FIELD MEASUREMENT OF METHANE AND CARBON DIOXIDE PRODUCTION BY CATTLE:
USE OF THE SULPHUR HEXAFLUORIDE (SF_6) TRACER GAS TECHNIQUE

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by

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Field Measurement of Methane and Carbon Dioxide Production by Cattle: Use of the Sulphur Hexafluoride (SF_6) Tracer Gas Technique

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Dinah Ama Boadi

A Thesis/Practicum submitted to the Faculty of Graduate Studies of The University of

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DOCTOR OF PHILOSOPHY

ABSTRACT

Three research trials were conducted to: a) validate the sulphur hexafluoride (SF_6) tracer gas technique (Tracer) in measuring methane (CH_4) and carbon dioxide (CO_2) production by cattle; b) to test the methodology in an outdoor pen setting using animal types, forage quality and feeding levels as test variables; c) to test the methodology in a pasture setting using grazing season (forage quality) and grain supplementation as the test variables. In the first study, CH_4 and CO_2 production measurements from six crossbred heifers (400 ±13.0 kg) using the Tracer were compared to estimates by respiration hood calorimetry (Cal). Methane production (130 ± 4.0 L d^{-1}) using Cal was not different ($P > 0.05$) from Tracer (137 ± 4.0 L d^{-1}). On the other hand, Tracer CO_2 production was 20% higher than Cal CO_2 production ($P < 0.01$). Animal-to-animal variation was significant using Tracer (11.7%) but not Cal (0.1%, $P > 0.05$). In the second study, cattle breed (Holstein (n = 6; 310 ± 15.3 kg)) vs. Charolais cross (n = 6; 310 ± 10.0 kg)), had no effect on CH_4 production ($P > 0.05$). Forage quality (High vs. Medium vs. Low) affected CH_4 (L d^{-1}) output with High = Medium >Low ($P < 0.05$), this observation was absent on restricted intake ($P > 0.05$). On ad-libitum feeding DMI was strongly correlated ($P = 0.0001$; r = 0.80) with CH_4 production. High quality forages yielded 42% and 32% less CH_4 (L kg^{-1} DOMI) than Low quality forages during ad-libitum and restricted feeding, respectively ($P < 0.05$). In the third study, eight beef steers (344.6 ± 6.4 kg) were assigned to legume-grass pastures (C; n = 4) or legume-grass pastures and a rolled barley supplement (S; n = 4) during the EARLY, MID and LATE periods of a grazing season. Grain supplementation reduced forage DMI by 11% ($P = 0.03$) and increased

TOMI by 14% ($P = 0.001$). There were no differences in CH_4 production (% TGEI) between S (6.4 ± 0.6%) and C (6.7 ± 0.6%) steers ($P = 0.7$). Energy lost as CH_4 (%TGEI), ranged from 4.7 to 8.4% (mean 6.5 ± 0.3%) during the grazing season. Steers on EARLY pastures had a 44% and 29% lower ($P < 0.05$) energy loss as CH_4 than animals on MID and LATE pastures. It is concluded that the SF_6 tracer gas technique provides an accurate measure of rumen CH_4 production by cattle in a free ranging environment. However the higher variation in measurements requires that more animals or more sampling days are needed to effectively identify differences between management treatments. Further studies are needed to verify CO_2 production from cattle using the SF_6 tracer gas technique.

DEDICATED

To my mother, Mrs. Vida K. Boadi, who took care of my kids so I could start and finish my Ph.D. program.

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FOREWORD

The research conducted for the purpose of this Ph.D. program has been written as the following three manuscripts. All manuscripts have been accepted by the Canadian Journal of Animal Science.

1. Validation of the sulphur hexafluoride (SF_6) tracer gas technique for measurement of methane and carbon dioxide production by cattle.
2. Methane production from dairy and beef heifers fed forages differing in nutrient density using the sulphur hexafluoride (SF_6) tracer gas technique.
3. Effects of grain supplementation on methane production of grazing steers using the sulphur hexafluoride (SF_6) tracer gas technique.

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ABBREVIATIONS

ADF	Acid detergent fibre
ADG	Average daily gain
BW	Body weight
CH_4	Methane
CO_2	Carbon dioxide
CP	Crude protein
DE	Digestible energy
DEI	Digestible energy intake
DM	Dry matter
DMI	Dry matter intake
DOMI	Digestible organic matter intake
GE	Gross energy
GEI	Gross energy intake
GHG	Greenhouse gas
IVDMD	In vitro dry matter digestibility
IVOMD	In vitro organic matter digestibility
NDF	Neutral detergent fibre
O_2	Oxygen
OMD	Organic matter digestibility
SF_6	Sulphur hexafluoride

TDMI	Total dry matter intake
TOMI	Total organic matter intake
TGEI	Total gross energy intake

1.0. GENERAL INTRODUCTION

Methane (CH_4), a colorless, odorless gas is produced as a by-product of microbial fermentation of feed in the gastrointestinal tract of ruminant animals. Methane is produced predominantly in the rumen (87%) and to a small extent (13%) in the large intestines (Murray et al. 1976; Torrent and Johnson 1994). Rumen CH_4 is primarily emitted from the animal by eructation. About 89% of the CH_4 produced in the large intestine is absorbed and excreted through the lungs; the remainder is excreted via the anus (Murray et al. 1976).

Methane production is a substantial loss of energy in the production of meat and milk by the ruminant. In general, CH_4 production in cattle constitutes about 2 -12% of dietary gross energy intake (Johnson and Johnson 1995). In recent years there has been renewed interest in CH_4 production by ruminants as a result of their contribution to global CH_4 emissions and its involvement in global warming. Methane gas is known to be the second most important greenhouse gas (GHG), contribution about 20% to global warming, with the largest contribution (63%) coming from carbon dioxide (CO_2) (Ruminant Livestock Efficiency Program (RLEP) 1997). The world's population of ruminants is estimated to contribute 79 million tonnes of CH_4 annually, which is about 20% of total atmospheric CH_4 emissions (Gibbs and Johnson 1994). Atmospheric CH_4 emissions, which have a warming potential of 21 fold greater than CO_2 are known to be increasing at a rate of 1% per year (Intergovernmental Panel on Climate Change (IPCC) 1994). It has been projected that global CH_4 emissions will increase by 15-38 % by the year 2025 (Houghton et al. 1992). Since CH_4 has a short atmospheric half-life of 11 years (RLEP 1997), any reduction in atmospheric CH_4

emissions will have a relatively immediate impact on global warming.

Research in the past two decades has identified factors such as the quality of diet fed (Moe and Tyrrell 1979); level of intake (Blaxter and Clapperton 1965); digesta passage rate and atmospheric temperature (Okine et al. 1989; von Keyserlingk and Mathison 1993); addition of unsaturated fatty acid (Czerkawski 1986; Dong et al. 1997a); species (Galbraith et al. 1998); and ionophore use (Thorton and Owens 1981; Mbanzamihigo et al. 1996), which influence the amount of CH_4 produced from ruminants. However, it has been difficult to assess the effectiveness of the above factors on CH_4 production under a commercial production setting, especially in pastured animals. This is because of the difficulties associated with gas measurement during grazing.

Measurements of ruminant CH_4 gas, have been made using respiratory calorimetry (McLean and Tobin 1987; Kelly et al 1994), isotopic tracers and *in vitro* techniques (Frances et al. 1993; Kung et al. 1992; Dong et al. 1997a; McMahon et al. 1999). Prediction equations and models relating CH_4 production to the characteristics of the feed, have also been established (Blaxter and Clapperton 1965; Moe and Tyrell 1979; Baldwin et al. 1987; Mills et al. 2001). Respiration calorimetry restricts animals from their natural environment for gas production measurements. *In vitro* techniques have been helpful as screening devices to predict CH_4 production however, it is difficult to extrapolate *in vitro* CH_4 production to cattle in a commercial production systems where rumen microbial populations and fermentation patterns are constantly changing due to feed selection and meal frequency.

The sulphur hexafluoride (SF_6) tracer gas technique uses SF_6, an inert non toxic gas, as a marker for quantifying CH_4 production from the mouth and nose. The technique enables direct measurement of CH_4 and evaluation of reduction strategies in free ranging animals (Johnson et al. 1994). There are variable validation reports comparing CH_4 estimates using the SF_6 tracer gas technique and the respiration calorimetry (Johnson et al. 1994; Ulyatt et al.1999). There is the need to confirm the accuracy of the SF_6 tracer technique, and to establish the measurement variability expected with its use in the field.

In ruminants, CO_2 occurs as a result of microbial fermentation as well as from metabolism in the body, and is exhaled through eructation and expiration (Duke 1993). It is known that the majority of eructed gases (over 90%) are inhaled via the trachea into the lungs, before exhalation to the atmosphere along with respiratory gas (Hoernicke et al. 1965; Young and Corbett 1972). It may then be possible to measure CO_2 production rate in expired and eructed gases by the SF_6 tracer gas technique, which has not been validated previously. Prediction of heat production from CO_2 production rates would allow energy expenditure to be estimated in grazing animals using the SF_6 tracer gas technique, to be determined without the need to restrain animals for respiration calorimetry.

The first experiment of the thesis research was conducted to validate the SF_6 tracer technique in measuring CH_4 and CO_2 production rates by cattle. The second experiment was conducted to test the SF_6 tracer technique in outdoor pen setting, to assess CH_4 output using animal types, forage quality and feeding levels as test variables. Finally, an experiment was undertaken to test the methodology in a pasture setting using grazing season (forage quality) and grain supplementation as the test variables.

2. 0. LITERATURE REVIEW

2.1. LIVESTOCK METHANE PRODUCTION

2.1.1. Global production estimates

It has been shown that 70% of global atmospheric CH_4 emissions are anthropogenic, or human related, while the rest of CH_4 emissions are from natural processes such as wetlands, oceans and fresh water (Westberg et al. 2001). The anthropogenic sources include coal mining, natural gas and petroleum industries, biomass burning, rice paddies, landfills, and fermentation in domestic animals and manure (Houghton et al. 1992). Animal production was estimated to contribute 20.4% of total atmospheric CH_4 emissions, and is considered to be the largest source of anthropogenic emission (Houghton et al. 1992). Thus making it a major target for reducing global CH_4 emissions. In the 1980's, Crutzen et al. (1986), estimated livestock CH_4 production to be 73.4 million tonnes (t) y^{-1} (Table 1). Crutzen et al. (1986) estimated that an additional 4 million t y^{-1} were contributed by wild ruminants, which is approximately 5% of that from domestic livestock. Estimates published by Gibbs and Johnson (1994) using 1990 global livestock census data show an increase in total global estimates of livestock CH_4 production to 79.2 million t y^{-1} even though animal numbers have not increased significantly over the decade (Table 1).

Ruminant livestock produce 97% of CH_4 generated by domestic animals (Johnson and Ward 1996). Cattle produce 73-74% of total livestock CH_4 emissions, while buffalo, sheep and goats produce 9-10%, 8% and 3.8-3.9% respectively of total animal emissions (Johnson and Ward 1996). Estimates of global CH_4 production from animals are based on the accuracy of estimating livestock CH_4 production from individual animals and animal populations in the different parts of world by the guidelines of the Intergovernmental Panel on Climate Change (IPCC,1995).

Table 1. Comparison of two estimates of global enteric CH_4 emissions by livestock[a]

	Crutzen et al. 1986 estimates		Gibbs and Johnson 1994 estimates		
Species	Head x 10^3	CH_4 (Tg y^{-1})[b]	Head x 10^3	CH_4 (Tg y^{-1})	% of total emissions
Cattle	1225	54.3	1279	58.1	73.4
Buffalo	124	6.2	141	7.7	9.7
Sheep	1137	6.9	1191	7.1	8.8
Goats	476	2.4	557	2.8	3.5
Camel	17	1.1	19	0.9	1.1
Pigs	774	0.9	857	1.1	1.3
Equine	117	1.7	119	1.7	2.1
Total		73.4		79.2	

[a] Data from Crutzen et al. (1986) and Gibbs and Johnson (1994).

[b] Tg = teragram = 10^{12} g = million tonne.

2.1.2. Canadian livestock methane production estimates

Current greenhouse gas (GHG) production from the Canadian agriculture sector (expressed in terms of its atmospheric warming potential [CO_2-equivalent]) are estimated at over 66 megatonnes (Mt) CO_2-equivalent, which represents 10% of Canada's total GHG production (Agriculture and Agri-Food Climate Change (AAFCC) Table Options Report 2000). Livestock production systems contribute about 58% of total production from agriculture with 28% associated with enteric fermentation and 30% related to manure handling, storage and land application (AAFCC 2000). Methane production by cattle is estimated to be 871,000 t annually, representing 96% of the CH_4 produced by livestock (1,057, 220 t y^{-1}; McAllister et al. 1996). Contribution by wild ruminants, has been estimated at 149,000 t y^{-1} but the accuracy of this estimate is questionable due to inaccurate population estimates and lack of measurements from wild ruminants (McAllister et al. 1996). Emissions from enteric fermentation in livestock production were 18 Mt CO_2-equivalent in 1996 and have increased by 14% since 1990 due to an increase in beef production (Neitzert et al. 1999).

Currently, livestock CH_4 production from enteric fermentation is estimated by multiplying the population of various animal categories by average emission factors for those categories (Table 2). The methodology is established in the guidelines of IPCC which uses default emission rates for cool climates (Neitzert et al. 1999). The IPCC emission factors are based on research conducted in the US, however the literature suggests that the type of animal, amount of feed, and environmental conditions can affect the amount of CH_4 produced and, therefore, may not be applicable to Canadian livestock production systems.

Table 2. Canadian methane emission factors for livestock and manure

Species	Enteric fermentation ($kg\ CH_4\ head\ year^{-1}$)	Manure management ($kg\ CH_4\ head\ year^{-1}$)
Cattle		
Bulls	75	1
Dairy cows	118	36
Beef cows	72	1
Dairy heifers	56	36
Beef heifers	56	1
Heifers for Slaughter	47	1
Steers	47	1
Calves	47	1
Other Livestock		
Swine	1.5	10
Sheep	8	0.19
Goats	8	0.12
Horses	13	1.4
Poultry		
Chickens	Not estimated	0.078
Hens	Not estimated	0.078
Turkeys	Not estimated	0.078

Source IPCC 1997, in Neitzert et al. (1999).

Methane emissions by cattle under Canadian feeding and management conditions, has been determined in several recent studies (McCaughey et al.1997; Kinsman et al.1997; McCaughey et al. 1999). Emissions from pastured steers (356 kg) ranged from 242-306 L d^{-1} or 63-80 kg hd^{-1} y^{-1} (McCaughey et al.1997), while CH_4 emissions from 511 kg lactating beef cows were 374 L d^{-1} or 98 kg hd^{-1} y^{-1} on alfalfa -grass pastures and 411 L d^{-1} or 107 kg hd^{-1} y^{-1} on grass pastures (McCaughey et al. 1999). Kinsman et al. (1997) found that lactating Holstein cows (600 kg) had a mean CH_4 production of 527 L d^{-1} or 139 kg hd^{-1} y^{-1} (See Appendix 10.0. for conversion factors). Comparison of actual emission rate data suggests an underestimation of some rates used by IPCC methodology. Information from these Canadian production studies are useful in developing a national database in which enteric CH_4 production estimates could be refined.

2.1.3. Units of expressing livestock methane production

Livestock CH_4 emission estimates have often been reported in Tg y^{-1} ; kg y^{-1} ; L d^{-1} and also by its warming potential when expressed on a CO_2-equivalent by environmentalists (Gibbs and Johnson 1994; Neitzert et al. 1999). Animal nutritionists often evaluate enteric fermentation losses as CH_4 on the basis of energy losses;(i.e., CH_4 as percentage of gross energy intake [GEI] or digestible energy intake [DEI]).

To producers, expression of CH_4 emission rates from livestock discussed in terms of weight or volume (g d^{-1}; kg y^{-1} or L d^{-1}), are of little relevance to productivity, since they do not appropriately describe the extent of feed energy lost as CH_4 when CH_4 reduction strategies are applied. For example, when productivity is improved with high quality diets, this may give rise to higher daily CH_4 production due to the increased amount of fermentable material presented to reticuloruminal microorganisms, however, the fractional CH_4 loss per unit weight gain or milk produced may be lower. Expression of CH_4 production from livestock in terms of efficiency (g CH_4 per kg gain or g CH_4 per kg milk) would enable a more targeted approach to minimization of adverse livestock impact on the environment.

2.2. METHANE PRODUCTION FROM MICROBIAL FERMENTATION

The conversion of organic matter to CH_4 in the rumen involves the integrated activities of different microbial species, with the final step effected by methanogenic bacteria (McAllister et al. 1996). Primary digestive microorganisms (bacteria, protozoa and fungi) hydrolyze proteins, starch and plant cell-wall polymers into amino acids and sugars. These simple products are then fermented to volatile fatty acids (VFA's), hydrogen (H_2) and CO_2 by both primary and secondary digestive microorganisms (Figure 1). Acetate, propionate and butyrate, which are the major VFA's, are then absorbed and utilized by the host animal.

Methane is formed by methanogens using mainly hydrogen (80%) and formate (18%) as substrates (Whitman et al. 1992); however, some methanogens such as *Methanosarcina* can produces CH_4 from methylamine, methanol or acetate (Vicini et al. 1987). The interaction of methanogens with other bacteria in the fermentation process allows methanogens to gain energy for their own growth, while the accumulation of H_2 and other

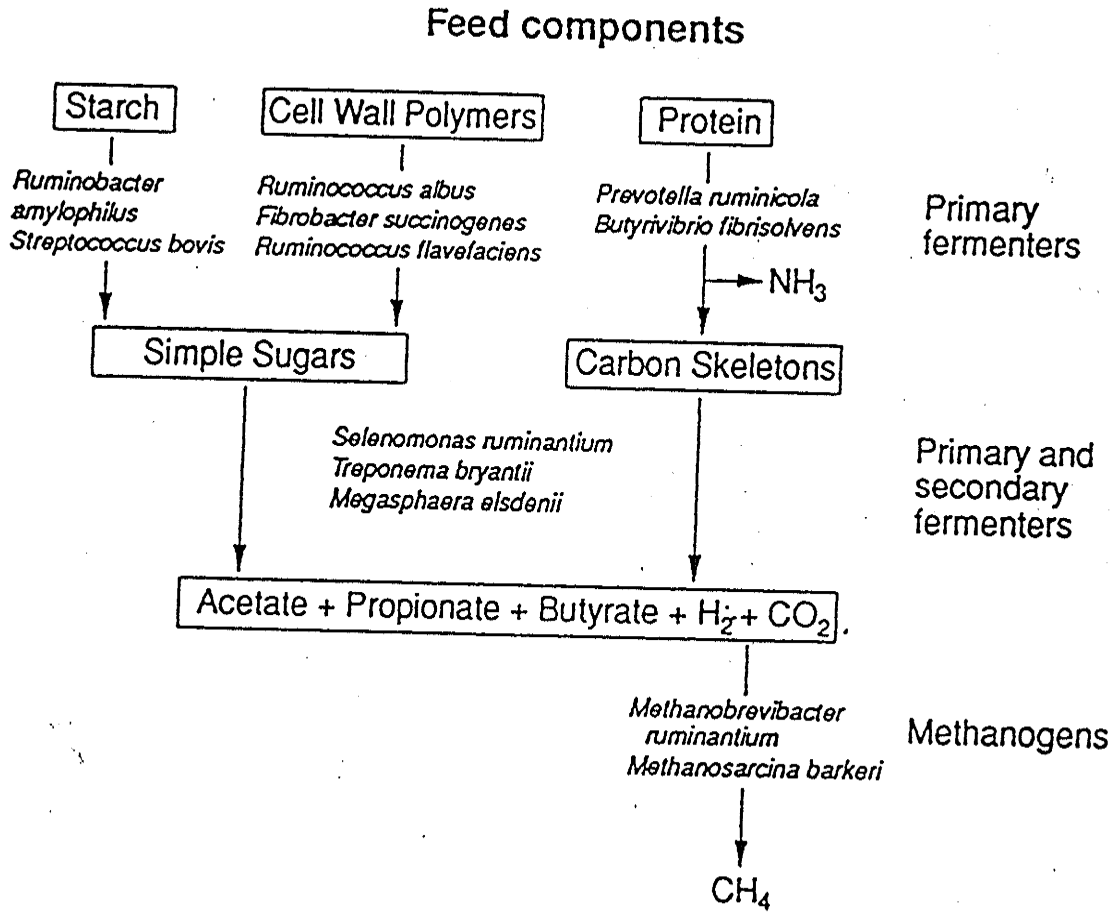

Figure 1. Microbial fermentation in the rumen (McAllister et al. 1996)

intermediates like formate are prevented, which benefits the growth of H_2-producing bacteria (Whitman et al. 1992).

Although methanogens are directly involved only in the very terminal stages of the fermentation, they are very important because they are capable of effectively utilizing electrons in the form of H_2 to reduce CO_2 to CH_4. Thus, in their absence, organic matter could not be degraded effectively in the gut (McAllister et al. 1996). However, since CH_4 has no nutritional value to the animal, its production represents a loss of dietary energy to the animal. Other exogenous compounds in the rumen such as unsaturated long chained fatty acids, sulphates and nitrates can also act as electron acceptors diverting electrons from CH_4 production (Baker 1997).

2.2.1 Methanogens

Sixty-six species of methanogens have been isolated from a variety of anaerobic habitats including sanitary landfills, acidic peat bogs, waterlogged soils, salt lakes, and the digestive tract of animals (Mackie et al. 1992). Methanogens represent a unique group of microorganisms, they possess three coenzymes which have not been found in other microorganisms. The three coenzymes are: coenzyme 420, involved in electron transfer in place of ferredoxin; coenzyme M involved in methyl transfer and factor B, a low molecular weight O_2 sensitive, heat stable coenzyme involved in the enzymatic formation of CH_4 from methylcoenzyme M (Jones et al. 1987).

Methanogens differ from almost all bacteria in not containing muramic acid in the cell wall; with the cell membrane lipids composed of isoprenoids ether-linked to glycerol or other carbohydrates (Jones et al. 1987). Analyses of the nucleotide sequence of the 16S ribosomal RNA indicates their very early evolutionary divergence from all other forms of life studied so far. They, therefore, have been classified in a different domain Archae (formerly Archaebacteria) within the kingdom Euryarchaeota (Jones et al. 1987).

Methanogens are nutritionally, fastidious anaerobes and grow only in environments with a redox potential below -300 mV (Stewart and Bryant 1988). Most methanogens grow at neutral pH, with an optimum between 6 and 8, however some species can thrive in extreme environments with pH extremes of 3 and 9.2 (Jones et al. 1987). Temperature-wise, two groups of methanogens are involved; the thermophilic species are active from about 45°C to 70°C and the mesophilic species are active between 35°C and 40°C (Bryant 1979).

In aquatic sediments and landfills, all organic carbon is converted to CO_2 and CH_4, because organic matter in these habitats is retained for a long period (weeks, months or years), allowing the slow growing aceticlastic methanogens to completely convert acetate, propionate and butyrate into CO_2 and CH_4 (Jones 1991). On the other hand, the turnover rate (1-2 d) of organic matter in the rumen is too short for complete anaerobic bio-conversion of carbon to CO_2 and CH_4, and the growth of the slow growing aceticlastic methanogens is inhibited.

2.2.2. Rumen methanogens

Five species of methanogens have been isolated in the rumen to date (McAllister et al.1996; Baker1999). These include *Methanobrevibacter ruminantium*; *Methanosarcrina bakeri*; *Methanosarcina mazei*; *Methanobacterium formicicum* and *Methanomicrobium mobile.* Only *Methanobrevibacter ruminantium* and *Methanosarcina bakeri* have been found in the rumen at populations greater than 10^6 mL^{-1} (Lovley et al. 1984), and are assumed to play a major role in ruminal methanogenesis (Stewart and Bryant 1988).

Methanobrevibacter ruminantium is a short, non-motile, Gram-positive coccobacilli present in high numbers (10^6 to 10^8 mL^{-1}). The organism uses hydrogen and carbon dioxide as the major source of electrons and carbon respectively for methanogenesis. It requires acetate as a major source of cell carbon and Coenzyme M as a cofactor for the terminal stage during methanogenesis (Jones et al. 1987). On the other hand, *Methanosarcina bakeri* which is found in high numbers in bio-reactors are present in lower numbers in the rumen (Vicini et al. 1987). The cells are Gram-positive, non-motile spheres which occur in large clusters. These organisms can utilize a large range of substrates for methanogenesis including, hydrogen, carbon dioxide, methanol, methylamines and acetate (Stewart and Bryant 1988).

In the rumen, methanogens have been found to be intimately associated with ciliate protozoa, often in structurally well defined granules or microbial consortia (McAllister et al. 1996; Baker 1999). Although methanogens are hydrophobic and therefore stick to feed particles, the attachment of methanogens to the protozoa appears to be dependent on the time of feeding the animal. Tokura et al. (1997) observed that the numbers of methanogens associated with protozoa were greatest soon after feeding (10 to 100 times pre-feeding levels), when the rate of fermentation is greatest. It was shown that the symbiotic relationship of methanogens and protozoa may generate 37% of rumen CH_4 emissions (Finlay et al. 1994). This association of methanogens with protozoa, can be used to reduce CH_4 emissions when protozoa are removed from the rumen through a process known as defaunation, which is discussed in a later section.

2.2.3. Substrates and energetics of methanogenesis

Methanogens use the process of formation of CH_4 to generate energy for growth. Substrates used in the process include H_2, CO_2, formate, acetate, methanol, methylamines, dimethyl sulfide and some alcohols (Jones 1991; Table 3). All species studied so far utilize H_2 to reduce CO_2 to CH_4, and as a result, hydrogen consuming methanogens (hydrogenotrophs) are found in all the types of anaerobic habitats (Jones 1991). From Table 3, it can be seen that the change in free-energy (ΔG) of the hydrogen using reaction is the most negative (-135 kJ mol^{-1} of methane); indicating a very great affinity for H_2 by methanogens, since the reaction is the most thermodynamically favored.

In the rumen, methanogens primarily use hydrogen, carbon dioxide and formate as substrates in methanogenesis. The unique biochemical ability of *Methanosarcina bakeri* to use methanol, methylamines and acetate in addition to carbon dioxide and hydrogen as substrates enables the slow growing *Methanosarcina* organisms to flourish in ruminants fed diets containing ingredients like molasses, that breakdown into methylamines, methanol and acetate, which can act as substrates for methanogenesis (Vicini et al. 1987).

Only two species; (*Methanosarcina* and *Methanosaeta*) are known to degrade acetate to CH_4. With the small change in free-energy (ΔG), this pathway represents an energetically unfavorable pathway for methanogens. The ΔG of this reaction (-31.0 kJ mol^{-1} of methane) is nearly equal to that required for synthesis of a molecule of ATP from ADP and inorganic phosphate (+31.8 kJ mol^{-1}; Jones et al. 1987). Thus, aceticlastic methanogenesis is not a lucrative way of generating energy when compared with methanogenesis from H_2 or formate for example (Table 3). Thus, if better growth substrates are available, methanogens will utilize acetate for biosynthesis and form CH_4 via a more energetically favorable pathway (Jones et al. 1987). This may explain why almost all methanogens can utilize H_2 to reduce CO_2 to CH_4, the most energy yielding pathway.

Table 3. Substrates and energetics of methane production[a]

Reactions	$\Delta G_0'$ (kJ mol^{-1} of methane)
Hydrogenotrophic reactions	
$4 H_2 + CO_2 \rightarrow CH_4 + 2H_2O$	-135.6
4 Formate $\rightarrow CH_4 + 3CO_2 + 2H_2O$	-130.1
4 (2-propanol) + $CO_2 \rightarrow CH_4$ + 4 acetone + $2H_2O$	- 36.5
Aceticlastic reaction	
Acetate $\rightarrow CH_4 + CO_2$	-31.0
Disproportionation reactions	
4 Methanol $\rightarrow 3CH_4 + CO_2 + 2H_2O$	-104.9
4 Methylamine + $3H_2O \rightarrow 3CH_4 + CO_2 + 4NH_4^+$	-75.0
2 Dimethyl sulfide + $2H_2O \rightarrow 3CH_4 + CO_2 + H_2S$	-73.8

[a] Data from Jones (1991)

2.2.4. Biochemistry of methanogenesis

The biochemical pathway of methanogenesis culminates in the reduction of a methyl group from one carbon-carrier coenzyme M, to CH_4 (Boone 1991). All methanogens share this final energy yielding step, although electrons for the reductive step may be obtained (depending on the species) from the oxidation of hydrogen, formate, methanol, methylamines or acetate. Most methanogens are able to reduce carbon dioxide through the formyl, methenyl, methylene and methyl stages to CH_4 (Rouviere and Wolfe 1988; Figure 2).

The role of six new coenezymes have been identified during methanogenesis. These coenzymes include methanofuran (MFR); tetrahydromethanoopterin (H_4MPT); cofactor F_{420}; cofactor F_{430}; coenzyme M (CoM) and 7 mercaptoheptanoylthreonine (HS-HTP) (DiMarco et al. 1990).

Formyl-methanofuran is the first stable product of CO_2 fixation with MFR (reaction 1). The formyl group is then transferred to H_4MPT a pterin, unique to methanogens catalyzed by formyl- MFR:H_4MPT formyl transferase (reaction 2). The formyl group is next converted to a methenyl group by the enzyme, 5, 10-methenyl-H_4MPT cyclohydrolase (reaction 3). The reduced deazaflavin coenzyme F_{420} donates electrons for reduction of the double bond of the methenyl group forming a methylene group. This reaction is catalyzed by the enzyme methylene- H_4MPT:coenzyme F_{420} oxidoreductase (reaction 4).

A similar oxido-reduction reaction is speculated to be involved in the reduction of methylene-H_4MPT to methyl-H_4MPT (reaction 5) (Rouviere and Wolfe 1988). Prior to reduction of the methyl group to CH_4, the methyl group of methyl-H_4MPT is transferred to

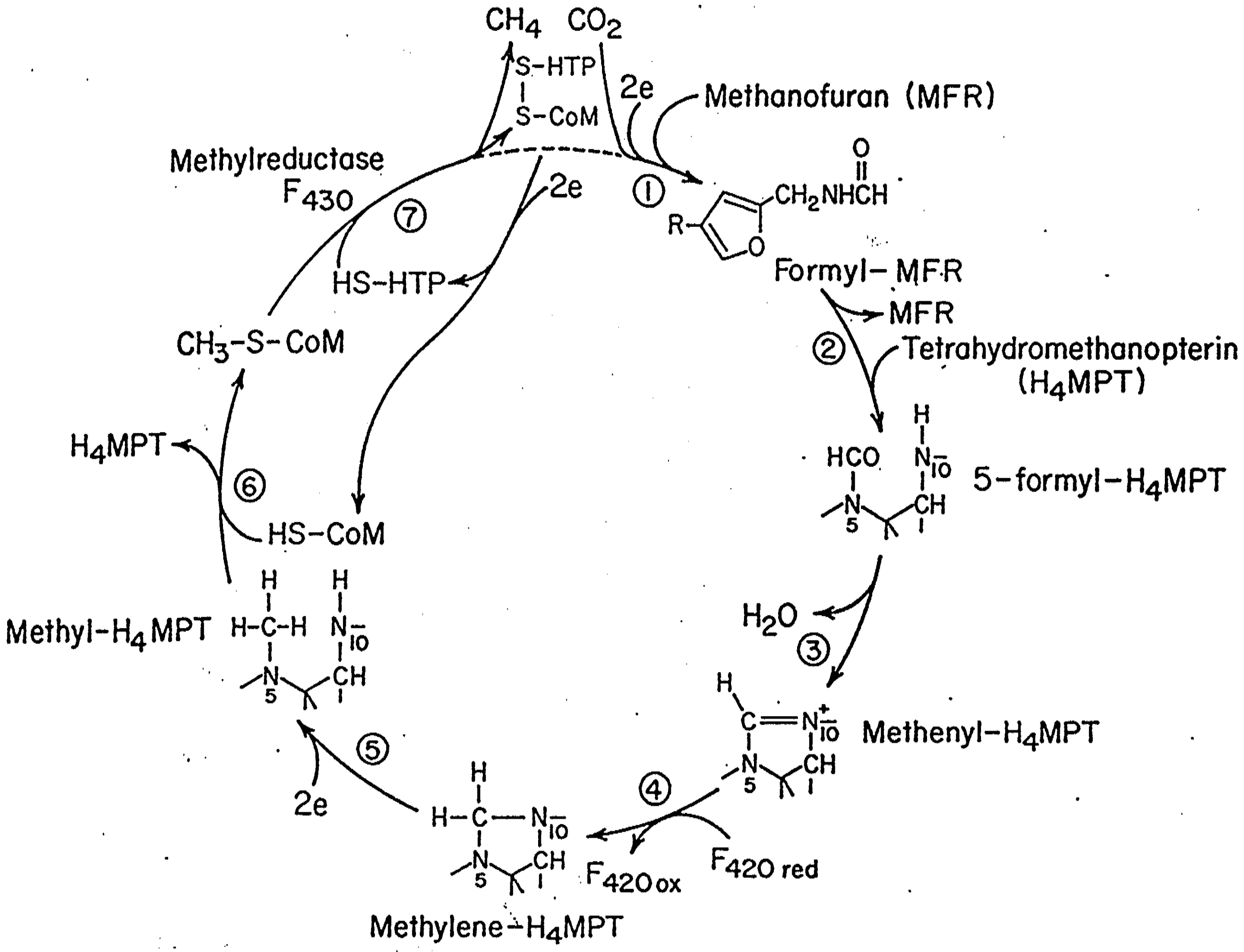

Figure 2. Pathway for methane production from CO_2 (Rouviere and Wolfe 1988)

coenzyme M (reaction 6). Methyl-CoM is then reduced to methane by methyl-coenzyme methyl reductase, a complex system involving proteins and a number cofactors such as F_{430}, ATP, HS-HTP, and FAD (reaction 7). This terminal reaction completes the cycle and is linked to the activation of carbon dioxide to form formyl-MFR.

Methane production from acetate also proceeds through the methlreductase system. Initially, acetate is activated to acetyl coenzyme A; acetyl coenzyme A is then metabolized to form an enzyme-bound carbon monoxide (CO) and a methyl group which is transferred to HS-CoM to form methyl-CoM via H_4MPT. Electrons for the reductive cleavage of methyl CoM to CH_4 are derived from oxidation of the enzyme-bound CO (Jones 1991).

Methanol and methylamines are converted to CH_4 either by direct reduction of the methyl group (via H_2) after transfer to HS-CoM via methyltransferases, or by a disproportionation reaction in which methanol is both oxidized and reduced. In both cases, some of the methanol or methylamine is oxidized to generate reducing equivalents for the subsequent reduction of the methyl group to CH_4 by the methylreductase system (Jones 1991).

2.2.5. Methanogens and cellulose fermentation

During the process of carbohydrate breakdown in the rumen, intracellular electron carrier coenzymes such as nicotinamide adenine dinucleotide (NAD) are reduced to NADH. However, NADH has to be reoxidized to NAD in order to continue the fermentation process. Different rumen microbes use different strategies or pathways for reoxidizing NADH, resulting in electron sink fermentation products such as butyrate, pyruvate, succinate, lactate and ethanol (Miller 1991; Figure 3). Ethanol is not a significant fermentation end product as a result of normal ruminal fermentation

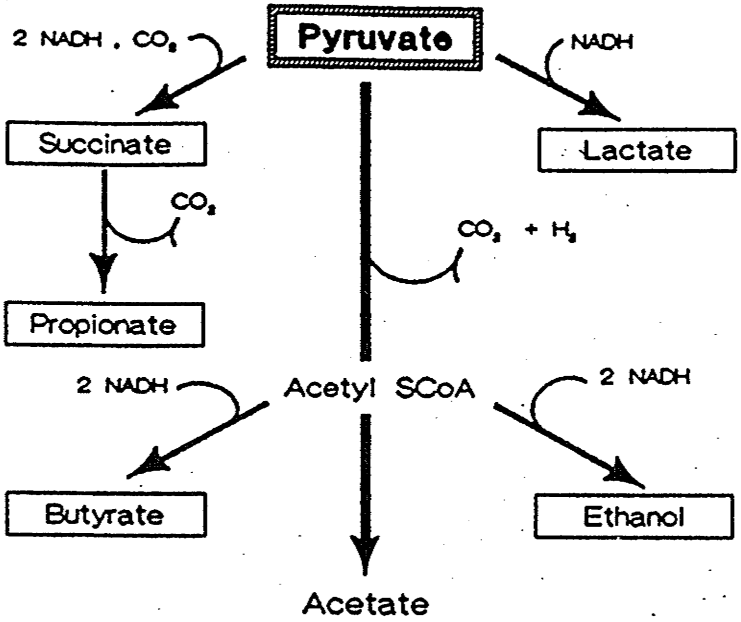

Figure 3. Oxidation of NADH and the fermentation of electron sink products by rumen microbes (Miller 1991)

All microbes, however, can oxidize pyruvate to acetyl coenzyme A (acetyl-SCoA), H_2 and CO_2. Acetyl-SCoA is converted to acetate, which is not an electron sink. In the reaction, H_2 is rather made available for methanogens. The end use of H_2 for CH_4 production by the methanogens enhances the energetic efficiency and extent of fibre digestion by other ruminal microorganisms, by preventing the accumulation of reduced nucleotides (e.g. NADH) through "interspecies hydrogen transfer" (Boone 1991; Williams et al. 1994).

"Interspecies hydrogen transfer" is the shuttling of reducing equivalents (NADH) from non methanogens to methanogens (Boone 1991). As a result of interspecies hydrogen transfer, the products of fermentation are altered by the presence of methanogens. A classic example of this transfer, can be seen in Figure 4.

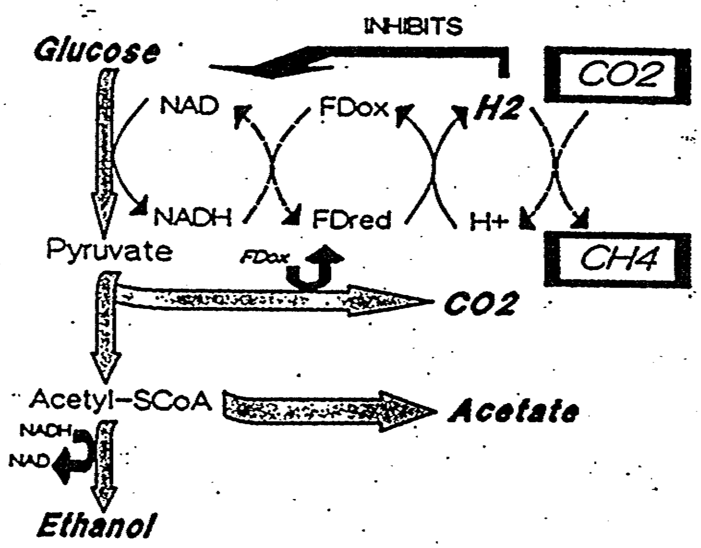

Figure 4. Fermentation products by *Ruminicoccus albus* in monoculture (Miller 1991)

A predominant rumen cellulolytic specie, *Ruminococcus albus*, produces ethanol, acetate, H_2 and CO_2 in monoculture, however it does not form ethanol in the rumen when co-cultured with a methanogen. *Ruminococcus albus* produces H_2 by oxidizing pyruvate to acetyl-SCoA, H_2 and CO_2 (Wolin and Miller 1988). Hydrogen does not inhibit the reaction because the enzyme pyruvate:ferredoxin oxidoreductase involved in the reaction has an oxidation potential near -400mV, close to that of H_2 (Wolin and Miller 1988).

Ruminococcus albus can also produce H_2 by oxidizing NADH to NAD and H_2, by the action of NADH:ferredoxin oxidoreductase and ferredoxin hydrogenase, but this reaction is thermodynamically unfavorable unless the H_2 partial pressure in the system is low (Glass et al. 1977). When H_2 accumulates as in pure cultures, *Ruminococcus albus* does not produce H_2 from NADH, but the NADH produced during glycolysis is reoxidized by the enzyme, alcohol dehydrogenase, to produce ethanol from acetyl-SCoA (Glass et al. 1977). When H_2 is removed by methanogens, H_2 is produced from NADH and acetyl-SCoA is completely transformed to acetate and the production of ATP, instead of the reduction of acetyl-SCoA to ethanol.

The maintenance of a low partial pressure of H_2 by methanogens, increases the production of acetate, and the ATP yield of both *Ruminococcus albus* and methaongens is enhanced (Wolin and Miller 1988). In co-cultures with methanogens, more ATP is available for the production of fibrolytic enzymes and cellulose digestion is accelerated in comparison with monocultures of cellulolytic bacteria (Pavlostatis et al. 1990). Wolin and Miller (1988) noted that *Ruminococcus albus* can increase ATP production from 3 to 4 moles per mole of glucose fermented with more acetate being produced and ethanol production being eliminated when methanogens are present.

Williams et al. (1994) have recently shown similar improvements in the digestion of xylans by co-culture of *Ruminococcus flavefaciens* and *Methanobrevibacter smithii* compared to *Ruminococcus flavefaciens* in monoculture. Other fermentative rumen organisms apart from cellulolytic bacteria, like fungi (Joblin et al. 1990) and protozoa (Finlay et al. 1994) that have different reactions for reoxidizing NADH can also participate in interspecies H_2 transfer interactions with methanogens. Similarly, co-culturing of rumen fungi with a variety of methanogens have been shown to decrease the formation of reduced fermentation products like ethanol, succinate and lactate, and enhanced digestion of cell-wall components (Joblin et al. 1990).

2.3. STRATEGIES THAT REDUCE RUMINANT METHANE PRODUCTION

It has so far been established, that CH_4 production is the terminal end use of hydrogen derived from the release of H_2 from reducing equivalents (NADH) and that methanogenesis plays an important role in normal rumen fermentation. However, it should be possible to reduce methanogenesis by inhibiting H_2 liberating reactions; promoting alternative reactions which accept H_2 during reoxidation of NADH or promote alternative H_2 - using reactions or routes for electron disposal. Strategies that reduce ruminal methanogenesis by these mechanisms are discussed below.

2.3.1. Alternate hydrogen acceptors

2.3.1.1. Addition of fats

Addition of lipids to ruminant diets to increase the energy density of diets, has been shown to depress CH_4 production (Dong et al. 1997a; Mathison 1997; Machmuller and Kreuzer 1999). Mathison (1997) reported that CH_4 production was reduced by 33% when 4% canola oil was added to a diet containing 85% concentrate in a feedlot study in Alberta. Kinsman et al. (1997) also observed that the addition of soybean oil at 3.4% of concentrate portion of TMR on DM basis, decreased CH_4 production by 8% in lactating Holsteins. The depression in CH_4 production with addition of fats has been attributed to the fact that unsaturated fatty acids can serve as electron acceptors during biohydrogenation (Hegarty 1999a). Saturated and unsaturated fatty acids have also been shown to have a toxic effect on ruminal microbes (Czerkawski 1986; Machmuller and Kreuzer 1999). Furthermore, long chain fatty acids are largely non-fermentable, and therefore they decrease the percentage of CH_4 that can be produced (Johnson and Johnson 1995).

The major problem associated with excessive fat addition (more than 5-6% of the ration) is depressed fiber degradation in the rumen, especially with forage-base diets (Mathison et al. 1998; Machmuller and Kreuzer 1999). Mathison (1997) observed that digestibility of fibre was reduced with the addition of canola oil, such that the ME content of the diet was not different from that of the control barley-based diet. Dong et al. (1997a) observed that of the three oils (coconut, canola, and cod-liver oil) used in an artificial rumen system, coconut oil was the most inhibitory to CH_4 production, and it correspondingly depressed fiber digestion the most. Although the addition of fats appears to reduce CH_4 production, their addition does not appear to result in increased energy availability to the animal, and therefore may not be a viable strategy.

2.3.1.2. Reduction of inorganic compounds

Inorganic sulfate and nitrates has been identified as possible competitors with methanogens for available H_2 (Czerkawski 1986; Nollet and Verstraete 1996), as shown in the following reducing equations:

$$SO_4^{-2} + 8e^- + 10H^+ \rightarrow H_2S + 4\,H_2O$$

$$NO_3- + 8e^- + 10H^+ \rightarrow NH_4^+ + 3\,H_2O$$

Reduction of sulfate and nitrate by microbes is thermodynamically more favorable than reduction of CO_2 to CH_4 by methanogens (McAllister et al. 1996; Hegarty 1999a). This is because sulfate and nitrate reducers are capable of using hydrogen at a lower partial pressure than methanogens, thus sulfate and nitrate reducers generally out compete methanogens (Mathison et al. 1998). Hegarty (1999a) noted that on a molar basis, methanogens and sulfate reducers consume an equal quantity of H_2. Therefore, decreasing CH_4 losses from sheep for example by 50% (0.75mol d^{-1}), would require the consumption of an equivalent 0.75 mol sulfate d^{-1} or 0.75 mol nitrate d^{-1}. However, Kandylis (1984) concluded that the consumption of sulfate-S at more than 0.3% in the diet would cause toxic effects in ruminants resulting from H_2S absorption through the lungs. Therefore, the consumption of 0.75 mol sulfate d^{-1} by sheep to reduce daily emission by 50% would certainly be fatal to the sheep. Thus, there is little potential of using sulfates as hydrogen sinks in diets to reduce methanogenesis.

Similarly, inorganic nitrates which are found in many forages can competitively reduce methanogenesis (Hergaty 1999a). Since nitrates are rapidly reduced to nitrite and subsequently reduced to ammonia, nitrates can in theory be used as a non-protein nitrogen source to ruminants. It is, however, toxic in high levels both as nitrate and after metabolism to nitrite in the rumen, because absorbed nitrites can oxidize hemoglobin to methemoglobin and interfere with oxygen transport (Mathison et al. 1998). Emerick (1988) concluded that diets containing 0.45% nitrate N are potentially toxic to ruminant animals. However to provide a complete alternative to electron disposal from fermentation, nitrate N would have to be added at a level of 4.7% of the digestible carbohydrate in the diet (Mathison et al. 1998). It can be concluded that the addition of nitrate in ruminant diets is not a feasible method for competitively reducing rumen CH_4 production.

2.3.1.3. Reductive acetogenesis

A long term technology which may hold some promise of diverting electrons from methanogens, is the production of acetic acid by acetogens (Joblin 1999). In the gut of termites and rodents, acetogens convert excess hydrogen to acetic acid, which is then utilized by the host (Joblin 1999). Acetogens have been isolated in the rumen, however they cannot compete effectively with methanogens for hydrogen ions, because they have a lower affinity for H_2 than methanogens (Greening and Leedle 1989).

Carbon flux studies in the rumens of sheep revealed that rumen acetogenesis occurs in the first 24 hr after birth, but is subsequently displaced by methanogens (Morvan et al. 1994), which easily out compete the acetogens for the low concentration of H_2 (-1uM) normally encountered in the rumen (Joblin 1999). Thus, methanogens have to be inhibited to allow H_2 pressure to rise before acetogenesis will be significant as an alternate H_2 sink in the rumen. Suppression of methanogens could be achieved by chemical (Van Nevel and Demeyer 1996), biological (Klieve and Hegarty 1999) or immunological means (Baker 1999) discussed in a latter section. Secondly, increasing the populations of acetogens through exogenous inoculations into the rumen, would be useful for competiting against methanogens (Joblin 1999), however, an attempt at inducing acetic acid by innoculation of acetogens was not successful (Immig et al. 1996).

2.3.2. Dietary practices

Decreasing H_2 production, which would reduced CH_4 formation, may be achieved by dietary practices which either reduces the total flow of organic matter through the fermentation pathway, or shifts the balance from acetate and H_2 production to propionate and butyrate pathways which are net consumers of H_2 (Hegarty 1999a). As a general rule there is an inverse relationship between CH_4 and propionic acid production in the rumen.

2.3.2.1. Diet quality

Interacting dietary factors such as the digestibility of the feed, the type of carbohydrates (soluble vs. cell wall) and carbohydrate:nitrogen ratio can affect the amount of CH_4 gas produced. Methane production levels can fall from a level of 6 -7% of GEI when forages are fed at maintenance to as low as 2-3% when high grain (corn) concentrates are fed at ad-libitum intake levels (Abo-Omar 1989). The very low CH_4 losses (2-3% of GEI) observed in corn- based diets, were not observed in studies using barley-based diets (8.5 to 12.0% of GEI) at similar levels of intake (Beever et al. 1989; Whitelaw et al. 1984). This indicates variability in response with different feed grains. The starch in barley grain is known to be more rapidly fermentable than for corn grain and, therefore, more CH_4 may be produced (Whitelaw et al. 1984).

Fermentation of structural carbohydrates results in higher CH_4, % of GEI, as a consequence of decreased rate of fermentation and passage rate, which favors a higher acetic:propionic acid production (Beever et al. 1989). The reverse is true in high grain diets fed at high intake levels, where there is a shift in fermentation pattern with increased passage rate and high rates of digestion which favors higher propionic acid production (Demeyer and Van Nevel 1975; Johnson and Johnson 1995). High digestion rates of grains will also lower ruminal pH, which inhibits the growth of methanogens (Stewart and Bryant 1988; Hegarty 1999a).

2.3.2.2. Feeding level and frequency

In general, the fractional CH_4 loss will decrease as daily intake increases (McAllister et al. 1996). Blaxter and Clapperton (1965) confirmed this relationship in 48 trials, showing that a negative regression exist between CH_4 production and feeding level. Generally, high intake results in increased passage rate of feed out of the rumen (McAllister et al. 1996). As a result of the increased passage rate, the extent of microbial access is decreased, which in turn reduces the extent and rate of ruminal dietary fermentation (Mathison et al. 1998). On the other hand, when highly digestible feeds (concentrates) are fed at restricted intakes, CH_4 losses, % GEI will increase (Whitelaw et al. 1984). However, highly digestible feeds are seldom fed at restricted levels in the feedlot industry and thus high fractional CH_4 losses are not encountered. The extent to which intake levels affect passage rate of roughages is proportionally less than with concentrate or mixed diets (Mathison et al. 1998). When diets with less than 25% concentrate are fed, the effect of intake on passage rate of roughages is limited (Galyean and Owens 1991).

Low frequencies of feeding tend to increase propionate production and lower CH_4 production (Sutton et al. 1986). This effect is associated with the lowering of the population of rumen protozoa and fungi, since multiple feedings prevent the drastic fluctuation in rumen pH that can be inhibitory to these microbes. For example, Kaufmann et al. (1980) showed that when a given level of concentrate was fed to cows twice a day, rumen pH fluctuated from 5.85 to 6.65, where as when cows were fed six times a day rumen pH ranged only from 6.15 to 6.40.

2.3.2.3. Retention time of digesta in the rumen

A reduction in CH_4 production is also observed when the mean residence time of feed in the rumen is reduced due to a concurrent decrease in ruminal digestion. Stainer and Davies (1981) reported that a 50% decrease in fluid dilution rate was associated with a 40% increase in CH_4 production in an *in-vitro* continuous culture. Okine et al. (1989) observed a 29% decrease in CH_4 production of cattle, when the fractional passage rate of particulate matter was increased 63% from 42 h to 26 h by placing weights in the rumen. About 28% of the variation in CH_4 production was attributed to the mean retention time.

2.3.2.4. Forage processing and preservation

Grinding or pelleting of forages to improve utilization in ruminants has been found to decrease CH_4 losses per unit of feed intake by 20-40% when fed at high intakes (Blaxter 1989). The lowered fiber digestibility and faster rate of passage associated with ground or pelleted forages can explain the decline in CH_4 production. However, fine grinding of forages, has not proved to be economical to dairy producers because of increased incidence of acidosis and lowered milk fat test, which is associated with lowered effective fibre from finely ground forages.

Chemical treatments such as the application of sodium hydroxide or ammonia to low quality forages have been shown to decrease CH_4 production per unit of product by about 10-15% (Johnson et al. 1996). Moss et al. (1994) noted that although the emission of CH_4 increases when low quality forages are treated chemically with sodium hydroxide or ammonia as a result of increased digestibility, the amount of CH_4 relative to digestible organic matter intake decreases. Chemical processing has not been widely accepted in the industry because it is not cost effective, and the caustic nature of the chemicals has negative environmental implications.

There is limited information with regard to the effects of forage preservation on CH_4 production. Methane production was shown to be lower when forages were ensiled than when dried (Sundstol 1981). This is because fermentation is reduced in the rumen with ensiled forages, and thus there is less fermentable material available in the rumen. Kirkpatrick and Steen (1999) observed differences between forage conserved as silage vs forages conserved by freezing on CH_4 production.

2.3.2.5. Forage species and maturity

Methane production in ruminants tend to increase with maturity of forage fed, and CH_4 yield from the ruminal fermentation of legume forages is generally lower than the yield from grass forages (McAllister et al. 1996). Fermentation of alfalfa silage was associated with a 0.5% of GEI decline in CH_4 production, compared to grass silage (Varga et al. 1985). Recent measurements of CH_4 production from grazing beef cows indicates a significantly lower fractional loss of CH_4 with alfalfa-grass pastures (7.1%) compared to grass-only pastures (9.5%) (McCaughey et al. 1999). The lowered CH_4 loss observed with legumes can be attributed to the lower proportion of structural carbohydrates in legumes and faster rate of passage of legumes, which will shift the fermentation pattern towards higher propionate production.

2.3.2.6. Grain supplementation of forages

Supplementation of forages with grain, especially at high intake levels improves the efficiency of feed utilization (Kirkpatrick and Steen 1999; Islam et al. 2000) by the provision of readily fermentable energy sources for microbes during fermentation. There is a reduction in the amount of energy lost as CH_4 associated with maintenance of the animal as a result of improved energy utilization; decreased acetate:propionate ratio and increased propionate production (Reis et al. 2001). The response of grain supplementation in reducing CH_4 production depends, however, on the type of forage in the basal diet, and the level of supplementation (Farverdin 1991). When silage diets were supplemented with three levels of flaked corn starch (0, 2 and 4 kg), the proportion of propionic acid increased for cows fed diets containing early cut grass silage, but no effects were found for cows fed late cut grass silage (Visser et al. 1998).

Apart from providing useful energy and reducing CH_4 production, grain supplements may have an adverse associative effect with the components of the forage diet by lowering digestibility of the forages. This is because ruminal pH is decreased with the addition of high levels of grain, which can inhibit the growth and action of cellulolytic bacteria (Allden 1981). Mould et al. (1983) showed that rumen pH was reduced from 6.6 (control) with the addition of whole barley (6.0), pelleted barley (6.2), whole corn (5.9) and pelleted corn (6.1) at 65% DM basis, to a hay based diet. They also observed that fibre digestion of the hay was depressed by 43%, 41%, 70% and 59%, respectively, compared to the control diet. Supplements containing highly digestible fibre such as soybean hulls have been shown to produce less negative associated effects than high-starch supplements (corn-soybean meal) in crossbred yearling cattle when fed at high feeding levels (0.8 to 1% of BW) with Bermuda grass (Garces et al. 1997). The authors observed no differences in supplement type at low feeding levels (0.4 to 0.5 % of BW). Also, it was shown that total tract organic matter digestibility (OMD) was lower ($P < 0.001$) in sheep fed corn-soybean meal than (54.8 % for low and 56.9% for high supplementation levels) than in sheep fed wheat middlings (57.4 % for low and 62.6% for high supplementation levels) or soybean hull (57.2 for low and 62.5% for high supplementation levels; Garces et al. 1997).

2.3.3. Ionophores

Ionophores are highly lipophilic substances which are able to shield and delocalize the charge of ions and facilitate their movement across membranes (Mathison et al. 1998). Monensin is the most commonly used and studied ionophore, with others such as lasalocid tetranasin, lysocellin, narasin, salinomycin and laidomycin also being used commerically. Ionophores which are added to ruminant diets to improve the efficiency of feed utilization have been shown to decrease CH_4 production. It was shown that in growing steers fed 4.1 kg d^{-1} of a low, medium, and high roughage diet with 200 mg d^{-1} supplemental monensin, CH_4 production was decreased by 16% at the low and medium roughage levels, and by 24% for the high roughage level (Thorton and Owen 1981).

The observed increase in propionate production and decrease in CH_4 production that accompanies monensin feeding has been associated with the selective reduction of Gram-positive ruminococci, and the proliferation of the Gram-negative bacteria which produce succinate and propionate (Ushida et al. 1985). Thus ionophores are not toxic to methanogens themselves, but to the bacteria that provide substrate to the methanogens.

There is evidence of an adaptation response of microbes to ionophores fed to cattle

(Abo-Omar 1989; Saa et al. 1993). However, in grain-fed sheep with repeated monensin addition, Mbanzamihigo et al. (1996) showed that the CH_4 production rates remained lower than controls for at least 35 days. Future potential, therefore, lies in the use of more persistent ionophores, and combinations of ionophores that can avoid microbial adaptation. However, even if there is some adaptation to monensin use, a reduction in CH_4 production from its use will occur because feed intake is reduced (Goodrich et al. 1984). Secondly, because of the positive effects of ionophores on feed efficiency, the continual use of ionophores as a strategy for reducing CH_4 production is economically justifiable.

2.3.4. Chemicals, enzymes and biological inhibitors

Chemical inhibitors such as bromoethanesulphonate (BES) is a potent inhibitor of methanogenesis because it is a structural analogue of the co-factor mercaptoethanesulfonic acid (HS- coenzyme M) used by methanogenic bacteria. (Taylor et al. 1974). Since HS-CoM is found only in methanogens, BES therefore works as a specific inhibitor when used in mixed microbial systems. Dong et al. (1997b) observed that BES depressed CH_4 production by 71% without significantly affecting organic matter digestibility and VFA concentrations in the artificial rumen (Rusitec). The reduction in CH_4 was attributed to the toxic effect of BES to methanogenic bacteria. However, a study in sheep showed that even though BES effectively depressed CH_4 production, its effectiveness persisted for just 3 days (Imming et al. 1995). It can be concluded that BES seems to be a good inhibitor of CH_4, however it may not play a significant role in reducing CH_4 on a commercial level.

Chlorinated methane analogues (chloroform, carbon tetrachloride, methylene chloride) and related compounds such as, tricloroethyl adipate, and pyromellitic diimide have been shown to inhibit methanogenesis (Bauchop 1967; Clapperton 1977; Linn et al. 1982). These chemicals inhibit methanogenesis through inhibition of methyl-CoM reductase. Since CH_4 analogues are directly toxic to methanogenic bacteria, their use results in decreased molar proportions of acetate and an increase in propionate. They, however, often decrease digestion and depress feed intake (Mathison et al. 1998). Also microbial populations in vivo have been found to adapt to or degrade many of these compound and favorable and long lasting effects on animal performance are limited (Demeyer et al. 1986).

Exogenous fibrolytic enzymes (cellulases and xylanases mixture) have the potential to improve utilization of fibrous feed (Dong et al. 1997b). When added to hay, organic matter digestibility was increased in an artificial rumen system (Rusitec). These positive effects of enzymes *in vitro* did not translate into reduced CH_4 production, or increased digestion rate or feed intake when fed to ewes (Dong et al. 1997b).

Opportunities exist for biological control of CH_4 using archeal viruses and bacteriocins specific to methanogens (Klieve and Hegarty 1999). Viruses are obligate pathogens, that can infect and lyse bacteria and archaea reducing the population of methanogens (Klieve and Hegarty 1999). To date, no attempt has been made to use these viruses to control methanogens, because of limited knowledge of the genetic diversity and viral susceptibility of methanogens and the host range of archeal viruses.

Bacteriocins on the other hand are bacteriocidal compounds that are generally peptide or protein in nature, are produced by bacteria, and potential exists for their use in inhibition of methanogens. It has been shown that Nisin, a bacterocin produced by *Lactococcus lactis* which has similar actions to monensin and widely used in the food industry as a preservative, stimulated propionate production, and reduced methanogenesis *in vitro* by 36% (Callaway et al. 1997). Whether this reduction is a direct effect of Nisin on methanogens or on bacteria supplying substrate to methanogens remains to be determined. Although they may be safe and can be incorporated into feed, a limitation may be the degree of stability of these peptides in the rumen environment, as rapid degradation could reduce the effectiveness of the compound (Klieve and Hegarty 1999).

In the past 3 years, researchers in Australia have vaccinated sheep with a number of experimental vaccine preparations against methanogens, so that the animals produce antibodies to methanogens (http://www.csiro.au). Methane production was reduced between 11 and 23%, and simultaneously productivity was improved in vaccinated animals suggesting an increase in propionate production as the alternative electron acceptor. No long or short term adverse effects on the sheep have been found. Researchers anticipate that commercial vaccines, when developed by year 2004, will be able to achieve a 3% gain in productivity and a 20% reduction CH_4 production.

It can be concluded that chemical, enzyme or biological inhibitors have potential *in vitro* and may be considered in future attempts to control CH_4 production, but the safety, adaptation of microbes to the inhibitors and negative effects of these on digestion and animal performance must be addressed. Consumers are becoming increasing concerned with the use of anti-microbials and antibiotics in livestock diets and thus may restrict the use of these inhibitors into the industry.

2.3.5. Defaunation

Defaunation which is the elimination of protozoa from the rumen by dietary or chemical agents, has been observed to reduce ruminal CH_4 production by about 20 to 50% depending on diet composition (Whitelaw et al. 1984; Van Nevel and Demeyer 1996). Whitelaw et al. (1984) observed a 50% decrease in CH_4 production following defaunation of cattle fed a barley-based diet, whereas Itabashi et al. (1984) did not detect any reduction in CH_4 production from defaunated goats fed forage diets. Protozoa in the rumen are associated with a high proportion of hydrogen ion production, and are closely associated with methanogens by providing a habitat for up to 20% of rumen methanogens (Stumm et al. 1982). It is assumed that there is a symbiotic hydrogen transfer between anaerobic protozoa and methanogens, which allows the protozoa to dispose hydrogen to methanogens and produce more acetate (Stumm et al. 1982).

The reduced ruminal methanogenesis observed with defaunation can be attributed to factors such as a shift of digestion from the rumen to the hind gut (Van Nevel and Demeyer 1996); lower rumen digestion of fibre (Veira 1986); the loss of methanogenic bacteria associated with protozoa (Hegarty 1999b). Defaunating agents or protozoa inhibitors are not currently available for commercial or practical use as many of the defaunating agents are toxic to the animal.

2.3.6. Improving animal productivity

In general when the productivity of the animal is improved, CH_4 production per unit of beef or milk is reduced. This is because the amount of feed energy associated with the maintenance of the animal, which is about 70-75% in beef cattle and 50% with dairy cattle is reduced (Mathison et al. 1998). This strategy is widely used in Canada's livestock production systems, where fewer animals are required to produce the same amount of product, therby reducing days to market and overall CH_4 production. Byers (1990) noted that 25% of the CH_4 loss associated with the production of beef in the US was associated with beef cows during the last 160 days of gestation, where there is often poor balance of diets. Improvement in reproductive efficiencies will minimize CH_4 emissions from unproductive animals. Anabolic implants have been used to improve growth and production of lean tissue in the beef industry. They reduce CH_4 production by reducing the % of feed energy required for maintenance, as well as feed energy for the production of fat tissues (Johnson et al. 1996). Implanted animals become more efficient at converting feed into lean tissues.

Improved productivity through the use of bovine somatotropin (bST) to stimulate lactation has been observed in US dairy cows. Enteric CH_4 (% GEI) production was reported to be reduced by 9% with the use of bST (Johnson et al. 1992). The use of bST in 603 kg Holstein cows consuming 21.6 kg dry matter d^{-1} and producing 37.1 kg milk d^{-1} reduced CH_4 production by 16% (Sechen et al. 1989). Consumer concern over the hormones residues in milk and meat products is preventing industry wide adoption in Canada.

In modeling lifetime CH_4 emissions of cattle in Northern Australia, McCrabb et al. (1998) found that finishing cattle for 2-5 months on a grain-based feedlot diet was associated with a 34-54% reduction in lifetime CH_4 production per kg of salable beef yield. Kurihara et al. (1998) have established a curvilinear relationship between live weight gain (LWG) and CH_4 production for Australian and Japanese beef cattle fed on grain-based diets. They also showed that an improvement of 0.1 kg d^{-1} in LWG for cattle with LWG of 0.5 kg d^{-1} by grain feeding was associated with 13% reduction in CH_4 production (g kg^{-1} LWG). In contrast, an improvement of 0.1 kg d^{-1} in LWG for cattle with a LWG of 1.1 kg d^{-1} was associated with a 5% reduction in CH_4 production (g kg^{-1} LWG^{-1}). This goes to show that improving LWG or productivity of poor pastures, or poor quality forages for cattle will lead to a more dramatic reduction in CH_4 production (g kg^{-1} LWG^{-1}) than for cattle on good quality pastures (e.g. legumes in grass mixes) or being fed grain. Similarly, a curvilinear relationship between CH_4 production (g CH_4 kg^{-1} milk) and milk production (kg milk d^{-1}) has been shown by Leng (1993), who indicated that Holstein cattle fed a high quality diet produced only 15% as much CH_4 L^{-1} of milk as native Indian cattle on traditional feed.

Strategic supplementation using by-pass protein, energy, mineral and protein supplements, to eliminate nutritional deficiencies (mostly associated with low quality feeds or tropical forage/pastures) will enhance productivity through increasing efficiency of feed utilization (Leng 1993). Nutrients, for example, in a molasses urea block lick given to ruminants fed low protein diets will ensure efficient digestion and lower CH_4 losses. Johnson et al. (1996) suggested CH_4 reductions per unit of product from strategic supplementation to be: molasses urea blocks (up to 40%); block plus escape protein (up to 60%); mineral, protein supplements (5-10%). It can be concluded that continuous improvement of both the level and efficiency of cattle production in the industry is an efficient and profitable available means of reducing CH_4 production.

2.3.7. Use of different species and breeds

There is little information on the comparative CH_4 production between species and between breeds. Terada et al. (1987) showed that CH_4 production by cattle fed forage- based diets at levels of 1.3-1.7 x maintenance requirements, were 8-12% higher than CH_4 yields by sheep fed a similar diet and level of feeding. Differences in CH_4 production (% of GEI) were also observed between bison (6.6%); wapiti (5.2%) and while-tailed deer (3.3%), when the species consumed 70, 87, and 68 g $kg^{-0.75} d^{-1}$ of alfalfa pellets, respectively (Galbraith et al. 1998). The relative order of decline in CH_4 production was explained to the concept of a reduction in rumen retention time of feed with smaller animals. Also, because the white-tailed deer is a browser /concentrate selector and has smaller relative rumen size, they generally produce lower CH_4. On the other hand, bison which is a roughage/ grass eater and has a larger rumen size to accommodate high roughage diets and therefore greater amount of CH_4 can be expected. Wapiti, which is an intermediate/mixed feeder animal, correspondingly had its CH_4 production in between the bison and white-tail deer. Body size has been shown to be a more important factor influencing feeding behavior and digestive physiology than digestive morphological differences of ruminant species (Gordon and Illius 1994). In comparing digesta mean retention time in eight browsers, seven intermediate, and eleven grazing African ruminants, Gordon and Illius (1994) showed that 99.6% of the variance was explained by a model with body mass and food type.

Although there is not much information on breed difference effects on CH_4 production, differences in rumen volume or size between breeds have been implicated in the differences in intake of poor quality roughages (Mould et al.1982). Mould et al. (1982) observed that voluntary intake relative to body weight of urea-supplemented barley straw by Friesian heifers was 42g kg $W^{-0.75}$, whereas that of similar quality urea -supplemented rice straw by small zebu cows in Bangladesh was 75g kg $W^{-0.75}$. The authors found that the filled gut of the zebu cattle was 33% of live weight compared with 18% for the western breed. It appears that the capacity to consume and utilize poor quality forages by the indigenous breed as a result of larger rumen volume and longer rumen retention time would increase fractional CH_4 losses. This was confirmed by Lal et al. (1987), who observed that CH_4 energy loss as % of GEI was higher in Holstein-Friesian x Harian cross cattle than in Holstein-Friesian cattle or buffalo fed on wheat straw based rations. Recent studies comparing the levels of CH_4 output (g d^{-1}) and CH_4 yield (% of GEI) for *Bos taurus* cattle in temperate Japan with Brahman cattle (*Bos indicus*) in tropical Australia showed no differences in emissions for cattle fed similar diets (Kurihara et al. 1998).

2.3.8. Environmental considerations

It is generally accepted that there would be a reduction in ruminal CH_4 production at lower temperatures and an increase in CH_4 production at higher temperatures based on the physiology of ruminant animals. Cold exposure generally results in lowered digestibility of feed in the rumen, because of faster passage rate through the gut. Kennedy and Milligan (1978) observed that CH_4 production was deceased by 30% due to cold exposure in sheep fed a pelleted diet hourly. They also observed that the ruminal passage rate constants of fluid and particulate matter increased by 54 and 68%, respectively in the cold environment. There was also a decline in volatile fatty acid production, a decrease in acetic acid and an increase in propionic acid production associated with cold exposed ruminants (Kennedy and Milligan 1978). There is evidence of reduced rumen motility associated with high environmental temperature (Mishra et al. 1970) and, therefore, CH_4 production might be expected to increase at higher temperatures. However, variable reports from the literature make it difficult to firmly establish the above general relationship between temperature and CH_4 production.

Rogerson (1960) observed variable effects of temperature between 20°C and 40°C on CH_4 production. He found that 50% more CH_4 was produced from cattle at 20°C with 6000 g d^{-1} feeding than at 4000 g d^{-1}. However, at 40°C CH_4 increased by only 25% with the higher feeding level, and at sub maintenance intake levels no effect of temperature was noted. Also, Von Keyserlingk and Mathison (1993) reported that CH_4 production was 25% higher in sheep housed at 4.7 °C than in sheep at 21 °C; fed 90 g of DM kg $BW^{-0.75}$ and 80g DM kg $BW^{-0.75}$, respectively, of a diet containing 50% chopped barley straw, 30% barley grain and 20% of a concentrate mixture. The authors explained this partly to an 8% increase in DM intake in the cold and, also, to the fact that the lambs in the trial may not have been truly cold stressed. When CH_4 production was expressed as a percentage of DEI, 14 % more CH_4 was formed in the cold environment. Similarly Dymtruk et al. (1995) measured higher CH_4 production in steers at -23.1°C than at 29.4°C when expressed as either % of GE and DE intakes. A firm conclusion, therefore, can not be drawn on the effects of environmental temperature on CH_4 production.

2.4. MEASUREMENT OF RUMINANT METHANE PRODUCTION

There is the need to measure or quantify CH_4 losses under a number of production situations, in order to develop and evaluate the effectiveness of mitigation strategies for ruminant CH_4 production. In the past, CH_4 measurement techniques have not been suitable for pastured animals. Measurement techniques relating to pastured animals have been developed in recent years (Johnson et al. 1994; Lockyer 1997; Leuning et al. 1998; Judd et al. 1999). Field CH_4 measurement techniques would ensure that databases of global emissions from domestic ruminants are refined to reflect actual commercial rearing conditions. Some CH_4 measurement techniques and prediction strategies are discussed below.

2.4.1. Respiration calorimetry

Respiration calorimetry measures heat production from respiratory gases; O_2

consumed, and CO_2 and CH_4 produced (McLean and Tobin 1987; Johnson and Johnson 1995). Methane can be measured using a closed circuit or an open circuit indirect calorimetry system, with the latter being the most frequently used (McDonald et al. 1995). The principle behind the open-circuit indirect calorimetry technique is that outside air is circulated around the animal's head, mouth and nose and the expired air by the animal is collected (McLean and Tobin 1987). Methane production is determined by measuring the total air flow through the calorimetry system and the difference in concentration between inspired and expired air. Various types and designs of animal respiration calorimeters have been described by Young et al. (1975); McLean and Tobin (1987); Miller and Koes (1988); Kelly et al. (1994).

In respiration calorimetry, animals have to be restricted in a chamber, a ventilated hood or have to wear a face mask in order for measurements to be taken (McLean and Tobin 1987). This makes it difficult to measure CH_4 production from grazing animals as their grazing behavior has to be interrupted. When the calorimetry system involves a chamber, both ruminal and hind gut CH_4 production can be estimated from the animal. When animals use the ventilated hood or wear a face mask, only ruminal CH_4 can be measured. Calorimetry using the face mask prevents animals from eating or drinking while CH_4 measurements are being taken. Respiration calorimeters are expensive to construct and animals must be trained to use the chamber, the ventilation hood or to wear a face mask. Secondly, gas measurements can be made on a few animals at the same time. The restriction of animal movement prevents the use of chambers, hoods and masks in pastured animals.

2.4.2. Prediction equations and models

Global and national estimates of CH_4 emissions derived using IPCC (1995) guidelines have been based on prediction equations and models. Crutzen et al. (1986) based their estimates of global ruminant CH_4 emissions on the relationship between feed intake and digestibility developed by Blaxter and Clapperton (1965):

$$\%CH_4\ (GEI) = 1.3 + 0.112D + L(2.37 - 0.05D)$$

where L is the level of feed intake and D is dry matter digestibility. The Blaxter and Clapperton (1965) prediction equation was developed from respiration calorimetry chamber experiments using mainly sheep, and is best suited in estimating CH_4 emissions when feed types and feeding levels are the same as those used to develop the model. The equation above predicts emission loss in the range of 5 to 8% of GEI, however observed CH_4 emissions from a wide range of feeds and animals varied from 2 to 12% of GEI (Johnson and Johnson 1995). Using an extensive database ($n = 452$), Johnson and Johnson (1995) showed that the ability of the Blaxter and Clapperton's equation to predict CH_4 emissions was weak. i.e. the relationship between predicted and observed CH_4 emissions was very poor ($r^2 = 0.23$). Moe and Tyrrell (1979) also proposed another equation for predicting CH_4 from feed soluble residue, hemicellulose and cellulose. The relationship was derived from measurements with cattle fed a high quality dairy ration as follows:

$$CH_4\ (MJ\ d^{-1}) = 3.406 + 0.510\ (\text{soluble residue}\ (kg\ d^{-1}) + 1.736\ (\text{hemicellulose}\ d^{-1}) + 2.648\ (\text{cellulose}\ d^{-1}).$$

In examining the accuracies of seven published equations for predicting CH_4 production, it was shown that the Moe and Tyrrell (1979) equation resulted in the lowest error of prediction with a variety of dairy cattle diets (Wilkerson et al. 1995). Although cellulose, hemicellulose and neutral detergent solubles are easy to measure in the laboratory, the equation was derived from respiration calorimetry measurements made from cattle fed high quality ration, and may not be applicable to cattle consuming poor quality forages.

Dynamic and mechanistic models to predict CH_4 from ruminants have also been established (Baldwin et al. 1987; Benchaar et al. 1998; Mills et al. 2001). The models predict the amount of CH_4 released due to microbial fermentation in the rumen as a result of the chemical characteristics of the diet. The equation of Moe and Tyrrell (1979) and Blaxter and Clapperton (1965) were shown to be poorer ($r^2 = 0.42$ and $r^2 = 0.57$, respectively) with errors of prediction equaling 33.72% and 22.93%, respectively (Benchaar et al. 1998). The authors showed that, with large variations in diet composition, mechanistic models allow the prediction of CH_4 production more accurately ($r^2 = 0.71$) than simple regression equations.

Both statistical and modeling approaches to predicting CH_4 emissions depend on data measured with respiration calorimetry for defining the relationship of energy intake and CH_4 production. A weakness in the use of chamber data is the question of the environment in which the animal is measured. The chamber is an artificial restricted environment, and the extent to which chamber results can be extrapolated to the range, pasture and feedlot situations is open to question. There is, therefore, a move toward refining ruminant CH_4 emission inventories. Directly measured CH_4 emission measurements using the SF_6 tracer gas technique from various classes of livestock in the United States have been established

(Westberg et al. 2001).

2.4.3. *In vitro* techniques

In vitro techniques simulate the fermentation processes in the rumen of animals by establishing cultures of mixed ruminal organisms. Batch and continuous culture fermentation such as the artificial simulation technique (Rusitec) have been used to study microbial digestion, rumen microbial populations, VFA's and also to measure CH_4 production (Czerkawski and Breckenridge 1977; Kung et al. 1992; Garcia Lopez et al. 1996; Dong et al. 1997b; McMahon et al. 1999).

In order to establish microbial cultures, fistulated animals are fed a typical diet of interest for a period of time and ruminal fluids are then collected post-feeding. Anaerobic conditions are always ensured by adding CO_2 during ruminal fluid collection. The ruminal fluids are then added to the diet of interest in incubation tubes (batch cultures) and fermentation allowed to proceed over a period of time (24 h), after which gases are measured (Kung et al. 1992; Garcia Lopez 1996). Rumen fluid temperature is maintained at 39°C during *in vitro* incubation. In continuous culture fermentation, fermentors with a 500 ml reaction vessel are used (Kung et al 1992; Garcia Lopez 1996), while the Rusitec, involves the use of four continuous fermentation vessels, allowing fermentation to proceed for longer period of time than in continuous cultures (Czerkawski and Breckenridge 1977; Dong et al. 1997b; McMahon et al. 1999).

The advantages of measuring CH_4 production with *in vitro* incubation systems is that it is lower in cost, more rapid and more repeatable than *in vivo* methods. However, the interpretation of *in vitro* CH_4 measurements to *in vivo* situations is not always valid. For example, exogenous fibrolytic enzymes were shown to improve fiber digestion using the Rusitec, however these positive effects for fibre digestion were not observed when enzyme treated forages was fed to ewes (Dong et al. 1997b). It was also observed that bromoethanosulphonate (BES) depressed CH_4 production by 71% in the Rusitec, however BES did not always depress CH_4 production *in vivo* in sheep (Dong et al. 1997b). Where effectiveness of BES was observed *in vivo* in sheep, it only persisted for 3 days (Immig et al. 1995). This implies the ability of the reticulorumen micro-flora to adapt to the presence of BES over time in the animal compared to the artificial rumen.

Some of the changes associated with *in vivo* fermentation come about gradually over a period of weeks rather than days and it is difficult to exert sufficient control with *in vitro* techniques. For example, in the Rusitec, it was shown that protozoal concentration decreased as fermentation progressed (Czerkawski and Breckenridge 1977). In the study of Dong et al. (1997b), rumen protozoa were not successfully cultivated using the Rusitec, due to exposure of vessels to atmospheric oxygen during the change of feed bags.

It can be concluded that the amount and activity of microbes may change under *in vivo* compared to *in vitro* conditions. The diet composition and amount of feed given to animals in *in vivo* evaluation and those used by donor animals for *in vitro* techniques may be different. It is therefore difficult to predict actual animal CH_4 losses from *in vitro* studies.

2.4.4. Isotopic tracer techniques

Isotopic tracer methods have also been used to estimate CH_4 production in ruminants (Murray et al. 1976; Frances et al. 1993). The method involved the use [^{3}H-] CH_4 and [^{14}C-] CH_4 in a ruminally cannulated animal (Murray et al. 1976). These authors used infusion lines to deliver the labeled gas to the ventral rumen and sampled gases in the dorsal section of the rumen. Using the specific gravity of the radio-labeled CH_4 gas, CH_4 production in respired gases and flatus were calculated (Murray et al. 1976).

Frances et al. (1993) used a single dose injection of tracer to estimate methanogenesis in the various compartments of the rumen and whole animal in non-steady state conditions which is pertinent to animals under once or twice daily feeding regime. The major limitation using isotopic tracers is the difficulty in preparation of infusion solutions because of the low solubility of CH_4 gas (Johnson and Johnson 1995).

2.4.5. Mass balance and micrometerological techniques

Measurement of CH_4 from groups of animals has also been documented (Kinsman et al. 1995; Lockyer 1997; Lassey et al. 1997; Leuning et al. 1998) using mainly mass balance methodologies. In brief, mass balance approach involves measuring the difference in CH_4 concentration coming into and exiting an enclosure (building or pen) containing a group of animals. Emissions of CH_4 are calculated from the volume of the air flow through the enclosure and the net concentration of CH_4. In studies using buildings (swine and indoor cattle facilities) as the enclosure, air flow is quantified by either measuring the air flow rate through exhaust vents using an anemometer or by employing a tracer gas (Kinsman et al. 1995; Westberg et al. 2001).

With grazing animals, Lockyer (1997) described a system in which air was drawn across animals enclosed in a 4.3 by 9.9 m polythene-clad tunnel placed over the pasture. Sheep (470 ha^{-1}) were enclosed for up to 10 days under the tunnel and CH_4 emission was estimated across vertical planes for incoming and outgoing air. Leuning et al. (1998) also described a mass balance approach in which animal were fenced in a 22 by 22 m enclosure (2818 sheep ha^{-1}) and gas was sampled from many ports on a frame-work up to 3.5 m high surrounding the enclosure. The advantage of these pasture techniques are that they can accommodate change in wind direction, however they require enclosing the animal at very high stocking rates. None of the mass balance methods measure CH_4 emissions from individual animals which have different digestion efficiencies and feeding behavior on pasture.

Micrometerological methods such as eddy correlation, eddy accumulation and gradient methods can be employed to measure CH_4 from a herd of animals on pasture (Andreae and Schimel 1989; Judd et al. 1999). Judd et al. (1999) measured CH_4 fluxes for 5 days across a paddock grazed by 20 sheep ha^{-1} using a gradient method. Samples of air were drawn from two heights (3.8 and 1.2 m) on a tower sited on the downwind boundary of the experimental area. Wind speed and direction were also measured from the tower. Disadvantages of this technique is that it is not flexible, as it requires a large fetch of undisturbed air on the upwind side of the sampling tower. Also, it can only be used on rainless days when the wind is in one direction, and it can be affected by movement of animals.

2.5. MEASUREMENT OF CARBON DIOXIDE PRODUCTION

Carbon dioxide is produced when feed material is oxidized during fermentation and also from body metabolism of the animal. Carbon dioxide is eliminated from the animal through eructation of fermentation gases and expiration from the lungs of that formed within the animal body (Duke 1993). Carbon dioxide measurement in livestock has primarily been conducted in order to estimate metabolic heat production (see Section 2.5.1).

Carbon dioxide production can be determined using closed or open circuit respiration calorimetry techniques (McLean and Tobin 1987; McDonald et al. 1995). The closed circuit calorimeters consist of an airtight container for the animal together with vessels holding absorbents for carbon dioxide and water vapor. The chamber incorporates devices for feeding and watering of animals. The oxygen used is replaces from a metered source, and at the end of the trial period, the CO_2 produced can be measured by weighing the absorbent (McLean and Tobin 1987). The main disadvantage of the closed circuit is that large quantities of absorbents are required, thus for a cow, 100 kg of soda lime would be needed each day to absorb carbon dioxide and 250 kg of silica gel to absorb water vapor (McDonald et al. 1995), thus the open circuit respiratory calorimetry systems have replaced the closed system in recent years.

In open circuit calorimetry, the amount of CO_2 produced is calculated by comparing the volume and composition of the air entering and leaving the chamber or hood. As the differences in composition between inlet and outlet must be kept small, very accurate measures of gas flow and composition are required. Various types and designs of animal respiration calorimetry have been described (Young et al. 1975; McLean and Tobin 1987; Miller and Koes 1988; Kelly et al. 1994). With respiratory calorimetry, animals have to be restrained for a period of time to allow measurements; this is not be suitable for long term measurement of CO_2 production in unconfined (grazing) animals.

To overcome this invasive method of restraining animals, tracheostomized animals were used to estimate CO_2 production and energy expenditure (Flatt et al. 1958; Young and Webster 1963) under grazing conditions. Tracheal cannulation techniques had limited success, as problems with the accumulation of mucus and a gradual development of swelling were observed in the trachea (McLean and Tobin 1987). All breathing through the normal nasopharyngeal route was cut off during measurements and additional CO_2 eructed from the rumen was not collected. A system known as the Mobile Indirect calorimetry was latter developed using a re-entrant tracheal cannula allowing inhalation via the nasopharynx and collection of eructed CO_2 (Young and Corbett 1972; Kelly et al. 1994).

Another method for determining CO_2 production rates in grazing animals is to continuously infuse radiocarbon in the form of ^{14}C sodium bicarbonate ($NAH^{14}CO_3$) into the jugular vein or peritoneal cavity of sheep (Corbett et al. 1971) or cattle (Havstad and Malachek 1982; Sanchez and Morris 1984). After 3 hrs of infusion of radio carbon, the level of radio carbon will be equilibrated in the body fluid and then body fluids such as urine, blood or saliva can be sampled. The rate of CO_2 production is estimated from the ratio of the rate of infused activity to the specific activity of body fluid.

Carbon dioxide has also been estimated using the doubled labeled water in the form of $^{2}H_2\,^{18}O_2$ isotopes (Schoeller and van Santen 1982; Nagy 1983) dosed into the pool of water in an animal or a man's body. The principle behind this technique is based on the observation that oxygen atoms in metabolically produced CO_2 equilibrate with oxygen atoms in the body's water via the action of carbonic anhydrase in the blood (Robbins 1993). Since O_2 is lost from the body as both carbon dioxide and water, whereas hydrogen is lost as water only, the difference in the respective decay of hydrogen and oxygen isotopes is a measure of carbon dioxide produced.

Techniques involving the use of isotopes are expensive, and animals will have to be restrained to collect body fluids. The solubility of isotopes in body fluids has been a challenge. Since hydrogen can be incorporated into new tissues especially fat, and into CH_4 production, the use of the double labeled water may not be appropriate for rapidly growing or fattening animals (Robbin 1993).

2.5.1. Estimation of heat production from respiratory gas exchange

One way of estimating heat production is from the measurement of respiratory gases, since the stochiometric relationship between heat production and gas exchange are constant for complete oxidation o f any organic compound (Robbins 1993). For example, with the oxidation of 1 mole of glucose, 6 moles of O_2 are consumed and 6 moles of CO_2 and 2.82 MJ of heat are produced.

$$C_6H_{12}O_2 + 6O_2 \rightarrow 6\,CO_2 + 6\,H_2O + 2.82\ \text{MJ}$$

Therefore, the metabolic heat production of an animal can be determined indirectly by measuring respiratory gas production (Young et al. 1975; McLean and Tobin 1987). It has been shown that the energy equivalent of O_2 is less variable when different substrates are metabolized and, therefore, the thermal equivalent of O_2 consumption (heat per L of O_2 consumed) rather than that of CO_2 production are used in indirect calorimetry to estimated heat production (McLean and Tobin 1987; McDonald et al. 1995).

The stochiometric ratio between carbon dioxide produced and atmospheric oxygen consumed by the animal is termed the respiratory quotient (RQ). The RQ from the equation above is 1, and can be determined from the ratio of 6 molecules of O_2 consumed and 6 molecules of CO_2 produced upon complete oxidation (Robbins 1993; McDonald et al. 1995). Fats are poor in molecular oxygen, as a result additional atmospheric O_2 must be consumed relative to CO_2 produced. The RQ of fat is lower (0.70), as shown in the oxidation of tripalmitin :

$$C_3H_5(OOC.C_{15}H_{31})_3 + 72.5\,O_2 \rightarrow 51CO_2 + 49\,H_2O + 32.02\ \text{MJ}$$

Proteins are incompletely oxidized in animals, because the body cannot oxidize nitrogen. For each gram of protein oxidized, 0.77 L of CO_2 is produced and 0.96 L of O_2 used giving and RQ of 0.8. Since animals oxidize a mixture of the fat, carbohydrate and proteins, the thermal equivalent needed to convert oxygen consumption to heat production can be determined by how much O_2 is consumed by each nutrient.

In ruminants part of the chemical energy in feed is converted to CH_4 and thus RQ is less than 1 (McLean and Tobin 1987). Therefore, heat calculation from respiratory exchange is corrected for this effect as shown in the Brouwer (1965) equation for calculating heat production from livestock:

$$M = (16.18 \times VO_2) + (5.02 \times VCO_2) - (2.17 \times VCH_4) - (5.99 \times N)$$

where M = metabolic heat production (kW); VO_2 = oxygen consumption (L); VCO_2 = carbon dioxide produced (L); VCH_4 = methane produced (L) and N = quantity of urinary nitrogen excreted (g). In open circuit calorimetry, the direct measurements made are ventilation rate, and the composition of inlet and outlet air. These values are used to calculate the quantities of gases consumed or produced. Heat production can therefore be determined by this simpler equation (McLean and Tobin 1987) mainly using oxygen since it contributes nearly the entire value of M (75%) in the equation above:

$$M = -20.5\, V_E \,\Delta FO_2$$

where M = metabolic heat production (kW); V_E = the expired air flow rate at standard temperature and pressure for dry air ($L\ s^{-1}$); ΔFO_2 = is the difference in O_2 concentration by volume between inhaled and expired air. It has been confirmed that from the measurement of carbon dioxide alone, heat production may be estimated with a ± 10 % accuracy, while heat production from oxygen alone may be estimate with a ± 1.2 % accuracy (McLean and Tobin 1987).

2.6. THE SULPHUR HEXAFLUORIDE TRACER GAS TECHNIQUE

The sulphur hexaflouride (SF_6) tracer gas technique, a non isotopic tracer technique developed by Johnson et al. (1994) allows for direct measurement of CH_4 production in individual unrestrained animals. The technique involves the use of an inert, odorless tracer gas, SF_6, which is released at a known rate into the rumen from a stainless steel permeation tube (12.5mm x 40 mm), inserted into the rumen. The permeation tube rate is calibrated for two months at 39°C in an incubator, prior to insertion into the rumen. A sample of air eructed and expired by the ruminant is collected using a specially constructed collection apparatus fitted onto a halter and neck strap (Figure 5; Johnson et al. 1994). The collection canister is evacuated prior to use and the rate at which gases are sampled from the mouth and nose is determined by the length and diameter of the capillary stainless steel tubing. The collection system is designed to deliver half of its volume during sampling ensuring a uniform sampling rate, i.e the canister pressure should read around half atmosphere (380 mm Hg) after a 24-h collection. To ensure this, the collection system is checked prior to sampling by hooking evacuated canisters onto the collection apparati, and allowing then to fill with atmospheric air over a 24-h period, while periodically checking the canister pressure (See Appendix B).

From the analysis of SF_6 and CH_4 concentrations in the collection canisters, using a gas chromatograph, CH_4 production can be calculated as follows:

$$CH_4\ (\mathrm{L\ min^{-1}}) = \text{Permeation tube } SF_6 \text{ release rate } (\mathrm{L\ min^{-1}}) \times [CH_4] / [SF_6]$$

where $[CH_4]$ and $[SF_6]$ are the concentrations of CH_4 and SF_6 in collection canisters after background concentrations of CH_4 and SF_6 have been deducted. The SF_6 gas accounts for dilutions as gases existing the animals mouth and nostrils are mixed with ambient air.

Therefore, the SF_6 tracer gas technique can be used under variable wind speeds in the field.

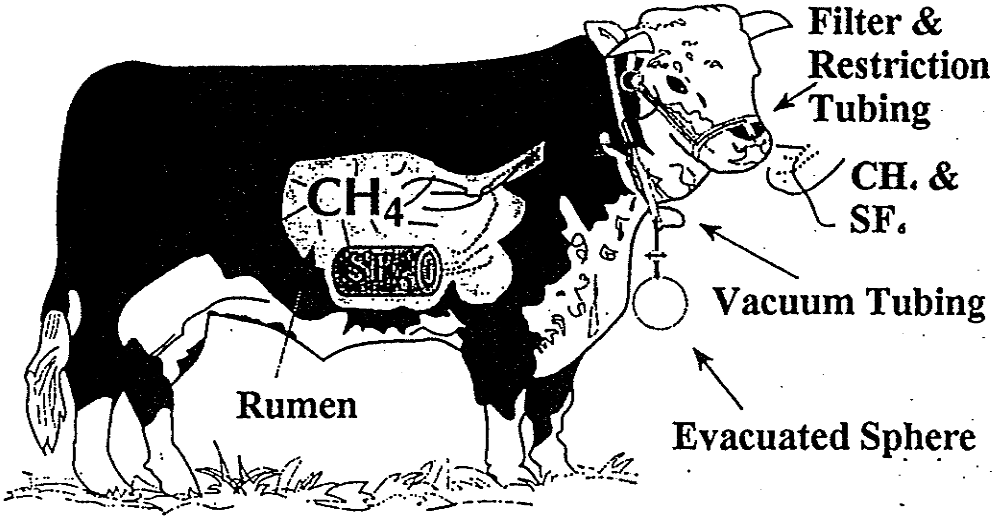

Figure 5. Illustration of the SF_6 tracer gas methodology (Johnson et al. 1994)

Estimates of CH_4 production using the SF_6 technique is dependent on the assumption that CH_4 and SF_6 are predominantly excreted via the mouth and nose. This is based on the study of Murray et al. (1976) who showed in sheep fed 800 g^{-1} lucerne chaff that 87% of CH_4 was produced in the rumen and 13% of CH_4 was produced in the lower digestive tract. Of the CH_4 produced in the lower digestive tract, 89% was absorbed into the blood stream, and expired from the lungs, where it is not distinguished from eructed CH_4 and can be accounted for by the SF_6 tracer technique.

The requirements of a tracer technique are that; the tracer is metabolically inactive (no impact on ruminal fermentation) and non toxic; the source of tracer is calibrated; and the tracer and tracee are well mixed in any sample (Ulyatt et al. 1999). Initial validation studies of these requirements showed that SF_6, an odorless, inert gas (146.05; molecular weight) which is detectable at very low concentration (1 ppt), is non toxic to animal or rumen microbes and had no effects on VFA concentrations and acetate:propionate ratios (Johnson et al. 1994).

The advantages of the SF_6 tracer technique includes its flexibility of use under pasture situation as well as in pen experiments. Gas data can be obtained from individual animals and measurements can be done simultaneously on several animals. This may allow the imposition and evaluation of CH_4 mitigation strategies, especially in grazing animals. The SF_6 tracer technique is also inexpensive compared to the construction of a respiration chamber. The SF_6 tracer technique however does not account for the hind gut CH_4 that is lost through the flatus; however, this is only 1.4% of total CH_4 produced. Animal would also have to be trained to wear gas collection apparatus.

2.6.1. Potential of the SF_6 tracer gas technique for indirect calorimetry

Early studies with tracheostomized ruminants have established that part of eructed gases from rumen fermentation (CH_4 and CO_2) are inhaled into the lungs before elimination to the atmosphere (Colvin et al.1957; Dougherty et al. 1962; Hoernicke et al. 1965;Young and Corbett 1972). Dougherty et al. (1962) collected 86 and 69% of eructed gas from the trachea of a Jersey cow and a Guernsey cow, respectively, while Hoernicke et al. (1965) observed that 71 to 99% of eructed gases were exhaled by the cranial trachea of tracheotomized cows.

Hornicke et al. (1965) have shown evidence of the interconnection of CH_4 and CO_2 production during digestion and metabolism of feed, and have established that the elimination of both CH_4 and CO_2 are from both the esophagus and the trachea (Figure 6). Since majority of CO_2 production are eructed and exhaled from the esophagus and trachea, CO_2 can be captured along with CH_4 in air samples collected by the SF_6 tracer gas technique provided the tracer mixes well with CO_2 gas.

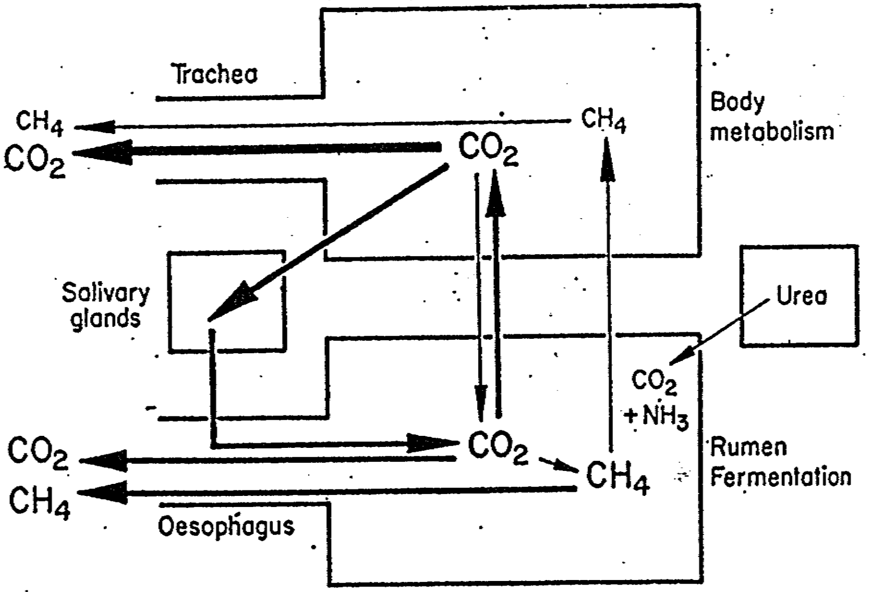

Figure 6. Scheme of the two gas-producing metabolic systems in ruminants, their outlets and inter-connections (Hoernicke et al. 1965)

McDonald et al. (1995) noted that heat production can be estimated with reasonable accuracy from CO_2 production in grazing animals without the need of restraining as employed in respiration calorimetry techniques. Energy expenditure from CO_2 production rates has been determined in grazing animals (Corbett et al. 1971; Harvstad and Malachek 1982; Sanchez and Morris 1984) where it is difficult to assess O_2 consumption, without restricting animals from grazing. If the SF_6 tracer gas technique can estimate CO_2 production rates in ruminants, then energy expenditure from the CO_2 production rate alone can be estimated with an accuracy of ± 10 % (McLean and Tobin 1987).

2.7. SUMMARY

Methanogenesis is an important final process in anaerobic fermentation of feed material. Methanogens remove excess hydrogen ions produced by other bacteria from rumen fluid, and enables the further breakdown of fibrous feeds in the rumen. However, depression of CH_4 production through strategies that provide alternate pathways for the disposal of H_2 ions, could benefit ruminant animals with an improved efficiency of energy utilization. Reduction in CH_4 production also would be beneficial for the environment by decreasing emission of this greenhouse gas.

Assessing the extent of CH_4 reduction under practical rearing conditions is important in evaluating the true potential of factors that can reduce CH_4 production from ruminants. Information derived from *in vitro*, prediction equations and chamber studies function to identify potential strategies, but cannot be extrapolated to predict losses under field conditions where feed selection, environmental conditions and dry matter intake are variable on the farm. The SF_6 tracer gas technique allows the flexibility of directly assessing variations in CH_4 production in cattle both in barns and on pasture. The identification and evaluation of mitigation strategies of livestock CH_4 production, could be assessed under a wide variety of production situation using the SF_6 tracer gas technique provided there is proper validation of this procedure. The SF_6 tracer gas technique's potential for measuring CO_2 production and heat production has not been previously considered, and if validated, energy expenditure can also be estimated for grazing animals using the SF_6 tracer gas technique.

3.0. RESEARCH HYPOTHESES AND OBJECTIVES

3.1. Hypotheses

The SF_6 tracer gas technique can be used to directly measure eructed and respired CH_4 and CO_2 production of cattle, and to evaluate factors contributing to improved animal efficiencies on pasture or other free-movement situations. The majority of eructed gases are inhaled into the lungs and along with CO_2 produced from metabolism are eliminated through the trachea and esophagus. Therefore, eructed and respired CH_4 and CO_2 production in air samples collected at the mouth and nose can be quantified using the SF_6 tracer gas technique.

3.2. Objectives

The general objectives of the thesis research were:

1) to determine if CH_4 and CO_2 production could be measured directly from cattle under their natural rearing conditions using the SF_6 tracer gas technique.

2) to evaluate factors that may reduce CH_4 production under production situations (outdoor pen and pasture).

To achieve this, three experiments were undertaken with the following specific objectives:

a) To validate the SF_6 tracer gas technique in measuring CH_4 and CO_2 production by cattle, by comparison of estimates with the open-circuit calorimetry using the ventilated hood.

b) To test the SF_6 tracer gas methodology in an outdoor pen setting using animal types, forage quality and feeding levels as test variables.

c) To test the SF_6 tracer gas methodology in a pasture setting using grazing season (forage quality) and grain supplementation as the test variables.

4.0. MANUSCRIPT I

VALIDATION OF THE SULPHUR HEXAFLUORIDE (SF_6) TRACER GAS TECHNIQUE FOR MEASUREMENT OF METHANE AND CARBON DIOXIDE PRODUCTION BY CATTLE

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4.1. ABSTRACT

Methane (CH_4) and carbon dioxide (CO_2) production from six crossbred yearling beef heifers (400 ± 13.0 kg) were measured, using the sulphur hexafluoride (SF_6) tracer gas technique (Tracer) and open-circuit hood calorimetry (Cal) to validate the tracer technique in estimating rumen CH_4 and CO_2 production in the field. Animals were individually fed a diet consisting of 50% barley concentrate and 50% alfalfa cubes at 1.3 x maintenance requirements daily. Heifers were divided into two groups for individual animal 24-h gas measurements by each method. Each group of heifers was rotated between the Cal and Tracer techniques for 6 consecutive days in an incomplete block design. Methane production ranged from 108 to 145 L d^{-1} (mean 130 ± 4.0 L d^{-1}) using the Cal technique, and 90 to 167 L d^{-1} (mean 137 ± 4.0 L d^{-1}) using the Tracer technique. The mean CH_4 production (L d^{-1}) was not different (P = 0.24) between the two methods. Carbon dioxide production with the Tracer technique was 20% higher than CO_2 production with the Cal technique ($P < 0.01$). The range of CO_2 production was 1574 to 2049 L d^{-1} (mean 1892 ± 74.0 L d^{-1}) by Cal, and 1541 to 3330 L d^{-1} (mean 2353 ± 74.0 L d^{-1}) by Tracer. Day-to-day variation in CH_4 production was not different within each method ($P > 0.05$), however, animal-to-animal variation (11.7%) was significant for the Tracer technique ($P = 0.04$) but not for the Cal technique ($P = 0.53$). There were no differences in variability ($P > 0.05$) between Cal and Tracer for CH_4 production. On the other hand, variability in CO_2 production was not equal ($P > 0.05$) between methods. Day-to-day variation in CO_2 production was significant using Cal, but not Tracer ($P > 0.05$). Animal-to-animal variation in CO_2 production was 1.6% and 11.8% by

Cal and Tracer techniques, respectively. It can be concluded that the SF_6 tracer technique accurately estimated rumen CH_4 production, but CO_2 production was 20% higher than estimates obtained by Cal. The study suggests that for CH_4 measurements using the SF_6 tracer technique, more animal numbers are needed than for Cal to reduce animal-to-animal variation.

Key words: Methane, carbon dioxide, SF_6 tracer technique, validation, cattle

4.2. INTRODUCTION

Methane (CH_4) production of cattle has often been measured using respiration calorimetry (Young et al. 1975; McLean and Tobin 1987) from which a number of prediction equations and models have been derived to estimate CH_4 production (Blaxter and Clapperton 1965; Holter and Young 1992; Wilkerson et al. 1995). Respiration calorimetry allows gases respired and eructed into a hood or chamber to be monitored and quantified, from which heat production of animals can then be indirectly assessed (McLean and Tobin 1987). Respiration calorimetry involves restricting animals from their natural environment, in order to estimate gas production thus making it inapplicable to grazing animals. As a result, information on CH_4 production of grazing ruminants which are exposed to variable environmental conditions and feed selection is limited.

The sulphur hexafluoride (SF_6) tracer gas technique, which was designed for free ranging animals, allows direct rumen CH_4 measurement without restricting animals from their natural environment and feeding behavior (Johnson et al. 1994). Also, measurements can be made simultaneously on several animals. The tracer gas technique uses an inert-non toxic gas, SF_6, as a marker. The tracer gas, released at a known rate in the rumen, allows the concentrations of eructed and respired gases from the mouth and nose to be quantified. The tracer can account for changes in dilution of expired gases associated with head or air movements, therefore applicable under variable wind speeds (Johnson and Johnson 1995). The SF_6 tracer gas technique however does not account for the small proportion (1.4 %) of hind gut CH_4 that is excreted through the anus (Murray et al.1976), and animals must be trained to wear a gas collection apparatus. There are variable reports on validation studies involving the SF_6 tracer gas technique and respiration calorimetry (Johnson et al. 1994; Ulyatt et al.1999). Some of the differences in reports can be attributed to measurements made with respiration calorimetry using the chamber, which accounts for rumen and all hind gut CH_4 losses, while the SF_6 tracer gas technique does not account for all the hind gut losses. There is the need to confirm that the SF_6 tracer gas technique can measure rumen CH_4 production, and to establish degree of variability expected with the former.

There is evidence that the majority of eructed gases from the rumen are inhaled via the trachea into the lungs before exhalation to the atmosphere along with respiratory gases (Colvin et al. 1957; Hoernicke et al. 1965; Young and Corbett 1972). It is, therefore, proposed that the SF_6 tracer technique also has the potential to measure CO_2 production rate. The determination of CO_2 production rates by the SF_6 tracer technique has not been previously considered and thus would require validation. Prediction of heat production from CO_2 production rates would allow energy balance studies in grazing animals using the SF_6 tracer technique.

The objectives of this study were to validate the SF_6 tracer technique in measuring CH_4 and CO_2 eliminated by cattle, with respiration calorimetry using the ventilated hood. A second objective was to establish the degree of variability associated with the SF_6 tracer technique measurements.

4.3. MATERIALS AND METHODS

4.3.1. Animals and Management

Six crossbred yearling beef heifers (400 ± 13.0 kg) (mean ± SD) were used to compare CH_4 and CO_2 production rates using the open-circuit calorimetry hood (Cal) and the SF_6 tracer gas technique (Tracer). The study was conducted at the Laird McElroy Environmental and Metabolic Center at the University of Alberta Research Farm, Edmonton, Alberta, from August 8 to 21, 1999.

Animals were individually fed once daily, a diet which consisted of 50% rolled-barley concentrate and 50% alfalfa cubes at 1.3 x maintenance requirements (Table 4). Dry matter intake of the diet ranged from 4.4-4.8 kg d^{-1} among animals. Animals were chosen from a previous experiment in which animals were consuming the same diet as shown in Table 4 for three months. They were adjusted to their ration allotment for one week prior to the start of gas collection. The level of feeding ensured that all feed was consumed and that intake relative to body weight was similar for all animals. Feeding began at 8:00 h, after which animals were moved from pens for gas sampling by both methods. Animals were cared for in accordance to the guidelines of the Canadian Council of Animal Care.

4.3.2. Experimental design and treatments

There were three days of gas collection from each animal by each method. Since a limited number of animals could be sampled by the calorimetry system, animals were randomly divided into 2 groups (3 animals each). Alternating for 6 days, one group of animals went into the ventilated hoods for calorimetry, and the other group of animals were put in an open ventilation facility (with a roof) having individual stalls for Tracer gas collection. The open ventilation facility was used for Tracer collection to prevent contamination of individual heifer air samples by the build up of background gases. A 24-h gas sampling occurred simultaneously by both methods, commencing shortly after feeding when all animals had settled down for sampling. The experiment was conducted as an incomplete block design, with days as blocks.

4.3.3. Methane and CO_2 determination by calorimetry

Methane and CO_2 production were determined using the open-circuit calorimetry system which employed a ventilated hood that enclosed the animal's head (Young et al. 1975). Animals could stand or lie tethered in a stall. Animals were adapted to the use of the ventilated hood during a previous experiment. Three 24-h collections were completed for each animal over the course of 6 days. Water was provided ad-libitum in the hood during sampling. Oxygen concentration was measured using a single-circuit Servomex paramagnetic oxygen analyzer (Model #540A, Crowbridge, UK). Carbon dioxide and CH_4 concentrations were measured with non-dispersion infrared analyzers (Model 880A, Rosemount Analytical Inc., La Habra, CA). All analyzers were calibrated at the beginning of each day's analyses as described by McLean and Tobin (1987). Air pressure and flow were recorded using a Foxboro electronic pressure and flow transmitter connected to a Strawberry Tree terminal

Panel T41 (Strawberry Tree Inc., Sunnyvale, CA.). The average flow rate over the six days of sampling was 237 ± 22.0 L min^{-1}. Lines from the animal hoods were switched using Ascoelectric switches (Ascoelcetric Ltd., Brantford ON). Methane, CO_2 and O_2 data were collected using the control setup of Workbench P.C 2.0 (Strawberry Tree Inc., Sunnyvale, CA.).

Before the experiment, a known amount of nitrogen gas was released into the calorimetry hoods to determine the recovery of the system. Nitrogen recovery factors were used to adjust CH_4, CO_2 and O_2 results (Galbraith et al. 1998). The mean calorimetry room temperature as animals entered and exited was 16 ± 1.0°C and 17 ± 0.8°C, respectively.

4.3.4. Methane and CO_2 determination by SF_6 tracer technique

Methane and CO_2 were sampled from each heifer for three 24-h periods using the SF_6 tracer gas technique (Johnson et al. 1994; See section 2.6). The rate of SF_6 release from the permeation tube is controlled by a permeable Teflon membrane (2 mm thickness) held in place by a stainless-steel Swaglok nut (See Appendix B). Each tube was charged with 0.260-0.300 mg of SF_6 at liquid nitrogen temperatures, and kept in an incubator at 39°C. Release rates of SF_6 were determined by measuring the weight loss of tubes for about 8 weeks to establish a steady pre-determined rate (See Appendix B). Sulphur hexafluoride release rates ranged from 250 to 500 ng min^{-1}. Based on a pre-trial test, and the study of Westberg et al. (2001), that showed the release rates of permeation tubes after removal from the rumen were similar ($P > 0.05$) to release rates prior to insertion into the rumen, the release rate of permeation tubes were assumed constant during the experimental period (See Appendix B).

Stainless steel permeation tubes containing SF_6 with known release rates were placed in the rumen (through the throat) using a speculum, a week prior to the start of the experiment. This allowed enough time for the tracer gas to equilibrate in the rumen. During this period, animals were trained to wear the gas collection apparati. Animals were moved into a squeeze chute daily for attachment and removal of collection apparati. In the open ventilation facilities, animals were placed in stalls, and not tethered during collection. Water was provided ad libitum during sampling.

Exhaled gas from the nose and mouth was drawn into pre-evacuated (30 mm Hg) stainless steel collection canisters (130-mm diameter), through a 900-mm capillary tubing (128µm i.d) with an in-line 15-µm filter and flexible nose piece fitted to a halter (McCaughey et al. 1999). The collection canister is suspended by a neck strap, attached to the halter apparatus with a quick connect fitting. Collection apparati were hung on the east and west side of the open ventilation facility each day to collect background air samples, which were used to correct expired gas concentrations. Collected canisters were pressure checked to identify blocked or leaking capillary systems to ensure data used represented a complete 24-h period. Spheres were then pressurized to 110 KPa with pure N_2 to prevent sample contamination prior to analyses, and to allow injection of gas samples into the sample loop of a gas chromatograph. The mean minimum and maximum temperature during Tracer sampling was 9 ± 1.2°C and 22 ± 2.2°C, respectively.

A gas chromatograph (Star 3600, Varian, Mississauga, ON) fitted with an electron capture detector was used for determining SF_6, and a flame ionization detector was used for determining CH_4 and CO_2 concentration in collected samples. Samples were analyzed in duplicate. The gas chromatograph was fitted with a Molecular Sieve 0.5nm (1800 mm) column for SF_6 and a Poropak QS (1800 mm) for CH_4 and CO_2. The column and injector temperatures were 35°C and 350°C respectively, and nitrogen was used as the carrier gas with a flow rate of 30 mL min^{-1}. Prepared standards were used to standardize the gas chromatograph for SF_6 (20 ppt, Scott-Marrin Inc., Riverside, CA); CH_4 (100 ppm; Supelco, Mississauga, ON) and CO_2 (1614 ppm; Matheson gas products Edmonton, AB) prior to sample analysis. Gas concentrations (SF_6, CH_4 and CO_2) were determined from peak areas and identified from their different retention times relative to the known standards.

Daily CH_4 and CO_2 production was calculated as follows (Johnson et al. 1994):

$$CH_4\ (L\ min^{-1}) = SF_6\ (L\ min^{-1}) \times [CH_4] / [SF_6]$$

$$CO_2\ (L\ min^{-1}) = SF_6\ (L\ min^{-1}) \times [CO_2] / [SF_6]$$

where SF_6 is the predetermined release rate from the permeation tube and $[CH_4]$, $[CO_2]$ and $[SF_6]$ are the concentrations of CH_4,CO_2 and SF_6 in samples after background concentrations have been subtracted from each animal sample (See Appendix A for data conversions and Appendix B for some background and animal gas data).

4.3.5. Chemical analyses

Feed samples were dried for 48 h in a forced draught oven at 60°C for dry matter (DM) determination. Samples were ground using a Wiley mill fitted with a 1 mm screen.

Dried samples were analyzed for crude protein (CP) using a Kjeltec 1030 auto analyzer (Tecator Inc., Herndon,VI; Association of Official Analytical Chemists [AOAC] 1990, method no. 984.13), and ash, method no. 942.05 (AOAC 1990). Acid detergent fibre (ADF) and neutral detergent fibre (NDF) were determined using an ANKOM 200 fibre analyzer (Fairport NY), with procedures described by Komarek (1993). Gross energy (GE) was determined using a Parr 1241 adiabatic bomb calorimeter.

4.3.6. Statistical analyses

Methane and CO_2 production data from both methods were analyzed by the analyses of variance using GLM in SAS Institute Inc, (1990) using the model:

$$Y_{ijk} = \mu + M_i + A_j + D_k + \epsilon_{ijk}$$

where Y_{ijk} = trait under consideration; μ = overall mean; M_i = methods with (i = 1,2); A_j = Animals with (j = 1...6) and D_k = Day with (k = 1...6); ϵ_{ijk} = experimental error term. Means were separated at the 5% level of significance using the probability of differences (PDIFF) options. The effect of day was further evaluated in each method. To compute day-to-day and animal-to-animal variations within each method, Type III expected mean squares were generated for the random effects (animal and day). To compare equality of variability of CH_4 and CO_2 production by the two methods, the residual variances from each method derived in the GLM were subjected to an F-test (Ratio of the larger error variance:smaller error variance) at $P = 0.05$.

4.4. RESULTS AND DISCUSSION

4.4.1. Methane production

Methane production ranged from 108 to 145 L d^{-1} (mean 130 ± 4.0 L d^{-1}) by the Cal method, and from 90 to 167 L d^{-1} (mean 137 ± 4.0 L d^{-1}) by the Tracer method. The mean CH_4 production (L d^{-1}) was not different ($P > 0.05$) between methods (Table 5). Also, there was no effect of day on CH_4 measurements within each method ($P > 0.05$; Figure 7). The average loss of GEI as CH_4 was similar by both methods ($P > 0.05$; Table 5), and values were within the range reported by growing animals (Johnson et al. 1994; McCaughey et al. 1997).

Johnson et al. (1994) showed that CH_4 produced by the SF_6 tracer technique (11.6 ± 0.7 L h^{-1}) compared to estimates from respiratory chamber measurements (12.9 ± 0.7 L h^{-1}) was about 90% of the values of chamber measurements. This is expected as the chamber method measures both rumen and hind gut CH_4, while the SF_6 tracer method and the ventilation hood method used in this study do not measure all hind gut CH_4. Similarly, it was observed in an experiment using 10 sheep fed chaffed lucerne that CH_4 emission estimated with the SF_6 tracer technique was 95% of respiratory chamber estimated emissions (Ulyatt et al. 1999). On the other hand Ulyatt et al. (1999), reported that estimates of CH_4 production made in the respiration chamber (7.7 ± 0.7 L h^{-1}) using five Friesian calves fed Rhodes grass, were twice ($P < 0.005$) estimates with the SF_6 technique either in pens (4.1 ± 0.4 L h^{-1}) or the SF_6 technique conducted concurrently in the chamber (4.0 ± 0.4 L h^{-1}). Although more CH_4 production can be expected from calorimetry using the chamber as opposed to the ventilation hood, the less than expected results of 50% more CH_4 from the chamber were attributed to the poor storage and transport of gases for analyses. To avoid loss and contamination of gases during transport of gases for analyses in our study, a positive pressure was created in the collected samples by pressurizing the spheres with nitrogen prior to transport. Also, gas samples were immediately analyzed upon arrival to avoid problems of leakage associated with long storage.

Average CH_4 production by individual animals ranged from 123 to 139 L d^{-1} with the Cal method, while the range was 108 to 149 L d^{-1} on the Tracer method (Figure 8). Mean CH_4 production for Animal #27 and #67 were significantly lower than for other animals using the Tracer technique. There were no differences ($P > 0.05$) in CH_4 production among animals using the Cal method (Figure 8).

Day-to-day variation of CH_4 production was not significant with the Tracer ($P = 0.93$) and Cal methods ($P = 0.20$). The lack of significant daily variations can be expected as the diet and amount fed was constant for all heifers in the trial. On the other hand, a significant animal-to-animal variation (11.7%) in CH_4 production was observed with the Tracer method, but not with the Cal method (0.1%; $P = 0.53$). The high animal-to-animal variation observed with the Tracer method agrees with recent observations using the SF_6 tracer technique for CH_4 production measurements (Lassey et al. 1997; Leuning et al. 1998; Ulyatt et al. 1999).

Ulyatt et al. (1999) noted that, in general, when the feed intake and the composition of the diet are similar for all animals, significant between-animal differences accounted for most of the variance with a lesser amount attributed to differences among days. There is, therefore, the need to include sufficient animals to detect differences between treatments and sufficient collection days per animal to minimize between day variation with the use of the Tracer method. In studies with grazing animals, between-animal and between-day variations in CH_4 production were strongly related to the amount and composition of pasture selected (Lassey et al. 1997; McCaughey et al. 1999). Lassey et al. (1997) observed much higher variation between animals (87%) and between collection days (13%) for mature dairy cows under grazing conditions. In testing the equality of variance in CH_4 production between Cal and Tracer, (at $F_{8/8\ (0.05)}$ value = 3.44), there were no significant differences between the residual variance of the two methods (F = 2.09). Following rigorous comparison of the traditional calorimetry technique using a ventilated hood and the SF_6 tracer technique which both measure respired gases, it can be concluded that the SF_6 tracer technique is accurate and can be used to measure rumen CH_4. However values using the SF_6 tracer technique need to be adjusted up in order to estimate total CH_4 production.

4.4.2. Carbon dioxide production

Carbon dioxide production ranged from 1574 to 2049 L d^{-1} (mean 1892 ± 74.0 L d^{-1}) by the Cal method, and from 1541 to 3330 L d^{-1} (mean 2353 ± 74.0 L d^{-1}) by the Tracer method. Tracer CO_2 production was 20% higher ($P < 0.01$) than Cal CO_2 production (Table 5). It is possible that the animals in the open ventilation facility during SF_6 tracer sampling were more active due to movement (non-tethered stalls) and were more excited (hooking up and removal of canister in the chute), which would contribute to the higher CO_2 production observed with the Tracer technique. Although majority of rumen gases go to the lungs before exhalation, there is no information about mixing of rumen and lung air, or mixing of CO_2 gas with the tracer gas.

Carbon dioxide production using the Tracer method was similar from day to day ($P = 0.77$) while daily differences ($P = 0.003$) were observed using the Cal method (Figure 9). Large variation was observed with the Tracer method, and as a consequence, small daily differences could not be detected whereas small variation in Cal made it possible to pick up difference in production on day 6. For a similar reason, day- to-day variation (CV = 4.1%) in CO_2 production was significant ($P < 0.05$) with the Cal method but not with the Tracer method (CV = 0.1 %; $P > 0.05$). Average CO_2 production by individual animals ranged from 1770 to 2022 L d^{-1} with the Cal method, while the range was 1894 to 2749 L d^{-1} on the Tracer method (Figure 10). Mean CO_2 production from Animal #61 and #67 were significantly lower than the other animals using the Cal technique, however, with the Tracer method there were no differences ($P > 0.05$) observed in CO_2 production among animals.

Animal-to-animal variation in CO_2 production was 11.8% using Tracer and 1.6% using Cal method ($P > 0.05$). In testing equality of the variance between the two methods, (at $F_{8/8\ (0.05)}$ value = 3.44), the residual variance for CO_2 production yielded significant differences (F = 92.3), which supports the lack of equality of estimates by the two methods for CO_2 production. Further investigation with the tracer method for CO_2 production will be needed to verify this observation.

4.5. CONCLUSIONS

It can be concluded that the SF_6 tracer technique accurately estimated rumen CH_4 production. Carbon dioxide production was 20% higher using the SF_6 tracer technique. Further studies to verify this observation with CO_2 production would be necessary. Animal to animal variation in CH_4 and CO_2 production was higher with the Tracer method, and this implies that more animals are needed for measurements using the Tracer method, than for Cal, to determine treatment differences.

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Table 4. The chemical analysis (DM basis) of diet[z] fed to heifers

	Barley concentrate[y]	Alfalfa cubes
CP (%)	15.1	16.5
ADF (%)	8 .0	34.9
NDF (%)	17.4	43.9
ASH (%)	6.6	10.9
GE (kJ g^{-1})	18.1	17.6
DMI (kg d^{-1})	2.3	2.3

[z] Dry matter intake of diet ranged from 4.4 - 4.8 kg d^{-1} among animals

[y] contained (DM basis): barley grain (78.4%); alfalfa-grass hay (10%); canola meal (7.0%); canola oil (2.2%); calcium phosphate (0.2%); calcium carbonate (1.3%); salt fortified (0.35%); Dynamate (0.3%) and Vitamin ADE + Rumensin (0.225%).

Table 5. Methane and CO_2 production by heifers (n=6) as measured using two sampling techniques (Means ± SE)

Trait	Calorimetry	SF_6 Tracer gas	*P*-value
CH_4 (L d^{-1})	130 ± 4.0	137 ± 4.0	0.24
CO_2 (L d^{-1})	1892 ± 74.0*a*	2354 ± 74.0*b*	<0.01
CH_4 (%GEI) [z]	6.3 ± 0.2	6.7 ± 0.2	0.23

[z] GEI = gross energy content of diet x DMI

*a-b*Means within a row with different letters differ ($P < 0.05$)

Figure 7. Daily CH_4 production of heifers (Mean ± SE; n=3) measured by Calorimetry (Cal) and SF_6 tracer technique (Tracer).

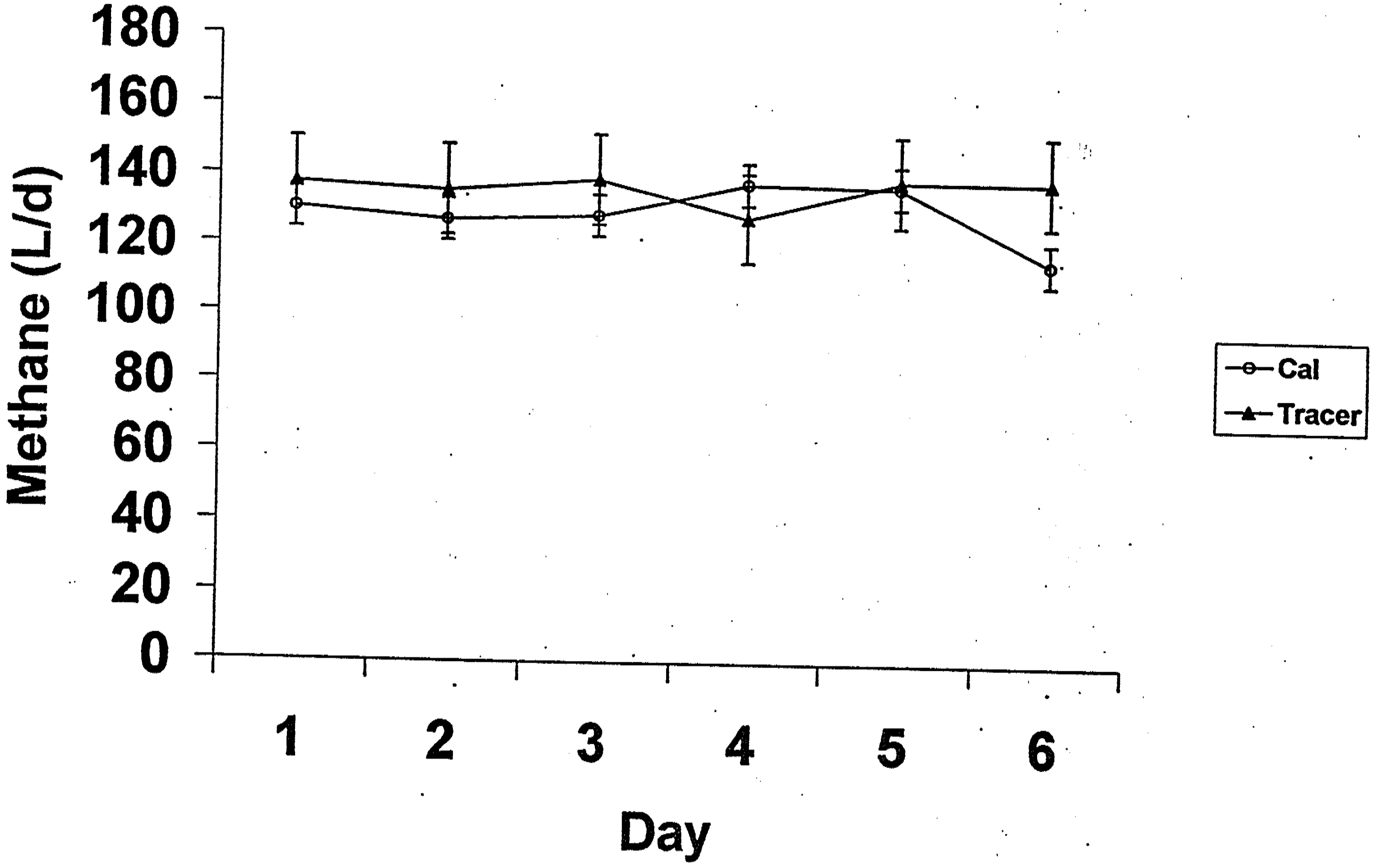

Methane (L/d)
180
160
140
120
100
80
60
40
20
0
1
2
3
4
5
6
Day
Cal
Tracer

Figure 8. Comparison of heifers' CH_4 production measured by Calorimetry (Cal) and SF_6 tracer technique (Tracer). *a-b*Means within a method with different letters differ($P < 0.05$).

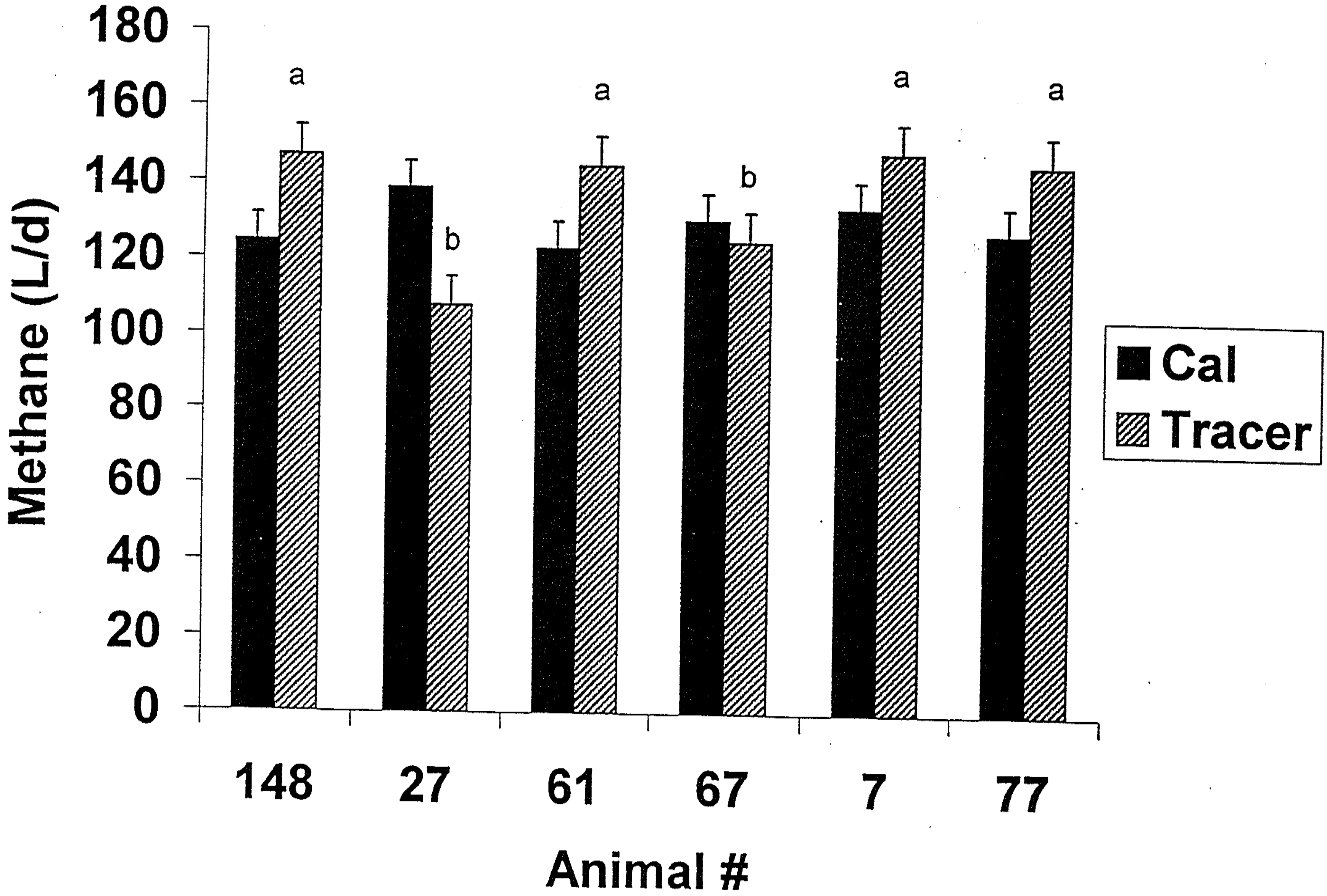

180
160
140
120
100
80
60
40
20
0
Methane (L/d)
a
b
a
b
a
a
148
27
61
67
7
77
Animal #
Cal
Tracer

Figure 9. Daily CO_2 production of heifers (Mean ± SE; n=3) measured by Calorimetry (Cal) and SF_6 tracer technique (Tracer).

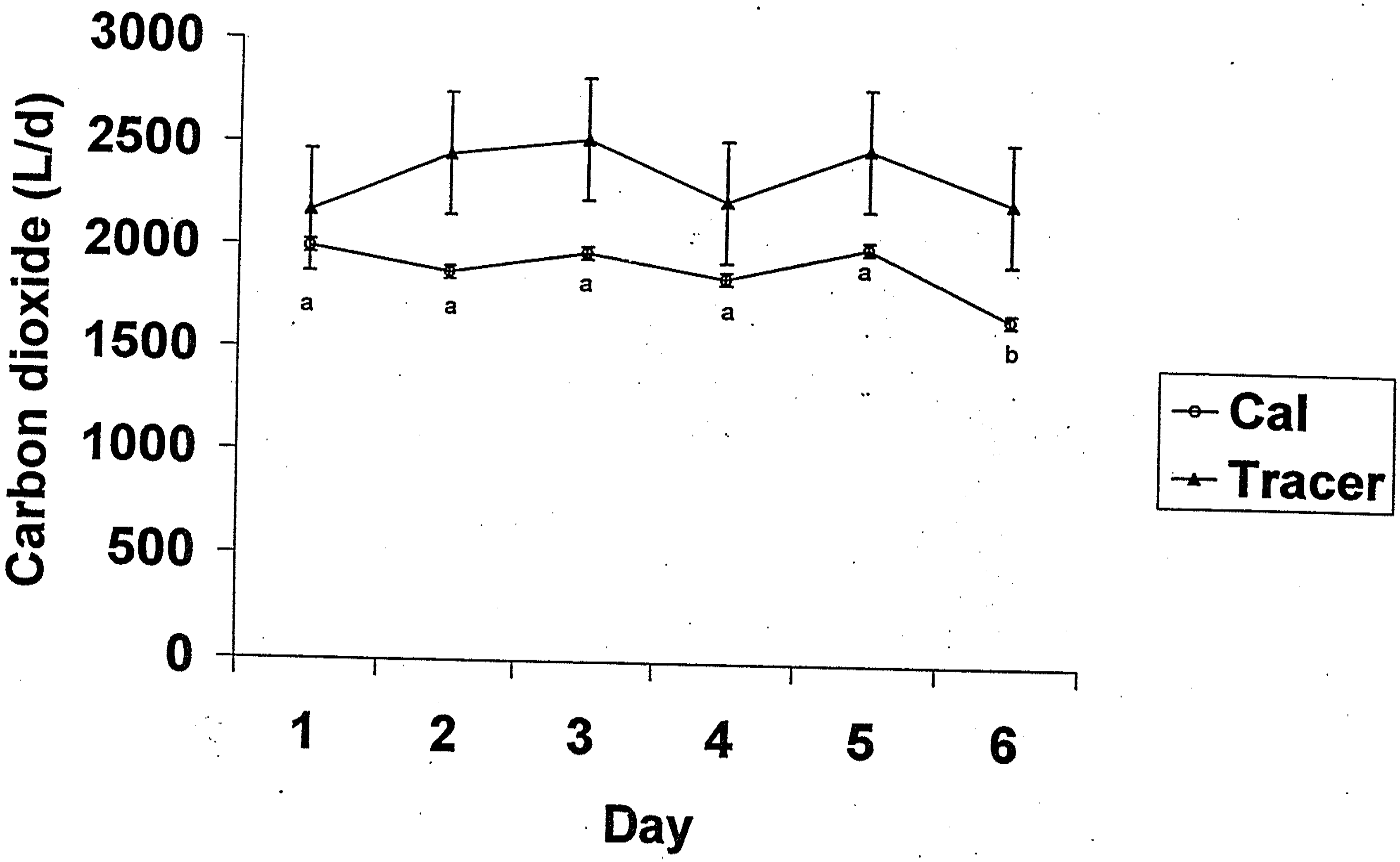
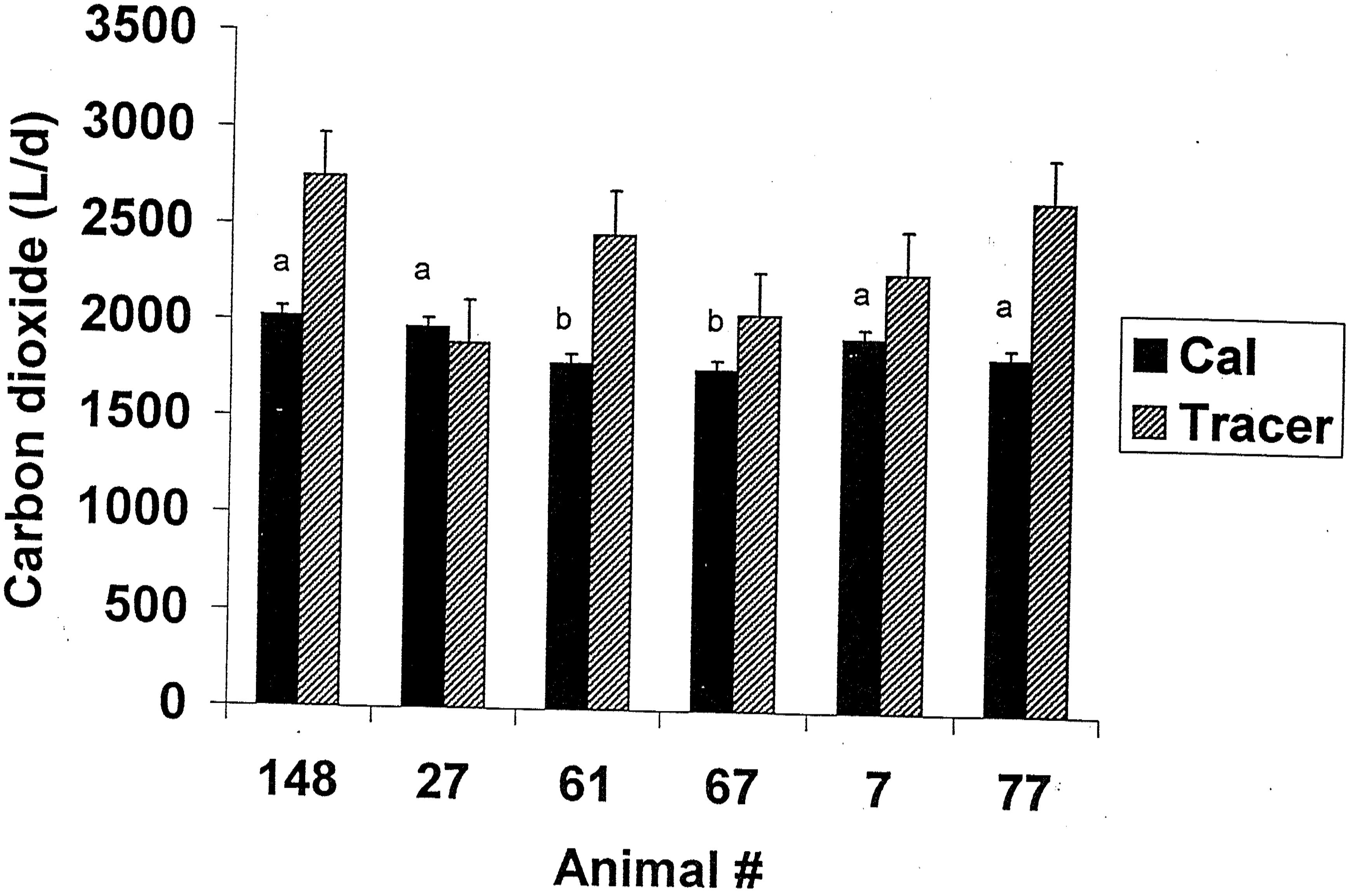

Carbon dioxide (L/d)
3000
2500
2000
1500
1000
500
0
a
a
a
a
a
b
1
2
3
4
5
6
Day
Cal
Tracer

Figure 10. Comparison of heifers' CO_2 production measured by Calorimetry (Cal) and SF_6 tracer technique (Tracer). *a*-*b*Means within a method with different letters differ($P < 0.05$).


3500
3000
2500
2000
1500
1000
500
0
Carbon dioxide (L/d)
a
a
b
b
a
a
148
27
61
67
7
77
Animal #
■ Cal
▨ Tracer

5.0. MANUSCRIPT II

METHANE PRODUCTION FROM DAIRY AND BEEF HEIFERS FED FORAGES DIFFERING IN NUTRIENT DENSITY USING THE SULPHUR HEXAFLUORIDE (SF_6) TRACER GAS TECHNIQUE

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5.1. ABSTRACT

The effect of cattle breed type (dairy [Holstein] versus beef [Charolais x Simmental]) and forage quality (High, Medium and Low) on methane production was measured under ad-libitum and restricted feeding conditions. The *invitro* organic matter digestibility (IVOMD) of the forage diets was High = 61.5%; Medium = 50.7% and Low = 38.5%. Each hay diet was fed to the animals in each of three periods in a 3 x 3 Latin squares replicated 4 times. Each period consisted of 23 days during which heifers were individually fed their assigned forage for 14 days on ad-libitum feeding. Following this, intake was restricted to 2% BW for 9 d. Methane production was measured for 5-24 h periods at both levels of intake using the SF_6 gas technique. Methane production was not different ($P > 0.05$) between Holsteins (238.0 ± 6.9 L d^{-1}) and Charolais crosses (228.6 ± 7.8 L d^{-1}) under either level of feeding. Methane production (L d^{-1}) was similar, from High and Medium quality forage ($P < 0.05$), but was lower ($P > 0.05$) on Low quality forages during ad-libitum feeding. This effect of forage quality on CH_4 production was absent during restricted feeding ($P > 0.05$). Day-to-day variation in CH_4 production was 26.9% and 27.1% on ad-libitum and restricted feeding respectively ($P < 0.05$), while animal-to-animal variation ($P < 0.05$) was 26.6% (ad-libitum) and 25.3% (restricted). On ad-libitum feeding, DMI was strongly correlated ($P = 0.0001$; $r = 0.8$) with CH_4 production (L d^{-1}), and accounted for 64% of daily CH_4 production variation. Methane (L kg^{-1} DOMI) was highest ($P < 0.05$) on low quality diets under both feeding regimes, and was not influenced ($P > 0.05$) by cattle type. Methane production as a % of GEI was not influenced by diet. It can be concluded, that the SF_6 tracer technique provides an accurate and precise measure of enteric CH_4 production directly from animals under production conditions. There were no differences in CH_4 production between the Holsteins and Charolais crossbreds, but the quality of forages affected CH_4 production under both ad-libitum and restricted feeding.

Key words: Methane, SF_6 tracer technique, cattle breed type, forage quality, level of intake.

5.2. INTRODUCTION

Methane production resulting from fermentation of feed in the gastrointestinal tract of ruminants represents a loss of dietary energy, which is typically about 2-12 % of gross energy intake (GEI) (Johnson and Johnson 1995). Methane gas production primarily depends on the quantity and quality of the feed that affects rate of digestion and rate of passage in the fermentation process (Van Soest 1982). Several other factors such as body size, gastrointestinal capacity, animal species, breed and environmental conditions can also influence the amount of CH_4 produced (McAllister et al. 1996), however, the exact interrelationships between these factors are unknown. There is limited information regarding the effects of breeds on CH_4 production. Lal et al. (1987) observed that energy losses as CH_4 were higher in Holstein- Friesian x Harian cross cattle than in Holstein-Friesian cattle in India.

Ruminant CH_4 production has received considerable attention in recent years due to its contributory role to atmospheric CH_4 production. Emissions from ruminants are estimated to contribute 16-20% of global atmospheric CH_4, of which 75% is produced by cattle (Crutzen et al. 1986). Ruminant CH_4 production has in the past been measured using respiration calorimetry from which prediction equations relating CH_4 production to dietary components have been derived (Blaxter and Clapperton 1965; Moe and Tyrrell 1979; Holter and Young 1992). Also in-vitro techniques (Czerkawski and Breckenridge 1977; Dong et al. 1997a) and isotopic methods (Murray et al. 1976; Frances et al. 1993) have been used. The use of respiration chamber, in-vitro techniques, isotopic method and prediction equations can not be related to cattle under production situations. This is because under some production situations, environmental temperature, meal size and frequency, and selection of feed components can affect intake, rate of digestion and retention time in the rumen. To ensure that appropriate reduction strategies are established for the industry, there is the need to refine CH_4 estimates under normal production conditions.

Recent advances in measurement methods such as the SF_6 tracer gas technique which uses an inert tracer gas source placed in the rumen of the animal allows direct measurement of CH_4 in unrestrained individual animals from samples of gases collected at the mouth and nose (Johnson et al. 1994). This technique does not measure all hind gut CH_4, which accounts for approximately 13% of total methane produced (Murray et al. 1976). However, 89% of hind gut methane is reabsorbed into the blood stream and expired through the lungs which can be collected. Thus, the SF_6 tracer gas technique may account for about 98.6% of total CH_4 production. The SF_6 tracer gas technique has been used on several animals simultaneously in their pens or while they graze (Lassey et. al 1997; McCaughey et al. 1999), however, animals have to be trained to wear a halter and collection canisters.

The objective of this study was to use the SF_6 tracer gas technique to measure and assess variations of CH_4 production in growing dairy and beef breeds being fed different forage at two levels of intake.

5.3. MATERIALS AND METHODS

5.3.1. Animals and management

Six Holstein (dairy; 310 ± 15.3 kg (mean ± SD)) and six Charolais x Simmental (beef; 310 ± 10.0 kg) yearling heifers (12 mo) were used in the study to assess the effects of cattle breed type (dairy vs beef), and forage quality at two levels of feeding on CH_4 production. It was expected that dairy heifers would consume more feed relative to body weight than beef heifers (NRC 1996), and this would influence CH_4 production. The experiment was conducted from February 3, 1998 to April 24, 1998 at the University of Manitoba Glenlea Research Station, 20 km south of Winnipeg, Manitoba. Animals were adapted to a grass hay diet for a 2-week period prior to the start of the trial. During this period, heifers were trained to wear halters with chain suspensions, to adapt them to wearing the CH_4 measuring apparatus later during the trial. Animals were held individually in open fronted pens with attached feeding troughs. Wood shavings were provided as bedding. The heifers were managed according to the guidelines of the Canadian Council of Animal Care.

5.3.2. Feeding

Three hay diets were chosen based on their invitro organic matter digestibility (IVOMD) and designated High (61.5%; legume/grass mixed hay); Medium (50.7%; grass hay) and Low (38.5%; grass hay) quality forage (Table 6). It was anticipated that diet would effect CH_4 energy yield during fermentation, because the rate and extent of digestion in the rumen would differ due to differences in digestibility and fibre content. Each hay diet was chopped and fed to the animals in each of three periods in a 3 x 3 Latin squares replicated four times.

Each period consisted of 23 days, animals received their respective diets ad-libitum (15% orts) for 14 days. During ad-libitum feeding, animals were adapted to their forages for 9 d, before 5- 24 h CH_4 collections began. Following ad-libitum feeding, DMI of heifers was restricted to 2% BW on the same diet (to ensure all feed was completely consumed) for 9 d, which included a 4-d adjustment period, followed by 5-24 h CH_4 collections. A 7-day adaptation was allowed between periods as animals switched diets.

Heifers were fed chopped hay once a day in the morning, and received 50 gm Hi C-N-Z (1:1) mineral/vitamins with selenium (Feed-Rite Ltd. Winnipeg, Manitoba) daily as a top dress. Trace-mineralized salt blocks and water were offered ad-libitum. Body weights were measured at the end of the ad libitum and restricted feeding phases of each period to adjust the amount of hay fed accordingly.

Hay lots were core-sampled prior to feeding in each period for chemical analyses (Table 6). Chopped hay offered and orts were weighed and sampled daily. Daily feed and ort samples were composited for each feeding level in each period, and sub-sampled for DM analysis.

5.3.3. Methane gas sampling and analyses

Methane gas was sampled using the SF_6 tracer gas technique (Johnson et al. 1994; See Manuscript I). Sulphur hexafluoride release rates ranged from 350 to 700 ng min^{-1} (See Appendix B). In each period, 24-h gas samples were collected from each animal for 5 d, at ad-libitum and restricted feeding levels. Heifers were restrained in a chute to remove and replace collection canisters. Gas collection apparati were hung on the east and west side of the pens to collect background air samples, which were used to correct expired gas concentrations.

Collected spheres were checked for pressure to identify blocked or leaking capillary systems to ensure data used represented a complete 24-h period. Spheres were then pressurized to 110 KPa with pure N_2, to prevent sample contamination prior to analysis, and to allow collection of samples for injection of gas samples into the sample loop of a gas chromatograph. A gas chromatograph (Star 3600, Varian, Mississauga, ON) fitted with electron capture and flame ionization detectors was used for determining SF_6 and CH_4, respectively. The gas chromatograph was fitted with a Molecular Sieve 0.5 nm (1800 mm) column and a Poropak QS (1800 mm) column for SF_6 and CH_4, respectively. The column oven temperature was 35°C and nitrogen was used as the carrier gas with a flow rate of 30 mL min^{-1}.

Samples were analyzed in duplicate. Prepared standards were used to standardize the gas chromatograph for SF_6 (20 ppt, Scott-Marrin Inc., Riverside, CA) and CH_4 (100 ppm; Supelco, Mississauga, ON) prior to sample analysis. Daily CH_4 production was calculated as follows (Johnson et al. 1994):

$$CH_4\ (\text{L min}^{-1}) = \text{Permeation tubes } SF_6 \text{ release rate } (\text{L min}^{-1}) \times [CH_4] / [SF_6]$$

where $[CH_4]$ and $[SF_6]$ are the concentrations of CH_4 and SF_6 in canisters after background concentrations have been deducted. The concentration of SF_6 was not detectable for two Charolais cross and one Holstein heifer in periods 2 and 3, and as such, CH_4 production could not be calculated for these animals.

5.3.4. Feed analyses

Feed and ort samples were dried for 48 h in a forced draught oven at 60°C for dry matter (DM) determination. Samples were ground using a Wiley mill fitted with a 1 mm screen. Dried samples were analyzed for crude protein (CP) using a Kjeltec 1030 auto analyzer (Tecator Inc., Herndon,VI; [Association of Official Analytical Chemists (AOAC) 1990], method no. 984.13), and ash using method no. 942.05 (AOAC 1990).

Acid detergent fibre (ADF) and neutral detergent fibre (NDF) were determined using an ANKOM 200 fibre analyzer (Fairport NY), with procedures described by Komarek (1993). Gross energy (GE) was determined using a Parr 1241 adiabatic bomb calorimeter. In vitro organic matter digestibility (IVOMD) was determined by the method of Tilley and Terry (1963) using bovine inoculum.

5.3.5. Statistical analyses

Methane production and DM intake in each feeding regime were analyzed by least square analyses of variance using GLM in SAS Institute Inc, (1990) using the model:

$$Y_{ijkl} = \mu + B_i + A_{j(i)} + D_k + P_l + (ADP)_{j(i)kl} + \epsilon_{ijkl}$$

where Y_{ijklm} = trait under consideration; μ = overall mean; B_i = breed with (i=1, 2); $A_{j(i)}$ = animals within breed, which was used as the error term to test for breed effect; D_k = hay diets with (k=1..3); P_l = period with (l=1..3); $ADP_{j(i)kl}$ = diet x period x animals within breed; which was used as an error term for the Diet and Period effect; ϵ_{ijklm} = experimental error term. Means were separated at the 5% level of significance using the probability of differences (PDIFF) option. To compute day to day and animal to animal variations, Type III expected mean squares were generated for the model in each feeding level, and the coefficient of variation (CV) calculated from root mean squares and means.

5.4. RESULTS AND DISCUSSION

Methane production ranged from 115.9 to 399.5 L d^{-1} (mean (SD) 229.0 ± 68.2 L d^{-1}) in the course of the study. Methane energy lost, % GEI, (See pp 158 for calculations) ranged from 4.6 to 9.4% (mean 6.9 ±1.2%) on ad-libitum feeding, while the range was 5.4 to 11.0% (mean 7.3 ±1.6%) on restricted feeding. The CH_4 energy yields in the study are comparable to values reported by McCaughey et al. (1997) for grazing steers (4.1- 5.2 % GEI) and forage-fed heifers (7.2 % GEI) (Johnson et al. 1994) using the SF_6 tracer gas method, and also for forage-fed heifers (7.1 % GEI) using respiration calorimetry (Johnson et al. 1994).

Contrary to expectation, DMI intake and corresponding CH_4 production (L d^{-1}, L CH_4 kg^{-1} DOMI or CH_4, % GEI) were not different ($P > 0.05$) between dairy and beef heifers for the two feeding levels (Table 7 and 8). There were no breed by diet interactions for intake and CH_4 production. This can be attributed in part to similar initial BW, and similar rate of gain ($P > 0.05$) during ad-libitum feeding ($P > 0.05$) for Holsteins (0.94 ± 0.13 kg d^{-1}) and Charolais cross (1.1 ± 0.13 kg d^{-1}) animals. On the other hand, Lal et al. (1987) observed that energy losses as CH_4 were higher in Holstein-Friesian x Harian cross cattle than in Holstein-Friesian cattle in India. This was attributed to the fact that the indigenous dairy cross had a larger gut capacity than the Holstein-Friesian; a result of adaptation to wheat straw-based diets. This resulted in a longer rumen retention time and increased CH_4 production. Galbraith et al. (1998) reported CH_4 losses of 6.6, 5.2 and 3.3% GEI for bison (*Bison bison*), wapiti (*Cervus elaphus*) and white-tail deer (*Odocoilleus virgianus*) respectively, when fed lucerne pellets, with the lowered trend of CH_4 production corresponding with a reduction in retention time with the smaller animals.

With respect to forage quality, daily DMI (kg d^{-1}); DOMI (kg d^{-1}) and GEI (kJ kg^{-1} d^{-1}) at ad-libitum declined ($P < 0.05$) as the quality of forage offered declined (Table 7). Methane production (L d^{-1}) for High and Medium diets were 27.8% and 29.8% higher, respectively, ($P < 0.05$) than the Low diet during ad-libitum feeding. A faster passage rate in the rumen is associated with more digestible forages. This increases intake, resulting in more fermentable substrate in the rumen and therefore higher CH_4 production (Johnson and Johnson 1995; McAllister et al. 1996). In contrast, CH_4 production per kg DOMI (L kg^{-1} DOMI) increased ($P < 0.05$) as the quality of forages decreased (Table 7).

During restricted feeding, DMI was lower by 5%, on the Medium and Low diets compared to the High diet ($P < 0.05$), while DOMI declined significantly as the quality of forages decreased (Table 8). Although similar amount of diet were fed on restricted feeding, animals still selected more of the high quality diet, not all the feed was consumed especially on the Low quality forages. There were no differences in CH_4 production (L d^{-1}) by diets during restricted feeding ($P > 0.05$), however CH_4 kg^{-1} DOMI was higher by 25% for Low versus Medium or High quality diets ($P < 0.05$). Lower fractional CH_4 losses with the High diet can be expected, as the lower proportion of structural carbohydrate content will change the fermentation pattern yielding less CH_4 (Moe and Tyrrell 1979). Similar findings were reported by Varga et al. (1985), who observed lower CH_4, % GEI in cattle consuming alfalfa silage (5.8%) as compared to orchard grass silage (6.3%). They suggested that the higher digestible organic matter content of legumes coupled with a faster rate of passage shifted fermentation against ruminal methanogenesis.

Day- to-day variation and animal to animal variation in intake and CH_4 production are shown in Table 9. There was a higher daily variation in DMI when animals were ad-libitum fed (22.5%) than when restricted fed (9.4%), because similar amounts of diets relative to body weight were offered on restricted feeding. A similar trend was also observed in animal to animal variations. Significant day to day variation and animal to animal variation for CH_4 production (L d^{-1}) were observed on ad-libitum and restricted feeding ($P < 0.05$). Variation in CH_4 production within individual animals ranged from 6.6% to 35.0% when fed ad-libitum, while it ranged from 14.4 % to 35.0 % on restricted intake. A high coefficient of variation was observed for day-to-day (26.9%) and animal-to-animal (26.6%) ad-libitum CH_4 emissions. Blaxter and Clapperton (1965) under controlled conditions and using 477 sheep experiments observed a low day-to-day variation (CV = 7.5%) and animal-to-animal variation (CV = 8.1%) in CH_4 production of sheep in calorimetry chambers fed similar diets and amounts. The more variable environmental and feeding conditions and the small number of animals used contributed to higher variation in our study.

Day to day variation can be related to such factors as DMI, and this was verified for cattle fed on ad-libitum basis, where CH_4 production was linearly correlated with DMI ($P = 0.0001$; $r = 0.80$). The strong correlation between CH_4 production rates and DMI suggests that DMI was a major determinant of variations in CH_4 emissions, accounting for 64% of the variation. As a result, a significant ($P = 0.0001$) linear regression of DMI on CH_4 production yielded a prediction equation of :

$$CH_4\,(\mathrm{L\ d^{-1}}) = 38.92 + 26.44\ \mathrm{DMI\ (kg)}\ (r^2 = 0.60).$$

On restricted feeding, where similar amounts of the diets were offered, there was a significant ($P = 0.034$) correlation between CH_4 (L d^{-1}) and DMI; r = 0.40), however, DMI explained only 16 % of daily CH_4 production. Lassey et al. (1997) using the SF_6 technique in measuring CH_4 emissions directly from 50 grazing sheep, observed a weak correlation between CH_4 emission rates and DMI; r = 0.37), suggesting that DMI was a relatively minor determinant of variation in CH_4 emissions, accounting for only 14% of the variance. They concluded that the intrinsic differences in sheep affected methanogenic response more than intake.

Animal-to-animal variation may be related to DMI, but could also include eating behavior of particular animals, variations in diet consumed relative to diet offered (selectivity of animals) and animal-to-animal variations in rumen capacity and digesta rate of passage (Van Soest 1982). The fact that variations in DMI on ad-libitum and restricted feeding levels were very different, but yielded similar animal-to-animal variation under both feeding regimes, suggests that rather than DMI, the digestive tract characteristics of the heifers and/or factors related to the SF_6 technique may be the major determinants of CH_4 variations during restricted feeding.

The direct measurement of CH_4 production from individual animals by the SF_6 technique allowed us to identify the least (8.2 ± 1.4%; CV = 17.5%) and most efficient (5.7 ± 1.3% CV = 22.9%) animals based on $CH_{4,}$ % GEI, in the course of the trial. The SF_6 technique, therefore, provides a useful tool in identifying and selecting animals under pastured or confined conditions based on their CH_4 production. However, more animals and sampling times may be needed to reduce variation observed in measurements. The technique can not quantify hind gut CH_4 production that was lost via the rectum, therefore, adjustments are needed on values to estimate total CH_4 production.

5.5. CONCLUSIONS

The SF_6 tracer technique provided a measure of CH_4 production directly from growing cattle fed various forage diets in a feedlot environment. In the study, breed had no effect on enteric CH_4 production, however, low quality forages increased fractional CH_4 losses during both ad-libitum and restricted feeding. Methane production could be predicted from DMI at ad-libitum feeding with DMI accounting for 64% of daily CH_4 production variations.

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Table 6. Chemical composition (DM basis) of forage treatments fed to heifers

Treatment	High	Medium	Low
Forage species	Legume/grass	Grass	Grass
Organic matter (%)	91.4	90.8	90.8
CP (%)	17.9	12.1	11.1
ADF (%)	31.8	38.7	43.2
NDF (%)	41.8	58.1	68.8
IVOMD (%)	61.5	50.7	38.5
GE (kJ g^{-1})	18.4	18.1	18.1

Table 7. The effects of breed and forage quality on intake and CH_4 production of heifers fed ad-libitum (LSmeans ± SE)

	BREED			DIET[z]			
Parameter	Charolais X (n=4)	Holstein (n=5)	*P*-value	High (n=9)	Medium (n=9)	Low (n=9)	*P*-value
Intake							
DMI (kg d^{-1})	8.4 ± 0.38	8.2 ± 0.34	0.68	9.7 ± 0.23*a*	8.9 ± 0.23*b*	6.3 ± 0.23*c*	<0.01
DOMI (kg d^{-1})	4.4 ± 0.12	4.1 ± 0.11	0.61	6.0 ± 0.14*a*	4.5 ± 0.14*b*	2.4 ± 0.14*c*	<0.01
GEI (kJ d^{-1})	152.6 ± 1.11	154.2 ± 1.01	0.81	185.6 ± 0.56*a*	159.3 ± 0.56*b*	116.2 ± 0.56*c*	<0.01
CH_4 production							
CH_4 (L d^{-1})	258.7 ± 11.89	258.0 ± 10.62	0.97	281.7 ± 13.35*a*	289.8 ± 13.35*a*	203.5 ± 13.35*b*	<0.01
CH_4 (L kg^{-1} DMI)	30.9 ± 1.76	31.7 ± 1.58	0.73	29.4 ± 1.83	32.5 ± 1.83	32.0 ± 1.83	0.46
CH_4 (L kg^{-1} DOMI)	64.5 ± 3.41	65.3 ± 3.05	0.87	47.8 ± 4.02*a*	63.7 ± 4.02*b*	83.2 ± 4.02*c*	<0.01
CH_4 (% GEI)	6.7 ± 0.33	6.7 ± 0.29	0.91	6.0 ± 0.38	7.1 ± 0.38	6.9 ± 0.38	0.14

[z] See Table 1.

*a-c*Means within factors in a row followed by different letters differ ($P < 0.05$).

Table 8. The effects of breed and forage quality on intake and CH_4 production of heifers during restricted feeding (LSmeans ± SE)

	BREED			DIET^z			
	Charolais X (n=4)	Holstein (n=5)	*P*-value	High (n=9)	Medium (n=9)	Low (n=9)	*P*-value
Intake							
DMI (kg d^{-1})	6.3 ± 0.20	6.1 ± 0.18	0.58	6.4 ± 0.07*a*	6.1 ± 0.07*b*	6.1 ± 0.07*b*	0.02
DOMI (kg d^{-1})	3.2 ± 0.02	3.1 ± 0.02	0.58	3.9 ± 0.03*a*	3.1 ± 0.03*b*	2.4 ± 0.03*c*	<0.01
GEI (kJ d^{-1})	112.4 ± 0.84	109.9 ± 0.75	0.61	117.5 ± 0.32*a*	107.8 ± 0.32*b*	107.8 ± 0.32*b*	<0.01
CH_4 production							
CH_4 (L d^{-1})	195.8 ± 13.10	213.2 ± 11.72	0.36	224.6 ± 16.19	193.3 ± 16.19	195.6 ± 16.19	0.35
CH_4 (L kg^{-1} DMI)	31.2 ± 1.89	34.6 ± 1.70	0.22	35.1 ± 2.43	31.6 ± 2.43	32.0 ± 2.43	0.56
CH_4 (L kg^{-1} DOMI)	63.3 ± 4.23	71.3 ± 3.79	0.17	56.6 ± 4.99*a*	62.2 ± 4.99*a*	83.1 ± 4.99*b*	0.01
CH_4 (% GEI)	6.9 ± 0.44	7.6 ± 0.39	0.24	7.6 ± 0.53	7.1 ± 0.53	7.1 ± 0.53	0.78

^{z}See Table 1.

a-b Means within factors in a row followed by different letters differ ($P < 0.05$).

Table 9. Variations in DMI and CH_4 production of heifers

Parameter	DMI (kg d^{-1})	CH_4 (L d^{-1})	CH_4 (% GEI)
	Day-to-day variation		
Ad-libitum intake			
mean ± SD	8.3 ± 1.9	257.9 ± 69.5	6.9 ± 1.2
cv (%)	22.5	26.9	17.8
Restricted intake			
mean ± SD	6.2 ± 0.6	202.0 ± 54.8	7.3 ± 1.6
cv (%)	9.4	27.1	21.5
	Animal-to-animal variation		
Ad-libitum intake			
mean ± SD	8.3 ± 2.1	257.9 ± 68.6	6.9 ± 1.2
cv (%)	25.1	26.6	17.7
Restricted intake			
mean ± SD	6.2 ± 0.6	202.0 ± 51.1	7.3 ± 1.6
cv (%)	8.8	25.3	21.5

6.0. MANUSCRIPT III

EFFECTS OF GRAIN SUPPLEMENTATION ON METHANE PRODUCTION OF GRAZING STEERS USING THE SULPHUR (SF_6) TRACER GAS TECHNIQUE

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6.1. ABSTRACT

The objective of the study was to examine the effect of supplemental grain on methane (CH_4) production of grazing steers. Eight beef steers (344.6 ± 6.4 kg) were assigned to legume-grass pasture (C; n= 4) or legume-grass pasture plus a rolled barley supplement (S; n = 4). In a completely randomized design with repeated measures, CH_4 output was measured for two 24-h periods, using the SF_6 tracer gas technique as steers entered (IN) and exited (OUT) paddocks. Two, 4 and 4 kg of rolled-barley grain was fed daily to S steers during the EARLY, MID and LATE periods of the grazing season, respectively. Supplementation reduced forage DMI by 11% ($P = 0.03$) and increased TOMI by 14% ($P = 0.001$). Daily CH_4 production was similar for C and S steers ($P > 0.05$). Methane production, increased ($P < 0.05$) from 256 L d^{-1} in the EARLY period to 364 L d^{-1} at the MID and 342 L d^{-1} at the LATE periods. Energy lost as CH_4, %TGEI, ranged from 4.7 to 8.4% (mean 6.5 ± 0.3%) during the grazing season, and there was no difference between S (6.4 ± 0.6%) and C (6.7 ± 0.6%) steers ($P = 0.71$). Methane production declined with grazing on high quality forages; steers on EARLY pastures had 44% and 29% lower ($P < 0.05$) energy loss as CH_4 than animals on MID and LATE pastures, respectively. There was also a 54% lower CH_4 loss when animals entered new paddocks relative to those exiting the paddocks ($P < 0.05$). It can be concluded that the effects of supplementation on CH_4 production were marginal in grazing steers. The study suggests that pasture quality plays a major role on the extent to which CH_4 production can be decreased with grain supplementation in grazing animals.

Key words: Methane, grazing steers, grain supplementation, pasture quality.

6.2. INTRODUCTION

Cultivated and native pastures are primary resources for beef production in Western Canada. As forages mature, there is decreased digestibility, related to decreased nitrogen and increased fibre and lignin content of the forage (Minson 1990). Reduced forage digestibility is accompanied by decreased forage intake, and an increased acetate:propionate ratio, which favors increased methane (CH_4) production per unit of forage consumed (McAllister et al. 1996).

The efficiency of gastrointestinal fermentation in ruminants, dictates the ratio between the end products formed, i.e., efficiency is high when there is optimum microbial growth relative to VFA produced (Van Soest 1982). Under such situations there is a high rate of digestion by microbes and lower CH_4 production (Leng 1993). Strategic supplementation can improve microbial efficiency by supplying the needed microbial growth factors (Leng 1993). Improvements in the efficiency of rumen fermentation resulting from urea and mineral supplementation have been shown to reduce the percentage of digested energy in low quality feeds fermented to CH_4 and also to reduce CH_4 produced per kg of gain or milk (Hennessy and Williamson 1990; Ward et al. 1993).

Most of the above studies have been conducted with penned animals fed poor quality forages. Limited studies have been conducted with grazing animals. This can be attributed to difficulties in obtaining CH_4 measurements while animals are grazing. The recent development of the sulphur hexafluoride (SF_6) tracer gas technique, allows direct measurement of individual grazing animals, without disturbance to their grazing behavior

(Johnson et al. 1994). McCaughey et al. (1997, 1999) have recently used the SF_6 tracer gas technique to measure the impact of grazing management on methane production by steers and lactating beef cows.

In temperate regions, where legumes are often incorporated in pastures, the lack of energy (rather than protein) is the most limiting nutrient for production of meat or milk by grazing animals (Allden 1981). The lack of sufficient energy can be attributed to declines in digestibility and intake with increased maturity (Minson 1990). The objective of this study was to use the SF_6 tracer gas technique to measure the effects of grain supplementation on CH_4 production of grazing steers.

6.3. MATERIALS AND METHODS

6.3.1. Animals

Eight Red Angus yearling steers, averaging 344.6 ± 6.4 kg (mean ±SD) were assigned to one of two dietary treatments to determine the effects of barley grain supplementation on CH_4 production during grazing. The selected steers were managed as part of a larger herd of 48 steers that were rotationally grazing on eight, 3.7-ha paddock pastures at the Brandon Research Station, Brandon, Manitoba, during the 1998 grazing season. Pastures consisted of alfalfa (*Medicago sativa* L.), and meadow bromegrass (*Bromus bieberstenii* Roem and Schult.). The proportion of legume:grass was determined at the beginning and end of CH_4 collection periods.

Animals started grazing pastures on May 5, 1998. They were adapted to grazing and supplemental feeding for six weeks prior to CH_4 collection. During the adaptation period, the test steers were halter trained, in order to get them accustomed to wearing the CH_4 collection apparatus. Steers had free access to water and a commercially prepared mineral supplement. Animals were cared for in accordance to the guidelines of the Canadian Council of Animal Care.

6.3.2. Experimental layout

The dietary treatments consisted of four steers (342.8 ± 6.4 kg (mean ±SD)) which grazed alfalfa and meadow bromegrass pastures (C), and four steers (346.5 ± 6.4 kg (mean ±SD)) which grazed the alfalfa-meadow bromegrass pastures, and were supplemented with steamed-rolled barley grain (S).

Three CH_4 sampling periods, EARLY (June 17-25, 1998); MID (July 20-29, 1998) and LATE (August 12-21, 1998) were selected in the grazing season to reflect changes in forage quality as the grazing season progressed. The beginning of each sampling period corresponded with animals grazing fresh paddocks. Methane production was measured from steers over two 24-h sampling times per sampling period: as they entered (IN) and exited (OUT) paddocks to reflect the changing pasture quality within paddocks during a sampling period. Animals were in paddocks for 10-12 days. The experiment was conducted as a completely randomized design with repeated measures.

6.3.3. Grain supplementation

Steers assigned as S received 2, 4 and 4 kg d^{-1} of steamed- rolled barley grain during the EARLY, MID and LATE grazing season, respectively. Animals were individually fed once a day (in the afternoon), using individual feeders located close to the pastures. Grain and orts were weighed and subsampled daily in each sampling period. Feed and ort samples were composited by period for future analyses.

6.3.4. Forage availability and botanical composition

Forage availability was determined at the beginning and end of each sampling period. Forage was clipped with electric grass shears, at a height of 50 mm within $0.25m^2$ quadrats. Ten randomly placed quadrats were sampled on each occasion. Dead material was separated from the forage, to ensure accurate estimates of current availability. Forage samples were separated by hand into legume and grass portions and dried at 50°C for 48 h. The mean dry weights of grass and legumes were used in calculating herbage mass (kg ha^{-1}), and ratios of legume:grass.

The available forage on offer during the EARLY sampling period was 2,932 kg DM ha^{-1} at IN and 1,308 kg DM ha^{-1} at OUT. The MID period herbage mass was 3,388 kg DM ha^{-1} at IN and 1,945 kg DM ha^{-1} at OUT. The LATE period herbage mass was 3,232 kg DM ha^{-1} at IN and 1,830 kg DM ha^{-1} at OUT. The alfalfa: meadow bromegrass ratio, DM basis, of pasture forage during the EARLY, MID and LATE sampling periods were 20.0:80.0 ; 33.1:66.9, and 37.6:62.4, respectively. The MID and LATE paddocks were secondary growths.

6.3.5. Forage quality

In each of the three sampling periods, three esophageal-fistulated steers were used to collect grazed forage samples for forage quality analyses. Fistulated steers were fasted overnight and allowed to graze in the sampling paddock for 20 min at both IN and OUT. Extrusa samples were obtained in canvas collection bags fitted around the cannulated areas.

Extrusa samples were squeezed through two layers of cheesecloth and the saliva fraction sampled and frozen immediately after separation. The saliva fraction was used to correct for organic matter losses of solid samples (Cohen 1979). The solid fraction of samples were immediately dried in a forced draught oven at 50°C for 48 h. Dried samples were ground using a Wiley mill fitted with a 1 mm screen, and later analysed for DM, OM, CP, ADF, NDF, GE, IVOMD and IVDMD.

6.3.6. Total and Forage intake

Total dry matter intake was determined by the following calculation (Burns et al. 1989):

$$\text{DMI (g d}^{-1}) = \text{Fecal DM output (g d}^{-1}) / 1\text{-(IVDMD/100)}$$

Fecal output was estimated using Cr_2O_3 controlled release capsules (Captec Ltd, Auckland, NZ), as described by Barlow et al. (1988). Each steer was dosed a week prior to the sampling period to establish a constant release of Cr_2O_3 in the gut. Fecal grab samples were collected daily from each steer for seven days representing, 48 h after paddock entry to 24 h after paddock exit. This allowed for lag time associated with passage rate and to ensure that intakes correspond accurately with CH_4 estimates taken at IN and OUT.

Fecal samples were packed with ice after collection, and frozen immediately. Fecal DM output was calculated as the ratio of Cr_2O_3 released (1.72 g d^{-1}):Cr_2O_3 concentration in feces (g g^{-1} DM). Forage intake of S steers was estimated by subtracting DMI intake of barley grain from total DMI.

6.3.7. Methane gas sampling and analyses

Methane gas was sampled using the SF_6 tracer gas technique (Johnson et al. 1994; See Manuscript I). Release rates of the permeation tubes used in this study ranged from 350 to 700 ng min^{-1} (See Appendix B). In each sampling time, 2 consecutive 24 h gas samples were collected from each animal. Steers were restrained in a chute to remove collection spheres prior to feeding of barley grain to prevent blockages of capillary lines with feed particles. New canisters were replaced after feeding and animals were then returned to pasture. Background air samples were collected each time animals were sampled by hanging the apparati on the north and south side fences around paddocks.

Collected spheres were pressure checked to identify blocked or leaking capillary systems to ensure data used represented a complete 24-h period. Spheres were then pressurized to 110 KPa with pure N_2, to prevent sample contamination prior to analysis, and to allow injection of gas samples into the sample loop of the gas chromatograph.

A gas chromatograph (Star 3600, Varian, Mississauga, ON) fitted with electron capture and flame ionization detectors was used for determining SF_6 and CH_4, respectively. The gas chromatograph was fitted with a Molecular Sieve 0.5 nm (1800 mm) column and a Poropak QS (1800 mm) column for SF_6 and CH_4 respectively. The column oven temperature was 35°C and nitrogen was used as the carrier gas with a flow rate of 30 ml min^{-1}. Samples were analysed in duplicates. Prepared standards were used to standardize the gas chromatograph for SF_6 (20 ppt; Scott-Marrin Inc., Riverside, CA) and CH_4 (100 ppm; Supelco, Mississauga, ON) prior to sample analysis. Daily CH_4 emissions were calculated as follows:

$$CH_4\,(L\ min^{-1}) = \text{Permeation tube } SF_6 \text{ release rate } (L\ min^{-1}) \times [CH_4] / [SF_6]$$

where $[CH_4]$ and $[SF_6]$ are the concentrations of CH_4 and SF_6 in samples after background concentrations have been deducted (See Appendix B).

6.3.8. Chemical analyses

Barley grain and ort samples were dried for 48 h in a forced draught oven at 60°C for dry matter (DM) determination. Fecal and saliva samples were freeze dried at -35°C (Genesis 25LE freeze dryer, Gardiner NY). Dried barley, forage and fecal samples were ground using a Wiley mill fitted with a 1 mm screen. Samples were analysed for crude protein (CP), using a Kjeltec 1030 auto analyser (Tecator Inc., Herndon,VI; [Association of Official Analytical Chemists (AOAC) 1990]) method no. 984.13 and ash using method no. 942.05 (AOAC 1990). Acid detergent fibre (ADF) and neutral detergent fibre (NDF) of grain and forage samples were determined using an ANKOM 200 fibre analyser (Fairport NY), with procedures described by Komarek (1993).

Gross energy (GE) was determined using a Parr 1241 adiabatic bomb calorimeter. In vitro dry matter digestibility (IVDMD) of forage and grain samples were determined by the method of Tilley and Terry (1963) using bovine inoculum. Chromium concentration in fecal samples was determined by atomic absorption spectrophometry (Model IL 551 AA/AE spectrophotometer), using air and acetylene flame (Williams et al. 1962).

6.3.9. Statistical analyses

Diet quality, intake and methane data were analysed by least square analyses of variance using GLM in SAS Institute Inc, (1990) in the model:

$$Y_{ijkl} = \mu + T_i + A_{j(i)} + P_k + (TP)_{ik} + (AP)_{j(i)k} + M_l + (TM)_{il} + (PM)_{kl} + (TPM)_{ikl} + \epsilon_{ijkl}$$

Where Y_{ijkl} = trait under consideration; μ = overall mean; T_i = dietary treatment ($i = 1,2$); $A_{j(i)}$ = animals within treatments, which was used as the error term to test for dietary treatment effect. P_k = sampling period in a grazing season ($k = 1..3$); $(TP)_{ik}$ = treatment x sampling period interaction. $(AP)_{j(i)k}$ = animals within treatment x sampling period interaction, which was used as an error term to test for sampling period and treatment x sampling period interaction. M_l = sampling time (IN and OUT) of paddocks ($l = 1,2$). $(TM)_{il}$ = treatment x sampling time interaction; $(PM)_{kl}$ = sampling period x sampling time interaction; $(TPM)_{ikl}$ = treatment x sampling period x sampling time interactions; ϵ_{ijkl} = experimental error term. Means were separated at the 5% level of significance using the probability of differences (PDIFF) option.

6.4. RESULTS AND DISCUSSION

6.4.1. Forage quality

The ADF content of forages consumed by esophageal fistulated steers at the Early period was 3% unit lower ($P < 0.05$) than the MID and the LATE periods, while the CP content remained uniform ($P > 0.05$) during the course of the grazing season (Table 10). This suggests that the CP content of forages, which ranged from 19.2% to 18% in the grazing season, would not have been a limiting factor to fermentation efficiency in that rumen microbes would be supplied with adequate protein for growth and digestion (Leng 1993).

Forage IVDMD on the other hand declined ($P < 0.05$) by 5% unit at the MID and the LATE periods. Forage quality changes during the grazing season were due to a small shift in species composition and physiological maturation of the plant. These results are within the range of values reported by McCaughey et al. (1997) for nutrient analyses of alfalfa- meadow bromegrass pastures. A more dramatic shift in forage quality was observed during the period of paddock occupation. There was a 17% unit increase ($P < 0.05$) in ADF content, a 14% and 17% unit decline in CP and IVDMD, respectively ($P < 0.05$), from the time animals entered to the time they exited paddocks (Table 10).

6.4.2. Intake

Concentrate feeding influenced the degree of change in forage DMI between IN and OUT sampling times ($P < 0.05$, Table 11). Forage intake was similar for S and C steers, when they entered a paddock, however at OUT, supplementation reduced ($P < 0.05$) forage intake by 31%. For every kg DM increase in barley grain intake, forage intake declined by 0.8 kg DM at OUT. In contrast, Meijs and Hoekstra (1984) observed that herbage intake declined with supplementation, when herbage availability was low, while at a high forage availability, the decline in herbage OM intake was high. In their study, forage quality was similar at both levels of herbage offered, while quality changes occurred as animals grazed in this trial.

Forage intake also was affected ($P < 0.05$) by treatment diets, sampling period and sampling time (Table 11). Average forage DMI declined ($P < 0.05$) by 11% in S steers. This is similar to the reduction in forage intake with concentrate supplementation observed by others (Penning et al. 1988; Faverdin et al. 1991). Forage intake (%BW) was observed to be the lowest ($P < 0.05$) at MID sampling period. Increasing concentrate offered and consumed at the beginning of the MID sampling period to 4 kg d^{-1} appears to be the cause for the decline in forage intake. This observation is consistent with other studies where a decline in forage intake with increasing level of concentrate supplementation has been observed (Meijs and Hoekstra 1984; Faverdin et al. 1991). Forage quality and quantity changes from the time animals entered to the time they left a paddock affected forage DMI the most, with a 51% decline in intake at OUT ($P < 0.05$). On average there was a 21% drop in digestibility of forages and a 39% increase in fibre content of forages contributing to the reduced DMI. Minson (1990) indicated that as plants mature there is a reduction in the concentration of digestible nutrients and an increase in structural content which increases gut fill and reduces intake.

It was observed that total DMI of S steers was higher ($P < 0.05$) than C steers at IN (Table 12). However at OUT, there were no differences ($P > 0.05$) in total DMI between S and C steers. This is because forage DMI did not drop at IN with supplementation. Supplemental grain increased ($P < 0.05$) TOMI by 14% in S steers (Table 12). Similar observation have been made in other studies (Young et al. 1980; Penning et al. 1988).

6.4.3. Methane production

Although S steers gained 57 kg more over 142 days of grazing than C steers ($P < 0.05$), CH_4 production (L d^{-1}, L kg BW^{-1}) was not different between the two diet treatments ($P > 0.05$; Table 13). This could suggest that a 14% increase in total DMI with supplementation was not sufficient to cause a significant change in CH_4 production.

Daily CH_4 production ranged from 256 L d^{-1} in EARLY period to 342 L d^{-1} in LATE period. These values were comparable to those observed by McCaughey et al. (1997) who reported daily CH_4 production values between 242.1 to 306.7 L d^{-1} from steers grazing pastures using the SF_6 tracer gas technique. Methane production varied between sampling periods, as a result of changes in forage quality and intake. McCaughey et al. (1999) also observed differences in CH_4 production between sampling periods from lactating beef cows grazing alfalfa-meadow bromegrass pastures. Variations in CH_4 production observed by McCaughey et al. (1999) were, however, attributed to changes in forage quality rather than voluntary intake, which was similar between sampling periods.

There were no effects ($P > 0.05$) of supplementation on CH_4 production expressed as L CH_4 kg $^{-1}$ TOMI or CH_4, % of TGEI by grazing steers (Table 13). Similarly, Visser et al. (1998) showed that, when silage diets were supplemented with three levels of flaked corn starch (0, 2 and 4 kg), the proportion of propionic acid increased (less CH_4) for cows fed diets containing early cut grass silage, however, no effects were found for cows fed late cut grass silage. The response of grain supplementation in reducing CH_4 production has been shown to depend on the type of forage in the basal diet, and the level of supplementation (Farverdin 1991).

Sampling period and time affected CH_4 production ($P < 0.01$), with the lowest CH_4 loss (%GEI) observed during the EARLY period and at entry into paddocks ($P < 0.01$; Table 13). The higher proportion of digestible nutrients and increased total digestible nutrient intake probably created a more rapid passage rate in the rumen and a shift in fermentation patterns towards less methanogenesis. McCaughey et al. (1999) observed that with higher intakes on alfalfa-grass pastures compared to grass-only pastures, energy loss through CH_4 production by lactating beef cows was 7.1% vs 9.5% of GEI, respectively ($P < 0.05$). Energy lost as CH_4, %TGEI ranged from 4.7 to 8.4% during the study with an overall mean loss of 6.5 ± 0.3 %, which is comparable to values reported by McCaughey et al. (1997) from steers grazing alfalfa- meadow brome pastures (4.1-5.2%) and Johnson et al. (1994) with forage-fed heifers (7.2%) using the SF_6 tracer gas technique.

6.5. CONCLUSIONS

There were marginal effects of grain supplementation on CH_4 production using the SF_6 tracer gas technique. Methane production was lowest at EARLY grazing and at entry into paddocks when forage quality was highest. The study suggests that pasture quality plays a major role on the extent to which CH_4 production can be reduced with grain supplementation in grazing animals.

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Table 10. Effect of time of grazing (Period) and length of time in a paddock (Time) on the nutrient analyses (DM basis) of esophageal masticates from alfalfa-meadow bromegrass pastures (LSmeans±SE)

Factors^z	NDF (%)	ADF (%)	CP (%)	ASH (%)	IVDMD (%)	GE (kJ g^{-1})
PERIOD (P)						
EARLY	50.5 ± 1.1	33.4 ± 0.7*b*	19.2 ± 0.4	10.2± 0.3*b*	75.5 ± 0.8*a*	18.4 ± 0.02*a*
MID	54.2 ± 1.5	36.5 ± 1.0*a*	17.9 ± 0.5	11.7± 0.4*a*	70.5 ± 1.1*b*	17.9 ± 0.02*b*
LATE	52.9 ± 1.4	36.6 ± 0.9*a*	18.2 ± 0.5	11.1± 0.4*a*	71.7 ± 0.9*b*	17.9 ± 0.02*b*
P-value	0.15	0.03	0.92	0.03	<0.01	0.01
TIME (T)						
IN	36.4 ± 1.1*b*	26.9 ± 0.7*b*	25.4 ± 0.4*a*	10.6 ± 0.3	81.0 ± 0.7*a*	18.8 ± 0.02*a*
OUT	68.6 ± 1.1*a*	44.0 ± 0.8*a*	11.4 ± 0.4*b*	11.4 ± 0.3	64.1 ± 0.8*b*	17.6 ± 0.02*b*
P-value	<0.01	<0.01	<0.01	0.09	<0.01	<0.01
(P) x (T)						
P-value	0.79	0.89	0.55	0.01	0.56	0.01

[z] Period (P) = EARLY (June17-25); MID (July 20-29) and LATE (Aug.12-21) period of a grazing season.
Time (T) = IN (entry) and OUT (exit) of paddocks.
a-b Means within each factor followed by different letters differ ($P < 0.05$).

Table 11. Effect of grain supplementation (Diet), time of grazing (Period) and time in a paddock (Time) on forage intake of steers (LSmeans ± SE)

Factors	DMI (kg d $^{-1}$)	DMI (% BW)	OMI (kg d $^{-1}$)
DIET[z]			
C	11.1 ± 0.3*a*	2.9 ± 0.1*a*	10.5 ± 0.3*a*
S	9.9 ± 0.3*b*	2.5 ± 0.1*b*	9.2 ± 0.3*b*
P-value	0.03	<0.01	0.05
PERIOD[y]			
EARLY	12.1 ± 0.4*a*	3.3 ± 0.1*a*	11.4 ± 0.4*a*
MID	8.5 ± 0.4*b*	2.1 ± 0.1*c*	7.9 ± 0.4*c*
LATE	11.0 ± 0.4*a*	2.6 ± 0.1*b*	10.2 ± 0.4*b*
P-value	<0.01	<0.01	<0.01
TIME[x]			
IN	14.1 ± 0.3*a*	3.6 ± 0.1*a*	13.3 ± 0.3*a*
OUT	6.9 ± 0.3*b*	1.7 ± 0.1*b*	6.3 ± 0.3*b*
P-value	<0.01	<0.01	<0.01
DIET x TIME			
C x IN	14.0 ± 0.4*a*	3.7 ± 0.1*a*	13.3 ± 0.4*a*
C x OUT	8.1 ± 0.5*b*	2.1 ± 0.1*b*	7.6 ± 0.5*b*
S x IN	14.2 ± 0.4*a*	3.6 ± 0.1*a*	13.3 ± 0.4*a*
S x OUT	5.6 ± 0.4*c*	1.4 ± 0.1*c*	5.1 ± 0.4*c*
P -value	<0.01	0.02	<0.01

[z] Diet: C = (alfalfa/grass pasture); S = (barley grain + alfalfa/grass pasture).

[y] Period defined in Table 10

[x] Time defined in Table 10.

a-*c*Means within each factor followed by different letters differ (P < 0.05).

Table 12. Effect of grain supplementation (Diet), time of grazing (Period) and time in a paddock (Time) on total intake[z] of steers (LSmeans ± SE)

Factors	Total DMI (kg d $^{-1}$)	Total DMI (% BW)	Total OMI (kg d $^{-1}$)
DIET[y]			
C	11.1 ± 0.3*b*	2.9 ± 0.1*b*	10.5 ± 0.3*b*
S	12.9 ± 0.3*a*	3.2 ± 0.1*a*	12.2 ± 0.3*a*
P-value	0.01	0.02	<0.01
PERIOD[x]			
EARLY	13.0 ± 0.4*a*	3.6 ± 0.1*a*	12.4 ± 0.4*a*
MID	10.2 ± 0.4*b*	2.6 ± 0.1*c*	9.6 ± 0.4*c*
LATE	12.7 ± 0.4*a*	3.0 ± 0.1*b*	12.0 ± 0.4*b*
P-value	<0.01	<0.01	<0.01
TIME[w]			
IN	15.6 ± 0.3*a*	4.0 ± 0.1*a*	14.8 ± 0.3*a*
OUT	8.4 ± 0.3*b*	2.1 ± 0.1*b*	7.8 ± 0.3*b*
P-value	<0.01	<0.01	<0.01
DIET x TIME			
C x IN	14.0 ± 0.4*b*	3.7 ± 0.1*b*	13.3 ± 0.4*b*
C x OUT	8.1 ± 0.5*c*	2.1 ± 0.1*c*	7.6 ± 0.5*c*
S x IN	17.2 ± 0.4*a*	4.3 ± 0.1*a*	16.3± 0.4*a*
S x OUT	8.6 ± 0.4*c*	2.1 ± 0.1*c*	8.1 ± 0.4*c*
P-value	<0.01	0.02	<0.01

[z] Total intake = Forage intake + barley grain intake.

[y] Diet: Con = (legume-grass pasture); Sup = (barley grain + legume-grass pasture).

[x] Period defined in Table 10.

[w] Time defined in Table 10.

a-c Means within each factor followed by different letters differ ($P < 0.05$).

Table 13. Effects of grain supplementation on methane (CH_4) production of grazing steers (LSmeans ±SE)

Factors	CH_4 (L d^{-1})	CH_4 (L kgBW^{-1})	CH_4 (L kg $^{-1}$ TDMI)	CH_4 (L kg $^{-1}$ TOMI)	CH_4 (% TGEI)
DIET^z					
C	310.5 ± 25.9	0.80 ± 0.06	30.8 ± 2.9	32.8 ± 3.1	6.7 ± 0.63
S	331.2 ± 24.6	0.81 ± 0.06	29.4 ± 2.7	31.3 ± 2.9	6.4 ± 0.59
P-value	0.58	0.91	0.73	0.73	0.71
PERIOD^y					
EARLY	256.3 ± 12.8*b*	0.70 ± 0.03*b*	21.7 ± 1.5*c*	22.8 ± 1.6*c*	4.7 ± 0.33*c*
MID	363.9 ± 11.8*a*	0.91 ± 0.03*a*	38.2 ± 1.7*a*	40.9 ± 1.8*a*	8.4 ± 0.36*a*
LATE	342.4 ± 11.8*a*	0.81 ± 0.03*a*	30.5 ± 1.5*b*	32.4 ± 1.6*b*	6.6 ± 0.33*b*
P-value	<0.01	<0.01	<0.01	<0.01	<0.01
TIME^x					
IN	325.5 ± 13.7	0.82 ± 0.04	22.1 ± 1.7*a*	23.2 ± 1.8*a*	4.6 ± 0.37*a*
OUT	316.2 ± 13.0	0.80 ± 0.04	38.2 ± 1.8*b*	40.9 ± 1.9*b*	8.5 ± 0.38*b*
P-value	0.63	0.46	<0.01	<0.01	<0.01

^{zyx}See Table 11.

a-*c*Means within each factor followed by different letters differ ($P < 0.05$)

7.0. GENERAL DISCUSSION

7.1. Assessment of the SF_6 tracer gas technique for gas measurement

7.1.1. Methane gas measurement

Methane production (L d^{-1}) measured by the SF_6 tracer gas technique was not different ($P = 0.24$) compared to estimates from respiration calorimetry using the ventilation hood (Chapter 4). This confirms that eructed and respired CH_4 gas from ruminants can be accurately determined using the SF_6 tracer gas technique in a free ranging situation as outlined in the thesis hypothesis (Chapter 3). Some previous studies have reported that measurements with the SF_6 tracer technique measured about 90-95% of gas measurements made with the respiratory calorimetry (Johnson et al. 1994; Ulyatt et al. 1999). This can be expected as the measure of comparison made in those studies involved the use of a respiratory chamber which measures both rumen and hind gut CH_4 gas production. In Chapter 4, CH_4 gas estimates by the SF_6 tracer gas technique were compared with estimates from the ventilation hood, which accounts for only respired and eructed CH_4.

The average loss of CH_4 expressed as %GEI, rather than L d^{-1} allows comparison of CH_4 production data with other studies in order to assess the accuracy of the SF_6 tracer gas technique in measuring energy lost as CH_4 by cattle. The research study in Chapter 4 showed that CH_4 production (%GEI) was similar ($P > 0.05$) using the SF_6 tracer gas technique and the ventilation hood. Methane production (%GEI) observed in Chapters 4, 5 and 6 were within the range of values reported for growing animals in other studies (Holter and Young 1992; Johnson et al. 1994; McCaughey et al. 1997). In Chapter 4, the mean CH_4 production

(%GEI) was 6.3 ± 0.2% using calorimetry and 6.7 ± 0.2% using the SF_6 tracer gas technique. In Chapter 5, CH_4 production (%GEI) from forage diets using the SF_6 tracer gas technique ranged from 4.6 to 9.4% (mean 6.9 ± 1.2%) on ad-libitum feeding, while it ranged from 4.7 to 8.4% (6.5 ± 0.3%) in steers grazing alfalfa-meadow bromegrass pastures (Chapter 6). The above mean values observed are comparable to CH_4 production losses of 6-7.2% on forage fed diets by growing animals using both the SF_6 tracer technique and respiratory calorimetry (Johnson et al.1994; McCaughey et al. 1997). The direct measurement of CH_4 production of individual animals by the SF_6 tracer technique, allowed the identification of the least and most efficient animal based on their digestion efficiencies (CH_4,%GEI) in pens and on pasture.

When a specific type of diet was offered at a constant amount (Chapter 4), day-to-day variation in CH_4 production was not significant with either the use of SF_6 tracer gas technique or the ventilation hood. On the other hand, significant and large day-to-day variation (CV = 27%) in CH_4 production was observed with the feeding of different qualities of forages and at two levels of intake (Chapter 5). This observation can be expected as DMI (CV = 22.5%) was highly variable, especially on ad-libitum intake. Dry matter intake was strongly correlated with CH_4 production (r= 0.8), and accounted for 64% of the variation in CH_4 production (Chapter 5).

Animal- to-animal variation in CH_4 production using the SF_6 tracer gas technique was significant and larger (CV =11.7%) than animal-to-animal variations using respiration calorimetry for gas measurement (Chapter 4). A higher coefficient of variation (26.6%) was observed between animals during ad-libitum (26.6%) than during restricted feeding (25.3%) in the second study (Chapter 5) using the SF_6 tracer gas technique. Animal-to-animal variation may be related to DMI, but could also be related to the eating behaviour of animals, variation in diet consumed relative to diet offered, and variation in rumen capacity and digesta passage rate (Van Soest 1982). In the second study, the higher animal-to-animal variation can be expected using the SF_6 tracer gas technique, as DMI was more variable, and there was more opportunity for selection of diet. Also, the various diets offered would impact differences in digestive efficiencies. It is possible that the low animal numbers, and some loss of gas measurements from some animals could also contribute to the high variations encountered in the study (Chapter 5). Similarly, high animal-to-animal variation has been observed with the use of the SF_6 tracer gas technique in other studies (Lassey et al. 1997; Leuning et al. 1998; Ulyatt et al. 1999). It can be concluded that these variations observed in the study are to be expected in the field with the use of the SF_6 tracer gas technique. The research study therefore suggests the need to include more animals and gas collection days to minimize variations in future experiments.

7.1.2. Carbon dioxide gas measurement

Measurement of CO_2 production using the SF_6 tracer technique was 20 % higher ($P < 0.01$) than CO_2 production from respiratory calorimetry using the hood (Chapter 4). It is possible that the higher activity of animals in the facilities provided, as a result of more opportunity for excitement and movement of animals contributed to the higher CO_2 production from body metabolism observed with the use of the SF_6 tracer gas technique. It has been reported that only 18% of expired CO_2 came from the rumen with the rest coming from body metabolism (Hoernicke et al. 1965). Although it has been established that CH_4 and

CO_2 travel together, and more than 90% of rumen gases go the lungs before exhalation (Hoernicke et al. 1965; Young and Corbett 1972), there is no information about mixing of CO_2 gas and the tracer gas. There could be the possibility of poor mixing of rumen and lung gases, resulting in preferential sampling of lung air, containing higher levels of CO_2 and lower levels of the tracer gas (higher CO_2: SF_6 ratio). Further studies are needed to verify the observations made in this research on CO_2 production measurements by the SF_6 tracer gas technique. This will be useful information for future measurements in the field and calculation of heat production. Future experiments that will assess CO_2 production with other methods apart from the respiratory calorimetry, will enable comparison of the SF_6 tracer gas technique with other methods of gas measurement.

Although mean CO_2 production by the SF_6 tracer gas technique was significantly higher than CO_2 production by the tracer method, animal-to-animal variation in CO_2 production (CV= 11.8%) were similar to those observed in CH_4 production (CV = 11.7%) suggesting a consistent variation to expect with measurements associated with the SF_6 tracer gas methodology for both CH_4 and CO_2 production. Also, CO_2 production measurements from all animals using the SF_6 tracer technique were consistently higher than measurements from the hood on each day of sampling (Figure 9). This consistency suggests that the SF_6 tracer gas technique holds promise for estimating CO_2 production, and for the calculation of heat production especially in pastured animals. McDonald et al. (1995) noted that for grazing animals, heat production can be estimated from CO_2 production alone with reasonable accuracy. In the past, it has been difficult to assess heat production and energy metabolism in pastured animals, and the SF_6 tracer gas technique shows potential for estimating heat production on pasture, where several pasture management strategies can be assessed on metabolism with some reasonable degree of accuracy and consistency.

7.2. Assessment of CH_4 reduction strategies using the SF_6 tracer gas technique

Animal type (Chapter 5), forage quality and quantity (Chapter 5 and 6), and grain supplementation (Chapter 6) were used as test variables in assessing changes on CH_4 production as measured by the SF_6 tracer gas technique. In Chapter 5, it was expected that dairy and beef cattle would have significant differences in feed intake and, therefore, differences in CH_4 production, however there were no effects of animal type on ($P > 0.05$) on intake or CH_4 production under either ad-libitum or restricted feeding. This can be attributed to the similar initial weights between the cattle types. Lal et al. (1987) observed that energy losses as CH_4 were higher in Holstein-Friesian x Harian cross cattle than in Holstein-Friesian cattle in India. This difference was attributed to the fact that the indigenous dairy cross has a larger gut capacity than Holstein-Friesian for intake of wheat straw based diets. It is possible that differences in gut capacity as a result of similar body size of improved breeds in the study were absent. Higher variations encountered with the use of the SF_6 tracer gas technique and lower animal numbers in the analyses of gas data, (Chapter 5) could possibly make it difficult to pick up treatment differences.

Despite the high variations in gas measurements by the SF_6 tracer technique, it is interesting to note, the effects of forage quality and quantity either offered or grazed on CH_4 production was significant ($P < 0.05$; Chapters 5 and 6). Methane (L kg $^{-1}$ DOMI) increased as the quality of forages declined ($P < 0.01$) under both ad-libitum and restricted intake as the quality of forages declined ($P < 0.01$) under both ad-libitum and restricted intake (Chapter 5). This is in agreement with the general trend of lower fractional CH_4 production with more digestible forages due to a faster passage rate of digesta in the rumen and lower proportion of structural carbohydrate content (Johnson and Johnson 1995; McAllister et al. 1996). The stage of grazing and time in a paddock influenced CH_4 production more dramatically ($P < 0.01$) than the effects of supplemental grain ($P > 0.05$; Chapter 6). Methane production (%GEI) was reduced by 45% when animals entered paddocks compared to when they exited at which time quality and quantity of forages were in short supply. Methane production was lowest ($P < 0.01$) during the early stages of the grazing season. The high proportion of digestible nutrients from forages during early stages of grazing and when animals entered the paddocks caused a shift in fermentation patterns towards less CH_4 production during these periods.

The quality and quantity of pasture on offer affected how grain supplementation influenced CH_4 production, thus there were marginal effects of grain supplementation on CH_4 production. This can be attributed to the interaction effects of grain and forage quality on total intake (Chapter 6). The study implies that forage quality plays a major role on the extent to which CH_4 production could be reduced by grain supplementation (Chapter 6). Overall, forage quality and quantity were the major variables that affect changes in CH_4 production as measured by the SF_6 tracer gas technique (Chapter 5 and 6). This confirms that diet quality and quantity are the major factors influencing the amount of CH_4 production in cattle (Johnson and Johnson 1995; Johnson et al. 1996).

7.3. Practical suggestions for the use of SF_6 tracer gas technique

The reliability of permeation tubes in releasing SF_6 at a steady and predictable rate is a critical factor in the success of the tracer methodology, and it is therefore suggested that permeation tubes be weighed weekly for about 8-10 weeks prior to trials to establish accurate and steady release rates. It is suggested that the amount of gas in permeation tubes be timed with duration of experiments; i.e lower tube release rates would be suitable for experiments of longer duration and vice versa. Tubes generally last between 10-12 mo, depending on release rates. The type and thickness of the Teflon membrane dictates the release rate of $SF_{6.}$ It has been observed that rates of less than 150 ng min^{-1} may allow tubes to last longer in the rumen, but the exhaled sample may have levels of SF_6 that are not detectable on the GC. Very high release rates of above 1000 ng min^{-1} (with the type of permeation tubes used in this study) are detectable on GC, but there is the possibility of expiry, depending on the duration of the trial. For example, a permeation tube filled with 0.300 mg of SF_6, and having a release rate of 1000 ng min^{-1} are expected to last about 4.5 mo. Generally 15-20% extra permeation tubes must be added to the number of tubes required, in order to have an adequate selection of tubes with optimal steady release rates.

Animals need to be adequately trained to wear gas collection apparatus prior to gas sampling. Halters with attached weights (chains) could be used in the training process. Rain, mud, manure and dry feed are potential obstacles that can block the capillary tubing of the gas collection equipment, therefore reducing success rates. Spare canisters, and gas collection harness must be on hand to replace blocked collection apparatus. On pasture, variable wind speeds dilute gas concentration, and it has been beneficial to use permeation tubes with higher release rates and to ensure that the position of the nose piece is closer to mouth and nose. This ensures that the tracer gas can be detected on GC. In winter, there is the possibility of freezing of the capillary tubing, and as such the tubing can be attached to the inside lining of the halter and insulated with inner lining of a car tire tubing.

In general, more animal and more sampling days are required to use SF_6 tracer gas methodology, as many external factors (feed, mud, environment, animals, housing facility) can impact the success of the gas collection procedure. Success rate of gas collection encountered has ranged from 75-95%. Proper care is needed on the part of the operator in collection, storage, transportation and analyses of gases. Sulphur hexafluoride gas is itself a greenhouse gas and therefore care should be taken to minimize amounts been released into the atmosphere. The amount placed in the permeation tubes (0.260-0.300 mg) is very little and therefore would not have any major impact on the environment.

8.0. CONCLUSIONS

It can be concluded that:

a) The SF_6 tracer gas technique can accurately estimate eructed and respired CH_4 production of individual cattle in pen and on pasture. The SF_6 tracer gas technique allows the characterization of digestive efficiencies among animals in the field.

b) Carbon dioxide production measurement by the SF_6 tracer gas technique was consistently higher than the measurements from respiratory calorimetry (hood). Further studies are needed to verify this observation.

c) Animal-to-animal variation in gas measurements using the SF_6 tracer gas technique were significantly higher than animal-to-animal variation using the respiratory calorimetry (hood). This implies that more animals may be needed for treatment comparisons using the SF_6 tracer gas technique than calorimetry to determine some treatment differences. However, a wider range of management treatments can be tested using the SF_6 methodology.

d) Animal type had no effect on CH_4 production during ad-libitum and restricted feeding of forages with different nutrient densities.

e) Low quality forages increase the fractional CH_4 production during both ad-libitum and restricted feeding.

f) There was no significant effect of grain supplementation on CH_4 production by grazing steers.

g) High quality forages during early grazing and at entrance into paddocks significantly reduced CH_4 production (%GEI). Pasture quality may play a major role on the extent to which grain supplementation can reduce CH_4 production of grazing animals.

9.0. LIST OF REFERENCES

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10.0. APPENDIXES

10.1. APPENDIX A

Data conversion and calculations of gas production rates

1. To calculate CH_4 production (litres min^{-1}) and CO_2 production (litres min^{-1})

Equation 1. CH_4 production (litres min^{-1}) = SF_6 release rate (litres min^{-1}) * Ratio [CH_4 (ng kg^{-1}) /SF_6 (ng kg^{-1})]

Equation 2. CO_2 production (litres min^{-1}) = SF_6 release rate (litres min^{-1}) * Ratio [CO_2 (ng kg^{-1}) /SF_6 (ng kg^{-1})]

a) Conversion of parts per million (ppm) to parts per trillion (ppt) or ng kg^{-1} : Mulitiplied by 1 x 10^6

b) Conversion of ng min^{-1} of permeation tube release rate into liters min^{-1} : Divided by 6.602 x 10^9

c) Conversion of litres min^{-1} to litres per day : Multiplied by 1440 mins (60 x 24 hr; or adjusted to exact sampling duration)

d) Mean daily concentration of background SF_6 (ppt) and CH_4 (ppm) and CO_2 (ppm) subtracted from each sample analyzed before conversions and equations applied

2. 1 litre CH_4 = 0.716 g CH_4
   1 litre CO_2 = 1.964 g CO_2
   1 litre SF_6 = 6.602 g SF_6
   1 litre CH_4 = 9.45 kcal
   1 g CH_4 = 13.2 kcal

3. To calculate CH_4 production (% of GEI)

   a) CH_4 production (kg y^{-1}) to CH_4 (litre day^{-1}): Multiply by 3.84.

   b) CH_4 production (litre day^{-1}) to CH_4 (g day^{-1}):Multiply by 0.716

   c) CH_4 (litre day^{-1}) into caloric value: Multiply by 9.45 kcal $litre^{-1}$

   d) CH_4 (kcal $litre^{-1}$) into kcal g^{-1} : Divide by 0.716

   e) CH_4 (%GEI) = [CH_4 (kcal g^{-1}) / (GEI (kcal g^{-1})] * 100

Analysis of variance (ANOVA) for gas data by calorimetry and SF_6 tracer technique in Manuscript I

CH_4

Source	**DF**	**SS**	**MS**	**F-value**	**Pr >F**
Method	1	431.04	431.04	1.47	0.24
Animal	5	1296.88	259.38	0.88	0.51
Day	5	377.61	75.52	0.26	0.93
Error	24	7059.14	294.13		

CO_2

Source	**DF**	**SS**	**MS**	**F-value**	**Pr >F**
Method	1	1913237.63	1913237.63	19.42	0.0002*
Animal	5	997731.74	199546.35	2.03	0.11
Day	5	442328.66	88465.73	0.9	0.5
Error	24	2364493.45	98520.56		

ANOVA for Intake and CH_4 data under two feeding regime (Manuscript II)

Ad-libitum feeding:

DMI

Source	**DF**	**SS**	**MS**	**F-value**	**Pr >F**
^{z}Breed	1	0.32	0.32	0.18	0.68
Animal (breed)	7	12.26	1.75	3.92	0.02*
Diet	2	54.18	27.09	60.55	0.0001*
Breed * Diet	2	1.71	0.86	1.92	0.19
Period	2	8.52	4.26	9.52	0.003*
Error	12	5.37	0.45		

^{z}Test of hypotheses using Type III MS for Animal (breed) as error term

CH_4 ($L\ d^{-1}$)

Source	**DF**	**SS**	**MS**	**F-value**	**Pr >F**
^{z}Breed	1	2.49	2.49	0.01	0.97
Animal (breed)	7	11842.4	1691.77	1.1	0.42
Diet	2	38813.34	19406.68	12.56	0.001*
Breed * Diet	2	91.53	45.77	0.03	0.97
Period	2	25198.15	12599.08	8.16	0.006
Error	12	18535.72	1544.64		

^{z}Test of hypotheses using Type III MS for Animal (breed) as error term

CH_4 (L kg $^{-1}$ DOMI)

Source	**DF**	**SS**	**MS**	**F-value**	**Pr >F**
[z]Breed	1	5.2	5.2	0.03	0.87
Animal (breed)	7	1318.93	188.42	1.35	0.31
Diet	2	5371.2	2685.6	19.22	0.0002*
Breed * Diet	2	74.46	37.23	0.27	0.77
Period	2	752.39	376.19	2.69	0.11
Error	12	1676.39	139.7		

[z]Test of hypotheses using Type III MS for Animal (breed) as error term

CH_4 (%GEI)

Source	**DF**	**SS**	**MS**	**F-value**	**Pr >F**
[z]Breed	1	0.01	0.01	0.01	0.91
Animal (breed)	7	7.93	1.13	0.89	0.54
Diet	2	5.86	2.93	2.29	0.14
Breed * Diet	2	0.13	0.06	0.05	0.95
Period	2	6.68	3.33	2.61	0.11
Error	12	15.32	1.28		

[z]Test of hypotheses using Type III MS for Animal (breed) as error term

Restricted feeding:

DMI

Source	**DF**	**SS**	**MS**	**F-value**	**Pr >F**
[z]Breed	1	0.16	0.16	0.34	0.58
Animal (breed)	7	3.31	0.47	10.83	0.0002*
Diet	2	0.51	0.25	5.81	0.02*
Breed * Diet	2	0.01	0.005	0.12	0.88
Period	2	4.11	2.06	47.12	0.0001
Error	12	0.52	0.04		

[z]Test of hypotheses using Type III MS for Animal (breed) as error term

CH_4 (L d $^{-1}$)

Source	**DF**	**SS**	**MS**	**F-value**	**Pr >F**
[z]Breed	1	2024.24	2024.24	0.98	0.35
Animal (breed)	7	14431.46	2061.64	0.91	0.53
Diet	2	5214.96	2607.48	1.15	0.35
Breed * Diet	2	1828.57	914.28	0.4	0.68
Period	2	12287.25	6143.63	2.71	0.11
Error	12	27250.87	2270.91		

[z]Test of hypotheses using Type III MS for Animal (breed) as error term

CH_4 (L kg $^{-1}$ DOMI)

Source	**DF**	**SS**	**MS**	**F-value**	**Pr >F**
[z]Breed	1	420.01	420.01	2.39	0.17
Animal (breed)	7	1230.64	175.81	0.82	0.59
Diet	2	3323.47	1661.73	7.71	0.007*
Breed * Diet	2	289.11	144.55	0.67	0.53
Period	2	1014.21	507.11	2.35	0.14
Error	12	2587.7	215.64		

[z]Test of hypotheses using Type III MS for Animal (breed) as error term

CH_4 (%GEI)

Source	**DF**	**SS**	**MS**	**F-value**	**Pr >F**
[z]Breed	1	3.6	3.6	1.62	0.24
Animal (breed)	7	15.52	2.22	0.92	0.52
Diet	2	1.22	0.61	0.25	0.8
Breed * Diet	2	1.64	0.82	0.34	0.72
Period	2	9.66	4.83	2.01	0.18
Error	12	28.8	2.4		

[z]Test of hypotheses using Type III MS for Animal (breed) as error term

ANOVA for Intake and CH_4 data (Manuscript III)

Forage DMI

Source	DF	SS	MS	F-value	Pr >F
^{z}Trt	1	15.26	15.26	7.75	0.03*
Animal (Trt)	6	11.82	1.97	0.83	0.56
^{y}Period	2	100.82	50.41	22.3	0.0001*
^{y}Trt * Period	2	3.44	1.72	0.76	0.49
Animal *Period (Trt)	12	27.11	2.26	0.96	0.52
Time	1	595.29	595.29	252.75	0.0001*
Period * Time	2	10.9	5.45	2.3	0.13
Trt * Time	1	20.8	20.8	8.8	0.009*
Trt * Period *Time	2	12.72	6.36	2.69	0.1
Error	17	40.2	2.36		

^{z}Test of hypotheses using Type III MS for animal(trt) as an error term

^{y}Test of hypotheses using Type III MS for animal*period(trt) as an error term

TOMI

Source	**DF**	**SS**	**MS**	**F-value**	**Pr >F**
[z]Trt	1	33.46	33.46	17.85	0.006*
Animal (Trt)	6	11.25	1.87	0.88	0.53
[y]Period	2	64.65	32.32	15.24	0.0002*
[y]Trt * Period	2	2.67	1.33	0.63	0.54
Animal *Period (Trt)	12	25.42	2.11	1	0.49
Time	1	553.92	553.92	261.11	0.0001*
Period * Time	2	8.4	4.2	1.98	0.17
Trt * Time	1	18.22	18.22	8.59	0.009
Trt * Period *Time	2	10.05	5.02	2.37	0.12
Error	17	36.06	2.12		

[z] Test of hypotheses using Type III MS for animal(trt) as an error term

[y] Test of hypotheses using Type III MS for animal*period(trt) as an error term

CH_4 (L d^{-1})

Source	**DF**	**SS**	**MS**	**F-value**	**Pr >F**
[z]Trt	1	4832.52	4832.52	0.33	0.58
Animal (Trt)	6	86889.02	14481.5	3.56	0.02*
[y]Period	2	93668.84	46834.42	20.96	0.0001*
[y]Trt * Period	2	2402.14	1201.07	0.54	0.6
Animal *Period (Trt)	12	26818.39	2234.87	0.55	0.85
Time	1	998.47	998.47	0.25	0.63
Period * Time	2	19476.71	9738.35	2.39	0.12
Trt * Time	1	557.35	557.35	0.14	0.72
Trt * Period *Time	2	10399.61	5199.8	1.28	0.3
Error	17	69243.62	4073.15		

[z]Test of hypotheses using Type III MS for animal(trt) as an error term

[y]Test of hypotheses using Type III MS for animal*period(trt) as an error term

CH_4(%GEI)

Source	**DF**	**SS**	**MS**	**F-value**	**Pr >F**
[z]Trt	1	1.38	1.38	0.16	0.7
Animal (Trt)	6	50.73	8.45	2.64	0.05*
[y]Period	2	101	50.5	15.79	0.0001*
[y]Trt * Period	2	9	4.5	2.58	0.12
Animal *Period (Trt)	12	20.92	1.74	0.54	0.86
Time	1	169.14	169.14	52.87	0.0001*
Period * Time	2	2.5	1.25	0.39	0.68
Trt * Time	1	3.85	3.85	1.2	0.29
Trt * Period *Time	2	2.48	1.24	0.39	0.68
Error	17	54.38	3.2		

[z]Test of hypotheses using Type III MS for animal(trt) as an error term
[y]Test of hypotheses using Type III MS for animal*period(trt) as an error term

10.1 APPENDIX B

Raw data showing the release rates of SF_6 in permeation tubes prior to insertion into the rumen and after removal from the rumen[z].

Permeation tube No.	Release rate (ng m^{-1}) prior to insertion into rumen	Release rate (ng m^{-1}) after removal from rumen
39	761.2	714.9
46	617.3	582.8
72	669.8	688.1
67	639.1	652.5
74	587.8	634.2
89	544.9	554.7
17	820.6	835.9

[z] Statistical analyses of data showed no significant differences ($P > 0.05$) between release rates prior to and after removal from the rumen.

Calibration of permeation tube (No.46) SF_6 release rates during incubation in air at 39°C (Manuscript 1).

Empty tube weight: 17.58810 g:Filled tube weight: 17.89045 g: SF_6 in tube= 0.302 mg

Dates	Incubation time (mins)	Tube weight (g)	Amount of SF_6 emitted (mg)	Release rate (ng m^{-1})
June 4, 1999	9966	17.88706	0.00339	340.15
June 11, 1999	10356	17.88300	0.00406	392.04
June 18, 1999	9724	17.87953	0.00347	356.85
June 25, 1999	10270	17.87569	0.00384	373.90
July 2, 1999	10260	17.87143	0.00426	415.20
July 9, 1999	10179	17.86736	0.00407	399.84
July 16, 1999	10003	17.86337	0.00399	398.88
July 23, 1999	10247	17.85921	0.00416	405.97

Calibration of permeation tube (No.66) SF_6 release rates during incubation in air at 39°C (Manuscript 2).

Empty tube weight: 18.55128 g:Filled tube weight: 18.84038 g: SF_6 in tube= 0.289 mg

Dates	Incubation time (mins)	Tube weight (g)	Amount of SF_6 emitted (mg)	Release rate (ng m^{-1})
Oct. 31, 1997	10268	18.82819	0.00615	598.90
Nov. 7, 1997	9973	18.82280	0.00539	540.46
Nov. 14, 1997	10356	18.81666	0.00614	592.89
Nov 21, 1997	9729	18.81154	0.00512	526.26
Nov 28, 1997	10258	18.80619	0.00535	521.54
Dec 5, 1997	9905	18.80110	0.00509	513.88
Dec 12, 1997	10173	18.79570	0.00540	530.82
Dec 19, 1997	9989	18.79028	0.00542	542.60

Calibration of permeation tube (No.62) SF_6 release rates during incubation in air at 39°C (Manuscript 3)

Empty tube weight: 17.71932 g:Filled tube weight: 17.97676 g: SF_6 in tube= 0.261 mg

Dates	Incubation time (mins)	Tube weight (g)	Amount of SF_6 emitted (mg)	Release rate (ng m^{-1})
April 6, 1998	10153	17.97676	0.00384	378.21
April 13, 1998	10205	17.97286	0.00390	382.17
April 20, 1998	9958	17.96894	0.00392	393.65
April 27, 1998	10314	17.96446	0.00448	434.36
May 4, 1998	9735	17.96058	0.00388	398.56
May 11, 1998	10289	17.95667	0.00391	380.02
May 19, 1998	10459	17.95221	0.00446	426.42
May 27, 1998	10170	17.94815	0.00406	399.21
June 4, 1998	9986	17.94430	0.00385	385.54

Some gas data used in Manuscript I (SF6 tracer gas technique)

Animal #	sf6(ppt)	sf6 (ppt)	ch4 (ppm)	ch4(ppm)	CO2(ppm)	CO2(ppm)
77	45.9768	45.0593	89.067	88.7133	1728.342	1734.682
67	111.605	112.2806	316.7995	317.022	4501.335	4488.164
61	21.2014	20.7187	97.9123	97.9473	1606.159	1602.622
bkd	0.8766	0.8123	0.686	0.6499	132.3606	129.7039
148	34.8524	34.5802	73.4369	73.6227	1482.64	1502.177
27	24.4321	24.4914	97.4469	96.9815	1724.497	1719.854
7	46.9245	47.8248	150.4229	149.6349	2573.024	2551.722
bkd	1.5757	1.42	1.1129	1.0155	151.3748	145.6122
77	33.3426	34.6435	77.4904	77.8799	1741.291	1726.057
67	74.4445	75.386	225.3735	225.3068	3545.738	3515.804
61	26.6642	26.5306	104.5882	104.0731	1968.629	1961.481
bkd	1.2149	0.8604	0.9857	0.6435	135.3408	129.395
148	37.4319	40.5169	92.9608	93.4625	1843.011	1876.838
27	7.2528	7.0432	23.0489	23.1379	544.8687	546.6086
7	65.6479	66.0471	211.8793	215.4678	3534.205	3454.333
bkd	0	0	0.8918	0.8326	145.7482	140.3593

bkd = background gas concentrations

Gas data used in Manuscript I

Animal #	Day	Method	CH4 (L/d)	CO2 (L/d)	Animal #	Day	Method	CH4 (L/d)	CO2 (L/d)
61	1	sf6	165.586	2508.36	7	1	cal	138.67	1920.74
61	5	sf6	130.855	2443.39	7	3	cal	123.224	1905.92
61	3	sf6	138.697	2455.54	7	5	cal	140.361	2007.5
67	5	sf6	144.745	2831.65	27	1	cal	142.953	1933.19
67	3	sf6	119.211	1804.36	27	3	cal	137.279	1981.09
67	1	sf6	111.654	1540.67	27	5	cal	135.753	2005.06
77	5	sf6	142.496	2211.67	148	1	cal	109.892	1978.32
77	3	sf6	159.866	3330.21	148	3	cal	126.243	2048.69
77	1	sf6	135.348	2455.41	148	5	cal	137.161	2038.85
7	2	sf6	147.985	2494.48	61	2	cal	127.79	1859.87
7	4	sf6	147.07	2315.96	61	6	cal	107.497	1683.2
7	6	sf6	151.778	2042.02	61	4	cal	134.104	1844.98
27	2	sf6	120.482	2093.62	67	4	cal	136.73	1853.45
27	4	sf6	90.1557	1633.04	67	2	cal	140.696	1883.89
27	6	sf6	113.005	1955.98	67	6	cal	114.582	1573.7
148	2	sf6	139.203	2768.16	77	2	cal	113.327	1883.8
148	4	sf6	148.266	2756.41	77	4	cal	144.593	1885.85
148	6	sf6	154.237	2722.11	77	6	cal	125.218	1775.76

Some gas data used in Manuscript II
AD-LIBITUM FEEDING

ANIMAL #	Diet	INJ 1 sf6(ppt)	INJ 2 sf6 (ppt)	INJ 1 ch4(ppm)	INJ 2 ch4 (ppm)
708	D1	no collection			
1G	D3	39.0712	39.1027	44.8319	47.364
74G	D2	24.4846	24.587	71.7824	70.2945
704	D2	10.3676	10.9533	20.6269	20.506
709	D3	nd	nd	3.2562	3.2513
20G	D1	22.5852	22.5045	42.3449	42.9659
Background		0	0	0.8913	0.8709

nd= not detected

ANIMAL #	Diet				
708	D1	no collection			
1G	D3	36.9444	37.4083	69.1201	67.8168
74G	D2	41.8601	42.3372	140.8303	140.0839
704	D2	12.942	12.7984	28.7077	27.3072
709	D3	10.7502	10.4224	19.7603	19.771
20G	D1	6.8407	7.4478	11.6716	12.0682
Background		0	0	1.7696	1.7557

ANIMAL #	Diet				
708	D1	9.3189	9.4637	27.3767	27.0166
1G	D3	70.1105	69.853	75.1225	74.6793
74G	D2	25.2978	25.2543	69.6356	71.0092
704	D2	14.6598	14.3941	36.6566	36.714
709	D3	10.0571	10.4746	18.6537	18.5991
20G	D1	7.5027	7.377	22.6557	22.639
Background		0	0	1.9224	1.83

ANIMAL #	Diet				
708	D1	7.7809	7.5142	22.6922	22.5114
1G	D3	36.7893	36.9032	78.065	78.2486
74G	D2	17.7557	17.4843	56.3476	56.336
704	D2	7.6623	8.0949	18.3892	19.549
709	D3	8.7606	8.228	16.3111	16.2294
20G	D1	24.3389	24.6278	48.487	48.363
Background		0	0	1.6038	1.6342

(Period 2)

ANIMAL #	Diet	INJ 1 sf6(ppt)	INJ 2 sf6 (ppt)	INJ 1 ch4(ppm)	INJ 2 ch4 (ppm)
705	D1	no collection			
702	D3	15.7326	15.9556	21.6346	22.1254
2113	D2	nd	nd	48.5149	49.1975
706	D2	2.0952	2.0952	38.4006	38.7629
26G	D1	12.9943	12.5708	34.7409	34.1447
NOTAG	D3	1.7642	1.7642	38.5117	37.9456
Background		0	0	1.4818	1.1421

ANIMAL #	Diet				
705	D1	no collection			
702	D3	15.4876	15.2193	22.691	22.8095
2113	D2	nd	nd	81.5647	80.4622
706	D2	4.2871	4.0723	59.1676	58.751
26G	D1				
NOTAG	D3	4.1209	4.4684	27.8725	26.2787
Background		0	0	2.1934	2.5947

ANIMAL #	Diet				
705	D1	9.0614	9.1097	29.3257	28.9072
702	D3	13.5588	12.7523	18.788	19.2237
2113	D2	nd	nd	89.6845	90.35
706	D2	7.1424	6.73	66.8816	67.3366
26G	D1	13.4374	14.9428	41.1943	41.0017
NOTAG	D3	3.8297	4.208	35.7128	30.9666
Background		0	0	2.3645	2.6287

ANIMAL #	Diet				
705	D1	10.222	10.6014	28.7005	28.9311
702	D3	14.1861	14.7899	22.7985	22.4836
2113	D2	nd	nd	84.4887	84.5857
706	D2	5.6245	6.2043	48.4205	47.5313
26G	D1	5.9625	6.2425	20.711	20.4992
NOTAG	D3	3.2282	3.6291	30.5692	31.2181
Background		0	0	2.8506	2.3947

Some gas data used in Manuscript II
RESTRICTED FEEDING (Period 1)

ANIMAL #	Diet	INJ 1 sf6(ppt)	INJ 2 sf6 (ppt)	INJ 1 ch4(ppm)	INJ 2 ch4 (ppm)
708	D2	19.3772	19.8047	42.9125	41.9203
1G	D1	83.7126	83.1814	147.5218	146.1938
74G	D3	29.1324	29.2238	58.4975	60.6153
704	D3	no collection			
709	D1	10.6476	10.619	26.3541	26.1555
20G	D2	45.4251	46.1747	74.9012	74.7948
Background		0	0	2.111	1.8965

nd=not detected

ANIMAL #	Diet				
708	D2	23.9915	24.2004	47.3636	47.4123
1G	D1	45.0065	44.9844	74.62	77.205
74G	D3	15.1132	14.788	25.157	25.4915
704	D3	no collection			
709	D1	9.1323	9.3439	21.0721	21.0059
20G	D2	60.792	59.797	69.8557	69.5878
Background		0	0	2.5864	2.3903

ANIMAL #	Diet				
708	D2	1.9681	1.9499	3.4101	3.3532
1G	D1	36.8214	36.7919	49.0758	46.9767
74G	D3	10.5059	10.7987	18.6082	18.4892
704	D3	no collection			
709	D1	59.548	59.6313	109.9452	109.3001
20G	D2	31.7569	31.7354	40.1067	39.9063
Background		0	0	1.8919	1.8726

ANIMAL #	Diet				
708	D2	14.2718	14.7413	28.2068	27.3891
1G	D1	41.9229	42.0038	52.3802	52.8974
74G	D3	15.9724	16.4085	29.0996	29.3081
704	D3	19.8358	19.7577	27.1639	25.6107
709	D1	44.5722	43.6434	82.6928	83.7383
20G	D2	9.2505	9.3071	12.856	12.7053
Background		0	0	1.4321	1.1653

ANIMAL #	Diet	INJ 1 sf6(ppt)	INJ 2 sf6 (ppt)	INJ 1 ch4(ppm)	INJ 2 ch4 (ppm)
705	D3	16.1004	16.321	25.0352	24.7746
702	D2	15.9421	16.4049	34.3972	37.3039
2113	D1	nd	nd	26.5096	23.6387
706	D1	no collection			
26G	D3	18.7774	18.8739	27.701	27.4089
NOTAG	D2	no collection			
Background		0		0.5913	0.5923

ANIMAL #	Diet				
705	D3	18.4967	18.7516	29.0138	28.9378
702	D2	26.4256	26.9814	35.7933	35.5138
2113	D1	nd	nd	46.1386	48.2695
706	D1	no collection			
26G	D3	no collection			
NOTAG	D2	no collection			
Background		0	0	0.6652	0.6692

ANIMAL #	Diet				
705	D3	8.9737	9.2624	13.356	13.3696
702	D2	20.1345	19.8686	26.3132	26.2207
2113	D1	nd	nd	22.9397	24.7313
706	D1	5.3	6.3096	170.1623	169.2419
26G	D3	no collection			
NOTAG	D2	2.1417	2.1354	26.07	25.8767
Background		0	0	1.111	1.194

ANIMAL #	Diet				
705	D3	3.0045	4.0795	5.8625	5.7339
702	D2	16.3792	15.975	26.0814	26.0363
2113	D1	nd	nd	11.3198	11.1643
706	D1	nd	nd	31.4006	31.0053
26G	D3	9.0807	9.4582	13.0323	17.1729
NOTAG	D2	no collection			
Background		0	0	0.967	0.9735

Some gas data (SF6 and CH4) used in Manuscript III

PERIOD II (JULY 20-29)

July 20/21 (IN)

S- steers	INJ 1 sf6(ppt)	INJ2 sf6(ppt)	INJ 1 ch4(ppm)	INJ 2 ch4(ppm)
429G	5.8196	5.9827	46.1706	45.9648
174G	3.105	3.1242	17.4407	17.742
234G	2.6274	2.0619	20.2951	20.0574
330G	3.1016	3.1054	17.8313	17.7384
C-steers				
293G	8.3135	8.591	32.7843	32.7414
229G	9.1228	9.274	46.7262	46.5916
285G	7.487	7.2223	55.8705	55.1459
189G	9.1082	9.1112	44.3343	44.3913
BKD	0	0	1.3456	1.45321

July 21/22 (IN)

S-steers	INJ 1 sf6(ppt)	INJ2 sf6(ppt)	INJ1 ch4(ppm)	INJ2 ch4(ppm)
429G	1.9826	1.7464	9.6502	9.7307
174G	3.3857	2.9614	17.6501	17.5118
234G	2.9701	2.8967	15.0191	14.8845
330G	3.1184	2.9072	19.2999	19.2614
C-steers				
293G	8.2129	7.9091	35.4491	35.2229
229G	4.6795	3.7956	25.8453	25.7234
285G	2.7028	2.7994	16.5312	16.4601
189G	5.3493	5.4757	24.9248	24.9862
BKD	0	0	1.2346	0.9988

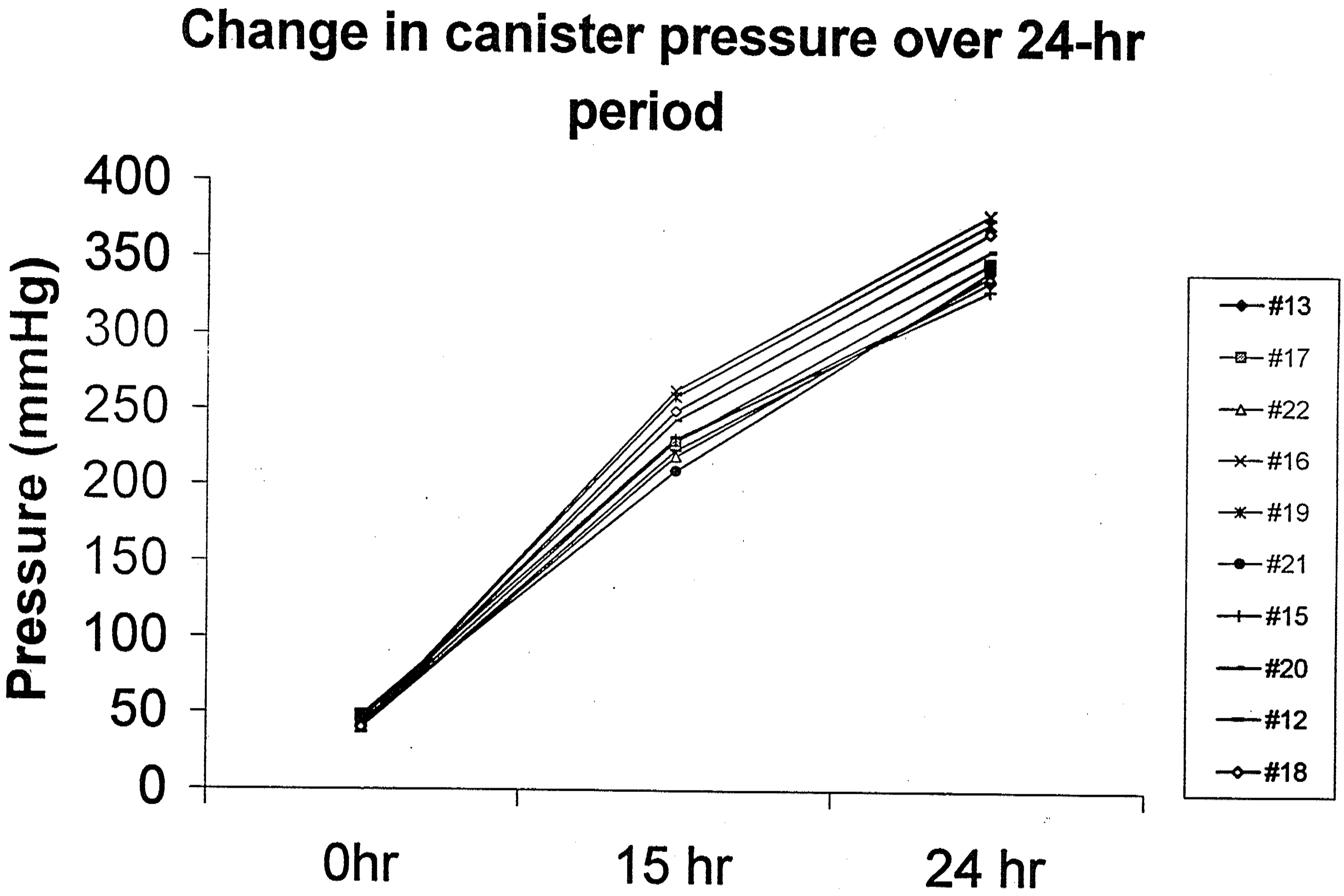
July 27/28 (OUT)

S- steers				
429G	2.3664	2.5581	9.6661	9.6229
174G	3.4605	3.8212	21.946	21.834
234G	7.635	7.4066	36.0618	35.8381
330G	5.7406	5.7669	30.5791	30.5835
C-steers				
293G	15.2797	15.5503	63.5118	63.6303
229G	3.8952	4.4422	24.993	26.4595
285G	7.3052	7.2234	40.3608	40.2035
189G	10.3149	10.9592	43.8987	43.4014
BKD	0	0	2.2421	2.9432

July 28/29 (OUT)

S-steers				
429G	2.2116	2.21	11.7554	11.8177
174G	6.6603	6.3341	32.6882	32.6489
234G	4.021	4.4004	32.0131	32.9786
330G	3.1273	2.9053	22.0124	21.7002
C-steers				
293G	8.925	8.9414	44.8738	44.609
229G	5.6871	5.5884	32.0214	30.6309
285G	6.9043	7.4123	43.3258	43.3027
189G	11.8666	11.4792	53.207	52.6995
BKD	0	0	2.453	2.1456

BKD= background gas concentrations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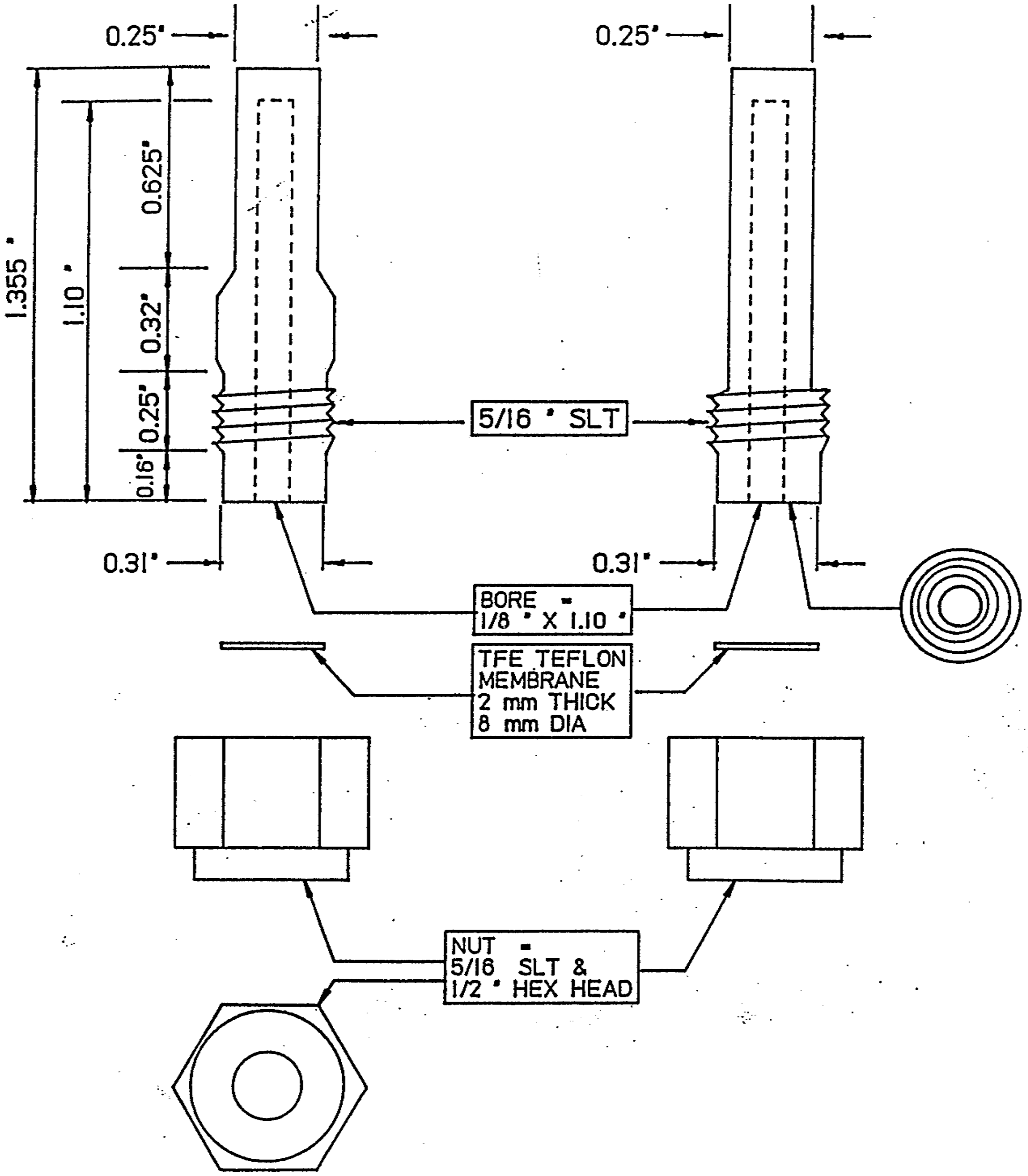

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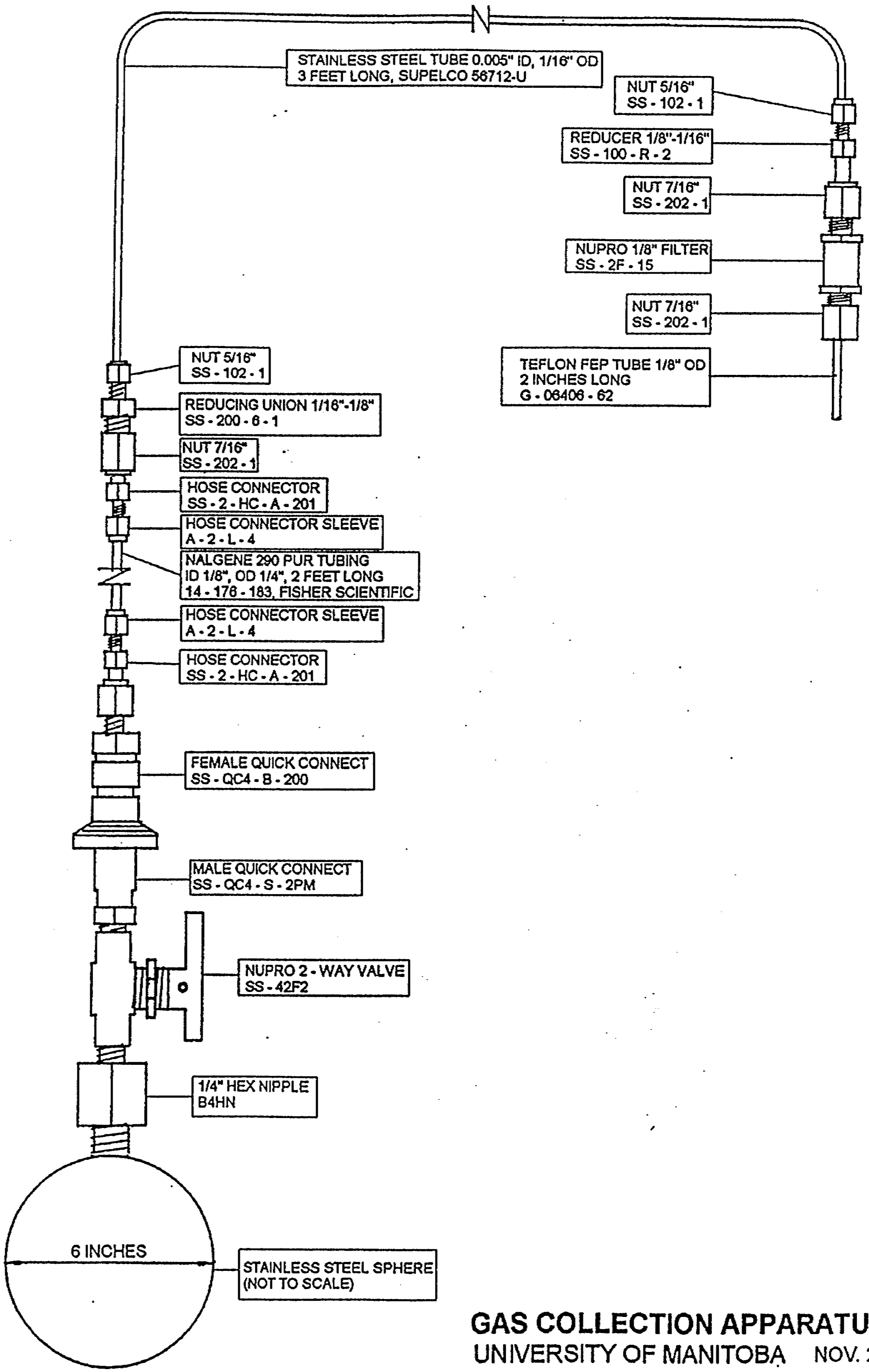
Figure 1		Drawn SRM
SF_6 PERMEATION TUBE		
Design	DR PAUL McCAUGHEY	



GAS COLLECTION APPARATUS

UNIVERSITY OF MANITOBA NOV. 2001