Feeding Time (2100 h vs. 0900 h) Effects on Feed Intake, Rumen Fermentation, Blood Metabolites, and Productivity of Lactating Holstein Cows

by

Akbar Nikkhah

A Thesis submitted to the Faculty of Graduate Studies of

The University of Manitoba

in partial fulfillment of the requirements for the degree of

DOCTOR OF PHILOSOPHY

Department of Animal Science

University of Manitoba

Winnipeg, Manitoba, Canada

THE UNIVERSITY OF MANITOBA

FACULTY OF GRADUATE STUDIES

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Feeding Time (2100 h vs. 0900 h) Effects on Feed Intake, Rumen Fermentation, Blood Metabolites, and Productivity of Lactating Holstein Cows

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To the Reader

ABSTRACT

Feeding at 2100 h vs. 0900 h was hypothesized to alter nutrient intake, digestion, and metabolism, and thus optimize productivity of lactating cows. The primary objective was to ascertain the effect of feeding at 2100 h vs. 0900 h in lactating cows on the major processes involving feed conversion into milk. The processes included feed ingestion and its 24-h patterns, rumen fermentation and its 24-h patterns, microbial protein synthesis, total tract nutrient digestion and nitrogen partitioning, circulating levels of blood metabolites and their 24-h patterns, and milk production and composition. This research was conducted in a metabolism unit under no heat stress. In the first study, four multiparous and four primiparous lactating Holstein cows were used in a 4 × 4 Latin square design in four 21-d periods. Each period consisted of 14 d of adaptation and 7 d of sampling. A total mixed ration (TMR) with forage to concentrate ratio of either 61.5:38.5 or 50.6:49.4 was fed once daily at either 2100 h or 0900 h. Feeding at 2100 h instead of 0900 h increased the amount of TMR consumed within 3 h after feeding but did not affect daily DMI. Feeding at 2100 h tended to increase rumen VFA at 4 h post-feeding. Feeding at 2100 h elevated blood levels of L-lactate and β-hydroxybutyrate (BHBA) at 2 and 4 h post-feeding, compared to feeding at 0900 h. Blood glucose at 2 h post-feeding was lower in the 2100 h-fed cows than in the 0900 h-fed cows. The altered 24 h patterns in feed intake, rumen fermentation, and blood metabolites by feeding at 2100 h were associated with increased milk fat yield in multiparous cows. In the second study, four multiparous and four primiparous cows were used in a cross-over experiment with two 42-d periods. The TMR had forage to concentrate ratio of 50.2:49.8. Each period had 21

d of adaptation. Four cows were ruminally cannulated. Results verified the findings of the first study that feeding at 2100 h instead of 0900 h increased feed intake within 3 h after feeding. There was an improved daily DMI in the 2100 h-fed primiparous but not in multiparous cows. Feeding at 2100 h increased rumen total VFA at 5 h, acetate at 5 and 6 h, and ammonia at 2 h post-feeding. Microbial protein synthesis and milk proportions of total short, medium, and long chain fatty acids did not differ between the 2100 h-fed and 0900 h-fed cows. The apparent total tract dry matter, nitrogen, and NDF digestibility was improved by feeding at 2100 h instead of at 0900 h. Consequently, milk fat and energy outputs were improved by feeding at 2100 h. Due to reduced fecal and urinary nitrogen excretions, cows retained greater nitrogen in the body when fed at 2100 h. Therefore, feeding Holstein cows at 2100 h instead of 0900 h altered post-feeding patterns in feed intake, rumen fermentation, blood metabolites, and in so doing, improved milk fat and nitrogen balance. Reduced fecal and urinary nitrogen loss by evening feeding suggests environmental benefits.

Key Words: feeding time, evening, morning, Holstein cow, milk yield, rumen, intake, digestion, metabolism

ACKNOWLEDGMENTS

The author will be grateful everlastingly to Dr. Kees Plaizier for his advice, genuineness, and company during this Ph.D. program. Words are inadequate. An effective approach to thank our teachers would be to responsibly contribute to the growth of our students. I acknowledge my committee members for their contributions to this learning process. I will remember Dr. Karin Wittenberg every time I develop a sound research hypothesis. I owe gratitude to Dr. Alma Kennedy whose advice led me to more profoundly appreciate the value of the clear communication of my knowledge in academia. For the timely completion of this degree, I am indebted to Dr. Shannon Scott who provided timely critiques. Gratitude goes to Dr. Gary Crow for his exceptional generosity in offering time for statistical advice. "Keep it simple, smooth, and tight". Imagine how much more logical our scientific communications could be if all truly grasped the statement by Dr. Roger Wilson. Thanks to Drs. James House, Martin Nyachoti, and Loreen Onischuk for furthering my knowledge, and Dr. Bill Guenter for his guidance prior to and during the candidacy examination. Thanks to Ms. Terri Garner, Ms. Janice Haines, Ms. Deanne Fulawka, Mr. Prakash Sharna, Ms. Karen Lim, Mr. Harry Muc, and Mr. Jason Neufeld for technical expertise and moral patience. Thanks to Mr. Dale Rosner, Ms. Heather Laurie, Mr. Berry Peril, and Mr. Aurele Chartier for help with animal management, Ms. Margaret Ann Baker, Ms. Ilse Frank, Ms. Cathy Plouffe and Ms. Claire Hutchinson for administrative skills, and graduate students for introducing memorable moments to my social life. This research was funded by the Dairy Farmers of Canada and Dairy Farmers of Manitoba. The author was the recipient of a National Scholarship from the Ministry of Science, Research, and Technology of Iran.

FOREWORD

This dissertation is written in manuscript style and is composed of eight chapters. The chapters include 1) General Introduction, 2) Literature Review, 3-6) findings of two multifaceted dairy studies in four separate manuscripts, 7) General Discussion, and 8) Final Conclusions. Chapters 3 and 4 present the data for the first major study, and Chapters 5 and 6 present the data for the second major study. The references are listed following Chapter 8. The author's major and original contributions to this Ph.D. dissertation involved designing and conducting the on-farm trials, data collection and laboratory experiments, statistical analysis, data documentation and interpretation, and preparing manuscripts for peer-review publication. The author has communicated and disseminated the findings in various national and international conferences both orally and as a poster. More than twelve abstracts were published in the Canadian Journal of Animal Science, Advances in Dairy Technology, Journal of Animal Science, and Journal of Dairy Science in 2005, 2006, and 2007. Chapters 2, 3, 4, 5, and 6 will be submitted as five separate full manuscripts for publication with A. Nikkhah as the first author.

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ABBREVIATIONS

AA = amino acid

A + B / P = (acetate + butyrate) / propionate

ADF = acid detergent fiber

BCS = body condition score

BCVFA = branched chain volatile fatty acids

BHBA = beta-hydroxy butyric acid

bST = bovine somatotropin

BUN = blood urea nitrogen

BW = body weight

CLA = conjugated linoleic acid

Co = cobalt

CP = crude protein

Cr = chromium

D = diet

DIM = days in milk

DM = dry matter

DMI = dry matter intake

ECM = energy corrected milk yield

FCM = fat corrected milk yield

F:C = forage to concentrate ratio

FF = feeding frequency

H = hour

HC = high concentrate

LC = low concentrate

MG = mammary gland

N = nitrogen

NB = nitrogen balance

NDF = neutral detergent fiber

NEFA = non-esterified fatty acids

NFC = non fiber carbohydrates

OM = organic matter

PAR = parity

PD = purine derivatives

PDV = portal-drained viscera

PSPS = Penn State Particle Separator

RDP = rumen degradable protein

SARA = sub-acute rumen acidosis

SD = standard deviation

SE = standard error

SH = sampling hour

SNF = solids non-fat

TF = time of feeding

TMR = total mixed ration

VFA = volatile fatty acids

CHAPTER 1

GENERAL INTRODUCTION

For maximum efficiency, the time of nutrient delivery to the rumen, splanchnic tissues, and the periphery needs to be synchronized with the endogenous rhythms in body metabolism. Diurnal rhythms in body metabolism are reflected in endogenous and exogenous patterns in blood levels of metabolites and hormones. Endogenous rhythms are regulated by the suprachiasmatic nuclei in the hypothalamous and not only by the environmental factors such as photoperiod and feeding time (Sehgal, 2004). For example, blood glucose in humans possesses endogenous rhythmicity (la Fleur et al., 2001). In contrast, exogenous rhythms are controlled by external agants. Blood urea is largely responsive to feeding and digestive function, and thus, is exogenously regulated (Piccione et al., 2003). It is important to determine if and to what extent feeding time can alter such diurnal patterns in the rumen and host's metabolism. Such knowledge will indicate the time of day when nutrients can be assimilated more efficiently for both productivity and body energy expenditure. Previous researchers (Kennedy et al., 2004; Small et al., 2004) have shown that evening rather than morning feeding improves beef cattle performance. No studies have investigated the chronobiological concept of feeding time in lactating dairy cows. The lactating cow is an exceptional mammal with uniquely high levels of feed intake and milk production (as high as 6× maintenance). Any chronobiological mediation of the rumen and intermediary metabolisms will have an enormous impact on milk secretion and tissue deposition. Ruminants have evolved to

ruminate mostly overnight, when the rumen possesses a greater fermentation capacity and volume, compared to day-time. Feeding in the evening, when ruminants have evolved to ruminate, may significantly alter post-feeding fermentation patterns. There is a lack of knowledge as to whether changing the feeding time can alter post-feeding patterns in feed intake. It is unknown if feeding time can alter post-feeding patterns in rumen fermentation and 24-h patterns in peripheral blood metabolites in lactating cows. Insight into such post-feeding patterns in ingestive, digestive, and metabolic indices is required to elucidate the mechanisms whereby feed delivery time mediates productive responses in ruminants. In humans and rats, for example, regulation of glucose metabolism and insulin sensitivity has been shown to heavily depend on time of day (la Fleur et al., 2001; Van Cauter et al., 1991). Humans are unable to metabolize or handle glucose as effectively in the evening as do they in the morning. This is because glucose tolerance and insulin sensitivity decreases as day progresses and evening begins (la Fleur et al., 2003). Such crucial knowledge leads to the suggestion that large evening meals may be avoided by humans seeking a reduced risk of type-2 diabetes and its consequent cardiovascular abnormalities. As ruminant specialists, we aim to open a new horizon into the chronobiological involvement of ruminant metabolism.

The studies described in this dissertation assessed the cow response to feeding at 2100 vs. 0900 h in the areas of 1) DMI and 24-h patterns in feed intake, 2) 24-h patterns in rumen pH and concentrations of volatile fatty acids and ammonia, as well as rumen volume and outflow rate, 3) total tract nutrient digestibility and N partitioning, 4) daily averages and 24-h patterns in peripheral blood metabolites, and 5) milk secretion. The literature will be reviewed for these factors. The animal and non-animal factors affecting

each factor will be evaluated. Also, the factors will be discussed in relation to time of feeding and time in a 24-h period. For instance, the external (diet properties and feeding strategies) and internal (endocrinological) factors affecting each factor will be described. In so doing, the areas where knowledge is lacking or inadequate will be highlighted.

CHAPTER 2

LITERATURE REVIEW

1. Feed Intake

1.1. An emphasis on ruminants

Feed costs account for about half the total management costs in a modern dairy farm (Schingoethe, 2002). However, this estimate of feed cost may be underestimated. When poor choices of feedstuffs or feeding strategies are not used, costs would rise because health and longevity of cows are compromised. To meet nutrient demands of a lactating cow at a certain milk yield and body growth, we need to accurately predict its feed intake (Roseler et al., 1997). Accurate prediction of feed intake requires a deep knowledge of both animal and non-animal factors affecting feed intake (Forbes, 1995; Poppi et al., 1994). Next, the contribution of such factors to feed intake control in certain productive and environmental situations must be quantified. Many factors affecting feed intake are still largely unknown. Even the magnitude of the factors which are known requires further quantification and modification for different production scenarios (Shah and Murphy, 2006). For these reasons, the prediction of feed intake remains a challenging task in livestock (Forbes, 2005). Predicting feed intake is possibly more challenging in ruminants than in non-ruminants. A multitude of variables such as rumen pH, osmolarity, and outflow rate affect the extent and rate of nutrient use by rumen microbes. More importantly, these variables interact with each other (Forbes, 1995). The nature of these interactions could be positive, negative, or additive, and their extent would be difficult to

measure in vivo. The accurate prediction of feed intake would necessitate accurate prediction of the rumen fermentation including pH, VFA and ammonia production, and microbial protein synthesis. Therefore, it is not surprising that what nutritionists and microbiologists project to obtain in terms of microbial yield as well as VFA production from computerized feeding programs may not be achievable in many on-farm scenarios. In addition, the post-rumen digestion differs in both capacity and efficiency between ruminants and non-ruminants (Harmon, 1992, 1993; McMichael, 1971; Sutton, 1971). Diets for ruminants are much more fibrous and contain less starch than diets for non-ruminants. As such, ruminants may not have developed as a high amylolytic capacity in the small intestine as non-ruminants. When starchy diets containing corn and sorghum grains are fed particularly to high-producing cows with high intake levels, the small intestine may receive a considerable amount of partially-hydrolyzed or intact starch (Ørskov, 1986; Owens, 1986). The small intestine in ruminants, however, may debatably not be able to efficiently assimilate the starch escaping the rumen (Huntington, 1997; Owens, 1986; Ørskov, 1986). Furthermore, the small intestinal nutrient assimilation in ruminants depends on several factors such as energy and protein intake (Harmon et al., 2004). Thus, numerous rumen and post rumen variables need to be quantified accurately before feed intake in ruminants can be reliably predicted. The current thesis introduces and examines the time of feeding (2100 h vs. 0900 h) as a mediator of 24-h patterns in feed intake of lactating dairy cows.

1.2. Feed Intake Regulation

1.2.1. Animal factors

1.2.1.1. Body weight and body fat

Nutrient demands rise as BW or more accurately metabolic BW increases (Rayburn and Fox, 1993). Growth affects feed intake (Quigley et al., 1986). Unlike multiparous cows which have already achieved the adult BW, primiparous cows are still growing (NRC, 2001). Sustained growth needs a balanced profile of nutrients, notably amino acids (AA) and energy (Lawrence, 2002). Growth will, thus, be expected to affect feed intake response to nutritional treatments. Body fat is another factor controlling feed intake. Kennedy (1953) proposed that animals control BW by controlling body fat. According to this lipostatic theory, increased body fat can depress feed intake. Such an effect appears to be mediated via leptin secreted by adipocytes and may be involved in feed intake regulation (Houseknecht et al., 1998). In a recent study using growing lambs, Tolkamp et al. (2006) suggested that body condition score (BCS), as an indicator of body fatness, can improve feed intake prediction models that are based on BW. The findings of Tolkamp et al. (2006) support the original lipostatic theory of Kennedy (1953). Earlier (Makela, 1956; Tayler, 1959), body fat, particularly in abdominal region, had been thought to limit the rumen capacity and reduce feed intake. Makela (1956) found that rumen contents were negatively related to the abdominal fat size in post slaughter cows. These studies, however, were unable to prove if the inhibitory impact of body fat was mediated maninly by the physical rather than metabolic constraints. Orr (1977) noticed that even when a highly digestible diet is fed, feed intake was lower in fat animals. These data would suggest that chemical constraints associated with body fat (such as leptin) may in part explain the lipostatic theory (Houseknecht et al., 1998).

1.2.1.2. Parity and lactation stage

Maintenance nutrient requirements are about 10-20% lower in primiparous compared to multiparous cows. The lower maintenance requirement would lower maintenance nutrient intake. Thus, at comparable productivity, primiparous cows would be expected to consume about 20% less feed than would multiparous cows (Ingvartsen and Andersen, 2000).

Lactating cows experience a dip in feed intake during the periparturient phase (3 weeks before until 3 weeks after calving; Drackley, 1999) with the minimum DMI occurring at calving. The decline in DMI starts even long before the last few weeks of pregnancy (Ingvartsen et al., 1997). The energy concentration of the diet has a significant impact on DMI response to pregnancy and calving. For instance, the decreased feed intake in late pregnancy is more pronounced at higher compared to lower dietary energy levels (Coppock et al., 1972). This may be due to stronger metabolic effects of high-energy diets on DMI (Illius and Jessop, 1996).

Parity may influence postcalving patterns in DMI (NRC, 2001). Primiparous cows tend to exhibit a slow rise in DMI over about 16 weeks postpartum, compared to multiparous cows. After the peak, DMI in primiparous cows remain almost constant but in multiparous cows DMI declines continuously (Figure 1a). The differences in postcalving DMI patterns between parities may stem from the different patterns in milk yield. Multiparous cows face a higher peak in milk yield followed by a more dramatic decrease towards the end of lactation. Primiparous cows, in contrast, have a more consistent milk yield pattern throughout lactation (Figure 1b). As a result, DMI curve will change accordingly.

It has been a question whether, and to what extent, feed intake pushes milk production or milk secretion drives feed intake. The latest NRC (2001) suggested that milk production drives feed intake. The NRC (2001) based its suggestion on the increased feed intake due to increased milk yield by bovine somatotropin. The application of bovine somatotropin in early lactation stimulates the mammary nutrient uptake and milk production in advance of increasing feed intake (Etherton and Bauman, 1998). Across parities, the peak in milk yield usually occurs at about 4-6 weeks postpartum, but the peak in DMI lags to occur at 10-14 weeks postpartum (Schingoethe, 2002). During the negative nutrient balance, the high-producing cows draw from their body reserves (fat, protein, calcium) to meet nutrient requirements (Bauman and Currie, 1980). This suggests that the elevated demand for nutrients at production peak drives the cow to increase DMI. However, such a driving force does not become apparent until after several weeks of increased milk yield (Bauman, 1992). Thus, in view of the literature, the author believes that the degree to which the milk yield stimulates feed intake varies across lactation. At higher production levels, DMI response should be more pronounced. The hypothetical positive impact of a given feeding strategy (such as time of feeding) on DMI is expected to be of greater magnitude in early lactation cows.

As an aside, there is a speculation that the high-yielding cows can produce more than the low-yielding cows because they can ruminate longer. The longer rumination enables the high-yielding cows to digest the feed more effectively (Phillips and Hecheimi, 1989).

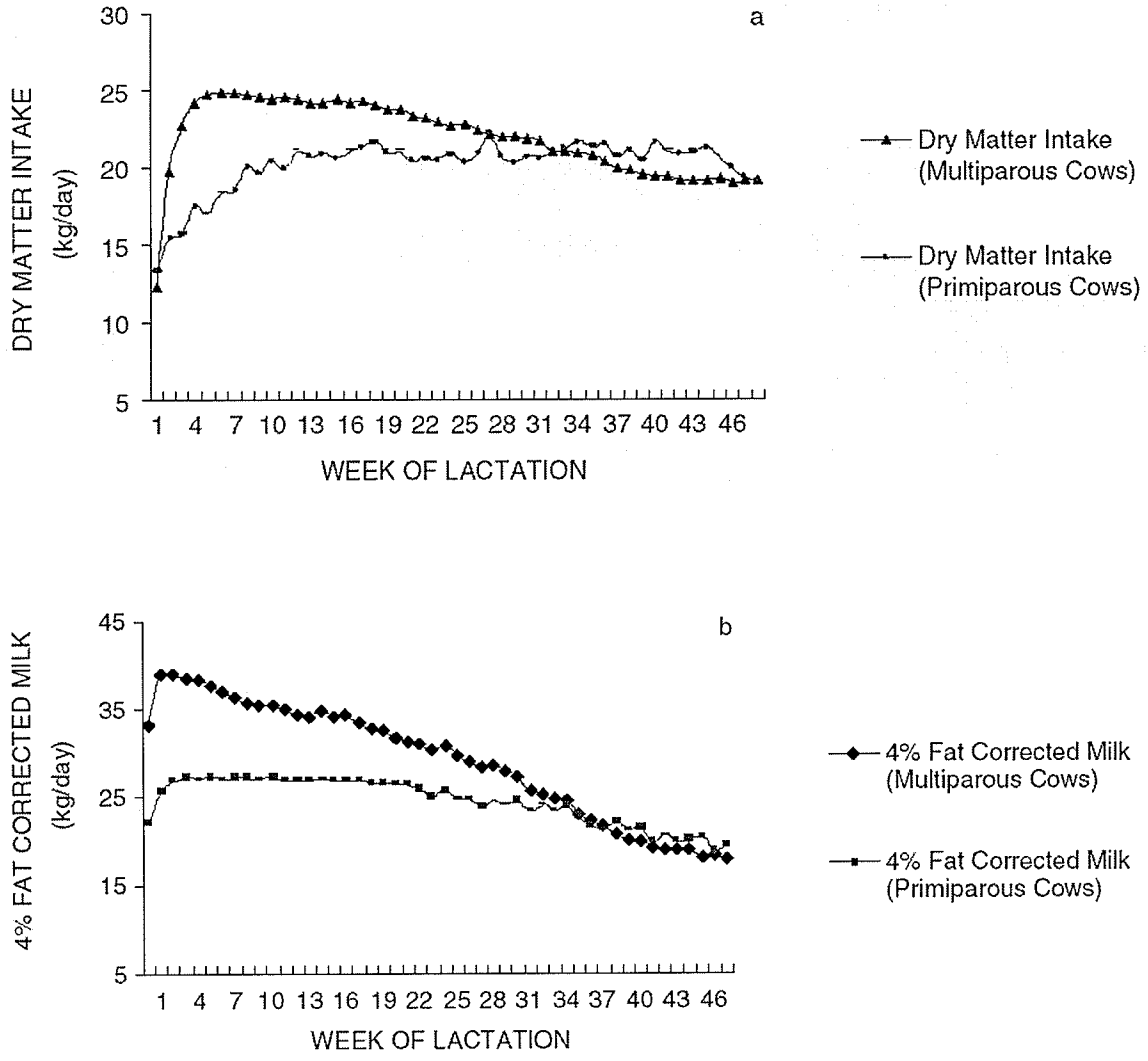


Figure 1. DMI (a) and milk yield (b) patterns of primiparous (first lactation) and multiparous (second or higher lactation) cows thoughout lactation (From NRC, 2001).

1.2.1.3. Physical and metabolic constraints

Over the last 15 years, several major physical and metabolic regulators of feed intake in ruminants have been emphasized. Ruminal fill (Allen, 1996, 2000; Forbes, 1995; Mertens, 1994) is one of the central regulators of DMI under certain circumstances such as when feeds with low digestibility are fed (Dado and Allen, 1995). The dietary

NDF, especially from forage, is a key contributor to reticulorumen fill. The greater NDF lowers the clearance rate of the rumen contents (Rayburn and Fox, 1993). Hence, the dietary NDF can be a key controller of feed intake in early and peak lactation cows that have not peaked in DMI or with limited rumen fiber pool (Allen, 2000). The NDF digestibility can significantly impact DMI (Oba and Allen, 1999). As NDF digestibility increases, the depressing effect of NDF on DMI weakens. Allen (2000) stated that DMI rose by 0.17 kg per unit rise in *in vitro* or *in situ* NDF digestibility. At higher NDF digestibilities, the NDF will have a smaller impact on rumen distension. Thus, factors affecting NDF digestibility will affect DMI.

Among the important metabolic constraints of appetite are rumen concentrations of volatile fatty acids (de Jong, 1981a; de Jong et al., 1981). Propionate injection into the portal vein has reduced feed intake in sheep (Anil and Forbes, 1980; Farningham and Whyte, 1993). Propionate rather than acetate seems to cause hypophagia (Allen, 2000). Insulin secretion (Grovum, 1995) and hepatic receptors (Anil and Forbes, 1980) have been proposed to mediate the hypophagic effects of propionate. In addition to the hepatic chemoreceptors, hepatic thermoreceptors may also control feed intake. Di Bella et al. (1981) heated the rat liver artificially and observed an increased chewing activity with reduced feed intake.

Feed intake is ultimately a psychological phenomenon integrating animal's abilities to cope with changes in diet composition and metabolic demands (Provenza et al., 1998). Thus, one must consider that the rumen or blood VFA is only one of many factors involved in feed intake (Forbes, 2005). Illius and Jessop (1996) suggested that imbalances in nutrient supply both in the rumen, postrumen, and hepatic evels can reduce

feed intake. They proposed that maximizing acetate use for lipogenesis needs a synchronous glucose supply (Figure 2). Glucose fuels lipogenesis by providing ATP and cofactors such as NADPH needed for fatty acid elongation (Illius and Jessop, 1996; Smith, 1971). Thus, even the high production rate of acetate, if accompanied by adequate supply of other nutrients, may not necessarily down-regulate feed intake. The framework of Illius and Jessop (1996) presumes that nutrient imbalances constrain feed intake via accumulation of excess metabolites such as acetate. Therefore, the animal targets a level of intake that minimizes nutrient imbalances. According to this framework, in the absence of adequate glucose, acetate will mount up and act as a hypophagic feedback.

β-hydroxybutyrate (BHBA) is another metabolite that can contribute to feed intake regulation. Subcutaneous administration of BHBA reduces feed intake in rats (Langhans et al., 1983; Scharrer and Langhans, 1990). The satiety signals may arise partly from direct oxidation of BHBA. Consequently, reducing equivalents or NADH accumulate in the mitochondria and depress feed intake (Langhans et al., 1985b). Unlike BHBA, subcutaneous administration of acetoacetate does not affect feed intake (Langhans et al., 1983). It seems, therefore, that the process of hepatic BHBA conversion to acetoacetate involving other metabolites and co-factors and not acetoacetate *per se* influences satiety (Forbes, 1995). The role of BHBA on feed intake regulation needs further research in ruminants.

Mayer (1953) was the first to suggest that blood glucose controls feed intake. Mayer (1953) indicated that the hypothalamus takes up glucose and thereby monitors and controls peripheral blood glucose. According to the Mayer's glucostatic theory, the hypothalamus controls blood glucose by controlling feed intake. Early trials with intra-

ruminal, intra-venous, or intra-cerebroventricular glucose infusion in sheep (Seoane and Baile, 1972), goats (Baile and Mayer, 1968; Baile and Mahoney, 1967) and cattle (Dowden and Jacobsen, 1960) demonstrated no effects of glucose on feed intake. Blood glucose and its diurnal fluctuations are considerably lower in ruminants than in non-ruminants (Bergman, 1983). Thus, blood glucose does not seem to be as significant in controlling feed intake in ruminants as it is in non-ruminants (Baile and Della-Fera, 1981). This is not surprising, because due to the extensive rumen fermentation of dietary carbohydrates, VFA and not glucose are the main digestion end-products absorbed across the gut in ruminants (Sutton, 1971). When high-starch diets containing corn and sorghum grains are fed, however, the amount of intact or partially hydrolyzed starch escaping the rumen may increase (Nocek and Tamminga, 1992). The intestinal starch and the resulting glucose may affect feed intake. The role of the absorbed glucose across small intestine on feed intake regulation requires has not been elucidated.

1.2.1.4. Hormones

Nutrient partitioning is mediated by a variety of hormones (Bauman and Currie, 1980). Hormones are involved in both short-term and long-term (Allen, 2000; Ingvartsen and Andersen, 2001) regulation of feed intake. The greatest fluctuations in body metabolism, nutrient partitioning, and feed intake usually occur around calving when levels of metabolic and reproductive hormones are highly variable in periparturient cows (Drackley et al., 1991; Ingvartsen and Andersen, 2001).

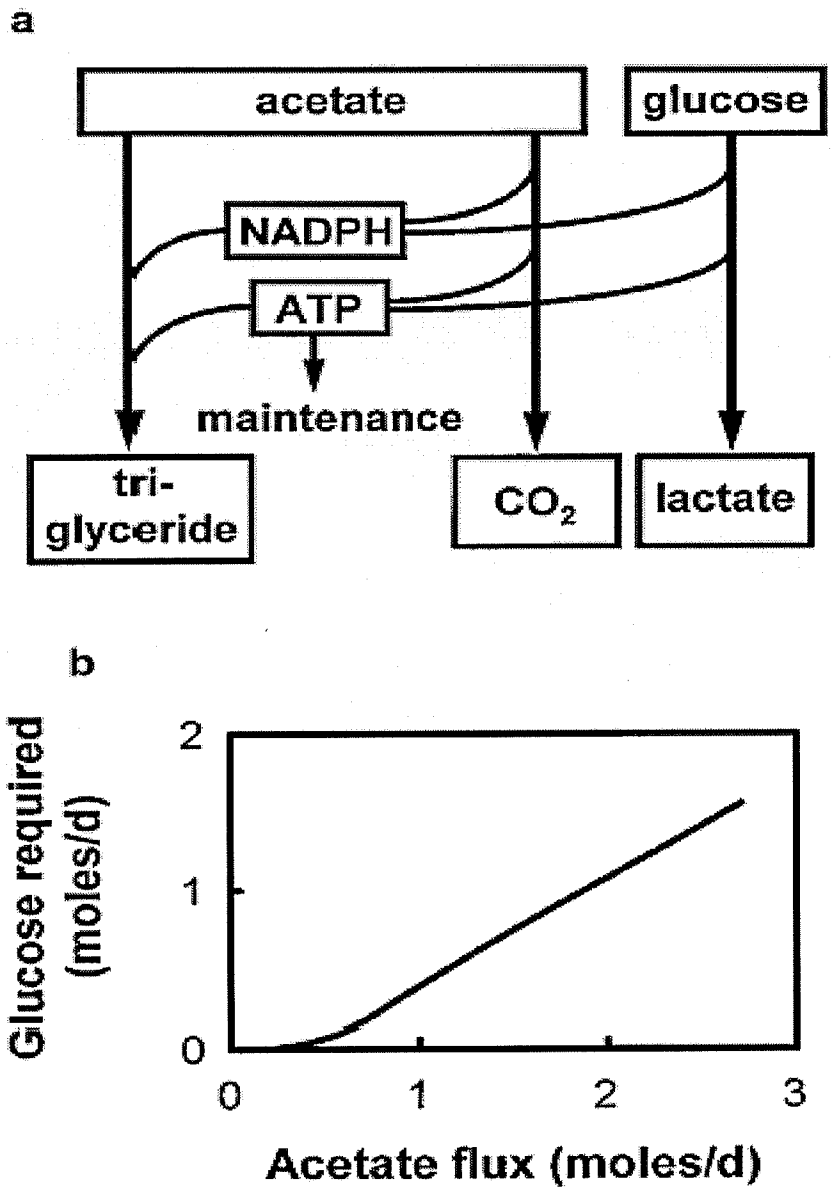


Figure 2. The effective acetate use by peripheral tissues requires adequate glucose. Pathways of acetate clearance (a) and glucose requirement for lipogenesis (b).
(From Illius and Jessop, 1996)

Estrogen depresses feed intake (Grummer et al., 1990) by acting primarily on the paraventricular nucleus of the hypothalamus (Butera and Beikirch, 1989). Insulin is another important hormone possessing both long-term and short-term effects on nutrient partitioning and feed intake (Brockman, 1976; Brockman and Laarveld, 1986). The long-

term roles of insulin in feed intake control relate to pregnancy and lactation (Bauman, 2000; Bauman and Currie, 1980). This occurs mainly during mid- and late-lactation when the cow tends to gain weight. Insulin is involved in both up- and down-regulation of feed intake in mammals. When compared to prepartum levels, insulin secretion drops substantively shortly after parturition (Ingvartsen and Andersen, 2001). Without the postpartum drop in insulin secretion rate, the cow would be unable to use body reserves and deal with insufficient DMI. The low postpartum insulin will additionally enable the cow to gradually increase DMI.

The short-term insulin effects on nutrient metabolism and partitioning, which are more relevant to the findings of this dissertation, initiate upon or even shortly before feeding or nutrient ingestion (Bassett, 1975). Seeing the fresh feed can cause a surge in insulin secretion in sheep and cattle (Bassett, 1975; Faverdin, 1986a; Vasilatos and Wangness, 1980). The higher postprandial insulin surge leads to greater glucose uptake by peripheral tissues. By increasing the peripheral glucose uptake, the postprandial insulin secretion may contribute to satiety (Anika et al., 1980). A higher postprandial insulin secretion and thus the increased peripheral glucose use may induce satiety signals. In contrast, insulin can stimulate feed intake in response to insufficient nutrient supply (Even and Nicolaidis, 1986). Insulin has also been reported to be associated with overconsumption in the rat (Brandes, 1977). It might be possible that the postprandial rise in insulin secretion in high-producing cows demanding much energy and N to sustain milk yield, may not necessarily depress DMI. Instead, due to high nutrient demand by the mammary gland, the postprandial insulin secretion might facilitate nutrient uptake by increasing feed intake.

Although studied in humans in as early as 1955 (Stunkard et al.), the role of glucagon in feed intake regulation in ruminants has not been delineated (NRC, 1987; Ingvartsen and Andersen, 2001). Intravenous glucagon induced satiety in humans. In contrast, intraperitoneal glucagons stimulated glucogenolysis but did not affect feed intake in rats (Geary and Smith, 1982a). Glucagon stimulates hepatic glucose production via both glycogenolysis and gluconeogenesis (Brockman, 1976). The hepatic glucose release does not appear to be the exclusive pathway whereby glucagon may affect satiety (Geary et al., 1981). For example, Geary and Smith (1982b) showed that increased blood glucose did not end the meal. The peritoneal use of rabbit glucagon antibodies in rats to reduce gluconeogenesis and blood glucose increased feed intake (Langhans et al., 1982). These data show that reduced blood glucose can induce hunger, but increased blood glucose may not induce satiety. Also, some evidence exists that exogenous glucagon reduces feed intake in sheep (Deetz and Wangness, 1981). Thus, the effect of glucagon on feed intake control seems to be mediated by other agents than only glucose. Future research is needed to elucidate the impact of glucagon on feed intake in dairy cows.

1.2.2. Dietary factors & feeding strategies

1.2.2.1. Physical and chemical properties of dietary fiber and forage to concentrate ratio

The diet fibre content can be defined by the dietary level of the cell wall (Blaxter et al., 1961) or more informatively by the cell wall and its physical properties (Mertens, 1997). The physical properties of dietary fiber include, but are not limited to, the cell contents such as starch, pectins and sugars, also fiber bulkiness, and its chemical integration with indigestible portions such as lignin and condensed tannins (Van Soest,

1994). Forage type, maturity at harvest, and post-harvest treatment can affect the physical and chemical properties of forage fiber. Khorasani et al. (2001) fed late-lactation cows either alfalfa silage or bromegrass silage at either 65 or 50% dietary inclusion rate. Feeding the diets with higher (43% NDF) compared to lower (40.5% NDF) F:C tended to reduce DMI. Robinson and McQueen (1997) manipulated fiber fermentability by feeding high and low quality alfalfa silage in midlactation cows. Fiber intake increased and DMI tended to increase as the TMR fiber fermentability increased. This shows that NDF fermentability affects DMI and, hence, can be used to predict DMI. Moreover, the interaction of dietary fiber and the stage of lactation impacts DMI. Friggens et al. (1998a) fed lactating cows a higher concentrate diet formulated to meet their nutrient requirements and a lower concentrate diet designed to limit energy intake. The intake of the higher concentrate diet depended on milk yield. The intake of the lower concentrate diet, however, did not change throughout lactation. This probably suggests a link between dietary concentrate level, and likely rumen capacity, with DMI (Conard et al., 1964). Knowledge of quantitative relationship between the dietary F:C and DMI is required before the F:C can be used to predict DMI at different stages of lactation. The impacts on DMI of diet physical properties must also consider fiber digestibility, animal production level, and lactation stage.

1.2.2.2. Feeding frequency & sequence

Feeding frequency (FF) has received a great deal of attention by ruminant nutritionists (Nocek, 1987; NRC, 2001). A multitude of factors such as dietary F:C, TMR vs. component feeding, cereal grain type, processing method, cow productivity, ambient

temperature, and dietary use of bST and their interactions mediate cow response to FF. Once the individual and interactive effects outlines above are determined, animal response to FF may be quantified.

French et al. (1990) showed no effects of feeding dietary concentrate either 2 or 12 times daily on DMI in early lactation cows. Dlivery of either a cubed or uncubed ration two or four time daily did not affect DMI in midlactation cows (Klusmeyer et al., 1990). Shabi et al. (1998) reported no differences in DMI of cows fed twice and four times daily. In another study, Shabi et al. (1999) found that DMI increased by four times compared to twice delivery of a corn-based TMR. They attributed the higher DMI of the more frequently fed cows to greater total tract DM digestibility. Dhiman et al. (2002) observed no significant effects of four times vs. once daily delivery of a corn grain-based TMR on DMI of midlactation cows. Monitoring the 24-h patterns in feed intake may help explain the underlying mechanisms of DMI response to FF. Recently, DeVries et al. (2005) showed that increasing FF from once to twice or from twice to four times daily increased average eating time per cow in group-fed lactating cows. The more frequently fed cows had a more even distribution of eating over the 24-h period. Also, feed sorting was reduced by the more frequent feed delivery. This may enhance fiber digestibility (Ørskov, 1999). Nevertheless, the eating activity reported by DeVries et al. (2005) was based on the number of cows present at the feed bunk and not the amount of feed consumed within a certain time interval by individual cows. Therefore, it remained unknown if FF affected DMI.

Reduced 24-h variation in eating may reduce 24-h variations in rumen pH (French and Kennelly, 1990; Shabi et al., 2002), which may reduce the risk of subacute rumen

acidosis and increase fiber digestibility (Nocek, 1987; Ørskov, 1999). However, before such a theoretical cascade turns into an on-farm reality, the more frequent feeding must prevent or reduce the time during which the rumen pH drops to the detrimental point to microbial metabolism. In the current thesis, the post-feeding patterns in rumen pH will be evaluated in 2100 h-fed and 0900 h-fed cows. This could determine if the post-feeding patterns in rumen pH depend on feed delivery time.

The feeding sequence of forage and concentrate is another variable affecting feed intake (Nocek, 1992; Robinson, 1989). Nocek (1992) observed a tendency for increased DMI when a mixture of grain and protein meal was delivered at 0700 h followed by forage delivery at 1000 h, compared to when forage was delivered first. Usually, offering forage prior to concentrate early in the morning is believed to form an amply developed rumen fiber mat, which can in turn stimulate salivation (Robinson, 1989) and may reduce the risk of subacute rumen acidosis (Voight et al., 1967). As a result of the controlled rumen pH, DMI may not drop (Allen, 2000). Again, before the effect of feeding sequence on DMI can be observed, the detrimental low rumen pH must occur.

1.2.2.3. Time of feeding (TF)

There is a lack of literature on the effect of TF on 24-h patterns in feed intake and feeding behavior of once-daily fed cows under normal ambient temperatures. Also, data on DMI response to TF under thermoneutral ambient temperatures is very scarce. Robinson et al. (1997) fed lactating cows 67% of the TMR at 0800 h and 33% of it at 1800 h. In addition they fed a protein supplement (about 15% of estimated DMI) at either 0830 h or 0030 h. Feeding the protein supplement at 0030 h instead of 0830 h increased

DMI of the protein supplement and numerically increased total DMI (16.92 vs. 15.94 kg/d). The numerical increase in DMI was associated with significant increases in rumen DM, OM, and CP digestibility. It was suggested that the midnight compared to morning delivery of the protein supplement stabilized rumen fermentation. Aharoni et al. (2005) fed lactating cows under heat stress four times during either the day or in the evening. The day-fed cows received the diet 30% at 0615 h, 20% at 1000 h, 25% at 1530 h, and 25% at 1900 h; whereas the evening-fed cows received the diet 20% at 0615 h, 30% at 1530 h, 25% at 1900 h, and 25% at 2100 h. Evening-fed group had no access to feed for 5.5 h during the day. Limiting the feed access during the day and shifting the majority of eating time to the evening hours reduced both DMI and energy expenditure. Under cold weather, feeding at 2000 h instead of 0900 h improved growth rate in beef steers without affecting DMI (Small et al., 2004). Kennedy et al. (2004) fed heifers at either 0900 or 2000 h in winter (<-15°C) and found no significant effects of TF on DMI. Evening feeding, however, improved feed efficiency during backgrounding period, compared to morning feeding. Ominski et al. (2002) exposed cows to 5-d phases of thermoneutral (24°C during the day and 20°C overnight) and heat stress (32°C) conditions followed by a 5-d recovery period. In the stress period, the temperature rose steadily up to 32°C between 0700 to 1000 h, remaining at 32°C until 1800 h, but it was reduced down to 20°C for the rest of the 24-h period. Despite no overall effect, DMI in the evening-fed cows was reduced during the heat stress phase (Ominski et al., 2002). Altogether, the DMI response to TF in the above-mentioned studies was most probably due to the changes in animal thermodynamics. Thus, it remains unknown if TF can control feed intake under thermoneutral conditions.

1.2.3. Diurnal patterns in ingestion behavior

1.2.3.1. Feed ingestion

Grazing occurs mainly around sunrise and sunset. In grazing sheep, the maximum jaw movement occurs in the late afternoon before sunset (Champion et al., 1994). Dairy cows exhibit three main grazing bouts of dawn, afternoon, and dusk (Rook and Huckle, 1997). However, the diurnal patterns in grazing appear to vary across seasons. In Western Australia with hot summers and mild winters, Arnold (1984) observed that the main summer grazing activity occurred during early morning and late afternoon with a smaller activity around midnight. Sheep and cattle had comparable grazing patterns in summer. In winter, however, the cattle showed intensive grazing activities not only in the morning and afternoon but also during the period from 2000-0100 h. Unlike in summer, in winter sheep had a progressive grazing activity from 0600 through 1700 with low activity in the evening. Under intensive feeding systems, dairy cows (Gottardo et al., 1999) and finishing bulls (Cozzi and Gottardo, 2005) fed only once daily in the morning exhibited two peaks in eating activity. The greatest one was right after feeding and another around dusk. The eating/grazing pattern has circadian patterns. Chilibroste et al. (1997) et al. (1997) allowed the cows to graze at 0900 h after an overnight starvation. The post grazing rumen DM pool size was much smaller than the afternoon rumen DM pool size of the previous day. Thus, cows seemed to stop the morning eating before reaching the maximum rumen capacity. The observation that rumen capacity peaks at dusk but not during the morning and afternoon grazing has been shown by others, as well (Thiago et al., 1992). Recently, Taweel et al. (2004) found that time spent eating during the dusk grazing bout was longer than that during the dawn and afternoon bouts. Moreover, during

dawn and afternoon bouts, the cows finished eating before reaching a rumen capacity which was reached during the dusk bout. Therefore, they concluded that the rumen fill has probably a more significant role in regulating the dusk feed intake compared to dawn and afternoon feed intakes. Cozzi and Gottardo (2005) showed that despite feeding adequate coarse corn silage as 44.9% of diet DM, finishing bulls selected the coarse particles for as long as 16-h post-feeding. It was thus concluded that such a late selection of the more structured TMR particles was an effort to optimize the intake of long roughage particles. In a beef study, Gregorini et al. (2006) showed that the herbage allocation time (0700 h vs. 1500 h) altered daily patterns in eating, ruminating, and idling behaviors in heifers. The heifers turned unto the ungrazed strip at 0700 h had more intense evening grazing with faster bite rates compared with the heifers allotted the herbage at 1500 h. The morning grazing was 36-39% of total daily grazing time in the latter group but only 25-28% in the former group. The nutritional status of the animal before feeding can affect the post-feeding eating intensity (Newman et al., 1994). Fasting, for instance, hastens eating by increasing the bite rate (Dougherty et al., 1989) and enlarging the bite mass (Newman et al., 1994). However, the data on the effects of feeding strategies on diurnal patterns in feed intake of non-grazing ruminants is scarce.

The large evening meals in grazing cows have partly been linked to an optimal foraging strategy (Rook et al., 1994; Penning et al., 1991; Taweel et al., 2004). The plant content of DM and water soluble carbohydrates rises and NDF decreases as day progresses (Burns et al., 2005; Orr et al., 2002; Taweel et al., 2006). The accumulation of highly-digestible nutrients in the evening is due to the daylight photosynthesis in plant leaves (Delagarde et al., 2000). This may partly explain why cattle, sheep, and goats

prefer the fresh forage grass and legumes harvested at sunset compared to that harvested at sunrise (Fisher et al., 1999, 2002). Burns et al. (2005) reported a higher in vitro true DM digestibility for PM-cut than for AM-cut alfalfa hay. Goats had greater DMI and greater total tract DM digestibility when they were offered the sunset-cut alfalfa hay instead of the sunrise-cut alfalfa hay. The grazing ruminants appear to learn the time of maximum plant nutrient quality, leading to a more intensive eating in the evening than in the morning (Taweel et al., 2004).

The lower ambient temperature at night than during the day may also contribute to the larger evening meals in grazing ruminants (Orr et al., 1997; Taweel et al., 2006). Additionally, ruminants attempt to optimize their nutrient intake profile by filling the rumen in the evening because usually little grazing occurs overnight (Penning et al., 1991). In so doing, microbial growth in the rumen would sustain by the time the morning meal would start. Ruminants have evolved adaptive patterns in ingestion, rumination, and digestion behaviors (Senn et al., 2000). Grazing has evolved to occur mostly during the day, particularly around sunset and sunrise, whereas rumination occurs mostly at resting time or overnight. Therefore, despite being incorporated into the intensive production systems, ruminants are still responsive to the signals induced by the environmental synchronizers such as light turn on and off as well as feed quality and availability.

The eating rate of both concentrate and hay in ad libitum-fed goats was higher during the dark period than during the light period. Also, goets perefered concentrate during dark rather than the light period (Senn et al., 1990). Thus, factors regulating feed intake may be of different type and magnitude between dark and light periods. Likewise, Durst et al. (1993) reported a nocturnal appetite for concentrate. In another study using

pygmy goats (Rossi and Scharrer, 1992), the meal size was associated with water intake more around the light-phase meals than the dark-phase meals. This shows that the factors affecting feed and water intake are of unequal type and magnitude between day and night (Johnson and Johnson, 1991). Senn et al. (1995) evaluated diurnal feed intake patterns in loose-housed lactating cows of the Holstein-Friesian, Simmental, and Jersey. Cows were fed once daily a diet composed of hay, corn silage, and grass silage in the morning. Regardless of the breed, all cows exhibited diurnal patterns in their eating activity with the maximum activity occurring between 0800-1300 h. Cows showed a night-time preference for the high-energy portion of the diet or grass silage. These authors stated that during the night ruminants prefer energy dense food. In a previous trial by the same group, Durst et al. (1993) found that cows preferred to eat more of the concentrate overnight. These findings implied that short-term, post-meal regulation of feed intake differ between day and night. As day progresses towards night, the cow faces a more negative nutrient balance, and thus, attempts to consume the highest energy in the shortest time (Senn et al., 1995).

The light-dark cycle is an important part of the animal environment (Piccione and Giovanni, 2002). In pigs, feed ingestion was shown to peak around the light turn-on and turn-off (Feddes et al., 1989). Turning lights on and off seems to act as an inducer, thus stimulating eating activity. This implies that animals can anticipate the time around which the lights are turned on and off. Feddes and DeShazer (1988, cited by Feddes et al., 1989) also showed that under fluctuating ambient temperatures, eating rate was the highest around 0500 h and 2100 h when lights were switched on and off, respectively. When the ambient temperature was kept constant over the 24-h period, the pigs ate most

intensely within 2-h after light-on and 3-h before light-off. This shows that diurnal variation in ambient temperature affect light-mediated feed intake. The fact that ruminants eat and graze most intensively around sunrise and sunset indicates that light intensity or wavelengths are important in stimulating feed intake (Linnane et al., 2001). The intense evening eating may be an interactive response to several factors rather than being solely related to light intensity. The anticipation of a non-eating or resting phase (overnight) could motivate the animal to fill the rumen to avoid hunger later during the night. Moreover, the more favorable environment reflected in higher herbage quality in the evening than in morning, feed preference, and social facilitation contribute to the intensive evening eating in grazing ruminants (Linnane et al., 2001; Provenza et al., 1998). The interaction between light intensity and the herbage quality may induce grazing beyond that achievable during the morning. As a result, the maximum rumen fill may not be achieved during the day. This emphasizes the notion that rumen fill is only one of a multitude of factors regulating feed intake (Linnane et al., 2001). Diurnal patterns in rumen digesta kinetics are still largely unexplored in non-grazing ruminants, particularly in response to feed delivery time.

1.2.3.2. Rumination

Rumination is associated with resting which occurs when the ruminant is relaxed. Cows usually ruminate between 6-8 h daily (Phillips, 2002). Rumination takes place mostly overnight, between 2000 h and 0800 h when little eating/grazing occur (Hancock, 1954; Gordon, 1958). The most intensive rumination occurs between 2200 h and 0600 h in day-fed animals (Phillips, 2002). Gordon and McAllister (1970) fed sheep twice daily

at 12-h intervals, exposed them to 12-h light and 12-h dark period, and observed diurnal rhythmicity in rumination. Thus, light did not seem to be a major cue affecting diurnal variation in rumination. The key factors regulating the initiation and cessation of rumination under different production systems yet to be fully described (Fraser and Broom, 2002). Gordon (1958) showed that rumination in sheep possessed diurnal patterns without a clear dependence on TF. In sheep fed at 1200, 2000, 0000, and 0800 h, the maximal rumination occurred in the early morning (0400-0800 h) and the minimum during the afternoon. The sheep fed at 0400 h and 1600 h peaked in rumination between 0000 h to 0400 h. When TF coincided with the time of greatest ruminating activity (0400-0800 h), feeding inhibited the rumination.

The rumen contents and rumination duration have been shown to be positively correlated in dairy cows (Lindstrom and Redbo, 2000). The greater rumen contents or greater rumen fiber load may stimulate bolus formation required for rumination. This stimulatory impact would be more pronounced when the meal is more fibrous because it can increase the intake of physically effective fiber and stimulate chewing activity. Welch and Smith (1968) offered rams an increasing amount of hay as either a single meal or continuous feeding. In both feeding systems, increasing the hay intake increased the rumination time. However, the positive response in rumination time to the hay intake was linear in single meal-fed rams but curvelinear in rams with continuous access to feed. Despite changing diurnal rumination patterns, shifting herbage allocation time from 0700 h to 1500 h did not affect total daily rumination time. However, the rumination and idling (no eating or ruminating activity) occurred mostly in the morning and afternoon after shifting grazing time from morning to the evening (Gregorini et al., 2006). Thus, feeding

time at 0700 h vs. 1500 h entirely changed diurnal rumination patterns without affecting total daily rumination time. Such information is lacking for cows fed once daily under intensive production systems.

2. Digestion

2.1. Diurnal patterns in rumen and post rumen environments

Feeding causes a major rapid increase in rumen osmolality within as little as 1-2 h. The increased osmality is caused by excessive introduction of ions and to some extent VFA, which is followed by a peak in rumen volume within 2-3 h (Ternouth, 1967). Diet fermentability and feed intake rate are two key factors affecting diurnal patterns in extent and rate of rumen metabolism (Lewis and McDonald, 1958), and subsequently the post rumen digestion (Harmon, 1993). Diurnal variations in rumen fermentation are expected to occur in parallel to the diurnal variation in the population and activity of rumen microbes (Michalowski, 1975). Researchers in New Zealand (D. P. Poppi, The University of Queensland, Australia, personal communication, 2006) observed noticeable diurnal variations in rumen pool and outflow rate in grazing sheep. Using grazing lactating ewes, Dove et al. (1988) reported 30% greater flow of abomasal digesta DM and OM during 2200-0200 h, compared with daily average. Also, Corbett and Pickering (1983) showed that diurnal patterns exist in rumen fill and abomasal digesta flow. Such consistent diurnal patterns in rumen fill and abomasal digesta flow have been attributed to diurnal patterns in rumination (Corbett and Pickering, 1983) and feed intake rate (Thomson et al., 1985). As indicated earlier, herbage becomes richer in nutrients as the day progresses. The more nutritious and palatable herbage in the evening than in the morning may

stimulates grazing/eating (Orr et al., 1997). This might partly explain why the rumen capacity in the evening faces a maximum that is usually not achieved during the day. Such diurnal patterns in herbage quality, eating rate, rumen fill, and digesta flow would, thus, contribute to diurnal patterns in nutrient delivery to the portal and subsequently to the peripheral blood. For instance, in ewes grazing perennial ryegrass, the abomasal flow of DM and non-ammonia N exhibited a 20-30% increase between 2300 h and 0300 h, when compared to the average daily flow (Dove et al., 1988). Under intensive feeding systems, a major increase in VFA production is usually observed shortly post-feeding, which is caused by increased microbial activity (Robinson et al., 1997; Rodriguez et al., 1997). However, it is not known if such post-feeding patterns in rumen fermentation depend on when the fresh feed is delivered to the ruminant.

2.2. Diurnal patterns in rumen metabolism

In once-daily fed cows, the rumen ammonia rises sharply within 1 to 3-h post-feeding, returning to the prefeeding level in 2-4 h (Gustafsson and Palmquist, 1993). With high dietary CP (19%), the rumen ammonia may not fall until shortly before the next day's feed delivery (Pereira and Armentano, 2000). In twice-daily fed cows, the post-feeding rise in the rumen ammonia occurs after each feed delivery (Robinson and McQueen, 1994). The rumen VFA rise gradually and peak at about 4-12 h post-feeding when the rumen pH is in its nadir (Nocek et al., 2002). The higher but shorter-lasting post-feeding surge in rumen ammonia than that in VFA is expected because the readily-fermentable carbohydrates stimulate microbial growth, leading to rapid N uptake. While the rumen ammonia is being assimilated into the microbial mass, the fermentation of the

more slowly degradable carbohydrates persists. As a result, the rumen VFA production continues to rise long after ammonia declines. As such, diurnal patterns in post-rumen flow of non-ammonia-N (Dove et al., 1988) and microbial amino acids (Robinson et al., 2002) are expected to change. Robinson et al. (1997) offered dairy cows a mixed diet twice a day with 67% offered at 0800 h and 33% at 1800 h. Also, they offered a protein meal equal to about 15% of DMI at either 0830 or 0030 h. Rumen pH was higher in the afternoon but lower in the night in cows receiving the protein meal at 0030 h than in cows receiving the protein meal at 0830 h. Rumen ammonia followed an opposite pattern. So, diurnal variations in rumen pH and ammonia were reversed with evening provision of the protein meal. Except for isobutyrate, the concentrations of rumen VFA remained higher for most of the 24-h period when protein meal was fed at midnight compared to when it was fed in the morning. The average daily levels of rumen acetate, propionate, butyrate, and total VFA were higher and ammonia was lower when the protein meal was fed at 0030 h instead of at 0830 h. With treatments comparable to those of Robinson et al. (1997) but in a 16-week lactation study, Moshtaghi Nia et al. (1995) found that rumen ammonia was lower between 0800 to 1100 h but higher between 0400 to 0800 h when the protein meal was offered at 0030 h instead of 0830 h. Diurnal patterns in rumen pH and VFA and their average daily concentrations did not show significant differences between groups. In grazing, lactating cows under a continuous stocking system on grass sward, Taweel et al. (2004) demonstrated that rumen acetate, propionate, and butyrate peaked around midnight. Rumen pH was lower at midnight than in the morning. Also, the rumen fiber pool was larger in the evening than during the day. Rumen ammonia exhibited two peaks at 1230 and 2230 h. These data suggest that the

rumen encounters a more extensive fermentation during the night-time. This implies that evening feed delivery time may offer opportunities to enhance nutrient use in the rumen and ultimately by the host ruminant.

3. Metabolism

3.1. Diurnal patterns of metabolites across portal-drained viscera, splanchnic tissues, and peripheral blood

3.1.1. Chronobiological definitions

Almost all forms of life have evolved to exhibit the physiological and behavioral patterns that are coordinated with their surrounding environment (Sehgal, 2004). This means that almost all biological processes such as biochemical reactions demonstrate patterns recurring at particular time intervals. Many principal rhythms of life are circadian which occur within approximately a 24-h period (Piccione and Giovanni, 2002). For instance, rest and activity, body temperature, digestive enzymes, and blood concentrations of some hormones and metabolites possess circadian rhythms (Sehgal, 2004; Van Cauter, 1990). Such rhythms are self-maintained and persist even in non-rhythmic environments. The circadian rhythms are endogenous driven by the biological clocks located in the hypothalamus and in the liver (Piccione et al., 2003). However, this should not leave the impression that external stimuli cannot affect the circadian rhythms but rather the circadian rhythms do not necessarily need any external cues to persist. There is an incorrect, interchangeable use of 'circadian' and 'diurnal' in the literature. The 'circadian rhythms' are by definition endogenous which recur over almost a 24-h period and do not require an external cue such as light intensity or feed delivery to be

sustained. The diurnal rhythms, in contrast, are 24-h rhythms which are easily altered by external factors and may not persist in the absence of an external cue (Sehgal, 2004; Piccione and Giovanni, 2002; Piccione et al., 2003). By altering the feeding time and photoperiod, one is very likely to alter the diurnal rhythms but not necessarily the circadian ones. Therefore, the potential exists to synchronize the occurrence of diurnal rhythms with that of circadian rhythms to manipulate nutrient use efficiency and partitioning, and thereby, to optimize nutrient metabolism. Using goats, Piccione et al. (2003) showed that daily rhythms of blood cholesterol persisted in the absence of once-daily feeding and did not respond to a 6-h advancement of feeding time. Such information is required as to other blood metabolites and hormones to evaluate the chronobiological significance of feeding time in nutrient partitioning and metabolism of high-producing ruminanys such as lactating cows.

3.1.2. Portal-drained viscera and splanchnic levels

The portal blood carries metabolites drained mostly from the rumen and the small and large intestines. These organs plus pancreas, spleen, and the associated fat and muscle tissues are called portal-drained viscera (PDV) (Lindsay and Reynolds, 2005). The PDV and liver form splanchnic tissues. Despite their smaller size relative to the rest of the body, the splanchnic tissues contribute to approximately 22-50% of total body oxygen use in cattle (Reynolds, 2002; McBride and Kelly, 1990). Therefore, the PDV as the major site of nutrient digestion can influence diurnal patterns in nutrient delivery to the portal vein. The liver will thus encounter diurnal patterns in the input of propionate, AA, lactate, and BHBA. Ortigues et al. (1996) showed that diurnal patterns in portal

blood metabolites occur in response to the diurnal patterns in feed intake, rumen fermentation, and post-rumen nutrient digestion and absorption. Except for propionate and ammonia, the liver has limited capacity for the uptake and metabolism of other metabolites such as acetate, lactate, and BHBA (Hueter et al., 1956; Armentano, 1992). As a result, diurnal patterns in the concentrations of acetate, lactate, and BHBA in the peripheral blood will most likely reflect the diurnal patterns in their portal concentrations (see Sutton et al., 1988).

3.1.3. Peripheral blood

3.1.3.1. Glucose

Blood glucose is known to vary diurnally in humans (Van Cauter, 1990). The diurnal variation in human blood glucose is attributed to diurnal patterns in 1) feed intake, 2) hepatic gluconeogenesis, 3) glucose tolerance, and 4) blood hormones such as adrenalin, glucagon, and coticosterone (la Fleur, 2003). Altogether, the diurnal variations in blood glucose of humans and rats are orchestrated by the internal clock located in the suprachiasmatic nucleus of the hypothalamus (Cailotto et al., 2005). For instance, blood glucose rises just before the beginning of the activity period, which is the early morning in humans and evening in rats. The early morning rise in blood glucose is recognized as "down phenomenon" occurring in non-diabetics (Arslanian et al., 1990). Such an early morning rise in blood glucose may be an arrangement by the suprachiasmatic nucleus preparing the body for the forthcoming activity period (la Fleur et al., 2001). Glucose tolerance or insulin sensitivity have been shown to decrease as day progresses, reaching a nadir around midnight (Van Cauter, 1990). The diurnal regulation of glucose metabolism

is meal-independent in humans (Van Cauter et al., 1989) and rats (la Fleur et al., 2001). When compared to humans, blood glucose is lower in ruminants (Bergman, 1983) partly because dietary carbohydrates undergo rumen fermentation before facing abomasal and intestinal assimilation. As a result, little intact or partially hydrolyzed soluble carbohydrates (5-29% of that consumed; Huntington et al., 2006; Sutton, 1971) enter the duodenum. Assuming an arrival of 1 kg starch in the small intestine, less than 60% has the potential to be fully digested before entering the large intestine (Huntington et al., 2006). In addition, the gut uses a considerable amount of glucose (Huntington and Reynolds, 1986). Thus, the glucose absorbed via the small intestine may not contribute to more than 20% of net hepatic glucose output (Wieghart et al., 1986).

Usually, about 20-35% of the total VFA produced in the rumen is propionate (Sutton, 1971). Ruminants rely on gluconeogenesis mainly from propionate and to a variable extent from AA (alanine and glutamine), lactate, and glycerol to meet their glucose demands (Danfær et al., 1995; Huntington, 1997). Gluconeogenesis occurs mostly in the liver and some in the kidney (Mayes, 2000). In non-ruminants, feeding can cause a rapid surge in the intestinal glucose absorption, and therefore, an abrupt glucose appearance in the peripheral blood (McMichael, 1971). Post-feeding blood glucose response is expected to be higher and earlier in non-ruminants than in ruminants. Apart from substrate availability, the post-feeding blood glucose response to feed delivery is regulated by the interactive effects of hormones such as insulin and glucagon.

In early lactation, twice-daily fed cows offered forage and concentrate separately exhibited a rise in blood glucose at 2.5-h post-feeding. The post-feeding rise in blood glucose was progressive and peaked at 7-h post-feeding (Eicher et al., 1999). The first

feed delivery time was 0630 h but the time of the second feed delivery was not reported. In the same paper but in another study, Eicher et al. (1999) found a significant post-feeding rise in blood glucose in cows fed roughage twice-daily and a concentrate <6 times daily. Furthermore, the blood glucose dropped between 1100 to 1330 h or between 5 to 7.5-h post-feeding. The first feed delivery was at 0600 h but the times of the other feed deliveries were not reported. These authors sampled the blood only four times daily at 0830, 1100, 1330, and 1600 h, which may not be enough for a complete monitoring of diurnal variation in blood metabolites. Sutton et al. (1988) offered lactating cows a diet with either 70 or 90% concentrate (DM-basis) twice daily at 0600 and 1645 h. Starting to decline before feeding, blood glucose continued to fall after each feed delivery. Meanwhile, blood insulin rose sharply. In cows fed the hay twice and the concentrate six times daily, such pronounced patterns in blood glucose were absent (Sutton et al., 1988). These data may suggest that the more frequent feeding reduces the net splachnic flux of propionate and lactate, and in so doing, may prevent a significant rise in hepatic glucose output. In beef cows, Coggins and Field (1976) offered about 5 kg silage at 0700 h and the rest of the diet at 1330 h. Blood glucose began to fall at 0900 h and declined further upon feed delivery at 1330 h hitting the nadir at 1700 h. Then, it went up overnight to reach the baseline at 0900 h. In another dairy study, Oba and Allen (2003) fed lactating cows two diets with about 32 and 21% starch (DM-basis) once daily at 1400 h. Blood glucose dropped upon feeding until 4 h post-feeding when it was at its nadir. At the same time, blood insulin rose to a peak at about 5-6 h post-feeding. The decline in blood glucose shortly before and after feeding in ruminants has been reported by others (Hove and Blom, 1973 in dairy cows; Bassett, 1974 in sheep; de Jong, 1981b in goats).

3.1.3.2. Urea

Blood urea has been shown to increase shortly post-feeding in goats (Piccione et al., 2003), dairy cows (Gustafsson and Palmquist, 1993), and beef cows (Coggins and Field, 1976). Lefcourt et al. (1999) demonstrated a diurnal rhythm for blood urea in lactating cows fed once daily at 0900 h. Blood urea peaked at 1100 h (2 h post-feeding) and then dropped until midnight after which it rose again. Plaizier et al. (2005) fed transition and early lactation cows twice daily at 0700 and 1300 h. Blood urea rose following the feed delivery at 0730 h but not after feed delivery at 1300 h. In early and mid lactation cows fed twice daily at 0700 and 1500 h, plasma urea rose after feeding at 0700 h and peaked between 1100-1200 h (Blum et al., 2000). Afterward, it dropped to its nadir at 0000 h. Therefore, it seems that the post-feeding rise in blood urea in twice-daily fed cows occurs in the first but not in the second feed delivery. Although post-feeding intake patterns were not measured, the post-feeding response in blood urea could be attributed to different amounts of feed eaten after different feed deliveries. Cows fed during the day usually eat little feed after midnight because fresh feed is absent (Ominski et al., 2002). As a result, the rumen fill decreases towards early morning and hunger develops. The considerable morning N consumption results in a rapid ammonia release in the rumen, promoting hepatic urea synthesis, which turns into a surge in blood urea (Gustafsson and Palmquist, 1993). This cascade may be less pronounced in subsequent feed deliveries.

3.1.3.3. Lactate

L-lactate is the predominant lactate isomer produced in the rumen. However, D-lactate is also produced when high-grain diets are fed (Harmon et al., 1985). Hepatic L-lactate flow originates from either the gut or non-splanchnic muscle tissues. The gut L-lactate is produced mostly by the rumen microbes or across the rumen wall via glucose, pyruvate, and propionate metabolism. D-lactate is synthesized by the rumen microbes and transferred to the liver to be used as a gluconeogenic or energy-generating substance (Giesecke and Stangassinger, 1992). In addition to the rumen, the non-splanchnic muscle tissues generate L-lactate as the end-product of anaerobic glucose oxidation. The resultant L-lactate travels to the liver to be converted to glucose which is then transferred back to the muscles to sustain normal muscle metabolism (Mayes, 2000). It is notable that the hepatic use of L-lactate (for oxidation or gluconeogenesis) is twice that of D-lactate (Armentano, 1992). In vitro studies (Donkin et al., 1991 cited by Armentano, 1992) have shown that L-lactate is used for gluconeogenesis with a lower rate which is not responsive to insulin, when compared to propionate. Thus, post-feeding insulin rise will probably not greatly reduce gluconeogenesis from lactate. Huntington (1997) presented L-lactate as the second most significant contributor to gluconeogenesis in fed lactating cows (16-17.5%) and beef steers (13.1%). In fasted cows, however, L-lactate contributed as much as 74.4% to the hepatic glucose synthesis (Huntington, 1990). These data indicate the significance of lactate in energy use by both mammary and non-mammary tissues. A sharp rise in blood L-lactate within 30 min of concentrate but not of hay delivery has been reported (de Jong, 1981a,b). The post-feeding rise in blood L-lactate was due to L-lactate buildup in the rumen after concentrate ingestion.

3.1.3.4. β-hydroxybutyrate (BHBA)

β-hydroxy butyrate is produced by the extensive metabolism of n-butyrate across the rumen wall (Reynolds et al., 1992). Of total n-butyrate infused into the rumen, 48-62% was released into the PDV and the rest were converted to acetoacetate, oxidized during absorption, or utilized by the rumen microbes (Kristensen et al., 2000). Through the alimentary and hepatic ketogeneses, the splanchnic tissues produce BHBA for peripheral oxidative use (Reynolds, 2001). Thus, any feeding strategy which augments rumen production of n-butyrate will subsequently contribute to a greater appearance of BHBA in the peripheral blood. If such a feeding strategy increases total VFA as well, blood insulin will rise (Godden and Weekes, 1981; Sutton et al., 1988). The high blood insulin facilitates BHBA use by peripheral tissues such as muscles (Jarrett et al., 1974; cited by Brockman and Laarveld, 1986). Thus, the energy status of the cow will improve with increased peripheral BHBA availability.

In goats offered a concentrate-based diet ad libitum, blood BHBA did not show any response to spontaneous meals (de Jong, 1981b). Plaizier et al. (2005) found higher blood BHBA during the day than overnight in lactating cows fed twice daily at 0700 and 1330 h. The high day-time BHBA mainly results from butyrate metabolism across the rumen wall so called "alimentary ketogenesis". The post-feeding surge in peripheral BHBA has also been observed by others (Coggins and Field, 1976; Blum et al., 2000; Sutton et al., 1988). With more frequent feeding, the post-meal increase in blood BHBA may be lower or even vanish (Sutton et al., 1988).

3.1.3.5. Non-esterified fatty acids (NEFA)

The NEFA levels in peripheral blood are usually used to evaluate the energy status of the animal. The blood NEFA can arise mainly from the lipolysis in adipose tissue. This is particularly important at times of negative energy balance when high-producing ruminants endure nutrient mobilization from the body reserves (Sidhu and Emery, 1972). At times of negative energy balance, if a given feeding strategy can reduce blood NEFA at any time of a 24-h period, it must have attenuated the metabolic stress on the animal (Grummer, 1993; NRC, 2001). The 24-h patterns in blood insulin and glucose are interrelated with the 24-h patterns in blood NEFA (Sutton et al., 1988). A feeding-induced rise in plasma insulin can be associated with a decrease in blood NEFA (Blum et al., 2000; Fröhli and Blum, 1988). An overnight rise in blood NEFA has been shown in lactating dairy and beef cows fed during the day (Blum et al., 2000; Coggins and Field, 1976; Frohli and Blum, 1988). In addition, if the time interval between the two milkings is longer overnight than during the day, the morning milk yield will proportionally increase, thereby increasing nutrient requirements. As a result, the risk of nutrient shortage in the night when fresh feed is not available will rise. Consequently, blood NEFA will need to rise to sustain milk secretion (Blum et al., 2000).

3.1.3.5. Insulin

Insulin is called the storage hormone because it stimulates glucose entry into the peripheral fat and muscle cells (Brockman, 1978). As mentioned previously, much less glucose crosses into the portal vein in ruminants compared to non-ruminants. In consequence, insulin would not have as significant impacts on hepatic glucose

metabolism in ruminants as it would in non-ruminants (Brockman et al., 1978). Nervous system, gut peptides, other pancreatic secretions, and nutrient absorption appear to be the main candidates in stimulating insulin release from the pancreas (Berthoud, 1984). The effects of the nervous system on insulin release regulation take place via sympathetic and parasympathetic neurons. The vision, odor, and flavor of the food can induce insulin secretion via activating the parasympathetic neurons in humans (Hales, 1971). The early 70's research (Bassett, 1971, 1972; McAtee and Trenkle, 1971; Trenkle, 1971, 1972) led to the proposal that neural impulses and gastrointestinal hormones are involved in the post-feeding insulin response to feed delivery in ruminants as well. Secretin and pancreozymin (cholecystokinin) stimulated insulin release in sheep (Trenkle, 1972). In another sheep study, Bassett (1974) observed that blood insulin in sheep rose sooner than did blood glucose, suggesting that glucose was not a major cause of the initial rise in post-feeding insulin release. Nevertheless, the ultimate increase in blood glucose may contribute to maintaining the high post-feeding insulin concentration. In goats fed ad libitum for a 3-h period daily, de Jong (1981a) showed a post-feeding rise in blood insulin, which probably was caused via VFA stimulation of the pancreatic β-cells. In his later study with goats, de Jong (1981b) again observed a post-meal rise in blood insulin; however, no such a peak was noticed in blood VFA. Thus, it was speculated that the nervous signals (rather than VFA) either directly or through the secretion of gut hormones may have resulted in the post-meal insulin response. A similar post-feeding rise in blood insulin has been shown (Godden and Weekes, 1981) in lambs fed chopped dry grass in two equal meals at 0900 and 1600 h. Lefcourt et al. (1996) monitored blood insulin every 15 min for 48 h in six lactating cows fed once daily at 0900 h. Blood insulin

exhibited distinct diurnal rhythms in all cows, peaking at 1745 h and fell to a nadir overnight or during the dark phase i.e., 2300-0700 h. A comparable zenith time in blood insulin was observed at 1830 h by Bines et al. (1983) and at 1800 h by Vasilatos and Wangness (1981). The 24-h pattern in peripheral blood insulin has been demonstrated to be closely linked to the 24-h patterns in feed intake. Blum et al. (1985) fed cows forage and concentrate separately and found that blood insulin rises sharply upon concentrate delivery at 0600 h and 1430 h and declines shortly thereafter, remaining lower overnight. Similarly, feed intake showed two major peaks (after both concentrate delivery), being considerably lower between 0200 to 0600 h. In this trial, the forage was offered ad libitum at 0600 h. The fact that blood insulin was higher in the afternoon but lower overnight would support the circadian patterns of peripheral insulin observed by Lefcourt et al. (1996). Overall, the 24-h patterns in peripheral blood insulin relate to the dietary content of readily fermentable carbohydrates (A. D. Kennedy; Department of Animal Science, University of Manitoba; personal conversation, January 2007).

4. Productivity

4.1. Effect of Time of Feeding on Milk Secretion

No study exists that has assessed the effect of TF on milk secretion in once-daily fed lactating cows under thermoneutral conditions. This is because TF has not previously been considered in relation to chronobiology of the ruminant metabolism. The literature lacks any hypothesis that attributes milk secretion of once-daily fed ruminants to evolutionary and endogenous patterns in the rumen, splanchnic, and peripheral metabolism. Thus, knowledge is lacking if time of day when TMR is delivered to the cow

(once-daily) can impact on the mammary nutrient availability and milk secretion. Robinson et al. (1997) delivered a protein supplement (equal to 15% of DMI) to lactating cows at either 0830 or 0030 h. Midnight instead of morning delivery of protein supplement improved milk fat via increased nutrient digestion in the rumen (Robinson et al., 1997). However, no such an effect was observed by Moshtaghi Nia et al. (2003), who fed cows a protein supplement at about 12% of DMI either at 0830 or 0030 h. These studies changed TF for only a small portion of the daily diet. More importantly, their main hypothesis was that midnight instead of morning delivery of the protein supplement would increase the duodenal recovery of the escape AA from the protein supplement, sparing them for milk secretion. This hypothesis was based on the assumption that rumen outflow rate would be higher and rumen AA digestibility would be lower in the night than during the day (Robinson et al., 1997). In contrast to their hypothesis, the rumen nutrient digestibility was even increased by protein delivery at 0030 h instead of at 0830 h (Robinson et al., 1997). In a 118-d lactation trial, Aharoni et al. (2005) fed heat-stressed lactating cows four times during either the day (30% at 0615 h, 20% at 1000 h, 25% at 1530 h, and 25% at 1900 h) or evening (20% at 0615 h, 30% at 1530 h, 25% at 1900 h, and 25% at 2100 h). Transferring the feed delivery times from the day into evening hours improved lactation persistency and reduced energy expenditure (Aharoni et al., 2005). The decline in energy expenditure was associated with a decline in feed intake which led to improved feed efficiency in evening-fed cows exposed to hot days (Aharoni et al., 2005). Recently, Kennedy et al. (2004) and Small et al. (2004) found that feed delivery at 2000 h instead of at 0900 h improved growth in beef heifers and steers under extremely cold winter temperatures (<-15°C). Diurnal patterns in feed intake, rumen fermentation,

and peripheral metabolites, however, were not studied in the above-mentioned experiments. Thus, it remained unclear if the improved growth of evening fed cattle was mainly due to greater efficiency of heat-increment use towards maintenance or due to changes in 24-h patterns of feed intake, rumen fermentation, and peripheral metabolism, or both. The major questions remain that if and via which mechanisms TF can affect and mediate productive responses in lactating cows under thermoneutral environments.

HYPOTHESES

Different times of feed delivery (0900 vs. 2100 h) alter 24-h nutrient availability. In so doing, feed delivery at 2100 h instead of 0900 h improves nutrient digestibility and alters nutrient partitioning in favor of milk secretion and body deposition and the expense of fecal and urinary excretions. As a result, milk yield will increase and nutrient excretion will decrease. The pathways are as following:

1) Evening (2100 h) instead of morning (0900 h) feed delivery will alter the post-feeding patterns of feed intake. A shift of intake to the evening will enhance rumen fermentation. The enhanced rumen fermentation will increase VFA production in the rumen and their absorption across the rumen wall. As a result, 24-h VFA, lactate, and BHBA supply to the portal circulation will increase. The enhanced metabolite release by the portal-drained viscera will subsequently alter the 24-h patterns in hepatic metabolism by increasing substrate supply. Consequently, peripheral blood levels of milk energy precursors such as BHBA, VFA and lactate will increase.

2) Based on the evolutionary concept, rumination occurs mostly overnight. This would imply a greater digestion capacity in the rumen in the night than during the day. Such a greater rumen volume and fermentation capacity in the night has been shown in grazing cows. Hence, the optimum rumen pH and microbial metabolism would be easier to achieve with the increased rumen fermentation if feed is delivered in the evening instead of the morning. Due to increased rumen capacity, feed delivery at 2100 h instead of at

0900 h will not depress rumen pH to the point at which the microbial rupture, endotoxin release, and systemic inflammatory response may occur (e.g., between 5.2 to 5.6 for at least 3-h daily).

3) Insulin stimulates nutrient storage by the peripheral tissues. Insulin does not have a major impact on hepatic gluconeogenesis from propionate, and has little effect on nutrient uptake by the mammary gland. The enhanced energy-producing substrates in the peripheral blood due to feed delivery at 2100 h will thus stimulate nutrient uptake by both mammary and non-mammary tissues such as adipose and muscle tissues. The result will be enhanced milk secretion and improved nutrient retention in the body.

4) Due to increased total tract nutrient digestibility, improved milk nutrient output, and induced nutrient retention in the body, nutrient excretion via both feces and urine will decrease. This implies a benefit to the environment.

SHORT-TERM OBJECTIVES

To determine and ascertain the effects of TMR delivery at 2100 h vs. 0900 h on:

1) Dry matter intake and diurnal patterns in feed intake, rumen pH and concentrations of VFA and ammonia.

2) Rumen volume, outflow rates, and retention times of fluid and solids.

3) Estimates of microbial protein synthesis based on the urinary excretion of purine derivatives (allantoin and uric acid).

5) Apparent total tract dry matter, nitrogen, NDF and ADF digestibility using total fecal collection technique.

6) Nitrogen partitioning into feces, urine, milk, and body retention.

7) Diurnal patterns in peripheral blood glucose, L-lactate, urea, BHBA, and NEFA.

8) Milk composition and yield of fat and protein plus milk fatty acid profiles.

LONG-TERM OBJECTIVES

1) Introduce TMR delivery time as a feasible feeding strategy to the dairy industry. This is hoped to improve feed intake, rumen fermentation, and thus productivity of lactating cows.

2) Launch a new insight into ruminant chronobiology. During the last three decades, such insight in humans has made a significant contribution to understanding the etiology of the major metabolic diseaeses (e.g., diabetes). The new vision underlines the significance of the time of feed delivery on feed ingestion, digestion, and metabolism in Holstein cows.

3) Promote future comparative studies using monogastrics and ruminants. Such studies would reveal the inter-species differences in 24-h patterns of feed intake and the intermediary metabolism in response to feeding time and diet composition. Livestock are used to feed humans. The public's concerns regarding the safety of animal foods and the shortage of natural resources such as feed ingredients fed to ruminants) are growing. Therefore, any inter-species difference will advance our knowledge to maximize nutritrient use efficiency by livestock, provide humans with safe food, and reduce nutrient excretion into the environment.

4) Highlight feed delivery time as an agent of feed intake regulation in ruminants. This will provide knowledge that could contribute to further improvements in feed intake prediction models in ruminants.

CHAPTER 3

Influence of Feed Delivery Time in Relation to Dietary Concentrate Level and Parity on Feed Intake, Rumen Fermentation, and Productivity of Lactating Cows

ABSTRACT

The objective of the present study was to determine the effects of feed delivery time, dietary concentrate level, and their interaction on dry matter intake, rumen fermentation and milk production. Four multiparous (days in milk = 83 ± 22, mean ± SD) and four primiparous (days in milk = 81 ± 23) cows received either a higher concentrate total mixed ration (TMR) with a forage to concentrate ratio (F:C) of 38.5:61.5 or a lower concentrate TMR with a F:C of 50.6:49.4. The TMR ware delivered either at 0900 h or at 2100 h. Treatments were arranged factorially (2 × 2) as a replicated 4 × 4 Latin square design with four 21-d periods. Each period had a 14-d adaptation phase. Rumen fluid was sampled at 0100 and 1300 h using an oral probe during week 3. Cows were not heat stressed during any time of the study (average 20.4°C and 68.1% RH). Diet and feed delivery time did not affect dry matter intake. Changing the feed delivery time from 0900 h to 2100 h increased ($P < 0.05$) milk fat percent from 2.5 to 2.9%, and numerically increased fat yield from 0.98 to 1.20 kg/d in multiparous cows, with no effects in primiparous cows. Time of feed elivery did not affect the daily averages in rumen pH and concentrations of volatile fatty acids and ammonia, milk protein, and body condition score. A tendency existed for a lower rumen pH and higher rumen VFA at 4 h after feed delivery in 2100 h-fed cows than in 0900 h-fed cows. Also, rumen butyrate and valerate

were higher at 4 h post-feeding when cows were fed at 2100 h. Decreasing the forage to concentrate ratio reduced rumen pH, acetate to propionate ratio and milk fat percent, and improved milk protein percent. Interactions between feed delivery time and dietary concentrate level were insignificant. Results suggest beneficial impacts of evening feeding on energy status of lactating cows. Parity influenced the effect of feeding time on cow productivity.

Key words: feed delivery time, concentrate level, evening feeding, lactating cow

INTRODUCTION

Feeding strategies for ruminants aim to optimize rumen fermentation (Hoover and Strokes, 1991), maintain metabolic balance (Reist et al., 2003), and reduce nutrient excretion into the environment (Tamminga, 1996). Optimizing the physical effectiveness (Mertens, 1997) and quantity (Khorasani et al., 2001) of dietary forages, forage to concentrate ratio (Reist et al., 2003), and frequency of feed delivery (Nocek, 1992, 1987; Philips and Rind, 2001; Cecava et al., 1990) are among strategies introduced previously.

The potential of evening feeding to attenuate heat stress and improve feed efficiency and lactation persistency has been evaluated in lactating cows (Aharoni et al., 2005) and ewes (Sevi et al., 2001). Evening feeding has also improved cattle growth (Kennedy et al., 2004). Nonetheless, provision of total mixed ration at different times in a 24-h period has received no attention in high-producing lactating cows under thermoneutral conditions. Robinson et al. (1997) attempted to increase rumen-escape protein by feeding a protein supplement at 0030 h instead of 0830 h. They hypothesized

that the rapid ruminal outflow rate during nocturnal fermentation would increase the rumen-escape protein. Unexpectedly, however, the rumen digestion and milk fat yield were increased in cows fed the protein supplement meal at 0030 h compared to 0830 h. The main hypothesis of the current study was, in contrast, based on biological timing of 24-h rhythms in rumen and blood metabolites (Piccoine, 2003). From a chronobiological perspective, the optimum animal performance may not be achieved in the absence of synchrony between optimum internal physiological state (24-h patterns in hormones and metabolites) and 24-h patterns of nutrient intake (feeding time and diet composition). Recently, this concept was practiced by improving the growth of evening-fed heifers and steers exposed to cold temperatures (Kennedy et al., 2004; Small et al., 2004). However, rumen fermentation was not monitored in these studies. Thus, it was unknown if the improved growth of evening-fed cattle was mainly due to greater efficiency of heat-increment use towards maintenance or due to changes in 24-h patterns of feed intake, rumen fermentation, and peripheral metabolism, or both. In heat-stressed lactating cows, afternoon and evening feeding deliveries were shown (Aharoni et al., 2005) to improve lactation persistency, compared to feed delivery distributed during the day. Nonetheless, it is unknown if evening feeding can alter post-feeding patterns in rumen fermentation and thereby improve cow performance under thermoneutral conditions.

The 24-h variations in insulin resistance and glucose tolerance have been demonstrated in humans and rats (la Fleur et al., 2001, Sehgal, 2004; Van Cauter et al., 1991). Because of reduced glucose tolerance in humans during the evening, it is recommended that large evening meals be avoided, especially when diabetes is a major concern (Sehgal, 2004). Endogenous diurnal variation in blood cholesterol, as an

indicator of liver metabolism, has recently been shown (Piccione et al., 2003). If such 24 h variations exist in rumen fermentation and cow metabolism as well, the time of feed delivery could have a significant impact on nutrient utilization and partitioning. The main objective of the current study was to determine the effects of feed delivery time (2100 vs. 0900 h), dietary concentrate level, and their interaction on feed intake, rumen fermentation, and milk yield and composition of lactating dairy cows.

MATERIALS AND METHODS

Experimental Design and Cow Management

Four multiparous (body weight (BW) = 652 ± 14 kg, body condition score (BCS) = 2.87 ± 0.14, days in milk (DIM) = 83 ± 22; mean ± SD) and four primiparous (BW = 667 ± 110 kg, BCS = 3.19 ± 0.66, DIM = 81 ± 23) Holstein cows were used in a double 4 × 4 Latin square design. The experiment had four 21-d periods. Each period had 14 d of adaptation followed by 7 d of data collection. Cows were housed indoors in eight individual stalls in the Metabolism Unit of the Glenlea Research Station, University of Manitoba from April through July, 2004. All animals had free access to fresh water. Except for the sampling period, cows were allowed 2 h of outdoor exercise every other day. The average air temperature and relative humidity of the metabolism unit were 20.4°C and 68.1%, respectively. The maximum air temperature of the metabolism unit did not go over 25°C at any time during the study. Cows were cared for according to the guidelines of the Canadian Council for Animal Care (CCAC, 1993).

Treatments were feeding either a higher concentrate (HC, 38.5:61.5 = forage to concentrate ratio) or a lower concentrate (LC, 50.6:49.4 = forage to concentrate ratio) diet either at 0900 h (morning) or at 2100 h (evening) (Tables 1, 2, 3). Total mixed rations (TMR) were prepared every morning using a Data Ranger Mixer (American Calan, Northwood, NH) with a Weigh Tronix head (model 1000, American Calan, Northwood, NH). Cows were fed *ad libitum*, allowing for about 5-10% orts. Cows were scored for body condition (Edmonson et al., 1989) and weighed at 0830 h at the beginning of the first experimental period and the end of each experimental period. Lights were turned on at 0345 h just before milking and turned off at 22:45 h.

Feed Intake and Analyses of TMR and Orts

Samples of both alfalfa silage and corn silage were taken once a week to check for any variations in DM content. This was to maintain dietary forage to concentrate ratio (F:C). The forage, TMR, and orts were sampled daily during the last 7-d of each period. The TMR and forage samples were pooled for each period. Orts were pooled weekly for each cow in each period. All feed and ort samples were dried at 60°C for 48-h to measure DM content. Dry matter intake (DMI) for each cow was calculated by multiplying the dry matter content of TMR and orts by their wet weight for every day of sampling weeks. Dried TMR and forage samples were ground using a Wiley mill (Thomas Wiley, Philadelphia, PA) to pass through a 1 mm screen and stored at -20°C until analyzed. All pooled TMR and forage samples were analyzed using the wet-chemistry procedures of AOAC (1997) for crude protein (CP, Kjeldahl method 984.13), ADF (method 973.18), ether extract (method 920.39), and ash (method 942.05). Alpha-amylase (Sigma no.

A3306: Sigma Chemical Co., St. Louis, MO) and sodium sulfate were used to determine NDF according to Van Soest et al. (1991) using an Ankom 200 Fiber Analyzer (Ankom Technology, Fairport, NY). Inductively coupled plasma emission spectroscopy (method 985.01; AOAC, 1997) was used to measure the levels of Ca, P, Mg, Na, and K using an Atom Scan 25 Plasma Spectrometer (Thermo Jarrell Ash Crop., Grand Junction, CO) after acid-digestion of the samples.

The Penn State Particle Separator (PSPS; Heinrichs, 1996) was used to determine particle size distribution of all TMR, forage, and ort samples. The PSPS had 3 screens including two top screens with diameters of 19 and 8 mm, respectively, one wire sieve with a diameter of 1.18 mm, and a bottom pan. After placing approximately 200 g of wet sample on the top screen, the PSPS was shaken 40 times, five times in each direction twice. The contents of each fraction were wet-weighed and the percent of each fraction was calculated (Heinrich, 1996) (Table 4).

Milking and Milk Analysis

Cows were milked twice daily at 0400 h and 1600 h in their stalls, and milk yields were determined using Tru-Test regulation meters at each milking for the entire experiment (Westfalia Surge, Mississauga, ON). Milk samples were collected into 50-ml vials for each cow from six consecutive milkings during collection weeks (week 3), preserved with 2-bromo-2-nitropropane-1,3-diol, and stored at 4°C. Samples were analyzed for milk components at the laboratory of Dairy Farmers of Manitoba (Winnipeg, MB, Canada) by near infrared using the Milk-o-Scan 303AB (Foss Electric,

Hillerød, Denmark). Energy corrected milk (ECM) was calculated using the following formula (DeFrain et al., 2006): (0.327 × kg milk) + (12.95 × kg fat) + (7.2 × kg protein).

Analyses of Rumen Fluid pH, VFA, and Ammonia

Rumen fluid samples were obtained twice daily during d 2 and d 4 of each collection period at 1300 h and 0100 h, respectively, corresponding to 4 h and 16 h post-feeding. A Geishauser oral probe (Duffield et al., 2004) was used to aspirate the rumen fluid. The initial 100 ml of the aspirated rumen fluid was discarded to minimize saliva contamination (Duffield et al., 2004). The pH of the second portion (100 ml) was measured immediately using an Accumet Basic 15 pH meter with an Accumet gel-filled polymer body combination pH electrode (Fisher Scientific, Fairlawn, NJ). Rumen fluid samples were then centrifuged at 1800 × *g* for 12 min and the supernatants were stored at -20°C. For VFA analysis, the frozen rumen fluid samples were thawed at room temperature. One half ml of 25% meta-phosphoric acid solution was vortexed with 2.5 ml of rumen fluid and tubes were placed in a -20°C freezer overnight. Upon thawing, tubes were centrifuged for 20 min at 3000 × *g* and 2 ml of supernatant were decanted into a clean dry vial. The samples were capped and placed into the autosampler device (Model 8100; Varian, Walnut Creek, CA) and VFA concentrations were determined by gas chromatography (Model 3400 Star; Varian) using a 1.83-m glass column (Model 2 to 1721; Supelco, Oakville, ON, Canada) (Erwin et al., 1961). The injector and detector temperatures were set at 170°C and 195°C, respectively, with initial and final column temperatures set at 120°C and 165°C, respectively. Ammonia concentration of rumen fluid samples was measured according to the method of Novozamsky et al. (1974).

Absorbance was read at 630 nm on a Pharmacia Biotech Ultraspec 2000 UV/visible spectrophotometer (Biochrom, Cambridge, UK).

Statistical Analyses

Data were analyzed using the MIXED Procedure of SAS (SAS Institute, 2003). The least square means estimation method was Restricted Maximum Likelihood. For production data, fixed effects included the time of feed delivery, concentrate level, concentrate level × time of feed delivery, parity, and two and three-way interactions between parity and treatments. The effects of cow within parity, and period were considered random. For rumen data, the effects of time of rumen sampling and its interactions with feed delivery time and diet were considered fixed. The PDIFF option of SAS (SAS Institute, 2003) was used to separate the different least square means. Residuals were tested for normality of distribution and homogeneity of variance. Fixed effects were declared significant at $P < 0.05$, and trends were discussed at $P < 0.10$. Standard errors presented were for the differences of least square means.

RESULTS AND DISCUSSION

Rumen Fermentation

Time of feed delivery did not significantly affect overall rumen pH and total VFA and ammonia (Table 5). Rumen pH tended to be lower ($P = 0.06$), total VFA, propionate ($P = 0.08$) and butyrate ($P = 0.05$) tended to be higher, and valerate ($P = 0.02$) and ammonia ($P < 0.01$) were higher at 4-h post-feeding in 2100 h-fed cows than in 0900 h-fed cows (Table 6). Rumen fermentation did not differ between parities. These results

suggest a more extensive rumen fermentation early post-feeding in 2100 h-fed cows compared to 0900 h-fed cows. Robinson et al. (1997) observed an increase in rumen VFA concentrations in cows fed a protein meal at 0030 h instead of 0830 h, leading them to conclude that there was a greater microbial growth by delivering a protein meal at 0030 h. The higher ammonia at 4 h post-feeding in 2100 h-fed cows could be due to their greater feed consumed within 3 h of 2100 h than 0900 h feeding (Chapter 4). The greater feed intake would subject greater N to microbial degradation, thereby increasing ammonia concentrations at 4 h after evening feeding. The greater ruminal substrate availability agrees with the higher total VFA at 4 h and lower VFA at 16 h post-feeding in 2100 h-fed cows compared to 0900 h-fed cows (Table 6).

The pH values represent the rumen conditions only at the time of sampling. The spot samples of rumen fluid do not consider diurnal variations in water intake, rumen pH, VFA concentrations, and fractional passage rate of rumen fluid (Allen, 1997; Dijkstra, 1993; Duffield et al., 2004). Nonetheless, the difference between the extents of rumen fermentation at 4 h post-feeding between the two diets was large enough to be detected by the spot rumen sampling (Table 5). Rumen fluid pH averaged across the two sampling times of 4 and 16 h post-feeding (Table 5) was lower ($P < 0.05$) in cows fed the HC diet than in cows fed the LC diet. The dietary effect on rumen fermentation agrees with others (Cecava et al., 1990; Khorasani and Kennelly, 2001) who reported a lower ruminal pH with HC than with LC diets.

Feeding the HC instead of the LC diet significantly increased ($P < 0.01$) the ruminal concentrations of propionate and valerate, but decreased ($P < 0.01$) the levels of acetate and butyrate. As a result, the acetate to propionate ratio was significantly lower

with the HC than with the LC diet. No differences ($P > 0.10$) were observed in ruminal concentration of ammonia between the two diets, indicating that LC diet may have provided sufficient fermentable carbohydrates for normal microbial growth as did HC diet. Feeding time did not significantly interact with diet concentrate level on rumen fermentation except for isovalerate.

Dry Matter Intake (DMI)

Neither feeding time ($P = 0.89$) nor the dietary concentrate level ($P = 0.96$) affected DMI (Table 7). Recently, Aharoni et al. (2005) reported a decline in DMI in response to afternoon and evening feedings instead of morning feeding under hot weather. Unlike the present study, they delivered the feed in four separate portions for evening-fed cows i.e., 20% at 0615 h, 30% at 1530 h, 25% at 1900 h, and 25% at 2100 h. Not surprisingly, such timing and frequency of feed delivery would change the diurnal feed intake patterns, and thus affect productivity. In a companion study (Chapter 4), we found that feeding time altered post-feeding patterns in feed intake. Cows fed at 2100 h consumed more feed within 3 h after feeding than cows fed at 0900 h (Chapter 4). Such a greater eating rate shortly after evening feeding was, however, followed by a slower eating rate between 3-6 h post-feeding, compared to morning feeding. As a result, total daily feed intake was comparable between evening and morning fed cows (Table 6). Using beef cattle, Small et al. (2004) and Kennedy et al. (2004) reported comparable DMI between morning and evening-fed cattle.

Similar DMI of cows fed HC and LC diets may indicate that the effects of dietary physical and chemical properties on DMI were comparable between the two diets.

Khorasani et al. (2001) reported a trend for greater DMI in cows fed a HC diet (F:C = 35:65) than in cows fed a LC (F:C = 50:50) diet. The average dietary NDF and the difference between the NDF of the two diets were greater in that study (37.2 vs. 46.4%) than in our study (28.6 vs. 31%). Therefore, a less pronounced impact of the diet on gut fill and DMI (Allen, 2000) was expected in the current experiment than in the study of Khorasani et al. (2001).

Milk Production and Composition

Milk yield was not influenced by TF ($P = 0.46$), dietary concentrate level ($P = 0.86$), and their interaction ($P = 0.71$) (Table 7). However, milk fat percent was higher ($P < 0.05$) and fat yield tended to be greater ($P < 0.10$) in 2100 h-fed multiparous cows than in 0900 h-fed multiparous cows (Table 8). These effects were not seen in primiparous cows (Table 8). The increased milk fat in multiparous cows may have related to higher rumen VFA at 4 h post-feeding when the TMR was delivered at 2100 h instead of 0900 h (Table 6). Similar to multiparous cows, primiparous cows had higher rumen VFA at 4 h post-feeding when fed at 2100 instead of 0900 h. However, the post-feeding rise in rumen VFA did not affect milk yield in primiparous cows. Multiparous cows had greater DMI ($P = 0.01$) and ECM ($P < 0.05$) than primiparous cows (Table 8). Thus, the energy balance of multiparous cows may have been more fragile, rendering them more responsive to dietary treatments, compared to primiparous cows. Furthermore, the orts from 2100 h-fed multiparous cows were finer ($P < 0.01$) than orts from 0900 h-fed multiparous cows (Table 8). The ort particle size did not differ ($P > 0.30$) between 2100 h-fed and 0900 h-fed primiparous cows. This could be due to the greater feed intake of

multiparous than of primiparous cows. The rumen pH was lower in multiparous cows than in primiparous cows. The lower rumen pH may have allowed multiparous cows to raise milk fat percent in response to feeding at 2100 h instead of at 0900 h. The trend (P = 0.06) for the higher milk percent of total solids (sum of lactose, fat, and proteins) in cows fed at 2100 h than in cows fed at 0900 h concurs with the increased milk fat percent.

In agreement with the current study, Khorasani and Kennelly (2001) reported no differences in milk yield of cows fed two diets with F:C of 25:75 and 50:50. The comparable milk yield between two diets can be explained by the similar DMI (Table 7). Likewise, Reist et al. (2003) reported comparable milk yields between cows fed a LC (F:C = 70:30) and HC diet (F:C = 50:50). Milk fat content was lower ($P < 0.01$) in cows fed the HC diet than in cows fed the LC diet (Table 7). The lower milk fat could be due to lower rumen acetate and butyrate concentrations and lower acetate to propionate ratio in HC-fed than in LC-fed cows (Table 5). Because of lower milk fat percent, fat-corrected milk yield was also lower ($P < 0.05$) in HC-fed than in LC-fed cows. These findings were in accord with the fact that greater amounts of readily fermentable carbohydrates can reduce cellulolytic activity, acetate to propionate ratio (France and Dijkstra, 2005), and milk fat (Van Soest, 1963). Total solids were also greater ($P < 0.05$) in the LC-fed cows than in the HC-fed cows, supporting the higher levels of milk fat in the LC-fed group. Feeding the HC diet instead of the LC diet increased ($P < 0.01$) milk protein content and tended to increase ($P = 0.05$) milk protein yield. Khorasani and Kennelly (2001) also reported higher milk protein content and yield for cows fed a HC diet than for cows fed a LC diet. The higher dietary fermentable carbohydrates in the HC diet, if provided with rumen degradable protein of a comparable degradation rate, could

potentially enhance rumen microbial protein synthesis (DePeters and Cant, 1992). The higher fermentable carbohydrates content of HC than of LC diet should have hence contributed to the increased milk protein in cows fed the HC diet. In addition, HC-fed cows had higher levels of rumen propionate than did LC-fed cows (Table 5). The greater propionate availability has been shown to increase the mammary nonessential AA uptake (Raggio et al., 2006), probably via sparing more AA in favor of protein secretion and at the expense of hepatic gluconeogenesis.

Changes in Body Weight and Body Condition Score

Feeding cows at 2100 h instead of 0900 h was associated with significant ($P < 0.05$) weight gain (Table 9). However, cows were weighed once daily at 0830 h which was at 23.5 h post-feeding for cows fed at 2100 h but 11.5 h post-feeding for cows fed at 0900 h. As a result, there was a longer time-lapse between feed delivery and weighing for cows fed at 0900 h than for cows fed at 2100 h. Time of feeding affects post-feeding patterns in feed intake and gut-fill (Chapter 4; Pritchard and Knutsen, 1995). Therefore, time of feeding relative to time of weighing can impact on BW measurements. If morning and evening fed animals are not weighed at a comparable time relative to feeding, changes in BW may partly represent the different amount of gut-fill in addition to true changes in tissue growth or loss. Kennedy et al., (0900 vs. 2000 h) Small et al., (0900 vs. 2000 h) and Schwartzkopf-Genswein et al. (0900 vs. 2100 h) (2004) all reported more BW gain by evening-fed compared to morning-fed beef cattle. Small et al. (2004) fed steers either at 0900 or at 2000 h, weighed the morning-fed steers at 0830 h or 23.5 h after feeding, but weighed the evening-fed steers at 1430 h or 18.5 h after feeding. Thus,

the effect of feeding time on weight gain was not fully separated from the effect of weighing time in the study of Small et al. (2004) as well. Schwarzkopf-Genswein et al. (2004) did not describe the weighing protocols used. Nonetheless, they also acknowledged the possibility that the positive weight gain response to evening feeding might be partly due to post-feeding effect of gut-fill. Unlike BW, body condition score was not influenced ($P > 0.70$) by feeding time. In a parallel study, a higher post-feeding peak in blood insulin of 2100 h-fed (22 IU/L) than of 0900 h-fed (12 IU/L) cows was found (Furedi et al., 2006a). Insulin stimulates nutrient deposition in peripheral tissues (Brockman and Laarveld, 1986). The higher insulin was expected to increase glucose, acetate, lactate and BHBA partitioning in favor of peripheral deposition. In so doing, the higher post-feeding insulin by evening vs. morning feeding has the potential to increase weight gain. Recently, Furedi et al. (2006b) weighed both 0900 h-fed and 2100 h-fed cows twice daily at 0830 and 2030 h in a 42-d lactation study. The BW gain of 2100 h-fed cows largely represented gut-fill. Furedi et al. (2006b) also found a thicker subcutaneous fat in 2100 h- fed cows than in 0900 h-fed cows. These data along with the results of the current study would point to the positive impact of evening feeding on energy efficiency. Diet had also an impact on BW changes. The HC-fed cows gained more BW ($P < 0.01$) and BCS ($P = 0.02$) than LC-fed cows (Table 9). Similarly, Oba and Allen (2003) found a BW gain response to feeding a higher instead of a lower starch diet. The positive BW gain response was associated with higher rises of plasma insulin in cows fed the higher starch diet (Oba and Allen, 2003).

CONCLUSIONS

Feeding at 2100 h instead of 0900 h increased milk fat percent and tended to increase fat yield in multiparous cows, but not in primiparous cows. The higher productive capacity of multiparous than of primiparous cows may have played a role in their milk fat response to evening feeding. Time of feeding did not affect milk protein. Rumen pH tended to be lower, total VFA and propionate tended to be higher, and butyrate, valerate, and ammonia were higher at 4-h post-feeding in 2100 h-fed cows than in 0900 h-fed cows. Feeding time did not interact with dietary concentrate level on cow performance. Results suggest that evening feeding can benefit lactating cows under thermoneutral conditions. Parity mediated cow response to feeding time.

Table 1. Forage and concentrate portions of the experimental diets (DM basis)

	Experimental Diets	
Diet ingredients	Higher Concentrate (HC)	Lower Concentrate (LC)
Alfalfa silage	15.87	20.96
Corn silage	22.67	29.66
Energy supplement	49.89	37.15
Protein supplement	11.57	12.23
Forage: concentrate ratio (F:C)	38.5 : 61.5	50.6 : 49.4

Table 2. The nutrient composition of corn silage, alfalfa silage, and total mixed rations (TMR) on a DM basis

	Forage		TMR[2]	
Nutrient[1]	Alfalfa Silage	Corn Silage	HC	LC
DM %	34.8 (1.9)	44.0 (1.1)	63.53 (0.79)	55.94 (0.92)
CP %	18.2 (2.1)	7.42 (0.6)	18.08 (0.3)	17.25 (0.58)
ADIP %	1.30 (0.24)	0.55 (0.13)	1.5 (0.22)	1.55 (0.13)
NFC[3] %	21.0 (5.6)	37.6 (3.5)	39.7 (1.34)	35.9 (2.5)
NDF %	47.1 (7.0)	47.4 (4.2)	28.6 (0.75)	33.8 (2.6)
ADF %	37.8 (4.4)	26.1 (0.6)	15.08 (0.25)	19.4 (0.78)
Ether extract %	2.9 (0.16)	2.3 (0.35)	5.85 (0.17)	5.29 (0.21)
Ash %	10.7 (1.1)	5.2 (0.54)	7.75 (0.37)	7.79 (0.37)
Ca %	1.36 (0.33)	0.27 (0.04)	1.09 (0.1)	1.1 (0.17)
P %	0.29 (0.06)	0.17 (0.02)	0.58 (0.1)	0.51 (0.1)
K %	2.87 (0.39)	1.19 (0.07)	1.22 (0.04)	1.03 (0.03)
Mg %	0.38 (0.06)	0.28 (0.02)	0.32 (0.01)	0.32 (0.02)
Na %	0.04 (0.01)	< 0.01	0.39 (0.03)	0.37 (0.03)

[1]n = 4 for each forage, (SD within brackets).

[2]HC = higher concentrate (61.5% of diet DM); LC = lower concentrate (50.6% of diet DM).

[3]Nonfiber carbohydrates = 100 – (NDF% + CP% + ether extract% + ash%).

Table 3. Ingredient composition of energy protein supplements (on a DM basis)

Ingredient	Energy Supplement	Protein Supplement
Rolled barley	54.0	—
Luprosil salt (calcium propionate)	0.2	—
Protein pellet[1]	1.8	—
Dairy supplement[2]	40.0	—
Tallow (feed grade rendered fat)	4.0	
Dried distillers grain	—	42.0
Fish meal	—	7.0
Canola meal	—	22.7
Soybean meal	—	20.0
Beet molasses	—	3.0
Niacin (Vit. B3)	—	0.3
Sodium bicarbonate	—	5.0

[1]Protein pellets contain 46.1% soybean meal, 2.6% wheat shorts, 40.0% canola meal, 5.0% oat hulls, 0.3% pellet binder, 1.0% cane molasses, and 5.0% corn gluten meal.

[2]Dairy supplement contains 0.13% vitamin ADE premix (Vit A, 16800 IU/kg; Vit D, 2215 IU/kg; Vit E, 75 IU/kg, DM basis), 0.13% trace mineral premix, 2.6% soybean meal, 0.06% selenium, 39.1% wheat shorts, 5.0% distillers grain, 17.5% canola meal, 15.0% ground wheat, 1.7% dicalcium phosphate, 1.6% salt, 2.0% dynamate, 0.3% pellet binder, 1.0% cane molasses, 3.7% calcium carbonate, and 10.0% corn gluten meal.

Table 4. Penn State particle size analyses of forage silages and TMR (SE within brackets)

PSPS sieving[1]	Silage		TMR[2]	
	Alfalfa	Corn	HC	LC
	% retained, as fed basis			
Top screen (> 19 mm)	17.2 (2.5)[a]	6.4 (1.6)[b]	3.7 (0.4)	4.9 (0.7)
Middle screen (> 8 mm)	46.2 (4.4)[b]	56.5 (2.4)[a]	34.4 (2.0)[b]	40.5 (1.4)[a]
Wire sieve (> 1.8 mm)	33.8 (2.4)	33.9 (2.3)	54.1 (1.9)[a]	48.0 (1.7)[b]
Bottom pan	2.8 (1.1)	3.2 (0.41)	7.8 (0.2)[a]	6.6 (0.3)[b]

[a,b]The values in each row with different superscripts for forages (corn silage vs. alfalfa silage) and TMR (HC vs. LC) and in top screen (> 19 mm) differ with $P < 0.05$, and for forages in middle screen (> 8 mm) with $P = 0.08$.

[1]Pooled n = 4 for each forage and TMR.

[2]HC = higher concentrate (61.5% of diet DM); LC = lower concentrate (50.6% of diet DM).

Table 5. Effects of diet and time of feeding (TF) on rumen fluid pH and concentrations of VFA and ammonia N[1]

	Diet[2]		TF			*P*-value		
Item	HC	LC	0900 h	2100 h	SE	Diet	TF	Diet × TF
pH	6.25	6.38	6.31	6.31	0.05	0.01	0.95	0.40
VFA, m*M*								
Total	120.0	121.7	123.0	119.0	3.8	0.66	0.22	0.19
Acetate (A)	69.3	76.5	74.9	71.0	2.5	0.006	0.13	0.15
Propionate (P)	35.1	28.2	32.1	31.2	1.3	<0.01	0.52	0.89
Butyrate	12.3	13.8	13.2	13.0	0.6	0.02	0.76	0.08
Isobutyrate	0.3	0.7	0.5	0.6	0.1	0.001	0.39	0.06
Isovalerate	0.9	1.2	1.0	1.1	0.05	<0.01	0.35	0.02
Valerate	1.6	1.3	1.5	1.4	0.07	<0.01	0.34	0.52
A:P	2.0	2.8	2.5	2.4	0.1	<0.01	0.31	0.21
NH_3 (mg/dL)	4.4	4.5	4.2	4.8	0.4	0.80	0.21	0.30

[1]Rumen fluid samples were taken twice a day at 0100 h and 1300 h corresponding to 4 and 16 h post-feeding, respectively for two non-consecutive days in each sampling week.

[2]HC = higher concentrate (61.46% of diet DM); LC = lower concentrate (50.62% of diet DM).

Table 6. Effect of post-feeding sampling hour (SH) in relation to time of feeding (TF) on rumen fluid pH and concentrations of VFA and ammonia N[1]

Item	TF					*P*-value	
	0900 h		2100 h				
Post-feeding hours	4 h	16 h	4 h	16 h	SE	SH	SH × TF
pH	6.29[b]	6.33[b]	6.17[c]	6.46[a]	0.06	0.009	0.001
VFA, m*M*							
Total	122.4[b]	123.9[b]	132.0[a]	105.0[c]	3.81	0.0005	0.001
Acetate (A)	74.7[ab]	75.0[ab]	79.3[a]	62.7[b]	2.50	0.001	0.002
Propionate (P)	31.6[b]	32.5[ab]	34.9[a]	27.5[c]	1.31	0.003	0.02
Butyrate	13.1[b]	13.3[ab]	14.8[a]	11.2[c]	0.87	0.003	0.01
Isobutyrate	0.49[b]	0.47[b]	0.41[b]	0.72[a]	0.14	0.01	0.14
Isovalerate	1.09[a]	1.07[a]	0.91[b]	1.11[a]	0.05	0.11	0.17
Valerate	1.38[b]	1.42[b]	1.59[a]	1.33[b]	0.09	0.03	0.10
A:P	2.49	2.43	2.39	2.35	0.12	0.97	0.57
Ammonia N (mg/dL)	4.34[b]	4.01[b]	6.00[a]	3.49[b]	0.63	0.02	0.002

[a,b,c] Different letters in the same row differ at $P < 0.05$.

[1]Rumen fluid samples were taken twice a day at 0100 and 1300 h respectively corresponding to 4 h and 16 h post-feeding for two non-consecutive days in each sampling week.

Table 7. Effects of diet and time of feeding (TF) on milk production and composition, feed intake and feed efficiency

	Diet[1]		TF			*P*-value		
Item	HC	LC	0900 h	2100 h	SE	Diet	TF	Diet × TF
DMI, kg/d	20.6	20.6	20.5	20.6	0.71	0.96	0.89	0.44
Milk yield, kg/d	36.9	37.1	36.6	37.3	0.92	0.86	0.46	0.71
3.5%FCM, kg/d	30.6[b]	33.3[a]	31.0	32.9	1.22	0.03	0.13	0.76
ECM, kg/d	27.1	29.1	27.3	29.0	1.03	0.07	0.10	0.58
Feed Efficiency	1.32[b]	1.41[a]	1.33	1.41	0.05	0.002	0.62	0.22
Milk Components								
Milk Fat %	2.55[b]	2.88[a]	2.57	2.76	0.11	0.001	0.10	0.98
Fat yield, kg/d	0.90[b]	1.06[a]	0.93	1.03	0.06	0.01	0.10	0.83
Milk protein, %	3.53[a]	3.36[b]	3.42	3.47	0.04	<0.001	0.23	0.18
Protein yield, kg/d	1.29[a]	1.24[b]	1.25	1.28	0.03	0.05	0.20	0.37
Milk SNF %	9.12	9.02	9.03	9.12	0.07	0.20	0.24	0.16
SNF yield, kg/d	3.35	3.34	3.30	3.39	0.09	0.87	0.30	0.42
Total solids %	11.60	11.90	11.61	11.88	0.13	0.02	0.06	0.46
Total solids yield, kg/d	4.25	4.40	4.23	4.43	0.13	0.27	0.15	0.49
Ort coarseness[2]	39.7[b]	54.3[a]	51.1[a]	42.9[b]	2.6	0.006	<0.001	0.94

[1]HC = higher concentrate (61.5% of diet DM); LC = lower concentrate (50.6% of diet DM).

[2]As-fed percentage of orts retained on two top screens (>8 mm) of the Penn State Particle Separator.

Table 8. Effect of parity and time of feeding (TF) on feed intake, feed efficiency, and milk production and composition

	Multiparous		Primiparous			*P*-value	
Item	0900 h	2100 h	0900 h	2100 h	SE	Parity	P × TF
DMI, kg/d	21.9[a]	22.3[a]	19.3[b]	19.0[b]	0.6	0.01	0.67
Milk Yield, kg/d	39.2	41.1	34.1	33.7	3.0	0.19	0.23
3.5% FCM, kg/d	32.8[ab]	37.3[a]	29.2[b]	28.6[b]	2.5	0.08	0.05
ECM, kg/d[1]	29.2[a]	32.8[a]	25.3[b]	25.2[b]	2.0	0.04	0.08
Feed Efficiency	1.33	1.47	1.31	1.32	0.11	0.83	0.48
Milk fat %	2.50[b]	2.91[a]	2.64[ab]	2.61[ab]	0.21	0.79	0.07
Fat yield, kg/d	0.98[ab]	1.20[a]	0.88[b]	0.86[b]	0.08	0.12	0.05
Milk protein %	3.49	3.52	3.35	3.41	0.14	0.56	0.66
Protein yield, kg/d	1.36[a]	1.42[a]	1.14[b]	1.14[b]	0.07	0.04	0.26
SNF %	9.19	9.19	8.86	9.04	0.18	0.39	0.22
SNF yield, kg/d	3.58	3.75	3.01	3.04	0.22	0.09	0.46
Total solids,	11.71	12.10	11.51	11.66	0.32	0.49	0.39
Total solids yield, kg/d	4.55	4.95	3.90	3.90	0.28	0.08	0.16
Ort coarseness[2]	53.9	40.9	48.4	44.8	4.6	0.82	0.08

[a,b]Different superscripts within the same row differ at $P < 0.05$.

[1]Energy corrected milk (3.5% fat and 3.2% protein).

[2]As-fed percentage of orts retained on sum of two top screens (>8 mm) of the Penn State Particle Separator.

Table 9. Effects of diet and time of feeding (TF) on body weight, body weight change, body condition score, and dry matter intake as a percent of body weight

	Diet		Time of feeding			*P*-value		
Item	HC	LC	0900 h	2100 h	SE	Diet	TF	Diet × TF
BW[1] (kg)	655.0	644.1	647.0	652.1	3.2	0.005	0.11	0.29
BW change[2] (kg/d)	0.31	-0.37	-0.31	0.26	0.25	0.02	0.04	0.65
BCS[3]	3.16	3.01	3.07	3.09	0.06	0.02	0.73	0.87
BCS change, in 21 d	0.16	-0.005	0.07	0.08	0.05	0.01	0.77	0.62

[1]Body weight.

[2]Values were calculated by dividing periodical changes in BW changes by 21 (number of days in each period).

[3]Body condition score (1-5 scale) (Edmonson et al., 1989).

CHAPTER 4

Response in Post-Feeding Patterns of Feed Intake and Peripheral Blood Metabolites to Feeding at 2100 h vs. 0900 h in Lactating Cows

ABSTRACT

The objective of the current study was to determine the effect of feeding time and forage to concentrate ratio on 24-h averages and patterns of feed intake and blood metabolites. Four multiparous (BW = 652 ± 14 kg, BCS = 2.87 ± 0.14, days in milk = 83 ± 22) and four primiparous (BW = 667 ± 110 kg, BCS = 3.19 ± 0.66, 81 ± 23 days in milk; mean ± SD) Holstein cows were used in a 4 × 4 Latin square design with a 2 × 2 factorial arrangement of treatments. A higher concentrate (HC, forage to concentrate ratio = 38.5 : 61.5) or a lower concentrate (LC, forage to concentrate ratio = 50.6 : 49.4) total mixed ration (TMR) was delivered at either 2100 h or 0900 h. The trial consisted of four 21-d periods. Each period had 14 d of adaptation and 7 d of sampling. Blood was sampled every 2 h for two 24 h periods during sampling weeks. Delivering the feed at 2100 h instead of at 0900 h increased the amount of feed consumed within 3 h post-feeding, from 26 to 37% of total daily intake. Dry matter intake over 24-h did not differ among treatments. Average daily plasma lactate tended to increase when TMR was offered at 2100 h instead of at 0900 h. Blood urea, lactate, and BHBA in both parities, but NEFA only in multiparous cows, exhibited significant 24 h patterns. The 2100 h-fed cows had lower blood glucose at 2 h post-feeding but higher blood lactate and BHBA at 2 h and 4 h post-feeding than did 0900 h-fed cows. Feeding the HC instead of the LC diet

increased daily averages of plasma glucose and lactate, and reduced BHBA. Average daily plasma NEFA was higher in primiparous than in multiparous cows. Results demonstrated that evening compared to morning feeding increased feed intake within 3 h of feeding, and thereby altered post-feeding patterns in blood metabolites. Time of feeding may, hence, affect peripheral nutrient availability in lactating cows.

Key words: Feeding time, 24-h pattern, blood metabolite, lactating cow

INTRODUCTION

Livestock physiology is influenced and regulated by external stimuli such as time of feed delivery and light-dark cycle (Piccione and Caola, 2002). The external stimuli can affect the 24-h patterns in feed intake and gastro-hepatic function (Piccione et al., 2003). The gut and liver are the specialized organs where nutrient assimilation occurs, thereby regulating substrate distribution throughout the body (Danfær, 1994; Sauvant, 1994). Hence, any factor that can affect diurnal patterns in nutrient flow to the gut and liver may in turn alter nutrient availability for peripheral tissues.

Fresh feed delivery determines diurnal patterns of eating activity in lactating cows (Philips and Rind, 2001; DeVries et al., 2003). Diurnal patterns in blood glucose, BHBA, and urea have also been observed in transition and early lactation cows (Plaizier et al., 2005; Sutton et al., 1988). Diurnal patterns in nutrient intake and blood hormones (e.g., insulin and GH) can explain the 24-h patterns in circulating blood metabolites (Vasilatos and Wangsness, 1981). Any changes in post-feeding patterns of feed intake would be expected to alter post-feeding patterns in rumen pH, VFA, ammonia, and likely the post

ruminal nutrient supply (Robinson et al., 1997, 2002; Chapters 4 and 6). The altered post-feeding and 24-h patterns in ruminal and intestinal nutrient delivery will consequently affect the hepatic release of glucose, acetate, AA, and BHBA (de Boer et al., 1985). As a result, the mammary and non-mammary (e.g., adipocytes) availability of nutrients will change.

Distributing feed deliveries in the afternoon and evening rather than during the day improved energy balance of lactating cows in hot environment (Aharoni et al., 2005). Robinson et al. (1997) observed an increase in rumen digestion and milk fat yield when a protein meal was fed at 0030 h instead of at 0730 h. Most recently, under normal ambient temperatures (20.4°C), we found an enhanced milk fat by feeding mutiparous cows at 2100 h instead of 0900 h (Chapter 3). When Blum et al. (2000) fed lactating cows twice daily at 0700 and 1500 h with equal amounts of feed at each delivery, blood glucose and NEFA declined after morning feeding but not after afternoon feeding. A postprandial rises in blood BHBA, urea and insulin occurred only after the 0700 h-feed delivery (Blum et al., 2000). Aharoni et al. (2005), Blum et al. (2000), and Robinson et al. (1997) did not monitor 24-h patterns in feed intake. It thus remains unexplored if the time of feed delivery alters the 24-h patterns in feed intake. It is therefore unknown if and to what extent the 24-h patterns in feed ingestion can alter the 24-h patterns in circulating blood metabolites. Knowledge of 24-h patterns in feed intake and blood metabolites would contribute to the understanding of the mechanisms mediating the productive responses to feeding time in once-daily fed cows.

Diurnal variations in glucose tolerance and insulin responsiveness have been shown in humans and rats (la Fleur et al., 2001; McNamara, 2004). In ruminants,

Piccione et al. (2003) recently found a nocturnal peak in body temperature. This may suggest that 24-h patterns in body metabolism exist in ruminants as well (Piccione et al., 2003). Also, evening instead of morning feeding has recently been shown to improve growth in beef steers (Small et al., 2004) and heifers (Kennedy et al., 2004). The hypothesis was that post-feeding patterns in rumen and body metabolism are mediated by feeding time. The objective of the current study was to determine the effect of delivering a higher or a lower concentrate total mixed ration at either 0900 h or 2100 h on 24-h patterns in feed intake and circulating blood metabolites in lactating Holstein cows.

MATERIALS AND METHODS

Cows and Experimental Design

Four multiparous (BW = 652 ± 14 kg, BCS = 2.87 ± 0.14, days in milk = 83 ± 22) and four primiparous (BW = 667 ± 110 kg, BCS = 3.19 ± 0.66, 81 ± 23 days in milk; mean ± SD) lactating Holstein cows were housed in tie stalls at the Dairy Metabolism Unit of Glenlea Research Station, University of Manitoba. All experimental procedures involving animals were according to the guidelines of the Canadian Council for Animal Care (CCAC, 1993). The average air temperature and relative humidity were 20.4°C and 68.1%, respectively. The maximum air temperature of the metabolism unit did not exceed 25°C at any time during the experiment.

Cows were offered either a higher concentrate diet (HC) with a forage to concentrate ratio (F:C) of 38:62 or a lower concentrate diet (LC) with a F:C of 49:51. The total mixed rations (TMR) were delivered at either 0900 h or 2100 h. The experimental

design was a double 4 × 4 Latin square with a 2 × 2 factorial arrangement of feed delivery time and dietary concentrate level. Each experimental period lasted for 21 d with 14 d of adaptation followed by 7 d of sampling and data collection. Cows were fed *ad libitum* allowing for between 5-10% orts, and had unlimited access to fresh water. Diurnal patterns in feed intake were monitored continuously using a data acquisition system within the metabolism unit (Grow-Safe Sys, Model 4000, Airdrie, AB). Total mixed rations (Table 1) were prepared every morning using a Data Ranger Mixer (American Calan, Northwood, NH) with a Weigh Tronix head (Model 1000, American Calan, Northwood, NH). Except for sampling weeks, cows were allowed for 2 h daily exercise (0700-0900 h). Milking was performed twice daily in the stalls at 0400 and 1600 h. Lights were on from 0345 until 2245 h. A more detailed description of the experimental conditions was given in Chapter 3.

Monitoring of 24-h Patterns in Circulating Blood Metabolites

The first day of each period, all cows were catheterized in the jugular vein. The next day, the catheters were flushed with a sterilized heparinised saline solution (0.9% NaCl, and 50 unit heparin/ml). Blood samples were drawn every 2 h for two 24 h periods during the sampling week. Each 24-h sampling period started at 0900 h and ended at 0900 h on the subsequent day. Catheters were flushed with 10 ml of heparinised saline solution between samplings to inhibit clot formation within the catheter and extension set. Blood samples were transferred into green-top vacutainer tubes with anti-coagulant (Na-heparin), immediately put on ice, and centrifuged at 3000 *g* for 20 min at 4°C to harvest the plasma. The plasma was immediately frozen at -20°C until metabolite

analysis. A BHBA reagent (Procedure No. 310-UV, Sigma Diagnostics, St. Louis, MO), and a NEFA kit (Randox Laboratories, Ardmore, Northern Ireland, UK) were used to measure plasma BHBA and NEFA concentrations using a BM/Hitachi 911 analyzer (Boehringer Mannhein, Mannhein, Germany). Plasma concentrations of glucose, lactate, and urea were determined using an automatic analyzer (Stat Profile ® Critical Xpress, 02454-9141 Nova Biomedical, Waltham, MA) equipped with enzymatic sensors.

Statistical Analyses

Considering the equally-spaced, repeated blood measures during each 24-h period, the Mixed Models Procedure of SAS (SAS Institute, 2003; Wang and Goonewardene, 2004) was used to analyze the data. The time or hour of sampling was the repeated factor and cow was the subject. The effects of diet, time of feeding (TF), parity, hour, and two-, three-, and four-way interactions were considered fixed. Random effects included period, day of sampling within period, cow within parity, and four-way interactions between diet, TF, and parity with cow within parity and day within period.

To account for the between-hour, within-cow correlation of the repeated measures and thus to minimize the risk from Type 1 statistical error (rejection of true null hypothesis), various covariance structures were tested (Wang and Goonewardene, 2004). Among seven different covariance structures tested (i.e., simple, compound symmetry, first-order autoregressive, heterogeneous compound symmetry, first-order heterogeneous autoregressive, ante-dependence, and unstructured), the first-order autoregressive fitted the best for BHBA, lactate, and glucose. The most appropriate covariance structure for NEFA and urea was first-order heterogeneous autoregressive.

To obtain normal distribution and alleviate the heterogeneity of variance for residuals, data were transformed. Box-Cox algorithm (Pelteir et al., 1998) was applied to all data to acquire the power of λ that minimized the correlation between the mean and standard error. Consequently, the most suitable transformation for each variable was obtained. For lactate and BHBA, λ was equal to zero, thus log-transformation was used (Pelteir et al., 1998). Square-root transformation was applied to urea since the λ was close to 0.5 (Steel et al., 1997). The powers of λ obtained for glucose and NEFA were -1.8 and -2, respectively, thus the following formula was used for transformation:

$$Y = Y^{\lambda} - 1 / \lambda \acute{y}^{\lambda-1}$$

Where, Y = observation, λ = Box-Cox power, $\acute{y}$ = geometric mean of observations. Before transformation, one unit was added to all NEFA values since there were a few zero values among the data. Tukey's multiple range test was used to compare the differences between least square means. Significant levels of fixed effects were declared at $P \leq 0.05$ and trends were discussed at $0.05 < P \leq 0.10$. The CONTRAST statement of SAS (SAS Institute, 2003) was used to test the polynomial trends in diurnal patterns of blood metabolites (Table 12). The mean and standard errors for original data were given in tables. Probability values reported were based on the analysis of transformed data. The original least square means (±SEM) were used to plot the 24-h variations in plasma metabolites.

RESULTS AND DISCUSSION

24-h Patterns in TMR Intake

The 24-h distribution of TMR intake was determined in 3-h intervals relative to time of TMR delivery. Feed delivery time altered ($P < 0.05$) the 24-h patterns of feed intake (Figure 3). The proportion of daily TMR intake consumed within 3 h of post-feeding was 37% for 2100 h-fed cows but only 26% for 0900 h-fed cows ($P < 0.05$). In cumulative terms, the amounts consumed between 0-6 h and 0-9 h post-feeding were comparable between both groups (data not shown). Parity and diet did not interact with time of feed delivery on 24-h patterns in feed intake ($P > 0.10$). Despite altering the post-feeding patterns of TMR intake, provision of TMR at 2100 h instead of 0900 h did not affect dry matter intake over 24 h.

Fresh feed delivery and milking are major determinants of diurnal patterns in feed intake of tie-stall-housed (Haley et al., 2000) and loose-housed (DeVries et al., 2003) lactating cows. The stimulatory effect of feed delivery on eating activity may persist even with four times daily feeding (DeVries et al., 2005). DeVries et al. (2005) reported that cows fed once daily at 0530 h spent less time eating compared to cows fed twice daily at 0530 and 1515 h. The longer eating time of cows fed twice than of cows fed once daily was mostly due to increased eating time between 2000 to 0600 h. However, when Phillips and Rind (2001) delivered fresh TMR either once daily at 0600 h or four times daily at 0600, 1000, 1400, and 1900 h to early-lactation cows in free stalls, the total eating time did not differ between treatments. However, cows fed four times daily tended to spend a longer time eating in the evening (1600-2000 h) than in the morning (0400-1200 h). The

results from the current study and the studies of DeVries et al. (2005) and Phillips and Rind (2001) suggest that dairy cows eat when they are offered the fresh feed and that the amount consumed after feed delivery depends on time of day. It is noteworthy that data from DeVries et al. (2005) and Philips and Rind (2001) did not quantify the proportional contribution of 1) the more frequent feed delivery and 2) evening feed delivery *per se*, to greater eating time. In addition, daily DMI was not measured by DeVries et al. (2005).

To our knowledge, the post-feeding pattern of feed intake in once-daily, evening-fed cows has not previously been studied. Phillips and Rind (2001) suggested that if cows anticipate the feeding time, they would exhibit a more pronounced peak in time spent eating upon fresh feed delivery. Thus, the possibility exists that cows may have anticipated the 2100 h-feed delivery in the current study. Plasma insulin was higher and plasma glucose was lower at 2 h post-feeding in 2100-fed cows than in 0900 h-fed cows (Furedi et al., 2006a). Higher insulin could mean a weakened action of glucagon, leading to reduced gluconeogenesis (Brockman et al., 1978). The intraperitoneal injection of glucagon antibodies to suppress glucagon's effect stimulates feeding in rats (Langhans et al., 1982), and the intravenous glucagon has been shown to reduce feed intake in sheep (Deetz et al., 1981). It is possible that the higher blood insulin and lower blood glucose at 2 h post-feeding in the 2100 h-fed cows than in the 0900 h-fed cows might have delayed the glucagon-driven satiety. This may have contributed to the increased feed intake within 3 h of the 2100 h-feeding. Moreover, anticipation of imminent "lights off" may have acted as an eating motivator in 2100 h-fed cows.

Average Daily Levels and 24-h Patterns of Circulating Plasma Metabolites

L-Lactate

Delivering the TMR at 2100 h instead of at 0900 h tended to increase ($P = 0.09$) plasma lactate across sampling times (Table 10). The HC diet produced a higher ($P < 0.05$) plasma lactate than did LC diet (Table 10). Plasma lactate exhibited more pronounced postprandial variation in 2100 h-fed cows than in 0900 h-fed cows (Figure 4). Significant effects of sampling hour ($P < 0.001$) and the interaction between time of feed delivery and sampling hour ($P < 0.001$) existed for plasma lactate (Table 10). The plasma lactate increased about 50% shortly after feeding in 2100 h-fed cows but there was no change in 0900 h-fed cows (Figure 4). Lactate can contribute to 10-20% of the glucose generated via gluconeogenesis in fed lactating cows (Danfær et al., 1995). Peripheral levels of lactate represent hepatic entrance load of lactate. Hepatic lactate influx originates from 1) the lactate absorbed across the gut and 2) the lactate resulting from peripheral anaerobic oxidation of glucose or other pyruvate precursors such as AA (Armentano, 1992). The portal lactate comes from both ruminal fermentation of starch and sugars and epithelial conversion of organic acids e.g., propionate (Giesecke and Stangassinger, 1980). As much as 50% of rumen propionate can be metabolized across the rumen wall (Reynolds, 2002). Using isolated ruminal epithelial cells, Baldwin and McLeod (2000) found that up to 15% of the propionate metabolized by the rumen epithelium may be used for lactate synthesis. Moreover, Lemosquet et al. (2004) observed a tendency for increased plasma lactate following ruminal infusion of propionate rather than intestinal infusion of glucose. Therefore, the nutritional strategies such as evening feeding that stimulate the post-feeding rumen production of VFA can

subsequently increase the hepatic lactate load. Given the fact that hepatic uptake of lactate is much lower than that of propionate (Armentano, 1992), the increased portal lactate is expected to elevate the circulating blood lactate. In addition, insulin tends to reduce hepatic metabolism of lactate in favor of peripheral availability (Brockman; 1984, 1985). Thus, the dramatic rise in plasma lactate of 2100 h-fed cows at 2-h post-feeding (Figure 4) was at least paerially due to high blood insulin at 2-h post-feeding. The higher plasma lactate shortly after feed delivery in 2100 h-fed cows was at least partially due to its alimentary supply. This explanation would be consistent with the increased feed intake within 3-h of feeding in 2100 h-fed cows (Figure 3).

The average daily plasma lactate was higher ($P = 0.05$) in HC-fed cows than in LC-fed cows (Table 10). The greater starch supply from the HC compared with the LC diet led to higher rumen propionate and lower pH (Chapter 3). The higher rumen propionate may have in turn increased the gut lactate delivery to the liver (Table 10). Likewise, feeding steers a higher concentrate diet resulted in net splanchnic release of lactate, but a lower concentrate diet was associated with net uptake of lactate (Huntington et al., 1996).

Urea

Time of feed delivery impacted 24-h patterns ($P < 0.001$), but not average daily levels, of plasma urea (Figure 5, Table 10). The HC delivery both at 0900 and 2100 h increased plasma urea only numerically at 2 h post-feeding. Subsequently, plasma urea decreased progressively until 6 h post-feeding in 2100-fed cows and until 12 h post-feeding in 0900 h-fed cows (Figure 5). Afterwards, it increased up to preprandial

baseline. In cows fed LC, however, plasma urea exhibited a significant rise ($P < 0.05$) at 2 h after 0900 h-feeding which lasted until 4 h post-feeding. It then dropped gradually until 10-12 h post-feeding ($P < 0.05$), reaching a nadir overnight, and then starting to rise 6 h before next feed delivery. The post-feeding rise in plasma urea in LC-fed cows occurred only at 2 h after 2100 h-feed delivery, but lasted until 6 h after 0900 h-feed delivery (Figure 5). While 0900 h-fed cows consumed less feed within 3 h of feed delivery than did 2100 h-fed cows, 0900 h-fed cows consumed greater TMR between 3-6 h post-feeding (Figure 3). This probably led to prolonged N availability and rumen ammonia production in 0900 h-fed cows. The post-feeding rise in blood urea has been shown by others in cows (Blum et al., 2000; Gustafsson and Palmquist, 1993; Plaizier et al., 2005) and goats (Piccione et al., 2003).

Provision of the HC diet instead of the LC diet tended ($P = 0.12$) to increase plasma urea (Table 10). The HC diet contained slightly higher CP content than LC diet (18.1 vs. 17.3%). Considering the similar DMI of cows between two diets (20.6 kg/d; Chapter 3), on average 170 g more CP was consumed by HC-fed cows than by LC-fed cows. The greater CP intake may lead to a greater ruminal ammonia production (Reynal and Broderick, 2005) and can debatably (see Firkins and Reynolds, 2005) increase the energetic costs due to hepatic urea formation. This suggests that the rate of ureogenesis was higher in HC-fed cows, supporting their higher plasma urea. The HC diet increased milk protein (Chapter 3), which may have been due to greater dietary starch, when compared to the LC diet. The greater starch increases diet fermentability, which may in turn increase rumen microbial protein synthesis (DePeters and Cant, 1992). As a result, more AA may be spared for milk protein secretion by HC than by LC diet.

Multiparous cows tended ($P < 0.10$) to have higher plasma urea than primiparous cows (Table 11). This could partly be attributed to the greater feed intake (or greater N intake) and more extensive rumen N degradation. Also, multiparous cows produced more milk protein than primiparous cows (1.39 vs. 1.14 ± 0.07 kg/d, $P = 0.04$). Therefore, multiparous cows they may have metabolized more AA in support of higher mammary demands, leading to greater hepatic urea output compared to primiparous cows.

BHBA

Plasma BHBA is seen as an indicator of energy status (Aeberhard et al., 2001; Eicher et al., 1999) and external stressors (Andersen et al., 2004) in dairy cows. The BHBA of gut origin seems, however, to receive less attention than does the hepatic BHBA in times of fat mobilization. In fed, unstressed ruminants, BHBA is mostly a product of butyrate metabolism across the rumen epithelium and offers a valuable energy source for peripheral tissues (Reynolds, 2002). The gut metabolism of butyrate provides energy to sustain the maintenance of visceral tissues, while serving the peripheral tissues with BHBA. Up to 90% of rumen butyrate can undergo either full oxidation or conversion to BHBA (Reynolds, 2002). Neither TF nor its interaction with dietary concentrate level affected daily averages of plasma BHBA (Table 10). However, the interaction of TF and sampling hour was significant ($P < 0.001$). Feeding at 2100 h increased ($P < 0.01$) plasma BHBA at 2 h post-feeding, followed by a rapid decline until 6 h post-feeding (Figure 6). In 0900 h-fed cows, plasma BHBA at 2 h post-feeding rose less than 50% compared to the prandial level. Plasma BHBA in 0900 h-fed cows remained at peak until 10 h after feed delivery when it declined. The 2100 h-fed cows

consumed a greater ($P < 0.05$) amount of feed (37 vs. 26% of daily intake) within 3-h of feed delivery than did 0900 h-fed cows (Figure 3). The greater feed intake shortly post-feeding resulted in greater rumen butyrate (14.8 vs. 13.0 m*M*) shortly after feed delivery (Chapter 3). The greater butyrate should have in turn increased butyrate transformation to BHBA across the rumen wall (Reynolds, 2002), thereby elevating BHBA appearance in portal and peripheral blood. An immediate postprandial rise in plasma BHBA has also been observed in twice-daily fed lactating cows (Blum et al., 2000; de Boer et al., 1985; Sutton et al., 1988). The post-feeding rise in plasma BHBA in the study of Blum et al. (2000) occurred only after feed delivery at 0700 h but not at 1500 h. The postprandial increase in blood insulin also occurred only after morning but not afternoon meal (Blum et al., 2000). Unlike the current study, the diurnal patterns in feed intake and rumen fermentation were not monitored by Blum et al. (2000). So, the possible effect of feeding behavior on 24-h patterns of blood metabolites remained unknown (Blum et al., 2000).

Feeding the HC diet instead of the LC diet reduced ($P < 0.01$) daily levels of plasma BHBA (Table 10). This was expected because the HC diet reduced rumen concentration of butyrate, compared with the LC diet (12.3 vs. 13.8 m*M*, $P < 0.05$; Chapter 3). A lower plasma BHBA in cows fed a HC diet than in cows fed a LC diet was also reported by Andersen et al. (2004). Sutton et al. (1988) observed a sharp post-feeding rise in plasma levels of VFA, BHBA, and insulin when a 90% concentrate diet was delivered twice instead of six times daily. Increased plasma VFA, BHBA, and insulin shortly after feeding were associated with reduced milk fat in the study of Sutton et al. (1988). In the current study, however, the higher blood lactate, BHBA, and propionate shortly after feeding in 2100 h-fed cows compared to 0900 h-fed cows were

accompanied by an elevated milk fat (1.03 vs. 0.93 kg/d; Chapter 3). The between-study differences in productive responses to postprandial rises in blood levels of milk precursors and insulin suggest that other hormones (e.g., glucagon and GH) might mediate increased milk fat evening feed delivery, as well.

NEFA

The mean daily levels of plasma NEFA were not altered by TF, dietary F:C, nor by their interaction (Table 10). However, plasma NEFA exhibited significant 24 h variation ($P < 0.01$), which was influenced by TF and parity (Table 11). Plasma NEFA showed a significant 24 h pattern in primiparous cows but not in multiparous cows (Figure 7). In multiparous cows, plasma NEFA tended ($P < 0.10$) to decrease at 2 h after 2100 h-feed delivery but not after 0900 h-feed delivery (Figure 7). In 0900 h-fed primiparous cows, plasma NEFA declined upon feed delivery until 4 h post-feeding, remaining at its nadir until 0100 h. Plasma NEFA then started to rise early in the morning to obtain its baseline at 0900 h (Figure 7). Most of the feed intake (>70%) in 0900 h-fed cows occured during the day (0900-2100, Figure 3). As a result, insulin pulses are expected to occur during the day, particularly after the first large morning meal (Furedi et al., 2006a; Sutton et al., 1998). Sutton et al. (1988) argued that intermittent pulses rather than average daily levels of insulin are responsible for altered nutrient partitioning in lactating cows. Plasma insulin increased shortly after feeding in 0900 h-fed cows (Furedi et al., 2006a). Such an immediate post-feeding insulin pulse may have in turn reduced the day-time plasma NEFA in 0900 h-fed primiparous cows (Figure 7). The rise in plasma NEFA after midnight in 0900 h-fed primiparous cows agrees with several other studies

(Blum et al., 2000; Fröhli and Blum, 1988; Plaizier et al., 2005; Sutton et al., 1988). The increasing trend in plasma NEFA later in the night can be explained by the lower feed intake (Figure 3), lower nutrient absorption, and thereby lower plasma insulin (Furedi et al., 2006a). Plasma NEFA in 2100 h-fed primiparous cows showed a tendency to decline for only 2 h post-feeding (Figure 7). The 2100 h-fed primiparous cows exhibited another fall in plasma NEFA at 10 h post-feeding (i.e., 0700 h), which was not observed in 0900 h-fed primiparous cows. The greater plasma insulin of 2100 h-fed than of 0900 h-fed primiparous cows at 6 to 8 h post-feeding (Furedi et al., 2006a) may have contributed to such a lower NEFA at 10 h post-feeding.

Dietary concentrate level and its interaction with TF did not affect 24-h patterns in plasma NEFA (Table 10). A lack of diet effect on average blood NEFA has also been reported by others (Andersen et al., 2004; Moorbey et al., 2002; Nielsen et al., 2003; Sutton et al., 1988). However, Nielsen et al. (2003) found that blood NEFA rose at 3 h before morning feeding, particularly for a high concentrate diet. In that study, the peak in plasma NEFA occurred at feeding time (1000 h) and was attributed to the lower energy intake overnight. Similarly, in the current study, plasma NEFA in 0900 h-fed cows peaked at 0900 h (i.e., at feed delivery time) and declined significantly by 4 h post-feeding. Insulin is a potent anti-lipolytic hormone (Brockman, 1978). Hence, the postprandial decline in plasma NEFA was most likely due to the insulin pulse at 2 h post-feeding (Furedi et al., 2006a).

The greater responsiveness of primiparous than of multiparous cows to TF could be attributed to their physiological state. Primiparous cows had greater BCS (3.19 vs. 2.87) and lower milk production (34 vs. 40 kg/d) than multiparous cows. This would

imply a stronger influence of insulin or storage hormone on body metabolism in primiparous than in multiparous cows. More notably, such an insulin-dependent growth of body tissues was superimposed on the greater body weight of primiparous than of multiparous cows (667 vs. 652 kg). Since feed delivery at 2100 h instead of 0900 increased post-feeding energy availability and blood insulin, we speculate that insulin partitioned a considerable amount of nutrients towards ongoing growth. As a result, improved energy status due to feed delivery at 2100 h instead of at 0900 h was also reflected in reduced blood NEFA upon feeding in primiparous cows. In multiparous cows, however, such an attenuated energy deficiency was reflected in improved milk secretion.

None of plasma samples of the current study had a NEFA level of >0.3 mEq/L when the mammary gland starts to directly take up the circulating NEFA for milk fat secretion (Nielsen et al., 2003). The NEFA levels obtained in the current trial were close to the lower end of normal range expected in bovine plasma (0.1-0.37 mEq/L). Thus, little impact of diurnal patterns in plasma NEFA on milk fat was expected.

CONCLUSIONS

Changing the time of TMR delivery from 0900 to 2100 h increased feed intake within 3 h of feeding by 2.4 kg/d. Feeding at 2100 h led to higher post-feeding peaks in blood lactate and BHBA, and lower blood glucose at 2 h post-feeding, when compared to feeding at 0900 h. The post-feeding patterns in blood urea were similarly affected by both times of feeding. The TMR delivery at 2100 instead of 0900 h tended to increase average

daily plasma lactate. In primiparous, but not multiparous cows, blood NEFA exhibited a pre feeding rise and a post-feeding decline, when feed was delivered at 0900 h instead of 2100 h. Results suggest that feeding at 2100 h instead of 0900 h increases feed intake and blood levels of energy-yielding metabolites shortly post-feeding. As a result, feed delivery at 2100 h can improve energy availability to the mammary and non-mammary tissues in lactating cows.

Table 10. Effects of diet (D), time of feeding (TF), sampling hour (H), and the interactions on circulating blood metabolites[1]

Item	Diet[2]		TF		SEM	*P*-value[2]						
	HC	LC	0900 h	2100 h		D	TF	D×TF	H	D×H	TF×H	D×TF×H
Lactate, mmol/L	0.70	0.65	0.65	0.69	0.03	*	†	NS	***	NS	***	NS
Urea, mmol/L	4.94	4.75	4.83	4.86	0.17	†	NS	NS	***	*	***	***
BHBA, umol/L	470.0	576.1	512.8	533.4	35	**	NS	NS	***	NS	***	NS
NEFA, mEq/mol	0.123	0.128	0.130	0.121	0.008	NS	NS	NS	NS	†	**	NS

[1]Least square means and standard errors are given for original data, but statistical significance levels are based on the analysis of transformed data. BHBA = β-hydroxybutyrate, NEFA = non-esterified fatty acids.

[2]HC = higher concentrate diet, F:C = 39:61; LC = lower concentrate diet, F:C = 49:51.

NS = not significant, $P > 0.15$.

† $0.05 < P \leq 0.12$.

* $0.01 < P < 0.05$.

** $P < 0.01$.

*** $P < 0.001$.

Table 11. Effect of parity (Par) and its interactions with treatments and sampling hour on blood metabolites[1]

Item	Primiparous	Multiparous	SEM	*P*-value[2]					
				Par	Par×D	Par×TF	Par × H	Par×D×H	Par ×TF×H
Lactate, mmol/L	0.67	0.67	0.04	NS	†	NS	†	NS	NS
Urea, mmol/L	4.66	5.03	0.196	†	NS	NS	NS	NS	NS
BHBA, umol/L	470.0	576.1	35	NS	NS	†	**	NS	NS
NEFA, mEq/mol	0.143	0.108	0.009	*	NS	NS	NS	NS	**

[1]Least square means and standard errors are for the original data, but the statistical significance levels are based on the analysis of transformed data. BHBA = β-hydroxybutyrate, NEFA = non-esterified fatty acids.

[2]D = diet (level of concentrate), H = hour of sampling, Par = parity, TF = time of feed delivery.

NS = not significant.

† $0.05 < P \leq 0.12$.

* $0.01 < P < 0.05$.

** $P < 0.01$.

*** $P < 0.001$.

Table 12. Diurnal variation of plasma metabolites over a 24-h period across treatments[1]

	Fixed effect of hour of sampling, contrasts, P^2 =				
Item	Linear	Quadratic	Cubic	Quatric	Quintic
Lactate, mmol/L	0.0008	0.78	0.0001	0.0032	0.0005
Urea, mmol/L	0.003	0.314	0.052	0.014	0.027
BHBA, umol/L	0.015	< .0001	0.081	0.469	< .0001
NEFA, mEq/L	0.284	0.033	0.71	0.051	0.574

[1]Blood samples were taken every 2 h between two subsequent 9 a.m. for two non-consecutive days during sampling week of each period, with a total of 13 samples per day. BHBA = β-hydroxybutyrate, NEFA = non-esterified fatty acids.

[2]Probability values are based on statistical analysis of transformed data.

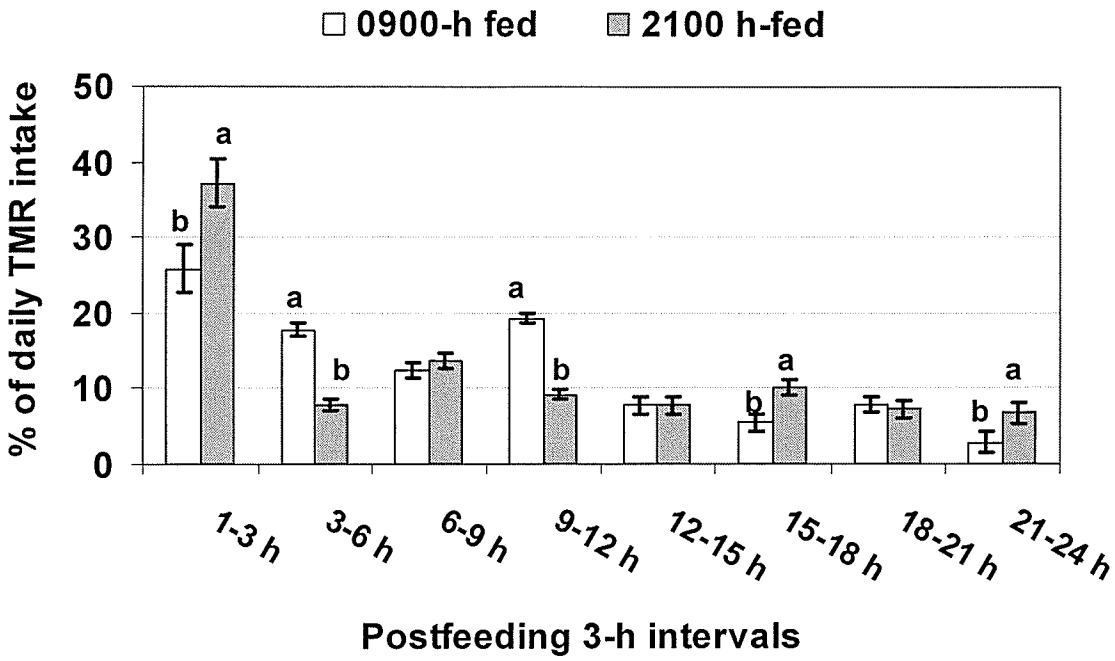


Figure 3. Post-feeding patterns of feed intake in cows fed either at 0900 h or at 2100 h. Within each 3-h, bars with different superscripts differ at $P < 0.05$.

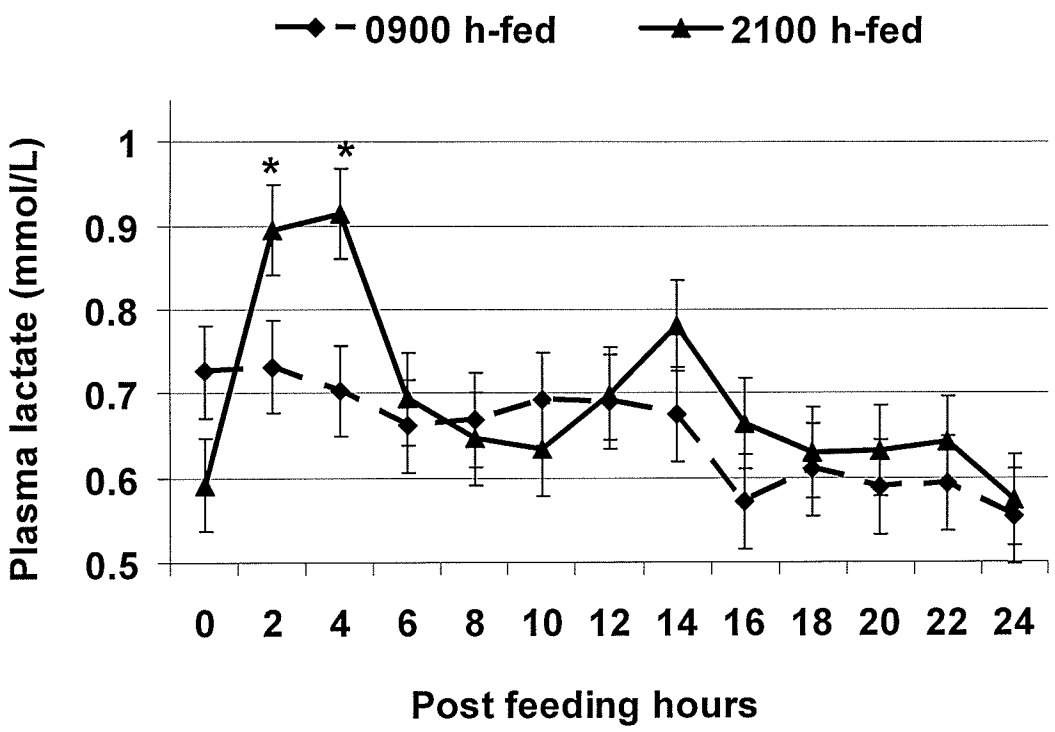


Figure 4. Post-feeding patterns of plasma lactate in cows fed either at 0900 h or at 2100 h. Within each sampling hour, * = $P < 0.05$.

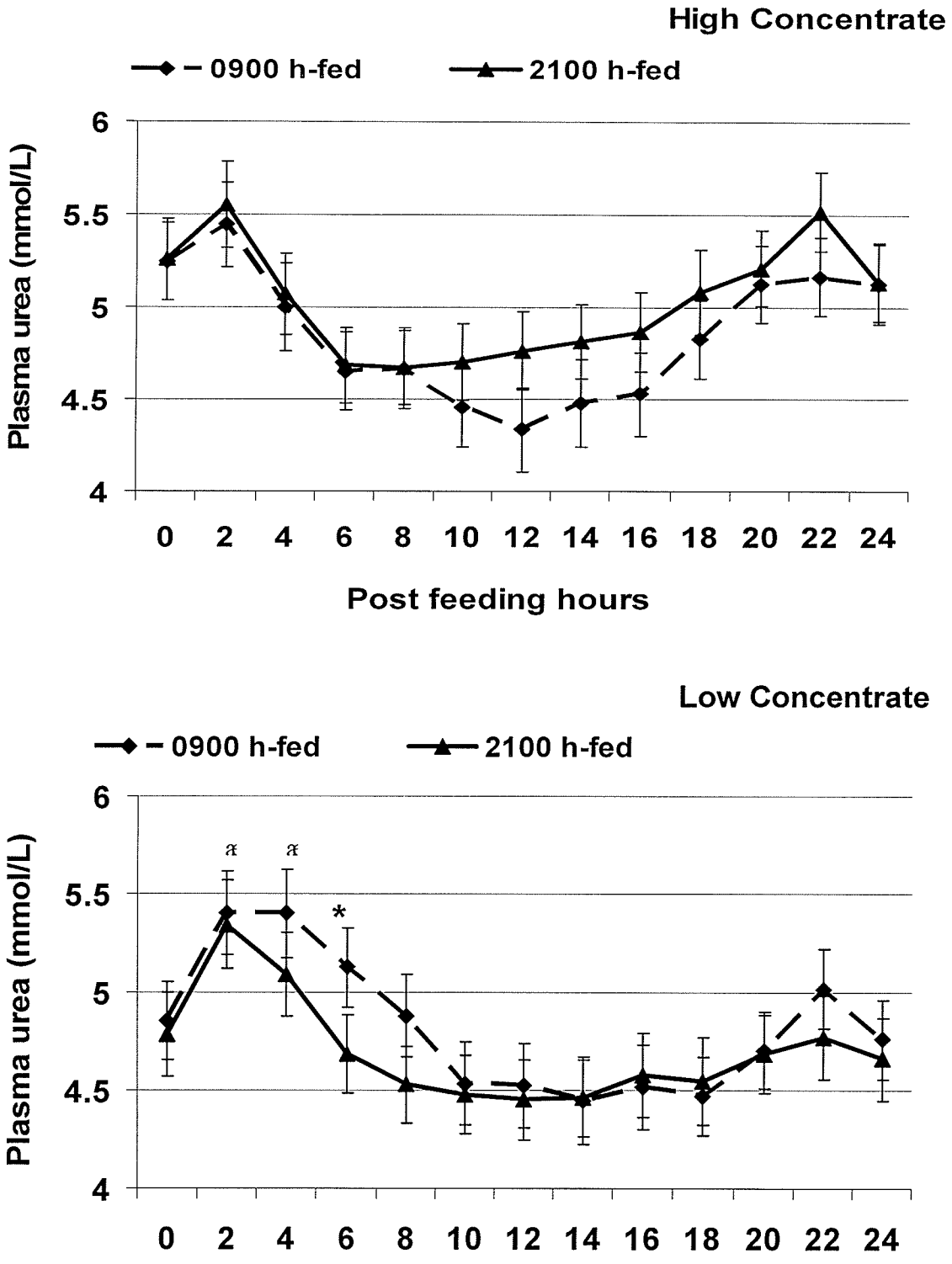


Figure 5. Post-feeding patterns in plasma urea of cows fed a higher (HC) or a lower concentrate (LC) diet either at 2100 or at 0900 h. Within each sampling hour, * = $P < 0.05$. æ = $P < 0.05$ for the comparisons of 0 vs. and 2- and 4-h post-feeding in LC diet.

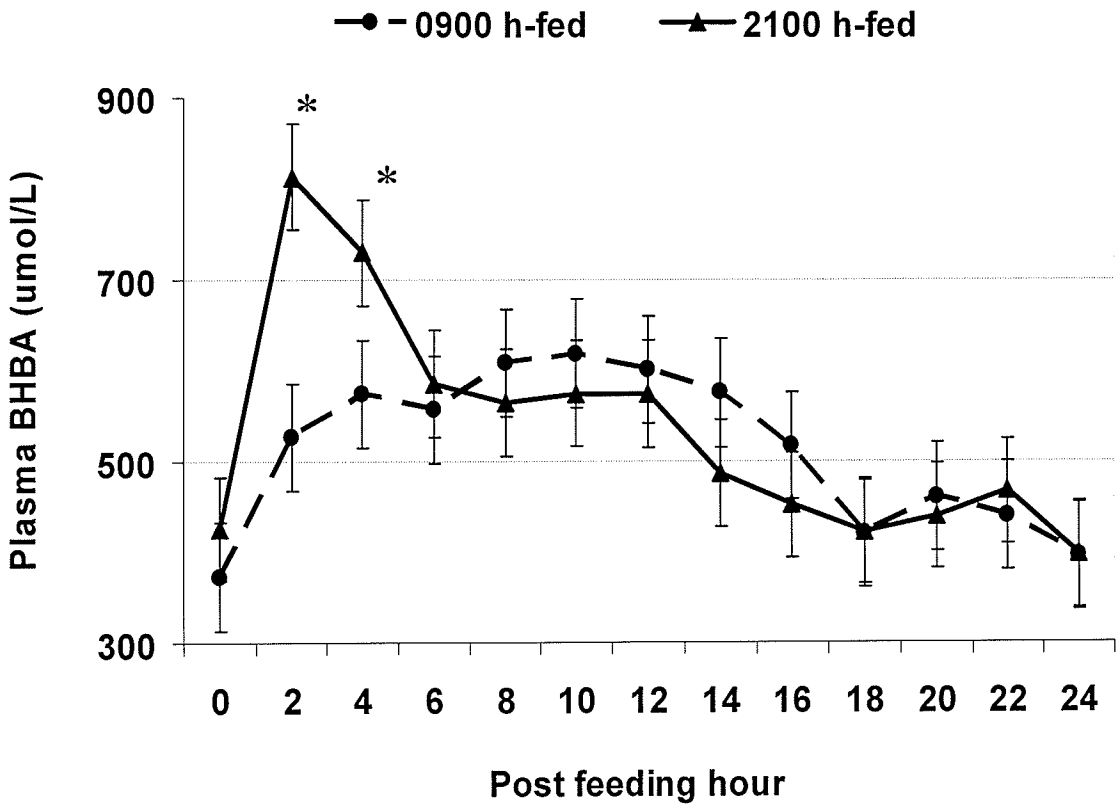


Figure 6. 24-h patterns of plasma BHBA in cows fed either at 0900 h or 2100 h. Within each hour, * = $P < 0.05$.

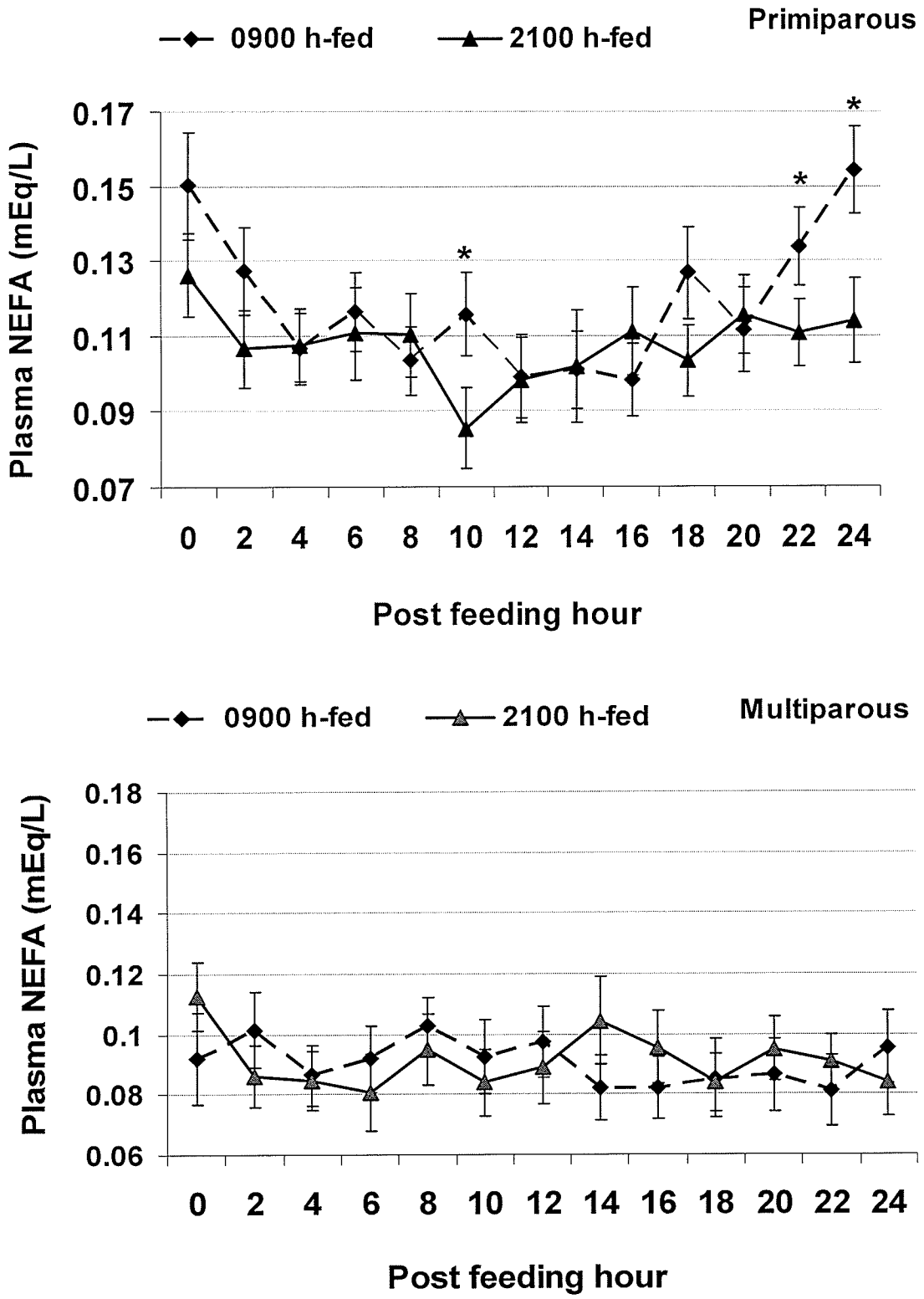


Figure 7. Post-feeding patterns of plasma NEFA in primiparous and multiparous cows fed either at 0900 h or at 2100 h. Within each hour, * = $P < 0.05$.

CHAPTER 5

Feed Delivery at 2100 h vs. 0900 h in Lactating Cows: Feed Intake, Rumen Fermentation, Nutrient Digestibility, Nitrogen Partitioning, and Productivity

ABSTRACT

The effects of providing a total mixed ration (TMR) at either 0900 h or 2100 h on feed intake, rumen fermentation, apparent total tract nutrient digestibility, nitrogen (N) partitioning and productivity of lactating cows were determined. Four multiparous (77 ± 25 days in milk at the beginning of the trial) and four primiparous (90 ± 33 days in milk at the beginning of the trial) Holstein cows were used in a cross-over design with two 6-week periods. Each period consisted of a 3-week adaptation. The TMR had a forage to concentrate ratio of 50.2:49.8 on a dry matter basis. Total fecal and urine were collected during week 4 of each period to measure total tract nutrient digestibility and N partitioning. Urinary excretion of purine derivatives (uric acid and allantoin) were used to estimate microbial protein synthesis. Provision of TMR at 2100 h instead of 0900 h increased dry matter intake in primiparous cows but did not affect it in multiparous cows. Feeding at 2100 h improved apparent total tract dry matter, crude protein and fiber digestibility, and N balance in all cows. Average daily rumen ammonia was lower in primiparous cows fed at 2100 h than in primiparous cows fed at 0900 h. Rumen propionate was lower and the acetate to propionate ratio was higher in 2100 h-fed multiparous cows than in 0900 h-fed multiparous cows. The evening-fed cows of both parities produced more fat-corrected yield (31.8 vs. 29.2 kg/d) and tended to produce

more energy-corrected milk (26.7 vs. 25.0 kg/d) than 0900 h-fed cows. Microbial protein synthesis and the milk proportions of total short, medium, and long chain fatty acids were not significantly affected by the treatments. Of N apparently digested in the total tract, evening instead of morning feeding reduced the proportion excreted via urine and secreted as milk in favor of body retention. As a result, N balance was improved by feed delivery at 2100 h. Results suggest that feed delivery at 2100 h instead of 0900 h can improve feed intake, nutrient digestibility, N balance and milk fat yield. Parity appeared to affect the impact of feed delivery time on cow performance, notably feed intake.

Key words: Evening feeding, feed delivery, rumen, N balance, lactating cow

INTRODUCTION

Feeding strategies for high-producing dairy cows are adopted to maintain rumen function and improve production persistency. Frequency and sequence of forage and concentrate delivery, dietary use of differently processed cereal grains, and maintaining physical effectiveness of dietary fiber are among feeding strategies underlined. Delivering fresh total mixed ration (TMR) at different times in a 24-h period under thermoneutral conditions, however, has received no attention. In hot (Aharoni et al., 2005; Reinhardt and Brandt, 1994) and cold (Kennedy et al., 2004) temperatures, evening instead of morning feeding has improved energy status of lactating cows and growing cattle.

Fresh feed delivery stimulates eating and thereby affects diurnal patterns in eating activity of lactating cows (DeVries and von Keyserlingk, 2005; Haley et al., 2000). Also,

evidence exists that post-feeding patterns of eating time and intensity (DeVries et al., 2005; Phillips and Rind, 2001) and of blood metabolites (Blum et al., 2000) differ between morning and evening feed deliveries in more than once-daily fed cows. We have recently shown that lactating cows can consume up to 30-70% of TMR within only 3 h of feed delivery (Chapters 4 and 6). Reinhardt and Brandt (1994) reported that Holstein steers fed ad libitum once daily for 1 kg/d weight gain also their feed within 3 h. The impact of feed delivery time on post-feeding patterns in feed intake, rumen fermentation, and cow metabolism are thus anticipated to be of greater magnitude when fresh feed is delivered once daily than more frequently. Dairy farmers who house their cows in the stalls prefer delivering the TMR only once daily, as it is less laborious than more frequent deliveries.

In our previous experiment using Latin square design with 21-d periods comprising a 14-d of adaptation, delivering the TMR at 2100 instead of at 0900 h increased rumen digestion and milk fat yield. The first objective of the current study was to establish such responses in rumen fermentation and milk fat with a 21-d adaptation to experimental conditions. Additional objectives were to determine microbial protein synthesis, total tract nutrient digestibility, N partitioning, and N balance in response to feed delivery at 2100 h vs. 0900 h.

MATERIALS AND METHODS

Experimental Design and Cow Management

Four multiparous [645 ± 75 kg body weight (BW); 77 ± 25 days in milk (DIM); mean ± SD] and four primiparous (576 ± 46 kg BW; 90 ± 33 DIM) lactating Holstein

cows were used in a cross-over design with two 42-d periods. Each period consisted of a 21-d adaptation phase. Four of the cows were rumen-cannulated. Cows were housed in tie-stalls at the Metabolism Unit of the Glenlea Research Station, University of Manitoba from September through December 2005. Cows received a TMR with a forage to concentrate ratio of 50.2:49.8 (DM basis, Table 13) *ad libitum* for the entire trial allowing for 5-10% orts. Cows had unlimited access to fresh water. During the adaptation periods, cows were allowed 2 h outdoor exercise every second morning. The average outside temperature and relative humidity during the sampling weeks were -3.7°C and 78.9%, respectively. Lights were turned on at 03:45 just before milking and turned off at 22:45 h. Cows were cared for according to the guidelines of the Canadian Council on Animal Care (CCAC, 1993).

Experimental treatments were offering a fresh TMR either at 0900 h or at 2100 h. The TMR was prepared daily in the morning using a Data Ranger Mixer (American Calan, Noothwood, NH) with a Weigh Tronix head (Model 1000, American Calan, Northwood, NH). The forage portion of the TMR was a 50:50 mixture of alfalfa silage and barley silage (DM basis). The concentrate portion consisted of premixed, separate energy and protein supplements (Chapter 3).

Sampling and Analysis of Feed, Orts, Fecal, and Urine

The TMR and orts of individual cows were sampled daily during week 4. Dry matter intake (DMI) for each cow was obtained by deducting the DM of orts from the DM content of the TMR offered. The TMR, alfalfa and barley silages, plus energy and protein supplements were sampled once a week on Thursday during the experiment. The

weekly changes in DM content of the forage silages were considered in fresh feed allocation to the cows to maintain the diet composition throughout the study. The TMR and forage samples were composited weekly for each period. The daily feed and ort samples were oven-dried at 60°C for 48-h to determine DM content. The dried samples were ground using a Wiley mill to pass through a 1 mm screen (Thomas Wiley, Philadelphia, PA) and stored at -20°C until analyzed. Total feces were collected using individual metal trays twice daily at 0900 and 2100 h during week-4. Fecal material from each cow was mixed thoroughly before taking about 1 kg representative sample twice daily. Samples were stored at -20°C for later nutrient analysis.

On the first day of week 4, urinary catheters were placed in the urethra 24 h before connection to the collection tubing (Wright et al. 1998). Total urine excretions were collected into polyester containers via indwelling bladder catheters was weighed twice daily at 0900 and 2100 h during week 4. To minimize N escape as ammonia, 100 ml of concentrated sulfuric acid was added to each urine container before each collection. A 50 ml sample of mixed urine was taken at each weighing and frozen at -20°C for later N determination. Another 2 ml sample of urine was taken, diluted 5 times in distilled water, and stored at -20°C for later analyses of purine derivatives (PD) including allantoin (Moscardini et al., 1998; Wright et al., 1998) and uric acid (Fujihara et al., 1987). The urinary excretion of allantoin and uric acid were used to estimate microbial protein flow to the duodenum (Mwenya et al., 2005).

The pooled TMR and forage samples were analyzed using the wet-chemistry procedures of AOAC (1990) for crude protein (CP, method 984.13), ADF (method 973.18), ether extract (method 920.39), and ash (method 942.05). Alpha-amylase and

sodium sulfite (Sigma no. A3306: Sigma Chemical Co., St. Louis, MO) were used to determine feed and fecal NDF according to Van Soest et al. (1991) using an Ankom 200 Fiber Analyzer (Ankom Technology, Fairport, NY). Inductively coupled plasma emission spectroscopy (AOAC, 1990) was used to measure the levels of Ca, P, Mg, Na, K, Cr, and Co using an Atom Scan 25 Plasma Spectrometer (Thermo Jarrell Ash Crop., Grand Junction, CO) after acid digestion of the samples.

Milking and Milk Analysis

Cows were milked twice daily at 0400 and 1600 h in their stalls. Milk yield at each milking was read using Tru Test regulation meters for the entire experiment (Westfalia Surge, Mississauga, ON). Milk was aliquoted into 50 ml vials at four consecutive milkings for all cows during the three sampling weeks. One milk sample was preserved with 2-bromo-2-nitropropane-1,3-diol, stored at 4°C, and analyzed for milk components at the laboratory of Dairy Farmers of Manitoba (Winnipeg, MB, Canada) by near infrared using the Milk-o-Scan 303AB (Føss Electric, Hillerød, Denmark). Another milk sample (10 ml) was taken with no preservative and frozen at -20°C for subsequent analysis of fatty acid profiles. Milk fatty acid profiles were determined using a gas chromatograph (Hewlett Packard HP5890A, Agilent Technologies, Inc., Santa Clara, CA), as described by Ward et al. (2002, 2003). The GC was equipped with a capillary column (0.25 mm ID, J&W Scientific HP88 100m, Agilent Technologies, Inc., Santa Clara, CA) with a film thickness of 0.2 μm. The injector and detector temperatures were set at 220°C and 290°C, respectively.

Analyses of Rumen Fluid pH, VFA, and Ammonia

The rumen pH of cannulated cows was monitored continuously during the sampling weeks. Rumen fluid from cannulated cows was sampled at 0, 2, 3, 4, 6, 8, 12, 16, 20, and 24 h after feed delivery twice a week during weeks 4 and 5 to study the 24-h patterns in rumen fermentation. In addition, rumen fluid from all cows was sampled twice at 1200 and 0000 h corresponding to 3 h and 15 h post-feeding on Thursday and Friday in week 5 of each experimental period. A Geishauser oral probe (Duffield et al., 2004) was used to aspirate the rumen fluid from non-cannulated cows. The initial 100 ml of rumen fluid aspirated was discarded to minimize saliva contamination and pH overestimation (Duffield et al., 2004). The pH of the second 100 ml aspirated was measured immediately using an Accumet Basic 15 pH meter with an Accumet gel-filled polymer body combination pH electrode (Fisher Scientific, Fairlawn, NJ). Upon measuring pH, rumen fluid samples were centrifuged at 1800 × *g* for 12 min, and the supernatants were stored at -20°C. For VFA analysis, frozen rumen fluid samples were thawed at room temperature and 0.5 ml of a 25% meta-phosphoric acid solution was added to 2.5 ml of rumen fluid. The tubes were vortexed and stored at -20°C overnight. Upon thawing, tubes were centrifuged for 20 min at 3000 × *g* and approximately 2 ml of supernatant were decanted into a clean dry vial. The samples were capped and put into the autosampler (Model 8100; Varian, Walnut Creek, CA). The VFA concentrations were determined by a gas chromatograph (Model 3400 Star; Varian) with a 1.83-m packed glass column (Model 2-1721; Supelco, Oakville, ON, Canada) (Erwin et al., 1961). The injector and detector temperatures were set at 170°C and 195°C, respectively. The respective initial and final column temperatures were 120°C and 165°C. Ammonia concentration of rumen

fluid samples was measured according to Novozamsky et al. (1974). Absorbance was read at 630 nm on a Pharmacia Biotech Ultraspec 2000 UV/visible spectrophotometer (Biochrom, Cambridge, UK).

Rumen Volume and Passage Kinetics of Fluid and Solids

Co-EDTA and Cr-mordanted alfalfa were used as the respective markers for measuring outflow rates of rumen fluid and solids in the cannulated cows. Both markers were prepared according to Uden et al. (1980). A total of 50 g Co-EDTA was dissolved in 300 ml of distilled water and infused into the rumen via the cannula at the time of feed delivery. Simultaneously, 300 g of Cr-mordanted alfalfa was introduced into 10 different sites of the rumen. Rumen fluid and solids were then sampled at 2, 3, 4, 6, 8, 12, 16, 24, 36, 48, and 72 h after marker introduction. The rumen concentrations of the markers were regressed against time using a first-order exponential equation to acquire the passage rates (slopes) (Maekawa et al., 2002). Rumen volume was calculated by dividing the amount of Co or Cr infused by the intercept of the equations for individual cows.

Weighing Protocol

To minimize influence of time of feed delivery on gut-fill and true BW changes, all cows were weighed twice daily at 0830 and 2030 h at the beginning and the end of each period. Since at the start of the trial all cows had been fed at 1100 h, only the morning BWs were used for the beginning of the study. For the rest of the trial, however, the average of morning and evening BWs were calculated for all cows. Cows were scored for body condition (Edmonson et al., 1989) at the beginning of each period and the end of

the second period. The body condition scoring system used had a 5-point scale, with a score of 1 being an emaciated cow and a score of 5 describing an obese cow (Edmonson et al., 1989).

Statistical Analyses

Data were analyzed as a linear MIXED MODEL (SAS Institute, 2003). The models for productivity and N partitioning data included fixed effects of treatment (time of feed delivery), parity (primiparous vs. multiparous), and the interaction of treatment with parity. Final repeated measures models of rumen data included additional fixed effects of sampling time, treatment × sampling time, parity × sampling time, and treatment × parity × sampling time. The effects of cow within parity and period were modeled as random. Least square means were estimated with the Restricted Maximum Likelihood (REML) method and degrees of freedom were of Between-Within (SAS Institute, 2003). The PDIFF option of SAS (2003) was used to separate the least square means. Normality of distribution and variance homogeneity of residuals were tested using Proc Univariate of SAS (SAS Institute, 2003). Fixed effects were declared significant at $P < 0.05$, and trends were discussed at $0.05 < P \leq 0.10$. Results were reported as least square means ± standard errors for the differences of least square means.

RESULTS

Dry Matter Intake (DMI) and Rumen Fermentation

The TMR delivery at 2100 h instead of 0900 h increased ($P < 0.05$) DMI in primiparous cows but not in multiparous cows (Table 19). Except for ammonia and the

molar percent of rumen propionate and (acetate + butyrate) to propionate ratio, parity did not interact with TF on rumen fermentation indices (Table 15). As a result, rumen fermentation data were presented across parities. Figure 8 shows the data where the effect of parity was significant.

Time of feeding and parity did not affect (P = 0.45) rumen pH (Table 15). Treatments did not affect rumen concentrations of total VFA, acetate, propionate, butyrate, isobutyrate, valerate, and lactate. The rumen concentration of isovalerate tended to be higher (P = 0.09) for cows fed at 0900 h than for cows fed at 2100 h. When expressed as molar proportion of total VFA, 2100 h-fed cows had higher (P = 0.01) rumen acetate than 0900 h-fed cows. The 2100 h-fed multiparous cows had lower rumen propionate (P < 0.01) than 0900 h-fed multiparous cows (Table 15). The acetate + butyrate to propionate ratio was higher (P < 0.01) in multiparous cows fed at 2100 h than in multiparous cow fed at 0900 h (Figure 8C). Molar percent of rumen propionate and the ratio of acetate + butyrate to propionate in primiparous cows did not differ between treatments (Figure 8C). In rumen-cannulated cows, the average daily rumen ammonia was lower (P < 0.05) in 2100 h-fed primiparous cows than in 0900 h-fed primiparous cows (Figure 8A). Multiparous cows fed at 2100 and 0900 h had comparable average daily rumen ammonia (Figure 8A). The 2100 h- instead of 0900 h-feeding did not impact rumen fluid and solids retention times, but increased (P = 0.07) rumen fluid volume (Table 15).

Apparent Total Tract Nutrient Digestibility and Nitrogen Partitioning

Providing the TMR at 2100 h instead of at 0900 h increased the apparent total tract digestibility of DM ($P < 0.01$), N ($P = 0.01$), and NDF ($P < 0.001$) (Table 16). The apparent total tract ADF digestibility was improved in primiparous cows (46 vs. 38.4%, $P < 0.01$) but not in multiparous cows (43.5 vs. 42.6%, $P = 0.67$) when feed was delivered at 2100 h instead of 0900 h (Table 16). Feedimg at 2100 h increased N intake in primiparous cows, but not in multiparous cows (Table 18). Microbial protein synthesis was not significantly affected by the treatments (Table 17). Feeding multiparous cows at 2100 instead of 0900 h reduced ($P < 0.01$) total N output (575.4 vs. 513.6 g). Althought total N intake was greater in primiparous cows fed at 2100 h than in primiparous cows fed at 0900 h ($P < 0.05$), the treatments did not affect total N output (Table 18). Feed delivery at 2100 h instead of 0900 h reduced ($P < 0.01$) daily urinary N excretion (177 vs. 194 g) in primiparous cows. When expressed as % of total N intake, urinary ($P = 0.06$) and fecal ($P = 0.01$) N excretions and milk N ($P = 0.03$) outputs were lower in cows fed at 2100 h than in cows fed at 0900 h (Table 18). Of total N apparently digested in the digestive tract, feeding at 2100 h instead of 0900 h reduced both urinary ($P = 0.05$) and milk ($P < 0.01$) N outputs (Table 18). Due to reduced fecal, urinary and milk N outputs, more N was retained ($P < 0.01$) by cows fed at 2100 h than by cows fed at 0900 h (55.9 vs. -3.5 g/d). Thus, TMR delivery at 2100 h improved N balance ($P = 0.01$, Table 18).

Milk Production and Composition, BW, and BCS

The time of TMR delivery did not impact ($P = 0.63$) actual milk yield (Table 19). Milk fat percent ($P = 0.06$) and yield ($P = 0.05$) were higher in 2100 h-fed cows than in

0900 h-fed cows. Feeding at 2100 h increased the proportion of $C_{18:0}$, but did not affect total proportions of short, medium, and long chain fatty acids in milk (Table 20). Treatments did not significantly affect milk protein percent and yield. Fat-corrected milk yield was increased ($P < 0.05$) by feeding at 2100 h (Table 19). Considering the changes in milk fat and protein percents, provision of TMR at 2100 h instead of at 0900 h tended to increase ($P = 0.08$) energy-corrected milk yield (ECM) (Table 19). Primiparous cows had higher ($P = 0.05$) milk SNF percent than multiparous cows. The changes in body weight and body condition score were not affected by feeding time (Table 19).

DISCUSSION

DMI and Rumen Fermentation

In the current study, TMR delivery at 2100 h instead of 0900 h increased DMI of primiparous cows under no heat stress. Robinson et al. (1997) reported an increase in intake of a protein meal when it was delivered at 0030 h instead of 0830 h. Under heat stress, however, Aharoni et al. (2005) and Ominski et al. (2002) reported a decline in DMI due to changing the feeding time from morning to evening. Of note, despite lower DMI, energy utilization efficiency was improved by evening instead of morning feeding (Aharoni et al., 2005). Unlike once-daily TMR delivery in our study, Aharoni et al. (2005) delivered the feed in four separate meals for evening-feeding treatment or 20% at 0615 h, 30% at 1530 h, 25% at 1900 h, and 25% at 2100 h. With such inter-study differences in feeding time and frequency, different DMI responses would not be surprising. Fresh feed delivery stimulates eating and thus dramatically impacts 24-h

patterns in eating time (DeVries et al., 2005) and feed intake (Chapter 4). Cows in the study of Ominski et al. (2002) underwent three consecutive 5-d phases of thermoneutral (24°C during the day and 20°C overnight), heat stress (32°C), and a thermoneutral recovery. As a result, carry-over effects from previous to following periods might have existed. This means DMI response in a given phase might have carried over an associative effect of the previous phase. Such an interactive scenario amongst three experimental phases (Ominski et al., 2002) may not necessarily reflect DMI response when environmental conditions remain comparable for the entire trial as was the case in the current study and in Robinson et al. (1997). The increased DMI in 2100 h-fed than in 0900 h-fed primiparous cows may have partly been due to increased TMR intake within 3 h of feed delivery (Chapter 6). Similarly, DMI in feedlot cattle was also increased by changing the feed delivery time from 0900 h to 2100 h (Schwartzkopf-Genswein et al., 2004). Phillips and Rind (2001) indicated that if cows anticipate the feed delivery time, they may show a more intense eating activity. There is a possibility that 2100 h-fed cows in the current study may have anticipated the feed delivery time and were, thus, craving the fresh feed. Lights were turned off at 2245 h or at 1 h and 45 min after evening feed delivery. Light can act as a regulator of animal metabolism (Piccione and Giovanni, 2002). Also, melatonin secretion usually increases during the dark period (Illnerova and Sumova, 1997). The increased nocturnal melatonin secretion has recently been shown to interact with diurnal variation in glucose metabolism in rats and humans (la Fleur et al., 2001; Lima et al., 1998). Lima et al. (1998) described that the night-time rise in blood glucose and insulin could, at least partly, be mediated by the night-time surge in melatonin secretion. Therefore, melatonin seems be involved in the evening insulin

resistance in non-ruminants. Additionally, glucose uptake and oxidation is known to contribute to satiety in mammals (Allen et al., 2005; Anika et al., 1980). Hence, a possible interaction amongst melatonin, insulin, and glucose uptake might have attenuated the satiety in 2100 h-fed cows compared to 0900 h-fed cows. However, this hypothesis needs to be tested in ruminants. Moreover, the rumen fill has been suggested (Taweel et al., 2004) to control feed intake differently during the day compared to overnight. The inhibitory effect of rumen fill on feed intake begins at a greater rumen volume overnight than during the day. This phenomenon may be attributed to the evolutionary diurnal patterns in rumination, rumen muscle contractions, and rumen fill. Ruminants have evolved to rest and ruminate mostly in the night when little grazing occurs (Hancock, 1954; Gordon and McAllister, 1970). As such, the night-time rumen fermentation would be more capacious than the day-time fermentation (Taweel et al., 2004). The greater feed intake, the larger rumen fluid volume, and the higher rumen VFA and ammonia shortly post-feeding in 2100 h-fed cows than in 0900 h-fed cows support such an evolutionary development of the 24-h patterns in rumen fermentation capacity.

The comparable average daily pH between cows fed at 2100 h and at 0900 h concurs with the observation by Robinson et al. (1997). In our preceding study (Chapter 3), feed delivery at 2100 h instead of at 0900 h did not affect average rumen pH either. The higher molar percent of acetate in 2100 h-fed than in 0900 h-fed cows (Table 15) agrees with Robinson et al. (1997). These authors observed a positive rumen acetate response to delivering a protein meal at 0030 h instead of at 0830 h. Therefore, the midnight delivery of the protein meal intensified rumen fermentation more than did its morning delivery (Robinson et al., 1997). Unlike our previous study where rumen

propionate was not altered by TF, feeding at 2100 h instead of 0900 h in multiparous cows reduced molar percent of propionate in the current trial (Table 15). The dietary NDF (39 vs. 32%) and ADF (27 vs. 17%) were considerably higher in the current study than that in the previous study (Chapter 3). The greater fiber can stimulate the cellulolytic bacteria, thereby directing the VFA profile towards more acetate than propionate (France and Dijkstra, 2005; Sutton, 1971). Hence, the increased intake of TMR within 3 h post-feeding in 2100 h-fed cows than in 0900 h-fed cows (Chapter 6) may have favored cellulolytics, thereby increasing the acetate to propionate ratio (Table 15).

The immediate post-feeding elevation in rumen ammonia was greater in 2100 h-fed than in 0900 h-fed cows. The higher peak in rumen ammonia could be due to increased N intake within 3 h of feeding. However, ammonia levels remained numerically lower afterwards, thus resulting in lower average daily ammonia in 2100 h-fed than in 0900 h-fed primiparous cows (Table 15). Evening rather than morning feeding in primiparous cows, thus, increased N supply to the rumen during the time when carbohydrate fermentation and microbial activity were increasing. We deduce that feed delivery at 2100 h instead of 0900 h may have offered a greater rumen capacity for a synchronous microbial access to N compounds, high-energy phosphate bonds, and carbon skeletons, in primipiparous than in multiparous cows. This may explain why daily rumen ammonia concentration was similar between the two feeding times in multiparous cows.

The rumen fluid volume was increased and the retention times of rumen fluid and solids were unaltered by TF. These data were consistent with the increased feed intake within 3 h of feed delivery in 2100 h-fed cows (Chapter 6). Primiparous cows had higher rumen solid outflow rate when fed at 2100 instead of 0900 h. This was not unexpected, as

primiparous cows fed at 2100 h had greater DMI than primiparous fed at 0900 h. The current study is the first uncovering the significant responses in rumen content kinetics to time of TMR delivery in lactating cows fed once daily. The comparable excretions of total purine derivatives (uric acid + allantoin) suggested that feeding time did not affect the rumen microbial protein synthesis (Table 17).

Apparent Total Tract Nutrient Digestion and Nitrogen Partitioning

The evening instead of morning feed delivery had a consistent positive impact on apparent total tract digestibility of dietary DM, N, and NDF (Table 16). Robinson et al. (1997) offered 67% of a TMR (13.94% CP) at 0080 h and 33% of it at 1600 h. Subsequently, they delivered a protein supplement (47.38% CP) equal to about 15% of expected daily DMI either at 0830 or at 0030 h. Cows consumed more of the protein meal when it was delivered at 0030 h instead of at 0830 h. Rumen digestibility of CP, DM, and organic matter were improved in that study. As well, total tract ADF digestibility tended to be greater and that of starch was greater when the protein meal was offered at 0030 h instead of at 0830 h (Robinson et al., 1997). These findings support the enhanced dry matter and NDF digestibility in 2100 h-fed cows in the current study. However, Robinson et al. (1997), observed no significant impacts of protein meal delivery time on total tract NDF and N digestibility. The differences between the two studies could be due to differences in experimental design, hours of feed delivery, and digestibility measurement techniques. Unlike the eight cows in our study, Robinson et al. (1997) used only four cows, resulting in inadequate statistical power to detect differences in fiber digestibility. In addition, the protein supplement fed by Robinson et al. (1997)

was only a small portion of the diet or 15% of the expected DMI, while we delivered the whole TMR either at 0900 or at 2100 h. Furthermore, Robinson et al. (1997) offered the TMR twice daily compared with once-daily feeding in our study. Unarguably, feed delivery stimulates bunk attendance and feed intake (DeVries et al., 2005; Chapters 4 and 6). Hence, offering only a small meal at 0030 h after the principal diet was delivered twice at 0800 and 1800 h (Robinson et al., 1997) may have induced a smaller impact on feed intake, rumen fermentation, and total tract nutrient digestion, when compared to offering the whole TMR at once in our study. Moreover, using total fecal collection in the current study enabled a stronger inter-treatment comparision when compared to methods using spot fecal sampling and markers employed by Robinson et al. (1997).

No ruminant study has determined N partitioning in response to time of feeding. We monitored N input via daily TMR intake and N output via total collection of feces, urine, and milk following a 21-d adaptation phase. The greater N intake of primiparous cows due to changing the TMR delivery time from 0900 to 2100 h was due to the greater DMI (Tables 18 and 19). Robinson et al. (1997) found that cows fed a protein-meal at 0030 instead of at 0830 h consumed more of the protein meal. Daily N input remained unchanged in multiparous cows when TMR was delivered at 2100 h instead of at 0900 most probably because DMI did also not change (Tables 18 and 19). Reduced portion of N intake excreted in feces due to feeding at 2100 h instead of at 0900 h (Table 18) was consistent with greater apparent total tract N digestibility in 2100 h-fed than in 0900 h-fed cows (Table 16). The decreased partitioning of N intake towards urine by TMR delivery at 2100 instead of at 0900 h (Table 18) could be explained by the lower overall daily rumen ammonia in 2100 h-fed compared to 0900 h-fed cows. The liver in ruminants

has immense capability to incorporate ammonia produced in the rumen and hindgut into urea (Reynolds, 1992). Hepatic urea has two major fates in ruminants. Urea is excreted in the urine but also a considerable amount can be recycled back into the gut, which continuously demands N for epithelial cell proliferation and maintenance. Much of the hepatic urea release, however, is excreted via urine when diets contain adequate N to meet requirements by rumen microbes and gut tissues (Huntington, 1989). To increase microbial growth, N delivery to the rumen requires adequate readily and potentially fermentable carbohydrates (Nocek and Russell, 1988). The TMR delivery at 2100 h instead of 0900 h may have reduced the total of urea excreted in the urine by decreasing hepatic ammonia load. This means that evening instead of morning feeding may have increased N retention by the peripheral and visceral tissues. The greater N retention due to feeding at 2100 h may have involved hormonal changes. This would be possible because the average daily rumen ammonia did not change in multiparous cows. Also, a higher plasma insulin was observed at 2, 6, and 8 h after feeding in 2100 h-fed than in 0900 h-fed cows, in a companion study (Furedi et al., 2006a). Insulin is known to stimulate nutrient use by non-hepatic, non-mammary tissues (Brockman, 1978; Brockman and Laarveld, 1986). In addition, comparable to our previous study (Chapter 4), the 2100 h-fed cows consumed more feed within 3 h of feeding than 0900 h-fed cows (Chapter 6). Increased feed intake of 2100 h-fed cows coincided with higher plasma insulin at 2-h post-feeding. Because of the increased feed intake within 3 h, the 2100 h-fed cows exhibited a numerically higher post-feeding plasma insulin (C. Furedi, unpublished). Thus, although not atatistically significant, the higher plasma insulin may have reduced gluconeogenesis from AA to favor protein synthesis. The TMR delivery at

2100 h instead of 0900 h reduced the proportion of the apparently digested N which was secreted in milk, but did not have a significant effect ($P = 0.17$) on daily secretion (g/d) of N in milk (Table 18). This could be explained by the trend for greater N intake due to feeding at 2100 h instead of 0900 h. In addition, the increased proportion of N retained in the body would automatically reduce the proportion secreted in milk even if the absolute amount of N secretion into milk does not change. This could in part explain why the daily milk N secretion did not change but its proportion of the apparently digested N was reduced by feed delivery at 2100 h. Due to reductions in daily percent of N intake excreted in feces and urine, N balance was improved by delivering the TMR at 2100 h instead of at 0900 h (Table 18).

Milk Production and Composition, and BW Changes

Increased milk fat yield was in agreement with our previous study. Robinson et al. (1997) found that delivering a protein-meal at 0030 instead of at 0830 h in twice-daily fed lactating cows increased milk yields of fat and energy. Aharoni et al. (2005) also found that delivering feed through the afternoon and evening hours improves energy status of heat-stressed lactating cows, when compared with feeding through the morning. Cows in the study of Aharoni et al. (2005) were exposed to high ambient temperatures, as opposed to thermoneutral conditions in the current study. Thus, the benefits of evening instead of morning feeding to energy metabolism of the cow are not mediated exclusively via improved thermodynamics. Acetate is a major substrate for milk fat synthesis and peripheral energy use in ruminants, as opposed to glucose in non-ruminants (Annison and Bryden, 1999). Among rumen VFA, acetate is used the least by splanchnic tissues and

hence is the only VFA appearing in significant levels in peripheral blood (Annison and Bryden, 1999; Brockman, 2005). Feeding at 2100 h instead of at 0900 h increased rumen concentration of acetate at 6 and 8 h post-feeding (Chapter 6). The molar percent of rumen acetate across the two sampling times of 0000 and 1200 h was also higher in 2100 h-fed than in 0900 h-fed cows (Table 15). The peripheral acetate availability appears to be the major regulator of the acetate uptake by the mammary gland (Brockman, 2005). The greater peripheral and mammary availability of acetate may have contributed to the improved milk fat yield in 2100 h-fed cows (Table 19).

Based on the original theory of Davis and Brown (1970, cited by Bauman and Griinari, 2003), a relationship exists between milk fat depression and milk content of *trans* octadecenoic acid (C18:1*trans*). In our study, 0900 h-fed cows had lower milk fat percent and yield compared to 2100 h-fed cows. However, *trans* isomers of C18:1 did not change between the two groups (Table 20). This suggests that the lower milk fat yield in 0900 h-fed cows was not related to the *trans* fatty acids of rumen origin.

In the rumen, linoleic acid (C18:2) can be biohydrogenated to stearic acid (C18:0). Linoleic acid is first converted to *cis-9, trans-11* CLA and then to C18:*1 trans-11* before stearic acid is produced (Bauman and Griinari et al., 2001). The mammary desaturation of C18:1 *trans-11* is the major contributor to milk *cis-9, trans-11* CLA (Corl et al., 2001). As a result, changes in milk fat content of *cis-9, trans-11* CLA and that of C18:1 *trans-11* are expected to parallel each other. Recently, using continuous cultures, AbuGhazaleh et al. (2005) confirmed that lower rumen pH (5.5 vs. 6.5) increases the oleic acid conversion to stearic acid. This means that a reduced rumen production of C18:1 *trans-11* and mammary synthesis of *cis-9, trans-11* CLA. Additionally, the lower

(5.5) compared to normal (6.5) pH reduced ^{13}C enrichment of C18:1 *trans-10* and abolished detection of *trans* isomers beyond C10 (AbuGhazaleh et al., 2005). Milk fat proportion of C18:1 *trans-11* and CLA *cis-9, trans-11* were comparable between 0900 h-fed and 2100 h-fed cows (Table 20). This was in accord with the comparable daily average of rumen pH between the treatments. It can thus be suggested that the lower rumen pH at 5-6 h post-feeding in the 2100 h-fed cows than in the 0900 h-fed cows did not significantly alter the biohyrogenation pathways of C18:2 in the rumen. Otherwise, the milk content of C18:1 *trans* and C18:2 *cis-9, trans-11* would have changed. Feeding at 2100 h did not affect milk content of C18:1 *trans* and CLA *cis-9, trans-11* (Table 20).

β-hydroxybutyrate was increased in peripheral blood at 2 and 4-h post-feeding by 2100 h instead of 0900 h feeding (Chapter 6). Rumen acetate was also higher at 5-6 h post-feeding in 2100 h-fed cows than in 0900 h-fed cows. β-hydroxybutyrate provides about one half of the initial four carbons required in *de novo* fat synthesis in the mammary gland (Bauman and Griinari, 2003). Also, acetate is the most important contributor to the mammary synthesis of short and medium chain fatty acids (Bauman and Griinari, 2003). Therefore, the increased post-feeding availability of acetate and BHBA was most likely responsible for the increased milk fat yield by TMR delivery at 2100 h instead of 0900 h.

The increased DMI and rumen acetate in 2100 h-fed compared to 0900 h-fed primiparous cows agree with the increased FCM and ECM (Table 19). Despite a smaller proportion of digested N secreted in milk rather than retained by the body, milk protein percent in primiparous cows was not affected by TF (Table 18). In multiparous cows, however, milk protein percent tended to decrease when TMR was delivered at 2100 h

instead of at 0900 h. The DMI in multiparous cows was not affected by treatments. In light of the similar DMI and increased proportion of retained N at the expense of milk N, the lower milk protein percent in 2100 h-fed than in 0900 h-fed multiparous cows would be expected. Moreover, the differences in N partitioning might be indicative of hormonal involvement in milk composition response to TF. In a parallel study (Furedi et al., unpublished), the post-feeding rises in plasma insulin were numerically higher in 2100 h-fed cows than in 0900 h-fed cows. The numerically higher post-feeding insulin in 2100 h-fed cows was associated with increased milk fat yield and improved N retention and balance (Chapter 3). Unlike our previous research (Chapter 3), the mediatory effect of parity on productive response to feed delivery time was apparent on milk protein but not on milk fat. The absence of a parity effect on milk protein in the first trial could be attributed to diet composition. The diets used in our first study had slightly higher CP (17.3-18.1 vs. 17.3%), considerably lower NDF (28-33 vs. 39%) and ADF (16-19 vs. 27%) and much higher NFC (36-40 vs. 29%) than the diet used in the present study. Higher CP and NFC would potentially favor microbial growth (Reynal and Boderick, 2005). The increased microbial protein supply would thus attenuate any parity-mediated decline in milk protein percent in response to dietary treatments e.g., feed delivery time. Also, the greater readily fermentable carbohydrates with lower dietary fiber (e.g., in our first study) can reduce rumen ammonia, hepatic ureogenesis, and urinary N. Alongside would be an increased proportion of the blood urea recycled into the rumen (Huntington, 1989). In consequence, the gut and liver outputs of AA may increase. If so, the parity-related fall in milk protein percent due to 2100 h-feed delivery may not be pronounced, as

was the case in our first project. It should be considered that other hormones such as glucagon and growth hormone might also be involved in the metabolic responses to TF.

Feed delivery time did not significantly affect the changes in BW and BCS. It is critical to indicate that obtaining the treatment-related changes in BW of adult ruminants is a challenging task. This is partly because significant 24-h patterns in feed intake and gut-fill not only exist but also vary amongst individual cows (individual cow data). Time of feed delivery interacts with the time of weighing on gut-fill, and thus, on true BW changes (Moshtaghi Nia et al., 1995; Pritchard and Knutsen, 1994). To minimize the confounding effect of gut-fill, cows were weighed twice daily at 0830 and 2030 h which were just before feed delivery at the beginning of both period and the end of the second period. Except for the beginning of the study, when all cows had been fed at 1100 h the previous day, the averages of morning and evening weights were used in the data analysis. The comparable BW gain between 2100 h-fed and 0900 h-fed cows was expected, as the greater DMI of 2100 h-fed than of 0900-fed cows was accomponied by greater milk fat yield (Table 19). A positive impact of evening instead of morning feeding on weight gain of beef cattle under freezing weather (Kennedy et al., Schwarzkopf-Genswein et al., Small et al., 2004) or in the summer (Pritchard and Knutsen, 1995) has been reported. Schwarzkopf-Genswein et al. (2004) did not describe the weighing protocol used. Small et al. (2004) fed steers either at 0900 or at 2000 h and weighed the morning-fed steers at 0830 which was at 23.5 h post-feeding, but weighed the evening-fed steers at 1430 h or 18.5 h post-feeding. It is notable that TF can alter post-feeding patterns in feed intake (Chapters 4 and 6; Pritchard and Knutsen, 1995) and thereby alters post-feeding patterns in gut-fill. Thus, the effect of TF on weight gain was,

at least partly, confounded by the effect of weighing time in the study of Small et al. (2004). Hence, to minimize the confounding effect of gut-fill on weight gain responses to TF, both evening-fed and morning-fed ruminants must be weighed at the same time relative to feed delivery or just before feeding. As acknowledged by Schwartzkopf-Genswein et al. (2004), the positive growth response to evening instead of morning feeding in beef studies may partly represent the different amount of gut-fill and not fully the true tissue deposition. In two recent dairy studies completed by our group (Furedi et al. 2006b; Chapter 3), such a confounding effect of gut-fill on true weight changes was shown in lactating cows, as well. However, this should not rule out the possible stimulatory effect of evening feeding on tissue growth (Small et al., 2004) because BW does not necessarily reflect the gains or shrinks in the body tissue mass (NRC, 2001). For example, recently, Furedi et al. (2006b) reported a thicker subcutaneous fat layer when lactating cows received a TMR at 2100 h compared to 0900 h.

CONCLUSIONS

Delivering a total mixed ration at 2100 h instead of at 0900 h increased DMI in primiparous cows. Nutrient demands for the continuing growth are superimposed on the requirements for milk secretion in primiparous cows. Such a growth obligation would usually not be faced by multiparous cows. Primiparous cows at high milk production levels are thus expected to be well responsive to evening feeding. Feeding at 2100 h instead of 0900 h increseed apparent total tract DM, CP, and NDF digestibility and did not affect microbial protein synthesis and the milk proportion of total short, medium, and long chain fatty acids. Feeding at 2100 h increased fat- and energy-corrected milk yields.

Of total N apparently digested, TMR delivery at 2100 h instead of at 0900 h reduced the proportions exctreted in the urine and secreted in milk in favor of body retention, thereby improving N balance. Reduced urinary and fecal N excretions by TMR delivery at 2100 h may have environmental implications.

Table 13. Forage and concentrate potions of the experimental diet

Diet ingredients	Dry matter basis
Alfalfa silage	25.1
Barley silage	25.1
Energy supplement[1]	39.2
Protein supplement[1]	10.6
Forage to concentrate ratio	50.2 : 49.8

[1]The supplements were those used in the first study (Chapter 3).

Table 14. Nutrient composition of silages and total mixed ration (TMR) on a DM basis

Item	Forage Silage		Concentrate		TMR
	Alfalfa	Barley	Energy suppl.	Protein suppl.	
DM %	57.5	26.5	87.9	88.8	52.4
CP	18.5	9.9	17.0	34.2	17.3
ADIP	1.4	1.4	0.29	6.5	2.5
NFC[1]	20.1	16.7	49.3	9.1	29
NDF	48.5	58.8	19.3	35.2	39.2
ADF	38.8	39.4	10.1	18.4	27.1
Ash	10.3	11.4	6.5	13.0	9.8
Ca	1.19	0.46	0.90	3.71	1.09
P	0.36	0.39	0.62	1.17	0.57
K	2.83	2.24	0.84	1.0	1.88
Mg	0.40	0.34	0.30	0.32	0.36
Na	0.09	0.07	0.27	1.48	0.32
Zn, ppm	31.7	37	116	70	71.7
Mn, ppm	39.8	41	113	53	74.5
Cu, ppm	11.5	9	31	9	15.2
Fe, ppm	311.0	522	198	408	322.0

[1]Nonfiber carbohydrates = 100 – (NDF% + CP% + EE% + ash%).

Table 15. Effects of time of feeding (TF) on indices of rumen fermentation[1]

Item	Time of feeding (TF)		SE	*P*-value		
	0900 h	2100 h		TF	Parity	TF × Parity
pH	6.33	6.28	0.06	0.45	0.52	0.18
VFA, m*M*						
Total	121.6	120.2	4.6	0.59	0.18	0.67
Acetate (A)	64.4	65.3	2.3	0.71	0.30	0.99
Propionate (P)	34.0	31.70	1.5	0.14	0.26	0.22
Butyrate (B)	18.7	17.8	0.8	0.35	0.07	0.94
Isobutyrate	1.7	1.6	0.13	0.52	0.78	0.93
Isovalerate	0.93	0.83	0.05	0.09	0.13	0.56
Valerate	2.04	1.95	0.09	0.29	0.12	0.86
Lactate	0.77	1.34	0.61	0.37	0.08	0.80
Molar %						
Acetate	52.9	55.0	0.72	0.01	0.71	0.34
Propionate	27.9	26.6	0.43	0.007	0.89	0.03
Butyrate	15.4	14.9	0.39	0.24	0.39	0.49
Isobutyrate	1.38	1.32	0.10	0.53	0.65	0.95
Isovalerate	0.77	0.70	0.03	0.03	0.36	0.20
Valerate	1.68	1.64	0.04	0.28	0.78	0.51
(A+B):P[2]	2.46	2.64	0.06	0.007	0.87	0.03
A/P	1.91	2.08	0.05	0.006	0.97	0.08
Ammonia, mg/dL	10.8	9.8	0.03	0.02	0.40	0.05
Rumen volume, L	89.5	106.8	7.7	0.07	0.06	-
Rumen fluid outflow rate, %/h	11.9	11.7	0.5	0.81	0.35	-
Retention time, h	8.2	8.9	0.7	0.36	0.40	-
Rumen solids outflow rate, %/h[3]	2.92	3.25	0.1	0.21	0.52	0.04
Retention time, h	32.8	31.4	1.8	0.52	0.53	0.11

[1]Rumen fluid samples were taken twice daily at 0000 and 1200 h corresponding to 3 and 15 h post-feeding. Data for rumen ammonia and outflow rates are for 4 rumen-cannulated cows. [2]Acetate + butyrate to propionate ratio.

Table 16. Effect of time of feeding (TF) and parity on apparent total tract nutrient digestibility[1]

	Multiparous		Primiparous			*P*-value		
	0900 h	2100 h	0900 h	2100 h	SE	TF	Parity	P × TF
Dry matter, %	60.8[b]	62.8[a]	60.2[b]	62.8[a]	0.65	0.004	0.75	0.70
N, %	63.5[b]	65.5[a]	62.7[b]	65.5[a]	0.69	0.01	0.73	0.57
NDF, %	45.2[b]	49.9[a]	44.5[b]	49.6[a]	0.66	<0.001	0.69	0.83
ADF, %	42.6[b]	43.5[b]	38.4[c]	46.0[a]	1.3	0.02	0.64	0.05

[1]Determined using total fecal collection technique.

[a,b,,c]Different superscripts within the same row differ at $P \leq 0.01$.

Table 17. Urinary purine derivative (PD) excretion and estimates of microbial protein synthesis in the rumen

Item	Feeding time (TF)		SE	*P*-value		
	0900 h	2100 h		TF	Parity	TF × Parity
Uric acid, mmol/d	52.8	48.6	2.2	0.12	0.43	0.15
Allantoin, mmol/d	454.6	421.4	28.9	0.30	0.34	0.57
Total PD, mmol/d	507.3	470.1	28.3	0.24	0.34	0.48
Absorbed PD, mmol/d	540.1	497.2	33.4	0.26	0.36	0.47
Microbial N synthesis, g/d	392.6	361.5	24.3	0.26	0.35	0.47

Table 18. Effect of time of feeding (TF) and parity on input, output, partitioning, and balance of N[1]

	Multiparous		Primiparous			*P*-value		
Item	0900 h	2100 h	0900 h	2100 h	SE	TF	Parity	P × TF
N intake, g/d	567.1	579.7	509.4[b]	562.1[a]	13.7	0.09	0.28	0.23
Urinary N, g/d	194.5[a]	177.1[b]	178.8	182.3	2.9	0.09	0.63	0.03
Fecal N, g/d	212.5	196.5	189.7	194.0	8.2	0.52	0.43	0.30
Urine N/Fecal N	0.90	0.92	0.96	0.95	0.03	0.85	0.42	0.72
Milk N, g/d	163.7	144.6	141.3	138.7	6.6	0.17	0.32	0.28
Total N output, g/d	575.4[a]	513.6[b]	509.8	515.0	3.5	0.003	0.18	0.002
% Of total N intake								
Urinary N	34.0	31.4	35.8[a]	32.6[b]	1.0	0.06	0.59	0.76
Fecal N	37.5	33.9	37.3	34.5	0.83	0.01	0.93	0.63
Milk N	30.2	24.0	27.8	24.9	1.3	0.03	0.79	0.30
N balance, g/d	-6.8	64.6	-0.33	47.2	10.4	0.01	0.79	0.34
% Of N apparently digested								
Urinary N excretion	54.9	47.9	57.2	49.6	2.2	0.05	0.70	0.91
Milk N secretion	47.9	36.6	44.4	37.9	1.7	0.006	0.77	0.23
N retention	-1.07	10.0	-0.9	9.1	1.2	0.003	0.82	0.45

[1]Determined using dry matter intake values and total fecal and urine collection technique.

[a,b]Within each parity, different superscripts refer to significant difference ot $P < 0.05$.

Table 19. Effect of time of feeding (TF) and parity on feed intake, milk production and composition, body weight (BW) changes, and body condition score (BCS)

	Multiparous		Primiparous			*P*-value		
Item	0900 h	2100 h	0900 h	2100 h	SE	TF	Parity	P × TF
Actual milk yield, kg/d	34.9	34.7	27.4	28.7	0.99	0.63	0.29	0.49
FCM[1], kg/d	32.1^{b}	34.8^{a}	26.3^{d}	28.8^{c}	1.09	0.04	0.13	0.91
ECM[2], kg/d	27.3^{a}	28.6^{a}	22.7^{c}	24.80^{b}	0.89	0.08	0.16	0.77
DMI, kg/d	20.4^{b}	20.6^{b}	18.5^{a}	20.4^{b}	0.48	0.04	0.25	0.17
Milk fat %	2.98^{b}	3.48^{a}	3.33^{ab}	3.58^{a}	0.18	0.06	0.56	0.50
Fat yield, kg/d	1.05^{b}	1.22^{a}	0.89^{c}	1.00^{b}	0.06	0.05	0.14	0.63
Milk protein %	3.12^{b}	2.82^{c}	3.47^{a}	3.49^{a}	0.06	0.11	0.13	0.09
Protein yield, kg/d	1.11	0.97	0.93	0.98	0.04	0.37	0.54	0.12
Milk SNF %	8.52^{b}	8.30^{c}	9.05^{a}	9.11^{a}	0.05	0.24	0.05	0.07
SNF yield, kg/d	3.04	2.93	2.47	2.60	0.10	0.90	0.34	0.31
BW change[3] (g/d)	-104	545	489	489	251	0.23	0.46	0.23
BCS[4]	2.84	2.75	2.84	2.81	0.16	0.11	0.85	0.39

[1]3.5% Fat-corrected milk = (.432 × kg/d milk yield) + (16.23 × kg/d fat yield).

[2]Energy-corrected milk = [(12.2× kg/d fat yield) + (5.65 × kg/d SNF yield) - (0.0752 × kg/d milk yield)].

[3]Values were calculated by dividing periodical changes in BW by the number of days (number of days in each period).

[4]Body condition score (1-5 scoring scale; Edmonson et al., 1989).

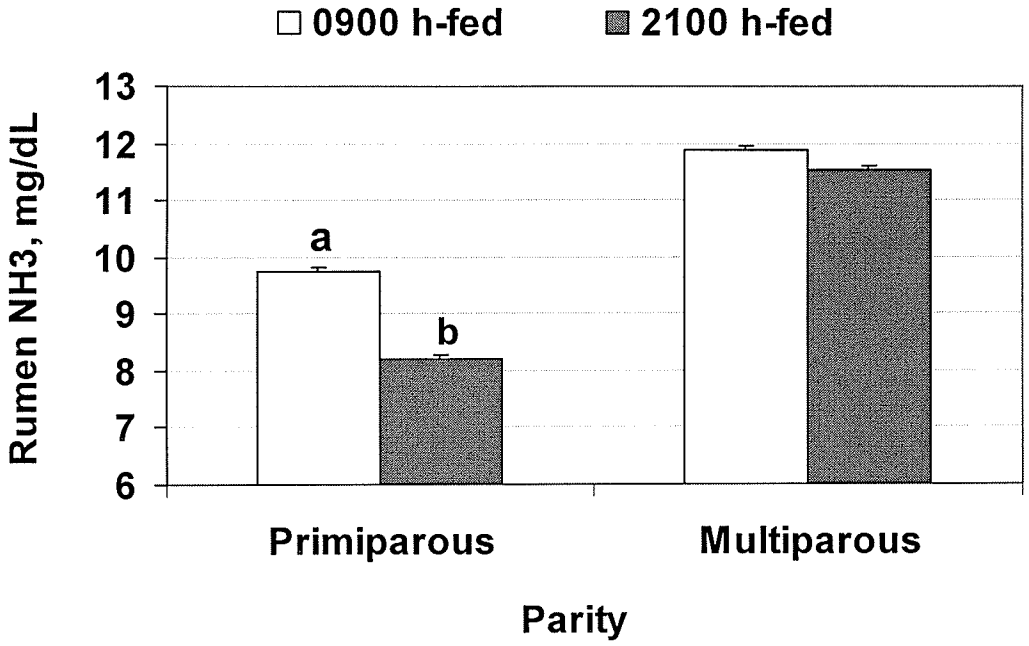
[a,b, c,d]Different superscripts within the same row differ at $P < 0.05$.

Table 20. Effects of time of feeding on milk fatty acid profiles[1]

Item	Feeding time (TF)		SE	*P*-value		
	0900 h	2100 h		TF	Parity	TF × Parity
8:0	0.52	0.42	0.06	0.10	0.22	0.19
10:0	1.70	1.58	0.05	0.01	0.45	0.01
12:0	2.37	2.08	0.14	0.05	0.32	0.82
12:1	0.07	0.06	0.003	0.01	0.77	0.43
13:0	0.09	0.08	0.003	0.01	0.94	0.93
13:1	3.98	3.65	0.14	0.03	0.42	0.72
14:0	8.64	8.71	0.41	0.86	0.27	0.03
14:1	0.82	0.83	0.12	0.91	0.91	0.55
15:0	0.95	0.94	0.02	0.88	0.25	0.61
15:1	0.016	0.02	<0.01	0.05	0.03	0.40
16:0	22.63	23.00	0.25	0.15	0.58	0.15
16:1 *t*	0.21	0.21	0.03	0.99	0.14	0.96
18:0	13.50	14.87	0.45	0.01	0.14	0.91
18:1 *trans-6*	0.057	0.061	<0.01	0.27	0.25	0.77
18:1 *trans-9*	2.16	1.03	0.62	0.10	0.33	0.06
18:1 *trans-11*	5.42	5.51	0.37	0.82	0.27	0.44
18:1 *trans*	8.27	5.94	0.48	0.88	0.15	0.91
18:1	27.8	27.9	0.48	0.88	0.15	0.91
18:2 *trans*	0.13	0.14	<0.01	0.56	0.43	0.87
18:3 *n-3*	3.14	3.01	0.06	0.03	0.59	0.97
18:3 *n-6*	0.29	0.30	0.01	0.18	0.49	0.22
19:0	0.22	0.23	<0.01	0.01	0.02	0.87
CLA, *cis9 trans11*	2.20	2.06	0.13	0.27	0.21	0.58
CLA, *trans10 cis12*	0.025	0.028	0.003	0.37	0.59	0.06
20:2	0.04	0.04	0.003	0.70	0.50	0.37
20:3	0.069	0.071	0.005	0.72	0.37	0.71
20:4	0.09	0.10	0.003	0.18	0.04	0.70
20:5	0.047	0.045	0.002	0.38	0.20	0.61
22:3	0.05	0.05	0.006	0.91	0.06	0.71
22:4	0.069	0.063	0.005	0.19	0.31	0.94
22:5	0.090	0.096	0.004	0.19	<0.01	0.44
22:6	0.089	0.090	0.003	0.71	0.16	0.42
SCFA[2]	9.96	9.48	0.51	0.36	0.09	0.55
MCFA[2]	34.65	34.56	0.41	0.83	0.99	0.04
LCFA[2]	54.37	54.98	0.54	0.27	0.42	0.28

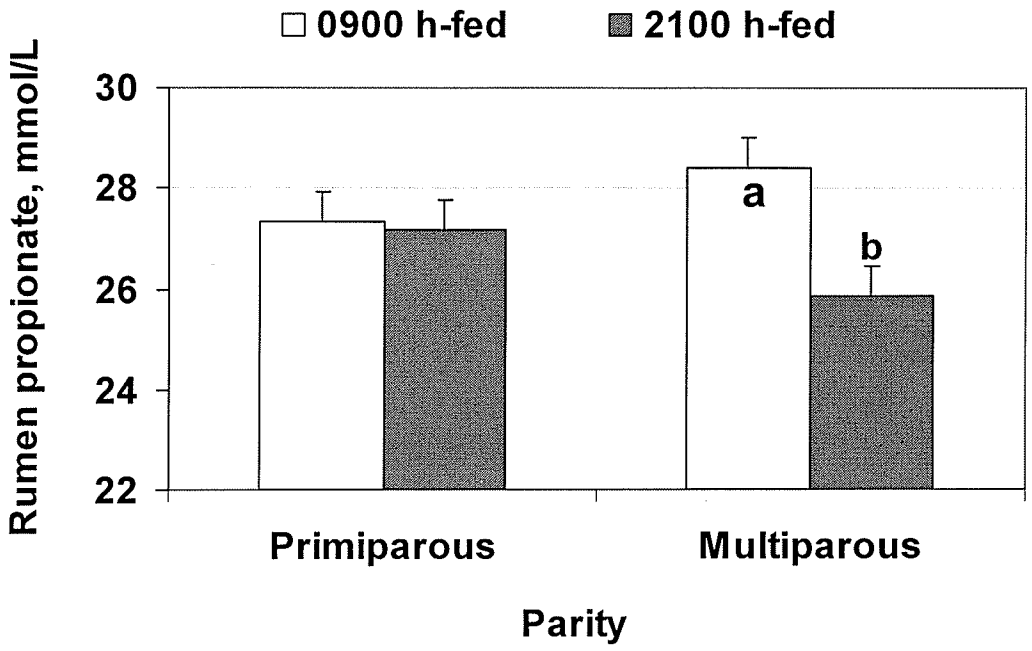
[1]The individual fatty acid peak area divided by the total fatty acids peak area multiplied by 100. About 92% of total fatty acids measured were reported.

[2]LCFA = long chain fatty acids or > C18; SCFA = short chain fatty acids or C4-C13; MCFA = medium chain fatty acids or C14-C17.

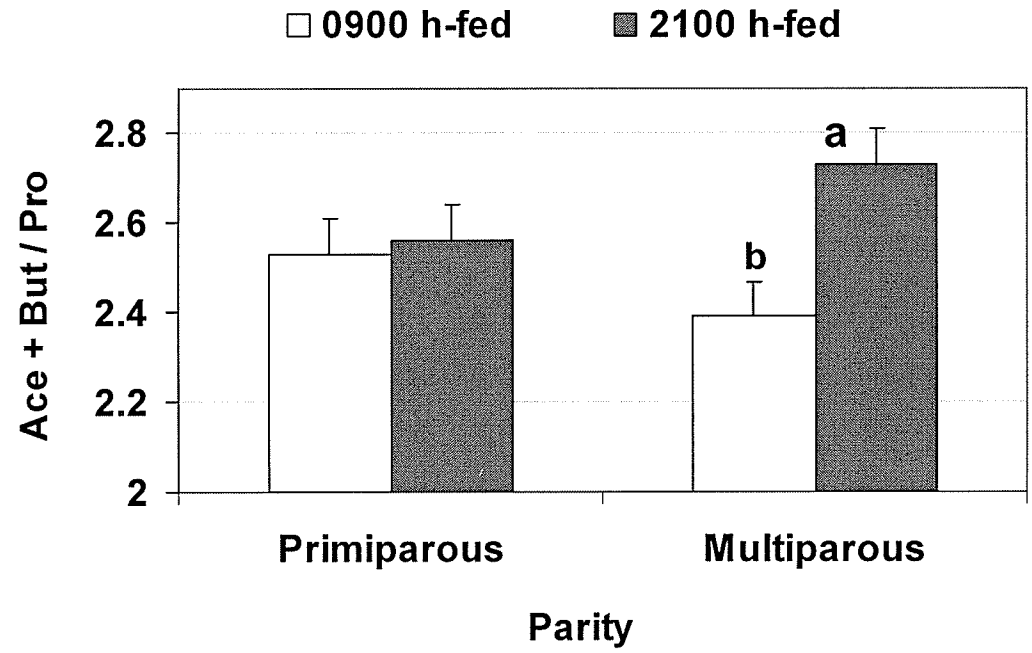


(A)

Figure 8. Parity-mediated response in average rumen ammonia (A), molar % of propionate (B) and acetate + butyrate to propionate ratio (C) to feed delivery time.


0900 h-fed
2100 h-fed
Rumen propionate, mmol/L
30
28
26
24
22
a
b
Primiparous
Multiparous
Parity

(B)


0900 h-fed
2100 h-fed
Ace + But / Pro
2.8
2.6
2.4
2.2
2
a
b
Primiparous
Multiparous
Parity

(C)

CHAPTER 6

Feed Delivery at 2100 instead of 0900 h Alters Post-Feeding Patterns of Feed Intake, Rumen Fermentation, and Blood Metabolites in Lactating Dairy Cows

ABSTRACT

The primary objective of the current study was to establish the 24-h patterns in feed intake, rumen fermentation, and blood metabolites in response to morning or evening feeding following 21-d adaptation periods. Four multiparous and four primiparous lactating Holsteins (82 days in milk at the beginning of the trial) were used in a cross-over design with two 6-week periods. Each period had a 4-week adaptation. A total mixed ration (TMR) containing 49.8% concentrate (dry matter basis) was delivered *ad libitum* at either 0900 or 2100 h. Feed intake in all cows and rumen pH in four cows were monitored continuously. Jugular blood was sampled via catheter every 2 h for 24 h during week 5 of each 6-week period. The proportion of daily TMR intake consumed within 3 h post-feeding was 55% in 2100 h-fed cows but 46% in 0900 h-fed cows. Cows fed at 2100 h had higher rumen total VFA at 6 h, higher acetate at 6 h and 8 h, and lower rumen pH at 5 h and 6 h post-feeding than cows fed at 0900 h. The acetate to propionate ratio was higher during 3-16 h after feeding in 2100 h-fed than in 0900 h-fed fed cows. Rumen ammonia was higher at 2 h but lower at 6 h post-feeding and remained numerically lower between 6 to 20 h post-feeding in 2100 h-fed than in 0900 h-fed cows. Feed delivery time did not affect daily averages of rumen pH and VFA, and plasma lactate and urea. Evening fed but not morning fed cows exhibited significant rises in

plasma lactate at 4 and 16 h post-feeding. Plasma urea increased shortly after feeding in morning fed cows but not in evening fed cows. Results indicate that time of feed delivery alters postprandial and 24-h patterns of feed intake, rumen fermentation, and peripheral blood metabolites. Time of feed delivery, hence, affects peripheral nutrient availability in lactating cows.

Key words: blood metabolite, evening feeding, feed intake, rumen, diurnal pattern

INTRODUCTION

Time of feed delivery is an external stimulator of digestive function and hepatic metabolism in ruminants (Piccione et al., 2003; Piccione and Caola, 2002; Taweel et al., 2004). Until most recently (Chapters 3, 4 and 5; Furedi et al., 2006a,b) time of providing total mixed ration (TMR) to dairy cows under thermoneutral conditions (e.g., <25°C) had received no attention. Studies with beef cattle have investigated the effect of feed delivery time under extremely cold temperatures (Kennedy et al. 2004; Small et al., 2004) and have found a positive effect of evening instead of morning feeding on growth.

Recent studies by our group using lactating cows (Furedi et al., 2006a,b; Chapter 4; Plaizier et al., 2005b) highlighted the feed delivery time as a mediator of post-feeding patterns in feed intake, rumen fermentation, and circulating metabolites. The TMR delivery at 2100 instead of 0900 h increased feed intake within 3 h of feeding, post-feeding rumen VFA concentrations, and postprandial lactate, BHBA, and insulin in the circulating blood. Consequently, milk fat and energy was enriched by feed delivery at 2100 h. In a follow-up study (Chapter 5), feed delivery at 2100 instead of 0900 h

increased feed intake within 3 h of feeding in all cows and DMI in primiparous cows. Also, feed delivery at 2100 h increased rumen volume, post-feeding peaks in rumen VFA and acetate, and total tract nutrient digestibility (Chapters 3 and 5). Consequently, milk fat and energy outputs were increased by feed delivery at 2100 h. Moreover, N excretion via feces and urine was reduced (Chapter 5). Hence, N balance was improved in 2100 h-fed cows compared to 0900 h-fed cows. The main objective of the present study was to establish the influence of feed delivery time on post-feeding patterns of feed intake, rumen fermentation, and circulating blood metabolites following a 21-d adaptation period.

MATERIALS AND METHODS

Experimental Design, Cows, and Treatments

Four multiparous (645 ± 75 kg BW; 77 ± 25 days in milk, mean ± SD) and four primiparous (576 ± 46 kg BW; 90 ± 33 days in milk) lactating Holstein cows were used in the Dairy Metabolism Unit of the Glenlea Research Station, University of Manitoba. Four of cows were fitted with rumen cannula. Cows were housed in tie-stalls and offered a TMR with a forage to concentrate ratio of 50.2:49.8% (DM basis) *ad libitum*. The average air temperature and relative humidity during the sampling weeks were -3.7°C and 78.9%, respectively. Experimental procedures involving animal surgery and care followed the guidelines of the Canadian Council on Animal Care (CCAC, 1993).

The TMR was delivered at either 0900 or 2100 h. The daily supply of TMR was adjusted based on the intake of individual cows in the previous day to allow for 5-10% orts. Cows had unlimited access to fresh water. The experimental design was a cross-over

with two 6-week periods. The first 3 weeks were for adaptation to experimental conditions followed by 3 weeks of sampling. The 24-h patterns in feed intake were monitored continuously using a data acquisition system within the metabolism unit (Grow-Safe System, Model 4000, Airdrie, AB). Every other morning during the adaptation weeks, cows were allowed 2 h of exercise. The TMR was prepared every morning. The dietary ingredients and chemical composition were given in Chapter 5. Cows were milked twice daily in their stalls at 0400 and 1600 h with lights remaining on from 0345 until 2245 h.

Continuous Monitoring of Rumen Fermentation

Rumen fermentation was monitored using four rumen-cannulated cows. Rumen pH was recorded every minute during the sampling weeks. Rumen fluid was sampled on Monday and Tuesday during week 4 at 0, 2, 3, 4, 6, 8, 12, 16, 20, 24 h after feeding to study 24-h patterns in rumen concentrations of volatile fatty acids (VFA) and ammonia. Upon sampling, rumen fluid samples were centrifuged at 1800 × *g* for 12 min and the supernatant was stored at -20°C. The frozen rumen fluid samples were later thawed at room temperature and 0.5 ml of 25% meta-phosphoric acid solution was mixed with 2.5 ml of the rumen fluid in plastic tubes and stored at -20°C overnight. The next morning, the mixtures were thawed again and centrifuged for 20 min at 3000 × *g*. Two ml of supernatant were then transferred to clean dry glass vials, capped, and placed onto the auto-sampler (Model 8100; Varian, Walnut Creek, CA). The concentrations of VFA were determined by gas chromatography (Model 3400 Star; Varian) using a 1.83-m packed glass column (Model 2-1721; Supelco, Oakville, ON, Canada) (Erwin et al., 1961). The

injector and detector temperatures were set at 170°C and 195°C, respectively, with initial and final column temperatures set at 120°C and 165°C, respectively. Ammonia concentration in rumen fluid samples was measured according to Novozamsky et al. (1974) and absorbance was read at 630 nm on a Pharmacia Biotech Ultraspec 2000 UV/visible spectrophotometer (Biochrom, Cambridge, UK).

Monitoring of 24-h Patterns in Circulating Blood Metabolites

All cows were catheterized in the jugular vein on the first day of week 5. The catheters were flushed with a sterilized heparin-saline solution (0.9% NaCl, and 50 units heparin/ml) and monitored for 24 h to ensure proper function before starting bleeding on the second day of week 5 at 0900 h. The jugular blood was sampled using a 20 ml syringe every 2 h for 24 h starting at 0090 h and ending at 0700 h of the next day. Immediately after each bleeding, all catheters were flushed with 10 ml of the heparin-saline solution to prevent clotting inside the catheter and the extension set. Upon sampling, blood was transferred into green-top vacutainer tubes containing anti-coagulant (Na-heparin) and placed on ice. Blood was centrifuged at 3000 × *g* for 15 min at 4°C and plasma was transferred to two 4- and 1.8-ml vials and frozen at -20°C. Plasma concentrations of lactate, and urea were measured using an automatic analyzer (Stat Profile ® Critical Xpress, 02454-9141 Nova Biomedical, Waltham, MA) equipped with enzymatic sensors.

Statistical Analyses

The amount of feed consumed within eight 3-h intervals (24-h phase) were expressed as percent of total daily TMR intake for individual cows. The amount ingested within each 3-h interval was compared between 2100 h-fed and 0900 h-fed cows using the Mixed Model Procedure of SAS (SAS Institute, 2003). The effects of TF, parity, and their interaction were fixed, and the effects of period and cow within parity were random. Repeated rumen and blood data were analyzed as a linear Mixed Model with the best fitted covariance structures (Little, 1998; Wang and Goonewardene, 2004). The model used for the rumen concentrations of metabolites included fixed effects of TF, parity, hour (of blood sampling), TF × parity, hour × parity, and TF × hour × parity. The effects of cow within parity, period, and TF × period × cow (parity) were considered random. Rumen pH was analyzed for three 24-h in each period. Therefore, the same fixed effects but with additional random effects of day within period, TF × period × hour × cow within parity, and TF × day within period × hour × cow within parity were modeled. To adjust for the different within-cow correlation of the repeated measures, the best fitted covariance structure was adopted for each metabolite (Wang and Goonewardene, 2004). Prior to variance analyses, normal distribution and variance homogeneity of residuals (Steel et al., 1997) were ensured using Proc Univariate of SAS (SAS Institute, 2003). Where significant, treatment means were separated using PDIFF option of SAS (SAS Institute, 2003). The significant levels of fixed effects were declared at $P < 0.05$ and trends were discussed at $0.05 < P < 0.10$.

RESULTS

24-h Patterns in Feed Intake

The 24-h distributions of TMR intake are presented in 3-h intervals relative to time of TMR delivery (Figure 9). The proportion of daily TMR intake consumed within 3 h post-feeding was 55% for 2100 h-fed cows but only 46% for 0900 h-fed cows (P = 0.06). While the proportion consumed within 6 h post-feeding was similar (P = 0.51) for 0900 h-fed (62.6%) and 2100 h-fed (65.7%) cows, within 9 h post-feeding, 2100 h-fed cows consumed greater percent of their daily TMR intake (80 vs. 73%, $P < 0.05$). By 15-h post-feeding, cumulatively, 0900 h-fed cows had consumed 96% but 2100 h-fed cows had consumed 90% of their daily intake ($P < 0.05$).

24-h Patterns and Daily Averages of Rumen pH and Metabolites

Rumen pH dropped until 6 h after feed delivery in 2100 h-fed cows and until 8 h after feed delivery in 0900 h-fed cows (Figure 10A). The 2100 h-fed cows had higher ($P < 0.05$) rumen VFA at 6 h and lower rumen pH at 5 h ($P < 0.01$) and 6 h ($P < 0.05$) post-feeding than 0900 h-fed cows (Figure 10A,B). In addition, cows fed at 2100 h had higher ($P < 0.05$) rumen acetate at 6 h and 8 h but lower propionate at 4 h post-feeding than did cows fed at 0900 h (Figure 11). Accordingly, acetate + butyrate to propionate ratio (A+B/P) was higher for most of the day in 2100 h-fed cows than in 0900 h-fed cows (Figure 12). The daily average of A+B/P was also higher in 2100 h-fed than in 0900 h-fed cows (Table 21). Of total VFA, the molar proportion of rumen propionate was lower ($P < 0.05$) in cows fed at 2100 h than in cows fed at 0900 h (Table 21). Comparable to

other VFA, rumen levels of butyrate also exhibited significant post-feeding rises with peaks at 6 h after feed delivery in both 2100 h-fed 0900 h-fed cows (Figure 10). Rumen butyrate in 0900-fed cows increased gradually upon feeding until 6 h after feeding; whereas rumen butyrate in 2100 h-fed cows rose between 0-2 h, plateaued between 2 and 4 h, and peaked at 6 h after feed delivery (Figure 12). Rumen ammonia at 2 h post-feeding was higher in 2100 h-fed cows than in 0900 h-fed cows, but it was lower ($P < 0.05$) at 6 h post-feeding and remained numerically lower between 6 to 20 h post-feeding in the 2100 h-fed cows than in the 0900 h-fed cows. As a result, the average daily rumen ammonia in 2100 h-fed cows was lower in 2100 h-fed cows (Table 21). Rumen concentrations of branched chain volatile fatty acids (BCVFA) showed a dramatic rise at 2 h post-feeding in both groups (Figure 13). However, rumen BCVFA returned to pre feeding baseline at 4 h post-feeding in 2100 h-fed cows but not in 0900 h-fed cows.

24-h Patterns and Daily Averages of Blood Metabolites

Plasma lactate and urea exhibited significant diurnal patterns ($P < 0.05$). Postprandial responses in plasma lactate, and urea were significantly altered by the time of feed delivery ($P < 0.05$). The 2100 h-fed but not 0900 h-fed cows showed significant rises in plasma lactate at 4 and 16-h post-feeding (Figure 14). Blood urea increased shortly after feeding in 0900 h-fed cows but not in 2100 h-fed cows (Figure 15). After peaking at 2 h post-feeding in 0900 h-fed cows, blood urea declined progressively until 10 h post-feeing. Blood urea in 2100 h-fed cows declined from 4-10 h post-feeding and rose sharply at 12 h post-feeding. Blood urea then remained higher until 2 h before the

next feed delivery, compared to 0900 h-fed cows (Figure 15). The TF did not affect the daily averages of lactate, and urea in the peripheral blood ($P > 0.05$, Table 22).

DISCUSSION

24-h Patterns in Feed Intake

The greater feed intake of 2100 h-fed cows compared to 0900 h-fed cows within 3 h after feed delivery supports the results of our previous study (Chapter 4). In the current study, 2100 h-fed cows consumed 55% and 0900 h-fed cows 46% of their daily intake within 3 h after feed delivery. In our previous study, 2100 h-fed cows ate 37% but 0900 h-fed cows only 26% of their daily intake within 3-h of feeding. Despite differences in the amount consumed within 3 h of feeding between two studies, 2100 h-fed cows consistently consumed more feed than did 0900 h-fed cows. Diets used in our previous study (Chapter 3) had lower NDF (28-33 vs. 39%) and ADF (15-19 vs. 27%) but higher non fiber carbohydrates (NFC) (36-40 vs. 29%) than did the diet in the present trial. Levels of fiber and readily fermentable carbohydrates are among important dietary factors contributing to feed intake regulation in lactating cows (Allen, 2000). Apparently, the higher dietary NDF in the current experiment was still below the range that could potentially down-regulate feed intake. The greater dietary NFC appears to have reduced the amount consumed shortly after feed delivery in the current study, compared to our previous study (Chapter 4). In that study, DMI did not differ between the two groups; whereas in the current study, DMI in primiparous but not in multiparous cows was greater when feed was delivered at 2100 h instead of 0900 h. In cows fed more than once

daily, eating time has been reported to increase in the evening compared to during the day (DeVries et al., 2005; Phillips and Rind, 2001). The time spent eating and not the amount of feed consumed within certain time periods were monitored in those studies. Thus, the effect of the time of day on feed intake could not be quantified. In beef cattle, Pritchard and Knutsen (1994) pointed out that evening vs. morning delivery of high-concentrate diets altered post-feeding patterns in feed intake. However, Pritchard and Knutsen (1994) also did not measure postprandial patterns in feed intake. They instead based their conclusions on within-day body weight changes in cattle fed either in the morning, in the evening, or in both morning and evening. Hence, results of the current study are the first to reveal the impact of feed delivery time on quantitative post-feeding patterns in feed intake of once-daily fed lactating cows. More appreciably, results of the current trial verify the findings of our previous experiment (Chapter 4). In support of the greater daily feed intake of 2100 h-fed than of 0900 h-fed primiparous cows in our study, Schwartzkopf-Genswein et al. (2004) also found a positive response in DMI of feedlot cattle to feed delivery at 2100 instead of at 0900 h. Diurnal patterns in feed intake were not monitored by Schwartzkopf-Genswein et al. (2004). Primiparous cows are still growing to achieve their mature body weight. Sustaining growth obligates additional nutrient demands. Schwartzkopf-Genswein et al. (2004) also used growing cattle. Tissue growth, thus, introduces a priority in nutrient partitioning towards body deposition. Such a growth priority superimposed on high milk production levels may have enabled the primiparous cows to increase DMI by feed delivery at 2100 h. In contrast, early and even mid lactation multiparous cows do not usually need as much nutrients for growth as do primiparous cows. Such a mediatory impact of growth on DMI response to evening

feeding applies to the study of Schwartzkopf-Genswein et al. (2004), as well. Additionally, energy-corrected milk yield was increased by 2.1 kg/d in primiparous cows but only by 1.3 kg/d in multiparous cows, when feed was offered at 2100 instead of at 0900 h. Such an increase in milk yield of primiparous cows may have been promoted by more DMI. Noteworthy, tissue deposition is a less energetically efficient process than milk secretion, thus needing more energy per each unit of product (Blaxter, 1989). Furthermore, feed delivery at 2100 h improved nitrogen retention and balance in all cows. Thus, it may be suggested that the increased DMI of primiparous in response to feeding at 2100 h was a collective effort to meet the obligation of growth and concomitantly maintain nutrient export to the mammary gland.

24-h Patterns in Rumen pH and Concentrations of Metabolites

Rumen pH showed a significant post-feeding decline lasting until 6-7 h in both groups. However, the post-feeding nadirs in rumen pH of 2100 h-fed cows were lower than that of 0900 h-fed cows. The greater decline in rumen pH of 2100 h-fed cows was most probably due to increased feed intake within 3 h after feed delivery. This is because greater feed intake provides greater fermentable organic matter to the rumen microbes. Since the rumen outflow rate did not differ between treatments (Chapter 5), the increased rumen fermentation and subsequently the lower rumen pH in 2100 h-fed cows, compared to 0900 h-fed cows, were not unexpected. Nevertheless, rumen pH rose between 8 and 15 h after feeding, leading the average daily rumen pH to remain comparable between treatments. Similarly, we had observed a lower postprandial decline in rumen pH of 2100 h-fed cows than of 0900 h-fed cows in a previous study (Chapter 3). Recently Taweel et

al. (2004) reported a lower rumen pH at midnight than during the day (0600-1000 h) in grazing dairy cows. Collectively, these data point to the more extensive night-time than day-time rumen fermentation. Continuous monitoring of rumen pH in the current study enabled us to substantiate our previous findings obtained with less frequent rumen sampling using a stomach tube. Due to saliva contamination, sampling the rumen fluid with the stomach tube may overestimate actual rumen pH, compared to the pH values obtained via cannulae (Duffield et al., 2004).

Rumen VFA concentrations peaked at 6-h post-feeding in both groups. However, the VFA peak was higher in 2100 h-fed cows than it was in 0900 h-fed cows (140 vs. 123 m*M*, $P < 0.05$). The higher peak in rumen VFA concurs with the lower nadir ($P < 0.05$) in rumen pH at 5 h (5.81 vs. 6.09) and 6 h (5.70 vs. 5.94) post-feeding in 2100 h-fed cows compared to 0900 h-fed cows. The higher VFA peak in 2100 h-fed than in 0900 h-fed cows was the result of the higher rumen acetate at 6 h post-feeding. Acetate is the largest contributor to total rumen VFA (Barcroft et al., 1944). In contrast, post-feeding peaks in rumen propionate and butyrate did not differ between treatments. As a result, the rumen ratio of (acetate + butyrate) to propionate (A+B/C) remained higher in 2100 h-fed cows than in 0900 h-fed cows between 3 to 8 h and at 16 h post-feeding. The higher A+B/C can be due to increased fibrolytic activity, leading to greater production of acetate and butyrate (France and Dijkstra, 2005). This reasoning gains support by the fact that TMR delivery at 2100 h instead of at 0900 h enhanced total tract fiber digestibility (Chapter 5). Moshtaghi Nia et al. (1995) and Robinson et al. (1997) fed a protein meal at 0030 instead of at 0830 h and found higher post-feeding rumen concentrations of total VFA and ammonia. As a result of the elevated rumen VFA after the midnight delivery of

the protein meal, the average daily rumen VFA was also increased (Robinson et al., 1997). These results agree with the higher peaks in rumen VFA and ammonia of the current study. As well, Taweel et al. (2004) reported higher rumen total VFA in the night than during the day in grazing cows. The larger night-time than day-time rumen fill has also been observed in grazing sheep (Thompson et al., 1985). The rumen volume was estimated to be larger in 2100 h-fed cows than it was in 0900 h-fed cows (Chapter 5). As the rumen retention fluid outflow rate and retention times of fluid and solids remained unchanged, a larger rumen volume could mean a more extensive rumen fermentation. This would be expected to increase rumen VFA production, particularly acetate, during the peak of rumen fermentation. Accordingly, VFA concentrations were higher in 2100 h-fed cows at 5 h post-feeding, compared to 0900 h-fed cows. Also, recently, Taweel et al. (2004) found a larger night-time than day-time rumen fiber pool in grazing dairy cows.

The higher rumen ammonia at 2 h post-feeding in 2100 h-fed cows than in 0900 h-fed cows was in accord with the increased feed intake, increased rumen volume, and elevated rumen VFA. To speculate, the lower concentrations of BCVFA at 4 and 6 h post-feeding in 2100 h-fed than in 0900 h-fed cows might have been due to temporary differences in rumen microbial populations. Such changes in microbial population are thought to have occurred due to increased feed intake and rumen fermentation shortly after feed delivery in 2100 h-fed cows. Rumen BCVFA originate mainly from microbial modification of dietary branched chain AA (Brockman, 2005). It might thus be postulated that TF altered AA transformation into BCVFA. Nonetheless, owing to the minor input

of BCVFA to the peripheral energy supply (Brockman, 2005), the inter-treatment differences may not significantly affect milk production.

24-h Patterns in Circulating Blood Metabolites

In agreement with our previous results (Chapter 4), plasma lactate showed a significant rise at 4 h post-feeding in 2100 h-fed cows but not in 0900 h-fed cows. Also similar to 24-h patterns in plasma lactate of 0900 h-fed cows in the previous project (Chapter 4), the 0900 h-fed cows experienced lower plasma lactate overnight than during the day (Figure 14). In addition to anaerobic oxidation of glucose by peripheral tissues, alimentary supply can be a major source of the circulating lactate in ruminants (Reynolds, 2003). Alimentary lactate is the lactate produced in the rumen due to microbial fermentation of readily-fermentable carbohydrates (Giesecke and Stangassinger, 1980), the lactate synthesized across the rumen epithelium via modification of organic acids such as propionate (Baldwin and McLeod, 2000; Giesecke and Stangassinger, 1980). The hepatic lactate use decreases when much propionate is available for gluconeogenesis (Armentano, 1992). As a result, more lactate would be expected to appear in peripheral blood at times of the greatest rumen propionate delivery or shortly after feeding (Ortrigues et al., 1996). Hence, the greater feed intake of 2100 h-fed cows than of 0900 h-fed cows supports the higher post-feeding peak in plasma lactate of 2100 h-fed cows. Circulating lactate can be utilized by the mammary and non-mammary tissues as an energy source (Giesecke and Stangassinger, 1980). Also, the mammary nutrient uptake appears to be more dependent on peripheral nutrient flow than a transitory hormonal regulation (Annison and Bryden, 1999; Griinari et al., 1997). As a result, milk fat and

energy outputs are expected to increase by increased mammary flow of nutrients. As such, milk production was increased by TMR delivery at 2100 h instead of 0900 h.

The circulating levels of plasma urea have been shown (Piccione et al., 2003) to depend mainly on digestive processes. Plasma urea tended to rise at 2 h post-feeding in 0900 h-fed cows. Rumen ammonia also rose drastically at 2 h post-feeding (Figure 13). Gastric ammonia is the predominant substrate for hepatic urea synthesis early post-feeding (Reynolds, 1992). Therefore, the peak in rumen ammonia can shortly be followed by a peak in circulating urea (Gustaffson and Palmquist, 1993). After the post-feeding peak, plasma urea in 0900 h-fed cows steadily declined until reaching a nadir at 12 h post-feeding, when it started to rise gradually to attain the baseline by next feed delivery. Unlike 0900 h-fed cows, 2100 h-fed cows did not show a post-feeding rise in plasma urea. The significant rise in plasma urea at 2 h of 0900 h but not of 2100 h TMR delivery may partly be attributed to the shorter-lasting peak in rumen ammonia in 2100 h-fed than in 0900 h-fed cows. Also, the rise in plasma urea at 10 h post-feeding in 2100 h-fed cows could be related to larger rumen volume and greater N intake within 3 h after feeding in 2100 h-fed cows compared to 0900 h-fed cows. Perhaps, the more extensive fermentation in 2100 h-fed cows may have led the rumen to recycle more of blood urea to sustain microbial growth and maintain the gut tissue metabolism. Since fecal and urinary N excretion were reduced by 2100 h feed delivery, the increased N and energy demands of the gut may in part explain the higher blood urea 10 h after feed delivery.

Despite consuming more feed within 3 h post-feeding, 2100 h-fed cows had rumen retention times of fluid and solids comparable to those of 0900 h-fed cows. The larger night-time than day-time rumen fill has also been reported in grazing cows

(Taweel et al., 2004) and sheep (Thompson et al., 1985). The reference to grazing ruminants is of importance because grazing occurs mostly around sunrise and sunset. Thus, the rumen kinetics would be expected to be comparable between the day and night, but it is not so. The above-mentioned pasture studies demonstrated that rumen kinetics of passage and digestion differ substantially between day and night. These results support the evolutionary fact that rumination has a circadian rhythm occurring mostly overnight (Gordon and McAllister, 1969). Hence, it may be suggested that offering the fresh feed in the evening when the ruminant has evolved to ruminate (and ensalivate) should increase nutrient digestibility via increased rumen volume and unaltered outflow rate. As such, it can be inferred that the capacity and duration of rumen fermentation were greater with feed delivery at 2100 h instead of at 0900 h. Under such a prolonged, capacious rumen fermentation, the gut metabolism would require greater energy and N to be sustained (Huntington, 1990). Gut tissues including the rumen, small and large intestines, pancreas, spleen and associated muscles possess high N turnover rates (Huntington, 1989, 1990). The greater N intake along with the greater N turnover rate by the gut tissues would mean greater ammonia, urea, and AA exchange amongst gut, liver, and peripheral tissues (Huntington, 1989). The greater N turnover results from the increased energy needs of the gut tissues and a need to remove the end products of N metabolism (Huntington, 1990). As a result, instead of being excreted as urea in the urine, ammonia along with organic acids (α-ketoglutarate, aspartate, and formate) serve both splanchnic and peripheral tissues as a source of N and energy. Reduced N partitioning towards urine in favor of body retention by feed delivery at 2100 h instead of at 0900 h (Chapter 5) lends support to the above logic. Huntington (1989) demonstrated that greater diet

fermentability increases gut N demand, leading to greater urea recycling into the gut via both saliva and plasma.

Feed delivery more frequent than once-daily has been shown to eliminate diurnal patterns in plasma urea (Folman et al., 1981). In the current study, TMR was delivered once daily. However, rumen ammonia remained at its peak from 2 to 6 h after 0900 h-feed delivery but was at its peak only 2 h after 2100 h-feed delivery (Figure 15). The higher blood urea over the 12-h preceding evening but not morning feeding was, thus, not related to rumen ammonia. Transamination involving aspartate, glutamate, glutamine, alanine, and glycine can significantly contribute to hepatic urea synthesis, especially during periods of elevated glucose demand (Krebs et al., 1979). Up to 40% of the hepatic glucose output in lactating cows can be derived from AA (Danfær et al., 1995). The AA use in glucose synthesis increases at greater AA availability or lower ruminal propionate and lactate supply (Danfær et al., 1995; Huntington, 1990). Accordingly, the higher plasma urea starting at 12 h post-feeding, when plasma urea was expected to be lower (see 0900 h-fed cows in Figure 15) in 2100 h-fed than in 0900 h-fed cows might reflect the hepatic use of AA for gluconeogenesis (Blum et al., 2000; Lefcourt et al., 1999). It is, however, unclear if such a possible role in hepatic N metabolism was stimulated by an increaed AA provision or by reduced propionate and lactate supply. Hormones and gut peptides can potentially affect N turnover in the liver (Reynolds, 1992). The larger rumen volume in 2100 h-fed cows than in 0900 h-fed cows might have increased N and energy demands by the gut tissues. This may have subsequently intensified N exchanges amongst the splancnhic tissues in 2100 h-fed cows, thereby contributing to their high blood urea through 12-h pre feeding.

CONCLUSIONS

Feed delivery at 2100 instead of at 0900 h increased feed intake within 3 h of feeding. Increased feed intake within 3 h of 2100 h-feed delivery was associated with increased DMI in primiparous but not in multiparous cows. Thus, parity or the physiological state of body or growth mediated DMI response to feed delivery time. Rumen pH was lower at 5 and 6 h, rumen VFA were higher at 6 h, and acetate was higher at 6, 8 and 12 h post-feeding in 2100 h-fed cows than in 0900 h-fed cows. Also, the daily average in the rumen (acetate + butyrate) to propionate ratio was higher in 2100 h-fed than in 0900 h-fed cows. Feed delivery at 2100 h instead of at 0900 h caused a post-feeding rise in blood lactate. Plasma urea was higher at 2-h after 0900 h-feeding but not 2100 h-feeding. Between 12 to 4 h pre-feeding, however, plasma urea was higher in 2100 h-fed cows than it was in 0900 h-fed cows. Results ascertained that feed delivery at 2100 h instead of 0900 h increased feed intake, rumen VFA, and blood lactate shortly post-feeding. Changing the feeding time form 0900 h to 2100 h may thus increase nutrient availability to splanchnic, mammary, and other peripheral tissues.

Table 21. Effects of time of feeding (TF), sampling hour (H), parity (P), and their interactions on rumen fermentation

Item	Primiparous		Multiparous			Fixed effects, P^3					
	0900 h	2100 h	0900 h	2100 h	SE[1]	TF	P	TF × P	H	P × H	TF× H[2]
Dry matter intake, kg/d	18.5[a]	20.4[b]	20.4	20.6	0.48	*	NS	NS	-	-	-
Rumen fermentation											
pH	6.26	6.28	6.21	6.16	0.07	NS	NS	NS	***	NS	***
VFA, m*M*	106.4	103.4	105.7	114.4	4.9	NS	NS	NS	***	*	NS
SCVFA, m*M*	3.85[b]	3.50[b]	4.21[a]	4.32[a]	0.17	NS	**	NS	***	†	NS
Acetate (A), m*M*	66.8	67.5	67.3	74.3	3.5	NS	NS	NS	**	*	NS
Propionate (P), m*M*	22.7[a]	20.7[ab]	20.4[b]	19.7[b]	0.96	NS	NS	NS	***	NS	NS
A+B/P	3.6[c]	3.9[bc]	4.1[b]	4.6[a]	0.15	*	†	NS	***	NS	NS
Butyrate (B), m*M*	13.7	12.8	15.6	14.7	1.2	NS	NS	NS	***	NS	NS
Ammonia, mg/dL	9.8[c]	8.2[d]	11.9[a]	11.5[b]	0.04	*	*	*	***	NS	†
Molar proportions											
Acetate	62.8[b]	65.5[a]	64.0[a]	65.0[a]	1.3	NS	NS	NS	***	NS	NS
Propionate	21.3[a]	19.9[b]	19.3[b]	17.4[c]	0.58	*	†	NS	**	NS	NS
Butyrate	15.8[ab]	14.7[b]	17.1[a]	15.6[ab]	1.04	NS	NS	NS	**	NS	NS

[1]Sandard errors are for the differences of least square means.
[2]Except for pH, hours of sampling for all rumen fermentation indices were expressed as post-feeding hours and not hours of day. Thus, a non-significant effect of TF× H means a significant effect of TF on 24-h patterns in rumen fermentation indices. The effects of TF on post-feeding patterns were illustrated in Figures 2, 3, 4, and 5.
NS = not significant i.e., $P > 0.15$. † = $0.05 < P < 0.10$. * = $0.01 < P \leq 0.05$. ** = $0.001 < P < 0.01$. *** = $P < 0.001$.
[a,b,c] Different superscripts within the same row differ at $P < 0.05$.

Table 22. Effects of time of feeding (TF), sampling hour (H), parity (P), and their interactions on circulating blood metabolites

Item	Time of feed delivery		SE[1]	Fixed effects [2], *P*					
	0900 h	2100 h		TF	P	TF × P	H	P × H	TF × H[2]
Lactate, mmol/L	0.67	0.72	0.03	NS	NS	NS	***	NS	NS
Urea, mmol/L	5.1	5.3	0.28	NS	NS	NS	***	NS	***

[1]Standard errors are for the differences of least square means.

[2]Hours of sampling were expressed as hours of day. Thus, a significant effect of TF × H corresponds to a significant effect of TF on 24-h patterns of blood metabolites. The effect of TF on post-feeding patterns in blood metabolites was shown in Figures 6, 7, and 8.

NS = not significant or $P > 0.19$. *** = $P < 0.001$.

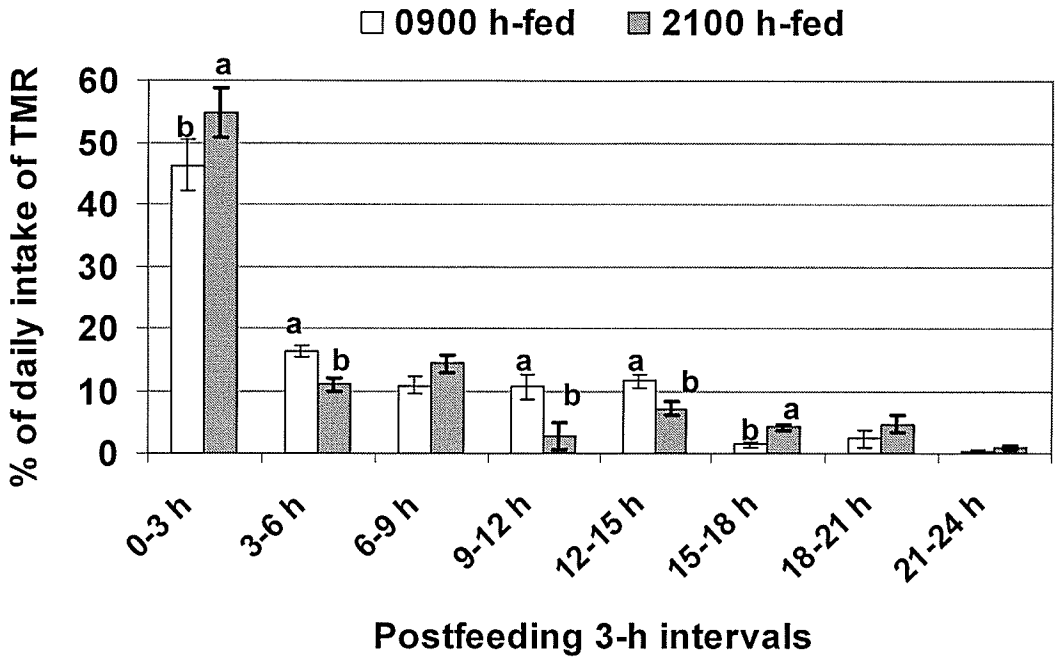


Figure 9. Post-feeding patterns of feed intake in cows fed either at 0900 h or at 2100 h. Bars with different superscripts within each 3-h interval differ at $P < 0.05$, except for 0-3 h where $P = 0.06$.

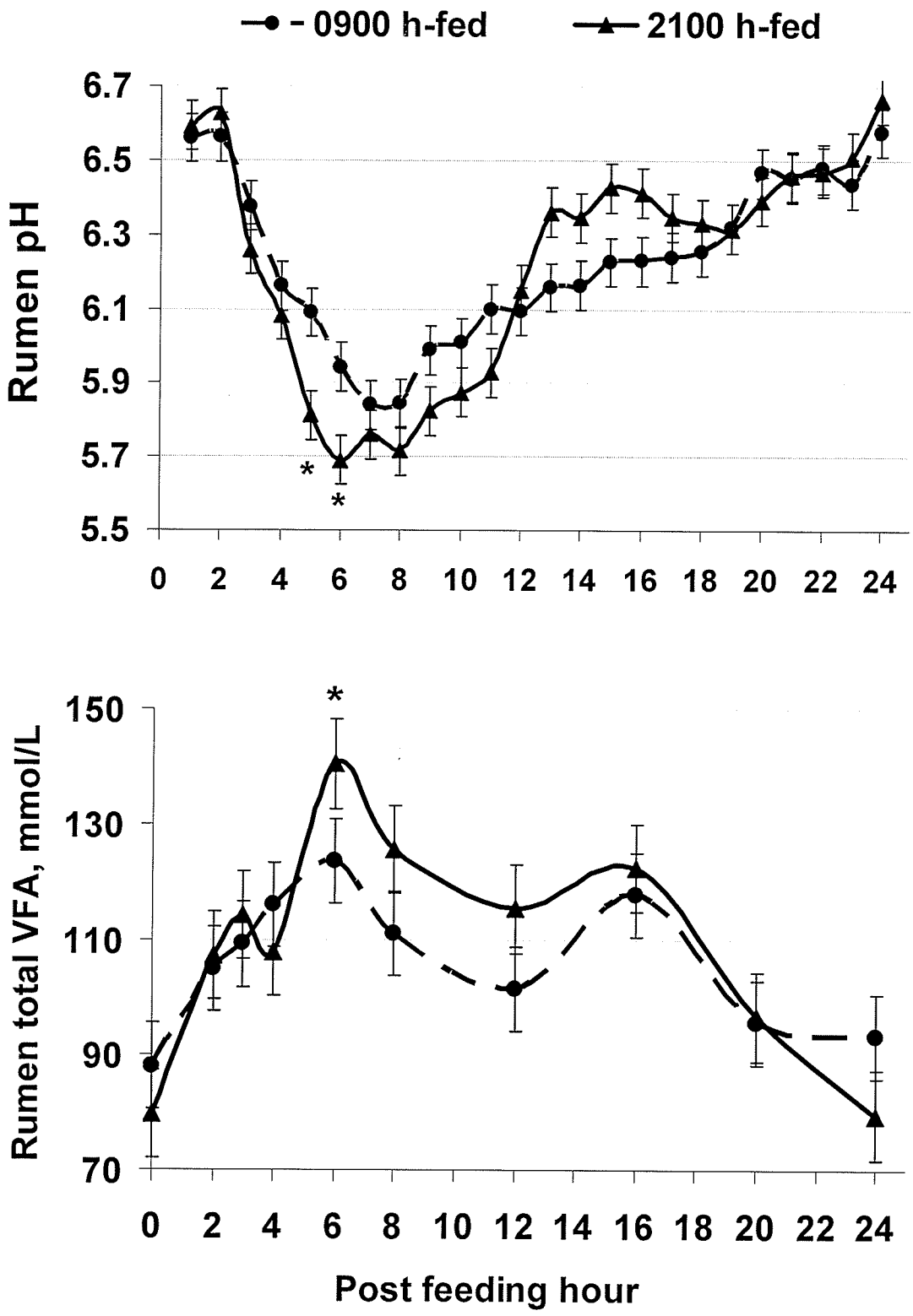


Figure 10. Post-feeding patterns of rumen pH (top) and total VFA (bottom) in cows fed either at 0900 h or at 2100 h. Within each hour, * = $P < 0.05$.

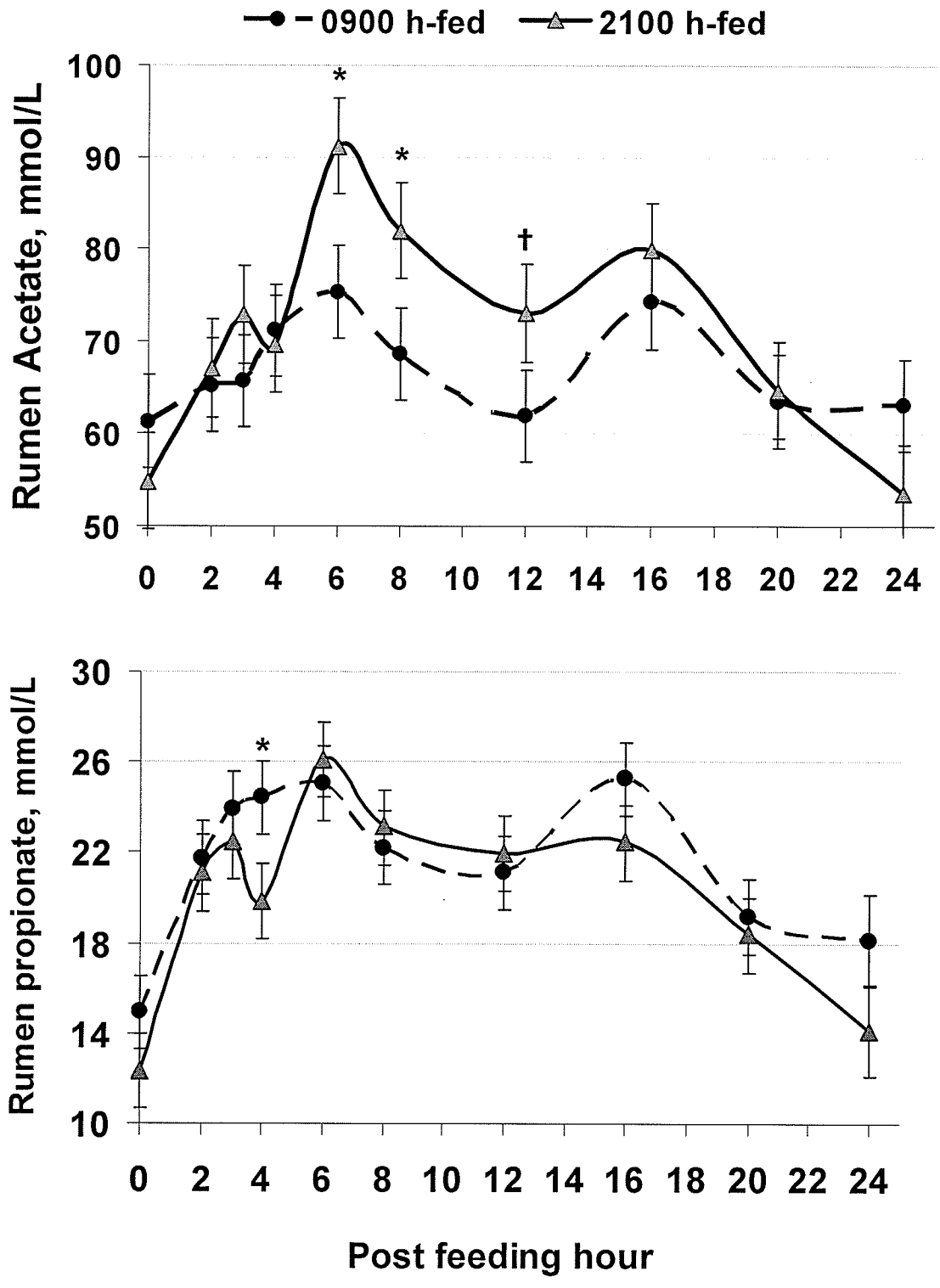


Figure 11. Post-feeding patterns of rumen acetate (top) and propionate (bottom) in cows fed at either 0900 h or 2100 h. Within each hour, * = $P < 0.05$; † = $P < 0.10$.

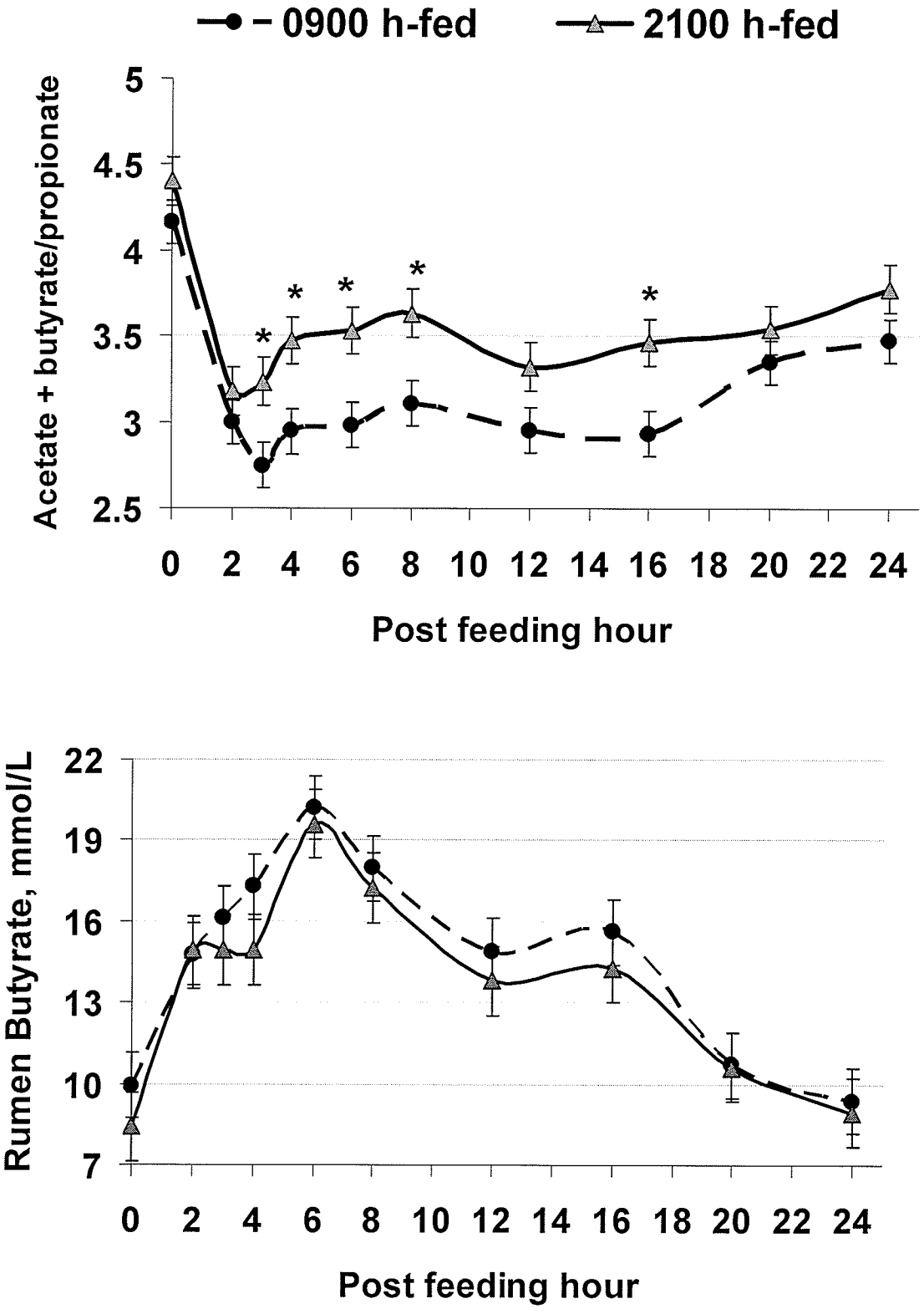


Figure 12. Post-feeding patterns in rumen acetate to propionate ratio (top) and butyrate (bottom) of cows fed either at 0900 h or at 2100 h. Within each hour, * = $P <$ 0.05.

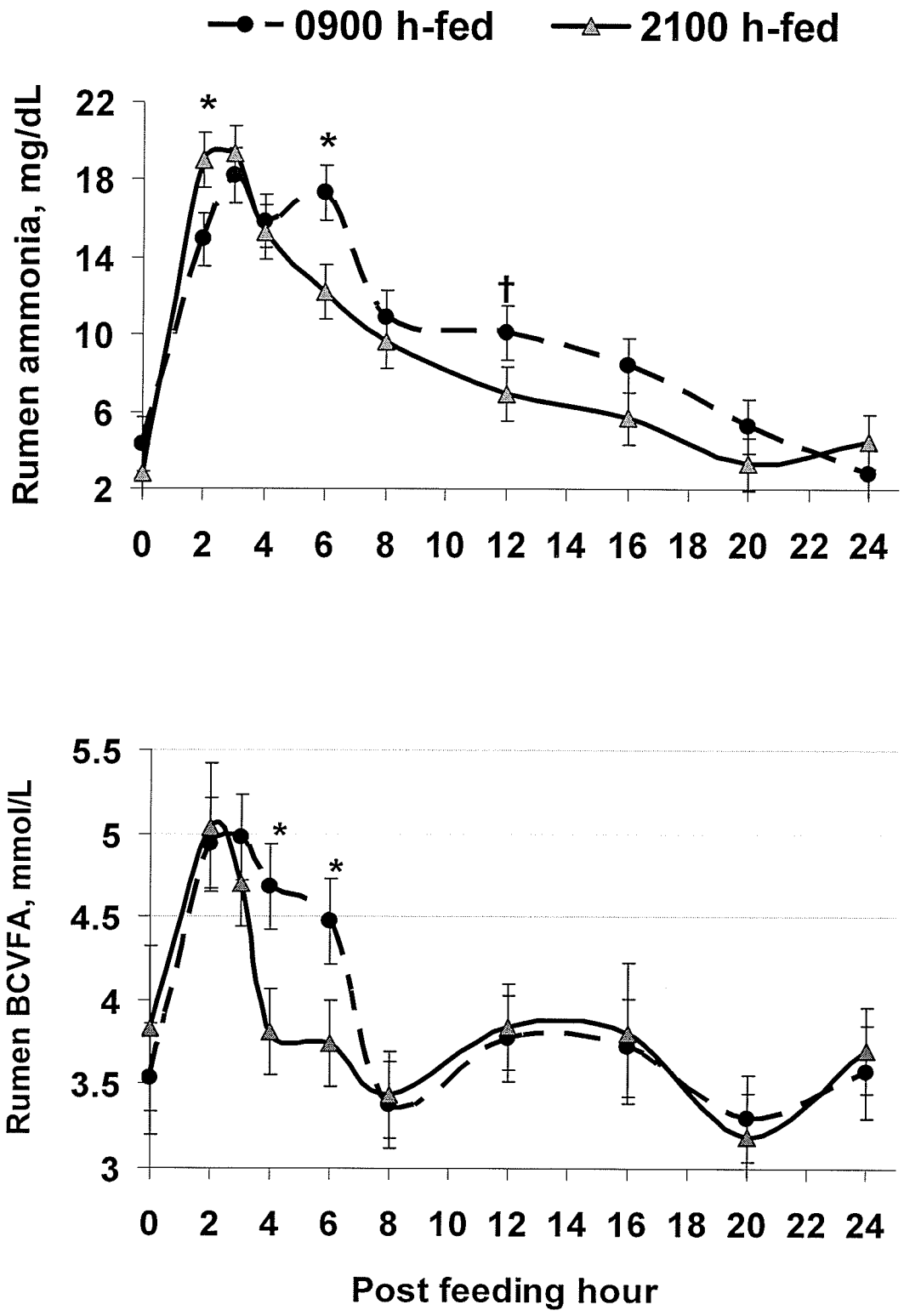


Figure 13. Post-feeding patterns of rumen ammonia (top) and branched-chain VFA (BCVFA, bottom) in cows fed either at 0900 h or at 2100 h. Within each hour, * = $P < 0.05$.

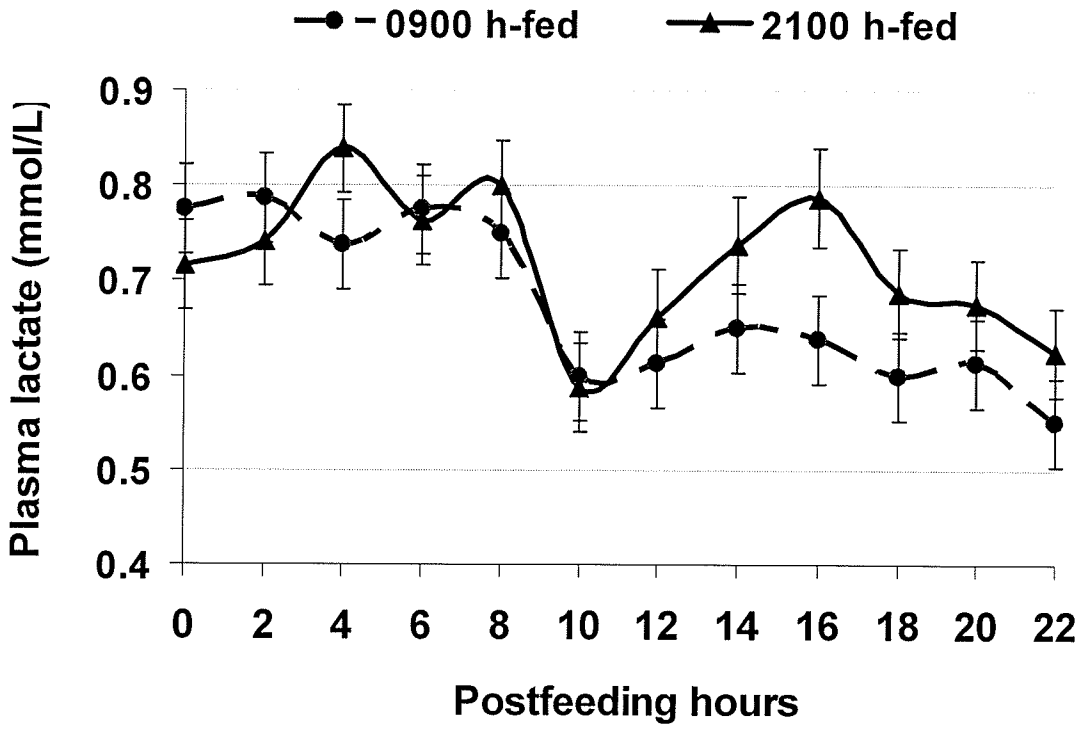


Figure 14. Post-feeding patterns of plasma lactate in cows fed either at 0900 h or at 2100 h. Arrows are feed delivery times. Within each hour, * = $P < 0.05$.

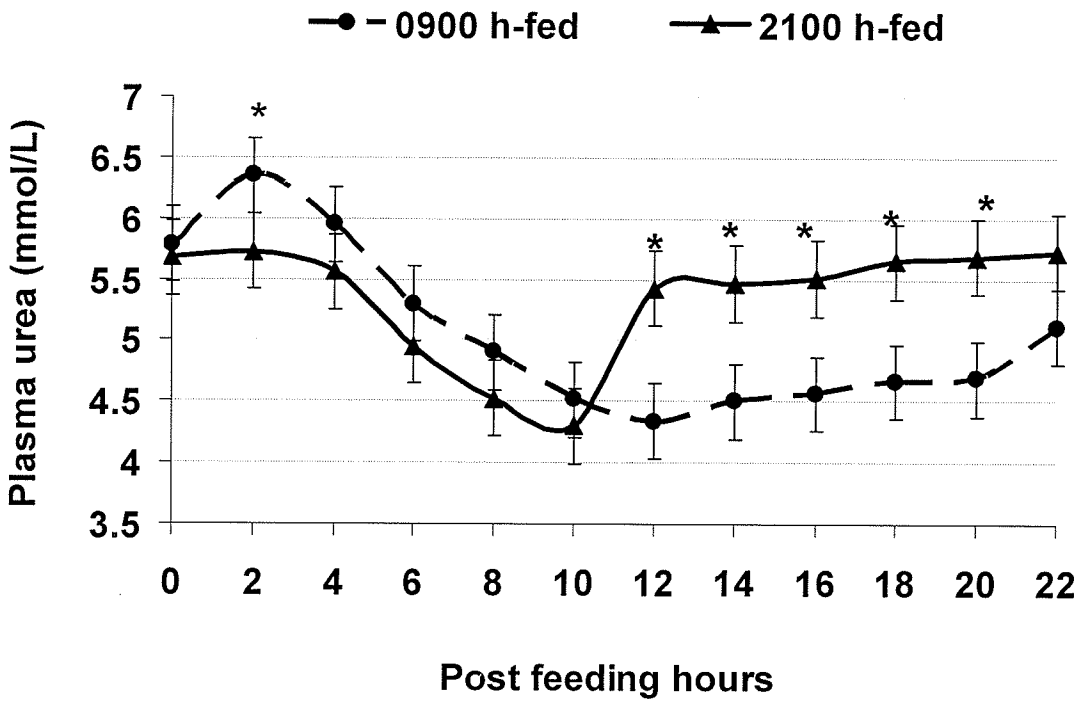


Figure 15. Post-feeding patterns of plasma urea in cows fed either at 0900 h or at 2100 h. Within each hour, * = $P < 0.05$.

CHAPTER 7

GENERAL DISCUSSION

The current thesis provided evidence that feed delivery at 2100 h instead of 0900 h alters the major steps involved in feed nutrient conversion to milk including **1)** feed intake, **2)** rumen fermentation and total tract nutrient digestion, **3)** peripheral blood metabolites, and **4)** milk secretion.

1) Diurnal patterns in feed intake. Feeding at 2100 h instead of 0900 h increased feed intake within 3-h of feeding in both studies (Chapters 4, 6). In the first study, within 3-h after feeding, the 2100 h-fed cows consumed 7.6 kg of DM but the 0900 h-fed cows only 5.3 kg of DM. In the second study and within the same time interval, the 2100 h-fed cows ate 11.2 kg but the 0900 h-fed cows 9.0 kg of DM. The TMRs in the first study contained on average 31.2% NDF and 37.8% NFC, whereas the TMR in the second project contained 39.2% NDF and 30% NFC (Chapters 3, 5). Two key points can be inferred from these results. First, regardless of parity and diet composition, feeding at 2100 h instead of at 0900 h consistently increased feed ingestion within 3 h after TMR delivery. Thus, diet composition did not have a major role in mediating the impact of time of feeding (TF) on stimulating feed intake within 3 h after feed delivery. Second, data provided a strong indication that once-daily fed lactating cows on average can eat as much as 50% of their daily intake within only 3 h after feed delivery (Chapter 6). Individual cow data showed that even some cows can eat as much as 70% of their daily

intake within 3 h after feed delivery. Notably, across the feeding times, the amount of feed consumed within 3 h after feeding was much less in the first study compared with the second study`. The difference could be due to the starchier and less fibrous nature of the TMR used in the first study project compared to the TMR used in the second study. The greater diet fermentability may depress feed intake via accumulated VFA and ammonia (Allen, 2000; Forbes, 2005). More importantly, the cow's ability to consume more feed following 2100 h than 0900 h feeding agrees with the notion that in high-producing ruminants, the rumen capacity or gut-fill may not be a key regulator of feed intake (Poppi et al., 1994; Ketelaars and Tolkamp, 1992; Taweel et al., 2004).

The greater appetite following feeding at 2100 h might also partly be due to the less-disturbed farm environment overnight than during the day. Lights were turned on at 0345 h and turned off at 2245 h or at 1:45 h post-feeding in the 2100 h-fed cows. The possible ability to anticipate the lights-off time by the 2100 h-fed cows may have contributed to such a rush in eating upon feeding. In the 0900 h-fed cows, on the contrary, feed delivery time was far from the time when lights were turned on and off.

Moreover, melatonin may regulate feed intake and glucose metabolism. Melatonin is involved with regulation of glucose metabolism in humans and rats (la Fleur et al., 2001; Lima et al., 1998; Picinato et al., 2002). For instance, exogenous melatonin consumed via drinking water boosts postprandial insulin response (la Fleur et al., 2001). Also, melatonin secretion is known to be induced by dark exposure (Illnerova and Sumova, 1997). In 2100 h-fed cows, feed was delivered shortly before lights-off or at 2245 h when melatonin secretion was expected to be high. In humans, reduced glucose tolerance is associated with elevated melatonin secretion (Van Cauter, 1998). Reduced

glucose tolerance is due to reduced insulin responsiveness and peripheral glucose uptake, which reflects a fall in glucose and insulin requirements (Arslanian et al., 1990; Van Cauter, 1990). If such a concurrence of reduced glucose tolerance and elevated melatonin secretion occurs in cows as well, the evening feed intake would be associated with increased blood levels of insulin and glucose. The elevated blood glucose could mean a decline in peripheral glucose uptake and insulin turnover (Lima et al., 1998). The peripheral metabolites such as glucose and VFA depress feed intake via the entry into the cells and not only staying in the blood (Forbes, 1995). Hence, factors reducing metabolite uptake and use by the peripheral tissues can consequently attenuate the metabolite-driven satiety. Accordingly, the presumed higher melatonin levels around feed delivery at 2100 h might have contributed to the reduced peripheral uptake of metabolites in favor of milk secretion. In so doing, the higher melatonin levels at night than during the day might have weakened the feed-driven satiety in the 2100 h-fed cows within 3 h after feeding, when compared with the 0900 h-fed cows. The above speculation would bear the possibility of a night-time glucose intolerance in lactating cows, as postulated by Furedi et al. (2006a). Milk energy output was greater in 2100 h-fed cows than in 0900 h-fed cows. This would indicate that shortly post-feeding, when feed intake and hepatic metabolite output were the greatest, milk nutrients precursors (such as lactate, glucose, and BHBA) were used by the mammary gland. The favored mammary nutrient use over non-mammary peripheral use might also indirectly suggest the existence of a night-time glucose intolerance. The concurrence of feed delivery and melatonin secretion might have contributed to the increased feed intake and blood insulin shortly post-feeding in 2100 h-fed cows than in 0900 h-fed cows.

Although blood glucagon was not measured, the higher post-feeding insulin could weaken glucagon's action, thereby reducing gluconeogenesis (Brockman et al., 1984). Reduced gluconeogenesis may reduce plasma glucose, as observed at 2 h post-feeding in 2100 h-fed cows compared to 0900 h-fed cows (Chapters 4, 6). Intravenous glucagon has been shown to depress feed intake (Deetz et al., 1981). The higher post-feeding insulin in 2100 h-fed cows than in 0900 h-fed cows (Furedi et al., 2006a) might have then reduced the glucagon-driven satiety in 2100 h-fed cows, thereby contributing to the higher feed intake of 2100 h-fed cows within 3 h of feed delivery.

The lights-off time can also be considered as a potential regulator of feeding behavior and nutrient metabolism (Sehgal, 2004). The 2100 h-fed cows were delivered the TMR at 2100 h or only 1:45 h before lights-off. Feed delivery shortly before the lights-off time may have hastened eating activity under light in anticipation of the lights-off time (Feddes et al., 1989). In addition, light can also influence animal metabolism (Piccione and Giovanni, 2002). I would thus infer that the sudden lights-off only shortly after feed delivery in 2100 h-fed cows may have acted as an inducer, thus strengthening their appetite, when compared with the 0900 h-fed cows. The combination of the two light-dependent stimuli (before and after lights-off) may have consequently played a role in greater TMR intake within 3 h of feeding in 2100 h-fed cows.

In the second study, the stimulated feed intake shortly post-feeding was associated with greater DMI in primiparous cows but not in multiparous cows (Chapter 5). Hence, parity seems to mediate the effects of TF on DMI if not on feed intake within 3-h of feed delivery. Primiparous cows face two major nutrient demands which are milk secretion and on-going body growth. Multiparous cows, however, do not require as much nutrients

for growth as do primiparous cows. Due to greater nutrient demand, primiparous cows are therefore expected to exhibit a more pronounced response to the factors that can stimulate feed intake. As a result, more nutrients will be available for milk secretion as well as tissue growth. The fact that 2100 h feed delivery did not increase DMI of primiparous cows in the first project could be attributed to higher diet fermentability (Allen, 2000). This suggests that metabolic constraints on DMI might be stronger than can be attenuated by the growth-driven nutrient demand in primiparous cows.

2) Total tract nutrient digestibility and diurnal patterns in rumen fermentation. Cellulose is the most abundant organic matter in the nature. Cellulose is also the major component of the NDF in plants. Diets fed to non-grazing lactating cows contain on average about 30-50% NDF (Van Soest, 1994). Therefore, a key strategy to improve cow productivity is to improve fiber utilization. Around 80-90% of fiber digestion occurs in the rumen, making this natural fermentor an obvious site for increasing fiber digestion. Any attempt to improve the ruminal fiber digestion would provide stabilized environment under which dietary N and starch can be utilized more efficiently. This is because the dietary degradable cell wall fiber is highly integrated with the fairly resistant phenolic compounds such as lignin and tannins. As a result, the cow needs to ruminate and re-chew the fiber mat (NDF) to reduce its particle size and release the within-cell soluble compounds. The reduced fiber particle size will additionally facilitate microbial adhesion needed for the effective fiber degradation in the rumen. The stimulated chewing would stimulate salivation, which would in turn increase rumen buffering capacity. Consequently, the likelihood of excessive VFA and lactate buildup, and thereby the risk

of SARA, will not increase despite the increased extent of rumen fermentation shortly post-feeding. Accordingly, delivering TMR at 2100 h instead of at 0900 h increased rumen VFA concentrations at 5 and 6-h post-feeding but did not reduce rumen pH to below the range detrimental to the rumen microbes (e.g., <5.6 for 3 h; Gozho et al., 2006). Increased rumen production of VFA at 5 and 6 h post-feeding in 2100 h-fed cows was associated with enhanced total tract DM, N, NDF, and ADF digestibility. Reduced fecal excretion of N and fiber was accompanied by a reduction in urinary N excretion, when cows were fed at 2100 h instead of at 0900 h. Decreased urinary N excretion in favor of body deposition in 2100 h-fed cows might be explained by the higher post-feeding blood insulin. Insulin does reduce gluconeogenesis from AA (Danfær et al., 1995), and thereby, can reduce ammonia production and hepatic urea formation. Due to reduced urinary and fecal N excretions, N balance was improved by feed delivery at 2100 h. Therefore, I infer that 2100 h instead of 0900 h feed delivery increased the extent of fermentation and metabolites release across the rumen into the portal blood, and in so doing, into the liver shortly post-feeding. The total tract digestibility was measured in the second but not in the first study. The post-feeding increases in rumen fermentation and blood metabolites along with the increased milk production were consistent between the two projects. This would suggest that 2100 h instead of 0900 h feed delivery must have enhanced total tract nutrient digestibility in the first study as well.

The present thesis demonstrated that in once-daily fed lactating cows, the post-feeding patterns in rumen VFA, ammonia, and pH can be altered by TF. Feed delivery at 2100 h instead of at 0900 h led to higher peaks in rumen ammonia and VFA and lower nadirs in rumen pH (Chapters 4, 6). The ratio of acetate to propionate remained higher for

most of the day in 2100 h-fed cows compared with 0900 h-fed cows (Chapter 6). Such different patterns in rumen fermentation between 2100 h-fed and 0900 h-fed cows were associated with, and in my opinion caused by, the greater feed intake within 3 h of feeding and larger rumen volume in 2100 h-fed cows (Chapters 3, 4, 5, 6).

It is assumed that more frequent feed delivery can reduce diurnal variation in rumen pH and stabilize microbial metabolism by reducing the risk of SARA (Nocek, 1992; Robinson, 1988; Robinson and Sniffen, 1985). However, before the more frequent feeding can be beneficial, the rumen fermentation must be compromised. Feeding lactating cows once-daily at 2100 h instead of 0900 h increased VFA and acetate concentrations at 5 and 6 h post-feeding without any excessive fall in rumen pH (Chapters 4, 6). Hence, less frequent feed delivery must not essentially be considered undesirable unless an in-depth knowledge of rumen biochemistry and feedstuff evaluation suggests so.

In the first study, rumen fluid was sampled only at 4 and 16 h post-feeding. Even with infrequent sampling, the more extensive rumen fermentation shortly post-feeding in 2100 h-fed than in 0900 h-fed cows was evidenced by the significant interaction of TF and rumen sampling time (Chapter 3). Rumen VFAs at 4 h post-feeing were higher in 2100 h-fed cows than in 0900 h-fed cows. There is a risk of saliva contamination and thus overestimation of true rumen pH when rumen fluid is sampled using the stomach tube (Duffield et al., 2004). Also, sampling of rumen fluid only twice a day does not allow studying of 24-h patterns in rumen fermentation. Continuous monitoring of rumen pH, VFA, and ammonia in the second study using cannulated cows established that the 2100 h instead of 0900 h feed delivery increased rumen fermentation without increasing

the risk of SARA (Chapter 5). The greater NDF and ADF digestibility and higher milk fat in 2100 h-fed than in 0900 h-fed cows in the second study (Chapter 5) demonstrated that rumen fermentation was not negatively affected in 2100 h-fed cows. Only with such a healthy rumen environment can fiber digestibility and milk fat be enhanced (Chapters 3, 5).

3) Peripheral blood metabolites and intermediate nutrient metabolism. An important finding was that post-feeding response in blood glucose was mediated mainly by time of feeding (TF). Cows fed at 2100 h showed a sharp post-feeding drop in blood glucose, whereas 0900 h-fed cows did not (Furedi et al., 2006a). This finding was substantiated in a subsequent study. The post-feeding decline in blood glucose was not a new observation (Oba and Allen, 2003; Blum et al., 1985, 2000) but the verity that the 2100 h and not the 0900 h feed delivery led to such a dramatic decline in blood glucose was novel. The decline in post-feeding blood glucose of 2100 h-fed cows was followed by an immediate dramatic rise (Furedi et al., 2006a). The decline in blood glucose had started even before feed delivery and was exacerbated upon feed delivery. The pre-feeding fall in blood glucose has similarly been shown in beef cows (Coggins and Field, 1976). The lower blood glucose at 2 h post-feeding in 2100 h-fed cows than in 0900 h-fed cows agrees with the higher blood insulin at 2 h post-feeding in 2100 h-fed cows (Furedi et al., 2006a). This is because insulin is an anti-gluconeogenic factor (Brockman, 1978). Hence, so long as blood insulin is high, the glucagon-induced gluconeogenesis may, at least partly, be suppressed. Oba and Allen (2003) speculated that the progressive decline in blood glucose until 4 h post-feeding is due to the use of glucose-6-phosphate in insulin-driven

glycogenesis. This speculation can be illuminated from two perspectives. From a general biochemical viewpoint, that assumption holds true because insulin can downregulate glucose-6-phosphatase, one of the four key enzymes regulating the hepatic gluconegenesis (Mayes, 2000). From a ruminant evolutionary standpoint, however, the speculation of Oba and Allen (2003) may need furtherance. Ruminants can maintain a much lower blood glucose than can non-ruminants such as non-diabetic humans (50-80 vs. 90-110 mg/dl). Generally, only 5-25% of the starch consumed can make it to the duodenum and thus little glucose is absorbed across the brush border in ruminants (Sutton, 1971; Huntington, 2006). In corn rich diet, the amount of escaped starch may be higher. As a result, little glucose directly enters the liver in ruminants shortly post-feeding (Huntington and Reynolds, 1986). The rumen propionate is the key precursor of the hepatic glucose synthesis in fed ruminants (Danfær et al., 1995). Such an evolutionary dependence on rumen propionate has probably resulted in a less responsive hepatic glucose metabolism to insulin in ruminants compared to in non-ruminants (Brockman, 1978). Additionally, unlike insulin, glucagon acts mainly on the liver and to a lower extent on peripheral tissues (Brockman, 1978). Evidence exists that insulin inhibits gluconeogenesis from AA, lactate, and glycerol but not from propionate (Brockman, 1985, 1990). Gluconeogenesis from propionate relies mainly on propionate availability and not insulin, which increases shortly post-feeding (Danfær et al., 1995). The small effect of insulin on hepatic metabolism shortly post-feeding will enable the cow to meet nutrient demands entailed by the mammary gland. As such, at times of high propionate availability (e.g., early post-feeding), insulin would not reduce hepatic glucose output because the mammary gland demands much glucose. Therefore, I would choose not to

not attribute the post-feeding decline in blood glucose of 2100 h-fed cows only to insulin, as blood glucagon, growth hormone, and corticosteroids were unknown. Unlike the periphery for insulin, the liver is the main site of action for glucagon (Brockman, 1976). Hence, since both insulin and glucagon can rise after feeding (Bassett, 1972), I deduce that insulin competes with glucagon and other hormones (e.g., growth horomone and corticosteroids) in determining the post-feeding response in blood glucose to feed intake. In addition, the pre-feeding nutritional status of the cow is influential in post-feeding insulin and glucose responses. For instance, Faverdin (1986b) found that intrajugular insulin at feed delivery decreased feed intake within 30 min of feeding in cows which had not been fed for the last 11 h, compared to no decrease in cows that were fed 4 h before the insulin treatment. This would suggest that insulin sensitivity increases as the absence of fresh feed is prolonged. Fasting or prolonged lack of access to fresh feed has caused a rapid rise in blood insulin upon feed delivery (Blum et al., 1988).

The higher blood insulin at 2 h post-feeding in 2100 h-fed cows, nonetheless, would not explain the progressive decline in blood glucose starting before feed delivery (Chapters 4, 6). The higher post-feeding rise in blood insulin could be in part due to rapid intake (Bassett, 1974b). Rapid feed intake is preceded by sensory stimuli such as sight and smell of fresh feed (Berthoud, 1984; Faverdin, 1986a). These stimuli contribute significantly to the surge in blood insulin immediately post-feeding. This process is called "cephalic phase insulin release", which has been well-demonstrated in ruminants (Faverdin, 1986a; Herath et al., 1999; Vasilatos and Wangness, 1980). The vagal branches of the abomasal, pyloric, and duodenal nerves mediate the insulin release during feed presentation (Herath et al., 1999). Therefore, insulin release does not essentially

require feed digestion and nutrient absorption to occur. This would imply that anticipating feed presentation by the cow may induce insulin response before feed delivery. If so, the pre feeding decrement in blood glucose may be explained. This may indicate that 2100 h-fed cows did anticipate feed presentation and lights-off time.

I must, however, point out that the interpretation of treatment effects solely based on peripheral levels of pancreatic hormones is open to scrutiny. The pancreatic hormones such as insulin and glucagons are initially secreted into the portal blood and are exposed to variable hepatic manipulation (Harmon, 1992). Hence, hormone levels in the peripheral blood may not fully characterize their concrete response to feeding strategies in the pancreas. Moreover, rapid eating *per se* can stimulate the secretion of gut hormones such as cholecystokinine and secretin, thereby eliciting an insulin response (Bassett, 1974; Trenkle, 1971). The increased blood insulin in 2100 h-fed cows (Furedi et al., 2006a) was hence in accord with the increased feed intake within 3 h after feeding.

The elevated hepatic glucose output represents the increased delivery of gluconeogenic substances such as propionate and lactate into the portal circulation. The increased propionate and lactate supply corresponds to the peak in rumen VFA production occurring between 2 and 8 h post-feeding (Stone, 2004). Thus, the marked rise in the post-feeding fluxes of glucogenic metabolites may justify the dramatic peak in blood glucose after 2 h post-feeding in 2100 h-fed cows. The absence of a post-feeding peak in blood glucose of 0900 h-fed cows suggests that the post-feeding rumen supplies of propionate and lactate were not as pronounced in the 0900 h-fed cows as were they in the 2100 h-fed cows. When feed is no longer fresh (overnight in 0900 h-fed cows and during the day in the 2100 h-fed cows), blood glucose increases (Plaizier et al., 2005;

Blum et al., 1988). The increased blood glucose at 10-14 h after feeding may partly be mediated via rumen propionate from the ongoing rumen VFA supply and later on by peripheral AA and lactate.

Regulation of glucose metabolism possesses a circadian rhythm in humans (la Fleur et al., 1999). During a constant intravenous glucose infusion for 30 h, a nocturnal rise in blood glucose occurred, peaking around mid-sleep. Thus, time of day is determining in how and to what extent glucose is metabolized by the periphery (Van Cauter et al., 1989; Van Cauter, 1990). This suggests that glucose tolerance in humans depends on both time of day regardless of sleep, and sleep regardless of time of day (Van Cauter et al., 1991). Thus, both the circadian clock (the hypothalamic suprachiasmatic nucleus; la Fleur et al., 1999) and light exposure (Challet et al., 2004) are responsible for regulation of the human blood glucose. The fact that blood glucose exhibits diurnal patterns regardless of feeding regimen (ad libitum feeding, scheduled frequent feeding, or fasting) (Van Cauter et al., 1989) indicates the endogenous nature of blood glucose regulation. The suprachaismatic nucleus contributes to blood glucose regulation independent of postprandial responses in feed intake (la Fleur, 2003). In the current studies (Furedi et al., 2006a, 2007), blood glucose exhibited significant pre and post-prandial responses to feed delivery at 2100 h but not at 0900 h. However, it was unknown if and to what extent such pre- and post-feeding responses were mediated by time of day regardless of TF. This is why the current thesis does not rule out the existence of endogenous rhythms in blood glucose of lactating cows.

The results indicate that pre- and post-feeding responses in blood glucose depend on when the fresh feed was delivered to the cows. In the first study, blood glucose did not

show any pre- or post-feeding responses to TF in the 0900 h-fed cows. This could mean that time of day had little impact of prandial changes in blood glucose of 0900 h-fed cows. However, in the 2100 h-fed cows, blood glucose progressively decreased from several hours before feeding until 2 h post-feeding when it was at a minimum. Afterward, it went up sharply to reach the baseline within 4 h post-feeding (Furedi et al., 2006a). Blood glucose in 2100 h-fed cows in the second study exhibited similar pre- and post-feeding responses. Based on the greater feed intake of 2100 h-fed cows than of 0900 h-fed cows shortly post-feeding, I suggest that pre- and postprandial patterns in feed intake were interrelated with the corresponding patterns in blood glucose. This suggestion gains support from the fact that the post-feeding insulin response was more pronounced in 2100 h-fed cows than in 0900 h-fed cows (Furedi et al., 2006a). The reliance of blood insulin responses on feeding has been shown in both ruminants (Lefcourt et al., 1999) and non-ruminants (la Fleur et al., 1999).

Feed delivery at 2100 h instead of at 0900 h across the two diets increased blood lactate by >1.5 times within 4 h of feed delivery (Chapter 4). In the first study, the 2100 h-fed cows had higher rumen VFA at 4 h post-feeding than did 0900 h-fed cows. Such a higher post-feeding rise in blood L-lactate of 2100 h-fed cows was verified in the second study (Chapter 6). Thus, the 2100 h- instead of 0900 h-feed delivery increased post-feeding provision of lactate, an energy source to the peripheral tissues (Giesecke and Stangassinger, 1980). The increased blood lactate could originate from the metabolism of propionate and glucose by the rumen as well as rapid microbial degradation of starch (Giesecke and Stangassinger, 1980). The greater feed intake or increased substrate

availability was thus expected to enhance the splanchnic lactate flux. The result would be a drastic rise in blood lactate shortly post-feeding, as was observed in 2100 h-fed cows.

Although both 0900 h-fed and 2100 h-fed cows received the TMR only once daily, the post-feeding rise in blood BHBA was more dramatic in 2100 h-fed cows (Chapter 4). The higher peripheral BHBA at 2-4 h post-feeding, when the splanchnic BHBA flux is maximal, would mean a greater energy availability to mammary and non-mammary tissues. In fed ruminants undergoing no major tissue mobilization, BHBA is mainly a product of butyrate modification across the gut epithelium. The metabolism of butyrate provides energy to sustain the maintenance of visceral tissues, while serving the periphery with BHBA (Reynolds, 2002). Up to 90% of rumen butyrate can undergo either full oxidation or conversion to ketones mainly BHBA (Reynolds, 2002). The augmented post-feeding rumen fermentation in 2100 h-fed cows should have stimulated n-butyrate modification by the rumen wall, thus increasing BHBA production at 2 and 4 post-feeding (Chapter 4). β-hydroxybutyrate contributes to about 10% of milk fat synthesis (Palmquist et al., 1969). It is also an energy source for peripheral tissues (Annison and Bryden, 1999b).

In the first study, the post-feeding rise in blood urea was comparable between 0900 h- and 2100 h-fed cows (Chapter 3). In the second study (Chapter 4), however, the post-feeding rise in blood urea occurred in the 0900 h-fed cows but not in the 2100 h-fed cows. In the 2100 h-fed cows, blood urea levels were not changed until 4 h post-feeding when it started to decline, reaching a nadir at 10 h post-feeding and returning to the feeding baseline at 12 h post-feeding (Chapter 4). This finding suggested that feeding may not necessarily induce a surge in blood urea. In ruminants, substantive N exchanges

occur amongst splanchnic tissues before the end-products of N metabolism appear as urea in the peripheral blood. Even after appearance in the peripheral blood, urea provided about 50% of N needed to sustain the gut maintenance (Huntington, 1989). The increased extent of the rumen fermentation is expected to increase N and energy demands by both rumen microbes and epithelia. Based on this reasoning, the gut in 2100 h-fed cows may have thus recycled back more of the hepatic urea output than the gut in 0900 h-fed cows. I, therefore, strongly believe that the interpretation of treatment differences or similarities in N metabolism would not be valid if made solely based on blood urea levels using spot blood samples.

The gut peptides such as cholecystockinin, glucagon-like-peptide-1, and somatostatin can depress feed intake (Silver and Morley, 1991; Spenser, 1986). The effect of the gut peptides on satiety or hunger seems to be mediated not solely by digestive processes but also by the central nervous system. Therefore, discussing the findings of the current thesis relative to gut peptides could not go beyond conjecture.

4) Milk production. It is known that milk secretion depends largely on mammary blood flow and substrate availability (Brockman, 2005). Feed delivery at 2100 h instead of at 0900 h increased post-feeding blood levels of BHBA and lactate drastically. Such a post-feeding rise likely occurred for acetate and propionate, as well (see Sutton et al., 1988 and Taweel et al., 2004). Over a 24-h period, blood lactate was higher in 2100 h-fed than in 0900 h-fed cows (Chapter 4). Also, blood insulin at 2 h post-feeding was higher in the 2100 h-fed cows than in the 0900 h-fed cows in both experiments (Furedi et al., 2006a, 2007), strengthening the above likelihood. In ruminants, VFA, and in particular

propionate and acetate, stimulate the post-feeding response in blood insulin (McAtee and Trenkle, 1971; Trenkle, 1970). Insulin stimulates nutrient storage and oxidation in peripheral tissues (Brockman, 1978). Insulin, however, does not have a major effect on the mammary uptake of milk precursors (Mcguire et al., 1995; Brockman and Laarveld, 1986; Laarveld et al., 1981, 1985). Hence, the marked post-feeding rises in blood VFA, BHBA, and lactate in the 2100 h-fed cows compared to the 0900 h-fed cows were expected to increase the mammary nutrient flux and promote metabolite deposition in non-mammary peripheral tissues. As such, the 2100 h- instead of 0900 h-feed delivery augmented milk fat yield and enhanced N retention (Chapters 3 and 5).

Feed delivery at 2100 h instead of at 0900 h did not change total proportion of either of short, medium, or long chain fatty acids in milk (Chapter 5). The proportion of C18:0 was higher in 2100 h-fed cows than in 0900 h-fed cows. The low rumen pH (5.5 vs. 6.5) has recently been shown (AbuGhazaleh et al., 2005) to increase the complete conversion of linoleic acid (C18:2) to stearic acid (C18:0). Despite comparable average daily rumen pH, the rumen pH at 5-6 h post-feeding was lower in 2100 h-fed cows than in 0900 h-fed cows. Thus, the lower post-feeding rumen pH might have favored the rumen production of C18:0, thereby contributing to its increased proportion in milk fat of 2100 h-fed cows.

The enhancement in total tract digestibility was about 2.4% for DM and N and 4.9% for NDF. Thus, dietary fiber digestion benefited the most from the 2100 h- instead of 0900 h-feeding, when compared to other nutrients. This emphasized the significance of increasing fiber digestibility in enhancing cow productivity and reducing nutrient excretion. Evidence exists that feed delivery at 2100 h instead of at 0900 h improves

growth in beef cattle (Kennedy et al., 2004; Small et al., 2004). In lactating cows, offering a protein supplement (about 15% of DMI) at 0030 h instead of at 0830 h increased rumen VFA and improved milk fat yield (Robinson et al., 1997). Nonetheless, there is no study to show the importance of the effect of the time of TMR delivery in once-daily fed lactating cows. As a result of feed delivery at 2100 h instead of at 0900 h, the post-feeding rumen acetate and total VFA as well as blood BHBA and lactate were increased (Chapters 4 and 6). Acetate and BHBA are the major contributors to *de novo* milk fat synthesis in the mammary gland. Therefore, it was consistent that 2100 h instead of 0900 h-feeding improved milk fat yield.

Feeding at 2100 h instead of 0900 h increased feed intake within 3 h of feeding. The amount consumed within 3 h of feed delivery was on average about 55% of the TMR offered. Based on the post-feeding rises in rumen VFA and blood metabolites, the impacts of feed delivery at 2100 h on rumen fermentation, total tract nutrient digestion, blood parameters, and milk secretion were all mediated most likely by the altered diurnal patterns in feed intake. In prmiparous cows, feeding at 2100 h increased digestible DMI by 1.67 kg which equals to 1.67 × 1.6 Mcal NE_L or 2.67 Mcal NE_L. Based on actual milk yield and milk percents of fat and protein (NRC, 2001), primiparous cows fed at 2100 h secreted 20.53 Mcal NE_L and primiparous cows fed at 0900 h secreted 18.94 Mcal NE_L as milk. Thus, feeding at 2100 h instead of 0900 h increased dietary NE_L availability by 2.67 Mcal/d while only 1.59 (20.53-18.94) Mcal was secreted in milk. This suggests that 1.08 Mcal NE_L was not used for milk production and was spent for internal energy expenditure by both sppanchnic and non-splanchnic peripheral tissues of primiparous cows. In multiparous cows, feeding at 2100 h increased digestible DMI by 0.54 kg or

0.86 Mcal NE_L (equal to 1.2 kg of 4% fat-crrected milk) while it reduced NEL secretion as milk by 0.25 Mcal/d. This suggests that feeding at 2100 h, although increased energy availability, did not partition it towards the mammary gland in multiparous cows. These findings indicate that parity affects the fate of the increased nutrients availability by evening feeding. Also, time of feeding is inferred to be a strategy to manipulate non-mammary nutrient partitioning.

CHAPTER 8

FINAL CONCLUSIONS

1) Feed delivery at 2100 h instead of at 0900 in tie-stall-housed lactating cows under no heat stress increased feed intake within 3 h after feeding by about 2.4 kg of dry matter. The increased feed intake shortly post-feeding was associated with comparable DMI in one study, but in another study led to greater DMI in primiparous and not in multiparous cows.

2) Feeding at 2100 h increased rumen VFA concentrations at 4-6 h post-feeding and rumen ammonia at 2 h post-feeding, compared to feeding at 0900 h.

3) The urinary excretion of purine derivatives (allantoin and uric acid) were not significantly affected by feeding time. This suggested that microbial protein synthesis did not significantly differ between the 2100 h-fed cows and the 0900 h-fed cows.

4) The peripheral blood BHBA and L-lactate at 2 and 4 h post-feeding were higher in cows fed at 2100 h than in cows fed at 0900 h. In the first but not in the second experiment, blood L-lactate across sampling times was higher in cows fed at 2100 h, as well. Blood BHBA across sampling times did not differ between treatments. Blood urea rose at 2 h post-feeding in both 2100 h-fed and 0900 h-fed cows in the first study. In the

second study, the post-feeding rise in blood urea occurred in the 0900 h-fed cows but not in the 2100 h-fed cows.

5) Feeding at 2100 h instead of 0900 h intensified rumen fermentation within 4-6 h post-feeding, increased rumen volume, but did not change rumen fluid outflow rate and the rumen fluid and solids retention times. Dry matter intake and rumen solids outflow rate was higher in 2100 h-fed than in 0900 h-fed primiparous cows. Feeding at 2100 h improved DM, N, NDF, and ADF total tract digestibility.

6) Feeding at 2100 h did not affect total proportions of short, medium, and long chain fatty acids in milk. This suggested that daily yield of the fatty acids with implications for human health (such as monounsaturated fatty acids and *cis-9, trans-11*CLA) was enhanced by changing feeding time, as milk fat yield was improved by feeding at 2100 h.

7) Because of increased total tract nutrient digestibility and peripheral availability of milk precursors (BHBA, L-lactate, and probably acetate), milk fat and energy outputs were enhanced by feeding at 2100 h. In addition, fecal and urinary N losses were reduced in favor of body N retention by feeding at 2100 h.

8) Feeding at 2100 h instead of at 0900 h altered diurnal patterns of feed intake. In so doing, feeding at 2100 h increased peripheral avaiablity of milk precursors including rumen VFA and blood metabolites. The altered post-feeding patterns in rumen and blood metabolites included a marked increase in rumen concentrations of VFA at 4-6 h post-

feeding and of blood metabolites at 2-4 h post-feeding. As a result of the improved nutrient digestibility and peripheral nutrient availability, milk fat yield was increased by feeding at 2100 h. In addition, N retention was enhanced. The less N excreted via feces and urine by feeding at 2100 instead of at 0900 h may have environmental implications.

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Appendix 1. Dietary ingredients of the high-concentrate (EXP1-HC) and low-concentrate (EXP1-LC) diets in the first experiment and the single diet in the second experiment (EXP2) (DM basis)

	Diets		
Diet ingredients	EXP1-HC	EXP1-LC	EXP2
Alfalfa silage	15.87	20.96	25.1
Corn silage	22.67	29.66	25.1
Rolled barley	26.94	20.06	21.17
Wheat shorts	7.80	5.81	6.13
Canola meal	6.12	2.78	5.14
Soybean meal	2.83	2.84	2.52
Ground wheat	2.99	2.23	2.35
Distillers grain	5.86	5.88	5.23
Corn gluten meal	1.996	1.486	1.57
Fish meal	0.81	0.856	0.74
Beet molasses	0.35	0.367	0.32
Cane molasses	0.20	0.1486	0.16
Tallow	1.99	1.49	1.57
Luprosil salt (Ca propionate)	0.099	0.074	0.078
salt	0.32	0.238	0.25
Dicalcium phosphate	0.34	0.252	0.27
Pellet binder	0.06	0.045	0.05
Dynamite	0.399	0.297	0.267
Selenium	0.012	0.009	0.009
Trace mineral supplement	0.026	0.019	0.02
Vitamins ADE	0.026	0.019	0.02
Niacin	0.034	0.037	0.032
Sodium bicarbonate	0.58	0.61	0.53
Protein pellet[1]	0.898	0.67	0.705
Calcium carbonate	0.7385	0.55	0.58

[1]Protein pellets contain 46.1% soybean meal, 2.6% wheat shorts, 40.0% canola meal, 5.0% oat hulls, 0.3% pellet binder, 1.0% cane molasses, and 5.0% corn gluten meal.