant species from previous studies using the SF_6 technique (Table 6.3). The power analysis indicated that the number of animals required to determine a 20% difference in CH_4 yield between treatment groups, with a power of 80%, was 4 cattle, 4 sheep and 9 deer (Table 6.3). However, the actual SD and coefficient of variation (CV) of CH_4 yield from this thesis, particularly for cattle and sheep, were greater than those used in the initial power analysis.

A retrospective power analysis was undertaken based on the actual SD obtained in each experiment. This analysis indicated that the number of animals used in each treatment group of all the experiments were too few to have an adequate probability of detecting a difference of 20% between means (except in Chapter 2; Table 6.3). It also showed that the percentage difference between two means required to obtain a statistical difference ($P < 0.05$), ranged from: 19 – 71% for cattle; 26 – 100% for sheep; and 17 – 31% for deer. This was a direct result of the high between- and within-animal variance in CH_4 yields. The high variability of the data in these studies was due to the low and variable concentrations of SF_6 in the collected breath samples.

The CV of the CH_4 yields in these studies were high for cattle and sheep (up to 44% and 57%, respectively; Table 6.4). Vlaming *et al*. (2008) reported that for cattle housed indoors, fed either lucerne chaff or a total mixed ration diet, the between- and within-animal CVs were similar and ranged from 8 to 18%. In addition, for grazing sheep Ulyatt *et al.* (1999) reported within-animal CVs of 2.2 to 42.6% and a between-animal CV of 17%, and Lassey *et al.* (1997) reported a flock CV of 2.4%, for CH_4 yields. The between-animal CV of deer presented here (17.2 – 22.0%) appears similar to the between-animal CV of adult hinds fed pasture indoors (21.0%; Swainson, 2004). It is not known why the SF_6

technique appeared to perform as expected in deer, yet lead to a high variability in the estimated CH_4 yields from sheep and cattle.

Table 6.3: Power analysis for determining the sample size required to detect a significant difference between two means with 95% confidence and a power of 80% and type 1 error (α) of 0.05 using the standard deviation (SD) from previous experiments.

Treatment/ species	Difference between means	SD	CV[4] (%)	Number animals required
Cattle	10	2.74[1]	8	7
	20			4
Sheep	10	1.53[2]	7	7
	20			4
Deer	10	2.82[3]	11	11
	20			9

[1] Ulyatt *et al*., 2002

[2] Woodward *et al.,* 2002

[3] Swainson, 2004

[4] Coefficient of variation

Table 6.4: Retrospective power analysis to determine the actual power obtained and the percentage difference required between two means to detect a significant difference of 20% with a power of 80% when based on calculated methane (CH_4) yield (g/kg dry matter intake). Also shown is the number of animals required to detect a statistical difference of 20% between two means for CH_4 yield (80% probability, and type 1 error (α) of 0.05).

	Treatment/ species	SD	CV (%)	Animals Planned	Actual animals used	Actual power	Animals required	% Difference
Chapter 2 Weaner deer experiment								
Months of age	4.5	2.42	18.3	20	20	92	15	17
	6.5	2.89	19.1	20	18	86	16	18
	9	3.35	19.7	20	16	79	17	20
	11.5	2.94	17.2	20	16	89	13	18
Chapter 3 Ewe monensin experiment								
Covariate	Control	3.87	20.9	10	6	29	19	37
	Treatment	6.08	30.7	10	9	24	38	43
Measurement 1	Control	6.67	31.9	10	7	17	41	51
	Treatment	5.85	32.1	10	6	14	42	57
Measurement 2	Control	9.72	51.9	10	9	11	107	73
	Treatment	5.42	31.6	10	6	14	41	56
Chapter 4 Sheep CH_4 mitigation experiment[1]								
Covariate measurement (all sheep fed the same diet and not receiving monensin or coconut oil)								
Forages	Chicory	6.02	27.2	20	17	54	30	27
	Pasture	9.07	39.2	20	19	33	62	37
Treatments	Control	8.70	34.0	8	8	17	47	51
	Monensin	4.86	22.9	8	8	35	22	34
	C. oil[2]	8.29	35.0	12	9	19	50	49
	M. + C. oil[3]	7.15	35.0	12	4	18	50	80

[1]Total number of animals used was 40 (Chapter 4).

[2]Coconut oil

[3]Monensin and coconut oil

Table 6.4 (continued): Retrospective power analysis to determine the actual power obtained and the percentage difference required between two means to detect a significant difference of 20% with a power of 80% when based on calculated methane (CH_4) yield (g/kg dry matter intake). Also shown is the number of animals required to detect a statistical difference of 20% between two means for CH_4 yield (80% probability, and type 1 error (α) of 0.05).

	Treatment/ species	SD*	CV (%)	Animals Planned	Actual animals used	Actual power	Animals required	% Difference
Measurement 1								
Forages	Chicory	12.30	57.1	20	15	14	129	60
	Pasture	10.66	37.3	20	18	34	56	36
Treatments	Control	18.27	56.9	8	6	7	129	100
	Monensin	7.75	30.8	8	5	12	39	61
	C. oil	11.43	43.3	12	11	16	75	54
	M. + C. oil	7.47	35.8	12	11	22	52	45
Measurement 2								
Forages	Chicory	12.61	48.6	20	16	19	94	50
	Pasture	14.34	44.5	20	18	25	79	43
Treatments	Control	16.88	49.9	8	7	9	99	80
	Monensin	11.48	42.1	8	7	11	71	68
	C. oil	16.94	48.9	12	9	11	95	63
	M. + C. oil	7.66	31.9	12	11	27	31	40
Chapter 5 Comparative species experiment								
Cattle	Summer	2.90	15.5	11	11	84	10	19
	Winter	10.58	44.2	11	7	10	78	71
Sheep	Summer	6.33	32.8	11	10	24	44	43
	Winter	3.99	22.0	11	10	47	20	29
Deer	Summer	3.46	20.6	11	9	48	18	29
	Winter	3.38	22.0	11	9	43	20	31

6.2.3.2 The SF_6 technique

Methane yields from both cattle and sheep were highly variable, apparently due to the poor collection of SF_6 in the breath samples. The poor collection of breath samples is normally related to the breath collection equipment, operator error and geometry of the inlet tube relative to the animal's nose and mouth (Ulyatt *et al.,* 1999). These factors do not appear to be responsible for the low concentrations of SF_6 gas collected and analysed in this thesis, as the air pressures in the yokes after collection were checked and samples discarded if they were not within the acceptable range. Furthermore, the concentration of CH_4 gas within the collected breath samples was within the expected range and less variable than SF_6. The molecular weight of SF_6 is nine times that of CH_4, which could potentially result in the poor mixing and unequal sampling of the two gases. Nevertheless, there is no evidence of this occurring (Ulyatt *et al.,* 1999; Johnson *et al.,* 2007).

Possible causes for the apparent start-stop behaviour of SF_6 gas released from the rumen, have not been previously reported. Suggested reasons include: (1) permeation tube moving around the rumen or out of the rumen; (2) SF_6 gas bubbling and somehow becoming trapped in the rumen or rumen material until it is released; (3) SF_6 gas escaping via another means, other than the mouth and nose; and (4) length of residence time in the rumen or age of the permeation tube.

The location of the permeation tubes cannot explain the lack of SF_6 gas detected in the breath sample in all instances. The location of the permeation tubes in the fistulated cattle used in the comparative species experiment (Chapter 5) was assessed at the end of the initial failed winter CH_4 measurement period and all tubes were present in the rumen (n = 4) or reticulum (n = 5) and in similar positions to those reported by Pinares-Patiño and Clark (2008).

The accumulation of SF_6 within the rumen due to the gas bubbling or becoming trapped by feed materials could explain the appearance and disappearance of SF_6 in the collected breath samples. A recent study by Lassey *et al.* (2011)

where SF_6 and CH_4 were measured every 20 minutes for 6 days showed that the excretion of SF_6 from the rumen of sheep is not uniform throughout the day and intermittent periods of low concentrations of SF_6 (only 2 x background) for some of the sheep were reported. This suggests that, while it is unusual to not detect SF_6 tracer gas in the daily collected breath samples, the intermittent release of SF_6, occurring for short time periods during the day, is not unusual.

The assumption that SF_6 gas does not flow out of the rumen was also challenged by Vlaming (2008), as SF_6 gas was emitted from the urine and faecal material of cattle, at very low concentrations. Nevertheless, this should not affect the efficacy of the SF_6 technique to estimate CH_4 emissions, as long as the gas is not stored in the animal. Validations of the SF_6 technique against total CH_4 emissions measured by calorimetry chambers, as reviewed by Pinares-Patiño and Clark (2008), suggest that differences in the mean CH_4 yield between the two techniques were statistically insignificant. Hammond *et al.* (2009), using the New Zealand CH_4 database, compared the variability of CH_4 yield in sheep fed ryegrass-based pasture, using the SF_6 and calorimetry chamber techniques. They found that, although the mean (± SD) CH_4 yields from both techniques appeared similar (SF_6 technique 23.4 ± 5.7 g CH_4/kg DMI and calorimetry 23.1 ± 2.9 g CH_4/kg DMI), there was greater variance around the mean of the CH_4 yields estimated using the SF_6 technique (CV = 24.4%) compared with calorimetry measurements (CV = 12.6%).

Another contributor towards the variability of estimated CH_4 emissions from ruminants, when using the SF_6 technique is the age of the permeation tube and length of time the permeation tube is in the animal. As presented in Chapter 2 the variation (standard error of the mean, SEM) of estimated CH_4 production during the last CH_4 measurement was approximately double that of the first CH_4 measurement. Differences between estimated CH_4 production when using either uncorrected (release rates determined pre-deployment only) or corrected release rates (pre-deployment release rates adjustment for SF_6 release following recovery at the termination of the experiment) of SF_6 were 9.4 to 20.5%. The percentage difference between estimated CH_4 production

determined by uncorrected or corrected release rates of SF_6 increased the longer the permeation tubes remained in the rumen of the deer. This highlights that the utmost care needs to be taken in determining SF_6 gas release rates. When possible, it is recommended that permeation tubes are recovered from animals at the termination of CH_4 measurements for the correction of SF_6 release rates in the rumen, especially in experiments were the permeation tubes may remain in the animal for more than two months.

6.3 CONCLUSIONS AND RECOMMENDATIONS FOR FUTURE RESEARCH

6.3.1 Conclusions

- The feeding of chicory reduced the CH_4 yield in sheep by 19% and indicates that herbs, as an alternative forage to pasture, could be an important tool for CH_4 mitigation in grazing systems.

- The reduction of CH_4 yield from sheep in response to monensin supplementation was inconsistent between the two studies presented here. This may have been due to differences in diet and/or monensin dose rate.

- Coconut oil did not reduce the CH_4 yield of sheep fed either fresh pasture or chicory, which may be a consequence of the low level of lipid supplementation.

- The combination of more than one CH_4 mitigation technology did not result in statistically significant additive reductions in CH_4 yield. However, the greater than expected variability in estimating CH_4 production, using the SF_6 technique, decreased the power of this experiment.

- Deer at 4.5 months of age had lower CH_4 yields than older deer. However, the age effect was impossible to isolate from effects of diet quality, deer seasonality or DMI. In addition the method used to measure the DMI of grazing animals could have resulted in an underestimation of CH_4 yield, particularly for the first measurement of CH_4 production.

- Differences in CH_4 yield were found between cattle, sheep and deer (cattle > sheep > deer). However, the calculated CH_4 yield from cattle was problematic and therefore may not be correct. No difference in CH_4 when expressed per unit of digestible DMI, were observed between sheep and deer, which suggests ability of the animal to digest the diet impacted on differences of CH_4 yield between species.

- The SF_6 technique used to determine CH_4 production in both sheep and cattle had greater variability between- and within- animals than previously reported. This was due to unusually low concentrations of SF_6 gas in the collected breath samples from sheep and cattle. No single explanation can be given to explain this.

6.3.2 Recommendations for future research

- The mechanisms by which the CH_4 yield in sheep was reduced in response to feeding chicory was not determined. Further research examining the dietary chemical composition and changes in digestive physiology and/or microbial populations is needed. In this thesis, chicory was fed as monoculture forage; however, in farm practice, forage swards consist of multiple plant species. The optimal proportion of chicory in the diet to achieve reductions in CH_4 yield and increase animal performance compared with pasture is not known and needs to be tested.

- The suitability of monensin as a CH_4 mitigation option, as well as its mechanism of action, for grazing ruminants needs to be explored.

- Further investigations into the possible effect of combining more than one potential mitigation agent on the CH_4 emissions of ruminants is needed to determine if combining more than one mitigation technology is feasible.

-It is recommended that the studies implying that CH_4 yield is influenced by animal age (Chapters 2) be repeated with suitable control animals, i.e. adult deer fed a similar diet at similar levels of feeding, alongside accurate measures of CH_4 production and DMI, to confirm the findings reported.

- Methane yield appeared to be affected by animal species. The reason for this difference between ruminant species is unclear and should be investigated under various dietary conditions (i.e. level of intake and composition of the diet). This is important as ruminant production systems in New Zealand typically involve more than one species.

- The accuracy of determining CH_4 production from ruminants is critical to the development of greenhouse gas inventories and mitigation technologies. The most suitable and accurate method to measure CH_4 should be used. The SF_6 technique is a useful tool for estimating CH_4 production, particularly for grazing ruminants. However, for continued use of this technique, the accuracy of establishing SF_6 release rates from permeation tubes needs to be improved, and a better understanding of the behaviour of the SF_6 gas whilst in the rumen is required.

ANNEX A

Decision analysis tree for the exclusion of methane emission data from statistical analysis – cattle and sheep.

Criteria were sought to define the range of sulphur hexafluoride gas (SF_6) (ppt [parts per trillion]), methane gas (CH_4) (ppm [parts per million]) concentrations and the ratio of SF_6:CH_4 from yokes that were typical of sheep and cattle. These criteria were applied to measurements of SF_6 and CH_4 gas concentrations from collected breath samples of cattle and sheep to help determine if estimates of daily CH_4 production measurements were sensible for statistical analysis. A decision tree (Figure A.1) was constructed to simplify the process of determining data inclusion/exclusion.

The data used to define the ranges presented in the decision tree for sheep and cattle were sourced from New Zealand Methane Database, from 2002 to 2007. This encompassed 15 independent sheep experiments totalling 3542 SF_6 and CH_4 data points and 17 independent cattle experiments totalling 5170 data points of SF_6 and CH_4. The descriptions of the distribution of the raw data, as shown in Table A.1, were by; minimum data point, lower quartile (25%), median (50%), upper quartile (75%) and maximum data points and percentiles (Figures A.3 to A.5), when data was ordered form lowest to highest; and the mean plus or minus (±) the standard deviation. No separation was made for experiment or treatment/diet.

The ranges presented in the decision tree for data inclusion/exclusion are based on 10% to 90% of the data when ranked in order, as shown in Table A.1 and Figure A.2 to A.5. This percentage was chosen, as it was similar to the mean plus or minus the standard deviation. In addition, Lassey *et al.* (1997) reported that CH_4 gas concentrations in the breath samples from sheep and cattle ranged from 21 – 207 ppm, with most concentrations within the range of 50 – 120 ppm, which gave the most reliable estimates of CH_4. This latter range reported by Lassey *et al.* (1997) is similar to the range of CH_4 gas

concentrations that resided within 10 – 90% of the data range presented here, i.e. sheep, 21 – 134 and cattle 21 – 151.

In the decision tree (Figure A.1), data was screened by the following steps;

1. Concentration of SF_6 gas, as this appeared to be the most variable of the two gases. If the concentration of SF_6 gas was deemed acceptable, then the data go to step 2, or if the data were unacceptable, then data were screened by 4c.

2. (2b) The ratio of the release rate of SF_6 gas from the permeation tube (PR) to the concentration of SF_6 gas from the collected breath sample. Because there is a large range of PR from the permeation tubes used, this step attempts to identify if the concentration of SF_6 gas in the breath sample is within an acceptable range for PR. If data were accepted, then they were taken to step 3; if data were rejected, they were excluded from the analysis.

3. The concentration of CH_4 gas collected in the breath sample was screened. If this was within the acceptable range, then data went to step 4; if rejected, data went to 4b.

4. (4b, 4c) The ratio of SF_6 to CH_4. The estimation of CH_4 is dependent on this ratio and by examining this ratio it helps to determine if the concentrations of both gases are likely to render sensible CH_4 production results. This is particularly important when breath samples may be diluted; for example, this may be due to the geometry of the inlet tube from the animal's nose or changes in weather conditions, i.e. wind, may dilute the concentrations of both SF_6 and CH_4 collected. However, the ratio of SF_6 to CH_4 is influence by the PR, as shown in Figures A.6 and A.7, therefore if this ratio falls outside the expected range, data may still be used (4c) as the ratio of PR to SF_6 concentration in the breath sample is acceptable (2, 2b).

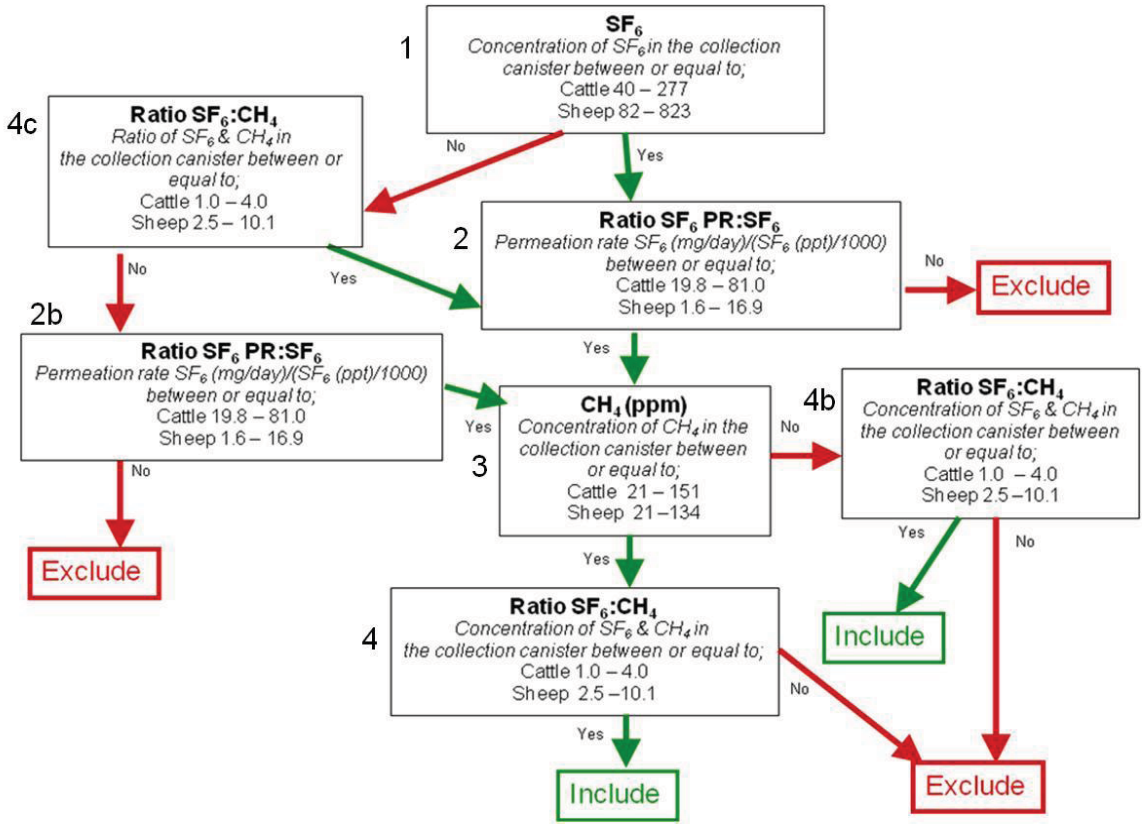


Figure A.1: Decision tree for the inclusion or exclusion of data based on the concentrations of the gases sulphur hexafluoride gas (SF6) and methane gas (CH4) from the collection canisters of sheep and cattle.

Table A.1: Description of the range of concentrations of sulphur hexafluoride gas (SF_6) and methane gas (CH_4) collected from the breath samples of sheep and cattle data in experiments from 2002 to 2007 using the SF_6 technique.

	Sheep[5]				Cattle[5]			
Data range (data point)	**SF_6[1]**	**CH_4[2]**	**Ratio SF_6:CH_4[3]**	**Ratio PR: SF_6[4]**	**SF_6**	**CH_4**	**Ratio**	**Ratio PR: SF_6**
All data ranked from lowest to highest								
Minimum	7.2	2	0.3	0.3	8	3	0.3	3.5
10th percentile (10%)	82	21	2.5	1.5	40	23	1.0	19.8
Lower quartile (25%)	165	37	3.8	2.4	75	35	1.3	29.8
Median (50%)	314	61	5.5	3.8	116	58	1.6	46.4
Upper quartile (75%)	544	95	7.8	7.7	188	104	2.5	63.5
90th percentile (90%)	823	134	10.1	16.9	277	151	4.0	81.0
Maximum	2833	383	53.5	101.6	1366	386	29.6	433.9
Mean	400	72	6.1	7.6	150	76	2.3	50.2
Standard deviation (SD)	336	50	4.1	10.8	134	58	2.3	30.9
Mean – SD	64	22	2	-3.3	16	18	0	19.3
Mean + SD	736	122	10.2	18.4	284	134	4.6	81.1

1SF6 parts per trillion (ppt).

2 CH4 parts per million (ppm)

3Ratio of SF6 (ppt):CH4 (ppm)

4Ratio of release rate of SF6 from permeation tube (PR) (mg/day): (SF6 (ppt)/1000)

5 Sheep n (experiments) = 15, n (data points) = 3542; cattle n (experiments) = 17, n (data points) = 5170. Data sourced from the New Zealand CH4 database.

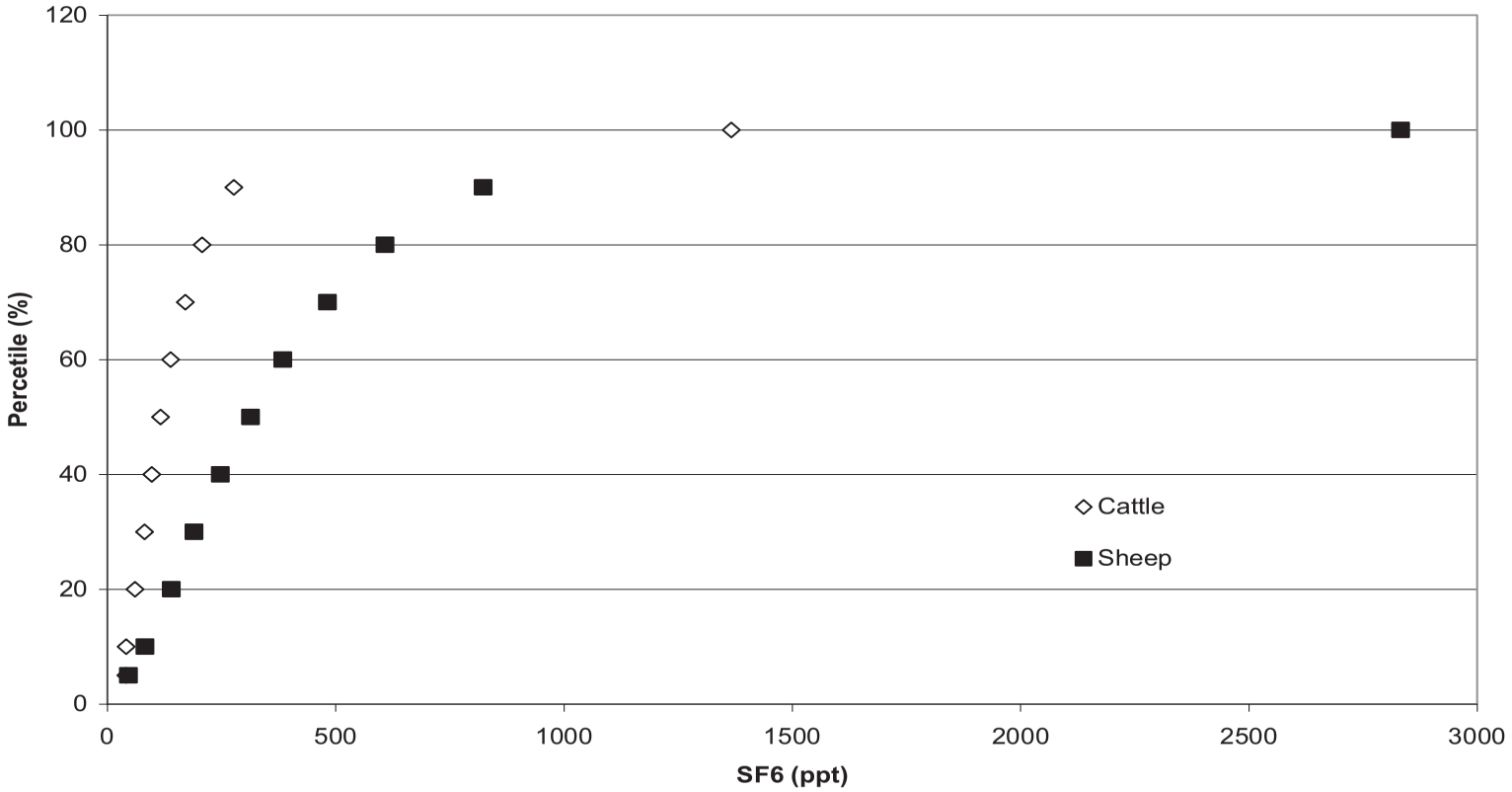


Figure A.2: Percentile (%) plot of sulphur hexafluoride gas (SF6) (parts per trillion (ppt)) concentrations as measured directly from sheep (n (experiments) = 15, n (data points) = 3542) and cattle (n (experiments) = 17, n (data points) = 5170) when data is ranked from lowest to highest.

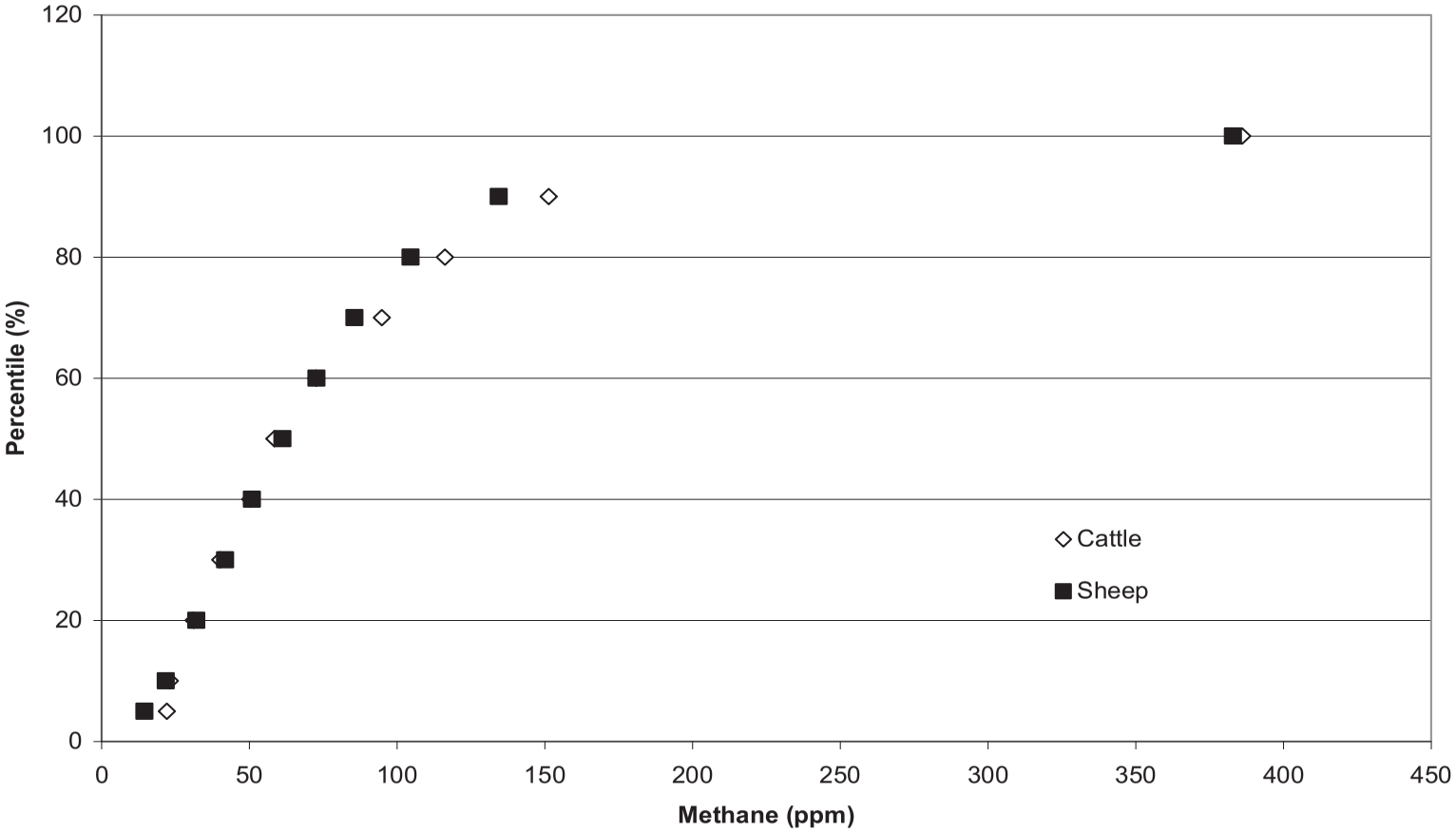


Figure A.3: Percentile (%) plot of methane gas (CH4) (parts per million (ppm)) concentrations as measured directly from sheep (n (experiments) = 15, n (data points) = 3542) and cattle (n (experiments) = 17, n (data points) = 5170) when data is ranked from lowest to highest.

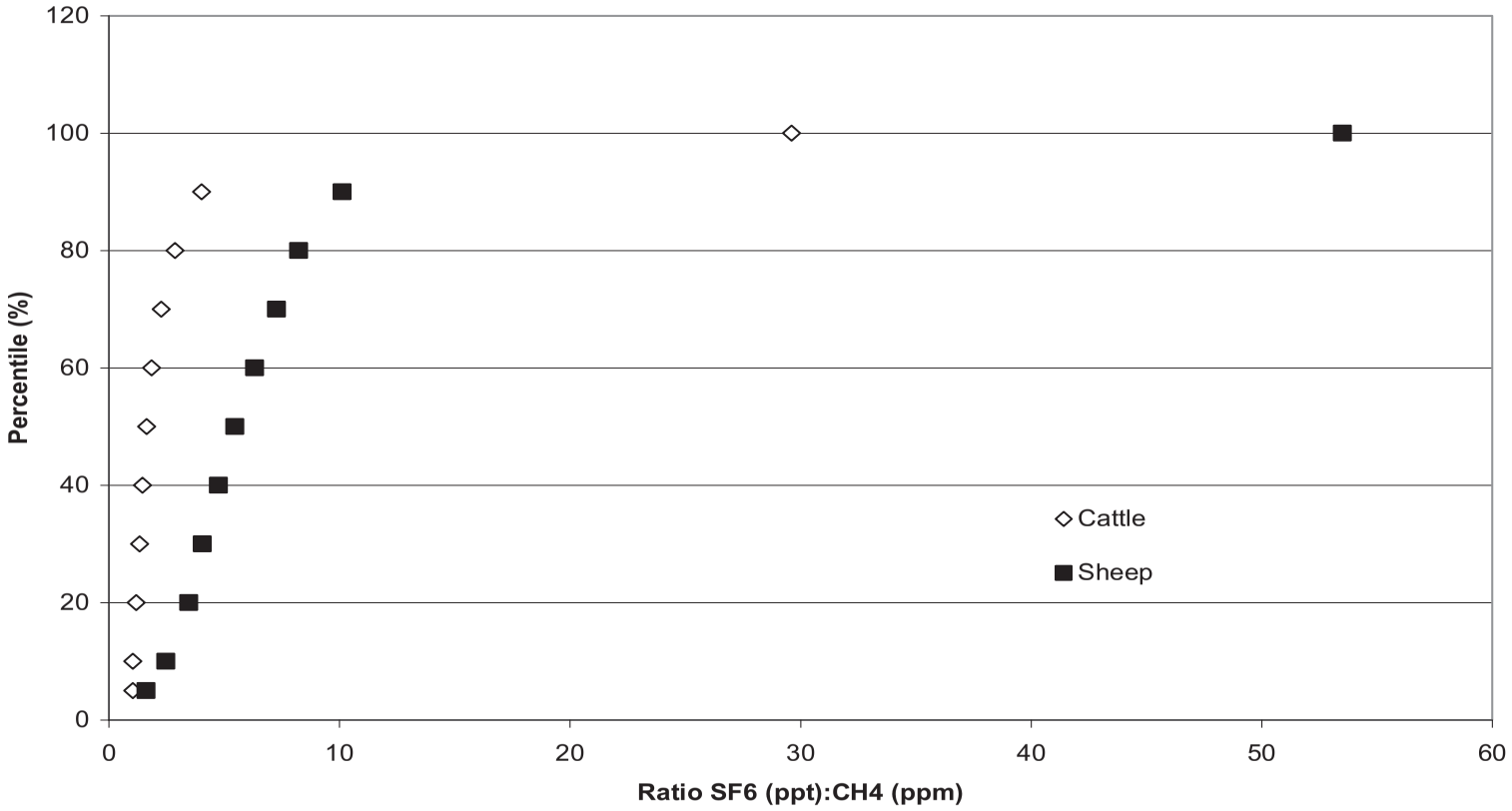


Figure A.4: Percentile (%) plot of the ratio of sulphur hexafluoride gas (SF_6) (parts per trillion (ppt)): methane gas (CH_4) (parts per million (ppm)) concentrations as measured directly from sheep (n (experiments) = 15, n (data points) = 3542) and cattle (n (experiments) = 17, n (data points) = 5170) when data is ranked from lowest to highest.

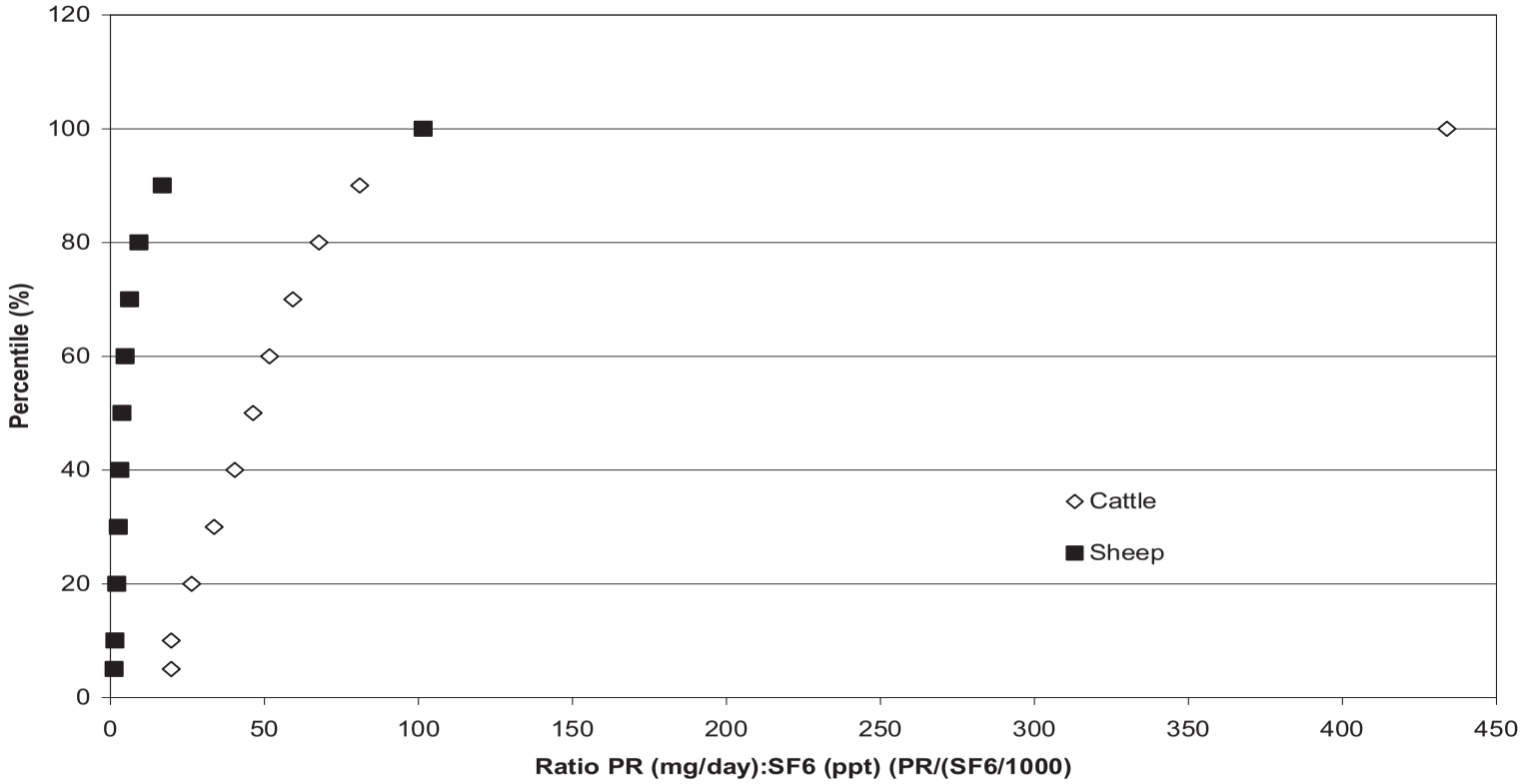


Figure A.5: Percentile (%) plot of the ratio of sulphur hexafluoride gas (SF_6) release from the permeation tube (PR) (mg/day): SF_6 (parts per trillion (ppt)) as measured directly from sheep (n (experiments) = 15, n (data points) = 3542) and cattle (n (experiments) = 17, n (data points) = 5170) when data is ranked from lowest to highest.

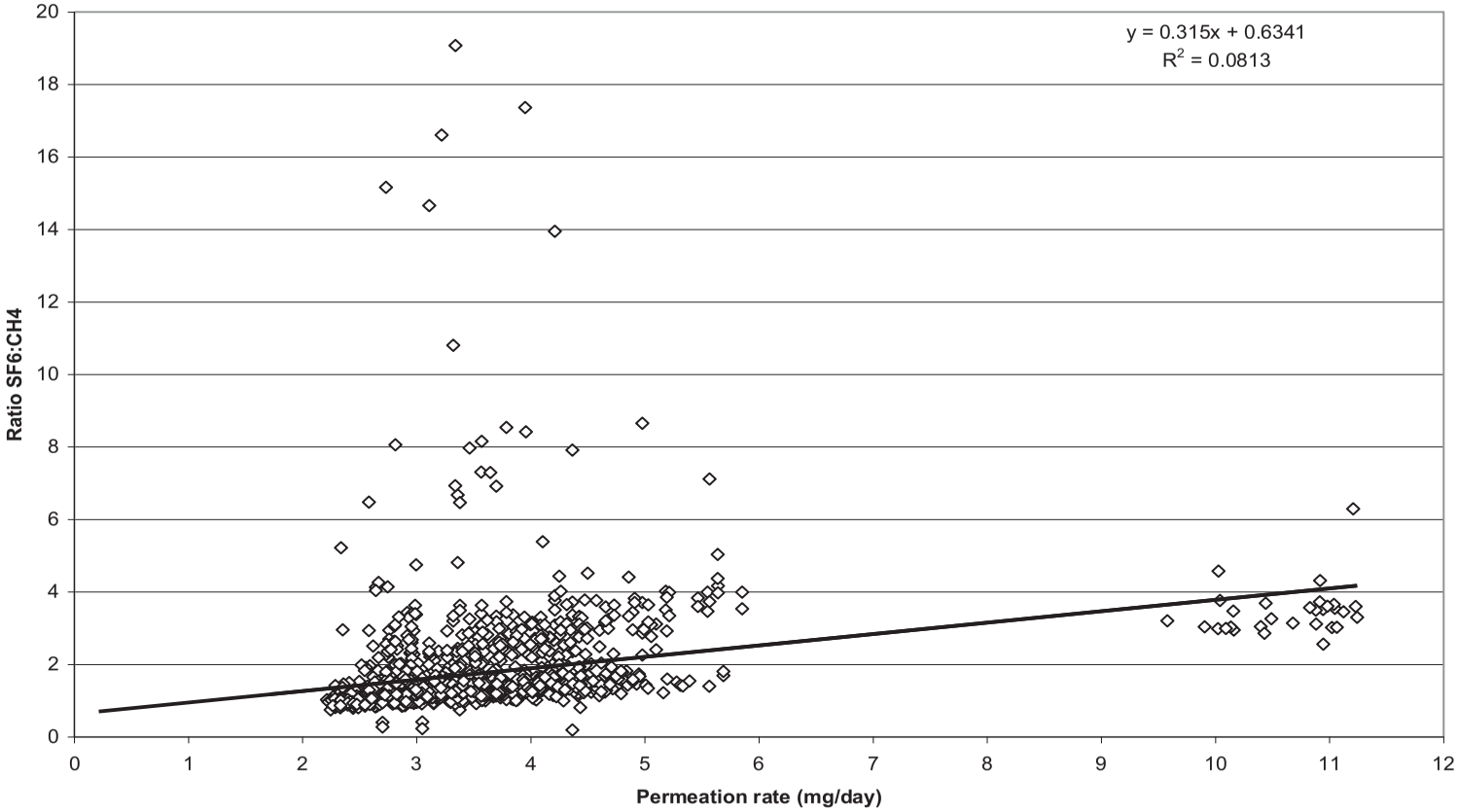


Figure A.6: Linear regression of release rate of sulphur hexafluoride gas (SF_6) from the permeation tube and the ratio of the gases SF_6: methane (CH_4) for cattle (n (experiments) = 17, n (data points) = 5170).

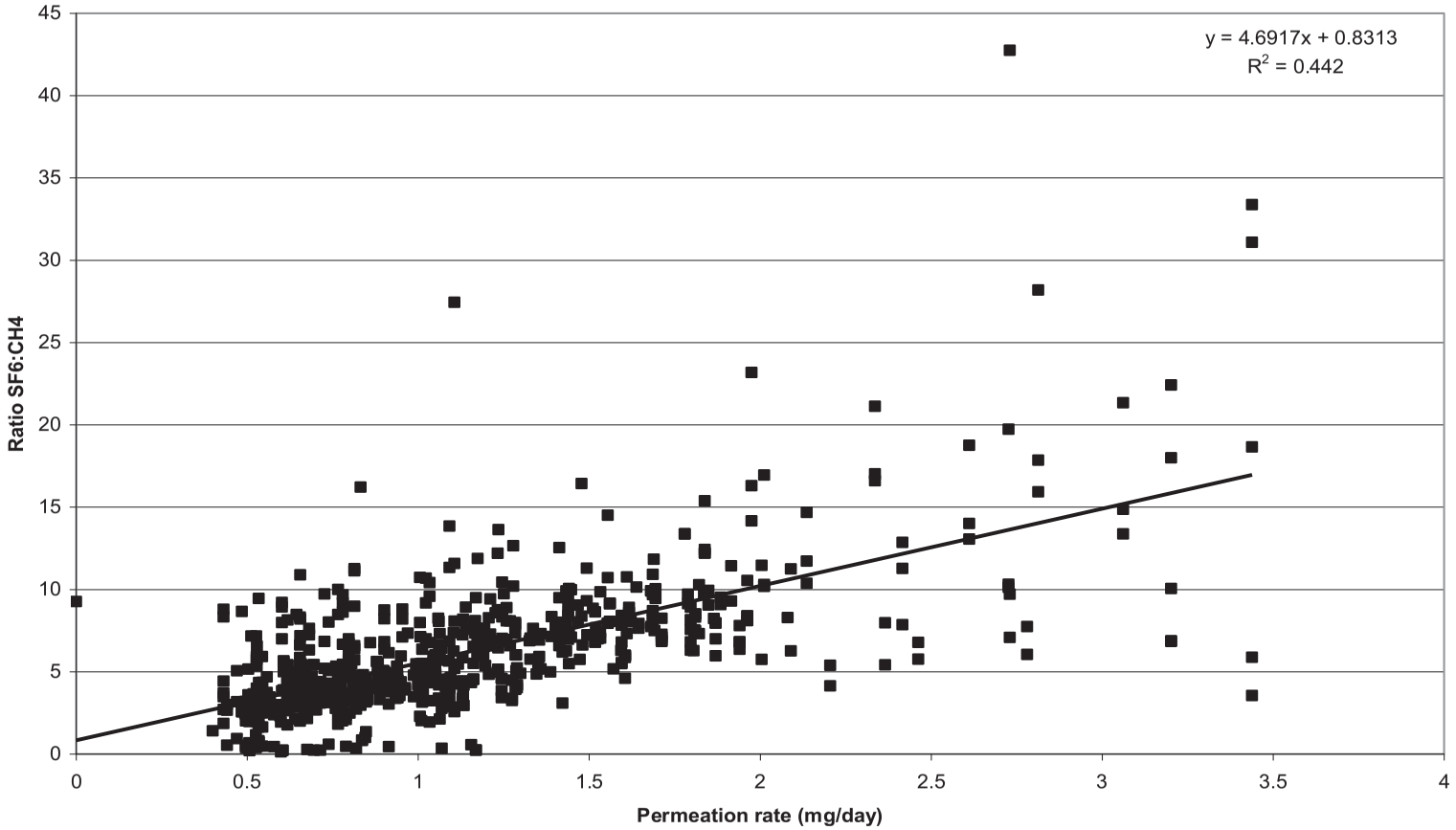


Figure A.7: Linear regression of release rate of sulphur hexafluoride gas (SF6) from the permeation tube and the ratio of the gases for sheep n (experiments) = 15, n (data points) = 3542).

CHAPTER 7

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