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EFFECTS OF ENVIRONMENTAL TEMPERATURE, DIET, AND FEEDING LEVEL ON METHANE PRODUCTION IN STEERS

by

ORYSIA IRYNA NATALKA DMYTRUK ©

A thesis submitted to the Faculty of Graduate Studies and Research in partial fulfillment of the requirements for the degree of MASTER OF SCIENCE

in

ANIMAL SCIENCE

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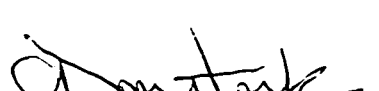

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The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies and Research for acceptance, a thesis entitled EFFECTS OF ENVIRONMENTAL TEMPERATURE, DIET, AND FEEDING LEVEL ON METHANE PRODUCTION IN STEERS submitted by ORYSIA IRYNA NATALKA DMYTRUK in partial fulfillment of the requirements for the degree of MASTER OF SCIENCE in ANIMAL SCIENCE.

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ABSTRACT

Twelve Hereford steers (373 ± 24 kg) were used to examine the effects of environmental temperature (-23, 19, or 29 °C), diet (100% hay or 90% concentrate and 10% hay), and feeding level (50 and 100 g $kg^{-0.75}d^{-1}$ of dry matter) on feed digestibility, digesta retention time, methane production, and heat production. The digestibility of acid detergent fiber was 17% lower (P=0.006) at -23 °C than 29 °C but the digestibilities of other dietary components were not influenced by temperature. Digestibilities were affected (P>0.05) by diet but not feeding level. No differences (P>0.05) in retention time of digesta in the rumen or total digestive tract were detected. The percentages of feed energy lost as methane were 5.2, 5.5, and 6.3% at 29, 19, -23 °C, respectively (P=0.05). Heat production of the steers was not affected (P>0.05) by temperature at the high feeding level but was greater at -23 than 19 or 29 °C at the maintenance feeding level.

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LIST OF ABBREVIATIONS

°C	Degree Celsius
ADF	Acid Detergent Fiber
B.C.	British Columbia
BCM	Bromochloromethane
CFC	Chlorofluorocarbon
CH_4	Methane
CO	Carbon Monoxide
Co-EDTA	Ethylenediaminetetraacetic acid chelates of Cobalt
Co-MRT	Cobalt - Mean Retention Time
CO_2	Carbon Dioxide
CP	Crude Protein
Cr	Chromium
CR-EDTA	Ethylenediaminetetraacetic acid chelates of Chromium
Cr-MRT	Chromium - Mean Retention Time
d	Day
DE	Digestible Energy
DM	Dry Matter
FAO	Food and Agricultural Organization
g	Gram
GE	Gross Energy
H_2	Hydrogen
HCl	Hydrochloric Acid
hr(s)	Hour(s)
kg	Kilogram
kJ	Kilojoule
L	Litre
lb	Pound
ME	Metabolizable Energy
MEI	Metabolizable Energy Intake
ml	Milliliter
mo	Month
mol	Mole
MRT	Mean Retention Time
N	Nitrogen
NaOH	Sodium Hydroxide
NDF	Neutral Detergent Fiber
NE	Net Energy
NOx	Nitrous Oxides
O_3	Ozone
OECD	Organization for Economic Co-operation and Development
$OH^{\bullet}$	Hydroxyl Radical
OM	Organic Matter

P	Probability
ppb	Parts Per Billion
PVC	Polyvinylchloride
SO_2	Sulfur Dioxide
Tg	Teragram = 1×10^{12}g
TM	Trace Mineralized
U.S.	United States of America
VFA	Volatile Fatty Acid
vol	Volume
wk	Week
wt	Weight
yr	Year

CHAPTER I -
INTRODUCTION

The concern over an increasing earth temperature due to the greenhouse effect was originally focused on carbon dioxide (CO_2) emissions. However, in the 1970s and 80s concern shifted to other gases such as methane (CH_4), nitrous oxide (NO_x), chlorofluorocarbons (CFC), and ozone (O_3) (Graedel and Crutzen 1989; Byers 1990; Drennen and Chapman 1991; Johnson and Johnson 1995). Scientists, in measuring Arctic and Antarctic ice cores, have found that over the last 300 years the concentrations of CH_4 and other greenhouse gases have increased exponentially (Warrick and Jager 1986). The current concentration of CH_4 is estimated to be approximately 1800 ppb (Khalil at al. 1993). At the current production rate of more than 500 Tg (1 Tg = 1 x 10^{12} grams), CH_4 is expected to cause 15 to 17% of the total global warming over the next 50 yr (IPCC 1992). The amount of CH_4 produced and knowledge of its effects have led to the examination of several CH_4 sources.

The sources of CH_4 are fairly well established. Examples of such sources are enteric fermentation, natural wetlands, biomass burning (eg. forest fires, land clearing), rice production, waste treatment, coal mining, fossil fuel exploration, transport, and combustion (eg. hydrocarbons) (Matthews and Fung 1987; Jaques 1991; Moss 1992; Johnson and Johnson 1995). The classification of the sources remains fairly arbitrary due to the fact that a clear line does not exist between natural CH_4 sources and those created through human intervention. On a world wide basis, the greatest contributor of CH_4

production is wetlands at 26%, followed by rice growing at 20%, all domestic animals at 15%, and the fossil fuel industry at 14% (Cohen 1992). Within each of the categories, the estimates are quite variable depending upon the method of CH_4 accounting. Cattle produce approximately 75% of the 15% of CH_4 produced by domestic animals with the majority of the remainder being produced by other ruminants such as water buffalo, sheep, and goats (Crutzen et al 1986; Moss 1992).

There are approximately 1.3 billion cattle globally, which produce a total of 65-100 Tg/yr of CH_4 or approximately 45 kg/animal/yr (Czerkawski 1969; Crutzen et al. 1986; Okine et al. 1989; Drennen and Chapman 1991; Moss 1992). However, due to the variability of these figures and the inability to measure some of the sources, the potential for an overestimation of some of the CH_4 sources exists. Byers (1990) suggests that an overestimation of CH_4 production of approximately 15% exists.

Animal nutrition research, in the past, has focused on finding methods to improve animal productivity and reducing CH_4 emissions, due to its energetic inefficiency, as opposed to because of its role in global warming (Johnson and Johnson 1995). The energetic inefficiency stems from the fact that energy, die to fermentation in the rumen, is released as CH_4 as opposed to being directed towards animal growth or productivity. However, due to the increased interest in CH_4 emissions and its direct effect on climate, recently more interest has been given to its potential contribution to climate change and impact on global warming.

Cattle lose approximately 6% of their dietary intake as eructated CH_4 (Johnson and Johnson 1995). This value varies between 2-3% and 10-16% depending on different factors such as feed quality (Johnson et al. 1972; Moss 1992; Hutcheson 1994). It has been documented in the literature that the amount of CH_4 lost from ruminants, particularly steers and sheep, is dependent upon factors such as alteration of environmental temperature, diet, and feeding level.

The literature is fairly limited with respect to the effect of temperature on CH_4 production in ruminants, particularly in warm temperatures, and few experiments have been conducted on more than two animals. Kennedy and Milligan (1978) observed that CH_4 production by sheep, in a cold environment, was decreased by 23%. Similarly, Graham et al. (1959) reported that as temperature decreased from 33°C to 8°C CH_4 production was reduced by 20%. This decreased CH_4 production was expected because the digesta passage rates increased, digestibility decreased, and microbial fermentation of digesta decreased in the cold (Lirette et al. 1988; von Keyserlingk and Mathison 1993; Young et al. 1989; Kennedy and Milligan 1978). Okine et al. (1989) reported that the previous relationship was consistent with a negative correlation between ruminal passage rate and CH_4 production. However, von Keyserlingk and Mathison (1993) found that more CH_4 was produced by sheep housed at 5°C than those housed at 21°C. This difference indicated that digesta passage rate was not the only factor affecting CH_4 production. Kennedy and Milligan (1978) and Kennedy et al. (1986) observed a

proportional decrease in acetic acid and increase in propionic acid in the cold environment. This indicated that temperature may also be a factor affecting volatile fatty acid production.

The diet type and quality also affects the amount of CH_4 produced from ruminants. Moss (1992) observed that CH_4 production increased proportionately with the amount of concentrate diet to the level of 60-80%, after which the level decreased. This decrease was also observed by Abo-Omar (1989), Carmean (1991), and Hutcheson (1994) with very high concentrate diets. Ørskov et al. (1968) and Van Soest (1982) extrapolated that the change in CH_4 production was due to a shift in the fermentation pattern in the rumen from acetate to propionate when a high concentrate diet is fed. This trend is reversed when a hay diet is fed (Fahey and Berger 1988).

Feeding level may be another factor affecting the relationship between environmental temperature and CH_4 production. Rogerson (1960) determined, using only a few animals, that CH_4 production in cattle was not affected by environmental temperature when the animals were fed at or below maintenance, but was reduced with increasing temperature and intake. The increased CH_4 production in sheep housed at a decreased temperature observed by von Keyserlingk (1992) was thought to be influenced by the effect of environmental temperature upon feeding level. Blaxter and Wainman (1961b) determined that CH_4 losses as a percentage of daily energy intake decreased with

an increasing feeding level. Blaxter and Clapperton (1965) confirmed this by determining that a negative relationship existed between CH_4 production and feeding level.

In light of the above observations, this study was designed to determine the effect of environmental temperature, diet, and feeding level on the amount of CH_4 produced from steers. The hypotheses to be examined in this study were: 1) the response of CH_4 production to temperature is curvilinear with CH_4 decreasing at both high and low temperatures with a peak at ambient temperature; 2) animals fed forage diets produce more CH_4 in cold temperatures than animals fed concentrate-based diets; 3) CH_4 production is not affected by cold temperatures at low feeding levels, while at high temperatures, feeding level has more effect on CH_4 production.

CHAPTER II -
LITERATURE REVIEW

2.1 Methane and the Greenhouse Effect

2.1.1. The Greenhouse Effect

The absorption and radiation of long-wave infrared energy of atmospheric gases, CO_2, CH_4, $N0_x$, $S0_2$, O_3, and CFC's, has been suggested as the cause for climatic change and global warming (Graedel and Crutzen 1989; Byers 1990; Johnson and Johnson 1995). This global warming phenomenon has been called the 'Greenhouse Effect'. The Greenhouse effect is the principle used to describe the phenomenon of trapping heat near the earth's surface. This phenomenon normally allows the earth which radiates energy at wavelengths and intensities characteristic of a body at -18°C, to maintain an average temperature some 33°C higher (Schneider 1989a).

Historically, CO_2 has been focused on as the primary greenhouse gas. However, more recently, the concern has shifted to the other gases, such as CH_4, NO_x, CFC's, and trophospheric O_3 (Drennen and Chapman 1991). The main reason for increased interest is that measurements between 1975 to 1985 suggested that non-CO_2 gases contributed approximately 50% to the warming effect. In the same period of time, the concentrations of CO_2, CH_4, and CFCs (depending on type of CFC) were estimated to increase by 4.6%, 11.0%, and 101% to 103%, respectively (Ramanathan 1988). The latter two increases are important because CH_4 and CFC trap 25 and 15,000 times more of the infrared radiation

than CO_2 and therefore have a greater impact on the Greenhouse Effect (Drennen and Chapman 1991).

Air trapped in Antarctic and Greenland ice sheets indicates a direct correlation between greenhouse gases, such as CH_4, and dramatic climate changes (Schneider 1989a). For example, ice core samples from Greenland, dating back 300 years, record an exponential increase in gas concentrations of trophospheric CH_4 (Warrick and Jager 1986). It has been suggested that CH_4 is neither destroyed in the trapped air nor diffuses out of deeply buried ice and therefore, CH_4 ice core values are considered to be accurate (Rasmussen and Khalil 1984). Measurements of preindustrial CH_4 trapped in the polar ice showed CH_4 concentrations remaining relatively stable at approximately 700 ppb until nearly 100 years ago when concentrations began to rise to the present levels of approximately 1800 ppb (Warrick and Jager 1986; Graedel and Crutzen 1989; Khalil et al. 1993). This exponential increase in concentration of atmospheric gases correlates with an increase in human population growth and other anthropogenic activities (Warrick and Jager 1986).

A precedent for reducing greenhouse gas emissions has been set by the Montreal Protocol. This protocol was the first substantive international agreement to reduce CFCs (Drennen and Chapman 1991). Also, other strategies were being negotiated ranging from "agreements on single gases, such as carbon dioxide, to forging comprehensive

agreements which establish composite allowable emission levels for several or all known greenhouse gases" (Drennen and Chapman 1991). These agreements which focused on emission reduction must have regulations which account for country dependent effects (Drennen and Chapman 1991). A weighting scheme was suggested to allow consistent reduction goals to be set. Setting goals provided counties with broad latitude as to how to achieve the target given the country's particular needs and cultural values. An international agreement which would focus reductions of CO_2 and other greenhouse gases was also suggested. The agreement would increase the importance of and need for inclusion of developing countries because of their contribution to CH_4 production from animal populations and rice paddy emissions.

2.1.2. Methane as a Greenhouse Gas

The awareness of the presence of CH_4 as a potentially problematic greenhouse gas began in the 1940s. In the past 300 years, since the beginning of the Industrial Revolution, CH_4 production has more than doubled; whereas CO_2 has increased by approximately 25% (Wuebbles and Edmonds 1991; Clark 1989). Total CH_4 in the earth's atmosphere is approximately 4000 Tg and the recorded total global emission of CH_4 to the atmosphere varies between 450 to 1000 Tg/yr (Khalil and Rasmussen 1983). It is calculated that 50-80 Tg more CH_4 yearly is emitted into the atmosphere than exceeds terrestrial and atmospheric oxidation (Khalil and Rasmussen 1983; Johnson and Johnson 1995). Atmospheric CH_4 gas has been increasing at a rate of 1.1% per year in this decade,

with an average residence time in the atmosphere of approximately 10 years (Khalil and Rasmussen 1983; Warrick and Jager 1986). At this rate, 15 to 17% of global warming over the next 50 years is expected to be caused by CH_4 (IPCC 1992).

The concern with CH_4 as a greenhouse gas lies in the fact that CH_4 is 25 to 30 times more potent as a greenhouse gas than CO_2. However, the CH_4 molecule has a much shorter atmospheric lifetime (Drennen and Chapman 1991; Rogers and Whitman 1991). Methane decays to CO_2 in 10 to 15 years whereas CO_2 has a lifetime of approximately 230 years (Lashof and Ahuja 1990).

2.1.3. Latitudinal and Seasonal Variations

A comparison of the measurements between Greenland ice cores and Byrd ice cores in Antarctica have found the concentration of CH_4 to be 10% higher in the Arctic ice cores. The latitudinal variations are most likely due to the larger land area and the abundance of land based methanogenic sources in the northern hemisphere (Rasmussen and Khalil 1983). Laboratory analysis of the trapped gases showed that seasonal CO_2 and CH_4 levels in the atmosphere vary in step with each other and the average local temperature. Methane concentrations in the northern hemisphere are at a minimum (2% lower) during the summer (June, July, August) and rise rapidly to the yearly maximum in the fall. The rapid increase in the fall suggests a large source of CH_4 is released in the fall

(Rasmussen and Khalil 1984). An explanation for the rapid increase of CH_4 production in the fall is that the seasonal sources present are so great that by late summer they have contributed enough CH_4 to overcompensate for the summer's depletion (Nisbet 1989). The seasonal cycles of CH_4 in the southern hemisphere are also lowest in the late summer and fall (February to May); however only sparse data exist for these locations.

2.1.4. Sources and Sinks of Methane

Global CH_4 sources have been estimated by researchers such as Khalil and Rasmussen (1983) and Bingemer and Crutzen (1987). This CH_4 budget can be divided into production sources and sinks of CH_4. Methane sources averaged about 425 Tg/yr and the sinks account for 340 Tg/yr. The discrepancy in the amount of CH_4 produced by the sources and absorbed by the sinks contributes to global warming.

2.1.4.1. Sinks

There are two main sinks for CH_4 in the environment: 1) photochemical oxidation by a tropospheric $OH^{\bullet}$ radical and 2) CH_4 decomposition by soil methanotrophic organisms in biomass, soils, and peatlands. The $OH^{\bullet}$ radical is the primary sink of CH_4, due to its high chemical reactivity (Graedel and Crutzen 1989). Approximately 85% of the CH_4 emitted into the atmosphere is destroyed by reacting with $OH^{\bullet}$ in the troposphere (Cicerone and Oremland 1988). The tendency towards a slow decline in the $OH^{\bullet}$ levels may add to the rate of CH_4 increase in the long term (Khalil and Rasmussen 1983). $OH^{\bullet}$

distribution is also influenced by the concentration of carbon monoxide (CO) in the atmosphere (Cicerone and Oremland 1988). Increased CO and CH_4 levels decreased the $OH^{\bullet}$ concentrations in the atmosphere (Cicerone and Oremland 1988).

The atmospheric $OH^{\bullet}$ levels vary seasonally in the northern and southern hemispheres. In addition to the natural seasonal variation of CH_4 production, the seasonality of production systems reduces the ability of $OH^{\bullet}$ to react with CH_4. During a northern summer and fall, the transport of $OH^{\bullet}$ from the northern to southern hemisphere is occurring while the destruction of CH_4 by $OH^{\bullet}$ is intense (Nisbet 1989). During the seasons where CH_4 self-cleaning diminishes in the southern hemisphere, the northern hemisphere experiences an increase in self-cleaning and vice versa.

The secondary sink for CH_4 in the environment is methanotrophic bacteria. The microorganisms limit the flux of CH_4 to the atmosphere from various ecosystems by a relatively slow rate of utilization of CH_4 from the atmosphere. Soil microbes are considered to be a sizeable global sink for CH_4 due to the fact that they remove the gas slowly from the atmosphere over a large area (Cicerone and Oremland 1988).

2.1.5. Classification of Sources

The literature describes two methods of classifying the sources of CH_4. The first classification system examines the distinction between natural and anthropogentic

sources and the second method describes the abiogenic and biogenic sources. The following sections will present the two classification systems.

2.1.5.1. Natural and Anthropogenic

Methane production can be partitioned between natural processes and anthropogenic activities. Natural sources produce 30% of the total annual production and the remaining 70% is accounted for by sources related to human activities (Moss 1992). Examples of natural sources are wetlands, lakes, tundra, wild fires, decay, termites, oceans, lakes, and wild animals (Jaques 1991). As free living wild ruminants are difficult to enumerate and sample and are not frequently studied. Wildlife, in gross CH_4 measurements are assumed to have a CH_4 production rate similar to domesticated species. Anthropogenic sources are those produced due to human activities. This category can be divided into three subgroups: 1) energy use, direct anthropogenic; 2) non-energy use, direct anthropogenic; and 3) indirect anthropogenic sources (Jaques 1991). The energy use sources encompass fossil fuel and biomass use, while the non-energy sources include the use of raw CO_2, incineration, cement production, biomass, landfills, manure, large animals, natural gas leaks, and coal mines. The indirect sources are more difficult to distinguish from some of the natural sources as they are seen as the by-product of activities not directly produced by humans but controlled by humans. These sources are agricultural lands, decay of wood products, fuel combustion, prescribed fires, and incineration.

2.1.5.2. Abiogenic and Biogenic

The second system of classification of CH_4 sources differs mainly in the allocation of CH_4 production from ruminants. However, this system can be divided into the sources of CH_4 produced from living matter (biogenic) or CH_4 not produced from living matter (abiogenic). The primary biogenic sources are rice paddies, ruminants, freshwater swamps and marshes (wetlands), and landfills. These sources produce CH_4 under strict anaerobic conditions through the microbial activity of mineralization of organic carbon (Matthews and Fung 1987; Moss 1992). Abiogenic sources are produced by activities such as biomass burning, coal mining, fossil fuel exploration, and transport (Matthews and Fung 1987). The abiogenic sources constitute 33% of the total global emissions (Moss 1992) and the biogenic make up the remaining portion.

2.1.6. Contributors of Methane Production

Many estimates of the amount of CH_4 produced are discussed in the literature. However, in examining the estimates a broad range of values emerges. The discrepancy in values most likely arises from the scale examined and the manner by which the sources are grouped. Although the most major sources of CH_4 have been identified, large uncertainties exist for estimates of the strength of the source (Matthews and Fung 1987). The uncertainty of CH_4 production values is exhibited in the variability of estimates

provided by Khalil and Rasmussen (1983) and Seiler (1984). The estimates provided for the annual CH_4 production were 390 - 765 Tg and 269 - 518 Tg, respectively.

On a world wide basis, wetlands are the single largest contributors of atmospheric CH_4 at 26%, followed by rice production at 20%, all domestic animals at 15%, and the fossil fuel industry at 14% (Cohen 1992). Wetland measurements range from 11-300 Tg/year with the majority of estimates being in the lower half of the range (Matthews and Fung 1987). The wide range of uncertainty and variability of the values reported regarding CH_4 production in wetlands is due to the lack of knowledge about wetlands and their emissions characteristics (Matthews and Fung 1987). An example of the variability is exemplified by the fact that in 75% of the tropics and subtropics flooding causes the soil to be methanogenic for at least of part of the year. Oceans, lakes, water logged tundra soils, humans, and herbivorous insects are estimated to produce a total of 7-22 Tg/year (Warrick and Jager 1986). Rice paddies have large variation in their production of CH_4 ranging from 30 to 220 Tg/year. The variability of these values is a result of incompletely recorded production rates for more than 95% of the total harvestable rice paddy area because most values from the Far East are not available (Warrick and Jager 1986). Natural aerobic soils produce 700 Tg/year but also decompose atmospheric CH_4 to a certain extent. (Warrick and Jager 1986). Solid wastes produce 10 Tg/year. Variability is also noted in the estimates for enteric fermentation. Khalil and Rasmussen (1983) and Seiler (1984) state that enteric fermentation produces 150±50 Tg and 72-99 Tg,

respectively. The enteric emissions from animals, particularly cattle, will be examined in more detail in the following section.

2.1.6.1. Animal Emissions

Several authors have provided estimates for global CH_4 production of animals. However, none of the authors provided a substantial amount of detail as to how the values were derived. Differences in the estimates were considered to have arisen because of differences in animal observations over time and differences in assumptions about emission rates by the animals.

The earliest estimated world wide production rates were published by Hutchinson (1949). He estimated, in the 1940s, that CH_4 emissions from large herbivores was 45 Tg/year (Crutzen et al. 1986). In 1974, Ehhalt calculated the global production of domestic ruminants to be 100 Tg for 1970. Crutzen (1983) estimated ruminant CH_4 production to be 60 Tg and Seiler (1984) suggested a value of 70-100 Tg (Crutzen et al. 1986). Cicerone and Oremland (1988) provided several estimates which varied from 72-99 Tg to 100-200 Tg.

Byers (1990) provides an assessment of global CH_4 production values from animals. He derives that beef, dairy, draft, and collateral cattle produce 7% of the total global CH_4 produced. Other domesticated and wild ruminants, and wild non-ruminants

produce 3.7%, humans account for approximately 1%, and termites, and other insects and invertebrates produce 0.6% and 2%, respectively. The estimated proportion of the contribution of cattle to the global CH_4 production suggested by Byers (1990) is lower than other estimates suggested in other literature. Moss (1992) and Jaques (1991) examine the proportion of CH_4 produced by domestic animals relative to other animals and humans. They estimate that domestic animals produce approximately 93% of the total CH_4 emitted into the environment by animals. Cattle, due to their size, energy intake, and numbers, are the major contributors producing 74% of animal CH_4 emissions. Sheep and goats produce approximately 13% and camels produce 1%. Non- ruminant emissions, predominantly horses and mules, produce 2%. Wild animals globally produce about 5% of total animal CH_4 (Moss 1992).

2.1.6.2. Cattle Emissions

As previously stated, animals produce approximately 15% of the total global CH_4 emissions. There are approximately 1.3 billion cattle in the world which produce a total of 65-100 Tg/yr. of CH_4. The broad range of estimates is due to the variability of cattle production between the developed and developing world. Approximately 76% of CH_4 emissions from animals occur in the northern hemisphere due to the latitudinal distribution of animals (Cicerone and Oremland 1988). An average annual CH_4 production rate of 45 kg/animal/yr was recorded due to the fact that cattle in the developed world produce 35 kg/animal and in the developing world 55 kg/animal of CH_4

is produced (Krause et al. 1992). Crutzen et al. (1986) based on FAO (Food and Agriculture Organization) numbers, and Cicerone and Oremland (1988) are in agreement with these CH_4 values estimates even though these two sources determine the values differently. Crutzen et al (1986) derived the numbers from an energetic perspective and Cicerone and Oremland based their calculations on a 500 kg cow averaging 200 L of CH_4/day.

Variable estimates of cattle CH_4 production have been reported in the literature. Reliable global estimates of CH_4 sources exist but are not consistent due to the difficulty of enumerating all cattle and the variability of CH_4 output between animals (Drennen and Chapman 1991). Moss (1992) reports that the global average of CH_4 produced by cattle falls within the range of 100 - 600 L CH_4/day. Okine et al. (1989) obtained measurements of CH_4 production of approximately 250 L/day/cow, or about 65 kg/year. Czerkawski (1969) estimated CH_4 production of cattle on a maintenance diet to be 150 - 170 L CH_4/day and on a production level diet to be 280 - 300 L CH_4/day. The higher production value was also shown to be approximately 360 L CH_4/day for dairy cattle in the U.S. (Drennen and Chapman 1991).

The reason for the wide ranging values associated with CH_4 production is primarily due to the better quality of feed used to feed 47% of the world's cattle in the developed world (Anastasi and Simpson 1993; Krause et al. 1992). Dairy cattle have the

highest input of quality and quantity of feed for milk production. In developing countries, a lower quality of feed and feed input is used and the animals are also used for draft purposes, not only for meat and dairy production (Krause et al. 1992).

2.1.6.3. Emissions from Animal Wastes

Animal wastes contribute 2.7% of global CH_4 emissions (Byers 1990). These emissions contribute substantially to the CH_4 emissions to the atmosphere if they are held in lagoons and allowed to decay anaerobically (Byers 1990). The potential for CH_4 production is also directly related to the amount of volatile solids present in the waste (Jaques 1991). Prior to determining the CH_4 production from waste, the factors of average live animal mass, total manure produced, total quantity of solids and volatile solids must be known as they affect the final values. In certain countries, such as India, where cattle are abundant, the manure is often collected and fermented in biogas digesters. These biogas plants are set up in the villages to supply clean fuel and save the manure, a rich source of crop nutrients, from burning (Singh and Gupta 1990). This is an efficient method of harnessing CH_4 and making it useful. It is important to note that animal wastes which are deposited on a pasture by grazing cattle make a negligible contribution to atmospheric CH_4 (Lodman, et al. 1991). The extent of CH_4 emissions from cattle wastes on pasture are dependent upon the temperature and moisture content of the environment and the manure. For example, more CH_4 is produced under conditions that tend to lead to the establishment of an anaerobic environment.

2.1.7. Overestimation of Importance of Livestock Contribution to the Greenhouse Effect

Cattle are viewed as a major contributor to atmospheric carbon imbalance and global warming due to their association with agricultural production. However, the contribution of CH_4 to the atmosphere by non-domesticated ruminants prior to intensive agricultural production is not usually considered. Richard Johnson, a soils specialist at the Alberta Environmental Centre, upon examining this situation, stated that "There were 65 million buffalo roaming the Canadian prairies less that 120 years ago. Today there are about 12 million cattle. It seems unlikely that the rapid increase in methane can be attributed to them" (Webster 1994). This statement suggests that although cattle are a major agricultural source of CH_4, the inputs and implications of other agricultural sources should be examined and considered equally important.

Agricultural production releases CO_2 indirectly due to the deforestation of land required to support livestock production and to make room for urbanized development. For example, in Latin America, beef production for export is reliant on tropical land clearing. Consumption of beef in the Organization for Economic Co-operation and Development (OECD) countries is linked to the loss of the tropical rain forest because of the importation of beef into developed countries (Krause et al. 1992). The burning of forests and grasslands for production, and agricultural wastes releases an additional 50-

100 Tg of CH_4 into the atmosphere (Rifkin 1992). Rifkin (1992) states that the amount of carbon in the biomass and in the soil humus of the world's forests is greater than the amount present in the atmosphere by 1.3 and 4 times, respectively. Therefore, when trees are cleared and burned for cattle pasture and the heavy machinery combusts its fuel, large amounts of CO_2 are emitted into the atmosphere. The trees which are not cleared are consumed by an increasing population of CH_4 producing termites. The contribution of such activities to the global carbon balance should not be attributed to cattle since it is a consequence of the increased need for human food and would occur even if there was no increase in cattle production.

Cattle are publicised as one of the sources which has created the greatest impact despite the fact that landfills and automobiles produce high levels of CH_4. This perception was shown by Hanson (1991) who stated that: "The methane content of the gas from Burn's bog Landfill in Delta B.C. is equivalent to that produced from 374,000 cattle but the province of B.C. only has 300,000 head of cattle distributed over millions of square miles while the Burn's Bog landfill is concentrated on only 2 square miles and is only one of many landfills in B.C. We appreciate the fact that man is creating a large amount of environmental damage but believe that focusing on trivialities such as methane produced from cattle detracts from the efforts which should be directed to important issues" (Cohen 1992).

The argument is also made that cattle management practices, in addition to the direct contribution of greenhouse gases through biomass burning and CH_4 production, contribute to the increased burning of fossil fuels which increase carbon emission to the atmosphere. For example, fossil fuels are used to operate mechanised agricultural machinery. Rifkin (1992) estimates that 1 gallon of gasoline is used in the production of 1 lb. of U.S. grain-fed beef. Similarly Byers (1990) stated that, "To put methane production for beef cattle in the U.S. in perspective, the methane produced in the course of producing a quarter lb. hamburger was modelled for cattle produced in an average (i.e. 18 mo.) U.S. beef cattle system. Total methane produced is about 80 kg to produce an 1100 lb. animal which results in about 480 lb. of lean retail product. This results in about 40 g of methane for a quarter lb. of hamburger. To put this in perspective, driving 6 miles each way to the store and back to purchase the sandwich in a well tuned car getting 25 mile/gallon would result in the production of 4200 g of carbon dioxide, or 100 times the amount of "greenhouse gas" as the methane generated in the production of the beef. Thus ~ 4200 g of carbon dioxide were produced to drive to the store to obtain a burger that 40 g of CH_4 were evolved to produce".

The importance of cattle as contributors to the CH_4 production has been overestimated by overlooking the source of CH_4's carbon content. The overemphasis of CH_4 from cattle production occurs because of: 1) an overestimation of ruminant inputs

into the carbon cycle and 2) the inclusion of new carbon into the cycle from terrestrial carbon stores.

The principle of carbon cycling can be applied to all biological sources of CH_4, such as rice paddies, cattle, wild animals, and termites. Drennen and Chapman (1991) uphold the view that ruminant CH_4 emissions are overestimated. They state that CH_4 from cattle originates from a carbon source which is currently in the carbon cycle. Therefore, CH_4 released from ruminants is not the same as CH_4 that is released from the anthropogenic sources such as natural gas pipeline leaks and coal mining. The anthropogenic sources are releasing CH_4 which was removed from the cycle tens of thousands of years ago. However, there is always some carbon released into the atmosphere which has not been previously released into the carbon cycle. The previously uncycled carbon accounts for a 6.9% increase in the net increase in atmospheric CH_4 (Drennen and Chapman 1991). However, when carbon cycling is ignored, then there is an 8.7 times overestimation of CH_4 production because all carbon released is considered to be previously unreleased carbon into the atmosphere.

Proper accounting of CH_4 produced globally may decrease the emphasis placed upon cattle to reduce the amount CH_4 produced. Examining the carbon cycle is an all inclusive method which provides an accounting of the global amount of CH_4 produced. The method is effective because it takes into consideration the origin of different carbon

compounds. Carbon products can be released by decomposition or burning through aerobic or anaerobic processes. Because the system is cyclical, increased carbon emissions though compounds such as CH_4 may at times overload the cycle. However, sinks of the carbon will attempt to compensate in order to maintain the balance. The greenhouse effect is occurring because of an imbalance due to increases in the aforementioned types of anthropogenic practices.

2.1.8. Difficulties in Reducing Methane Production from Cattle

The perception exists that it is important to limit the consumption of beef in order to reduce livestock production of CH_4. However, a reduction in global CH_4 production is difficult to achieve only by decreasing cattle numbers (Krause et al. 1992). Krause et al. (1992) state that such a reduction would require a 50% decrease in beef consumption in the developed world to offset the population related growth of CH_4 in the developing world assuming an unchanging per capita beef consumption. For this to be achieved, herd size would have to be limited to the amount required for dairy production. The maximum decrease that could realistically be achieved is 20% of present levels by the year 2100 (Krause et al. 1992).

An overall decrease of 20% of the current CH_4 concentration is required for the atmosphere to reach a stable concentration (Krause et al. 1992). In order for this goal to be achieved, a decrease in cattle CH_4 production, biomass burning, rice paddy production,

landfills, and natural gas and mining losses would need to occur. Mitigative actions would have to be put into place to provide the public with less reason to target the cattle industry. Some mitigative actions proposed are: capturing emitted CH_4 in from animals in feedlots and intensive indoor systems, managing the dietary factors which affect CH_4 production, increasing the productivity of cattle through the use of ionophores, and proper accounting of the carbon cycle (Krause et al. 1992).

2.1.9. Factors Influencing Methane Production from Cattle

Methane is produced as a result of fermentation of carbohydrates in the ruminant digestive system (Jaques 1991). The gases from the microbial fermentation of plant material in the ruminal compartment of the stomach exit the animal through eructation with a gas composition of approximately 65% CO_2, 27% CH_4, and trace other gases (Drennen and Chapman 1991). The rate of CH_4 production is dependent on physiological and dietary factors. Some of the factors influencing CH_4 production in the ruminant are temperature, diet type, feeding levels, digesta passage rates, volatile fatty acids (VFA) composition, and chemical additives to the diet.

2.1.9.1. Temperature

The literature is fairly limited with respect to the effect of temperature on CH_4 production in ruminants, particularly in warm temperatures, and few experiments have been conducted on more than two animals. Kennedy and Milligan (1978) observed that

CH_4 production by sheep, in a cold environment, was decreased by 23%. Similarly, Graham et al. (1959) reported that as temperature decreased from 33°C to 8°C CH_4 production was reduced by 20%. Several studies indicate that CH_4 production may not always decrease when ruminants are in cold environments. von Keyserlingk and Mathison (1993) reported that CH_4 production in sheep at 4.7°C was 25% higher than those at 21°C due to an 8% increase in DM intake in the cold. When the results were expressed as a percentage of dietary digestible energy, 14% more CH_4 was produced in the cold. Rogerson (1960) found that CH_4 production was not consistent between the temperatures of 20 and 40°C. Methane production decreased at high temperatures and high feed intakes but was not affected by temperature at submaintenance intakes.

2.1.9.2. Diet Type

The quantity and nature of the diet largely determines the amount of energy lost in the form of CH_4 (Rogerson 1960). Grain is incorporated into the forage based diets in order to maximize the energy intake of the animals, especially for those required to maintain high levels of production. The energy retrieved from slowly fermented materials is influenced more by a short turnover time in the rumen than readily-fermentable dietary components (Mertens 1977). Research has demonstrated that CH_4 yields have been noted to decrease proportionately with increases in concentrate in the diet to the level of 60-80%, after which CH_4 yields decline abruptly (Moss 1992). The decrease in CH_4 yields is

due to a shift away from acetic acid, the methanogenic precursor, towards propionic acid as a byproduct of carbohydrate digestion.

By examining the effect of a poor quality hay and the addition of a cotton cake and barley meal concentrate supplement, it was concluded that CH_4 losses increased with an improving nutritional level (Rogerson 1960). Methane yields have been found to be greater for high digestibility diets as opposed to those with lower digestibility diets (Moss 1992). Methane losses may be reduced to 2-3% of gross energy intake by feeding high grain concentrates (≥90% concentrate) at near ad libitum intake levels (Abo-Omar 1989; Carmean 1991; Hutcheson 1994; Johnson and Johnson 1995). Methane production was also found to increase proportionally with an increase in the dietary roughage when steers were fed varying proportions of hay and corn at maintenance or twice maintenance (Blaxter and Wainman 1964). Kujawa (1994) determined CH_4 losses fell to 4 to 5% of GE intake when beetpulp was fed at high intakes. Johnson et al. (1972) noted that ruminant CH_4 losses range between 8 and 10% of the gross energy (GE) intake.

Van Soest (1982) extrapolates that a high grain diet and/or the addition of soluble carbohydrates results in a shift in fermentation pattern in the rumen to a reduction in the acetate to propionate ratio. These changes are also associated with a more hostile

environment for the methanogenic bacteria in which passage rates are increased, ruminal pH is lowered, and certain populations of protozoa, ruminal ciliates, and methanogenic bacteria may be eliminated or inhibited (Eadie et al. 1970; Krumholtz et al. 1983). Forage-based diets, on the other hand, affect CH_4 yield indirectly depending upon stage of maturity, method of preservation, species, and climate in which they are grown (McAllister 1996). Methane yield from leguminous forages was found to be generally lower than that produced from grass forages (Varga et al. 1985).

2.1.9.3. Feeding Level

Blaxter and Wainman (1961a) noted that CH_4 losses as a percentage of daily energy intake decreased with an increase in feeding level. In sheep, there was a tendency for CH_4 production to be higher at submaintenance levels with a rate of decline of CH_4 production with increasing feeding levels. This rate of decline was greater in sheep than in cattle on similar rations (Blaxter and Wainman 1961b). The results of this study was confirmed by Blaxter and Clapperton (1965) who determined though 48 trials that a negative regression existed between CH_4 production and feeding level. They stated that although the absolute amount of CH_4 produced increased with an increase in feeding level, the amount of CH_4 produced with increasing feeding level decreased slightly with each increase.

Rogerson (1960) noted similar results where an increased temperature and high level of feed intake decreased CH_4 production while feeding levels at maintenance or lower did not have a noticeable effect on CH_4 produced. At 20°C, approximately 50% more CH_4 was produced at a 6000g feeding level when compared to a 4000 g level. However, at 40°C, only 25% more CH_4 was produced at the higher intake level. Kennedy and Milligan (1978) deduced from the partitioning of CH_4 production, that for sheep at a constant feed intake, fermentation activity was decreased by 1/3 due to cold exposure.

Blaxter (1967) noted a decrease of 12-30% of the amount energy lost as CH_4 per unit feed consumed when intake was increased from maintenance to twice maintenance. More CH_4 is produced per 100 kcal feed consumed with a high digestibility feed at maintenance and increasing feed intake of such feed depresses CH_4 production per unit feed consumed more than with low digestibility feeds (Crutzen et al. 1986). As a percentage of the energy intake, CH_4 production was at 9.2% at a maintenance feeding level and was decreased to 5.3% when the feeding level was increased by 75% for high grain diets (Mathison, unpubl.). Methane yields were determined to be independent of digestibility at twice maintenance and decreased from 6 to 5% of GE intake at three times maintenance when the digestibility of the feed increased from 60 to 90% (Crutzen et al. 1986).

2.1.9.4. Passage Rates and Retention Time

In cold environments, due to increased passage rates, decreased rumen retention time, and reduced microbial fermentation of the digesta, a decline in the production of CH_4 has been noted (Lirette et al. 1988; Delfino and Mathison 1991). The increased rate of passage of digesta decreases the microbial degradation of ingesta and markedly reduces the digestion of more fibrous food materials (Young et al. 1989). The increased passage rate also augmentes the quantity of ingesta that passed through the reticulorumen and reduced feed digestability. In order to compensate for this, there is usually a stimulation of appetite in the cold. Kennedy and Milligan (1978) determined that in cold environments the rumen passage rate constants of fluid and particulates of sheep increased by 54 and 68%, respectively, with a concomitant 30% decrease in CH_4 production. These results were confirmed by Okine et al. (1989) when a 29% decrease in CH_4 production and a 63% increase in fractional passage rate of particulate matter were observed in steers with weights added to the rumen. The depression in digestibility with lowered ambient temperature appeared to be rectilinear in two experiments where sheep were given long, chopped, or pelleted hay at fixed intakes and temperatures from -8 to 18°C (Westra and Christopherson 1976; Nicholson et al. 1980).

2.1.9.5. Volatile Fatty Acids

Johnson et al. (1972) found that the ruminal molar percentage of acetate significantly decreased when CH_4 was suppressed. At the same time, propionate and

butyrate molar percentages increased, although a greater increase in propionate was noted. Thus, there was a narrowing of the acetate:propionate and acetate:butyrate ratios. These changes are also achieved by offering ruminants ad libitum access to diets rich in starch (Ørskov et al. 1968).

The addition of readily fermentable carbohydrates in the diet at maintenance levels increases the ciliate population in the rumen (Bonhomme 1990). The increase in CH_4 production observed in such instances is most likely due to an increase in hydrogen transfer between rumen ciliates and methanogens (Krumholz et al 1983). Removal of the ciliates in sheep and steers reduces the amount of CH_4 regardless of the diet fed to the animals and is accompanied by a reduction of acetate and butyrate as end-products of protozoal fermentation (Whitelaw et al. 1984; Kreuzer et al 1986)

2.1.9.6 Chemical Additives to the Ruminant Diet

The control of CH_4 production can potentially enhance the productivity of the ruminant animal since the ruminant loses approximately 8-10% of GE intake through CH_4 production (Johnson et al. 1972). Chemical additives to the ruminant diet can be categorized into two forms: chemical alteration of feed through additives or ensiling and chemical additives for inhibition of methanogenesis. As previously stated, CH_4 production tends to be increased in mature dried forages (Armstrong 1960; Sundstol 1981). Ensiled forages have lower CH_4 yields than dried, legume forages and also lower

than grass forages (Moss 1992) Thus, low quality forages which are upgraded have a higher nutritive value and have a greater CH_4 yield than high quality forages which are poorly preserved. Methane losses were found to be higher for coarsely chopped rather than finely ground or pelleted diets (Moss 1992; Hironaka et al. 1996). Moss et al. (1994) concluded that low quality forages can be upgraded by chemical additives, such as NaOH or ammonia, and that such upgrading increases the volume of CH_4 produced by wethers but decreases total CH_4 production relative to digestible OM intake.

Rumen methanogenesis has been documented to be inhibited by halogenated CH_4 analogues, such as bromochloromethane (BCM), and ionophore antibiotics, such as monensin and lasalocid (Johnson et al. 1972; Durand 1982; Delfino and Mathison 1991). These compounds, in addition to reducing CH_4, result in a concomitant increase in propionate production. The CH_4 analogues are directly toxic to methanogenic bacteria and increase the production of gaseous H_2, while ionophores transfer electrons from methanogenesis to propionate production without producing H_2 (Demeyer et al. 1986). Other compounds, such as saturated and unsaturated fatty acids, sulphated monounsaturated alcohols, chloral hydrate, branched chain carboxylic acids, and chloroform, have also been examined for their use as anitmethanogenic substances with varying results (Czerkawski et al. 1966; Johnson et al. 1972; Ørskov 1975). Stanier and Davis (1981) in Demeyer et al. (1986) reported that CH_4 production was inhibited more by analogues on high roughage diets because more CH_4 is produced on high fiber diets.

Ruminal fiber digestion is not impaired by such analogues; while monensin may inhibit some fiber digestion as well as depress rumen feed protein degradation and protozoal activity. Johnson et al. (1972) found that BCM suppressed CH_4 production in steers almost completely for 3-6 hrs after feeding without markedly affecting ruminal pH. Methane production gradually recovered up to prefeeding levels by 15 hr. Johnson et al. (1972) found that dietary fat was not an efficient inhibitor of CH_4 production when fed at 3.3% of the steers daily high concentrate ration. Chemical defaunation of the rumen may also be considered as a method of reducing CH_4 production. However, despite the increase in net microbial N yields under such circumstances, rumen protein, fiber degradation, and retention times were depressed along with methanogenesis.

While the antimethanogenic properties of chemicals are useful, the side effects of their use must be considered. The use of CH_4 inhibitors must be judicious as the microflora are able to adapt to the inhibitors over long periods of time therefore resulting in only short term inhibition (Ørskov 1975). Also, some inhibitors affect the digestion of other dietary components and depress feed intake.

2.2. Effects of Environmental Temperature on Cattle

2.2.1. Temperature and Digestibility

Graham et al. (1959) noted that at medium and high nutritional levels an increase in 10 °C of environmental temperature resulted in a 1% increase in apparent digestibility

of feed energy in sheep. Digestibility in sheep that were fed long, chopped hay, or pelleted hay at fixed intakes decrease by approximately 0.20 and 0.15 percentage units for every °C decreased in ambient temperature (Kennedy et al. 1986). Westra and Christopherson (1976) reported a decrease in DM digestibility in sheep of 0.19 digestibility units per °C, and Kennedy et al. (1982) and Kelly et al. (1989) noted a reduction in sheep on a basal diet of brome grass hay of 0.23 and 0.26 digestibility units per °C. It would appear that the decline in digestibility with decreasing temperatures is slightly less in steers than in sheep. Christopherson (1976) determined that for steers DM digestibility was decreased by 0.08% per °C decrease in temperature.

Christopherson and Kennedy (1983) observed that the digestibility of forage diets, which tend to ferment slowly, appeared to be more susceptible to changes in the rate of passage brought about by their exposure to different temperatures than concentrate diets. This was based on an experiment done by Young and Degen (1981) where the digestibility of a pelleted barley-alfalfa diet was not affected by low temperatures. Similarly, Young and Degen (1981), Williams and Innes (1982), Kennedy et al. (1982), Christopherson and Kennedy 1983, and McBride and Christopherson (1984) determined that cold exposure did not affect the digestibility of all-concentrate diets. Kennedy et al. (1982) determined that for a rapidly fermented, all-concentrate barley-canola meal diet in wethers, temperature did not influence digestibility. Christopherson (1976) and McBride (1982) in two separate experiments determined that digestibility of diets consisting of

50% chopped hay and 50% concentrate was influenced by temperature in cattle and that the digestion of a 75% concentrate, 25% pelleted hay diet was affected for lactating ewes. Therefore, the digestibility of slowly fermenting forage diets appeared to be more susceptible to temperature related changes in motility and rate of passage of digesta than concentrate-based diets. Graham (1967) did not find a temperature effect on digestion when sheep were exposed to 10°C and given a diet of alfalfa:wheat chaff.

The effects of warm temperatures on ruminant feed utilization have not been examined as extensively as cold temperatures. Research also indicates that the opposite physiological and digestive effects of cold temperature conditions occur in warm temperatures. Graham et al. (1959) determined that with increases in environmental temperature, the energy lost in feces decreased resulting in a concomitant increase in amount of energy apparently absorbed. Blaxter and Wainman (1961a) and Warren et al. (1974) reported that warm temperatures (above 25°C) increased feed digestibility in cattle. Davis and Merilan (1960) indicated that an increase in digestibility and a decrease in ad libitum feed intake occurred as a result of exposure to high temperatures. Other authors have also reported increased digestibility in cattle exposed to heat (Blaxter and Wainman 1961; Warren et al. 1974). The increased feed digestibility was supported by Attebery and Johnson (1969) who showed that high environmental temperatures reduced rumen motility and reduced volatile fatty acid production in the rumen in cattle.

Several experiments, such as those by Warren et al. (1974), Colditz and Kellaway (1972), and Nicholson et al. (1980), confirmed a physiological basis for and influence of environmental temperature on the digestibility of sheep and cattle. Exposure to cold temperatures rapidly affects digestive functions within the first few hours or days (Gonyou et al. 1979). Christopherson (1976) reported that periods of reduced digestibility in steers were generally associated with increased resting metabolic rates. Christopherson and Kennedy (1983) also noted the variability in the interaction between temperature and digestibility. This interaction was considered to be affected by the animal's insulation. Larger digestibility changes were noted for animals with less fleece insulation or smaller body size. There was thus no effect of treatment temperature on digestibility of bromegrass pellets for well insulated sheep in the study of Christopherson and Kennedy (1983).

2.2.2. Feeding Level and Temperature

Rogerson (1960) observed that the apparent digestibility of dietary energy decreased at a high feeding level. Net energy (NE) per unit food decreases with increasing intake (Blaxter and Wainman 1961b). The decline in NE is due to the decline of digestion and fermentation of food with increasing intake. It was noted that net energy per unit of food decreased with increasing intake due to a decline in digestibility and to the increased efficiency of oxidative processes active at and below maintenance (Blaxter and Wainman 1961b).

Graham et al. (1959) concluded that there were no significant differences in the apparent digestibility of feed due to environmental temperature when feeding level increased from submaintenance to maintenance but noted a decrease in digestibility at a high feeding level for closely clipped sheep. Kennedy and Milligan (1978) reported that a decrease in digestibility was associated with increased food intakes in cold environments at high feeding levels. However, more OM was apparently digested in the lower digestive tract of animals in cold environments than in animals at warm temperatures.

Kennedy et al. (1986) examined the effects of cold exposure of sheep on voluntary feed intakes. The study found that there was a lag time in the increase in feed intake which varied with diet type. However, passage rates and digestive tract motility responded immediately. Therefore, Kennedy et al. (1986) concluded that the response of the digestive tract to temperature affected the accommodation of larger feed intake as opposed to being driven by voluntary feed intake.

2.2.3. Passage Rate, Retention Time, and Digestibility

Variations in passage rate of feed have been agreed upon to be the major cause of the positive relationship between digestibility and temperature. Delfino and Mathison (1991) noted that a cold environment increased passage rates of digesta leading to the reduction of digestive efficiency. Westra and Christopherson (1976) determined that

depression of digestibility occurred because of faster passage of dietary residues through the digestive tract in cold exposed animals treated with digesta markers. Similar results were reported with sheep and cattle in studies by Christopherson and Kennedy (1983) and Warren et al. (1974). However, the shorter turn over time in the rumen affected the degradation of readily fermentable diets less than the more slowly fermented materials (Mertens 1977).

Consolidated results from studies by Westra and Christopherson (1976), Kennedy et al. (1976), Kennedy and Milligan (1978), Kennedy et al (1982), and Kennedy (1985) as reported in Kennedy et al. (1986) showed that for closely shorn sheep at an ambient temperature of 20°C the average retention time in the total digestive tract of digesta solid markers was considerably lower than (by, on average, 6.5 h) for cold exposed animals fed bromegrass pellets. Chopped brome hay, reed canary grass hay, alfalfa hay, red clover hay, and pelleted reed canary grass were affected to a lesser extent (0.9-3.1 h.).

Kennedy et al. (1976), Kennedy and Milligan (1978), and Weston (1977) showed the cold environment to produce a decrease in rumen fluid volume and an increased dilution rate of the fluid marker ^{51}Chromium-EDTA (Cr-EDTA). Kennedy (1985) showed that medium sized particles (Cr), general particulate (^{103}Ru), and fluid Cobalt-EDTA (Co-EDTA) marker fractions did not always demonstrate the same proportional shifts in cold temperatures. They surmised that these differences indicated that the

passage rate of a chopped legume diet's medium sized particles increased more relative to the small particles; while the reverse was true for chopped grass diets.

In studies with sheep, a decrease in the total retention time during cold exposure was mainly due to the ruminal retention time of digesta with little alteration in the postruminal retention time. Kennedy et al. (1976) and Kennedy and Milligan (1978) demonstrated that increased passage rates decrease OM degradation in the rumen while concomitantly increasing the efficiency of microbial digestion and increasing the quantity of digesta escaping degradation.

Changes in digestive function are associated with increased gut motility, increased rate of passage, and increased circulating thyroid hormone level (Westra and Christopherson 1976; Kennedy et al. 1977). Lirette et al (1988) determined that increased feed intake in colder environments was related to a rise in the mean daily frequency of reticuloruminal contractions. They also determined that cold stress increased the frequency of contractions in the rumen and omasum and produced a non-significant increase in reticular motility. Increased forestomach motility would be expected to increase the rate of passage of small particles from the rumen by mixing, sorting and fluid propulsion (Christopherson and Kennedy 1983). High environmental temperatures have been noted to reduce rumen motility and alter microbial fermentation in cattle (Attebery and Johnson, 1969; Mishra et al. 1970).

2.2.5. Volatile fatty Acids

The volatile fatty acid (VFA) ratio of acetate to propionate, in the rumen, has been shown to change with an alteration in the diet type from high concentrate to high fibre. Fahey and Berger (1988) state that the acetate: propionate ratio decreases as the forage:concentrate ratio decreases.

Research has indicated that VFAs may be influenced by environmental temperatures due to metabolic processes (Kelley et al. 1967; Weldy et al. 1964). Kennedy and Milligan (1978) reported an increase in the molar proportion of propionate in a cold environment. They surmised that this change would have a positive impact on an animal's energy supply by minimising the cold induced decrease in metabolizable energy and by increasing glucogenic precursors. Theoretically, there should be an enhancement of available productive energy and relief of ketotic stress.

Volatile fatty acid production and ruminal pool sizes declined in proportion to the reduction in OM digestion in sheep exposed to cold temperatures (Kennedy et al. 1976; Kennedy and Milligan 1978). Total rumen VFA concentrations were not consistently increased or decreased in the cold but, changes in relative proportions have been observed and were characterized by decreases in acetic acid and increases in propionic acid as the fermentation of cell wall constituents decreased (Kennedy et al. 1986). Volatile fatty acid

and CH_4 production were correlated with the amount of OM digested in the stomach in the study of Kennedy and Milligan (1978).

Kelley et al. (1967) observed that very high temperatures influence the physical and metabolic activity of the rumen. When temperatures increased from 1.6 to 37.7°C with unadapted Holstein cows, the total VFA concentrations in the rumen decreased significantly from 153 to 66 mEq/L; and acetate increased, and propionate and butyrate decreased as a percentage of total VFA.

CHAPTER III -
EFFECT OF ENVIRONMENTAL TEMPERATURE , DIET, AND FEEDING LEVEL ON METHANE PRODUCTION IN STEERS

3.1 Materials and Methods

3.1.1. Experimental Design

A 3 x 2 x 2 factorially designed experiment, which examined the effects of temperature (-23, 19, and 29°C), diet (100% hay, and 10% hay and 90% concentrate) and feeding level (maintenance and twice maintenance) on 12 steers, was conducted from August 25 to December 5, 1993. Within each combination of environmental temperature and feed, one steer was initially fed at maintenance and the other at twice maintenance. At the completion of the measurements at these feeding levels, steers were changed to the other feeding level and measurements were repeated so that there would be two observations per animal per treatment. Steers were then re-randomized and the trial was repeated. At the completion of all measurements, a total of four measurements per animal were obtained for the assigned diets and environment at each of the two feeding levels. Prior to the main periods, a 2 wk adaptation period was allowed for acclimatization to the new feeds and environments. There was a 1 wk period of adjustment within each of the main trials to new feeding levels. Experimental periods were 37 d in length.

The experiment was conducted in accordance with the Canadian Council of Animal Care guidelines and under the auspices of the Faculty of Agriculture, Forestry, and Home Economics Animal Care Committee.

3.1.2. Animals and Housing

Twelve Hereford steers with a means body weight of 373±24 kg were moved from the Ellerslie Research Station, where they had been adapted to metabolic crates and respiration hoods, to the Laird W. McElroy Environmental and Metabolic Research Center at the Edmonton Research Station. Upon arrival, the animals were held for approximately 1 wk in an outside pen with a covered shed. During this period, the steers were fed 3 kg d^{-1} of rolled barley grain per steer in addition to ad libitum good quality hay. The steers were dewormed with a subcutaneous injections of Ivomec (Merck AgVet, Kirkland, Que.).

Steers were blocked by weight and randomly allocated into three groups of four to ensure equal weights within environmental groups. The steers were housed in three temperature environments: hot (29±0.8°C), ambient (19±2.1°C), and cold (-23±3.8°C). Steers at ambient temperature were introduced to their environment immediately; steers in the hot room were introduced into the room and the heat was increased by 5°C every 2 d; and steers in the cold chamber were introduced at an initial temperature of 0°C and acclimatized by decreasing the environmental temperature 5°C every 2 d.

For passage rate fecal and digestibility collections, steers were housed in metabolic crates (183 x 107 x 218 cm) throughout the experimental trials. During 24 h calorimetry measurements animals were tied in a stall with their heads in calorimetric

hoods (170 x 76 x 254 cm). The hoods were constructed of plywood on four sides with a front panel fitted with a plexiglas window which allowed light into the chamber and access for feeding. A polyvinyl neck drape extended from the back of the hood and was tied around the steer's neck to prevent excessive air movement out of the hood.

In the interval between experimental periods, while either being accustomed to different feeding levels or re-acclimatized to different environments, steers were exercised every few d. Steers in the ambient environment were exercised in floor pens for approximately 4 h. Occasionally, however, they were housed overnight in individual pens with controlled individual feeding. Steers in the hot room were exercised in the stable at the ambient temperature for a maximum of 3 to 4 h in order to prevent the disruption of the acclimatization of the steers to their environment. In cold environments, steers were exercised in smaller refrigerated rooms at a temperature of -20°C, two at a time, for approximately 4 h. Between replications of trials, all steers were weighed and placed in an outdoor pen for exercise for 17d. At this time, the steers were fed a hay and grain diet similar to that consumed upon arrival at the Research Centre.

3.1.3. Feeding

All steers were individually fed in metabolic crates or in the calorimetric hoods on the floor stalls, and received trace mineralized salt free choice and ad libitum water. The hay was a good quality chopped alfalfa hay and concentrate (Table 1). The hay diet was offered at feeding levels of 59 and 118 g $kg^{-.75}d^{-1}$ at the maintenance and twice

maintenance feeding levels, respectively. Corresponding values for grain were 41 and 83 g $kg^{.75}d^{-1}$. The steers were fed twice daily at 0800 and 1600 h. Feed intakes were gradually increased over a 7 day period; the grain diet intake was increased 500g d^{-1} to allow for rumen microbial adjustment whereas the hay was increased more rapidly. During the accustomization period, the animals that did not consume all of the daily allocated increase were maintained at the same level for the following day. Once on full feed, no adjustments were made to dietary intake. All steers reached the desired level of feed at the twice maintenance level except steer No. 209 in the second experimental period. He was removed from the experiment due to an initial incident of severe bloat and subsequent chronic bloating.

During the digestibility and calorimetry collections, orts were collected daily and 5% of the sample was selected and frozen at 20°C for further analysis. Pooled representative feed samples were taken daily throughout each experimental period. The pooled samples were ground through a 1 mm screen and stored for analysis.

3.1.4. Volatile Fatty Acid (VFA) Collections

Collections of rumen fluid for volatile fatty acid measurements were started at 1300 h d-15 of the experimental period. All 12 steers were sampled within approximately 2 h, except for the first experimental period which took longer (approximately 6 h). Steers were held in a squeeze chute, and a stomach tube and a manual suction pump were used to obtain approximately 200 mL of rumen fluid. Some samples contained a large

proportion of saliva. Ruminal contents were strained through four layers of cheesecloth to obtain the liquid fraction. A 4 mL sample of the liquid fraction was immediately pipetted into a vial containing 1 mL of 25% (wt vol^{-1}) phosphoric acid to prevent VFA loss. The vials were frozen at -20°C.

3.1.5. Fecal Passage Rate Determinations

Immediately after collection of samples for VFA determination (d-15), a single pulse dose of 50 g Cr-mordanted ground alfalfa hay and 20 g Co-EDTA was drenched into the animal using a flexible 1.7 m PVC tube. There was some variability in the total amount of marker received by each steer due to loss during dosing.

The sampling of fecal material commenced 10 h after the completion of the marker dosing and continued for 5 d. The samples were collected from the steers in the metabolic creates by rectal sampling at designated times. The schedule for collection of fecal samples was as follows: 0, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 33, 44, 51, 60, 69, 77, 94.5, and 130 hours. Immediately after collection, the samples were frozen, at -20°C.

3.1.6. Fecal and Urine Collections

Digestibility of the diet and N retentions were measured by collecting feces and urine for 6 d (from d-17 to d-22). To prevent volatilization of ammonia, 500 mL of 6 N

hydrochloric acid was added daily to the urine collection trays to acidify the urine. Fecal output was collected every 24 h and a 2% sample was taken and frozen at -20°C. A sample equivalent to 1% of the urinary output was frozen at -20°C.

In the week following the collections, the pooled fecal samples were thawed and dried in foil trays at 110°C to a constant weight for 24-48 h, depending upon the size of the sample. A sample for use in the determination of total fecal DM, CP, NDF, ADF, and lignin was subsequently ground through a 1 mm screen and stored for further analysis.

3.1.7. Respiration Measurements

The 24 h calorimetry measurements began 2 d after the completion of the digestibility trial (d-25 to d-28). Respired gases from two animals in each of the hot and cold rooms were measured on d-25. The remaining two animals in each of the hot and cold rooms were sampled on d-26. On d-27 and d-28, the animals at the ambient temperature were sampled two at a time. The collections in the next sampling period involved the ambient and cold rooms being sampled together for the first two d, followed by 2 d of sampling in the hot room. This procedure was repeated for the second period.

The steers were introduced into the hoods in the morning of the day of the run, between 0800 and 1000 h and removed the following morning. A polyvinyl neck drape was tied around each steer's neck to prevent substantial air movement out of the hood and to ensure a slightly negative air pressure in the hood. Animal behavior in the hoods did

not vary substantially from their behavior in the metabolic crates. The closure and the pressure in the hoods was checked regularly throughout the experimental period. While in the hoods, the steers were fed twice daily. The 0800 h feeding was placed in the hood at the beginning of the calorimetry measurements and the steers were fed again at approximately 1530 h. Respiration data was not collected from the open hood during feeding.

Oxygen concentration in the respired air was measured with a Taylor Servomex oxygen analyzer (Taylor Servomex LTD, model OA. 184, Crowborough, England). The production of carbon dioxide and CH_4 production were measured with Beckman non-dispersed infrared analyzers (Beckman Instruments LTD, Model #864, Fullerton, C.A., USA). Flows of respired gases were measured by a Fisher and Porter variable area flow meter rotometer (Fisher and Porter LTD, Toronto, Canada). The data were collected with a Datalogger (Data Electronics Corp., model DT100, Melbourne, Australia) every 10 s and averaged for 3 min over a 15 min interval for each of the steers. An automatic switching device was used to switch between the animals within the same room at 15 min intervals. Room temperature and barometric pressure were measured daily. Room air gases were routinely collected to correct for instrumental drift and changes in composition of room air.

The equipment was calibrated prior to run, and at approximately 0900, 1600 and 2300 h during the run. The concentrations of the standard calibration gases were 0.999%

CO_2, 0.1018% CH_4, and 19.83% O_2. Outside air was also used as the other standard gas in the calibration procedure. Calorimetry equipment was calibrated with the nitrogen injection technique (Young et al. 1984) at the end of every complete run. Oxygen consumption, CO_2, and CH_4 production were determined using techniques described by Young et al. (1984). Heat production (kJ s^{-1}) was calculated by: -20.5 x (expired air flow rate) x (difference in O_2 concentration by volume between inspired and expired air) (Maclean and Tobin 1987).

3.1.8. Chemical Analyses

Dry matter content of the diets and feces was determined by drying 1 g samples in a 110°C oven for a minimum of 4 h to a constant weight. Determination of nitrogen content of the diets and feces and urine samples was by the Kjeldahl procedure (AOAC 1984, procedure #2.057). An adiabatic bomb calorimeter (Parr Instrument Company, Inc., Moline, Illinois, USA) was used to determine the energy content of the feed, urine, and feces. Duplicate 5 mL urine samples were freeze-dried in 3x8 cm plastic bags prior to determination of energy content. Four empty plastic bags were combusted to attain a baseline caloric content of the bags. Neutral and acid fibre, as well as lignin, in feed and fecal samples was determined by a procedure outlined by Van Soest et al. (1991).

Volatile fatty acids (VFA) were determined by gas chromatography. The VFA samples were thawed, a prepared internal standard (4 methyl valeric acid) was added, and peaks measured with a Varian Model 3700 Gas Chromatograph (Walnutcreek, CA). The

samples were taken by split injection, with 22:1 ratio, in an auto sampler (Varian Model 8035 Auto Sampler, Walnutcreek, CA). A Stabelwax - DA column (30m x 0.25 mmID x 0.5 μm film) (Restek Corp., Bellefonte, PA) was used in the chromatograph along with a flame ionization detector (FID) with a flow rate of 30 mL min^{-1} of high purity hydrogen and 300 mL min^{-1} of air. The samples were volatized into the high purity helium carrier gas using flow rates of approximately 1.0 mL min^{-1}. The helium makeup gas had a flow rate of 30 mL min^{-1}. Outputs were analyzed by the Schimadzu EZChrom Chromatography Data System (Schimadzu Sci. Instruments, Inc. Ver. 2.X, Columbia, MD, USA).

3.1.9. Marker Analysis and Calculation of Mean Retention Times

Fecal samples, containing Cr and Co markers, were ground through a 1 mm screen prior to analysis for chromium and cobalt. Duplicate subsamples (0.5 and 1 g) were taken from each of the fecal samples and the samples were digested in 4M HNO_3, in a 75°C oven, for 24 h for each 0.25 g of material (Reese et al. 1994). After this, the samples were cooled, a solution containing ammonium hydroxide, EDTA, lithium hydroxide, and calcium hydroxide was added, and the samples were once again digested for 18-24 h, cooled, and filtered through Whitman #41 filter paper for this procedure. Blank samples were also prepared with unlabeled fecal material. The chromium and cobalt content in the samples was measured with a Perkin-Elmer 4000 Atomic Absorption Spectrometer (Norwak, CT). The mean retention time of digesta in the reticulorumen, as well as intestinal transit time were analyzed according to a procedure

developed by Reese et al. (1995) using information derived from Cr-mordanted and Co-EDTA digesta markers.

3.10. Statistical Analysis

The data were analyzed according to a 3 x 2 x 2 factorial experimental design. The analysis of the procedure was carried out using GLM procedure on SAS (1988) for the temperature, diet, and feeding level main effects. Twelve steers were sampled per period with the exception of one animal which was removed from the second half of the experiment. Due to this removal, least squared means were used. Body weight, included in the model as a covariate, was analyzed to observe the relationship to body weight. Body weight was used as a covariate, however it was removed from the model if it was not significant ($P>0.05$). All means were also separated by the Student Neumann Keul test (SAS, 1988).

The temperature by diet, temperature by feeding level, and diet by feeding level interactions were analyzed using simple means. Environmental temperature and feed type main effects and their interactions were tested against animal nested within the feed by temperature by replication effect. The feeding level effect and its interactions were tested against the residual error.

3.2. Results

On d-35 of the experiment, one steer in the cold environment bloated severely and continued to chronically bloat. This animal was removed from the second half of the trial. All other animals remained healthy throughout the experiment. The animals acclimatized to the environment through shivering thermogenesis and by growing a thicker hair coat. The assessment of shivering and hair coat growth was assessed visually.

3.2.1. Apparent Digestibilities

All apparent digestibilities were slightly numerically higher in the 29 °C environment than in the -23 °C or 19 °C, however differences were only significant for acid detergent fiber (ADF) ($P=0.006$) (Table 2). As expected, the dry matter (DM), gross energy (GE), and crude protein (CP) digestibilities of the concentrate diet were greater than that of the hay diet (80.9 vs. 63.9%; 80.3 vs. 64.6%; and 77.5 vs. 72.1%, respectively ($P=0.0001$)). However, neutral detergent fiber (NDF) and ADF digestibilities for the hay diet were 9 % and 76 %, respectively ($P<0.05$), greater than for the grain diet. Significant interactions for apparent digestibilities were not observed for temperature by diet, temperature by feeding level, or diet by feeding level.

3.2.2. Digesta Flow

Differences were not observed ($P>0.05$) in mean particle (CrMRT) or liquid (CoMRT) retention time in the rumen, mean retention of 'average' digesta in the different

compartments of the digestive tract, or in mean intestinal transit time among the different temperature groups (Table 3). Diet and feeding level also did not affect ($P>0.05$) mean retention time. However, the generally accepted pattern of retention of digesta in the digestive tract for shorter periods of time at higher feeding levels was apparent in the data. The interactions between temperature, diet, and feeding level also were not significant ($P>0.05$).

3.2.3. Methane and Urinary Losses

Daily CH_4 production (kJ $Kg^{-0.75}$) was not influenced by environmental temperature or feeding level ($P>0.05$) (Table 4). However, CH_4 production was higher at -23 °C than at 29 °C when expressed as both a percentage of GE ($P=0.05$) and digestible energy (DE) ($P=0.02$). Methane production was influenced by diet with steers fed the hay diet producing 78% more CH_4 daily than the grain diet ($P=0.0004$). Methane production as a percentage of GE was, however, not influenced by diet. However, CH_4 production as a percentage of GE and DE were higher at the maintenance feeding level ($P=0.0001$). Diet by feeding level interactions were significant for daily CH_4 production ($P<0.001$). Daily CH_4 production decreased with feeding level when grain was fed but increased ($P<0.05$) when hay was fed. Decreases in CH_4 production with increasing feeding level were also greater for grain than hay when CH_4 production was expressed as a percentage of GE and DE ($P=0.0003$ and $P=0.003$, respectively).

Urinary energy losses were highest at -23 °C (P=0.01) and lowest at 19 °C (Table 4). Urinary energy losses when expressed as a percentage of DE was 59% higher at -23 °C than at 19 °C. Significant differences (P<0.05) in daily urinary energy were also noted for diet and feeding level; more urinary energy was lost when hay was fed and losses were highest at the twice maintenance level. No differences due to feeding level were detected, however, when urinary energy was expressed as a % of GE or DE. No temperature by diet, temperature by feeding level, or diet by feeding level interactions were detected for urinary energy losses.

3.2.4. Ruminal Volatile Fatty Acid Proportions

The molar proportion of acetate, propionate, isobutyrate, butyrate, isovalerate, valerate and caproic acid in rumen fluid of steers held at different temperatures were not affected by temperature nor was the acetate:propionate ratio (Table 5). As expected, the proportion of acetate was 41% (P=0.0001) higher for the hay diet than for the grain diet and propionate was 84% (P=0.0001) higher for the grain diet. The molar proportion of acetate was also 14% higher at the maintenance feeding level whereas that of propionate was 57% higher at the twice maintenance level. The acetate:propionate ratio was also affected by diet (P=0.0001) and feeding level (P=0.0005). With the exception of isobutyrate for both factors and valerate for feeding level, the molar percentage of the other VFA were all significantly affected by diet and feeding level (P>0.0001).

There were no significant temperature by diet or temperature by feeding level interactions. The interaction of diet by feeding level was not significant for isobutyrate, valerate, and caproic acid molar proportions but was significant for acetate (P=0.003), propionate (P=0.0001), butyrate (P=0.007), isovalerate (P=0.002), and the acetate:propionate ratio (P=0.008). Generally, feeding level had little effect on molar proportions in the hay diet but had large influences when the grain diet was fed.

3.2.5. Energy Partitioning

The ME intakes, heat production, retained energy, fat, and protein were calculated from the average daily feed intakes during the calorimetry measurements (Table 6). There were no differences in ME intake of steers at the different temperatures. ME intake was 15% higher for the hay diet than the grain (P=0.001) and was significantly different between the two feeding levels (P=0.0001).

Heat production was 20% higher (P=0.02) for steers at -23 °C than at either 29 °C or 19 °C. Diet had no effect on heat production. Similar to ME intake, heat production was significantly higher in steers fed at the twice maintenance feeding level (P=0.004). The temperature by feeding level interaction was significant (P=0.005); at -23 °C there was actually a small decrease in heat production with increased feeding level.

Total retained energy and energy retained as fat and protein were lowest for the steers at -23 °C and highest for the steers at 29 °C (Table 6). Similarly, retained energy,

fat, and protein were higher (P=0.0001), for the steers fed at the twice maintenance feeding level than for those fed at maintenance. However, diet was only found to be significant (P=0.001) in terms of a difference in retained protein with steers fed the hay diet having a higher retention of protein. No significant differences were noted for the temperature by diet and diet by feeding level interactions. The temperature by feeding level interaction was significant for retained energy (P=0.005) and retained fat (P=0.002). There was a larger increase in energy retention with increased feeding level at -23 °C than at the other two temperatures.

3.3 Discussion

3.3.1 Apparent Digestibilities

In this study, despite the fact that the results were not significant for temperature, a decline of 0.06 percentage units per °C in DM digestibility for the steers was determined between -23 and 29 °C (Table 2). Upon examining the change in digestibility between 19 to 29 °C environments, a decrease of 0.27 percentage units per °C was noted. These results are similar to those of Miaron and Christopherson (1992) where a decrease of 0.44 percentages units in digestibility for steers per degree °C temperature decrease was measured in the range of 10 to 28 °C, but no effect of temperature on digestibility was seen between 10 and -10 °C. In another experiment, Christopherson (1976) determined that for steers DM digestibility was decreased by 0.08 percentage units per °C decrease in temperature. The temperature-related decrease in digestibility appears to be due to an increase in passage rate in the cold which reduces the time available for microbial digestion of the feed particles (Christopherson and Kennedy 1983).

Kennedy et al. (1986) found a decrease in digestibility of approximately 0.15 and 0.20 percentage units per °C for sheep fed long, chopped, or pelleted hay. Westra and Christopherson (1976) reported a decrease in DM digestibility in sheep of 0.19 digestibility units per °C, and Kennedy et al. (1982) and Kelly and et al. (1989) noted a reduction in sheep on a basal diet of brome grass hay of 0.23 and 0.26 digestibility units per °C.

Several researchers have shown that cold exposure did not affect the digestibility of all-concentrate diets (Young and Degen 1981; Kennedy et al. 1982; Christopherson and Kennedy 1983; McBride and Christopherson 1984; and Williams and Innes 1982). This would occur as concentrate diets are more readily digested in a shorter period of time. Christopherson (1976) and McBride (1982), in two separate experiments, determined that the digestibility of concentrate diets were less susceptible to temperature related changes in motility and passage rate than slow fermenting forage diets. These differences indicated a potential diet by temperature interaction. Such an interaction was not noted in this experiment.

Feeding level had no influence on diet digestibility in this study despite the fact that digestibility normally decreases as the amount of food consumed increases (National Academy of Sciences-National Research Council 1988). The hay was of good quality which may have influenced the results for this diet. With respect to the concentrate diet, Delfino and Mathison (1991) determined that the digestibility of a barley-based diet was not affected by intake level when the feeding levels were below two times maintenance.

3.3.2 Digesta Flow

Although retention times of particles and liquid in the digestive tract, retention times of average digesta in different compartments of the digestive tract, and intestinal transit times were not significantly affected by temperature, diet, feeding level, or their

interactions, the mean retention time of digesta in the rumen did show an 23% decline as the temperature was decreased from 19 to -23 °C (Table 3) which is in agreement with the literature (Table 3). Miaron and Christopherson (1992) reported a 21% decrease in mean retention time of particulate matter in the rumen of steers as the temperature declined from 10 to -10 °C. Dilution rate of rumen fluid numerically decreased in the cold in their experiment in contrast with our results. These researchers did find a non-significant increase in rumen retention times as the temperature moved from 10 to 28 °C which is in contrast with the numerical differences in our trial. The results of our study are also in agreement with the findings by Christopherson and Kennedy (1983), Westra and Christopherson (1976), Kennedy et al. (1976,1977, 1982), and Kennedy and Milligan (1978) who have concluded that exposure to cold environments resulted in an increased passage rate of digesta (i.e. decreased retention time) from the reticulorumen. The cold environment also stimulated a decrease in rumen fluid volume and increased the dilution rate of the Cr-EDTA fluid marker in the studies of Kennedy et al (1976), Kennedy and Milligan (1978), and Weston (1977). High environmental temperatures are associated with reduced rumen motility (Attebery and Johnson 1969; Mishra et al. 1970). However, any such effect did not cause increased retention times in our experiment.

Although retention time of digesta in the rumen was numerically less at 29 °C than at 23 °C, digestibilities numerically increased in response to the temperature rise. This is in contrast with other studies which have shown that digestibility of feeds decrease with decreased retention time in the cold environments. This difference in trend

of results may indicate a differing response of digestibility to digesta retention times at higher temperatures but is more likely related to diet quality. Mertens (1977), Kennedy et al. (1976), and Kennedy and Milligan (1978) observed that a shorter retention time affected the degradation of readily fermentable diets less than more slowly fermentable diets.

The non-significant decrease in retention times with an increase in feeding level is consistent with the trend presented in the literature. As an example, Blaxter et al. (1956) showed that the retention time of digesta in the digestive tract was reduced at higher feeding levels leading to a concomitant reduction in digesta fermentation and digestion.

Differences were observed between retention time of Cr mordanted particles and Co-EDTA liquid fraction in the total digestive tract in our experiment. The liquid fraction of the digesta passed through the digestive tract faster than the particulates. Such a difference was also noted in steers by Miaron and Christopherson (1992). Kennedy (1985) found that labeled medium sized particles and the liquid fraction did not always demonstrate proportional shifts in retention time in response to changing temperatures.

3.3.3 Volatile Fatty Acids

We could not detect any influence of temperature on molar proportions of VFA in this experiment (Table 5). Several studies have indicated that VFA production may be altered due to the influences of temperature on metabolic processes. Kennedy et al.

(1976) observed that the cold environment was characterized by a proportional decrease in acetic acid and increase in propionic acid even though the differences between these VFA were not significant. Kennedy and Milligan (1978) suggested that the increase of the molar proportion of propionate in a cold environment, which they observed in their experiment, allowed the animal to minimize the cold induced decrease in metabolizable energy and increase the production of glucose. Kelley et al. (1967) determined that the VFA concentrations decreased and concentrations of acetate and propionate were reduced by 50% and 72%, respectively, when subject to higher temperatures, such as 38 °C. However, the Holstein cows were exposed to the extreme temperatures and sampled without an adaptation period.

Diet was noted to significantly affect the proportions of acetate and propionate in this study. As expected, the ratio of acetate to propionate increased when more forage was in the diet (Fahey and Berger 1988). Volatile fatty acids were also altered by feeding level, with the proportion of propionate increasing and acetate decreasing as the feeding level was increased from 50 to 101 g $kg^{-0.75}$ d^{-1} . Kennedy and Milligan (1978) observed significant differences between low and high feeding levels in both cold and warm environments with the higher feeding levels producing the least amount of acetate and the greatest proportion of propionate. The effect of feeding level on VFA levels is most likely due to the interdependence of retention time in the rumen and feeding level. At a maintenance feeding level, the molar proportion of acetate is expected to be greater than

at a higher feeding level, regardless of the level of concentrate or hay in the diet, as demonstrated in our work.

3.3.4. Methane and Urinary Losses

Methane production (kJ $kg^{-.75}$) was numerically higher (24%) for steers housed at -23 °C than those at 29 °C (Table 6). For the steers held at -23 °C CH_4 losses accounted for 6.3% of the GE intake whereas losses in steers at 29 °C were 5.2% of the GE intake (P=0.05). However, it must be noted that in the cold, due to their increased metabolic requirements, the steers could have consumed an ad libitum feeding level which was higher than twice maintenance to meet their energy requirements. This increase in intake may have decreased the level of CH_4 production as a percentage of GE intake. This result (i.e. a 21% increase in CH_4 production in the cold as compared with the hot environment) was consistent with a previous study done in our laboratory (von Keyserlingk and Mathison 1993). In that study, for shorn sheep held at 5 °C, 6.4% of the GE intake was accounted for in the CH_4 losses and at 21 °C CH_4 losses were 5.6% of GE intake. This difference was equivalent to a 14% increase in CH_4 in the cold.

Additional literature on the effects of temperature on CH_4 losses, particularly in the warm environment, is limited. In three of four studies, estimates of CH_4 production were based on measurements of only two animals (Graham et al. 1959; Rogerson 1960; Blaxter and Wainman 1961a, b). Graham et al. (1959) reported that when temperature was decreased from 33 °C to 8 °C at fed intakes of 1,200 or 1,800 g d^{-1}, CH_4 production

in adult sheep was reduced by 20%. However, at restricted intake levels (600 g d^{-1}) CH_4 production was not affected by temperature. Blaxter and Wainman (1961b) determined that CH_4 production was not affected by temperature at below maintenance feeding levels, but noted a decrease in CH_4 production with temperature for one steer housed in the cold and fed above maintenance.

Rogerson (1960) noted that temperature effects on CH_4 production were variable between 20 °C and 40 °C. Approximately 50% more CH_4 was produced at 20 °C at the 6000 g level than the 4000 g level. However, at 40 °C CH_4 production increased only by 25% at the higher feeding level. He also observed that CH_4 production was reduced at lower temperatures at feeding levels above maintenance, whereas at feeding levels below maintenance temperature had no effect. Kennedy and Milligan (1978), in an experiment in which they partitioned CH_4 production between the rumen and the lower digestive tract in sheep, observed that CH_4 production was decreased by approximately 30% due to cold exposure. However in their experiment sheep were fed a pelleted diet hourly and the dilution rate of liquid in the rumen was increased by 69% in the cold environment which would reduce the time for CH_4 production in the rumen. However, the changes in dilution rate and retention time were not as large in our experiment, and our steers were fed hay twice daily. Therefore, their results are expected to differ from ours.

The possibility that our results, suggesting an increase of CH_4 production in the cold, might have been influenced by our methods needs to be examined. Since hoods

were used, we only measured CH_4 which appeared in respired air thus no measure of the gas emitted through the anus was made. This may mean that our CH_4 production values are underestimated. Murray et al. (1976) have, however, reported that even though 13% of the total CH_4 produced in the animal was produced in the cecum, a large portion of CH_4 produced in the lower digestive tract entered the blood and was emitted in respired air. Kennedy and Milligan (1978) reported that the fraction of total CH_4 produced in the rumen was 0.81 in the warm and 0.68 in the cold. If anything, then, in our experiment we would expect that there would be a greater difference between CH_4 production in the warm and cold if we had measured total CH_4 production (respired + feces) by placing the animals in a respiration chamber.

The results acquired in this and the previous study (von Keyserlingk and Mathison 1993), indicate that instances exist where increased CH_4 production in steers housed in a cold environment. An explanation for this increase is not readily apparent. Based on the amount of substrate available for fermentation and the physiological responses to temperature, less CH_4 should have been produced in the cold because digestibility was lower thereby releasing less substrate for rumen fermentation. Also, a trend towards reducing mean retention time of digesta normally reduces the amount of CH_4 produced. Okine et al (1989) reported a negative correlation between passage rates and CH_4 production; CH_4 production decreased by 29% when the mean retention time in the rumen decreased by 63%. In our experiment, the 21% increase in CH_4 production in the cold occurred when there was a 8% decrease in retention time of digesta in the rumen.

Moreover, changes in fermentation pattern did not account for the greater proportion of CH_4 being produced in the cold since acetate was non-significantly decreased and propionate was increased whereas there is usually a negative correlation between CH_4 production and the proportion of propionate in rumen fluid.

More CH_4 was produced daily when steers were fed a hay diet than when the concentrate diet was fed (82.8 kJ $^{d-1}$ vs. 46.5 kJ $^{d-1}$, P>0.0001). These results were expected because more acetate and less propionate is produced when hay diets are metabolized and thereby resulting in an increased level of CH_4 produced. The slowly fermented hay diet is influenced more by a change in retention time in the rumen than a readily fermentable concentrate diet (Mertens 1977; Moe and Tyrrell 1979). Very high concentrate diets (90%+ concentrate), as was fed in this study, can result in CH_4 losses of as low as 2-3% of GE at high feeding levels (Abo-Omar 1989; Carmean 1991; Hutcheson 1994). We also noted that CH_4 production was 3.4% of GE intake when the 90% concentrate diet was fed at twice maintenance.

Although numerically more CH_4 is produced at the higher feeding level than at the lower feeding level, CH_4 production per unit gross energy consumed was decreased by 53% at the higher feeding level (Table 4). Blaxter and Wainman (1961a) and Blaxter and Clapperton (1965) determined though 48 trials that a negative regression existed between CH_4 production and feeding level expressed as a percentage of GE. Blaxter

(1967) noted a decrease of 12-30% of the amount of energy lost as CH_4 per unit feed consumed when the feeding level was increased to twice maintenance.

Urinary energy losses were increased when environmental temperature decreased. The increased energy lost in the urine occurs due the increased urea excretion associated with lower protein deposition in the steers in the cold (Table 6). These results were in agreement with those found by von Keyserlingk and Mathison (1993) who determined that urinary energy losses increased with decreasing temperature. Graham et al. (1959) reported that the energy content of the urine decreased significantly with increasing temperature at low feeding levels. However, Rogerson (1960) found that not influenced greatly by temperature and Blaxter and Wainman (1961a) found that temperature did not influence energy losses in the urine. Diet also affected the amount of energy lost in the urine in our experiment; generally urinary energy losses are greater when hay-based diets are fed.

In this study, daily urinary energy losses increased with increasing feeding level. Blaxter and Wainman (1961b) determined that urinary energy losses were increased with an increased feeding level.

3.3.5 Energy Partitioning

As expected, heat production was greater at -23 °C than for either of the other two temperatures. Heat production increased by 61% ($P<0.05$) as the temperature was

decreased from 19 to -23 °C at the maintenance feeding level. Shivering was noted as one mechanism the animals used to maintain their body temperature. Miaron and Christopherson (1992) measured a 32% increase in heat production when the temperature for steers fed at 1.3 times maintenance was decreased from 10 to -10 °C. In contrast with our results where heat productions were similar at 19 and 29 °C, Miaron and Christopherson (1992) found a 21% decrease in heat production as the temperature was increased from 10 to 28 °C. It is unlikely that the steers were below their critical temperature at 10 °C, rather the authors suggested that the higher temperatures may have been associated with a reduction in secretion of certain anabolic and catabolic hormones and a slowing of the metabolic processes. An experiment conducted by Blaxter and Wainman (1961a) on only two steers showed that, at the maintenance feeding level, maximum energy retention occurred at 25 °C, i.e. heat production was increased at higher temperatures, but there was no statistically significant increase in heat production when the temperature was reduced to 5 °C and subsequently to -5 °C.

In contrast with results obtained at maintenance, heat production was not affected by temperature at a feeding level of twice maintenance. The lack of increase in heat production at the higher temperature was also found by Rogerson (1960). Comparison of the effects of low environmental temperatures on heat production in the steers at low and high intakes confirm that the heat increment of feeding associated with the extra feed intake is useful in keeping the animal warm when the temperature is below the critical temperature of the animal.

3.3.6 Conclusion

The digestibility measurements, heat production, urinary losses, and energy retention all behaved as expected when related to temperature, diet, and feeding level. Similarly, although differences in mean retention time of digesta in the rumen were not significant for any of the factors or their interactions, the numerical differences showed a decrease in retention time in the cold. Volatile fatty acids were not significantly altered by temperature but were affected by feeding level. Methane production was increased when a hay diet was fed and when the feeding level was increased to twice maintenance. In contrast with inferences drawn from previous research, CH_4 was increased in the steers housed in the cold environment. The explanation for this phenomenon is not readily apparent since a lower production of fermentable substrate would be predicted on the basis of a reduction in the retention time in the rumen. Further experimentation centering on factors such as effect of environment on protozoal populations, effect of water temperature on rumen metabolism, and feeding level in relation to maximal DM intake are necessary to determine the causes of this difference. These findings, and those of von Keyserlingk and Mathison (1993) could lead to a shift in the understanding of CH_4 production from ruminants.

Table 1. Ingredients and composition of hay and concentrate dry matter

Item	Concentrate	Hay
Ingredients (% Dry Matter)		
Alfalfa Hay	--	100
Barley Grain	92.4	--
Molasses, Beet	1.9	--
Soybean Meal	3.4	--
Calcium Phosphate	0.25	--
Calcium Carbonate	1.1	--
Salt, TM[z]	0.62	--
Vitamin ADE[y]	0.31	
Composition		
Dry matter content (%)	90.64	90.81
Gross energy (MJ/kg)	18.25	18.39
Acid detergent fibre (%)	5.56	28.68
Neutral detergent fibre (%)	18.89	42.83
Crude protein (%)	13.62	16.73

[z]Trace mineralized salt contained 99% NaCl, 0.004% Co, and 0.007% I.
[y]Vitamin premix contained 10,000,000 IU Vitamin A, 1,000,000 IU Vitamin D_3, and 10,000 IU Vitamin E per kg.

Table 2. Apparent digestibilities (%) of dry matter, gross energy, crude protein, neutral detergent fiber, and acid detergent fiber

Item	n[y]	Wt[z] kg	DMI[z] g/kg$^{.75z}$	Apparent Digestibility (%) DM[z]	GE[z]	CP[z]	NDF[z]	ADF[z]
Temperature[x]								
29°C	16	371	75.4	74.3	74.3	76.2	54.9	45.9a
19°C	16	369	75.5	71.6	71.6	74.7	48.0	40.2ab
-23°C	14	376	75.6	71.3	71.4	73.6	48.5	38.3b
SE		7.25	0.05	0.81	0.71	0.63	1.87	2.11
Probability		0.75	0.17	0.19	0.32	0.47	0.38	0.006
Diet								
Hay	24	367	88.8a	63.9b	64.6b	72.1b	52.7a	52.9a
Grain	22	377	62.2b	80.9a	80.3a	77.5a	48.2b	30.0b
SE		5.79	0.04	0.70	0.70	0.87	1.29	1.72
Probability		0.24	0.0001	0.0001	0.0001	0.0001	0.04	0.0001
Feeding level								
Maintenance	23	372	50.4b	71.9	72.1	74.8	50.2	40.6
Twice Maintenance	23	372	100.6a	72.9	72.8	74.8	50.7	42.3
SE		5.88	0.04	0.49	0.52	0.58	1.51	1.23
Probability		1.00	0.0001	0.21	0.39	0.98	0.82	0.38
Temperature x diet								
29°C x hay	8		89	67.5c	66.4c	73.0c	55.6a	55.0a
29°C x grain	8		62	82.9a	82.2a	79.4a	54.3a	36.8b
19°C x hay	8		89	63.0c	63.7c	71.8c	51.8ab	53.5a
19°C x grain	8		62	80.2ab	79.5ab	77.6ab	4b4.2	26.6c
-23°C x hay	8		89	63.0c	63.6c	71.6c	50.8ab	50.2a
-23°C x grain	6		62	78.9b	78.2bc	74.5bc	43.1b	22.6c
SE			10.0	1.08	1.12	1.27	2.60	3.03
Probability			0.08	0.0001	0.0001	0.0001	0.006	0.0001
Temperature x feeding level								
29°C x maintenance	8		50b	74.2	74.3	76.5	56.2	46.0
29°C x twice maintenance	8		100a	74.4	74.3	75.9	53.8	45.8
19°C x maintenance	8		50b	71.1	71.2	74.8	47.1	40.2
19°C x twice maintenance	8		101a	72.2	72.0	74.6	48.9	43.8
-23°C x maintenance	7		52b	69.2	69.4	72.7	46.8	37.6
-23°C x twice maintenance	7		103a	70.4	70.3	72.9	48.2	39.1
SE			5.43	3.42	3.18	1.64	2.83	5.65
Probability			0.0001	0.86	0.84	0.53	0.11	0.84
Diet x feeding level[w]								
Hay x maintenance	12		59c	62.9b	64.0b	72.3b	52.4	51.2a
Hay x twice maintenance	12		118a	64.9b	65.2b	71.9b	53.1	54.5a
Grain x maintenance	11		41d	81.0a	80.2a	77.4a	47.8	29.8b
Grain x twice maintenance	11		83b	80.6a	79.9a	77.4a	47.4	29.1b
SE			0.057	0.94	0.98	1.09	2.37	2.75
Probability			0.0001	0.0001	0.0001	0.0003	0.20	0.0001

[z]Abbreviations: Wt, weight; DMI, dry matter intake; DM, dry matter; CP, crude protein; NDF, neutral detergent fibre; ADF, acid detergent fibre.

[y]Number of animals in each treatment.

[x]Least squares means shown only for temperature, diet, and feeding level, the remaining are simple means

[w]Diet by feeding level interaction was significant for DMI at 0.0001.

Table 3. Mean retention time (h) and transit time (h) in the digestive tract.

Item	n[y]	CrMRT[z] (h)	CoMRT[z] (h)	TT[z] (h)	MRT1[z] (h)	MRT2[z] (h)	MRT3[z] (h)
Temperature[x]							
29°C	16	51.6	31.9	11.0	17.2	7.7	1.0
19°C	16	52.6	37.3	11.8	20.2	4.9	2.6
-23°C	14	48.7	30.5	10.0	15.5	3.9	1.1
SE		2.14	2.00	0.82	1.41	1.52	0.49
Probability		0.68	0.39	0.36	0.29	0.20	0.11
Diet[x]							
Hay	24	52.0	32.7	10.5	17.0	5.7	1.2
Grain	22	50.0	33.8	11.4	18.3	5.2	1.9
SE		1.74	1.63	0.67	1.15	1.24	0.40
Probability		0.59	0.78	0.29	0.56	0.79	0.26
Feeding level[x]							
Maintenance	23	53.1	34.9	10.8	18.9	7.4	1.8
Twice Maintenance	23	48.8	31.6	11.1	16.4	3.6	1.3
SE		1.92	2.20	0.57	1.29	0.98	0.35
Probability		0.28	0.45	0.76	0.33	0.08	0.45
Temperature x diet[w]							
29°C x hay	8	53.0	32.1	10.3	16.4	7.5	1.1
29°C x grain	8	53.7	34.8	11.5	17.9	8.8	0.9
19°C x hay	8	49.1	33.9	10.6	17.3	3.6	1.8
19°C x grain	8	54.9	37.0	12.9	23.5	5.8	2.4
-23°C x hay	8	54.7	33.5	10.9	17.1	7.4	1.2
-23°C x grain	6	44.3	29.3	9.9	14.7	1.5	0.9
SE		3.49	3.59	0.99	2.39	2.41	0.66
Probability		0.30	0.82	0.28	0.29	0.30	0.57
Temperature x feeding level[w]							
29°C x maintenance	8	54.5	32.1	10.4	17.2	9.0	1.4
29°C x twice maintenance	8	52.2	34.9	11.4	17.1	7.3	0.6
19°C x maintenance	8	51.0	34.5	11.5	19.3	4.4	2.0
19°C x twice maintenance	8	51.6	35.6	12.2	20.2	4.4	2.1
-23°C x maintenance	7	54.5	36.0	11.2	18.3	7.1	0.6
-23°C x twice maintenance	7	44.6	25.8	9.8	13.0	2.0	1.8
SE		3.54	3.41	1.03	2.43	2.43	0.64
Probability		0.49	0.39	0.61	0.48	0.43	0.34
Diet x feeding level[w]							
Hay x maintenance	12	54.0	34.2	10.6	17.9	7.4	1.2
Hay x twice maintenance	12	50.3	32.1	10.6	16.0	4.7	1.5
Grain x maintenance	11	52.7	34.0	11.4	18.5	6.4	1.4
Grain x twice maintenance	11	49.6	33.7	11.7	18.8	5.10	1.4
SE		02.94	2.91	0.84	2.02	2.04	0.55
Probability		0.68	0.95	0.71	0.75	0.75	0.98

[z]Abbreviation: CrMRT, the mean retention time for chromium mordanted fiber in the digestive tract; CoMRT, the mean retention time for cobalt-EDTA in the digestive tract; TT, the time for flow through the non-mixing component of the digestive tract; MRT1, the retention time of average digesta in the forestomach; MRT2, the digesta retention time in compartment 2 (abomasum or cecum-proximal colon); MRT3, the digesta retention time in compartment 3 (abomasum or cecum-proximal colon).

[y]Number of animals within each treatment.

[x]Means used are least squared means.

[w]Means used are simple means.

Table 4. Daily methane and urinary energy losses.

Item	n[y]	Methane			Urine		
		kJ $kg^{-0.75}$	% GE[z]	% DE[z]	kJ $kg^{-0.75}$	% GE[z]	% DE[z]
Temperature[x]							
29°C	16	63.2	5.2b	7.0b	33.9ab	2.4ab	3.4ab
19°C	16	66.9	5.5ab	7.7ab	32.2b	2.2b	3.2b
-23°C	14	78.7	6.3a	8.9a	48.1a	3.6a	5.1a
SE		6.35	0.45	0.61	3.26	0.29	0.45
Probability		0.14	0.05	0.02	0.01	0.01	0.01
Diet							
Hay	24	82.8a	5.5	8.5	48.5a	3.0	4.7a
Grain	22	46.5b	5.8	7.3	27.6b	2.5	3.1b
SE		5.18	0.37	0.49	2.63	0.24	0.37
Probability		0.0004	0.32	0.66	0.0001	0.13	0.005
Feeding level							
Maintenance	23	66.5	7.4a	10.2a	27.6b	2.9	4.2
Twice Maintenance	23	72.4	3.9b	5.5b	48.1a	2.5	3.6
SE		4.14	0.24	0.32	2.76	0.23	0.33
Probability		0.35	0.0001	0.0001	0.0001	0.25	0.23
Temperature x diet							
29°C x hay	8	73.2ab	4.6	7.0	52.7ab	3.4ab	5.1ab
29°C x grain	8	53.1b	5.5	6.7	20.9c	1.8c	2.2d
19°C x hay	8	81.2ab	5.4	8.5	39.3bc	2.3bc	3.7bcd
19°C x grain	8	52.7b	5.5	6.9	24.7c	2.2bc	2.7cd
-23°C x hay	8	94.1a	6.1	9.5	59.0a	3.7a	5.8a
-23°C x grain	6	65.3ab	6.7	8.6	36.8bc	3.5ab	4.5abc
SE		9.62	0.92	1.24	5.65	0.35	0.52
Probability		0.03	0.77	0.47	0.0001	0.0009	0.0001
Temperature x feeding level							
29°C x maintenance	8	61.1	6.8a	9.1ba	25.5bc	2.6b	3.6b
29°C x twice maintenance	8	64.9	3.6b	5.0b	42.2bac	2.2b	3.1b
19°C x maintenance	8	64.4	7.1a	10.0a	19.2c	2.1b	3.0b
19°C x twice maintenance	8	69.4	3.8b	5.4b	44.8ba	2.4b	3.4b
-23°C x maintenance	7	74.9	8.1a	11.6a	38.1bac	4.1a	5.9a
-23°C x twice maintenance	7	88.7	4.6b	6.7bc	60.7a	3.1a	4.6ba
SE		10.83	0.64	0.87	5.98	0.38	0.62
Probability		0.54	0.0001	0.0001	0.0003	0.01	0.02
Diet x feeding level[w]							
Hay x maintenance	12	69.9b	6.4b	10.0a	34.3b	3.1	4.9a
Hay x twice maintenance	12	95.8a	4.5c	7.0b	62.3a	2.9	4.4ba
Grain x maintenance	11	62.8b	8.3a	10.3a	19.7c	2.6	3.3ba
Grain x twice maintenance	11	49.8b	3.4c	4.2c	33.5b	2.2	2.8b
SE		7.40	0.47	0.68	4.02	0.35	0.52
Probability		0.0007	0.0001	0.0001	0.0001	0.31	0.02

[z]Abbreviations: GE, gross energy; DE, digestible energy.
[y]Number of animals in each treatment.
[x]Least squares means shown only for temperature, diet, and feeding level, the remaining are simple means
[w]Diet by feeding level interactions were significant for CH_4: kcal/kg ^{75}, GE, and DE at 0.004, 0.0003, and 0.003, respectively.

Table 5. Concentrations of volatile fatty acids in the rumen

Item	n[y]	A:P ratio	Molar Percentage A	P	IB	B	IV	V	C
Temperature[x]									
29 °C	16	2.8	59.9	26.6	0.7	10.0	1.0	1.6	0.2
19 °C	16	3.2	61.2	24.5	1.0	10.8	0.9	1.5	0.1
-23 °C	14	2.8	59.4	26.5	0.8	10.9	0.9	1.5	0.1
SE		0.22	0.89	1.55	0.23	1.02	0.07	0.08	0.03
Probability		0.34	0.43	0.42	0.68	0.87	0.67	0.58	0.57
Diet									
Hay	24	3.9a	70.3a	18.2b	1.0	8.5b	0.8b	1.1b	0.1b
Grain	22	2.0b	50.0b	33.5a	0.6	12.6a	1.1a	1.9a	0.2a
SE		0.18	0.60	1.26	0.19	0.83	0.06	0.07	0.03
Probability		0.0001	0.0001	0.0001	0.24	0.007	0.03	0.0001	0.01
Feeding level									
Maintenance	23	3.5a	64.1a	20.1b	0.8	12.3a	1.2a	1.4	0.1b
Twice Maintenance	23	2.4b	56.3b	31.6a	0.9	8.8b	0.7b	0.6	0.2a
SE		0.17	0.59	1.01	0.21	0.85	0.09	0.08	0.03
Probability		0.0005	0.0001	0.0001	0.73	0.01	0.002	0.10	0.03
Temperature x diet									
29 °C x hay	8	3.9a	70.0a	18.3b	0.8	8.7	0.9	1.2b	0.1
29 °C x grain	8	1.7b	49.7b	34.9a	0.6	11.3	1.1	2.0a	0.3
19 °C x hay	8	4.1a	71.1a	17.9b	1.3	8.0	0.6	1.0b	0.1
19 °C x grain	8	2.3b	51.3b	31.0ba	0.7	13.5	1.3	1.9a	0.2
-23 °C x hay	8	3.9a	69.8a	18.4b	0.9	8.8	0.9	1.1b	0.1
-23 °C x grain	6	1.8b	49.0b	34.6a	0.6	12.9	0.9	1.9a	0.2
SE		0.39	2.27	3.48	0.35	1.70	0.19	0.14	0.06
Probability		0.0001	0.0001	0.0002	0.29	0.17	0.14	0.0001	0.12
Temperature x feeding level									
29 °C x maintenance	8	3.3	62.6	21.3	0.8	12.3	1.3ba	1.6	0.1
29 °C x twice maintenance	8	2.3	57.2	31.9	0.6	7.7	0.7ba	1.6	0.3
19 °C x maintenance	8	3.9	66.7	17.6	0.7	12.3	1.3a	1.4	0.1
19 °C x twice maintenance	8	2.5	55.7	31.3	1.4	9.3	0.6b	1.6	0.2
-23 °C x maintenance	7	3.4	62.9	21.4	0.8	12.5	1.0ba	1.3	0.1
-23 °C x twice maintenance	7	2.3	55.9	31.6	0.7	9.2	0.8ba	1.7	0.1
SE		0.51	4.30	4.10	0.36	1.71	0.17	0.22	0.06
Probability		0.19	0.43	0.06	0.68	0.24	0.01	0.88	0.05
Diet x feeding level[w]									
Hay x maintenance	12	4.1a	71.5a	17.4c	0.8	8.4b	0.8b	1.1c	0.1b
Hay x twice maintenance	12	3.7a	69.1a	19.0cb	1.3	8.7b	0.8b	1.1c	0.1b
Grain x maintenance	11	2.9b	56.6b	22.8b	0.8	16.3a	1.6a	1.7b	0.1b
Grain x twice maintenance	11	1.0c	43.4c	44.2a	0.5	8.8b	0.6b	2.1a	0.3a
SE		0.23	1.07	1.53	0.28	1.10	0.11	0.10	0.04
Probability		0.0001	0.0001	0.0001	0.22	0.0001	0.0001	0.0001	0.0002

[z]Abbreviations: A:P ratio, acetic acid:propionic acid ratio; A, acetic acid; P, propionic acid; IB, isobutyric acid; B, butyric acid; isovaleric acid; V, valeric acid; C, caproic acid.

[y]Number of animals in each treatment.

[x]Least squares means shown only for temperature, diet, and feeding level, the remaining are simple means.

[w]Diet by feeding level interactions were significant for A:P, A, P, B, IV at 0.008, 0.0003, 0.0001, 0.007, 0.002, respectively.

Table 6. Daily ME intake, heat production, and retained energy, fat, and protein (kJ $kg^{-0.75}$).

Item	n[y]	ME Intake[s]	Heat[z]	Retained Energy		
				Total[z]	Protein[z]	Fat[z]
Temperature[x]						
29 °C	16	882	799b	113a	88ab	24a
19 °C	16	816	799b	75ab	93a	-16ab
-23 °C	14	841	962a	-121b	46b	-167b
SE		25.5	42.9	50.7	8.07	48.9
Probability		0.18	0.02	0.005	0.003	0.01
Diet						
Hay	24	908a	874	50	95a	-44
Grain	22	786b	833	-6.3	57b	-63
SE		20.8	34.9	41.3	6.6	39.9
Probability		0.001	0.37	0.28	0.001	0.69
Feeding level						
Maintenance	23	535a	778b	-217b	23b	-242b
Twice Maintenance	23	1159a	933a	623a	128a	136a
SE		20.7	32.4	35.8	6.6	33.5
Probability		0.0001	0.004	0.0001	0.0001	0.0001
Temperature x diet						
29 °C x hay	8	941	761	163	100	66
29 °C x grain	8	828	799	58	75	-16
19 °C x hay	8	891	833	92	121	-29
19 °C x grain	8	741	799	58	67	-4
-23 °C x hay	8	981	991	-100	67	-167
-23 °C x grain	6	786	946	-159	25	-188
SE		126.3	79.9	119.2	23.0	103.3
Probability		0.87	0.28	0.42	0.14	0.50
Temperature x feeding level[w]						
29 °C x maintenance	8	586b	651b	-71b	29c	-100a
29 °C x twice maintenance	8	1184a	941a	297a	146a	151a
19 °C x maintenance	8	489b	636b	-79b	42c	-121a
19 °C x twice maintenance	8	1142a	958a	230a	142a	88a
-23 °C x maintenance	7	531b	1025a	-489c	3c	-494b
-23 °C x twice maintenance	7	1159a	920a	238a	96b	142a
SE		40.7	62.3	72.4	13.3	66.9
Probability		0.0001	0.0001	0.0001	0.0001	0.0001
Diet x feeding level						
Hay x maintenance	12	590c	795	-201b	38c	-243b
Hay x twice maintenance	12	1222a	954	305a	151a	155a
Grain x maintenance	11	473d	734	-201b	13c	-213b
Grain x twice maintenance	11	1096b	925	201a	105b	93a
SE		27.9	62.3	70.3	10.9	66.5
Probability		0.0001	0.04	0.0001	0.0001	0.0001

[y]Abbreviations: ME intake, metabolizable energy intake; heat, heat production; energy, retained energy; protein, retained protein; and fat, retained fat;

[z]Number of animals within each treatment.

[x]Least squares means shown only for temperature, diet, and feeding level, the remaining are simple means

[w]Temperature by feeding level interactions were significant for heat, energy, and fat at 0.002, 0.005, and 0.002, respectively.

CHAPTER IV -
GENERAL DISCUSSION

The effect of environmental temperature, diet type, and feeding levels on ruminants has been well reviewed in the literature. The general effects and trends of cold, ambient, and warm temperatures on digestibility confirmed in this trial were that cattle exhibit an increased heat production and decrease in digestive capacity and retention time in the cold.

In this study, CH_4 production from steers was 21% higher at -23 °C than at 29 °C. This finding did not concur with the hypothesis that methane production was expected to be reduced at both high and low temperatures; although, CH_4 production behaved as hypothesized at the high temperature, it increased, instead of decreased, at the low temperature.

The increase in CH_4 production is also contradictory to the common belief that CH_4 production should fall in the cold. Unfortunately, the there are only two previous experiments concerning this subject in which more than two animals were used. In the Kennedy and Milligan (1978) study with sheep, a 30% reduction in CH_4 production was noted in the cold. Their experiment does have limitations, however, in that the sheep were fed an abnormal diet consisting of pellets, feed was provided hourly, and CH_4 production was only measured over a 3 hour period. In contrast, von Keyserlingk and Mathison (1993) measured CH_4 production in sheep fed a diet consisting of 50% barley straw and 50%

concentrate over a 24 hour period and obtained a 14% increase in the portion of GE lost as CH_4 in the cold. There were enough differences between these two trials, then, to warrant this further examination into the effect of environment on CH_4 losses in this study.

The CH_4 production measurements are as accurate as the equipment was routinely calibrated and heat production values were within the expected range. It must be noted that some minor complications were experienced during the calorimetry sampling at -23 °C as the moisture exhaled by the steers tended to freeze inside the 'respired gas collection PVC hose' and had to be cleared out regularly. As samples were continuously being drawn out of the calorimetry hood and the volume passing through the hose was periodically reduced, the CH_4 values determined for the cold environment may be slightly underestimated, instead of overestimated.

The explanation for the increase in CH_4 production in the cold is not readily apparent. Upon examining the amount of substrate available for fermentation and the physiological and metabolic responses to temperature, less CH_4 should have been produced. Digestibility of the diets was lower in the cold temperature which indicated that less of the diet was available for fermentation to CH_4. The nonsignficant decrease in mean retention time would also be consistent with a decrease in CH_4 production in the cold. Ruminal VFA proportions would also suggest that CH_4 production could have been reduced, rather than increased, in the cold. Unfortunately other factors which are known to influence CH_4

production were not examined in this experiment. It is known, for example, that CH_4 production is greater when protozoa are present in the rumen. It is not inconceivable that environmental conditions may affect protozoal populations inside the rumen although no data was found to support this hypothesis. Another possibility which may have affected the results, is the drinking water temperatures which occurred during the experiment. Although water temperatures were not measured, it is expected that the temperatures would have risen gradually in the warm environment from about the 15 °C temperature of tap water and fall in the cold temperature to a temperature of less than 10 °C, as controlled by the submersible water heaters which were used. Another factor which could have affected CH_4 production is the increased blood flow to internal organs in the cold which Miaron and Christopherson (1992) observed.

Another hypothesis of the study was that forage diets in the cold environment would produce more CH_4 than concentrate diets. This hypothesis was also rejected. There was no overall difference in CH_4 production (percentage of GE intake) between hay and concentrate diets. In fact, more CH_4 was produced with the concentrate diet, than with the hay diet, at the maintenance feeding level. The slight reduction in CH_4 production with the concentrate diet at the twice maintenance feeding level was not significant. However, if CH_4 production is expressed as a percentage of digestible energy, energy losses as CH_4 were, in fact, less with the concentrate diet at the twice maintenance feeding level.

The CH_4 losses as a percent of GE decreased by 47% when the feeding level was increased from maintenance to twice maintenance. This decrease was expected because increased digesta in the digestive tract would tend to increase the passage rate in the rumen. The increase in passage rate decreases the time for fermentation and decreases the amount of CH_4 produced. The hypothesis relating to CH_4 production not being affected by temperature at a low feeding level at cold temperatures while having more of an effect at high temperatures was also not proven. There was no interaction of feeding level and temperature on CH_4 production (expressed as a percentage of GE).

The ME as a percent of GE was decreased by 8% at -23 °C in comparison with 29 °C. This decrease in ME as a percentage of GE and the 20% increase in heat production in the cold the animals would have had a food requirement greater than the animals in the warm. The effect of cold environments on the animal's efficiency of feed utilization was, therefore, greater than expected because of the increased CH_4 production in the cold.

Based on the aforementioned conclusions, this study may have some broad implications regarding CH_4 and the Greenhouse Effect. If CH_4 production does increase in cold environments, the contribution of domestic animals (primarily, cattle and sheep) would increase in the northern hemisphere, particularly when temperatures drop below freezing. This could alter the estimate calculated by Cicerone and Oremland (1988) that approximately 76% of the global CH_4 emissions from all animals, including cattle, occurs

in the northern hemisphere. The increase may be minimal on a global scale as cattle contribute approximately 15% of the total global CH_4 production (Drennen and Chapman 1991). However, this alteration in the level of CH_4 production should not affect the institution of mitigative actions to reduce CH_4 production from other sources because each action reducing CH_4 will affect the rate at global warming could occur. Some of the potential mechanisms would be to harness CH_4 emitted from domesticated animals and animal wastes, to increase awareness of the dietary factors affecting CH_4 production, and to employ mechanisms which would decrease CH_4 production and increase cattle productivity (Krause et al. 1992).

Other studies should be conducted to allow for better prediction of total global CH_4 emissions. Studies of this nature would better allow scientists to estimate CH_4 production and help reduce its impact as a contributor to the Greenhouse Effect. These studies should examine and quantify CH_4 production from both domestic and non-domestic animals, in addition sheep and cattle.

With regards to studies dealing specifically with ruminant CH_4 production, the increased CH_4 production at cold temperatures, as found in this study and von Keyserlingk and Mathison (1993), needs to be better examined. An investigation of nature of the ruminal microorganisms and their response to temperature, diet, and feeding level may be useful in determining the mechanism controlling CH_4 production at extreme temperatures. In

addition to the cold environment, future research could also build upon the research of Blaxter and Clapperton (1965) who noted a decrease in CH_4 production at high temperatures. A higher temperature would have to be reached than was achieved in this experiment. This would aid in the understanding of CH_4 production at the animals upper critical temperature.

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