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HEAT INCREMENT AND METHANE PRODUCTION BY

MUSKOXEN FED BROWSE

Presented to the Faculty

of the University of Alaska Fairbanks

in Partial Fulfillment of the Requirements

for the Degree of

DOCTOR OF PHILOSOPHY

By

James Patrick Lawler

B.S., M.S.

August 2001

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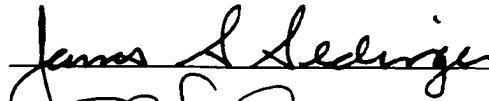
HEAT INCREMENT AND METHANE PRODUCTION BY

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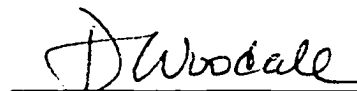
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ABSTRACT

Many browse species contain anti-herbivory compounds that deter consumers by their toxicity or digestive inhibitory effects. Animals that consume browse are assumed to pay a detoxification energy cost, which increases the heat increment of feeding (HIF). Ruminants also lose potentially metabolizable energy as methane (CH_4); but browse may lower CH_4 production. I hypothesized that increases in energy loss to HIF by animals eating browse could be offset by a reduction in energy lost via CH_4 production. Muskoxen eat both graminoids and browse and are considered to be energetically conservative due to their existence in a sparse arctic environment. These traits make them ideal for energetic studies. Muskoxen were fasted for 24 h and then fed a test meal composed of hay mixed with graded percentages of one of three browse species (Willow: *Salix alaxensis, S. pulchra*, birch: *Betula nana*). Browse consisted of twigs in winter and leaves in the summer. Heat increment of feeding and CH_4 production were estimated with an indirect calorimeter. Addition of woody twigs or leaves of birch to hay diets tended to depress HIF following the test meal. Woody twigs and leaves of willow added to hay diets tended to increase HIF. Woody browse tended to lower CH_4 production when fed at > 20% of the meal. Leafy browse had variable effects on CH_4 production; *S. alaxensis* was stimulatory, *S. pulchra* was inhibitory, while *B. nana* showed not consistent pattern. Generally, CH_4 production by muskoxen was low at 2.0-3.2% of GE intake when compared with estimates for sheep and cattle (2-12% of GE intake). Although diets high in fermentable carbohydrates stimulated methane production, secondary compounds apparently had a suppressing effect as deduced from the relation of

in vitro digestibility to methane production. Given the low overall CH_4 production in muskoxen, and the inconsistency of the relationship of CH_4 to HIF, it is unlikely that significant gains in energy retention are made by reductions in CH_4 production through browse consumption.

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ACKNOWLEDGMENTS

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In addition to my committee, John Bryant of the Institute of Arctic Biology, University of Alaska Fairbanks, was instrumental in shaping my thinking about plant chemistry and the way plants and animals interact. He also provided valuable lab space and instruction on proanthocyanidin assays. Rich Kedrowski of the Chemical Nutritional Laboratory at the University of Alaska Fairbanks, was very patient in instructing me on various forage analyses, and provided valuable time and space in the Chemical Nutritional Lab for me to complete my work. Tom Clausen of the Chemistry Department, University of Alaska Fairbanks, generously provided space and instruction is his laboratory for the extraction of tannins.

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INTRODUCTION

Muskoxen (*Ovibos moschatus*), and caribou and reindeer (*Rangifer tarandus*) are the northern-most distributed large herbivores. They live, and in some cases thrive, in environments where plant growth can be limited to two months of the year, plant species diversity is low, and plant productivity is limited and variable (White *et al.* 1981; Klein 1986). To survive under conditions imposed by the arctic requires animals to have evolved unique suites of behavioral, nutritional, and physiological traits. The ability to make efficient use of energy would be a great advantage under the conditions imposed by the arctic. Muskoxen and caribou have achieved a high degree of success. Twenty five thousand years ago the arctic and subarctic were much more cosmopolitan environments for large herbivores. Steepe bison (*Bison priscus*), mammoths (*Mammuthus* spp.), wild horses (*Equss* spp.), helmeted muskoxen (*Boötherium bombifrons*), western camels (*Camelops hesternus*), saiga (*Saiga tatarica*), caribou and muskoxen all shared the northern landscape (Gutherie 1982). Of this original host of large herbivores, only muskoxen and caribou remain.

In comparison to caribou, muskoxen are the more conservative of the two northern-most species. Muskoxen are relatively sedentary, and females especially are unlikely to travel great distances in search of high quality food. Certainly long distance seasonal movements, such as those exhibited by caribou, are not part of the ecology of muskoxen. Presumably then, the combination of a sparse environment coupled with a relatively sedentary life style would constrain muskoxen to be energetically conservative. It is thought that caribou and muskoxen survive the long arctic winters by lowering their

nutrient requirements, continuing to forage, and making use of body reserves stored during the short snow-free season (Tyler & Blix 1990).

Muskoxen have demonstrated a certain amount of flexibility in their feeding niche. Based on observed muskox diets in the Canadian high arctic and digestive ability of captive animals, muskoxen have been classified as grazers. Adamczewski (1995) demonstrated the ability of muskoxen to handle and digest a high-fiber, low-quality diet. Indeed, in Adamczewski's study, muskoxen out-performed the consummate grazer, the cow. Conversely, other authors have classified muskoxen as intermediate mixed feeders (Dehority 1984; Hofmann 1988). Observed diets in southern portions of muskoxen range, such as Alaska, show muskoxen will readily consume browse. Browse species found on muskoxen ranges are typically defended against mammalian herbivores through a host of plant chemical compounds. These compounds function either as digestive inhibitors, or as toxins. Why then, would an animal that functions so well as a grazer voluntarily consume browse species with anti-nutritional qualities?

Energy may be lost at numerous steps in the conversion of energy consumed to metabolic energy (Fig. 1). This thesis focuses on four aspects of energetics in muskoxen: 1) seasonal energy expenditure; 2) the measurement of the heat increment of feeding; 3) the heat increment of feeding of natural diets; and 4) and the loss of energy to CH_4 production on natural diets. I hypothesize that muskoxen have evolved to maximize the efficiency of conversion of dietary energy to metabolic energy and therefore attempt to minimize inefficiences.
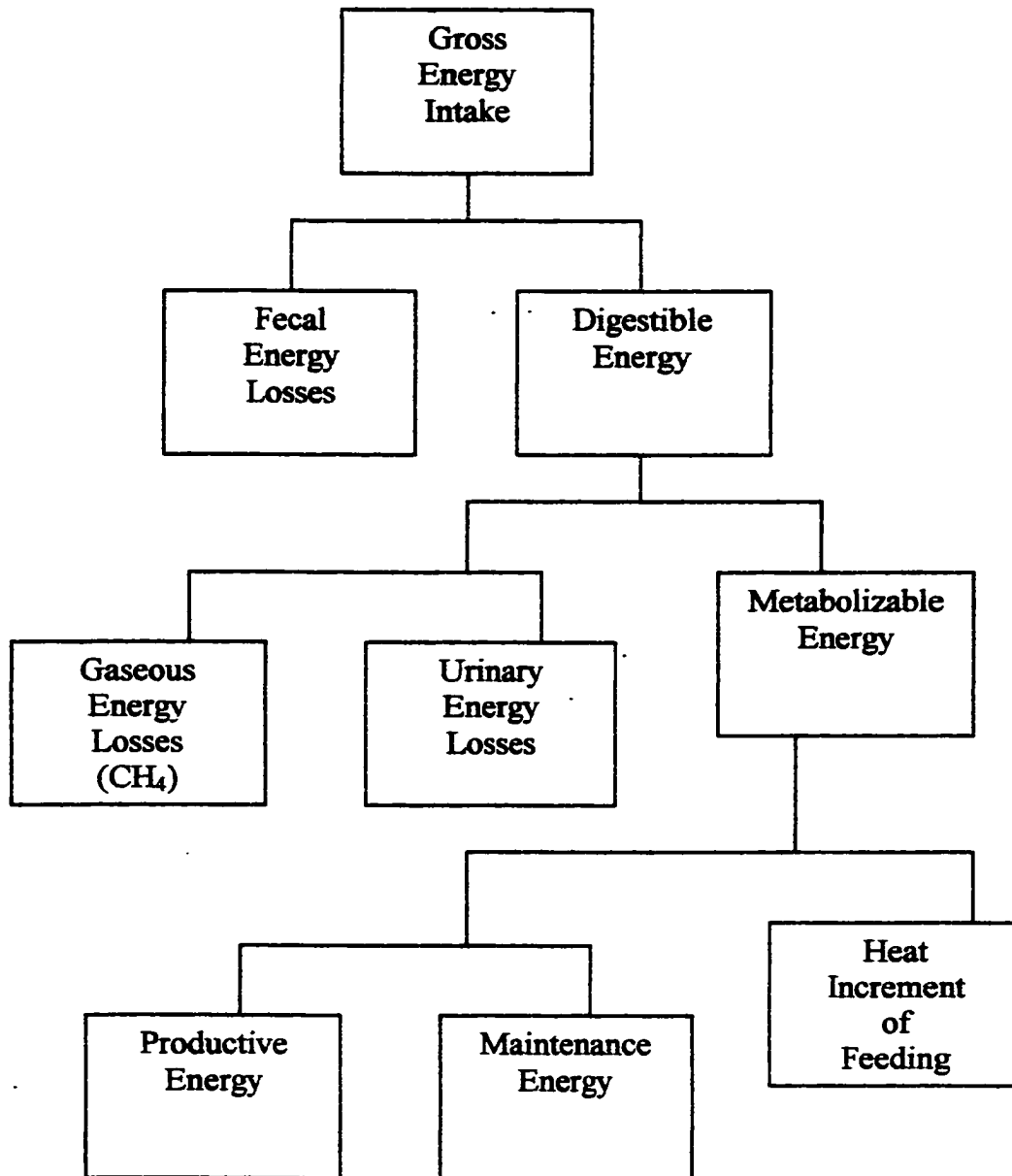
Fig. 1. Flow Diagram of energy intake and partitioning of energy in ruminants based on Kleiber (1975) and Webster (1983). This thesis focuses on CH₄ energy losses, heat increment of feeding, and maintenance energy requirements.

The most direct means to address seasonal change in availability of energy is to change the requirements for energy. Down regulation of metabolism has been proposed as an adaptation to limited winter food supply by many authors (see Wood *et al.* 1962; Mautz *et al.* 1992). Problems with methodology however, have caused confusion in studies examining seasonal metabolism. Mautz *et al.* (1992) and Jensen *et al.* (1999) indicated that differences in seasonal metabolic rates in white-tailed deer (*Odocoileus virginianus*) could be explained by measurements made outside the thermoneutral zone and that HIF was known to substitute for cold-induced thermogenesis (Jensen *et al.* 1999). I therefore investigated thermoneutrality and seasonality in this study (Chapters 1 and 2).

The heat increment of feeding (HIF) is an increase in energy expenditure associated with the intake of a meal. Heat increment of feeding is an important aspect of wildlife nutritional ecology owing to it's role in seasonal energy balance (Kleiber 1975; Robbins 1993), thermoregulation (Jensen *et al.* 1999), and association with detoxification of plant secondary compounds (Van Soest 1982; Foley 1987; Robbins 1993). A common problem in physiological studies of wildlife is sample size. Seldom are there more than a few study animals. This problem is compounded in situations using wild forage. Plant material is difficult to procure and changes in plant phenology dictate small windows of time in which to conduct experiments. Given these constraints, I reassessed the potential of a single-meal approach for estimation of heat increment of feeding (Chapter 2).

In many portions of their range, muskoxen avidly consume browse. In particular, willows (*Salix spp.*), including leaves in summer and twigs in winter, are an important

component of muskox diets (see Klein 1992). In contrast, although dwarf birch (*B. nana*) is found on many muskoxen ranges (Bos 1967; Jingfors 1981; Robus 1981; O'Brien 1988; Wilson 1992), it is not reported as an important dietary component. In this study, I examined HIF in muskoxen consuming a hay (*Bromus inermus*) based diet mixed with graded levels of woody and leafy browse. I tested a null hypothesis that the addition of browse to a graminoid based diet would not alter the heat increment of feeding (Chapter 3).

Methane (CH_4) production causes a significant loss of dietary energy to ruminants yet little is known of dietary controls over CH_4 production in wild ruminants. Between 2 and 15% of the utilizable energy of a diet can be lost as CH_4 in the conversion of dietary energy to metabolizable energy in domestic ruminants (Leng 1991). This inefficiency in energy conversion is puzzling, particularly in instances where energy conservation appears to be critical to the life history of the species. In domestic ruminants, CH_4 production can be altered through ration manipulation (see Leng 1991; Johnson & Ward 1996; Van Nevel & Demeyer 1996; for review). The fermentation of browse is thought to reduce CH_4 production (Robbins 1993) but little evidence is available to support this conviction. I suggest browse consumption in wild muskoxen could improve the efficiency of energy utilization through a reduction in CH_4 production, provided the other major loss of energy, heat increment of feeding, is not affected by browse consumption (Chapter 4). I suggest that although the anti-herbivory components of browse, both leafy (summer) and woody (winter), may bring about an increase in the HIF due to the cost of detoxification, this energy loss may be offset by lower CH_4 production.

Each of the four chapters in this thesis are manuscripts that have either been submitted for publication in professional journals, or are intended to be submitted for publication in professional journals. Consequently, throughout these manuscripts, I have retained the original author designations as a footnote to the title page of each chapter. I have also included individual abstracts, introductions, and conclusions for each chapter. References from each chapter however, have been consolidated into a single section at the end of the thesis. Robert G. White contributed to the inception of this study and the development of the research design, and R. Terry Bowyer made substantial contributions to the statistical analysis in Chapter 2. Nonetheless, the thesis I wrote myself and I am responsible for its contents. Each chapter addresses a discrete aspect of energy metabolism in muskoxen. Chapter 1 investigates the influence of season on energy metabolism in muskoxen and documents the timing of seasonal shifts in energy metabolism. Chapter 2 develops a methodology to measure the heat increment of feeding which allows the investigation of the affects of a diversity of diets on energy metabolism in situations where a limited number of study animals are available. Chapter 3 evaluates the effects of woody and leafy browse species on the loss of energy in muskoxen from the heat increment of feeding. Chapter 4 evaluates the effects of woody and leafy browse species relative to the loss of energy in muskoxen from CH_4 production. Results and conclusions from the individual chapters are consolidated and presented in a Synopsis.

CHAPTER 1: Seasonal Changes in Metabolic Rates in Muskoxen Following Twenty-Four Hours of Starvation[1]

Abstract: Timing of seasonal trends in post-prandial energy expenditure (EE) was measured in muskoxen (2 males and 1 female) given a standardized meal followed by a 24-26 h starvation during 10 months over the course of a year. EE was significantly lower in winter than summer. CH_4 production (E_{CH4}) was reversed with winter highs and summer lows. Ratio of E_{CH4}:EE indicates a change in dietary efficiency but this difference was not associated with a seasonal shift in RQ, which was constant. The main increase in EE from winter to summer occurred between April and May and the summer to winter decrease between August and September.

Key words: *Ovibos moschatus*, energy expenditure, seasonality, methane.

Introduction

A seasonal lowering of resting metabolic rate typifies a north temperate adaptation to limiting winter food supply in a cold environment (Wood *et al.*, 1962; Feist & White, 1989). Nilssen *et al.* (1994) documented a significantly lower resting

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[1] Lawler JP & White RG (1997) Seasonal changes in metabolic rates in muskoxen following twenty-four hours of starvation. Rangifer **17**, 135-139.

metabolic rate for *ad. lib.* fed and starved muskoxen (*Ovibos moschatus*) during the winter compared to summer. However, timing of the down regulation in winter and up regulation in summer was not determined. The objective of this study was to determine the timing of seasonal trends in metabolism of muskoxen given a standardized meal followed by a 24-26 h starvation.

Materials and methods

Energy expenditure was measured in two intact males and one intact non-pregnant female muskoxen during 10 months over the course of a year. All were 3 year olds and had been used in similar metabolic trials since yearlings. Animals were calm during metabolic trials. Before and after each trial, animals were maintained in a brome pasture (*Bromus inermus*) with *ad. libitum* access to brome hay. Twice a week, animals were offered high-protein pellets (Quality Texture, Alaska Mill and Feed, Anchorage) fed at a daily rate of 14 g (DM)•kg $BW^{-0.75}$. At the start of an experiment each animal was brought into an unheated barn, offered 1 standard meal of 50% chopped brome hay and 50% pellets fed at 10 g (DM) •kg $BW^{-0.75}$ and then starved for 24-26 h. Each was then placed in an open circuit metabolic chamber (White *et al.*, 1984) to measure oxygen consumption, and carbon dioxide and methane production at 2 min intervals over a 2 h period. Temperature and barometric pressure was logged by computer. Energy expenditure (EE) and methane energy loss (E_{CH4}) was calculated from gas

concentrations, and flow rates (Kokjer, 1981). Calculations were performed using the entire 2 h interval. Therefore, differences in EEs associated with different activities were not calculated. Respiratory quotient (RQ) was calculated as CO_2 expired/O_2 consumed. Differences in energy expenditure were assessed by a two-factor analysis of variance. Sources of variance for the analysis were season, animal, and animal by season. Significance was determined at a 5 percent confidence level.

Results and discussion

EE was significantly lower in winter (November through March) than summer (June, July, August) (Fig. 1.1 and Table 1.1, $P < 0.001$). Mean EE ($n = 3$) was lowest in April at 343 ± 15 (SEM) kJ•kg $BW^{-0.75}$ (1.11 ± 0.03 W per kg) and highest in June at 457 $\pm$ 16 kJ•kg $BW^{-0.75}$ (1.45 ± 0.05 W•kg). The 33% difference was significant ($P = 0.006$) but less divergent than the seasonal values for 7-10 h starved animals reported by Nilssen *et al.* (1994) who found winter and summer metabolic rates of 0.86 ± 0.10 and 1.74 ± 0.27 W•kg respectively (a 49% difference). The difference in winter-summer extremes more closely resemble the 30% difference recorded by Nilssen *et al.* (1994) for 6 d starved animals (winter values of 0.62 ± 0.07 W•kg and summer values of 0.77 ± 0.03 W•kg). Values reported for our muskoxen, represent both lying and standing-active animals, whereas those for Nilssen *et al.* (1994) are for the lying period. Low EEs
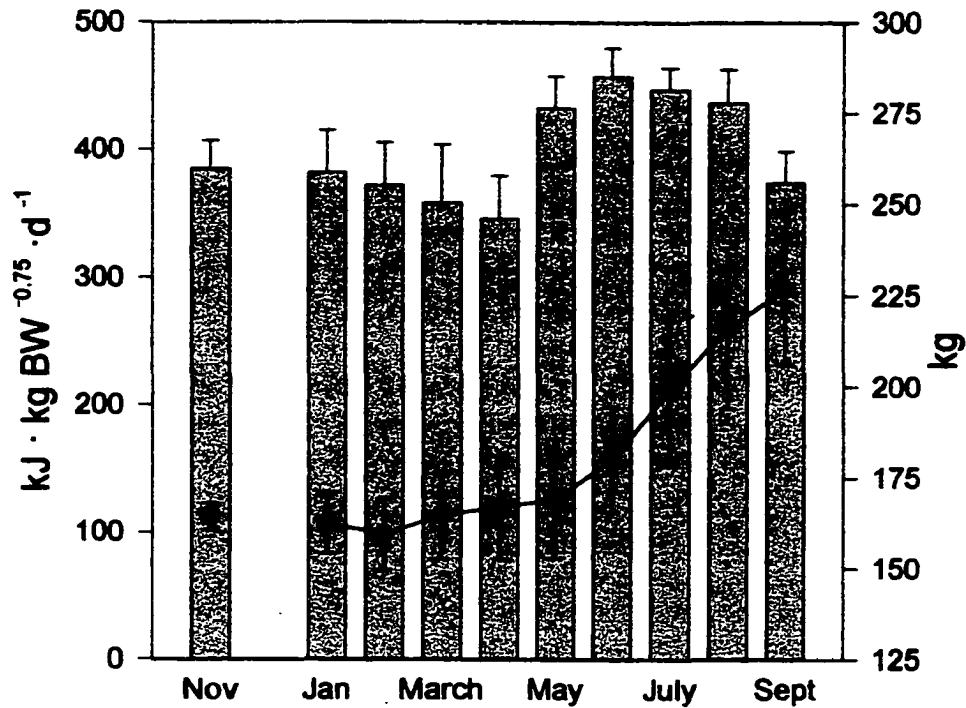
Fig. 1.1. Seasonal trends in mean $\pm$ SE energy expenditure (columns) and mean body weight (line) of 3 muskoxen following a standardized meal and 24 hours of starvation from November 1993 through September 1994 at the Large Animal Research Station, Fairbanks, Alaska. Seasonal patterns of summer highs and winter lows in energy expenditure and body weight were not synchronized.

Table 1.1. Comparison of winter 1993-1994 with summer 1994 mean standardized energy expenditures, methane production and RQ of three 3 year old muskoxen at the Large Animal Research Station, Fairbanks, Alaska.

	Summer[1]		Winter[2]		
	mean	SEM	mean	SEM	P
Daily Energy Expenditure[3]	447	8.5	370	5.6	<0.001
Energy Expenditure ($W \cdot kg^{-1}$)	1.40	0.03	1.20	0.02	<0.001
CH_4 Energy[3]	17.0	1.16	24.0	0.92	0.001
CH_4 Energy / Energy Expenditure	0.038	0.003	0.063	0.002	<0.001
RQ	0.88	0.01	0.90	0.01	NS

[1] June ($n = 3$: Where n is the number of trials per month), July ($n = 3$) and August ($n = 3$)
[2] November ($n = 3$), January ($n = 15$), February ($n = 14$) and March ($n = 19$)
[3] $kJ \cdot kg \, BW^{-0.75} \cdot d^{-1}$

occurred when body mass was at maintenance (November through April) and highest

values during a period of weight gain (June through September)(Fig. 1.1). Summer and

winter mean values were 53% and 27% respectively above the predicted BMR using the

Kleiber equation (Kleiber, 1975).

CH_4 production showed a significant but reverse seasonal pattern with winter

highs and summer lows (Fig. 1.2 and Table 1.1, $P = 0.03$). Mean daily E_{CH4} loss after the

standardized starvation was lowest in June at 15 ± 2.6 kJ•kg $BW^{-0.75}$ and highest in

January at 28 ± 2.1 kJ•kg $BW^{-0.75}$ ($P = 0.02$) (Fig. 1.2). Ratio of E_{CH4}:EE indicates a

change in dietary efficiency (Fig. 1.3) but this difference was not associated with a

seasonal shift in RQ which was constant at 0.88.

Variation exists between individuals and between seasons in voluntary levels of

dietary intake, passage rate, and digestibility in muskoxen (Holleman *et al.*, 1984; White

et al., 1984; Adamczewski *et al.*, 1994). Each of these factors alter the heat increment

of feeding and make it difficult to determine when a ruminant animal will reach a post-

absorptive state (Blaxter, 1962). Usually methane production would be low or non-

existent when ruminants are truly post absorptive, thus our estimate of significant

production shows heat increment is present. However, in some species, starving an

animal to the point that it reaches a post-absorptive state has been shown to cause

hyperactivity and restlessness (Robbins, 1994). These factors and those imposed by

animal welfare concerns make it difficult to achieve the conditions required to directly

measure BMR in large ruminants.
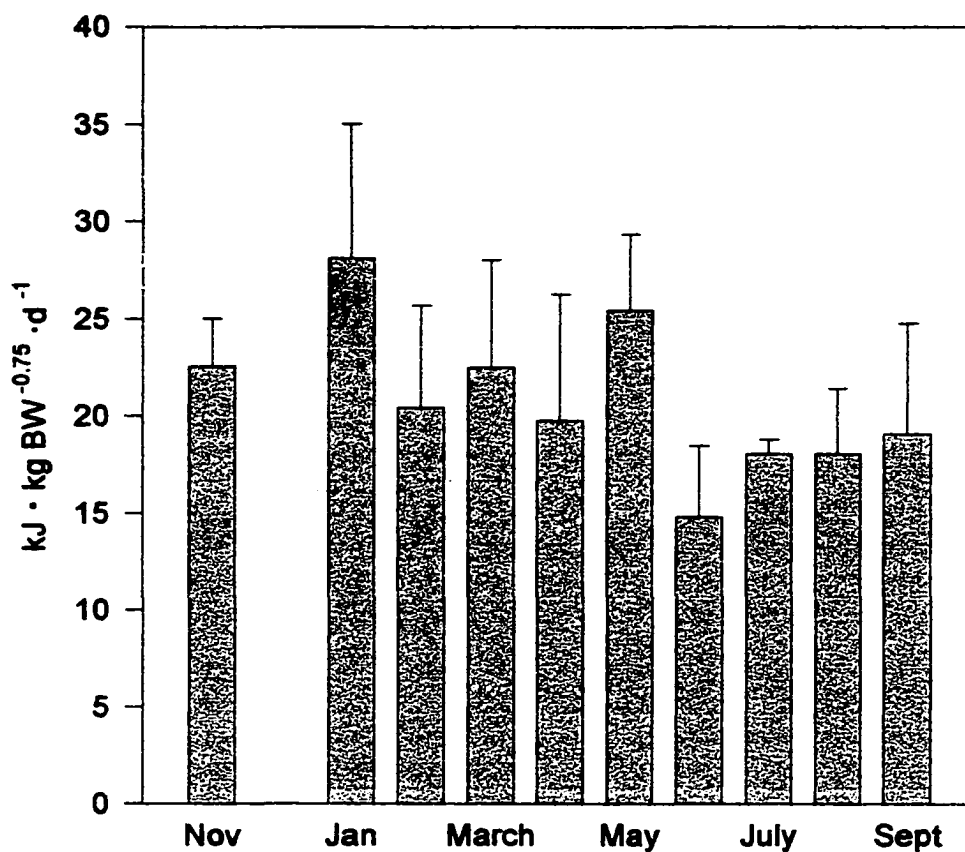
Fig. 1.2. Trends in mean ± SE CH_4 energy loss of 3 muskoxen following a standardized meal and 24 hours of starvation from November 1993 through September 1994 at the Large Animal Research Station, Fairbanks, Alaska. Methane energy loss was higher in winter than in summer.
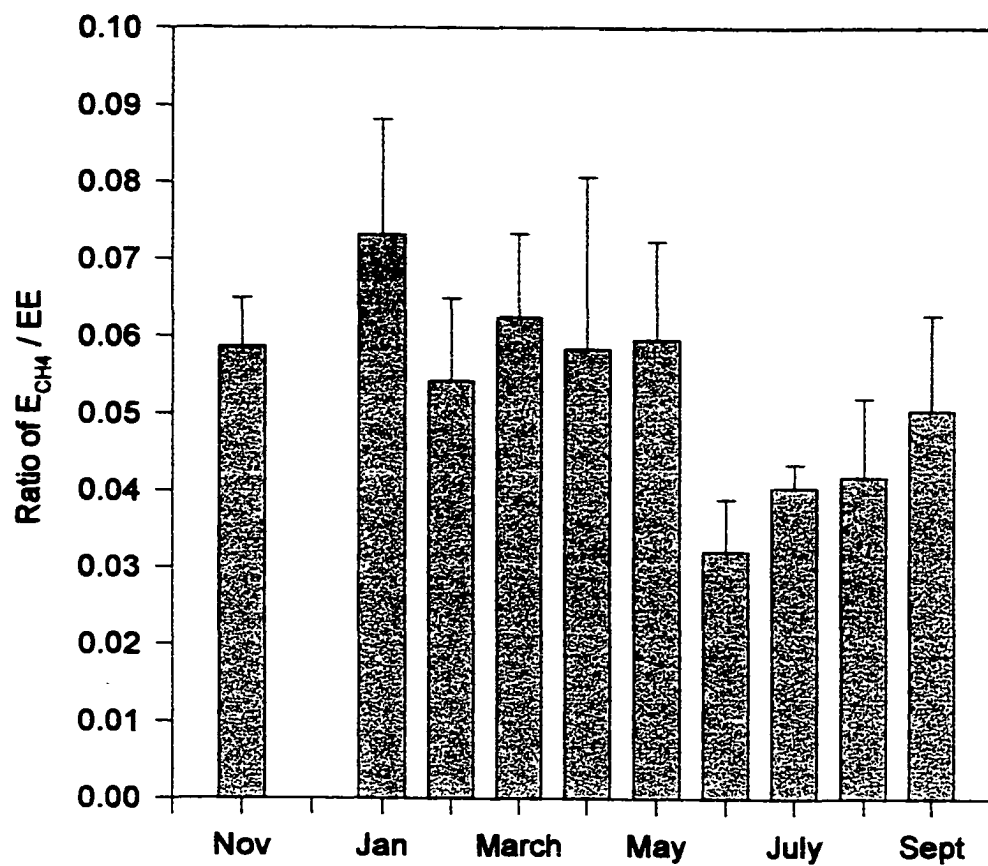
Fig. 1.3. Ratio of monthly mean methane energy loss to monthly mean heat energy expenditure indicating changes in dietary efficiency. Muskoxen at the Large Animal Research Station, Fairbanks, Alaska were more efficient in summer 1994 then in winter of 1993-1994.

An alternative approach to long-term starvation is to measure EE under standardized conditions (Blaxter & Boyne, 1982), with the realization that some effects of heat increment may be carried over to add an indeterminate contribution to the variability in EE. As depicted by trends in body weight (Fig. 1.1), muskoxen were at maintenance levels in winter, but in positive energy balance in the summer. Thus the seasonal pattern in EE for these 24 h starved muskoxen, even when preceded by a standardized meal, undoubtedly reflect some residual heat increment and other metabolic effects associated with body weight gain and food intake. However, the 33% shift in seasonal extremes in this study closely resembles that for long-starved muskoxen (Nilssen *et al.*, 1994), which suggests that endogenous regulations could partially contribute to these seasonal EEs. Nilssen *et al.* (1994) argue that the 30% decrease in EE in long starved muskoxen is a down regulation and not due to heat increment.

For the first time, we report on the timing of seasonal changes. The main increase in EE from winter to summer occurred between April and May and the summer to winter decrease between August and September (Fig. 1.1). Possible cues that initiate seasonal shifts include seasonal photoperiod and forage quality and quantity. Under the conditions of this study in Fairbanks, Alaska (latitude 65° N) the month of April provides a summer cue, while the month of August provides that for winter, the month after and the month before equinox respectively. From a natural history viewpoint, it is significant that the initiation of calving occurs in April, and initiation of the rutting season, and a

shift of nutrient partitioning in adult females from milk production to body reserves,

occurs in early August (White *et al.*, 1989), precisely when we observe significant shifts

in seasonal EE.

CHAPTER 2: Determination of Heat Increment of Feeding in a Large Ruminant: The Muskox[1]

Abstract: We evaluated procedures to allow repeated measures of the heat increment of feeding (HIF). We modified traditional procedures by withholding food from test animals for only 24 h and reducing the size of the meal to that typical of a single feeding event. We used the muskox (*Ovibos moschatus*) as the test animal, chopped brome hay (*Bromus inermis*) as the test meal, and an 8 h post-feeding HIF recovery period. To ensure thermal regulation did not obscure estimates of HIF, we investigated effects of date and temperature on energy expenditure (EE). We subtracted the energy cost of standing (87 ± 11.1 kJ•kg $BW^{-0.75}$• d^{-1}, an increase of $21 \pm 2.7\%$ over bedded) from observed EE during the measurement period. We evaluated three methods to estimate HIF under these conditions: i) a constant baseline value (EEp) was subtracted from the EE following the meal; ii) a linearly declining baseline was subtracted from the EE following the meal; and iii) results from method (ii) were extrapolated to time ∞ by assuming HIF responded exponentially. Estimates of HIF expressed as a fraction of metabolizable energy intake in winter were 0.07, 0.19, and 0.38 for methods (i) through (iii), respectively. In summer, estimates were 0.06, 0.20, and 0.29 for methods (i) through (iii), respectively. Methods (i), (ii) and (iii) all gave repeatable measures of HIF.

Methods (i) and (ii) gave lower estimates of HIF than predicted based on values reported in the agricultural literature. Method (iii) tended to overestimate HIF compared with predictions but estimates were within the range of reported values.

Key words: Muskox, metabolic rate, heat increment of feeding, dietary induced thermogenesis.

Introduction

Heat increment of feeding (HIF) is an important component of seasonal energy balance (Kleiber, 1975; Robbins, 1993), thermoregulation (Jensen et al., 1999), and association with detoxification of plant secondary compounds (Van Soest, 1982; Foley, 1987; Robbins, 1993). Although digestibility of native forages by wild ruminants is well studied (Van Soest, 1982; Robbins, 1993), estimates of the efficiency with which energy is retained as net or productive energy are few. This dearth of information for wild ruminants is related to the large amounts of forage that must be harvested and fed to test animals, as well as to problems of animal confinement and the expense of metabolism stalls and calorimetric equipment. Energy lost as heat must be measured by gaseous exchange to allocate metabolizable energy (ME) between nonproductive heat production (the heat increment of feeding, HIF) and net energy (NE) used for maintenance, milk, protein and fat deposition, and work (Brody, 1945; Blaxter, 1962). The fraction of ME retained as NE is termed the efficiency in use of metabolizable energy, k (Blaxter, 1962). Small changes in k can have profound effects on productivity (Blaxter, 1962; White,

1983). Forage structure, plant species, and plant chemistry, which stimulate metabolism

in the detoxification process, all can affect k (Agricultural Research Council, 1980; Van

Soest, 1982; Robbins, 1993; Foley, 1987; Foley & McArthur, 1994; Iason & Murray,

1996). HIF and k are interrelated mathematically: $k = 1-(HIF/MEI)$, where MEI is

metabolizable energy intake. For domestic ruminants, k often is estimated by measuring

energy retention at two levels of intake – at and above maintenance. Each diet is fed for

approximately 2 weeks (Agricultural Research Council, 1980). This procedure is an

adaptation of the "Kellner" method (Brody, 1945). HIF and NE also can be measured on

a single meal fed to a post-absorptive animal (i.e., an animal at BMR), as in the "Rubner"

method (Brody, 1945). This latter approach reduces the amount of food needed for

determination of HIF to approximately daily requirements for maintenance (Blaxter,

1962). Nonetheless, values for HIF measured by reference to a fasted animal at BMR

may be lower than those recorded for an animal fed at maintenance (Brody, 1964; Ferrell

et al., 1986). The utility of post-absorptive ruminants for measurement of HIF is

therefore questionable. The single-meal approach has been used extensively in

nonruminant species, including man, to investigate the relative roles of carbohydrates,

lipids, amino acids, and proteins in stimulating heat production; the process is termed

dietary induced thermogenesis (Rothwell & Stock, 1983). For ruminants, however,

repeatedly subjecting test animals to prolonged fasts (2-10 days depending on animal

weight and pre-fast levels of food intake [Marston, 1948; Ferrell et al., 1986]) is not

conducive to routine assay. In addition, the 50-70 h period required to recover all HIF of

a full daily meal can be a severe logistical constraint.

In the search for a technique that could be used to estimate HIF and k for wild forages by ruminants, we reassessed the potential of the single-meal approach. Because Brody (1945), Marston (1948), and Webster (1979) report on the shortcomings of using BMR as a baseline to measure HIF and k, we argue that the period of fasting should be reduced. Specifically, the fasting period should be sufficiently short that dietary nutrients are still being absorbed from the gut, thereby minimizing mobilization of body protein for gluconeogenesis. At BMR, ruminants must catabolize tissue protein to meet glucose requirements, which may result in an endogenous heat increment (Chowdhury & Ørskov, 1997) causing an overestimate of basal metabolism and consequently a spuriously low estimate of HI of the food (Brody, 1945). The fasting period also must be sufficiently long to result in repeatable measures of baseline for energy expenditure. We suggest the amount of test food given should approximate a normal meal, and ideally, the measurement period should recover the entire heat increment of that meal. As a test of the modified single-meal procedure, mean estimates of HIF and k should be within the range of measurements for established test forages.

We selected a study organism that is a generalist ruminant that consumes a diverse and seasonally changing diet to test the modified single-meal procedure. The muskox (*Ovibos moschatus*) meets this criteria by consuming both graminoids (White et al., 1984; Eisfeld, 1990; Adamczewski et al., 1994a) and woody browse (Tener, 1965; Bos, 1967; Jingfors, 1981; Robus, 1981). In addition, the muskox is easily trained to confinement (Flood et al., 1984; Frisby et al., 1984; White et al., 1984). For comparison with extant data, we sought a plant species that was known to be low in plant secondary

compounds because processing of those compounds may be energetically costly (Foley &
McArthur, 1994; Iason & Murray, 1996). Many agricultural grasses meet that
requirement (Van Soest, 1982). In the north, smooth brome-grass (*Bromus inermis*)
makes high-quality and palatable hay that has been used in digestibility and rate of
passage studies in muskoxen (Holleman et al., 1984; White et al., 1984) and is similar in
fiber composition to pasture hays used in trials with domestic ruminants (Van Soest,
1982).

A complication in the assessment of HIF and k is that metabolic rates of northern
ruminants may change seasonally (e.g., caribou [*Rangifer tarandus*], McEwan &
Whitehead, 1970; moose [*Alces alces*], Regelin et al., 1985, Renecker & Hudson, 1986,
Schwartz et al., 1991; roe deer [*Capreolus capreolus*], Weiner, 1977; elk [*Cervus
elaphus*], Pauls et al., 1981; muskoxen, Nilssen et al., 1994; Lawler & White, 1997).
Work by Mautz et al. (1992) and Jensen et al. (1999) however, indicated that differences
in seasonal metabolic rates in white-tailed deer (*Odocoileus virginianus*) could be
explained by measurements being made outside the thermoneutral zone and that HIF was
known to substitute for cold-induced thermogenesis (Jensen et al., 1999). We therefore
investigated thermoneutrality and seasonality in our study.

Traditionally, additional energy for standing compared with bedding is assumed
to be minimal (Blaxter, 1962) or not included in estimates of HIF (Mautz et al., 1992;
Jensen et al., 1999). Energy costs of standing are not traditionally subtracted using the
continuous feeding technique to estimate energy balance (Agricultural Research Council,

1980). Therefore, we measured the energy cost of standing in muskoxen to determine the effect of standing cost on estimation of HIF and k.

We estimated HIF corrected for the energy cost of standing using 3 methods. Method (i)--HIF was estimated by subtracting mean pre-feeding energy expenditure (EEp) from post-feeding EE in 2 min increments and positive values were summed over an 8 h observation period. In some preliminary trials, we noted that the observed EE during the 6th and 7th hour post-feeding were less than EEp. This observation led to our 2nd technique. Method (ii)--HIF was estimated by subtracting a linearly decreasing EEp from observed EE following a meal. Given that the HIF of the meal may not have been recovered in the 8 h period, we extrapolated recovery to time ∞ (t_∞), which constituted the 3rd method. Method (iii)--nonlinear least-squares regression was used to fit a curve to the cummulative HIF over time and extrapolated to t_∞. HIF of the meal was considered complete (i.e., HIF fully recovered) when the fitted curve reached an asymptote.

The objective of this study was, therefore, to test a protocol that allowed estimation in a large ruminant of the HIF of a small quantity of forage over a short measurement period. As a part of that test, we investigated the influence of date and temperature on EE in muskoxen. We develop a technique for correcting EE estimates for the energy cost of standing. Seasonal differences in EE and RQ and changes in EE and RQ within trials also were explored for insights into seasonal metabolism of muskoxen.

Methods

Approach

All experiments were conducted, at the Large Animal Research Station (LARS) of the Institute of Arctic Biology at the University of Alaska Fairbanks, Fairbanks USA (64° 50'N, 147° 43'W). Muskoxen were taken from pasture and fed a standardized meal of 50% brome hay and 50% Quality Texture Ration (QTX, Alaska Mill and Feed, Anchorage, Alaska, USA) at 10 g dry matter (DM)•kg body weight (BW)$^{-0.75}$. Animals were then fasted for 24-26 h before being placed in a metabolism chamber. Data collection commenced approximately 0.5 h later once gases within the chamber had equilibrated. Oxygen consumption and CO_2 and CH_4 production were determined at 2 min intervals for the next 10 h. Behavior was monitored remotely with a video camera placed within the chamber. Animals had free access to water in summer and to snow in winter while in the chamber. During the first 2 h of the metabolic trial, muskoxen were offered no food and baseline values for EE were determined. At 2 h, the experimental meal of 100% chipped brome hay was made available by opening the lid of a feed bin built into the chamber. EE was monitored for the next 8 h. Meals were offered at 10 g DM•kg BW$^{-0.75}$ and all meals were consumed entirely.

Diet composition

Dry-matter content of hay was determined by drying at 60°C for 48 h. Neutral detergent fiber (NDF), acid detergent fiber (ADF), and acid detergent lignin (ADL) were determined sequentially, and hemicellulose and cellulose calculated by subtraction

(Goering & Van Soest, 1970). Total nitrogen (N) and carbon content was determined by combustion in an N-analyzer system (LECO CNS-2000 elemental analyzer, St Joseph, MI). A bomb calorimeter was used to determine gross-energy content (Parr 1108 oxygen combustion bomb, Moline, IL). Apparent digestibility (D) of brome hay was assumed to be 0.74 (range 0.73-0.76) based on previous research by Holleman et al. (1984). The ratio of metabolizable energy to digested energy of the hay was assumed to be 0.84 (Agricultural Research Council, 1980). Where k = 0.55 + 0.24 D (Graham, 1966), the expected k for hay is 0.73, indicating that HIF expressed as a fraction of ME should be approximately 0.27 (i.e., 1- k).

Metabolic trials and study animals

During winter 1994, three 2-year old muskoxen were used (2 males and 1 female) in 54 metabolic trials as part of a related study. Each animal was evaluated on 100% hay diet on 2 occasions resulting in 6 total metabolic data sets--the timing of each trial was randomly distributed within the larger data set. During summer 1995, a second cohort of muskoxen was used, which ranged in age from 1 to 2 years (5 males and 3 females). Twenty-eight total metabolic trials were conducted in summer with 8 study animals evaluated on 100% hay on one occasion. All muskoxen used during the metabolic studies were sexually intact. All females were nonpregnant and nonlactating.

When muskoxen were not being used in trials, they were maintained in pastures dominated by smooth brome grass. Muskoxen had ad libitum access to brome hay during all seasons. All animals involved in this study had been habituated to

confinement in the metabolic chamber as young animals. Only trials during which animals remained calm and unagitated throughout the 10 h trial were used for analyses. The University of Alaska Fairbanks, Institutional Animal Care and Use Committee (IACUC #93-01) approved protocols for this experiment and care of these animals and protocols were in keeping with methods approved by the American Society of Mammalogists (Animal Care and Use Committee, 1998) for research on captive mammals.

Indirect calorimetry

The metabolism chamber (Fig. 2.1) measured 2.1 x 2.1 x 1.5 m (6,450 L). A small fan within the chamber kept the air continually mixed. Outdoor air was drawn through the chamber by a rheostatically controlled vacuum motor (National Super Service Co. model M-1, Toledo, OH). Rate of air flow was controlled by a balance of the rheostat and an adjustable gate valve to maintain a constant flow ($\pm$ 2.8% during a 10 h experiment). Rate of air flow (L•min^{-1} at standard temperature and pressure) was continuously monitored with a mass flowmeter (Hastings model STH-759K, Hampton, VA.). A sample of this air stream was drawn through calcium sulfate (Drierite) and analyzed for oxygen concentration (Applied Electro-chemistry model S-3A, Ametek, Inc., Pittsburgh, PA), carbon dioxide (Applied Electro-chemistry model CD-3A), and methane (Infrared Industries model 703D, Santa Barbara, CA). Outdoor air and 3 standard gases were used to calibrate analyzers. Calibration occurred at the start of each metabolic trial and at approximately 2 h intervals throughout experiments. A data-
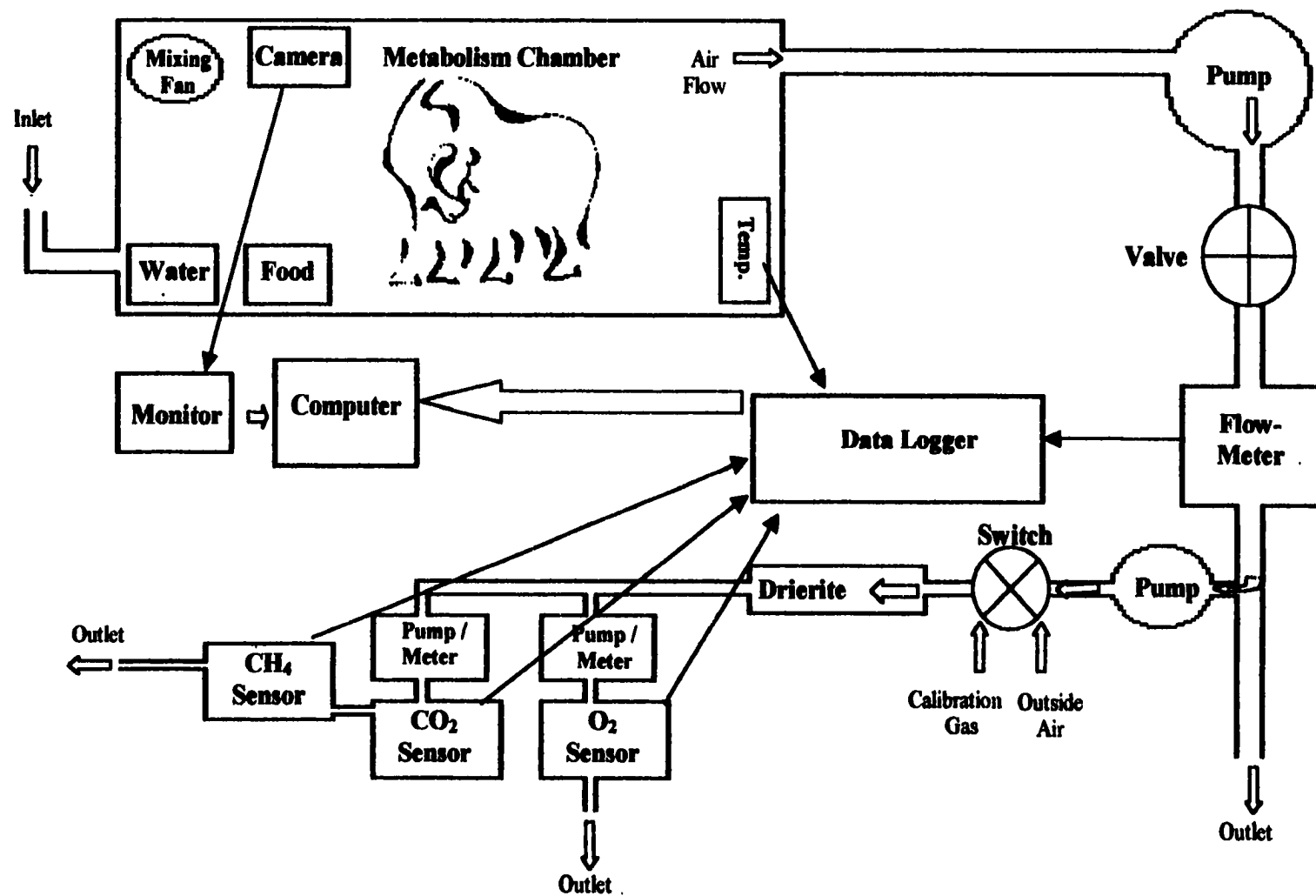
Fig. 2.1. Diagram of the indirect calorimeter system used to determine energy expenditure at the Large Animal Research Station, Fairbanks, Alaska. Concentrations of O_2, CO_2, CH_4 and air out flow rate from a metabolic chamber were monitored continuously and values stored using a data acquisition system linked to a computer. Energy expenditure was calculated from the gaseous exchange. Relative sizes of the muskox and objects are not to scale.

acquisition system (Omega model 481, Quebec, Canada) sampled the rate of gas flow and humidity, temperature, barometric pressure and gas concentrations in the airstream leaving the chamber at 2 min intervals. Data were recorded and stored on a computer. Animal activity was monitored by remote camera and was recorded by an observer every 2 min in synchrony with the data acquisition system (Altmann, 1974).

Calculation of energy expenditure and Respiratory Quotient

EE was calculated by multiplying the volume of oxygen consumed during the trial by the thermal equivalent of oxygen at the measured respiratory quotient (Brody, 1964) with the following equation (Marston, 1948):

$$EE \ (kcal/h) = (B)(4.686) + (1.23[A/B - 0.707]),$$

where B is oxygen consumption $(mL \cdot h^{-1})$, and A is carbon dioxide production $(mL \cdot h^{-1})$. These metabolic rates were then expressed as kilojoules (1 kcal = 4.184 kJ, Agricultural Research Council, 1980) per min $(kJ \cdot min^{-1})$ and corrected to a metabolic body-weight basis (Kleiber, 1975) $(kJ \cdot kg \ BW^{-0.75} \cdot min^{-1})$ and on a daily metabolic body weight basis $(kJ \cdot kg \ BW^{-0.75} \cdot d^{-1})$. RQ was determined as carbon dioxide production $(mL \cdot h^{-1})$/oxygen consumption $(mL \cdot h^{-1})$. EE and RQ were determined at 2 min intervals continuously over 10 h, from 2 h prior to feed access and for an additional 8 h following presentation of the meal.

Temperature and thermoneutrality

Because of large body size and whole-body insulation, no cold induced thermogenesis was expected at laboratory ambient temperatures for muskoxen in winter. Hence, no substitution of HIF for cold thermogenesis was expected because animals would be within their thermoneutral range. We tested this prediction, along with an alternative--EEp may be influenced by date (i.e., photoperiod). We used regression analysis to determine whether chamber temperature (T_{Ch}), Julian date, or both influenced EEp. The effect of date and temperature on metabolic rate in muskoxen was examined with the 1 h EEp values from the 54 trials in winter and the 28 trials in summer. Winter trials were conducted between 9 January and 15 April 1994, and summer trials were between 16 July and 13 August 1995. Temperatures within the metabolism chamber ranged from 13°C to 8°C during winter experiments, and between 13°C and 21°C during summer trials.

Energy cost of standing

The energetic cost of standing compared with bedding was determined from a subset of trials not used in the determination of the HIF. Conditions for inclusion in this data set were EE values for an animal bedded for a minimum of two turnover times of the metabolic chamber (approximately 25 min), either before or after the bedding event, and EE values for an animal standing for at least 1.4 turnover times (approximately 18 min). Five of 48 trials met these criteria in winter 1994. One animal in the winter trials had 3 trials in which those criteria were met. In that instance, one trial was chosen at random

for inclusion in the analysis. Data sets consisted of the change in energy expenditure to go from bedded to standing, and the change in energy expenditure to go from standing to bedded. In summer 1995, 3 of 20 trials met criteria for calculation of standing compared with bedded energy expenditure. Each estimate was from a different animal, which resulted in 3 estimates of the energy cost of standing for summer. Different animals were used in the winter and summer trials.

All HIF trials used the mean energetic cost of standing to adjust for the additional energy to stand in comparison to bed. Following the initiation of a standing event, gases within the metabolic chamber were allowed to turnover one time before the experimentally determined cost of standing was subtracted from observed EE. Following cessation of a standing event, cost of standing was subtracted from observed EE for a period representing one turnover time. After one turnover time, the residual contribution of standing was assumed to decline in an exponential fashion. Thus, the bedded energy expenditure (EE corrected) was estimated at 2 min intervals as:

$$EE \text{ corrected} = EE - S \; e^{-TT(Ta - Tb)},$$

where EE = observed energy expenditure (kJ•kg $BW^{-0.75}$ •2 min^{-1}), S = energy expended in standing over bedding, e = base of the natural logarithm, TT = turnover time of the metabolic chamber, Ta = current time in the trial (time at which EE was collected), and Tb = time animal lay down + 1 turnover time.

Calculation of heat increment of feeding (HIF)

HIF was calculated by three methods. Method (i)—2 min mean EE during the 1 h prior to the meal was considered baseline and was subtracted from each successive 2 min EE following the meal. The positive values of EE-EEp were summed over the 8 h time period to estimate the HIF (Fig. 2.2):

$$HIF\ (i) = \Sigma\ (EE\ following\ meal - EEp).$$

Method (ii)—baseline EE (EEb) was assumed to decline linearly throughout the 8 h measurement period. The decline was estimated from the mean EE 1 h before feeding and the lowest 20 min EE during the final 1 h of the trial (Fig. 2.2):

$$HIF\ (ii) = \Sigma\ (EE\ following\ meal - linearly\ declining\ EEb).$$

Method (iii)—cumulative HIF (cHIF) at time ∞ (cHIF$_{(t)}$) was determined from the data generated from method (ii). Because HIF would be complete at time ∞ (t∞), we used nonlinear regression to fit a curve of cHIF to time after feeding to determine the asymptote of cHIF (cHIF$_{(t)}$), assuming the asymptote was reached exponentially at rate r(h^{-1})(Fig. 2.3):

$$HIF\ (iii) = cHIF_{(t)} + cHIF_{(t0)}\ e^{-rt}.$$

Estimates of r and cHIF$_{(t)}$ were obtained by nonlinear weighting of residuals based on a Poisson distribution of residuals with SAAM II (Version 1.02, ©SAAM Institute, University of Washington, Seattle, WA, USA).
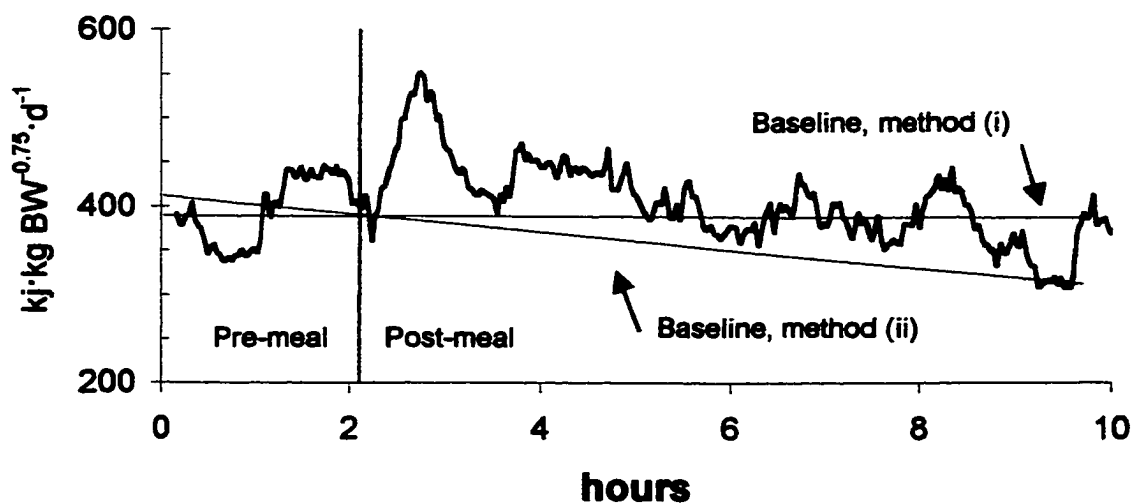
Fig. 2.2. A 10 h metabolic trial at the Large Animal Research Station, Fairbanks, Alaska illustrating 2 methods used to calculated HIF. Line (i) is a baseline value determined as the mean energy expenditure during the hour before the presentation of the meal. Line (ii) is a projected baseline assuming energy expenditure is declining in a linear manner throughout the trial. HIF was considered to be the energy expenditure following a meal above each of these 2 lines.
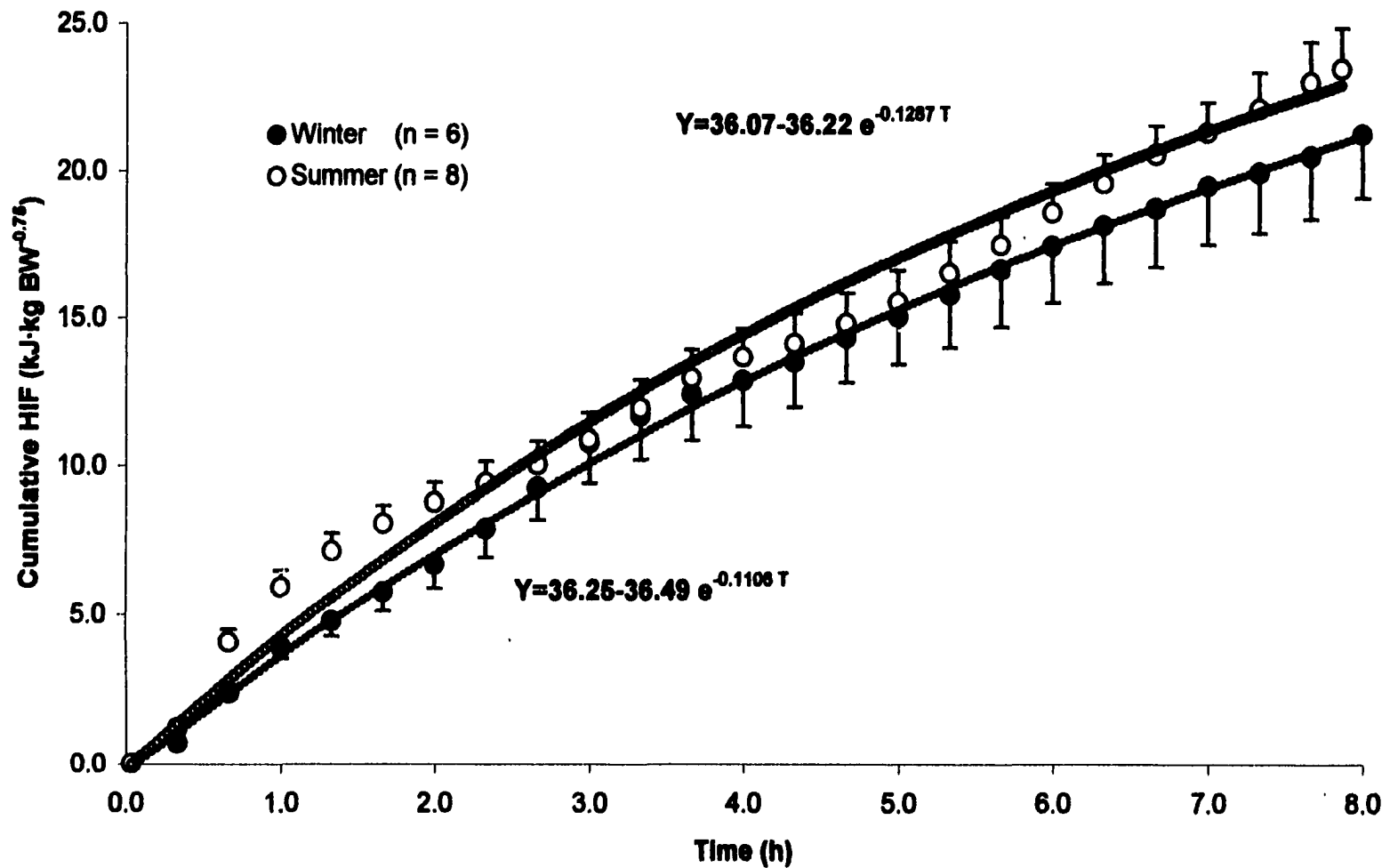
Fig. 2.3. Mean ($\pm$ SE) cumulative HIF of muskoxen in the 8 h following the presentation of a meal of 100% brome hay fed at 10 g·kg BW$^{-0.75}$·d^{-1}. Values presented are corrected to metabolic body size (kg BW$^{0.75}$) and corrected for the energy cost of standing. Metabolic trials examining HIF were conducted at the Large Animal Research Station, Fairbanks, Alaska during the winter of 1994 and the summer of 1995.

All methods for calculating HIF were applied to post-meal values for energy for: 1) observed data; 2) observed data corrected for energy cost of standing; 3) observed data corrected for energy cost of standing and metabolic body size; and 4) observed data corrected for energy cost of standing, metabolic body size, and metabolizable energy intake. HIF estimates were evaluated mathematically as:

$$HIF_{IA} = (HIF \text{ method } (x) - HIF_{pred.}) / HIF_{pred}$$

Where HIF_{IA} = index of accuracy of HIF estimate relative to predicted values, x = the HIF method being evaluated (i, ii, or iii), and $HIF_{pred.}$ = HIF predicted from diet composition. Evaluated HIF estimates were corrected for the energetic cost of standing, metabolic body size, and metabolizable energy intake.

Recovery of the heat increment

We assessed whether the observation period was sufficient to recover the heat increment. For method (i), we fitted a linear model to the post-feeding 2 min EE data for each animal, and estimated the time at which post-feeding EE = EEp (termed X_o). Recovery should be complete if $X_0 < 8$ h. For method (ii), HIF (ii) estimated at 8 h was compared with that at $t\infty$, i.e. HIF (iii). Recovery was complete if HIF (ii) approximated HIF (iii). For method (iii) complete recovery was assumed once cHIF reached its asymtote.

Seasonality

In each of the 3 methods, BMR was not subtracted to estimate HIF, so no between-season differences in HIF and k would be expected. Subtraction of EEp (method (i)) or EEb (method (ii) and method (iii)) accounted for seasonal and other factors that may have affected resting metabolism in muskoxen. To test that prediction, HIF values for summer and winter from each of the 3 methods were compared. In addition, RQs were examined between seasons as an indication of digestive state of animals.

Statistical analyses

All statistical tests were performed with SPSS for windows (Version 7.0.2, SPSS Inc., Chicago Illinois). We tested the hypothesis that EEp was not affected by temperature or date using regression analysis and stepwise multiple regression (Zar, 1996). For entry of independent variables into the stepwise multiple regression model, we used a P-to-enter of ≤ 0.05, and a P-to-remove of ≥ 0.10. The correlation matrix was examined to determine if date or temperature was more highly correlated to EEp. We controlled for multicollinearity by examining variance inflation factors and we evaluated model selection with adjusted multiple coefficients of determination. We also examined the partial regression coefficients to determine if either date or temperature had a greater influence on EEp. The proportion of variability not explained when one of the

independent variable was held constant in the regression model was examined with partial correlation coefficients.

We tested the hypothesis of equal energy expenditure for bedding and standing, and for equal energy expenditure for bedding before and after the standing event using repeated-measures general linear models (von Ende, 1993; Zar, 1996). To test for changes in EE and RQ during metabolic trials, we also used repeated-measures general linear models. For tests that involved energy expenditure, values corrected and uncorrected for the energy cost of standing were included in the model. Season was included in these repeated-measures analyses as a between-subject factor. Contrasts were used to test for changes in RQs during trials using the 1 h pre-meal mean RQ as a control (von Ende, 1993).

Seasonal differences in the pattern of post-feeding EE were examined with repeated-measures general linear models by evaluating the length of time between EE peaks in summer and winter. The time between each sequential peak was considered a within-subject variable for that specific trail resulting in 6 within-subject factors per metabolic trial. Season was a between-subject factor. Data sets used in repeated-measures analyses were examined for sphericity with the Mauchly sphericity test. Results of all statistical analyses were assumed to be significant when $P \leq 0.05$.

Percent recoveries of HIF values were arcsine square root transformed before analysis (Zar, 1996). We used t-tests to investigate seasonal differences in time of recovery of the HIF of method (i). For method (ii), we used a repeated-measures general linear model to test the hypothesis of no seasonal difference in: (1) time of recovery of

HIF between summer and winter; and (2) rate of HIF recovery between summer and winter.

Evaluation of the 3 methods for determining HIF was conducted with the log-likelihood ratio goodness of fit test (Zar, 1996). Log-likelihood ratio goodness of fit tests were first applied to the entire data set and then to seasonal data sets. HIF estimates based on methods (i), (ii) and (iii) were compared to HIF estimates from predictive equations based on the composition of the hay diets. HIF estimates for methods (i), (ii), and (iii) were compared with values for the: 1) observed data; 2) observed data corrected for energy cost of standing; 3) observed data corrected for energy cost of standing and metabolic body size; and 4) observed data corrected for energy cost of standing, metabolic body size, and metabolizable energy intake. Estimates of HIF were catagorized as "predicted", "less than predicted", and "more than predicted". HIF estimates were classified as predicted if they were within $\pm$ 10% of the predicted HIF, and more or less than predicted accordingly. Because Robbins (1993) reported the standard deviation of net energy coefficient (i.e., k) of diets fed to ruminants at maintenance was 10%, we considered this an appropriate range for our evaluation. Following log-likelihood tests, Z tests (Zar, 1996) were used to further differentiate between HIF estimation methods and to examine seasonal differences in HIF estimates.

Results

Body weight

Mean monthly weight of individual muskoxen ranged between 148 kg to 182 kg from January to April 1994 ($n = 3$). Mean winter monthly weight increased from 163 kg in January to 165 kg in April (Fig. 2.4). During June - September 1995 ($n = 8$) mean monthly weight of individuals ranged from 96 kg to 200 kg. Mean summer monthly weight increased from 127 kg in June to 142 kg in September. The 2 kg gain in weight during winter months was minor in comparison with the 15 kg gain over summer and early autumn (Fig. 2.4).

Feed composition and intake

Composition of brome hay was similar between study periods (Table 2.1) characterized (winter and summer, respectively) by moderate crude protein (N*6.25 = 12 and 10%), high cell solubles (41 and 32%), and low acid detergent lignin (2.8 and 3.3%). Predicted dry matter digestibility (DMD) from the detergent analysis equation of Van Soest & Moore (1965) was 0.83 and 0.86, and the summative equation of Van Soest (1965) gave DMDs of 0.70 and 0.68 respectively, for winter and summer forage. Mean gross energy intake (GEI) during winter trials was 8136 kJ (SE = 207.0 kJ; $n = 6$). In the summer, GEI was 6961 kJ (SE = 471.9 kJ; $n = 8$). Seasonal differences in energy intake were largely caused by body weight because hay was fed on a metabolic body weight basis (10 g feed·kg $BW^{-0.75}$). Overall, mean gross energy intake on a metabolic body

Table 2.1. Chemical analysis and energy content of hay used in metabolic trials during the winter of 1994 and the summer of 1995 at the Large Animal Research Station, Fairbanks, Alaska. Hay samples were assayed in triplicate and values presented are mean percentages ($\pm$ SE). Apparent digestible energy (DE) was assumed to be 0.74 of GE, metabolizable energy (ME) was assumed to be 0.84 of DE, k (the efficiency of use of metabolizable energy) was assumed to be equal to 0.55 + 0.24 DE, and the heat increment of feeding (HIF) was assumed to be ME - k.

%	Winter 1994	Summer 1995
Dry matter	88.7 ($\pm$ 0.33)	81.0 ($\pm$ 3.00)
Organic matter	93.8 ($\pm$ 0.54)	94.8 ($\pm$ 0.03)
Neutral-detergent fiber	58.7 ($\pm$ 0.41)	67.5 ($\pm$ 0.08)
Acid-detergent fiber	32.2 ($\pm$ 0.31)	36.6 ($\pm$ 0.01)
Hemicellulose	26.5 ($\pm$ 0.28)	30.8 ($\pm$ 0.08)
Cellulose	29.1 ($\pm$ 0.28)	33.2 ($\pm$ 0.01)
Acid detergent lignin	2.8 ($\pm$ 0.1)	3.3 ($\pm$ 0.02)
Ash	0.43 ($\pm$ 0.06)	0.26 ($\pm$ 0.05)
Nitrogen	1.9 ($\pm$ 0.28)	1.6 ($\pm$ 0.03)
Carbon	44.0 ($\pm$ 0.1)	43.3 ($\pm$ 0.02)
Gross energy (kJ/g)	17.79 ($\pm$ 0.02)	18.08 ($\pm$ 0.02)
Predicted values:		
DE (kJ/g)	13.16 ($\pm$ 0.01)	13.38($\pm$ 0.02)
ME (kJ/g)	11.05 ($\pm$ 0.01)	11.24 ($\pm$ 0.01)
K[1] (kJ/g)	8.07 ($\pm$ 0.01)	8.17 ($\pm$ 0.01)
HIF (kJ/g)	2.98 ($\pm$ 0.01)	3.07 ($\pm$ 0.01)

[1]K is the fraction of metabolizable energy retained as net energy and is termed the efficiency in use of metabolizable energy.

weight basis was 178 kJ•kg $BW^{-0.75}$ ($n = 14$). Assuming in vivo DMD of 0.74 based on previous studies for hay of similar quality fed young muskoxen, and a metabolizability of 0.84, mean daily MEI in winter and summer, respectively, was 5,057 kJ (SE = 125.8 kJ; $n = 6$) and 4,327 kJ (SE = 286.3 kJ; $n = 8$). Expressed per unit metabolic body weight, mean MEI during all trials was 112 kJ•kg $BW^{-0.75}$ (SE = 0.2 kJ•kg $BW^{-0.75}$; $n = 14$). Using the predictive equation of Graham (1974; k = 0.55+0.24 * DMD), we derived an estimate for k of 0.73 for the efficiency in use of metabolizable energy of the diet (Table 2.1). Predicted net energy (k * ME) available to the animal for work or productive processes of hay diets ranged from 3,437 to 3,967 kJ in winter (mean = 3,690 kJ; SE = 94) and from 2,502 to 4,260 kJ in summer (mean = 3,343 kJ; SE = 233 kJ). Predicted HIF ranged from 1,294 to 1,493 kJ in winter (mean = 1,389 kJ; SE = 35 kJ) and from 937 to 1,595 kJ in summer (mean = 1,251 kJ; SE = 87 kJ). These predicted values of HIF were used for comparison to HIF estimates from each of our three methods.

Chamber temperature and thermoneutrality

Mean temperature during winter trials from 9 January to 15 April, 1994 was 1.4°C (SD = 4.8°C). Chamber temperatures increased significantly throughout that period ($F_{[1, 53]}$ = 16.232, $P < 0.001$, $r^2 = 0.23$). Chamber temperatures predicted by regression were –5.2°C in the first January trials, and 2.4°C at the last trial in April, a 7.2°C change (Table 2.2). EEp was weakly but significantly associated with temperature ($F_{[1, 53]}$ = 4.309, $P = 0.043$, $r^2 = 0.08$). Observed temperatures varied between –13.2 and

Table 2.2. Linear regression models evaluating relationships between metabolic rates $(kJ \cdot kg\ BW^{-0.75} \cdot d^{-1})$, chamber temperature (°C) and Julian date for metabolic trials conducted between 9 January and 15 April 1994 ($n = 54$) at the Large Animal Research Station, Fairbanks, Alaska.

Dependent Variable	Model	r^2	Adj. r^2	F	P
Metabolic rate	$\hat{Y} = 387.323 - 0.433\ date - 1.052\ temperature$	0.148	0.116	4.526	0.015
Metabolic rate	$\hat{Y} = 393.4 - 0.5146\ date$	0.136	NA	8.377	0.006
Metabolic rate	$\hat{Y} = 361.27 - 2.326\ temperature$	0.075	NA	4.299	0.043
Temperature	$\hat{Y} = -5.8751 + 0.079\ date$	0.234	NA	16.232	<0.001

r^2, coefficient of multiple determination; Adj. r^2, coefficient of multiple determination adjusted for the number of independent variables in the equation; NA, not applicable.

8°C. EEp predicted from regression analysis for these temperatures was 392 kJ·kg BW$^{-0.75}$·d^{-1} and 343 kJ·kg BW$^{-0.75}$·d^{-1} respectively; a 49 kJ·kg BW$^{-0.75}$·d^{-1} decrease in EEp as temperature increased (Table 2.2). EEp also was significantly related to date of the trial ($F_{[1, 53]}$ = 8.273, P = 0.006, r^2 = 0.14). Predicted EEp of the first trial was 389 kJ·kg BW$^{-0.75}$·d^{-1} and by the last day was 340 kJ·kg BW$^{-0.75}$·d^{-1}; a seasonal decrease of 49 kJ·kg BW$^{-0.75}$·d^{-1}. Including both date and temperature in a full regression model did not improve the model ($F_{[2, 52]}$ = 4.526, P = 0.015, R^2 adj = 0.12). Stepwise regression analysis produced a model that included date (P = 0.039) but excluded temperature (P= 0.400) based on partial-F-tests. Results from the correlation matrix, indicate date (r = -0.37) was more highly correlated to EEp than was temperature (r = -0.27). Standardized partial regression coefficients were -0.31 for Julian date and -0.12 for temperature. The proportion of variability not explained when temperature was excluded from the full model was minor (partial correlation coefficient = -0.117).

Mean temperature during summer trials from 16 July to 13 August, 1995 was 17.4°C (SD = 1.8°C). Temperature did not rise or fall over this period ($F_{[1, 27]}$ =1.025, P = 0.32). Neither date ($F_{[1, 27]}$ =1.707, P = 0.20) nor temperature ($F_{[1, 27]}$ =0.854, P = 0.36) appeared to alter EEp in trials during summer. Multiple regression did not indicate a significant relationship when temperature and date were both included in the regression model ($F_{[2, 26]}$ = 1.078, P = 0.36, R^2 adj < 0.01).

Energy cost of standing

Muskoxen expended a mean 373 kJ·kg $BW^{-0.75}$·d^{-1} (SE = 16.6 kJ·kg $BW^{-0.75}$·d^{-1}; n = 6) while bedded in winter, and 472 kJ·kg $BW^{-0.75}$·d^{-1} (SE = 12.9 kJ·kg $BW^{-0.75}$·d^{-1}; n = 5) while bedded in summer. Standing required 450 kJ·kg $BW^{-0.75}$·d^{-1} (SE = 25.4; n = 3) in winter, and 573 kJ·kg $BW^{-0.75}$·d^{-1} (SE = 14.7; n = 3) in summer. In 6 data sets included in these calculations (3 each in summer and winter), measurements of bedding bouts were made before the standing event in 5 instances. In 6 instances, measurements of bedding bouts were made after the standing event. A repeated-measures general linear model did not indicate a significant difference in bedded energy expenditure when measured either before or after standing events ($F_{[1, 3]}$ = 3.181, P = 0.172; Table 2.3). No difference was detected between winter (n = 6) and summer (n = 5) estimates of EE stand – EE bed ($F_{[1, 3]}$ = 0.059, P = 0.823). Including summer and winter values, the mean cost of standing over bedding was 87 kJ·kg $BW^{-0.75}$·d^{-1} (SE = 11.1) or expressed as a fraction of EE while bedded, the mean energy cost of standing was 21% (Table 2.4).

Animals spent comparable percentages of the trial standing in summer (mean = 26%, SD = 2.1%, n = 3) and winter (mean = 21%, SD = 5.1%, n = 3). Variation in length of time standing was greater in summer than in winter with animals standing from a range of 20 – 30% of the trial in summer compared to 18 –23 % of the trial in winter.

Energy expenditure

Energy cost of standing increased the mean-corrected EEp by 9.1% in winter HIF trials and by 6.8% in HIF trials during summer (Table 2.5). In the terminal 1 h of

Table 2.3. Estimates of the energy cost of standing for muskoxen at the Large Animal Research Station, Fairbanks, Alaska. Energy expenditure for muskoxen while bedded were subtracted from that while standing. Bedded values were determined by allowing a 25 minute stabilization period (2 chamber turnover times) either before or after a standing event, and then calculating a 20 minute mean. Standing values were determined 20 minutes following the initiation of the standing event. Values are for 3 muskoxen in the winter of 1994 and a different 3 muskoxen in the summer or 1995.

Season	Energy to Stand $kJ \cdot kg \, BW^{-0.75} \cdot d^{-1}$		Mean	Mean Increment
	(Bed $\rightarrow$ Stand)	(Stand $\rightarrow$ Bed)	$kJ \cdot kg \, BW^{-0.75} \cdot d^{-1}$	(%)
Winter	93.7 (n=3)	59.2 (n=3)	76.5[b]	21
Summer	85.3 (n=2)	107.9 (n=3)	96.6[b]	21
Mean	89.5[a]	83.6[a]		
		Grand Mean	86.6	21

Repeated-measures general linear models indicated no statistical difference between the change in energy to go from standing to bedded before and after the bedding event (a) or between winter and summer values (b).

Table 2.4. Species comparison of the incremental cost of standing over bedded.

Species	Increment (%)	Reference
Muskox	21	Current study
Roe deer	22	Weiner 1977
Pronghorn	21	Wesley *et al.* 1973
Caribou calves	12	Luick and White 1982
White-tailed deer	20	Silver *et al.* 1977
Bighorn sheep	18	Chappel and Hudson 1979
Reindeer	10	White and Yousef 1977
Elk	19	Gates and Hudson 1978
	15	Pauls *et al.* 1981
	25	Pauls *et al.* 1981
Moose calves	35	Renecker *et al.* 1978
Moose	25	Regelin *et al.* 1981
	21	Renecker and Hudson 1983

*Table modified from S. G. Fancy and R.G. White, 1984.

Table 2.5. Mean (± SE) values for pre-meal energy expenditure (EEp) and terminal energy expenditure (EEt). Muskoxen were fasted for 26 h and EEp was measured for 1 h, they were then fed a meal and EE was followed for the next 8 h. The final h of this trial was EEt. Values presented are un-corrected and corrected for the energy cost of standing. We also present summer values following correction for seasonal affects. All metabolic trials were conducted at the Large Animal Research Station, Fairbanks, Alaska. Trials were conducted during the winter of 1994 and the summer of 1995.

	Winter[a] (n = 6)		Summer[a] (n = 8)		Summer (n = 8)
	With Standing[b]	Without Standing[b]	With Standing[c]	Without Standing[c]	Corrected for season and standing
EEp (kJ•kg BW$^{-0.75}$•d^{-1})	396 (±10.8)	363 (±10.9)	499 (±20.9)	467 (±13.8)	299 (±13.8)
EEt (kJ•kg BW$^{-0.75}$•d^{-1})	357 (±8.9)	329 (±8.8)	441 (±16.0)	403 (±13.8)	337 (±13.8)

[a,b,c] Repeated –measures general linear models were used to test for differences between EE estimates corrected and uncorrected for standing, to test for differences between summer and winter values, and to test for differences between summer values corrected for seasonal effects and winter values. Values within a column with a common superscript are significantly different.

metabolic trials, energy cost of standing increased the mean-corrected energy expenditure (EEt) by 8.5% and 20% in the winter and summer HIF trials, respectively. Values corrected for the energy cost of standing were significantly different from uncorrected values ($F_{[1, 24]}$ = 30.692, P < 0.001; Table 2.5) and there was no difference between seasons in the effect of correcting for the energy cost of standing ($F_{[1, 24]}$ = 1.929, P = 0.178).

Summer and winter values of EEp were greater than EEt values ($F_{[1, 24]}$ = 30.692, P < 0.001; Table 2.5). Seasonal variation in EEp and EEt was significant ($F_{[1, 24]}$ = 55.888, P < 0.001). When the mean seasonal difference in EEp, namely 104 kJ.kg BW$^{-0.75}$.d^{-1}, was subtracted from the summer values of EE$_t$, seasonal differences for EEt disappeared ($F_{[1, 24]}$ = 1.618, P = 0.228).

RQ

Following 26 h of fasting, 1 h mean pre-feeding RQ during winter trials was 0.93 (SE = 0.026, n = 6) and during summer trials was 0.84 (SE = 0.017; n = 8). During the 1 h block in which the meal was offered and consumed, winter mean RQ was 1.01 and summer RQ was 0.90. Mean RQ in the terminal 1 h of the trials was 0.99 (SE = 0.027) in winter and 0.92 (SE = 0.030) in summer. Between-season variation in RQ was significant ($F_{[1, 12]}$ = 15.810, P = 0.002).

Within metabolic trials, RQ varied significantly ($F_{[2, 24]}$ = 4.907, P = 0.016). Mean RQ made a comparable increase from 1 h pre-meal to the 1 h time block that included the meal in winter (0.08) and summer (0.06). Mean RQ was greater during the

terminal 1 h of the trial in comparison to the mean pre-meal RQ in both winter and summer (by 0.06 and 0.08 respectively). In winter, mean RQ was 0.02 lower in the final 1 h of the trial in comparison to the feeding 1 h block RQ. In summer, the pattern was reversed with an increase in mean RQ of 0.02 from the 1 h meal block to the terminal 1 h block. Post-hoc contrasts, with the 1 h pre-meal mean RQ as a control, resulted in significant differences in the change in RQ from 1 h pre-meal to 1 h meal RQ ($F_{[1, 12]}$ = 15.424, P = 0.002), and between 1 h pre-meal and 1h terminal RQ ($F_{[1, 12]}$ = 6.631, P = 0.027).

Pattern of post-feeding energy expenditure

EE increased immediately following feeding and peaked at about 30 min with mean increases in EE of 35% and 50% for winter and summer respectively (Fig. 2.4 and 2.5). Following the peak, EE declined but showed irregular peaks partially attributable to standing. When standing EE was subtracted, each individual exhibited bursts of EE that were regularly spaced at approximately 70 min; this cyclical behavior is discernable in the mean curves (Fig. 2.4 and 2.5). Within-trials, this regularity was surprisingly consistent ($F_{[5, 5]}$ = 2.805, P = 0.141). Although the mean time between peaks in winter (68 min, SE = 4.9 min) was similar to summer (70 min, SE = 3.5 min) a season * time between peaks interaction indicated this was a significant difference ($F_{[5, 5]}$ = 5.579, P = 0.041).

When the mean seasonal difference in EEp (104 kJ•kg $BW^{-0.75}$ •d^{-1}) was subtracted from the mean summer EE trial, a seasonal effect in the amplitude of bursts of
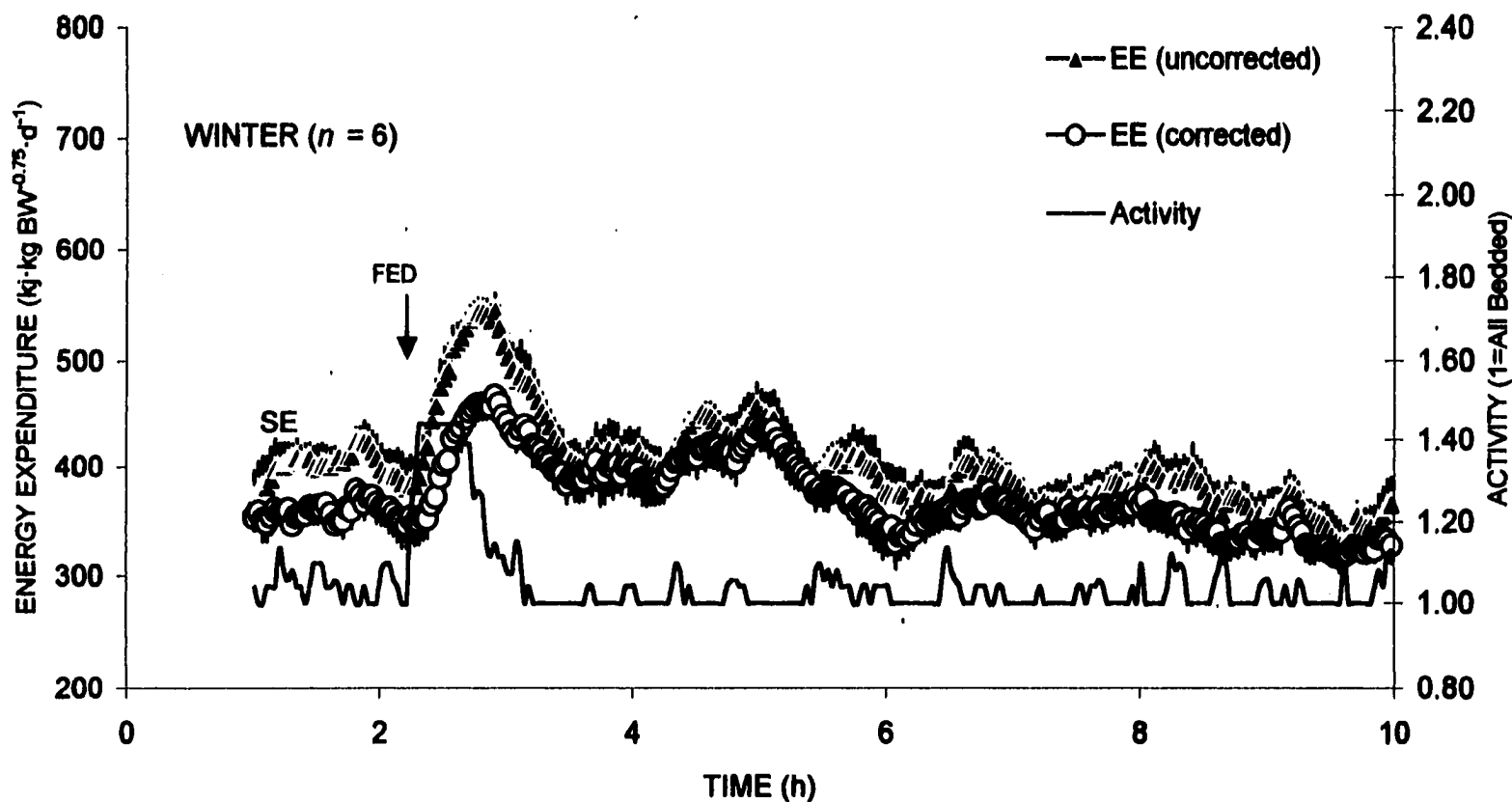
Fig. 2.4. Mean energy expenditure and activity of muskoxen over a 10 h metabolic trial during winter ($n = 6$). The top line depicts mean energy expenditure uncorrected for standing. The middle line depicts mean energy expenditure following a correction for standing. The bottom line depicts the categorical value of specific activities ranging from 1 (bedded) to 1.4 (feeding). Two hours after the start of the trial, the animals were fed a meal of 100% hay fed at 10 g•kg $BW^{-0.75}$. All trials were conducted at the Large Animal Research Station, Fairbanks, Alaska during the winter of 1994 and the summer of 1995.
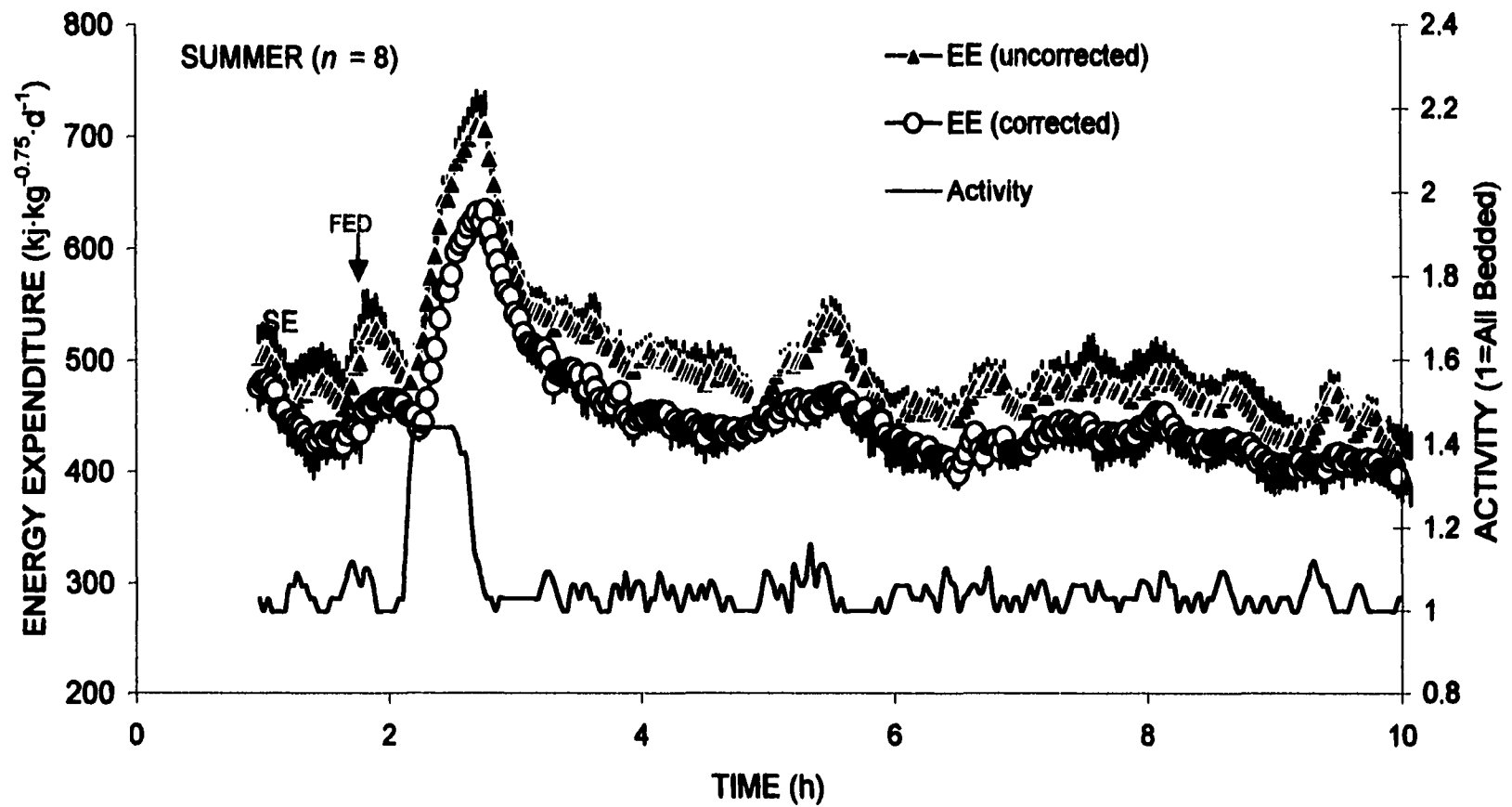
Fig. 2.5. Mean energy expenditure and activity of muskoxen over a 10 h metabolic trial during summer ($n = 8$). The top line depicts mean energy expenditure uncorrected for standing. The middle line depicts mean energy expenditure following a correction for standing. The bottom line depicts the categorical value of specific activities ranging from 1 (bedded) to 1.4 (feeding). Two hours after the start of the trial, the animals were fed a meal of 100% hay fed at 10 g•kg $BW^{-0.75}$. All trials were conducted at the Large Animal Research Station, Fairbanks, Alaska during the winter of 1994 and the summer of 1995.

EE over the first 5 h post-feeding occurred (Fig. 2.6). In winter, peak EEs at positions 2, 3, 4 (95, 150 and 203 min, respectively) were of equivalent magnitude, following a dominant first peak at 32 min. In summer, the first EE peak at 31 min was dominant, with minor contributions made by peaks at positions 3 and 4 (195 and 263 min, respectively).

Recovery of the heat increment

Method (i)—recovery of HI was assumed to be complete as EE returned to baseline (EEp). This recovery occurred at essentially the same time during both seasons ($t = 0.119$, df $= 8.5$, $P = 0.91$): 4.8 h (SE $= 0.26$ h; $n = 6$) in winter (Fig. 2.4) and 4.9 h (SE $= 0.79$ h; $n = 8$) in summer (Fig. 2.5). Method (ii)—cHIF increased exponentially (Fig. 2.3) with time and did not reach a asymtote within the 8 h of this study. Mean recoveries at 8 h, expressed as a percent of that at t_∞ [i.e. method (iii)], were lower in winter (mean $= 54\%$, SE $= 7.1\%$; $n = 6$) than summer (mean $= 75\%$, SE $= 5.3\%$; $n = 8$; $F_{[1, 13]} = 6.366$, $P = 0.027$). Although HI approached an asymtote at a slower rate in winter (mean $= 0.104$ h^{-1}, SE $= 0.019$ h^{-1}; $n = 8$) than summer (mean $= 0.175$ h^{-1}, SE $= 0.032$ h^{-1}; $n = 6$), the between-season differences were not significant ($F_{[1, 13]} = 3.085$, $P = 0.104$). Method (iii)—HIF (iii) is the value at t_∞ when all of the HI was predicted to be recovered. As demonstrated by the percent recovery of HIF for Method (ii), the majority of the HIF is recovered more quickly in summer than winter.
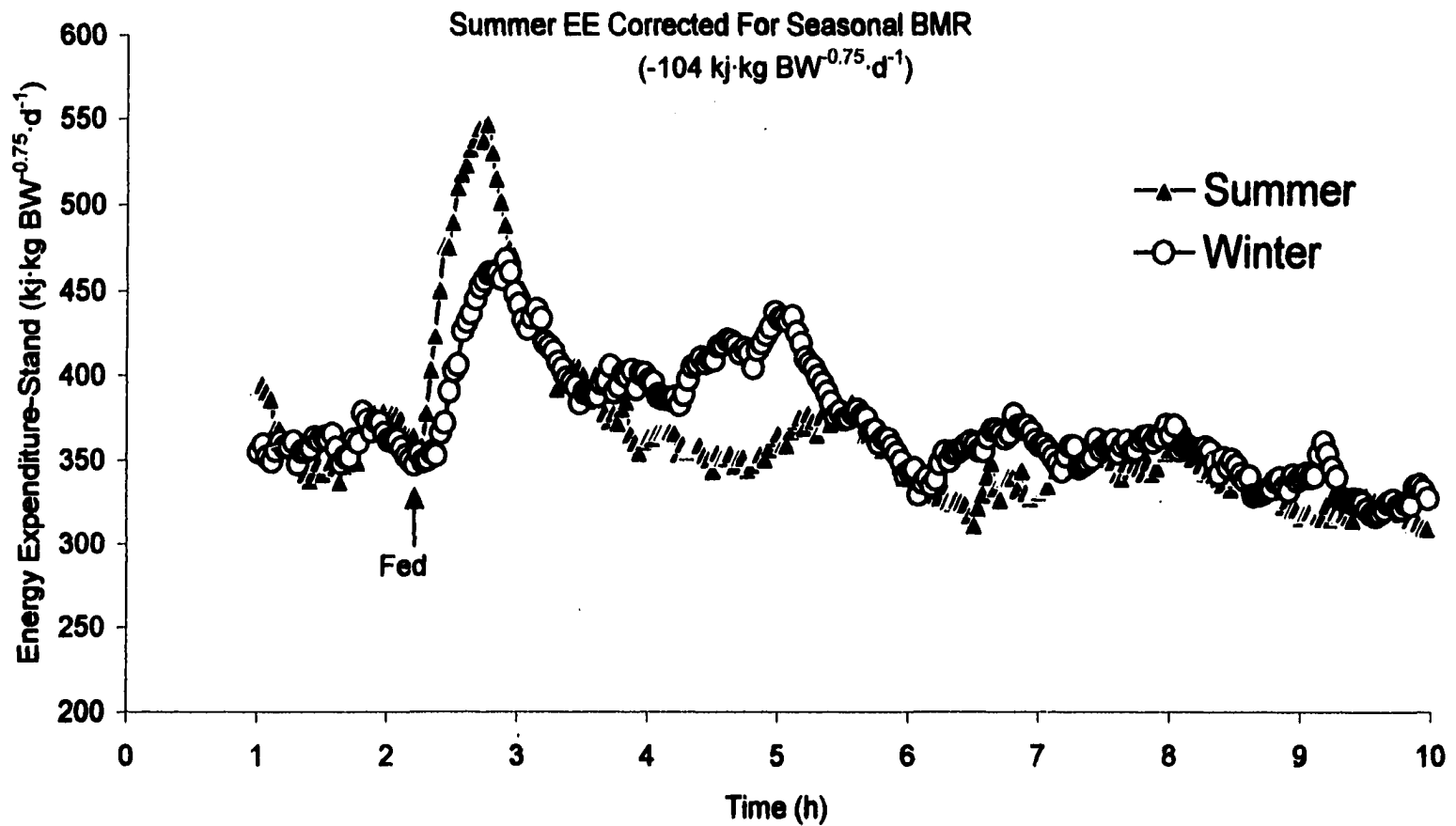
Fig. 2.6. Mean energy expenditure and activity of muskoxen over a 10 h metabolic trial during winter 1994 (n = 6) and summer 1995 (n=8). Both sets of values have been corrected to eliminate the energy cost of standing and the summer values have been adjusted to compensate for the seasonal difference in 24 h fasted metabolic rates. Two hours after the start of the trial, the animals were fed a meal of 100% hay fed at 10 g•kg $BW^{-0.75}$. All trials were conducted at the Large Animal Research Station, Fairbanks, Alaska.

Heat increment of feeding

After adjusting for metabolic body weight and correcting for the energy cost of standing, individual estimates of HIF (i) ranged from 4.4 to 12.9 kJ•kg $BW^{-0.75}$ in winter and from 3.5 to 8.9 kJ•kg $BW^{-0.75}$ in summer (Table 2.6). As a fraction of metabolizable energy intake, HIF estimated from method (i) ranged from 0.04 to 0.117 in winter and from 0.031 – 0.079 in summer (Table 2.7). Following the correction for the energy cost of standing, mean HIF calculated from method (i) produced a 6% increase over EEp in winter and a 4% increase in summer.

Range for individual estimates of HIF (ii), after correcting to metabolic body weight and correcting for the energy cost of standing, in winter was 14 - 27 kJ•kg $BW^{-0.75}$ and in summer was 18 - 30 kJ•kg $BW^{-0.75}$ (Table 2.6). As a fraction of metabolizable energy intake, HIF from method (ii) ranged from 0.124 to 0.244 in winter and from 0.162 to 0.270 in summer (Table 2.7). Mean HIF derived from method (ii), corrected for the energy cost of standing, represented an increase of 17% over EEp in the winter and a 14% increase in summer.

Following correction for metabolic body weight and the energy cost of standing, the range for individual estimates of HIF (iii) was 23 - 53 kJ•kg $BW^{-0.75}$ in winter and 20 - 55 kJ•kg $BW^{-0.75}$ in summer (Table 2.6). As a fraction of metabolic energy intake, HIF from method (iii) ranged from 0.204 to 0.482 in winter and from 0.178 to 0.490 in summer (Table 2.7). HIF derived from method (iii), and following the correction for standing, represented an increase of 34% over EEp in the winter and a 20% increase in the summer.

Table 2.6. Mean, standard deviations (SD), and coefficients of variation (CV) values for
the Heat Increment of Feeding (HIF). Values presented are corrected for the energy cost
of standing. Results for HIF method (i) were derived using a constant energy expenditure
baseline over 8 h, results for HIF method (ii) were derived using a linearly declining
baseline over 8 h, and results for HIF method (iii) were derived using the results from
method (ii) extrapolated as an exponentially decaying function to time ∞. All metabolic
trials examining HIF were conducted at the Large Animal Research Station, Fairbanks,
Alaska during the winter of 1994 and summer of 1995.

	Winter ($n = 6$)			Summer ($n = 8$)		
	Mean	SD	CV	Mean	SD	CV
Method (i)						
kJ	366	139.4	0.38	236	56.1	0.24
kJ·kg $BW^{-0.75}$	8.0	3.02	0.38	6.3	1.94	0.31
Method (ii)						
kJ	973	257.2	0.26	896	114.6	0.13
kJ·kg $BW^{-0.75}$	21.2	5.26	0.25	23.7	3.99	0.17
Method (iii)						
kJ	1906	543.3	0.29	1218	240.0	0.20
kJ·kg $BW^{-0.75}$	42.0	12.19	0.29	33	10.81	0.33

Table 2.7. Mean (± SEM) Heat Increment of Feeding (HIF) as a fraction of gross energy intake (HIF/GEI), digestible energy intake (HIF/DEI) and metabolizable energy intake (HIF/MEI). Results for HIF method (i) were derived using a constant energy expenditure baseline over 8 h, results for HIF method (ii) were derived using a linearly declining baseline over 8 h, and results for HIF method (iii) were derived using the results from method (ii) extrapolated as an exponentially decaying function to time ∞. HIF values were corrected for the energy cost of standing. All metabolic trials examining HIF were conducted at the Large Animal Research Station, Fairbanks, Alaska during the winter of 1994 and summer of 1995.

	Winter ($n = 6$)			Summer ($n = 8$)		
	HIF/GEI	HIF/DEI	HIF/MEI	HIF/GEI	HIF/DEI	HIF/MEI
Method (i)	0.05 (± 0.007)	0.06 (± 0.009)	0.07 (± 0.011)	0.04 (± 0.004)	0.05 (± 0.005)	0.06 (± 0.006)
Method (ii)	0.12 (± 0.012)	0.16 (± 0.016)	0.19 (± 0.019)	0.13 (± 0.008)	0.18 (± 0.011)	0.20 (± 0.018)
Method (iii)	0.23 (± 0.028)	0.32 (± 0.038)	0.38 (± 0.045)	0.18 (± 0.021)	0.25 (± 0.029)	0.29 (± 0.034)

A log-likelihood test indicated significant differences in the performance of methods (i), (ii), and (iii) as estimators of HIF ($G_{[4]} = 121.63, P < 0.001$; Fig. 2.7) when compared to predicted values. Method (i) consistently underestimated HIF. Methods (ii) and (iii) produced expected estimates of HIF with similar frequency (14.3% and 12.5% of the trials, respectively). When summer and winter estimates of HIF were examined separately, method (ii) and method (iii) performed significantly differently from each other ($G_{[4]} = 29.18, P < 0.001$ and $G_{[4]} = 421.2, P < 0.001$ for summer and winter, respectively; Fig. 2.8). Two-sample z-tests for proportions indicated that there was no significant difference in the frequency of underestimation of HIF when comparing summer values to winter values for method (ii) ($Z = 1.63, P = 0.10$) or method (iii) ($Z = 0.78, P = 0.44$).

An evaluation of an index of accuracy for each method of determining HIF again showed that method (i) consistently underestimated HIF in comparison to predicted values (Table 2.7). Method (ii) also underestimated HIF but to a lesser degree. Method (iii) overestimated HIF in comparison to predicted values but this tendency was stronger in winter than summer. Repeatability or precision of Methods (i), (ii), and (iii) are best accessed by examining the coefficients of variation for mean values of HIF methods (Table 2.8). Based on that measure of precision, method (ii) resulted in the most repeatable, and therefore precise measure of HIF, and results from methods (i) and (iii) were comparable.
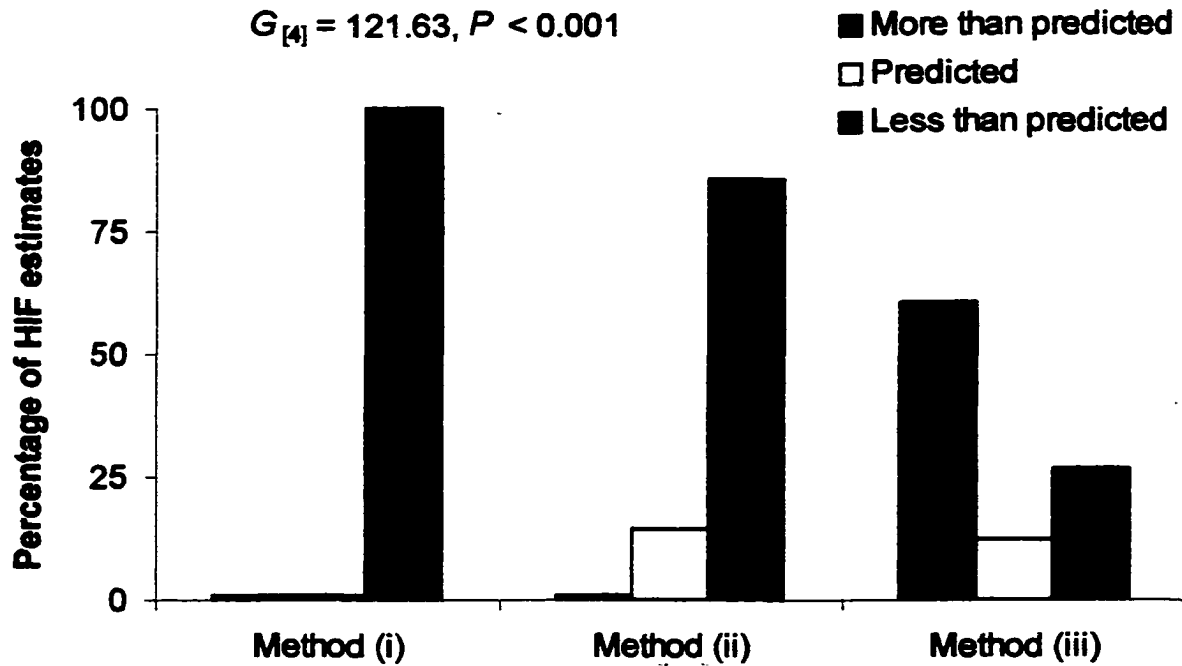
Fig. 2.7. Evaluation of HIF estimates using methods (i), (ii) and (iii) compared to predicted HIF based on the composition of the hay diets. HIF estimates for methods (i), (ii), and (iii) were compared using values for;1) observed data, 2) observed data corrected for energy cost of standing, 3) observed data corrected for energy cost of standing and metabolic body size, and 4) observed data corrected for energy cost of standing, metabolic body size, and metabolizable energy intake. HIF estimates were classified as predicted if they were within the $\pm$ 10% range of the predicted HIF, and more or less than predicted accordingly. HIF estimates are for 3 muskoxen during the winter of 1994 and the summer of 1995 at the Large Animal Research Station, Fairbanks, Alaska.

Summer 1995

$G_{[2]} = 29.18, P < 0.001$

■ More than predicted
□ Predicted
■ Less than predicted

Percentage of HIF estimates

100

75

50

25

0

Method (ii)   Method (iii)

Winter 1994

$G_{[2]} = 29.18, P < 0.001$

■ More than predicted
□ Predicted
■ Less than predicted

Percentage of HIF estimates

100

75

50

25

0

Method (ii)   Method (iii)
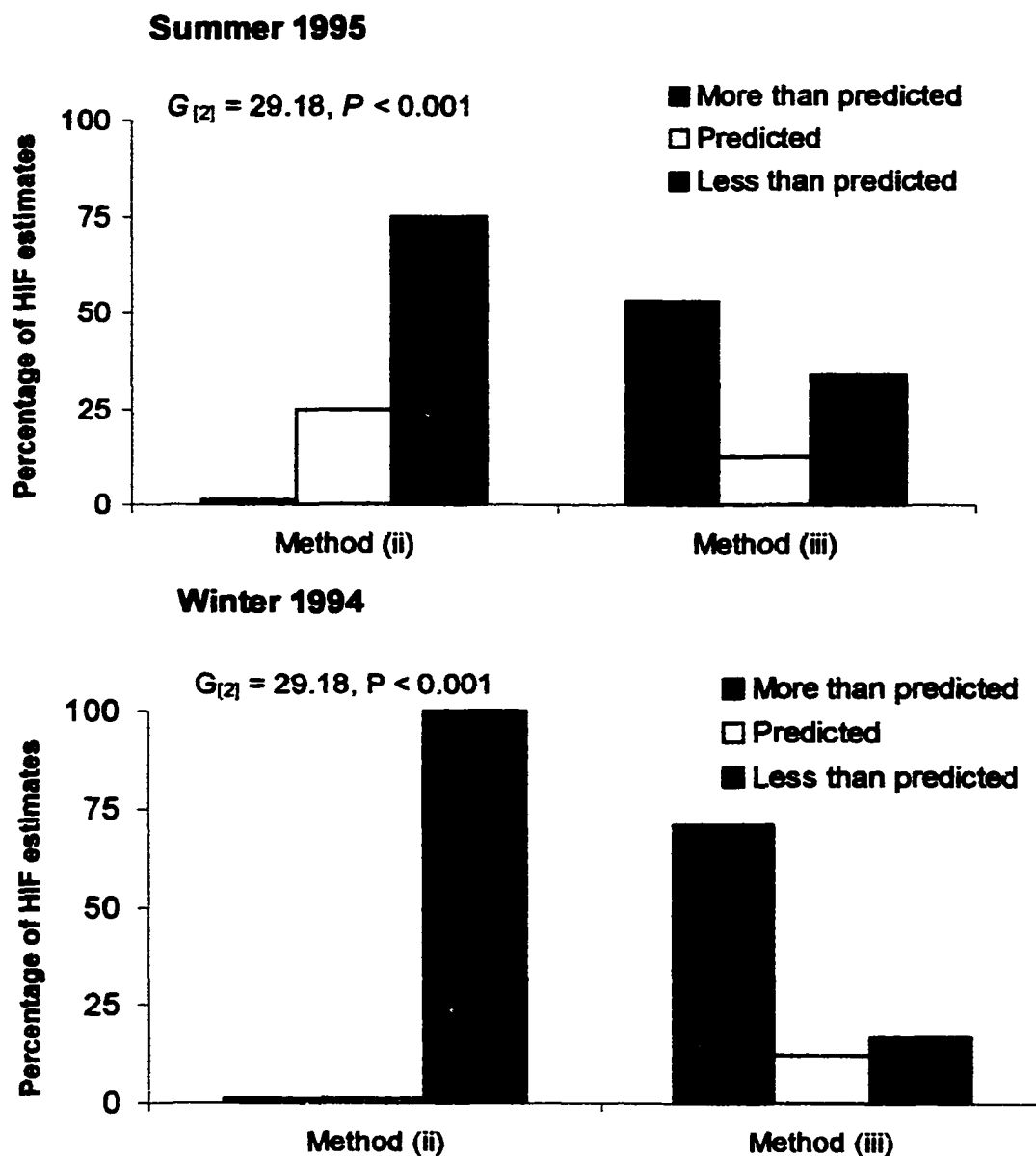
Fig. 2.8. Evaluation of summer and winter HIF estimates using methods (ii) and (iii) compared to predicted HIF based on the composition of the hay diets. HIF estimates for methods (ii), and (iii) were compared using values for the; 1) observed data, 2) observed data corrected for energy cost of standing, 3) observed data corrected for energy cost of standing and metabolic body size, and 4) observed data corrected for energy cost of standing, metabolic body size, and metabolizable energy intake. HIF estimates were classified as predicted if they were within the $\pm$ 10% range of the predicted HIF, and more or less than predicted accordingly. HIF estimates are for 3 muskoxen during the winter of 1994 and the summer of 1995 at the Large Animal Research Station, Fairbanks, Alaska.

Table 2.8. Index of accuracy for the Heat Increment of Feeding (HIF) calculated using one of three methods in comparison to HIF predicted from the composition of the feed. HIF values were corrected for the energy cost of standing, metabolic body sizes of animals, and metabolizable energy intake. Results for HIF method (i) were derived using a constant energy expenditure baseline over 8 h, results for HIF method (ii) were derived using a linearly declining baseline over 8 h, and results for HIF method (iii) were derived using the results from method (ii) extrapolated as an exponentially decaying function to time ∞. All metabolic trials examining HIF were conducted at the Large Animal Research Station, Fairbanks, Alaska during the winter of 1994 and summer of 1995. The index was calculated as: (HIF method (x) – predicted HIF) / predicted HIF. Mean values and their coefficients of variation (CV) are presented.

	Winter $(n = 6)$	Summer $(n = 8)$
Method (i)		
Mean	- 0.74	- 0.79
CV	0.135	0.080
Method (ii)		
Mean	- 0.30	- 0.22
CV	0.570	0.609
Method (iii)		
Mean	0.37	0.08
CV	1.080	4.413

Heat increment in relation to energy expenditure

During the 8 h collection period following the presentation of the meal and following correction for the energy cost of standing, the cumulative EE was 5,697 kJ (SE = 299.2, $n = 6$) and 6,022 kJ (SE = 357.6, $n = 8$) for winter and summer, respectively. Adjusting EE to metabolic body size (MBS) resulted in individual cumulative EEs in winter ranging from 109 to 134 kJ•kg $BW^{-0.75}$ (mean = 124 kJ•kg $BW^{-0.75}$, SE = 4.1 kJ•kg $BW^{-0.75}$, $n = 6$) and in summer from 135 to 170 kJ•kg $BW^{-0.75}$ (mean = 148 kJ•kg $BW^{-0.75}$, SE = 4.2 kJ•kg $BW^{-0.75}$, $n = 8$).

Following the correction for standing, values for individual estimates of HIF (i) / cumulative EE ranged in winter from 0.04 - 0.10 (mean = 0.06, SE = 0.009, $n = 6$) and in summer from 0.02 – 0.06 (mean = 0.04, SE = 0.004, $n = 8$). In relation to cumulative EE, HI recovered over 8 h as measured by method (ii), ranged in winter from 0.13 to 0.21 (mean = 0.17, SE = 0.013, $n = 6$) and in summer from 0.13 to 0.22 (mean = 0.15, SE = 0.011, $n = 8$). Because HIF estimated by Method (iii) was an extrapolation beyond 8 h, no empirical comparison with cumulative EE was possible.

Discussion

Estimates of the efficiency of use of metabolizable energy of forages used by wild ruminants are few because the quantity of forage that must be harvested for such trials is unrealistically high. Techniques for estimating HIF and k must start by accounting for other factors that affect metabolic measurements in ruminants including: a) forage quality, which exerts strong controls over k (Agriculture Research Council, 1980;

Robbins, 1990), b) chamber temperatures below the thermoneutral range that influence

EE when some HI is used to meet thermogenic costs (Mautz et al., 1992; Jensen et al.,

1999), c) season, and the associated changes in physiology and nutrition (Regelin et al.,

1985; Nilssen et al., 1994; Worden & Pekin, 1995; Jensen et al., 1999), and d) activity,

particularly the energy cost of standing (Fancy & White, 1985; Mautz et al., 1992).

Brome hay used in this study was of equivalent quality throughout the study

(Table 2.1). We did not expect the slightly higher fiber content, and slightly higher gross

energy content of hay in the summer to translate into higher HIF estimates in summer

compared to winter. Our results supported this prediction as HIF values were, for the

most part, lower in summer than in winter (5 of 6 mean estimates; Table 2.6).

Controlling for differences in MEI by expressing HIF values as a fraction of MEI (Table

2.7) also resulted in estimates of HIF that were either equivalent across seasons, or

greater in winter than summer (HIF method (iii)).

The upper and lower critical temperatures for postnatal muskoxen is known (Blix

et al., 1984) but not for older muskoxen. Extrapolation from coat insulation and body-

mass allometry predict a lower critical temperature below $-40^{\circ}C$ (Feist & White, 1989),

considerably below the lowest chamber temperature ($-13^{\circ}C$) of this study. Therefore, we

anticipated no use of HIF for thermogenesis. In muskoxen, EE following 26 h of fasting

was not affected by temperature, Julian date, or their interactions during summer. In

winter, EEp declined as temperatures became warmer but also was related significantly to

Julian date. Indications from the stepwise multiple regression analysis, however,

indicated that date was a better predictor of EEp than was temperature. That conclusion

was supported by the correlation matrix, the standardized regression coefficient, and the partial correlation coefficient for temperature.

An integral part of the heat increment of feeding is the nutritional and physiological state of the animal and, for northern species, the time of year of the measurement. Our finding of a significantly lower EE in the last hour of the metabolic trial in comparison to the 24 h fasted animal (EEp) was not unexpected given the findings of Chowdhury & Ørskov (1997); once glucogenic precursors are no longer being absorbed, and glycogen stores are depleted, a ruminant must use protein to meet its endogenous glucose requirements, which brings about an increase in EE that constitutes an endogenous HI. We suggest that this endogenous HI is then suppressed by glucogenic components of the meal; thus, the post-meal EE can then decrease, relative to EEp, to the extent that EEt<EEp. Consequently, measurements of HIF need to be made before fasting induced gluconeogensis. Within this context, an appropriate measure of HIF should be short enough that nutrients are still being absorbed from the gut, yet long enough to result in repeatable measures of the HIF. Ideally, all of the HI from a particular meal would be recovered.

The nutritional and physiological state of our study animals varied between seasons, which was manifested in seasonal trends in weight, EE values and RQs. Seasonal weight stasis in muskoxen during winter indicates animals were at maintenance levels of MEI even when hay was offered ad libitum. Weight gained during summer and early autumn was associated with a 104 kJ·kg $BW^{-0.75}$ ·d^{-1} increase in EEp. We have shown previously that this increase in food intake occurs between April and May (Lawler

& White, 1997). Those differences were associated with a doubling in voluntary food

intake from winter to summer (White et al., 1984; Holleman et al., 1984). Seasonal

differences in RQs indicated a difference in substrate metabolism between summer and

winter. Following 24 h of fasting and during the 1 h feeding time block, winter RQs

indicated muskoxen were metabolizing a higher proportion of carbohydrate to fat in

comparison with summer values. That outcome is consistent with a slower metabolic rate

(Nilssen et al., 1994; Lawler & White, 1997), a slower passage rate (Holleman et al.,

1984; Adamczewski, 1994a), and may be indicative of a seasonal change in rumen

microbes. Our conclusion is supported by seasonal patterns of EE (Fig. 2.6). Thus,

measures of the heat increment and net-energy values for feeds in winter may have little

relevance in summer.

Seasonal patterns of EE and voluntary forage intake may cause changes in basal

metabolism in wildlife species. The observation that long-term fasted animals in summer

can exhibit metabolic rates similar to those expected from post-absorptive animals in the

winter does not indicate that BMR remained the same, but rather that metabolic rates

vary with dietary intake and resulting gut activity. To argue otherwise would require

consideration of gut activity as something other than an endogenous energy requirement.

To avoid problems associated with seasonal changes in BMR, energy expended with

consumption of different meals might best be compared with baseline values, which are

allowed to vary seasonally and that do not artificially depress metabolic rates through

long-term fasts. This practice would tend to obscure seasonal differences that are a valid

portion of a species "basal" metabolism. Our methods fulfill these objectives by allowing a baseline metabolism to be determined for each trial.

Variation in activity levels, either between seasons or among individual animals, has a high potential of obscuring variation in HIF. Muskoxen in this study used 21% more energy standing than when bedded. Correcting EE values for standing had a significant effect on EEp and EEt in both summer and winter. Correcting metabolic trials for the additional cost of standing, however, is a cumbersome process. Given the effort required, researchers need to evaluate the appropriateness of applying corrective measures to their data. In situations where animals spend little time active, or in trials with comparable activity levels, correcting for activity may be unnecessary.

Our observations that EEt can be less than the EEp indicates that use of a static baseline [i.e. method (i)] is inappropriate for absolute measures of HIF because a portion of the energy released from the test meal would be attributed to the residual heat increment of the previous meal, resulting in an underestimate of HIF. Alternatively, if animals were more active during the pre-feeding than the post-feeding periods, a similar decline in EE could occur. We reject this latter possibility because quantification of activity at 2 min intervals showed that the animals were, if anything, less active pre-feeding. Also, the relative relation of EEp to EEt remained unchanged whether data were corrected for standing. Because the test meal affected baseline EE, this result would place a limitation on simply subtracting EEp as a baseline for the estimation of HIF.

We suggest that a criterion for measuring HIF is a measurement period short enough that nutrients were still being absorbed from the gut. During the 8 h post feeding

measurement period in these series of trials, this criteria was met because RQ values during the terminal 1 h indicated carbohydrates versus fats were being metabolized (winter mean RQ = 0.99; summer mean RQ = 0.92). Methods (i) and (ii) both meet this criterion because they are limited to the 8 h period. Method (iii) met this criterion because the trajectory of the extrapolated HIF values is determined during the 8 h trial, before fasting induced gluconeogenesis.

A second criterion for the usefulness of a technique is that it is long enough to result in repeatable measures of the HIF and recovers all of the expected heat increment in the study period. The protocol and technique used to determine EE and the HIF in this study gave repeatable estimates of EEp and the total EE over 8 h post-feeding both with and without correction for the energy cost of standing, and methods (i), (ii), and (iii) all produced repeatable estimates of HIF and K. Certainly, the criterion of recovering most of the theoretical HIF is met when EEp is subtracted from EE [method (i)], because we have already noted that HIF was assumed to be complete when the post-meal EE was equal to EEp, and this was met at just under 5 h following the meal. The sliding baseline approach [method (ii)] resulted in recoveries of heat increment of 54 and 75% of that predicted at t_∞ [(method iii)] for winter and summer respectively. This result suggests method (ii) underestimates measures of HIF. Because method (iii) is an extrapolation to t_∞, this method, by definition, recovers the entire HIF.

The final criterion for assessing these 3 methods for measuring HIF is that the mean estimate of HIF and k for a standard forage is within the range of traditionally determined values for the food type. Because we have no measure of the

metabolizability of energy in this hay by muskoxen, we used a value for a similar diet in agriculture systems for which 84% of digestable energy is metabolizable (Agricultural Research Council, 1980). Based on that value and a brome hay digestibility of 0.74 (Holleman et al., 1984), efficiency in use of ME for maintenance (k) is expected to be approximately 0.73 (Graham, 1966), which gives an HIF/MEI ratio of 0.27. Estimates of HIF were assumed to fall within the range of traditionally determined estimates if they were within the $\pm$ 10% range of the predicted HIF. Using method (i) the HIF/MEI estimate during winter was 0.07 and during summer was 0.06. These values are clearly lower than those predicted with the traditional method. With the sliding EEp technique [method (ii)], estimates of HIF/MEI during winter and summer were 0.19 and 0.20, respectively. Those values also were lower than predicted. Nonetheless, k estimated by method (ii) at 0.82 and 0.80 for winter and summer, respectively, was within the range reported for high quality hay (Agricultural Research Council, 1980). For method (iii), the HIF to MEI ratio asymptote values of 0.38 and 0.29 during winter and summer, respectively, were higher than predicted, but fell within the range reported for high quality hay (Agricultural Research Council, 1980). Likewise estimates of k, 0.62 in winter and 0.71 in summer, were well within the reported values for high quality hay.

In conclusion, the determination of EE following the consumption of a standardized single meal over an 8 h period and following 24 h of fasting has advantages over multi-day experiments with animals continuously fed. In wildlife studies, logistical constraints limit the number of study animals and the ability of the animals to withstand days of restraint may be limited. The amount of wild forage needed for a continuous

feeding trial often cannot be harvested and stored. We suggest this single meal and 10 h trial approach yields repeatable estimates of HIF and would be useful in wildlife studies when evaluating wild forages. We suggest methods (i) and method (ii) result in highly repeatable EE measurements and both would be appropriate techniques for measuring relative changes in EE associated with consumption of a meal or in studying the temporal dynamics of HIF, but absolute estimates of HIF are low in comparison to predicted HIF values. Method (iii) results in repeatable measures of HIF but tends to over estimate HIF in comparison to predicted values based on previous work done with wildlife species (Robbins, 1993). Nonetheless, in comparison to published values for domestic species consuming high quality hay, method (iii) provides an appropriate measure of absolute HIF and is the preferred analysis to evaluate the absolute size of the HIF.

Chapter 3: Effect of Browse on the Heat Increment of

Feeding in Muskoxen[1]

Abstract: We investigated heat increment of feeding in muskoxen (*Ovibos moschatus*) consuming experimental diets of graded levels of three browse species mixed with brome hay (*Bromus inermis*). Browse species used were *Salix alaxensis*, *Salix planifolia* subspecies *pulchra*, and *Betula nana*. A commercially available textured concentrate feed was also included to assess the possibility that browse may act as a concentrate feed. Browse was fed as chipped woody twigs in winter and as freshly stripped leaves in summer mixed with hay and energy expenditure was determined by gas exchange. A repeated-measures general-linear model was used to examine energy expenditure of muskoxen before and after consumption of experimental diets by dividing the 10 h trial into 2 h time blocks. Energy expenditure peaked in the 2 h time block that included the meal and declined thereafter. Rate of decline in energy expenditure following the meal varied significantly with browse and hay mixes compared with the 100% hay diet for a series of winter metabolic trials that included the twigs of *Salix alaxensis* and *S. pulchra* ($P = 0.015$), and for the series of summer trials that included hay mixed with leaves of *S. alaxensis*, *S. pulchra*, *B. nana*, and a textured concentrate ($P = 0.024$). The heat increment index was estimated as the net 8 h energy expenditure and although no

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[1] Lawler, JP, White, RG. Effects of Browse on the Heat Increment of Feeding in Muskoxen. In prep. Canadian Journal of Zoology.

differences were statistically detectable in experimental diet mixes, energy losses in

winter were highest for woody browse species with the highest concentrations of

proanthocyanidin (*S. alaxensis* and *S. pulchra*). In contrast, there was no apparent effect

of proanthocyanidin on heat increment during summer metabolic trials with leafy

browse. During summer, net energy loss tended to be inversely related to acid detergent

lignin levels, which were high in *B. nana* and low in *S. pulchra* and *S. alaxensis*.

INTRODUCTION

The heat increment of feeding (HIF) is an increase in energy expenditure (EE)

associated with the intake of a meal. In ruminants, energy is expended as heat with

ingestion, rumination, and fermentation arising from rumen microbial activity, gut tissue

metabolism, and increased metabolism in tissues other than the digestive tract,

respectively (Hudson and Christopherson 1985). Gut digestive processes include the

energetic costs of gastric, pancreatic, bilary and intestinal secretion. Energy is needed for

transporter and enzyme up-regulation and for biochemical transformation of metabolic

products (Secor and Diamond 1995). Energy is also necessary for the synthesis of body

tissue. In some situations, energy may be required to absorb, detoxify and excrete

ingested plant secondary metabolites (Foley et al. 1995; Illius and Jessop 1995).

HIF is positively correlated with the metabolizable energy of the diet (Blaxter

1962; Webster 1983), mass of the meal (Secor and Diamond 1995; Chappell et al. 1996;

Rosen and Trites 1997), and metabolizable energy intake (Sedinger et al. 1992).

Nonetheless, given the many processes occurring in a ruminant with the ingestion of a

meal, precise measurements of the amount of energy lost from a meal as heat are difficult

to make and highly variable (Blaxter 1962).

For energetic studies, arctic ruminants are ideal because these large herbivores are

adapted to environments where efficient use of dietary energy is critical (White 1983;

Klein 1986; Tyler and Blix 1990; Klein 1992). Muskoxen (*Ovibos moschatus*), and

caribou and reindeer (*Rangifer tarandus*) are the only ruminants native to the high arctic.

Muskoxen are relatively sedentary, and especially females are unlikely to travel long

distances in search of high quality forages (Biddlecomb 1992; Klein 1992; Staaland et al.

1997). These factors presumably constrain muskoxen to be energetically conservative.

In addition, because of variation observed in muskoxen diets, a wide range of diets

exhibiting a wide spectrum of digestive characteristics can be examined. Muskoxen are

capable of subsisting on low-quality diets of hay (White et al. 1984; Eisfeld 1990;

Adamczewski et al. 1994a; Adamczewski et al. 1994b). In many portions of their range,

however, browse (sometimes considered a concentrate feed) is an important component

of their diet. Willows (*Salix* spp.) in particular, including leaves in summer and twigs in

winter, have been used heavily by muskoxen in Alaska (Bos 1967; Jingfors 1981; Robus

1981; O'Brian 1988; Wilson 1992), Canada (Tener 1965; Oakes et al. 1992; Shaefer

1995), and Greenland (Thing et al. 1987; Staaland and Olsen 1992; Forchhammer 1995;

Nelleman 1997). Robus (1981) noted that in the 3 major locations used by muskoxen in

her study area, muskoxen productivity was highest in the area with the greatest

abundance of willow.

In this study, we examine HIF in a wild ruminant consuming a natural diet. We tested the null hypothesis that the addition of browse to a graminoid based diet would not alter the HIF. In addition, we tested the null hypotheses that HIF would not vary by the type of browse consumed with a graminoid based diet, or by the percent of browse consumed. To test these hypotheses, we measured effects of consuming graded levels of woody browse, leafy browse, and a textured concentrate on HIF in muskoxen relative to a graminoid-based diets.

MATERIALS AND METHODS

Approach and study animals

We determined EE following a meal in captive muskoxen fed diets with graded levels of browse. Each animal was trained to spend up to 10 h in an indirect open-circuit calorimeter after being fed a standard ration followed by 1 day without food (see *Metabolic experiments*).

Muskoxen were housed, and all metabolic experiments were conducted, at the Large Animal Research Station (LARS), Institute of Arctic Biology, University of Alaska Fairbanks, USA (64° 50'N, 147° 43'W). In winter 1994, mean ± SD weight of the 3 muskoxen at the start of the metabolic trials was 163 ±11.0 kg with a range of 148-183 kg. During summer 1995, mean weight of 9 muskoxen was 121 ±28.1 kg and their weights ranged from 83-187 kg. These same muskoxen averaged 158 ± 30.2 kg and ranged from 115-212 kg during winter of 1996.

The protocol for this study was to withhold food from muskoxen for 24-26 h before sealing them in a respiration chamber. Data collection commenced approximately 0.5 h later, once gases within the chamber had equilibrated. Animals had free access to water during summer and snow during winter. During the first 2 h of the metabolic trial, muskoxen were offered no food and base line values for EE were determined. At 2 h, the experimental meal was made available by opening the cover over a feed bin and gaseous exchange was measured for the next 8 h (Fig. 3.1).

All animals involved in this study had been habituated to confinement in the metabolic chamber as young animals. Only trials in which animals remained calm and unagitated throughout were used for analyses. In winter 1994, three 3-y-old muskoxen were used (2 males and 1 female) for the metabolic experiments. These same muskoxen were used in winter 1995 as 4-y-olds. In summer 1995 and winter 1996, a second cohort of nine muskoxen ranging in age from 2 to 3-y were used (6 male and 3 female; Table 3.1). All muskoxen used during the metabolic studies were sexually intact. Females were not pregnant or lactating. When muskoxen were not being fasted or in the respiration chamber, they were maintained in pastures dominated by smooth brome (*Bromus inermis*), foxtail (*Hordeum jubatum*), lambs quarter (*Chenopodium album*), knot weed (*Polygonum aviculare*), and shepherds purse (*Capsella bursa-pastoris*). During all seasons, muskoxen had access to feeders with brome hay. Protocols for this experiment and care of these animals were approved by the University of Alaska, Fairbanks, Institutional Animal Care and Use Committee (IACUC # 93-01) and protocols were in
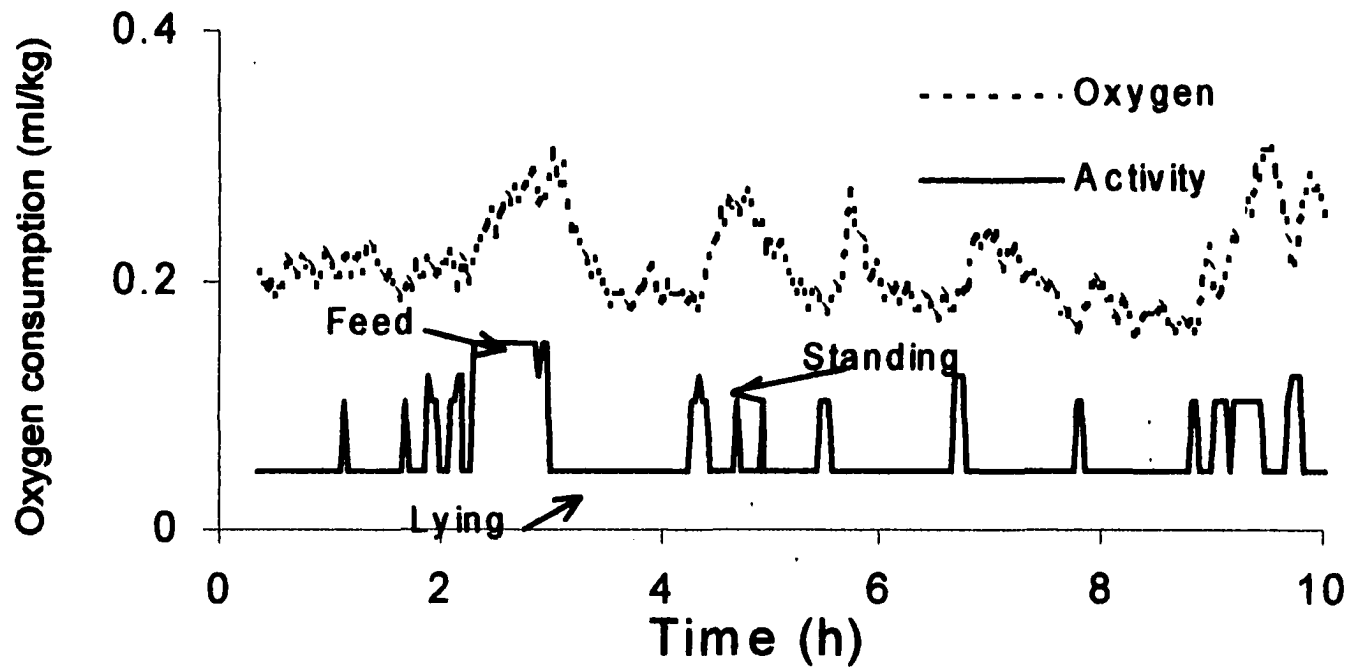
Fig. 3.1. Temporal changes in oxygen consumption for a muskox at the Large Animal Research Station, Fairbanks, Alaska, 14 January 1994 as measured with an indirect calorimeter. Also shown is the activity profile during the 10 h trial. This muskox was fed a meal (10 g DM•kg $BM^{-0.75}$) of 60% *S. pulchra* and 40% hay 2 hours into the metabolic trial.

Table 3.1. Individual muskox and diets used during metabolic trials in relation to year and season, Large Animal Research Station, Fairbanks, Alaska, USA.

Season and Year	Muskox identity	Age (Y)	Experimental diet addition	Percent of diet mixture [b]
Winter 1994	Shumagin [c]	3	*S. alaxensis* (twigs)	0, 20, 40, 60, 80
	Tundra	3	*S. pulchra* (twigs)	0, 20, 40, 60, 80 [b]
	Sparbo	3		
Winter 1995	Shumagin [c]	4	Concentrate	0, 20, 50
	Tundra	4		
	Sparbo	4		
Summer 1995	Damien	2	Concentrate	0, 20, 40, 60
	Terezy	2	*S. alaxensis* (leaves)	0, 20, 40, 60
	Salix [c]	2	*S. pulchra* (leaves)	0, 20, 40, 60
	Scooter [c]	2	*B. nana* (leaves)	0, 20, 40, 60
	Carter	2		
	Saydy [c]	3		
	Devon	2		
	Bathurst	2		
	Gaston	3		
Winter 1996	Damien	2	*B. nana* (twigs)	0, 20, 40, 60
	Terezy	2		
	Salix [c]	2		
	Scooter [c]	2		
	Carter	2		
	Saydy [c]	3		
	Devon	2		
	Bathurst	2		
	Gaston	3		

[a] Based on a DM content.
[b] Remaining DM percentage is hay.
[c] Female

keeping with methods approved by the American Society of Mammalogists (Animal Care and Use Committee, 1998) for research on captive mammals.

Diets and feeding levels

Experimental diets during winter 1994 consisted of hand-clipped twigs of *Salix alaxensis* or *S. planifolia* subspecies *pulchra* (*S. pulchra*) passed through a chipper (Sears, Roebuck and Co., 8 hp craftsman, Chicago, IL) and mixed by hand with chipped brome hay. Each *Salix* species was fed during the metabolic trials at 20, 40, 60, and 80% dry matter (DM) of the meal with the remaining percentage composed of hay. In addition, metabolic trials were conducted with 100% hay. During winter 1996, we followed the same procedure using *Betula nana* twigs (plant nomenclature follows Hultén, 1990), but the 80% twig concentration treatment was eliminated from trials due to a high rejection rates for 80% browse during the winter of 1994. All twigs were gathered during the season in which they were fed and each species was collected in only one location near Fairbanks Alaska, (64° 46'N, 147° 28'W). Twigs were stored outdoors until fed and in both years, and temperatures never exceeded freezing. All twigs collected were current annual growth, and were collected from the top of mature growth-form plants.

During summer 1995 (16 July – 13 August), freshly stripped leaves of *S. alaxensis*, *S. pulchra*, and *B. nana* were hand mixed (each species separately) with chipped hay and fed at 20, 40 and 60% DM with the remaining percentage composed of chipped hay. Twigs with the leaves attached were gathered 1-6 h before the start of a

metabolic experiment and leaves were hand stripped immediately before the animal was sealed in the chamber. Experiments involving leaves were restricted to mid-summer to minimize changes in diet quality because of changing plant phenology. Leaves were collected from current annual growth from the top of mature plants.

Quality Texture Ration, Alaska Mill and Feed, Anchorage, Alaska, a grain and corn-based textured concentrate, was added to hay forming the experimental diets during a separate set of metabolic experiments in February and March 1995, and as part of the experimental set that included leaves during summer 1995. This concentrate was mixed with chipped hay and fed at 0, 20, and 50% DM during winter 1995 and at 0, 20, 40 and 60% DM during summer 1995. The remainder of those diets was composed of chipped hay.

In an effort to standardize metabolic states before the meal, each animal was brought in from pasture, weighed, given a standardized meal of 50% DM hay and 50% DM textured concentrate, and then fasted for the next 24-26 h. The 1-d fasting period served the dual purpose of standardizing digestive states of the animals and ensuring animals would have an appetite for the experimental diets.

All experimental diets, as well as standardized diets, were fed as a single meal at 10 g dry matter (DM) per kg body mass $(BM)^{-0.75}$. This feeding level was determined from a series of preliminary trials. Following 24 h without food, muskoxen were expected to readily consume everything offered in one feeding bout.

Analytical methods

The dry matter (DM) content of twigs, leaves, hay, and concentrate used during all metabolic trials was determined by drying the material at 60°C for 48 h. Neutral detergent fiber (NDF), acid detergent fiber (ADF), and acid detergent lignin (ADL) were determined sequentially (Goering and Van Soest 1970), and hemicellulose and cellulose estimated by subtraction. Total nitrogen and carbon content of all feeds were analyzed by combustion in an N analyzer system (LECO CNS-2000 elemental analyzer, St Joseph, MI). Gross energy (GE) was determined by combustion in a bomb calorimeter (Parr 1108 oxygen combustion bomb, Moline, IL).

Tannins from all leaves and twigs were extracted with 80% acetone and purified by filtration through Sephadex LH20 as described by Clausen et al. (1990). These purified tannins were used as standards for evaluating proanthocyanidin concentrations in each browse type and each browse species. Concentrations of proanthocyanidin were measured following the assay outlined by Martin and Martin (1982) and described by Bryant (1987). Feeds were freeze dried for 48 h and then ground through a Wiley mill with an 850-µm screen. A 150 mg sample was extracted twice sequentially in 20 ml of 50% aqueous methanol at 95°C for 10 min. The supernatant was diluted to 50 ml and a 0.300 ml aliquot of this was added to 3.0 ml Butanol / HCl / ferrous sulfate solution and refluxed for 2 h at 95°C. Light absorbence at 550 nm was read on a UV/Vis spectrophotometer (Perkin Elmer Lambda 1 series, Oak brook, IL). Each feed sample was analyzed in triplicate.

Indirect calorimetry

An open-circuit respiration chamber was used to measure oxygen consumption and carbon dioxide and methane production of muskoxen during 10 h trials. Outdoor air and 3 standard gases were used to calibrate analyzers. Calibration occurred at the start of each metabolic experiment and at approximately 2-h intervals throughout. A data acquisition system linked to a computer recorded flow rate, humidity, temperature, barometric pressure, and gas concentrations in the airstream leaving the metabolic chamber in 2-min increments (Fig. 3.1). Animal activity was monitored by remote camera. Activity was categorized for analysis as bedded or active (standing, feeding, drinking, and scratching), and was recorded with scan sampling (Altmann 1974) every 2-min in synchrony with the data acquisition system.

Metabolic experiments

Fifty-four metabolic experiments were conducted from 9 January to 15 April 1994. Three young muskoxen were evaluated on 9 different experimental diets (Table 3.1). Each diet was randomly selected and offered to each muskoxen on 2 occasions. Each animal was fasted for 24-26 h and spent the next 10-12 h in the calorimeter. Following the experiment, each animal was allowed 3 d before being involved in a new experiment.

From 16 July to 13 August 1995, 28 trials were conducted with 9 different animals (Table 3.1). Thirteen different experimental diets were offered during that period, and each animal was evaluated on 100% hay and from 1 to 3 of the experimental

diet mixes. Mixed diets were randomly assigned to animals. The experimental protocol was similar to that conducted in winter 1994 in that each animal was fasted for 24-26 h and each metabolic experiment lasted from 10-12 h. There was a minimum of 3 d between metabolic experiments for individual animals.

From 5 January to 21 January 1996, 17 trials were conducted with the same animals used during summer 1995 (Table 3.1). During these trials, each animal was evaluated on 100% hay and one of three different dietary mixes. The experimental protocol followed that of summer 1995.

Textured concentrate was used during metabolic experiments in February and March 1995, and concurrently with the leafy browse diets in summer 1995 (Table 3.1). Textured concentrate was included in these metabolic experiments as a standard for a concentrate feed. The 3 animals used during metabolic trials in winter 1994 were evaluated on 2 levels of textured concentrate dietary mixes from 15 February to 7 March 1995. Nine animals involved in metabolic experiments during summer 1995 were evaluated on 3 levels of textured concentrate dietary mixes.

Energy calculations

Energy expenditure in the 8 h following the presentation of the meal was calculated by multiplying the volume of oxygen consumed during the trial by the thermal equivalent of oxygen at the measured respiratory quotient (Brody 1964). Those metabolic rates were then expressed as kilo joules (kj) of daily EE on the basis of metabolic body mass (Kleiber 1975)(EE, $kJ \cdot kg\ BM^{-0.75} \cdot h^{-1}$).

To estimate the net contribution of meal-derived EE (HIF), we determined baseline or pre-meal estimates of EE. That measurement was made for 2 h at the end of a 24-h fast just before presentation of the experimental meal. Energy expenditure (EE) attributed to a particular meal was calculated for the 8 h following presentation of the meal by difference and expressed as a HIF index; HIF index = after meal EE - before meal EE. Although not appropriate for deriving absolute measures of the HIF (Chapter 2), this method of determining HIF is appropriate for comparing relative changes in EE following meals composed of different foods and results in repeatable measurements. In addition, this method is simple relative to other methods of calculating HIF relying on only a single variable. Potential problems regarding assumptions of the rate of change in EE beyond the 8 h metabolic trials are avoided as is the assumption that the test meal does not influence the EE during the final 1 h of the trial.

Statistical analysis

To ensure established baseline values did not reflect variance in activity patterns between types of browse (type) or percentages of browse fed (percent), pre-meal patterns of activity were examined with a general linear model (GLM-general factorial, SPSS 7.0 for Windows, 1995; SPSS Inc., Chicago, IL). Fixed factors used in this model were browse type and percent of browse fed. Animal was included, where appropriate, as a random effect (winter 1994 trial, and winter textured concentrate trail).

Variability in HIF between type of browse, and between percent of diet composed of browse can be characterized by differences in the response patterns of HIF production

by browse type and browse percentage, or by differences in net EE values. We examined

variation in the temporal pattern of HIF by dividing the entire feeding and post-feeding

period (8 h) into four 2-h blocks (within-subject variable) and testing for differences in

time blocks with a repeated-measures GLM (repeated measures, SPSS 7.0 for Windows,

1995). This test uses multivariate techniques to test for differences within a response

variable (time blocks), and analyzes within and between-subject variable in a series of

ANOVAs. We tested hypotheses regarding changes in response patterns of HIF

following a meal or "parallelism" (slope), and tested hypotheses regarding net HIF

following the meal or "levels" (intercept) (vonEnde 1993). Factors included in the model

were muskoxen (where appropriate), browse type, and percent of browse consumed.

Activity was included in the model as a covariate.

Variance-covariance matrices for with-in subject factor were examined with the

Mauchly test for sphericity. Where the sphericity assumption was not met, the degrees of

freedom of the F statistic were adjusted according to the severity of this violation with a

Greenhouse-Geisser estimator (von Ende 1993). When significant variation occurred, the

Tukey method (HSD) of post-hoc multiple comparisons was used to compare means of

factor levels. In all analyses, groups were considered significantly different if $P < 0.05$.

Observed power of all hypothesis tests was examined to determine the probability

of rejecting the null hypothesis (no difference in means between factors) when it was

false. Partial eta-squared results for each test were examined to evaluate the strength of

association between browse type, percent of browse in the diet, and their interaction,

with the HIF index. Partial eta-squared is the proportion of the total variability in the

dependent variable that is accounted for by the variation in the independent variable and is similar to partial coefficients of determination from standard multiple regression analysis (SPSS 1991).

RESULTS

Diet analysis

Hay was high in fiber, with high NDF (65.9%) and ADF (35.9%), relative to other forages (Table 3.2). Twigs were lower in NDF (59.8%) but contained higher amounts of ADF (45.6%) than did hay. Both textured concentrate and leaves had relatively low percentages of NDF (22.1 and 31.5%, respectively) and ADF (6.5 and 21.6%, respectively).

Although fiber content was high in both hay and twigs, composition of the fiber differed. Hay fiber was largely cellulose (32.5%) and hemicellulose (29.5%) with small amounts of lignin (3.6%). Twig fiber also was largely cellulose (24.4%) but contained more lignin (21.7%) and, correspondingly, less hemicellulose (13.5%) than hay fiber. Leaf fiber was almost evenly split between cellulose (10.1%), hemicellulose (9.9%) and lignin (10.6%). Textured concentrate was unique in that it had higher amounts of hemicellulose (16.3%) than cellulose (5.7%) and insubstantial amounts of lignin (1.0%).

Percent N was highest in textured concentrate (3.1%) followed by leaves (1.8%) and hay (1.8%). Twigs were lowest in N (1.0%). GE was highest in twigs (21.2 kJ/g)

Table 3.2. Chemical composition and gross energy of twigs, leaves, hay and textured concentrate fed to muskoxen during the course of metabolic experiments at the Large Animal Research Station, University of Alaska, Fairbanks. Metabolic trials using these feeds were conducted from 9 January to 15 April, 1994 (twigs of *S. alaxensis* and *S. pulchra*), 16 July to 13 August, 1995 (leaves of *S. alaxensis*, *S. pulchra* and *B. nana*, and textured concentrate), 15 February to 7 March 1995 (textured concentrate), and 5 to 21 January, 1996 (twigs of *B. nana*).

Component[a]	Twigs						Leaves					
	S. alaxensis	SE	S. pulchra	SE	B. nana	SE	S. alaxensis	SE	S. pulchra	SE	B. nana	SE
Dry matter	53.8	0.86	52.5	0.87	56	2	39	3.34	42.8	2.06	41.5	4.29
Organic matter	96.1	0.18	97.2	0.15	98.5	0.01	88.8	0.90	96.2	0.08	96.7	0.09
Neutral-detergent fiber	53.5	1.28	59.2	1.22	66.6	0.62	30.2	0.56	26.1	0.06	38.3	1.25
Acid-detergent fiber	41.3	1.15	46.6	0.96	48.9	0.18	20.5	0.54	17.3	0.14	27.1	0.96
Hemicellulose	12.1	0.37	12.6	0.51	15.8	0.37	9.6	0.20	8.8	0.15	11.2	0.33
Cellulose	25.9	0.93	25.8	0.73	21.6	0.33	12.5	0.43	8.6	0.08	9.2	0.49
Acid detergent lignin	15.7	0.43	20.7	0.77	28.6	0.18	5.5	0.21	8.7	0.15	17.6	0.56
Proanthocyanidin	15.0	0.87	14.5	1.29	6.1	0.46	9.2	0.71	11.7	1.94	8.4	2.26
Crude Protein	5.8	0.28	6.2	0.22	7.2	0.15	10.5	0.64	13.6	0.31	9.9	0.06
Gross energy (kj/g)	20.1	0.03	20.5	0.04	23.0	0.01	17.9	0.01	19.6	0.04	21.2	0.02
Ether extract	2.8	0.30	1.9	0.21	2.0	0.07	2.8	0.21	1.3	0.32	2.9	0.32
Cell Content	46.5	1.08	40.8	1.10	33.4	0.62	69.8	0.45	73.9	0.20	61.7	0.98

Component[a]	Hay						Textured Concentrate					
	Winter 94	SE	Summer 95	SE	Winter 96	SE	Winter 94	SE	Summer 95	SE	Winter 96	SE
Dry matter	88.7	0.33	81	3.00	89.3	0.33	89.3	0.33	88.5	1.50	89	-
Organic matter	93.8	0.54	94.8	0.03	95.1	-	93.0	0.34	97.7	0.37	91.6	0.12
Neutral-detergent fiber	58.7	0.41	67.5	0.08	71.9	0.18	22.8	0.34	20.0	0.45	23.5	0.19
Acid-detergent fiber	32.2	0.31	36.6	0.01	39.0	0.35	6.8	0.21	6.2	0.12	6.4	0.12
Hemicellulose	26.5	0.28	30.8	0.08	31.1	0.12	16.0	0.39	13.8	0.57	19.1	5.52
Cellulose	29.1	0.28	33.2	0.01	35.1	0.09	5.9	0.08	5.3	0.06	5.9	0.07
Acid detergent lignin	2.8	0.10	3.3	0.02	4.5	0.16	0.70	0.11	0.9	0.01	1.4	0.11
Proanthocyanidin	ND		ND		ND		ND		ND		ND	
Crude Protein	11.8	1.90	10.2	-	11.2	0.10	18.0	0.38	19.6	-	20.1	0.10
Gross energy (kJ/g)	17.8	0.02	18.1	0.02	18.5	0.06	17.6	0.02	18.0	0.02	18.0	0.03
Ether extract	1.5	0.57	1.0	0.36	ND		3.0	0.40	5.2	0.52	ND	
Cell Content	41.3	0.38	32.5	1.41	28.1	0.18	77.2	0.34	80.0	0.48	76.5	5.02

[a]All values (except dry matter and gross energy) are expressed as a percentage of dry matter.

ND, not determined.

NDFs and ADFs were determined sequentially.

followed by leaves (19.6 kJ/g) but energy content varied considerably among species

(Table 3.2). GE in hay (18.1 kJ/g) was comparable to that in textured concentrate (17.9

kJ/g). Proanthocyanidin content of browse species did not exhibit a consistent seasonal

trend. Overall, winter twigs had a higher percentage proanthocyanidin than did summer

leaves (11.9 and 9.8%, respectively), but this pattern did not hold for *B. nana* (Table 3.2).

Feed rejection

During winter 1994, 11 of 54 metabolic trials were removed from the analysis due

to animals failing to consume the entire meal (Table 3.3). When 80% of the

experimental meal offered consisted of woody browse, muskoxen failed to consume the

entire diet 67% of the time and this rejection was equally divided between the 2 species

of *Salix* (each was rejected on 4 of 6 occasions). Twenty-five percent of the meals fed at

60% woody browse resulted in feed rejection (*S. alaxensis* twice and *S. pulchra* once).

At the 80% level of browse, amount of feed rejected was similar between the 2

Salix species at 0-43% of the food offered (means $\pm$ SD of 12.2 $\pm$ 6.50% for *S. alaxensis*

and 16.7 $\pm$ 7.28% for *S. pulchra*). At the 60% browse level, rejection ranged from 0-16%

(4.8 $\pm$ 3.08% for *S. alaxensis* and 1.7 $\pm$ 1.67% for *S. pulchra*).

Because of high rejection rates associated with browse fed at 80% of the diet

during winter 1994, this feeding level was eliminated from further metabolic trials. All

meals offered during metabolic trials summer 1995 were consumed entirely.

Nonetheless, one trial with 60% *S. pulchra* leaves was terminated soon after the meal

Table 3.3. Meals during winter 1994 at the Large Animal Research Station, Fairbanks, Alaska for which a portion of the meal was rejected. Muskoxen were taken from pasture, fed a standardized meal and starved for 24-26 h. They were then placed in a metabolic chamber and offered one of nine different diets. Diets were composed of hay and chipped woody twigs. Hay was fed as 100% of the diet and mixed with *S. alaxensis* and *S. pulchra* at 20, 40, 60, and 80% of the diet. All experimental diets were mixed on a dry matter (DM) basis. Hay was assumed to be 89% DM, *S. alaxensis* was assumed to be 54% DM, and *S. pulchra* was assumed to be 53% DM. All diets were offered to muskoxen at 10 g·kg $BM^{-0.75}$.

Animal	Browse species	0% Browse (No. of trials) Offered / Rejected[a]	20% Browse (No. of trials) Offered / Rejected[a]	40% Browse (No. of trials) Offered / Rejected[a]	60% Browse (No. of trials) Offered / Rejected[a]	80% Browse (No. of trials) Offered / Rejected[a]
Schumigan	*S. alaxensis*	2 / 0	2 / 0	2 / 0	2 / 0	2 / 2
	S. pulchra	NA[b]	2 / 0	2 / 0	2 / 0	2 / 1
Tundra	*S. alaxensis*	2 / 0	2 / 0	2 / 0	2 / 1	2 / 1
	S. pulchra	NA[b]	2 / 0	2 / 0	2 / 0	2 / 1
Sparbo	*S. alaxensis*	2 / 0	2 / 0	2 / 0	2 / 1	2 / 1
	S. pulchra.	NA[b]	2 / 0	2 / 0	2 / 1	2 / 2
All Muskoxen	*S. alaxensis*	6 / 0	6 / 0	6 / 0	6 / 2	6 / 4
	S. pulchra	NA[b]	6 / 0	6 / 0	6 / 1	6 / 4
	TOTAL	6 / 0	12 / 0	12 / 0	12 / 3	12 / 8

[a] Number of times diet was offered / number of times a portion of the diet was rejected.

[b] The 0% browse level (100% hay) was offered to each muskoxen during 2 metabolic trials.

was offered because of a restless animal, and that trial was eliminated from analysis. Because only one trial at this feeding level was scheduled, no data are available for the 60% browse level for *S. pulchra*.

During winter 1996, only one trial was rejected (*B. nana* was offered at 60% of the diet and 67% of the meal was rejected). This trial was eliminated, leaving 2 trials of 60% *B. nana* for analysis. All metabolic experiments that included the textured concentrate as the experimental additive to the hay diet were consumed in their entirety.

Pre-meal levels of activity

We report activity as the percent of time the animal spent bedded during a particular 2-h time block. During winter 1994, animals involved in the metabolic trials spent a mean of 92% (SE = 1.2 %, n = 43) of their time bedded before being fed (h 25 and 26 since food withdrawal). The winter trials with textured concentrate were similar in that muskoxen spent 91% (SE = 1.1 %, n =15) of their time bedded. Animals were more active before their meal in the remaining metabolic trials. During summer 1995, muskoxen were bedded 86% (SE = 1.9 %, n = 28) of the time before experimental meals that included leafy browse and textured concentrate, and 85% (SE = 2.0%, n = 16) of the time bedded before meals that included twigs during winter 1996.

Activity level before meals did not vary significantly among individual animals (winter 1994, P = 0.934; winter 1995 P = 0.600) in either set of metabolic experiments where animal was included as a factor. Likewise, no significant pre-meal variation in activity was detected between differing browse types offered in the experimental diets (*P*

= 0.932, 0.585, 0.131, 0.476 for winter 1994, summer 95, winter 96, and winter textured concentrate trials, respectively) or between different percentages of browse consumed in the diets (P = 0.134, 0.630, 0.470, 0.498 for winter 1994, summer 95, winter 96, and winter textured concentrate trials, respectively) in any of the metabolic experiments. Nevertheless, a significant type * animal interaction (P = 0.002) was observed during the textured concentrate trials in winter when one animal spent less time bedded (74%) before consuming 100% hay than the other two (89 and 97%).

Energy expenditure

Mean baseline EE was higher in summer than winter. During the winters of 1994, 1995 and 1996, mean energy expenditure was 15.3 kJ•kg $BM^{-0.75}$•h^{-1} (SE = 0.22 kJ•kg $BM^{-0.75}$•h^{-1}, n = 55), 14.5 kJ•kg $BM^{-0.75}$•h^{-1} (SE = 0.38 kJ•kg $BM^{-0.75}$•h^{-1}, n = 15), and 16.7 kJ•kg $BM^{-0.75}$•h^{-1} (SE = 0.64 kJ•kg $BM^{-0.75}$•h^{-1}, n = 17), respectively, before the meal. In summer 1995, mean energy expenditure was 20.7 kJ•kg $BM^{-0.75}$•h^{-1} (SE = 0.70 kJ•kg $BM^{-0.75}$•h^{-1}, n = 28) before the meal.

Following presentation of the meal, EE peaked in the 2-h time block, which included consumption of the meal, and declined in the next 3 periods in all instances (Fig. 3.2 and 3.3). By the forth (6-8 h) time block, EE was below pre-meal values for all diet mixes except textured concentrate trials in winter, and 60% browse in winter 1994 and 1996 (Fig. 3.2 and 3.3).
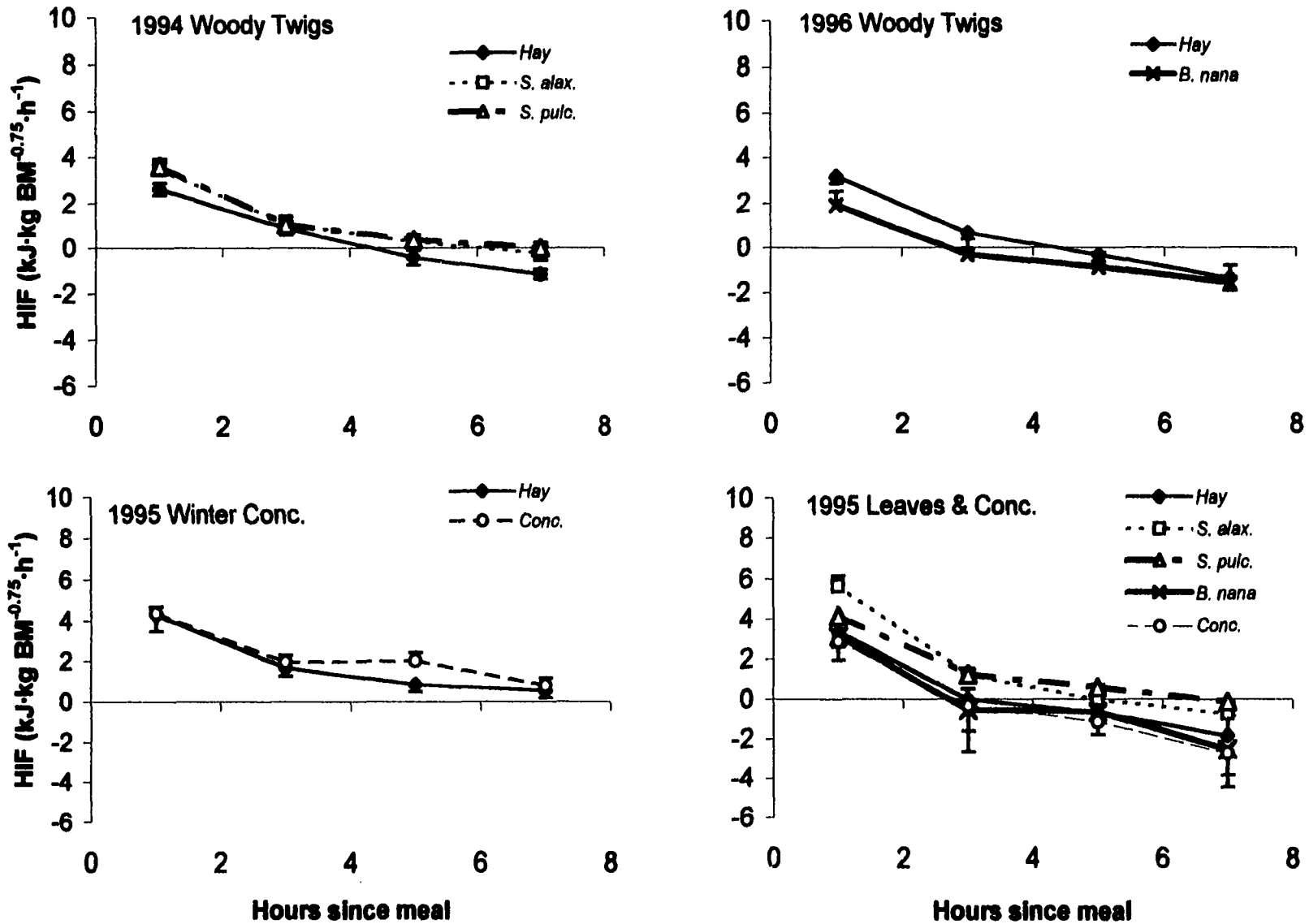
Fig. 3.2. Effects of type of browse (*S. alaxensis, S. pulchra* and *B. Nana*), and textured concentrate supplement (Conc.) on heat increment of feeding in muskoxen at the Large Animal Research Station, Fairbanks, Alaska. Values presented are net energy expenditure above a baseline value determined following 24 h without food. Mean + SE.
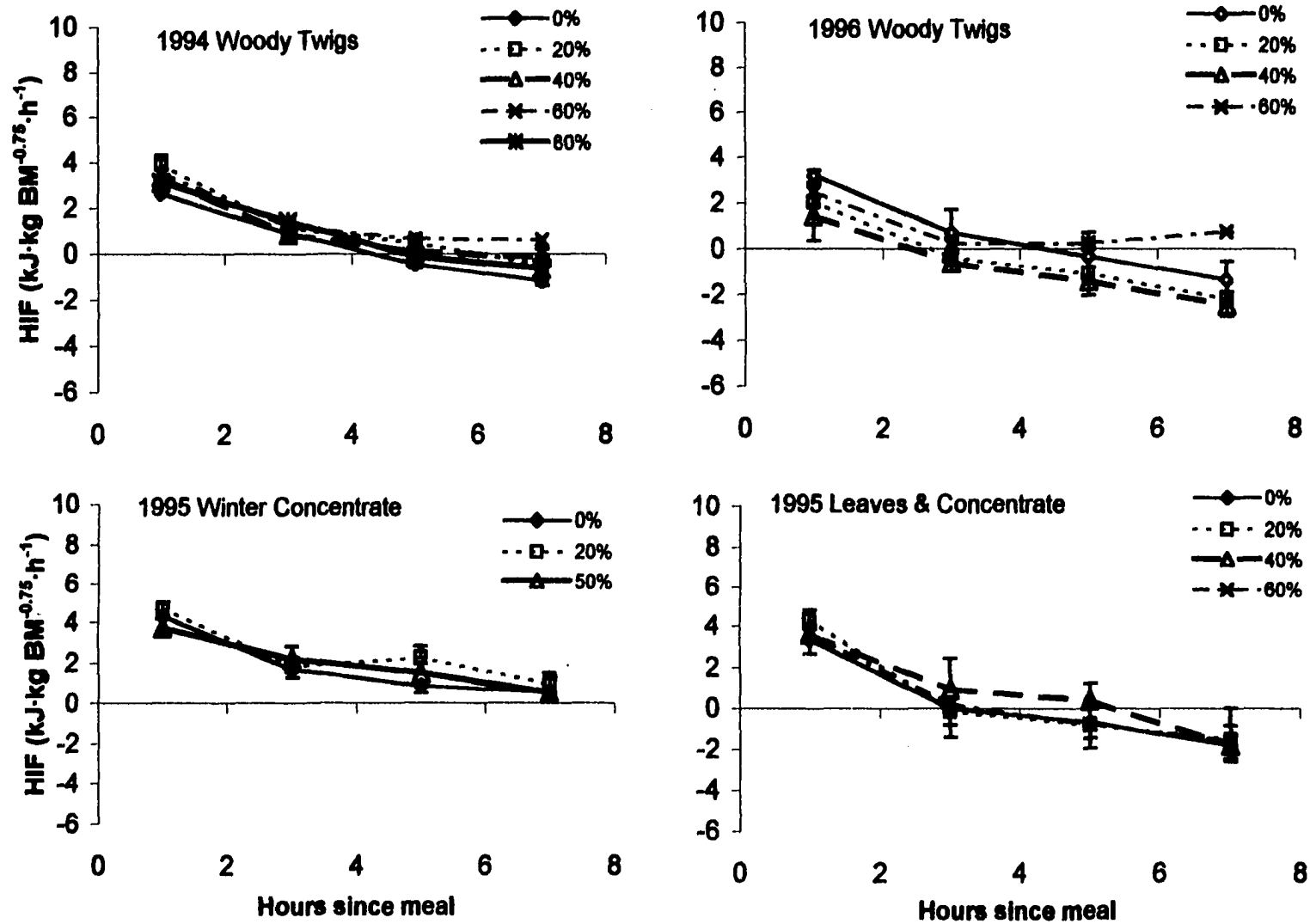
Fig. 3.3. Effects of dietary level (percent) of browse or textured concentrate supplement (Conc) on heat increment of feeding in muskoxen at the Large Animal Research Station, Fairbanks, Alaska. Values presented are net energy expenditure above a baseline value determined following 24 h without food. 0% browse is 100% hay. Mean + SE.

Differences in rate of decline in EE (parallelism hypothesis) in the 8 h following

the presentation of a meal were identified with repeated-measures ANOVA during winter

1994 and summer 1995 (Table 3.4). In winter 1994, all browse percentages contributed

to the significant time * percent of browse in the diet interaction, and post hoc tests failed

to identify a specific percent browse as responsible for that outcome. Separate analyses

of S. alaxensis twigs and S. pulchra twigs were incapable of statistically distinguishing

between energy expenditure associated with specific percent browse (Fig. 3.4). Those

diets with intermediate levels (20 and 60% of Salix) of winter woody browse however,

tended to have higher net energy expenditure in the initial 2 h block that included the

meal when compared with other levels of percent browse in the diet (Fig. 3.4). Those

diets also sustained a higher level of net EE later in the trial (Fig 3.3). During summer

1995, sample sizes of 1 or 2 for each specific browse type * percent browse level

prevented hypothesis testing of individual browse types to investigate the time * type *

percent browse interaction identified during that set of metabolic trials. Based on

differences in fiber and chemical composition of the leafy browse in comparison with

textured concentrate, we re-analyzed the summer 1995 data set without data for textured

concentrate. The time * type* percent browse interaction was again significant ($P =$

0.002). In addition, the time * type interaction approached significance ($P = 0.093$). As

in winter 1994 trials, intermediate levels (40%) of Salix leaves maintained higher levels

of net EE in later time periods than did higher and lower leaf-hay mixtures (Fig. 3.4).

Table 3.4. Summary of repeated-measures GLM results used to evaluate HIF in muskoxen at the Large Animal Research Station, University of Alaska, Fairbanks. Individual animal was included as a covariate in the winter of 1994 and 1995. Results were considered significant if $P \leq 0.05$. Because two GLMs were constructed for winter 1996 twigs, and winter 1995 textured concentrate, a $P \leq 0.025$ was considered significant for individual tests to achieve an overall significance level of $P \leq 0.05$ within that trial series.

			Wint. 1994 Twigs[a]	Summ. 1995 Leaves and Conc.[a]	Wint. 1996 Twigs[a]	Wint. 1995 Conc.[a]
Changes in HIF index over 8 h	Browse type	d.f.	1, 17	3, 15	1, 13	1, 8
		F	0.01	1.24	0.49	2.66
		P	0.932	0.330	0.497[b]	0.142[b]
		Part. eta squared	.00	.20	0.04	0.25
	Browse percent	d.f.	3, 17	2, 15	3, 11	2, 5
		F	0.15	0.17	0.80	1.10
		P	0.932	0.847	0.519[c]	0.403[c]
		Part. eta squared	0.03	.02	0.18	0.31
	Type x percent	d.f.	3, 17	5, 15		
		F	0.29	1.14		
		P	0.834	0.382		
		Part. eta squared	0.05	0.28		
Changes in rates of EE over 8 h	Time x browse type	d.f.	3, 51	9, 45	3, 39	3, 24
		F	0.83	1.05	0.85	0.74
		P	0.483	0.415	0.477[b]	0.538[b]
		Part. eta squared	0.05	0.17	0.06	0.09
	Time x browse percent	d.f.	9, 51	6, 45	9, 33	6, 15
		F	2.61	1.64	1.21	0.38
		P	0.015*	0.158	0.325[c]	0.881[c]
		Part. eta squared	0.32	0.18	0.25	0.13
	Time x type x percent	d.f.	9, 51	15, 45		
		F	0.78	2.16		
		P	0.640	0.024*		
		Part. eta squared	0.12	0.42		

* Significant result.

[a] Diets consisted of hay mixed with: 1) winter 1994: woody twigs of *S. alaxensis* and *S. pulchra*; 2) summer 1995: leaves of *S. alaxensis, S. pulchra* and *B. nana*, and textured concentrate; 3) winter 1996: woody twigs of *B. nana*; and 4) winter concentrate: textured concentrate. Percentages of browse and textured concentrate added to the hay were; winter 1994: 0, 20, 40, 60, and 80% DM; 2) summer 1995: 0, 20, 40, and 60% DM; 3) winter 1996: 0, 20, 40, and 60% DM; and 4) winter concentrate: 0, 20, and 50%.
[b] Reduced model without percent of browse in the model.
[c] Reduced model without browse type in the model.
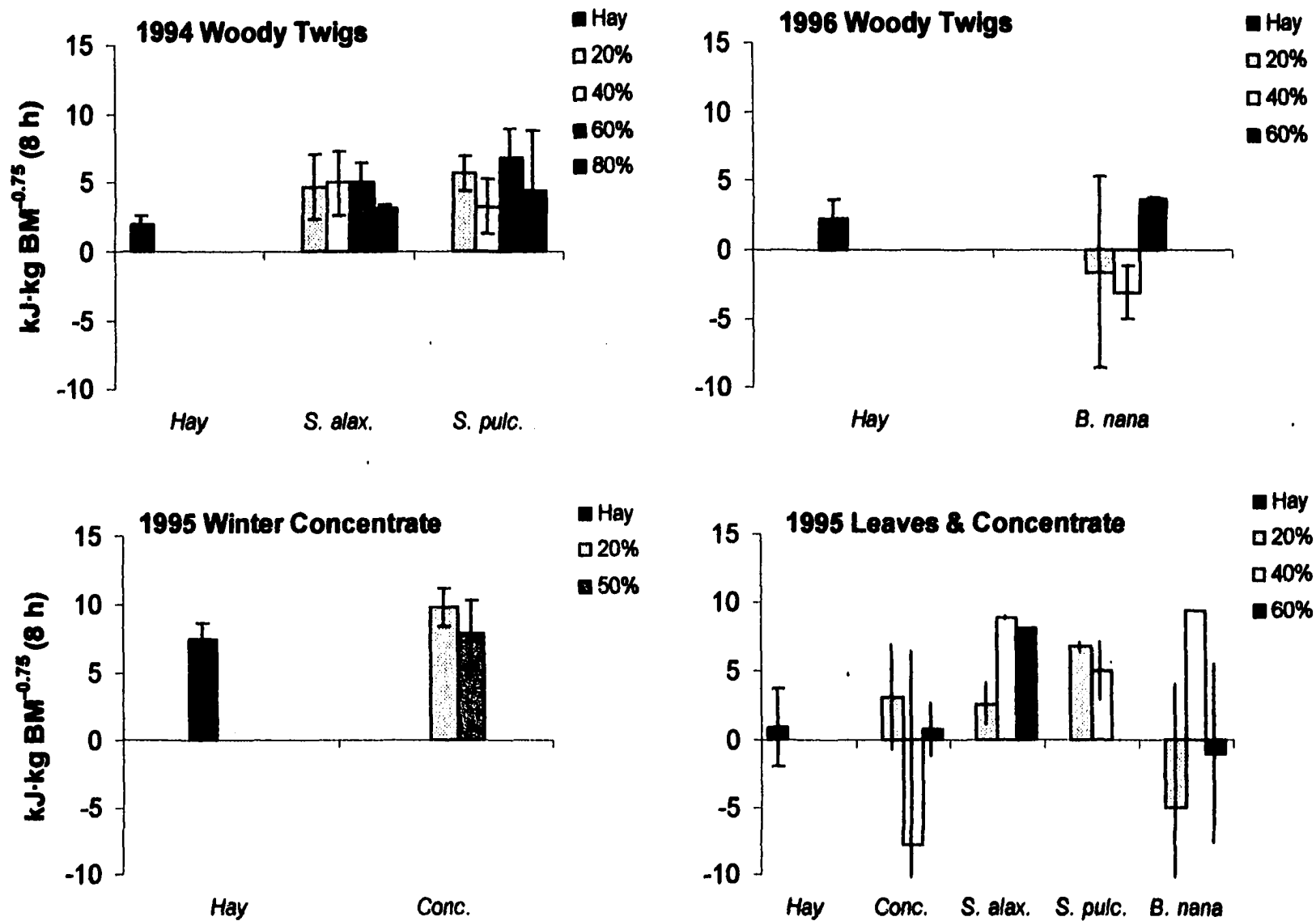
Fig. 3.4. Energy expenditure by type of browse and percent of the diet made up of browse (remainder of the diet was hay) for muskoxen at the Large Animal Research Station, Fairbanks, Alaska. Values presented are net energy expenditure above a baseline value determined following 24 h without food. Values depicted are means ± SE (error bar where $n > 3$), mean ± range (vertical bar: where $n = 2$), or the value from a single trial (no bar: where $n = 1$).

Net values for EE above baseline in the 8 h following presentation of the experimental meals were not significantly affected by the main effects of browse type consumed, browse percent, or by the browse type * percent interaction in any of the four complete data sets (Table 3.4). No significant effect of percent browse in the diet was detected when *S. alaxensis* and *S. pulchra* were analyzed separately. Results from the re-analysis of the data set from summer 1995, excluding textured concentrate, did not alter the results from the complete data set.

Relatively large amounts of variability in EE within browse types consumed and within percentage of browse consumed resulted in low statistical power to detect variation associated with diet (Table 3.5). This is particularly true in the winter of 1996 and the winter textured concentrate trials where a combination of sample size and variance made any results from hypothesis tests regarding rates of EE or net EE inconclusive.

Results from partial eta-squared for type of browse consumed and the percentage of browse consumed indicated relatively large effects on total HIF index during winter textured concentrate trials (Table 3.4). Likewise, relatively large effects on total HIF index were attributed to the percent of the diet composed of *B. nana* twigs during winter 1996. The interaction of browse type and percentage of the diet composed of browse had large effects on total HIF during summer 1995 when leaves of both *Salix* sps. and *B. nana* leaves were part of experimental diets.

Table 3.5. Observer power (1-β) of GLM-repeated measures tests used to examine HIF in muskoxen at the Large Animal Research Station, Fairbanks, Alaska. All results from the winter of 1994 and the winter concentrate values are from 3 muskoxen. Results from the summer of 1995 and winter of 1996 are from 9 muskoxen. Observed power computed using α = 0.05.

		Winter 1994 Woody Twigs[a]	Summer 1995 Leaves & Conc. [a]	Winter 1996 Woody Twigs[a]	Winter Conc. [a]
Changes in HIF index over 8 h (level)	Browse type	0.051	0.266	0.099[b]	0.301[b]
	Percent browse	0.071	0.071	0.170[c]	0.154[c]
	Browse type x browse percent	0.094	0.296		
Changes in rates of energy expenditure over 8 h (parallelism)	Time x browse type	0.217	0.451	0.217[b]	0.184[b]
	Time x percent browse	0.904	0.566	0.486[c]	0.123[c]
	Time x browse type x percent browse	0.337	0.922		

[a] Diets consisted of hay mixed with browse or textured concentrate. Browse consisted of: 1) winter 1994: woody twigs of *S. alaxensis* and *S. pulchra*; 2) summer 1995: leaves of *S. alaxensis*, *S. pulchra* and *B. nana*, and textured concentrate; 3) winter 1996: woody twigs of *B. nana*; and 4) winter concentrate: textured concentrate. Percentages of browse or textured concentrate added to hay were; 1) winter 1994: 0, 20, 40, 60, and 80% DM; 2) summer 1995: 0, 20, 40, and 60% DM; 3) winter 1996: 0, 20, 40, and 60% DM; and 4) winter concentrate: 0, 20, and 50%.
[b] Reduced model without percent of browse in model.
[c] Reduced model without browse type in model.

Likewise, results from partial eta-squared for type of browse consumed during all series of trials indicated relatively large effects on rate of decline of HIF index following the test meal (Table 3.4). During summer 1995, type of browse and the interaction between type of browse and percent of browse consumed also had large effects on the rate of decline of the HIF index.

DISCUSSION

Browse consumption and rejection

Muskoxen were fasted for 26 h and then offered meals equal to 10 g DM·kg $BM^{-0.75}$, approximately 26% of the *ad libitum* level (White et al. 1984). Even so, meals were not fully consumed when they contained a high percentage of woody browse (60 to 80%). Species of browse chosen for this experiment during the winter of 1994 (*S. alaxensis* and *S. pulchra*) are important winter forage for muskoxen in southern portions of their range (Bos 1967; Jingfors 1981; Robus 1981; O'Brien 1988). Nevertheless, our results suggest that muskoxen have an upper tolerance to preferred browse in a given meal or foraging period. Thus, to maintain meal size, it appears to us that consumption of a browse species must be balanced with consumption of non-browse plant material.

Although *B. nana* commonly occurs in some muskoxen ranges (Bos 1967; Jingfors 1981; Robus 1981; O'Brien 1988; Wilson 1992), rarely is this species reported as food for wild muskoxen and is considered non-preferred. The low preference of *B. nana* as a forage for muskoxen may be due to the resinous quality of *B. nana*. Forage

palatablity for numerous wildlife species has been shown to be negatively correlated with resin content (Bryant and Kuropat 1980). Hence, consumption of diets that contained up to 60% *B. nana* leaves and twigs is intriguing in this regard with only one trial during winter 1996 with 60% *B. nana* not fully consumed.

Consumption of diets containing large percentages of *B. nana* therefore may be a result of the sequence in which experimental meals were offered to individual animals. In winter 1994, when only 3 muskoxen were available for this study, muskoxen were exposed repeatedly to species of 2 woody browse (*S. alaxensis* and *S. pulchra*). In contrast, 9 muskoxen used in experiments during winter 1996 were exposed to browse (*B. nana*) on one occasion only. Animals during winter 1994 potentially could associate post-ingestive consequences to a particular meal and responded accordingly the next time a meal was offered.

Influence of textured concentrate and browse on energy expenditure

Baseline values for EE were established after 24 h of fasting. Activity for energetic analysis in this experiment was classified into one of two states. Animals were either bedded or active. While bedded, animals were either completely still or possibly ruminating and EE from these activities could be expected to be relatively constant. The active state however, included standing, feeding, drinking, scratching and other behaviors. Those activities can require very different EEs (Fancy and White 1985; Robbins 1993) and have the potential to add variability to our assessment of energy expenditure associated with activity. The significant browse type consumed * animal

interaction in textured concentrate trials during winter and the large amount of variability

in those trials indicates this may have been a problem and may have obscured variation in

EE associated with diet.

The change in EE that we observed with the addition of browse to a graminoid

based diet during winter 1994 and summer 1995 could be caused by 3 processes. The

most straight-forward explanation for the increase in HIF would be an increase in intake

of metabolizable energy (Blaxter 1962; Webster 1983) with the addition of browse.

Increasing dietary levels of metabolizable energy causes HIF to increase in a curvilinear

fashion such that successive increases in metabolizable energy cause successively greater

increases in HIF (Webster 1983). That outcome is a result of the greater efficiency of

utilization of fermentation products for maintenance requirements in comparison with

lipogenesis (Blaxter 1962).

A second potential explanation for the changes we observed in EE with the

addition of browse to a gramionoid based diet is a change in the products of fermentation

resulting in a change in efficiency in metabolism of the meal (Blaxter 1962; Van Soest

1982). Forages with a greater proportion of soluble carbohydrates (i.e., leafy browse) in

comparison with fiber and nondigestible components (i.e., woody browse) produce more

volatile fatty acids. Plant solubles result in a higher proportion of propionate production

relative to acetate and methane production in comparison to fiber fermentation (van

Hoven and Boomker 1985). Propionate is the only one of the major volatile fatty acids

produced in the rumen that is glucogenic (Van Soest 1982). Increased metabolism of

acetate requires gluconeogenesis from protein precursors and, therefore, constitutes a loss

in efficiency. Glucose is needed for production of NADPH for fatty acid synthesis (Blaxter 1962; Webster 1983). In addition, the carbon and hydrogen that would have gone into methane production is now channeled into propionate production and thereby increases energy available to the ruminant (van Hoven and Boomker 1985). In this study however, changes in the products of fermentation as a consequence of cell soluble / fiber ratios is not tenable. Some of the highest net EE measurements were noted with leaves of *S. alaxensis* and *S. pulchra* (Fig. 3.2) that have relatively large amounts of cell contents (Table 3.2). In addition, woody twigs of *B. nana* that are high in cellulose and hemicellulose (Table 3.2) were associated with the lowest net EE (Fig. 3.2).

A final explanation for the changes we observed in EE following a meal would be costs associated with the detoxification of plant secondary metabolites. Despite the widespread occurrence of condensed tannins in many species of browse and the role attributed to these compounds as defensive agents, little is known about the effect of consuming phenolics (or browse) on the EE of the whole animal (Iason and Murray 1996). Once absorbed from the gut, some phenolic compounds cause an uncoupling of oxidative phosphorylation leading to greater oxygen consumption (Singleton and Kratzer 1969). Elimination of absorbed tannins is accomplished by oxidation, reduction or hydrolysis followed by conjugation with an endogenous molecule creating a water-soluble, highly ionized molecule that is easily filtered by the kidneys (Foley et al. 1995). Energy is required at each of these steps, and additional energy may be lost in the conjugated metabolite that is excreted (Iason and Murray 1996). In addition, the animal may acquire an acid load from this process that must then be buffered. In this study,

higher concentrations of proanthocyanidins in winter woody twigs corresponded to greater HIF values. In contrast, the relationship of proanthocyanidin to HIF was not consistent for summer leafy browse.

When viewed in the context of the ecology of muskoxen, the results indicate muskoxen are well-adapted grazers (Adamczewski et al. 1994a; Adamczewski et al. 1994b), but also show that browse species are metabolized efficiently even though these herbivores are not anatomically well adapted to a strict diet of browse (Hofmann 1988). Willows are a known preferred browse species, whereas *B. nana* is not. Based on these observations, we would predict a diet that included moderate levels of *Salix* to be the most energetically advantageous to the animal.

We demonstrated that addition of browse to a graminoid based diet in a single small meal was capable of influencing muskoxen EE during winter 1994 and summer 1995. Changes in metabolizable energy, relative proportions of volatile fatty acids, and secondary plant metabolites all have the potential to influence EE following a meal. Relative importance of each of those factors to the energy balance of the animal likely changes with the type (leaf or twig) and species of browse, as well as the proportion of browse in the diet. This hypothesis is supported by the observation that no sequential trend in EE occurred for any of the browse species with increasing levels of dietary browse. Ruminants may be capable of offsetting minor costs associated with consuming browse containing secondary plant metabolites with improved ratios of volatile fatty acid production in the rumen or higher intakes of metabolizable energy.

Chapter 4: Effects of Browse on Methane Production in Muskoxen

Abstract: Loss of energy as methane (CH_4) was determined in muskoxen (*Ovibos moschatus*) fed graded levels of woody browse (*S. alaxensis*, *S. pulchra*, and *B. nana*) in winter, leafy browse (*S. alaxensis*, *S. pulchra*, and *B. nana*) in summer, and textured concentrate in winter and summer mixed as a ration with brome (*Bromus inermis*) hay. I evaluated the energetic importance of CH_4 production by comparing it with energy loss from an index of the heat increment of feeding. After 24 h without food, mean CH_4 production was 7.1 $ml \cdot kg^{-1} \cdot h^{-1}$ during three winters and 8.2 $ml \cdot kg^{-1} \cdot h^{-1}$ during one summer. In the 8 h following presentation of a meal (fed at 10 g per kg body mass$^{-0.75}$ [$BM^{-0.75}$]), mean CH_4 production was 11.0 $ml \cdot kg^{-1} \cdot h^{-1}$ during winters and 11.7 $ml \cdot kg^{-1} \cdot h^{-1}$ during summer. Energy lost as CH_4 in the fed animal (7.2-10.1% of energy expenditure) represented a 45% increase compared with that after 24 h without food (5.2-6.9% of energy expenditure). Methane energy represented a substantial percentage (20 –124%) of a heat increment of feeding index during both winter and summer. Methane production by muskoxen fed woody or leafy browse was lower than that predicted from the agriculture literature (2.0-3.2% of gross energy intake) suggesting an overall low CH_4 production by muskoxen. Rate of CH_4 production was significantly higher when textured concentrate was in the diet ($P = 0.021$). Net production of CH_4 in the 8 h following the

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experimental meal was significantly lowered by percentage of twigs in diets ($P = 0.012$)

during one winter but not in others. Methane production did not consistently relate to

heat increment index for browse types or textured concentrate in either the winter or the

summer. Regression analysis failed to identify a significant relationship between CH_4

production and either the heat increment index or mean RQ. Stepwise multiple

regression that included the independent variables cellulose, hemicellulose, lignin,

proanthocyanidin, crude protein, ether extract, digestibility, and gross energy content of

fed diets identified *in vitro* dry matter digestibility as the single important predictor of

CH_4 production ($r^2 = 0.16$, $P < 0.001$).

INTRODUCTION

Methane (CH_4) is a potentially significant loss of dietary energy to ruminants yet

little is known of dietary controls over CH_4 production in wild animals. Between 2 and

15% of the utilizable energy of a diet can be lost as CH_4 in the conversion of dietary

energy to metabolic energy (Blaxter and Clapperton, 1965; Leng, 1991). This

inefficiency in energy conversion is puzzling, particularly in instances where energy

conservation appears to be a critical component of the life history of the species.

Measuring production of CH_4 in wild ruminants is difficult. Rather than directly

measuring CH_4, many energetic studies or wild ruminants use a predictive equation

developed by Blaxter and Clapperton (1965) to estimate this energy loss as:

CH_4 (% of GE consumed) = 3.67 + 0.062 apparent GE digestibility,

where GE = gross energy. This equation is based on cattle and sheep, fed at maintenance, utilizing a number of mixed agricultural-based diets. Numerous experiments have indicated that estimates of CH_4 production derived from the Blaxter and Clapperton equation can overestimate actual CH_4 production by cattle by as much as 30% (Murray et al., 1975). For, moose (*Alces alces*), the only northern species for which there are published values, Schwartz et al. (1987) measured CH_4 energy loss at 2.1-4.8% of GE intake, which was considerably lower than the values predicted from the Blaxter and Clapperton equation (6.5-8.4%).

In addition to GE intake, other dietary components affect CH_4 production in ruminants. These include digested carbohydrate portions of the diet (cellulose, hemicellulose, and "soluble residue"; Moe and Tyrrell, 1979), particular lipids (Czerkawski et al., 1966; Van Nevel and Demeyer, 1996), and crude protein (Leng, 1991; Moss et al. 1994). Tannins of low molecular weight are toxic to methanogens and may be another dietary component important in influencing CH_4 production in ruminants (Field et al., 1990).

I hypothesize that in energetically demanding environments such as the arctic, wild ruminants should evolve strategies to minimize loss of CH_4. This reduction could either be accomplished by genetic or physiological controls over alimentary function or through diet selection. Hackstein *et al.* (1996a and 1996b) demonstrated that phylogeny influences the presence of methanogenic bacteria in the alimentary tract of mammals and other animals. Presence of methanogens is a shared primitive characteristic that has been lost in some taxa. Ruminants have retained that character, but under harsh

environmental conditions, there could be evolutionary pressure to minimize energy loss to methanogens. Indeed, hind-gut fermentation in rock ptarmagain (*Lagopus mutus*) produces little CH_4 in comparison to domestic ruminants (Gasaway, 1976). In domestic ruminants, CH_4 production can be altered through ration manipulation (Leng, 1991; Johnson and Ward, 1996; Van Nevel and Demeyer, 1996; for review). Fermentation of browse is thought to reduce CH_4 production (Robbins, 1993), but little evidence is available to support that hypothesis. If browse in fact lowers CH_4 production, wild ruminants could minimize CH_4 production through diet choice.

Foraging in the Arctic presents ruminants with nutritional challenges and the need for energy efficiency (White, 1983; Klein, 1986; Tyler and Blix, 1990; Klein, 1992). Muskoxen are well adapted grazers (*sensu* Hofmann, 1988) capable of subsisting on low-quality hay (White et al., 1984; Eisfeld, 1990; Adamczewski et al., 1994a; Adamczewski et al., 1994b), but also capable of existing on a browse-based diet. Willows (*Salix* spp.) in particular are heavily used in many locations (Bos, 1967; Jingfors, 1981; Wilson, 1992; Oakes *et al.* 1992; Shaefer and Messier, 1995; Forchhammer, 1995; Nelleman, 1997). I hypothesize browse consumption in wild muskoxen improves efficiency of energy utilization through a reduction in CH_4 production, provided the other major loss of energy, the heat increment of feeding, is not effected by browse consumption.

The fundamental hypothesis tested was that adding browse, either woody twigs in the winter or leafy browse in the summer, to a graminoid-based diet would alter CH_4 production. I predicted that CH_4 production would vary by species of browse consumed and by the percentage of diet composed of browse. Energy loss as CH_4 expressed as a

proportion of GE was compared with that predicted by the equation of Blaxter and

Clapperton, (1965). Finally, I investigated the potential to predict CH_4 production in

muskox from dietary fiber, crude protein, GE, *in vitro* dry matter digestibility (IVDMD),

ether extract, and proanthocyanidin concentrations.

MATERIALS AND METHODS

Approach

Muskox were placed in a stall and fed a standardized meal. They were then

fasted for 24 h before being placed into a respiration chamber. Outdoor air was drawn

through the open-circuit respiration chamber. Rate of air flow (l/min. at standard

temperature and pressure) was monitored continuously with a mass flowmeter. A sample

of the air stream was drawn through calcium sulphate (Drierite) and analyzed for oxygen

(O_2), carbon dioxide (CO_2) and CH_4 concentrations over 10-h (Fig. 4.1). A data

acquisition system linked to a computer recorded flow rate, humidity, temperature,

barometric pressure, and gas concentrations in the airstream leaving the metabolic

chamber in 2-min intervals. During the first 0.5 h in the respiration chamber, muskox

settled into their new environment, and gases within the chamber were allowed to

equilibrate (roughly 2.5 turnovers of the volume of the chamber). Data collection

commenced following that equilibration period. During the first 2 h of the metabolic

trial, muskoxen were offered no food. At 2 h, an experimental meal was offered, at 10

$g\cdot kg\,BM^{-0.75}$, and gaseous exchange was monitored for 8 h (Chapters 2 and 3). Following
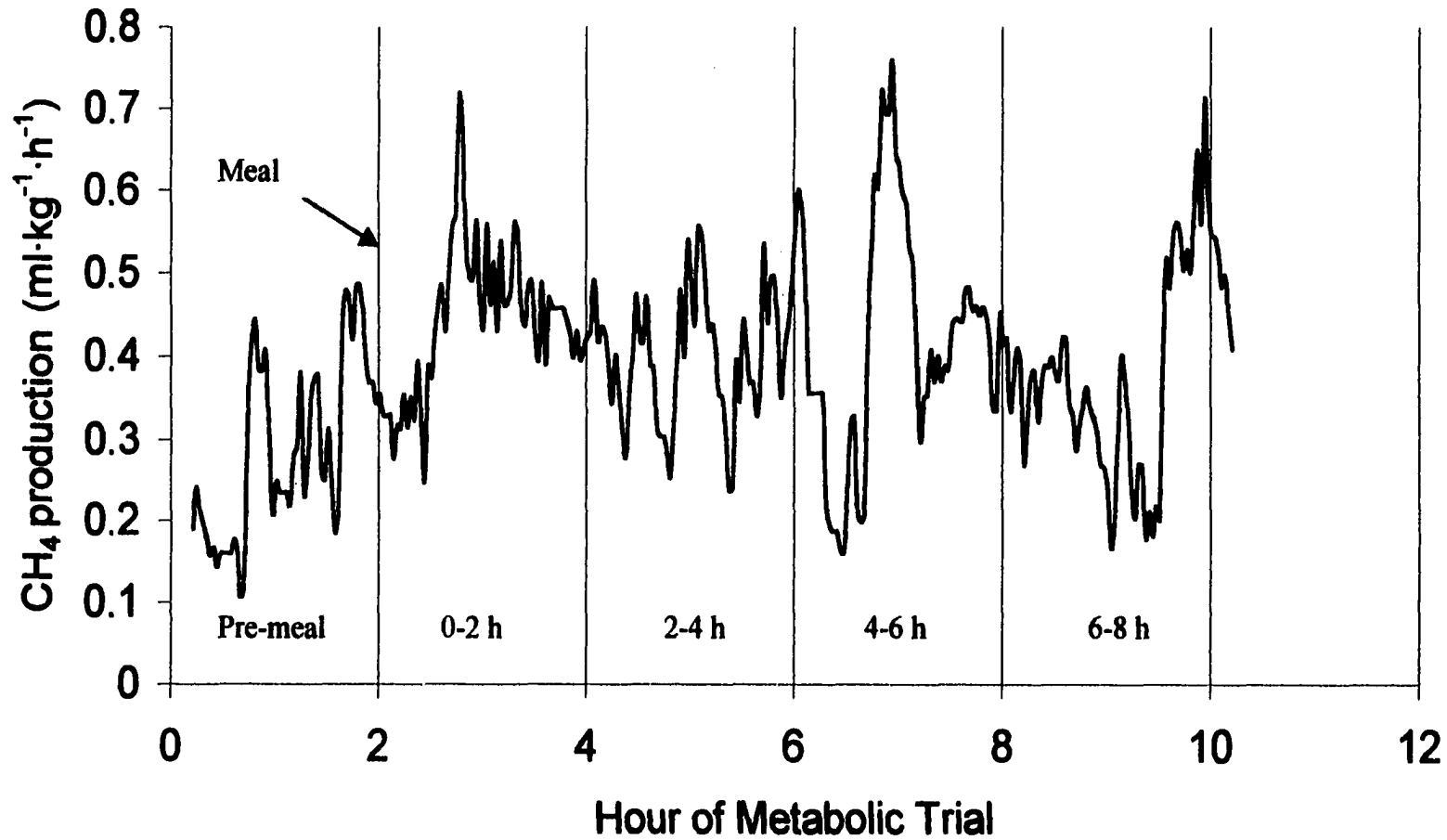
Fig. 4.1. An example of methane production measured with indirect calorimetry. This particular trial was conducted on March 6, 1994 at the Large Animal Research Station, Fairbanks, Alaska. Total methane produced over the entire 10 h trial is depicted. The muskoxen was fed a meal 2 h into the trial. For analysis, methane produced in 2 h intervals was converted to an hourly rate.

a metabolic trial, 3 d passed before the animal was again brought in from pasture and the process repeated.

Study Animals

Muskoxen were maintained and all metabolic experiments were conducted at the Large Animal Research Station (LARS), Institute of Arctic Biology, University of Alaska, Fairbanks (64°50'N, 147°43'W). Three 3-year-old muskoxen were used during the winter of 1994 and again in the winter of 1995 as 4-year-olds. Nine muskoxen, seven 2-year-olds and two 3-year-olds, were used for metabolic experiments in summer 1995 and again in January 1996 (Table 4.1). All experimental animals were sexually intact and none were pregnant or lactating. All animals used in this study were habituated to confinement in the metabolic chamber. Only trials in which animals remained calm throughout were used during analysis. When muskoxen were not being withheld from food or in the respiration chamber, they had ready access to feeders with smooth brome hay (*Bromus inermis*), and pasture dominated primarily by smooth brome. Protocols for this experiment and care of these animals were approved by the University of Alaska Fairbanks, Institutional Animal Use and Care Committee (IACUC # 93-01), and protocols were in keeping with methods approved by the American Society of Mammalogists (1998) for research on captive mammals.

Table 4.1. Experimental browses, experimental mixtures, and number of muskoxen used in a series of 4 metabolic trials at the Large Animal Research Station, University of Alaska Fairbanks, Fairbanks, Alaska, USA.

Season	No. and sex of Muskoxen Used	Experimental Browse	% Browse Mixed[a] With Hay
Winter 1994	2 Males / 1 Female	S. alaxensis (twigs) S. pulchra (twigs)	0, 20, 40, 60, 80 [b]
Winter 1995	2 Males / 1 Female	Textured Concentrate	0, 20, 50 [b]
Summer 1995	6 Males / 3 Females	Textured Concentrate S. alaxensis (leaves) S. pulchra (leaves) B. nana (leaves)	0, 20, 40, 60 [b]
Winter 1996	6 Males / 3 Females	B. nana (twigs)	0, 20, 40, 60 [b]

[a] Diets offered on a DM basis.
[b] Remaining DM percentage is hay.

Diets and Feeding Levels

During winter 1994, experimental diets consisted of hand-clipped twigs of *Salix alaxensis* or *S. pulchra* passed through a chipper and mixed by hand with brome hay passed through the chipper. Individual browse species were fed during the metabolic trials at 0, 20, 40, 60, and 80% dry matter (DM) of the meal with the remainder composed of hay (Table 4.1). During winter1996, I followed the same procedure using *Betula nana* twigs (plant nomenclature follows Hultén, 1990), but in this instance, the 80% twig concentration was eliminated from the trial. All twigs were collected in the same vicinity and were fed during the season in which they were gathered. Twigs were stored outdoors where temperatures never exceeded freezing. Twigs were current annual growth collected from the top of mature growth-form shrubs.

During summer 1995, twigs of *S. alaxensis*, *S. pulchra*, and *B. nana* with the leaves attached were gathered 1-6 h before the start of a metabolic experiment, and leaves were hand stripped immediately before the animal was sealed in the chamber. Freshly stripped leaves were hand mixed with chipped hay and fed at 20, 40 and 60% DM with the remainder being chipped hay (Table 4.1). Experiments were restricted to mid-summer (16 July – 13 August) to minimize phenological changes in browse quality. Leaves were stripped from current annual growth collected from the top of mature shrubs.

Textured concentrate was included in trials to facilitate comparison with data derived from cattle and sheep (Blaxter and Clapperton, 1965; Moe and Tyrrell, 1979; Leng, 1991; Moss et al., 1994; Johnson and Ward, 1996), and because leafy browse has

been considered a concentrate feed (Hofmann, 1988). Textured concentrate was used during metabolic experiments in winter 1995, and in conjunction with the browse experiments in summer 1995 (Table 4.1). Textured concentrate was mixed with chipped hay and fed at 0, 20, 40, 50 and 60% DM with the remaining percentage being the chipped hay.

Feed Analytical Methods

The DM content of leaves, twigs, hay and textured concentrate used during all metabolic trials were determined by drying the material at 60°C for 48 h. Neutral detergent fiber (NDF), acid detergent fiber (ADF), and acid detergent lignin (ADL) were determined sequentially and hemicellulose and cellulose determined by subtraction (Goering and Van Soest, 1970). Nitrogen content of all feed types was analyzed by combustion in a LECO elemental analyzer. Crude protein was calculated as nitrogen content of the diet times 6.25. Gross energy was determined by combustion with a Parr adiabatic bomb calorimeter. Proanthocyanidin concentrations were determined with the proanthocyanidin assay described by Martin and Martin (1982). Tannins from each browse species (Clausen et al., 1990) in each season (i.e., woody twigs in winter and leaves in summer) were purified and used as standards for evaluating proanthocyanidin concentrations in browse. Crude fat content was estimated by ether extract in a soxhlet apparatus. Mean values for DM, fiber, crude protein, GE, proanthocyanidin content and crude fat for each forage type were used to calculate values for dietary mixes.

Dry-matter disappearance of all feeds used in metabolic trials was determined

with the *in vitro* dry matter digestibility (IVDMD) method of Tilly and Terry (1963).

The source animal for rumen liquor was a fistulated muskoxen kept in the same pastures

as animals used during metabolic experiments. This fistulated muskoxen had free access

to brome hay and was fed a mixture containing chipped twigs and leaves of all browse

species used during metabolic trials mixed with textured concentrate. This mixture was

fed daily for 2 weeks before collection of rumen fluid. All feed was removed from the

animal 12 h before collection of rumen liquor. Values for IVDMD were determined for

experimental mixtures as well as pure samples of each forage type. Digestibility values

reported are means from three replicates.

Metabolic Experiments

Three young muskoxen were evaluated on 9 experimental diets from 9 January to

15 April, 1994 ($n = 54$; Table 4.1). Diets were offered in random order, and each diet

was offered to each muskox on 2 occasions. Nine animals were used for metabolic

experiments from 16 July to 13 August, 1995 ($n = 28$; Table 4.1). Ten experimental diets

were offered during that period, and each animal was evaluated on 100% hay, and from 1

to 3 of the experimental diet mixes. Mixed diets were randomly assigned to animals. A

minimum of 3 d was allowed between metabolic experiments for individual animals.

From 5 January to 21 January 1996, metabolic trials ($n = 17$) were conducted with the

same animals used during summer 1995 (Table 4.1). Each animal was evaluated on

100% hay and 1 of 3 dietary mixes.

Textured concentrate was used during metabolic experiments in February and March 1995, and concurrent with experimental diets in summer 1995 (Table 4.1). Three animals that were used during metabolic trials in winter 1994 were evaluated on 100% hay and two diets with textured concentrate from 15 February to 7 March 1995 ($n = 15$). Nine animals involved in the metabolic experiments during summer 1995 were evaluated on 100% hay and 3 textured concentrate mixes ($n = 14$).

Energy Calculations

Oxygen consumption and CO_2 production were used to calculate energy expenditure (Chapter 2 and 3) and respiratory quotient (RQ) of the animal (Brouwer, 1965). Energy lost as CH_4 was determined by multiplying the volume of CH_4 produced by its thermal equivalent (39.45 $kJ \cdot L^{-1}$ CH_4; Brouwer, 1965). Mean CH_4 production and energy expenditure in the 2-h before presentation of the experimental meal were considered baselines for estimating net losses of CH_4 energy and as a heat increment index (Chapter 2) following feeding (i.e., meal-derived heat increment = post-meal energy expenditure minus baseline energy expenditure, and meal-derived CH_4 loss = post-meal CH_4 production minus baseline CH_4 production). All calculations were made in 2-min increments. For statistical analysis, CH_4 production was estimated in 2-h blocks by summing net production during those periods. Net CH_4 and heat increment index were estimated for the entire 8 h following the meal in the same manner.

Statistical Analysis

Statistical tests were performed with SPSS for windows (Version 7.0.2, SPSS Inc., Chicago Illinois). Repeated-measures, general-linear models (GLM) were used to examine variability in CH_4 production during each series of metabolic trials. The response curve of CH_4 production following experimental meals was examined as a rate process by dividing the entire 8-h following presentation of a meal into four 2-h blocks [within-subject variable (Fig. 4.1)] and testing for differences among time blocks. Between-subject variables used in analysis of woody browse in winter 1994 were individual animal, species of browse (type), and percentage of the experimental diet composed of browse. Between-subject variables used in analysis of leafy browse in summer 1995 were type and percent of browse. Because of small sample sizes, two repeated-measures, general-linear models were used for analysis of trials for summer textured concentrate, winter textured concentrate, and woody browse in winter 1996. In the first model, I used the between-subject variable, type of browse. In the second model, I used the between-subject variable, percent of browse. In those instances where more than one GLM model was used for analysis, the α level was adjusted accordingly (i.e., α corrected = desired α/number of GLM models used for analysis).

Profile analyses (von Ende 1993), using both univariate (ANOVA) and multivariate (MANOVA) tests, were used to examine hypotheses regarding differences in the pattern of CH_4 production following the meal or "parallelism" (slope), and to test hypotheses regarding net differences in CH_4 production following presentation of the

meal or "levels" (intercept) of the response curves. The Mauchly test for sphericity was used to examine the variance-covariance matrices for the within-subject factor. In instances where the sphericity assumption was not met, degrees of freedom for the F statistic were adjusted according to the severity of this violation with a Greenhouse-Geisser estimator (von Ende, 1993). Differences in within-subject and between-subject factors were examined by transforming factor-level means into sets of contrasts. Transformed variables were analyzed with ANOVA to determine which factor levels were responsible for the variation (von Ende, 1993).

Low statistical power is a common problem when using repeated-measures designs (von Ende, 1993). As a consequence, some authors have suggested using a larger α level in situations where low power is suspected (Stevens, 1992). Because of a small numbers of trials for each browse species at each percentage level of the diet, there was an increased potential for problems with power in those metabolic experiments. Therefore, results for repeated-measures GLM were considered significantly different if $P \leq 0.10$, to avoid making a β error. In addition, I examined partial-eta squared results to evaluate the strength of association with CH_4 production of browse type, percent of browse, and the interaction of browse type and percent in the diet. Partial eta-squared can be defined as; SSH / (SSH + SSE), where SSH and SSE are the sums of squares for the hypothesis and error terms, respectively. Partial-eta squared is the proportion of the total variation in the dependent variable that is accounted for by the variation in the independent variable (SPSS, 1991). Partial-eta squared is similar to partial coefficients

of determination from standard multiple regression analysis in that it is useful in evaluating effect size (SPSS, 1991).

I used multiple regression (Zar, 1996) to investigate CH_4 loss as a function of the heat increment index from the meal and mean RQ in the 8-h following presentation of the meal. Individual series of metabolic trials were analyzed separately. Individual animal was included in the model during winter 1994 and during concentrate trials during winter1995, by using indicator variables. Values for CH_4 and heat-increment index that entered in regression models were expressed on a body-mass basis (BM^{-1}). I examined variance-inflation factors (VIF) to assess multicollinearity. Variance-inflation factors > 10 were considered problematic (Chatterjee and Price, 1991). I examined the standardized partial-regression coefficients to determine which of the independent variables had greater influence on CH_4 loss.

To investigate CH_4 loss as a function of chemical composition of forages fed during metabolic experiments, I used multiple regression and stepwise multiple regression (Zar, 1996). The initial regression model was composed of dietary components traditionally used to predict CH_4 production (Blaxter and Clapperton, 1965; Moe and Terrell, 1979): intake of cellulose, hemicellulose, lignin, proanthocyanidin, crude protein, IVDMD, GE, and ether extract. Because of potential multicollinearity, a second model was developed that used NDF in lieu of cellulose, hemicellulose and lignin, but included proanthocyanidin, crude protein, IVDMD, GE, and ether extract. Both sets of independent variables were then modeled with stepwise multiple regression to develop a "best" model. Trial series (i.e., winter 1994, summer 1995, etc.) were coded

as indicator variables and included in all regression models as independent variables. For entry of independent variables into the stepwise multiple regression, I used a P-to-enter of ≤ 0.05, and a P-to-remove of ≥ 0.10. The correlation matrix was examined to determine which of the independent variables was most highly correlated to CH_4 loss and to identify potential problems with multicollinearity. I also examined variance inflation factors to control for multicollinearity. I compared models with adjusted multiple coefficients of determination. I examined the standardized partial regression coefficients to determine which of the independent variables had the greatest influence on CH_4 loss.

Results

Diet Analysis

Neutral detergent fiber content for hay and twigs were 65.9% dry matter (DM) and 59.8% DM, respectively. Twigs contained a higher proportion of ADF (45.6% DM) than did hay (35.9% DM). Textured concentrate and leaves both contained relatively low amounts of fiber, with NDF values of 22.1% DM and 31.5% DM, respectively, and ADF values of 6.5% DM and 21.6% DM, respectively (Table 4.2).

Fiber in hay was largely composed of cellulose (32.5% DM) and hemicellulose (29.5% DM) with small amounts of lignin (3.6% DM). Twig-fiber also was largely cellulose (24.4% DM) but contained more lignin (21.7% DM) and, correspondingly, less hemicelluolose (13.5% DM). Leaf-fiber had comparable proportions of cellulose (10.1% DM), hemicellulose (9.9% DM), and lignin (10.6% DM). Textured concentrate

Table 4.2. Chemical composition and gross energy of twigs, leaves, hay and textured concentrate fed to muskoxen during the course of metabolic experiments at the Large Animal Research Station, University of Alaska Fairbanks, Fairbanks, Alaska, USA. Metabolic trials using these feeds were conducted from 9 January to 15 April 1994 (twigs of *Salix alaxensis* and *S. pulchra*), 16 July to 13 August 1995 (leaves of *S. alaxensis*, *S. pulchra* and *Betula nana*, and textured concentrate), 15 February to 7 March 1995 (textured concentrate), and 5 to 21 January 1996 (twigs of *B. nana*).

Component	Twigs						Leaves					
	S. alaxensis	SE	S. pulchra	SE	B. nana	SE	S. alaxensis	SE	S. pulchra	SE	B. nana	SE
Dry matter	53.8	0.86	52.5	0.87	56	2	39	3.34	42.8	2.06	41.5	4.29
Organic matter	96.1	0.18	97.2	0.15	98.5	0.01	88.8	0.90	96.2	0.08	96.7	0.09
Neutral-detergent fiber	53.5	1.28	59.2	1.22	66.6	0.62	30.2	0.56	26.1	0.06	38.3	1.25
Acid-detergent fiber	41.3	1.15	46.6	0.96	46.9	0.16	20.5	0.54	17.3	0.14	27.1	0.96
Hemicellulose	12.1	0.37	12.6	0.51	15.8	0.37	9.6	0.20	8.8	0.15	11.2	0.33
Cellulose	25.9	0.93	25.8	0.73	21.6	0.33	12.5	0.43	8.6	0.08	9.2	0.49
Acid detergent lignin	15.7	0.43	20.7	0.77	28.6	0.18	5.5	0.21	8.7	0.15	17.6	0.56
Proanthocyanidin	15.0	0.87	14.5	1.29	6.1	0.46	9.2	0.71	11.7	1.94	8.4	2.26
Crude Protein	5.8	0.28	6.2	0.22	7.2	0.15	10.5	0.64	13.6	0.31	9.9	0.06
Gross energy (kJ/g)	20.1	0.03	20.5	0.04	23.0	0.01	17.9	0.01	19.6	0.04	21.2	0.02
Ether extract	2.8	0.30	1.9	0.21	2.0	0.07	2.8	0.21	1.3	0.32	2.9	0.32
Cell Content	46.5	1.08	40.8	1.10	33.4	0.62	69.8	0.45	73.9	0.20	61.7	0.98

Component	Hay						Textured Concentrate					
	Winter 94	SE	Summer 95	SE	Winter 96	SE	Winter 94	SE	Summer 95	SE	Winter 96	SE
Dry matter	88.7	0.33	81	3.00	89.3	0.33	89.3	0.33	88.5	1.50	89	-
Organic matter	93.8	0.54	94.8	0.03	95.1	-	93.0	0.34	97.7	0.37	91.6	0.12
Neutral-detergent fiber	58.7	0.41	67.5	0.08	71.9	0.18	22.8	0.34	20.0	0.45	23.5	0.19
Acid-detergent fiber	32.2	0.31	36.6	0.01	39.0	0.35	6.8	0.21	6.2	0.12	6.4	0.12
Hemicellulose	26.5	0.28	30.8	0.08	31.1	0.12	16.0	0.39	13.8	0.57	19.1	5.52
Cellulose	29.1	0.28	33.2	0.01	35.1	0.09	5.9	0.08	5.3	0.06	5.9	0.07
Acid detergent lignin	2.8	0.10	3.3	0.02	4.5	0.16	0.70	0.11	0.9	0.01	1.4	0.11
Proanthocyanidin	ND		ND		ND		ND		ND		ND	
Crude Protein	11.8	1.90	10.2	-	11.2	0.10	18.0	0.38	19.6	-	20.1	0.10
Gross energy (kJ/g)	17.8	0.02	18.1	0.02	18.5	0.06	17.6	0.02	18.0	0.02	18.0	0.03
Ether extract	1.5	0.57	1.0	0.36	ND		3.0	0.40	5.2	0.52	ND	
Cell Content	41.3	0.38	32.5	1.41	28.1	0.18	77.2	0.34	80.0	0.48	76.5	5.02

ND, not determined.
All values (except dry matter and gross energy) are expressed as a percentage of dry mass.
NDFs and ADFs were run sequentially.

contained more hemicellulose (16.3% DM) than cellulose (5.7% DM) and negligible lignin (1.0 ± 0.13% DM) (Table 4.2).

Crude protein content was highest in the concentrate (19.4% DM), followed by leaves (11.3% DM) and hay (11.3% DM), and twigs (6.3% DM). Gross energy was highest in woody (21.2 kJ·g⁻¹) and leafy (19.6 kJ·g⁻¹) browse but energy content varied considerably among species. Gross energy content of hay (18.1 kJ·g⁻¹) was similar to that of the concentrate (17.9 kJ·g⁻¹). Proanthocyanidin content of woody twigs in winter and leafy browse in summer also varied considerably among species, but woody twigs tended to have a higher levels (11.9% DM) than did leaves (9.8% DM) (Table 4.2).

IVDMD increased as textured concentrate increased in the diet (Fig. 4.2). In contrast, increasing levels of twigs in the diet steadily reduced IVDMD in diets with the woody browse species. Increasing the levels of leaves in the diet also reduced IVDMD (Fig. 4.2). The IVDMD of leaves from *S. alaxensis* and *B. nana* was greater than for twigs of the same species. The reverse was noted for *S. pulchra* for which twigs were more digestible than leaves at the same percentage of diet.

Methane Production

After 24 h without food, mean pre-meal rates of CH_4 production for muskoxen were 6.9 ml·kg⁻¹·h⁻¹ (SE = 0.27 ml·kg⁻¹·h⁻¹, n = 55), 6.1 ml·kg⁻¹·h⁻¹ (SE = 0.58 ml·kg⁻¹·h⁻¹, n = 15), and 8.2 ml·kg⁻¹·h⁻¹ (SE = 0.42 ml·kg⁻¹·h⁻¹, n = 17) during winter 1994, 1995 and 1996, respectively. The mean pre-meal rate of CH_4 production for summer 1995 was 8.2
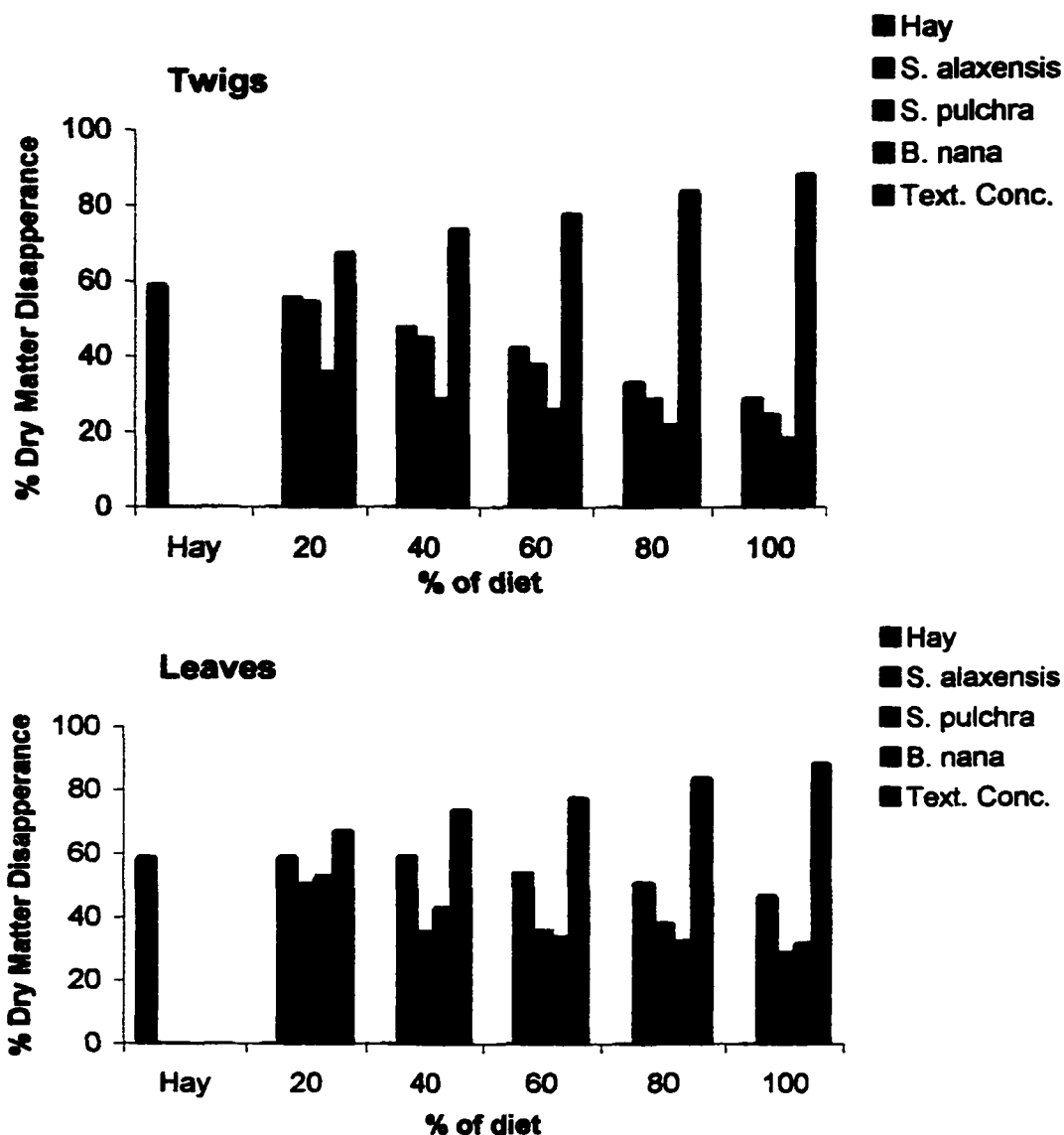
Fig. 4.2. *In vitro* dry matter disappearance of winter woody twigs and summer leafy browse of *S. alaxensis*, *S. pulchra*, and *B. nana* mixed with hay (*Bromus inermis*), 17 April, 1996. This figure indicates the level of browse included in the diet (i.e., 0% indicates a diet composed of 100% hay and 0% browse). Dry matter disappearance of a textured concentrate is included on each graph as a standard. The source animal for the rumen liquor was a fistulated muskoxen. This muskoxen was maintained at the Large Animal Research Station, University of Alaska Fairbanks, Fairbanks, Alaska, USA.

$ml \cdot kg^{-1} \cdot h^{-1}$ (SE = 0.48 $ml \cdot kg^{-1} \cdot h^{-1}$, n = 29). In the 8 h following the presentation of

experimental meals, mean rates of CH_4 production were 10.5 $ml \cdot kg^{-1} \cdot h^{-1}$ (SE = 0.32

$ml \cdot kg^{-1} \cdot h^{-1}$, n = 43), 10.7 $ml \cdot kg^{-1} \cdot h^{-1}$ (SE = 0.58 $ml \cdot kg^{-1} \cdot h^{-1}$, n = 15), and 11.9 $ml \cdot kg^{-1} \cdot h^{-1}$

(SE = 0.44 $ml \cdot kg^{-1} \cdot h^{-1}$, n = 16) during winter 1994, 1995 and 1996, respectively, and 11.7

$ml \cdot kg^{-1} \cdot h^{-1}$ (SE = 0.41 $ml \cdot kg^{-1} \cdot h^{-1}$, n = 27) during summer 1995.

Following presentation of the experimental meal, energy loss as CH_4, expressed as

a percentage of energy expenditure of the animal, increased by about 45% (Table 4.3).

The increase in CH_4 production was a substantial portion of energy loss following

experimental meals compared to the heat increment index of the meal (Table 4.3).

Relative to GE intake, the increase in CH_4 production was only approximately one third

(28-42%) of predictions using the Blaxter and Clapperton (1965) equation (Table 4.3).

Rate of CH_4 production during metabolic trials peaked in the time interval that

included the meal, and declined toward baseline thereafter in all instances (Table 4.4;

Fig. 4.3 and 4.4). Adding textured concentrate to hay diets had a significant effect on

rate of CH_4 production (parrallelism hypothesis) during summer (Table 4.5). Post-hoc

contrasts indicated a significant difference in rate of CH_4 production between the 0-2 h

interval and the 2-4 h interval. Effect of different percentages of textured concentrate,

and addition of *B. nana* to experimental diets also approached significance (Table 4.5).

During winter 1994, altering percentage of woody twigs in experimental diets

resulted in significantly different net production of CH_4 (Table 4.5). Post-hoc contrasts

were not capable of discriminating between different percentage levels. In trials that

Table 4.3. Seasonal methane energy loss from muskoxen at the Large Animal Research Station, University of Alaska Fairbanks, Fairbanks, Alaska, USA. Methane energy loss is expressed as a percentage of the energy expenditure during trials, heat increment during trials, as a percentage of the gross energy intake of experimental meals and, as predicted by the Blaxter and Clapperton equation (1965).

Methane Energy loss		Winter 1994	Winter 1995	Winter 1996	Summer 1995*
% of Heat Increment					
Premeal:	Mean	6.4	6.3	6.9	- 5.2
CH_4/EE^a	SE	0.21	0.43	0.26	0.31
(Total)	n	55	15	17	29
Post-meal (0-8 h):	Mean	9.2	9.8	10.1	7.2
CH_4/EE^a	SE	0.23	0.34	0.26	0.22
(Total)	n	43	15	16	27
Meal:	Mean	124.0	39.5	80.0	20.2
meal CH_4/meal HII^b	SE	48.26	4.05	33.0	11.33
	n	43	15	16	27
Meal effect:					
Post-meal/premeal		44	56	46	38
% of GE intake					
% GE intake:	Mean	2.2	3.2	2.1	2.0
meal CH_4/meal GE	SE	0.13	0.21	0.19	0.18
	n	43	15	16	27
Predicted^c % of GE intake:	Mean	6.6	7.7	6.4	7.2
predicted CH_4/meal GE	SE	0.08	0.11	0.22	0.14
	n	43	15	16	27
Observed/predicted		33	42	33	28

*Values from the summer 1995 include trials with leaves as part of the dietary mix, and trials with textured ration as part of the dietary mix.
[a] EE = total energy expenditure.
[b] HII = heat increment index. The energy attributed to the meal.
[c] Blaxter and Clapperton, 1965.

Table 4.4. Mean and standard error (SE) methane production by muskoxen at the Large Animal Research Station, University of Alaska Fairbanks, Fairbanks, Alaska, USA. Methane production in each season is summarized in 2 h intervals. Methane production in 8h intervals is summarized by type of browse included in the meal, and the percent of the diet made up of browse. Mean methane production is expressed as $ml \cdot kg^{-1} \cdot h^{-1}$.

	Winter 94[a]		Summer 95[b]		Winter 96[c]		Winter QTX[d]		Summer QTX[e]	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Time Since meal										
0 - 2 h	5.3	0.27	5.1	0.45	4.8	0.35	7.2	0.35	7.4	0.48
2 - 4 h	3.6	0.23	2.7	0.38	4.2	0.37	4.2	0.30	3.9	0.45
4 - 6 h	2.8	0.28	2.2	0.69	3.0	0.45	4.3	0.58	3.4	0.89
6 - 8 h	2.2	0.30	2.4	0.68	2.4	0.39	3.9	0.32	2.2	0.76
Diet Mix*										
Hay	4.1	0.53	3.3	0.66	3.6	0.43	4.1	0.38	3.3	0.60
Text. concentrate							5.0	0.31	4.5	0.70
S. alaxensis twigs	3.4	0.32								
S. pulchra twigs	3.4	0.30								
B. nana twigs					3.8	0.43				
S. alaxensis lvs.			3.3	0.83						
S. pulchra lvs.			2.4	0.88						
B. nana lvs.			3.2	0.81						
Percent Browse*										
0[f]	4.1	0.53	3.3	0.66	3.7	0.41	4.2	0.39	3.3	0.60
20	4.6	0.36	3.0	0.85	4.3	0.61	4.5	0.52	4.3	1.20
40	3.3	0.36	3.2	0.84	3.8	0.60			5.5	1.20
50							6.0	0.84		
60	2.8	0.45	3.0	1.07	2.6	0.83			3.8	1.20
80	2.5	0.62								

[a] *n*=43. Experimental diets were mixes of hay and winter twigs of *S. alaxensis* and *S. pulchra*. Twigs were added to hay as 0, 20, 40, 60, and 80% DM of the diet.

[b] *n*=23. Experimental diets were mixes of hay and summer leaves of *S. alaxensis*, *S. pulchra*, and *B. nana*. Leaves were added to hay as 0, 20, 40, and 60% DM of the diet.

[c] *n*=16. Experimental diets were mixes of hay and winter twigs of *B. nana*. Twigs were added to hay as 0, 20, 40, and 60% DM of the diet.

[d] *n*=15. Experimental diets were mixes of hay and a corn based concentrate. Textured concentrate was added to hay as 0, 20 and 50% DM of the diet.

[e] *n*= 14. Experimental diets were mixes of hay and textured concentrate. Textured concentrate was added to hay as 0, 20, 40 and 60% DM of the diet.

[f] 0% browse is equivalent to 100% hay.

* Mean methane production 8 h after the experimental meal.

10   1994 Woody Twigs   ◆—Hay
                              ··◻··S. alex.
8   —▲— S. pulc.

$ml \cdot kg^{-1} \cdot h^{-1}$

6

4

2

0
0   2   4   6   8

10   1996 Woody Twigs   ◆—Hay
                              —✕—B. nana
8

6

4

2

0
0   2   4   6   .8

10   1995 Winter Concentrate   ◆—Hay
                              —●— Conc.
8

$ml \cdot kg^{-1} \cdot h^{-1}$

6

4

2

0
0   2   4   6   8
         Hours since meal

10   1995 Leaves & Concentrate ◆—Hay
                              ··◻··S. alex.
8   —▲— S. pulc.
                              —✕—B. nana
6   —●—Conc.

4

2

0
0   2   4   6   8
         Hours since meal
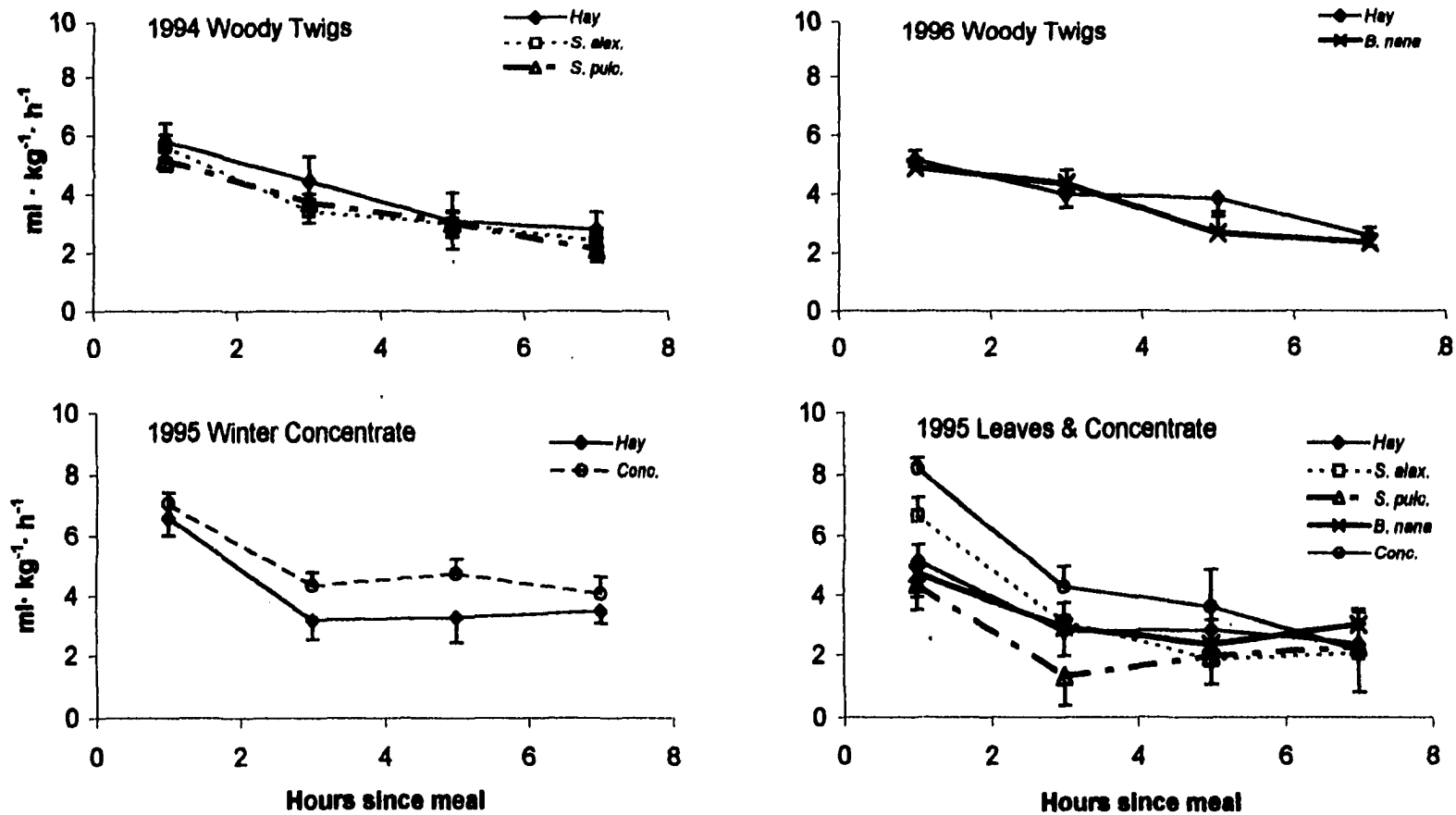
Fig. 4.3. Effects of type of browse (S. alaxensis, S. pulchra, and B. nana) and textured concentrate supplement (Conc.) on methane production over time in muskoxen at the Large Animal Research Station, University of Alaska Fairbanks, Fairbanks, Alaska. Values (mean ± SE) presented are net methane above a baseline determined following 24-26 h without food.

1994 Woody Twigs

ml·kg⁻¹·h⁻¹ → $ml \cdot kg^{-1} \cdot h^{-1}$

- ◆ 0%
- □ 20%
- △ 40%
- ✕ 60%
- ✶ 80%

1996 Woody Twigs

- ◆ 0%
- □ 20%
- △ 40%
- ✕ 60%

1995 Winter Concentrate

- ◆ 0%
- □ 20%
- △ 50%

1995 Leaves & Concentrate

- ◆ 0%
- □ 20%
- △ 40%
- ✕ 60%

Hours since meal

Hours since meal

Fig. 4.4. Effects of dietary level (percent) of browse or textured concentrate (Conc.) on methane production over time in muskoxen at the Large Animal Research Station, University of Alaska Fairbanks, Fairbanks, Alaska. Values (mean $\pm$ SE) presented are net methane production above a basline value determined following 24 h without food. The 0% browse level is 100% hay.

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Table 4.5. Summary of repeated-measures GLM results evaluating methane production in muskoxen at the Large Animal Research Station, University of Alaska Fairbanks, Fairbanks, Alaska. Individual animal was included as a covariate in the winters of 1994 and 1995. Results were considered significant if $P \leq 0.10$ for the winter 1994 woody twig data set and the summer 1995 leaves data set. Because two general linear models were constructed for winter 1996 woody twigs, winter 1995 textured concentrate, and summer 1995 textured concentrate, a $P \leq 0.05$ was considered significant for individual tests to achieve an overall significance level of $P \leq 0.10$ within that trial series.

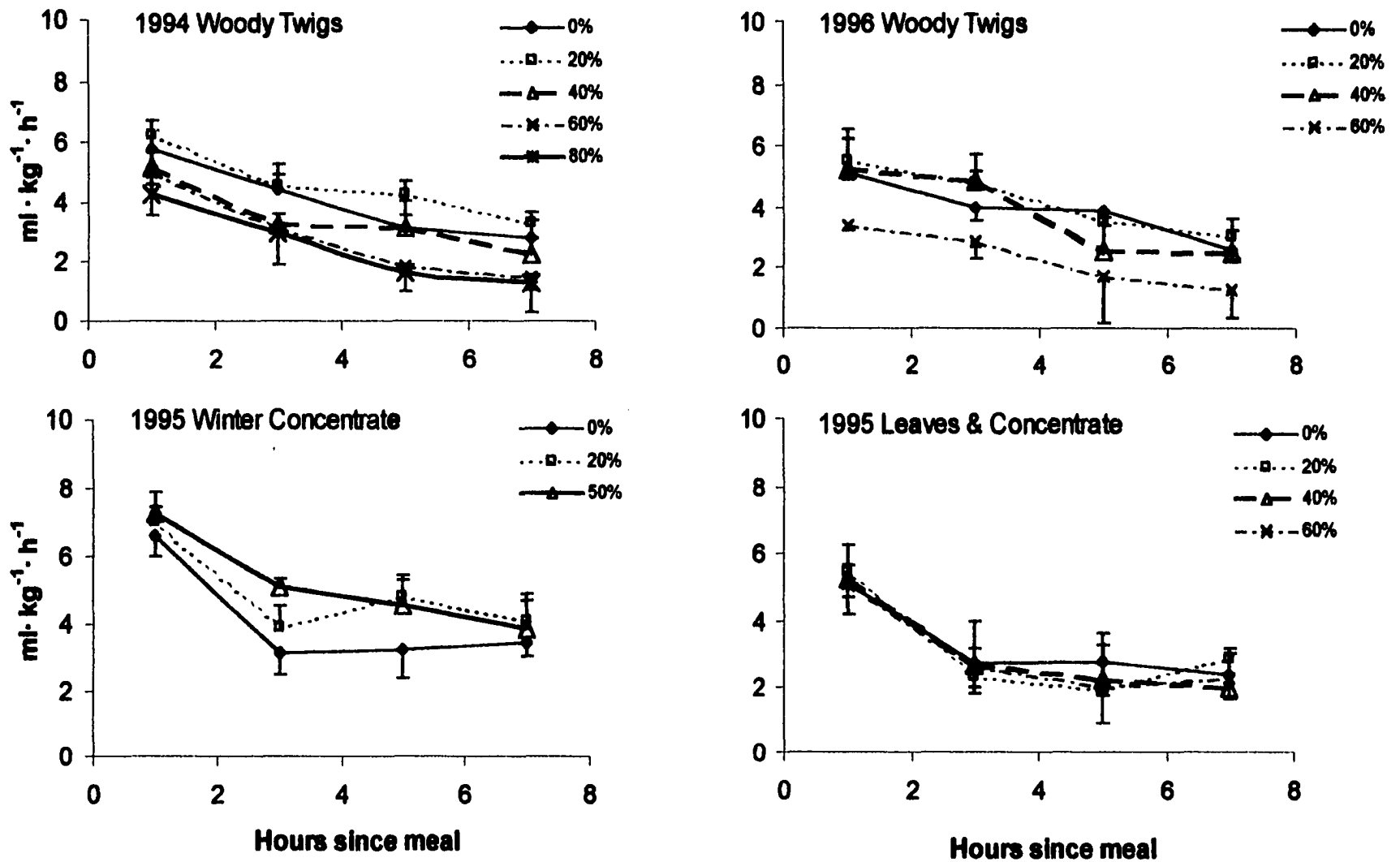|			Wint. 1994 Twigs[a]	Summ. 1995 Leaves[a]	Wint. 1996 Twigs[a]	Wint. 1995 Conc.[a]	Summ. 1995 Conc.[a]
Changes in net CH₄ produced over 8 h	Browse type	d.f.	1, 18	2, 13	1, 14	1, 9	1, 12
		F	1.01	0.49	0.35	3.82	2.10
		P	0.329	0.625	0.566[b]	0.083[b]	0.173[b]
		Part. eta squared	0.05	0.07	0.02	0.30	0.15
	Browse percent	d.f.	3, 18	2, 13	3, 12	2, 6	3, 10
		F	4.84	0.10	1.66	1.62	1.07
		P	0.012*	0.907	0.227[c]	0.273[c]	0.404[c]
		Part. eta squared	0.45	0.02	0.30	0.35	0.24
	Type x percent	d.f.	3, 18	3, 13			
		F	0.19	0.58			
		P	0.900	0.641			
		Part. eta squared	0.03	0.12			
Changes in rates of CH₄ produced over 8 h	Time x browse type	d.f.	2.3, 40.7	3.1, 20.4	2.3, 31.7	2.6, 23.4	2.3, 28
		F	1.35	1.33	2.45	0.77	4.20
		P	0.272	0.290	0.096[b]	0.506[b]	0.021*[b]
		Part. eta squared	0.07	0.17	0.15	0.08	0.26
	Time x browse percent	d.f.	6.8, 40.7	3.1, 20.4	6.8, 27.1	4.2, 12.5	6.7, 22.2
		F	0.48	0.44	0.973	0.74	2.04
		P	0.836	0.735	0.469[c]	0.588[c]	0.097[c]
		Part. eta squared	0.07	0.06	0.20	.20	0.38
	Time x type x percent	d.f.	6.8, 40.7	4.7, 20.4			
		F	0.54	1.28			
		P	0.793	0.312			
		Part. eta squared	.08	0.23			

* Significant result.

[a] Experimental diets fed consisted of hay mixed with browse or textured concentrate. Browse consisted of : 1) 1994: Winter woody twigs of *S. alaxensis* and *S. pulchra*. 2) 1995: Summer leaves of *S. alaxensis*, *S. pulchra* and *B. nana*. 3) 1996: Winter woody twigs of *B. nana*. 4) Winter and summer 1995: Corn based textured concentrate. Percentages of browse added to the hay were; 1) 1994: 0, 20, 40, 60, and 80% DM. 2) 1995: 0, 20, 40, and 60% DM. 3) 1996: 0, 20, 40, and 60% DM. 4) Winter 1995 textured concentrate: 0, 20, and 50%. 5) Summer 1995 textured concentrate: 0, 20, 40, and 60%.

[b] Reduced model without percent of browse in the model.

[c] Reduced model without browse type in the model.

included twigs of either *S. alaxensis* or *S. pulchra*, however, CH_4 production tended to be less when browse constituted 60 or 80% of the diet (Fig. 4.4). During winter trials with textured concentrate, percentage of textured concentrate in experimental diets resulted in a significant increase in net production of CH_4 (Tables 4.4 and 4.5).

Variability in CH_4 production was high, both within browse types and within percentages of browse offered. Observed statistical power during metabolic trials tended to be low (Table 4.6).

Results from partial eta-squared results indicated relatively large effects on rates of CH_4 production during many of the trials, which was attributable to the variables of browse type and percent of the diet composed of browse (Table 4.5). Proportion of total variability in rate of CH_4 production that could be attributed to the type of browse was 17, 15, and 26% for leaves in summer 1995, twigs in winter 1996, and textured concentrate in summer 1995, respectively. Proportion of total variability in rate of CH_4 production attributed to percentage of browse consumed was 20% for leaves in summer 1995 and textured concentrate in winter 1995, and 38% for textured concentrate in summer 1995. The interaction of type of leaves in summer 1995 with the percentage of leaves fed, accounted for 23% of total variability in rate of CH_4 production.

Likewise, partial eta-squared results indicated relatively large effects on total CH_4 produced, attributable to the variables of browse type and percentage of the diet composed of browse (Table 4.5). Consuming textured concentrate as part of the experimental diet explained 30 and 15% of the variability in the total CH_4 produced from

Table 4.6. Observed power (1-β) results from GLM-repeated measures analysis used to examine methane production in muskoxen at the Large Animal Research Station, University of Alaska Fairbanks, Fairbanks, Alaska. All results from the winter of 1994, and the winter concentrate values are from 3 muskoxen. Results from the summer of 1995, winter of 1996, and the summer QTX values are from 9 muskoxen. Power computed using α = 0.10.

		Winter 1994 Woody Twigs[a]	Summer 1995 Leaves[a]	Winter 1996 Woody Twigs[a]	Winter 1995 Concentrate[a]	Summer 1995 Concentrate[a]
		1-β	1-β	1-β	1-β	1-β
Changes in net CH_4 produced over 8 h	Browse type	0.253	0.070	0.024	0.298	0.392
	Percent browse	0.909	0.015	0.474	0.360	0.334
	Browse type x browse percent	0.146	0.117			
Changes in rates of CH_4 produced over 8 h	Time x browse type	0.414	0.442	0.149	0.079	0.836
	Time x percent browse	0.290	0.211	0.471	0.291	0.766
	Time x browse x percent	0.315	0.491			

[a] Experimental diets fed consisted of hay mixed with browse or textured concentrate. Browse consisted of : 1) 1994: Winter woody twigs of *S. alaxensis* and *S. pulchra*. 2) 1995: Summer leaves of *S. alaxensis*, *S. pulchra* and *B. nana*. 3) 1996: Winter woody twigs of *B. nana*. 4) Winter and summer 1995: Corn based textured concentrate. Percentages of browse added to the hay were; 1) 1994: 0, 20, 40, 60, and 80% DM. 2) 1995: 0, 20, 40, and 60% DM. 3) 1996: 0, 20, 40, and 60% DM. 4) Winter 1995 textured concentrate: 0, 20, and 50%. 5) Summer 1995 textured concentrate: 0, 20, 40, and 60%.

[b] Reduced model without percent of browse in model.

[c] Reduced model without browse type in model.

the meal during winter 1995 and summer 1995, respectively. Percentage of browse or textured concentrate consumed explained 45, 30, 35, and 24% of the variability in the total CH_4 produced following the meal for winter twigs 1994, winter twigs 1996, winter textured concentrate 1995, and summer textured concentrate 1995, respectively.

Methane production did not show a consistent relationship to the heat increment index of the meal for any browse species (Fig. 4.5). The lack of significance for predictive equations based on the heat increment index and RQ, indicate a low proportion of variation in CH_4 production explained by those regression models (R^2 adj) (Table 4.7). Only the regression from textured concentrate trials during winter 1995 approached significance. Results from the correlation matrix for this regression indicated the heat increment index from the meal was strongly correlated to CH_4 production ($r = 0.61$), but also indicated a high degree of correlation among some of the independent variables. In particular, an individual animal was strongly correlated to the heat increment index ($r = 0.77$), and RQ ($r = -0.56$). Variance inflation factors for this model indicate that this multicollinearity may not have been overly detrimental to the model (VIF = 2.6, 1.5, 3.3 and 1.4 for heat increment, RQ, first indicator for trial series, and second indicator for trial series, respectively).

Better fits were achieved with regression models of CH_4 production as a function of dietary composition and digestibility (Table 4.8). All models tested indicated significant linear relationships. Eliminating the single digestibility measurement (IVDMD) from the independent variables (Model 2; Table 4.8) did little to model fit in comparison to the full model with all independent variable entered (Model 1; Table 4.8).
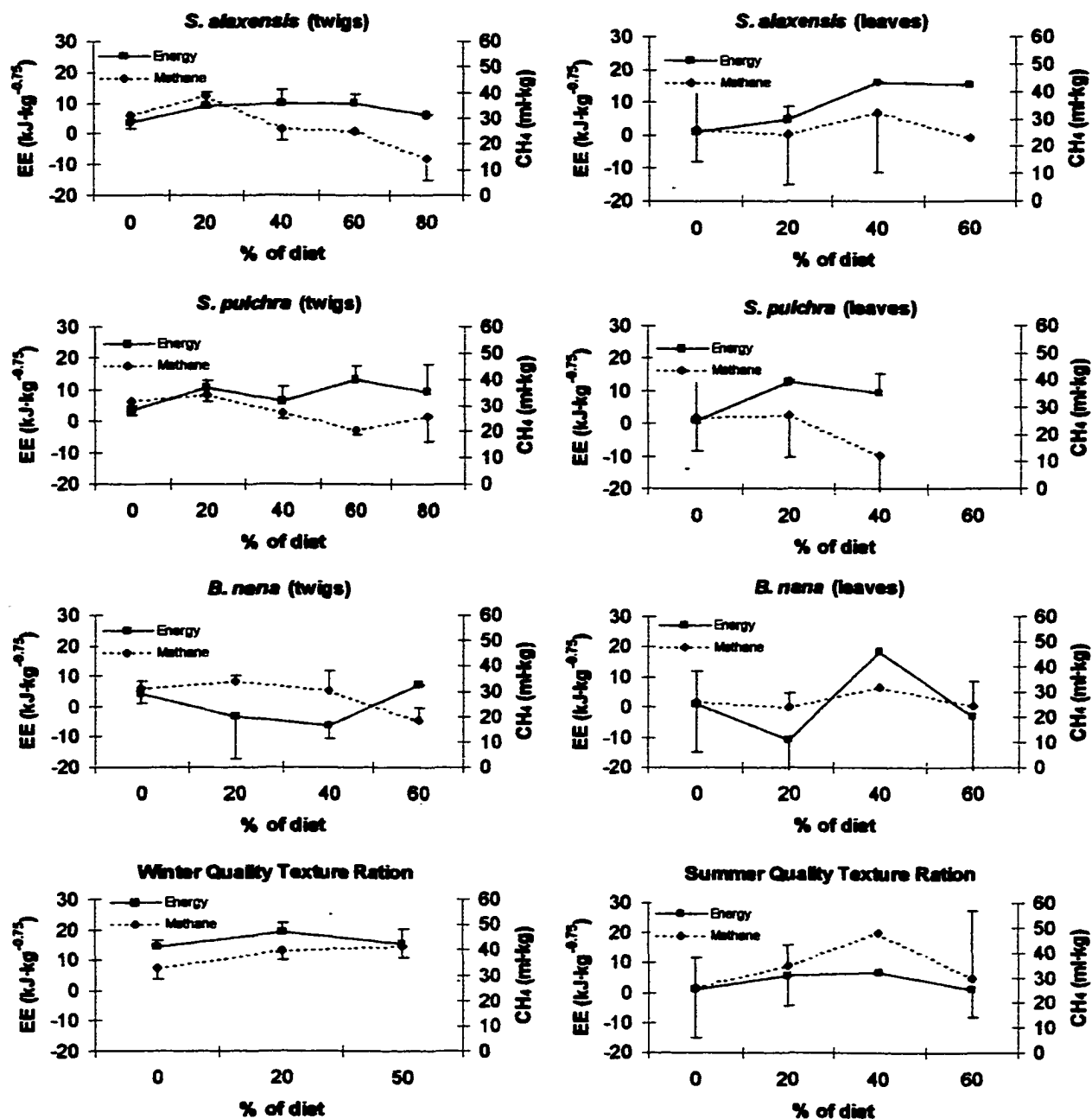
Fig. 4.5. The relationship of methane production to heat increment of different experimental diets as determined using an indirect calorimeter at the Large Animal Research Station, University of Alaska Fairbanks, Fairbanks, Alaska. Experimental diets were fed at 10 g • kg$^{-0.75}$ BM. Diets consisted of chipped hay mixed with graded levels of either chipped twigs or hand stripped leaves. Diets were fed inside a calorimeter following a 24 h fast.

Table 4.7. Linear regression models evaluating relationship between the meal derived heat increment index ($j \cdot g^{-1}$ body mass) and mean respiratory quotient (RQ) following the experimental meal, to meal derived methane production ($ml \cdot g^{-1}$ body mass). Values for methane production (CH_4), heat increment index (HII), and RQ were gathered from muskoxen at the Large Animal Research Station, University of Alaska Fairbanks, Fairbanks, Alaska. In winter 1994 trials and winter 1995 trials, individual study animal ($n = 3$) were included in the model using indicator variables (ind 1 and ind 2). In summer 1995 and winter 1996, individual animals ($n = 9$) were not included in models.

Trial series	Model	d.f.	R^2	Adj. R^2	F	P
Winter 1994 woody twigs ($n = 43$)	$CH_4 = -0.030 + 0.002(HII) + 0.061(RQ) + 0.005(ind\ 1) + 0.001(ind\ 2)$	4, 42	0.10	0.01	1.11	0.367
Summer 1995 Leaves and text. conc. ($n = 28$)	$CH_4 = 0.083 + 0.005(HII) - 0.058(RQ)$	2, 27	0.10	0.03	1.40	0.266
Winter 1996 woody twigs ($n = 17$)	$CH_4 = -0.026 + 0.004(HII) + 0.056(RQ)$	2, 16	0.07	-0.06	0.540	0.594
Winter 1995 text. conc. ($n = 15$)	$CH_4 = 0.079 + 0.046(HII) - 0.067(RQ) - 0.015(ind\ 1) - 0.001(ind\ 2)$	4, 14	0.531	0.343	2.83	0.083

d.f., degrees of freedom; R^2, coefficient of multiple determination; Adj. R^2, coefficient of multiple determination adjusted for the number of independent variables in the equation.

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Table 4.8. Linear regression models evaluating relationship between diet composition and quality, to meal derived CH_4 production (ml·kg^{-1} body mass). Methane production was measured on muskoxen at the Large Animal Research Station, University of Alaska Fairbanks, Fairbanks, Alaska. Models were developed with data from winter 1994 ($n = 43$), summer 1995 ($n = 28$), winter 1995 ($n = 15$), and winter 1996 ($n = 17$). Individual data sets were coded and included as variables in models.

Model [a]	d.f.	R^2	Adj. R^2	F	P
Model 1: $CH_4 = 291.8 - 0.485(CELL) + 0.537(HEMI) + 0.393(LIG) - 0.214(PRO) - 0.786(CP) + 2.408(ETH) + 0.030(DMD) - 13.756(GE) + 2.332(SET1) - 6.836(SET2) +3.384(SET3)$	11, 102	0.30	0.21	3.50	<0.001
Model 1A: $CH_4 = 15.8 + 0.059(DMD)$ [b]	1, 102	0.16	NA	19.53	<0.001
Model 2: $CH_4 = 301.2 - 0.428(CELL) + 0.471(HEMI) + 0.366(LIG) - 0.244(PRO) - 0.643(CP) + 2.469(ETH) - 14.203(GE) + 1.788(SET1) - 6.961(SET2) + 3.172(SET3)$	10, 102	0.30	0.22	3.86	<0.001
Model 2A: $CH_4 = 86.1 - 3.525(GE) + 0.189(CP)$ [b]	2, 102	0.18	0.16	10.90	<0.001
Model 3: $CH_4 = 102.1 - 0.020(FERM) - 0.114(DEF) + 0.004(CP) + 1.359(ETH) - 3.942(GE) + 0.613(SET1) - 3.525(SET2) + 5.169(SET3)$	8, 102	0.24	0.18	3.71	0.001
Model 3A: Same as 2A					
Model 4: $CH_4 = 6.59 + 2.411(DMD*GE)$ [c]	1, 102	0.18	NA	22.37	<0.001
Model 5: $CH_4 = 19.7 + .043(RES) + 0.260(HEMI) - 0.192(CELL)$ [d]	3, 102	0.13	0.11	5.09	<0.003

d.f degrees of freedom; R^2, coefficient of multiple determination; Adj. R^2, coefficient of multiple determination.
[a] Independent variables: (constant), neutral detergent fiber (NDF), cellulose (CELL), hemicellulose (HEMI), lignin (LIG), proanthocyanidin (PRO), crude protein (CP), ether extract (ETH), *in vitro* dry matter disappearance (DMD), gross energy (GE), trial indicator #1 (SET1), trial indicator #2 (SET2), trial indicator #3 (SET3), fermentable fiber (FERM), defensive agents (DEF), and soluble residue (RES). Independent variables were entered as grams dry matter consumed with the exception of GE (kJ consumed) and indicator variables.
[b] Results of stepwise selection of variables from primary models 1, 2 or 3 (P-to-enter of ≤ 0.05 and a P-to-remove of ≥ 0.10).
[c] Model developed using percent digestible gross energy as suggested by Blaxter and Clapperton (1965).
[d] Model developed using cellulose, hemicellulose, and soluble residue (neutral detergent solubles - crude protein and ether extract) as suggested by Moe and Tyrrell (1979).

Combining fermentable cell wall components (cellulose and hemicellulose) into a single

variable, and defense agents (proantocyanidin and lignin) into a single component did

little to the predictiveness of the model (Model 3; Table 4.8). Stepwise selection on

Model 1 included only IVDMD as a predictor of CH_4 production (Model 1A; Table 4.8).

For Model 2, stepwise procedures selected GE and crude protein as predictors of CH_4

production. The predictive model, based on agricultural diets, with the independent

variables suggested by Blaxter and Clapperton (model 4) (1965), performed as well as

the two models produced from stepwise variable selection (Models 1A and 2A). Model

5, based on the findings of Moe and Tyrrell (1979) for agricultural diets, performed the

poorest of all models tested.

Although the correlation matrices indicated IVDMD of experimental diets was

the most highly correlated independent variable to CH_4 production ($r = 0.403$), other

dietary components also were correlated (Table 4.9). Independent variables that were

strongly correlated with CH_4 production included GE ($r = -0.381$), crude protein ($r =$

0.371), proanthocyanidin ($r = -0.344$), lignin ($r = -0.309$), and hemicellulose ($r = 0.261$)

(Table 4.9).

Many independent variables also were correlated strongly (Table 4.9). Variance-

inflation factors for Models 1 indicated collinearity problems in every independent

variable with the exception of the indicator variables. The range of VIFs for Method 1

were from 549.0 (hemicellulose) to 17.6 (lignin). In Model 2, problems with

multicollinearity also were apparent with the largest VIF values for hemicellulose (VIF =

523.8), and only one diet component had a VIF <10 (ether extract VIF = 7.3). The

Table 4.9. Correlation matrices of chemical, energy, and digestibility characteristics of experimental diets used during muskox metabolism trials at the Large Animal Research Station, University of Alaska Fairbanks, Fairbanks, Alaska during the winters of 1994, 1995, 1996, and the summer of 1995. Experimental diets were hay mixed with graded levels of woody browse, leafy browse, or a corn based, textured concentrate. Browse species used were *S. alaxensis*, *S. pulchra*, and *B. nana*. Levels of intake of these browse species was 20, 40, 60 and 80% of the dry matter intake.

	CELL	HEMI	LIG	PRO	CP	ETH	DMD	GE	CH$_4$
CELL	1.00	0.893	0.063	-0.142	0.636	0.282	0.591	-0.245	0.133
HEMI	0.893	1.00	-0.255	-0.539	0.799	0.148	0.746	-0.434	0.261
LIG	0.063	-0.255	1.00	0.768	-0.332	0.348	-0.524	0.848	-0.309
PRO	-0.142	-0.539	0.768	1.00	-0.446	0.401	-0.485	0.568	-0.344
CP	0.636	0.799	-0.332	-0.466	1.00	0.518	0.935	-0.581	0.371
ETH	0.282	0.148	0.348	0.401	0.518	1.00	0.409	0.027	0.136
DMD	0.591	0.746	-0.524	-0.485	0.935	0.409	1.00	-0.769	0.403
GE	-0.245	-0.434	0.848	0.568	-0.581	0.027	-0.769	1.00	-0.381
CH$_4$	0.133	0.261	-0.309	-0.344	0.371	0.136	0.403	-0.381	1.00

CELL= cellulose, HEMI = hemicellulose, LIG = lignin, PRO = proanthocyanidin, CP = crude protein, ETH = crude fat, DMD = in vitro dry matter digestibility, GE = gross energy, and NDF = neutral detergent fiber.

problem of multicollinearity was much reduced in Model 3 by combining hemicellulose and cellulose, and lignin and proanthocyanidin. In Model 3, only protein had a VIF >10 (protein VIF = 11.9).

Discussion

Methane Production in Muskoxen

Estimated CH_4 production in muskoxen was within the lower range of values reported for ruminants. Net CH_4 loss in muskoxen was between 2.0 and 3.2% of GE intake, which compares favorably to values for moose which lost 3.1-4.8% of dietary GE when fed on pelleted forage, and 2.1% when consuming mixed browse (Schwartz et al., 1987). Bison (*Bison bison*), North American elk (*Cervus elaphus*), and white-tailed deer (*Odocoilus virginianus*) consuming sun-cured alfalfa pellets lost CH_4 equivalent to 6.6, 5.2, and 3.3%, respectively, of GE intake (Galbraith et al., 1998). In cattle, CH_4 energy losses relative to GE intake ranged from 2 to 12% (Johnson and Ward, 1996). Much variation in CH_4 production in ruminants is attributed to factors that influence rate of passage of food. Those factors included level of intake, physical properties of the forage, and digestibility (Blaxter and Clapperton; 1965, Moe and Tyrrell, 1979; Johnson and Ward, 1996). Results from Galbraith et al. (1998) indicate that morphology and body size also may be a factor, because the large roughage-grazer (bison) had a slower rate of passage than did a medium sized intermediate-mixed feeder (elk), and both had slower rates of passage than did a small browser-concentrate selector (white-tailed deer).

Muskoxen are most commonly classified as grazers (Hofmann, 1988; Adamczewski et al., 1994a; Adamczewski et al., 1994b), yet rates of CH_4 production as a proportion of GE intake were more consistent with those of browsers.

The Influence of Browse on CH_4 Production

Significant changes in rates of CH_4 production following the consumption of experimental meals occurred during textured concentrate trials in summer 1995. Large effects (partial eta-squared) on the rate of CH_4 production and on the net CH_4 production were attributed to having textured concentrate in the diet and to the percentage of the diet composed of textured concentrate during winter 1995 and summer 1995. A surprising aspect of these results was the tendency for higher initial rates of CH_4 production and higher net production of CH_4 on those diets with textured concentrate (Fig. 4.3 and 4.4). Muskoxen used in my experiment had been maintained primarily on brome hay. Textured concentrate, relative to hay could be characterized as low in fiber (including both cellulose and hemicellulose), high in crude protein, and small in particle size. In addition, textured concentrate has a faster passage rate in muskoxen compared to hay (Holleman et al., 1984). Diets high in concentrate reduce CH_4 production in cattle (Moss, 1992; Leedle and Greening 1988). Because muskoxen were not maintained on concentrate, perhaps concentrates lowered pH in the rumen, and increased the proportion of easily fermentable carbohydrates, both of which inhibit methanogens (Van Soest, 1982; Van Nevel and Demeyer, 1996; Leng, 1991). Methanogens are the most sensitive

group of rumen microorganisms (Van Soest, 1982), leading to the expectation that the most likely short-term change in CH_4 production would be a decrease rather than an increase in production. Conversely, CH_4 production is positively correlated with increasing apparent digestibility of the diet at maintenance levels of intake (Blaxter, 1965). In conditions imposed by this study, the improved digestibility of the diet was likely more important in determining CH_4 output during trials with textured concentrate than was fiber content, or composition of the fiber.

Net CH_4 produced from the meal varied significantly among diets differing in percentage of woody twigs of *S. alaxensis* and *S. pulchra* added to hay diets during winter 1994. Although not statistically significant, *B. nana* added to hay diets in winter 1996 resulted in a decline in net CH_4 production following the meal. Also there were large effects on rate of CH_4 production from percentage of the diet composed of *B. nana* twigs during winter 1996. Adding leaves of *S. alaxensis, S. pulchra* or *B. nana* to the diet during summer 1995 substantially decreased net CH_4 production. Leaves and twigs added to hay diets tended to result in lower rates and lower net production of CH_4 (Fig 4.3 and 4.4). My results are consistent with expectations that CH_4 production was based on physical and chemical properties of browse.

Robbins (1993) suggested that CH_4 production from fermentation of browse would be low. Results from leafy browse trials are consistent with lower leaf fiber to cell content in my study in comparison with hay, and the correspondingly higher proportion of readily fermentable carbohydrates. Increasing the proportion of readily fermentable carbohydrates speeds passage rate (Van Soest, 1983). Woody browse, although

comparable with hay in overall fiber content, had much higher proportions of lignin, a non-fermentable and largely indigestible component of fiber. In addition, lignin content in woody browse has faster passage rates (Van Soest, 1983). Both factors tend to decrease CH_4 production (Van Soest, 1983). In leaves and twigs, the presence of plant secondary metabolites also may lead to a decrease in CH_4 production. Detoxification of secondary metabolites can lead to acidosis (Foley 1995; Illius and Jessop 1995), which would result in a hostile environment for rumen methanogens. Twigs and leaves of all browse species offered during trials contained measurable quantities of phenolics (i.e., proanthocyanidins). Some phenolic compounds have been shown to be toxic to rumen bacteria (Akin, 1982; Chesson et al. 1982; Borneman et al. 1986; Varel & Jung, 1986; Field et al., 1990), and this too could be detrimental to rumen methanogens.

Methanogenic bacteria are highly sensitive to changes in rumen condition yet the only strong evidence for a decline in methanogens in this study was the decrease in CH_4 production between 20 and 60% woody browse (Salix) during winter 1994. Because I fed pulsed meals separated by at least 3 d on hay, adaptation by the rumen biota did not likely allow rapid detoxification of plant secondary compounds. Other possibilities exist for the lack of a statistically significant effect including: 1) variability in CH_4 production in muskoxen is large enough to mask effects; 2) methodology did not allow sufficient time for long-term diet adaptation; 3) the particular phenolics, or other plant secondary compounds, in these browse species were not detrimental to rumen methanogens; 4) muskoxen were capable of metabolizing the phenolics, or other plant secondary compounds, in woody and leafy browse, and; 5) quantity of phenolics, or other plant

secondary compounds, consumed, either by the amount offered or by the amount

voluntarily consumed by the animals, was insufficient to produce a response.

Prediction of CH₄ Production from Animal Metabolism and Feed Composition

Linear models for predicting CH_4 production developed from heat increment

index and RQ values performed poorly based on R^2 ajd. values (Table 4.7). This was

unexpected and may result from variation in the heat increment index, and measurements

of CH_4 production. Although ruminal CH_4 production is relatively constant measured

CH_4 production changes with activity (Blaxter 1962) and with patterns of eructation

(Blaxter and Clapperton, 1965) resulting in short-term variation in emission patterns (Fig.

4.1). Methane production is a function of methanogenic bacteria metabolism. Numerous

factors can influence methanogens but these bacteria are the single source of CH_4. In

contrast, variation in energy expenditure following a meal may result from changes in

metabolism by rumen flora (including methanogens), components of animal metabolism

associated with heat increment of feeding, and activity.

Diet constituents performed poorly as independent variables predicting CH_4

production by regression equations. Blaxter and Clapperton (1965) combined data from

trials in which cattle and sheep had been fed diets of hay, concentrate and mixed diets,

and reported a strong relationship between CH_4 production, expressed as a percentage of

GE consumed, and apparent digestibility of dietary energy (residual standard deviation =

0.71). Moe and Tyrrell (1979) predicted CH_4 production as a percent of GE for cattle fed

concentrate, corn, barley, oats, beet pulp, dried brewers grain, dried distillers grain, and

wheat bran. With this wide variety of feed types, three variables, namely soluble residue, hemicellulose, and cellulose, explained much of the variation in CH_4 production ($r^2 = 0.67$). These predictive equations performed poorly with muskoxen consuming leaves, woody browse, and textured concentrate ($r^2 = 0.24$-0.30). When applied to CH_4 values in this study, the Blaxter and Clapperton equation overestimated CH_4 production almost three fold. Therefore, estimates of CH_4 production in wildlife species based on these models are likely to be erroneous.

The influence of browse on energetics of muskoxen

The addition of browse to a graminoid-based diet in a single small meal was capable of influencing CH_4 production by muskoxen in some circumstances. Nevertheless, my hypotheses that adding browse to diets would reduce CH_4 production by muskoxen was only supported during winter 1994, when woody twigs of *S. alaxensis* and *S. pulchra* were added to meals. My hypothesis was rejected in summer 1995 with leaves of *S. alaxensis*, *S. pulchra*, and *B. nana*, and in winter 1995 with woody twigs of *B. nana*. During leafy browse trials in summer 1995, and trials in winter 1996, data were inconclusive because of small sample sizes and sufficient variability in CH_4 production between trials resulting in a poor ability to discriminate between browse species and percentages of browse included in the diet. The combination of large effect size (partial eta-squared), and low observed power in metabolic trials leads to the conclusion that there is a strong potential for significant changes, both statistically and biologically, in CH_4 production because of consumption of browse.

This study showed that CH_4 production by muskoxen as a percentage of gross energy intake was substantially lower than that observed in domestic ruminants. Thus it may be difficult to detect effects of browse in lowering CH_4 production. My hypothesis that reduction in CH_4 energy loss could offset potential costs of detoxification of plant secondary compounds in browse species could be neither substantiated nor rejected. As indicated in chapter 3, the heat increment index used in this study underestimates the true heat increment of feeding. My finding that CH_4 energy loss and the heat increment index were of similar magnitude (Table 4.3) suggests further studies on both effects are warranted. Diet digestibility, gross energy content and crude protein levels were shown to affect the heat increment index and CH_4 production (chapter 3, Table 4.9), although variance accounted for was low (Table 4.8). This indicates that in this series of trials, antiherbivory compounds (i.e., proanthocyanidins) were not the primary factor influencing CH_4 production. The effect of browse on lowering IVDMD however, and the significant association of CH_4 production with IVDMD suggest an indirect role for plant secondary compounds as these are known to suppress DMD in *in vitro* systems (Person et al., 1980; Trudell et al., 1980).

Synopsis

Muskoxen (*Ovibos moschatus*) evolved in an environment typified by low plant productivity and highly seasonal shifts in quality and availability of forage (White *et al.* 1981; Klein 1986). Winter conditions at high latitudes may last for 9-10 months of the year and extreme cold may persist for extended periods. Snow and ice may make forage unavailable and the forage that is available is often low in nutrients and high in structural carbohydrates (Staaland & Olesen 1992). During the short growing season, muskoxen must store body reserves for the ensuing season of restricted food availability (White *et al.* 1989; Adamczewski 1995) while meeting seasonal energy and nutrient demands for reproduction and lactation (White *et al.* 1989; Parker *et al.* 1990).

Muskoxen have adopted a conservative strategy to cope with the rigors of the arctic. A relatively large body size coupled with excellent insulation serves to minimize energy needed for heat production for homeostasis (Adamczewski 1995). Energy conservation is apparent in their sedentary lifestyle. Based on these observations, diet selection and processing of this forage should then also reflect energy saving strategies. To be most efficient, strategies should be flexible and capable of responding to seasonal changes in forage availability.

Consistent with these hypotheses, Holleman *et al.* (1984) and Adamczewski *et al.* (1994a) noted slower passage rates in muskoxen for forage in winter in comparison to summer. Winter passage rates of recalcitrant fiber is among the slowest recorded for ruminants and may have contributed to the greater efficiency of digestion of low quality

hay in muskoxen compared to cattle (Adamczewski *et al.* 1994b). White *et al.* (1984) and Adamczewski *et al.* (1994a) documented lower voluntary food intakes in winter in comparison with summer and correspondingly lower maintenance energy requirements. Seasonal weight stasis in muskoxen has been documented in numerous studies (White *et al.* 1984; White *et al.* 1989; Groves 1992; Adamczewski *et al.* 1992; Adamczewski *et al.* 1994a). My goal with this thesis was to further investigate energy efficiencies in muskoxen and explore the role diet selection has on energy efficiencies.

Seasonal lowering of resting metabolic rate would be an advantageous adaptation to limiting winter food supply in a cold environment (Wood *et al.* 1962; Feist & White 1989). Nilssen *et al.* (1994) reported a 49% difference in winter and summer metabolic rates in muskox that had food withheld for 7-10 h, and a 30% difference in metabolic rate following 6 d without food. However, timing of the down regulation in winter and up regulation in summer was not determined. I documented a 33% difference in winter and summer metabolic rates in muskoxen following 24 h without food (Chapter 1). The main increase in EE from winter to summer occurred between April and May and the summer to winter decrease between August and September. The timing of seasonal shifts in metabolic rates for muskoxen correspond both to major changes in photoperiod and food availability. Initiation of calving occurs in late April, and temperatures can be expected to be moderating with increased solar influx in the months following the vernal equinox. In August, muskoxen begin to rut, nighttime temperatures are consistently below freezing and plants have translocated nutrients to their roots (Chapin *et al.* 1975). In August, adult females shift nutrient partitioning from milk production to body reserves (White *et*

al. 1989), precisely when I observed the significant down-regulation in fasting energy expenditure.

In Chapter 2, I confirm the seasonal shift in fasted metabolic rate and present evidence that this shift is due to changes in basal metabolism rather than changes in efficiencies of diet metabolism as expressed by the heat increment of feeding (HIF). Controlling for differences in metabolic energy intake by expressing HIF values as a fraction of metabolic energy intake resulted in estimates of HIF that were either equivalent across seasons, or greater in winter in comparison to summer. Therefore, efficiency in use of hay diets was unchanged with season, or potentially, efficiency improved in the winter.

Metabolic measurements outside of the thermoneutral zone have the potential to obscure seasonal differences in metabolic rates (*sensu* Mautz *et al.* 1992). Muskoxen in this study did not show a metabolic response to temperature, date, or a date-temperature interaction during summer. In winter, energy expenditure declined as temperatures became warmer, but declined also with advancing Julian date (i.e., with increasing photoperiod). Stepwise regression analysis indicated date was a better predictor of energy expenditure following a 24 h fast than was temperature and this conclusion was supported by the correlation matrix, the standardized regression coefficient, and the partial correlation coefficient for temperature. Therefore, seasonal changes in fasting metabolism of muskoxen were endogenous, or a product of pre-fasting level of feed intake, and not a product of experimental temperatures.

Variation in activity levels, either between seasons or among individual animals, has a high potential of obscuring variation in metabolic rate and in HIF. The energy cost of standing can be high (Fancy & White 1985) and constitutes an important component of ecological energetics. I estimated the energy cost of standing as 21% higher than when bedded (Chapter 2). The energy cost of standing did not vary by season and was similar to estimates for other wild ruminants (Fancy & White 1985).

Correcting energy expenditure for standing costs had a small but significant effect on the magnitude of the HIF (Chapter 2). The process is time consuming however, and required continuous monitoring of experimental animals. Given the effort required, researchers need to evaluate the appropriateness of applying corrective measures to their data. In situations where animals spend little time active, or in trials with comparable activity levels, correcting for standing costs is unnecessary.

Following 24 h of fasting and during a 1 h feeding time block, winter respiratory quotients indicated muskoxen were metabolizing a higher proportion of carbohydrate to fat compared to summer values (Chapter 2). That outcome is consistent with a slower metabolic rate (Nilssen et al. 1994; Lawler & White 1997), a slower passage rate (Holleman et al. 1984; Adamczewski 1994a), and again indicates a slower metabolic rate in winter compared with summer.

Based on these observations, the traditional method of fasting ruminants to a post absorptive state to measure efficiency of utilization of forage may negate aspects of seasonal metabolism that should be considered in baseline estimates of HIF. The observation that long-term fasted animals in summer can exhibit metabolic rates similar

to those expected from post-absorptive animals in the winter does not indicate that BMR remained the same, but rather that metabolic rates vary with dietary intake and resulting gut activity. Energy expended with consumption of different meals therefore might best be compared with baseline values which are allowed to vary seasonally and values not artificially depressed through long-term food deprivation.

I evaluated three methods to estimate HIF using a muskoxen fasted for 24 h, fed a meal typical of a single feeding bout. Fasted muskoxen were placed in a respiration chamber and a prefeeding EE (EEp) was determined over 2 h. At the end of the 2 h prefeeding period, the experimental meal was offered and EE was followed for the next 8 h. Heat increment of feeding was estimated by: i) subtracting a constant baseline value (EEp) from the EE following the meal; ii) subtracting a linearly declining baseline from the EE following the meal; and iii) extrapolating results from method (ii) to time ∞ by assuming HIF responded exponentially (Chapter 2). Methods (i), (ii) and (iii) all gave repeatable measures of HIF. Methods (i) and (ii) gave lower estimates of HIF than predicted. Method (iii) tended to overestimate HIF compared with predicted estimates but estimates were within the range of reported values for high quality grass hay. Although method (iii) was the most appropriate method for estimating absolute values of HIF, all three methods allowed comparisons of relative changes in HIF associated with different diets. Method (i) was chosen to examine natural forages as an index of HIF from diets consumed by muskoxen because it was relatively simple in comparison to methods (ii) and (iii), and because it made no assumptions beyond the 8 h post-feeding HIF recovery period.

I investigated two aspects of energy efficiency in muskoxen consuming woody and leafy browse, HIF (Chapter 3), and CH_4 production (Chapter 4). Between 3 and 12 % of the gross energy of a diet may be lost as HIF (Blaxter 1962), and between 2 and 12% of the gross energy of a diet can be lost as CH_4 in the conversion of dietary energy to metabolic energy (Johnson & Ward 1996). Heat increment of feeding (HIF) is important owing to the role it plays in seasonal energy balance (Kleiber 1975; Robbins 1993), thermoregulation (Jensen et al. 1999), and association with detoxification of plant secondary compounds (Van Soest 1982; Foley 1987; Robbins 1993). I assumed that detoxification costs could be measured as an increase in the HIF. In ruminants, browse is thought to inhibit methane (CH_4) production (Robbins 1993). Thus ruminants may balance detoxification costs with energy savings by a reduced CH_4 production, when consuming browse.

Muskoxen are well adapted grazers (sensu Hofmann 1988) capable of subsisting on low quality hay (White et al. 1981; White et al. 1984; Eisfeld 1990; Adamczewski et al. 1994a; Adamczewski et al. 1994b) but they also regularly consume browse. Willows (Salix spp.) in particular are heavily used in many locations. Although birch (B. nana) commonly occurs in some muskoxen ranges, rarely is this species reported as food for wild muskox. Based on these observations, I predicted a diet that included moderate levels of Salix to be energetically advantageous to muskox in comparison with a 100% graminoid diet, and a diet that included moderate levels of B. nana to be less energetically advantageous in comparison with a 100% graminoid diet. Consequently, I

tested a null hypothesis that the addition of browse to a graminoid based diet would not alter the HIF (Chapter 3) or CH_4 (Chapter 4) in muskoxen. In addition, I hypothesized that HIF or CH_4 would not vary with the type of browse consumed with a graminoid based diet or by the percent of browse consumed.

Experimental meals were not fully consumed when they contained a high percentage of woody or leafy browse (60 to 80%) of either the willow species or the birch species (Chapter 3). This result suggests that muskoxen will consume only up to a certain level of a browse species in a given meal or foraging period. Alternatively, consumption of browse species must be balanced with consumption of other plant material.

I detected statistically significant changes in HIF following meals with the addition of browse to a graminoid based diet during winter1994 and in summer 1995. In winter 1994, diets that contained moderate levels of *Salix* woody browse (20-60%) tended to have higher HIF than did the 100% hay diet. In summer 1995, diets that contained moderate levels of *Salix* leafy browse also tended to have higher HIF than did the 100% hay diet. No trend in HIF was detectable for those diets that contained winter woody twigs and summer leafy browse of birch.

Estimated levels of CH_4 production in muskoxen eating brome hay diets were lower (2.0 to 3.2% of GE intake) than expected (Blaxter & Clapperton 1965) for sheep and cattle eating the quality hay fed at maintenance energy requirement (6.4 to 7.7% of GE intake) but were within the range of values reported for wild ruminants fed browse (Robbins 1993). A statistically significant variance in net CH_4 produced from meals was

detected during the winter 1994, where moderate to high levels (40 to 80%) of browse in the diet suppressed CH_4 production. Large effects on CH_4 production were noted from adding leaves of willows and birch to the diet during summer 1995, with stimulated CH_4 production from *S. alaxensis*, supressed CH_4 production from *S. pulchra*, and variable CH_4 production from *B. nana* in comparison to CH_4 production from 100% brome hay. Likewise, twigs of *B. nana* did not affect CH_4 production in comparison to 100% brome hay during winter 1996. Overall, woody browse added to hay diets tended to result in lower rates and lower net production of CH_4.

Changes in HIF and CH_4 production could be brought about by three possibilities; changes in level of energy intake, changes in the products of fermentation, and consequences of secondary metabolite ingestion. An increase in metabolizable energy intake (Blaxter 1962; Webster 1983), and an increase in apparent digestibility (Blaxter 1962) would increase both HIF and CH4 production, respectively. A second possibility for changes in HIF and CH_4 production, is change in the end-products of rumen fermentation resulting in changes in efficiency in use of metabolizable energy of the meal (Blaxter 1962; Van Soest 1982). Forages with a greater proportion of soluble carbohydrates in comparison to fiber and non-digestible components (i.e., leafy browse) produce a higher proportion of propionate relative to acetate and hence methane production (van Hoven & Boomker 1985). The ratio of propionate to acetate also influences energy efficiency through gluconeogenesis, as propionate is the only major volatile fatty acid produced from rumen fermentation that is glucogenic (Van Soest 1982). Protein precursors are needed for gluconeogensis when insufficient propionate is

available (Van Soest 1982). A third and final explanation for the changes we observed in

HIF and CH_4 production following a meal is the consequence associated with the

detoxification of plant secondary metabolites. Energy is required in the process of

detoxifying plant antiherbivory compounds (Iason & Murray 1996). Alternatively,

detoxification of secondary metabolites could result in a hostile environment for rumen

methanogens, thereby eliminating or reducing CH_4 energy loss (Akin 1982; Chesson *et

al.* 1982; Borneman *et al.* 1986; Varel & Jung 1986; Field *et al.* 1990). Whereas

elimination of methanogens may result in improved efficiency of diet metabolism,

elimination of other components of the rumen flora, such as cellulolytic bacteria

(Chesson *et al.* 1982; Borneman *et al.* 1986; Varel & Jung 1986), through ingestion of

plant secondary compounds, may reduce overall efficiency of diet use. To examine

influences of browse on rumen digestion and CH_4 production, I tested models in which

CH_4 production was predicted from dietary nutrient components and digestibility

(Blaxter & Clapperton 1965; Moe and Tyrrell 1979), and in turn investigated the

influence of browse on the IVDMD of brome hay (Chapter 4).

Addition of browse, whether leafy or woody, reduced IVDMD of hay based diets.

In contrast, the addition of textured concentrate increased IVDMD of hay based diets.

My models of CH_4 production, based on *in vitro* dry matter digestibility experiments and

forage composition did not favor energy content, fermentation products, or plant

secondary metabolites as unifying principals explaining consequences of ruminant

ingestion of woody browse. Changes in metabolizable energy intake, relative proportions

of volatile fatty acids, and secondary plant metabolites all have the potential to influence

the HIF and CH_4 production following a meal. The relative importance of each of these factors to the energy balance of the animal likely changes with the type (leaf or twig) and species of browse as well as the proportion of browse in the diet. Thus, ruminants may be capable of offsetting minor costs associated with consuming browse containing secondary plant metabolites with improved ratios of volatile fatty acid production in the rumen or higher intakes of metabolizable energy Greater intakes of metabolizable energy could be accomplished either through ingestion of plants minimally protected by secondary metabolites, or by increasing the quantity of forage consumed.

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