EFFECTS OF PERIPARTUM PROPYLENE GLYCOL SUPPLEMENTATION ON NITROGEN METABOLISM, BODY COMPOSITION AND GENE EXPRESSION FOR THE MAJOR PROTEOLYTIC SYSTEMS IN SKELETAL MUSCLE IN TRANSITION DAIRY COWS

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ABSTRACT

Early-lactating dairy cows mobilize body protein, primarily from skeletal muscle, to provide amino acids which are directed towards gluconeogenesis and milk protein synthesis. Propylene glycol (PG) is a precursor of ruminal propionate, and our hypothesis was that its dietary inclusion could attenuate skeletal muscle wasting by reducing amino acid-driven gluconeogenesis. The major objectives of this study were to delineate the effects of pre- and post-partum PG supplementation in transition dairy cows on whole-body nitrogen (N) balance, urinary 3-methylhistidine (3-MH) excretion, body composition, and gene expression profiles for the major protein degradation pathways in skeletal muscle. Sixteen pregnant cows (7 primiparous and 9 multiparous) were paired based on expected calving dates and then randomly assigned within each pair to either a basal diet (control) or basal diet plus 600 mL/d of PG (PG). Diets were fed twice daily for ad libitum intake, and PG was fed in equal amounts as a top dress. All measurements were conducted at 3 time intervals starting at d -14 ± 5, d 15 and d 38 relative to calving. Propylene glycol had no effect ($P > 0.05$) on whole-body N balance, urinary 3-MH excretion, and body composition. However, N balance was lower ($P < 0.001$) at d 15 and d 38, compared to d -14. Urinary excretion of 3-MH was lower at d -14 than at d 15 ($P = 0.01$) and d 38 ($P = 0.001$). Supplemental PG had no effect ($P > 0.05$) on body weight (BW), and all components of empty BW. On average, cows fed both diets mobilized 19 kg of body fat and 14 kg of body protein between d -14 and d 38. Supplemental PG had no effect on mRNA abundance in skeletal muscle for m-calpain ($P = 0.96$) and 14-kDa ubiquitin-carrier protein E2 (14-kDa E2) ($P = 0.54$); however, PG supplementation down-regulated mRNA expression for μ-calpain at d 15 ($P = 0.02$), and tended to down-regulate mRNA expression for ubiquitin at d 15 ($P =$

0.07) and proteasome 26S subunit-ATPase at d 38 (P = 0.097). Relative to calving, mRNA abundance for m-calpain (P = 0.02) and μ-calpain (P = 0.005) were higher at d 15 compared to d -14 and d 38. Messenger RNA abundance for ubiquitin (P = 0.07) and 14-kDa E2 (P = 0.005) were lower at d 38 compared to d 15. In summary, these results demonstrate that up-regulation of the Ca^{2+}-dependent and ubiquitin-mediated proteolytic pathways are the mechanisms by which skeletal muscle wasting occurs in early-lactating cows. In addition, dietary supplementation with PG may down-regulate some of these proteolytic pathways, thereby potentially attenuating undesirable skeletal muscle wasting.

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14-kDa	14-kDa ubiquitin-conjugating enzyme E2
3-MH	3-methylhistidine
ADF	Acid detergent fiber
BHBA	β-hydroxybutyrate
BW	Body weight
CP	Crude protein
DIM	Days in milk
DM	Dry matter
DMI	Dry matter intake
EBW	Empty body weight
GAPDH	Glyceraldehyde-3-phosphate
GE	Gross energy
LW	Live weight
mRNA	Messenger ribonucleic acid
N	Nitrogen
NDF	Neutral detergent fiber
NEFA	Non-esterified fatty acids
PUN	Plasma urea nitrogen
TMR	Total mixed ration
UPP	Ubiquitin proteasome pathway
USV	Urea space volume
VFA	Volatile fatty acids

1.0 GENERAL INTRODUCTION

There is constant turnover of maternal protein reserves in dairy cows as they move through their lactation cycle. This continuous turnover of protein reserves enables the cow to adapt to the nutritional challenge which occurs during the transition period (Komaragiri et al., 1997). The transition period is a period spanning 6 weeks, starting from 3 weeks pre-partum and ending 3 weeks postpartum, during which dry matter intake (DMI) cannot meet maternal nutrient requirements for maintenance and milk production (Drackley, 1999). In order to maintain milk output in early lactation, the shortfall in nutrient intake is made up by the catabolism of maternal reserves (NRC, 1989), as 41 to 90 kg of maternal fat and up to 24 kg of protein reserves are mobilized (Erdman and Andrew, 1989). The majority of protein mobilization in transition cows occurs in skeletal muscle as a result of a reduction in protein synthesis and an increase in protein degradation, which, ultimately causes net mobilization of skeletal muscle protein (Bell et al., 2000). Several methods which include nitrogen (N) balance determination (Plaizier et al., 2000a), quantification of urinary 3-methylhistidine (3-MH) excretion (Plaizier et al., 2000b) and body composition measurement (Andrew and Erdman, 1995) are used to indirectly demonstrate protein catabolism in skeletal muscle. Despite the use of these various methods to indirectly quantify skeletal protein mobilization, the exact cellular mechanisms which lead to muscle wasting in transition cows remain to be elucidated.

As a result of the overwhelming demand for glucose for milk lactose synthesis during early lactation, the mobilization of body protein enables the cow to repartition amino acids towards gluconeogenesis (Bauman and Currie, 1980). Propionate is the major precursor for gluconeogenesis (Drackley et al., 2001); however, limited feed intake during early lactation limits ruminal propionate supply to the liver, hence raising the requirement for alternative gluconeogenic precursors. Amino acids derived from skeletal muscle proteolysis are an important alternative source of glucose carbon during early lactation. Overton et al. (1998) observed an increase in alanine contribution to hepatic gluconeogenesis during the first 3 weeks post-partum and postulated that the increase in amino acid-fueled gluconeogenesis, which occurs during the transition period, is greater than at any other time during the lactation cycle. However, although mobilization of maternal amino acids and fatty acids is necessary to augment the inadequate dietary nutrient supply, excessive mobilization can lead to an increase in the incidence of metabolic disorders, poor reproductive and lactation performance, impaired animal health and welfare, and increased veterinary costs (Drackley, 1999; Varga, 2004). Therefore, it is imperative to prevent excessive mobilization of maternal protein and fat reserves. One approach that has been investigated to achieve this goal is the provision of supplemental gluconeogenic precursors, such as propylene glycol (PG). Propylene glycol is a precursor of ruminal propionate (Nielsen and Ingvartsen, 2004) and its dietary inclusion has been shown to increase plasma glucose (Miyoshi et al., 2001; Shingfield et al., 2002) and insulin (Grummer et al., 1994; Christensen et al., 1997), while decreasing plasma non-esterified fatty acid (NEFA) and β-hydroxybutyrate (β-HOB) concentrations (Grummer et al., 1994; Christensen et al., 1997). However, the effects of PG in attenuating skeletal muscle wasting by potentially reducing amino acid-

driven gluconeogenesis, through its provision of an alternate gluconeogenic precursor, in transition dairy cows is unknown. Therefore, this thesis research was conducted to investigate the impact of dietary PG supplementation on nitrogen metabolism, body composition and gene expression for the major proteolytic systems in skeletal muscle in transition dairy cows.

2.0 LITERATURE REVIEW

2.1 The transition period

Genetic selection has been the major driver of the improvement in milk yield over the years, leading to today's high-yielding dairy cow (VandeHaar and St-Pierre, 2006). The estimated 2% increase in milk yield per year, in the past 25 years, has also led to challenges in nutrient supply to match milk production in the high yielding dairy cow, especially during the transition period (Eastridge, 2006). The transition period, which starts 3 weeks pre-partum and continues until 3 weeks post-partum, is the most challenging in a dairy cow's lactation cycle (Grummer, 1995). The transition from being a dry cow to a lactating cow is usually not smooth given the acute changes which occur in the physiology, metabolism, nutrition and environment of the transition dairy cow (Goff and Horst, 1997; Drackley, 1999). Therefore, up to 75% of metabolic disorders and infectious diseases occur within a month of calving (LeBlanc et al., 2006).

Meeting the nutritional needs of the transition dairy cow has been recognized to be of paramount importance in enabling a smooth transition, as nutrition is intricately linked to the occurrence of diseases and metabolic disorders post-partum, and lactational performance (Overton and Waldon, 2004; LeBlanc et al., 2006; Goff, 2006). However, feeding the transition cow is a big challenge as nutrition is influenced by many factors

including the physiological state (Goff and Horst, 1997) and endocrine status of the cow (Grummer, 1995), which also change during the transition period.

During late pregnancy, nutrient requirements for the whole gravid uterus and mammary gland development increase. Maternal energy and protein requirements for fetal growth increase by 30 to 50% (Bell, 1995). Despite this increase in nutrient requirements as parturition approaches, there is a 30 to 35% decrease in DMI (Grummer, 1995), which can be partly attributed to changes in the endocrine status of the cow (Ingvartsen and Andersen, 2000). At parturition, there is a drastic increase in nutrient requirements for colostrum production and the synthesis of milk components during the subsequent lactation further increases the demand for nutrients (Goff and Horst, 1997). Following calving, there is an increase in DMI. However, the nutrient requirements for lactation increase at a faster rate than DMI, such that, nutrient supply only matches nutrient demand around week 5 post-partum (DeFrain et al., 2004). Therefore, in order to maintain milk output in early lactation, the shortfall in nutrient intake is made up by the catabolism of body fat and protein (NRC, 1989).

2.2 Mobilization of body fat

The mismatch between energy demand and dietary energy supply leads to a negative energy balance in high-yielding dairy cows during early lactation (Schröder and Staufenbiel, 2006). Therefore, to offset the energy deficit, and maintain lactation, the cow will catabolize a considerable amount of body fat. Estimates indicate that 41 to 90 kg of body fat (Erdman and Andrew, 1989) primarily from intramuscular, subcutaneous and internal fat (Gibb et al., 1992), are mobilized during early lactation.

Mobilization of body fat is a normal process which enables the cow to augment the energy supply as the mobilized body fat avails energy for milk production to the cow (Bauman and Currie, 1980). However, excessive mobilization of body fat elevates plasma non-esterified fatty acid (NEFA) concentration and increases its uptake by the liver, thereby leading to fatty liver syndrome and ketosis (Drackley, 1999). Over-mobilization of body fat also impairs reproductive performance (Lucy, 2001). Several management strategies arising from the extensive research on lipid mobilization, which include feeding glucogenic diets (van Knegsel et al., 2007), have been shown to limit body fat mobilization.

2.3 Mobilization of body protein

Relatively fewer studies on body protein catabolism have been conducted. Failure to consume adequate protein to meet maternal amino acid requirements for milk protein synthesis and hepatic gluconeogenesis during the first 2 weeks postpartum leads to transition cows mobilizing tissue protein (Bell et al., 2000). However, body protein mobilization is more restricted and occurs for a shorter time compared to body fat mobilization (Tamminga et al., 1997). Therefore, up to 24 kg of body protein (Erdman and Andrew, 1989) are mobilized during early lactation as tissue protein synthesis may be suppressed and the proteolytic rate increased (Bell et al., 2000). In lactating ruminants, skeletal muscle is recognized as the major site for protein mobilization (Bell et al., 2000), but few studies have investigated protein metabolism in this tissue during lactation (Baracos et al., 1991; Meijer et al., 1995; Komaragiri et al., 1997; Kokkonen et al., 2005). Skeletal muscle protein mass has been reported to fall in early-lactating dairy cows (Komaragiri et al., 1997) and goats (Baracos et al., 1991). In transition dairy cows,

the decrease in the *longissimus lumborum* muscle diameter, which starts within the last week pre-partum and continues during early lactation, indicates protein mobilization in skeletal muscle (Kokkonen et al., 2005).

2.4 Characterization of skeletal muscle wasting in transition dairy cows

There is paucity of information on skeletal protein breakdown (Taillandier et al., 2004). This is due to the complex nature of skeletal muscle breakdown which makes it difficult to characterize and, thus, there are no precise procedures to quantify muscle wasting *in vivo* (Taillandier et al., 2004). However, there is extensive use of different indirect methods to show body protein catabolism in dairy cows. These indirect methods include measuring N balance, urinary excretion of 3-MH, and changes in empty body protein content using isotope dilution techniques.

2.4.1 Nitrogen balance

Nitrogen balance, which shows the relative changes in body N status, is determined by calculating the difference between dietary N intake and N excreted in urine, feces and milk (Goode, 1981; Spanghero and Kowalski, 1997). When dietary N intake is higher than the sum of N loss in urine, feces and milk, animals will retain N (Barboza and Parker, 2006). On the contrary, dietary N intake which does not surpass N loss necessitates mobilization of body N reserves. High yielding dairy cows are invariably in negative N balance during early lactation which leads to catabolism of body protein (Maltz and Silanikove, 1996; Plaizier et al., 2000a).

Despite N balance providing a useful indication of N status in dairy cows, its determination is usually fraught with errors associated with sample collection,

preparation and analysis (see Spanghero and Kowalski, 1997). Following a meta-analysis, Spanghero and Kowalski (1997) showed the errors associated with N balance determination and pointed out some of the origins of these errors in the different studies. During sample collection, there can be volatile N loss, especially from feces. Urine N losses can also occur, although the use of strong acids can curtail these losses. Further N losses can occur during fecal drying, as some methods, including freeze drying, have been reported to lead to N losses. Dermal N losses as scarf and hair are often ignored as they are not easy to quantify, although they contribute to N output. All these losses in N which can be unaccounted for in N balance studies can result in an underestimation of N output. Analytical errors can occur when determining milk N content, as milk N has two fractions, the protein and non-protein fractions. Milk N analysis using infra red does not separate these fractions, which is of importance, as the protein fraction should be used for N balance calculations. Therefore, use of milk N values from infra red analysis, if not corrected for the non-protein fraction, can overestimate N output. All these sources of errors are partly responsible for the variation in N status and the magnitude of N balance, in the different studies in lactating dairy cows, as different sample collection and analysis conditions are used (Spanghero and Kowalski, 1997). However, despite the possible flaws associated with N balance determination, it is still an important method to indirectly show maternal protein catabolism in dairy cows, provided precautions are taken to minimize errors (Spanghero and Kowalski, 1997).

2.4.2 Urinary 3-MH excretion

Changes in urinary excretion of 3-MH can indirectly show the changes occurring in the myofibrillar protein mass in dairy cows (Harris and Milne, 1981). Actin and

myosin, which make up skeletal muscle, contain 3-MH which is a modified amino acid which does not have a specific tRNA molecule required for its addition to muscle protein during protein synthesis (Long et al., 1975). Post-translational methylation of histidine results in the formation of 3-MH. The lack of a specific tRNA molecule leads to 3-MH being quantitatively excreted in urine when skeletal muscle protein is degraded, as it can neither be re-incorporated into muscle protein during translation nor be further broken down. Therefore, the observed elevation of urinary of 3-MH excretion post-calving compared to the pre-calving period suggests that there is an increase in degradation of skeletal muscle protein during early lactation in high yielding dairy cows (Plaizier et al., 2000b; Doepel et al., 2002).

2.4.3 Changes in body composition

Mobilization of maternal body reserves including body protein during early lactation result in changes in body composition (Rastani et al., 2001). These changes in body composition which occur during the periparturient period can be determined by isotope dilution techniques (Reid et al., 1955; Agnew et al., 2005). The use of the different isotope dilution techniques to estimate body composition relies on the assumption of a high correlation between empty body water content of an animal, and the empty body fat, protein, and ash contents (Reid et al., 1955; Andrew and Erdman, 1995). Empty body water content is determined by dilution of an infused isotope in blood, before empty body fat, protein, and ash contents are estimated regressively (Andrew and Erdman, 1995). Andrew and Erdman (1995) were able to track the changes in body protein content of dairy cows during the dry period, early, and late lactation using the deuterium oxide and urea dilution techniques.

The different indirect methods to quantify body protein mobilization in periparturient cows provide an insight on the homeorhetic response which involves skeletal muscle protein (Bauman and Currie, 1980; Drackley et al, 2001). However, there is not much known in terms of the cellular and molecular homeorhetic responses which occur during the transition period (Drackley et al., 2001). The underlying cellular proteolytic mechanism(s) responsible for the increased proteolysis of myofibrillar protein in skeletal muscles of ruminants in negative energy and protein balance during the periparturient period remain(s) to be delineated, and this was a major objective of this thesis research.

2.5 Cellular protein degradation systems in skeletal muscle

The cellular protein degradation machinery in skeletal muscle consists of the ubiquitin-proteasome pathway (UPP), calcium (Ca^{2+})-dependent proteases (calpains), lysosomal proteases (cathepsins), caspaces and matrix metalloproteinases (Taillandier et al., 2004). These proteolytic systems are involved in the breakdown of myofibrillar proteins, which make up the bulk of skeletal muscle, and the sarcoplasmic and stroma proteins (Goll et al., 2007). However, the UPP and Ca^{2+}-dependent proteases are considered to be the main proteolytic systems in skeletal muscle under many catabolic states, as they are thought to work in concert to catabolize muscle proteins (Wray et al., 2002; Goll et al., 2007). Therefore, the UPP and Ca^{2+}-dependent proteases could be involved in the cellular homeorhetic response in transition dairy cows which leads to the breakdown of skeletal muscle protein to avail amino acids to support milk production (Fig 2.1).

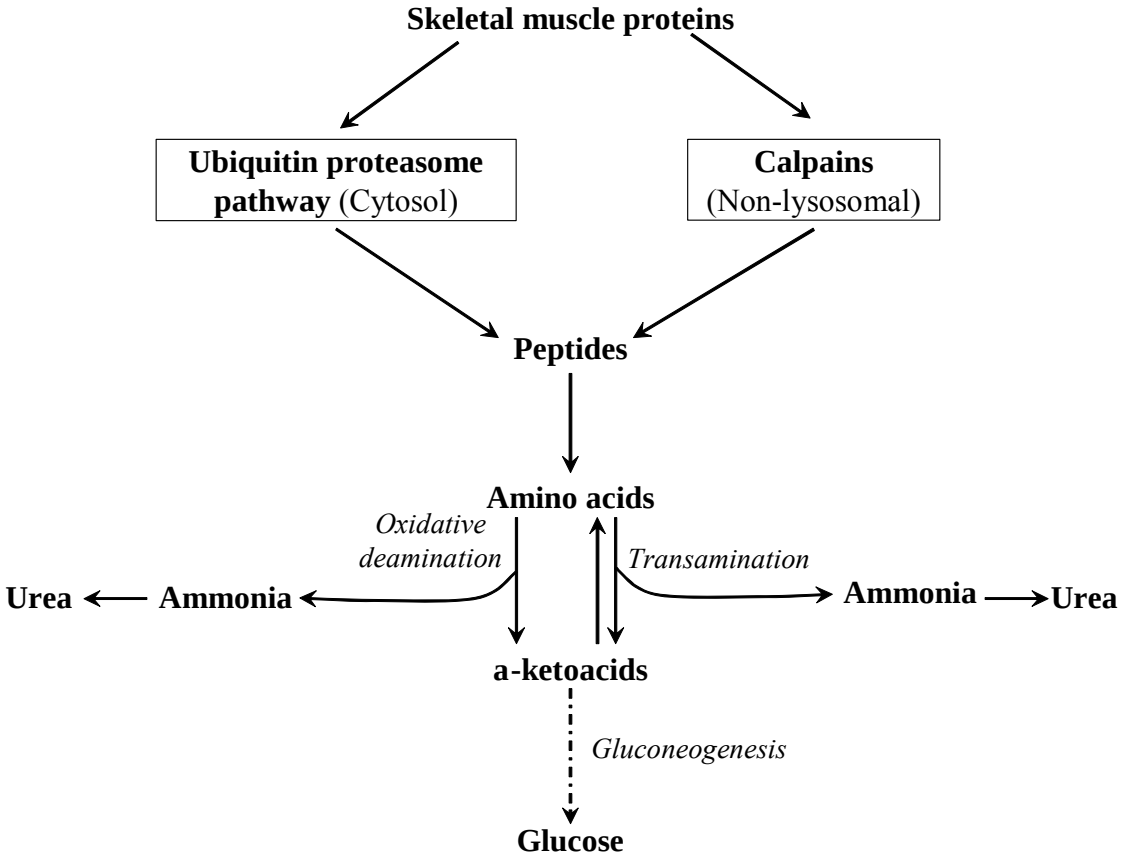


Figure 2.1. The proteolytic systems which could be responsible for skeletal muscle protein catabolism to provide amino acids for hepatic gluconeogenesis during the transition period.

2.5.1 The ubiquitin-proteasome pathway

The UPP is an ATP-dependent pathway, which is multi-step, and consists of a number of enzymes (Mitch et al, 1996; Solomon and Goldberg, 1996). The majority of cellular proteins including the skeletal muscle proteins, actin, myosin and troponin, are degraded by the UPP, which according to Goll et al. (2007) degrades over 80 to 90% of proteins in cells. Despite being the major cellular proteolytic system, the UPP cannot disassemble actomyosin and myofibrils (Solomon and Goldberg, 1996; Goll et al., 2007). Therefore, other proteolytic systems are involved in the break down of these protein complexes, which avails substrates for breakdown by the UPP (Goll et al., 2007).

Degradation of cellular proteins by the UPP occurs in two major steps which are ubiquitination of the target protein and catabolism by the 26S proteasome (Taillandier et al., 2004). Ubiquitination involves a series of steps catalyzed by 3 enzymes families. During the initial ATP-dependent step, ubiquitin is activated by the sole member of the first enzyme family, ubiquitin-activating enzyme (E1). Once activated, ubiquitin is translated to any one of a family of ubiquitin conjugating enzymes (E2), which include the 14-kDa ubiquitin-conjugating enzyme (14-kDa E2). At this stage, ubiquitin can either be transferred to the target protein by E2, thus forming a ubiquitinated target protein, or is linked to other ubiquitin molecules to form a chain of tandem-repeat units of ubiquitin molecules in a step that requires any one of a family of the third enzyme, ubiquitin-protein ligase (E3). Tagging of the target protein enables its recognition by the 26S proteasome which is a proteolytic complex composed of several components including the proteasome 26S subunit, ATPase. Degradation of the tagged protein then occurs in the 26S proteasome in a process which requires energy.

The UPP has been shown to result in the increased muscle wasting in fasted rats (Kee et al., 2003) and in dairy cows with metabolic acidosis (Mutsvangwa et al., 2004). Clowes et al. (2005) observed an increase in mRNA concentrations for 14-kDa E2 and proteasome subunit C9, which was positively correlated to the amount of body protein catabolized. However, it is not known whether the UPP is involved in the increase in muscle protein wasting in dairy cows during early lactation.

2.5.2 Calpains

Calpains are Ca^{2+}-activated proteases, which include the two isoforms, μ-calpain and m-calpain, and their inhibitor, calpastatin (Goll et al., 2003). Calpains are involved in muscle wasting as they mainly disrupt the myofibrillar protein structural integrity (Huang and Forsberg, 1998; Goll et al., 2003; Goll et al., 2007). Disruption of myofibrillar structure is achieved by degradation of titin and nebulin, which are myofibrillar proteins (Goll et al., 2003). Other proteins involved in maintaining the myofibrillar structure, which include desmin, tropomyosin and C-protein, are also degraded by calpains. Therefore, once structural stability is lost, there is release of actin and myosin molecules, polypeptides and polypeptide fragments (Goll et al., 2003). Given the inability of calpains to degrade the released actin and myosin molecules and the other polypeptides, other proteolytic systems, mainly the UPP, play a significant role in breaking down these proteins to amino acids (Solomon and Goldberg, 1996; Goll et al., 2003). Therefore, calpains are thought to set-off myofibrillar protein catabolism (Wray et al., 2002; Goll et al., 2007).

Calpains have been shown to be involved in increased muscle wasting in different catabolic states including fasted rats (Arakawa et al., 1983) and rainbow trout

(Salem et al., 2005), where the nutrient deficit increased body protein mobilization. However, the contribution of calpains to muscle wasting during the periparturient period when high-yielding dairy cows cannot meet their nutrient requirements from the dietary nutrient supply is unknown.

2.6 Detrimental effects of excessive mobilization of body protein

The dynamic nature of maternal protein reserves reflect a metabolic adaptation which enables cows to "weather the storm of nutritional challenges" which occur during the lactation cycle (Piersma and Lindström, 1997; Schwilch et al., 2002). During the transition period, the loss of body protein, mainly skeletal muscle protein, result in the channeling of mobilized body amino acids towards milk protein synthesis and hepatic gluconeogenesis, which enables milk production to continue despite the limited dietary nutrient supply (Bauman and Currie, 1980; Clowes et al., 2005).

Despite maternal body loss being an adaptive mechanism, there is a threshold beyond which it has detrimental effects (Clowes et al., 2003). Beyond this upper critical limit, the amount and rate of protein mobilization cannot provide adequate substrates to support milk synthesis and, thus, there will be a decline in milk production and milk protein content (Jones and Stahly, 1999; Clowes et al, 2003). In lactating sows, loss of over 9 to 12% of maternal protein mass led to a reduction in milk production and milk protein content (Clowes et al., 2003). In dairy cows, maternal protein loss in excess of 15% has been reported to reduce milk production and milk fat content, and maintain milk protein content below the expected levels (Botts et al., 1979).

Excessive mobilization of body protein also leads to impaired reproductive performance. Amino acids from mobilized skeletal muscle protein have to be deaminated (Butler, 1998) or transaminated (Berg et al., 2002) before their carbon skeletons enter the transcarboxylic acid cycle (TCA) where they ultimately yield energy (Figure 2.1). Deamination and transamination result in the production of ammonia (NH_3), which the body excretes as urea, as it is toxic (Butler, 1998). However, urea production is an energy dependent process. Therefore, over-mobilization of body protein to avail amino acids for hepatic gluconeogenesis during early lactation also increases the energy required to get rid of the NH_3 produced during transamination of amino acids, further exacerbating the energy deficit (Formigoni and Trevisi, 2003). Ultimately, the energy deficit will negatively affect fertility (Butler, 2005). The production of urea when skeletal muscle-derived amino acids are transaminated can lead to an increase in plasma urea concentration (Jorritsma et al., 2003). The increase in plasma urea concentration can have detrimental effects on reproductive performance, as elevation of PUN or MUN over 19 mg/dL reduces pregnancy rates in dairy cows (Butler et al., 1996). Although NH_3 that is produced is ultimately converted to urea, some of the NH_3 ends up in the uterine environment where, together with urea, it has a negative impact on reproductive performance (Butler, 2005). In lactating sows, reduction in ovarian function occurs when over 9 to 12% of maternal protein mass is catabolized (Clowes et al., 2003).

The impaired lactation and reproductive performance which occur in lactating animals is a regulatory mechanism which will avert further loss of body protein and, thus, maintain a specific minimum amount of maternal protein mass (Clowes et al., 2003). It is not known if this mechanism involves down-regulation of any proteolytic system so as to prevent excessive loss of maternal protein in transition cows. However,

there is a clear need for feeding strategies to reduce body protein loss during early lactation, especially for the high-yielding dairy cows in Canadian herds.

2.7 Feeding strategies to prevent over-mobilization of maternal body reserves

To prevent the problems associated with excessive mobilization of maternal body reserves, several feeding strategies are used on dairy farms. The decrease in DMI as parturition approaches leads to the nutrient deficit which expedites mobilization of body fat and protein. Therefore, nutritional strategies have been centered on increasing the nutrient density of periparturient cow diets to counter the detrimental effects associated with the decline in DMI, which occurs as parturition approaches (Park et al., 2002).

Catabolism of maternal body reserves has been shown to be attenuated by increasing the nutrient density of periparturient cow diets (Doepel et al., 2002; Vandehaar et al., 1999; Minor et al., 1998; Komaragiri et al., 1998; van Knegsel et al., 2007). An improvement in lipid metabolism, as reflected by lower plasma NEFA and liver triglyceride concentrations around calving, have been reported after increasing energy density (Doepel et al., 2002) or both energy and protein density (Vandehaar et al., 1999) in the pre-partum ration. Feeding a diet high in non-fiber carbohydrates during the periparturient period led to a decrease in plasma NEFA and BHBA concentrations pre- and post-partum, and a decrease in liver triglyceride and an increase in liver glycogen concentrations post-partum, possibly due to the increase in propionate production (Minor et al., 1998). Addition of lipids to pre-partum diets tended to reduce body fat mobilization (Komaragiri et al., 1998). However, increasing the energy density of transition diets by adding lipids generally does not result in beneficial effects on lipid

metabolism (see Overton and Waldron, 2004). In a recent study, van Knegsel et al. (2007) showed that glucogenic diets are more effective than lipogenic diets in reducing mobilization of body fat in transition dairy cows as they are better insulin secretagogues.

Several studies (Putman and Varga, 1998; Moorby et al., 2000; Doepel et al., 2002; Komaragiri and Erdman, 1997; Komaragiri et al., 1998) have been carried out to determine whether dietary manipulation improves N metabolism in periparturient cows. An increase in the protein density of the pre-partum diet led to an increase in protein intake and, thus, an improvement in N balance pre-partum (Putman and Varga, 1998). Moorby et al. (2000) also observed an improvement in N balance pre-partum with high protein pre-partum diets, although there were no beneficial carry-over effects during early lactation. Increasing the protein density of pre-partum diets led to a decline in urinary 3-MH excretion pre-partum, which indirectly indicated a reduction in muscle protein degradation (Doepel et al., 2002). However, the high protein density pre-partum diets did not attenuate muscle wasting post-partum as urinary 3-MH excretion was not reduced (Doepel et al., 2002). In a study by Komaragiri and Erdman (1997), mobilization of body protein was not suppressed by increasing the protein density of pre-partum diets since no changes occurred in empty body protein content post-partum when crude protein (CP) and ruminally undegradable protein (RUP) content of pre-partum diets was increased. Addition of dietary fat to pre-partum diets tended to increase mobilization of maternal protein reserves (Komaragiri et al., 1998). Therefore, based on these studies on N metabolism in periparturient cows, there has not been much success in attenuating body protein catabolism during early lactation. There is a clear need for other nutritional strategies to limit body protein mobilization in high producing dairy cows during early lactation. The ideal strategy should aim to avail energy to the cow

and, thus, reduce the magnitude of the negative energy balance during early lactation (Juchem et al., 2004).

2.8 Energy metabolism during the transition period

Ruminal and hindgut fermentation result in production of volatile fatty acids (VFA), with propionate being the major gluconeogenic precursor (Seal and Reynolds, 1993). Propionate contributes an estimated maximum of 32 to 73% to hepatic gluconeogenesis, whilst amino acids from dietary intake and skeletal protein mobilization contribute 10 to 30%. However, propionate supply during the transition period is limited (Drackley et al., 2001).

Despite the increase in glucose demand towards parturition, the starch content in dairy cow diets, and ultimately propionate supply, is reduced starting at 4 to 6 weeks before calving, which is the beginning of the far-off dry period (Dann et al., 2006). Drying-off entails a change-over from the high concentrate lactation diet to the high fiber far-off dry period diet (Goff and Horst, 1997). The change in the type of substrate or feed available for fermentation also leads to changes in the rumen microbes (Yokohama and Johnson, 1988). The lactate-producing and -utilizing bacteria, which are the most abundant during the lactation stage, are replaced by the pre-dominantly cellulose-digesting and methane-producing bacteria. The second phase of the dry period or the close-up period starts 3 weeks before calving (Dann et al., 2006). During this period, cows are gradually introduced to the lactation diet so as to allow the rumen to adapt to the high concentrate diet (Park et al., 2002). However, the close-up period coincides with the decline in DMI, which further lowers propionate supply for glucose synthesis. At calving, cows are fed an early lactation diet which is higher in concentrate

than the close-up period diet, until 21 to 28 days in milk (DIM) (Rabelo et al., 2003). The starch content of this early lactation diet is also limited compared to the diet fed at peak lactation. These changes in the diet during the close-up period and at calving result in a second transition in the rumen microbial populations, which is a reversal of what happens at drying-off (Goff and Horst, 1997). Despite the decrease in the cellulolytic bacteria and increase in the lactate-producing bacterial populations being rapid, the increase in the lactate-utilizing bacteria lags behind. The asynchronous increase in the lactate-producing and lactate-utilizing bacteria populations can result in an accumulation of lactate in the rumen during early lactation. The high fiber diet introduced when cows are dried-off also result in the loss of up to 50% of the absorptive area of the rumen when rumen papillae size and density are reduced (Dirksen et al., 1985). At parturition, introduction of the high concentrate diet leads to a concomitant increase in rumen papillae size and density. However, full rumen absorptive capacity is achieved 4 to 6 weeks post-partum (Dirksen et al., 1985). Therefore, coupled with the lag in lactate-utilizing bacteria proliferation, which leads to ruminal lactate accumulation, the limited capacity to absorb the VFAs produced during microbial fermentation can lead to rumen acidosis during the periparturient period (Goff and Horst, 1997; Nocek, 1997). The increase in intake of the high concentrate early lactation diet leads to an increase in the production of propionate in the rumen. However, the increase in production of propionate when DMI increases, possibly does not translate to an increase in the propionate supply to the liver as a result of compromised rumen absorptive capacity.

The disparity between propionate supply and demand during the periparturient period results in a glucose deficit, which Drackley et al. (2001) estimated to be up to 500 g/d during early lactation. The inadequate propionate supply leads to an increase in the

contribution of other glucogenic precursors, mainly amino acids, from dietary sources and mobilized body protein to make up for the glucose shortfall (Bell et al., 2000) as the net contribution of propionate and glucogenic amino acids to hepatic gluconeogenesis is dictated by their availability (Danfær et al., 1995). The increase in amino acid-fueled gluconeogenesis during early lactation was shown by Overton et al. (1998), who observed almost a two-fold increase in the amount of alanine partitioned towards glucose synthesis during early lactation. Therefore, part of the estimated catabolism of 1,000 g of body protein daily within the first 7 to 10 DIM (Bell et al., 2000) is driven by milk lactose synthesis. The mobilized amino acids are also used for milk protein synthesis as shown by Wilson et al. (1988) who estimated that, besides 24% of lactose in milk being derived from mobilized amino acids, 34% of casein is also synthesized from body protein during early lactation.

The deficit of diet-derived gluconeogenic precursors which occurs during the transition period results in the catabolism of body protein in transition dairy cows. Therefore, it would seem plausible that provision of a glucogenic compound (e.g. propylene glycol; PG) to transition cows could reduce the amount of amino acids required for gluconeogenesis, especially the amino acids from degradation of skeletal muscle since the ability of the cow to mobilize protein is considerably limited.

2.9 Supplementing transition cow diets with propylene glycol

Propylene glycol is a glucogenic compound which is extensively used to treat ketosis. A proportion of PG that is administered is absorbed from the rumen whilst the remainder of the PG is dehydrated in the rumen resulting in the production of propanal (Kristensen and Raun, 2007). Further metabolism of propanal which occurs in the rumen

yields propanol and propionate. Therefore, PG, propanal, propanol and propionate are the metabolites which are extracted and metabolized by the liver. Figure 2.2 illustrates the metabolism of PG in the rumen and liver of dairy cows. Metabolism of PG in the hepatocytes, which is a slow process, mainly leads to the production of L-lactate, whilst oxidation of some of the extracted propanol yields propionate. The propionate produced in the rumen and the liver, and the L-lactate from propanol oxidation, can ultimately yield energy when they enter the transcarboxylic acid (TCA) cycle (Nielsen and Ingvartsen, 2004). Several studies (Grummer et al., 1994; Miyoshi et al., 2001; Butler et al., 2006) have shown the elevation in blood glucose concentration when PG is administered, which has been mainly attributed to its glucogenic properties. Propylene glycol could possibly reduce the glucose-deficiency driven muscle wasting in transition dairy cows. Bartley and Black (1966) and Freetly and Klindt (1996) observed a reduction in hepatic gluconeogenesis following infusion of exogenous glucose intravenously in cows and ewes, respectively, which showed that the liver has the capacity to reduce oxidation of the different gluconeogenic substrates, including amino acids, in the presence of an alternative glucose source. Therefore, provision of PG, which is an alternative glucogenic source, could reduce the amount of amino acids required for gluconeogenesis, especially the amino acids that are derived from the degradation of skeletal muscle (Figure 2.2). The elevation in blood glucose concentration when PG is fed to cows result in the elevation of blood insulin concentration (Christensen et al., 1997). Insulin secretion results in a reduction in skeletal muscle protein catabolism in mature animals (Gelfand and Barrett, 1987; Rooyackers and Nair, 1997), although the exact mechanisms involved have not been fully elucidated (Fawcett et al., 2001).

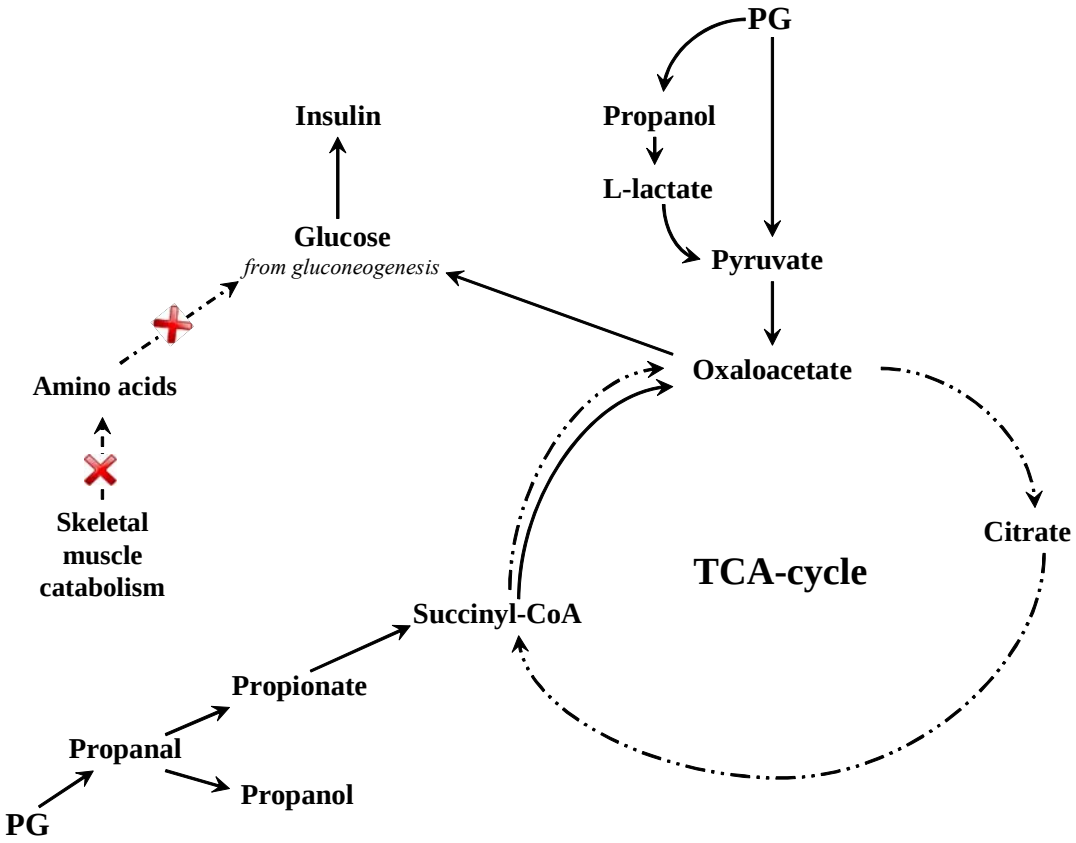


Figure 2.2. Metabolism of propylene glycol in the rumen and liver of transition dairy cows, and the possible mechanisms which could lead to propylene glycol reducing skeletal muscle protein catabolism during the transition period.

(Adapted from Nielsen and Ingvartsen, 2004; Kristensen and Raun, 2007)

However, in a study using goats, Larbaud et al (1996) concluded that the effect of insulin on muscle wasting could be at the gene transcription level after observing a reduction in the levels of mRNA encoding ubiquitin when insulin concentration in blood was elevated. Messenger RNA expression for 14-kDa E2 was down-regulated by insulin in rat skeletal muscle *in vitro* (Wing and Banville, 1994). Therefore, it is plausible that the increase in insulin concentration when PG is administered can reduce muscle wasting in transition dairy cows by down-regulating the expression of genes encoding the proteolytic enzymes.

2.10 Impact of propylene glycol on feed intake, blood metabolites, and milk production and composition

Considerable research has been conducted on feeding PG to ruminants, going as far back as the work by Johnson (1954). However, most of the work with PG has focused on determining its impact on feed intake, blood metabolites, lipid metabolism, and milk production and composition.

2.10.1 Feed intake

Supplementing PG in pre- and post-partum cow diets can affect DMI. There has been a lot of concern when feeding PG to dairy cows, especially when it is mixed into the TMR, as it is unpalatable (Johnson, 1954). The adverse effect of PG on feed intake, which could be dose dependent, has been noted when over 500 g/d of PG have been mixed into lactating cow rations (Dhiman et al., 1993; Miyoshi et al., 2001). In studies by Cozzi et al. (1996) and Christensen et al. (1997) there was no decline in DMI when less than 500 g/d of PG was mixed into the TMR. To prevent the decrease in DMI when

supplementing PG, the methods of choice is orally drenching the PG (Miyoshi et al., 2001), as drenching over 1,000 g of PG/d has been shown not to affect DMI (Studer et al., 1993).

2.10.2 Blood metabolites

Propylene glycol is a glucogenic precursor. Several studies (Miyoshi et al., 2001; Shingfield et al., 2002; Butler et al., 2006) have shown the gluconeogenic property of PG, as reflected by an elevation in blood glucose when it was fed to dairy cows. The elevation in blood glucose concentration when PG is fed to dairy cows has also been shown to lead to a concomitant increase in blood insulin concentration (Grummer et al., 1994; Christensen et al., 1997; Miyoshi et al., 2001; Butler et al., 2006). Insulin is anti-lipolytic (McCann and Hansel, 1986), and the elevation of blood insulin leads to a reduction in mobilization of body fat, as reflected by the observed reduction in blood NEFA concentration (Grummer et al., 1994; Christensen et al., 1997; Miyoshi et al., 2001; Butler et al., 2006). Propylene glycol supplementation also reduces liver triglyceride (TG) concentration during early lactation (Studer et al, 1993; Butler et al., 2006). The reduction in liver TG concentration is due to the insulin-mediated anti-lipolytic effect of PG, as there is a positive correlation which exists between blood NEFA concentration and uptake of NEFA by the liver (Bell, 1980). Dietary inclusion of PG has also been shown to reduce blood BHBA concentration (Grummer et al., 1994; Christensen et al., 1997; Butler et al., 2006). The reduction in blood NEFA concentration when PG is included in diets leads to the reduction in blood BHBA concentration, as BHBA is a product of NEFA oxidation (Vazques-Anon et al., 1994;

Busato et al., 2002). Therefore, the dietary inclusion of PG can result in beneficial effects on lipid metabolism in periparturient cows (Butler et al., 2006).

2.10.3 Milk production and composition

An extensive review of the literature on PG supplementation for dairy cows indicates that PG supplementation does not generally increase milk production or alter milk composition (Nielsen and Ingvartsen, 2004). However, in a study by Butler et al. (2006), although PG supplementation did not increase milk production, it tended to reduce milk fat. According to Nielsen and Ingvartsen (2004), the milk fat depression could have resulted from the reduction in plasma NEFA concentration, as NEFA are precursors for milk fat synthesis. Another possible explanation is the reduction in the supply of acetate for milk fat synthesis, as the dietary inclusion of PG can alter the ruminal acetate to propionate ratio (Nielsen and Ingvartsen, 2004). Butler et al. (2006) also reported an increase in milk lactose concentration, possibly linked to the improved energy balance, when PG was fed to periparturient cows, although milk lactose concentration is not easily altered by dietary manipulation (Patton et al., 2004).

To my knowledge, there is no information on the impact of PG supplementation on N balance, urinary 3-MH excretion and gene expression for the major proteolytic systems, in transition dairy cows, and it was a major objective of this thesis research to fill that information gap.

2.11 CONCLUSIONS

Given the progress in genetic selection, high-yielding dairy cows in Canadian dairy herds cannot consume adequate nutrients to match their milk production during

early lactation. Therefore, high-yielding dairy cows are in a negative glucose balance during early lactation, which partly drives mobilization of mainly skeletal muscle protein reserves, which avails amino acids for glucose production. However, the exact proteolytic systems which lead to catabolism of skeletal muscle protein, an adaptive mechanism which reduces the nutrient deficit during early lactation, are unknown. It is imperative to reduce muscle wasting in high-yielding dairy cows, as over-mobilization of maternal protein reserves has a negative impact on lactation and reproductive performance. Feeding propylene glycol to transition dairy cows has often been shown to improve their lipid metabolic status, through its elevation of blood glucose and insulin concentrations. However, the impact of propylene glycol, an alternative gluconeogenic precursor, which can reduce the glucose deficit during early lactation, on nitrogen metabolism, body composition and gene expression for protein degradation systems in skeletal muscle, is unknown.

2.12 HYPOTHESES

The hypotheses for the experiment described in this thesis were:

1) Body protein loss during early lactation in dairy cows can be ameliorated by administering PG in pre- and post-partum diets.

2) Up-regulation of genes encoding the major protein degrading systems is associated with body protein loss during early lactation in high-yielding dairy cows.

2.13 OBJECTIVES

The objectives of this study were to:

1) Determine the impact of PG supplementation during the periparturient period on body composition, whole-body N balance and urinary 3-MH excretion; and

2) Characterize the changes in gene expression profiles for the major protein degradation systems in skeletal muscle during early lactation, and the impact of PG supplementation on these changes in high-yielding dairy cows.

3.0 EFFECTS OF PERIPARTUM PROPYLENE GLYCOL SUPPLEMENTATION ON NITROGEN METABOLISM, BODY COMPOSITION AND GENE EXPRESSION FOR THE MAJOR PROTEOLYTIC SYSTEMS IN SKELETAL MUSCLE IN DAIRY COWS.

3.1 INTRODUCTION

Typically, voluntary feed intake in dairy cows declines by as much as 30 to 40% around calving compared with intake during the early dry period (Grummer, 1993). At the onset of lactation, the dairy cow mobilizes body adipose and protein reserves in order to support the energy requirements for high milk production in early lactation. Although body fat depots are recognized as the main source of energy reserves, the catabolism of both body fat and protein contribute to nutrient requirements in early lactation (NRC, 2001). During this period, body fat mobilization ranges from 41 to 90 kg (Erdman and Andrew, 1989) and protein mobilization ranges from 21 to 24 kg (Komaragiri et al., 1997). Therefore, in addition to being in a negative energy balance dairy cows also experience a negative nitrogen (N) balance in early lactation (Plaizier et al. 2000a).

Protein mobilization is driven by the overwhelming demand for glucose for milk lactose and protein syntheses during early lactation, and results in the repartitioning of amino acids towards gluconeogenesis by the cow (Bauman and Currie, 1980). Low feed

intake in early lactation, relative to the animal's energy demands, limits the contribution from ruminal propionate to the liver's gluconeogenic precursor pool. Given that propionate is the major precursor for gluconeogenesis, it is possible that its limited supply to the liver increases the requirement for amino acids derived from skeletal muscle proteolysis to become an alternative source of glucose carbon. Although skeletal muscle is the major site for protein mobilization, only a few studies have investigated protein metabolism in this tissue during lactation (Meijer et al., 1995; Komaragiri et al., 1997). Skeletal muscle protein mass has been shown to decrease in early-lactating dairy cows (Komaragiri et al., 1997); however, to our knowledge, the major protein degradation pathways that are responsible for skeletal muscle proteolysis in transition dairy cows have not been characterized.

Although the mobilization of protein reserves is necessary to augment the inadequate dietary supply of energy, excessive mobilization can lead to increased incidence of metabolic disorders, and poor reproductive and lactational performance (Drackley, 1999). One possible approach to limit body protein catabolism could be to provide supplemental gluconeogenic precursors such as propylene glycol (PG) that are rapidly absorbed from the rumen for gluconeogenesis in the liver. Propylene glycol is a precursor of ruminal propionate (Nielsen and Ingvartsen, 2004). Dietary inclusion of PG has been shown to increase plasma glucose (Miyoshi et al., 2001), and to decrease plasma non-esterified fatty acid (NEFA) and β-hydroxybutyrate (BHBA) concentrations (Grummer et al., 1994). However, the effect of PG in attenuating skeletal muscle wasting by reducing amino acid-driven gluconeogenesis in transition dairy cows is unknown. Therefore, we hypothesized that skeletal muscle proteolysis during early lactation in dairy cows can be attenuated by administering PG in pre-partum and post-

partum diets. The study was designed to determine the impact of PG supplementation during the periparturient period on whole-body N balance, urinary 3-MH excretion and body composition. A second objective was to determine the changes in gene expression profiles for the major protein degradation pathways in skeletal muscle during early lactation and the impact of PG supplementation on these changes.

3.2 MATERIALS AND METHODS

3.2.1 Experimental Design and Animals

Sixteen pregnant cows (7 primiparous and 9 multiparous) at the Greenbrae Dairy Research Facility (University of Saskatchewan) were used in a randomized complete block design. The experiment was carried out from May to December 2006. Two treatment diets were fed to individual cows in tie-stalls from d -7 ± 5 to d 45 relative to calving. Sample collection for determination of body composition, nitrogen (N) balance, urinary 3-MH excretion, and gene expression for the major proteolytic pathways, was carried out at 3 time intervals at d -14 ± 5, d 15 and d 38 relative to calving. The first collection period (d -14 ± 5) was the pretreatment period. The experimental procedures used were all approved by the University of Saskatchewan Animal Care Committee (UCACS Protocol No. 20040048) and were in accordance with the Canadian Council of Animal Care guidelines (Ottawa, ON).

3.2.2 Experimental Treatments and Cow Management

The 16 pregnant cows were paired based on their expected calving dates and randomly assigned to either a basal diet (control; n = 8) or basal diet plus 600 mL/d of

PG (Glycol-P, Vétoquinol, Quebec City, Canada; n = 8) from d -7 to d 45 relative to calving. The amount of PG fed was based on previous research of Grummer et al. (1994) who observed linear and quadratic effects on plasma glucose, insulin, NEFA and BHBA when 0, 307, 613, and 919 g/d of PG were fed, so it was decided to use an intermediate dose of PG. The same control diet was fed as a total mixed ration (TMR) pre- and post-partum and the ingredient and chemical composition of the diet is given in Table 3.1. Cows were fed daily for ad libitum intake at 0830 and 1600 h. Propylene glycol (300 mL) was top-dressed onto the TMR and then thoroughly mixed with the TMR for both the morning and afternoon feeding. The amount of the TMR the cows consumed and the refusals were recorded daily.

Cows were housed in individual tie-stalls a week before the pre-treatment period. Following the first collection period (pre-treatment period), the cows were moved into individual maternity pens, where they were kept until they calved. A day after calving, the cows were put back in the individual tie-stalls, where they remained until the end of the experiment at d 45 relative to calving.

Table 3.1. Average ingredient and chemical composition of basal diet.

Ingredient Composition, % DM basis	
Barley silage	32.4
Hay	16.3
Dehydrated alfalfa	7.8
Barley	17.1
Wheat	1.0
Oats	1.5
Molasses	0.6
Soybean Meal	8.1
Canola Meal	7.2
Wheat Distillers Dried Grains	2.2
Corn Gluten Meal	1.5
Golden Flakes[1]	0.3
Canola Oil	0.5
Mineral-Vitamin Mix[2]	2.3
Niacin-Magnesium Mix[3]	0.04
Cobalt-Iodized Salt	0.5
Sodium Bicarbonate	0.5
Ground Limestone	0.04
Dynamate[4]	0.2
Chemical Composition, DM basis	
Dry Matter, %	55.4
Organic Matter, %	92.6
Crude Protein, %	17.3
Fat, %	2.8
NDF, %	38.2
ADF, %	23.4
NE_L[5], Mcal/kg	1.63

[1]Dried fat supplement (Malaysian Palm Oil) distributed in Western Canada by Prairie Micro-Tech Inc., Regina, Saskatchewan.

[2]Supplied 22 mg manganese, 30 mg zinc, 8 mg copper, 0.2 mg selenium, 5278 I.U. vitamin A, 864 I.U. vitamin D_3 and 14 I.U. vitamin E per kg of control diet. The mix also contributes 0.07 % magnesium, 0.23 % calcium, 0.12 % phosphorus, 0.11 % sodium and 0.18 % chloride to the control diet. Prepared by Federated Cooperatives Ltd., Saskatoon.

[3]Supplied 0.13 g of niacin and 0.04 g of magnesium per kg control diet.

[4]Supplied 0.07 % sulfur, 0.06 % potassium, 0.04 % magnesium per kg control diet (International Minerals and Chemical Corp., Mundelein, ILL).

[5]Calculated using NRC (2001).

3.2.3 Sample Collection

3.2.3.1 Body Composition Determination

Body composition measurement was determined using the urea dilution technique as described by Agnew et al. (2005). Briefly, on d 1 of each measurement period, each cow was weighed at 1400 h. On the day 2, the cow was fitted with a temporary jugular polyethylene catheter (0.86 mm I.D. x 1.32 mm O.D.; Scientific Commodities Inc., Lake Havasu City, AZ) to allow for urea infusion and blood sampling. This was followed by collection of a 10 mL blood sample into a vacutainer tube containing lithium heparin (Becton Dickinson, Franklin Lakes, NJ) to determine background plasma urea nitrogen (PUN). A pre-determined volume of a 20% w/v urea solution (reagent grade urea dissolved in 9 g/L isotonic saline) which provided 130 mg urea per kg of LW was then infused via the jugular catheter over 3 min. Following infusion, the catheter was flushed with saline. A blood sample was collected via the jugular catheter at 12 min after the mean infusion time. The blood samples that were collected were immediately placed on ice and centrifuged at 1,800 × *g* for 15 min to obtain plasma, which was stored at – 20°C for later analysis of plasma urea nitrogen (PUN).

3.2.3.2 Determination of Nitrogen Balance, Urinary 3-methylhistidine Excretion and Total Tract Nutrient Digestibilities

Nitrogen balance and total tract nutrient digestibility determinations were conducted using 5-d total urine and feces collection periods. On day 2, following determination of body composition, each cow was fitted with an indwelling bladder

catheter (Bardex Foley Catheter, 75-mL capacity balloon; C. R. Bard Inc., Covington, GA) using the method described by Crutchfield (1968). On day 3, at 0800 h, the indwelling bladder catheters were then connected to pre-weighed 20-L polyethylene containers used for urine collection. The 20-L polyethylene containers contained 150 mL of concentrated hydrochloric acid to prevent nitrogen loss and microbial growth. Daily urine production was weighed at 0800 h, thoroughly mixed, and a 2.5% sub-sample was collected and stored at – 20°C for later N and 3-MH analysis. A 2-mL aliquot of urine was also collected, diluted 1:5 with distilled water and stored at – 20°C for later analysis of urea N. Fecal collection was performed using a steel tray placed behind each cow in a tie-stall. On a daily basis, the feces produced were thoroughly mixed at 0800 h and put in pre-weighed containers to determine total output. A 5% fecal sub-sample was collected and stored at – 20°C for later analysis. A TMR and orts sub-sample was collected daily during each N balance determination period and stored at – 20°C for further analysis.

3.2.3.3 Muscle Tissue Collection

On d 8 of each measurement period, muscle tissue was obtained by needle biopsy from the longissimus muscle (*m. longissimus lumborum*) as described by Bergström et al. (1974). Briefly, a 5 cm^2 area of skin on the hind limb (roughly 10 cm from the rib and 8 cm from the backbone) was shaved and cleaned with iodine (Rougier, Mirabel, QC, Canada). A local anesthetic (20 g/L of lidocaine without epinephrine; Vetoquinol, Lavaltrie, QC, Canada) was then injected subcutaneously without penetrating the muscle tissue, before a 2-cm scalpel incision was made. A sterile biopsy

needle (7 mm i.d.) was inserted into muscle to approximately 5 cm to collect 100 to 200 mg of muscle tissue. The collected muscle tissue was snap-frozen in liquid nitrogen and stored at -80°C pending RNA analysis. The incision was then stapled after sampling.

3.2.3.4 Milk Sampling

Milk sampling was carried out on 3 consecutive days within each N balance determination period post-partum. On each collection day, milk was collected into plastic vials containing 2-bromo-2-nitropropane-1-2-diol as a preservative. The milk samples from the morning (0430 h) and afternoon (1530 h) milkings were pooled proportionally based on milk yield and stored at 4°C before being sent to the Provincial Milk Testing Laboratory (Saskatchewan Agriculture, Food and Rural Revitalization, Regina, SK).

3.2.3.5 Blood Sampling

Blood samples for determination of plasma glucose, insulin and urea nitrogen (PUN), and serum non-esterified fatty acids (NEFA) and β-hydroxybutyrate (BHBA) concentrations were collected weekly at 0900 h from the coccygeal vein starting on d -14 ± 5 and ending on d 45 ± 0 relative to calving. Blood samples for plasma were collected using 10-mL vacutainer tubes containing lithium heparin (Becton Dickinson, Franklin Lakes, NJ), whilst 10-mL vacutainer tubes without heparin (Becton Dickinson, Franklin Lakes, NJ) were used for serum. The collected blood samples were immediately placed on ice and then processed by centrifugation at 1,800 × g for 15 min to obtain plasma and serum which were then stored at – 20°C until later analysis.

3.2.4 Sample Analysis

All samples were analyzed in duplicate.

3.2.4.1 Feeds, Feces and Urine Analysis

Preceding analysis, the frozen feed, urine and fecal samples were thawed and pooled per total collection period for each cow. The feed and fecal samples were oven-dried at 55°C for 48 h to determine dry matter content (AOAC, 1990) and then ground through a 1-mm screen using a Christy-Norris mill (Christy and Norris Ltd., Chelmsford, England). The ground feed and fecal samples were then analyzed for N using the macro-Kjeldahl procedure, acid detergent fiber (ADF) and fat (AOAC, 1990) and neutral detergent fiber (NDF) (Van Soest et., 1991). Urine was analyzed for N using the macro-Kjeldahl procedure (AOAC, 1990). A diacetyl monoxime-based method was used to analyze urine urea N using a urea nitrogen kit (Stanbio Laboratory, Boerne, TX, USA).

3.2.4.2 Urinary 3-Methylhistidine Analysis

Urinary 3-MH was quantified using reverse-phase high-performance liquid chromatography (HPLC). Sample preparation was as described by Min et al. (1992). Briefly, 475 µl of urine (total collections samples) and 25 µl of 50 µg/mL L-ornithine hydrochloride (internal standard) were mixed with 500 µl of 0.7 M perchloric acid (PCA) before centrifugation at 18,000 *g* for 5 min (Microfuge 18, Beckman, Coulter, Germany). The supernatant (500 µl) was mixed with 500 µl of 0.25 M potassium hydroxide (KOH) to neutralise the sample, before centrifugation at 18,000 *g* for 5 min.

The supernatant (50 µl) was collected and then added to 50 µl of the derivatization reagent and 500 µl of 0.4 M sodium borate (pH 11). The mixture was vortexed for 30 sec and then filtered using a 0.45 µm syringe filter before injection of 10 µl onto the column. To make the derivatization reagent, 50 mg of o-phthaldialdehyde (OPA) were dissolved in 4.5 mL of methanol (MeOH) before addition of 50 µl of 3-mercaptopropionic acid (MPA) and 450 µl of 0.4 M sodium borate (pH 11). Seperation of compounds was carried out on a reverse-phase column (Luna 2.5 µm C18 (2)-HST, 100 × 3.0 mm, Phenomenex, Torrance, CA, USA) using an Agilent HPLC system (Agilent 1100 series, Agilent Technologies, Waldbronn, Germany). A Shimadzu fluorescence detector (RF-551, Spectrofluorometric detector, Shimadzu, Columbia, MD, USA) was used for detection. The mobile phase consisted of two buffers (A and B). Buffer A consisted of 12.5 mM potassium phosphate and 5% MeOH (pH 9) whilst buffer B consisted of 12.5 mM potassium phosphate and 20% acetonitrile (ACN) (pH 9). Both buffers were filtered and degassed before use. The gradient was A to B in 60 min, which was followed by a 5-min column wash using 75% ACN. The flow rate was 0.75 mL/min and the 3-MH derivative was detected at an excitation wavelength (E_x) of 330 nm and emission wavelength (E_m) of 450 nm.

3.2.4.3 Analysis of mRNA levels for µ-calpain, m-calpain, ubiquitin, 14-kDA ubiquitin conjugating enzyme E2 and proteasome 26S subunit, ATPase in skeletal muscle

Frozen muscle tissue was analyzed for µ-calpain, m-calpain, ubiquitin, 14-kDa ubiquitin-carrier protein E2 (14-kDa E2), proteasome 26S subunit, ATPase and glyceraldehyde-3-phosphate dehydrogenase (GAPDH; a housekeeping gene) transcript

abundance. Muscle tissue samples were pulverized with a mortar and pestle under liquid nitrogen before a 30-mg sub-sample was used for total RNA extraction using an RNeasy Mini Kit (Qiagen, Mississauga, ON) and subsequent RNase-free DNase (Qiagen) digestion. The extracted RNA was quantified with PicoGreen (Molecular Probes, Eugene, OR) using a fluorometer (Fluoroscan Ascent FL, Themo Labsystems). Extracted RNA (45 ng) was reverse transcribed to cDNA using SuperScript III First-Strand Synthesis System (Invitrogen, Carlsbad, CA) prior to determination of gene transcript abundance by real-time quantitative PCR (qPCR) (iCycler iQ Real-Time PCR detection system, BioRad) using SYBR Green fluorescence detection. The primers (Table 3.2) used for m-calpain, ubiquitin and 14-kDa ubiquitin-carrier protein E2 (14-kDa E2) were designed using Beacon Designer (PREMIER Biosoft International, Palo Alto, CA) whilst the primers used for GAPDH (Mena et al. 2002), μ-calpain (Juszczuk-Kubiak et al. 2004) and proteasome 26S subunit, ATPase (Guo et al. 2005) were reported previously. Primer specificity was confirmed by sequencing (data not shown) of the relevant PCR product following standard PCR amplification of cDNA from bovine muscle using conditions similar to qPCR (Table 3.3). The real-time qPCR reaction mixture used for each gene consisted of 12.5 μl Platinum SYBR Green qPCR SuperMix-UDG (Invitrogen, Burlington, ON), 0.5 μl of each primer (25 μM), and 1.0 μl template cDNA, and was made up to 25 μl. The qPCR amplification conditions specific for each gene are summarized in table 3.3. To prepare standard curves for each target gene, bovine muscle cDNA was amplified using relevant qPCR primers, gel extracted (Invitrogen, Burlington, ON) and quantified using PicoGreen (Molecular Probes, Eugene, OR).

Table 3.2. Quantitative real-time PCR primers.

Gene name	Accession #[1]	Primer sequences	
		Forward (5' – 3')	Reverse (5' – 3')
μ-calpain	AF248054	TTCAGGCCAATCTCCCCGACG	GATGTTGAACTCCACCAGGCCCAG
m-calpain	XM_864105	GGAGGAAGAGGACGAGGAC	TTGCTGAGGTGGATGTTGG
Ubiquitin	NM_174133	GCCGCACTCTTTCTGATTACAAC	CGTTCTCGATGGTGTCACTGG
14-kDa ubiquitin-carrier protein E2	BC109502	TGGACCACAAGGAACACCGTATG	TGCAGCGTCACCATTGAGAGG
Proteasome 26S subunit, ATPase	BC102595	TGTCCGAGAGAATCGCTACA	TAGGTTTGGGGAAAGAGGCT
GAPDH[2]	NM_001034034	GCCTGGAGAAACCTGCCAAGTATG	TGGTCCTCAGTGTAGCCTAGAATGC

[1]Accession # = GenBank accession number.

[2]GAPDH = glyceraldehyde-3-phosphate dehydrogenase.

Table 3.3. Quantitative real-time PCR conditions.

	Denaturation step (40X)[1]		Annealing step (40X)		Extension step (40X)	
Gene name	T (°C)	Time (sec)	T (°C)	Time (sec)	T (°C)	Time (sec)
GAPDH[2]	95.0	30	62.0	30	72.0	30
μ-calpain	95.0	30	62.0	30	72.0	30
m-calpain	95.0	30	60.0	30	72.0	30
Ubiquitin	95.0	30	55.0	30		
14-kDa ubiquitin-carrier protein E2	95.0	30	56.0	30		
Proteasome 26S subunit, ATPase	95.0	30	58.0	30		

[1]40X = 40 cycles; T = temperature.

[2]GAPDH = glyceraldehyde-3-phosphate dehydrogenase.

Transcript abundance for each gene was determined by interpolating the threshold cycle for cDNA against standard curve constructed from gel purified amplicons over the range of 10^1 to 10^7 copies (copy number calculated based on molecular weight). The results are presented as target gene transcript abundance normalised to GAPDH transcript abundance.

3.2.4.4 Blood Analysis

A diacetyl monoxime-based method was used to determine plasma urea concentration using a urea nitrogen kit (Stanbio Laboratory, Boerne, TX, USA). Plasma glucose and urea nitrogen were determined using an enzymatic-colorimetric glucose and a colorimetric urea nitrogen kit respectively (Stanbio Laboratory, Boerne, TX, USA). Plasma for insulin analysis was first radio-iodinated (Greenwood et al., 1963) using a crystalline bovine insulin standard (Lilly Research Laboratories, Indianapolis, IN, USA; lot no. 615-70N-80) before analysis of the radio-iodinated insulin by double antibody radio immunoassay (Brockman, 1979). Serum NEFA and BHBA were analyzed at the Animal Health Laboratory (Guelph, ON, Canada).

3.2.4.5 Milk Analysis

Analysis of milk CP (N × 6.38), lactose and fat was performed using an infrared analyzer (Foss System 4000, Foss Electric, Hillerød, Denmark). A Beckman analyzer (Beckman instruments, CA) was used to quantify milk urea nitrogen (MUN).

3.2.5 Calculations and Statistical Analysis

Urea space volume (USV_{12}, kg) was then calculated by dividing the amount of urea infused by the change in PUN (difference in PUN concentration between the pre- and post-infusion samples). Multiple regression prediction equations (Table 3.4) developed by Agnew et al. (2005) for dairy cows were then used to estimate empty body weight and protein, lipid, energy, water and ash contents using urea space volume at 12 min (USV_{12}), urea space volume at 12 min as a proportion of empty body weight ($USV_{12/EBW}$), live weight and parity.

Energy balance (**EB**) was calculated as net energy intake (**NEI**) – net energy requirement (**NER**). Net energy intake was determined as average daily DM intake x the calculated NE_L value of the diet. For PG-fed cows, this calculation of NEI included the estimated energy contribution of PG (2.83 Mcal/d). For pre-fresh cows, NER was calculated as net energy for maintenance ($\mathbf{NE_M}$) + net energy for pregnancy ($\mathbf{NE_P}$), where NE_M and NE_P (Mcal/d) where calculated using NRC (2001) equations. For lactating cows, NER was calculated as NE_M + net energy for lactation (NE_L). Net energy for lactation (Mcal/d) was calculated as (9.29 x fat yield/d) + (5.71 x protein yield/d) + (3.95 x lactose yield/d; NRC, 2001).

All animals were included in the statistical analysis (control, $n = 8$; PG, $n = 8$). All pretreatment data (d -14 ± 5) were analyzed using a *t*-test to determine if there were any dietary treatment differences. The pretreatment means for urine nitrogen (N) were different; therefore, the pretreatment data were used as covariates. Feed intake and digestibility, blood metabolite, N balance data (except for urine N), urinary 3-MH, body composition and gene expression data were analyzed as a completely randomized block design with repeated measures using the PROC MIXED procedure of SAS (2004). The

variance-covariance error structure with the lowest Akaike's and Bayesian information criteria was used for repeated measures analysis for each of the measurements. Treatment was considered as a fixed effect, and week of sampling (blood metabolites) or day (relative to calving) as the repeated measure. Milk production and composition data were analyzed as a completely randomized block design using the PROC MIXED procedure of SAS (2004). Treatment, time or treatment × time effects were declared significant at $P < 0.05$ and trends at $0.05 \leq P < 0.10$.

Table 3.4. Multiple regression equations used to estimate empty body weight, crude protein, lipid, ash, gross energy and water using urea space, live weight and parity.

Regression equations[1]		
Empty Body Weight (kg)	=	$0.133(USV_{12}) + 0.687(LW) - 21.0$
Crude protein (kg)	=	$0.036(USV_{12}) + 0.105(LW) + 5.0$
Lipid (kg)	=	$- 0.065(USV_{12/EBW}) + 0.184(LW) - 15.2$
Ash (kg)	=	$0.019(USV_{12}) + 0.029(LW) + 1.29(Parity) - 0.4$
Gross energy (MJ)	=	$- 2.405(USV_{12/EBW}) + 11.89(LW) - 1651$
Water (kg)	=	$0.111(USV_{12}) + 0.330(LW) + 42.0$

[1]Regression equations were developed and validated for use in lactating dairy cows by Agnew et al. (2005).

USV_{12} = Urea space volume at 12 min (kg), LW = Live weight, $USV_{12/EBW}$ = Urea space volume at 12 min as a proportion of EBW (g/kg).

3.4 RESULTS AND DISCUSSION

3.4.1 Nutrient intakes and digestibilities

Dietary addition of PG had no effect ($P = 0.22$) on DM intake; as a consequence, OM ($P = 0.40$), N ($P = 0.51$), ADF ($P = 0.62$) and NDF ($P = 0.57$) intakes were unaffected by PG supplementation (Table 3.5). A preponderance of previous studies has reported that PG supplementation does not alter DM intake in early-lactating dairy cows (Miyoshi et al., 2001; Pickett et al., 2003; Nielsen and Ingvartsen, 2004). Propylene glycol is unpalatable, and its addition into a TMR, as in the present study, masks its negative effects on feed intake. Christensen et al. (1997) also found no negative effect of mixing PG into the TMR on DMI, although a smaller dose of PG was used in that study (307 to 341 mL/d) compared to the current study. However, Dhiman et al. (1993) observed a 10% reduction in feed intake when PG was added to the TMR of mid-lactation cows, but the PG dose used in that study (688 g/d) was larger than that used in the present study. Across dietary treatments, DM intake was higher at d 15 and d 38 compared to d -14 ($P < 0.001$); post-partum, DM intake was higher at d 38 compared to d 15 ($P < 0.001$; Table 3.5). These changes in DM intake were expected because the 3-wk period preceding calving is characterized by a reduction in feed intake (McNamara et al., 2003). However, post-partum, there is a gradual increase in DM intake which peaks around 75 days-in-milk (Moallem et al., 2000). The increase in DM intake at d 15 and d 38 also led to a concomitant increase ($P < 0.001$) in OM, ADF and NDF intakes (Table 3.5).

Table 3.5. The effect of propylene glycol administration on dry matter (DM), organic matter (OM), acid detergent fiber (ADF), neutral detergent fiber (NDF) and nitrogen intakes and digestibilities in transition dairy cows.

	Treatment[1]			*P* value[2]		
Item	Control	PG	SEM	Trtmt	Time	T × T
Dry matter						
Intake, kg/d						
d -14 (Pre-treatment)[3]	9.7[a]	10.1[a]	1.9			
d 15	15.2[b]	14.7[b]	1.9	0.22	<0.0001	0.23
d 38	19.9[c]	17.8[c]	1.9			
Digestibility, %						
d -14 (Pre-treatment)	63.9	64.9	0.8			
d 15	63.6	65.0	0.8	0.06	0.61	0.96
d 38	64.7	65.6	0.8			
Organic matter						
Intake, kg/d						
d -14 (Pre-treatment)	9.0[a]	9.4[a]	1.8			
d 15	14.1[b]	13.6[b]	1.8	0.40	<0.0001	0.39
d 38	18.4[c]	16.5[c]	1.8			
Digestibility, %						
d -14 (Pre-treatment)	65.3	66.3	0.8			
d 15	64.8	66.1	0.8	0.06	0.47	0.97
d 38	66.0	66.9	0.8			
ADF						
Intake, kg/d						
d -14 (Pre-treatment)	2.56[a]	2.78[a]	0.45			
d 15	3.59[b]	3.48[b]	0.48	0.62	<0.0001	0.25
d 38	4.72[c]	4.31[c]	0.48			
Digestibility, %						
d -14 (Pre-treatment)	43.6	46.7	1.7			
d 15	38.8	40.8	2.1	0.10	0.08	0.72
d 38	41.2	42.1	1.2			
NDF						
Intake, kg/d						
d -14 (Pre-treatment)	4.23[a]	4.62[a]	0.79			
d 15	5.88[b]	5.63[b]	0.79	0.57	<0.0001	0.16
d 38	7.57[c]	6.90[c]	0.79			
Digestibility, %[4]						
d -14 (Pre-treatment)	45.8[a]	49.7[a]	1.5			
d 15	42.1[b]	43.8[b]	1.5	0.003	0.02	0.80
d 38	42.6[ab]	44.6[ab]	1.5			

Nitrogen						
Intake, g/d						
d -14 (Pre-treatment)	230.9[a]	240.4[a]	18.8			
d 15	400.5[b]	392.6[b]	30.3	0.51	<0.0001	0.41
d 38	506.7[c]	465.1[c]	30.1			
Digestibility, %						
d -14 (Pre-treatment)	70.0	70.5	0.7			
d 15	69.1	69.9	0.7	0.14	0.57	0.75
d 38	69.3	70.8	0.7			

[a,b]Treatment means within columns with different superscripts differ ($P < 0.05$).

[1]PG = Propylene glycol.

[2]Trtmt = Treatment; T × T = Treatment × Time interaction.

[3]Pretreatment values were not different ($P > 0.05$).

[4]Superscripts for NDF digestibility show only the changes with time although there was a treatment effect.

Supplementing pre- and post-partum diets with PG tended to increase apparent DM (P = 0.06) and OM (P = 0.06) digestibility. There was an increase in NDF (P = 0.03) digestibility when PG was supplemented; however, PG had no effect on N (P = 0.14) and ADF (P = 0.10) digestibility (Table 3.5). Others (Cozzi et al., 1996; Shingfield et al., 2002) reported that PG supplementation did not alter nutrient digestibility. It is not clear why PG supplementation increased NDF digestibility in the present study. Numerically, cows fed PG ate 0.4 to 2.1 kg/d less feed at d 15 and d 38 respectively, when compared to cows not receiving PG. The lower feed intake could have resulted in a longer rumen retention time and, thus, an increase in fibre digestion (Colucci et al., 1982). The tendency for higher DM and OM digestibilities with PG supplementation lends support to this suggestion. Across dietary treatments, there were no changes in apparent DM (P = 0.61) and OM (P = 0.47) digestibility with time; however, ADF digestibility tended (P = 0.08) to decrease post-partum compared to d -14, whilst NDF digestibility was 4.85 percentage points higher (P = 0.01) and 4.21 percentage points higher (P = 0.02) before calving compared to d 15 and d 38, respectively (Table 3.5). Doreau et al. (1990) also observed a decrease in fibre digestion post-partum compared to the pre-partum period, which they partly attributed to the changes in DM intake as there was a small increase in DM intake post-partum. In the current study, average DM intake (across treatments) increased by 51 and 94 % at d 15 and d 38, respectively, compared to d -14, which could have possibly led to a lower rumen retention time and, thus, a decline in fibre digestibility (Colluci et al., 1982).

3.4.2 Blood metabolites

The blood metabolite data summarized in Table 3.6 is for the time corresponding to the N balance determination periods whilst Figures 3.1, 3.2, 3.3, 3.4 and 3.5 show the weekly data. Propylene glycol supplementation had no effect on plasma glucose (P = 0.93), insulin (P = 0.65), serum NEFA (P = 0.81), BHBA (P = 0.57) and PUN (P = 0.29) (Table 3.6). Cozzi et al. (1996) also found no effect of mixing either 200-mL or 400-mL of PG into a TMR on blood glucose and urea, and plasma NEFA and insulin in cows during mid-lactation. Christensen et al. (1997) restricted feed intake in cows and heifers to mimic the conditions during early lactation when nutrient intake cannot meet maternal nutrient requirements. In that study, mixing 341 mL/d and 307 mL/d of PG into the TMR fed to cows and heifers, respectively, did not increase glucose concentration or reduce NEFA and BHBA concentrations, although it increased insulin concentration. However, orally drenching 296, 592 or 887 mL of PG in feed-restricted heifers during the pre-partum period resulted in an increase in glucose and insulin concentrations, and reduced NEFA and BHBA concentrations (Grummer et al., 1994). Orally drenching 500 mL of PG/d had no effect on glucose and NEFA concentrations at d -10, although it increased glucose and reduced NEFA concentrations at d 2 and d 25 relative to calving (Butler et al., 2006). In that study by Butler et al. (2006), orally drenching PG also tended to increase insulin concentration at d -10 and d 2 and increase insulin concentration at d 25 relative to calving and reduce BHBA concentration. Given the differences in the efficacy of PG in affecting blood parameters when either orally drenched or mixed into the TMR, it appears orally drenching PG is more effective at increasing glucose and insulin concentrations and reducing NEFA concentration compared to mixing into the TMR (Christensen et al., 1997; Cozzi et al., 1996).

Table 3.6. The effect of propylene glycol administration on blood metabolites in transition dairy cows.

	Treatment[1]			*P* value[2]		
Item[3]	Control	PG	SEM	Trtmt	Time	T × T
Glucose, mmol/L						
d -14 (Pre-treatment)[4]	3.78[a]	3.78[a]	0.37			
d 15	3.28[b]	3.17[b]	0.34	0.93	<0.0001	0.66
d 38	3.15[b]	3.21[b]	0.34			
Insulin, uU/mL						
d -14 (Pre-treatment)	17.32[a]	18.69[a]	4.69			
d 15	4.35[b]	3.76[b]	0.60	0.65	0.0002	0.30
d 38	6.42[c]	9.12[c]	1.67			
NEFA, mmol/L						
d -14 (Pre-treatment)	0.31[a]	0.26[a]	0.08			
d 15	0.61[b]	0.59[b]	0.14	0.81	0.02	0.95
d 38	0.38[a]	0.38[a]	0.10			
BHBA, umol/L						
d -14 (Pre-treatment)	576.3[a]	540.0[a]	60.7			
d 15	717.2[b]	1040.2[b]	179.6	0.57	0.04	0.28
d 38	690.1[ab]	645.1[ab]	118.4			
PUN, mg/dL						
d -14 (Pre-treatment)	12.3	12.8	0.7			
d 15	12.0	13.0	0.7	0.29	0.06	0.93
d 38	13.6	14.3	0.7			

[a,b]Treatment means within columns with different superscripts differ ($P < 0.05$).

[1]PG = Propylene glycol.

[2]Trtmt = Treatment; T × T = Treatment × Time interaction.

[3]NEFA = non-esterified fatty acids; BHBA = beta-hydroxybutyrate; PUN = plasma urea nitrogen.

[4]Pretreatment values were not different ($P > 0.05$).

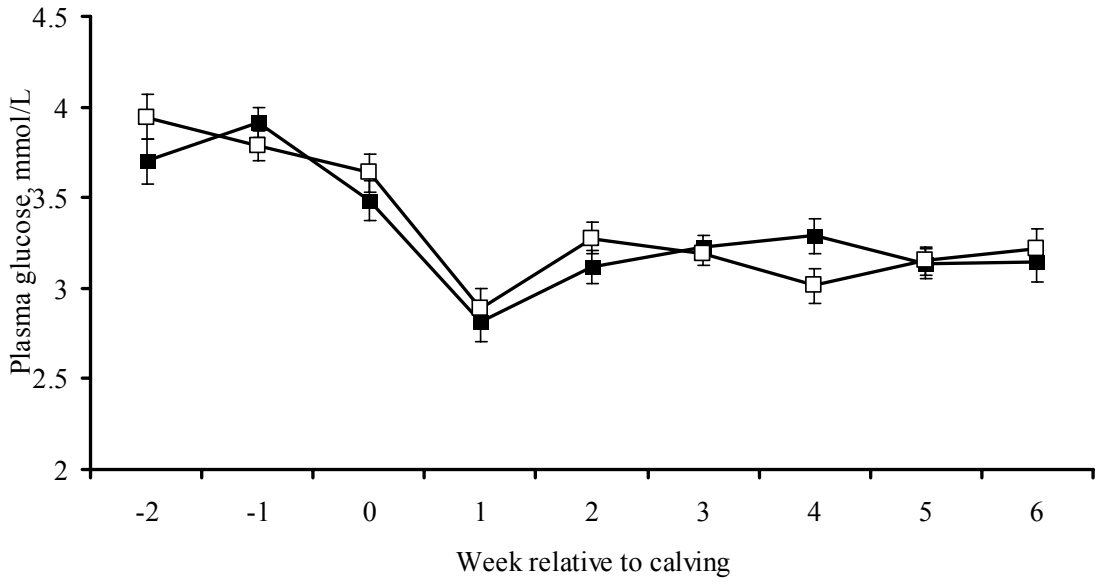


Figure 3.1. Plasma glucose concentrations from d -14 to d 42 relative to calving in cows fed the control (□) or control plus propylene glycol (■) diets.

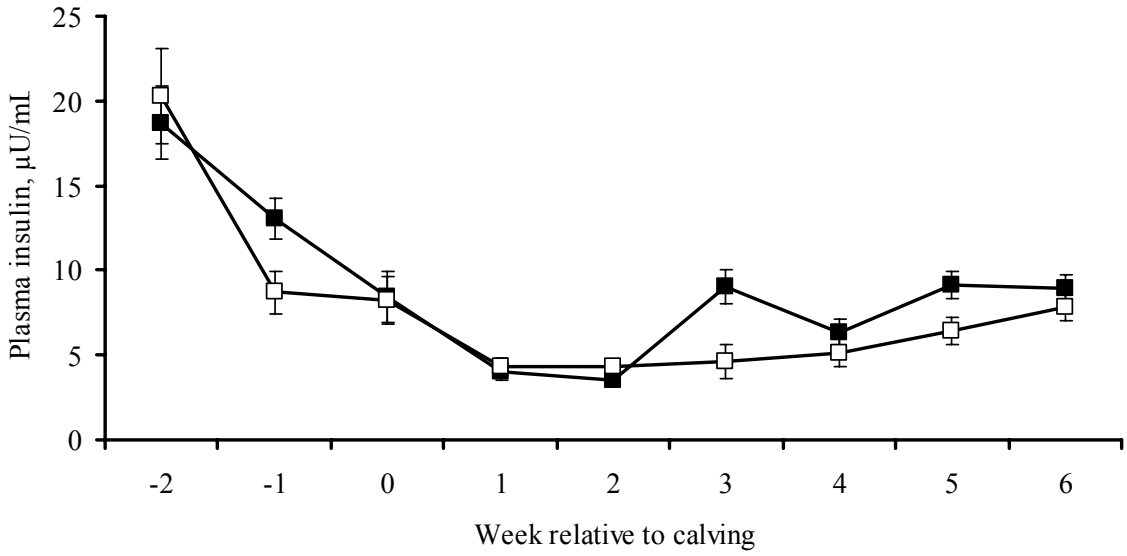


Figure 3.2. Plasma insulin concentrations from d -14 to d 42 relative to calving in cows fed the control (□) or control plus propylene glycol (■) diets.

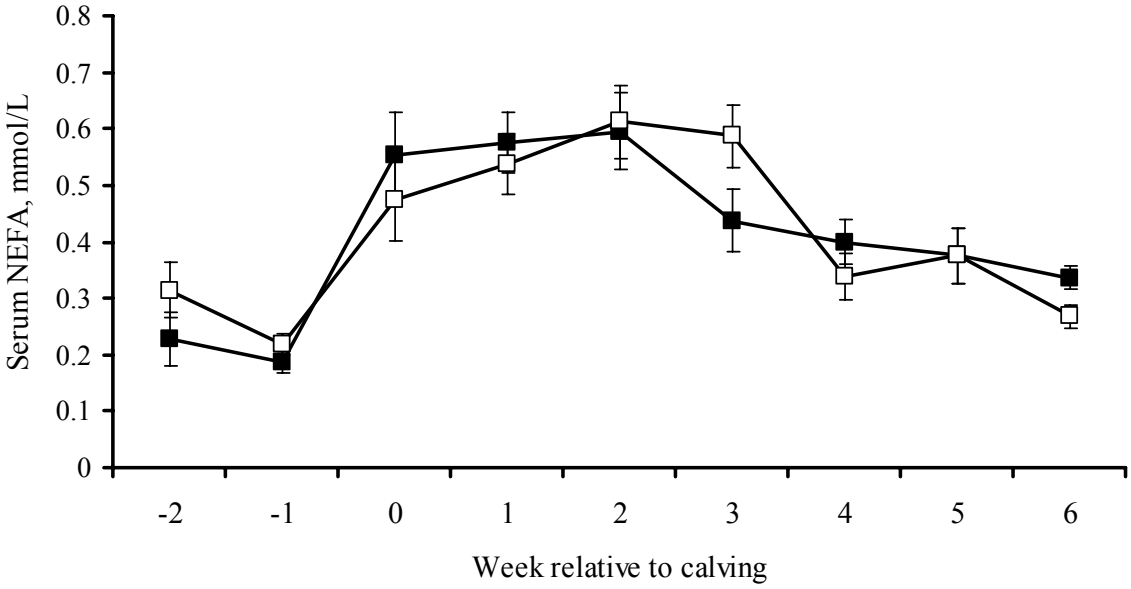


Figure 3.3. Serum NEFA concentrations from d -14 to d 42 relative to calving in cows fed the control (□) or control plus propylene glycol (■) diets.

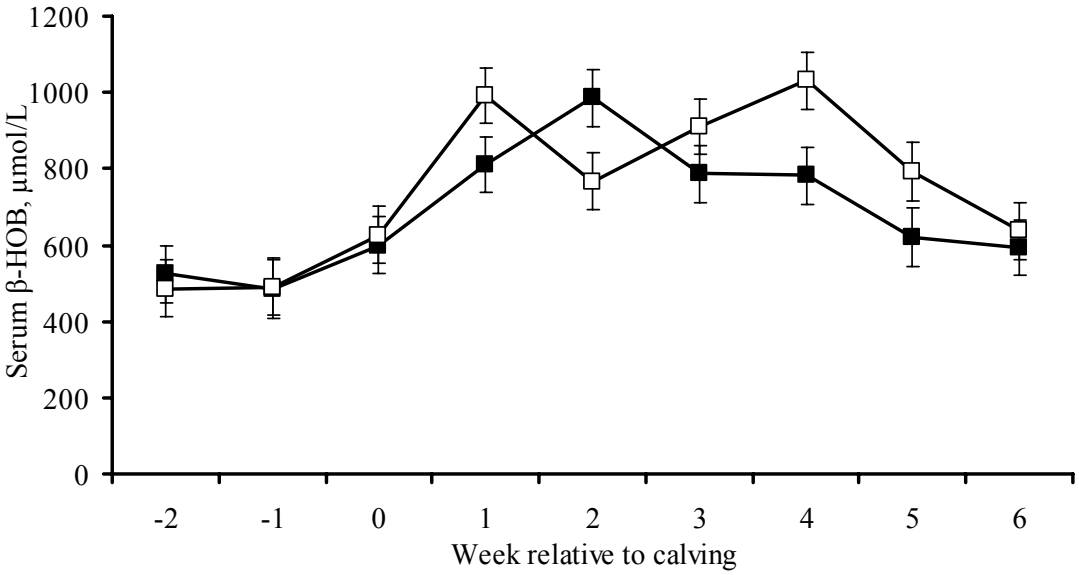


Figure 3.4. Serum BHBA concentrations from d -14 to d 42 relative to calving in cows fed the control (□) or control plus propylene glycol (■) diets.

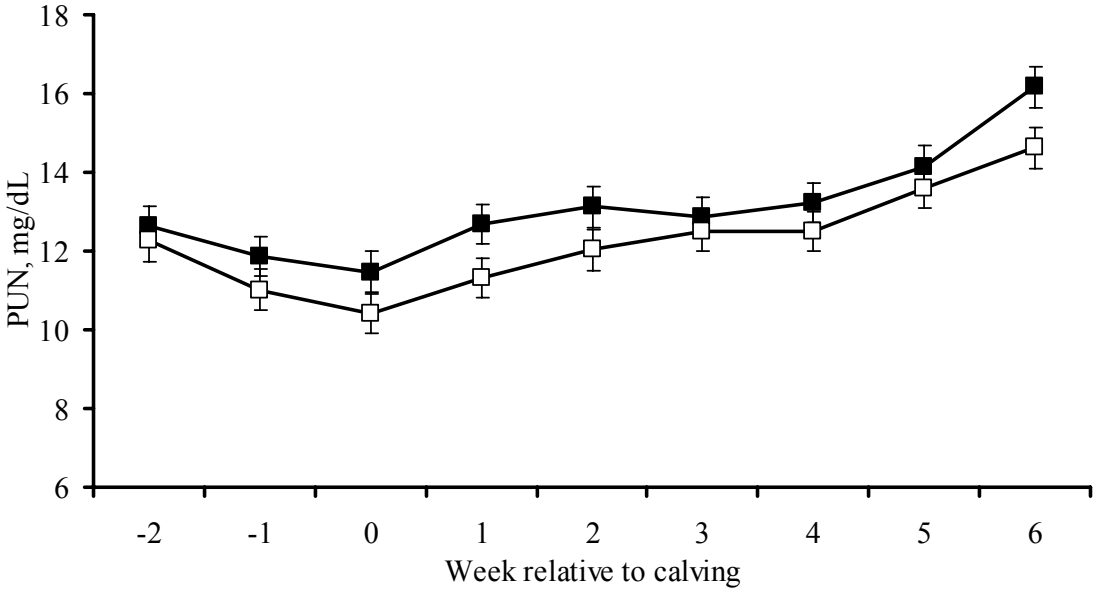


Figure 3.5. PUN concentrations from d -14 to d 42 relative to calving in cows fed the control (□) or control plus propylene glycol (■) diets.

Propylene glycol was mixed with the TMR in the current study, thus leading to its slower absorption and metabolism compared to studies where PG was orally drenched, and this could have ultimately led to the imperceptible changes in glucose, insulin, NEFA and BHBA concentrations (Cozzi et al., 1996).

In an attempt to curtail the glucose deficit during early lactation, other researchers have also focused on the impact of different glucogenic substrates, including glycerol (DeFrain et al., 2004; Ogborn, 2006), and calcium or sodium propionate (Goff et al., 1996; Beem, 2003; DeFrain et al., 2005) on energy metabolism during the transition period. Supplementing either 430 or 860 g/d of glycerol (on a DM basis) by mixing it into the ration fed to transition cows from d -14 to d 21 post-partum had no effect on plasma glucose, insulin, and BHBA concentrations pre-partum (DeFrain et al., 2004). Post-partum, supplementing 430 g/d of glycerol led to a decline in blood glucose concentrations at d 7, and BHBA concentration at d 14 and d 21, and a gradual increase in plasma insulin concentrations, whilst supplementing 860 g/d of glycerol led to a drastic decrease in glucose concentration from d 14 to d 21, and elevated BHBA concentrations from d 7, whilst insulin concentrations were unaffected (DeFrain et al., 2004). These observations led DeFrain et al. (2004) to conclude that glycerol is not an ideal glucogenic supplement for mixing into transition dairy cow diets as it can exacerbate the glucose deficit and result in increased ketogenesis during early lactation. Ogborn (2006) orally drenched transition cows with crude glycerine, providing an estimated 625g of glycerol/d during the first 5 days post-partum, which led to a reduction in DMI and resulted in no changes in plasma glucose and NEFA concentrations. Ogborn (2006) also reported a tendency of the orally drenched glycerol to increase BHBA concentration, which further brought questions whether it was

worthwhile supplementing glycerol in transition dairy cows. Goff et al. (1996) fed Jersey cows 268 g of propionate, as a calcium propionate paste at calving and 12 hr after calving and reported lower NEFA and BHBA concentrations 24 hr after calving compared to the control cows. Given these observations, Goff et al. (1996) concluded that calcium propionate supplementation can reduce the energy deficit which occurs during the transition period. On the contrary, some studies have shown no beneficial effects of propionate supplements in transition dairy cows. Top-dressing 120 g of propionic acid/d (contained in a 1:1 mixture of sodium and calcium salts of propionate) and then mixing it with the TMR from d -14 to d 21 relative to calving did not change plasma glucose, insulin, NEFA and BHBA concentrations (DeFrain et al., 2005). Supplementing 113 g of calcium propionate as a top-dressing in transition dairy cow diets resulted in no changes in plasma glucose and BHBA concentrations (Beem, 2003). Therefore, available evidence in literature indicates that metabolic responses to dietary supplementation with various glucogenic precursors in transition dairy cows have been inconsistent.

Across dietary treatments, plasma glucose and insulin, and serum NEFA and BHBA concentrations changed with time. Plasma glucose concentrations (Figure 3.1) decreased as calving approached and the lowest glucose concentration was observed at week 1 post-partum. Thereafter, glucose gradually increased, but concentrations remained lower ($P < 0.001$) post-calving than pre-calving. Vazque-Anon et al. (1994) reported a 25% decrease in plasma glucose concentration at week 1 post-partum, before a gradual increase by week 2 post-partum. The decrease in blood glucose concentration in early lactation is related to an increase in glucose demand for milk lactose synthesis, coupled with a reduced availability of diet-derived gluconeogenic precursors due to a

low DM intake (Doepel et al., 2002). As expected, plasma insulin concentration (Figure 3.2) gradually decreased as calving approached, leading to the low concentration from week 1 until week 6 post-partum. Given the anti-lipolytic effect of insulin, the low insulin concentration post-partum could have led to an increase in body fat mobilization (McCann and Hansel, 1986). Serum NEFA concentrations (Figure 3.3) increased (P = 0.01) at calving and remained elevated until week 3 post-partum, before gradually decreasing. Studies by Vazquez-Anon et al. (1994) and Rastani et al. (2000) also showed similar changes in blood NEFA concentrations in periparturient cows. Mobilization of body fat avails energy to the cow when dietary intake is insufficient to meet requirements (McNamara, 1991). Therefore, the elevation in serum NEFA concentration during early lactation in this study indicates an increase in the mobilization of body fat to augment the inadequate dietary nutrient supply. Serum BHBA concentrations (Figure 3.4) also increased (P = 0.006) dramatically from wk -1 to wk 1, and then remained elevated until wk 6. The negative energy balance during early lactation leads to the increase in mobilization of body fat and a concomitant increase in production of BHBA due to the oxidation of the mobilized NEFAs (Vazques-Anon et al., 1994). Despite the NEFA and BHBA concentrations following the same pattern, BHBA remained elevated until week 4 post-partum compared to week 3 for NEFA, and this lag in BHBA concentration is an indication that increased ketone production is driven by an increase in body fat mobilization (Busato et al., 2002).

Across dietary treatments, PUN concentrations (Figure 3.5) decreased as calving approached, before gradually increasing post-partum. The increase in PUN post-partum could have been as a result of an increase in DMI post-partum, as PUN is positively correlated to DMI and CP content of a diet (Broderick and Clayton, 1997). Despite the

increase in PUN concentration post-partum in this study, it did not exceed 19 mg/dL, which is the threshold beyond which reproductive performance is impacted negatively (Butler et al., 1996).

3.4.3 Milk production and composition

Cows on the PG treatment received 600 mL/d of PG. Assuming total absorption from the gut, this amount of PG would be equivalent to an additional intake of 2.825 Mcal/d of net energy of lactation (NE_L; as calculated by Miyoshi et al., 2001). Based on this additional NE_L intake, one would expect a positive response in milk production, which was not the case in the present study (P = 0.36) (Table 3.7). Our results are corroborated by observations made by Butler et al. (2006) who also found no positive effect of an additional 2 Mcal of energy/d supplied by PG on milk yield during the transition period. It is plausible that the PG dose used in the current study did not increase the NE_L content of the diet sufficiently to elicit a response in milk production (Nielsen and Ingvarten, 2004). However, it is noteworthy that cows fed PG produced 1.4 to 1.8 kg/d less milk compared to control cows and, although this difference was not statistically significant, it is consistent with the numerically lower DM intake for cows fed PG.

Butler et al. (2006) reported a tendency of orally drenched PG to reduce milk fat content in association with an increase in plasma insulin and a decline in plasma NEFA concentrations. The reduction in plasma NEFA concentration due to the insulin-mediated anti-lipolytic property of PG (Christensen et al., 1997) has been implicated in causing milk fat depression (Nielsen and Ingvartsen, 2004).

Table 3.7. The effect of propylene glycol administration on milk production and composition in transition dairy cows.

	Treatment[1]			*P* value[2]		
Item	Control	PG	SEM	Trtmt	Time	T × T
Milk yield, kg						
d 15	35.3	33.9	4.2	0.36	0.14	0.90
d 38	38.2	36.4	4.2			
Fat, %						
d 15	4.12	4.24	0.23	0.50	0.06	0.89
d 38	3.64	3.83	0.22			
Fat yield, kg/d						
d 15	1.42	1.46	0.20	0.81	0.53	0.85
d 38	1.38	1.39	0.20			
Protein, %						
d 15	2.84	2.72	0.08	0.11	0.88	0.89
d 38	2.84	2.70	0.08			
Protein yield, kg/d						
d 15	1.01	0.93	0.12	0.13	0.31	0.78
d 38	1.08	0.97	0.12			
Lactose, %						
d 15	4.57	4.58	0.06	0.52	0.48	0.66
d 38	4.58	4.62	0.06			
Lactose yield, kg/d						
d 15	1.61	1.57	0.18	0.52	0.16	0.92
d 38	1.75	1.68	0.18			
Milk urea N, mg/dL						
d 15	13.6	14.3	0.9	0.74	0.64	0.62
d 38	14.5	14.3	0.9			

[1]PG = Propylene glycol.

[2]Trtmt = Treatment; T × T = Treatment × Time interaction.

This arises from the reduction in the availability of NEFA, which the mammary gland can extract from blood and incorporate into milk fat. This hypothesis would explain why there were no changes in milk fat content ($P = 0.50$) and milk fat yield ($P = 0.81$) in this study as there was no increase in plasma insulin and decrease in serum NEFA concentration after PG supplementation. However, results in other studies do not support this hypothesis. Despite PG supplementation reducing NEFA concentration, there was no corresponding reduction in milk fat content during early lactation (Pickett et al., 2003; Hoedemaker et al., 2004). According to Nielsen and Ingvartsen (2004), the other possible explanation for the milk fat depression during early lactation when PG is supplemented is the reduction in the ruminal production of acetate, which is the key metabolite required for mammary gland milk fatty acid synthesis.

Propylene glycol supplementation did not affect milk protein content ($P = 0.11$) and milk protein yield ($P = 0.13$) (Table 3.7), supporting observations from previous studies (Pickett et al., 2003; Hoedemaker et al., 2004). The diet-derived glucogenic substrate deficit, mainly propionate, which occurs due to the inadequate DMI during early lactation, can cause an increase in the amount of amino acids used for gluconeogenesis (Bell et al., 2000). Feeding PG during early lactation can augment the dietary glucogenic precursor supply, potentially reducing the need for amino acids to contribute carbon skeletons for gluconeogenesis. However, the reduction in the glucogenic substrate deficit when PG is supplemented might not substantially improve the energy balance and reduce amino acid-fueled gluconeogenesis, given the magnitude of the energy deficit during early lactation (Nielsen and Ingvartsen, 2004). Ultimately, it is likely that there was no increase in the availability of amino acids which could be used

for milk protein synthesis in the current experiment, resulting in no changes in milk protein content (Nielsen and Ingvartsen, 2004).

In the current study, there was no effect of PG on milk lactose content ($P = 0.52$) and milk lactose yield ($P = 0.52$). Milk lactose content has been reported not to be easily altered by dietary manipulations (Patton et al., 2004). Mixing 200 to 688 g of PG/d into the TMR during mid-lactation did not alter milk lactose content (Dhiman et al., 1993; Cozzi et al., 1996). However, mixing 495 g of PG/day into the concentrate (Fisher et al., 1973) and orally drenching 500 mL PG/d (Butler et al., 2006) have been shown to increase milk lactose content during early lactation reflecting an improvement in the energy balance (Patton et al, 2004).

As expected, there was 8% increase ($P = 0.14$) in milk production from d 15 to d 38 (Table 3.7). There were no changes in milk fat yield ($P = 0.53$), milk protein content ($P = 0.88$), milk protein yield ($P = 0.31$), milk lactose content ($P = 0.48$) and milk lactose yield ($P = 0.16$) with time; however, milk fat content tended to decrease with time ($P = 0.06$; Table 3.7). The increase in milk production during early lactation in Holsteins is accompanied by a decrease in milk fat content (Stanton et al., 1992). Milk urea N did not change with time ($P = 0.64$). However, the MUN concentration did not exceed 19 mg/dL and, thus, did not impair reproductive performance (Butler et al., 1996).

3.4.4 Nitrogen balance and urinary 3-MH excretion

Propylene glycol had no effect on N intake ($P = 0.51$), fecal N ($P = 0.33$), milk N ($P = 0.12$), N retention ($P = 0.74$), and urinary excretion of 3-MH ($P = 0.16$) (Table 3.8).

Table 3.8. The effect of propylene glycol administration on N balance, and urinary excretion of 3-methylhistidine in transition dairy cows.

	Treatment[1]			*P* value[2]		
Item[3]	Control	PG	SEM	Trtmt	Time	T × T
N intake, g/d						
d -14 (Pre-treatment)[4]	230.9[a]	240.4[a]	18.8			
d 15	400.5[b]	392.6[b]	30.3	0.51	<0.0001	0.41
d 38	506.7[c]	465.1[c]	30.1			
Fecal N, g/d						
d -14 (Pre-treatment)	69.2[a]	71.1[a]	4.7			
d 15	122.8[b]	118.5[b]	8.7	0.33	<0.0001	0.28
d 38	156.1[c]	136.5[c]	10.0			
Urine N, g/d						
d -14 (Covariate)						
d 15	184.6	167.9	12.8	0.35	0.09	0.93
d 38	202.5	187.5	12.8			
Milk N, g/d						
d 15	157.6	145.9	18.7	0.12	0.30	0.76
d 38	170.0	152.7	18.6			
N retention, g/d[5]						
d -14 (Pre-treatment)	27.7[a]	17.5[a]	5.6			
d 15	-57.4[b]	-49.7[b]	11.1	0.74	<0.0001	0.62
d 38	-14.7[c]	-19.3[c]	9.4			
3-MH, mmol/d						
d -14 (Pre-treatment)	1.87[a]	1.52[a]	0.28			
d 15	2.39[b]	1.71[b]	0.33	0.16	0.001	0.32
d 38	2.76[b]	2.08[b]	0.41			

[a,b]Treatment means within columns with different superscripts differ ($P < 0.05$).

[1]PG = Propylene glycol.

[2]Trtmt = Treatment; T × T = Treatment × Time interaction.

[3]3-MH = 3-methylhistidine.

[4]Pretreatment values were not different ($P > 0.05$).

[5]A conversion factor of 6.38 was used in calculating the milk N content for N retention determination.

N retention = N intake – N output (N output at d -14 = urine + feces; N output at d 15 and 38 = urine + feces + milk).

To our knowledge, there are no published studies in which the effects of PG supplementation on N balance and urinary 3-MH excretion have been investigated in early-lactating dairy cows. As PG is a gluconeogenic precursor, we had anticipated that its provision in the diet would decrease the need for skeletal muscle-derived amino acids to drive gluconeogenesis, thus potentially enhancing N balance and decreasing urinary 3-MH excretion. Our results do not support that suggestion, presumably because the dose of PG used in the present study was inadequate to elicit a response. In addition, PG was administered via the TMR, thus allowing a progressive absorption of PG which may have diluted any potential response. These suggestions are partly supported by the lack of effect of PG supplementation on blood glucose concentrations and energy balance.

Across dietary treatments, nitrogen intake was lower at d -14 compared to d 15 ($P < 0.001$) and d 38 ($P < 0.001$); post-partum, N intake was higher at d 38 compared to d 15 ($P < 0.001$). The increase in N intake at d 15 and d 38 was due to the increase in DM intake as lactation progressed. Fecal N was lower at d -14 compared to d 15 ($P < 0.001$) and d 38 ($P < 0.001$). Post-partum, Fecal N was higher ($P = 0.002$) at d 38 compared to d 15. The increase in Fecal N was due to the increase in N intake (Kiran and Mutsvangwa, 2007). Urine N excretion tended ($P = 0.098$) to increase at d 38 compared to d 15. Nitrogen retention was positive at d -14. This is in contrast to the study by Maltz and Silanikove (1996) where they found N balance to be negative at 2 wk pre-partum. This disparity can be explained by the different pre-partum diets fed and, thus, N intakes. The pre-partum diet fed by Maltz and Silanikove (1996) contained 10% CP (on DM basis) and the cows consumed 122 g N/d whereas in the current study, the pre-partum diet contained 17% CP (on a DM basis) and the N intake was 230 g N/d. Post-partum, the negative N retention values in the current study were in the range

reported by Maltz and Silanikove (1996) with similar CP contents of the post-partum diets. During early lactation, the capacity to produce milk exceeds the ability to consume adequate nutrients to keep up with milk production in high yielding dairy cows (Botts et al., 1979). Therefore, the cow tries to bridge the nutrient gap by mobilizing maternal reserves. The negative N retention at d 15 and d 38 in the current study was a result of the nutrient deficit during early lactation which led to the mobilization of body protein to support milk production (Plaizier et al., 2000a). Nitrogen retention was more negative at d 15 than at d 38 ($P < 0.05$). Given the increase in DMI and, thus, nutrient supply as lactation progresses, the nutrient deficit during early lactation is transient. Therefore, as lactation progresses, N balance meliorates and there is a reduction in body protein mobilization (Plaizier et al., 2000a).

Across dietary treatments, urinary excretion of 3-MH, a commonly used indicator of the extent of myofibrillar protein degradation (Vissers et al., 2003), was lower at d -14 compared to d 15 ($P = 0.01$) and d 38 ($P =0.001$; Table 3.8). The elevation of urinary 3-MH excretion post-partum suggests an increase in the breakdown of myofibers in skeletal muscle as a result of the nutrient deficit which occurs in high-yielding dairy cows (Motyl and Barej, 1986; Plaizier et al., 2000b). Urinary 3-MH excretion at d 15 was not different from d 38. Motyl and Barej (1986) also found no differences in urinary 3-MH excretion at different time periods from d 5 to d 70 after calving. In a study by Tamminga et al. (1997), there was an increase in catabolism of body protein up to wk 4 after calving. However, by wk 5 post-partum, there was protein accretion. Therefore, the reduction in skeletal muscle breakdown as lactation progresses should also lead to a reduction in urinary excretion of 3-MH (Plaizier et al., 2000b). Despite a larger proportion of urinary 3-MH excretion in cattle being derived from the

breakdown of skeletal muscle, non-skeletal muscle can contribute up to 20% of urinary 3-MH excretion (Plaizier et al., 2000b). The contribution of non-skeletal muscle can be of significance in transition dairy cows as a result of uterine involution post-partum, since uterine smooth muscle contains 3-MH (Tian and Noakes, 1991). Uterine involution in dairy cows occurs up to 45 days post-partum (Jainudeen and Hafez, 2000) and the cow has to breakdown around 2,350 g (on a dry weight basis) of uterine tissue (Kaidi et al., 1995). Urine collection for the last 3-MH determination period in the current study was carried out from d 38 to d 45 postpartum, and it coincided with the end of the involution period, which is the time when a large proportion of uterine tissue is degraded (Plaizier et al., 2000b). Therefore, there could have been an increased contribution of uterine tissue to urinary 3-MH excretion at d 38, resulting in higher urinary 3-MH excretion compared to d 15. Tian and Noakes (1991) attempted to determine if there was a relationship between 3-MH concentration in peripheral circulation and uterine involution, and they concluded that no correlation existed in Holstein cows. However, the plasma 3-MH concentration around d 14 and d 38 were not different in that study.

The cows in the present study excreted 1.7, 2.1 and 2.4 mmol/d of 3-MH in urine at d -14, d 15 and d 38 respectively. Cows in the study by Motyl and Barej (1986) excreted 1.52 and 1.20 mmol/d of 3-MH at d 5 to d 10 and d 45 to d 50 respectively, which was lower than in the current study. The level of production is one of the factors which will determine the amount of body protein mobilized during early lactation (Komaragiri and Erdman, 1997). The higher milk production in the current study (36 kg/d) compared to 23 kg/d from d 5 to d 10 and 29 kg/d from d 45 to d 50 reported by Motyl and Barej (1986), could have led to a larger nutrient deficit in the current study,

therby necessitating the breakdown of a greater amount of skeletal muscle proteins. Plaizier et al. (2000b) reported excretion of 2.48 mmol/d of 3-MH in urine from d -10 to d -3 relative to calving, and 4.11 mmol/d from d 3 to d 9 post-partum, which was almost double the amount at d 15 in the current study. The greatest amount of body protein is mobilized during the first 10 d of lactation and, thereafter, there is a reduction in body protein degradation (Bell et al., 2000). Therefore, Plaizier et al. (2000b) quantified urinary 3-MH excretion during a period when there is intensified breakdown of skeletal muscle protein, thereby possibly explaining the higher excretion of 3-MH.

3.4.5 Body Composition

Despite the possible anti-lipolytic (Christensen et al., 1997) and maternal protein sparing properties of PG (Bell et al., 2000), feeding 600 mL of PG/d as part of the TMR did not reduce mobilization of maternal body fat (P = 0.30) and protein (P = 0.36) (Table 3.9). This could have been a result of the PG supplemented in the current study having no impact on the energy status of the transition cows as reflected by the lack of a reduction in serum NEFA concentration and milk fat content (Ingvartsen, 2006). Therefore, this could have resulted in a similar energy deficit and, thus, similar losses of body protein and fat reserves between cows fed the control diet and those fed PG. Propylene glycol supplementation had no effect on BW, empty BW (P = 0.33), and empty BW contents of GE (P = 0.30), ash (P = 0.52), and water (P = 0.36). As in the current study, Moallem et al. (2007), Kokkonen et al. (2005) and DeFrain et al. (2004) did not observe any beneficial effect of supplementing glucogenic precursors in transition cow diets on body composition.

Table 3.9. The effect of propylene glycol administration on body composition and energy balance in transition dairy cows[1].

	Treatment[2]			*P* value[3]		
Body Component[4]	Control	PG	SEM	Trtmt	Time	T × T
BW, kg						
d -14 (Pre-treatment)[5]	767.2[a]	740.8[a]	56.8			
d 15	695.2[b]	668.4[b]	55.8	0.30	<0.0001	0.99
d 38	663.9[c]	639.3[c]	54.5			
Empty BW, kg						
d -14 (Pre-treatment)	606.1[a]	589.0[a]	50.0			
d 15	550.5[b]	529.6[b]	49.5	0.33	<0.0001	0.96
d 38	525.0[c]	503.2[c]	48.4			
Lipid, kg						
d -14 (Pre-treatment)	125.9[a]	121.0[a]	10.5			
d 15	112.6[b]	107.7[b]	10.3	0.30	<0.0001	0.99
d 38	106.9[c]	102.4[c]	10.0			
CP, kg						
d -14 (Pre-treatment)	112.6[a]	110.1[a]	9.0			
d 15	103.4[b]	99.9[b]	9.0	0.36	<0.0001	0.89
d 38	99.0[b]	95.1[b]	8.7			
GE, Mcal						
d -14 (Pre-treatment)	1784.8[a]	1709.7[a]	161.6			
d 15	1580.2[b]	1504.2[b]	158.6	0.30	<0.0001	0.99
d 38	1491.2[c]	1421.4[c]	155.0			
Ash, kg						
d -14 (Pre-treatment)	38.7[a]	38.1[a]	4.6			
d 15	35.8[b]	34.6[b]	4.6	0.52	<0.0001	0.78
d 38	34.3[b]	32.8[b]	4.6			
Water, kg						
d -14 (Pre-treatment)	378.6[a]	370.8[a]	28.2			
d 15	349.8[b]	338.8[b]	28.0	0.36	<0.0001	0.89
d 38	336.1[b]	323.9[b]	27.3			
Water:Ash						
d -14 (Pre-treatment)	9.8	9.8	0.4			
d 15	9.8	9.8	0.4	0.96	0.41	0.84
d 38	9.9	9.9	0.4			
Water:CP						
d -14 (Pre-treatment)	3.36[a]	3.37[a]	0.02			
d 15	3.38[b]	3.40[b]	0.02	0.34	<0.0001	0.86
d 38	3.40[c]	3.41[c]	0.02			
NEI[6], Mcal/d						
d -14 (Pre-treatment)	15.77	16.63	2.07			
d 15	24.81	26.94	2.43	0.44	<0.0001	0.58
d 38	32.38	32.09	2.44			

NER[6], Mcal/d						
d -14 (Pre-treatment)	15.15	14.73	0.95			
d 15	35.90	36.04	1.83	0.87	<0.0001	0.90
d 38	36.09	35.65	1.75			
EB[6], Mcal/d						
d -14 (Pre-treatment)	0.62	2.01	0.97			
d 15	-11.09	-8.32	1.69	0.34	<0.0001	0.43
d 38	-3.72	-3.45	1.77			

[a,b]Treatment means within columns with different letters differ ($P < 0.05$).

[1]Body composition was determined using the urea dilution technique (Agnew et al., 2005).

[2]PG = Propylene glycol.

[3]Trtmt = Treatment, T × T = Treatment × Time interaction.

[4]BW = Body weight, Empty BW = empty body weight, CP = crude protein, GE = gross energy.

[5]Pretreatment values were not different ($P > 0.05$).

[6]NEI = net energy intake (calculated as average daily DM intake x the calculated NE_L value of the diet; for PG-fed cows, this included estimated energy contribution of PG), NER = net energy requirement (for pre-fresh cows, NER was calculated as net energy for maintenance [NE_M] + net energy for pregnancy [NE_P], where NE_M and NE_P [Mcal/d] where calculated using NRC [2001] equations. For lactating cows, NER was calculated as NE_M + net energy for lactation [NE_L]. Net energy for lactation (Mcal/d) was calculated as (9.29 x fat yield/d) + (5.71 x protein yield/d) + (3.95 x lactose yield/d), EB = energy balance (calculated as NEI – NER).

Besides failing to improve energy balance, supplementing 500 g of PG/d during the first 3 weeks post-partum did not reduce body condition loss (Moallem et al., 2007). Kokkonen et al. (2005) measured the depth of subcutaneous fat, *longissimus lumborum* muscle diameter and plasma 3-MH during the transition period and concluded that adding 1 kg of a glucogenic supplemement containing PG to the concentrate fed to transition cows does not reduce catabolism of body fat and protein. Feeding either 430 g or 860 g of glycerol/d as part of the TMR had no effect on BW and body condition loss at d -21, d 0 and d 21 relative to calving (DeFrain et al., 2004).

Across dietary treatments, empty BW and its components changed with time (Table 3.9). As expected, there was a reduction in BW at d 15 ($P < 0.01$) and d 38 ($P < 0.01$) compared to d -14. On average, cows lost 102 kg of BW from d -14 to d 38 relative to calving. This was lower than the average loss of 133 kg of BW, during the same period from 2 weeks pre-partum to 5 weeks post-partum as reported by Komaragiri and Erdman (1997) in cows of similar initial BW. Changes in empty BW followed the same pattern as BW and there was an average loss of 83 kg of empty BW. Body lipid content decreased ($P < 0.001$) by 19 kg from d -14 to d 38, which lies within the range of 15 to 60 kg of body fat mobilized within the first 8 weeks post-partum as reported by Chilliard et al. (1991). However, the estimated loss of 19 kg body fat was lower than in other studies (Komaragiri and Erdman, 1997; Komaragiri et al., 1998). The cows in the studies by Komaragiri and Erdman (1997) and Komaragiri et al. (1998) had higher initial lipid content ranging from 143 to 159 kg, compared to 123 kg in the present study; in addition, their milk production averaged 41 kg/d which was higher than the 36 kg/d observed in the present study. Body fat mobilization (rate and extent) is a function of body fat reserves and milk production, among other factors, as body fat mobilization

is positively correlated to body fat reserves at calving and milk production during the subsequent lactation (Komaragiri and Erdman, 1997). Therefore, the lower estimated loss of 19 kg in this study could be due to the smaller body fat reserves pre-partum and lower milk production post-partum compared to the studies by Komaragiri and Erdman (1997) and Komaragiri et al. (1998).

Body protein content was lower at d 15 compared to d -14 ($P < 0.001$). However, body protein content was only numerically lower ($P = 0.07$) at d 38 compared to d 15 (Table 3.9). Other studies (Tamminga et al., 1997; Komaragiri and Erdman, 1997) have shown similar results where body protein reserve depletion is restricted, with repletion occurring by week 5 post-partum, whilst body fat mobilization continues for an extended period. The different studies on body protein mobilization are not always in agreement in terms of whether body protein accretion or depletion occurs and the magnitude of the change in body protein reserves during early lactation (Komaragiri et al., 1998). The differences in these studies result from the differences in the amount and composition of diets the cows were fed and their level of production (Andrew et al., 1994). Andrew et al. (1994) found no changes in protein reserves during early lactation, which is in contrast to the loss of 21 kg during the first 5 weeks reported by Komaragiri and Erdman (1997). The estimated loss of body protein was 14 kg from d -14 to d 38 in the present study, and it was in agreement to the 12 kg of protein mobilized by week 5 post-partum as reported by Komaragiri et al. (1998).

Body gross energy (GE) content was higher at d -14 compared to d 15 ($P < 0.001$) and d 38 ($P < 0.001$), indicating mobilization of body fat and protein to meet nutrient demands for milk production as lactation progresses. The change in body GE content was - 291 Mcal, and it was lower than - 605 Mcal reported by Komaragiri and

Erdman (1997) during the same time period. However, the loss of body GE in this study is comparable to values by Chilliard et al. (1991), who calculated a loss of 258 Mcal in cows fed a high energy concentrate compared to 329 Mcal for cows fed a low energy diet during the first 8 weeks post-partum. The average milk production in the current study was 36 kg/d whilst it was 30 kg/d in the study by Chilliard et al. (1991), which were both lower than 41 kg/d reported by Komaragiri and Erdman (1997). Therefore, the magnitude of the energy deficit in dairy cows as dictated by such factors as nutrient intake and milk production will determine the amount of body reserves mobilized during early lactation.

Across dietary treatments, cows had a higher ash content at d -14 compared to d 15 ($P < 0.001$) and d 38 ($P < 0.001$; Table 3.9). The production of colostrum at parturition and the synthesis of milk components during the subsequent lactation result in deficits of calcium, phosphorus, magnesium and potassium during early lactation. Therefore, this mineral deficit during early lactation could have resulted in the mobilization of body minerals in the current study (Kume et al., 2001; Kume et al., 2003).

There has to be a constant relationship between empty body water, and empty body fat, protein and ash for predictions using the dilution techniques to be valid (Reid et al., 1955). Moulton (1923) reported that in mature cattle, the composition of the fat-free matter (water, protein and ash) remains relatively constant and, according to Reid et al. (1955), the fat-free matter composition is constant even in growing cattle. However, a considerable amount of animal factors affect the fat and fat-free matter composition in dairy cows and this poses a lot of challenges when trying to predict empty BW content and its components using the urea dilution technique (Agnew et al., 2005). Physiological

status is one of the main factors which can result in changes in composition of the fat and fat-free matter in dairy cows (Andrew and Erdman, 1995). Therefore, Andrew and Erdman (1995) stated that the changes in the fat-free matter in dairy cows caused by these various factors should be taken into account when using prediction equations developed from dilution techniques. The prediction equations used in this study were derived from a study by Agnew et al. (2005) and had been validated for use in lactating cows. The empty body water to CP ratio changed with time ($P < 0.05$), whilst the empty body water to ash ratio did not change with time ($P > 0.05$). The empty body water to CP ratio was highest at d 38 ($P < 0.05$) and lowest at d -14 ($P < 0.05$). Andrew and Erdman (1995) also reported an increase in the empty body water to CP ratio during early lactation in dairy cows and attributed this change to either the mobilization of body protein reserves or the increase in the volume of body water imposed by milk production. In the same study by Andrew and Erdman (1995), the empty body water to ash ratio did not change with physiological stage, which is in agreement with the current study.

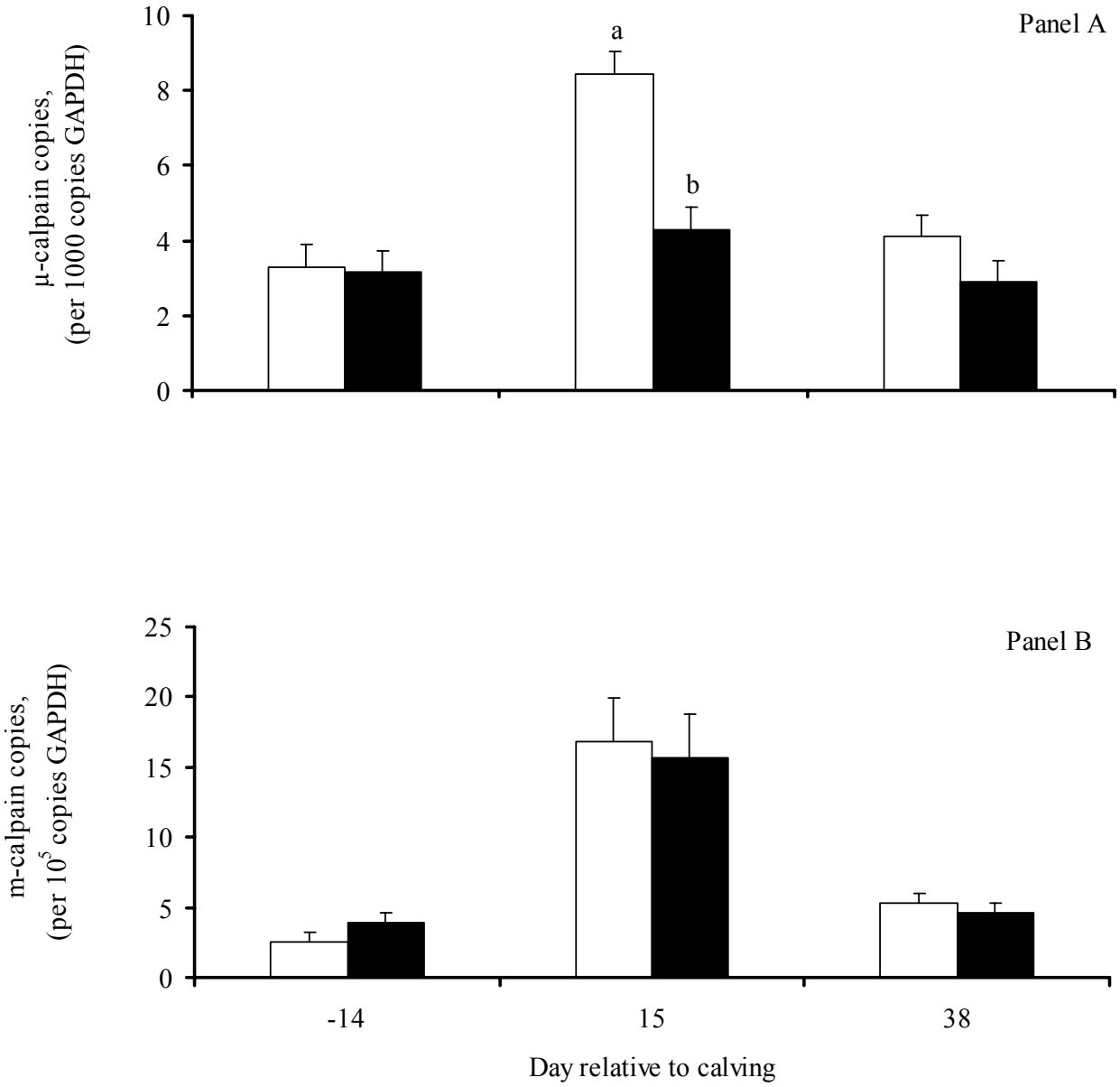
Across dietary treatments, pre-partum (d -14) NEI was slightly higher that NER; consequently, pre-fresh cows were in a slight positive energy balance (Table 3.9). After calving, cows were in a negative EB, which reached a nadir of -8.32 to 11.09 Mcal/d at d 15 (Table 3.9). Negative EB in early lactation is expected, as cows often fail to consume adequate DM to meet nutrient demands for milk production (NRC, 2001). Others (Rastani et al., 2001; Doepel et al., 2002) have reported EB nadir of -12.9 to -16 Mcal/d within the first 2 wk of lactation.

3.4.6 Gene Expression Profiles for the Major Protein Degradation Pathways

A major objective of this study was to determine the changes in the levels of mRNA encoding the Ca^{2+}-dependent proteases (μ- and m-calpain) and components of the multi-enzyme ubiquitin proteasome pathway (UPP) (ubiquitin, 14-kDa E2 and proteasome 26S subunit, ATPase) and the impact of PG on theses changes during early lactation. For over 50% of enzymes in cells, enzyme activity is modulated after transcription (Goll et al., 2007). However, despite the large number of studies on gene expression for the different proteolytic enzymes in different animal models, only a few studies has determined the correlation between mRNA abundance and protein activity, which is of importance given the post-trascriptional and post-translational modifications which can occur. An increase in μ- and m-calpain expression was coupled with an increase in calpain catalytic activity in raibow trout (Salem et al., 2004). In studies in rats under different catabolic states (Price et al., 1996; Bailey et al., 1996), the elevation of mRNA encoding ubiquitin and the proteasome subunits C3, C5 and C9, was associated with an increase in muscle protein degradation, thereby indicating that there is a positive correlation between gene expression and enzyme activity. However, the correlation between mRNA levels, and enzyme levels and enzyme activity for μ-calpain, m-calpain, ubiquitin, 14-kDa E2 and proteasome 26S subunit, ATPase in dairy cows is unknown. In the current study, although enzyme concentration and enzyme activity were not quantified, other variables which occur downstream of gene expression including changes in empty body protein content, N-balance and urinary 3-MH were determined.

As expected, expression of μ-calpain was up-regulated at d 15 (P = 0.005) compared to d -14 before being down regulated to pre-calving levels by d 38 (Figure 3.6).

Figure 3.6. Levels of mRNA for μ-calpain (Panel A) and m-calpain (Panel B) in cows fed the control (open bars) or control plus propylene glycol (PG) (solid bars) diets. Level of μ-calpain mRNA was lower at d 15 (P = 0.02) in cows fed PG compared to control cows. Across dietary treatments, levels of μ-calpain (P = 0.003) and m-calpain (P = 0.02) mRNA were greater at d 15 compared to d - 14 and d 38.

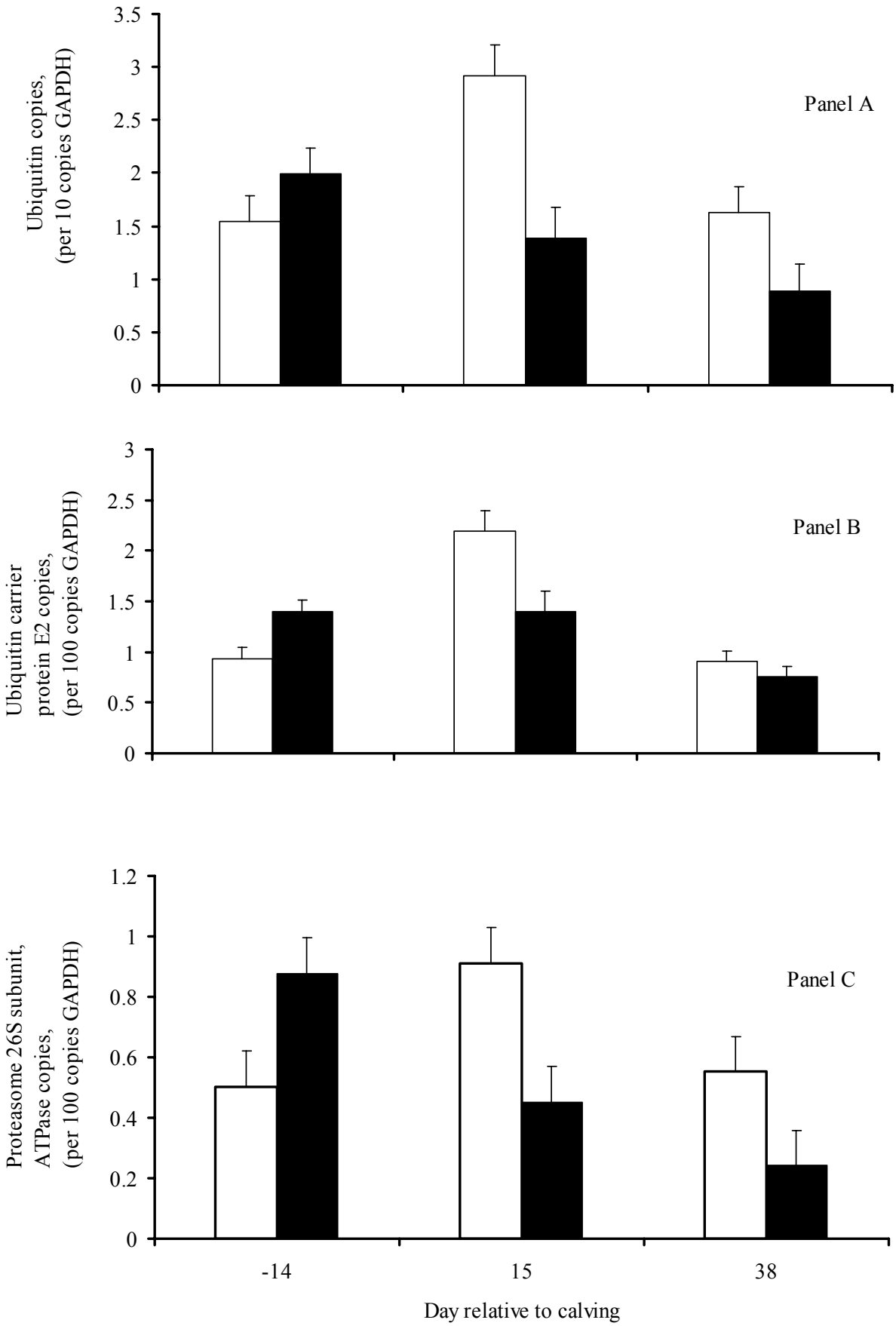


Similarly, m-calpain was also up-regulated at d 15 ($P = 0.02$) compared to d -14 before being down regulated to pre-calving levels by d 38 (Figure 3.6). For skeletal muscle wasting to occur, the complex myofibrillar structure has to be disrupted (Goll et al., 2007). μ- and m-calpain have been shown to break down the proteins that maintain the myofibrillar structure and, thus, are involved in the first step which leads to skeletal muscle wasting as they avail the myofilaments which are degraded by the UPP (Goll et al., 2007). Several studies have also shown an increase in μ- and m-calpain expression during muscle wasting in different animal models. There was an increase in mRNA levels for m-calpain in rats (Voisin et al., 1996) and humans (Mansoor et al., 1996), and both μ- and m-calpain in rainbow trout (Salem et al., 2004) during muscle wasting. The increase in μ- and m-calpain expression at d 15 relative to calving coincided with the elevation of urinary 3-MH excretion. 3-MH is a constituent of the muscle proteins, actin and myosin, and its excretion in urine is indicative of the breakdown of skeletal muscle (Harris and Milne, 1981). Nitrogen balance was also at its most negative at d 15 compared to d -14 and d 38 relative to calving which indicates the N deficit during early lactation. Therefore, the fact that there was an increase in μ- and m-calpain expression at a time when there was an elevation of urinary 3-MH excretion and N balance was at its most negative, is an indication that the nutrient deficit during early lactation led to the up-regulation of μ- and m-calpain. It is speculated that μ- and m-calpain have an important role in the homeorhetic response in transition dairy cows as upregulation of these genes results in an increase in skeletal muscle wasting so as to avail amino acids for milk production at a time when the dietary nutrient supply is inadequate to meet requirements.

Propylene glycol supplementation had no effect (P = 0.96) on m-calpain expression, but it lowered μ-calpain expression (P = 0.02) at d 15 relative to calving (Figure 3.6). Propylene glycol, which is a glucogenic precursor, could have improved the glucose balance leading to a reduced contribution of amino acids to hepatic gluconeogenesis and, thus, the possible sparing of skeletal muscle protein as reflected by the down-regulation of μ-calpain at d 15. However, other indirect measures of body protein breakdown, including m-calpain expression, N balance, urinary 3-MH excretion and changes in body composition did not show any reduction in body protein mobilization during early lactation.

There was a 22% increase (P = 0.63) in mRNA abundance for ubiquitin at d 15 compared to d -14 (Figure 3.7). However, mRNA abundance for ubiquitin tended (P = 0.07) to be lower at d 38 compared to d 15. Messenger RNA abundance for 14-kDa E2 was higher by 55% (P = 0.22) at d 15 than at -14. However, 14-kDa E2 abundance was lower at d 38 compared to d 15 (P = 0.005). Ubiquitination is the first step in a cascade of events, in which both ubiquitin and ubiquitin conjugating enzyme are involved, leading to the breakdown of proteins by the ATP-dependent 26S proteasome (Goll et al., 2007). In a study by Medina et al. (1995), an increase in ubiquitin mRNA expression was shown to lead to an increase in ubiquitin abundance and increased muscle loss in rats which had either been starved or had their soleus denervated. There was an increase in total and myofibrillar protein degradation, and an elevation of urinary 3-MH excretion, which was coupled with an increase in ubiquitin mRNA levels in rat skeletal muscle during sepsis (Tiao et al., 1994).

Figure 3.7. Levels of mRNA for ubiquitin (Panel A), ubiquitin-carrier protein E2 (Panel B), and proteasome 26S subunit, ATPase (Panel C) in cows fed the control (open bars) or control plus propylene glycol (PG) (solid bars) diets. Levels of ubiquitin mRNA tended to be lower ($P = 0.07$) at d 15 in cows fed PG compared to control cows. Levels of proteasome 26S subunit, ATPase mRNA tended to be lower ($P = 0.097$) at d 38 in cows fed PG compared to control cows. Across dietary treatments, levels of mRNA for 14-kDa ubiquitin-carrier protein E2 were lower ($P = 0.005$) at d 38 compared to d 15.


Ubiquitin copies, (per 10 copies GAPDH)
3.5
3
2.5
2
1.5
1
0.5
0
Panel A
Ubiquitin carrier protein E2 copies, (per 100 copies GAPDH)
3
2.5
2
1.5
1
0.5
0
Panel B
Proteasome 26S subunit, ATPase copies, (per 100 copies GAPDH)
1.2
1
0.8
0.6
0.4
0.2
0
Panel C
-14
15
38
Day relative to calving

Mansoor et al. (1996) also found an increase in muscle ubiquitin and 14 k-Da E2 mRNA expression in humans experiencing muscle wasting as reflected by a negative N balance, increased endogenous leucine flux (which is indicative of increased whole body protein loss) and elevated urinary 3-MH excretion. In the current study, the increase in ubiquitin expression at d 15 and numerically higher expression of 14-kDa E2 at d 15 compared to 38 coincided with the possible increased availability of myofilaments for breakdown due to the action of μ- and m-calpain. Therefore, the up-regulation of ubiquitin expression could have led to the increased tagging of proteins destined for breakdown by the UPP. This would have, ultimately, resulted in an increase in the contribution of skeletal muscle-derived amino acids to hepatic gluconeogenesis and milk protein synthesis during early lactation, which is supported by the observed negative N balance, elevated urinary 3-MH excretion and low body protein content during early lactation. Although the mRNA abundance of ubiquitin and 14-kDa E2 changed with time, there was no effect of time on proteasome 26S subunit, ATPase expression (Figure 3.7). All components of the ubiquitin-dependent system are involved in various steps that regulate this pathway, but their relative importance in these regulatory steps has not been elucidated. Therefore, the physiological importance of the lack of response in mRNA abundance for the proteasome 26S subunit, ATPase, in parallel with the changes in mRNA abundance for the other components of the system, is unknown. It is plausible that the lack of change in proteasome 26S subunit, ATPase expression with time could have been a result of how that gene is regulated. Despite finding an increase in gene expression of several components of the 26S proteasome in fasted mice, there were no changes in other components (Jagoe et al., 2002).

Supplementing pre- and post-partum diets with PG tended (P = 0.07) to down-regulate ubiquitin expression with time (Figure 3.7), as ubiquitin mRNA abundance tended to be reduced (P = 0.07) at d 15 relative to calving. Propylene glycol supplementation also tended (P = 0.06) to reduce proteasome 26S subunit, ATPase expression with time, as mRNA abundance for proteasome 26S subunit, ATPase tended to be lower (P = 0.097) at d 38. However, 14-kDa E2 expression was not reduced (P = 0.54) by PG supplementation. The tendency of PG supplementation in pre- and post-partum diets to reduce mRNA abundance for ubiquitin and proteasome 26S subunit, ATPase suggests that PG can possibly reduce the degradation of skeletal muscle protein by the UPP during early lactation. However, the possible skeletal muscle-sparing effect of PG is not consistent with our observations of accompanying indirect measures of body protein degradation.

4.1 GENERAL DISCUSSION

The first objective of this study was to determine the impact of supplementing PG in pre- and post-partum diets on nutrient intake and digestibility, blood metabolites, milk production and composition, body composition, whole-body N balance and urinary 3-MH excretion. Pre- and post-partum PG administration had no effect on nutrient intake and digestibility, blood metabolites, and milk production and composition. To my knowledge, this was the first study to determine the effect of PG on body composition, whole-body N balance and urinary 3-MH excretion in transition dairy cows. The glucose deficit during early lactation leads to an increase in oxidation of amino acids derived from the diet and maternal protein reserves. Our hypothesis was based on the assumption that provision of an alternative glucose source could reduce the glucose deficit during early lactation and, thus, spare amino acids derived from maternal protein reserves in high yielding dairy cows. Propylene glycol supplementation did not affect body composition, whole-body N balance and urinary 3-MH excretion. The lack of an effect of PG on body composition, whole-body N balance and urinary 3-MH excretion, which are indirect measures of body protein catabolism, indicated that the PG supplemented did not reduce mobilization of maternal protein reserves. The failure of PG to attenuate muscle wasting is supported by the fact that PG supplementation did not lead to an improvement in glucose status, although the plasma glucose concentrations in

the current study are just a snap shot of a very specific point in time and did not necessarily reflect whole body glucose production.

Failure of PG supplementation in changing gross indices of energy and protein metabolism in the current study could have been due to a number of reasons. During the transition period, the magnitude of the negative energy balance is large. Based on the intake and estimated energy density of the diet fed, the variation in energy intake in the current study was approximately 3.42 Mcal/d, which was greater than the estimated energy contribution of PG (2.825 Mcal/d). Therefore, the PG dose used in this study failed to substantially improve the energy status of the transition dairy cows given the magnitude of the negative energy balance and variation in energy intake. The lack of any beneficial response in energy balance in the current study could also be related to the method of PG administration, an intermediate dose, 600 mL of PG/d, was mixed into the TMR. In contrast to our study, Grummer (1994) and Butler et al. (2006) observed an improvement in the metabolic status reflected by an increase in plasma glucose and insulin and a decrease in plasma NEFA and BHBA when a similar dose of PG was orally drenched. Therefore, further studies which use a higher dose of PG which would potentially result in a substantial improvement in the glucose status of transition dairy cows should be carried out. The PG should also be orally drenched, as the efficacy of PG on improving metabolic status is higher when it is oral drenched compared to mixing it with the TMR (Cozzi et al, 1996; Christensen et al., 1997).

Prior to this study, although it was known that there is an increase in skeletal muscle catabolism during the transition period, the exact protein degradation systems involved in this process had never been delineated. The major contribution of the current study was characterization of the changes in mRNA levels for components of the Ca^{2+}-

dependent and ubiquitin-mediated proteolytic systems. Results from this study demonstrated the up-regulation of the Ca^{2+}-dependent and ubiquitin-mediated proteolytic pathways during early lactation, which was supported by the concomittant changes in body composition, whole-body N balance and urinary 3-MH excretion. The increase in urinary 3-MH excretion from d -14 to d 38 was accompanied by a decrease in empty body protein content, indicative of catabolism of skeletal muscle protein. The negative N balance during early lactation also indirectly showed that body protein was being catabolized. Therefore, the up-regulation of the Ca^{2+}-dependent and ubiquitin-mediated proteolytic pathways could be the mechanism by which skeletal muscle catabolism occurs in early-lactating cows. In the current study, only mRNA concentrations were determined. However, enzymes in cells can be controlled after transcription, and, a correlation might not exist between transcript abundance and enzyme activity. Therefore, further studies in which enzyme concentrations and most importantly enzyme activity are quantified would substantially add value to the transcript abundance data. The second part of the second objective was to determine if feeding PG, a gluconeogenic precursor, would reduce muscle wasting at the gene expression level. Despite having no effect on the other indirect measures of body protein catabolism, PG supplementation down-regulated some of the proteolytic pathways. The reason for this discrepancy is not known. However, given that transcript abundance measurement occurs at the molecular level, it could be more sensitive in detecting the changes in protein metabolism compared to the other indirect measurements, which occur at the organismal level.

4.2 GENERAL CONCLUSION

During early lactation, the observed decrease in body protein content, increase in urinary 3-MH excretion and negative N balance reflected an increase in body protein mobilization to support lactation. Propylene glycol supplementation did not attenuate muscle wasting as it had no effect on these indirect measures of body protein catabolism. μ- and m-calpain, and components of the ubiquitin-mediated proteolytic pathway were also up-regulated during early lactation, at a time when there was an increase in maternal protein catabolism. Therefore, up-regulation of μ- and m-calpain, and components of the ubiquitin-mediated proteolytic pathway is associated with catabolism of body protein in transition dairy cows. However, dietary supplementation with PG may down-regulate Ca^{2+}-dependent and ubiquitin-mediated proteolytic pathways, thereby potentially attenuating undesirable skeletal muscle wasting.

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