

## ABSTRACT

Title of Dissertation: A STUDY OF GLUCOSE METABOLISM AND KETOSIS DEVELOPMENT IN PERIPARTURIENT COWS USING A MECHANISTIC MODEL

Juen Guo, Doctor of Philosophy, 2005

Dissertation Directed By: Associate Professor, Richard A. Kohn,  
Department of Animal and Avian Sciences

Periparturient cows are susceptible to ketosis. An animal trial was conducted to evaluate the effect of a transition diet on production performance and ketone body (**KB**) accumulation. The transition diet was fed from 14 days before expected parturition to 14 days after calving. The energy density and nonstructural carbohydrate content in the transition diet was lower compared to the lactation diet, but higher compared to the non-lactating cow diet. Production performance was not affected by transition diet. Plasma glycerol may be an important contributor to gluconeogenesis during the periparturient period. Feeding a transition diet around parturition was associated with greater mobilization of adipose tissue and greater exposure to KB in early lactation.

Data from the animal trial were used to develop a mechanistic model to quantify the interrelationship between glucose and lipid metabolism in periparturient cows. The driving variables of the model were dry matter intake, feed composition, calf birth weight, milk production, and milk components. The response variables were

body fat content and concentrations of plasma glucose, glycerol, nonesterified fatty acids and total KB. Comparison of model predictions to data collected in an independent experiment revealed that the model over-predicted glucose and KB concentrations by 0.62 and 0.37 mM, respectively. Calf birth weight, dry matter intake, milk yield, and body condition score were increased by one standard deviation to estimate the model response in KB formation. The responses to the increases in the model parameters (e.g. the rate of fat mobilization) were evaluated to identify the likely critical control points in the animal. The model demonstrated that utilization rate of nonesterified fatty acids has a greater impact on KB concentrations in the first few days of lactation than the other parameters tested in the model. Glucose deficiency was closely related to the rate of fat mobilization. And, the excessive KB could result from elevated fat mobilization for glycerol to compensate for the negative glucose balance in periparturient cows. It is important to avoid overfeeding during the pre-lactation period to prevent ketosis development.

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PERIPARTURIENT COWS USING A MECHANISTIC MODEL

By

Juen Guo

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Advisory Committee:  
Professor Richard A. Kohn, Chair  
Professor Robert R. Peters, Co-Chair  
Professor Brian J. Bequette  
Professor Thomas W. Castonguay  
Professor Larry W. Douglass  
Professor Richard A. Erdman

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## List of Abbreviations

ACAC	acetoacetate
ADF	acid detergent fiber
ATP	adenosine triphosphate
AUC	area under the curve
BCS	body condition score
BHBA	$\beta$ -hydroxybutyrate
BW	body weight
CP	crude protein
DIM	days in milk
DM	dry matter
DMI	dry matter intake
KB	ketone bodies
mM	milli-molar (concentration)
Mol	mole(number, mass)
NDF	neutral detergent fiber
NEFA	nonesterified fatty acids
NEL	net energy for lactation
NSC	nonstructural carbohydrates
TMR	total mixed ration(s)
VFA	volatile fatty acids

# Chapter 1: Literature Review

## Introduction

Nutrition and management of cows during the transition period have received increased attention in recent years as researchers and field nutritionists have recognized the importance of this critical period. Health problems during the periparturient period can easily erase the entire profit potential of an individual cow in that lactation (Drackley, 1999). Usually, the transition period is defined as 3 weeks prepartum until 3 weeks postpartum. The primary challenge faced, even by healthy cows, is a sudden and marked increase in nutrient requirements for milk production, at a time when dry matter intake (**DMI**), and thus nutrient supply, lags far behind (Bell, 1995).

Failure to adequately meet this challenge, coupled with other stressors associated with parturition and adjustments to lactation, no doubt compromises lactation performance and may result in a range of health problems including ketosis, milk fever, retained fetal membranes, metritis and displaced abomasum. These diseases mainly affect dairy cow productivity in three ways: 1) by reducing reproductive efficiency, 2) by shortening the expected length of productive life (i.e., by increasing culling rate), and 3) by lowering milk yield (Rajala, 1998).

Among diseases found in the periparturient cows, the incidence of clinical ketosis typically ranges from 1.5 to 15 % of cows, but determining the incidences of subclinical ketosis is much more difficult. Herds with minimal intensity of ketosis detection usually report low incidence rates. And, estimates worldwide vary from 6 to 62% (Drackley, 1997).

The consequences of ketosis include lower milk yield, higher incidence of other disorders, increased reproductive problems, decreased herd life, and increased costs. In a 500-cow California dairy herd, Deluyker et al. (1991) found that occurrence of clinical ketosis during the first 21 days of lactation reduced peak milk yield by 2.7 kg per day and decreased milk yield by 253 kg during the first 120 days of lactation. Even subclinical ketosis may cause substantial losses of milk yield during early lactation (Andersson, 1988). Cows that develop ketosis are at an increased risk of developing other health problems (Borsberry et al., 1989; Cobo-Abreu et al., 1979; Dohoo et al., 1984; Erb et al., 1985). For example, ketosis was found to increase the risk for left-displaced abomasum by as much as 13.8 times (Correa et al. 1993). Reproductive performance also suffers as the degree of ketonemia and incidence of ketosis increase (Andersson, 1988; Gustafsson and Emanuelson, 1996).

## Definition of Ketosis

Ketosis refers to a condition in which ketone bodies (**KB**) accumulate to high concentrations in the blood of cows. Clinical ketosis is usually described subjectively as early lactation cows with diminished appetite, hard or dry feces, decreased milk yield, rapid weight loss, and elevated KB in urine, blood, milk, or breath. Subclinical ketosis can be defined objectively and can be reliably measured across herds. A definition of subclinical ketosis is ‘a condition marked by increased levels of circulating KB without the presence of clinical signs of ketosis (Duffield, 2000). A threshold concentration of 1.4 mM of  $\beta$ -hydroxybutyrate (**BHBA**) defines subclinical ketosis. Acetoacetate (**ACAC**) is also one of the major KB found in the blood. Blood concentrations of ACAC above 0.36 mM or the sum of ACAC plus BHBA of greater than 0.97 mM represents subclinical

ketosis. These cut-points are not very well defined. Each dairy farm defines ketosis a little bit differently, which makes it difficult to compare the incidence and prevalence of ketosis.

Ketosis is usually categorized into three types (Holtenius and Holtenius, 1996). Type I ketosis is classic, and is referred to as underfeeding ketosis. Cows that are 3 to 6 weeks postpartum are at their highest milk energy outflow, and they simply cannot keep up with energy demands because of some deficiency in nutritional management. Blood KB concentrations may become very high and blood glucose concentrations very low. Type II ketosis is named after type II diabetes mellitus, its metabolic counterpart. The concentrations of both insulin and glucose are high, and insulin resistance is also a characteristic of type II ketosis. Blood KB concentrations are not as high in type II ketosis as for type I. Type II ketosis is diagnosed in a dairy herd when a high incidence of subclinical or clinical ketosis is observed in cows in the first two weeks of lactation, combined with a high prevalence of elevated blood nonesterified fatty acid (**NEFA**) concentrations. Butyric acid silage ketosis is related to feeding ketogenic silages (Tveit et al., 1992). Hay crop silages that are chopped too wet tend to favor growth of *Clostridium* *sp.* bacteria, which ferment some carbohydrates to butyric acid instead the desired lactic acid. About 450 to 950 g of butyric acid will reliably induce clinical ketosis in nearly any early lactation cow (Oetzel, 2003). Each type has a different etiology, however, as there is overlap between the categories, and herds may have a combination of the three types. According to early literature, ketosis occurs most commonly during the third or fourth week after calving (Baird, 1982); however, in herds fed total mixed rations (TMR) nowadays, the greatest incidence is during the first two weeks after calving (Drackley,

1997). Because of the importance of the transition period as discussed previously, the current study focuses on the occurrence of ketosis in the first three weeks of lactation.

## Theories of Ketosis

Development of hepatic ketogenesis is related to 1) substrate (NEFA) supply to the liver, 2) the activity of carnitine acyltransferase I (EC 2.3.1.21) for promotion of fatty acyl-CoA entry into the mitochondria for acetyl-CoA synthesis and metabolism, and 3) the intra-mitochondrial activity of 3-hydroxy-3-methylglutaryl-CoA synthase (HMG-CoA synthase), which is the regulatory step in conversion of acetyl-CoA to KB (Hegardt, 1999). Various theories about ketosis development have remained unchanged over the years. One theory is that high rates of gluconeogenesis during the negative energy balance of the periparturient period enhance ketogenesis as a result of depletion of oxaloacetate from the mitochondria for cytosolic gluconeogenesis (Krebs, 1966). During ketosis less oxaloacetate would be available for condensation with acetyl-CoA, and, hence, more acetyl-CoA would be diverted towards KB formation (Baird et al., 1968). One objection to this theory for the regulation of ketogenesis is that it is the intra-mitochondrial, rather than whole-cell, concentration of oxaloacetate that is likely to be of importance in determining the rate of entry of acetyl-CoA into the citric acid cycle because the enzymes of this cycle are located within the mitochondria. The intra-mitochondrial concentration of oxaloacetate need not change necessarily in parallel with the whole-cell concentration.

Another theory is that regulation of ketogenesis could occur at the point of entry of NEFA into the mitochondria (Williamson, 1979). The entry rate of NEFA can be determined by the concentration of malonyl-CoA, which inhibits carnitine acyltransferase

I (EC 2.3.1.21). Malonyl-CoA is the product of the acetyl-CoA carboxylase (EC 6.4.1.2) reaction, and in the rat its concentration is proportional both to carbohydrate status and to the rate of lipogenesis (McGarry et al., 1977; 1978). However, it is unlikely that malonyl-CoA could be important in the intrahepatic regulation of ketogenesis in the cow, because the rate of lipogenesis is low in bovine liver (Ballard et al., 1969).

Another theory, arising from the classical principles of respiratory control, is that substrate oxidation and adenosine triphosphate (ATP) synthesis proceed only as fast as needed to supply ATP for endergonic reactions within the cell. Ketogenesis was proposed as a thermogenic process, in which long-chain fatty acids could continue to be metabolized to KB without being subjected to limitations by ATP turnover. Such a process in ruminants would help to explain why oxidation rates continue to increase in bovine hepatocytes as media NEFA concentration is increased (Cadorniga-Valino et al., 1997). However, support for this theory has not materialized, and the *in vivo* significance is unclear, even in rats (Berry et al., 1983). Furthermore, because the ratio of ACAC to BHBA increases as hepatic ketogenesis increases, it seems unlikely that this mechanism could be operative in ruminants (Heitmann et al., 1987).

Development of ketosis in cows in a fat condition may differ from the account described above. Because the hormonal environment in early lactation favors mobilization of adipose tissue, one might speculate that in fat cows the initial step in the etiology of ketosis is mobilization of an excessive quantity of NEFA. Appetite may be depressed in these fat cows so that negative energy balance develops, which in turn would lead to rapid mobilization of fat.

Although the above theories differ from each other in some ways, it has been widely accepted that milk production drives nutrient needs of dairy cows. Rapidly increasing milk production after parturition greatly increases demands for glucose for milk lactose synthesis, at a time when feed intake has not reached its maximum. An imbalance between the supply versus the demand for glucose may occur. In this case cows are likely to be in negative energy balance, which in turn leads to mobilization of fatty acids from adipose tissue. Oxidation of fatty acids provides energy, thus lessening the demand for glucose. Excessive mobilization of fatty acids causes an excessive rate of ketogenesis.

### Excessive Mobilization of Adipose Tissue

According to the theories discussed above, a suggested solution to ketosis would be the feeding of lipids to cows in early lactation in order to improve negative energy balance. Cows are in the most severe negative energy balance the first week following calving (Grummer and Carroll, 1991). However numerous trials indicate that supplemental fat is not as beneficial as one might predict for reducing ketosis (Grummer and Carroll, 1991; Chilliard, 1993; Ruegsegger and Schultz, 1985; Jerred et al., 1990; Hoffman et al., 1991; Schingoethe and Casper, 1991; Grummer et al., 1995).

Likewise, the above theories cannot give a reasonable explanation for excessive lipid mobilization. At day 4 postpartum cows were predicted to mobilize about 10.7 moles of NEFA from lipid tissue (Pullen et al., 1989). This amount of NEFA is the equivalent to approximately 30 Mcal/d, which is about 2.5 times the calculated negative energy balance of these cows. Uptake of NEFA by the liver probably exceeds hepatic demands for ATP synthesis in which case the liver would need to dissipate energy as heat



through peroxisomal pathway (Grum et al., 1994, 1996; Piot et al., 1998), to esterify the excess NEFA (Kleppe et al., 1988) or to convert it to KB (Hegardt, 1999). On the other hand, all the metabolic reactions occurring in a living creature are regulated to achieve balance and economy (Nelson, 1999). It is a paradox that cows do not mobilize as much fat as needed to meet this energy deficit in the periparturient period.

## Glucose Metabolism in Periparturient Cows

The answer to the above paradox may relate to the characteristics of ruminant glucose metabolism. Because much of the dietary carbohydrate is fermented in the rumen, little glucose is absorbed directly from the digestive tract. Dairy cows rely almost exclusively on gluconeogenesis in the liver to meet their glucose requirements. The rapid increase in milk production after parturition greatly increases the demand for glucose for milk lactose synthesis, and occurs at a time when feed intake has not yet reached its maximum. Limited feed intake during the early lactation means that the supply of propionate for glucose synthesis is limited. Supplying adequate glucose for milk synthesis is considered to be the greatest metabolic challenge to cows during early lactation (Drackley, 1993). Evidence from non-ruminant species indicates that the rate of hepatic ketogenesis from NEFA is determined both by the rate of NEFA supply and by the carbohydrate status of the liver (Williamson, 1979). Although data for effects of carbohydrate nutrition on the incidence of ketosis are still inconclusive (Grummer, 1995), most nutritionists agree that sufficient nonstructural carbohydrates (NSC) must be present to provide adequate energy, in the form of propionate, for glucose synthesis and to suppress synthesis of KB.

## Difficulties in studying Ketosis

Although researchers have attempted to establish biological relationships between nutrient status and increased KB in blood, the periparturient period is very poorly understood in comparison with our knowledge of cows during and after peak lactation. To date, factors that trigger the onset of ketosis and the mechanism of ketosis are still not clear. And there is a very small base of literature to make conclusions as to how to feed transition cows (Grummer, 1995).

Several factors contribute to this small knowledge base. Periparturient cows are in a homeorhetic state, which is characterized by non-steady state. Many metabolic events occur rapidly with most adaptations probably completed within about 20 days around parturition (Drackley, 1999). Measurements during this time, fraught with a high degree of variability, make analysis more difficult to detect differences statistically. In addition, the way in which experiments are designed and the results of those experiments are difficult to analyze. Currently almost all the experimental results published have dealt with either the average values across one week or month, or daily values at intervals of one week or month regardless of how quickly and rapidly metabolic events occur within the transition period. In doing so, a lot of dynamic and quantitative information is lost. Therefore, it is necessary to approach the complicated problems during the transition period with a quantitative and dynamic approach.

Because the transition period presents problems too complex for empirical approaches, a mechanistic model is needed. All the metabolic events and their interrelationships are described by mathematical expressions in the model. By running the model we are able to simultaneously consider all these events and their relationships,

and directly analyze flows of nutrients. Therefore, such a model could be used to identify the risk factors for development of ketosis, and to improve feeding management during the transition period.

## Conclusion

Nutrient imbalances occur during the transition period. The interaction between glucose and lipid metabolism is involved in ketosis development. The non-steady state must be considered in approaching the problem of periparturient ketosis.

Research is needed to further investigate the effect of NSC on ketosis development and the interrelationship between glucose and lipid metabolism in periparturient cows.

## Chapter 2: Effect of Transition Diet on Production Performance and Metabolism in Periparturient Dairy Cows

### Introduction

Suboptimal transition from the late gestation period to lactation can impair production and reproductive performance, and cause economic losses (Drackley, 1999). It has been suggested that feeding additional nonfiber carbohydrate before parturition may allow ruminal microorganisms to adapt to high concentrate diets and promote the development of ruminal papillae (Dirksen et al., 1985). The transition period is the most stressful time in the production cycle because of depressed feed intake, and endocrine and metabolic changes at parturition. Changing feed from the diet for non-lactating cow to the diet for lactation on the day of calving may exert additional stress on fresh cows. Therefore, it may be beneficial to feed periparturient cows with a transition diet, which has less forage than the diet for non-lactating cows, but more than the diet fed to cows in peak lactation. On the other hand, feeding diets with high energy density prepartum resulted in a greater decline in DMI as parturition approached (Minor et al., 1998). In addition, feeding a transition diet, with a lower energy density than the lactation diet, may exacerbate the period of negative energy balance when milk yield is increasing substantially in early lactation. The effect of transition diet on periparturient metabolism is not well documented in the literature, and there is little evidence to guide dairy producers on how to feed their periparturient cows.

Establishing clear guidelines requires a comprehensive understanding of biochemical events occurring during the transition period. The fetus and uterus demand

more glucose in late gestation and milk synthesis requires more glucose after calving (Bell, 1995). In addition, the depressed DMI and mobilization of adipose tissue in early lactation result in elevated concentrations of NEFA and KB in plasma. Glycerol is also released during lipolysis, and is a precursor for glucose. But the role of glycerol has not been studied as much as NEFA. Glycerol has been measured in only a few studies (Lomax and Baird, 1983; Reynolds et al., 2003), and explanations of those data were limited and based on qualitative information or extrapolation.

Most of available data describing the metabolism during transition in dairy cows are based on a few measurements obtained over a large interval of time such as a week or longer. The steady-state assumption behind those measurements does not hold in the periparturient period. This period is characterized by homeorhetic adjustments to accommodate parturition and lactation (Bauman and Currie, 1980), some of these adjustments occurring in a very short period of time, such as the sharp rise in blood glucose concentrations (Tucker, 1985). The non-steady state in transition cows is poorly characterized. Measurements of blood metabolites should employ more frequent sampling to capture the dynamic changes in the periparturient period. The objectives of this study were to characterize metabolism during the transition period, to study the glucogenic role played by glycerol, and to evaluate the effects of transition diet on periparturient metabolism and production performance.

## Materials and Methods

### Cows, Diets, and Treatments

The experiment was conducted at the University of Maryland Central Research Farm in Clarksville MD from August 22, 2004 until January 15, 2005. All procedures with animals were reviewed and approved by the University of Maryland Animal Care and Use Committee. Twenty eight multiparous Holstein cows were blocked according to parity and expected calving date and assigned at random to one of the two groups: treatment or control. From twenty eight days before expected calving, animals were fed a non-lactating cow diet (Table 2.1) for 14 d. One-half of the cows remained on the non-lactating cow diet as the control group, and the other half was fed a transition diet (Table 2.1) until 14 d after calving as the treatment group. A lactation diet was offered to control cows after calving and to treatment cows 14 d after calving.

### Measurements

Diets were fed once daily in the morning as TMR. The amount of TMR offered and refusals were measured daily. Samples of TMR were obtained on Monday, Wednesday, and Friday throughout the trial, dried at 55 °C for 120 h, ground through a Wiley mill (1-mm screen; Arthur H. Thomas, Philadelphia, PA), composited weekly, and analyzed for starch (STA-20, Sigma Chemical Co., St. Louis, MO), sequentially analyzed for neutral detergent fiber (**NDF**), acid detergent fiber (**ADF**), and lignin (Mertens, 2002), ash (550 °C for 24 h), NDF-CP and ADF-CP (Licitra et al., 1996), and CP (AOAC, 1990) on a dry matter basis. The non-lactating cows were weighed before feeding and the lactating cows were weighed after morning milking on Monday, Wednesday, and Friday.

Body condition score (**BCS**) was judged by two persons on Wednesdays on a five-point scale (where 1 = thin to 5 = fat) at 0.25 unit increments (Edmenson et al., 1989). Calf weights were recorded before first colostrum was fed.

Cows were milked twice daily, and milk production was recorded at each milking. Morning and evening milk samples were obtained on Monday, Wednesday, and Friday, and analyzed by Lancaster DHIA (Manheim, PA) for fat, protein, and total solids on a Bentley 300, milk urea nitrogen on a Bentley Chemspec 150 and by our lab for lactose (Bergmeyer et al., 1983).

Blood samples were taken by puncture of coccygeal vein/artery using 20-gauge needles and Vacutainer tubes containing sodium fluoride (Becton Dickson, Franklin Lakes, NJ) before and 3.5 h after morning feeding on Monday, Wednesday, and Friday. To justify the coccygeal vein sampling regime, the first 22 cows entering the study were fitted with a sterile jugular catheter (0.04 cm i.d. and 0.08 cm o.d.) on the Thursday between 5 and 12 days in milk (**DIM**); patency was maintained by 3.6 % citrate in physiological saline solution. On the next day, jugular blood was taken hourly from 7:00 h to 19:00 h. Samples were immediately placed on ice, and within 30 min, centrifuged at  $1000 \times g$  for 10 min. Plasma was stored on dry ice, and transported to the laboratory within nine hours. Upon arrival plasma samples were analyzed for ACAC (Harano et al., 1983). Acetone was determined by gas chromatography (model 6890; Agilent Technologies Inc., Wilmington, DE) in a 2-mm glass column packed with Carbowax 1176 (Supelco Inc., Bellefonte, PA). Helium was used as the carrier gas at a flow rate of 20.0 ml/min, and the injector, column, and detector temperature were 220, 60, and 200 °C respectively. The rest of split samples were stored at -25 °C until later

analyses for NEFA (NEFA-C kit Vaco Chemicals USA, Richmond, VA), glucose (kit – 510, Sigma Chemical Co., St. Louis, MO), glycerol (GY105, Randox, San Diego, CA) and BHBA (Harano et al., 1983). Intra-assay CV was less than 5 %.

### Statistical Analyses

The effects of days receiving the transition diet prepartum, and pretreatment body weight (**BW**) and BCS were initially analyzed as covariates, and the effects of expected due dates and parity initially were analyzed as block factors. The covariates and block factors were not significant and were subsequently excluded from the final model. The measurements were analyzed by analysis of variance using PROC MIXED (SAS, 1999). The statistical model was:

$$Y_{ijk} = u + P_i + T_j + C_{k(j)} + P_i \times T_j + \epsilon_{ijk}$$

where  $Y_{ijk}$  = observations for dependent variables;  $u$  = overall mean;  $P_i$  = effect of time: the last 17 days of gestation, the first 14 DIM, and from d 15 to d 21 postpartum;  $T_j$  = effect of treatment;  $C_{k(j)}$  = random effect of cow within treatment;  $P_i \times T_j$  = interaction between time and treatment;  $\epsilon_{ijk}$  = residual error. The covariance between residuals within cow was modeled as compound symmetry determined by goodness of fit measures.

The concentrations for coccygeal blood metabolites before and after morning feeding within a day were averaged to reduce daily variation. Area under the curve (**AUC**) for blood metabolites was calculated using trapezoidal rule (Jones, 1997). Agreement of sampling regimes between coccygeal and jugular veins was examined using the statistical procedures by Bland and Altman (1986). The maximum acceptable difference was defined as the upper limit of 95 % confidence interval for the difference



between the blood samples drawn from jugular and coccygeal veins at the same time. Significance was declared at  $P < 0.05$ , unless otherwise noted.

## Results

### Composition of Diets

Ingredient and chemical compositions of diets are presented in Table 2.1. The energy values of the diets were calculated to be 1.54, 1.71, and 1.77 Mcal/kg for non-lactating, transition, and lactation diets considering the discount factors based on total digestible nutrient intake above maintenance (NRC, 2001). The differences in energy densities between transition and lactation diets resulted from different high-moisture corn and corn silage contents in the two diets. Likewise, NFC and starch contents were greater in the lactation diet compared to the transition diet. All the other nutrients were consumed in quantities sufficient to meet NRC requirements (2001).

### Health and Calf Weight

Thirty cows were initially selected for the trial, but two cows from the treatment group failed to complete the trial. One cow on the transition diet was diagnosed with clinical ketosis at day 2 after calving, and administered i.v. with 1000 cc of 5 % dextrose at day 2 and 3 postpartum. Another cow received the transition diet for only 3 days before calving, and delivered twin calves. Unfortunately, the data from the ketotic cow could not be collected during the critical time points when ketosis was evident. The data from this ketotic cow and the one that calved early were not included in statistical analysis. The incidence of the other health problems for each treatment appeared to be in

a normal range, but could not be accurately assessed in a trial of this size. Calf weights at birth were not affected by the transition diet ( $P = 0.53$ ).

#### Dry Matter Intake, Body Weight, Body Condition Score, and Energy Balance

The average amount of time before calving was 17 days (minimum = 12 d; maximum = 26 d) for cows receiving the transition diet. Thus, the data from the last 17 days of gestation were used to determine prepartum treatment effect. Precalving DMI was greater for the treatment group compared with the control ( $P = 0.002$ ; Table 2.2; Figure 2.1). Postcalving DMI did not differ between the treatment and control groups while on treatment in the first 14 DIM ( $P = 0.44$ ) and after treatment from day 15 to day 21 of lactation ( $P = 0.23$ ). Likewise, animals fed the transition diet had greater energy intake during the prepartum period compared to animals in the control group (24.9 vs. 18.8 Mcal/d;  $P < 0.01$ ), but there was no difference postcalving ( $P > 0.10$ ). All the cows were in positive energy balance before calving and in negative energy balance after calving (Figure 2.2). The treatment cows had a greater energy balance prepartum (10.0 vs. 4.2 Mcal/d;  $P < 0.01$ ), and a lower negative energy balance in the first 14 DIM (-8.9 vs. -5.8 Mcal/d;  $P = 0.03$ ).

Initial BW and BCS were similar between the treatment and control groups ( $P > 0.10$ ; 758 (SE = 16.2) kg, and 2.76 (SE = 0.126) respectively). No treatment effect was observed for change in BW and BCS throughout the trial ( $P > 0.15$ ; Table 2.2).

#### Milk Yield and Composition

In the previous lactation, the 305-d mature-equivalent milk yields, and fat and protein percentage did not differ between the two groups ( $P > 0.10$ ), and were 4276 (SE =

838) kg, 3.92 (SE = 0.08) %, 2.98 (SE = 0.03) % respectively. No treatment effect was found for milk yield or milk components in the first 21 DIM ( $P > 0.05$ ; Table 2.3). The patterns of milk yield were similar between the treatment and control groups (Figure 2.3).

### Blood Metabolites

The concentrations of the glucose, NEFA, glycerol, ACAC, BHBA, and acetone at 0 and 3.5 h after morning feeding from coccygeal vein, and at each hour within 12 h postprandial from jugular vein were presented in Figure 2.4. The average concentrations of acetone at 0 and 3.5 h after morning feeding from coccygeal vein were different from the averages at each hour within 12 h postprandial from jugular vein ( $P < 0.05$ ). The averages of ACAC were also different between tail and jugular vein plasma samples ( $P < 0.05$ ). The differences between jugular and coccygeal vein blood drawn at the same time were presented in table 2.4.

The profiles of glucose concentrations in the control and treatment groups were shown in Figure 2.5. In both groups, plasma glucose concentrations increased at calving and then dropped dramatically after calving. In the last 17 days of gestation, AUC for glucose in the treatment group was greater than that in the control group ( $P < 0.05$ ; Table 2.5).

The NEFA concentrations in all cows remained relatively constant in late gestation, and began to increase as parturition approached. These concentrations declined for 10 days after parturition (Figure 2.5). The average NEFA concentrations in the third week of lactation remained elevated compared to the concentrations before 17 days relative to calving ( $P < 0.01$ ) in both groups. Cows in the treatment group had greater AUC for NEFA in the third week of lactation compared with the control group ( $P =$

0.004; Table 2.5). Changes in plasma glycerol concentrations followed similar patterns to those in NEFA (Figure 2.5). Feeding transition diets increased AUC for glycerol across the third week of lactation ( $P = 0.02$ ; Table 2.5). At day 3 postpartum, the average concentration of NEFA was 0.8 mM in the treatment group. About 11.8 mol of NEFA and 3.9 mol of glycerol could be released from lipid tissue according to the empirical model:

$$\text{Entry rate of fatty acid} = 130 + 0.732 \times \text{NEFA} \quad (R^2 = 0.49; \text{Pullen 1989})$$

where entry rate = entry rate of fatty acid in  $\text{mol} \times \text{h}^{-1} \times \text{BW kg}^{-1}$ ; NEFA = plasma concentration of NEFA in  $\mu\text{M}$ . Assuming all the glycerol was converted into glucose, about 354 g of glucose could be synthesized from glycerol by gluconeogenesis at day 3 postpartum.

Plasma concentrations of ACAC followed a similar pattern during the periparturient period as those of acetone and BHBA (Figure 2.5). The treatment group had greater AUC for ACAC than the control group in the first 14 DIM ( $P = 0.04$ ; Table 2.5) and in the third week of lactation ( $P = 0.04$ ). For all the cows, the ratios of ACAC and acetone to BHBA postpartum were greater than those prepartum ( $P < 0.01$ ). The increases in the ratios occurred between 5 d before and after calving (Figure 2.6).

## Discussion

### Production Performance

Feeding a diet high in NSC before calving resulted in increased DMI and energy intake in agreement with other studies (Rabelo et al., 2003; Keady et al. 2001; Holcomb et al. 2001; Minor et al. 1998). With high NSC content, ruminal dry matter

(DM) digestibility is higher allowing for faster ruminal absorption and evacuation and consequently higher DMI. The treatment cows in our study were in a more positive energy balance prepartum and a more negative energy balance postpartum compared with the control cows. The prepartum difference in energy balance resulted from the greater energy intake in the treatment group. The postpartum difference may result from the slightly higher milk yield in the treatment group.

A positive effect of increasing dietary energy density precalving on milk yield and milk constituents had been reported in some studies (Keady et al., 2001; McNamara et al., 2003). However such an effect had not been observed in other experiments (Holcomb et al., 2001; Mashek and Beede, 2000). Many factors may contribute to the ambiguity of the response such as variations in cow parity, BCS, basal diet, or the genetic potential of the cows (McNamara et al., 2003). Feeding a high-concentrate diet after parturition increased milk yield (McNamara et al., 2003). The response to postcalving concentrates also depended on the prepartum dietary energy density (McNamara et al., 2003). In the current study, the treatment cows received a diet with greater energy density precalving and lower energy density postcalving compared to the control cows. The application of the transition diet both before and after parturition may be the reason that no significant effect had been detected on milk yield and components in the present study.

#### Blood Samples from Coccygeal and Jugular Veins

Average concentration of a blood metabolite across a period of time within a day would be more representative than a single measurement at a specific time point. Blood sampling from the tail vein has become a widely-used technique because it

produces less disturbance, requires less restraint, and access is easier to obtain compared to jugular vein samples. Thus, the average concentration of blood metabolites from coccygeal vein at 0 and 3.5 h after feeding was used in the present study. To justify the representation, the averages of blood metabolite concentrations from the coccygeal vein at 0 and 3.5 h after feeding were compared to the averages of hourly concentrations across 12 hours of jugular vein samples, using the difference between coccygeal and jugular blood drawn at the same time as a reference. The difference in blood sampling regime between coccygeal and jugular vein is significant only for acetone and ACAC, which may be related to the complicated metabolism in peripheral tissues and to the relatively low energy requirements of the tail.

#### Area Under the Curve

Blood metabolites are highly regulated and coordinated to meet body requirements. However, blood metabolites, especially blood glucose, showed considerable variation after parturition. It is difficult to detect any treatment effect at any specific time point due to high variation associated with those measurements. In the present study, AUC, which is a measure of exposure to a blood metabolite across a period of time, was used to account for the non-steady state.

#### Blood Glucose

Cows in the treatment group had greater AUC for glucose before parturition than cows in the control group. Rukkwamsuk et al. (1999) reported that high energy diets increased concentrations of plasma glucose in the last week of gestation. However, Baird et al. (1980) infused propionate in multiparous non-lactating cows and observed no

change in blood glucose. The decrease in glucose concentration in the control cows during the last 21 d of gestation may result from fetal growth and mammary gland development. The increase in glucose concentrations at calving was observed in the present study as well as by Studer et al. (1993). Glucose concentrations at calving are mainly mediated by cortisol and glucagons (Tucker, 1985). Sampling time relative to calving may affect glucose concentration measurement. After calving, glucose concentrations were lower compared with prepartum concentrations in the treatment group, as has been reported before (Vazquez-Anon et al., 1994; Greenfield et al., 2000). However, this pattern was not observed in the control cows, probably because of the lower glucose availability before parturition.

#### Blood Nonesterified Fatty Acids and Glycerol

Feeding a transition diet lowered NEFA concentrations in the late gestation period (Minor et al. 1998). As parturition approached, NEFA concentrations increased (Dann et al., 1999), and peaked after parturition. The magnitude of adipose tissue mobilization after parturition was inversely related to the DMI before parturition (Holtenius et al., 2003). The NEFA concentrations in plasma reflect the rate of adipose mobilization (Pullen et al., 1989). In the current study, greater AUC for NEFA in the treatment cows indicated that less adipose tissue was mobilized in the control cows after calving. This decrease in fat catabolism could be explained by the lower energy density in the transition diet compared with the lactation diet. The slightly greater milk yield in the treatment group may be another explanation. Force feeding cows during the prefresh transition period reduced the magnitude of NEFA increase, but did not completely eliminate it (Bertics et al., 1992). These observations indicated that part of the plasma

NEFA is hormonally induced. Cows undergo tremendous endocrine changes during and immediately after calving. Most of those changes are also involved in adipose mobilization such as mediated by cytokines, catecholamines, estradiol, and somatotropin. In the present study no dietary effect on plasma NEFA was observed in the first 14 DIM possibly because the dietary effect was overwhelmed by the change in endocrine status. The dietary effect on NEFA was only found in the carryover period (the third week of lactation), perhaps because the effect of the endocrine changes weakened.

The treatment effect on plasma glycerol was also observed in the carryover period but not in the first 14 DIM probably for the same reason as for NEFA. Glycerol, as well as NEFA, released into the blood predominantly reflects fat mobilization. The potential need for glycerol as glucose precursor has been reviewed (Bell, 1995; Drackley et al., 2001). In the present study, concentrations of glycerol peaked immediately after calving, and remained high during the first 21 DIM compared to the prepartum concentrations. Measurements of liver pyruvate carboxylase (EC 4.1.1.31) mRNA indicated an increased gluconeogenesis in days following parturition (Greenfield et al., 2000). Reynolds et al. (2003) reported that net liver removal of glycerol increased greatly in early lactation. In the current study, approximately 354 g of glucose could come from glycerol via fat mobilization, which would account for 24 % of milk lactose synthesis at day 3 postpartum in the treatment group. This estimate agrees with Bell (1995) that approximately 15 to 20 % of the glucose demand may be provided by plasma glycerol at day 4 postpartum. Although all amino acids, except for lysine and leucine, are also gluconeogenic substrate, alanine and glutamine account for 40 to 60 % of the glucogenic



potential of all the amino acids (Bergman and Heitmann, 1978). However, pyruvate may be the ultimate source of the carbon skeleton for glutamine and alanine synthesis in vivo (Wolfe, 2001), and a majority of pyruvate is produced by glucose and propionate metabolism in ruminants. Therefore the contribution of glycerol may be critical to glucose metabolism during the first few days of lactation.

The greater AUC for glycerol in the treatment cows indicated that feeding a transition diet around parturition resulted in greater adipose mobilization compared with the control cows. Glucose and lipid metabolism are often studied in isolation. It has been widely accepted that fat mobilization in periparturient cows resulted from negative energy balance. At day 3 postpartum, the treatment cows were estimated to mobilize about 11.8 mol of NEFA from lipid tissue according to the empirical model by Pullen et al. (1989). This amount of NEFA is the equivalent of 33 Mcal/d, about 4.4 times the calculated negative energy balance of those cows. Any living creature is a self-regulating chemical engine, continually adjusting for maximum economy (Nelson, 1999). The explanation of why cows did not mobilize only as much fat as needed to meet energy requirements may relate to the characteristics of ruminant glucose metabolism. During the periparturient period, depressed DMI and increased fetal growth and milk synthesis could result in a severe negative glucose balance. Glycerol has several advantages over amino acids for gluconeogenesis. First, periparturient cows are already in negative protein balance, excessive gluconeogenesis from amino acids could further exaggerate protein deficiency. Second, glycerol enters into the metabolic pathway more closely to glucose than do glucogenic amino acids. Third, lipolysis results in elevated NEFA and KB, while deamination increases ammonia; comparatively ammonia is more toxic than KB to

animals. Finally, NEFA and KB can be used by many peripheral tissues to conserve glucose, whereas excessive ammonia can only be discharged via urea genesis which consumes more energy. The contribution of glycerol to gluconeogenesis is largely dependent on the amount of adipose mobilized (Drackley et al., 2001). The profiles of NEFA and glycerol in the current study indicated that glucose negative balance and maximum gluconeogenesis from glycerol occurred mainly in the first week of lactation. This is in agreement with the previous observation that hepatic glycogen storage, an indicator of carbohydrate status, began to replete by day 7 postpartum (Vazquez-Anon et al., 1994). Therefore excessive lipid mobilization could be caused, not by negative energy balance per se, but by negative glucose balance.

### Blood Ketone Bodies

In the first 21 DIM, the cows fed the transition diet had greater AUC for ACAC, and thus exposed to more KB and were more susceptible to ketosis compared with the control cows. The lower concentrate in the transition diet compared with the lactation diet may account for the larger AUC for KB in the treatment cows. The increases in KB concentrations in this study as well as in the others (Dorshorst and Grummer, 2002; Greenfield et al., 2000) are probably related to adipose mobilization and elevated hepatic metabolism when DMI is depressed (Dorshorst and Grummer, 2002).

The ratio of ACAC to BHBA increased as total KB increased (Menaha et al., 1967). The proportion of ACAC to BHBA is in equilibrium with NAD/NADH ratio in a reaction catalyzed by BHBA dehydrogenase (EC 1.1.1.30; Williamson et al., 1967). Conversion of NAD to NADH, mediated through glycolysis, may depend on glucose availability in the cytosol. When there is less glucose available for glycolysis, there

would be less NADH generated, which would increase the ratio of ACAC to BHBA. In ketotic cows, the ratio of ACAC to BHBA is high (Heitmann et al., 1987), and NAD/NADH is high in the cytosol. In the present study, the increase in ACAC/BHBA ratio implies that glucose deficiency developed within 5 d of parturition, and the cows remained in a negative glucose balance for the remaining of the experiment. However, a more rigorous testing of this hypothesis would require use of a mathematical model to quantify flow of metabolism in the periparturient cows.

## Conclusion

Production performance was not affected significantly by offering a transition diet. Plasma glycerol may be an important contributor to gluconeogenesis in the first few days of lactation. Feeding a transition diet around parturition is associated with more mobilization of adipose tissue and greater exposure to KB in the early lactation.

## Implications

Glucose and lipid metabolism in periparturient cows is characterized by non-steady state condition and is affected by many factors: feed intake, milk synthesis, fat mobilization, and ketogenesis. The complicated metabolic events and non-steady state condition make it difficult to integrate available information to better understand ketosis development. A mechanistic model capable of evaluating many kinds of data simultaneously and dynamically should be employed to study periparturient metabolism.

Table 2. 1 Ingredient and nutrient composition of diets offered. Non-lactating diet (offered 28 d prior to expected calving), transition diet (offered 14 d prior to expected calving through 14 DIM for the treatment group), and lactation diet (offered after calving).

Ingredient	Diets			of
	Non-lactating	Transition	Lactation	
	-----%			
Alfalfa-wheat silage	41.9	23.4	24.9	
Timothy hay	30.0	-----	-----	
Corn silage	27.9	41.7	18.7	
Corn, high moisture	-----	16.7	37.5	
Soybean 49	-----	7.5	7.5	
Soybean roasted	-----	6.7	6.7	
Salt and premix	1.2 <sup>1</sup>	4.0 <sup>2</sup>	4.0 <sup>2</sup>	
Chemical composition				
DM as fed	42.9	43.2	50.7	
NDF	53.1	35.2	29.9	
ADF	32.3	19.3	15.7	
Lignin	5.5	4.2	2.4	
CP	10.9	16.8	16.8	
NDF-CP <sup>3</sup>	0.26	0.28	0.27	
ADF-CP <sup>4</sup>	0.10	0.10	0.06	
Ether Extract	2.8	4.0	4.1	
Starch	9.2	25.1	28.6	
Ash	8.0	5.8	6.4	
NFC <sup>5</sup>	25.7	39.0	43.7	
NE <sub>L</sub> <sup>6</sup>	1.54	1.71	1.77	

<sup>1</sup> Contained 23.8 % CP, 5.0 % NaCl, 1.7 % P, 4.7 % Mg, 0.05 % K, 8 mg/kg Se, 330 KU/kg vitamin A, 82.6 KU/kg vitamin D, 2.6 KU/kg vitamin E.

<sup>2</sup> Contained 29.84 % CP, 5.36 % Ca, 6.1 % NaCl, 0.9 % P, 2.1 % Mg, 0.59 % K, 5.25 mg/kg Se, 130 KU/kg vitamin A.

<sup>3</sup> Neutral detergent insoluble crude protein.

<sup>4</sup> Acid detergent insoluble crude protein.

<sup>5</sup> Nonfiber carbohydrates. Calculated by difference: 100 - [(NDF - NDF-CP) + CP + Ether Extract + Ash].

<sup>6</sup> Calculated according to the NRC (2001).

Table 2. 2 Effect of a transition diet from 17 d prior to calving through 14 DIM on DMI, BW, BCS and energy balance.

	Prepartum <sup>1</sup>				Postpartum <sup>2</sup>				Carryover <sup>3</sup>			
	TRT <sup>4</sup>	CON <sup>5</sup>	SEM	<i>P</i>	TRT	CON	SEM	<i>P</i>	TRT	CON	SEM	<i>P</i>
Number of cows	14	14	-----	-----	14	14	-----	-----	14	14	-----	-----
DMI, kg/d	14.8	12.2	0.51	0.002	16.4	16.2	0.44	0.75	21.3	20.2	0.65	0.23
DMI, % BW	1.90	1.66	0.083	0.048	2.37	2.51	0.077	0.20	3.21	3.25	0.110	0.77
Energy intake <sup>6</sup> , Mcal/d	24.9	18.8	0.78	<0.001	28.1	28.7	0.77	0.63	37.6	35.7	1.16	0.26
Energy balance <sup>6</sup> , Mcal/d	10.0	4.2	0.89	<0.001	-8.9	-5.8	0.98	0.03	-6.5	-5.2	1.21	0.45
BW <sup>7</sup> , kg	780	746	22.3	0.29	698	650	22	0.13	672	629	22	0.17
BW change <sup>8</sup> , kg	9.4	4.0	6.95	0.57	-22.5	-25.7	6.10	0.71	-16.6	-10.1	3.5	0.20
BCS <sup>9</sup>	2.89	2.63	0.126	0.15	2.30	2.22	0.11	0.45	2.32	2.20	0.107	0.41
BCS change <sup>10</sup>	-0.08	-0.03	0.032	0.24	-0.38	-0.31	0.042	0.25	-0.11	-0.09	0.04	0.75

<sup>1</sup> Last 17 days of gestation period.

<sup>2</sup> First 14 days of lactation period.

<sup>3</sup> From d 15 to d 21 of lactation period.

<sup>4</sup> TRT = Treatment. The treatment cows were offered a transition diet in the last 17 days of gestation and the first 14 days of lactation, and a lactation diet from d 15 to d 21 of lactation.

<sup>5</sup> CON = Control. The control cows were offered a non-lactating cow diet in the last 17 days of gestation, and a lactation diet in the first 21 DIM.

<sup>6</sup> Calculated according to the NRC 2001.

<sup>7</sup> Prepartum: Mean BW from 3 wk precalving to calving; Postpartum: Mean BW from calving to 2 wk postcalving. Carryover: Mean BW in the third week of lactation.

<sup>8,10</sup> Prepartum: Difference between pretreatment and calving data; Postpartum: Difference between calving data and data at 2 wk postcalving; Carryover: Difference between the data at 2 wk postcalving and the data at 3 wk postcalving.

<sup>9</sup> Body condition score, 1=thin, 5=fat. Prepartum: Mean BCS at 3 wk precalving; Postpartum: Mean BCS at 2 wk postcalving. Carryover: Mean BCS at 3 wk postcalving.

Table 2. 3Effect of a transition diet from 14 d prior to expected calving through 14 DIM on calf weight, milk yield, and composition.

	Postpartum <sup>1</sup>				Carryover <sup>2</sup>			
	TRT <sup>3</sup>	CON <sup>4</sup>	SEM	<i>P</i>	TRT	CON	SEM	<i>P</i>
Number of cows	14	14	-----	-----	14	14	-----	-----
Calf weight, kg	44.6	43.5	1.26	0.53	-----	-----	-----	-----
Milk yield, kg/d	33.4	32.9	1.4	0.79	41.1	40.3	1.4	0.68
4 % FCM, kg/d	37.2	34.5	1.5	0.21	41.7	39.0	1.6	0.25
Fat, %	4.54	4.33	0.11	0.20	4.06	3.82	0.17	0.33
Fat, kg/d	1.57	1.43	0.07	0.17	1.69	1.53	0.08	0.19
Protein, %	3.70	3.49	0.11	0.13	2.94	2.87	0.06	0.39
Protein, kg/d	1.27	1.15	0.05	0.10	1.20	1.15	0.04	0.50
Lactose, %	4.24	4.16	0.05	0.31	4.48	4.31	0.07	0.11
Lactose, kg/d	1.47	1.37	0.06	0.23	1.85	1.74	0.07	0.31
SNF <sup>5</sup> , %	5.33	5.32	0.05	0.93	5.68	5.65	0.07	0.77
SNF, kg/d	1.84	1.75	0.07	0.41	2.33	2.28	0.09	0.69
MUN <sup>6</sup> , mg/dl	8.1	9.3	0.54	0.11	8.8	8.7	0.54	0.96

<sup>1</sup> First 14 days of lactation period while on transition or control diet.

<sup>2</sup> From d 15 to d 21 of lactation period while all cows on the same lactation diet.

<sup>3</sup> TRT = Treatment. The treatment cows were offered a transition diet in the last 17 days of gestation and the first 14 days of lactation, and a lactation diet from d 15 to d 21 of lactation.

<sup>4</sup> CON = Control. The control cows were offered a non-lactating cow diet in the last 17 days of gestation, and a lactation diet in the first 21 DIM.

<sup>5</sup> Solids, Non-Fat.

<sup>6</sup> Milk urea nitrogen.

Table 2. 4. The concentrations of glucose, NEFA, glycerol, ACAC, BHBA, and acetone from jugular and coccygeal vein drawn at the same timea.

	n	Acetone	ACAC	BHBA	Glucose	NEFA	Glycerol
Jugular, mM	22	0.10	0.06	0.44	2.92	0.47	0.04
Tail, mM	22	0.08	0.07	0.49	3.10	0.45	0.03
Difference, mM	22	0.02	0.01	0.05	0.18	0.02	0.01
Difference <sup>b</sup> , %	22	3	1	3	1	1	7
SED, mM	22	0.024	0.007	0.225	0.812	0.156	0.013

<sup>a</sup> From cows between 5 and 12 DIM. Blood was drawn before morning feeding.

<sup>b</sup> Difference calculated as (Jugular-Tail)/(Jugular/2+Tail/2) × 100 %.

Table 2. 5. Effect of a transition diet from 14 d prior to expected calving through 14 DIM on AUC for different time windows for glucose, NEFA, glycerol, ACAC, BHBA, and acetone.

Concentration <sup>4</sup>	Prepartum <sup>1</sup>			Postpartum <sup>2</sup>			Carryover <sup>3</sup>					
	CON <sup>5</sup>	TRT <sup>6</sup>	SEM	P	CON	TRT	SEM	P	CON	TRT	SEM	P
NEFA, mM	0.25	0.23	0.034	0.72	0.43	0.53	0.052	0.15	0.22	0.36	0.034	0.004
Glucose, mM	2.96	3.86	0.278	0.048	3.25	3.31	0.126	0.75	3.23	3.44	0.124	0.22
Acetone, mM	0.03	0.04	0.003	0.12	0.08	0.09	0.008	0.21	0.06	0.07	0.008	0.33
Glycerol, mM	0.02	0.02	0.002	0.27	0.03	0.03	0.003	0.18	0.01	0.02	0.002	0.03
BHBA, mM	0.32	0.39	0.035	0.17	0.45	0.54	0.043	0.16	0.38	0.46	0.037	0.14
ACAC, mM	0.03	0.04	0.004	0.15	0.06	0.08	0.007	0.04	0.05	0.08	0.009	0.04

<sup>1</sup> Last 17 days of gestation period.

<sup>2</sup> First 14 days of lactation period

<sup>3</sup> From d 15 to d 21 of lactation period.<sup>3</sup> From d 15 to d 21 of lactation period.

<sup>4</sup> Average concentrations calculated as AUC/d per period.

<sup>5</sup> CON = Control. The control cows were offered a non-lactating cow diet in the last 17 days of gestation, and a lactation diet in the first 21 DIM. Number of cows = 14.

<sup>6</sup> TRT = Treatment. The treatment cows were offered a transition diet in the last 17 days of gestation and the first 14 days of lactation, and a lactation diet from d 15 to d 21 of lactation. Number of cows = 14.



**Figure 2.1.** Dry matter intake around the time of calving (pooled SEM = 1.41 ) for cows fed non-lactating prepartum and lactation diets postpartum (control, ◆) or a transition diet from 17 d prior to calving through 14 DIM (treatment, ▲). Each dot represents the average of two contiguous days. The treatment group had greater DMI in the last 17 days of gestation than the control group ( $P = 0.002$ )

**Figure 2.2.** Energy balance around the time of calving (pooled SEM = 1.60) for cows fed non-lactating prepartum and lactation diets postpartum (control, ◆) or a transition diet from 17 d prior to calving through 14 DIM (treatment, ▲). Each dot represents the average of two contiguous days. The treatment cows had a greater energy balance in the last 17 days of gestation (10.0 vs. 4.2 Mcal/d;  $P < 0.01$ ), and a lower negative energy balance in the first 14 DIM (-8.9 vs. -5.8 Mcal/d;  $P = 0.03$ ) than the control cows.

**Figure 2.3.** Milk yield during the first 21 DIM (pooled SEM = 1.2 ) for cows fed non-lactating prepartum and lactation diets postpartum (control, ◆) or a transition diet from 17 d prior to calving through 14 DIM (treatment, ▲). Each dot represents the average of two contiguous days.

**Figure 2.4.** The concentrations of plasma glucose, NEFA, glycerol, ACAC, BHBA, and acetone at each hour throughout a 12-h period from jugular vein (◆) and at 0, 3.5 h after feeding from coccygeal vein (●) in lactating cows between d 5 and 12 DIM (pooled SEM = 0.13, 0.05, 0.004, 0.029, 0.054, and 0.026 mM for glucose, NEFA, glycerol, ACAC, BHBA, and acetone respectively). The average concentrations of tail

samples at 0, 3.5 h after feeding were different from those of coccygeal samples at each hour throughout a 12-h period for ACAC and acetone ( $P < 0.05$ ).

**Figure 2.5.** Change in plasma glucose, NEFA, glycerol, ACAC, BHBA, and acetone around the time of calving for cows fed non-lactating and lactation diets prepartum and postpartum (control, ◆) or a transition diet from d -17 through d 14 relative to calving (treatment, ▲). Each dot represents the average of two contiguous days (pooled SEM = 0.30, 0.08, 0.004, 0.0139, 0.071, and 0.0135 mM for glucose, NEFA, glycerol, ACAC, BHBA, and acetone respectively). In the last 17 days of gestation AUC for glucose in the treatment group was greater than that in the control group ( $P = 0.048$ ). In the first 14 days of lactation, AUC for ACAC in the treatment group was greater than that in the control group ( $P = 0.04$ ). Between day 15 and day 21 postpartum, AUC for ACAC, NEFA, and glycerol in the treatment group were greater than that in the control group ( $P < 0.05$ ).

**Figure 2.6.** Ratio of plasma ACAC to BHBA and acetone to BHBA around the time of calving for cows fed non-lactating and lactation diets prepartum and postpartum (control, ◆) or a transition diet from d -17 through d 14 relative to calving (treatment, ▲). Each dot represents the average of two contiguous days (pooled SEM = 3.0, and 2.5 respectively). For all the cows, the ratios of ACAC and acetone to BHBA postpartum were greater than those prepartum ( $P < 0.01$ ).

Figure 2. 1 Dry matter intake around the time of calving

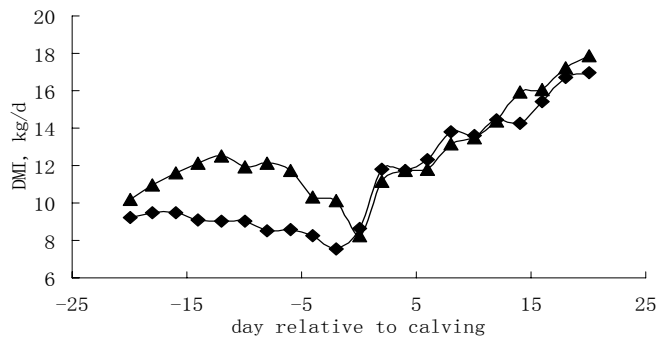


Figure 2. 2 Energy balance around the time of calving

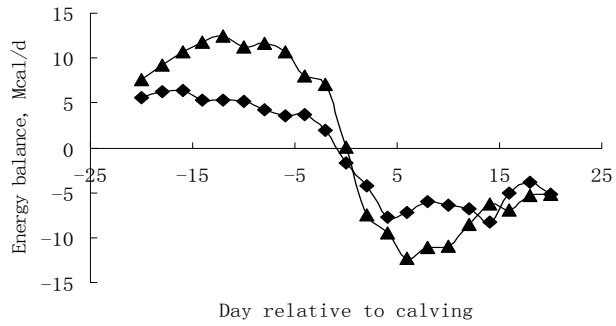


Figure 2. 3 Milk yield during the first 21 DIM

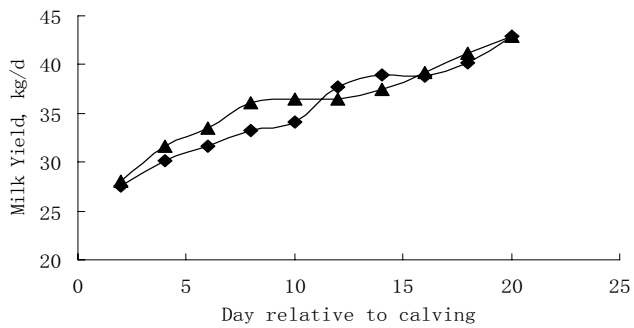


Figure 2. 4 The concentrations of plasma glucose, NEFA, glycerol, ACAC, BHBA, and acetone from jugular vein and coccygeal vein

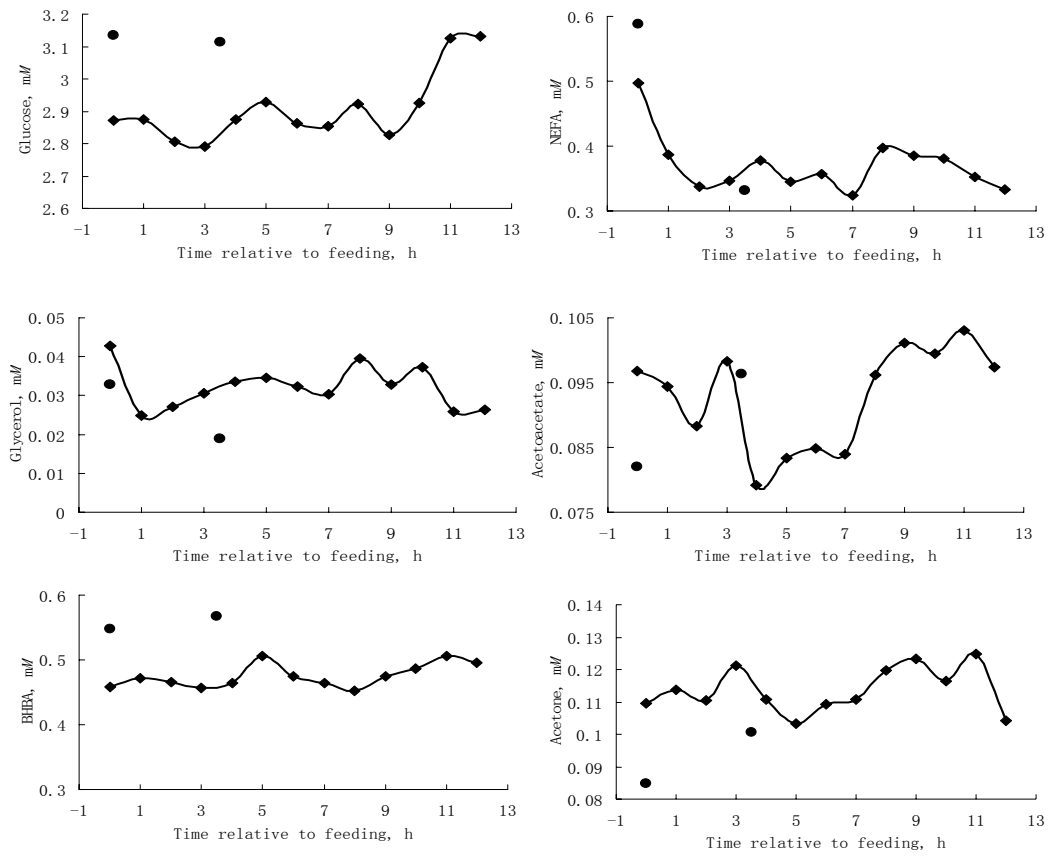


Figure 2. 5 Change in plasma glucose, NEFA, glycerol, ACAC, BHBA, and acetone around the time of calving

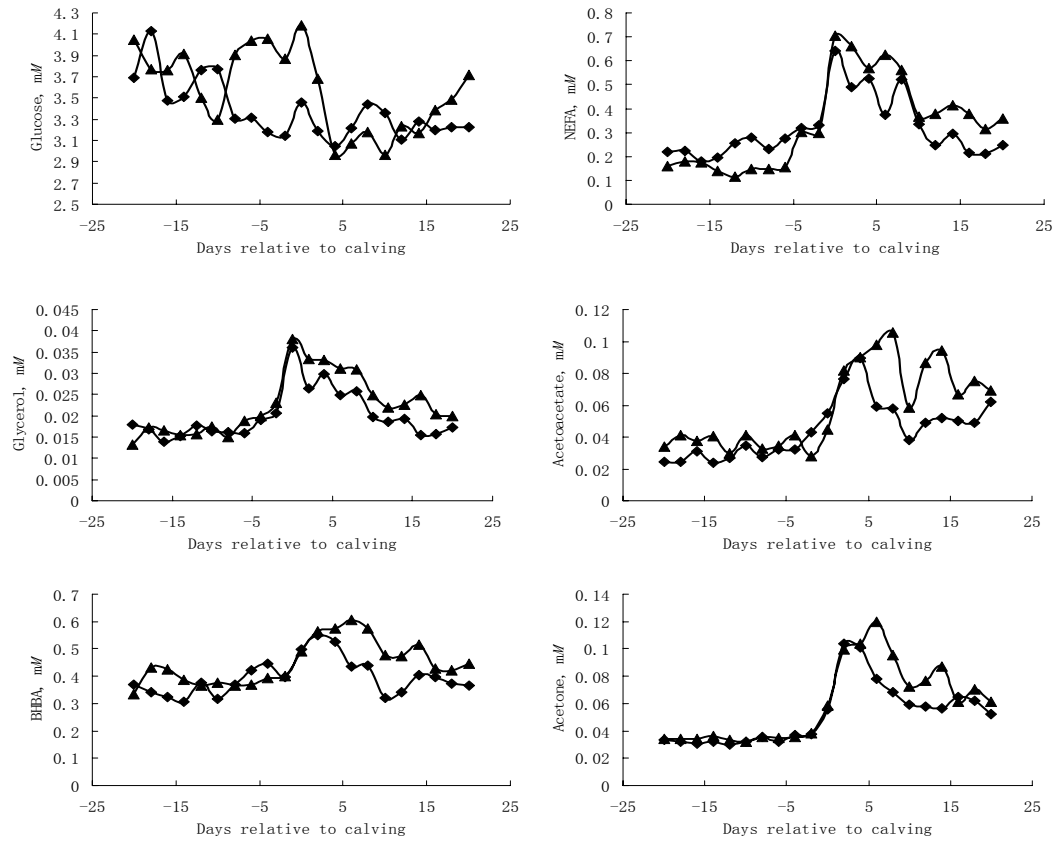
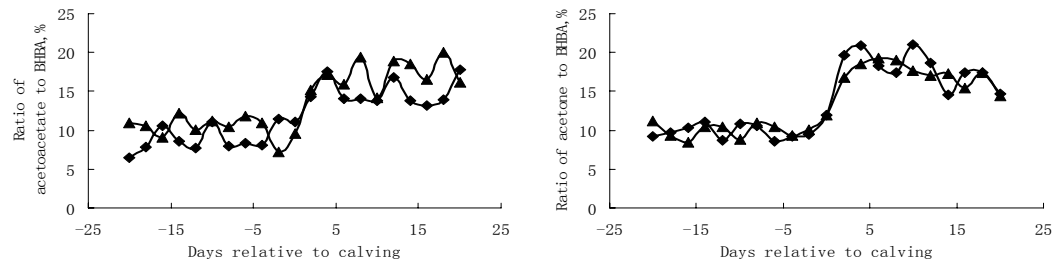


Figure 2. 6 Ratio of plasma ACAC to BHBA and acetone to BHBA around the time of calving



## Chapter 3: Modeling Glucose and Lipid Metabolism in Periparturient Cows

### Introduction

Ketosis causes economic losses in dairy herds directly by decreasing milk production and indirectly by increasing the risk for other periparturient diseases. Available data indicate that ketosis is closely related to fat mobilization and energy balance. However, cows mobilize more adipose tissue compared to the calculated energy balance (Bell, 1995), and fat supplementation may increase NEFA release from adipose tissue (Grummer and Carroll, 1991; Chilliard, 1993), and did not affect plasma BHBA (Skaar et al., 1989). On the other hand, the demand for glucose is increased greatly by fetal growth, development of mammary gland, and milk synthesis while DMI is depressed around parturition. Glycerol may be an important contributor to glucose synthesis during the periparturient period (Guo et al., submitted). Administration of propylene glycol, a glucose precursor, decreased NEFA and BHBA concentrations (Studer et al., 1993). Thus, ketosis development may result from the interaction between glucose and lipid metabolism, but not from energy deficiency per se. We hypothesize that fat mobilization is related to glucose deficiency, and excessive KB could result from elevated fat mobilization for glycerol to compensate for the negative glucose balance in periparturient cows.

The contribution of glucose and lipid metabolism to the energy economy of the animal is highly variable and is affected by many factors: feed intake, body fat

reserve, milk synthesis, maintenance energy requirements, and energy balance (McNamara, 1991). Although researchers have attempted to understand biological relationships between nutrient status and KB concentrations in the blood, the cause of ketosis is still poorly understood to date, not only because of the extremely complicated metabolism but also because of the homeorhetic state of periparturient cows. The complex interaction of metabolic events makes it difficult to integrate available information to study the mechanism of ketosis. In addition, homeorhesis, characterized by a non-steady state, results in great variations associated with the measurements during the periparturient period. These two factors have led to a need to define the timing, magnitude, rate, and regulation of glucose and lipid metabolism simultaneously in periparturient cows. For these reasons, we developed a dynamic, mechanistic model to quantify flow of metabolites in the periparturient cow.

The objectives of this study were to test the hypotheses: 1) fat mobilization in periparturient cows is equivalent to glucose deficiency; and 2) excessive KB may result from elevated fat mobilization for glycerol to compensate for the negative glucose balance.

## Materials and Methods

### Data

The developmental data set used to parameterize the model was described previously (Guo et al., submitted). Twenty eight multiparous Holstein cows were fed a non-lactating cow diet prepartum and a lactation diet postpartum. One half of

the cows were used as the control group. A transition diet was fed to the other half as the treatment group for 14 d prior to expected calving date until 14 d postcalving. On the day of calving, cows undergo a dramatic stress and change in endocrinal status. Nutrient requirements for the fetus and for labor are not known. Thus the data on the day of calving were not included in the model parameterization.

### The Model

A representation of the model is shown in Figure 3.1 and Tables 3.1 and 3.2. The driving variables were dry matter intake, feed composition, calf birth weight, milk production, and milk components. The response variables were body fat content and the concentrations of plasma glucose, glycerol, NEFA and KB.

***Response Variables.*** The glucose concentrations depended on the rate of glucose synthesis from glycerol, the rate of glucose utilization by peripheral tissues, and the rates of gluconeogenesis from propionate and protein, and the rates of glucose utilization for fetal growth and milk synthesis (Table 3.2). Removal of glycerol by the liver (Reynolds et al., 2003) and gluconeogenesis (Greenfield et al., 2000) increased greatly following parturition. The model assumed that glycerol released from fat mobilization was completely utilized for glucose synthesis. Glucose consumed by peripheral tissues was expressed as a function of glucose concentration in plasma and metabolic body weight (Table 3.2). Glucose consumed by peripheral tissues depends on the three factors: endocrine status, tissue sensitivity to hormones (Pettersson et al., 1993) and glucose availability (Yki-Jarvinen et al., 1987). The exact mathematical relationship between the glucose consumption and



the three factors is not known. An arbitrary equation was selected to approximately represent that relationship in the model (Table 3.2). In the equation, the dependence of peripheral consumption on glucose availability was defined as the ratio of plasma glucose concentrations to a reference concentration (3.15 mM). The power 'Q' to that ratio was assigned to 3 prepartum and to 4 postpartum. The biological meaning of the power 'Q', which is not known, is presumably related to endocrine status and tissue sensitivity to hormones.

The glycerol concentrations depended on fat mobilization and the rate of gluconeogenesis from glycerol in the model. Lipogenesis in adipose tissues is inhibited in periparturient cows, and glycerol released by lipolysis could not be reutilized in situ (Chilliard, 1993). Thus, glycerol used for lipogenesis in adipose tissues was not accounted for in the kinetics of glycerol concentrations, but was considered glucose consumption by peripheral tissues in the model.

Total body fat (kg) depended on the rate of adipose mobilization (kg/d per kg BW) and glucose deficiency (ratio glucose supply from feed to glucose demand per day) in the model. Adipose tissue in dairy cows is highly regulated and has been developed to support the physiologic states of pregnancy and lactation so that nourishment of the young can be ensured (Pond, et al., 1989). Toward the end of gestation, the dominant state becomes catabolism and is accompanied by rapid loss of stored lipid in early lactation (McNamara, 1991). As the concentrations of glycerol and NEFA began to increase around d 7 prepartum (Guo et al., submitted), the model assumed that lipolysis and lipogenesis were in equilibrium before d 7

prepartum, and lipogenesis had completely ceased in adipose tissues after d 7 postpartum. The metabolism of adipose tissues is also highly related to energy balance (McNamara, et al., 1987). However fetal growth and milk synthesis cannot take direct advantage of energy from NEFA. As energy balance involves lipid and glucose metabolism, negative energy balance may not be as accurate as a metabolic description as negative glucose balance. A negative relationship between glucose deficiency and lipid mobilization could exist during the periparturient period. Thus, the change in body fat was assumed to depend on glucose deficiency termed as the ratio of glucose demand to supply in the model.

The change in plasma NEFA was expressed as a balance between its utilization and fat mobilization in the model. Uptake and oxidation of NEFA by the liver and extrahepatic tissues are directly related to plasma concentrations (Pethick et al., 1983). Thus, the model assumed the rate of NEFA utilization (mmol/d per mM NEFA concentration) followed first order kinetics. Acetate from rumen fermentation may enter fatty acid synthesis as acetyl-CoA or through carboxylation to malonyl-CoA; alternatively, it may enter the citric acid cycle through condensation with oxaloacetate. Adipose tissues and mammary gland are the main sites of lipogenesis in dairy cattle (Beita and Nizzi, 1997). The fatty acids from de novo synthesis are incorporated into triglyceride, not directly released into plasma in the form of NEFA. Plasma NEFA concentrations are not influenced by plasma acetate concentrations (Sato et al., 1999). Lipogenesis was assumed to be in equilibrium with lipolysis before d 7 prepartum, and had completely ceased in adipose tissues after d 7

postpartum in the model as discussed previously. Therefore, the effect of the acetate from rumen fermentation on fat mobilization and NEFA concentrations was not considered in the model.

The KB concentrations depended on the ketogenesis rates from NEFA and butyrate (mmol/d per mM NEFA or butyrate concentration), and the rate of KB utilization (mmol/d per mM KB concentration). The equation of Pullen et al. (1989) relating oxidation rate and plasma concentration of NEFA predicts the rapid oxidation of approximately 35 % of NEFA entry rate. Mammary uptake of NEFA accounts for an additional 17 % of NEFA turnover (Bell, 1995). It is likely that a considerable fraction of mobilized NEFA is diverted to ketogenesis. Hepatic ketogenesis is regulated by substrate supply to the liver, and by the activities of ketogenic enzymes (Hegart, 1999). Given that the activities of ketogenic enzymes are not limited in healthy cows, the model assumed that 50 % of NEFA was incompletely oxidized to KB by the liver. Britton and Krehbiel (1993) infused different concentrations of butyrate and found that 14 % of the butyrate was metabolized into KB. In the model, 14 % of butyrate from rumen fermentation was assumed to be converted into KB by rumen epithelium. The rate of KB utilization is proportional to their circulating concentrations (DeFronzo and Ferrannini, 2001), and was represented as a first order reaction in the model.

***Model Structure.*** The highest priority for glucose requirement was assigned to milk synthesis and fetal growth in the model. What is known supports this assumption that during pregnancy the flow of nutrients across the placenta and

into the developing mammary gland are under control of hormones from the conceptus instead of the dam (Bauman and Currie, 1980). Glucose utilization by peripheral tissues was reduced in late gestation and early lactation (Bauman and Elliot, 1983). Uterine uptake accounted for approximately half of maternal glucose supply (Bell, 1995). Mammary glucose uptake on the day after parturition was nine times that on d 7 to 9 prepartum (Davis et al., 1979).

Maternal strategies for accommodating the glucose requirements for conceptus and mammary gland include the changes in both glucose and lipid metabolism (Bell, 1995). Periparturient cows mobilized much more adipose tissue compared to the calculated energy balance. The explanation of why cows did not mobilize only as much fat as needed to meet energy requirement may be related to the characteristics of glucose metabolism in ruminants. During the periparturient period, depressed DMI and increased fetal growth and milk synthesis could result in a severe negative glucose balance. Glycerol has several advantages over amino acids for gluconeogenesis (Guo et al., submitted). Excessive lipid mobilization could be caused not by negative energy balance per se, but by negative glucose balance. In the model, the hypothesis on the interrelationship between glucose and lipid metabolism was that glucose deficiency could lead to a mobilization of adipose tissue to supply the needed glucose precursor – glycerol; concurrently fatty acids would be released into blood and predispose cows to ketosis.

***Parameters Derived From Published Literature.*** The rate of propionate production from rumen fermentation was calculated by DMI and feed composition

(Murphy et al., 1982; Table 3.2). The rumen adaptation index in the model represented the shift to grain fermentating in rumen from the high-forage diet of the non-lactating period. The propionate-producing microbes are assumed to increase when the transition or lactation diet is fed. It takes about 10 days for a cow to adapt to a high-grain diet (Coe et al., 1999). The shift depends mainly on two factors: substrate availability, and microbial population. The index was derived from the logistic growth equation (Chanter, 1976):

$$dW/dt = k \times W \times S$$

where  $W$  is microbial population at time of  $t$ ;  $k$  is a growth rate constant;  $S$  is substrate availability for microbial growth, which after integration and rearrangement gives

$$W = W_0 \times W_f / (W_0 + (W_f - W_0)^{-ut})$$

where  $W_0 = 1$ ;  $W_f = 100$ ;  $u = 0.95$ . The values for  $W_0$  and  $W_f$  were arbitrarily assigned only to represent the change in propionate-producing microbes. The coefficient  $u$  corresponds to the rate of adaptation of rumen microbes to the high-grain diet, and it was assigned the value 0.95 which corresponds to a 10-day adaptation period. In reality, some of the propionate is metabolized by rumen epithelial, and some starch may escape from rumen fermentation and be absorbed in the form of glucose. As the two factors counteract each other, the contribution of the escape starch to glucose production was not considered and all the propionate was assumed to be converted into glucose by the liver in the model.

The rate of butyrate production was estimated in the same way as the rate of propionate production in the model (Table 3.2).

The rate of gluconeogenesis from protein was estimated by the amount of catabolized protein. Catabolism of 100 g of protein was assumed to give rise to 58 g of glucose (Dukes, 1993). Before parturition, one third of metabolizable protein was catabolized (NRC 2001) assuming that all the metabolizable protein came from feed. After parturition the cows were in a state of negative protein balance, and some of the catabolized protein came from endogenous sources. The rate of catabolized protein postpartum was estimated by urinary nitrogen excretion as:

Urinary Nitrogen (g/d) =  $0.026 \times \text{BW (kg)} \times \text{Milk Urea Nitrogen (mg/dl)}$  (Kohn et al., 2002).

The rate of glucose utilization for fetal growth was derived from the energy requirement which was estimated from the calf birth weights according to NRC (2001). In the non-lactating period, about 0.775 of 2.336 Mcal/d of energy required for fetal growth came from glucose and lactate (Bell, 1995). Presumably, one third of the energy requirement was provided in the form of glucose.

Mammary glucose requirement was estimated by milk yield and lactose concentrations. Lactose production in milk accounted for 55 to 78 % of the glucose uptake by the mammary gland (Cant et al., 1993; Mackle et al., 2000). In the model the efficiency of glucose uptake for lactose synthesis was assumed to be 66.5 % which is the average of 55 and 78 %.

***Parameters Determined By Best Fit.*** The parameters listed in Table 3.3 were optimized by a modified Powell Algorithm for each individual cow to find the minimum sum of squares for deviations between observed data and model predictions (Scientist, 1995). The model predictions for the response variables were calculated using the DMI, feed composition, calf birth weight, milk yield, and milk components from the development data set as the model input. The initial values for the response variables were adapted from the pretreatment measurements from the developmental data set. Two criteria (Neal et al., 1983) were applied in parameterizing the model. First the predictions of the model must approximate observed data. Second, biological realism should not be violated.

### Statistical Analysis

In the analysis for mean bias, linear bias, and RMSPE, the mean parameter values for all cows (Table 3.3) in the developmental data were used to calculate model predictions, compared against observations. A mean bias for model predictions was declared if residual (observed – predicted) values were significantly different from zero. Linear bias for model predictions was evaluated by regression analysis for residuals against model predictions, and was declared significant if

$$\text{Max} (|B(P_{\min} - P_{\text{ave}})|, |B(P_{\max} - P_{\text{ave}})|) \leq 1.95 \times \delta_e \text{ (St-Pierre, 2004)}$$

where B is the slope of residual regression against model predictions;  $P_{\min}$ ,  $P_{\max}$ , and  $P_{\text{ave}}$  are the minimum, maximum, and mean of model predictions respectively; and  $\delta_e$  is standard error of means from published literature. Root mean square prediction error (**RMSPE**) was calculated from the following equation (Bibby et al., 1977):

$$\text{RMSPE} = \text{square root of } (\Sigma (\text{observed} - \text{predicted})^2 / n).$$

Statistical significance was declared at  $P < 0.05$ .

## Results

Parameter values are shown in Table 3.3. The rates of fat mobilization and KB utilization postpartum were significantly greater than those prepartum ( $P < 0.05$ ,  $P < 0.01$  respectively).

Residual analysis for the developmental data was conducted by comparing model predictions with residuals (observed – predicted) for the response variables (Figures 3.4 and 3.5). The model over-predicted glycerol concentrations with a mean bias of 0.001 mM ( $P < 0.05$ ; Table 3.4). Linear biases for glycerol and KB predictions were observed ( $P < 0.05$ ). No mean bias or linear bias was found for body fat predictions ( $P > 0.05$ ). The standard error of model predictions was 19, 43, 48, 36, and 4 % of mean predictions for glucose, glycerol, NEFA, KB, and body fat predictions.

The predicted values for plasma glucose, glycerol, NEFA, and KB followed a similar pattern to the data observed in the animal trial (Figure 3.2). The agreement between the predicted and observed KB was further demonstrated in a ketotic cow as an extreme case (Figure 3.3). One cow was diagnosed with clinical ketosis and her data were excluded from data analysis in the previous paper (Guo et al., submitted) and from model parameterization in the present paper. At d 2 and 3 postpartum, she was administrated i.v. with 1000 cc of dextrose (5 %). The KB



concentration increased from 0.6 to 0.9 mM, and then decreased from 0.9 to 0.25 mM after the dextrose treatment had stopped. The model predictions for KB concentrations appeared to agree with the observed values reasonably well.

According to the model prediction, peripheral tissues consumed more glucose in the last 21 days of gestation compared to the first 21 DIM with a surge around parturition. Glucose consumed by peripheral tissues was greater for the treatment cows from d 15 prepartum to d 10 postpartum compared to the control cows (Figure 3.6). The predicted glucose balances differed in the patterns between the treatment and control groups (Figure 3.7). Before parturition, the treatment cows had a greater glucose balance. However, after parturition the control cows had a greater glucose balance compared to the treatment cows. According to the model prediction, glycerol provided 12 % and 17 % of the glucose demand in the control and treatment group respectively, and the treatment group mobilized more glycerol for gluconeogenesis from adipose tissues after parturition compared to the control (Figure 3.8).

## Discussion

### Physiological Basis of the Model

The model assumed that glucose was the limiting nutrient during the periparturient period. In ruminants, glucose supply is met mainly by gluconeogenesis, an inefficient pathway compared with hydrolysis of starch in nonruminant animals. On the other hand, fetal growth in the late gestation (House

and Bell, 1993) and milk synthesis after parturition increased dramatically during the periparturient period. Most of the carbon required for fetal growth and metabolism is supplied by glucose and lactate (Bell, 1995). Lactate utilization for gluconeogenesis primarily represents recycling of carbon because most of circulating lactate is formed either during catabolism of glucose by peripheral tissues or by partial catabolism of propionate by visceral epithelial tissues (Drackely et al., 2001). Around parturition, the demand for glucose is increased greatly by fetal growth, development of mammary gland, and milk synthesis, at a time when feed intake has been depressed. Thus, glucose balance between supply and demand could play a critical role in the orchestration of the entire metabolism in periparturient cows.

### Glucose

Although the equation of glucose utilization by peripheral tissues did not fully represent the biological mechanism, the model predicted blood glucose concentrations without any bias. Glucose utilization by peripheral tissues is regulated mainly by plasma insulin, tissue responses to insulin (Pettersen et al., 1993) and glucose availability (Yki-Jarvinen et al., 1987). The model predicted that more glucose was consumed by peripheral tissues prepartum compared to the postpartum period with a surge around parturition. Plasma insulin decreases as the cow progresses from late gestation to early lactation with an acute surge at parturition (Kunz et al., 1985). The model predicted pattern of glucose consumption is in agreement with the profile of plasma insulin in the periparturient period. A

difference in peripheral glucose utilization between the treatment and control groups was also predicted by the model. Before parturition the difference may result from the greater glucose availability and greater insulin concentrations in the treatment groups compared to the control group as reported previously (Guo et al., submitted). Feeding a high-concentrate diet during the late gestation period increased plasma insulin concentrations, and this effect carried over into early lactation (Holcomb et al., 2001). The carryover effect may be responsible for the difference between the treatment and control groups after parturition as the model predicted. According to the model predictions, the amount of glucose consumed by peripheral tissues ranged from 0.07 to 0.04 prepartum, and from 0.05 to 0.04 mol/d per kg<sup>0.75</sup> BW postpartum in the control cows. A turnover rate of glucose in ruminants under basal conditions had been reported by Baldwin (1995) between 0.03 and 0.05 mol/d per kg<sup>0.75</sup> BW. Compared to the data by Baldwin (1995), the over-prediction may be caused by the two factors: first, the cows were not under the basal condition; second, during the non-lactating period glucose may be used to synthesize triglyceride in adipose tissues.

### Glycerol

A mean bias was observed for glycerol concentration predictions; however the absolute value was only 0.001 mM, which is biologically insignificant relative to the glycerol concentrations under the basal condition. A linear bias was also observed for glycerol predictions. The linear bias was mainly caused by a few of the residuals when glycerol predictions were above the normal range. In the process of

hexoneogenesis, blood glycerol can be directly incorporated into galactose for lactose synthesis in the mammary gland (Sunehag et al., 2002). The linear bias for glycerol predictions may be caused by the hexoneogenesis which was not considered in the model. Contribution of glycerol to gluconeogenesis ranged from 15 to 20 % of the glucose demand at 4 d postpartum (Bell, 1995), which agrees well with the range from 12 to 17 % as predicted by the current model.

### Body Fat Content

The result of model parameterization showed that the postpartum rate of fat mobilization was greater than the prepartum rate. Lipolytic pathways are highly regulated by insulin, glucagons, epinephrine, norepinephrine, somatotropin, prolactin, estrogen, progesterone, glucocorticoids, thyroid hormones, and also possibly adenosine (Vernon, et al., 1991). The change in the endocrine profiles and sensitivities to those hormones greatly enhanced fat mobilization in the early lactation compared to the late gestation (Bell, 1995).

Using the ratio of glucose demand to supply as the coefficient of glucose deficiency, the model successfully predicted body fat content with no bias. The justification for prediction of fat mobilization was further supported by the precision of body fat predictions. The precision and accuracy of body fat predictions indicated that glucose deficiency is closely related to the rate of adipose mobilization in periparturient cows.

### Nonesterified Fatty Acids

The endocrine status differs greatly before and after parturition. However, according to the model parameterization, the prepartum rate of NEFA utilization was not significantly different from the postpartum rate, probably because NEFA metabolism in the liver of dairy cows is less responsive to hormonal control than is the metabolism in laboratory species (Cadorniga-Valino et al., 1997). In the model, the postpartum rate of NEFA utilization rate was 0.2236 (mmol/d per mM NEFA concentration) corresponding to 7.1 mol of NEFA by oxidation, ketogenesis, and milk fat synthesis at 5 d postpartum in the control group. Since the NEFA pool had only one input or fat loss, and one output or utilization in the model, the NEFA utilization rate should be approximately equal to NEFA entry rate. At 5 d postpartum when the average NEFA concentration was 0.523 mM in the control group, the empirical model (Pullen, et al., 1989) predicted about 7.9 mol/d of the NEFA entry rate, which agreed well with the current model prediction.

### Ketone Bodies

In the model, the prepartum rate of KB utilization was significantly different from the postpartum rate. The difference is in agreement with the results published by Heitmann, et al. (1987) that the uptake rate of BHBA by the uterus is only half of that by the hindquarters in pregnant sheep; however, the mammary gland utilizes BHBA at rates similar to or greater than in hindquarters of lactating sheep. The pre- and post-partum rates of KB utilization rates were 0.31 and 0.47 mmol/d per mM KB concentration respectively, corresponding to 7.5 mol per day in late

gestation and 17.5 mol at d 5 postpartum in the control cows. Based on the data in sheep (Heitmann et al. 1987), the cows in the control group utilized 4.6 mol of KB per day in late gestation, and 12.5 mol of KB at d 5 postpartum when corrected for BW. In fed ruminants, the utilization rates of KB could range from 3.4 to 8.3 mol/d, according to the KB turnover rate of  $0.026 \text{ mmol} \times \text{min}^{-1} \times \text{kg}^{-0.75}$  (Baldwin, 1995).

Compared with the published data, the utilization rates of KB in the model were slightly greater for two reasons. First, the data by Heitmann et al. (1987) did not include acetone metabolism. The quantitative information on acetone metabolism is extremely limited for dairy cows. In rats, after administration of radio-labeled acetone by stomach tube or by injection, a demonstrable amount of radioactivity in glycogen, urea, cholesterol, fatty acids, amino acids, heme as well as a substantial amount radio-labeled carbon were recovered in exhaled carbon dioxide (Price and Rittenber, 1950). Second, the ketolytic pathway occurs in extrahepatic tissues via two reversible reactions, that include activation of ACAC to ACAC coenzyme A, and the creation of acetate coenzyme A. The exchange between the ACAC and acetate coenzyme A pools creates a technical artifact known as 'pseudoketogenesis' that hinders the precise estimation of in vivo KB flux (Fink et al., 1988).

There is a significant linear bias for KB predictions in the model. The bias may result from acetone metabolism as discussed above. In addition, as KB levels increase, an increased proportion of KB is lost in the urine or via breathing. The ratio of KB excreted via urine (mg/d) to blood concentration (mg/dl) is less than 42

in humans under normal conditions, while the ratio is about 56 in an untreated diabetic patient (Nelson, 1999). Another possibility may come from peroxisomal oxidation which is not considered in the model. The peroxisomal pathway, an auxiliary pathway to mitochondrial oxidation, may be induced when hepatocellular influx of NEFA is increased (Drackley et al., 2001).

Although, the current model represented glucose and lipid metabolism under normal condition, the KB profile after therapeutic glucose infusion was successfully simulated in a ketotic cow. Clinical ketosis occurs when there is a failure of the homeostatic mechanisms regulating the glucose and fat metabolism. The therapeutic approach is to reestablish the normal homeostasis (Herdt, and Emery, 1992). The agreement between the model prediction and KB profile after therapeutic treatment further supports the proposed interrelationship between glucose and lipid metabolism simulated in the model.

## Conclusion

Using DMI, feed composition, calf birth weight, milk yield, and milk components as driving variables, the model can predict the changes in body fat content, and plasma glucose, glycerol, NEFA, and KB during the periparturient period. When using this model to quantify metabolite flows, glucose deficiency was closely related to the rate of fat mobilization. The excessive KB could result from elevated fat mobilization for glycerol to compensate for the negative glucose balance in periparturient cows.

Table 3. 1 Principal symbols used in the model.

Symbol	Definition	Unit
t	Day relative to calving	d
Adi	Rumen adaptation index	%
$P_{(\text{propionate})}$	Production rate of propionate from rumen fermentation	mol/d
$P_{(\text{butyrate})}$	Production rate of butyrate from rumen fermentation	mol/d
$P_{(\text{glucose, protein})}$	Production rate of glucose from metabolizable protein	mol/d
$\text{Fetus}_{(\text{glucose})}$	Glucose required for fetal growth per day	mol/d
$\text{Milk}_{(\text{glucose})}$	Glucose required for milk synthesis per day	mol/d
$\text{Peri}_{(\text{glucose})}$	Glucose utilized by peripheral tissues per day	mol/d
$[\text{Glucose}]_p$	Glucose concentration in plasma	mM
$[\text{Glycerol}]_p$	Glycerol concentration in plasma	mM
$[\text{NEFA}]_p$	NEFA concentration in plasma	mM
$[\text{KB}]_p$	Total ketone body concentration in plasma	mM
Fat	Body fat content	kg
$U_{(\text{peri, glucose})}$	Utilization rate of glucose by peripheral tissues	mol/d per kg <sup>0.75</sup> BW
$P_{(\text{fat, adipose})}$	Mobilization rate of fat from adipose tissues	kg/d per kg body fat
$P_{(\text{glucose, glycerol})}$	production rate of glucose from glycerol	mmol × mmol <sup>-1</sup>
$T_{(\text{fat, adipose})}$	Turnover rate of adipose tissues before 7 d prepartum	kg/d per kg body fat
$U_{(\text{NEFA})}$	Utilization rate of NEFA	mmol/d per mM
$U_{(\text{KB})}$	Utilization rate of ketone bodies	mmol/d per mM



Table 3. 2 Model equations.

Prediction	Equation
$Adi^1$	$1 \times 100 / (1 + (100 - 1)^{(-0.95 \times \text{day})})$
$P_{(\text{propionate})}^2$	$DMI \times (\text{coefficient1} + (\text{coefficient2} - \text{coefficient1}) \times Adi)$
$P_{(\text{butyrate})}$	$DMI \times (\text{coefficient3} + (\text{coefficient4} - \text{coefficient3}) \times Adi)$
$P_{(\text{glucose, protein})}^3$	$DMI \times MP \times 0.33 \times 58 \% / 180$ (prepartum) $MUN \times BW \times 0.0259 \times 6.25 \times 0.58$ (postpartum)
$Fetus_{(\text{glucose})}^4$	$Fetus \text{ ME requirement} / (3 \times 3.75 \times 180)$
$Milk_{(\text{glucose})}^5$	$Milk \text{ yield} \times [Lactose]_m / 342 \times 2 \times 1.5$
$Peri_{(\text{glucose})}^6$	$U_{(\text{peri, glucose})} \times ([Glucose]_p / [Glucose0])^Q \times BW^{0.75}$
$dFat^7$	$-P_{(\text{fat, adipose})} \times fat \times (Fetus_{(\text{glucose})} + Milk_{(\text{glucose})} + Peri_{(\text{glucose})}) / (P_{(\text{propionate})} / 2 + P_{(\text{glucose, protein})})$
$d[Glucose]_p^8$	$(P_{(\text{propionate})} / 2 + P_{(\text{glucose, protein})} - Fetus_{(\text{glucose})} - Milk_{(\text{glucose})} - Peri_{(\text{glucose})}) \times 1000 / CO + P_{(\text{glucose, glycerol})} \times [Glycerol]_p$
$d[Glycerol]_p$	$dFat / 866 \times 10^6 / CO - 2 \times P_{(\text{glucose, glycerol})} \times [Glycerol]_p$ (after 7 d prepartum) $U_{fat} \times fat \times 10^6 / 866 / CO - 2 \times P_{(\text{glucose, glycerol})} \times [Glycerol]_p$ (before 7 d prepartum)
$d[NEFA]_p$	$dFat \times 10^6 / 866 \times 3 / CO - U_{(NEFA)} \times [NEFA]_p$ (after 7 d prepartum) $T_{(\text{fat, adipose})} \times fat \times 10^6 / 866 / CO \times 3 - U_{(NEFA)} \times [NEFA]_p$ (before 7 d prepartum)
$d[KB]_p$	$0.5 \times U_{(NEFA)} \times [NEFA]_p \times 4.375 + 0.14 \times P_{(\text{butyrate})} \times 1000 / CO - U_{(KB)} \times [KB]_p$

<sup>1</sup> Day represents days since diets had been changed.

<sup>2</sup> Coefficient1 and 3: propionate and butyrate produced by 1 kg of dry diet.

Coefficient 2 and 4: propionate and butyrate produced by 1 kg of concentrate diet.

VFA production by 1 kg of DM fermented in rumen calculated by Murphy's model (1982).

<sup>3</sup> MP: metabolizable protein estimated by NRC(2001); 0.33: percentage of MP for catabolism; 58 %: catabolism of 100 of protein gives 58 g of glucose (Dukes, 1993).  $MUN \times BW \times 0.0259$ : estimation of urinary nitrogen excretion, where MUM is milk urea nitrogen (Kohn et al, 2002)

<sup>4</sup> Fetus ME requirement estimated by NRC(2001); One third of energy requirement for fetus provided by glucose (NRC, 2001); 1 g of glucose gives rise to 3.75 kcal of energy.

<sup>5</sup>  $[Lactose]_m$ : lactose concentration in milk; Mammary glucose requirement is 1.5 times that required for lactose synthesis (Cantet et al., 1993; Mackle et al., 2000).

<sup>6</sup>  $[Glucose0] = 3.15 \text{ mM}$  as reference concentration of plasma glucose.  $Q = 3$  (prepartum),  $Q = 4$  (postpartum).

<sup>7</sup>  $dFat$  was set to zero prior to d 7 prepartum.

<sup>8</sup>  $CO$ : Cardiac output =  $472 \text{ L} \times BW^{-0.75} \times d^{-1}$  (Baldwin, 1995).  $P_{(\text{glucose, glycerol})} = 0.5$ : Plasma glycerol was completely utilized by gluconeogenesis.

Table 3. 3 The mean values of the parameters (n = 28)

Parameter <sup>1</sup>	Prepartum (SD)	Postpartum (SD)	SED	P <sup>2</sup>
U <sub>(peri, glucose)</sub>	0.039 (0.0166)	0.042 (0.0276)	0.0067	0.47
T <sub>(fat, adipose)</sub>	0.009 (0.0031)			
P <sub>(fat, adipose)</sub>	0.012 (0.0057)	0.015 (0.0059)	0.0013	0.04
U <sub>(NEFA)</sub>	0.188 (0.07892)	0.224 (0.0775)	0.0245	0.16
U <sub>(KB)</sub>	0.31 (0.118)	0.47 (0.167)	0.045	<0.01

1 U<sub>(peri, glucose)</sub>: Utilization rate of glucose by peripheral tissues (mol/d per kg<sup>0.75</sup> BW).

T<sub>(fat, adipose)</sub>: Turnover rate of adipose tissues before 7 d prepartum (kg/d per kg body fat).

P<sub>(fat, adipose)</sub>: Mobilization rate of fat from adipose tissues (kg/d per kg body fat)

U<sub>(NEFA)</sub>: Utilization rate of NEFA (mmol/d per mM NEFA concentration).

U<sub>(KB)</sub>: Utilization rate of KB (mmol/d per mM KB concentration).

2 Differences between the pre and postpartum values were tested by paired t-test.

Table 3. 4 Residual analysis for the developmental data set (from 28 cows)

Prediction	n	Mean	RMSPE <sup>1</sup>	CV	M bias <sup>2</sup>	L bias <sup>3</sup>	$\delta^4$
[Glucose] <sub>p</sub> , mM	472	3.41	0.663	19 %	-0.02	0.17	0.326
[Glycerol] <sub>p</sub> , mM	469	0.022	0.009	43 %	-0.001*	0.021*	0.003
[NEFA] <sub>p</sub> , mM	470	0.33	0.162	48 %	-0.01	0.07	0.092
[KB] <sub>p</sub> , mM	467	0.49	0.180	36 %	-0.02	0.44*	0.096
Fat, kg	56	69.2	2.96	4 %	-0.5	1.6	3.90

<sup>1</sup> Root mean square prediction error.

<sup>2</sup> Mean bias: Mean observation-mean prediction.

<sup>3</sup> Linear bias: analyzed according to (St-Pierre, 2004).

<sup>4</sup> Average values of SEM from Rajala (1998), Putnam et al. (1999), Nocek (1997), Bigner et al. (1996), Enjalbert et al. (2001), Greenfield et al. (2000), McNamca (1991), and Reynolds et al. (2003).

\* P < 0.05.

**Figure 3.1.** A model of glucose and lipid metabolism in periparturient cows.

**Figure 3.2.** Time courses of the blood metabolites during periparturient period. ▲ observed values in the control group, ■ observed values in the treatment group, ----- -- predicted values for the control group, ——— predicted values for the treatment group. The cows in the control group were fed a dry diet preclaving and a lactation diet postcalving. The cows in the treatment group were fed a transition diet in the last 17 days of gestation and the first 14 days of lactation.

**Figure 3.3.** Time courses of total plasma KB in a ketotic cow during periparturient period. ■ observed, ——— model predicted. The cow was diagnosed with clinical ketosis and given 1000 cc of 5 % dextrose at d 2 and 3 postpartum. Data around parturition were missing due to weather condition.

**Figure 3.4.** Plots of residual values against model predictions. Residual = observed – predicted. ▲ identified as outliers, not used by regression analysis. Glucose:  $Y=0.65-0.18 \times X$ ; Glycerol:  $Y=0.006 - 0.313 \times X$ ; NEFA:  $Y=0.007 - 0.054 \times X$ ; KB:  $Y= 0.21 - 0.44 \times X$ .

**Figure 3.5.** Plot of residual values against mode predicted body fat. Residual = observed – predicted.  $Y = 3.48 - 0.06 \times X$ .

**Figure 3.6.** Model prediction for glucose consumed by the peripheral tissues. ▲ control group, ■ treatment group. The cows in the control group were fed a dry diet preclaving and a lactation diet postcalving. The cows in the treatment group were fed a transition diet in the last 14 days of gestation and the first 14 days of lactation.

**Figure 3.7.** Estimated glucose balances during the periparturient period. ▲ control group, ■ treatment group. The cows in the control group were fed a dry diet preclaving and a lactation diet postcalving. The cows in the treatment group were

fed a transition diet in the last 14 days of gestation and the first 14 days of lactation. Glucose balances were calculated by propionate from feed, catabolized protein, fetus growth, milk synthesis, and peripheral tissue requirement.

**Figure 3.8.** Model prediction for the contribution of glycerol from fat mobilization to glucose synthesis in the periparturient cows. ▲ control group, ■ treatment group. The cows in the control group were fed a dry diet precalving and a lactation diet postcalving. The cows in the treatment group were fed a transition diet in the last 14 days of gestation and the first 14 days of lactation.

Figure 3. 1 A model of glucose and lipid metabolism in periparturient cows.

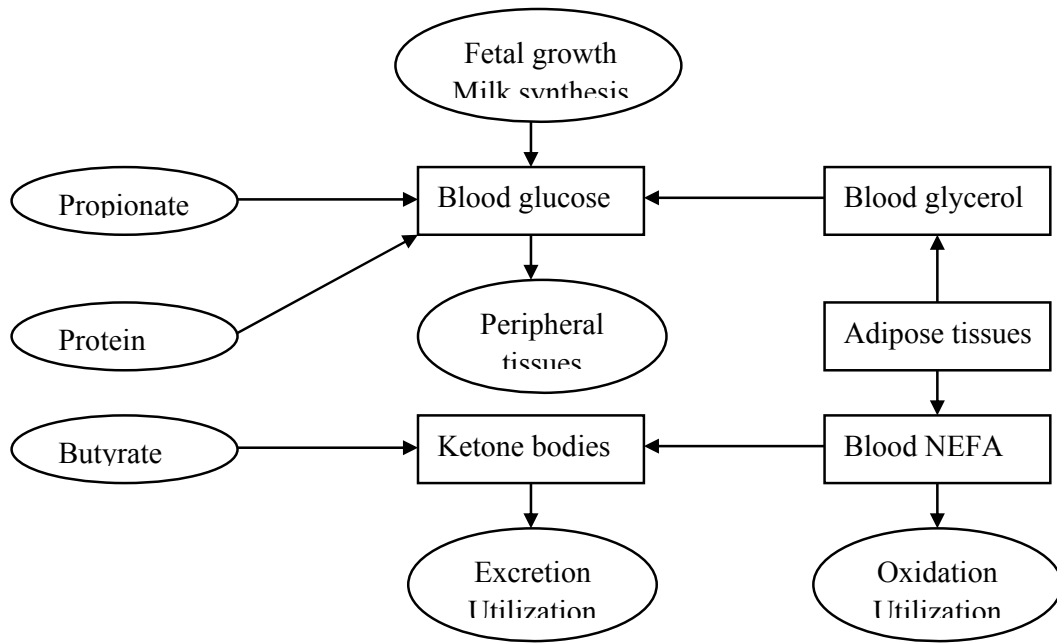


Figure 3. 2 Time courses of the blood metabolites during periparturient period.

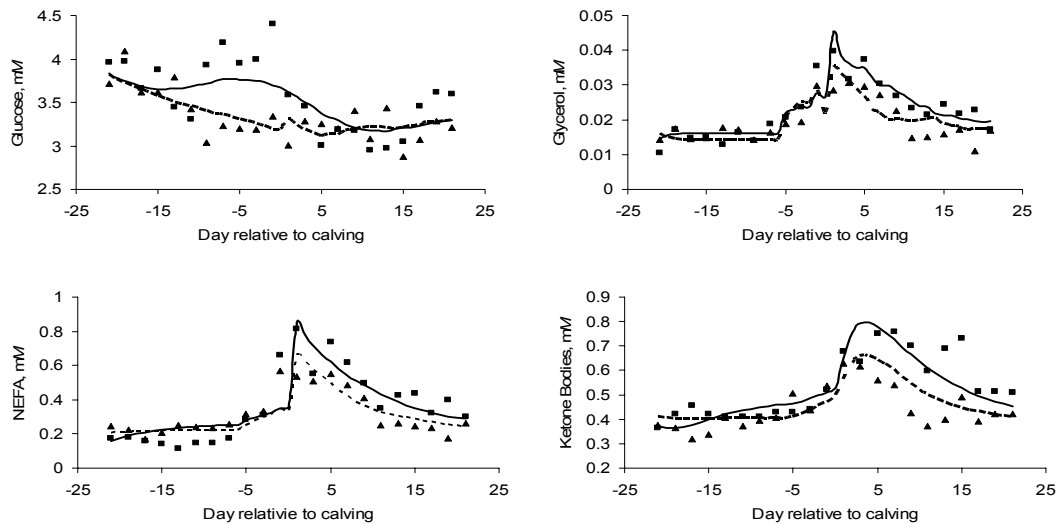


Figure 3. 3 Time courses of total plasma KB in a ketotic cow during periparturient period.

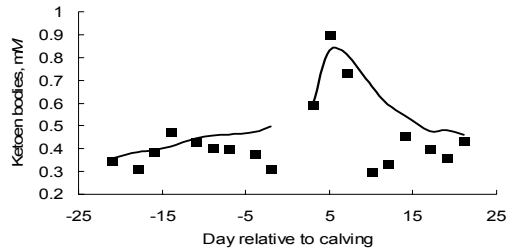


Figure 3. 4 Plots of residual values against model predictions.

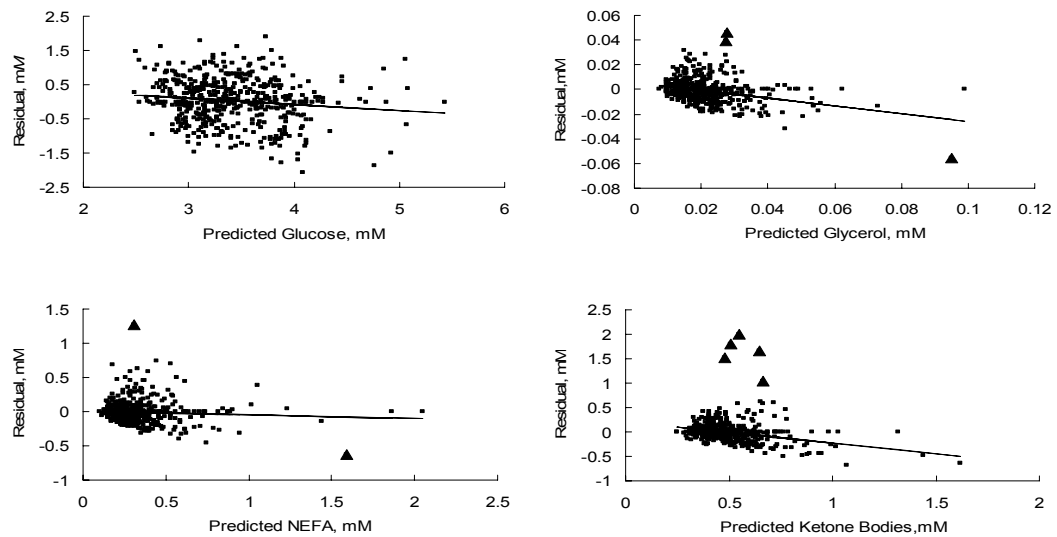


Figure 3. 5 Plot of residual values against mode predicted body fat.

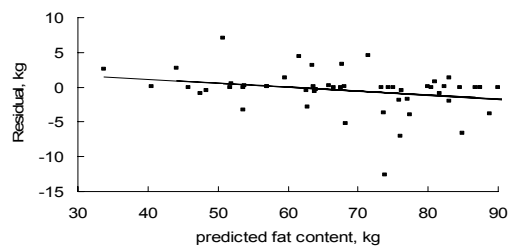


Figure 3. 6 Model prediction for glucose consumed by the peripheral tissues.

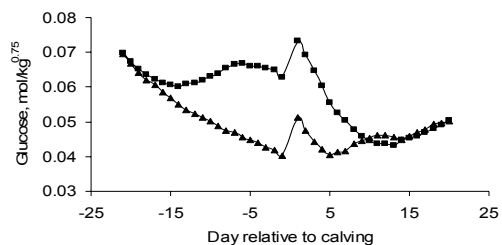


Figure 3. 7 Estimated glucose balances during the periparturient period.

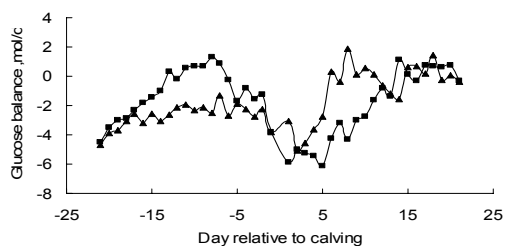
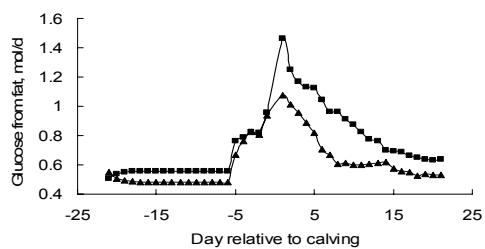


Figure 3. 8 Model prediction for the contribution of glycerol from fat mobilization to glucose synthesis in the periparturient cows.



## Chapter 4: Evaluation of a Mechanistic Model on Glucose and Lipid Metabolism in Periparturient Cows

### Introduction

Dairy cows are susceptible to ketosis during the periparturient period. The incidence of ketosis usually can be decreased through improved nutrition and feeding management. Many factors are involved in the development of ketosis including milk yield, BCS, and DMI (Drackley, 1997). High producing cows are more susceptible to ketosis than low producing ones (Baird, 1982). Overconditioned cows are at greater risk for ketosis (Fronk et al., 1980). Other evidence suggests that nutrient intake during early lactation is critical to minimize the incidence of ketosis (Lean et al., 1994). However, these factors are closely interrelated. Cows that have high DMI usually produce more milk. Cows that are fat or overconditioned at calving may be at risk for lower feed intake (Treacher et al., 1986), and lower milk yield (Gearhart, et al., 1990). The interrelationship makes it difficult to tell the relative importance of feed intake, body condition, and milk yield to nutrition management.

Minimizing the incidence of ketosis requires a comprehensive understanding of the metabolic processes that occur during the periparturient period. The susceptibility to ketosis is associated with many metabolic processes like fat mobilization, KB utilization, NEFA utilization, and glucose consumption by peripheral tissues (Guo et al., submitted). The homeorhetic state during the



periparturient period and the interaction between these metabolic processes make it difficult to identify the contribution of each process to the KB profile.

A compartmental model was developed to quantitatively and dynamically describe the glucose and lipid metabolism in periparturient cows (Guo et al., unpublished). In the current study this model was used to provide quantitative estimates of the impact of the above metabolic processes, and production performance on ketosis development, insight into the potential for manipulating periparturient metabolism to decrease the incidence of ketosis, and the most promising means to do so.

The objectives of the current study were to evaluate the accuracy and precision of the model with data collected in an independent experiment, to determine the relative importance of dry matter intake, calf birth weight, milk yield, and BCS to nutritional management, and to identify critical metabolic processes for ketosis development.

## Materials and Methods

### Data Sets

The independent data from an animal trial by DeFrain et al (unpublished) was used to evaluate the accuracy and precision of the model. In the animal trial, thirty Holstein cows were used to evaluate the effects of feeding glycerol from d 14 prepartum to 21 DIM. Treatments were: 0.86 kg/d of corn starch (control), 0.43 kg/d

corn starch + 0.43 kg/d glycerol (low glycerol), or 0.86 kg/d glycerol (high glycerol), topdressed, and hand-mixed into the upper 1/3 of the daily ration.

The following integration and conversion was made to transform the independent data into the form of the driving variables and response variables of the model. The production rates of propionate and butyrate production from the control diet in the independent data were estimated by feed composition and DMI according to Murphy et al (1982). Rumen fermentation of glycerol supplemented diets could not be appropriately estimated by Murphy's model which was developed for typical diets. The rates of propionate and butyrate production from low and high glycerol diets were extrapolated from the control diet by the ratios of volatile fatty acids (VFA) concentrations in rumen fluid. In the independent data, glycerol and KB concentrations were not measured. The KB concentrations were estimated by the BHBA concentrations and the ratio of KB to BHBA. The ratio was set to 1.18 prepartum and 1.34 postpartum (Guo et al., unpublished). The initial value for glycerol concentration was assigned to 0.03 mM adapted from Guo et al. (unpublished). The initial values for the rest of response variables were the pretreatment measurements from the independent data.

The data from the control group in the developmental data set (Guo et al., unpublished) were used as reference data for behavior and sensitivity analysis instead of the independent data for the following reasons. First, the nutrition management for control animals in the developmental data set was more representative to the current dairy industry than that in the independent study.

Second, there were no glycerol data in the independent study. Lipolysis resulted in the release of glycerol and NEFA. Glycerol could not be reutilized in situ (Chilliard, 1993) but NEFA could be. Thus plasma glycerol was considered to be a reflection of actual lipolysis. Third, only plasma BHBA was measured in the independent data, not the total KB concentrations.

In the developmental and independent data, body fat content was not actually measured, but was estimated by the equation:

$$\text{Body fat (kg)} = \text{BW} \times 0.817 \times (\text{BCS} \times 7.54 - 3.77) / 100$$

where  $\text{BW} \times 0.817$  was the estimate of empty BW (NRC, 2001).

### Model Evaluation

The accuracy and precision were determined by comparing the residuals (observed – predicted) to predicted values. The model predicted values were calculated from DMI, feed composition, VFA concentrations of rumen fluid, calf birth weights, milk yield, milk composition, BW, and BCS from the independent data. The observed data were the concentrations of blood metabolites and body fat contents from the independent data. The accuracy and precision of the model were evaluated by the root mean square prediction error (**RMSPE**):

$$\text{RMSPE} = \text{square root of } (\Sigma (\text{observed} - \text{predicted})^2 / n) \quad (\text{Bibby et al., 1977}).$$

A mean bias for model predictions was declared if mean of residual (observed – predicted) values was significantly different from zero. Linear bias for model predictions was evaluated by regression analysis of residuals against model predictions. Statistical significance was declared at  $P < 0.05$ .

The behavior of the model was observed when DMI, calf birth weight, milk yield, or BCS was increased by one standard deviation. The standard deviations were adapted from the reference data. The body fat loss and blood metabolite changes in the AUC across the last 21 days prepartum and the first 21 days postpartum were compared to the reference data and were evaluated to assure that the model contained the provisions adequate to simulation of response relationships observed in studies.

We also evaluated how sensitive the model is to estimates of model parameters by changing each parameter by one standard deviation. The values for the model parameters and the standard deviation associated with them were adapted from the reference data. The model responses of fat loss and the AUC for blood metabolites were evaluated to identify the key parameters that have great impact on the response variables of the model.

## Results and Discussion

### Residual Analysis

The RMSPE for glucose predictions was 1.00 mM, which was 24 % of mean prediction (Table 4.1). The variation could result from many factors like stress, glucocorticoid, and adrenergic agents (Bell, 1995) which are not considered in the model. The model overestimated ( $P < 0.01$ ) plasma glucose concentrations by 0.62 mM accounting for 38.8 % of total prediction error. The difference in the feed composition between the trial for model development and model evaluation may

cause different patterns in rumen fermentation. However the same coefficients from Murphy's model (1982) had been used to estimate the VFA production, which may result in the mean bias for glucose predictions. A linear bias was also observed ( $P < 0.01$ ) for the glucose predictions (Figure 4.1) accounting for 24.5 % of total prediction error (Table 4.1). This linear bias may have resulted from feed intake as the glucose residuals were negatively related to DMI ( $P < 0.01$ , Figure 4.2). In the independent trial, starch and/or glycerol were topdressed with TMR, which may cause a pulse manner in NSC digestion. Consequently, as feed intake increased, more starch and/or glycerol may escape from the rumen fermentation and end up in the small intestine. It had been reported that infusion of starch directly into the small intestine did not increase glucose appearance in portal drained viscera (Nocek, and Tamminga, 1991). The starch and/or glycerol disappeared in the small intestine may not end up in the form of plasma glucose, which resulted in the over-prediction for plasma glucose concentrations by the model as DMI increased.

No bias was observed in NEFA predictions ( $P > 0.05$ ). The RMSPE for NEFA and KB predictions were 0.238 and 0.527 mM which were 60 and 81 % of mean prediction respectively. In the model, the KB compartment was downstream to the NEFA compartment, which was downstream to the glucose compartment. The prediction error from the upstream compartments could pass downward and accumulate in the downstream compartments resulting in relatively high prediction errors associated with NEFA and KB. The model over-predicted KB concentrations by 0.373 mM ( $P < 0.01$ , Table 4.1). The same reason for overestimating glucose

predictions may also be responsible for the main bias for KB as discussed previously. In addition, a different analysis method was used to determine BHBA concentrations in the independent data set from that used in the development data set. The KB concentrations in the independent data set were not measured directly, but were estimated from the ratio of BHBA to ACAC and acetone which would contribute to some of the error.

The RMSPE for body fat prediction was 7.4 kg which is 6% of mean prediction (Table 4.1). No bias was observed for body fat predictions ( $P > 0.05$ ; Figure 4.1).

#### Behavioral Analysis

An analysis was carried out to assure the model is capable of simulating the response relationships observed in studies, and to compare the importance of different factors on the animal response. The model responses to increasing DMI, fetal weight, milk yield, and BCS were consistent with published results as described below. The concentrations of plasma KB were positively related to milk yield and BCS, and negatively related to feed intake (Table 4.2). Greater intake increases release of insulin (Holcomb et al., 2001), which modulates mobilization of body fat and increases glucose precursor supply, which decreases KB synthesis. Overconditioned cows are at high risk of ketosis development (Fronk et al., 1980). Cows with high milk production have relatively low glucose, high NEFA and BHBA concentrations in plasma and are at high risk of ketosis development (Drackley, 1997).

The 22.3% increase in BCS had little impact on plasma glucose concentrations. However, glucose concentrations were positively related to DMI, and negatively related to fetal weight and milk yield (Figure 4.3). The model responses to 9.8 % increase in postpartum DMI and 12.7 % increase in milk yield were 4.2 and 4.3 % changes in glucose AUC respectively (Table 4.2). The similar magnitudes of the model responses to DMI and milk yield implied that the plasma glucose concentrations are equally affected by DMI and milk yield during the periparturient period. The response of fat loss to DMI and milk yield was similar to that of glucose. The model response of glucose concentrations to fetal weight prepartum was not as intense as it was to milk yield postpartum. The lower intensity of the prepartum response probably results from the fact that most hypoglycemia is developed post-calving in cows, not before parturition in contrast to the occurrence of hypoglycemia in sheep.

The change in BCS had a greater impact on plasma glycerol concentrations compared with DMI, fetal weight, and milk yield, as well as on NEFA, KB (Figure 4.3), and body fat loss (Table 4.2). Overfeeding during pre-lactation period leads to deposition of body fat and overcondition at calving (Fronk et al., 1980; Grummer et al., 1995). Fat cows are prone to increased adipose sensitivity, which is the tendency to mobilize body fat rapidly under stresses like calving or underfeeding (Oetzel, 2003). Fat cows usually have a high concentration of blood NEFA and BHBA (Fronk, 1980). Excessive mobilization of fat not only increases concentrations of NEFA (Rukkwamsuk et al., 1999) and KB (Oetzel, 2003), but also enhances fat

infiltration in liver, and depresses appetite (Oetzel, 2003). The rate of hepatic ketogenesis from NEFA is determined both by supply rate of NEFA and by carbohydrate status of the liver (Williamson, 1979). Carbohydrate insufficiency is expressed by a decrease in glycogen concentration and gluconeogenesis in the liver (Baird and Heitzman, 1971). The rate of gluconeogenesis was decreased when hepatocytes were infiltrated with lipids (Cadorniga-Valino et al., 1997). If the carbohydrate status is low, the proportion of assimilated NEFA transformed into KB will increase (Baird, 1982). Ketotic cows had a higher percentage of fat in their livers than did healthy cows, and that the extent of fatty infiltrations was positively correlated with the concentrations of KB in the blood (Grohn et al., 1983). Ketosis is observed more frequently in fat cows than in normal cows (Andrews et al., 1991). Thus, it is important to avoid overfeeding during the pre-lactation period to prevent ketosis.

Before parturition, cows were in positive energy balance, and most of KB was produced by rumen epithelia from feed fermentation. The origination of prepartum KB could result in a positive relationship between KB concentrations and DMI. This relationship is in agreement with the result that the AUC for KB was positively related to DMI during the prepartum period (Table 4.2; Figure 4.3). After parturition, this relationship is attenuated by adipose mobilization. As DMI increased after parturition, less body fat was mobilized and less KB was produced from incomplete fatty acid oxidation. In the model, the importance of DMI, fetal



weight, milk yield, and BCS was evaluated partially. The interaction among the three was not considered in the behavior analysis.

### Sensitivity Analysis

The goal of sensitivity analysis is to identify the key control points in glucose and lipid metabolism contributing to the development of ketosis. The rate of glucose utilization by peripheral tissues had greater impact on the glucose concentrations in plasma than body fat mobilization rate (Figure 4.4). Glucose concentrations are affected by the rate of fat mobilization in a relatively indirect way compared to the rate of glucose utilization by peripheral tissues. High lipolysis rate may increase mitochondrial NADH and contribute more energy to the cellular energy charge, resulting in reduced demand for energy from glycogenolysis (Sprriet and Watt, 2003).

Although the rate of fat mobilization is the main factor affecting glycerol concentrations (Figure 4.4) and fat loss (Table 4.3) during the periparturient period, increased rate of glucose utilization by peripheral tissues could also result in elevated glycerol concentrations in the first few days of lactation (Figure 4.4). The interrelationship between peripheral glucose utilization and increased glycerol concentrations in plasma may be caused by glucose deficiency as described below. Carbohydrate metabolism in early lactation is dominated by the mammary requirement for glucose, mostly for lactose synthesis (Bell 1995). Estimates of the supply of glucose relative to demand for glucose by Overton (1998) indicated a glucose deficiency after calving. This glucose deficiency must be made up by

increased gluconeogenesis from endogenous amino acids, and glycerol, and by decreased glucose consumption by peripheral tissues. Early lactation (2 to 4 wk postpartum) is characterized by a moderate degree of insulin resistance in peripheral tissues, thereby sparing of glucose and promoting the mobilization of NEFA, glycerol, and amino acids (Bell, 1995). However, in the first few days of lactation, glucose deficiency could be exaggerated by peripheral tissues. Uptake of plasma glucose by peripheral tissue is related to insulin concentrations, tissue sensitivity to insulin (Pettersson et al., 1993), and glucose concentrations (Yki-Jarvinen et al., 1987). Tissue sensitivity to insulin is defined as the insulin concentration required to produce a half-maximal response (Kahn, 1978). Insulin resistance is primarily due to decreased sensitivity, not due to maximal response in the peripheral tissues of late-pregnant ewes (Pettersson et al., 1993). Accordingly, in spite of insulin resistance, a great amount of glucose could be utilized by peripheral tissues if the concentrations of insulin and glucose were elevated. Although plasma concentrations of insulin and glucose decrease during the periparturient period, a surge occurs in insulin (Kunz et al., 1985) and glucose concentrations (Tucker, 1985) at parturition. The concentrations of glucose and insulin remain high in the first few days of lactation after the surges. Thus, peripheral tissues may utilize a greater amount of glucose, which makes the glucose deficiency even worse in the first few days of lactation compared to the rest of the periparturient period. On the other hand, gluconeogenesis from propionate and protein could have reached its limitation/potential because DMI is depressed and cows are in a state of negative protein balance in the first few days

of lactation. Contribution of plasma glycerol to gluconeogenesis depends on fat mobilization (Drackley et al., 2001). Glucose deficiency could lead to elevated mobilization of adipose tissue to increase gluconeogenesis from glycerol. Therefore, glucose utilization by peripheral tissues has a great impact on the concentrations of plasma glycerol in the first few days of lactation.

Plasma NEFA, another byproduct of lipolysis, could be oxidized or converted to KB by the liver, reesterified to triglyceride in lipid tissue, incorporated into milk fat in mammary gland, thus, the concentrations of plasma NEFA are greatly affected by NEFA utilization rate. The impact of fat mobilization rate and peripheral glucose consumption rate on NEFA was similar to that on glycerol (Figure 4.4).

The model simulation demonstrated that plasma KB concentrations are dependent mainly upon fat mobilization and KB utilization rates (Table 4.3). All but a few tissues, like the liver and brain, can use KB due to the presence of BHBA dehydrogenase. The impact of fat mobilization rate on KB before parturition is not as intensive as that after parturition (Table 4.3) because fatty acids from lipolysis are not the main source of prepartum KB as discussed previously. The susceptibility to ketosis is not only dependent on total exposure to KB during a period of time as measured by AUC, but also on the maximal concentrations of KB the animal suffers. The results of model simulation indicated that the peak of KB concentrations in the first few days of lactation could also be affected by NEFA utilization rate and peripheral glucose consumption rate (Figure 4.4). Increased rate

of glucose consumption by peripheral tissues results in a severe glucose deficiency compared to the normal rate of glucose consumption, and glucose deficiency could lead to elevated mobilization of adipose tissue as discussed previously. Hepatic ketogenesis is not only regulated by the activities of carnitine palmitoyltransferase-1 (EC 2.3.1.21) and 3-hydroxy-3-methylglutaryl-CoA synthase (EC 2.3.3.10), but also by NEFA supply to the liver (Hegardt, 1999). Increased fat mobilization and increased NEFA utilization provide more NEFA for ketogenesis compared to the normal fat mobilization and NEFA utilization. In addition, the ratio of ACAC to BHBA reached a maximum on about day 5 postpartum (Guo et al., unpublished). Thus, the first few days of lactation could be the most critical time for periparturient cows because ACAC is more toxic than BHBA. Therefore, the susceptibility to ketosis is closely related to the glucose consumption by peripheral tissues, and NEFA utilization rate in the first few days of lactation.

## Limitations and Applications

The model was based on the hypothesis that glucose deficiency promoted fat mobilization in periparturient cows in excess of that required for energy. The robustness of the model relies on the accurate estimates of glucose supply to the system. Murphy's model (1983) had been used to estimate glucose supply from rumen fermentation. However, this model is empirical, and rumen fermentation is affected by many factors like feeding regime, particle size, and physical stage, which are not accounted for in the model. Therefore, the estimates of glucose supply into the model are associated with an unknown variation.

As the NSC content increased in the diet, more propionate would be produced from rumen fermentation. Total nutrient intake is a combination of both nutrient density and DMI. The model would respond to a high-NSC diet similarly to increasing DMI. The increased glucose supply from an extra 1.5 kg of DMI is equivalent to an increase in NSC content by 9% in the diet. Thus, the impact of feed composition was not evaluated in the current study.

In the developmental and independent data, body fat content was estimated from BCS and empty BW (NRC, 2001). In early lactation, body reserves are mobilized to compensate for nutrient in balance. At the same time, the proportion of visceral tissues is increased to adapt to the increasing DMI. Thus empty BW could not be accurately estimated by  $BW \times 0.817$  during this period of time. In addition, BCS is a subjective measurement and always associated with human error. Therefore, BW and BCS should be measured by the same protocol as described previously to minimize error in the model prediction.

## Conclusions

Overall, the model is valuable for studying the homeorhetic state of glucose and lipid metabolism in periparturient cows. However, the driving variables of the model should be measured or determined the same way as the model was developed. The profiles of blood metabolites could be simulated and evaluated under various circumstances such as different production levels, feed intakes, and initial BCS at calving.

The model evaluation indicated that it is important to avoid overfeeding in late-gestation period. During the first few days of lactation, the rate of NEFA utilization had a great impact on KB concentrations in periparturient cows.

Table 4. 1 Model evaluation for accuracy and precision by the independent data.

Prediction	n	Mean <sup>2</sup>	RMSPE <sup>3</sup>	CV	Mean bias		Linear bias		Residual Error <sup>7</sup>
					Bias <sup>4</sup>	Error <sup>5</sup>	Bias <sup>6</sup>	Error	
				(%)		(%)		(%)	(%)
Glucose, mM	153	4.3	1.00	24	-0.62**	38.8	-1.02**	24.5	36.7
NEFA, mM	134	0.39	0.238	60	-0.037	2.5	0.370	1.7	95.8
KB <sup>8</sup> , mM	137	0.65	0.527	81	-0.373**	50.1	-0.515	1.0	48.9
Fat, kg	47	119	7.4	6	-1.4	3.6	-0.03	1.3	95.1

<sup>1</sup> The independent data were adapted from Defrain, et al. (unpublished).

<sup>2</sup> Mean of model predictions

<sup>3</sup> Root mean square prediction error = square root of  $(\sum (\text{observed} - \text{predicted})^2 / n)$  (Bibby et al., 1977).

<sup>4</sup> Mean observation – mean prediction.

<sup>5</sup> Percentage of total prediction error explained by bias.

<sup>6</sup> Slope of residual on prediction.

<sup>7</sup>  $100 - [\text{error (percentage) mean bias} + \text{error (percentage) linear bias}]$ .

<sup>8</sup> Plasma ketone bodies.

\*\*  $P < 0.01$ .

Table 4. 2 Model responses to the increased DMI, calf weight, milk yield, and BCS

	% Change in driving variable <sup>1</sup>	Predicted responses of AUC <sup>2</sup> for blood metabolites and fat loss (%) <sup>3</sup>				
		Glucose	Glycerol	NEFA	KB <sup>6</sup>	Fat loss
Prepartum <sup>4</sup> DMI	15.6	3.5	-1.5	-0.8	3.2	-4.6
Calf weight	10.8	-1.0	0.4	0.2	0.1	1.1
Prepartum BCS	22.3	0.3	21.8	18.1	10.9	23.3
Postpartum <sup>5</sup> DMI	9.8	4.2	-3.1	-2.4	0.2	-3.5
Milk Yield	12.7	-4.3	3.1	2.3	1.7	3.5
Postpartum BCS	22.3	0.4	20.3	13.6	9.1	23.1

<sup>1</sup> Increased by one standard deviation. The standard deviations were adapted from Guo et al. (unpublished).

<sup>2</sup> Area under the curve.

<sup>3</sup> The model responses were expressed as a percentage change in response variables compared to the reference data.

<sup>4</sup> Prepartum: the last 21 days of gestation.

<sup>5</sup> Postpartum: the first 21 days of lactation.

<sup>6</sup> Plasma ketone bodies.



Table 4. 3 The impact of increasing the model parameters on AUC for blood metabolites and body fat loss.

Parameter <sup>1</sup>	% Change in parameter <sup>2</sup>	Predicted responses of AUC <sup>3</sup> for blood metabolites and fat loss (%) <sup>4</sup>				
		Glucose	Glycerol	NEFA	KB	Fat loss
Prepartum <sup>5</sup> U <sub>(peri, glucose)</sub>	42.6	-7.7	0.5	0.3	0.1	1.5
Prepartum P <sub>(fat, adipose)</sub>	48.7	0.1	15.0	7.8	2.5	46.5
Prepartum U <sub>(NEFA)</sub>	42.0	NA <sup>7</sup>	NA	-23.8	6.0	NA
Prpartume U <sub>(KB)</sub>	38.8	NA	NA	NA	-25.2	NA
Postpartum <sup>6</sup> U <sub>(peri, glucose)</sub>	65.3	-7.8	5.3	4.1	3.1	6.0
Postpartum P <sub>(fat, adipose)</sub>	40.3	0.6	29.2	20.0	13.7	33.0
Postpartum U <sub>(NEFA)</sub>	34.7	NA	NA	-21.7	3.0	NA
Postpartum U <sub>(KB)</sub>	35.4	NA	NA	NA	-23.8	NA

<sup>1</sup> U<sub>(peri, glucose)</sub>: Utilization rate of glucose by peripheral tissues (mol/d per kg<sup>0.75</sup> BW).

T<sub>(fat, adipose)</sub>: Turnover rate of adipose tissues before 7 d prepartum (kg/d per kg body fat).

P<sub>(fat, adipose)</sub>: Mobilization rate of fat from adipose tissues (kg/d per kg body fat)

U<sub>(NEFA)</sub>: Utilization rate of NEFA (mmol/d per mM NEFA concentration).

U<sub>(KB)</sub>: Utilization rate of KB (mmol/d per mM KB concentration).

<sup>2</sup> Increased by one standard deviation. The standard deviations were adapted from Guo et al. (unpublish).

<sup>3</sup> Area under the curve.

<sup>4</sup> The model responses were expressed as a percentage change in response variables compared to the reference data.

<sup>5</sup> Prepartum: the last 21 days of gestation.

<sup>6</sup> Postpartum: the first 21 days of lactation.

<sup>7</sup> Impact of the parameter was not considered in the model.

**Figure 4.1.** Residual analysis for model prediction. Plot of residual (observed – predicted) against model predictions. Glucose: mean bias  $Y = -0.62$  ( $P < 0.01$ ); linear bias  $Y = 3.73 - 1.02 \times X$  ( $P < 0.01$ ), NEFA: mean bias  $Y = -0.037$  ( $P = 0.07$ ); linear bias  $Y = -0.183 + 0.370 \times X$  ( $P = 0.13$ ); KB: mean bias  $Y = -0.373$  ( $P < 0.01$ ); linear bias  $Y = -0.037 - 0.515 \times X$  ( $P = 0.10$ ); body fat: mean bias  $Y = -1.4$  ( $P = 0.20$ ); linear bias  $Y = 2.54 - 0.03 \times X$  ( $P = 0.43$ ).

**Figure 4.2.** Plot of glucose residuals (observed – predicted) against DMI.  $Y = 0.374 - 0.035 \times X$  ( $P < 0.01$ ).

**Figure 4.3.** Model responses to increasing DMI, calf weight, milk yield, and BCS by one standard deviation. — control,  $\Delta\Delta\Delta\Delta\Delta$  increased DMI,  $\circ\circ\circ\circ\circ\circ$  increased calf weight/milk yield,  $\square\square\square\square\square\square$  increased BCS. The standard deviations were adapted from Guo et al. (unpublished).

**Figure 4.4.** The impact of increasing model parameters by one standard deviation on plasma metabolites. — control,  $\Delta\Delta\Delta\Delta\Delta$  increased rate of NEFA utilization,  $\circ\circ\circ\circ\circ\circ$  increased rate of fat mobilization,  $\square\square\square\square\square\square$  increased rate of glucose consumption by peripheral tissues, \*\*\*\*\* increased rate of KB utilization. The standard deviations were adapted from Guo et al. (unpublished).

Figure 4. 1 Residual analysis for model prediction.

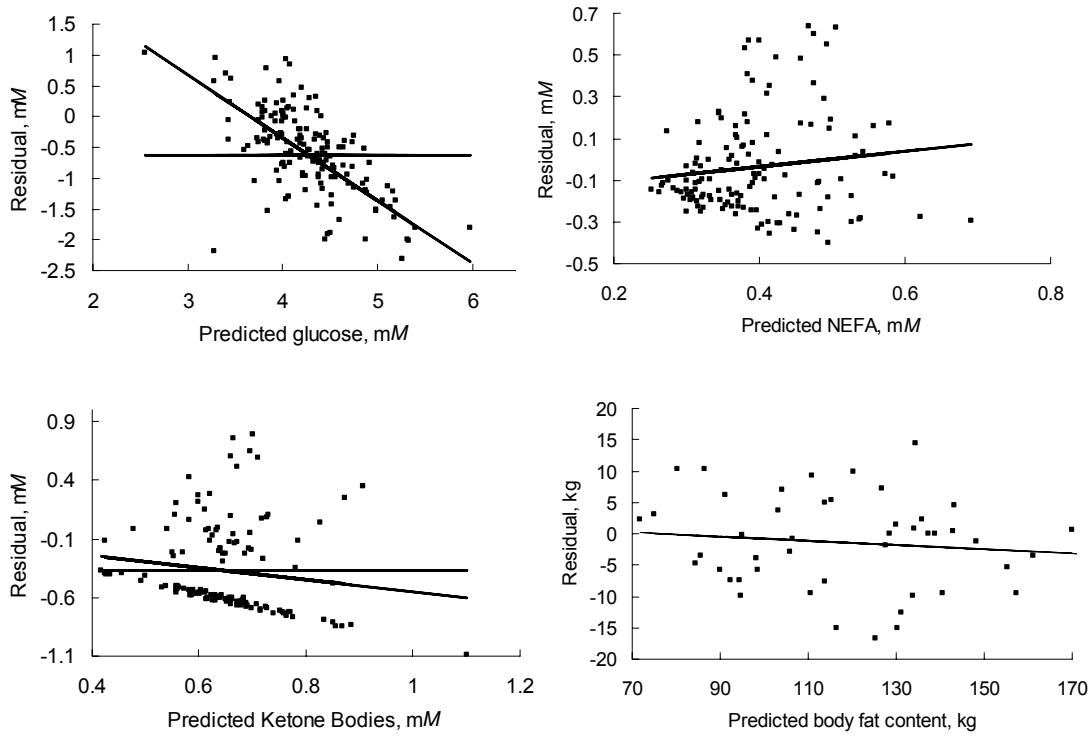


Figure 4. 2 Plot of glucose residuals (observed – predicted) against DMI.

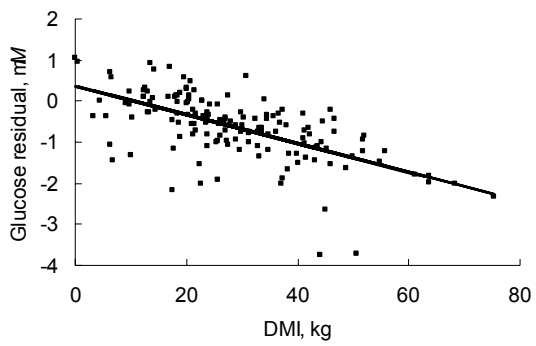


Figure 4. 3 Model responses to increasing DMI, calf weight, milk yield, and BCS.

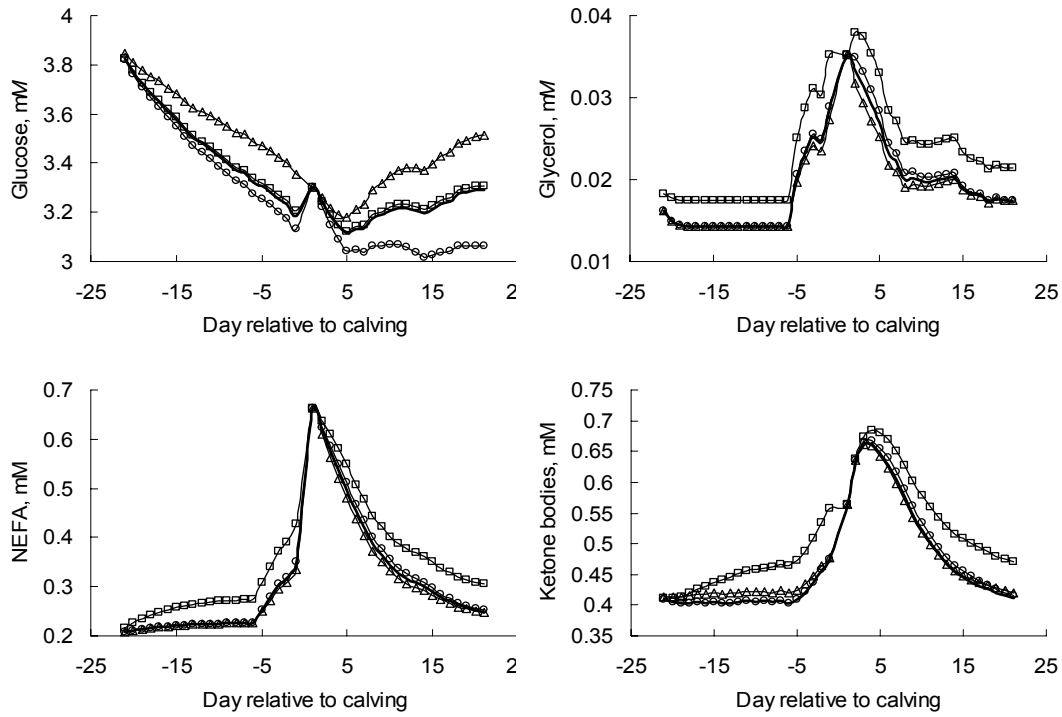
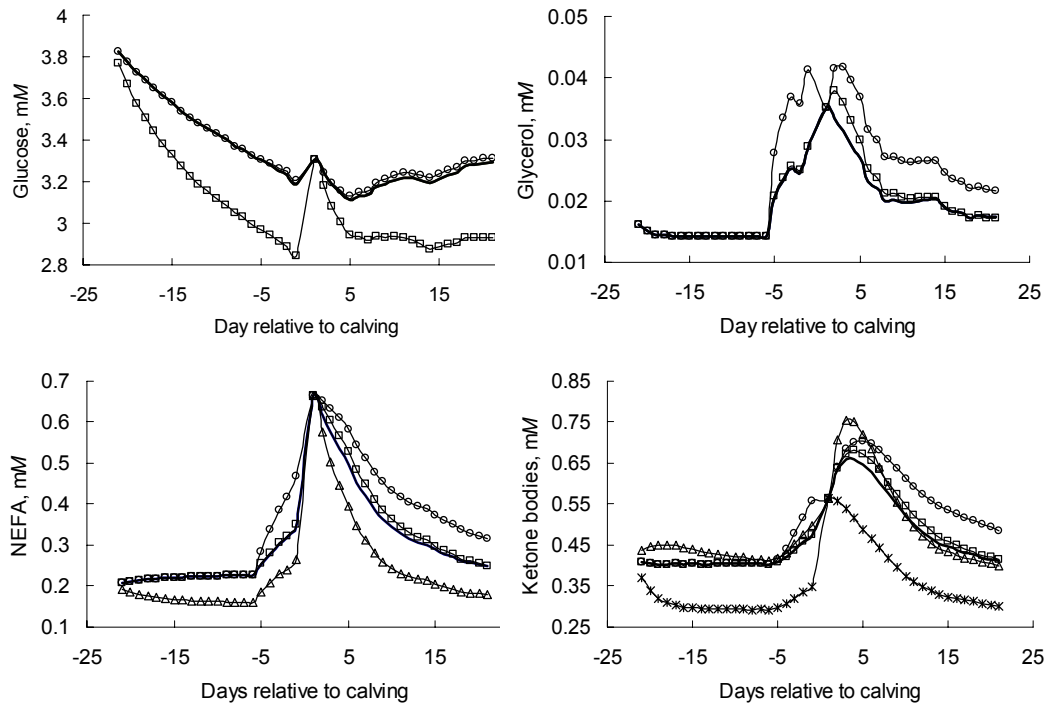


Figure 4. 4 The impact of increasing model parameters on plasma metabolites.



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