Methane emissions from farmed red deer

A thesis in partial fulfillment of the requirements for the
degree of Master of Science in Animal Science
at Massey University, Palmerston North.

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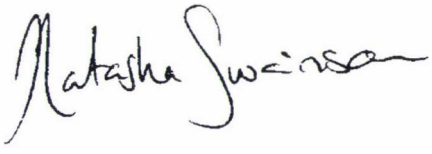
DECLARATION

The studies presented in this thesis were completed by the author while a post-graduate student in the Institute of Food Nutrition and Human Health, College of Sciences, Massey University, Palmerston North, New Zealand. This is all my own work and the views presented are mine alone. Any assistance received is acknowledged in the thesis.

I officially state that the contents of the thesis have not been submitted for any other degree and are not currently being submitted for any other degree. I certify that to the best of my knowledge, any help received in preparing this thesis, and all sources used, have been acknowledged in the thesis.



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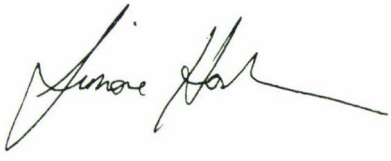

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ABSTRACT

Methane (CH_4) is one of the end products of fermentation of ingested feed by the microbial population residing in the foregut of ruminants. It represents a potential loss of 2-12% of gross energy consumed, and is a potent greenhouse gas. The objective of this study was to firstly measure methane emissions for the first time using the sulfur hexafluoride tracer technique in red deer (*Cervus elahus*) grazing ryegrass-based pasture *(Lolium perenne*) and secondly, to compare methane emissions of deer grazing chicory (*Cichorium intybus*) and plantain (*Plantago lanceolata*) with those grazing ryegrass-based pasture.

Methane production per day and per kg of dry matter intake (DMI) was measured using the sulfur hexafluoride tracer technique coupled the with η-alkane technique for feed intake estimation in 25 red deer grazing ryegrass-based pasture, chicory or plantain in March and May of 2003. Methane production per unit DMI obtained in this study (37.8 g / kg DMI) was approximately 75-80% greater than values used in the New Zealand National Greenhouse Gas Inventory for dairy cows and sheep, and estimated for deer grazing ryegrass-based pastures. Deer grazing chicory and plantain in March exhibited lower methane emissions per kg DMI compared with ryegrass-based pasture. However, in May methane emissions per kg of DMI from plantain was similar to pasture, which were both higher compared with chicory. The variability and accuracy of results obtained for estimated DMI using the alkane technique was questioned, and a lack of published information regarding methane production by red deer provided few possible explanations for the apparently high methane emissions. This prompted the initiation of an indoor study where DMI could be accurately measured concurrently with methane production using 12 animals from the grazing study.

Mean methane production per kg DMI of 12 mature hinds housed individually indoors in metabolism cages and fed fresh ryegrass-based pasture in August 2003 was 22.5 g CH_4/kg DMI. This figure was similar to published results obtained from sheep and cattle on similar diets and was 42% lower than the grazing study in autumn. This latter result emphasises the importance of

obtaining accurate individual DMI measurements with which to express methane emissions per unit feed intake.

Estimated dry matter intakes using the double n-alkane technique have not previously been validated against actual intakes for red deer, or for deer fed fresh forages. Therefore, the third experiment attempted to validate the use of this technique with rumen-fistulated, castrated red deer stags housed indoors and fed either fresh ryegrass-based pasture or plantain, while concurrently measuring methane production. Indirect estimation of DMI using the double n-alkane technique underestimated actual DMI of pasture by 23.5% and overestimated actual DMI of plantain by 13.9%. These results indicate that the estimation of DMI by the double n-alkane technique was possibly not valid for comparisons between treatments, and across experiments or animal species. The impact on methane emissions of the inaccurate estimation of DMI by the double n-technique resulted in methane production from deer fed pasture being overestimated by 11.0 g CH_4/kg DMI and an underestimation of methane production of 4.8 g CH_4/kg DMI for deer fed plantain.

Findings of this thesis suggest that the measurement of methane from grazing and/or forage-fed animals should be conducted under conditions where DMI can be measured accurately, otherwise comparisons of methane production across treatments, experiments or species may be invalid. The latter two studies indicate that methane production of forage-fed red deer is similar to published values for sheep and cattle. However, this should be confirmed by direct comparisons where all species are fed the same diet, methane measurements are conducted over the same time period using identical methods, and feed intake can be accurately determined.

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LIST OF ABBREVIATIONS

%	percentage
°C	degrees Celsius
/ (/kg)	per (per kilogram)
¼	one-quarter
⅔	two-thirds
ADF	acid detergent fibre
ANOVA	analysis of variance
ADP	adenosine diphosphate
ATP	adenosine triphosphate
BW	body weight
CH_4	methane
CO_2	carbon dioxide
CP	crude protein
CRC	controlled release capsule
CT	condensed tannin
D	digestible
d	day
DM	dry matter
DMI	dry matter intake
et al.,	and others
etc.	et cetera
Expt.	experiment
Fd	ferredoxin
FOR	fractional outflow rate
g	gram
GE	gross energy
GEI	gross energy intake
Gg	gigagram (10^9 g)
GHG	greenhouse gas (es)
H_2	hydrogen
hr (s)	hour (s)
ha	hectare

hd	head
HWSC	hot water soluble carbohydrates
kg	kilogram
kJ	kilojoules
kPa	kilopascal
l	litre
LW	liveweight
LWG	liveweight gain
m	metre
m^2	metres squared
MAF	Ministry of Agriculture and Forestry
ME	metabolizable energy
mg	milligram
min	minute
MJ	mega joule
ml	millilitre
mm	millimetre
N	nitrogen
n	number of observations
η	η-alkane
N_2O	nitrous oxide
n/a	not available
NAD	nicotinamide adenine dinucleotide
NDF	neutral detergent fibre
NDFI	neutral detergent fibre intake
NIR	near-infrared reflectance
O_2	oxygen
OM	organic matter
OMI	organic matter intake
R.	*Ruminococcus*
RFC:SC	ratio readily fermentable carbohydrate: structural charbohydrate
SF_6	sulphur hexafluoride

vs.	versus
$W^{0.75}$	metabolic liveweight

CHAPTER 1. Review of literature

1.1 Introduction

The objectives of this review of the literature were to establish the role methane (CH_4) plays in global warming, the agricultural industry sector's contribution to greenhouses gases, but with a focus on methane in New Zealand, and current research into methane emissions from ruminants in New Zealand focusing on potential methane emissions from deer.

1.2. Global warming and methane

The earth's surface retains heat through the presence of an atmospheric gaseous insulation layer. Some of the gases present in this layer are referred to as *greenhouse gases* and are responsible for absorbing solar infrared radiation in the atmosphere (Moss *et al.*, 2000). The accumulation of greenhouse gases (GHG) related to human activity, such as carbon dioxide (CO_2), nitrous oxide (N_2O), methane and CFCs within the earth's atmosphere, are proposed to contribute to an increase in the earth's average temperature (global warming). The rates of the estimated accumulation of the greenhouse gases are shown in Table 1.1. To prevent or slow this process, which is predicted to result in major ecological changes to ecosystems, a reduction in the emission of these greenhouse gases emitted from human activity is required. Global warming is predicted to increase the earth's average temperature from 0.5 °C to 2.5°C by the year 2030 (Moss *et al.*, 2000).

The actual increase in global temperatures due to the accumulation of methane is not clearly defined. However, it is estimated that CH_4 resulting from human activity has increased in atmospheric concentration by 140% since the pre-industrial era (Howden & Reyenga, 1999) (Table 1). It is also recognised that methane has a global warming potential that is twenty-one times greater than CO_2. Therefore, it is predicted that methane may contribute to approximately 20% of global warming (Howden & Reyenga, 1999). Methane's atmospheric lifetime is shorter than that of carbon dioxide (12 years versus 5-200 years) (Ravindranath & Sathaye, 2002), any positive effects of reducing methane emissions will be obtainable within a short time period.

Table 1.1. Atmospheric volume mixing ratios of main agricultural greenhouse gases during the last glacial, the pre-industrial Holocene and from 1900 to 1990. Also shown are the observed annual atmospheric growth rates in 1990, and atmospheric residence times (Crutzen, 1995).

	CO_2 ppmv[a]	CH_4 ppmv	N_2O ppbv
Last glacial (≈ 18000 yr BP)	195	0.35	244
Pre-industrial	280	0.79	260 or 288
1900	296	0.97	292
1960	316	1.27	296
1970	325	1.42	299
1980	337	1.57	303
1990	354	1.72	310
Annual increase	1.8 (0.5%)	0.015 (0.9%)	0.8 (0.25%)
Atmospheric residence Time/years	50-200	9	130

[a]ppmv = parts per million by volume = 10^{-6}; ppbv = 10^{-9}; pptv = 10^{-12}

1.2.1. Global methane emissions

Methane emissions (Figure 1.1) from human activity may account for 70% of total methane emissions, where the remaining 30% arises from natural sources such as natural wetlands and oceans and lakes (Moss *et al.*, 2000). A large proportion (approximately ¼ to ⅔) of the CH_4 from anthropogenic sources is a result of agricultural activities and, of that, 16 to 19% of CH_4 arises from farmed ruminants (Clark, 2002; Moss *et al.*, 2000). However methane emissions from wild ruminants remains largely undefined.

As illustrated in Figure 1.1, the total methane production from all sources exceeds the methane sinks in the environment by 12%. The two major methane sinks in the environment are the reaction of methane with hydroxyl radicals in

the atmosphere and the second is by methanotrophic bacteria (Moss *et al.*, 2000; O'Hara *et al.*, 2003).

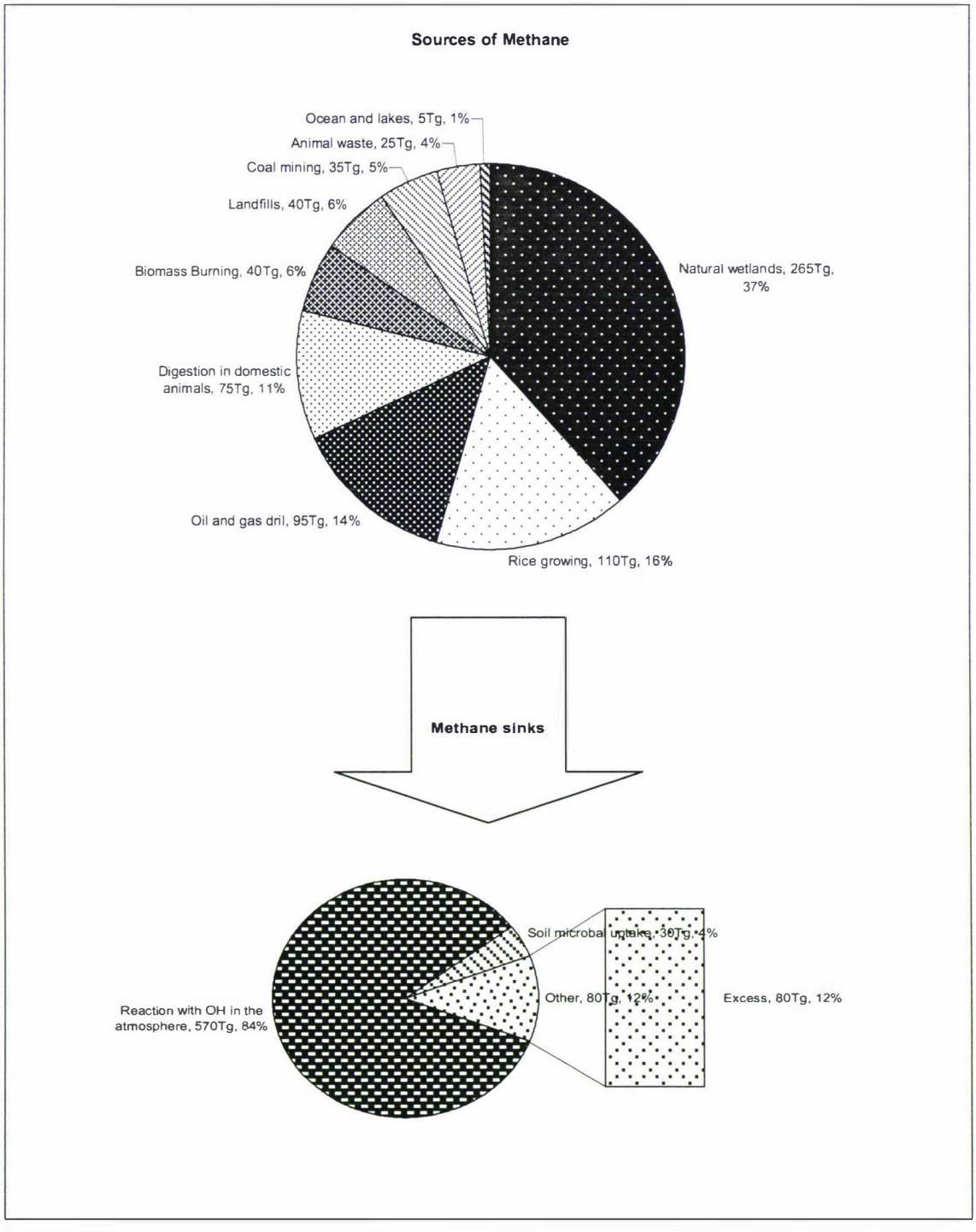

Figure 1.1. Sources and sinks of methane on the earth and in the atmosphere (Moss *et al.*, 2000).

1.2.2. Methane emissions from New Zealand and New Zealand agriculture

Methane in 2001 as CO_2 equivalent accounts for 38% of total New Zealand GHG emissions (Table 1.2). Emissions from grazing ruminants account for 88% of the total methane emissions, and approximately 98% of methane from the New Zealand agricultural sector (NIR, 2003; O'Hara *et al.*, 2003). The high percentage of GHG emissions due to livestock for New Zealand (37.5%) (NIR, 2003) and Australia (12%) renders them unique among developed countries, most of which have a significantly smaller percentage of their emissions from livestock, for example United States of America (3.3%), United Kingdom (4%), Canada (3.8%), and Japan (0.8%) (Howden & Reyenga, 1999).

Table 1.2. Summary of New Zealand's Greenhouse Gas Emissions (NIR., 2003)

	1990		2001		
Gas	CO_2 Equi[1]	% total	CO_2 Equi*	% total	% Change
CO_2[†]	25,266.88	40.9	32,430.19	44.6	28.35
CH_4	25,600.37	41.5	27,065.36	37.5	5.72
N_2O	10,281.46	16.5	12,576.17	17.4	22.32
Total[†]	61,754.10	100	72,379.13	100	17.2

*CO_2 equilvent Gg (gigagram, 10^9 g)

[†] Without land use change and forestry

The ratification of the Kyoto Protocol by New Zealand means GHG emissions in 2008 will be required not to exceed 1990 levels (Howden & Reyenga, 1999). Therefore, it is important that firstly there is a creation of accurate inventories based on animal numbers and rates of methane emissions by ruminant livestock (section 1.2.4) and secondly, that there is research into sustainable methods for reducing methane production (section 1.4) under New Zealand pastoral farming conditions.

1.2.3. Current research into enteric methane emissions

Research initiated to determine the enteric methane production from grazing sheep and dairy cattle has been conducted using the ERUCT (Emissions from Ruminants Using a Calibrated Tracer) method, where the tracer gas employed was sulphur hexafluoride (SF_6), commonly referred to as the SF_6 technique (Ulyatt *et al.*, 1997) developed by Johnson *et al.*, (1994). Enteric methane emissions from sheep and cattle when measured using the SF_6 method are comparable to methane emissions measured by open-circuit respiration calorimetry (Pinares-Patino, 2000; Boadi *et al.*, 2002).

The SF_6 method is based on three major assumptions as described below. Firstly, the SF_6 technique relies on the collection of two gases, SF_6 and CH_4. It is assumed that emission of SF_6 exactly simulates the emission of CH_4 and that the rate of the dilution of the gases is the same. Grounds for this assumption rely on the notion that the mixing of both gases is dominated by turbulent diffusion than by molecular diffusion, therefore gases of different weight experiencing the same degree of turbulence will have the same rate of diffusion and are not affected by molecular diffusion (Johnson *et al.*, 1994). The molecular weight of SF_6 is nine times that of methane (Ulyatt *et al.*, 1999), which could result in different rates of diffusion of the gases. However, as reported by Ulyatt *et al.*, (1999), there does not seem to be evidence of different molecular weight affecting the sampling of expired gases.

Secondly, it is assumed that in measuring enteric methane from ruminants, SF_6 is released from the permeation tube at a constant rate for the life of the tube. The rate of SF_6 release from the permeation tubes is calibrated by regular weighing of the tube in the laboratory for a period (ideally two months) at 39°C (Ulyatt *et al.*, 1999). It is assumed that the rate of SF_6 release is also constant once the permeation tube enters the rumen. Ulyatt *et al.*, (1999) examined the rate of release of permeation tubes maintained in the laboratory for 500 days and found the rate of SF_6 release was slightly curvilinear, with a faster rate of release at the start compared with the end of the measurements. The release rate of SF_6 from the permeation tube retrieved from animals post-experiment after 235 days has been found to be reduced by 20% compared with the pre-

experimental release rate (Ulyatt *et al.,* 1999. There was no suggestion to whether the change in release rate was due to the curvilinear relationship of SF_6 or if the rumen itself affects the release rate. However, it was noted that recovered permeation tubes were encrusted with microbial deposits and digesta had penetrated around the lock-nut of the tube (Ulyatt *et al.*, 1999).

The third assumption of the SF_6 technique is that the major route for methane excretion is via the mouth and nose. However, this is based upon one study conducted with four sheep fed 800g of lucerne chaff per day (Ulyatt *et al.*, 1999). This study showed that 87% of methane was produced in the rumen and the remaining 13% in the lower digestive tract, and that 98% of the methane produced in the gastrointestinal tract was excreted via the mouth and 2% in flatus. It was also shown that of the methane produced in the lower intestine 89% was absorbed and expired (Murray *et al.*, 1976). In the validation of the SF_6 technique it has been found that emissions measured using the SF_6 technique were within 90-95% of the values obtained from respiratory chambers. This supports the assumption that the main route of methane expiration is via the mouth and nose (Pinares-Patino, 2000; Boadi *et al.*, 2002). However, this is based on a limited number of trials, and there is very little known about species differences and influences of diet or season upon the sites of methane production and excretion.

Generally, published results have found that when grazing ryegrass-based pasture (perennial ryegrass (*Lolium perenne*)/white clover (*Trifolium repens*) 80:20), sheep produce less methane (CH_4 g / kg DMI) than do cattle and that there is some seasonal variation in methane emissions, which are expected to be related to seasonal changes in pasture chemical composition (Ulyatt *et al.*, 2002b) (Table 1.3.). Studies have found the production of methane per unit digestible dry matter intake (DDMI) is fairly similar for sheep and dairy cows and a common figure of 26 g CH_4/ kg DDMI could therefore be used for breeding ewes and dairy cows (Ulyatt *et al.*, 2002b). Currently, there are no published methane emissions for either grazing beef cattle or deer using the SF_6 method in New Zealand.

Table 1.3. Methane measurements using the SF6 technique from different classes of ruminant livestock, under grazing conditions fed ryegrass-based pasture ([1]Ulyatt et al., 2002a; [2]Ulyatt et al., unpublished; [3]Lassey et al., 1997; [4]Pinares-Pantiño, 2000; [5]Pinares-Pantiño et al., 2003a; [6]Pinares-Pantiño et al., 2003b; [7]Waghorn et al 2002; In O'Hara et al., 2003).

	Pasture	BW (kg)	Dig (%)	DMI (kg/d)	CH_4 (g/d)	CH_4/DMI (g/kg)	CH_4/DDMI (g/kg)	MY
Ewes	PR/WC:Sept[1]	54	82	1.51	30.6	20.3	24.7	6.1
	NOV[1]	54	72	1.46	33.2	22.7	31.5	6.6
	Mar[1]	62	75	1.35	27	20.1	27.0	6.1
	Jul[1]	66	82	1.89	27.9	15.1	18.5	4.6
Wethers	PR/WC:Mar[3]	37	75	1.27	18.9	15.0	19.8	4.6
	PR/WC:Apr[2]	38	80	1.39	19.3	13.8	17.4	4.2
	PR/WC:Apr[4]	41	81	1.70	21.9	12.9	15.9	3.9
	PR/WC:Jun[4]	40	80	1.41	24.1	17.3	21.7	5.2
	PR/WC:Nov[5]	46	76	1.62	29.9	19.8	26.0	6.0
	PR/WC:Oct[6]	45	82	2.07	30.4	15.2	18.6	4.5
	PR/WC:Nov[6]	48	77	1.81	34.3	19.8	25.9	6.0
	PR/WC:Jan[6]	52	79	2.26	30.4	14.0	17.9	4.2
	PR/WC:Feb[6]	53	82	2.42	31.2	13.7	16.7	4.1
Cows	PR/WC:Sept[1]	475	82	19.3	431	22.4	27.3	6.8
	PR/WC:Mar[3]	483	77	12.9	263	20.4	26.4	6.2
	NZ pasture/Sep[7]	497	82	17.2	307	18.0	22.0	5.3
	Dec[7]	519	79	17.0	376	22.2	28.3	6.6
	Mar[7]	519	78	15.0	353	23.8	30.4	7.0
*	OS pasture/Sep[7]	588	82	17.7	267	15.1	18.4	4.5
	Dec[7]	601	74	17.6	345	19.9	26.8	6.0
	Mar[7]	594	78	16.3	379	23.4	30.1	6.9

* OS represents cows of overseas genetics

BW = body weight, Dig= DM digestibility, CH4 = methane, DMI = dry matter intake, DDMI = digestible dry matter intake, MY methane yield (MJ/100 MJ gross energy intake). Dominant pasture species; PR = perennial ryegrass and WC = white clover.

1.2.4. Livestock methane emissions and inventory

Methane emissions per kilogram of dry matter intake for ruminant livestock as used in the New Zealand National Greenhouse Gas Inventory created by MAF (Ministry of Agriculture and Forestry) are based upon research results using the SF_6 method conducted in New Zealand (section 1.2. 3.). For inventory purposes adult sheep are said to produce 20.9g CH_4 / kg DMI, sheep less than 1 year 16.8 g CH_4 / kg DMI and adult dairy cattle 21.6 g CH_4 / kg DMI (NIR, 2003). However, as not all classes of stock have had methane measured under grazing conditions, it is assumed that all dairy and beef cattle are represented by the value for adult dairy cattle, and similarly all adult sheep are represented by the value for adult ewes. Adult deer are assumed to emit the average of adult sheep and dairy cattle (21.25 g CH_4 / kg DMI) (NIR, 2003).

Inventory estimates of enteric methane production from the Sheep, Dairy, Beef and the Deer Industries show that the total enteric methane has decreased by nearly 10% from 1990 to 2000 (Clark, 2002; NIR, 2003). As discussed by Clark (2002), this is assumed to reflect the current trends of livestock numbers in the four livestock industries, reflecting current industry trends.

Prior to the research using the SF_6 technique, methane emissions for inventory purposes were based upon the Baldwin mathematical model, which estimates methane production based upon feed intake, the chemical composition of the diet and results from calorimetry chamber work. It has been found that the Baldwin model has a tendency to overestimate methane production by 20-30% as compared with data gained using the SF_6 technique (Clark, 2002; NIR, 2003). Therefore, there may be significant behavioural and environmental factors which affect the production of methane that are not accounted for in the Baldwin mathematical model and are present under New Zealand farming conditions.

1.2.5. Methane emissions and deer

Enteric methane production of deer from 1990 to 2000 was predicted to double, based upon increased livestock numbers of the deer industry (section 1.5.1).

Methane production from adult deer is estimated at 22.6 kg / animal / year (de Klein *et al.*, 2002) or (21.25 g CH_4 / kg DMI) (NIR, 2003). Total methane production by deer is expected to double again by 2010, if the current expected growth as predicted by Clark, (2002) in the deer industry continues. However the current growth of the deer industry has declined (section 1.5.1). Unlike sheep and cattle, there are no published data on methane production from grazing deer using the SF_6 method.

A limited number of experiments have measured methane production from deer housed in calorimetry chambers. Semiadi *et al.*, (1998) found that methane production of deer consuming a diet with an organic matter digestibility (OMD) of 77%, averaged 16.4 g CH_4 / kg DMI and 19.7 g CH_4 / kg DMI for red deer (*Cervus elaphus*) and sambar (*Cervus unicolor*), respectively, when fed at maintenance. Methane production was similar when deer were fed twice energy maintenance, with mean values of 15.2 g CH_4 / kg DMI and 22.0 g CH_4 / kg DMI for red deer and sambar respectively. However, there was no mention of a species difference in methane production. Galbraith *et al.*, (1998) examined the methane production of bison (*Bison bison*), wapiti (*Cervus* elaphus) and white-tailed deer (*Odocoileus virginianus*). It was observed that wapiti produced 16.8 g CH_4 / kg DMI and white tail deer produced 10.8 g CH_4 / kg DMI. A significant interspecies difference of methane production was observed, where bison lost the greatest proportion of gross energy intake (GEI) to methane, while white-tailed deer lost the least. For both bison and wapiti there was found to be a seasonal effect of methane production from February/March (late winter) to May/June (early summer), where methane production as a percentage of gross energy intake was found to decrease.

Methane measured in calorimetry chamber experiments suggests that methane production from deer could be lower than the estimated value (21.25g CH_4 / kg DMI) used currently in the inventory (section 1.2.4) and lower than the methane production of sheep and cattle per kilogram of dry matter intake. The above data suggests that some species of deer may also exhibit seasonality in methane production, as found for wapiti but not white-tailed deer (Galbraith *et al.*, 1998).

1. 3. Methanogenesis

1. 3. 1. Digestion in the rumen

Ruminants themselves, like all animals, are unable to digest cellulose and hemi-cellulose due to a lack of the appropriate enzymes. However, by forming a symbiotic relationship with micro-organisms, bacteria, protozoa, and fungi, within the gastrointestinal tract, they are able to indirectly gain energy from fibrous materials. Microbial organisms reside within the foregut (rumen-reticulum) and the hindgut, that is the large intestine and caecum, where plant material is fermented.

Fermentation of substances within the rumen and hindgut is typically an oxidative process, where catabolic reactions release energy for microbial anabolic reactions. However, in the rumen this process occurs in an anaerobic environment. Therefore, the transfer of electrons and protons must be to acceptors other than oxygen (Demeyer & Van Nevel, 1974; Russell & Wallace, 1997). Irrespective of genus and species, there is a common biochemical pathway of plant breakdown and fermentation by ruminal microbes. The first stage involves the breakdown of plant polymers to monomers and oligomers primarily by exogenous microbial enzymes. The products of hydrolysis are not readily detectable in the rumen as they are quickly engulfed by microbes and converted to pyruvate in intracellular metabolism, in the second stage of the fermentation process (McDonald *et al.*, 1995).

Products of the fermentation process, particularly in the second stage of fermentation, which cannot be reutilised by the microbes, are waste products. The host animal (the ruminant) is able to absorb and gain energy from some of these waste products, for example the volatile fatty acids (VFA's: propionate, butyrate and acetate). However, some of the end products of the fermentation process are not able to be utilised by the host animal and must be removed from the rumen, in particular the gases CO_2, and methane (as described in section 1.2.3).

1.3.2. Methanogens

Ruminant enteric methane production is attributed to a group of anaerobic microbes commonly called *bacteria*, known as *methanogens*, which more closely resemble eukaryotes, than they do to true bacteria (Stewart *et al.*, 1997) and are classified as Archae or Archaebacteria (McAllister *et al.*, 1996). Approximately, 66 separate methanogenic species have been isolated from anaerobic environments; five of these species are found to be important within the rumen (McAllister *et al.*, 1996; Stewart *et al.*, 1997). Only two of these species (*Methanobrevibacter ruminantium* and *Methanosarcicina* sp) are thought to reside in the rumen at concentrations greater than 1×10^6 mL^{-1} (McAllister *et al.*, 1996). The establishment of a population of methanogens within the rumen of newborn lambs is considered to occur within 30 hours of birth, while eructation of calves begins four weeks after birth (Johnson & Johnson, 1995). This suggests that methanogens, whilst lacking the population density of other microbes, may be a fundamental component of the microbial population (McAllister *et al.*, 1996).

The growth of methanogens is substantially slower than that of fermentative rumen bacteria (Miller & Wolin, 2001). The rapid turnover rate of fibre within the rumen effectively prevents methanogens from being able to convert organic matter to CO_2 and methane, however primary and secondary fermenters within the rumen produce the end products required (CO_2, hydrogen and formate) (section 1.2.3) to produce methane (McAllister *et al.*, 1996).

The removal of protozoa from the rumen has been found to influence methane production. This may be because ciliate protozoa are associated with some species of methanogens within the rumen. It is estimated that between 9 – 25 % of methanogens are associated with protozoa, either externally or as endosymbionts (Newbold *et al.*, 1996; Takahashi, 2001). The colonisation of the ciliates in part is dependent upon the partial hydrogen concentration of the rumen, as there is a greater degree of association of methanogens with ciliates in low concentrations of hydrogen than after a feeding event. This has been suggested to occur because protozoa are a major source of hydrogen within the rumen (Takahashi, 2001).

1.3.3. Methanogenesis

Methane produced in the rumen serves as an important sink for hydrogen. The build-up of hydrogen within the rumen is reported to slow the fermentation process by changing the rumen environment (McAllister *et al.*, 1996). Therefore, although methanogens do not directly contribute to the digestion of fibre, the rate of fibre breakdown is enhanced in the presence of methanogens as they effectively reduce the build-up of reduced nucleotides (e.g. NADH) via interspecies hydrogen transfer (McAllister *et al.*, 1996).

1.3.3.1. Hydrogen transfer in the rumen

Interspecies hydrogen (H_2) transfer in the context of the rumen refers to H_2 produced in the fermentation process, which is instantaneously used in reactions by H_2-utilising microbes, predominantly methanogens under low partial pressure of H_2 (Miller, 1995; Wolin *et al.*, 1997). Hydrogen is produced by the re-oxidation of reduced co-factors in the presence of H_2-utilising microbes. As a general rule, co-factors such as NAD, are reduced when energy (ATP) is released from a substrate (Czerkawski, 1986).

The release of energy in the fermentation process by substrate phosphyorylation occurs in two key reactions. These reactions are (1) the NAD-linked dehydrogenation of glyceraldehyde-3-phosphate and (2) pyruvate lyase reactions (Demeyer & Van Nevel, 1974). A common example to show the release of energy and influence of H_2-utilizing bacteria on the fermentation process for these two reactions is the fermentation pathway used by a key celluolytic bacterium, *Ruminococcus albus* (Figure 1.2). In the dehydrogenation of glyceraldehyde-3-phosphate NAD is reduced to NADH, consequently protons and electrons are transferred to ferredoxin (fd). In reaction 2 the electrons and protons are passed directly to fd; from fd they are then transferred to flavodoxin and related compounds, resulting in the release of H_2. H_2 is released into the surrounding environment (rumen) in the presence of H_2-utilising microbes, and acetate is produced as the major product of fermentation. This is commonly seen when *R. ablus* is co-cultured with methanogens or another H_2-utilising microbes (Glass *et al.*, 1977; Wolin *et al.*, 1997).

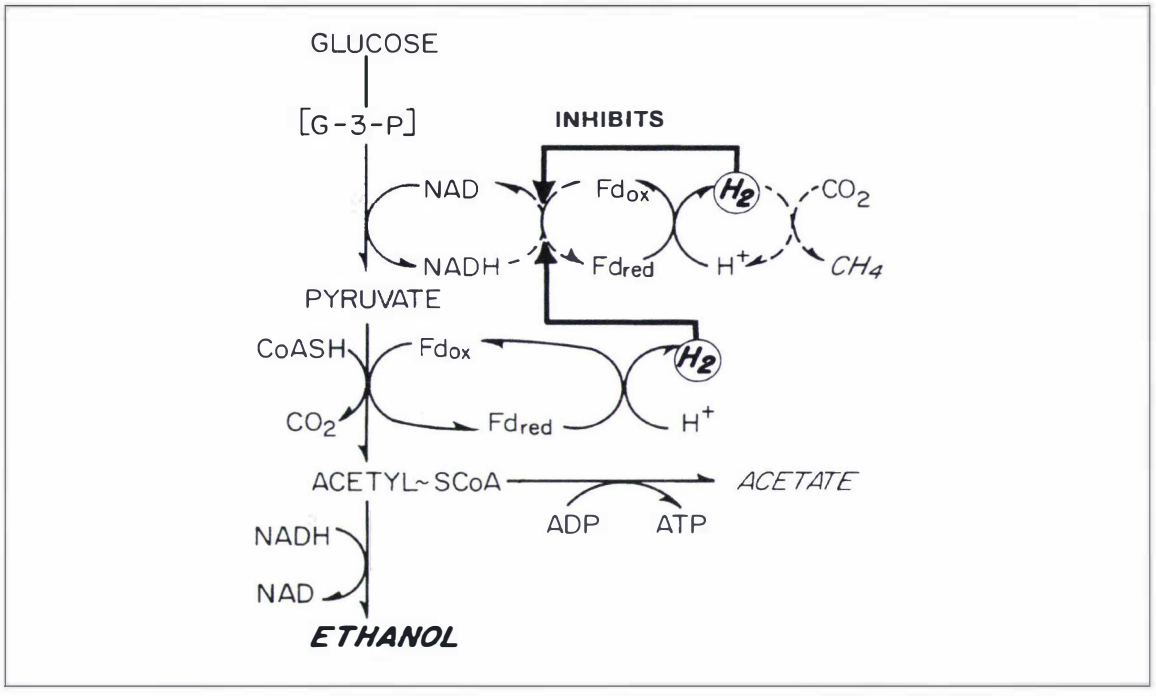

Figure 1.2. Hexose fermentation by *Ruminococcus albus* in the absence and presence of methanogens. In mono-culture, *R. albus* degrades glucose to acetate, ethanol, H_2, and CO_2. Hydrogen inhibits its formation from NADH. In co-culture with methanogens, NADH is used to reduce protons to H_2 and the final products are acetate and CH_4 (Fd = ferredoxin) (Miller, 1995)

Alternatively, in the absence of H_2-utilising microbes, as in the monoculture of *R. albus,* the H_2 release from reactions 1 and 2 results a higher partial pressure of hydrogen, which acts to inhibit the re-oxidation of NADH by transfer of electrons and a proton to fd (figure 1.2). Thus, NADH is reoxidised by dehydrogenase, resulting in acetyl-coA being reduced to ethanol or lactate (figure 1.2). Therefore, methanogens maintain a low partial pressure of hydrogen (less than 0.1 kPa) by reducing carbon dioxide to form methane, which results in the continued release of hydrogen (Miller, 1995; Wolin *et al.*, 1997). However, if the partial pressure of H_2 increases above 0.1 kPa, the re-oxidation of NADH to NAD plus H_2 via ferredoxin, becomes thermodynamically unfavourable (Bauchop & Mountfort, 1981; Miller, 1995)

Consequently, the energy gained by the fermenting microbes, is higher in the presence of H_2-utilising microbes (4 ATP) than when H_2-utilising microbes are

not present (3 ATP), and in addition energy is gained by methanogens when they reduce CO_2 to methane (Wolin e*t al.*, 1997). Therefore, the fermentation process is more efficient in terms of energy gained by the rumen microbes in the presence of methanogens and other H_2-utilizing microbes such as acetagens (Hegarty, 1999).

The bacterium *R. albus* used in this example is not the only major fermenting microbe affected by the partial pressure of hydrogen. In general, these microbes when grown in monoculture produce products such as lactate, ethanol and succinate. However, when they are co-cultured with methanogens or H_2-utilising microbes, these products are significantly reduced and the concentration of acetate is increased. Ethanol and lactate are not important intermediate substrates in the fermentation process, unlike succinate, which is an important intermediate in the formation of propionate. Therefore, H_2-utilising microbes can influence the ratio of acetate to propionate. Interestingly, microbes whose products result in the formation of butyric acid do not seem to be affected by changes in the partial pressure of hydrogen whether or not H_2-utilising are present (Miller, 1995).

1.4. Mitigation

The reduction of gross energy intake lost to methane may result in the redirecting of that energy to increased growth and production performance of ruminants. Methane mitigation strategies will play an important role in New Zealand being able to meet Kyoto protocol requirements upon compliance of the agreement in 2008, where total enteric methane emissions from ruminants may need to be reduced. Joblin, (1999) has identified three major sites of microbial intervention to reduce methane production (Figure 1.3). The first site of microbial intervention is to alter the microbes involved in digestion and to alter the formation of hydrogen within the rumen. The second site is to provide alternative sinks for hydrogen and the third is to reduce the population of methanogens in the rumen. Potential methods of methane mitigation include the supplementation of chemical feed additives or dietary manipulation.

Possible options for reducing enteric methane production have been reviewed extensively (Baker, 1999; Hegarty, 1999; Lee *et al.*, 2000; Moss *et al.*, 2000; Pinares-Patino, 2000; Ulyatt, Clark *et al.*, 2002; O'Hara *et al.*, 2003), therefore a summary of methane mitigation options discussed will be concerning methane mitigation by manipulating production systems, intake and dietary components of forages fed to animals.

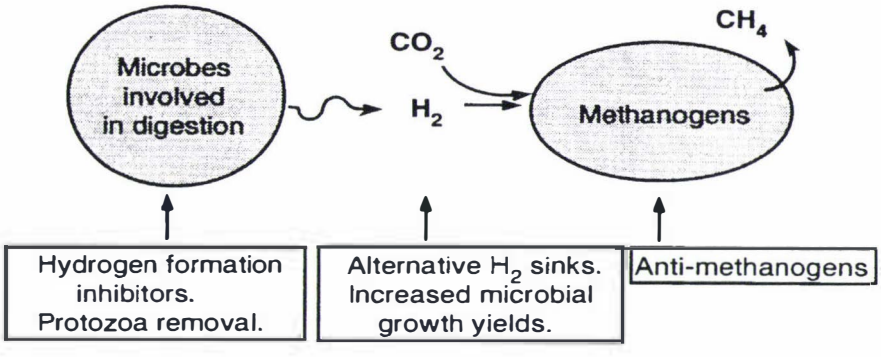

Figure 1.3. Possible sites of microbial-intervention for lowering ruminant methane (Joblin, 1999).

1.4.1. Production systems

By increasing farm production efficiency, methane emission from animals per unit of production is able to be reduced per unit of output (meat, milk, wool etc.). For example, if animal productivity is measured as live weight gain (LWG) and plotted against methane production per LWG (g/kg) (figure 1.4) a negative curvilinear relationship appears (Howden & Reyenga, 1999; Kurihara *et al.*, 1997). This curvilinear relationship results from the nonlinear relationship of feed intake and growth, which is a result of the maintenance requirement of the animal becoming proportionally smaller as intake above maintenance increases (Howden & Reyenga, 1999). An example of increasing production efficiency to reduce methane production is the New Zealand sheep industry, which has been able to decrease overall methane emissions by 14.7% from 1990 to 2001 (NIR, 2003) by decreasing animal numbers. Nevertheless, by increasing the animal production efficiency this industry has been able to maintain total production as lambing percentages from 1990 to 1999 have increased by 12% and similarly

lamb and sheep slaughter weights have increased by 12 and 11% respectively (Clark, 2002).

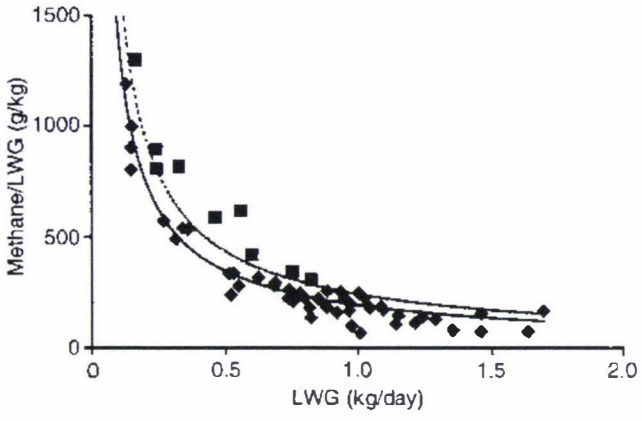

Figure 1.4. Change in efficiency of liveweight gain (LWG) in terms of methane emissions with increasing rate of liveweight gain (LWG) for *Bos indicus* eating a tropical forage diet (■), and *B. taurus* and *B. indicus* on a high grain diet (◆). Relationships are shown for grain diets (-) and tropical forage diets (---) (Howden & Reyenga, 1999).

Increases in production efficiency to reduce methane emissions must be viewed with caution, as increased production performance often coincides with increased animal intake (section 1.3.2). Increased animal production and intake will create decreases in methane per product gain or per kg of DMI, however as animal intake is increased there will be an increase in methane production per animal or per day (Howden & Reyenga, 1999). Therefore, methods of increasing production efficiency that will not increase DMI intake may need to be investigated as discussed by Howden & Reyenga, (1999).

1.4.2. Feed intake

Dry matter intake of ruminants has been found to be related to methane production, where as intake increases methane production is found to decrease per kg of DMI (Figure 1.5). However, methane production per day has an overall increase as intake increases (Blaxter & Clapperton, 1965; Johnson &

Johnson, 1995; Kurihara *et al.*, 1997) The major route in which intake is thought to affect methane production is via passage of feed particles out of the rumen (Moss *et al.,* 2000). Therefore, under circumstances of increased rumen particle outflow, methane production per kg of DMI should be reduced. In agreement with this (Pinares-Patino *et al.*, 2003) found that methane production in sheep housed indoors was positively correlated with the rumen pool size of organic matter (OM), OM intake and rumen fill, but negatively correlated to the fractional outflow of particles from the rumen.

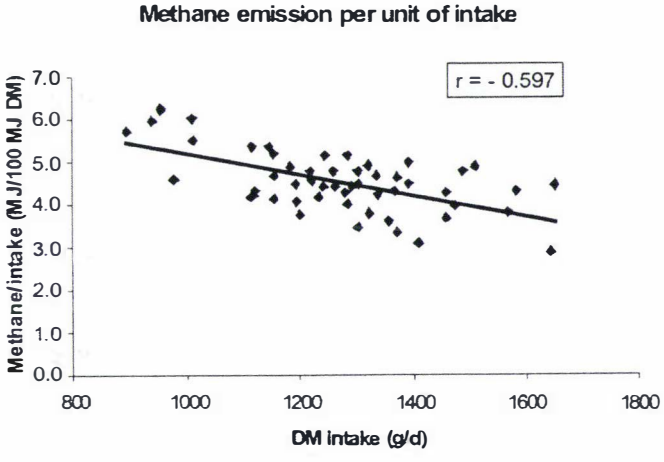

Figure 1.5. Methane emissions per unit of feed intake plotted against DM intake in sheep grazing the same pasture (Lassey *et al*, 1997; In O'Hara *et al.*, 2003).

The relationship of decreasing methane production per kg of DMI remains true except when the diet is of low apparent digestibility as shown in Figure 1.6 (Blaxter & Clapperton, 1965). This therefore suggests that methane production may also be influenced by apparent digestibly, the nutrient components of the diet, and the digesta passage rate. Red deer fed chicory *(Cichorium intybus)* have been found to exhibit a faster particle outflow rate (Kusmartono *et al.*, 1997) possibly due to a greater rate of particle breakdown (Kusmartono *et al.*, 1996) compared with ryegrass-based pasture (*Lolium perenne*), therefore the feeding of chicory may be useful as a mitigation tool in deer and other

ruminants. Waghorn et al., (2002) found that methane emissions of sheep fed chicory was reduced by up to 41% per kg of DMI as compared with pasture.

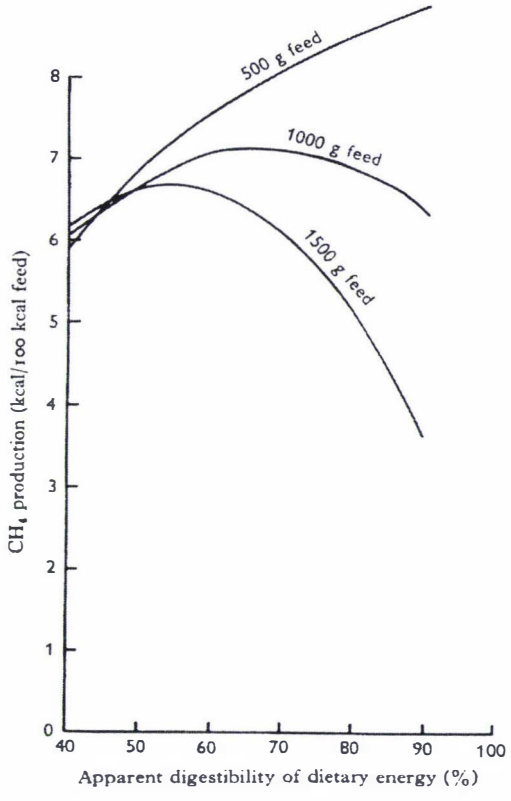

Figure 1.6. Estimated CH_4 production by sheep and cattle receiving constant amounts of feed, at three different levels of feeding and apparent digestibility (Blaxter & Clapperton, 1965).

1.4.3 Apparent digestibility

The apparent digestibly (either as organic matter or as energy) of feeds fed to ruminants has been found to affect the methane production of animals per unit of feed intake. As the digestibility of a given feed increases, the proportion that may be fermented by the microbial populations also increases, therefore resulting in methane production increases (Moss *et al.*, 2000). Blaxter & Clapperton (1965) found that methane production of sheep and cattle increases with increasing apparent digestibility (energy) when animals are fed roughages, pelleted or milled feeds and mixed diets. However, it was also found that when intake increased at each level of apparent digestibility the methane produced (kcal/100 kcal feed) decreased for diets of medium and high digestibility. The methane production of animals consuming diets of low digestibility was not

affected by the level of intake (Figure 1.6). Pinares-Patino *et al.*, (2001) found that when ten rumen-cannulated sheep were fed lucerne chaff the apparent digestibility of cellulose and the apparent mean retention time of the diet was positively related to methane emissions expressed as a percentage of gross energy.

1.4.4. Dietary manipulation

The manipulation of the dietary components of feeds fed to alter methane emissions may be an effective method for methane mitigation. However, the manipulation of single nutrient components without dramatically changing other components of the diet is difficult in mixed ration diets and is almost impossible to achieve for animals grazing forages.

Johnson and Johnson, (1995) suggested that there were two broad diet-related mechanisms by which methane emissions from ruminants could be manipulated. Firstly, the amount of dietary fibre that was available for fermentation in the rumen could be manipulated to effect carbohydrate fermentation and the rate of passage through the rumen, which would also include absolute feeding levels (section 1.4.2). The second mechanism of manipulation may be by the regulation of hydrogen and methane production via the manipulation of the ratio of volatile fatty acids produced as a result of fermentation. Much of the research investigating the affects of forages and chemical composition upon methane emission rates has been based upon conserved forages or total mixed ration diets.

The proportion of each type of carbohydrate (cellulose, hemicellulose and soluble carbohydrates) present in forages fed to ruminants has been found to influence methane emissions (Moe & Tyrrell, 1979; Holter & Young, 1992). The proportion of digested cellulose is predicted to result in methane emissions that are three and five times greater than the digestion of hemicellulose and soluble carbohydrates respectively. Methane production tends to be related to the carbohydrate type fermented and the time spent in the rumen, as soluble carbohydrates tend to pass through the rumen and are digested in the small

intestine (Moe & Tyrrell, 1979). However, the proportion of carbohydrates in forages and total mixed rations influenced methane production only at intakes greater than 1.5 maintenance energy (Moe & Tyrrell, 1979).

By changing the sources and proportion of carbohydrates, that is the ratio of structural and soluble carbohydrates, by the feeding of forages with a reduced proportion of structural fibre and a greater content of readily fermentable carbohydrates or cereal grains, a lower rumen pH may result, leading to an increased proportion of propionate being produced. This may result in unfavourable conditions for methanogens and hence methane production (Johnson & Johnson, 1995; Lee *et al.*, 2000). The manipulation of acetate to propionate to decrease methane emissions should be viewed with caution. For example, Hoskin *et al.* (1995) found that deer fed chicory compared with those fed ryegrass-based pasture had a greater ratio of acetate: propionate. However, Waghorn *et al.* (2002) found that the methane emissions per kg DMI of sheep housed indoors was reduced by up to 40% when fed chicory compared with those animals fed pasture, the ratio of acetate:propionate was not investigated in this study.

The increasing concentration and digestibility of the chemical components ADF (acid detergent fibre), and NDF (Neutral detergent fibre) have been found to be positively related to methane emissions when expressed as proportion of gross energy,. In contrast increasing crude protein and dietary fat digestibility in the diet is negatively related to methane emissions as a percentage of gross energy (Moe & Tyrrell, 1979; Holter & Young, 1992). Similary some secondary plant compounds, such as condensed tannins, have also been shown to be negatively related to methane production. Waghorn *et al.,* (2002) showed that forages containing condensed tannins such as sulla (*Hedysarum coronarium*) and lotus (*Lotus pedunculatus*) fed to sheep reduced methane production per kg of DMI by up to 50% compared with sheep fed ryegrass-based pasture, of which 16% of the reduced methane emissions was due to condensed tannins. Other secondary compounds found in alternative forages such as sesquiterpene lactones have not been evaluated in their potential role for methane mitigation.

Table 1.4. Methane emissions from sheep and dairy cows fed a range of diets indoors, determined with the SF6 tracer technique (Pinares-Pantino, 2000; [2]Waghorn et al., 2002a; [3]Waghorn et al., 2002b; [4]Woodward et al., 2002; In O'Hara et al., 2003).

	Diet	BW (kg)	Dig (%)	DMI (kg/d)	CH_4 (g/d)	CH_4/DMI (g/kg)	CH_4/DDMI (g/kg)	MY
Wethers	Pasture[1]	57	73	1.26	25.0	19.8	27.3	6.0
	Lucerne hay[1]	59	59	1.08	18.7	17.3	29.2	5.2
	Lucerne hay[1]	43	64	1.18	18.8	15.9	24.8	4.8
	Pasture[2]	33	74	1.12	28.7	25.7	34.7	7.8
	Lucerne[2]	38	71	1.47	30.2	20.6	29.0	6.2
	Sulla[2]	38	73	1.50	26.3	17.5	24.1	5.4
	Sulla/lucerne[2]	38	71	1.67	31.8	19.0	26.7	5.8
	Chicory[2]	35	79	1.12	18.1	16.2	20.4	4.9
	Sulla[2]	36	63	1.17	20.5	17.5	27.7	5.3
	Chicory/Sulla[2]	36	71	1.37	23.1	16.9	23.8	5.1
	Red Clover[2]	44	76	1.76	31.2	17.7	23.4	5.4
	Chicory/ red clover[2]	36	77	1.36	26.8	19.7	25.6	6.0
	Lotus[2]	40	70	0.94	10.8	11.5	16.4	3.5
	Lotus (+PEG)[2]	40	76	0.94	12.9	13.8	17.3	4.2
Ewe Lambs	Lucerne hay[1]	35	64	0.90	11.5	14.8	23.1	4.4
Dairy cows	NZ/TMR: Sept[3]	544	72	21.4	422	20.3	27.3	5.6
	Dec[3]	583	81	19.7	435	22.0	27.3	6.1
	Mar[3]	626	80	19.0	423	22.3	27.9	6.4
	*OS/TMR: Sept[3]	610	75	27.9	446	16.0	21.5	4.4
	Dec[3]	646	80	23.0	448	19.5	24.3	5.4
	Mar[3]	693	78	21.4	452	21.4	27.1	6.2
	Ryegrass[4]		66	10.7	260	24.6	37.3	7.2
	Sulla[4]		83	13.1	253	19.5	23.5	6.1

BW = body weight, Dig= DM digestibility, CH4 = methane, DMI = dry matter intake, DDMI = digestible dry matter intake, MY methane yield (MJ/100 MJ gross energy intake), *OS = cows of overseas genetics, TMR = total mixed ration, PEG = .polyethylene glycol MW 3350.

1.5.1 The New Zealand deer industry

Currently New Zealand is the world's largest supplier of farmed venison, and exports approximately 90% of its total production. The majority of venison is exported to Europe, particularly Germany which receives over 50% of total venison exports. The other main product from the deer industry is velvet antler, which is exported to Asia, namely South Korea (MAF, 2003).

The population of farmed deer in New Zealand was predicted to be approximately between 1.7 million and 2 million animals as at June 2003 (Figure 1.7) with approximately three-quarters of this estimate comprising of hinds. However, the present (January 2004) estimates for growth of the deer industry due to current economic events are that annual growth has slowed substantially to approximately 5% (M. J. Loza personal communication, 2004) from previous estimates ranging from 9-14% (Barry *et al.*, 2002). To maintain and improve production performance in the deer industry, gains must be made through improved genetics, nutrition and health management in a sustainable manner without reliance on feed additives and drugs (Parker & Loza, 2003).

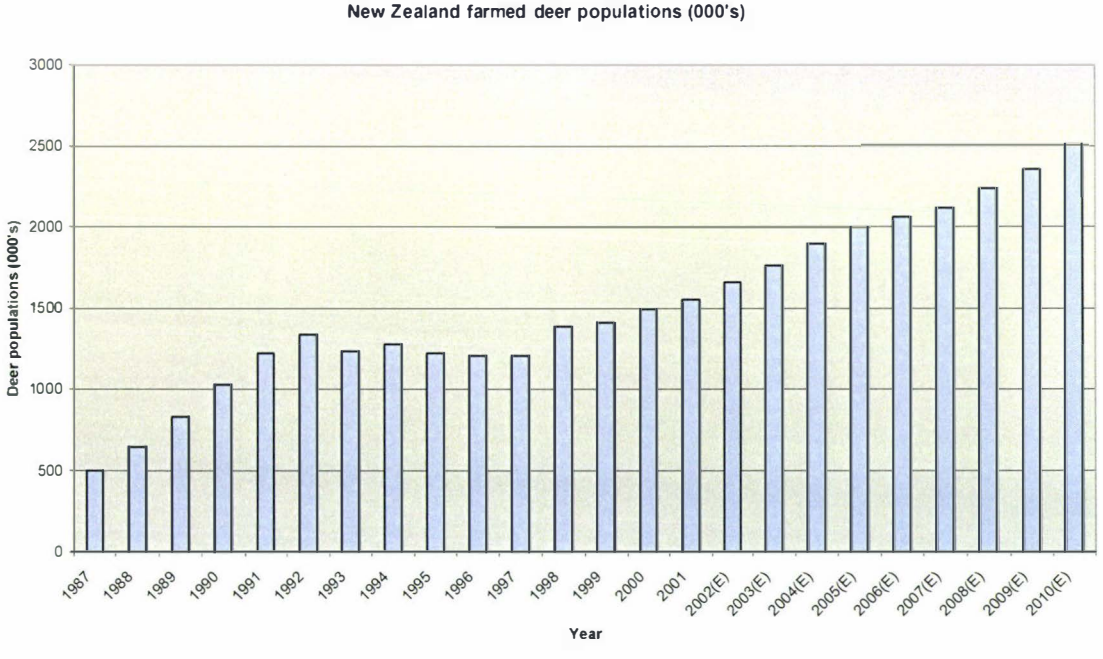

Figure 1.7. Population growth from 1987 and estimated growth to 2010 (M. J. Loza, January 2004, unpublished).

1.5.2. Deer, energy requirements and forages

Deer in New Zealand are predominantly grazed on pastures (commonly perennial ryegrass-based pasture) that have traditionally been fed to sheep and cattle. However, the suitability of ryegrass-based pasture for optimal performance of farmed deer is questionable for the reasons outlined below.

Those deer that are commonly farmed in New Zealand (red deer and wapiti) have evolved as intermediate feeders (Hofmann, 1985) (Figure 1.8). Concentrate and intermediate feeders that evolved earlier than the roughage feeders are assumed to be less efficient at utilising energy from fibrous feeds, as the rumen tends to be less developed and less able to selectively delay food passage. In contrast, the more developed rumen of roughage feeders, concentrate and intermediate feeders are thought to be better suited to a diet of plants lower in fibre and higher in soluble carbohydrate, protein, fat and oil, (such as legumes, and herbs) than the grasses normally consumed by roughage feeders.

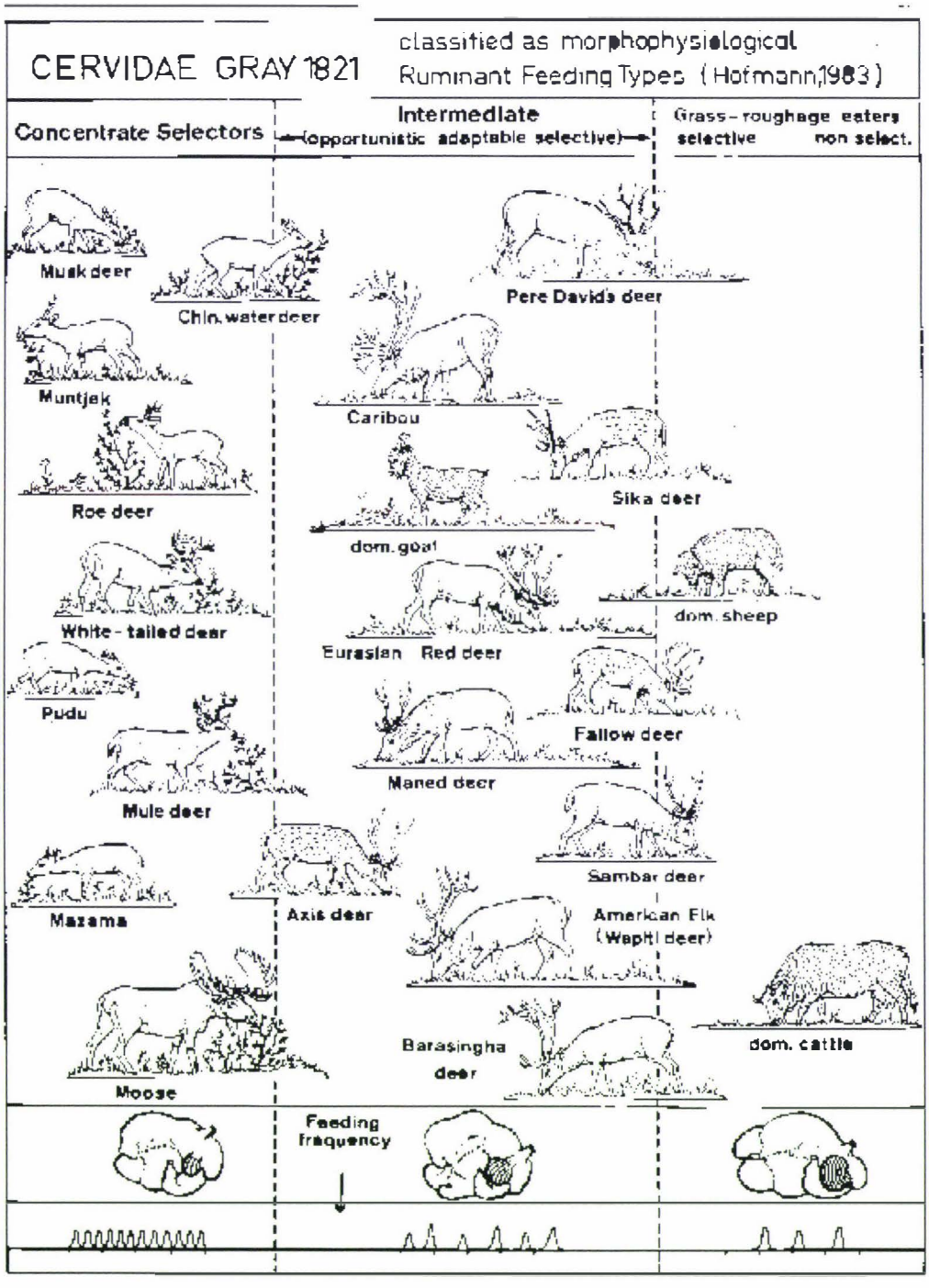

Figure 1.8. Classification of the Cervidae on the basis of morphophysiological feeding types. The position of a species is based on typical structures of the digestive tract and/or on feeding behaviour/forage selection. The baseline indicates flexibility in adaptation; from left to right the ability to utilise fibrous roughage increases (Hofmann, 1985).

The reproductive cycle, as dictated by seasonal control mechanisms of temperate deer species, has a marked effect on the annual cycle of feed demand. This distinct cycle of feed demand of deer results in a peak in demand in summer, of 47 MJME/kg DM for an average lactating red deer hind, compared with 22 MJME/g DM for winter maintenance of the same pregnant hind (Moloney, 2003). Weaner hinds and stags also show a similar pattern in feed intake as reflected in their seasonal pattern of growth which ranges from 150g per day live weight gain (LWG) in winter compared with 250- 350 g/day LWG in the following spring (Moloney, 2003; Stevens & Corson, 2003). As illustrated in Figure 1.9 the peak in feed demand of both lactating hinds and growing animals lags behind the peak of pasture growth in the spring by 2-3 months.

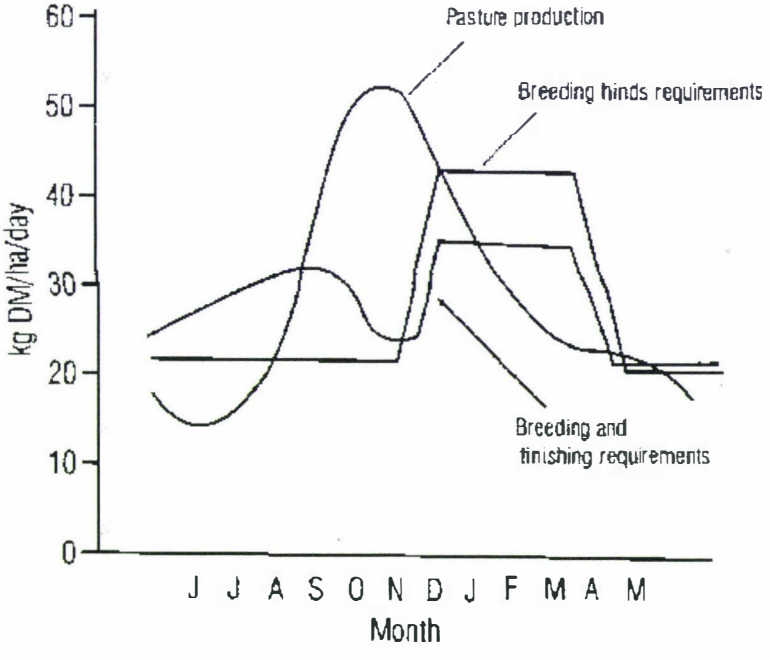

Figure 1.9. Seasonal variation in pasture production and animal requirements in deer production systems in perennial ryegrass/white clovers pastures (Moloney, 2003).

An indication of the severity of the production inefficiency of New Zealand deer farms has been illustrated by a study by Audigé (1995) where it was found that only 15% (ranging from 0 to 40%) of weaner stags reached target weights of 92

kg body weight or 50 kg carcass weight by their first year. Average growth rates were found to be 118, 96 and 206 grams per day for autumn, winter and spring respectively. In an attempt to meet feed demands of deer in late spring and summer alternative forages have been evaluated. Specialist forages include the summer and winter brassica crops, legumes such as red clover, lotus species or lucerne, and herbs such as chicory (Moloney, 2003).

The feeding value[1] of a limited number of specialist forages has been evaluated in deer compared with ryegrass-pastures, which have been reviewed by Barry *et al.*, (2002) and Hoskin *et al.*, (2003). The feeding value of chicory, red clover (*Triolium pratense*), sulla (*Hedysarum coronarium*) and birdsfoot trefoil (*Lotus corniculatus*) was greater than ryegrass-based pasture, with the indicator of feeding value in these studies being liveweight gain. Liveweight gain of deer fed alternative forages, compared with ryegrass-based pasture, have been up 55%, 23%, and 30% for autumn, spring and summer, respectively (Hoskin *et al.*, 2003). The higher feeding value or growth rates of deer fed these forages in autumn compared with growth rates in spring represents a decrease in the feeding value of ryegrass-based pasture in autumn rather than an increase of feeding value of the specialist forages from spring to autumn (Barry et al., 2002). The increased growth rates from deer fed specialist forages can result in 90-100% of stags meeting target liveweights for venison production by 1 year of age, resulting in an increase of farm productivity (Barry *et al.*, 2002).

Many of the specialist forages that have been evaluated have been found to contain secondary plant compounds, such as condensed tannins, endophyte alkaloids, coumestans, isoflavones, sesquiterpene lactones, iridoid glycosides and phenolic glycosides (Barry *et al.*, 2002; Hoskin *et al.*, 2003). The presence of some of these secondary plant compounds such as condensed tannins and sesquiterpene lactones have indicated some nutritional benefit for ruminants

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[1] Feeding value is 'defined as the animal production obtained from grazing a forage under unrestricted conditions, and its components are voluntary feed intake, the digestive process (including percentage digestion and retention time in each section of the digestive system), and the efficiency of utilization of digested nutrients' (Barry *et al.*, 2002).

and particularly deer. However the impact of secondary plant compounds in isolation on feeding values is not known (Hoskin *et al.*, 2003). One factor that may contribute to the greater feeding value of specialised forages could be that secondary plant compounds have been shown to have anthelmintic properties against parasites of deer (Hoskin, 1998) and other ruminants (Hoskin *et al.*, 2003). Therefore, it is likely that the reduced parasite burden of deer fed forages containing secondary plant compounds, such as chicory in the farmed situation compared with those fed ryegrass-based pasture will exhibit greater animal production (liveweight gains) on the same DMI allocation, as less protein and energy is required for maintenance (i.e. maintaining bodyweight) and there is likely to be a reduced nutrient demand for combating parasite infection.

1.6. Conclusion and Requirements for further research

1.6.1. Methane accounts for 38% of New Zealand's greenhouse gas emissions, of which 88% arises from ruminant livestock in the microbial fermentation of ingested feed.

1.6.2. Methane emissions of grazing adult sheep, sheep less than 1 year and dairy cows have been measured using the SF_6 technique. However, no measurements have yet been conduced using all classes of sheep and dairy cattle, beef cattle, and deer. To provide an accurate set of methane emissions measurements from ruminant livestock, methane emissions need to be measured from all species and classes of livestock.

1.6.3. Due to the increase of the farmed deer population estimated in 2002 of approximately 10% per annum, methane emissions of red deer were predicted to double from 2000 to 2008, therefore it was thought that deer would contribute to a large proportion of the methane emissions from ruminant livestock above 1990 levels. However, the current growth of the deer industry has sharply declined to approximately 5% per annum.

1.6.4. Grazing red deer are suggested to produce methane per kg of DMI that is similar to the production by grazing sheep and dairy cattle. This suggestion is based upon a limited number of studies that have measured methane production of deer in calorimetry chambers.

1.6.5. Temperate deer species, which are farmed in New Zealand, have been established to exhibit strong seasonal cycles of voluntary food intake, energy requirements, reproductive annual cycles and digestive physiological changes; it is not known if these seasonal changes will affect methane emission levels throughout the year. Methane emissions from wapiti, as measured in a calorimetry chambers, showed that methane as a percentage of GE decreased from early summer to autumn, therefore it is suggested that the methane emissions of grazing temperate species of deer will change with time (season).

1.6.6. The dietary components of ruminant feeds have been shown to affect methane emissions, however as much of this research has been conducted using conserved or total mixed rations, there is a need for similar research to be undertaken with fresh forages.

1.6.7. The feeding of alternative forages to reduce methane emissions can provide a methane mitigation option that is free of feed additives and drugs. Sheep and dairy cows have shown reduced methane emissions per kg of DMI when fed chicory, sulla, and lotus species. Further research needs to be conducted to test other forages for methane mitigation potential and to discover if other ruminant species also reduce methane emissions in response to being fed these forages.

1.6.8. Ryegrass-based pasture has been found not to be suited for maximum animal production performance of deer though out the entire year,

especially in summer, therefore to optimise the genetic potential for animal production specialist forages such as chicory, red clover and forages crops are being utilised. Some of these specialist forages have been found to be of greater feeding value compared with ryegrass-based pasture, however feeding values, rumen kinetics and apparent digestibility research has been conducted in a limited number of forages and in a restricted number of trials. There needs to be more research investigating the potential of forages already employed to feed deer, and the potential of new forages species, with the aim of increasing animal productivity.

CHAPTER 2. Methane production from farmed red deer grazing perennial ryegrass pasture, chicory, or plantain.

2.1. Introduction

In signing the Kyoto agreement New Zealand has made its commitment to the reduction of greenhouse gas emissions. In the period 2008 -2010, following ratification of this agreement, New Zealand will be financially responsible for the emission of greenhouse gases above the baseline year of 1990. Methane accounts for 38% of the total greenhouse gases produced by NZ, where enteric methane emissions from grazing ruminants account for 88% of the total methane emissions, and 99% of methane from the agricultural sector (NIR, 2003). Because of animal numbers, the NZ sheep industry is the largest single contributor. However, in the last decade estimated methane production from the deer industry has increased by 50% and is expected to approximately double again by 2008 (Clark, 2002).

In the official New Zealand national inventory, the enteric methane production of deer is estimated to be 21.25 g CH_4 per kg dry matter intake (DMI), which is an average of the values used for adult sheep (20.9 g CH_4/kg DMI) and adult dairy cows (21.6 g CH_4/kg DMI) (NIR, 2003). A limited number of published studies have measured methane production from deer using calorimetry chambers and have indicated that methane production from red deer might be expected to be lower than that from both adult sheep and dairy cows (Galbraith *et al.*, 1998; Semiadi *et al.*, 1998).

Semiadi *et al.*, (1998) found that red deer and sambar deer fed at maintenance produced an average of 16.4 g CH_4 / kg DMI and 19.7 g CH_4 / kg DMI respectively, when consuming a diet of 77% organic matter digestibility (OMD). When fed twice maintenance they were found to produce 15.2 g CH_4 / kg DMI and 22.0 g CH_4 / kg DMI for red deer and sambar respectively, there was no mention of a species difference of methane production. Galbraith *et al.*, (1998) examined the methane production of bison, wapiti and white-tailed deer. It was observed that wapiti produced 16.8 g CH_4 / kg DMI and white-tailed deer

produced 10.8 g CH_4 / kg DMI. A significant interspecies difference of methane production was observed, where bison lost the greatest proportion of gross energy (GE) to methane and white-tailed deer the least. Both bison and wapiti were found to exhibit seasonal differences whereby methane production per kg DMI decreases from February/March to May/June with the greatest change in methane production exhibited by bison, then wapiti deer. The change in methane production was 3.9% GE and 2.4% GE, respectively.

Farmed deer in New Zealand are grazed largely upon perennial ryegrass/clover pastures developed for grazing by sheep and cattle. However, due to strong seasonality of feed intake and growth, later calving compared with sheep and cattle and the high dietary preference for legumes and herbs of the temperate deer species farmed in New Zealand (Hoskin *et al.*, 2003) deer are less suited to perennial ryegrass-based pastures than are sheep and cattle. Beneficial effects on deer health and production from grazing alternative forages such as chicory, which produce large quantities of high nutritive value herbage in summer and autumn, have been well documented (Barry, 1998; Barry et al., 2002; Moloney, 2003), with some effects being attributed to the presence of secondary compounds (Hoskin *et al.*, 2003).

Forages such as sulla, birdsfoot trefoil and lotus major containing secondary compounds such as condensed tannins have been found to reduce methane emissions from sheep and cattle by up to 50 and 28%, respectively (Woodward et al., 2001; Woodward et al., 2002). Condensed tannins (CT) have been found to alter the breakdown and fermentation of protein and fibre in the digestive tract of ruminants through changes in the microbial population and growth (Woodward *et al.*, 2001; Barry *et al.*, 2002). Waghorn *et al.*, (2002) found that CT significantly reduced methanogenesis by 16% and methane production per kg DDMI (digestible dry matter intake) was reduced in sheep by up to 50% (16.4 g CH_4/DDMI) when fed lotus major containing CT of 5.3 g /kg, compared with those fed ryegrass-based pasture. Sheep fed chicory were found to have reduced methane production of up to 41% (20.4 g CH_4/DDMI) compared with sheep fed ryegrass-based pasture. Chicory in this study was not reported to

contain CT, however other studies have reported a low presence of CT in chicory of 1.7g/ kg DM (Barry, 1998) and 2.80 g /kg (Hoskin e*t al.*, 1995).

The primary objective of this experiment was to measure enteric methane output from red deer grazing ryegrass-based pasture using the SF_6 technique in order to provide deer-specific data for the New Zealand national inventory of ruminant methane emissions. A secondary objective was to initiate an investigation into the effect of the alternative forage herbs, chicory and plantain, on the methane production of grazing red deer.

2.2. Materials and methods

2.2.1. Experimental design

An experiment to measure methane production from farmed red deer grazing different forage species was conducted at Massey University Deer Research Unit, Palmerston North, New Zealand. Twenty red deer hinds and five castrated stags were randomly allocated to one of three treatment forages, perennial ryegrass-based pasture (*n* = 12 hinds and *n* = 1 castrated stag), chicory or plantain (both *n* = 4 hinds and 2 castrated stags) in a repeated measures experimental design conducted over two periods; late summer (3-7th March 2003; Period 1) and late autumn, (27th May – 1st June 2003; Period 2).

2.2.2. Animals

Twenty red deer hinds of mixed age (4.76 ± 2.45 age in years; 115.0 ± 13.10 liveweight in kg) and five hand-reared castrated stags (8 ± 6.60 age in years; 143.7 ± 37.42 liveweight in kg), four with rumen and abomasal cannulae and one that were non-surgically modified, were used. All the hinds and four stags were used for methane measurements, the remaining rumen fistulated stag on pasture was used to measure alkane capsule release rate only.

Initially twenty-five hinds were selected from the commercial herd, based upon previous good behaviour during routine handling. These animals, plus four of the castrated stags, were accustomed to the methane measuring equipment

(halters, harnesses and yokes) and the handling procedures daily for two weeks. Any animals that became stressed or did not accept the equipment or handling procedures were omitted from future work (*n*=3), leaving a total of 20 hinds for use in the experiment and two randomly selected hinds for use as pre-trained spares.

Hinds and castrated stags were randomly allocated to the three treatment groups based on age and weight at the start of Period 1 according to Table 1, with 13 deer grazing pasture (12 hinds, 1 stag) and 6 deer (4 hinds, 2 stags) grazing both chicory and plantain.

Table 2.1. Number of animals in each treatment group, mean and standard deviation expressed for age and weight.

Treatments	Hinds	Castrated stags	Age (years)	Liveweight March (kg)	Liveweight May (kg)
Pasture	12	1*	4.64 ± 2.5	121.2 ± 25.5	110.2 ± 24.8
Chicory	4	2	5.67 ± 4.2	125.5 ± 23.9	117.0 ± 19.6
Plantain	4	2	6.83 ± 5.4	113.8 ± 11.0	106.2 ± 17.5

* Not used for methane measurements.

Prior to the start of each period animals were grazed on permanent perennial ryegrass-based pasture. Animals were allocated to their treatment groups ten days before the start of methane collection to allow for diet adaptation. Animals were given ad libitum access to water. From Period 1 to Period 2 three animals (1 castrated stag and 2 hinds) were dropped out and replaced with trained spares due to either health problems, behavioural problems or poor sampling of SF_6. Animals were weighed on the day of treatment allocation and again at the start and end of the methane measurement period.

2.2.3. Forages and Grazing Management

The forages grazed were: 1.20ha (3 paddocks) established perennial ryegrass (*Lolium pernne* cv. Nui)/white clover (*Trifolium repens* cv. Huia) pasture, sown December 1992 or earlier; 1.21ha (3 paddocks) chicory (*Cichorium inybus* cv. Grasslands Puna), sown November 2001 and 1.24ha (2 paddocks) narrow-leaved plantain (*Plantago lanceolata* cv. Ceres Tonic), sown November 2002. Urea was applied to pasture, plantain and chicory paddocks from March to May with a total application of 25 kg N (nitrogen) per hectare. All three plantain paddocks were mechanically topped once between March and May to control reproductive growth, however chicory and pasture paddocks were not topped.

Deer were rotationally grazed throughout the dietary adjustment and methane measurement periods. Herbage allowances were set at 10 kg "edible" dry matter (DM) /deer/day to allow for unrestricted feed intake. Within each period the deer did not graze each paddock more than once. Following Period 1, non-experimental stock were used to remove excess herbage to a constant residual.

2.2.4. Forage Sampling and Measurements

Pre- and post-grazing herbage mass was measured by taking cuts to soil level from six quadrats (1.5m^2) from each paddock for DM determination (60°C, 48 hrs); enabling calculation of grazing days (Semiadi *et al.*, 1993) according to the allowance set. Samples for botanical composition from cuts to soil level beside each quadrat were dissected into grasses, clover (red and white together), dead matter and weed (pasture), and for plantain and chicory, stem and leaf (separately), clover, dead matter and weed. Each component was separately oven-dried and weighed. The herbage allowance was calculated by subtracting the proportion of "unedible" dead, weed (all forages) and stem (chicory and plantain only) from the total herbage mass to give the "edible" herbage mass.

Each day during the methane measurement period, feed offered (randomly cutting 20 x 20 cm squares down to soil level until 500g wet weight forage was collected) and hand-plucked samples estimating deer diet selected were taken daily (Semiadi *et al.*, 1993; Kusmartono *et al.*, 1996) washed, and stored at minus 20°C for chemical analysis. At the end of each experimental period the

feed offered and diet selected samples were pooled, and oven dried for 48 hrs at 60°C.

2.2.5. Methane measurement

Enteric methane production from 24 animals (20 hinds and 4 castrated stags) was determined using the SF_6 tracer method, as developed by Johnson *et al.*, 1994) and modified according to Ulyatt *et al.* (1999; 2002b). Hinds were fitted with modified sheep halters and body harnesses to which gas collection yokes were fitted at the base of the deer's neck, attached to the body harness with velcro™, as shown in plates 2.1, 2.2 and 2.3. Animals were accustomed to the wearing of the halters, harness, yokes and the handling procedures in the removal of harnesses and yokes over a training period of two weeks. The yokes were made from high-pressure PVC and had a total volume of 2.5 L. Each deer had a brass SF_6 permeation tube inserted orally into the rumen approximately seven days before the start of methane collection in March; the same permeation tube was used in May. Deer that were employed in May that were not in the March trial had permeation tubes inserted seven days before the start of methane collection. The permeation tubes contained the sulphur hexafluoride (SF_6) tracer gas, which was emmitted at an assumed constant rate, as determined by the incubation at 37°C and regular weighing of each tube prior to its insertion into the rumen (Ulyatt *et al.*, 1999).

Plate 2.1. Deer wearing methane collection equipment, grazing ryegrass-based pasture in March 2003.

Plate 2.2. Deer wearing methane collection equipment while grazing plantain in May 2003.

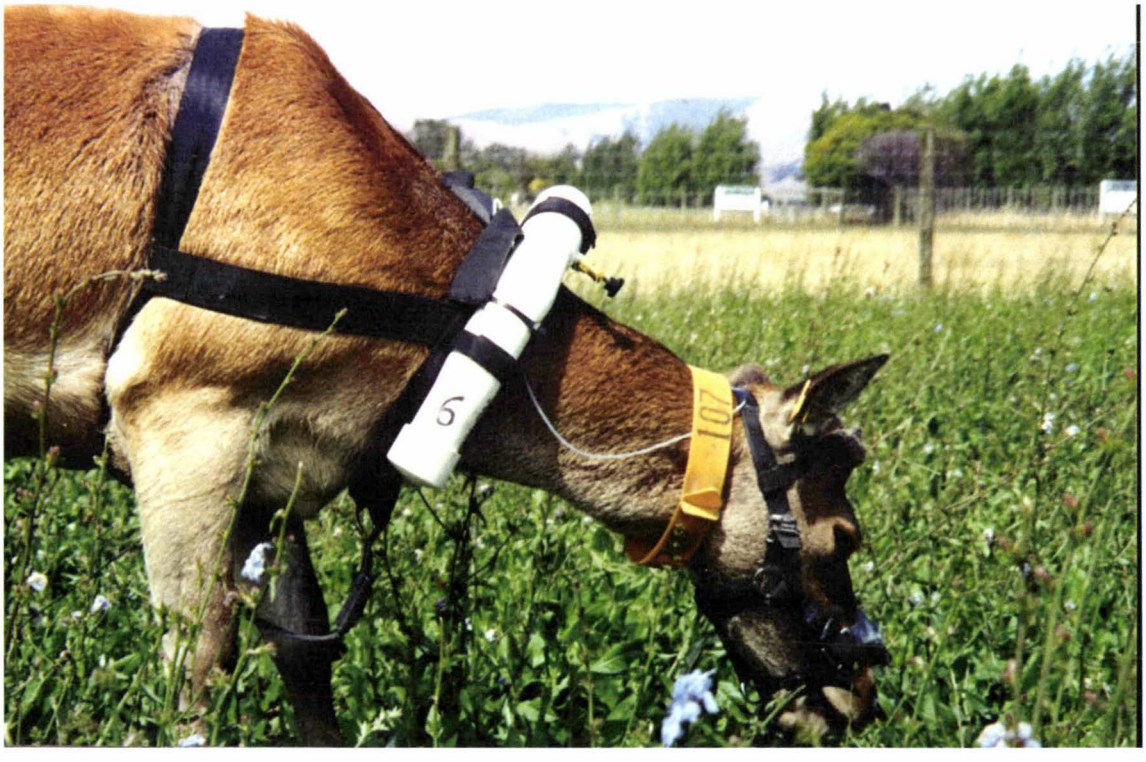


Plate 2.3. Deer wearing methane collection equipment, grazing chicory in March 2003.

The collection of the SF_6 and expired gases occurred over a period of five days, each day's collection occurring over a twenty-four hour period, where four days of successful sampling was required. At the start of each 24 hr collection period an evacuated yoke (-100 kPa) was placed on the animal's neck and attached to the body harness. Expired air was sampled from above the nose, via a nylon tube connecting to a capillary tube (0.76–1.27mm in diameter) resulted in restricting the airflow to approximately 0.7 ml/min restricted to the evacuated yoke. On the removal of the yoke after 24 hrs the sample collected averaged between 1 – 1.5 L. On the removal of the yokes the pressures were checked to ensure that a sample was collected. If the yoke held no pressure or was still evacuated, the halter of that animal was changed as this indicated broken or blocked tubing. In the proximity of each treatment paddock which was being grazed whilst methane measurements were being taken, a background yoke which sampled the ambient air was placed on the fence at the approximate height of the yokes worn by the deer.

Methane and SF_6 were determined by gas chromatography (Hewlett Packard 5890 Seris II) fitted with a stainless steel Molesive packed column (Alltech C-5000) and using a flame ionisation (CH_4) and electron-capture (SF_6) detectors (Ulyatt *et al.*, 2002). To standardize the gas chromatograph for SF_6 and CH_4 three prepared standards of high, medium and low concentrations SF_6 and CH_4 were used. Standards were run at the start and end of the sample run, and in addition, the medium standard was run at regular intervals through the sampling period to ensure consistent column performance. Methane production was calculated using the formula below (Equation 1) as developed by Johnson *et al.*, (1994).

$$CH_4 \text{ production (g/day)} = \text{Perm.tube } SF_6 \text{ release rate (g/day)} \times \left([CH_4]_{yoke} - [CH_4]_{background} / [SF_6]_{yoke} - [SF_6]_{background}\right) \quad \text{(Equation 1)}$$

2.2.6. Voluntary feed intake

The double n-alkane procedure was used to estimate voluntary intake (Dove & Mayes 1996; Dove, *et al.*, 1996; Gedir & Hudson, 2000). Seven days before the start of methane measurements animals were dosed with alkane controlled release capsules (CRC). The capsules were manufactured for growing cattle (Captec, Nufarm Ltd., New Zealand) containing 4 g of η-dotriacontane ($C_{32}H_{66}$) plus 4 g η-hexatriacontane ($C_{36}H_{74}$), and all alkane capsules used were from the same batch. Hinds and the nonsurgically modified castrated stag were administered the capsules orally. However, the four cannulated castrated stags, at least one from each treatment, had alkane capsules inserted into the rumen via the rumen cannulae. These alkane capsules were attached to a string so that during the methane measurement period the disappearance of the remaining matrix of the capsule could be determined daily to calculate release rates. Due to difficulties arising from administering capsules designed for growing cattle of 100kg minimum and not deer of 80kg minimum, one animal in Period 1 and three animals in Period 2 were dosed with two sheep-sized capsules as used by Stevens, (2003). Sheep capsules contain 1 g of η-dotriacontane and 1 g of η- hexatriacontane. Dry matter intake was estimated, as in the equation below (equation 2), from the daily dose rate and the dietary and faecal concentrations of the dosed even-chain alkane (η-dotriacontane, $C_{32}H_{66}$), and the adjacent natural odd-chain alkane (η-monotriacontane, $C_{31}H_{64}$).

$$Intake = \frac{F_i}{F_j} D_j \left(H_i - \frac{F_i}{F_j} H_j \right)$$

(Equation 2)

In the equation *Hi* and *Fi* represent the herbage and faecal (respectively) concentrations of the odd-chain alkane, *Hj* and *Fj* are the even chain equivalents and *Dj* is the daily dose of the even chain alkane. The daily dose rate was determined by the disappearance rate of the alkane matrix over time, as measured daily in the morning from the rumen cannulated animals. However, for those deer that received sheep capsules the daily dose rate as

suggested by the supplier was used. Faecal samples were collected by rectal grab samples from every animal once daily in the morning during the measurement period. Faecal samples were oven dried for 48 hrs at 60°C and ground in a coffee grinder. The daily samples of faeces, feed offered and feed selected (dried and ground) were then combined per animal and per forage and analysed for the alkanes, η-dotriacontane and η-monotriacontane, via gas chromatography as described by Dove *et al.* (1996), with the following modifications; industrial-heptane was used instead of analytical grade and saponification took place in an oven rather than on heating blocks.

2.2.7. Laboratory Analyses

Following oven drying, forages were ground to pass a 1 mm sieve (Willey Mill, USA). Organic matter (OM) content was measured by ashing the samples in a furnace at 500°C for 16hrs. Total N was determined by the Dumas method (Leco CNS 2000 Model 602 600 200, USA) and it was assumed that the conversion factor of nitrogen to crude protein is 6.25. Neutral detergent fibre (NDF), acid detergent fibre (ADF), hemi-cellulose, cellulose and lignin contents were analysed following the detergent procedures of Van Soest (1994). Cellulose was calculated as ADF less lignin and hemicellulose was calculated NDF less ADF. Hot water soluble carbohydrate (HWSC) and pectin were analysed using boiling water, and reflux in ammonium oxalate respectively, as described by Bailey & Ulyatt, (1970). Gross energy was determined through heat of combustion using an adiabatic bomb calorimeter (Gallenkamp Autobomb, Watson Victor Ltd, UK). Extractable and bound condensed tannins were determined by the Butanol/HCL procedure of Terrill *et al.* (1992). Organic matter digestibility and metabolisable energy (MJ ME) of the forages were estimated using near infra red spectroscopy (NIRS), where forage samples of 0.5-1.0g are exposed to an electro-magnetic scan of a spectral wavelength range of 1100 to 2500 nm (near infrared), the reflected energy is then measured by the instrument (feedTech, Agresearch, New Zealand) (Corson *et al.*, 1999).

2.2.8. Statistical analysis

Methane and DMI data were analysed using the mixed model procedure in SAS (SAS, 1998). Type of forage (ryegrass-based pasture, chicory and plantain) and time of measurement (March versus May) were fixed effects in the model, and time of measurement was used as a repeated measurement with compound symmetry covariance structure. The random statement included deer within forage type. The model is shown in Equation 3 below.

$$Y_{ijk} = \mu + F_i + D_{j(i)} + T_k + (F \times T)_{ik} + e_{ijk}$$

(Equation 3)

Where μ = overall mean; F_i = fixed effect of forage type (i = 1 to 3); $D_{j(i)}$ = random effect of deer within forage type (j = 1 to 12 or j = 1 to 6); T_k = fixed effect of time of measurement analysed as repeated measurement (k = 1 to 2); $F_i \times T_k$ = fixed effect of interaction between forage type and time of measurement; e_{ijk} = random residual error, assumed to be normally distributed.

The model for testing the effect of pasture sampling strategy ('feed offered' versus 'feed selected') on DMI, OMI, GEI, methane per kg DMI and methane % of GEI included type of forage (ryegrass-based pasture, chicory, and plantain), time of measurement (March and May), and method of sampling ('feed offered' versus 'feed selected') as fixed effects in the model including all interactions. The random statement comprised deer within forage type.

Chemical compostion of forages where possible were analyzed using the mixed model procedure in SAS (SAS, 1998). Type of forage (ryegrass-based pasture, chicory and plantain) and time of measurement (March versus May) were fixed effects in the model, and time of measurement was used as a repeated measurement with compound symmetry covariance structure. The random statement included sample within forage type. The model is shown in Equation 4 below.

$$Y_{ijk} = \mu + F_i + S_{j(i)} + T_k + (F \times T)_{ik} + e_{ijk}$$

(Equation 4)

Where μ = overall mean; F_i = fixed effect of forage type (i = 1 to 3); $S_{j(i)}$ = random effect of sample within forage type (j = 1 to 2); T_k = fixed effect of time of measurement analysed as repeated measurement (k = 1 to 2); $F_i \times T_k$ = fixed effect of interaction between forage type and time of measurement; e_{ijk} = random residual error, assumed to be normally distributed.

Values reported for methane, DMI and nutrient composition of forages are reported as least square means, as stated, all other values reported in the tables are mean values, standard error of the mean (SEM), difference (SED) or standard deviation (STD) are reported as stated. Significance was declared at $P \leq 0.05$, and a trend was reported if $0.05 < P \leq 0.10$. All mean comparisons were by Fisher's least significant difference method after a significant treatment main effect was detected.

2.3. Results

2.3.1. Botanical composition and dry matter

The three treatment forages differed significantly in their botanical composition (BC), however no significant effect of period on BC was observed. The clover content of pasture was significantly lower compared with chicory (P = 0.009), but not plantain (P = 0.33). The clover content of chicory was significantly greater than plantain (P = 0.004). Chicory had a higher proportion of weeds than both pasture (P = 0.04) and plantain (P = 0.03), which were similar. The proportion of dead matter in pasture compared with either plantain or chicory did not differ significantly. However, the proportion of dead material in chicory was significantly greater than in plantain (P = 0.03). The leaf content of pasture was significantly greater than chicory (P = 0.0001), but not plantain (P = 0.11). The proportion of leaf in chicory was significantly less than for plantain (P = 0.0001), probably due to the higher proportion of stem in chicory compared with plantain (P = 0.02).

Table 2.2. The botanical composition of the three treatment forages grazed by hinds in March and May.

% DM	Pasture (n=3)			Chicory (n=3)			Plantain (n=2)			*P*-values		
	March	May	SED	March	May	SED	March	May	SED	Forage	Time	F × T[2]
Stem	-	-		19.7	19.7	6.1	1.0	1.0	7.4	0.02	1.0	1.0
Clover	2.0	2.0	1.8	6.7	6.7	1.8	0.5	0.5	2.2	0.007	1.0	1.0
Weed	1.7	1.3	2.7	6.3	6.3	2.7	0.5	0.5	3.3	0.05	0.9	1.0
Dead	22.7	22.3	6.8	31.0	31.0	6.8	16.0	16.5	8.3	0.07	0.99	1.0
Leaf[1]	73.7	74.3	5.5	36.3	36.3	5.5	82.0	81.5	6.7	0.0001	0.99	0.99

[1]The leaf proportion of pasture contain both stem and leaf.

[2]Forage type by time.

The dry matter percentage (DM%) of all three forages is shown in Table 2.3. and decreased from March to May, with the greatest difference occurring in pasture.

Table 2.3. Mean dry matter percent and standard deviation of forages, feed offered and feed selected, as sampled during the methane measurement period.

	Pasture (n=18)		Chicory (n= 18)		Plantain (n= 12)	
	Mean	STD	Mean	STD	Mean	STD
Feed Offered						
March	24	0.02	17	0.01	16	0.03
May	13	0.01	10	0.002	11	0.01
Feed Selected						
March	22	0.03	11	0.02	15	0.01
May	13	0.01	10	0.01	10	0.01

2.3.2. Chemical composition of forages

The chemical composition of feed offered and feed selected are presented in Tables 2.4a and 2.4b, respectively. Unless stated all forages are presented as a percentage of dry matter, the means of chemical components that were able to be analysed with statistics are presented as least square means and all errors are the standard error of the difference of the mean.

2.3.2.1. Feed offered

The DM percentage of OM, NDF, hemicellulose, cellulose, crude protein and gross energy present in ryegrass-based pasture grazed by animals in March and May was greater ($P < 0.05$) than both chicory and plantain. The HWSC of pasture also tended to be greater than that of chicory ($P = 0.06$), but not of plantain ($P = 0.5$). In contrast, the percentage of ADF, lignin, and readily fermentable carbohydrate: structural carbohydrates (RFC:SC) in the DM of

ryegrass-based pasture was less ($P < 0.05$) than both chicory and plantain. The DM percentage of pectin, gross energy, cellulose, and hemicellulose of chicory were greater than plantain ($P < 0.05$). However chicory had reduced concentrations of RFC:SC, ADF, and lignin ($P < 0.05$), where both HWSC ($P = 0.07$) and NDF ($P = 0.08$) tended to be less as compared with plantain.

The chemical components, pectin and gross energy, in all forages changed with time ($P < 0.01$). The DM percentage of HWSC, crude protein, RFC in the DM of ryegrass-based pasture, chicory and plantain all increased with time. In contrast, the proportion of OM, NDF, ADF and cellulose decreased with time for all forages. The hemicellulose content of pasture increased with time, while it decreased in both chicory and plantain, in contrast the DM percentage of lignin in pasture decreased while it increased in both chicory and plantain.

2.3.2.2. Feed Selected

The organic matter, NDF, cellulose, hemicellulose, HWSC, and gross energy of pasture selected were greater than both chicory and plantain ($P < 0.05$), where the DM percentage of RFC:SC, pectin crude protein, ADF and lignin was less than those of plantain and chicory ($P < 0.05$). The DM proportion of crude protein, gross energy, pectin, RFC:SC in chicory selected was greater than plantain ($P < 0.02$). However, the concentration of NDF, ADF, cellulose, HWSC and lignin of chicory was less than plantain selected ($P < 0.05$).

Overall there was a significant effect of time of all chemical components of the forages ($P < 0.05$), except for cellulose and ADF ($P > 0.10$). The RFC:SC, HWSC and crude protein of all forages increased with time; in contrast, the OM content of pasture and plantain deceased, but not the OM percentage of chicory ($P = 0.10$), the lignin content of chicory and plantain also decreased, but not pasture ($P = 0.15$). The NDF proportion of chicory decreased with time, however there was no significant change of pasture ($P = 0.2$) or plantain ($P = 0.2$). In contrast the pectin proportion of both pasture and plantain and plantain increased with time, however the pectin in chicory decreased ($P = 0.0003$). The

gross energy of both pasture and chicory increased, however there was no significant change in the gross energy of plantain ($P = 0.5$).

2.3.2.3. Feed offered versus feed selected

The method of forage sampling (not tabulated) was significant ($P < 0.02$) for the DM percentages of all of the chemical components except for organic matter ($P = 0.4$) and HWSC ($P = 0.7$). The proportion of NDF, ADF, cellulose, and hemicellulose represented a greater proportion of DM when sampled using the feed offered technique compared with the feed selected technique. In contrast the DM proportion of lignin, crude protein, RFC:SC, pectin, and gross energy were represented in smaller proportions when forages were sampled by the feed offered technique.

Table 2.4. a & b. Chemical composition of the forages fed during the methane measurement period for feed offered (3a) and feed selected (3b) (% DM).

2.4. a. Feed offered

	Pasture			Chicory			Plantain			*P*-values		
% DM	March	May	SED	March	May	SED	March	May	SED	Forage	Time	F × T
OM	92.5	91.5	0.18	87.6	86.1	0.19	87.7	86.0	0.19	0.0001	0.0002	0.115
ADF	29.7	24.6	0.73	31.0	24.9	0.36	32.8	30.5	0.36	0.02	0.0006	0.01
NDF	57.6	56.2	0.49	46.5	36.4	0.48	45.4	39.2	0.49	0.0001	0.0002	0.002
Hemicellulose *(b)*	30.4	32.7	0.32	14.7	10.7	0.28	13.2	9.4	0.28	0.0001	0.002	0.001
Cellulose *(b)*	24.2	21.3	0.59	24.3	15.8	0.40	19.7	13.5	0.40	0.006	0.0002	0.009
Lignin	3.8	2.5	0.38	7.3	9.7	0.43	12.8	16.3	0.39	0.0001	0.0006	0.0003
Pectin *(a)*	1.3	1.8	0.33	7.5	6.6	0.17	5.3	5.5	0.17	0.0023	0.62	0.032
HWSC *(a)*	10.0	11.1	0.84	4.6	10.5	0.54	4.9	14.5	0.54	0.093	0.0007	0.007
Ratio RFC:SC (a/*b*)[1]	0.20	0.23	0.03	0.30	0.64	0.02	0.31	0.88	0.02	0.004	0.0001	0.001
Crude protein	13.1	24.3	0.44	14.2	21.6	0.36	11.3	13.3	0.36	0.0009	0.0001	0.001
Condensed tannins	0.20	0.13		0.33	0.42		0.19	0.20				
OM digestibility [2]	58.3	75.5		70.8	78.6		69.6	73.9				
ME (MJ /kg DM)[2]	8.5	10.8		10.2	11.2		10.0	10.8				
GE (MJ/kg DM)	18.37	18.98	0.12	17.87	17.81	0.12	17.25	17.11	0.12	0.001	0.15	0.04

[1] Ratio of readily fermentable carbohydrate: structural carbohydrates. [2]Estimated using NIR.

2.4. b. Feed selected

	Pasture			Chicory			Plantain			P-values		
% DM	March	May	SED	March	May	SED	March	May	SED	Forage	Time	F × T
OM	92.0	91.3	0.16	86.8	86.5	0.16	87.3	87.2	0.16	0.0001	0.02	0.18
ADF	25.2	22.5	1.1	23.1	20.7	1.1	30.0	31.7	1.1	0.003	0.2	0.12
NDF	55.4	56.1	0.52	32.0	29.4	0.52	40.9	40.0	0.52	0.0001	0.05	0.04
Hemicellulose (b)	30.2	33.7	1.3	8.9	8.6	1.3	11.0	8.3	1.3	0.0002	0.8	0.09
Cellulose (b)	21.4	19.9	1.2	8.1	10.1	1.2	12.2	14.2	1.2	0.002	0.33	0.21
Lignin	3.8	2.6	0.7	15.0	10.9	0.7	17.7	17.5	0.7	0.0001	0.008	0.02
Pectin (a)	0.9	1.2	0.04	8.1	7.2	0.04	5.3	5.7	0.04	0.0001	0.07	0.0005
HWSC (a)	9.8	12.2	0.41	4.2	12.3	0.41	3.8	15.9	0.41	0.006	0.0001	0.001
Ratio RFC:SC (a/b)[1]	0.21	0.25	0.02	0.66	0.98	0.02	0.37	0.94	0.02	0.0009	0.0001	0.0005
Crude protein	16.3	28.4	0.37	20.8	26.9	0.37	14.0	15.8	0.37	0.0006	0.0001	0.0007
Condensed tannins	0.17	0.06		0.39	0.34		0.38	0.28				
OM digestibility [2]	nd	nd		nd	nd		nd	nd				
ME (MJ /kg DM)[2]	nd	nd		nd	nd		nd	nd				
GE (MJ/kg DM)	18.82	19.64	0.06	18.14	18.84	0.06	17.46	17.41	0.06	0.001	0.0008	0.004

[1]Ratio of readily fermentable carbohydrate: structural carbohydrate, [2]Estimated using NIR., nd not determined

2.3.3 Body weight and live weight change

The live weights of the deer are reported in Table 2.5. Despite *ad libitum* access to feed, deer lost weight in both March and May, with animals losing significantly more weight in March than in May (405 g/d versus 253 g/d, P = 0.006) during the trial period. The greater weight loss in March could reflect the lower feed quality of the forages in March and greater stress associated with intensive handling and wearing of collection equipment compared with the second run in May. There was found to be no significant difference of weight loss with forage type.

Table 2.5 Average body weight of deer at start of experimental periods and body weight change.

	Pasture			Chicory			Plantain			*P*-values		
	March	May	SED	March	May	SED	March	May	SED	Forage	Time	F × T
BW, kg[1]	108.5±10.7	103.8±9.5	-	120.2±25.2	117.0±19.6	-	108.7±10.6	106.7±17.5	-	-	-	-
BW change, (g/d)	-291	-240	67	-514	-326	96	-410	-192	92	0.34	0.006	0.27

[1]Mean ± standard deviation

2.3.4 Dry matter intake

Dry matter intake (DMI) was estimated using the double n-alkane procedure, where n-monotriacontane ($C_{31}H_{64}$) was used as the adjacent alkane to n-dotriacontane ($C_{32}H_{66}$), as it had a higher content than n-tritriactane ($C_{33}H_{68}$). The contents of these alkanes are shown in Table 2.6. It was found that the alkane content of chicory was less than that of either plantain or pasture. The alkane capsules used also contained hexatricontane ($C_{36}H_{74}$) and the two adjacent alkanes. However, these could not be used to calculate intake as they were either very low or not detectable. The difference in the alkane content of feed offered and feed selected was not great for plantain and ryegrass-based pasture, but there was found to be up to a 50% difference in chicory offered and selected. Gross analysis of feed offered and selected samples analysed by both Lincoln University and Dexcel laboratories, were similar, as shown in table 2.7.

Table 2.6. The alkane content of n-monotriacontane ($C_{31}H_{64}$), n-dotriacontane ($C_{32}H_{66}$) and n-tritriactane ($C_{33}H_{68}$) of the three forage treatments (ryegrass/clover, chicory and plantain) for March and May (n = 2).

Alkane,	*Pasture*				*Chicory*				*Plantain*			
mg/100gDM	March		May		March		May		March		May	
	FO	FS	FO	FS	FO	FS	FO	FS	FO	FS	FO	FS
$C_{31}H_{64}$	27.1	24.5	22.1	23.6	9.9	5.2	3.8	2.7	25.2	27.8	38.2	48.2
$C_{32}H_{66}$	1.1	1.1	1.2	1.2	0.8	0.6	0.5	0.5	2.8	3.2	3.3	4.1
$C_{33}H_{68}$	9.1	9.7	15.4	16.0	1.2	0.9	1.0	0.7	9.1	10.6	13.7	16.7

FO = Feed offered, FS = Feed selected

Table 2.7 Gross analyses of η-alkanes (C_{31} to C_{33}) for all forges for both feed selected and feed offered as analysed by both Lincoln University and Dexcel laboratories.

			η - alkanes (mg/100g)			
			C_{31}	C_{32}	C_{33}	Analysed at;
Feed Offered	March	Pasture	27.1	1.1	9.1	*Dexcel*
			25.5	1.0	9.2	*Lincoln*
		Chicory	9.9	0.8	1.2	*Dexcel*
			8.3	0.5	1.0	*Lincoln*
		Plantain	25.2	2.8	9.1	*Dexcel*
			24.7	2.6	9.1	*Lincoln*
	May	Pasture	22.1	1.2	15.4	*Lincoln*
		Chicory	3.5	0.3	0.7	*Lincoln*
		Plantain	38.2	3.3	13.7	*Lincoln*
Feed Selected	March	Pasture	24.5	1.1	9.7	*Lincoln*
		Chicory	4.0	0.4	0.6	*Lincoln*
		Plantain	27.8	3.2	10.6	*Lincoln*
	May	Pasture	23.6	1.2	16.0	*Lincoln*
		Chicory	4.6	3.3	1.9	*Lincoln*
		Plantain	45.2	4.1	4.1	*Lincoln*

The DMI in May for plantain was based upon five observations instead of six, because for one animal the dosed alkane was non-detectable. It was assumed that this animal lost the capsule. DMI was calculated using the alkane concentrations as shown in Table 2.6 for both feed offered and selected. It was found that estimated DMI of all forages was influenced by the method used to collect the forage samples, with the mean for feed offered (2869 ± 124 (SEM) g/d) being significantly lower than for feed selected (3756 ± 124 (SEM) g/d) (P = 0.0001), as shown in Table 2.8. However, a closer examination of the interaction of forage and method found that this difference was largely driven by differences within the chicory treatment, as there was a significant effect of

method and forage (P = 0.0001), whereas there was no significant effect for either plantain or pasture (P = 0.8 and P = 0.7 respectively).

Similar relationships were also found for organic matter intake (OMI), the percentage of gross energy lost as methane, gross energy intake and methane produced per kg of DMI with use of feed offered vs. feed selected values. There was found to be no effect of forage sampling method when examining the interaction of method by time, or time by method and forage. There was no significant effect of sampling method for plantain and pasture, and the DMI of chicory using data from feed selected samples was not biologically feasible for either March or May (5204 ± 284 (SEM) g/d and 9010 ± 284 (SEM) g /d respectively). Therefore the results for the DMI of feed offered only, are presented in Table 2.9.

Table 2.8. Effect of the method of forage sampling, either feed selected or feed offered, on estimated dry matter intake and methane production per unit of intake of deer grazing ryegrass/clover, plantain or chicory.

	Pasture			Chicory			Plantain			*P*-values	
	Feed offered	Feed selected	SED	Feed offered	Feed selected	SED	Feed offered	Feed selected	SED	Method	Forage x Method
DMI, g/d	1959	1991	137	4322	7107	193	2238	2173	203	0.0001	0.0001
OMI, g/d	1879	1820	140	3741	6125	198	1948	1898	207	0.0001	0.0001
GEI, kJ/d	38253	38318	2900	77050	131152	4102	38518	37903	4302	0.0001	0.0001
CH_4/DMI, g/kg	37.5	36.8	2.1	18.7	10.4	2.9	26.2	25.6	3.2	0.05	0.08
CH_4, % of GEI	10.9	10.5	0.43	5.8	3.1	0.92	8.4	8.1	1.01	0.029	0.12

Dry matter intake for the three treatment forages are shown in Table 2.9. There was a significant main effect of forage species on mean DMI of grazing deer (P = 0.0001). The intake for ryegrass-based pasture, chicory and plantain was 1948 g/d, 4321 g/d and 2258 g/d respectively, where chicory was significantly greater than pasture and plantain (P = 0.0001). The mean DMI in March (2313 g/d) was significantly lower than in May (3371 g/d) (P = 0.0001). The DMI of pasture and plantain in March compared with May were similar (P >0.10), whereas the DMI of chicory significantly (P = 0.0001) increased from March to May. The DMI of deer consuming pasture in both trials was significantly lower than that of animals grazing chicory during both March (P = 0.02) and May (P = 0.0001). The DMI of deer grazing pasture in March (P = 0.04), but not May was significantly lower than those grazing plantain. The DMI of deer grazing chicory and plantain did not differ statistically in March, however in May the DMI of deer grazing chicory was significantly greater than for plantain (P = 0.0001).

Results for OMI were similar to DMI, with a highly significant main effect of forage type (P = 0.0001). The average OMI of pasture, chicory, and plantain was 1791, 3741 and 1963g/d respectively, where pasture and plantain were similar and lower than chicory (P = 0.0001). The intake of OM for March (2057 g/d) was lower than for May (2340 g/d) (P = 0.0001). The interaction of forage and time for OMI was similar to DMI, however plantain tended to be greater than pasture in March (P = 0.08).

Gross energy intake (GEI) per day followed the same pattern as both OMI and DMI. GEI averaged 36.4, 77.1 and 38.8 MJ/d for pasture, chicory and plantain with significant effects of forage type (P = 0.0001) and time (P = 0.0001), where for May GEI (41.1 MJ/d) was greater than for March (38.8 MJ/d). The time effect seems to be driven by the significant difference of chicory (P = 0.0001) as plantain and pasture were not significantly different with time (P = 0.14). Similarly to OMI and DMI, the interaction of forage with time showed that the GEI of chicory was significantly higher in March compared with pasture (P = 0.03), but not plantain (P = 0.6), and the GEI of plantain tended to be greater than that of pasture (P = 0.09). In May the GEI of deer grazing pasture was similar to that of plantain (P = 0.41), but was significantly lower than that of

chicory (P = 0.0001). The GEI of chicory was also significantly greater (P = 0.0001) than the GEI of plantain.

2.3.5. Methane production

A summary of methane production is presented in Table 2.7 for methane production per day and per kg DMI, with individual animal data presented in Appendix 2.5 Table 2.10. For one animal in the plantain group in March it appeared that the permeation tube may have been lost as there was no detection of SF_6. This animal was replaced for the May period, but then a different animal from the same group had to be omitted from the May period because only one day of successful measurement of methane production was obtained. Total methane production (g/d) did not differ significantly according to forage grazed (P = 0.13), where methane averaged 71.4, 68.5 and 56.8 g/d for pasture, chicory and plantain, respectively. However, methane production was significantly different with time (P = 0.05), where methane production in May (70.6 g/d) was greater than in March (60.6 g/d). There was found to be no significant forage type by time interaction for methane production.

Methane production calculated as gross energy (GE, kJ/d) output also did not differ with forage grazed (P = 0.13). However, the effect of time was significant (P = 0.05), with more GE as methane produced during May (3882 kJ/d) than during March (3330 kJ/d).

Methane as a proportion of body weight differed significantly with time (P = 0.02), with a greater proportion of methane produced per kg of body weight (BW) per day in May (0.66 g/kg BW/d) than in March (0.54 g/kg BW/d). There was a trend towards lower methane production per unit bodyweight for the herbs compared with pasture (P=0.08), which was 0.67, 0.59 and 0.53 g/kg BW/d for pasture, chicory and plantain respectively. There was no significant time by forage type interaction (P = 0.5).

In contrast, methane production when expressed as a proportion of DMI showed no significant effect of time (P = 0.73). However, there was found to be a significant effect of forage type (P = 0.0001), where the average methane

production over March and May was 37.7, 18.7 and 26.7 g/DMI/d for pasture, chicory and plantain, respectively. Methane production per kg DMI from deer grazing pasture in March was similar to that produce in May (P = 0.81). In contrast, there was found to be a significant difference in methane production (g/kg DMI) for both chicory and plantain with time. Chicory had higher emissions of methane production in March (P = 0.03) compared with May, whereas the reverse was true for plantain (P = 0.008). In March the methane emissions from the deer grazing pasture were higher compared with those grazing chicory (P = 0.02) and plantain (P = 0.0009), with no significant difference observed between the two herbs. However, in May, methane emissions (per kg DMI) from pasture did not differ significantly from those from plantain, but were significantly greater than were those from chicory (P = 0.0001), with chicory emissions significantly less than plantain (P = 0.0009). Over all It was found that methane (g/kg DMI) production from deer grazing pasture was significantly greater than for both chicory (P = 0.0001) and plantain (P = 0.009), but chicory tended to be less than plantain (P = 0.08). A significant time by forage type interaction was found (P = 0.0045).

A significant forage type effect (P = 0.0003) and forage by time interaction (P = 0.004) was found for the percentage of GEI lost as methane, however no effect of time was found. The average percentage of GEI lost to methane was 11.1, 8.6 and 5.8% for ryegrass-based pasture, plantain and chicory respectively, where ryegrass-based pasture was significantly higher compared with both chicory (P = 0.0001) and plantain (P = 0.05). The proportion of GEI lost as methane for chicory was significantly greater than for plantain (P = 0.04). The interaction of time and forage was driven by differences between periods for both herbs (chicory; P = 0.03: plantain P = 0.006), but not pasture (P = 0.51). In March, the percentage of GEI lost to methane from chicory was less than from pasture (P = 0.02), but similar to plantain (P = 0.25). However, in May, less GEI as methane was lost from those deer grazing chicory compared with plantain (P = 0.0005) and ryegrass-based pasture (P = 0.0002). In May, the percentage of GEI lost to methane for ryegrass-based pasture and plantain was similar (P = 0.64).

Table 2.9. Intake and methane production for red deer grazing ryegrass-based pasture, chicory and plantain.

	Pasture			Chicory			Plantain			*P*-values		
	March	May	SED	March	May	SED	March	May	SED	Forage	Time	F × T
DMI, g/d	1828	2067	206	2615	6029	287	2499	2017	316	0.0001	0.0001	0.0001
OMI, g/d	1690	1892	180	2290	5192	251	2191	1735	276	0.0001	0.0001	0.0001
GEI, kJ/d	33591	39248	3655	46727	107373	5098	43113	34502	5603	0.0001	0.0001	0.0001
CH_4, g/d	68.2	74.7	6.20	65.3	71.7	8.68	48.2	65.4	9.44	0.13	0.05	0.62
CH_4, g/kg BW/d	0.63	0.71	0.06	0.56	0.62	0.08	0.43	0.64	0.09	0.08	0.02	0.46
CH_4, kJ GE/d	3749	4109	341	3593	3946	477	2649	3595	518	0.13	0.05	0.62
CH_4/DMI, g/kg	38.5	37.0	5.28	25.5	12.0	5.55	17.6	35.7	6.12	0.0001	0.74	0.005
CH_4, % of GEI	11.52	10.72	1.22	7.85	3.70	1.72	5.63	11.49	1.89	0.0003	0.75	0.004

2.4 Discussion

This study has directly measured methane production from grazing red deer for the first time. A mean value of 71.4 g CH_4 / d or 37.7 g CH_4/kg DMI/d was obtained for deer grazing pasture. For New Zealand inventory purposes, and to compare methane production between species of differing body size and feed consumption where direct comparative trials have not been published, methane production per unit feed intake has been used (NIR, 2003). For grazing trials, such comparisons are dependent on accurate, repeatable measurement of both methane production and feed intake, and this limitation will be discussed below. However, the value for methane production per unit dry matter intake obtained for grazing deer in this study is approximately 75-80% greater than values used in the inventory for dairy cows (21.6 g CH_4/kg DMI, (NIR, 2003)), sheep (20.9 CH_4/kg DMI, (NIR, 2003)) and estimated for deer (21.25, (NIR, 2003)) grazing ryegrass-based pastures. The values obtained in this study for deer fall outside the range of values published for sheep and cattle grazing ryegrass-based pasture (12.9 – 23.8 g CH_4/kg DMI (O'Hara *et al.,* 2003). Methane production per kg of body weight of sheep (0.57 g CH_4 /kg BW (O'Hara et al., 2003)) and dairy cows (0.63 g CH_4 /kg BW (O'Hara et al., 2003)) was similar to the average value for deer for grazing chicory (0.59 g CH_4 /kg BW) and plantain (0.53 g CH_4 /kg BW). However, methane production of deer-grazing ryegrass-based pasture (0. 67 g CH_4 /kg BW) was higher.

There does not seem to be any obvious explanation for the apparently greater production of methane per kg of DMI from deer compared with published values for other ruminant livestock. However, this study did not provide a direct comparison between ruminant species grazing the same pastures at the same time, comparisons of methane production between species using data from this study should be undertaken with caution. Prudence should also be used when comparing measurements from calorimetry chambers to grazing trials. The calorimetry chamber is an artificial environment, therefore should not be directly compared with methane measurements per day or per kg of DMI measured from grazing animals (Clark, 2002; Johnson *et al.*, 1994). However, validation studies have found methane production from grazing sheep and cattle to fall

within 90-95% of chamber measurements (Johnson *et al.*, 1994; Pinares-Patino, 2000; Boadi *et al.*, 2002).

Species comparative differences in, and possible effects of, digestive physiology and passage rate on methane production may explain a higher methane production by deer compared to sheep. Pinares-Patino *et al.*, (2003) found that methane production by sheep housed indoors was positively correlated with the rumen pool size of organic matter, organic matter intake and rumen fill, but negatively correlated to the fractional outflow of particles from the rumen. It was also found that methane production could be predicted by OM pool size and the molar percentage of butyrate present in rumen fluid. Despite a higher voluntary intake of deer compared with sheep, Domingue *et al.* (1991) showed that deer maintained an average total rumen pool size throughout the year that was smaller than that of sheep by 17%, whilst maintaining a greater FOR (percent per hour) than sheep in both summer and winter. So if methane production is positively correlated with rumen pool size and negatively correlated with the fractional outflow rate (FOR) of particles according to the evidence presented by Pinares-Patino *et al.* (2003), then according to evidence presented by Domingue *et al.* (1991), deer should produce less methane than sheep, in contrast to the results of the present study. However, looking more closely at the results of Domingue *et al.* (1991) reveals the faster FOR of deer seemed to be a function of a greater FOR of liquid rather than of particulate matter. Also the DM rumen pool of deer was found to be greater than that of sheep in summer (43 vs 37 g / kg $W^{0.75}$, respectively) and smaller than that of sheep in winter (31 vs. 40 g / kg $W^{0.75}$, respectively). This emphasises the need for direct comparative studies of methane production by different farmed ruminant species.

Methane production expressed per unit GE and DMI from deer grazing chicory was reduced compared with pasture. However, the proportion of reduced methane may be overestimated due to overestimation of feed intake, especially in May where deer were estimated to consume approximately 6 kg of chicory (DM). Previous studies of deer indoors have found that deer readily consume more chicory than pasture, but the consumption of chicory was 2.25 kg for

castrated stags (Kusmartono *et al.*, 1997) and it is unlikely non-lactating hinds would be able to consume 6 kg DM. However, it was estimated -using intra-ruminal chromium slow release capsules- that lactating hinds in the grazing situation ate 6.3 kg/d DM chicory (Kusmartono *et al.*, 1996). It is assumed that intake for chicory in May using the alkane technique was overestimated. The estimation of intake may result from the very low alkane content of chicory found for the alkanes used to estimate intake. The intake of the deer in March seemed reasonable; however, the accuracy of the double η-alkane technique to measure intake of chicory should be questioned. There do not seem to be any published data measuring the DMI of chicory using the n-alkane technique. Published data have shown that actual feed intake was overestimated by 6.1% when feed intake was calculated using the double n-alkane when gestating wapiti hinds were fed a cubed dried alfalfa-based diet (Gedir & Hudson, 2000), but these results can hardly be compared to feeding a low DM, fresh forage such as chicory.

The measured consumption of ryegrass-based pasture by deer in this study was found to be within previously published values. Contrary to the seasonal cycle of voluntary feed intake of temperate deer, which reduces from summer to winter (Domingue *et al.*, 1991; Rhind *et al.*, 1998), deer in this study in March consumed 13% less DM per day than in May. The low consumption of ryegrass-based pasture in March may reflect the high fibre and proportion of DM of the forage, which could have restricted intake due to rumen fill (Forbes, 1977; McDonald *et al.,* 1995). The low apparent digestibility of the pasture treatment may reflect the unusually dry summer, which occurred during this experiment (NIWA, 2004).

In a comparative study of deer, sheep and goats (Domingue *et al.*, 1991) it was found that deer exhibited greater seasonality in rumen pool size, DMI, FOR and apparent digestibility than did either sheep or goats. Deer consumed more DM in summer than did sheep, and were able to digest fibre more efficiently than were than sheep (P = 0.09) in summer due to an increase in rumen pool size. These findings by Dominque *et al.,* (1991) suggested that methane production from deer may exhibit seasonal variation. In the current study, there was no

clear indication of a shift in methane production due to seasonality, which is in contrast to the findings of Galbraith et al., (1998), where Northern Hemisphere wapiti were found to have a seasonal decrease in methane production from February/March to May/June. Methane production (g/d) per day of deer grazing pasture was higher in May than in March, which may reflect the increased consumption of DM in May compared to March. Grams of methane production per day from deer grazing chicory and plantain were also greater in May as compared to March, when deer grazing chicory, like those grazing pasture, were estimated to have a greater DMI in May. However, those deer grazing plantain were estimated to have a reduced DMI in May compared to March. The effect of seasonality of methane production in this study may be confused with the estimation of intake as the accuracy of the DMI is under question.

Investigation into the effect of grazing alternative forages on methane production of grazing deer showed that when taking an average of both periods, deer grazing chicory and plantain produced 50 and 41%, respectively, less methane per kg DMI than did deer grazing perennial-ryegrass based pasture. This effect was more pronounced in March, particularly for plantain, possibly due to drought-mediated effects on forage chemical composition, discussed below. Sheep fed chicory indoors were found to have decreased methane production of up to 40% compared with those animals fed pasture (Waghorn *et al.*, 2002).

In the same study, it was found that CT significantly reduced methanogenesis by 16% and methane production was reduced in sheep by up to 50% (16.4 g CH_4/DDMI) when fed lotus (*Lotus pedunculatus)* containing CT of 5.3 g /kg. Sheep fed chicory showed a 41% (20.4 g CH_4/DDMI) reduction in methane emissions per kg DMI compared to sheep fed pasture (20.4g CH_4/DDMI), however, no CT were detected in the chicory fed to sheep. In the current study, concentrations of CT found in chicory and plantain were similar to that found in pasture, and therefore were unlikely to be responsible for any differences between forages. Condensed tannins have been found to alter the breakdown and fermentation of protein and fibre in the digestive tract of ruminants through changes in microbial populations and growth (Barry *et al.*, 2002; Woodward *et*

al., 2001). Deer grazing chicory compared with ryegrass-based pasture have shown a tenfold (93.3%) decrease in the population of methanogens (cells per ml) in the rumen, additionally the methanogen colonies were also reported to appear to be different when compared with animals grazing ryegrass-based pasture and plantain (N. Walker, personnal communication, 2003). However, this may be due to other nutritional factors besides CT. Sheep have also been shown to reduce methane production by 16% and 44% when fed lucerne and *Lotus pendunculatus* respectively compared with sheep fed pasture (Woodward *et al.*, 2001). Cattle grazing *Lotus corniculatus* and sulla were found to produce an average of 30% less methane per kg DMI than were cattle consuming pasture (Woodward *et al.*, 2002; Woodward *et al.*, 2001), whereas cows fed sulla had a reduction of gross energy intake lost in the form of methane of 1.1% compare with those fed pasture (Woodward *et al.*, 2002).

Aside from the influence of DMI, the findings of the current study suggest that deer grazing chicory and plantain had reduced methane production as expressed per unit DMI or percentage of GEI and this could possibly be associated with the following points regarding current understanding of how nutritional factors influence methane production. Feeding of forages with a reduced proportion of fibre and a greater content of readily fermentable carbohydrates, such as cereal grains, tends to result in a lower rumen pH and a greater proportion of propionate being produced (Johnson & Johnson, 1995; Lee *et al.*, 2000). However, the manipulation of acetate to propionate to decrease methane emissions should be viewed with caution. For example Hoskin *et al.*, (1995) found that deer fed chicory compared with those fed ryegrass-based pasture showed a greater ratio of acetate: propionate. However, this has not been established for sheep or cattle. Under these conditions, there tends to be more methane produced (Lee et al., 2000; Benchaar *et al.*, 2001). An increased rate of passage is negatively correlated with methane production (McAllister *et al.*, 1996; Benchaar *et al.*, 2001).

The reduced methane production per kg DMI from deer grazing chicory compared with pasture is possible for nutritional reasons, as previous studies with deer have shown that chicory has a greater ME and high ratio of readily

fermentable to structural carbohydrates compared with pasture (Barry, 1998; Hoskin *et al.*, 1995; Kusmartono *et al.*, 1996). Deer consuming chicory have also been shown to have a greater rate of rumen outflow than have deer fed ryegrass-based pasture (Kusmartono *et al.*, 1996). Little is known about the ruminal digestion of plantain. However, the higher estimated OM digestibility in March and increased ratio of readily fermentable carbohydrates to structural carbohydrates of plantain compared to pasture suggests that nutritive factors could have influenced methane production.

In conclusion, methane emissions per kg of DMI of red deer appear to be greater than those values of sheep and cattle, and appear to be affected by forage type fed. However, estimated DMI used in this study may not be representative of actual DMI values of grazing red deer, consequently these results should be viewed with caution. Due to the uncertainty of the estimated DMI, concurrent measurements of methane and accurate measurements of DMI need to be undertaken to either support or disprove the results of this experiment.

2.5. Appendix

Table 2.10. Methane emissions (g) per day (a) and per kg of DMI (b) of individual red deer feed ryegrass-based pasture, chicory and plantain over the entire methane measurement period (March and May).

(a) Methane grams per day.

		March (days)								May (days)						
Treatment	**Deer**	**1**	**2**	**3**	**4**	**5**	**6**	**Mean**	**STD**	**1**	**2**	**3**	**4**	**5**	**Mean**	**STD**
Pasture	**54**	60.7	73.1	65.7	70.9	62.0		66.48	5.42	52.3	55.1	54.0	66.4	58.9	57.3	5.61
	^206									n/a	n/a	n/a	66.4	55.7	61.1	7.57
	323	n/a	100.2	88.7	98.8	98.2		96.48	5.25	75.2	67.8	55.9	67.0	72.7	67.7	7.43
	411	72.2	75.2	70.9	73.7	68.20		73.00	2.68	66.2	71.2	57.6	74.3	70.0	67.9	6.43
	730	58.2	56.3	58.8	59.3	n/a		58.15	1.31	93.2	127.7	111.9	132.4	136.8	120.4	17.88
	836	n/a	40.2	83.3	42.3	49.3		53.78	20.06	48.6	47.1	37.3	54.1	54.7	48.4	7.02
	840	61.8	55.3	64.3	59.9	57.1		59.68	3.60	66.9	n/a	82.8	101.5	n/a	83.7	17.32
	853	58.8	63.8	58.9	61.0	61.6		60.82	2.08	66.5	65.7	98.6	67.1	113.2	82.2	22.23
	908	69.2	82.4	83.9	96.4	79.0		82.18	9.80	90.6	84.2	77.0	115.0	116.7	96.7	18.14
	938	58.0	68.5	69.7	72.7	70.8		67.94	5.77	n/a	67.2	57.2	82.8	78.6	71.5	11.56
	942	61.5	60.8	60.5	66.1	65.4		62.86	2.67	n/a	71.1	n/a	71.1	59.7	67.3	6.58
	945	73.0	79.4	81.0	88.9	78.9		80.24	5.71	54.2	n/a	n/a	42.2	79.0	58.5	18.77
	^953	65.6	72.6	69.9	69.6	65.4		68.62	3.08							
	Mean	63.90	68.98	71.30	71.63	68.72		69.19		68.19	73.01	70.26	78.36	81.45	73.55	
	STD	5.74	15.38	10.49	16.39	13.09		12.13		15.88	22.99	24.05	25.81	28.10	19.77	
Chicory	**68**	n/a	63.0	60.5	67.1	70.0		65.15	4.23	43.2	56.2	65.3	86.6	102.8	70.8	23.87
	455	47.9	63.8	56.2	64.0	65.5		59.48	7.42	41.7	39.9	54.4	72.5	76.5	57.0	16.98
	732	49.5	n/a	60.5	n/a	65.8		58.60	8.31	45.0	23.0	64.3	98.8	102.1	66.6	34.17
	859	n/a	n/a	50.9	63.6	57.9		57.47	6.36	n/a	89.5	62.7	89.2	107.8	87.3	18.56
	Deer A	51.3	64.0	66.6	52.3	65.2		59.88	7.44	30.3	98.8	63.3	83.2	99.3	75.0	28.98
	Deer L	87.4	84.1	90.8	84.4	110.0		91.34	10.78	55.9	65.0	61.8	82.2	104.0	73.8	19.52
	Mean	59.0	68.7	64.3	66.3	72.4		65.3		43.2	62.1	62.0	85.4	98.8	71.8	
	STD	19.0	10.3	14.0	11.6	18.8		13.0		9.1	28.9	3.9	8.7	11.2	10.0	
Plantain	**46**	19.1	16.5	25.2	31.8	30.2	30.6	25.57	6.48	60.1	47.3	50.0	51.1	49.7	51.6	4.93
	424	n/a	58.7	81.3	67.4	80.4	76.3	72.82	9.62	88.9	79.3	92.2	92.2	102.1	90.9	8.18
	***612**	n/a	3477.1	952.3	2275.2	2172.1	3025.6	2380.46	963.45							
	***408**									99.4	97.3	73.2	118.0	106.0		
	862	n/a	40.1	n/a	27.2	42.9	39.2	37.35	6.95	n/a	n/a	n/a	n/a	60.5	60.5	0.00
	T2	91.5	42.9	48.9	62.8	68.4	65.3	63.30	17.02							
	Deer P	n/a	n/a	33.7	38.4	42.2	52.5	41.70	8.00	45.0	49.8	n/a	55.6	77.7	57.0	14.45
	Deer S									n/a	12.3	24.3	39.1	41.7	29.4	13.71
	Mean	55.30	727.06	228.28	417.13	406.03	548.25	436.87		73.35	57.20	59.93	71.20	72.95	57.89	
	STD	51.19	1537.39	405.30	910.41	865.39	1213.76	952.32		25.16	32.65	29.36	32.82	26.97	22.10	

^ Deer 206 replaced deer 953 in May. * Deer 408 replaced deer 612 in May. n/a no sample collected.

(b) Methane grams per kg DMI.

			March (days)								May (days)							
Treatment	**Deer**	**DMI (g/d)**	**1**	**2**	**3**	**4**	**5**	**6**	**Mean**	**STD**	**DMI (kg/d)**	**1**	**2**	**3**	**4**	**5**	**Mean**	**STD**
Pasture	**54**	1708.6	35.5	42.8	38.5	41.5	36.3		38.9	3.17	2477.80	21.1	22.2	21.8	26.8	23.8	23.1	2.27
	^206										3002.7	n/a	n/a	n/a	22.1	18.5	20.3	2.52
	323	1615.52	n/a	62.0	54.9	61.2	60.8		59.72	3.25	1452.60	51.8	46.7	38.5	46.1	50.0	46.6	5.12
	411	2030.7	35.6	37.0	34.9	36.3	33.6		35.48	1.32	2155.30	30.7	33.0	26.7	34.5	32.5	31.5	2.98
	730	2101.6	27.7	26.8	28.0	28.2	n/a		27.67	0.62	2434.40	38.3	52.5	46.0	54.4	56.2	49.5	7.34
	836	1502.6	n/a	26.8	55.4	28.2	32.8		35.79	13.35	1537.80	31.6	30.6	24.3	35.2	35.6	31.4	4.56
	840	1179.2	52.4	46.9	54.5	50.8	48.4		50.61	3.05	1771.20	37.8	n/a	46.7	57.3	n/a	47.3	9.78
	853	1975.5	29.8	32.3	29.8	30.9	31.2		30.79	1.05	1872.80	35.5	35.1	52.6	35.8	60.4	43.9	11.87
	908	1850.9	37.4	44.5	45.3	52.1	42.7		44.40	5.29	2731.00	33.2	30.8	28.2	42.1	42.7	35.4	6.64
	938	1759.9	33.0	38.9	39.6	41.3	40.2		38.60	3.28	1880.90	n/a	35.7	30.4	44.0	41.8	38.0	6.15
	942	2059.1	29.9	29.5	29.4	32.1	31.8		30.53	1.30	1670.40	n/a	42.6	n/a	42.6	35.7	40.3	3.94
	945	1954	37.4	40.6	41.5	45.5	40.4		41.06	2.92	1809.60	30.0	n/a	n/a	23.3	43.7	32.3	10.37
	^953	1884.5	34.8	38.5	37.1	36.9	34.7		36.41	1.63								
	Mean	1801.84	35.33	38.89	40.74	40.41	39.35		39.16		2066.38	34.43	36.58	35.02	38.69	40 09	36.64	
	STD	268.56	6.87	9.90	9.97	10.40	8.87		9.02		492.57	8.28	9.22	11.22	11.26	12.74	9.38	
Chicory	**68**	2346.5	n/a	26.8	25.8	28.6	29.8		27.8	1.80	6437.94	6.7	8.7	10.1	13.5	16.0	11.0	3.71
	455	2501.99	19.1	25.5	22.5	25.6	26.2		23.8	2.97	4992.54	8.4	8.0	10.9	14.5	15.3	11.4	3.40
	732	2891.72	17.1	n/a	20.9	n/a	22.8		20.3	2.88	5508.40	8.2	4.2	11.7	17.9	18.5	12.1	6.20
	859	2771.99	n/a	n/a	18.4	22.9	20.9		20.7	2.29	5831.29	n/a	15.3	10.8	15.3	18.5	n/a	3.18
	Deer A	2876.5	17.8	22.2	23.2	18.2	22.7		20.8	2.59	7305.16	4.1	13.5	8.7	11.4	13.6	10.3	3.97
	Deer L	2300.4	38.0	36.6	39.5	36.7	47.8		39.7	4.68	6097.62	9.2	10.7	10.1	13.5	17.1	12.1	3.20
	Mean	2614.85	23.02	27.79	25.03	26.40	28.36		25.51		6028.83	7.31	10.07	10.38	14.35	16.49	11.38	
	STD	265.87	10.02	6.16	7.49	6.91	10.05		7.51		798.11	1.98	4.03	1.01	2.20	1.92	0.78	
Plantain	**46**	1866.2	10.2	8.8	13.5	17.0	16.2	16.4	13.70	3.47	753.92	60.1	47.3	50.0	51.1	49.7	51.6	4.93
	424	3369.1	n/a	17.4	24.1	20.0	23.9	22.6	21.61	2.86	2171.23	88.9	79.3	92.2	92.2	102.1	90.9	8.18
	***612**	1861.2	n/a	1868.2	511.7	1222.4	1167.0	1625.6	1278.99	517.65		99.4	97.3	73.2	118.0	106.0	98.8	16.42
	***408**										n/a							
	862	2711.8	n/a	14.8	n/a	10.0	15.8	14.5	13.77	2.56	3870.04	n/a	n/a	n/a	n/a	60.5	60.5	0 00
	T2	3400.7	26.9	12.6	14.4	18.5	20.1	19.2	18.61	5.01								
	Deer P	1986.4	n/a	n/a	17.0	19.3	21.2	26.4	20.99	4.03	1789.49	45.0	49.8	n/a	55.6	77.7	57.0	14.45
	Deer S										1409.65	n/a	12.3	24.3	39.1	41.7	29.4	13.71
	Mean	2532.5667	18.6	384.4	116.1	217.9	210.7	287.5	227.9		1998.87	73.35	57.20	59.93	71.20	72.95	64.71	
	STD	731.93495	11.78855	829.4917	221.1482	492.1412	468.5149	655.5758	514.9159		1169.61	25.16	32.65	29.36	32.82	26.97	25.87	

^ Deer 206 replaced deer 953 in May. * Deer 408 replaced deer 612 in May. n/a no sample collected.

Chapter 3: Methane production of red deer housed indoors and fed fresh perennial ryegrass-based pasture.

3.1. Introduction

For the purposes of the New Zealand Greenhouse Gas Inventory, methane production was measured from mixed-age red deer hinds grazing a ryegrass-based pasture in autumn 2003, using the SF_6 technique for the first time in deer, as described in Chapter 2. It was found that hinds produced an average of 71.5 grams per day and 37.8 g per kg DMI of methane per animal. These emissions, when expressed per kilogram of DM eaten, were 75 and 80% greater than mean values for cattle and sheep (respectively), reported by the New Zealand Greenhouse Gas Inventory. The values of methane emissions of sheep and dairy cows used in the New Zealand Greenhouse Gas Inventory are exclusively based upon all the published and unpublished experiments conducted in New Zealand using the SF_6 method (NIR, 2003). The emissions recorded in Chapter 2, expressed per kg DM intake, were also approximately double that of average methane emissions from red deer housed in calorimetry chambers, when fed a pelleted concentrate diet at maintenance or twice maintenance (Semiadi *et al.*, 1998).

The SF_6 technique has been used extensively for measuring methane production from sheep and cattle. Validation of the method has resulted in methane emissions using SF_6 being at least 90-95% of methane emissions measured in respiratory chambers (Johnson *et al.*, 1994; Pinares-Patino, 2000; Boadi *et al.*, 2002). The double n-alkane technique has also been used extensively for estimating the feed intake of grazing sheep and cattle, in conjunction with methane measurements using the SF_6 technique (Robertson & Waghorn, 2002; Ulyatt *et al.*, 2002b; Woodward et al., 2002). The double η-alkane technique was used in the experiment described in Chapter 2 to estimate feed intake, from which the methane emissions per unit feed intake were derived. However, this experiment raised some doubt as to the accuracy of the double n-alkane technique, particularly for deer grazing chicory. There have been no published reports validating the use of the double η-alkane

technique in red deer fed fresh forages. However, a Canadian study by Gedir and Hudson, (2000), found that the intake of wapiti hinds when fed an alfalfa-based compound diet using intra-ruminal controlled-release devices (CRD), was slightly overestimated by 6.1%, when using alkanes η-dotriacontane, ($C_{32}H_{66}$), and the adjacent natural odd-chain alkane η-monotriacontane, ($C_{31}H_{64}$).

It is assumed that the SF_6 technique is more reliable at determining methane emissions than the double η-alkane technique is at estimating feed intake. Therefore, the accurate measurement of individual feed intakes combined with methane measurements, using the SF_6 technique, of animals previously used for the determination of methane emissions in the grazing situation should assist in elucidating the high methane emission values obtained in Chapter 2. Therefore, the objective of this experiment was to accurately measure the feed intake of deer housed individually indoors when fed fresh pasture, whilst concurrently measuring enteric methane production using the SF_6 technique.

3.2. Materials and methods

3.2.1. Experimental design

Methane production was measured using 12 mixed-age red deer hinds also used previously in the grazing experiment and housed indoors whilst fed perennial ryegrass-based pasture. The experiment was conducted in Palmerston North, New Zealand at Massey University's deer metabolism facility from 20th August to 5th September 2003.

3.2.2. Animals

The 12-mixed age red deer hinds (102.5 ± 10.3 live weight kg and 4.6 ± 2.6 years old) used in the indoor experiment were from the commercial herd and had been previously used for the methane measurements described in Chapter 2. Hinds were pregnancy tested using ultrasound (Bingham *et al.,* 1990) on the 22nd May. Ten hinds were confirmed as being pregnant at this time, with two

hinds unable to be confirmed pregnant. As at 20th August hinds were at 90 ± 3.57 days' gestation, according to days' gestation estimated by ultrasound on the 22nd May. The finish date for this experiment of 5 September was set to be before the hinds went into their third trimester of pregnancy. The Massey University Animal Ethics Committee, with the condition that hinds were removed from the metabolism cages on entering the third trimester of pregnancy, gave approval for the experiment.

Hinds were selected on temperament from behaviour during the previous methane experiment and perceived likelihood of adaptation to metabolism cages. However, hinds from the ryegrass-based pasture treatment of the grazing trial were preferred over hinds from other treatment groups to allow a better comparison of measurements made whilst grazing and indoors on a similar diet.

As none of the hinds had previously been housed in metabolism cages, all hinds underwent a training period. However, due to this occurring in mid-winter when pasture supply was limited, hind's diet was supplemented with lucerne chaff ('Chaffage', The Great Hage Company) and deer-nuts whilst on a maintenance pasture grazing allocation prior to initial housing. Hinds were housed individually in metabolism cages indoors and fed solely chaff and nuts for 5 days. Deer were then returned to the grazing situation and fed ad libitum pasture only for 3 days. Then they were brought back inside for an 11-day adjustment period to handling procedures and experimental diet before the start of the methane measurements, during which time they were fed cut ryegrass-based pasture as described below. Three days before the start of the measurement period methane halters were fitted. During methane measurements the size of the metabolism cage was adjusted using a sliding cage side to prevent animals from turning around and tangling tubing. However, animals were not prevented from lying down during this time.

3.2.3. Diet and intake

During the experiment animals were fed a tetraploid perennial ryegrass-based pasture (cv. Quartet), which was cut daily using a sickle bar mower, in the morning before the first feeding, and stored at an ambient temperature (at approximately 11°C), for the afternoon feeding.

Hinds were fed ad libitum during the methane measurement period, with 15 kg wet weight or 2.07 kg DM offered per animal per day. This ad libitum feeding level was determined during the adjustment period, where the feed offered daily was increased until individual deer consumption reached a plateau. Deer were fed twice a day, 0830 hr and 1630 hrs, when deer received half of their daily ration. All animals also received ad libitum access to water.

Total wet feed intake was determined by weighing the wet feed offered less the feed refused from the bin and sweepings. Sweepings were any pasture in the cage, water bucket or on the floor (all pooled) spilt by the deer during the interval from one feeding to the next, but pooled per animal per day for weighing.

3.2.4. Forage sampling

During the methane measurement period triplicate 200g samples of feed offered were taken daily for DM determination and a 200g sample of feed offered taken daily for both botanical composition and chemical analysis. Feed refused samples for each deer were taken from well-mixed feed remaining in the bins only, as sweepings were likely to be slightly contaminated with hair and dust. Of the individual feed refused daily, triplicate 200g samples were taken for DM determination and two 200g samples were taken and pooled separately per animal for botanical composition and chemical analysis. DM samples were dried daily at 100°C for 24 hrs. The samples of botanical composition were dissected into grasses, legumes, weed and dead. Each component was oven dried at 60°C for 24 hrs.

3.2.5. Laboratory Analyses

The samples for chemical composition analysis were stored frozen until oven drying (60°C for 48 hrs) and grinding. Samples were ground to pass a 1 mm sieve (Willey Mill, USA). Forage samples were analysed as described in Chapter 2, section 2.3.7. Metabolic energy, starch and soluble sugars and organic matter digestibility were estimated by NIR, whereas organic matter, NDF, ADF, lignin and carbohydrates were analysed by wet chemistry (as per chapter 2).

3.2.6. Methane measurement

Enteric methane production was determined using the SF_6 tracer method, as developed by Johnson et al., (1994) and modified according to Ulyatt et al., (1999) and Ulyatt et al., (2002b) as described in Chapter 2 section 2.3.5. The yokes were attached externally at the rear of the cages (Plate 1). Sampling of the ambient air was done by the placement of four yokes and halters in the centre of each wall of the deer shed, at approximately the same height as the deer. The four background yokes were not all equidistance from deer or sources of ventilation. Windows and air vents along the walls of the shed -as well as two roof extraction fans- provided ventilation of the shed, which was important to maintain mixing of the ambient air in the shed and avoid the build-up of methane or SF_6 in pockets throughout the shed. All hinds used in the experiment already had a permeation tube inserted into the rumen from the grazing trials; it was assumed that the permeation tubes were still emitting a constant rate of SF_6.

Plate 3.1 Hind housed in a metabolism cage and wearing methane-collecting apparatus, with the yoke attached near the rear of the sliding door of cage.

3.2.7. Statistical analysis

Data were analysed using the mixed model procedure in SAS (SAS, 1998). Type of forage (ryegrass-based pasture) and time of measurement (grazing experiment (Chapter 2) vs. indoor experiment) were fixed effects in the model, and the experiment was conducted by way of a repeated measurement with compound symmetry covariance structure. The random statement included deer within forage type. The model is in Equation 1 below.

$$Y_{ijk} = \mu + F_i + D_{j(i)} + E_k + (D \times E)_{ik} + e_{ijk}$$

(Equation 1)

Where μ = overall mean; F_i = fixed effect of forage type (i = 1); $D_{j(i)}$ = random effect of deer within forage type (j = 1 to 12); E_k = fixed effect of experiment of measurement analysed as repeated measurement (k = 1 to 3); $D_i \times E_k$ = fixed

effect of interaction between deer and experiment of measurement; e_{ijk} = random residual error, assumed to be normally distributed.

Values reported for the comparison of the experimental data are least square means; all other values reported are the mean and either the standard error of the mean (SEM) or standard deviation of the sample (STD), as stated. Significance was declared at $P \leq 0.05$, and a trend was reported if $0.05 < P \leq 0.10$. All mean comparisons were by Fisher's least significant difference method after a significant treatment main effect was detected.

3.3. Results

3.3.1. Forages

Table 3.1, shows that the pasture offered was found to have a high proportion of ryegrass leaf and very little clover, weed or dead matter. The ryegrass was in a totally vegetative state with no reproductive stem material present. There was some dietary selection of the pasture components, as the feed refused by the hinds appeared to have a greater proportion of dead material and clover and a lower leaf proportion than the feed offered, suggesting that hinds selected against the dead and clover components of the pasture.

Table 3.1. Botanical composition of pasture offered and feed refused.

	Botanical composition %DM			
	Leaf	clover	Weed	Dead
Feed offered (n=15)	88.3	0.3	0.0	11.3
Feed refused				
Deer (n=10)				
46	81.8	0.9	0.0	17.3
54	71.3	0.5	0.0	28.1
206	69.8	0.6	0.0	29.6
411	83.6	0.8	0.0	15.5
455	84.1	0.4	0.0	15.5
730	80.8	0.5	0.0	18.7
836	83.8	0.2	0.0	15.9
853	80.9	0.7	0.0	18.3
908	59.8	0.6	0.0	39.6
938	75.2	0.6	0.0	24.2
942	82.2	0.7	0.0	17.1
954	82.7	0.6	0.0	16.7
Mean[1]	78 ± 2.2	0.59± 0.17	0.0	21.4 ± 6.2

[1]Mean ± STD

The nutritional components of the pasture fed and refused are shown in Table 3.2. The ryegrass pasture fed to the hinds was estimated, by NIR, to have an organic matter digestibility of 80.1% and a metabolisable energy content of 11.3 MJ per kg of DM. There were found to be only minor differences between the nutrient composition of pasture offered and refused. The average proportion of DM of the pasture offered was 13.8%.

Table 3.2. Nutrient composition of pasture offered and refused during the methane measurement period.

% DM	Feed offered[3] *(n = 4)*	Feed refused[3] *(n = 12)*
Organic Matter	87.7 ± 0.9	85.0 ± 0.8
NDF	45.0 ± 1	45.0 ± 1.0
ADF	26.0 ± 0.7	27.1 ± 1.0
Lignin[1]	1.72	-
Hemicellulose	19.1 ± 1.25	17.9 ± 0.41
Cellulose	24.2 ± 0.7	25.4± 1.0
Carbohydrates[5]	8.7 ± 0.75	7.2 ± 0.3
SSS[2, 4]	10.7 ± 1	-
Crude protein	16.8 ± 0.8	16.0 ± 0.2
Organic matter digestibility[2]	80.1 ± 0.7	-
ME (MJ /kg DM)[2]	11.3 ± 0.1	-
GE (MJ/kg DM)[1]	18.02	-

[1] Composite samples, [2] Estimated by NIR, composite sample.

[3] Mean ± standard error of the mean

[4] SSS = Starch and soluble sugars

[5] Carbohydrates includes starch and soluble sugars

3.3.2. Dry matter intake

The average amount of pasture offered per day was 2.07 kg, which resulted in deer being offered 23.4 MJME per day. Deer were found to consume an average of 1.55 ± 0.3 kg of DM per day, resulting in an estimated metabolisable energy intake of 17.5 ± 3.4 MJ per day. Deer grazing pasture in the grazing

experiment were estimated to consume an average of 1.97 kg DM per day, which was significantly (P = 0.0001) greater than the deer in the indoor trial (Table 3.4). Dry matter intake of deer in both the grazing experiment and indoor experiment are shown in Appendices 3.6, Table 3.6.

3.3.3. Animals

Two animals from the grazing experiment (Chapter 2) who were allocated to the pasture treatment were not included in the indoor experiment because behaviour traits rendered them unlikely to become adapted to being housed indoors without becoming highly stressed. The exclusion of these animals from the indoor experiment was not thought to affect the mean methane production per day as the methane measured per day from these two animals were found to be randomly distributed amongst the treatment group in May, although one animal was an outlier in March. To maintain the constant treatment numbers, two deer that were allocated to the other treatment groups in the grazing experiment were used as replacements for the deer that were excluded from the indoor experiment. The mean methane measured from these deer was also found to be randomly distributed amongst the treatment group. Therefore, it was assumed that the presence of these deer did not alter the mean methane production (CH_4/d) of the treatment group. The mean methane emissions as per day for each individual animal are shown in Appendices 3.6, Figure 3.1.

Animals were found to lose an average of 51 ± 156 (STD) grams bodyweight per day over the adjustment and methane measurement periods combined, with the largest proportion of weight loss accruing in the adjustment period when hinds lost an average 943 ± 329 g/day. Bodyweight loss for the grazing experiment (Section 3.3.5, Table 3.4) was significantly (P = 0.0001) greater (-267.62g/d) than for animals housed indoors. The interaction of deer and experiment showed that all deer, except one, in both experiments had a significantly greater weight loss in the grazing experiment. However, as animals were pregnant in the indoor experiment they were expected to be gaining weight due to fetal growth, in contrast to the animals in the grazing experiment, where methane was measured pre and post mating, and the live weights of the deer were not likely to be affected by pregnancy.

Table 3.3. Mean (± SEM) daily methane production and dry matter intake (DMI) of hinds fed ryegrass pasture indoors.

Deer	46	54	206	411	455	730	836	853	908	938	942	945	Mean	SEM
Daily CH_4, g/d														
Day 1	34.1	30.4	54.5	15.4	26.9	36.6	19.6	27.2	n/a	n/a	20.1	48.8	31.4	3.6
Day 2	28.8	n/a	51.5	24.2	26.9	39.6	30.6	28.6	41.0	n/a	29.6	45.7	34.7	2.6
Day 3	34.9	31.1	45.8	29.0	18.7	35.0	32.3	30.1	43.8	24.4	31.8	29.1	32.2	2.1
Day 4	36.6	37.3	69.4	30.5	27.3	45.4	37.8	33.1	47.8	27.2	34.5	n/a	38.8	3.5
Day 5	34.0	33.9	51.5	30.8	29.0	44.2	28.5	35.0	49.9	34.8	40.0	25.3	36.4	2.4
Mean CH_4, g/d	33.7	33.2	54.5	26.0	25.8	40.1	29.8	30.8	45.6	28.8	31.2	37.2	34.7	2.9
Standard deviation	2.9	3.1	8.9	6.5	4.0	4.6	6.6	3.2	4.0	5.4	7.3	11.8	-	-
Mean DMI, kg/d	1.46	1.49	1.61	1.49	1.32	1.28	1.28	1.43	1.97	1.54	1.52	1.69	1.55	0.05
Mean NDFI, g/d	585	671	725	671	594	576	526	644	887	693	684	761	668	27
CH_4/DMI, g/kg	23.1	22.3	33.8	17.4	19.5	31.3	23.3	21.5	23.1	18.7	20.5	22.0	23.0	1.4
CH_4/NDFI, g/kg	57.6	49.3	75.2	38.7	43.4	69.6	56.7	47.8	51.4	41.6	45.6	48.9	52.2	3.2
CH_4, g/kg BW/d	0.38	0.37	0.57	0.26	0.24	0.37	0.34	0.33	0.38	0.29	0.33	0.40	0.36	0.02
CH_4, kJ GE/d	1854	1826	2998	1430	1419	2206	1639	1694	2508	1584	1771	2046	1915	134
CH_4, % of GEI	7.0	6.8	10.3	5.3	6.0	9.6	7.1	6.6	7.1	5.7	6.5	6.7	7.1	0.4

n/a No sample collected

3.3.4 Methane production

Methane production per day is shown in Table 3.3, with an average methane production per day of 36.4 g recorded. When methane production was expressed per kg of DM eaten, the average methane production was 23.0 g CH_4/kg DMI, with methane production ranging from 17.4 g CH_4/kg DMI to 33.7 g CH_4/kg DMI for individual deer. The average gross energy lost as methane was 1915 KJ per day, therefore resulting in a mean value of 7.1% of the gross energy intake being lost as methane.

3.3.5 Deer grazing pasture versus deer housed indoors

Dry matter intake and methane production of deer housed indoors compared with deer grazing pasture is shown in Table 3.4. The DM intakes of deer grazing pasture were found to be greater than when the same deer were housed indoors and fed cut pasture (Chapter 2, section 2.4.4). The average estimated organic matter digestibility, metabolizable energy, CP and NDF of the pasture offered in the grazing experiment were 66.9%, 9.7 MJ kg, 18.7 % DM and 56.9 % DM, respectively. In comparison, metabolisable energy, CP and NDF of the pasture offered in the grazing experiment were 80.1%, 11.3 MJ kg, 16.8 % DM and 45.0 % DM, respectively.

Methane production as measured from animals housed indoors (34.7 g/d) was found to be significantly lower than during the grazing experiment (71.4 g/d). Methane production expressed per kg of DM eaten was significantly (P = 0.0001) lower for animals housed indoors (22.5 g/kg DMI/d) compared with grazing (37.3 g/ kg DMI/d).

When looking at individual animal data for animals fed pasture in both experiments (Appendix 3.6, Table 3.6), all animals except one produced significantly (P = 0.0001) lower methane per day when housed indoors compared with grazing. When expressed as grams of methane per kg of DMI,

methane production from all animals, except two, exhibited significantly lower methane (P = 0.02) production indoors.

Table 3.4. A comparison of dry matter intake, body weight, body weight change and methane production of deer firstly grazing, and then being fed perennial ryegrass-based pasture indoors.

	Grazing[1]	Indoor[2]	SED	Expt[4] P-values	Expt[4] x Deer P-values
Body weight, kg	106.08	101.96	0.34	0.0001	0.0001
Body weight change, g/d	-267	-51	10	0.0001	0.0001
DMI, g/d	1973	1557	37.5	0.0001	0.0001
GEI kJ/d	36902	28060	724	0.0001	0.0001
CH_4, g/d	71.5	34.7	2.5	0.0001	0.0015
CH_4/DMI g/kg	37.3	22.5	1.2	0.0001	0.0001
CH_4, % of GEI	11.0	6.9	0.4	0.0001	0.0001
NDFI, g/d	1121	700	19	0.0001	0.0001
CH_4/NDFI, g/kg	65.5	50.0	2.23	0.0001	0.0001
CH_4, g/kg BW/d	0.67	0.34	0.02	0.0001	0.15

[1]Grazing experiment, chapter 2

[2]Indoor experiment, this chapter.

[4] Expt, represents grazing vs. indoor experiment

Neutral detergent fibre intake (NDFI) -which represents the amount of cellulose, hemicellulose, and lignin in the forage intake per day- was significantly lower in the indoor experiment (700 g/d) than in the grazing experiment (1121 g/d) (P = 0.0001), with all animals having a significantly lower intake of NDF when housed indoors compared with grazing. Methane per kg of NDFI was also found to be significantly lower in the indoor experiment compared with the grazing experiment. Methane expressed per kg of body weight was significantly lower in the indoor experiment (P = 0.0001), however the interaction of deer with experiment was not significant (P = 0.15).

3.4. Discussion

Methane produced per day from hinds indoors was 51% less per day and 38% less per kilogram of dry matter eaten than for hinds grazing pasture (Chapter 2). Methane emissions per kg of DMI were 27% greater than the calorimetry chamber measurements of Semiadi *et al.*, (1998), and were closer to the estimated value (21.25 g CH_4/ kg DMI) used in the inventory (NIR, 2003). Table 5 shows methane production data from grazing trials of ruminants consuming ryegrass-based pastures compared with indoor trials. This table shows that for sheep and cattle, respectively, methane production measured indoors was 45 and 18% higher than in the grazing situation, with no obvious explanation for the difference (O'Hara *et al.*, 2003). This is in stark contrast to the current results from deer.

Table 3.5. Summary of experiments measuring methane using the SF_6 technique in NZ from ewes, wethers and cows grazing or housed indoors fed a ryegrass-based pasture (Lassey *et al.*, 1997; Lassey *et al.*, unpublished; Pinares-Pantiño, 2000; Ulyatt *et al.*, 2002a; Waghorn *et al.*, 2002; Woodward *et al.*, 2002; Pinares-Pantiño, 2003a; Pinares-Pantiño, 2003b; In O'Hara *et al.*, 2003).

			Dig[1] (kg)	DMI (kg/d)	CH_4 (g/d)	CH_4/DMI (g/kg)	CH_4/DDMI (g/kg)	CH_4, % GE
Ewes	*(n=4)*[4]	*Grazing*	77.8 ± 2.6[2]	1.55 ± 0.1	29.7 ± 1.4	19.6 ± 1.6	25.4 ± 2.7	6.0± 0.41
		Indoors	-	-	-	-	-	-
Wethers	*(n=9)*	*Grazing*	78.6 ± 1.0	1.77 ± 0.14	26.6 ± 1.9	15.7 ± 0.9	20.0 ± 1.2	4.7 ± 0.3
	(n=2)	*Indoors*	73.5 ± 0.5	1.19 ± 0.07	26.8 ± 1.9	22.8 ± 2.9	31.0 ± 3.7	6.9 ± 0.9
Cows	*(n=8)*	*Grazing*	79 ± 1.0	16.6 ± 0.7	340.1 ± 20.5	20.7 ± 1.0	26.2 ± 1.4	6.1 ± 0.3
	(n=1)	*Indoors*	66	10.7	260	24.6	37.3	7.2
Deer[3]	*(n=1)*	*Grazing*	66.9	1.95	71.5	37.8	55.0	11.12
	(n=1)	*Indoors*	80.1	1.55	34.7	23.0	28.0	7.1

[1]Dig = DM digestibility, [2] mean ± (SE), [3] This thesis, [4] n, represents the number of experiments.

There is no obvious explanation for the difference of methane production of grazing animals versus those housed indoors. In this study, the indoor experiment compared with the grazing experiment was found to have less methane:SF_6. When using the equation developed by Johnson *et al.*, (1994) an increase of SF_6 in the ratio of methane: SF_6 will effectively result in a depression of calculated methane emissions, where a decrease of SF_6 will cause an elevation of calculated methane. Accumulative factors that could possibly result in some differences in calculated methane emissions and the measured concentration of SF_6 and methane are suggested as follows. Firstly, when measuring enteric methane from ruminants, it is assumed that SF_6 is released from the permeation tube at a constant rate for the life of the tube as described in Chapter 1, section 1.2.3. As there was a lower concentration of SF_6 collected in the yokes in the grazing trial, there could be some unknown factor in the rumen that could have either depressed that rate of SF_6 release from the permeation tube or the rumen. However, it is unlikely that the rumen and rumen environment of deer differ sufficiently either over time or from those of sheep and cattle to depress SF_6 enough to cause an inflated estimate of methane emissions

Secondly, the SF_6 technique relies on the collection of two gases. It is assumed that emission of SF_6 exactly simulates the emission of CH_4 and that the rate of the dilution of the gases is the same. Grounds for this assumption depend on the idea that the diffusion of gases is more affected by turbulence than by molecular weight, therefore gases of different weight experiencing the same degree of turbulence will have the same rate of diffusion (Johnson *et al.*, 1994). The molecular weight of SF_6 is nine times that of methane (Ulyatt *et al.*, 1999), which could affect the efficiency of the collection of either of the gases, as reported by Ulyatt *et al.*, (1999). There does not seem to be evidence of different molecular weight affecting the sampling of expired gases. However, the different weight of the gases may affect the ratio of SF_6 and methane in the ambient air, as collected by background yokes. In the equation used to calculate methane emissions the concentration of SF_6 and methane is corrected against the concentration of SF_6 and methane in the surrounding environment. Therefore if there is stratification of the gases, the concentrations collected by

the background yokes will depend on their placement in respect to the animals and all other sources of methane and SF_6. As SF_6 is heavier than methane, SF_6 could accumulate around the animal and cage while methane, a lighter gas, dissipates more quickly, especially in the metabolism cages, which would result in a higher concentration of SF_6 collected by the animal yokes, resulting in a calculated decrease in methane compared with grazing animals. To account for the accumulation of SF_6 around the animal in a second indoor trial (Chapter 4) background yokes were placed so that air inside the cages was collected. There was found to be no difference of calculated methane using ambient air from either within the cages or from the backgrounds placed in the shed.

Another assumption of the SF_6 technique is that the major route for methane excretion is via the mouth and nose, however this assumption is based upon one study conducted with four sheep fed 800g of lucerne chaff per day (Murray *et al.*, 1976; Ulyatt *et al.*, 1999) (Chapter 1, section 1.2.3). However, this has been done in a limited number of trials, and there is very little known about either species differences or the effect of diet or season upon the sites of methane production and excretion. It has been established by Domingue *et al.*, (1991) that deer have a greater seasonal change of DMI and digestive physiology than do sheep (as discussed below). Therefore, it could be suggested that the proportion of methane produced in the rumen or lower digestive tract may change with time (season), which could result in a change of the proportion of methane expired or excreted. If a greater proportion of methane is excreted as flatus, some of this may be picked up in the background yokes, especially in an indoor experiment, which would then be subtracted from the concentration of methane in the animal yokes, further decreasing the calculated methane production. It is also not known if the nutrient component of the diet (e.g. organic matter digestibility and fibre content) affects the site of digestion and/or the site of methane production. Therefore, it could be suggested that the combined change of digestive physiology in response to seasonality and a change in feed quality- from 69% to 80% organic matter digestibility in the indoor experiment- could have resulted in a shift in the site of methane production and possibly the proportion of methane expired via the mouth and nose. Research needs to be conducted examining the sites of

methane production and the proportion of methane expired via the mouth and nose.

Pasture (tetraploid) consumed in the indoor experiment compared with that fed in grazing experiment (diploid) was higher in estimated apparent digestibility and had a lower fibre content (NDF). Digestibility and fibre have been shown to affect the methane production of animals. Increasing the fibre content when animals are fed maintenance or above maintenance causes a shift in the ratio of propionate and acetate production towards increased acetate production, which has been shown to increase the production of methane (Blaxter & Clapperton, 1965; McAllister *et al.*, 1996). Blaxter and Clapperton (1965) have also shown that methane production is expected to decrease with increasing digestibility; presumably, this may be a response to the decreasing fibre and increasing soluble sugars in feeds with higher apparent digestibility. Thus, it may be reasonable to suggest that the higher digestibility and lower fibre content of the pasture fed indoors, compared with pasture fed to the grazing animals, may have, in part, resulted in the lower methane production per animal and per unit of feed eaten.

Deer are highly seasonal in voluntary feed intake, where the intake and nutrient requirements peak in summer when lactating adult hinds would be expected to require between 47-49 MJ ME per day (Fennessy et al., 1981; Shin et al., 2000; Mulley, 2003) and consume approximately 4 kg DM per day. However, in winter nonlactating hinds are expected to consume 1.7 kg DM or require 22 MJ ME per day. Domingue *et al.*, (1991) found that the digestive physiology of deer exhibits seasonal changes and this may also partly account for some of the differences observed between the grazing trial and indoor trial. Deer were found to increase their total rumen pool size from winter to summer, leading to no decrease in apparent digestibility with greater feed intake as occurs in sheep and goats. It was also found that the fractional outflow rate (FOR) of particles and liquid from the rumen in deer changed significantly from summer to winter. This led to the overall conclusion by Domingue *et al.* (1991) that deer exhibit a greater seasonal change in intake and digestive physiology than sheep. Methane production from sheep has been correlated with rumen pool size and

FOR. Pinares-Patino *et al.*, (2003) found that methane production is positively correlated to the rumen pool size of organic matter and negatively correlated to the rate of FOR. This suggests that seasonal changes in the digestive physiology of deer may be associated with seasonal changes in methane production. Therefore, further indoor research needs to be undertaken to investigate the seasonal production of methane in deer and the digestive physiological changes that occur, which may result in changes of methane production.

Given that the average dry matter intake of hinds indoors was 1.55 kg DM per day, then hinds were consuming an estimated average metabolisable energy intake of 17.5 MJ per day. This is lower than the recommended 24 MJ per day for maintenance (Fennessy *et al.*, 1981; Shin *et al.*, 2000) and 28 MJ ME per day (Jermy, 2003; Mulley, 2003) for hinds in the second trimester of pregnancy and consuming approximately 2.0 kg DM per day during mid pregnancy and 2.3 kg DM in late pregnancy (Shin *et al.*, 2000) . However, hinds were expected to consume less than they would outside, as they are not subjected to winter environmental conditions, and are not searching for food, and may not require as much energy for maintenance in these respects. However, during the housing period the deer lost on average 51 ± 156 g/d bodyweight, suggesting that on average the level of feed intake achieved in this experiment was below maintenance for these animals. Therefore, despite prior training and adjustment to housing, given the short housing intervals involved, it is most likely that stress at housing and intensive handling procedures prevented some animals from achieving a maintenance level of feed intake. Blaxter and Clapperton, (1965) found that on very low apparent digestible diets (~40-60%) the amount of feed consumed by an animal is almost independent of the methane production (kcal/100 kcal feed). However, when animals are fed diets with a greater proportion of the dietary energy being apparently digestible, intake above maintenance has a negative effect on methane production (kcal/100 kcal feed). Therefore, although the animals apparently ate more in the grazing trial, the low estimated digestibility of the organic matter may have resulted in methane production being higher than expected for DMI consumed.

Results from the indoor experiment imply that methane production data from both housed and grazing deer should be viewed with caution when applied to the inventory of GHG. However the methane measured suggests that methane production from deer is greater than the estimated value of 21.3 g CH_4/kg DMI (NIR, 2003). Both these experiments should be repeated, preferably at several times during the year in the same animals, to confirm the current findings and investigate the possible influence of seasonality on methane production.

3.5. Appendix

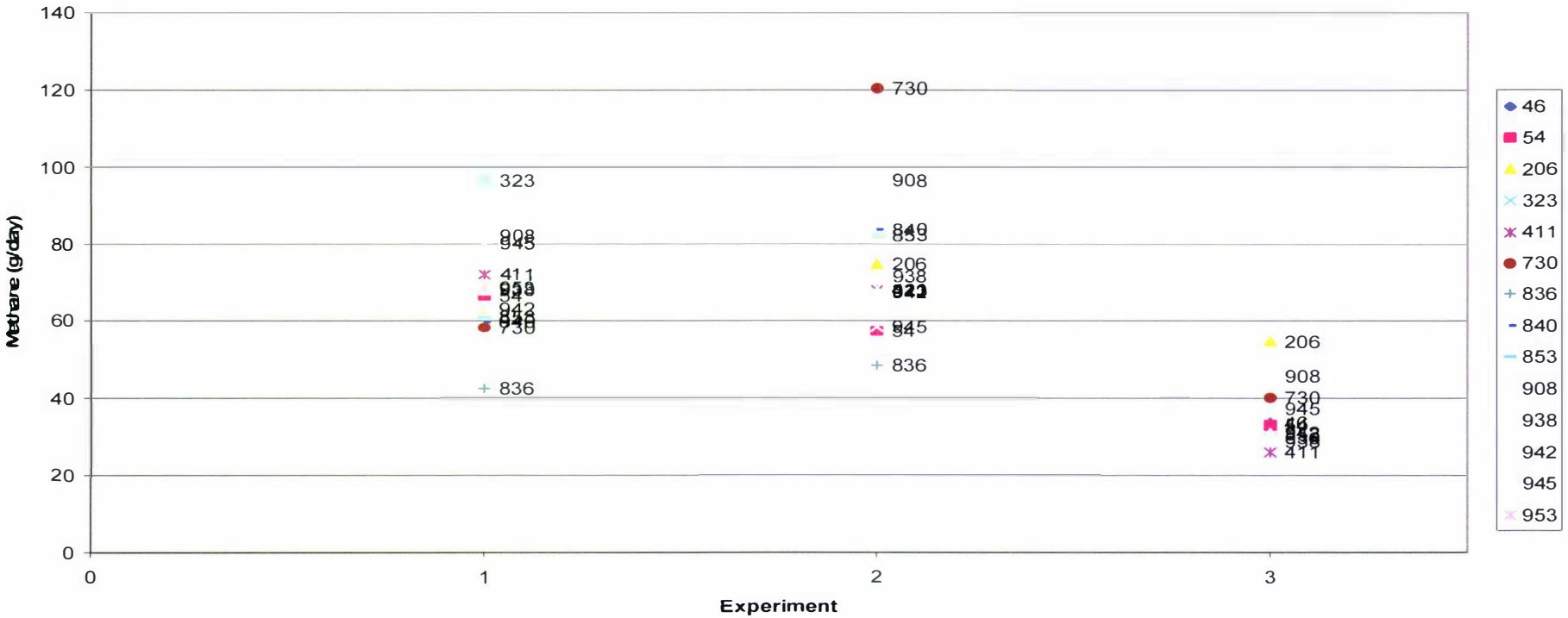


Figure 3.1. Mean enteric methane emissions (g per day) from deer fed pasture from the grazing and indoor experiments. 1 and 2 represent the grazing experiment where 1 represents the trial in March and 2 represents the trial in May and 3 the indoor experiment. Deer 323 and 840 were not included in the indoor experiment and were replaced with deer 46 and 455.

Table 3.6. A comparison of the DMI, methane production per day and per kg of DMI of deer feed pasture in the grazing and indoor experiments.

	DMI (g)				Methane (g/d)				Methane/DMI (g/kg)			
Deer	Grazing	Indoor	SED	*P*-values	Grazing	Indoor	SED	*P*-values	Grazing	Indoor	SED	*P*-values
46[1]	-	1464			-	34.7			-	23.2		
54	2093	1538	129	0.0001	61.9	33.2	7.9	0.0004	31.0	21.6	3.9	0.017
206	3003	1612	148	0.0001	74.7	54.5	11.2	0.073	24.9	33.7	5.5	0.11
323[1]	1534	-			80.5	-			52.5	-		
411	2093	1494	129	0.0001	69.9	25.98	7.3	0.0001	33.5	17.4	3.6	0.0001
455[1]	-	1318			-	25.8			-	19.9		
730	2268	1718	129	0.0001	92.73	40.2	7.4	0.0001	39.8	23.6	3.7	0.0001
836	1520	1280	129	0.06	45.8	29.8	7.4	0.0332	30.1	24.1	3.6	0.11
840[1]	1475	-			68.7	-			49.4	-		
853	1925	1434	129	0.0002	71.5	30.8	7.3	0.0001	37.3	23.4	3.6	0.0002
908	2291	1970	129	0.0136	89.5	45.6	7.9	0.0001	39.9	22.8	3.9	0.0001
938	1820	1646	129	0.18	69.5	28.8	8.9	0.0001	38.3	17.0	4.4	0.0001
942	1865	1524	129	0.009	64.5	31.2	7.6	0.0001	34.2	20.3	3.8	0.0003
945	1882	1688	129	0.13	72.1	37.2	8.2	0.0001	37.8	23.2	4.1	0.0005

[1]Deer 323 and 840 were not included in the indoor experiment and were replaced with deer 46 and 455.

Chapter 4: Validation of the double η-alkane procedure to estimate the dry matter intake of red deer fed fresh pasture or plantain.

4.1 Introduction

Accurate determination of dry matter intakes of individuals or groups of animals is essential to the measurement of methane emissions and nutritional research to enable valid comparisons across treatments, trials and species. The dry matter intake of animals housed indoors can be determined by the simple measurement of feed offered less feed refused. However, the housing of animals indoors may cause behavioural changes which could influence normal intake as compared with the grazing situation. In addition, when feeding fresh forages indoors, the simple act of harvesting the forage and presenting it in the cut form is therefore likely to give different results from the grazing situation where animals harvest the plant material themselves. The estimation of the feed intake of individual grazing animals is difficult and indirect measurements are normally employed, such as indigestible markers, for example chromium oxide, or indigestible components of the feed eaten such as lignin or alkanes.

The method of using n-alkanes, saturated long-chain hydrocarbons of plant cuticular wax, named the *double n-alkane technique*, has been developed and refined by Dove and Mayes, (1991, 1996). This technique employs both naturally occurring η-alkanes (odd-chain alkanes) of forage species and orally-administered synthetic (even-chain) alkanes. To accurately measure intake this method relies on the assumptions that herbage samples taken for laboratory analyses are representative of the forage consumed and that the dosed alkane and its adjacent naturally occurring alkane are recovered in the faeces in similar proportions (Dove & Mayes, 1991). Faecal recovery of alkanes has been found to be incomplete due to limited absorption of the alkanes in the digestive tract. However, it is assumed that modification or absorption of the alkanes is small, as faecal recoveries of dosed alkanes and their adjacent naturally occurring alkane are similar (Dove & Mayes, 1991; Dove *et al.*, 2002). Furthermore, Hendricksen *et al.*, (2002) found that the recovery rates of adjacent n-alkanes

were highly variable when brahman-cross cattle were fed several diets containing tropical grasses (buffel-grass fresh or as hay) and lucerne hay. The low n-alkane content of the lucerne hay (C_{35} <10mg/kg) used by Hendrickson *et al* (2002) also resulted in poor calculation of the faecal recovery rates and hence intake.

The accurate measurement of intake is important in the measurement of methane emissions, as methane per kg DMI forms the basis of the inventory of ruminant greenhouse gas emissions and allows for comparison of methane emissions between trials and animal species. The double n-technique has been used as a measurement of intake in studies measuring the methane production of grazing sheep and cattle (Robertson & Waghorn, 2002; Ulyatt *et al.*, 2002b; Woodward *et al.*, 2002). However, when intake was measured during concurrent measurements of methane production using η-alkanes in red deer grazing ryegrass-based pasture, chicory and plantain (Chapter 2) the accuracy of the intake measurements was questioned, due to the high estimation of the DMI of chicory.

Currently there are no published data validating the measurement of the intake of red deer consuming fresh forages as measured by the double n-alkane technique. However, validation studies have been conducted with fallow and wapiti deer fed dried, alfalfa-based diets. The actual intake of nine male fallow deer, eight months of age, fed lucerne chaff was found to be not significantly (P=0.05) different from estimated intake. However, numerically the DMI of the fallow deer was underestimated by 11%, as deer fed lucerne chaff actually consumed 394g/d, and estimated DMI showed that deer consumed 354g/d (Ru *et al.*, 2002). In contrast, the measurement of intake vs. estimated intake of eight wapiti hinds fed an alfalfa-based compound diet overestimated the true intake by 6.1% (C_{31}:C_{32}) and 2.2% (C_{33}:C_{32}) (Gedir & Hudson, 2000).

Due to uncertainty concerning the accuracy of estimated intakes of grazing red deer (Chapter 2), the overestimation of intake by wapiti hinds (Gedir & Hudson, 2000) and underestimation of intake by fallow deer (Ru *et al.*, 2002) using the double η-alkane technique, and the fact that this technique has not been

validated for deer consuming fresh forages, the validation of this technique in red deer fed fresh forage is needed. Therefore, the objectives of this study were to evaluate the double η-alkane technique for estimating the dry matter intake of housed adult red deer fed either ryegrass-based pasture or plantain, while also concurrently measuring methane production.

4.2 Materials and methods

4.2.1 Experimental design

The validation of the double η-alkane technique was conducted at the deer metabolism facility at Massey University's Deer Research Unit, September to October 2003. The six week experiment involved six adult castrated red deer fed ryegrass-based pasture and plantain in two periods in a cross-over design, with each period lasting 3 weeks. Five animals were employed in the validation of the η-alkane technique and methane emissions were concurrently measured using the SF_6 technique in 6 animals.

4.2.2 Animals

Six adult hand-reared castrated red deer stags were used (8.7 ± 6.1 (STD) years of age and weighing 146.6 ± 55.6 (STD) kg), five of which had rumen cannulae (four also with abomasal cannulae), and one of which was non-surgically modified. Animals were randomly allocated to one of two groups: Group A (*n*=4) in the first period were fed pasture and Group B (*n*=2) were fed plantain, with the diets changed over for the second period. Unequal allocation of animals to groups was because plantain supply at the trial start was potentially limiting. One animal in Group A, who was nonsurgically modified, was used to measure methane production only. Animals in group B took longer to adapt to housing than did animals in group A, therefore period 1 for group B was repeated following period 1 once feed intakes had stabilised. Each period consisted of three weeks as shown below;

Period 1 and 2

Week 1. Animals grazing their allocated diets outdoors ad libitum.

Week 2. Adjustment period for animals to housing in metabolism crates and fed cut treatment diet forage ad libitum.

Week 3. Measurement period.

4.2.3 Diets and actual intakes

The forages fed consisted of permanent perennial ryegrass (cv Nui), white clover (cv Huia) pasture (ryegrass-based pasture) and pure narrow-leaved plantain (cv Ceres Tonic). For the first week animals were grazed outside *ad libitum* on their allocated forage diet. When housed indoors in metabolism cages (weeks two and three of each period), animals were fed fresh forage cut daily with a sickle bar mower at 1300 hrs and stored at ambient temperature (at approximately 11°C), for the afternoon and morning feeding. The level of feeding used during housing was determined in week two of period one, where the feed offered daily was increased until individual deer consumption reached a plateau. This took approximately 2-3 days. In week 3 of period 1 all animals on both diets were set to a constant DM allowance based upon the group mean, maximum daily intake of the plantain group, for which mean *ad libitum* intake in week 2 of period one was slightly lower than for the pasture group. This was in order to try to have animals on both diets in the same period offered the same amount of DM, and to maintain this level of feed intake across both periods. Hence deer in period two were offered the same level of dry matter of the new diet as they received in period one. Deer were fed twice daily at 0900 and 1630h, and received half their daily ration at each feeding. Animals had *ad libitum* access to water at all times.

Feed intake was determined by weighing the feed offered less the feed refused from the bin and sweepings. Sweepings were any forage in the cage, water bucket or on the floor (all pooled) spilt by the deer during the interval from one feeding to the next, but pooled per animal per day for weighing.

4.2.4 Forage Sampling

During the measurement period triplicate 200g samples of feed offered were taken daily for DM determination and a 200g sample of feed offered taken daily for botanical composition, chemical analysis, and alkane analysis. Feed refused samples for DM and chemical analysis were taken from the well-mixed feed remaining in the bins only, as sweepings were likely to be slightly contaminated with hair and dust. Of the daily individual feed refusals, triplicate 200g samples were taken for DM determination and two 200g samples were taken and pooled separately per animal for botanical composition, chemical and alkane analysis. Triplicate samples (200g) of sweepings were also taken for DM determination, as the DM content of the sweepings was likely be different compared with that of feed refusals in the bin. DM samples were dried daily at 100°C for 24 hr. The samples of botanical composition were dissected into grasses, legumes, weed and dead for pasture and plantain leaves, plantain reproductive stem, legumes, weed and dead for the plantain sward. Each component was oven dried at 100°C for 24 hr.

4.2.5 Laboratory Analyses

The samples for chemical composition were stored frozen prior to freeze drying and grinding. Samples were ground to pass a 1 mm sieve (Willey Mill, USA). Forage samples were analysed as described in Chapter 2, section 2.3.7. However, no samples were analysed by NIR. Dry matter apparent digestibility was calculated using Equation 1.

$$\text{DM digestibility} = \frac{(\text{DM feed offered} - \text{feed refused}) - \text{DM faeces}}{(\text{DM feed offered} - \text{feed refused})}$$

(Equation 1)

4.2.6 Methane measurements

Methane emissions were measured from all six animals, four of which had been used for methane measurements in the grazing experiment (Chapter 2) and already had SF_6 permeation tubes present in the rumen. The two animals that had not been previously used for methane measurements underwent training prior to this experiment to become accustomed to wearing the methane collection halters and harness. These animals had permeation tubes inserted into the rumen seven days before the first set of measurements. Enteric methane production was determined using the SF_6 tracer method, as developed by Johnson *et al.*, (1994) and modified according to Ulyatt *et al.*, (1999) and Ulyatt *et al.*, (2002b) as described in Chapter 2 section 2.3.5, and with the appropriate modifications as described in Chapter 3 to adapt the method for deer housed indoors.

4.2.7 Voluntary Feed Intake – double n-alkane technique

The double n-alkane procedure was used to estimate voluntary intake (Dove & Mayes, 1996; Gedir & Hudson, 2000) from five cannulated castrate stags. Seven days before the start of methane measurements animals had alkane controlled release capsules inserted into the rumen via rumen cannulae. The capsules used in this experiment were all from the same batch, which was also the same batch as used in the grazing experiment. Capsules were manufactured for growing cattle (Captec, Nufarm Ltd., New Zealand) containing 4 g of η-dotriacontane ($C_{32}H_{66}$) plus 4 g η-hexatriacontane ($C_{36}H_{74}$). The alkane capsule was attached to a string so that during the methane measurement period the disappearance of the remaining matrix of the capsule could be measured to determine the daily release rate. Dry matter intake was estimated, as in equation 2 below, from the daily dose rate and the dietary and faecal concentrations of the dosed even-chain alkane (η-dotriacontane, $C_{32}H_{66}$), and the adjacent natural odd-chain alkane (η-monotriacontane, $C_{31}H_{64}$).

$$Intake = \frac{F_i}{F_j} D_j \left(H_i - \frac{F_i}{F_j} H_j \right)$$

(Equation 2)

In the equation *Hi* and *Fi* represent the herbage and faecal (respectively) concentrations of the odd-chain alkane, *Hj* and *Fj* are the even chain equivalents and *Dj* is the daily dose of the even chain alkane. The daily dose rate was determined per animal by the disappearance rate of the alkane matrix over time, as measured daily at 1300 hrs from the five rumen-cannulated animals. Total faecal output was weighed daily 0900 hrs (coinciding with feeding). Faecal samples for alkane analysis were collected once daily (0900 hrs) during the measurement period from isolated faecal samples.

Faecal samples were oven dried for 48 hrs at 60°C and ground in a coffee grinder. The pooled feed offered forages samples for each diet were oven dried and ground to pass a 1 mm sieve (Willey Mill, USA). The daily samples of faeces, (dried and ground) were then combined per animal and per forage. Faecal and forage samples were then analysed for the alkanes, η-dotriacontane ($C_{32}H_{66}$), and η-monotriacontane ($C_{31}H_{64}$), via gas chromatography as described by Dove et al., (1996), with the following modifications. Industrial-heptane was used instead of analytical grade and saponification taking place in an oven rather than on heating blocks.

4.2.8 Statistical analysis

Alkane validation – Data were analysed by SAS (SAS 1998) by simple ANOVA. Type of forage (pasture or plantain), period the animals were fed each forage, and methods of intake measurement were fixed effects of the model. The model is equation 2.

$$Y_{ijk} = \mu + F_i + P_j + M_k + (M \times F)_{ik} + e_{ijk}$$

(Equation 2)

Where μ = overall mean; F_i = fixed effect of forage type (i = 1 to 2); P_j = fixed effect of period (j =1 to 2); M_k = method of DMI (k = 1 to 2); $(M \times F)_{ik}$ = fixed effect of interaction between forage type and time of measurement; e_{ijk} = random residual error, assumed to be normally distributed.

Methane emissions - Data were analysed by SAS (SAS 1998) by simple ANOVA. Type of forage (pasture or plantain), and period the animals were fed each forage. The model is shown in equation 3.

$$Y_{ijk} = \mu + F_i + P_j + (F \times P)_{ik} + e_{ijk}$$

(Equation 3)

Where μ = overall mean; F_i = fixed effect of forage type (i = 1 to 2); P_j = fixed effect of period (j =1 to 2); $(P \times F)_{ik}$ = fixed effect of interaction between forage type and time of measurement; e_{ijk} = random residual error, assumed to be normally distributed.

Values reported for the comparison of the experimental data are least square means, all other values reported are the mean and either the standard error of the mean (SEM) or standard deviation of the sample (STD), as stated. Significance was declared at $P \leq 0.05$, and a trend was reported if $0.05 < P \leq 0.10$. All mean comparisons were by Fisher's least significant difference method after a significant treatment main effect was detected.

4.3 Results

4.3.1 Botanical composition

Table 4.1 shows the botanical composition of feed offered and feed refused for ryegrass-based pasture (a) and plantain (b). The botanical composition of the feed offered and refused showed a tendency for animals to select against dead material for both forages. When fed plantain, five out of six animals showed a strong selection for leaf material over reproductive stem material. Pasture was in a totally vegetative state, with no reproductive stem material present.

Table 4.1. The botanical composition of feed offered and feed refused of ryegrass-based pasture (a) and plantain (b).

a. Ryegrass- based pasture

	Botanical composition %DM				
	Leaf	Stem	Clover	Weed	Dead
Feed offered[1]	94.5 ± 2.1	-	2 ± 1.4	0	3.5 ± 0.7
Feed refused					
Deer P	91	-	4	2	4
Deer A	92	-	1	2	5
Deer G	91	-	2	1	6
Deer L	92	-	2	0	6
Deer T	44	-	6	0	50
Deer S	84	-	8	0	7

[1]Average of period 1 and 2

b. Plantain

	Botanical composition %DM				
	Leaf	Stem	Clover	Weed	Dead
Feed offered[1]	45 ± 17.0	48 ± 11.3	0.5 ± 0.7	2 ± 1.41	4.5 ± 3.54
Feed refused					
Deer P	44	45	1	1	9
Deer A	27	69	0	0	3
Deer G	11	85	0	1	3
Deer L	32	66	0	0	2
Deer T	19	71	0	2	7
Deer S	26	59	0	3	11

[1]Average of period 1 and 2

4.3.2 Chemical composition of forages

The mean forage chemical composition, as shown in Table 4.2, (% of DM) over both periods showed that pasture had a greater NDF, hemicellulose, cellulose and gross energy content than plantain (P <0.001), whereas in contrast the OM, ADF, lignin, HWSC, and pectin contents were lower in pasture compared with plantain (P < 0.005). The higher concentration of pectin and HWSC in plantain contributed to a greater ratio of readily fermentable carbohydrate to structural carbohydrates for plantain (0.77) compared with pasture (0.25) (P <.0001).

All chemical components of the feed offered to deer on both diets changed significantly across periods (P < 0.03), except for pectin. This is probably a reflection of the rapid growth and development of both forage species during the mid spring season, despite efforts with agronomic management to maintain herbage quality from period 1 to period 2.

Table 4.2. Chemical composition and apparent dry matter digestibility of forages fed to deer.

	Pasture			Plantain			*P* - values		
% DM	Period 1	Period 2	SE	Period 1	Period 2	SE	Forage	Period	F x P
Organic matter	88.1	89.5	0.29	91.1	92.8	0.29	0.0004	0.006	0.67
ADF	23.2	25.1	0.27	29.6	22.5	0.26	0.0021	0.0007	<.0001
NDF	41.0	43.9	0.07	43.1	32.9	0.07	0.0001	0.0001	0.0001
Hemicellulose *(b)*	17.8	18.8	0.27	13.5	10.4	0.27	<.0001	0.015	0.0016
Cellulose *(b)*	21.9	23.2	0.26	22.4	14.9	0.26	0.0001	0.0003	<.0001
Lignin	1.24	1.99	0.123	7.18	7.62	0.123	<.0001	0.028	0.367
Pectin *(a)*	1.27	1.61	0.041	4.10	3.49	0.041	<.0001	0.086	0.002
Hot water soluble carbohydrate (a)	7.66	9.63	1.26	14.38	22.25	1.26	0.0015	0.018	0.079
Ratio RFC:SC *(a/b)**	0.22	0.27	0.03	0.51	1.02	0.03	<.0001	0.0009	0.002
CP, % DM	24.4	19.0	0.08	12.7	11.5	0.08	0.0001	0.0001	0.0001
Gross energy (MJ /kg DM)	19.16	18.7	0.03	18.5	18.5	0.03	0.0003	0.0013	0.0019
DM apparent digestibility	81.07	78.61	1.72	67.01	72.02		0.0001	0.4377	0.0267

* Ratio of readily fermentable carbohydrate: structural carbohydrates

4.3.3 Dry matter intake - calculated and measured intake

For DMI of the five deer used for the η-alkane validation, a significant main effect of forage ($P = 0.0001$) and period ($P = 0.01$) were found, where DMI was greater in period 1 (1634 ± 50 g/d) compared with period 2 (1452 ± 50 g/d). The overall average DMI of plantain (1750 ± 0.05 g/d) was greater than that of pasture (1328 ± 50 g/d). A significant method of intake determination by forage interaction was found ($P = 0.0001$), as illustrated in Figure 4.1.

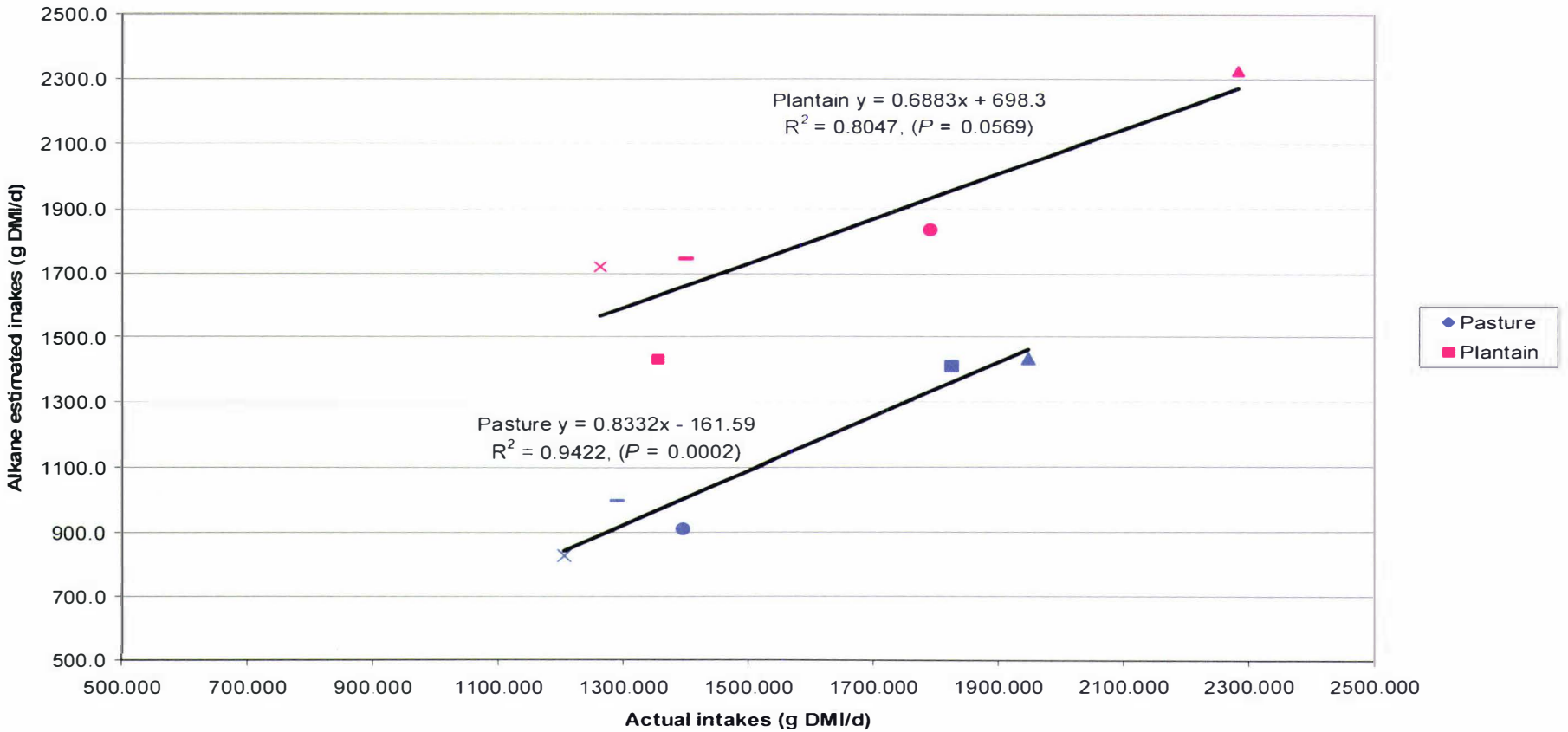


Figure 4.1. Dry matter intakes (g/d) for deer fed ryegrass-based pasture and plantain, where DMI is measured (actual) or estimated using the double η-alkane technique. Individual deer are represented by differing symbols, Deer P (–), Deer A (■), Deer G (×), Deer S (•), and Deer T (▲), where deer fed each forage is denoted by colour.

The actual mean (± SEM) dry matter intake of the five deer consuming pasture and plantain was 1531 ± 73 and 1658 ± 73 g/d, respectively. The mean (± SEM) dry matter intakes as calculated by the double η-alkane technique were 1125 ± 73 and 1857 ± 73 g/d for pasture and plantain respectively (Table 4.3) . It was found that the mean intake of animals when consuming pasture was significantly (P = 0.0002) underestimated by 23.5 ± 7.6 (STD) % using the double n-alkane technique. In contrast, the mean DMI of plantain tended to be overestimated 13.9 ± 15.2 (STD) % (P < 0.06). Figure 4.1 shows the linear relationship of actual DMI vs. estimated DMI and the significance of the difference of estimated and actual DMI for each forage fed. Although a strong relationship between actual and estimated DMI was found (R^2 = 0.9; P = 0.0002) for pasture and plantain (R^2= 0.7; P < 0.06), on average the estimated DMI of animals fed pasture was significantly less than actual DMI, whereas in contrast the DMI of plantain was overestimated.

Table 4.3. Dry matter intake of individual deer as measured (actual) or calculated using the double η-alkane technique (alkane).

	Ryegrass-based pasture			Plantain		
	Actual[1] (g/d)	Alkane (g/d)	Alkane % Actual	Actual[1] (g/d)	Alkane (g/d)	Alkane % Actual
Deer P	1243 ± 239	997	80.2	1473 ± 359	1746	118.5
Deer A	1823 ± 250	1410	77.3	1431 ± 341	1433	100.1
Deer G	1172 ± 335	829	70.7	1248 ± 309	1721	137.9
Deer T	1960 ± 276	1433	73.1	2267 ± 428	2325	102.6
Deer S	1242 ± 609	910	73.3	1662 ± 380	1836	110.5
Mean	1488 ± 373	1116 ± 285	74.9 ± 3.8	1616 ± 392	1812 ± 324	113.9 ± 15.2

[1]n = 5

4.3.4 Herbage concentrations, dose rates and faecal recovery rates of natural and synthetic alkanes

The concentration of alkanes present in each forage are shown in Table 4.4. The natural alkane content of C_{31} in plantain was slightly higher than in pasture, but the converse was found for C_{33} and C_{35} .

Table 4.4. The η-alkane concentrations present in forage species offered to deer.

	η-alkane concentrations of feed offered (mg/g)				
	C_{31}	C_{32}	C_{33}	C_{35}	C_{36}
Pasture					
Period 1	0.167	0.009	0.118	0.011	0.000
Period 2	0.197	0.009	0.115	0.011	0.000
Plantain					
Period 1	0.257	0.016	0.068	0.009	0.000
Period 2	0.222	0.014	0.059	0.007	0.000

The dose rates of alkanes were calculated for each individual animal and are shown in Table 4.5 in comparison with the manufacturer's recommended dose rates. Dose rates per animal were found by measuring the disappearance of the matrix of the alkane capsules over the five day measurement period and calculating the slope of the line of best fit, for each animal (shown in Appendix 4.5, Figure 4.5). The mean rate of matrix disappearance had a strong negative linear relationship to the increasing number of days that the alkane capsules were in the rumen (pasture R^2 = 0.99 and plantain R^2 = 0.99). The disappearance of the matrix in the animals fed plantain was greater than those fed ryegrass-based pasture (P = 0.0005). There was also found to be some

variation around the mean rates of disappearance as represented by the standard error of the mean, shown in Figure 4.2. The variation of the mean disappearance rates of the matrix from the CRC seemed to be driven by a significant difference of deer by forage (P = 0.0001) interaction, where deer all deer except Deer P showed a greater rate (P < 0.001) of matrix disappearance when fed plantain compared with ryegrass-based pasture.

Table 4.5. Calculated and recommended dose rates of synthetic alkanes, η-dotriacontane ($C_{32}H_{66}$) and n-hexatriacontane ($C_{36}H_{72}$).

	Pasture (mg/d)		Plantain (mg/d)	
	C_{32}	C_{36}	C_{32}	C_{36}
Deer P	170.65	180.49	204.36	216.14
Deer A	174.76	184.84	182.63	193.16
Deer G	173.45	183.45	206.61	218.52
Deer T	180.57	190.98	218.22	230.81
Deer S	176.82	187.01	182.45	192.97
Mean ± STD	175.7 ± 4.14	185.4 ± 3.94	198.9 ± 15.79	210.3 ± 16.7
Deer T[2]	-	-	160.16	138.68
Deer S[2]	-	-	131.12	169.39
Captec[1]	200	211.5	200	211.5

[1] Captec, manufacture's recommended dose rates for calculating intake.

[2] Alkane recovery rates from group B, period 1, which was later repeated due to poor adaption of these animals to housing expressed by low and variable feed intakes. DMI of plantain for Deer T and Deer S was 319 ± 326 (STD) g/d and 197 ± 179, respectively.

The average dose rate of η-dotriacontane ($C_{32}H_{66}$) and η-hexatriacontane ($C_{36}H_{72}$) (table 4.5) when animals were fed pasture was below that of the stated manufacturer's dose rate. However, the real and manufacter's dose rates were similar for animals fed plantain. The dosage rates of $C_{32}H_{66}$ ranged from 182.45 mg/d to 218.22 mg/d, and $C_{36}H_{72}$ ranged from 193.16 to 230.81 mg/d. During the initial period 1, group B was slow to adapt to housing and hence DMI was low and variable leading to low dose rates of $C_{32}H_{66}$ compared with when period 1 was repeated.

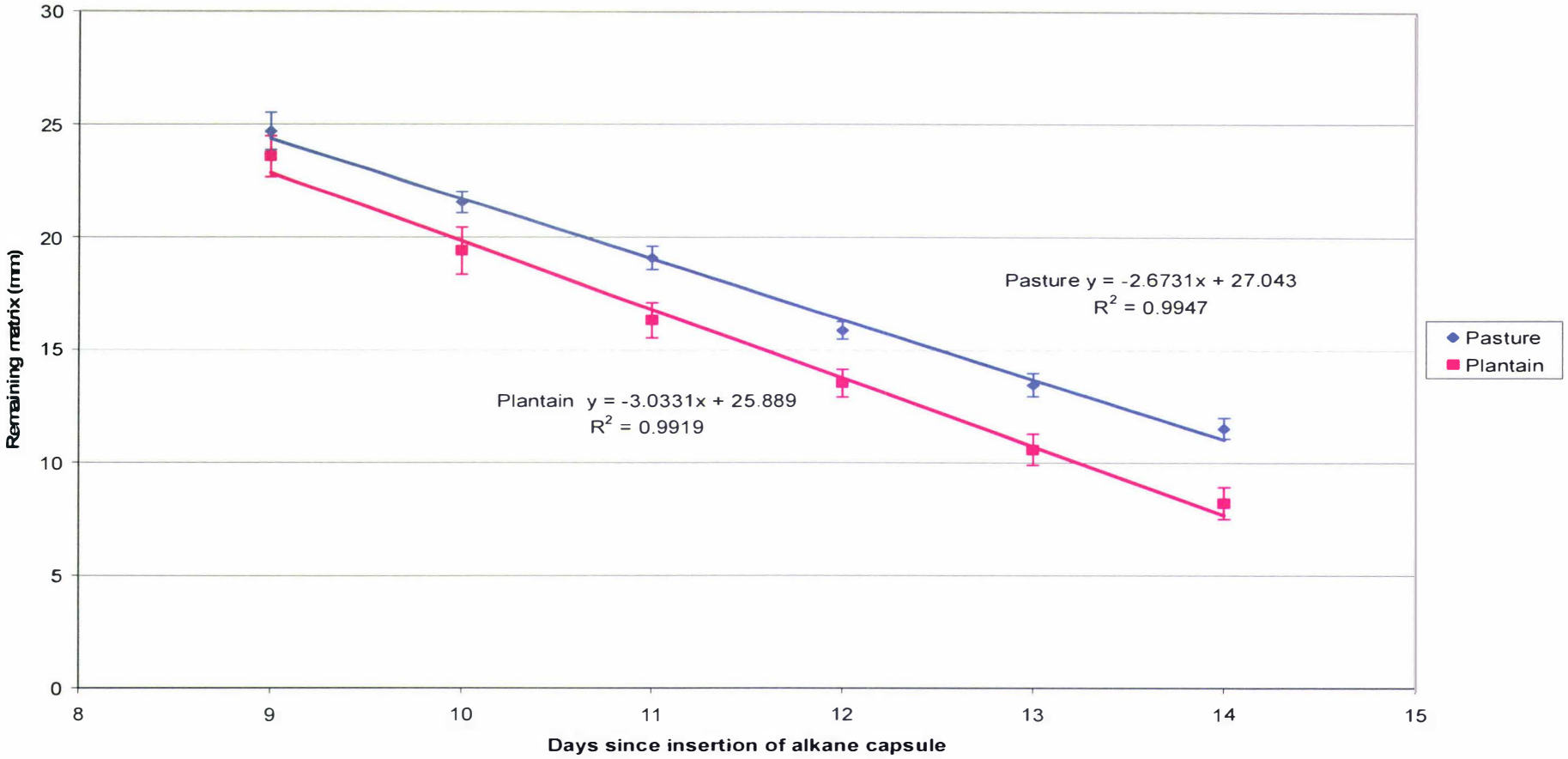


Figure 4.2. Mean matrix disappearance rates of animals fed ryegrass-based pasture and plantain. Error bars represent the standard error of the mean.

The faecal recovery rates of both natural and dosed alkanes are presented in Table 4.6 and Figure 4.3. The recovery rates of individual alkanes tended to be differ significantly ($P < 0.07$), which agrees with the weak linear relationship of faecal recovery rate and alkane carbon length (Figure 4.3). There was also a significant affect of period, where in period 1 the mean recovery rate was 92.5% and period 2 was 117.2%, however this may be an affect of the greater number of animals fed plantain in period 2. Alkanes used to calculate intake in both Chapter 2 and the current experiment were η-dotriacontane ($C_{32}H_{66}$), and η-monotriacontane ($C_{31}H_{64}$). However, the recovery rates of C_{32}, C_{31} and others in Table 4.6 differed from one another ($P = 0.04$), and were lower in pasture-fed animals (92.5%) compared with plantain-fed animals (119.7%) ($P = 0.001$). The majority of alkane faecal recovery for plantain-fed animals were above 100%, and particularly for C_{32}, recovery rates of pasture-fed animals were also calculated to be greater than 100%. There was a high degree of individual animal variability in the alkane faecal recovery data from both pasture and plantain-fed animals, as denoted by the standard error of the mean shown by error bars in figure 4.3.

Table 4.6. The faecal recovery rates of dosed and naturally occurring η-alkanes in pasture (a) and plantain (b).

(a) Pasture

	Faecal recovery (%)				
	C_{31}[a]	C_{32}[ab]	C_{33}	C_{35}	C_{36}[b]
Deer P	78	98	82	88	89
Deer A	81	103	88	95	96
Deer G	75	107	80	93	89
Deer T	85	106	94	105	101
Deer S	76	113	81	87	105
Mean ± STD	79 ± 4.1	105 ± 5.5	85 ± 5.9	94 ± 7.2	96 ± 7.1

[a] Alkanes used to calculate intake. [b] Dosed alkanes.

(b) Plantain

	Faecal recovery (%)				
	C_{31}[a]	C_{32}[ab]	C_{33}	C_{35}	C_{36}[b]
Deer P	136	111	159	172	90
Deer A	130	136	155	168	107
Deer G	139	105	187	202	88
Deer T	122	109	14	150	98
Deer S	117	123	13	141	114
Mean ± STD	129 ± 9.3	117 ± 12.7	106 ± 85.0	167 ± 23.5	99 ± 11.1

[a] Alkanes used to calculate intake. [b] Dosed alkanes.

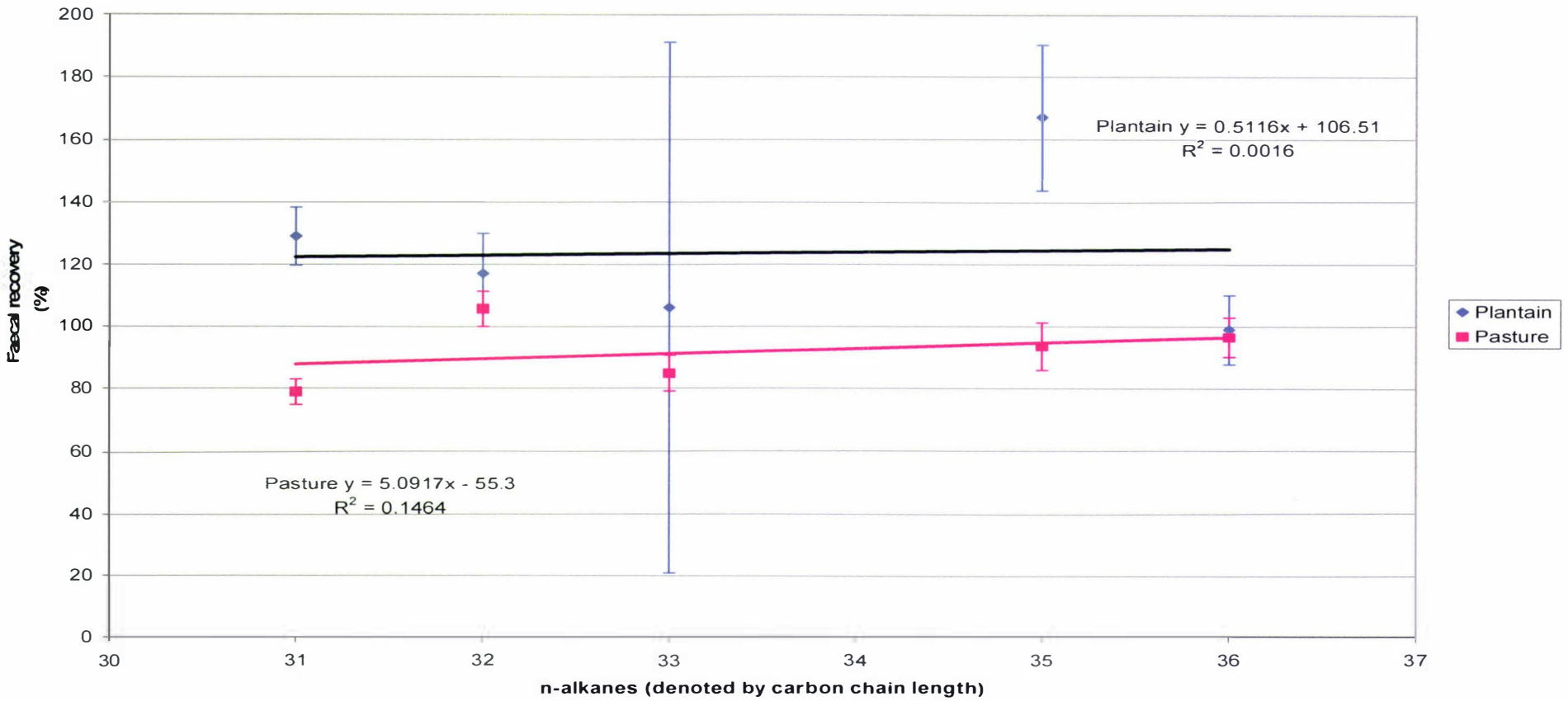


Figure 4.3. Mean faecal recovery rates of dosed and naturally occurring η-alkanes, of deer fed pasture or plantain, error bars represent the standard error of the mean.

4.3.4 Methane production

No significant main effects of forage type (P = 0.3) or period (P = 0.2) were found for daily methane production grams per day, (shown in Table 4.7). However, there was a significant forage by period interaction (P = 0.0001). In period 1 methane emissions per day from animals fed ryegrass-based pasture (35.1 ± 1.8 g/d) were less than from animals fed plantain (43.4 ± 2.5 g/d) (P = 0.009), with the converse true in period 2 (ryegrass-based pasture 48.5 ± 2.8 g/d; plantain 35.5± 1.8 g/d; P = 0.0003), thus suggesting an animal effect.

Methane production per kg actual DMI of deer fed ryegrass-based pasture and plantain is shown in Table 4.7 and was similar for both forages (P = 0.8). Actual dry matter intakes and methane emissions for individual deer are shown in Appendix 4.5, Table 4.8.

Table 4.7 Methane production and actual DMI from deer when fed either ryegrass-based pasture or plantain.

	Pasture[1]		Plantain[1]		P – values		
	LSM	SEM	LSM	SEM	Forage	Period	P x F
DMI (g/d)	1548	85.25	1722	85.25	0.2704	0.2495	0.0064
CH_4 (g/d)	41.8	1.64	39.46	1.54	0.2294	0.3047	0.0001
CH_4/DMI (g/kg)	25.6	1.79	25.0	1.67	0.8002	0.3085	0.5346

[1] Methane emissions and DMI are measured from all six animals.

Methane emissions per kg DMI using estimated and actual DMI from the five deer that were used for both measurements were compared to determine the effect of the method of intake measurement on methane emissions (Figure 4.4). Methane emissions per kg DMI from pasture were significantly (P = 0.0001) overestimated by 11 g CH_4/kg DMI when DMI was calculated using the alkane technique. When deer were fed plantain it was found that methane emissions per kg DMI were significantly (P < 0.04) underestimated by 4.77 g CH_4/kg DMI.

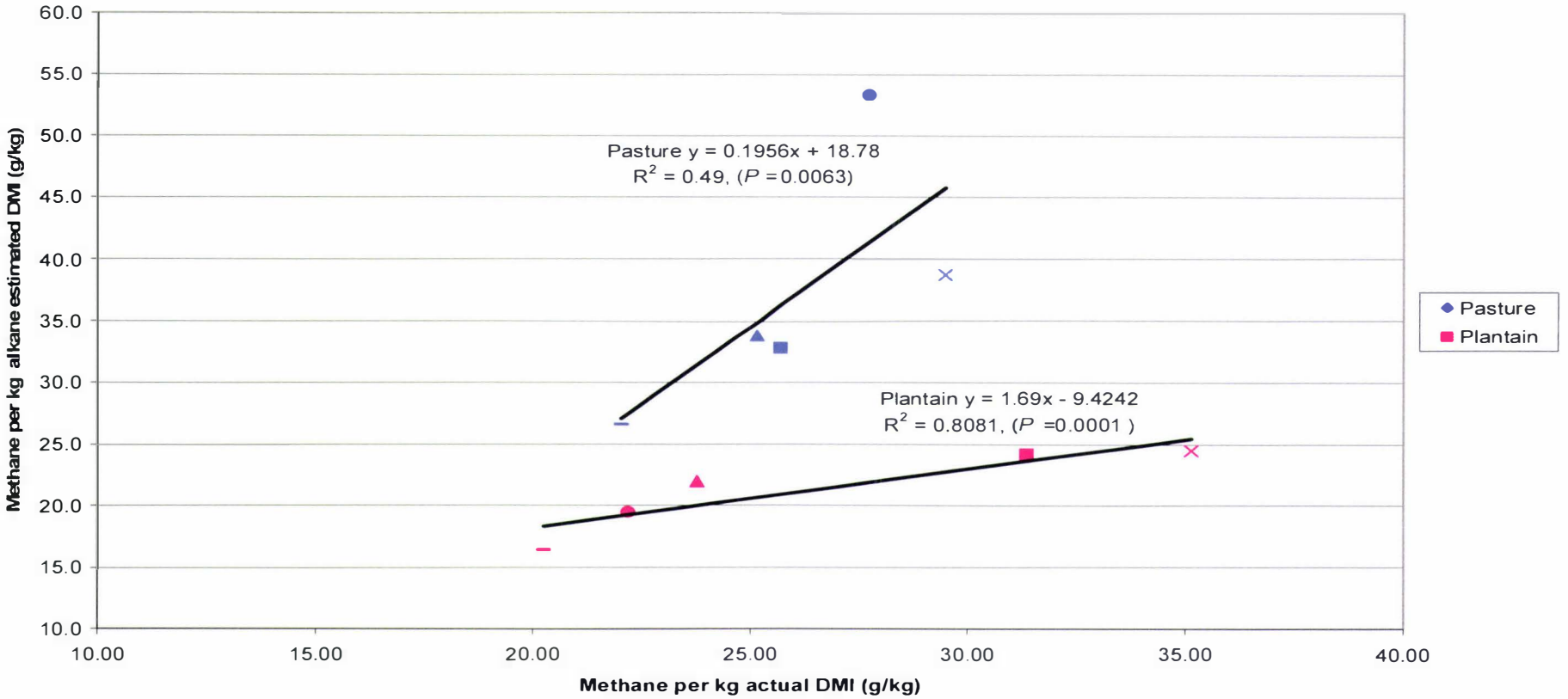


Figure 4.4. Methane emissions per kg of DMI for deer fed ryegrass-based pasture and plantain, where DMI is measured (actual) or estimated using the double η-alkane technique. Individual deer are represented by differing symbols, Deer P (–), Deer A (■), Deer G (×), Deer S (•), and Deer T (▲), where deer fed each forage is denoted by colour.

4.4 Discussion

The dry matter intake of deer consuming pasture was underestimated (23.5%) and the DMI of plantain was overestimated (13.9%) when DMI was calculated by the n-alkane technique as compared with actual intakes. This therefore influences methane production per kg DMI such that methane production was overestimated when animals were fed pasture by 11 g CH_4/kg DMI and underestimated by 4.8 g 11 g CH_4/kg DMI when animals were fed plantain. This highlights the need for accurate measures of feed intake to be taken in conjunction with measurements of methane emissions, for methane production per unit DMI to be a valid means of expressing methane emissions. It also throws doubt on differences in methane production per unit feed intake that have been reported for ruminants grazing different forage species, including those presented in Chapter 2, where the η-alkane method has been used to estimate feed intake.

The findings of this experiment are in contrast to validation trials conducted with sheep and cattle as reported by Dove and Mayes, (1991, 1996) and fallow deer (Ru *et al.*, 2002), where intakes calculated by the n-double alkane technique were representative of actual intakes. However, it is important to emphasise that these trials were conducted largely on dried diets, not fresh forages. Recent trials in New Zealand with dairy cattle (H. Clark, personal communication, 2004) and sheep (Krause, 2002 unpublished) showed that estimated intakes using the alkane technique were erratic compared with real intakes. Krause (2002 unpublished) found that the intakes of sheep fed white clover were significantly underestimated by 17% (P = 0.0002), but other studies report overestimations ranging from 3.6-12.6% (Gedir & Hudson, 2000; Valiente *et al.*, 2003).

The double n-alkane method is reliant on two main assumptions according to Dove and Mayes, (1991, 1996). Firstly, that the herbage subsample taken to represent feed offered has a representative η-alkane profile of the forage actually eaten by the animal. In the current study animal selection of the components of pasture was not substantial. However, animals actively selected

against the stem proportion of the plantain forage, hence the feed offered sample that was taken to be analysed for its alkane profile may not have been representative of the alkane profile that was consumed by animals fed plantain. Therefore, future experimentation of this type should analyse both feed offered and feed refused samples for alkane content to determine the actual intake of natural plant alkanes to allow for diet selection, or intakes should be restricted to the point where no feed is refused. The concentration of the naturally occurring η- alkane C_{31} in the feed offered of both ryegrass-based pasture and plantain was considered high enough that it would not created large errors in the calculation of intake. However, the low n-alkane concentrations of C_{35}, if used to calculate intake, may result in large errors in the estimation of intake as it was suggested by Hendricksen *et al.*, (2002) that alkane concentrations less than <0.01mg/g would result in poor calculation of faecal recovery rates and intake.

The second assumption of the n-alkane technique is that the rate of alkane faecal recovery is similar for both the dosed alkane and the natural alkane used to calculate intake, even if faecal recovery rates are not complete. If faecal recovery rates are dissimilar there can be 'an error of 1.25% in the estimated intake for each percentage difference in recovery between the alkane pair' (Dove & Mayes, 1996). As there was up to a 26% difference in faecal recovery of C_{31} and C_{32} of pasture and 12% difference in plantain-fed animals in the present study, differences of recovery rates are suggested to impact heavily on accuracy of estimation of intake. The faecal recovery of the dosed alkane, C_{32} was found to average over 100% for both pasture and plantain and the faecal recovery rate of C_{31} was also over 100% for plantain, but not pasture. Krause, (2002 unpublished) also reported that for three of the six sheep used in that validation study there were dosed alkane (C_{32}) recoveries above 100%. It was also found by Krause (2002 unpublished) that there was a 22% difference between the recovery of C_{31} and C_{32}. Published studies have also shown that faecal recovery rates can exceed 100% of either dosed or natural intake of alkanes (Dove *et al.,* 2002; Valiente *et al.,* 2003). However, reasons for alkanes exceeding 100% were not addressed in these reports. The dissimilarity of the rates of faecal recovery and the recovery rates of both the dosed and naturally

occurring η-alkanes over that of either the dose rates or herbage intake suggested that η-alkanes may not be inert in the digestive tract.

The incomplete recovery of alkanes has been acknowledged by Dove and Mayes (1991, 1996) and is the first suggestion in the literature that alkanes are not inert in the digestive tract. Reasons for the dissimilarity, and high percentage of alkane recovered in the faeces of the current study are suggested below. In a study by Mayes in 1988 as cited in Doves & Mayes (1991) and Hendrickson *et al.,* (2002) with sheep, a disappearance of alkanes occurred in the small intestine of the ruminant. This study by Mayes in 1988 (as cited in Doves & Mayes, 1991) suggested that the microbes of the foregut did not influence dosed or natural alkanes as there was very little loss of the alkanes until the small intestine. Straight chain hydrocarbons were found by McCarthy (1964) to be directly transformed to fatty acids of the same carbon length, in goats, chicken and rats. In contrast, Ohajuruka & Palmquist (1991) found evidence for ruminal loss of C_{32} in dairy cattle, indicating the there may be some rumen modification and/or disappearance of alkanes. It is also possible that a small proportion of alkanes recovered in the faeces of ruminants is endogenous in origin or due to the saturation of unsaturated hydrocarbons consumed in the diet (Mayes *et al.,* 1986; Doves & Mayes, 1991). Fungi, yeast and bacteria have been found to both utilise and synthesise n-alkane (McKenna & Kallo, 1956). However, it has not been established if these species reside in the digestive tract of ruminants. The findings in this study, together with those studies reported, highlight the need for research investigating the breakdown, digestion and metabolism of η-alkanes by ruminants and the microbes within the digestive tract when fed both fresh forages and conserved forages.

The hypothesis that the dosed η-alkanes (even-chain η-alkanes) may behave differently from the odd chain alkanes (Dove & Mayes, 1991), may contribute to the dissimilarity of alkane faecal recovery rates as found with red deer. The even chain η-alkanes have been established to remain in the particulate phase of the digesta. However, 30-40% of the dosed even chain alkanes have been found associated with the liquid phase of the digesta (Doves & Mayes 1991).

The association of the dosed alkanes with the liquid phase of the digesta may in deer account for some of the dissimilarity of the faecal recovery rates. Domingue *et al.* (1991) found that the outflow of liquid and particulate matter in the rumen of deer is markedly different from that of sheep, with deer having a greater outflow rate of liquid than sheep in both summer and winter, but with more of a difference in summer than in winter. However, the particulate matter outflow rate of deer is slower than of sheep, therefore suggesting there is selective retention of the particle component of the digesta. This therefore suggests that in red deer the large difference in the faecal recovery rates of dietary and dosed η-alkanes could be created by the faster excretion rate of dietary alkanes associated with the liquid phase of the digesta than of dosed alkanes associated with particles.

The finding that the mean dosage rates of alkanes, which are a function of matrix disappearance rate in the rumen, in pasture-fed animals were lower than the manufacturer's recommended rates is consistent with the findings of Ru *et al.,* (2002). In contrast, the mean dose rates of alkanes in plantain-fed animals were similar to the dose rates recommended by the manufacturer. Therefore, the assumption that a common dose rate across forage treatments exists is not supported. There was also a considerable range of dose rates between animals. Dove et al., (2002) claimed that the dose rates of slow release capsules were not affected by the level of feeding or feeding frequency. Animals in group B that had very low DMI of plantain in the initial period 1 (which was repeated), had very low dose rates of synthetic η-alkanes compared to when they ate normally in the repeated period, thus suggesting that dosage rates between animals consuming different amounts of the same diet may not be similar.

Dose rates were calculated from the slope of the rate of disappearance of the alkane matrix over time. The variability in the rate of matrix disappearance in this study was considerable compared with that reported by Dove *et al.*, (2002) and probably caused the high variability of dose rates found in this study. The rates of matrix disappearance from those animals in the grazing experiment were also found to be different between and within forage (Appendix 4.5, Figure

4.6). The findings of this study imply that individual dose rates of hinds and stags in the grazing experiment (Chapter 2) may also have been as variable as reported here, and this may have contributed to inaccuracies in feed intake estimation. However, as release rates have only been measured in this study and Chapter 2 in rumen fistulated deer, the question remains as to whether this is representative of release rates in non-fistulated deer. Dove *et al.*, (2002) indicated that there was no difference between surgically and nonsurgically modified sheep in dosage rates.

Ideally, the validation of the double n-alkane technique for use in grazing animals should be conducted in the grazing situation as both the fresh diet and the grazing behaviour may influence the estimation of DMI. As there is no way to determine accurate individual intakes of grazing animals, a compromise is made to validate the technique using a fresh forage indoors, but this does not allow for unknown behaviour differences of deer housed indoors or grazing forages. Diet, due to digestion effects, is more likely than behaviour to influence the accuracy of the technique.

The findings of this experiment suggest that the estimation of intake using alkanes is highly variable and of poor accuracy and concurrent measurements with methane may significantly over or underestimate methane production per kg DMI eaten. This is of considerable concern when applied to the New Zealand National Inventory of Greenhouse Gas Emissions. The under and overestimation of estimated intakes for ryegrass-based pasture and plantain suggest there may be an effect of forage on the calculation of intake that warrants further investigation, especially with deer consuming fresh forages.

4.5. Appendix

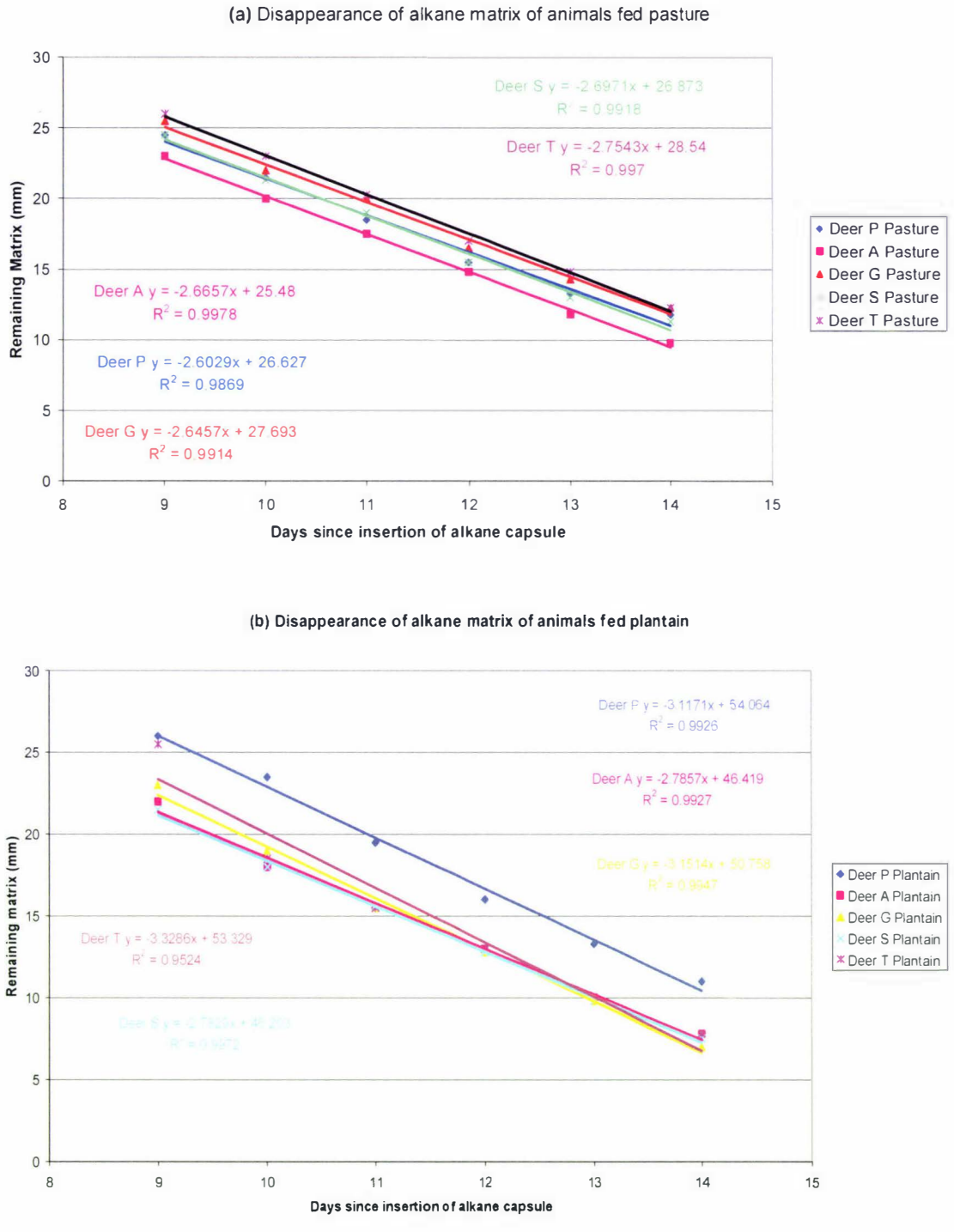
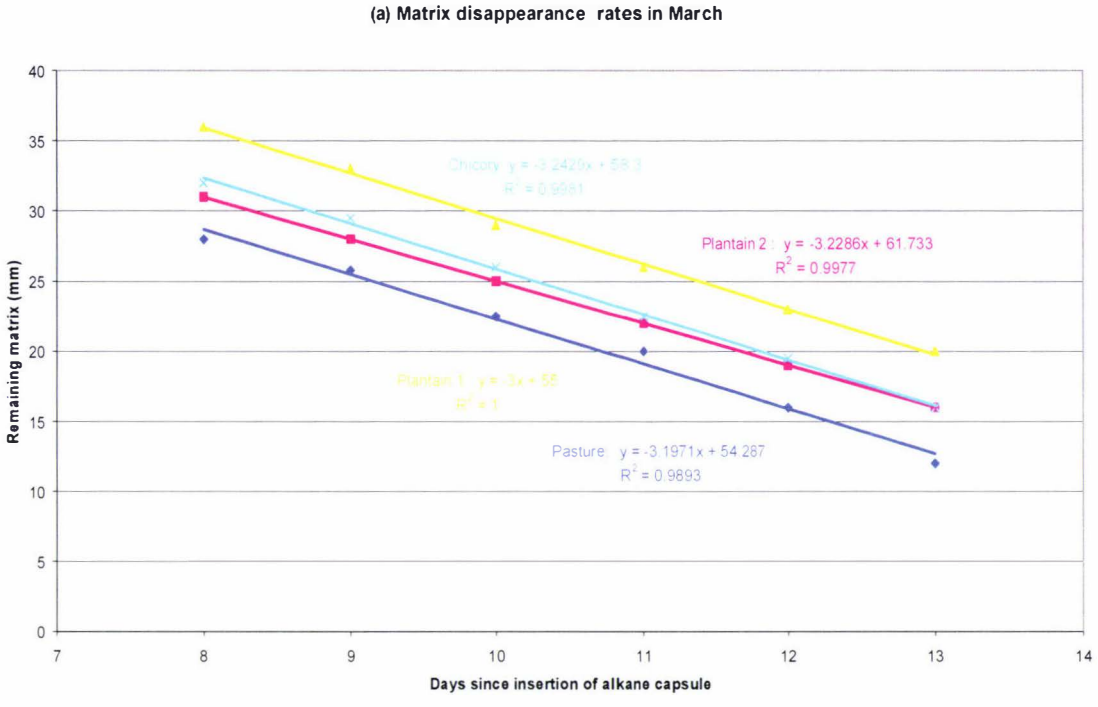


Figure 4.5. The disappearance rates of the alkane matrix of individual deer when fed ryegrass-based pasture (a) of plantain (b).



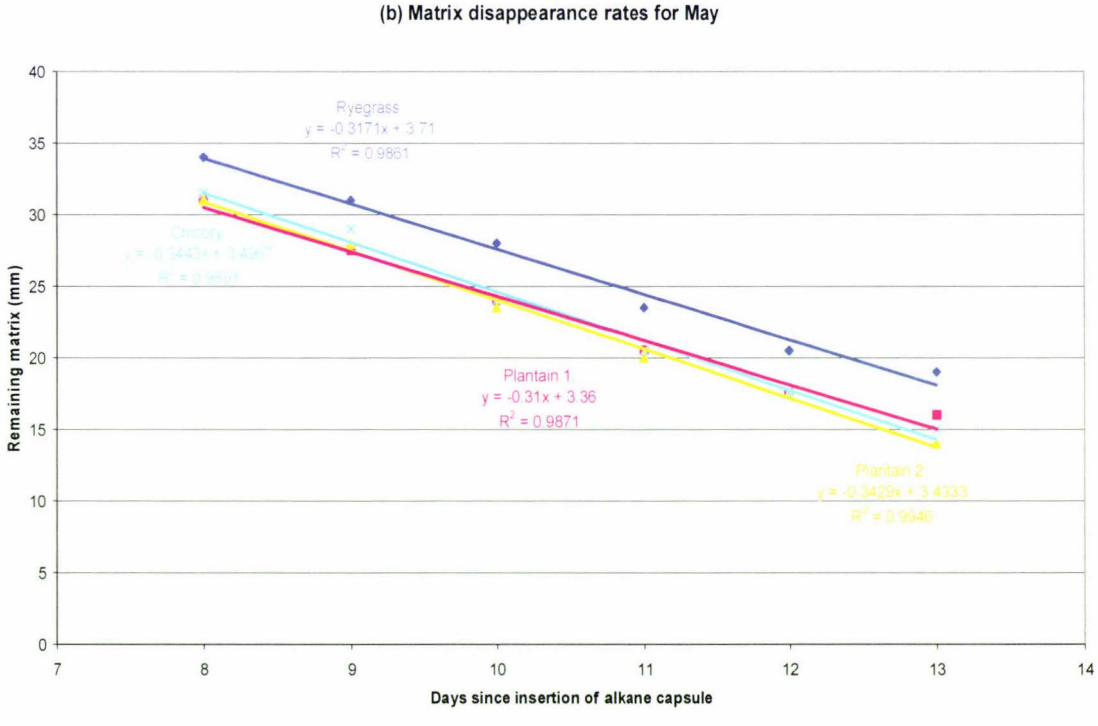


Figure 4.6. The disappearance rates of the alkane matrix in March (a) and May (a) from the fistulated red deer used to calculated dosage rates of η-alkanes for animals used in the grazing trial (chapter 2).

Table 4.8. Dry matter intakes and methane emissions per day and per kg DMI based upon actual intakes for deer when fed pasture and plantain.

	Pasture		Plantain		*P* - values		
	LSM	SEM	LSM	SEM	Forage	Deer	D x F
Intake g/d	1515	67.36	1641	67.36	0.1904	0.0001	0.1335
Deer P	1243.2	165.0	1472	165.0			
Deer A	1823	165.0	1340	160.0			
Deer G	1172	165.0	1248	160.0			
Deer L	1554	165.0	1862	165.0			
Deer T	1960	165.0	2264	165.0			
Deer S	1338	165.0	1661.6	165.0			
CH_4 (g/d)	39.58	0.99	38.198	0.96	0.3244	0.0001	0.0001
Deer P	26.51	2.31	28.61	2.31			
Deer A	46.22	2.31	34.61	2.31			
Deer G	32.07	2.31	42.12	2.31			
Deer L	35.64	2.31	37.03	2.58			
Deer T	48.47	2.31	51.12	2.31			
Deer S	48.55	2.98	35.71	2.31			
CH_4/DMI (g/kg)	25.78	1.51	25.43	1.46	0.9449	0.0184	0.4874
Deer P	22.04	3.51	20.25	3.51			
Deer A	25.70	3.51	31.37	3.51			
Deer G	29.50	3.51	35.62	3.51			
Deer L	23.33	3.51	19.38	3.92			
Deer T	25.16	3.51	23.79	3.51			
Deer S	27.74	4.54	22.19	3.51			

Chapter 5: General Discussion

5.1 Introduction

Methane production per day and per kg of DMI measured from 20 mixed-age hinds and 4 castrated stags grazing ryegrass-based pasture, chicory and plantain in March and May of 2003 (chapter 2), did not support the hypothesis that methane emissions per kg of DMI from red deer would be similar to those of adult sheep and dairy cows. Methane production per unit dry matter intake obtained for grazing deer in this study was approximately 75-80% greater than published mean methane emissions from sheep and cattle fed perennial-ryegrass-based pasture. However, the hypothesis that methane emissions per kg of dry matter would be reduced in deer grazing forage herbs compared with perennial ryegrass-based pasture was supported by this study. The accuracy of these results was questioned, however, because of concerns regarding the accuracy of the estimated DMI determined using the double n-alkane technique. This prompted the initiation of an indoor study where intake could be accurately measured concurrently with the methane production of hinds.

Methane emissions from an indoor study (Chapter 3) contrasted with the results of the grazing experiment and supported the hypothesis that methane production from red deer is similar to that from sheep and cattle, thus indicating, that the calculated intake using the double η-alkane technique was inaccurate. The validation of this technique (Chapter 4) confirmed this, as DMI of pasture and plantain was significantly under and overestimated, respectively, which significantly impacted on methane emissions per kg of DMI when calculated using estimated intakes compared to actual intakes.

5.2 Methane production by red deer

Figure 5.1 shows methane emissions measured using the SF_6 technique from red deer on both a daily and per unit feed intake basis measured in all three studies. This is the first such data from red deer and deer fed fresh forages. Methane production per unit DMI obtained from grazing deer in the first study

(37.8 g/kg DMI) is approximately 75-80% greater than values used in the National Inventory for dairy cows (21.6 g CH_4/kg DMI, (NIR, 2003), sheep (20.9 CH_4/kg DMI, (NIR, 2003) and estimated for deer, based on (21.25, (NIR, 2003) grazing ryegrass-based pastures and 39% greater than values obtained from both indoor studies presented in this thesis.

The hypothesis that deer produce less methane per unit feed intake than do sheep and cattle is not supported by all three studies, as study one (Chapter 2) found higher emissions for deer than published values for sheep and cattle and studies two (Chapter 3) and three (Chapter 4) found similar emissions to reported values for other ruminant livestock. However, such comparisons are based on the assumption, used by the National Inventory, that the ^{13}C-alkane technique used to estimate feed intake in most of the reported studies is an appropriate technique that is accurate in all species and across different diets. This assumption is not supported by the results of the current studies and is discussed in more depth in section 5.3. In addition it should be highlighted that the studies presented here were not robustly designed to test the hypothesis that methane emissions from deer differed from those of other ruminants, and further study is required to do this by direct comparisons where all species are fed the same diet, methane measurements are conducted over the same time period using identical methods and feed intake can be accurately determined.

No effect of seasonality was observed for methane production per kg DMI in the deer used in the studies reported here and conducted over the period from late summer to mid-spring, despite evidence to indicate that the digestive physiology of deer is highly seasonal (Domingue *et al.*, 1991). Rumen pool size and digesta passage rate has been found to change more with season in red deer compared with sheep (Domingue *et al.*, 1991), and these aspects of digestive physiology are known to affect methane production (Pinares-Patino *et al.*, 2003). It is not known how or if changes in digestive physiology would influence methane production in deer over time or compared with other

ruminant species, and this warrants further investigation in appropriately designed comparative experiments.

The double η-alkane technique for estimating DMI was validated against actual intakes in the final study, as discussed in section 5.3, while concurrently measuring methane emissions. The impact on methane emissions of the inaccurate estimation of dry matter intake by the double n-technique in this experiment resulted in methane production from deer fed pasture overestimated by 30.1% and from deer fed plantain being underestimated by 16.0%. The implications of these results mean that the grazing experiment needs to be repeated, but in such a way that DMI intakes can be accurately measured.

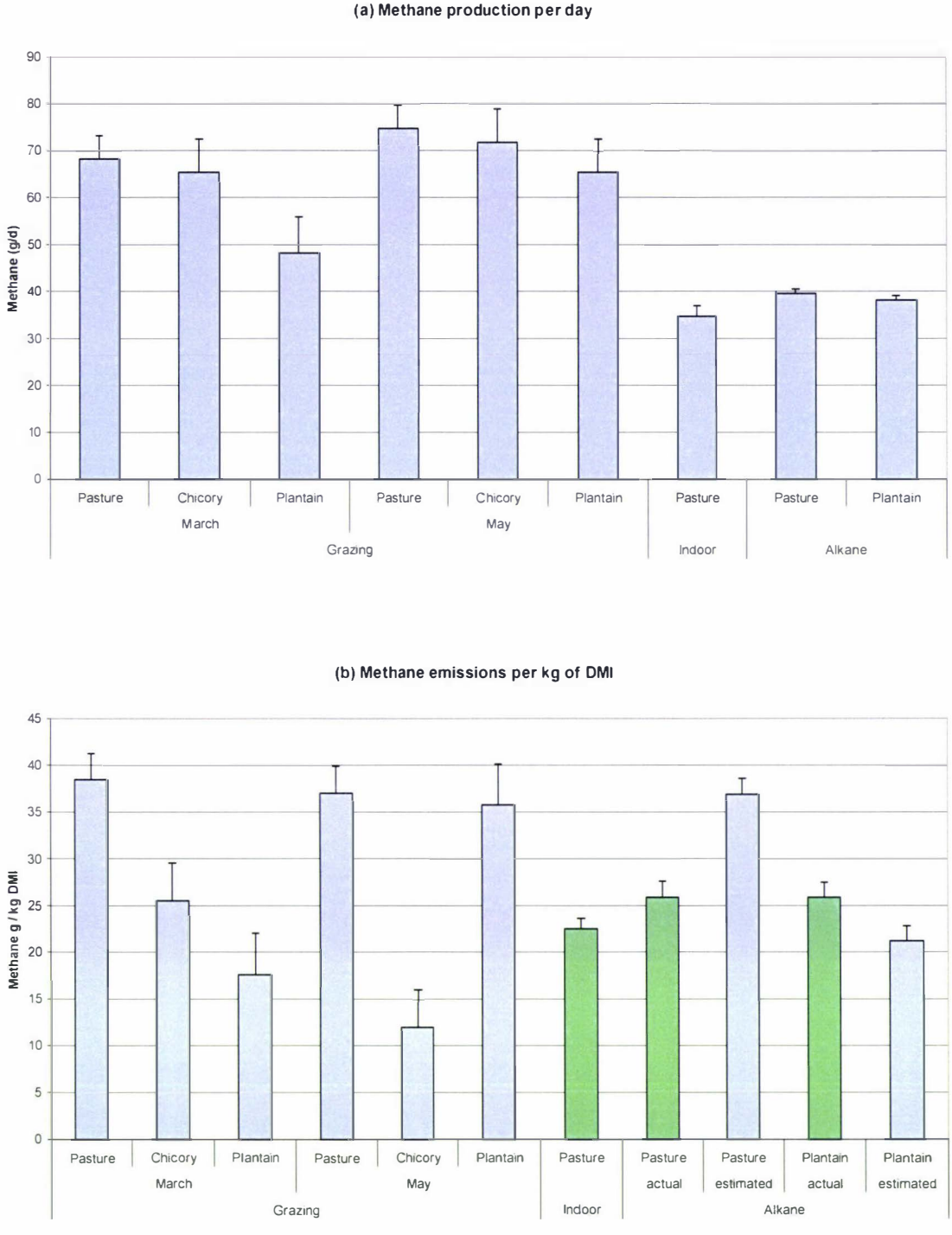


Figure 5.1. Methane production per day (a) and per kg of DMI (b) from red deer across three experiments, Grazing experiment (Chapter 2), Indoor experiment (Chapter 3), Alkane validation experiment (Chapter 4). Error bars represent the standard error of the least square mean. Dry matter intakes that are estimated by the double n-alkane method are represented in blue (■), measured intakes are represented by the green bars (■).

5.2 Effect of forage species on methane production

Deer grazing chicory and plantain in March and deer grazing chicory in May appeared to have lower methane emissions compared with those grazing ryegrass-based pasture. Waghorn *et al.,* (2002) observed that methane production from sheep fed chicory was reduced compared with those fed ryegrass-based pasture. Forage herbs, such as chicory, when fed to deer have been established to have a significantly greater feeding value compared with deer fed ryegrass-based pasture (Barry 1998; Hoskin *et al.*, 2003). Components of the feeding value of forages, as defined by Barry (1998), are voluntary feed intake, digestive processes (including the percentage digestion and retention time in the digestive tract). Deer when fed chicory have been found to exhibit greater voluntary feed intake, lower rumen pH and a faster particle outflow rate out of the rumen (Hoskin *et al.*, 1995; Kusmartono *et al*, 1996, 1997). These are characteristics that have been established to influence methane production of ruminants (Blaxter & Clapperton 1965; Johnson & Johnson 1995; Pinares-Patino *et al.*, 2003) as described in Chapter 1, section 1.4. The effect of secondary plant compounds on methane production is uncertain, however the concentration of condensed tannins in chicory was low and therefore is not thought to have influenced methane emissions, as similar to the findings of Waghorn *et al.*, (2002). However, the level by which methane production was reduced in deer fed chicory or plantain in the current study may have been overestimated, especially for chicory, due to the very high estimated dry matter intakes (Figure 5.2).

5.3 Dry matter intake

Estimated and/or actual dry matter intakes for the three experiments are shown in Figure 5.2. The indoor validation of the double η-alkane technique supported suspicions that this method may not have accurately represented actual intakes of deer in the grazing study. In the indoor study pasture dry matter consumption was underestimated by 23.5% and plantain was overestimated by 13.9%. The DMI of deer grazing chicory in the May trial were considered higher than biologically feasible (6kg DM /hd /day), even though actual intakes of deer grazing chicory could not be compared to

estimated intakes in this study. This was probably related to the low n-alkane content of chicory. Existing established alternative marker techniques for estimating DMI of animals grazing forage herbs such as chicory, for example use of chromic oxide, do not seem to offer a better solution as similar high estimated DMI's have been reported in lactating hinds grazing chicory using this technique (6.3kgDM/ hd/ day) (Kusmartono *et al.*, 1996). The validation of the η-alkane technique should be extended to validate the use of the n-alkane technique for estimation of the DMI of chicory and other fresh forages including legumes.

It is believed that from the findings of the n-alkane validation study, alkanes within the digestive tract are not as inert as suggested by Dove and Mayes (1991, 1996). Dove and Mayes suggested that alkanes were inert in the digestive tract as there was a similar recovery of dosed and synthetic η-alkanes in the faeces. In contrast, this study showed that faecal recovery rates of η-alkanes were highly variable. This may indicate either digestion, metabolism of alkanes by the animal or microbial populations within the digestive tract. However, this may be in part due to the greater variability of calculated dose rates and measured disappearance rates of the matrix of the controlled release capsules used as compared with those reported by Dove *et al.,* (2002). Dose rates of η-alkanes were found to be different between pasture and plantain, suggesting that for any treatment forages the alkane dose rates need to be calculated from measured matrix disappearance rates, not manufacturer's dose rates. However, inter-animal variability was also found to be high, suggesting that a mean or representative dose rate gained from one or more animals may not be able to be used across all animals in a treatment group.

Differences of faecal recovery rates of η-alkanes may also be associated with the unique digestive physiology of deer (Domingue *et al.,* 1991). Dove & Mayes, (1991) hypothesised that 30-40% of the dosed η-alkanes were associated with the liquid phase of the digesta, whereas natural η-alkanes remained associated with the particulate phase of the digesta. Deer have a

rapid passage rate of the liquid phase compared with the particulate phase of digesta in comparison to sheep (Domingue *et al.*, 1991). Therefore the double η-alkane technique may be more unsuitable for accurate measurements of dry matter intake in deer, compared with other ruminant livestock species.

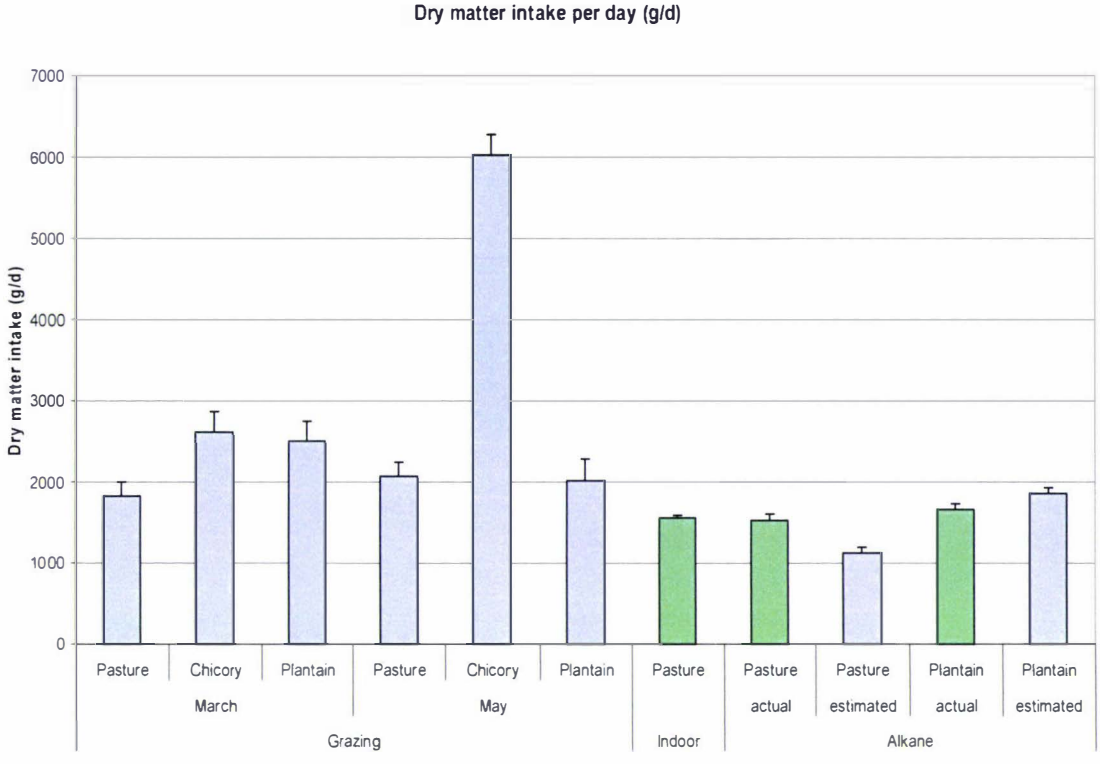


Figure 5.2. Dry matter intake from red deer across three experiments, Grazing experiment (Chapter 2), Indoor experiment (Chapter 3), Alkane validation experiment (Chapter 4), error bars represent the standard error of the least square mean. Dry matter intakes that are estimated by the double n-alkane method are represented in blue (■), measured intakes are represented by the green bars (■).

5.4 Conclusions and recommendations for future research

Conclusions

- Methane emissions per kg DMI from red deer appear to be similar to those from sheep and cattle based upon indoor studies where intake was directly measured.

- The feeding of chicory compared with pasture could reduce methane production per kg of DMI. In contrast, it appears that methane emissions per kg of DMI were not reduced in two out of three occasions when animals were fed plantain compared with ryegrass-based pasture.

- The estimation of DMI by the double n-alkane technique seems to be highly variable and of poor accuracy and concurrent measurements with methane production may significantly over or underestimate methane production per kg of DMI.

Recommendations for future research

- To confirm that methane emissions per kg of DMI of red deer grazing ryegrass-based pasture are similar to those of sheep and cattle, methane measurements need to be repeated and under conditions where direct comparisons of ruminant species can be made.

- The measurement of methane emissions from deer needs to be repeated for chicory and evaluated for other specialised forages that are used commercially or that may be potentially be used in deer grazing systems, to asses methane mitigation properties of these forages.

- The validation of the η-alkane technique implies that for future research where accurate individual or group estimates are required to be able to compare results across treatments, experiments and species, dry matter intakes need to be directly measured.

LIST OF REFERENCES

1. Audigé, L. J. M. (1995). *Deer herd health and production profiling.* Unpublished PhD, Massey University.
2. Bailey, R. W., & Ulyatt, M. J. (1970). Pasture quality and ruminant nutrition II. Carbohydrate and lignin composition of detergent-extracted residues from pasture grasses and legumes. *New Zealand Journal of Agricultural Research, 13*, 591-604.
3. Baker, S. K. (1999). Rumen methanogens, and inhibition of methanogenesis. *Australian Journal of Agricultural Research, 50*, 1293-1298.
4. Barry, T. N. (1998). The feeding value of chicory *(Cichorium inybus)* for ruminant livestock. *Journal of Agricultural Science, 131*, 251-257.
5. Barry, T. N., Hoskin, S. O., & Wilson, P. R. (2002). Novel forages for growth and health in farmed deer. *New Zealand Veterinary Journal, 50*(6), 244-251.
6. Bauchop, T., & Mountfort, D. O. (1981). Cellulose fermentation by a rumen anaerobic fungus in both the absence and presence of rumen methanogens. *Applied and Environmental Microbiology, 42*(6), 1103-1110.
7. Benchaar, C., Pomar, C., & Chiquette, J. (2001). Evaluation of dietary strategies to reduce methane production in ruminants: A modelling approach. *Canadian Journal of Animal Science, 81*, 563-574.
8. Bingham, C., Wilson, P. R., & Davies, A. S (1990). Real-time ultrasonography for pregnancy diagnosis and estimation of foetus age in farmed red deer. *Veterinary Record. 125,* 102-106.
9. Blaxter, K. L., & Clapperton, J. L. (1965). Prediction of the amount of methane by ruminants. *British Journal of Nutrition, 19*, 511-522.
10. Boadi, D. A., Wittenberg, K. M., & Kennedy, A. D. (2002). Validation of the sulphur hexafluoride (SF_6) tracer gas technique for measurement of methane and carbon dioxide production by cattle. *Canadian Journal of Animal Science, 82*, 125-131.

11. Clark, H. (2002). Ruminant methane emission measurements and estimates - from gut to globe. *Proceedings of the New Zealand Society of Animal Production, 62*, 206-210.

12. Corson, D. C., Waghorn, G. C., Ulyatt, M. J., & Lee, J. (1999). NIRS: Forage analysis and livestock feeding. *Proceedings of the New Zealand Grassland Association, 61*, 127-132.

13. Crutzen, P. J. (1995). The role of methane in atmospheric chemistry and climate. *Ruminant physiology: digestion, metabolism, growth and reproduction. Proceedings of the eighth international symposium on ruminant physiology.*

14. Czerkawski, J. W. (1986). *An introduction to rumen studies.* United Kingdom: Pergamon Press.

15. de Klein, C., Boswell, C., Drewry, J., McDowell, R., & Muirhead, R. (2002). *Deer and the Environment. A review of current knowledge to identify knowledge gaps and research priorities. Prepared for DEEResearch.* www.DEEResearch.org.nz

16. Demeyer, D. I., & Van Nevel, C. J. (1974). Methanogens, an integrated part of carbohydrate fermentation, and its control. *Paper presented at the Proceedings of the IV International symposium on ruminant physiology,* Sydney, Australia.

17. Domingue, F. B., Dellow, D. W., Wilson, P. R., & Barry, T. N. (1991). Comparative digestion in deer, goats and sheep. *New Zealand Journal of Agricultural Research, 34*, 45-53.

18. Dove, H., & Mayes, R. W. (1991). The use of plant wax alkanes as marker substances in studies of the nutrition of herbivores: A review. *Australian Journal of Agricultural Research, 42*, 913-953.

19. Dove, H., & Mayes, R. W. (1996). Plant wax components: A new approach to estimating intake and diet composition in herbivores. *Journal of Nutrition, 126*, 13-26.

20. Dove, H., Mayes, R. W., & Freer, M. (1996). Effects of species, plant part and plant age in the n-alkane concentration in the cuticular wax of pasture plants. *Australian Journal of Agricultural Research, 47*, 1333-1347.

21. Dove, H., Mayes, R. W., Lamb, C. S., & Ellis, K. J. (2002). Factors influencing the release rate of alkanes from an intra-ruminal, controlled-release device, and the resultant accuracy of intake estimation in sheep. *Australian Journal of Agricultural Research, 53*, 681-696.

22. Fennessy, P. F., Moore, G. H., & Corson, I. D. (1981). Energy requirements of red deer. *Proceedings of the Society of Animal Production, 41*, 167-173.

23. Forbes, J. M. (1977). Interrelationships between physical and metabolic control of voluntary food intake in fattening, pregnant and lactating mature sheep: A model. *Animal production, 24,* 91-101.

24. Galbraith, J. K., Mathison, G. W., Hudson, R. J., McAllister, T. A., & Cheng, K. J. (1998). Intake, digestibility, methane and heat production in bison, wapiti and white-tailed deer. *Canadian Journal of Animal Science, 78*, 681-691.

25. Gedir, J. V., & Hudson, R. J. (2000). Estimating dry matter digestibility and intake in wapiti (*Cervus elaphus canadensis)* using the double *n*-alkane ratio technique. *Small Ruminant Research, 36*, 57-62.

26. Glass, T. L., Bryant, M. P., & Wolin, M. J. (1977). Partial purification of ferredoxin from *Ruminococcus albus* and its role in pyruvate metabolism and reduction of nicotinamide adenine dinucleotide by H_2. *Journal of Bacteriology, 131*(2), 463-472.

27. Hegarty, R. S. (1999). Mechanisms for competitively reducing ruminal methanogenesis. *Australian Journal of Agricultural Research, 50*, 1299-1305.

28. Hendricksen, R. E., Reich, M. M., Robertson, R. F., Reid, D. J., Gazzola, C., Rideout, J. A., & Hill, R. A. (2002). Estimating the voluntary intake and digestibility of buffel-grass and lucerne hays offered to Brahman-cross cattle using n-alkanes. *Animal Science, 74*, 567-577.

29. Hofmann, R. R. (1985). Digestive physiology of the deer- Their morphysiological specialisation and adaption. In P. F. Fennessy & K. R. Drew (Eds.), *Biology of Deer Production* (pp. 393-408): The Royal Society of New Zealand.

30. Holter, J. B., & Young, A. J. (1992). Methane prediction in dry and lactating Holstein cows. *Journal of Dairy Science, 75*, 2165-2175.

31. Hoskin, S. O. (1998). *Internal parasitism and growth of farmed deer fed different forage species.* Unpublished PhD thesis, Massey University, Palmerston North, New Zealand.

32. Hoskin, S. O., Barry, T. N., & Wilson, P. R. (2003). The role of plants containing secondary compounds in sustainable deer farming - a review. *Wrightson Seeds Deer Nutrition Symposium: The nutrition and management of deer on grazing systems. Proceedings of a New Zealand Grassland Association (Inc) Symposium*, Lincoln University, New Zealand.

33. Hoskin, S. O., Stafford, K. J., & Barry, T. N. (1995). Digestion, rumen fermentation and chewing behaviour of red deer fed fresh chicory and perennial ryegrass. *Journal of Agricultural Science, 124*, 289-295.

34. Howden, S. M., & Reyenga, P. J. (1999). Methane Emissions from Australian livestock: implications of the Kyoto Protocol. *Australian Journal of Agricultural Research, 50*, 1285-1291.

35. Jermy, C. (2003). *Industry overview from an international prespective.* Paper presented at the Wrightson seeds deer nutrition symposium: The nutrition and management of deer on grazing systems, Lincoln University.

36. Joblin, K. N. (1999). Ruminal acetogens and their potential to lower ruminant methane production. *Australian Journal of Agricultural Research, 50*, 1307-1313.

37. Johnson, K., Huyler, M., Westberg, H., Lamb, B., & Zimmerman, P. (1994). Measurement of methanr emissions from ruminant livestock using a SF_6 tracer technique. *Environmental Science and Technology, 28*, 359-362.

38. Johnson, K. A., & Johnson, D. E. (1995). Methane emissions form cattle. *Journal of Animal Science, 73*, 2483-2492.

39. Krause, M. (2002). Methane production from sheep grazing white clover and from sheep fed white clover in indoor and outdoor crates. *Unpuplished*

40. Kurihara, M., Shibata, M., Nishida, T., Purnomoadi, A., & Terada, F. (1997). *Methane production and its dietary manipulation in ruminants*. Tyoko: Japan Scientific Press.

41. Kusmartono, Barry, T. N., Wilson, P. R., Kemp, P. D., & J, S. K. (1996). Effects of grazing chicory *(Chichorium intybus)* and perennial ryegrass *(Lolium perenne)*/white clover (*Trifolium repens)* pasture upon the growth and voluntary feed intake of red and hybrid deer during lactation and post-weaning growth. *Journal of Agricultural Science, 127*, 387-401.

42. Kusmartono, Shimada, A., & Barry, T. N. (1997). Rumen digestion and rumen outflow rate in deer fed fresh chicory *(Cichorium intybus)* or perennial ryegrass *(Lolium perenne). Journal of Agricultural Science, 128*, 87-94.

43. Kusmartono, Shimada, A., Stafford, K. J., & Barry, T. N. (1996). Intra-ruminal particle size reduction in deer fed fresh perennial ryegrass (*Lolium perenne*) or chicory *(Chichorium intybus). Journal of Agricultural Science, 127*, 525-531.

44. Lee, S. C., Lee, H. J., & Oh, Y. K. (2000). Methane Production from enteric fermentation in ruminants. *Journal of Animal Science, 13* (Special Issue), 171-181.

45. Loza, M. J. (2003). *Annual conference speeches*. www.deernz.org/

46. MAF., (2003). *Deer Monitoring Report.* Ministry of Agriculture and Forestry.

47. Mayes, R. W., Lamb, C. S., & Colgrove, P. M. (1986). The use of dosed and herbage n-alkanes as markers for the determination of herbage intake. *Journal of Agricultural Science, 107,* 161-170.

48. McAllister, T. A., Okine, E. K., Mathison, G. W., & Cheng, K. J. (1996). Dietary. environmental and microbiological aspects of methane production in ruminants. *Canadian Journal of Animal Science, 76*, 231-243.

49. McCarthy, R. D. (1964). Mammalian metabolism of straight-chain saturated hydrocarbons. *Biochimica Et Biophysica ACTA, 84,* 74-79.

50. McDonald, P., Edwards, R. A., Greenhalgh, J. F. D., & Morgan, C. A. (1995). *Animal Nutririon, fifth edition*: Longman.

51. McKenna, E. J., & Kallio, R. E. (1965). The biology of hydrocarbons. *Annual Review of Mircobiology, 19,* 183-205.

52. Miller, T. L. (1995). Ecology of methane production and hydrogen sinks in the rumen. Ruminant physiology: Digestion, metabolism, growth and reproduction. *Proceedings of the eighth international symposium on ruminant physiology.*, Ferdinand Enke Verlag Stuttgart.

53. Miller, T. L., & Wolin, M. J. (2001). Inhibition of growth of methane producing bacteria of the ruminant forestomach by hydroxymethylglutaryl~SCoA reductase inhibitors. *Journal of Dairy Science, 84*, 1445-1448.

54. NIWA, (2004). *Climate Summary 2003.* www.niwa.co.nz

55. Moe, P. W., & Tyrrell, H. F. (1979). Methane production in dairy cows. *Journal of Dairy Science, 62*, 1583-1586.

56. Moloney, S. (2003). Designing pastures for modern deer farming systems. *Proceedings of the New Zealand Society of Animal Production, 63*, 274-279.

57. Moss, A. R., Jouany, J.-P., & NewBold, J. (2000). Methane production by ruminants: its contribution to global warming. *Annuals of Zootechnology, 49*, 231-253.

58. Mulley, R. C. (2003, 8-9 November). *The feed requirements of adult red deer.* Paper presented at the Wrightson seeds deer nutrition symposium: The nutrition and management of deer on grazing systems, Lincoln University.

59. Murray, R. M., Bryant, A. M., & A, L. R. (1976). Rates of methane production in the rumen and large intestine of sheep. *British Journal of Nutrition, 36*, 1-14.

60. Newbold, C. J., Ushida, K., Morvan, B., Fonty, G., & Jouany, J. P. (1996). The role of ciliate protozoa in the lysis of methanogenic archaea in rumen fluid. *Letters in Applied Microbiology, 23*, 421-425.

61. NIR. (2003). *National Inventory Report 1990-2001.*

62. Ohajuruka, O. A., & Palmquist, D. L. (1991). Evaluation of *n*-alkanes as digesta markers in dairy cattle. *Journal of Animal Science, 69,* 1726-1732.

63. O'Hara, P., Freney, J., & Ulyatt, M. J. (2003). Abatement of agricultural non-carbon dioxide greenhouse gas emissions. A study of research requirements. *Report prepared for the Ministru of Agriculture and Forestry on behalf of the Covenor, Ministeral Group on Climate Change, the Mintester of Agriculture and the Primary Industries Council.*

64. Parker, W. J., & Loza, M. J. (2003). Deer industry expansion and wealth creation. *Wrightson Seeds Deer Nutrition Symposium: The nutrition and management of deer on grazing systems. Proceedings of a New Zealand Grassland Association (Inc) Symposium,* Lincoln University, New Zealand.

65. Pinares-Patino, C. S. (2000). *Methane emission from forage-fed sheep, a study of variation between animals.* Unpublished PhD Thesis, Massy University, Palmerston North.

66. Pinares-Patino, C. S., Ulyatt, M. J., Holmes, C., Barry, T. N., & Lassey, K. R. (2001). *Some rumen digestion characteristics and methane emission in sheep.* Paper presented at the Energy metabolism in animals. Proceedings of the fifthtenth symposium on energy metabolism in animals.

67. Pinares-Patino, C. S., Ulyatt, M. J., Lassey, K. R., Barry, T. N., & Holmes, C. (2003). Rumen function and digestion parameters associated with differances between sheep in methane emissions when feed chaffed lucerne hay. *Journal of Agricultural Science, 140*, 205-214.

68. Ravindranath, N. H., & Sathaye, J. A. (2002). *Climate change and developing countries*: Lluwer Academic Publishers.

69. Rhind, S. M., McMillien, S. R., Duff, E., Hirst, D., & Wright, S. (1998). Seasonality of meal patterns and hormonal correlates in red deer. *Physiology and Behavior, 65*(2), 295-302.

70. Robertson, L. J., & Waghorn, G. C. (2002). Dairy industry perspectives on methane emissions and production from cattle fed pasture or total mixed rations in New Zealand. *Proceedings of the New Zealand Society of Animal Production, 62*, 213-218.

71. Ru, Y. J., Miao, Z. H., Glatz, P. C., & Choct, M. (2002). Predicting feed intake of follow deer (*Dama Dama)* using alkanes as a maker. *Asian-Australian Journal of Animal Science, 15*(2), 209-212.

72. Russell, J. B., & Wallace, R. J. (1997). Energy-yielding and energy consuming reactions. In P. N. Hobsen & C. S. Stewart (Eds.), *The rumen microbial ecosystem, second edition* (pp. 246-282): Blackie Academic & Professional.

73. SAS (1998) SAS® User's Guide: Satatistics, Version 7th. 1998. SAS Inst., Inc., Cary, NC.

74. Semiadi, G., Barry, T. N., Wilson, P. R., Hodgson, J., & Purchas, P. W. (1993). Growth and venison production from red deer (*Cervus elaphus)* grazing red clover *(Trifolium pratense)* or perennial ryegrass (*Lolium perenne*)/white clover (*Trifolium repens*) pasture. *Journal of Agricultural Science, 121*, 265-271.

75. Semiadi, G., Holmes, C., Barry, T. N., & Muir, P. D. (1998). The efficiency of utilization of energy and nitrogen in young sambar *(Cervus unicolor)* and red deer *(Cervus elaphus). Journal of Agricultural Science, 130*, 193-198.

76. Shin, H. T., Hudson, R. J., Gao, X. H., & Suttie, J. M. (2000). Nutritional requirements and management for farmed deer - review. *Asian-Australian Journal of Animal Science, 13*(4), 561-573.

77. Stevens, D. R. (2003). Project: Improving spring growth of weaner deer using supplemental roughage. *Final reprot Prepared for DEEResearch LTD.*

78. Stevens, D. R., & Corson, D. C. (2003). Effects of winter feed type on feed intake during the transition back to spring pasture. Wrightson Seeds Deer Nutrition Symposium: The nutrition and management of deer on grazing systems. Proceedings of a New Zealand Grassland Association (Inc) Symposium, Lincoln University.

79. Stewart, C. S., Flint, H. J., & Bryant, M. P. (1997). The rumen bacteria. In P. N. Hobsen & C. S. Stewart (Eds.), *The rumen microbial ecosystem* (Second Edition ed., pp. 10-72). New York: Blackie Academic & Professional.

80. Takahashi, J. (2001). Nutritional manipulation of methanogenesis in ruminants. *Asian-Australian Journal of Animal Science, 14* (Special Issue), 131-135.

81. Terrill, T. H., Rowen, A. M., Douglas, G. B., & Barry, T. N. (1992). Determination of extractable and bound condensed tannin concentrations in forage plants, protein concentrate meals and cereal grains. *Journal of the Science of Food and Agriculture, 58*, 321-329.

82. Ulyatt, M. J., Baker, S. K., McCrabb, G. J., & R, L. K. (1999). Accuracy of SF_6 Tracer technology and alternatives for field measurements. *Australian Journal of Agricultural Research, 50*, 1329-1334.

83. Ulyatt, M. J., Clark, H., & Lassey, K. R. (2002a). Methane and climate change. *Proceedings of the New Zealand Grassland Association, 64*(153-157).

84. Ulyatt, M. J., Lassey, K. R., Martin, R. J., Walker, C. F., & Shelton, I. D. (1997). Methane emission from grazing sheep and cattle. *Proceedings of the New Zealand Society of Animal Production, 57*, 130-133.

85. Ulyatt, M. J., Lassey, K. R., Shelton, I. D., & Walker, C. F. (2002b). Seasonal variation in methane emission from dairy cows and breeding ewes grazing ryegrass/white clover pasture in New Zealand. *New Zealand Journal of Agricultural Research, 45*, 217-226.

86. Valiente, O. L., Delgado, P., de Vega, A., & Guada, J. A. (2003). Validation of the *n*-alkane technique to estimate intake, digestibility, and diet composition in sheep consuming mixed grain:roughage diets. *Australian Journal of Agricultural Research, 54*, 693-702.

87. Van Soest, P. J., (1994). *Nutritional Ecology of the Ruminant second edition, 230-252*. Cornell University Press, USA.

88. Waghorn, G. C., Tavendale, M. H., & Woodfield, D. R. (2002). Methanogenesis from forages fed to sheep. *Proceedings of the New Zealand Grassland Association, 64*, 167-171.

89. Wolin, M. J., Miller, T. L., & Stewart, C. S. (1997). Microbe-microbe interactions. In P. N. Hobsen & C. S. Stewart (Eds.), *The rumen microbal ecosystem, second edition* (pp. 467-491).

90. Woodward, S. L., Waghorn, G. C., Lassey, K. R., & Laboyrie, P. G. (2002). Does feeding sulla (*Hedysarum coronarium*) reduce methane emissions from dairy cows. *Proceedings of the New Zealand Society of Animal Production, 62*, 227-230.

91. Woodward, S. L., Waghorn, G. C., Ulyatt, M. J., & Lassey, K. R. (2001). Early indications that feeding *Lotus* will reduce methane emissions from ruminants. *Proceedings of the New Zealand Society of Animal Production, 61*, 23-26.