Use of urine pH to predict incidence of ketosis in transition dairy cows

Amy Elizabeth Beem

Follow this and additional works at: https://digitalcommons.lsu.edu/gradschool_theses

Part of the Animal Sciences Commons

Recommended Citation

USE OF URINE PH TO PREDICT INCIDENCE OF KETOSIS IN TRANSITION DAIRY COWS

A Thesis

Submitted to the Graduate Faculty of the
Louisiana State University and
Agricultural and Mechanical College
in partial fulfillment of the
requirements for the degree of
Master of Science

in

The Interdepartmental Program in Animal and Dairy Sciences

by

Amy Elizabeth Beem
B. S., Louisiana State University, 2000
December, 2003
# TABLE OF CONTENTS

LIST OF TABLES ............................................................................................................. iii

LIST OF FIGURES ............................................................................................................ iv

ABBREVIATION KEY ..................................................................................................... vi

ABSTRACT ...................................................................................................................... vii

CHAPTER 1. INTRODUCTION ........................................................................................ 1

CHAPTER 2. REVIEW OF LITERATURE ...................................................................... 3
  2.1 Background ......................................................................................................... 3
  2.2 Nutritional Management ................................................................................... 4
  2.3 Etiology of Ketosis............................................................................................ 6
  2.4 Biochemistry of Ketosis.................................................................................... 9
  2.5 Prevention and Treatment of Ketosis ............................................................. 12
  2.6 Summary......................................................................................................... 17
  2.7 Objectives ....................................................................................................... 18

CHAPTER 3. MATERIALS AND METHODS .............................................................. 19
  3.1 Experimental Design, Dietary Treatments and Animal Care ......................... 19
  3.2 Sample Collection and Analytical Procedures ................................................ 21
  3.3 Statistical Analysis.......................................................................................... 22

CHAPTER 4. RESULTS AND DISCUSSION ................................................................24
  4.1 Chemical Analysis of Experimental and Lactation Diets............................... 24
  4.2 Lactational Performances and Bodyweights ................................................... 24
  4.3 Mean Urine and Plasma Metabolites.............................................................. 33
  4.4 Prepartum/Postpartum Urine and Plasma Metabolites................................. 43
  4.5 Correlations of Urine Parameters.................................................................... 45

CHAPTER 5. SUMMARY AND CONCLUSIONS .......................................................... 51

REFERENCES .................................................................................................................. 52

APPENDIX. CORRELATION COEFFICIENTS FOR URINE PARAMETERS FOR EACH COW . 56

VITA .................................................................................................................................. 57
LIST OF TABLES

Table 3-1. Composition of the normal prepartum energy, high prepartum energy, and lactation diets ..............................................................20

Table 4-1. Least squares means of chemical analysis for normal prepartum energy, high prepartum energy, and lactation diets .........................25

Table 4-2. Least squares means of lactational performance and body weights of cows fed diets with different prepartum energy concentrations with or without supplemental calcium propionate ........................................ 26

Table 4-3. Least squares means of urine pH and metabolites in urine and plasma for cows fed diets with different prepartum energy concentrations with or without supplemental calcium propionate during the entire trial and both pre and post calving ........................................................................34

Table 4-4. Correlations of urine parameters for cows fed diets with different prepartum energy concentrations with or without supplemental calcium propionate ...............49
LIST OF FIGURES

Figure 4-1. Least squares means for DMI for cows fed diets with normal or high prepartum energy concentrations with or without supplemental calcium propionate.........................................................28

Figure 4-2. Least squares means for milk production for cows fed diets with normal or high prepartum energy concentrations with or without supplemental calcium propionate..............................................................................................................29

Figure 4-3. 4% FCM for cows fed diets with normal or high prepartum energy concentrations with or without supplemental calcium propionate.................................................................30

Figure 4-4. Bodyweights for cows fed diets with normal or high prepartum energy concentrations with or without supplemental calcium propionate.................................................................32

Figure 4-5. Least squares means of urine pH of cows fed diets with normal or high prepartum energy concentrations with or without supplemental calcium propionate.................................................................35

Figure 4-6. Least squares means of urine pH of cows fed diet with or without supplemental calcium propionate.................................................................................................................................36

Figure 4-7. Least squares means of urine acetoacetate concentrations of cows fed diets with or without supplemental calcium propionate.........................................................................................37

Figure 4-8. Least squares means of urine acetoacetate concentrations of cows fed diets with normal or high prepartum energy concentrations and with or without supplemental calcium propionate.................................................................38

Figure 4-9. Least squares means for urine BHBA concentrations for cows fed diets with normal or high prepartum energy concentrations with or without supplemental calcium propionate.................................................................................................40

Figure 4-10. Least squares means of urine BHBA concentration of cows fed diets with or without supplemental calcium propionate.................................................................................................41

Figure 4-11. Least squares means for plasma BHBA concentration of cows fed diets with normal or high prepartum energy concentrations and with or without supplemental calcium propionate.................................................................................................42

Figure 4-12. Plasma glucose concentrations for cows fed diets with normal or high prepartum energy concentrations with or without supplemental calcium propionate.................................................................................................44
Figure 4-13. Scatter plot of urine BHBA and pH concentrations and the regression line between these values from cows fed diets with normal or high prepartum energy concentrations and with or without supplemental calcium propionate..............46

Figure 4-14. Scatter plot of urine pH and acetoacetate concentrations and the regression line between these values from cows fed diets with normal or high prepartum energy concentrations and with or without supplemental calcium propionate.....47

Figure 4-15. Scatter plot of urine BHBA and acetoacetate concentrations and the regression line between these values from cows fed diets with normal or high prepartum energy concentrations and with or without supplemental calcium propionate..........................................................48
ABBREVIATION KEY

4% FCM = 4% fat corrected milk

ADF = Acid detergent fiber

ADIN = Acid detergent insoluble nitrogen

BHBA = β-hydroxybutyrate

BWT = Bodyweight

CP = Crude protein

DIM = Days in milk

DM = Dry matter

DMI = Dry matter intake

IV = Intravenous

NEFA = Non-esterified fatty acids

NDF = Neutral detergent fiber

OAA = Oxaloacetate

PDE = Prepartum dietary energy

SCC = Somatic cell count

SCCS = Somatic cell count score

VFA = Volatile fatty acids
ABSTRACT

Forty-one (10 primiparous and 31 multiparous) Holstein cows were used in an experiment to determine the effects of prepartum dietary energy (PDE) and supplemental Ca-propionate on the incidence of ketosis in transition dairy cows and to evaluate the usefulness of urine pH as a predictor for ketosis. Treatment factors were prepartum dietary energy concentration (normal vs. high) with or without 113.5g Ca-Propionate/day (Nutro Cal®, Kemin Industries, Inc., Des Moines, IA). All cows were fed the same basal diet postpartum. Ca-propionate supplementation continued postpartum. Cows were individually fed and dry matter intakes (DMI) were measured daily. Milk production was measured and sampled at each milking for 3 weeks postpartum and analyzed for % fat, % protein, and SCC (somatic cell count). Blood samples were collected prior to the afternoon milking 3x/wk and analyzed for β-hydroxybutyrate (BHBA) and glucose concentrations. Urine was collected 3x/wk and analyzed for pH, acetoacetate, and BHBA. Treatment did not affect mean DMI, milk yield, milk component production or percentage, 4% FCM, SCCS, BWT, urine pH, urine acetoacetate, urine BHBA, plasma BHBA, or plasma glucose. There was a statistical interaction of Ca-propionate supplementation and week for urine pH and BHBA. Correlation coefficients between urine BHBA, urine pH, and urine acetoacetate were not useful for prediction of ketosis across all cows. However, they were numerically higher when restricted to data from an individual cow. Correlation coefficients between BHBA and acetoacetate concentrations in urine were significant. These data suggest that PDE and Ca-propionate supplementation had no effect on the incidence of ketosis, however Ca-propionate supplementation allowed cows to recover faster from ketosis postpartum. The use of
urine pH was not a useful tool for the prediction of ketosis across a herd, but may be a useful indicator of ketosis when compared within an individual animal. Due to factors unrelated to treatment diets, there was a high incidence of health disorders such as retained fetal membranes, metritis, displaced abomasums, and laminitis were observed during this trial that probably affected any observed treatment responses.
CHAPTER 1. INTRODUCTION

The transition period for dairy cattle is usually defined as three weeks prepartum to three weeks postpartum (Block and Sanchez, 2000; Drackley, 1999). The majority of diseases and disorders that a cow endures are during this period with the most susceptible time being centered around parturition. For the purposes of this discussion, a disease will have a causative organism as its origin and a disorder will involve metabolic breakdown. Abnormal conditions traditionally associated with the transition period include hypocalcemia, retained fetal membranes, hypoglycemia, displaced abomasum, metritis, and ketosis (Drackley, 1999; Goff and Horst, 1997). All of these diseases and disorders can be either primary or secondary to another condition.

Several events occur during the transition period allowing little time for self-recovery and contribute to the cow's susceptibility to disorders during this period. Maintenance of a fetus, parturition, up to a 30% decrease in DMI and severe increase in nutrient demands due to lactation, all within a few weeks of each other create an extremely metabolically stressful environment (Block and Sanchez, 2000; Goff and Horst, 1997). Metabolic demands for the cow and fetus increase as pregnancy progresses (NRC, 2001). However, metabolic demands for the production of colostrum are much greater than those of the fetus and further contribute to the cow's negative energy balance (Goff and Horst, 1997).

Because this is an extremely stressful time that has a large impact on the subsequent lactation, numerous researchers have studied different aspects of this period. This review will discuss the benefits and demands of transition cow programs, as well as aspects of good nutritional management in relation to the transition period. Also, several
elements of ketosis such as etiology, biochemical characteristics, methods of prevention, and treatments will be presented in relation to their effect on the transition cow.
CHAPTER 2. REVIEW OF LITERATURE

2.1 Background

Some dairy producers have started to implement transition cow programs and have had marked success in decreasing the incidence of health diseases and disorders. These programs outline specific procedures that producers can implement to minimize diseases, disorders, and causes of stress to cows during the transition period while maximizing profitability (Spain, 2000). Programs developed for the dry period are to prepare cows for the intense events of calving and lactation. Dry cow treatments include the prophylactic use of antibiotics, vaccinations for \textit{E. coli} and reproductive diseases, foot care, stress management, body condition management, and implementation of proper data keeping to aid in effective monitoring. Spain (2000) has developed a program titled the "100 day contract" which involves stringent monitoring, prevention, and treatment regimes for cows during the dry period through peak lactation.

Fresh cow programs target the few days after parturition when susceptibility to problems is at its peak. They include very close monitoring and aggressive treatment of diseases and disorders. Fresh cow programs implement preventative techniques such as early doses of glucose precursors and calcium (Drackley, 1999). Fresh cow programs also recommend that the calving environment be clean, cow body condition be evaluated, and daily body temperature monitored. Programs include timely doses of supportive treatments such as energy supplements, calcium, fever reducers, and vitamins. Use of the California Mastitis Test postpartum is encouraged for early detection and treatment of mastitis. Assigning cows to groups relative to calving date aids in record keeping (Pharmacia & Upjohn, 2001a b c). Because these programs involve stringent
observation, diagnosis, and treatment of any problems during the first few days after parturition, they increase management and labor costs to the producer (Drackley, 1999). Under the best management conditions, metabolic disorders such as ketosis can still occur and often remain undetected in the subclinical stages. Therefore, even when prevention methods for ketosis are being practiced, methods for prompt diagnosis of ketosis are still needed.

2.2 Nutritional Management

A high plane of nutrition is needed after parturition to supply the high demands of lactation (Drackley, 1999). Often the cow cannot physically consume enough feed to meet her energy requirements and must rely on body stores. Good nutritional management is successful at reducing the potential incidence of metabolic disorders faced by the cow. Nutritional management should include management of the ruminal environment, feed bunk management, and proper ration formulation. Cows are generally fed a low energy diet during the dry period that promotes growth of cellulolytic bacteria, thus decreasing the population of bacteria that produce propionate, a glucogenic precursor (NRC, 2001). The diets generally fed to dairy cows postpartum contain a higher concentration of energy than diets fed during the dry period. Abrupt changes to the diet of dairy cattle such as the change to a high-energy diet at calving result in alterations to the fermentation balance due to shifts in the proportions of microbial species present. Energy dense diets cause more acid production in the rumen and result in less mastication and rumination, which produces less saliva to act as a buffer, thus lowering the pH of the rumen (Owens and Goetsch, 1988). Cellulolytic bacteria are less tolerant of the lower pH and their numbers decrease, while more tolerant species that are
able to metabolize concentrate feeds faster begin to increase in number. With an abrupt diet change the adaptation period of microbial population change can take up to one week resulting in decreased ruminal productivity. Therefore, a gradual diet change beginning three weeks prior to parturition allows the rumen environment to remain efficient throughout the new diet transition. Beginning three weeks before calving, shifting gradually from the close-up dry cow ration to the lactation ration will reduce stress associated with a diet change (Goff and Horst, 1997; Van Soest, 1994). However, implementation of this practice must be done carefully. By increasing the amount of concentrate to increase the diet's energy density, the risk of causing ruminal acidosis increases. Ruminal acidosis lowers DMI and can cause laminitis that further predisposes cattle to transition period diseases and disorders, thus lowering milk yield (Spain, 2000). To maximize DMI insure adequate feed bunk space, proper feed bunk sanitation, and encourage the practice of pushing up feed several times a day (Cheeke, 1999). Finally, when formulating the diet maintain an adequate amount of fiber for proper rumen motility and function while considering the cow's energy demands and relying on body stores for some of the cows energy needs (Van Soest, 1994).

Strict feed management and proper maintenance of body condition during the dry period is important. Fat accumulation in the liver during the dry period causes the organ to be less efficient for fat mobilization. Because of the physiological stresses on the cow and concurrent decrease in DMI, threat of fat accumulation in the liver increases about 2 weeks prepartum to just after parturition. During this time hepatic triglycerides increase to four to five times the usual level (Grummer, 1993). When the rate of triglyceride
synthesis is higher than what the liver can export as very low-density lipoprotein, fatty liver occurs (Grummer, 1993; Rukkwamsuk et al., 1998).

Over feeding cows during the dry period can increase their risk of developing ketosis during the transition period (Grummer, 1993). Obese cows have a greater predisposition for developing fatty liver and have a harder time recovering metabolically from hypophagia during parturition, which can put the cow at greater risk for ketosis (Rukkwamsuk et al., 1998). Grummer (1993) reported the rate of triglyceride formation in the liver is directly proportional to the concentration of plasma nonesterified fatty acids (NEFA). Rukkwamsuk et al. (1998) reported both hepatic triglyceride and plasma NEFA levels were higher in overfed cows.

2.3 Etiology of Ketosis

Ketosis can occur as clinical or subclinical, with each having subgroups of primary and secondary. When the disorder is associated with observable signs it is termed clinical. Some of these signs include: severe decreases in DMI, sweet smell of breath, rapid loss of body condition, lethargy, ataxia, head down, arched back, obsessive licking or grooming especially of the forearms, appetite for metal, chewing on stalls, and in some cases, nervousness, trembling, confusion, and in very rare cases coma and death (Baird, 1982). The cow’s body temperature will remain normal if there is no other existing agent to cause a fever. Biochemical characteristics of clinical ketosis include a minimum serum BHBA concentration of 27 mg/dl (Duffield, 2000), total urine ketone levels greater than 20 mg/dl (Kronfeld, 1980), elevated blood NEFA levels of greater than 2.0 mg/dL (Bergman, 1971), and accumulation of fat in the liver (Baird, 1982).
Subclinical ketosis occurs when there are no outward observable signs. However, the cow's blood serum BHBA levels are elevated slightly above normal, beginning at 10.4 mg/dl (Duffield, 2000). There is usually a slight decrease in milk production and DMI. The prevalence of subclinical ketosis worldwide has been reported to range from 8.9% to 34%, while the incidence of clinical ketosis during lactation varies from 2% to 15% (Baird, 1982). Subclinical ketosis is especially detrimental to the cow and because there is a decline in productivity without any apparent signs to warrant treatment. Slight decreases in DMI and milk yield as a result of subclinical ketosis may not be noticed by the producer, however over time those slight milk yield losses can add up to a significant profit loss. Subclinical ketosis also affects subsequent lactations which never allows the producer to receive the full milking potential of an otherwise unaffected, healthy cow (Duffield, 2000).

Primary and secondary denote the etiology of the disorder. Primary ketosis occurs when the cow is ketotic with no other signs of disorder or stress. Secondary ketosis results due to some other predisposing condition. There is disagreement in the literature concerning the existence of primary ketosis. As an example, Kronfeld (1971) concurs with the theory that a cow's ketone level spontaneously elevates, causing hypoglycemia and a subsequent decrease in DMI. However, Fox (1971) disagrees with this theory and argues that there is a lack of finding the preceding disorder, which secondarily causes ketosis. Baird (1982) uses spontaneous ketosis and primary ketosis interchangeably and defined both as ketosis caused only by a decrease in carbohydrate supply available to the cow. For clarity, spontaneous ketosis will be defined in this manuscript as ketosis that occurs with adequate energy intake and no predisposing
factors. Primary ketosis will denote ketosis caused only by a decrease in carbohydrate supply available to the cow.

There are discrepancies about which metabolic pathways are the most important in ketosis. Not one, but several metabolic pathways appear to be involved in the development of ketosis and disruption of any one of them can cause ketosis. The pathway usually disrupted in ketosis is the TCA cycle. A build up of acetyl-CoA results when it is not oxidized in the TCA cycle, either due to 1) a lack of propionate to sufficiently fuel the TCA cycle, or 2) the inability of the liver to efficiently metabolize lipids during negative energy balance. The particular circumstances presented to the cow before and during the onset of the disorder determine which metabolic pathways are disrupted in that particular case.

The event common to all cases of ketosis is low carbohydrate supply. Though milk production declines in all cases of fasting, milk production declines slower during the early lactation period (Baird, 1982). The cow's metabolic substrates and pathways need to be balanced to maintain metabolic order. A disruption of any part can cause a cow to be susceptible to ketosis (Kronfeld, 1980). Obvious disruptions are feed restriction due to hypophagia associated with calving, reduced DMI associated with sickness, injury, or poor quality feed, or the inability to consume enough feed to meet the energy demands of lactation. Deficiency in DMI reduces the amount of precursors available for efficient microbial production of volatile fatty acids (VFA) for energy for the cow and the gluconeogenic precursors needed for high milk production.

Though little glucogenic precursors come from this source, it should be noted that the triglycerides of a cow's body stores contain about 1-2% methyl-branched-chain and
odd chain fatty acids (Van Soest, 1994). Methyl-branched-chain and odd chain FA are oxidized in the same manner as even chain FA, except the last sequence leaves a 5 carbon fatty acyl-CoA which is cleaved leaving an acetyl-CoA and propionyl-CoA. The acetyl-CoA leaves to bind with oxaloacetate (OAA) in the TCA cycle while energy and biotin are needed to convert the propionyl-CoA to D-methylmalonyl-CoA. The D-methylmalonyl-CoA is isomerized to its L-stereoisomer then is rearranged to form succinyl-CoA, which eventually enters the TCA cycle (Nelson and Cox, 2000; Voet and Voet, 1995).

2.4 Biochemistry of Ketosis

When a cow is in negative energy balance, resulting low insulin levels initiate the mobilization of amino acids and lipid due to a decrease in energy supply for the cow (Fahey and Berger, 1988). Glycogen and amino acids mobilized are used for energy and glucogenic precursors that enter the TCA cycle. However, most of the amino acids mobilized from muscle breakdown of a cow in early lactation will be used for milk protein synthesis.

Because insulin is not required for uptake of glucose by the mammary gland, glucose is continually absorbed by the mammary gland even when blood glucose levels are dangerously low (Baird, 1982). The mammary gland’s high demand for glucose coupled with insufficient carbohydrate supply causes blood levels of glucose to drop and glucagon levels to increase. An increase in glucagon triggers lipase, which increases lipid breakdown for energy. To be mobilized, triglycerides are hydrolyzed into NEFA and glycerol, which provide energy. An increased level of NEFA from lipid degradation actually exaggerates the severity of ketosis. Stress usually associated with parturition or
sickness can elevate corticosteroids and epinephrine, which also cause the release of NEFA from adipose tissue (Drackley, 1999). The NEFA are carried through the blood and transported to the liver while the glycerol is used in active tissue to produce ATP via the glycolytic pathway. In the liver, the cleaved NEFA travel to the mitochondria via carrier-mediated transport by carnitine. Once the NEFA is in the mitochondria, it undergoes β-oxidation to form acetyl-CoA and fatty acyl-CoA. The fatty acyl-CoA is fed back into β-oxidation for further degradation until the entire NEFA has been dissected into acetyl-CoA. This available acetyl-CoA must be condensed with OAA to be utilized via the glycolytic pathway. In ruminants OAA formation is largely from propionate. Because DMI is depressed in ketosis, propionate production is usually reduced. Therefore, there may not be sufficient OAA present to condense with the acetyl-CoA units produced and allow them to efficiently enter the TCA cycle (Van Soest, 1994). These excess acetyl-CoA bind together to form acetoacetate that is converted to the ketones BHBA, acetoacetic acid, and acetone (Guyton and Hall, 2000). In this situation, the cow is unable to utilize the ketones fast enough causing them to spill over from the bloodstream into other bodily fluids. As ketonemia develops, the cow’s nervous system is affected and causes a subsequent decrease in DMI, which further escalates the ketotic state.

Although it is quite uncommon in current production settings, feeding poorly fermented silage can cause ketosis due to the high content of butyrate it contains. The cow consumes the silage and cannot metabolize the butyrate fast enough to compensate for its high intake. Butyrate builds up in the rumen, diffuses across the rumen wall and
enters the bloodstream. As excess butyrate is converted to BHBA in the liver, it spills over into the bloodstream causing ketosis (Adler et al., 1958).

A more probable cause of ketosis is starvation. Here a negative energy balance due to a decreased consumption of feed combined with high milk production triggers the mobilization of adipose stores resulting in an eventual build up of ketones. Metabolic priority is given to the mammary gland for glucose causing rapid mobilization of fat for energy to support other bodily functions (Baird, 1982). However, ketosis does not usually occur in first lactation heifers, probably because they have not yet reached their full milking potential (Baird, 1982).

Ketosis may be hereditary (Duffield, 2000; Schultz, 1971). However, cows affected by ketosis also tend to be high milk producers (Duffield, 2000; Schultz, 1971). Because milk production is heritable, ketosis may not be hereditary, but rather associated with high milk production (Schultz, 1971). As the dairy industry continues to select for high milk production, the incidence of ketosis may rise, emphasizing the need for early detection and treatment (Duffield, 2000). However, average milk production has risen over 2,800 kg/cow/year in the United States from 1982 to 2002 (USDA, 2003) without any reported increase in the incidence of ketosis. As an example, Duffield (2000) relied upon estimates of incidence of ketosis by Baird (1982). This implies that something other than increased milk production is responsible for any genetic link concerning ketosis.

2.5 Prevention and Treatments of Ketosis

Development of ketosis decreases milk production at the time of sickness and suppresses the cow's potential for maximum milk production for the current and all
subsequent lactation periods (Baird, 1982; Drackley, 1999). Elevated blood ketones associated with ketosis have been shown to inhibit lymphocyte proliferation and therefore suppress immune function (Franklin and Young, 1991). Since ketosis usually occurs during the transition period, and immune function is suppressed during this time, ketosis may increase the risk of developing infectious disease (Goff and Horst, 1997). Therefore prevention and treatment of ketosis is very important.

It has been estimated that subclinical ketosis reduces milk yield 300 to 450 kg per lactation (Duffield, 2000). Less milk produced and treatment for the condition cost the producer money, time and effort (Drackley, 1999). The total cost of ketosis including replacement costs, culling, milk loss, and treatment has been reported to be approximately 200 Canadian dollars per case (Duffield, 2000). By monitoring for ketosis for three days postpartum, cases can be detected earlier, which will reduce the amount of profit lost (Drackley, 1999; Spain, 2000). Monitoring increases labor and requires detection methods, but profits saved by early detection of ketosis can exceed the amount of monitoring costs.

Methods for monitoring ketosis include detection of an acetone smell on the cow's breath, milk or urine as well as a decrease in the cow's DMI with concentrate typically left uneaten (Fox, 1971). Methods also include routine testing for the presence of elevated levels of ketones in the urine, blood or milk (Fox, 1971). Simple reagent test strips can be used in the field for detection of elevated ketones in the urine (Magers and Tabb, 1979) or milk, while blood chemistries are the most definitive for detection (Duffield, 2000). It has been reported (Duffield, 2000; Kronfeld, 1980; Schultz, 1968) that low specificity, difficulty in interpreting a positive result and the difficulty of
collection makes the use of urine ketone concentrations for diagnosing ketosis in transition dairy cattle less popular than milk ketone concentrations among producers. However, Fox (1971) reported that using urine to detect ketosis is more sensitive during cases of subclinical ketosis in which milk may yield a negative test. Urine ketone concentrations may be useful as a general screening tool for ketosis prevention and detection because cows producing a negative test can be ruled out for ketosis (Schultz, 1968, Schultz, 1971).

There are several methods for the treatment of ketosis (Bergman, 1993; Kronfeld, 1980). Treatments are often used in combination because rarely is one method consistently successful in treating all cases (Bergman, 1993; Kronfeld, 1980). Any treatment of ketosis that has been caused by a preceding problem should include proper evaluation and therapy for the originating condition (Kronfeld, 1980). Cows may overcome ketosis with no treatment (Bergman, 1973; Bergman, 1993). However, overcoming the disorder without treatment prolongs the duration of ketosis resulting in a more substantial decrease in milk production from the cow (Bergman, 1993). Therefore, failing to treat a ketotic cow is costly to the producer. Any treatment that can raise or at least maintain blood sugar could in theory be used as a ketotic treatment, but those treatments that are the most effective, inexpensive, and easy are the methods most commonly used (Bergman, 1993). Many of the substances used for treatment of ketosis are also used for prevention.

An increase in DMI can help prevent or reverse ketosis by increasing the amount of glucogenic precursors available to the animal (Schultz, 1971). Increasing palatability
of the diet, frequent feeding, regular exercise and reduction of stress may increase the 
amount of feed consumed (Bergman, 1993; Schultz, 1971).

Oral administration of either propylene glycol or glycerol is commonly used as an 
initial treatment for ketosis. Propylene glycol is converted to pyruvate and glycerol. 
Pyruvate is utilized via the glycolytic pathway, thus supplying substrate for hepatic 
gluconeogenesis (Bergman, 1973, Kronfeld, 1980). Glycerol is utilized via the glycolytic 
pathway in active tissue (Bergman, 1973). Along with its use for treatment of ketosis, 
feeding propylene glycol has been examined for its effectiveness in the prevention of 
subclinical ketosis (Emery et al., 1964). Propylene glycol was shown to decrease milk 
ketone levels and increase milk production. Both glycerol as a treatment therapy and 
propylene glycol as either a preventative or treatment therapy prove effective due to their 
high body retention rate and low rumen degradation (Bergman, 1993; Emery et al., 
1964).

Intravenous (IV) glucose can be given as a first treatment, but is usually given as 
a second method when previous oral doses of propylene glycol or glycerol were not 
sufficient for reversing ketosis (Bergman, 1993, Kronfeld, 1980). An effective oral 
treatment is usually attempted first because it is less invasive than administration via IV, 
provided the case is not severe. Administration of glucose orally is not effective because 
almost the entire dose is fermented by the ruminal microbes (Bergman, 1993; Kronfeld, 
1980). Acute cases of ketosis may need a more invasive therapy such as IV glucose 
administration to rapidly increase blood glucose concentrations (Kronfeld, 1980). 
Restoring normal levels of blood glucose in the animal allows the central nervous system
to function normally thereby preventing severe complications of ketosis such as coma and death.

Though the mechanism of action is not fully understood, large amounts of nicotinic acid inhibits lipolysis thus decreasing the output of ketone bodies by the liver (Grummer, 1993; Schultz, 1968; Waterman et al., 1972). The most probable mechanism appears to be that niacin increases hepatic fat stores (Skaar et al., 1989). Niacin inhibits hepatic fat export in humans (Grundy et al., 1981), and may function similarly in ruminants. Oral administration of nicotinic acid was evaluated by Waterman et al. (1972) as a treatment for both subclinical and clinical cases of ketosis in lactating dairy cattle. Nicotinic acid was shown to decrease ketone levels. However, within 10 hours of treatment with nicotinic acid in subclinical cases and within 20 hours for the clinical cases, blood ketone levels began to rise to an even higher level than what was measured before treatment. Glucose levels rose after treatment but then declined sharply nearly 12 hours and again nearly 72 hours after treatment for both clinical and subclinical cases of ketosis. Waterman et al. (1972) did report that no relapses of ketosis occurred in those treated with nicotinic acid, however the period associated with very high ketone levels after treatment could be thought of as a relapse. Nicotinic acid may be considered as a short-term treatment for ketosis. However, the rebound phase associated with post-treatment may reduce the value of nicotinic acid as a treatment for ketosis. Schultz (1971) examined the effects of feeding nicotinic acid as a preventative measure. By continuing to add nicotinic acid to the ration after the levels of NEFA dropped, ketone levels spiked but then tapered off to a normal level with no relapses of ketosis occurring after the feeding of nicotinic acid (Schultz, 1971).
Glucocorticoids, primarily cortisol, are released during stress or can be given to increase the supply of energy to the animal. Glucocorticoids cause the breakdown of triacylglycerols releasing fatty acids for β-oxidation and glycerol that enters the TCA cycle. Glucocorticoids also cause the breakdown of muscle tissue to provide amino acids for gluconeogenesis and cause the breakdown of glycogen from the liver, which enters the TCA cycle. These products that enter the TCA cycle become precursors for gluconeogenesis in the liver (Guyton and Hall, 2000; Nelson and Cox, 2000). This method is attractive because it lasts much longer than an IV dose of glucose (Bergman, 1993; Fox, 1971).

Using propionate as a treatment has been shown to increase blood glucose levels (Schultz, 1971). Sodium propionate has been used as an effective treatment by providing propionate to the cow. However, if inhaled, sodium propionate can cause significant damage to lung tissue (Fox, 1971). Other salts of propionic acid such as calcium propionate may decrease the risk of adverse health effects. Lipker and Schlatter (1997) and Stokes and Goff (2001) reported decreased incidence of ketosis when Ca-propionate was fed during the entire transition period or as a bolus at calving. Feeding an increased amount of grain will also promote the production of propionate by shifting the rumen's population of microbes to those that ferment grain into propionate (Schultz, 1968).

Cobalt has been used as a treatment (Fox, 1971) and for prevention (Schultz, 1971) for ketosis. Vitamin B₁₂, which is synthesized by ruminal microbes, requires cobalt for its production. The conversion of propionate to succinate requires vitamin B₁₂ proving that a cobalt deficiency would be detrimental (Schultz, 1968; Schultz, 1971). Propionate travels in the blood to the liver where it binds to an acetyl-CoA to become
propionyl-CoA. Propionyl-CoA is carboxylated to form methylmalonyl-CoA. Methylmalonyl-CoA requires methylmalonyl-CoA mutase for its rearrangement to succinyl-CoA, which enters into the TCA cycle. Functioning as a coenzyme, vitamin B₁₂ is necessary for the function of methylmalonyl-CoA mutase (Fahey and Berger, 1988; Guyton and Hall, 2000; Nelson and Cox, 2000). Therefore, cobalt supplementation to prevent ketosis is to minimize any impact of vitamin B₁₂ deficiency and allow for optimal conversion efficiency of propionate to succinate.

2.6 Summary

As ketosis sets in and an overload of ketones is produced in the liver, they begin to spill into the bloodstream for subsequent mobilization (Baird, 1982; Guyton and Hall, 2000). When the bloodstream becomes saturated with acidic ketones, blood pH is threatened and must remain stable for normal metabolic function (Guyton and Hall, 2000). Blood pH changes very little because along with pulmonary expiration of CO₂ and acetone, the blood has an efficient chemical acid-base buffer system and an ability to remove acids when their blood concentrations reach dangerously high levels (Bergman, 1993; Guyton and Hall, 2000). One method for acid removal is via renal filtration into the urine. Once in the urine, ketones begin to alter urinary pH because the renal system has little buffering capacity, and the only way of removal of acid is through urination (Guyton and Hall, 2000). Because blood pH levels fluctuate minimally, urinary pH may be a more useful diagnostic measure for the presence or severity of ketosis particularly during subclinical stages. With this in mind, an experiment was designed to determine if urinary pH could be used as a diagnostic tool for ketosis in transition dairy cows.
2.7 Objectives

The objectives of this study were to 1) evaluate the ability of Ca-propionate supplementation to aid in the prevention of ketosis, 2) evaluate the impact of prepartum dietary energy concentration on incidence of ketosis and 3) test if urine pH can be used as a sole measurement to accurately predict ketosis in transition dairy cows.
CHAPTER 3. MATERIALS AND METHODS

3.1 Experimental Design, Dietary Treatments and Animal Care

Forty-one Holstein cows (mean BWT = 630.55 kg) were grouped by parity and anticipated parturition date and used in a randomized complete block designed experiment to 1) evaluate the ability of Ca-propionate supplementation to aid in the prevention of ketosis, 2) evaluate the impact of PDE concentration on incidence of ketosis and 3) test if urine pH can be used as a sole measurement to accurately predict ketosis in transition dairy cows. All animal handling was completed under protocols approved by the LSU Agricultural Center’s Institutional Animal Care and Use Committee.

Cows began the trial 3 weeks prior to their anticipated parturition date. Cows within each group were assigned to one of four treatments that were arranged as a 2 x 2 factorial. Factors were prepartum dietary energy concentration (normal vs. high) with or without 113.5g Ca-Propionate/day (Nutro Cal®, Kemin Industries, Inc., Des Moines, IA). Cows were fed treatment diets from the time they began the study until parturition. After parturition, all cows were fed the same diet with Ca-propionate supplementation continued as assigned prepartum. Cows were housed together while on trial with access to bedded free stalls, a 0.20235 ha pasture, and fresh water at all times. Body weight of all cows was recorded weekly.

Cows were fed at 0800 h. Diets (Table 3-1) were fed as total mixed rations with supplemental Ca-propionate top-dressed. Individual as offered feed intakes were measured daily using electronic feeding gates (American Calan, Inc., Northwood NH). Diets were sampled daily. Samples were stored frozen (-20°C) for subsequent nutrient
Table 3-1. Composition of the normal prepartum energy, high prepartum energy, and lactation diets.

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Normal energy</th>
<th>High energy</th>
<th>Lactation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>% of DM</td>
<td></td>
<td>% of DM</td>
</tr>
<tr>
<td>Alfalfa Hay</td>
<td>—</td>
<td>—</td>
<td>17.07</td>
</tr>
<tr>
<td>Bermudagrass Hay</td>
<td>49.98</td>
<td>8.16</td>
<td>—</td>
</tr>
<tr>
<td>Corn Silage</td>
<td>10.00</td>
<td>40.82</td>
<td>29.01</td>
</tr>
<tr>
<td>Ground Corn</td>
<td>15.02</td>
<td>20.41</td>
<td>10.24</td>
</tr>
<tr>
<td>Protein Concentrate¹</td>
<td>25.00</td>
<td>30.61</td>
<td>29.01</td>
</tr>
<tr>
<td>Whole Cottonseed</td>
<td>—</td>
<td>—</td>
<td>13.65</td>
</tr>
<tr>
<td>Sodium bicarbonate</td>
<td>—</td>
<td>—</td>
<td>1.02</td>
</tr>
</tbody>
</table>

¹22.16% corn, 56.16% soybean meal, 10.85% dolomitic limestone, 5.42% monocalcium phosphate, 5.42% trace mineral salt.
analysis. Orts for each cow were sampled weekly and frozen (-20°C) for later DM determination. Stored samples of ingredients, diets and orts were analyzed for DM (AOAC, 1980), the ingredients and diets were then ground to pass a 5mm screen. Dry, ground samples of diets were composited by week on an equal dry weight basis prior to nutrient analysis. Dry, ground samples of ingredients were composited by month prior to nutrient analysis. Samples of ingredients and diets were analyzed for Ash, N (AOAC, 1980), ADIN (AOAC, 1980; Van Soest et al., 1991), NDF, and ADF (Van Soest et al., 1991). After parturition, all cows were fed a constant diet (Table 3-1). The prepartum normal and high-energy diets were formulated to contain 105 and 145 % respectively of the estimated net energy requirements of a mature 748 kg dry Holstein cow and consuming 14.51 kg of DM/day (NRC, 2001). The postpartum diet was formulated to meet the requirements of a mature 658 kg lactating Holstein cow that is 15 DIM producing 23 kg/day of 3.5 % fat and 3.3 % true protein milk and consuming 13.52 (NRC, 2001).

3.2 Sample Collection and Analytical Procedures

Milk was sampled beginning on day three relative to parturition to avoid sampling colostrum, and production was recorded at each milking. Milk samples were analyzed for % fat, % protein, and SCC by the Louisiana Dairy Herd Improvement Laboratory (Baton Rouge, LA).

Blood samples were obtained by venipuncture from a coccygeal vessel 3x/week during weeks -3, -2, -1, +1, +2, +3, relative to parturition. Plasma was isolated from blood samples immediately after collection and frozen (-20°C) for subsequent analysis. Using commercial spectrophotometric kits, plasma was analyzed for glucose.
concentrations (Sigma Tech Bull. No.315; Sigma Chemical, St. Louis, MO). Plasma was then composited by week and analyzed for BHBA levels using a commercial reagent test kit (Sigma Diagnostics, St. Louis, MO, procedure No. 310-UV).

Samples of urine from each cow were collected 3x/week during weeks -3, -2, -1, +1, +2, +3, relative to parturition by gentle massage of the perineum. Immediately after collection, urine pH was recorded, and acetoacetate concentrations were measured using Ketostix® test strips (Bayer Corporation Diagnostics Division, Elkhart, IN, Magers and Tabb, 1979). Urine samples were then frozen (-20°C) for later laboratory analysis of BHBA (Sigma Diagnostics; St. Louis, MO, procedure No. 310-UV).

3.3 Statistical Analysis

Chemical analysis of the normal prepartum energy, high prepartum energy, and postpartum diets were analyzed using ANOVA and Fisher's LSD. Data for BWT, urine pH, urine acetoacetate, and urine and plasma BHBA were analyzed by ANOVA using a mixed model which included terms for fixed effects of prepartum dietary energy level, Ca-propionate supplementation, week, and their interactions. Cow nested in the interaction of Ca-propionate and dietary energy level was included as a random variable that was used as the error term for the main effects. Week was modeled as a repeated term that was assumed to be correlated within cow using a constant covariance structure. Data for DMI and milk production data were analyzed by ANOVA using a mixed model, which included terms for fixed effects of prepartum dietary energy level, Ca-propionate supplementation, week or day, and their interactions. Cow nested in the interaction of Ca-propionate and dietary energy level was included as a random variable that was used as the error term for the main effects. Week was modeled as a repeated term that was
assumed to be correlated within cow using a type I autoregressive covariance structure. Pearson correlation coefficients within urine measurements (acetoacetate concentrations, BHBA, and pH) were calculated across all cows, for each cow, by diet type, for Ca-propionate supplementation, and by their interactions. All calculations were performed using SAS (SAS Institute Inc., 1990).
CHAPTER 4. RESULTS AND DISCUSSION

4.1 Chemical Analysis of Experimental and Lactation Diets

The high-energy prepartum diet had lower DM ($P < 0.01$; Table 4-1), NDF ($P < 0.01$), and ADF ($P < 0.01$) than the normal prepartum energy diet, but there were no differences in the contents of total N or ash between the high-energy prepartum diet and the normal energy prepartum diet. The high-energy prepartum diet had lower ADIN ($P = 0.01$; Table 4-1) and bound CP ($P = 0.01$) than the normal energy prepartum diet but had a greater amount of available CP ($P = 0.01$) than the normal energy prepartum diet. These results are expected because compared to the normal energy prepartum diet, more concentrate in the ration is necessary to formulate energy dense diets (as seen with the high-energy prepartum and lactation diets), and therefore these diets contain less fiber to maximize the amount of calories consumed by the cow without having to increase the amount of feed consumed. The decreased amount of fiber in the high-energy prepartum diet also reflects the decreased amount of bound CP usually associated with higher amounts of dietary lignin (Van Soest, 1994). Because total N was not different between the prepartum diets and bound CP was lower in the high-energy prepartum diet, available CP increased in the high-energy prepartum diet. Analysis of the lactation diet can be seen in Table 4-1.

4.2 Lactational Performances and Bodyweights

The DMI was not effected by PDE ($P = 0.93$; Table 4-2), Ca-propionate ($P = 0.23$), or their interaction ($P = 0.84$). Rigout et al. (2003) also reported no differences in DMI for cows ruminally supplemented with propionate. Similarly, Skaar et al. (1989) reported no differences in DMI when feeding increased amounts of energy during the transition
Table 4-1. Means of chemical analysis for normal prepartum energy, high prepartum energy, and lactation diets.

<table>
<thead>
<tr>
<th>Item</th>
<th>Normal energy</th>
<th>High energy</th>
<th>Lactation</th>
<th>SEM</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>DM, %</td>
<td>65.18</td>
<td>43.43</td>
<td>48.35</td>
<td>0.41</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>-----------------</td>
<td>---------------</td>
<td>-------------</td>
<td>-----------</td>
<td>------</td>
<td>---------</td>
</tr>
<tr>
<td>% of DM</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>N</td>
<td>2.21</td>
<td>2.48</td>
<td>2.28</td>
<td>0.10</td>
<td>0.12</td>
</tr>
<tr>
<td>CP(^1)</td>
<td>13.82</td>
<td>15.52</td>
<td>14.23</td>
<td>0.61</td>
<td>0.12</td>
</tr>
<tr>
<td>ADIN(^2)</td>
<td>0.31</td>
<td>0.23</td>
<td>0.24</td>
<td>0.02</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>Bound CP(^3)</td>
<td>1.93</td>
<td>1.44</td>
<td>1.51</td>
<td>0.13</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>Available CP(^4)</td>
<td>11.90</td>
<td>14.08</td>
<td>12.90</td>
<td>0.63</td>
<td>0.04</td>
</tr>
<tr>
<td>NDF</td>
<td>51.65</td>
<td>40.99</td>
<td>42.10</td>
<td>1.01</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>ADF</td>
<td>25.42</td>
<td>17.97</td>
<td>23.51</td>
<td>0.65</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>Ash</td>
<td>6.45</td>
<td>7.63</td>
<td>7.27</td>
<td>1.27</td>
<td>0.77</td>
</tr>
<tr>
<td>NE(_L) Mcal/kg(^5)</td>
<td>1.63</td>
<td>1.85</td>
<td>1.83</td>
<td>—</td>
<td>—</td>
</tr>
</tbody>
</table>

\(^1\) CP = N x 6.25  
\(^2\) ADIN = acid detergent insoluble nitrogen  
\(^3\) Bound CP = ADIN x 6.25  
\(^4\) Available CP = CP - Bound CP  
\(^5\) Calculated from NRC (2001)
Table 4-2. Least squares means of lactational performance and body weights of cows fed diets with different prepartum energy concentrations with or without supplemental calcium propionate.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Normal Energy</th>
<th>High Energy</th>
<th>SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(-) Ca-prop</td>
<td>(+) Ca-prop</td>
<td>(-) Ca-prop</td>
</tr>
<tr>
<td>DMI (kg), prepartum</td>
<td>9.5</td>
<td>8.7</td>
<td>9.9</td>
</tr>
<tr>
<td></td>
<td>12.6</td>
<td>10.3</td>
<td>13.3</td>
</tr>
<tr>
<td>Milk, kg</td>
<td>26.6</td>
<td>20.8</td>
<td>25.3</td>
</tr>
<tr>
<td>4% FCM, kg</td>
<td>26.4</td>
<td>21.7</td>
<td>26.4</td>
</tr>
<tr>
<td>Fat, %</td>
<td>4.1</td>
<td>4.3</td>
<td>4.3</td>
</tr>
<tr>
<td>kg</td>
<td>1.1</td>
<td>0.9</td>
<td>1.1</td>
</tr>
<tr>
<td>Protein, %</td>
<td>2.7</td>
<td>2.7</td>
<td>2.9</td>
</tr>
<tr>
<td>kg</td>
<td>0.7</td>
<td>0.6</td>
<td>0.8</td>
</tr>
<tr>
<td>SCCS</td>
<td>3.1</td>
<td>4.3</td>
<td>4.1</td>
</tr>
<tr>
<td>BWT (kg), prepartum</td>
<td>631.3</td>
<td>679.9</td>
<td>694.9</td>
</tr>
<tr>
<td></td>
<td>571.1</td>
<td>585.5</td>
<td>606.9</td>
</tr>
</tbody>
</table>
period. There were no significant interactions of PDE ($P = 0.88$), Ca-propionate ($P = 0.43$) or their interaction ($P = 0.85$; Figure 4-1) with time for DMI. However, DMI trends reflected those typical for transition cows (Lyford and Huber, 1988).

Supplemental Ca-propionate tended to decrease milk production ($P = 0.15$; Table 4-2), however, PDE ($P = 0.78$) and the interaction of PDE and Ca-propionate ($P = 0.55$) did not effect milk production. This is in contrast to data presented by Schultz (1971) and Stokes and Goff (2001) who reported that milk production increased with the addition of Ca-propionate postpartum. Additionally, Goff et al. (1996) and Melendez et al. (2002) reported no difference in milk yield with the addition of Ca-propionate postpartum.

Increased energy prepartum (Skaar et al., 1989) and infusion of propionate into the rumen postpartum (Rigout et al., 2003) have been shown to increase milk production. Though not significant, cows supplemented with Ca-propionate had a numeric decrease in DMI, possibly causing the slight decrease in total milk production. There were no significant interactions of PDE ($P = 0.91$), Ca-propionate ($P = 0.71$) or their interaction ($P = 0.94$; Figure 4-2) with time for total milk yield.

Since there were no differences in milk fat percentage, 4% FCM was not affected by PDE ($P = 0.83$), or the interaction of PDE and Ca-propionate ($P = 0.83$). However, there was a tendency for Ca-propionate to decrease 4% FCM production ($P = 0.14$). This is most likely due to the numeric decrease in total milk production from cows fed Ca-propionate. There also were no significant interactions of PDE ($P = 0.45$), Ca-propionate ($P = 0.17$) or their interaction ($P = 0.84$; Figure 4-3) with time for 4% FCM.

Milk fat percentage was not affected by PDE ($P = 0.75$; Table 4-2), Ca-propionate ($P = 0.70$), or their interaction ($P = 0.53$). Milk fat production was not affected by PDE
Figure 4-1. Least squares means for DMI for cows fed diets with normal or high prepartum energy concentrations with or without supplemental calcium propionate.
Figure 4-2. Least squares means for milk production for cows fed diets with normal or high prepartum energy concentrations with or without supplemental calcium propionate.
Figure 4-3. 4% FCM for cows fed diets with normal or high prepartum energy concentrations with or without supplemental calcium propionate.
(P = 0.68), or the interaction of PDE and Ca-propionate (P = 0.86), but Ca-propionate supplementation tended to decrease milk fat production (P = 0.10). Although there were no changes in milk fat percentage observed, there was a slight numeric decrease in milk fat yield. This is probably associated with the slight numeric decrease in milk yield. Skaar et al. (1989) also reported no change in milk fat percentage or yield with energy supplementation prepartum. However, Rigout et al. (2003) reported a decrease in milk fat percentage and yield with ruminal supplementation of propionate postpartum.

Milk protein percentage was not affected by PDE (P = 0.58; Table 4-2), Ca-propionate (P = 0.62) or their interaction (P = 0.73). Milk protein production was not affected by PDE (P = 0.53) or the interaction of PDE and Ca-propionate (P = 0.83), but Ca-propionate tended to decrease milk protein production (P = 0.10). Rigout et al. (2003) and Skaar et al. (1989) reported similar results with supplemental energy prepartum and ruminal supplementation of propionate postpartum, respectively.

The SCCS was not affected by PDE (P = 0.40), Ca-propionate (P = 0.22) or their interaction (P = 0.43). The mean SCCS ranged from 3.1 to 4.3. However, a large incidence of uterine infections was observed in these cows and this systematic infection may have been artificially increasing the shedding of somatic cells into the milk although we have no way to determine if this is true.

Mean BWT was not affected by PDE (P = 0.33), Ca-propionate (P = 0.72) or their interaction (P = 0.30). Similarly, there were no interactions of treatments with time (P > 0.30; Figure 4-4) indicating that all cows gained and lost similar amounts of weight before and after parturition, respectfully.
Figure 4-4. Bodyweights for cows fed diets with normal or high prepartum energy concentrations with or without supplemental calcium propionate.
4.3 Mean Urine and Plasma Metabolites

Mean urine pH was not effected by Ca-Propionate ($P = 0.99$; Table 4-3) or the interaction of diet and Ca-Propionate ($P = 0.90$), but the high-energy diet tended to decrease urine pH ($P = 0.06$). There were no significant interactions of PDE ($P = 0.95$), or the interaction of PDE and Ca-propionate with time ($P = 0.17$; Figure 4-5) for urine pH. However there was an interaction of Ca-propionate supplementation with week ($P = 0.06$; Figure 4-6) for urine pH. Urine pH of cows not fed supplemental Ca-propionate tended to remain stable as compared to the urine pH of cows that were supplemented with Ca-propionate.

Mean urine acetoacetate concentrations were not affected by diet ($P = 0.70$; Table 4-3), Ca-Propionate ($P = 0.89$) or their interaction ($P = 0.59$). There were no significant interactions of PDE ($P = 0.92$), Ca-propionate ($P = 0.15$; Figure 4-7) or the interaction of PDE and Ca-propionate ($P = 0.17$; Figure 4-8) with time for urine acetoacetate concentrations. Although not statistically significant, by week 3 postpartum urine acetoacetate concentrations of cows supplemented with Ca-propionate began to fall while urine acetoacetate concentrations of cows not supplemented with Ca-propionate were still rising. This suggests that supplemental Ca-propionate allowed cows to recover faster from ketosis than if Ca-propionate was not supplemented. If urine acetoacetate concentrations had been monitored past 3 weeks postpartum, the effect of Ca-propionate supplementation may have been more readily observed.

Mean urine BHBA levels were not affected by diet ($P = 0.75$; Table 4-3), Ca-propionate ($P = 0.99$) or their interaction ($P = 0.21$). There were no significant interactions of PDE ($P = 0.78$), or the interaction of PDE and Ca-propionate ($P = 0.07$;
Table 4-3. Least squares means of urine pH and metabolites in urine and plasma for cows fed diets with different prepartum energy concentrations with or without supplemental calcium propionate during the entire trial and both pre and post calving.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Normal Energy</th>
<th></th>
<th>High Energy</th>
<th></th>
<th>SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(-) Ca-prop</td>
<td>(+) Ca-prop</td>
<td>(-) Ca-prop</td>
<td>(+) Ca-prop</td>
<td></td>
</tr>
<tr>
<td>Urine pH</td>
<td>Overall</td>
<td>8.0</td>
<td>8.0</td>
<td>7.9</td>
<td>7.7</td>
</tr>
<tr>
<td></td>
<td>Pre</td>
<td>8.0</td>
<td>8.1</td>
<td>7.9</td>
<td>7.9</td>
</tr>
<tr>
<td></td>
<td>Post</td>
<td>8.0</td>
<td>7.9</td>
<td>7.8</td>
<td>7.7</td>
</tr>
<tr>
<td>Urine acetoacetate, mg/dl</td>
<td>Overall</td>
<td>15.2</td>
<td>17.6</td>
<td>16.2</td>
<td>12.2</td>
</tr>
<tr>
<td></td>
<td>Pre</td>
<td>3.5</td>
<td>1.7</td>
<td>2.5</td>
<td>2.2</td>
</tr>
<tr>
<td></td>
<td>Post</td>
<td>25.7</td>
<td>35.4</td>
<td>29.1</td>
<td>23.9</td>
</tr>
<tr>
<td>Urine BHBA, mg/dl</td>
<td>Overall</td>
<td>6.6</td>
<td>15.7</td>
<td>18.1</td>
<td>8.8</td>
</tr>
<tr>
<td></td>
<td>Pre</td>
<td>0.8</td>
<td>0.7</td>
<td>1.0</td>
<td>1.0</td>
</tr>
<tr>
<td></td>
<td>Post</td>
<td>12.1</td>
<td>32.8</td>
<td>35.2</td>
<td>17.7</td>
</tr>
<tr>
<td>Plasma BHBA, mg/dl</td>
<td>Overall</td>
<td>7.9</td>
<td>8.3</td>
<td>9.6</td>
<td>7.2</td>
</tr>
<tr>
<td></td>
<td>Pre</td>
<td>6.1</td>
<td>4.5</td>
<td>5.5</td>
<td>5.8</td>
</tr>
<tr>
<td></td>
<td>Post</td>
<td>9.7</td>
<td>12.2</td>
<td>13.7</td>
<td>8.6</td>
</tr>
<tr>
<td>Plasma glucose, mg/dl</td>
<td>Overall</td>
<td>53.74</td>
<td>54.30</td>
<td>55.23</td>
<td>54.78</td>
</tr>
<tr>
<td></td>
<td>Pre</td>
<td>56.91</td>
<td>58.32</td>
<td>60.24</td>
<td>57.57</td>
</tr>
<tr>
<td></td>
<td>Post</td>
<td>51.23</td>
<td>50.28</td>
<td>50.22</td>
<td>51.58</td>
</tr>
</tbody>
</table>
Figure 4-5. Least squares means of urine pH of cows fed diets with normal or high prepartum energy concentrations with or without supplemental calcium propionate.
Figure 4-6. Least squares means of urine pH of cows fed diet with or without supplemental calcium propionate.
Figure 4-7. Least squares means of urine acetoacetate concentrations of cows fed diets with or without supplemental calcium propionate.
Figure 4-8. Least squares means of urine acetoacetate concentrations of cows fed diets with normal or high prepartum energy concentrations and with or without supplemental calcium propionate.
Figure 4-9) with time for urine BHBA. However, there was a significant interaction of Ca-propionate ($P = 0.02$; Figure 4-10) with time for urine BHBA concentrations. Ca-propionate supplementation decreased levels of urine BHBA after 2 weeks postpartum. This suggests that supplemental Ca-propionate allowed cows to recover from ketosis more quickly than if Ca-propionate was not supplemented. If urine BHBA concentrations had been monitored past 3 weeks postpartum, the effect of Ca-propionate supplementation may have been more clearly observed.

Mean plasma BHBA levels were not affected by diet ($P = 0.90$; Table 4-3), Ca-Propionate ($P = 0.62$) or their interaction ($P = 0.49$). There were no significant interactions of PDE ($P = 0.73$), Ca-propionate ($P = 0.34$) or the interaction of PDE and Ca-propionate ($P = 0.32$; Figure 4-11) with time for mean plasma BHBA concentrations. Rigout et al. (2003) reported a decrease in plasma BHBA when propionate was supplemented to cows postpartum. Also, Goff et al. (1996), Melendez et al. (2002) and Stokes and Goff (2001) reported a decrease in plasma BHBA levels when cows were supplemented with Ca-propionate postpartum. Though not significant, numerical differences were observed. Cows supplemented with Ca-propionate and fed high PDE had lower plasma BHBA levels compared to those fed normal PDE and not supplemented with Ca-propionate. This may suggest that cows fed high PDE produced more ruminal propionate and thus were more metabolically adapted to use the supplemented propionate. Cows fed normal PDE without proper transition to the lactation diet are less adapted to utilize supplemental Ca-propionate.

39
Figure 4-9. Least squares means for urine BHBA concentrations for cows fed diets with normal or high prepartum energy concentrations with or without supplemental calcium propionate.
Figure 4-10. Least squares means of urine BHBA concentration of cows fed diets with or without supplemental calcium propionate.
Figure 4-11. Least squares means for plasma BHBA concentration of cows fed diets with normal or high prepartum energy concentrations and with or without supplemental calcium propionate.
It should be noted that during the prepartum period, levels of urine acetoacetate, urine BHBA and plasma BHBA were at very low levels. This implies that there was no incidence of ketosis. During the postpartum period, those levels were much higher showing the risk of ketosis to be much higher after parturition because of the increased energy demands placed on the cow immediately after calving.

Mean plasma glucose levels were not affected by diet ($P=0.55$, Table 4-3), Ca-propionate ($P=0.97$) or their interaction ($P=0.76$). There were no significant interactions of PDE ($P=0.78$), Ca-propionate ($P=0.87$) or the interaction of PDE and Ca-propionate ($P=0.35$, Figure 4-12) for mean plasma glucose concentrations. Rigout et al. (2003) reported no change in plasma glucose when propionate was supplemented to cows postpartum. Also, Melendez et al. (2002) and Stokes and Goff (2001) reported no change in plasma glucose levels when cows were supplemented with Ca-propionate postpartum. Because an animal’s system aggressively maintains relatively constant plasma glucose to ensure survival (Guyton and Hall, 2000), these results were expected.

4.4 Prepartum/Postpartum Urine and Plasma Metabolites

Typically during the transition period major differences in blood and urine metabolites occur prepartum vs. postpartum, therefore, urine and plasma metabolites prepartum and postpartum were evaluated separately to further describe those changes present in the cows relative to treatment (Table 4-3). Prepartum urine pH was not affected by supplementation of Ca-propionate ($P=0.33$) or the interaction of PDE and Ca-propionate ($P=0.45$), however, increased PDE decreased urine pH prepartum ($P=$
Figure 4-12. Plasma glucose concentrations for cows fed diets with normal or high prepartum energy concentrations with or without supplemental calcium propionate.
Postpartum urine pH was not affected by PDE \((P = 0.20)\) Ca-propionate \((P = 0.46)\) or the interaction of PDE and Ca-propionate \((P = 0.87)\). Neither prepartum nor postpartum urine acetoacetate concentrations were affected by PDE \((P = 0.86; P = 0.74)\), supplementation of Ca-propionate \((P = 0.47; P = 0.86)\) or the interaction of PDE and Ca-propionate \((P = 0.62; P = 0.55)\). Similarly, neither prepartum nor postpartum urine BHBA concentrations were affected by PDE \((P = 0.45; P = 0.80)\), supplementation of Ca-propionate \((P = 0.86; P = 0.92)\) or the interaction of PDE and Ca-propionate \((P = 0.96; P = 0.23)\). Neither prepartum nor postpartum plasma BHBA concentrations were affected by PDE \((P = 0.63; P = 0.94)\), supplementation of Ca-propionate \((P = 0.33; P = 0.65)\) or the interaction of PDE and Ca-propionate \((P = 0.13; P = 0.23)\). Neither prepartum nor postpartum plasma glucose concentrations were affected by PDE \((P = 0.44; P = 0.96)\), supplementation of Ca-propionate \((P = 0.70; P = 0.94)\) or the interaction of PDE and Ca-propionate \((P = 0.23; P = 0.69)\).

4.5 Correlations of Urine Parameters

Urine BHBA concentrations and urine pH \((r = -0.54; \text{Figure 4-13})\) and urine pH and urine acetoacetate concentrations \((r = -0.51; \text{Figure 4-14})\) were both negatively correlated across all cows. However, the relationship does not appear useful as a predictor of ketosis. Urine BHBA and acetoacetate concentrations were highly correlated \((r = 0.83; \text{Figure 4-15})\) which was expected since acetoacetate is an intermediate in the formation of and a metabolite of the breakdown of BHBA (Guyton and Hall, 2000).

Correlation coefficients within treatment (Table 4-4) were greater than those across all cows. This suggests that knowing more information about a cow in the
Figure 4-13. Scatter plot of urine BHBA and pH concentrations and the regression line between these values from cows fed diets with normal or high prepartum energy concentrations and with or without supplemental calcium propionate.
Figure 4-14. Scatter plot of urine pH and acetoacetate concentrations and the regression line between these values from cows fed diets with normal or high prepartum energy concentrations and with or without supplemental calcium propionate.
Figure 4-15. Scatter plot of urine BHBA and acetoacetate concentrations and the regression line between these values from cows fed diets with normal or high prepartum energy concentrations and with or without supplemental calcium propionate.
Table 4-4. Correlations of urine parameters for cows fed diets with different prepartum energy concentrations with or without supplemental calcium propionate.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Normal Energy</th>
<th></th>
<th>High Energy</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(-) Ca-prop</td>
<td>(+) Ca-prop</td>
<td>(-) Ca-prop</td>
<td>(+) Ca-prop</td>
</tr>
<tr>
<td>BHBA (mg/dl) / pH</td>
<td>-0.14924</td>
<td>-0.65243</td>
<td>-0.71848</td>
<td>-0.28798</td>
</tr>
<tr>
<td>BHBA (mg/dl) / Acetoacetate (mg/dl)</td>
<td>0.88339</td>
<td>0.81087</td>
<td>0.91610</td>
<td>0.77630</td>
</tr>
<tr>
<td>pH / Acetoacetate (mg/dl)</td>
<td>-0.34659</td>
<td>-0.63855</td>
<td>-0.70034</td>
<td>-0.42665</td>
</tr>
</tbody>
</table>
transition period (such as diet type) may increase the usefulness of urine measurements for predicting incidence of ketosis.

Correlation coefficients within each cow (appendix) were generally higher for all cows and indicate that urine parameters are most useful for predicting incidence of ketosis for individual animals. These data suggest that comparing urine BHBA concentrations or urine pH measurements to those previously recorded for that particular cow, may allow them to be used as predictors of ketosis. Though monitoring of urine parameters for each transition cow is labor intensive and will require the purchase of monitoring equipment, prevention of subclinical ketosis not only reduces the risk of the development of other disorders, but also prevents the damaging effects ketosis has on subsequent lactations. Also, transition and fresh cow programs for successful management during the transition period recommend more intensive monitoring of transition cows.
CHAPTER 5. SUMMARY AND CONCLUSIONS

Supplementation of Ca-propionate during the transition period did not significantly alter the incidence of ketosis in Holstein cows. Milk production as well as milk protein synthesis tended to decrease with the supplementation of Ca-propionate. However, supplementation of Ca-propionate during the transition period allowed cows to recover faster from ketosis postpartum than not having received supplementation at all. Feeding a high-energy prepartum diet to Holstein cows tended to increase urine pH, but prepartum dietary energy level had no significant effect on total milk production, milk components or on the incidence of ketosis. The interaction of Ca-propionate supplementation and prepartum dietary energy level had no significant effect on the incidence of ketosis, total milk production or milk components in Holstein transition cows. The use of urine pH to predict ketosis is not useful within a herd, but when used within an individual animal, urine pH may prove useful as a predictor of ketosis in transition dairy cows.

Due to factors unrelated to treatment diets there was a high incidence of health disorders such as retained fetal membranes, uterine infections, displaced abomasums, and laminitis during this trial that may have taken precedence over any treatment effects. However, with good management, including close transition cow monitoring such as daily individual urine pH observations, Ca-propionate supplementation may be useful to prevent ketosis, and urine pH may be used as a screening tool for detection of those cows not at risk for developing ketosis.
REFERENCES


Lipker, T. and L. Asclatter. 1997. The ability of NutroCAL to maintain ketone levels below that observed in control animals. Kemin Technical Information. Kemin Industries, Inc., Des Moines, IA.


### APPENDIX. Correlation Coefficients for Urine Parameters for Each Cow

<table>
<thead>
<tr>
<th>Cow</th>
<th>BHBA/pH</th>
<th>BHBA/acetoacetate</th>
<th>PH/acetoacetate</th>
</tr>
</thead>
<tbody>
<tr>
<td>H528</td>
<td>0.16438</td>
<td>0.84935</td>
<td>-0.91190</td>
</tr>
<tr>
<td>H537</td>
<td>-0.26135</td>
<td>0.95542</td>
<td>-0.51776</td>
</tr>
<tr>
<td>H543</td>
<td>-0.28103</td>
<td>NA^1</td>
<td>NA</td>
</tr>
<tr>
<td>H544</td>
<td>0.24538</td>
<td>NA</td>
<td>0.76482</td>
</tr>
<tr>
<td>H545</td>
<td>0.05965</td>
<td>0.74084</td>
<td>-0.23062</td>
</tr>
<tr>
<td>H574</td>
<td>-0.22676</td>
<td>0.42695</td>
<td>-0.27821</td>
</tr>
<tr>
<td>H583</td>
<td>0.21652</td>
<td>0.41925</td>
<td>-0.30421</td>
</tr>
<tr>
<td>H607</td>
<td>0.40524</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>H611</td>
<td>0.33973</td>
<td>0.36000</td>
<td>-0.01316</td>
</tr>
<tr>
<td>H613</td>
<td>-0.68695</td>
<td>0.92287</td>
<td>-0.55045</td>
</tr>
<tr>
<td>H616</td>
<td>-0.19048</td>
<td>0.95456</td>
<td>-0.29487</td>
</tr>
<tr>
<td>H617</td>
<td>-0.23799</td>
<td>0.39185</td>
<td>-0.83062</td>
</tr>
<tr>
<td>H621</td>
<td>-0.64188</td>
<td>0.84982</td>
<td>-0.67605</td>
</tr>
<tr>
<td>H623</td>
<td>0.18619</td>
<td>0.60752</td>
<td>-0.20751</td>
</tr>
<tr>
<td>H634</td>
<td>0.16796</td>
<td>0.99514</td>
<td>NA</td>
</tr>
<tr>
<td>H635</td>
<td>0.81700</td>
<td>0.99658</td>
<td>-0.83519</td>
</tr>
<tr>
<td>H647</td>
<td>0.24161</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>H649</td>
<td>-0.06399</td>
<td>0.92880</td>
<td>-0.29015</td>
</tr>
<tr>
<td>H654</td>
<td>0.08900</td>
<td>0.83596</td>
<td>-0.40557</td>
</tr>
<tr>
<td>H658</td>
<td>0.53909</td>
<td>0.98070</td>
<td>0.47847</td>
</tr>
<tr>
<td>H659</td>
<td>-0.55749</td>
<td>0.70535</td>
<td>-0.35039</td>
</tr>
<tr>
<td>H661</td>
<td>0.25644</td>
<td>0.61004</td>
<td>NA</td>
</tr>
<tr>
<td>H662</td>
<td>0.58018</td>
<td>0.34206</td>
<td>-0.12666</td>
</tr>
<tr>
<td>H663</td>
<td>0.15931</td>
<td>0.82013</td>
<td>-0.01926</td>
</tr>
<tr>
<td>H666</td>
<td>-0.88490</td>
<td>0.98316</td>
<td>-0.78395</td>
</tr>
<tr>
<td>H669</td>
<td>0.35734</td>
<td>NA</td>
<td>-0.36525</td>
</tr>
<tr>
<td>H673</td>
<td>-0.64120</td>
<td>0.94070</td>
<td>-0.44589</td>
</tr>
<tr>
<td>P078</td>
<td>-0.61938</td>
<td>0.97403</td>
<td>-0.58560</td>
</tr>
<tr>
<td>P087</td>
<td>-0.22972</td>
<td>0.88408</td>
<td>-0.32896</td>
</tr>
<tr>
<td>P108</td>
<td>-0.80422</td>
<td>0.99542</td>
<td>-0.56357</td>
</tr>
<tr>
<td>P109</td>
<td>0.13182</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>P114</td>
<td>-0.67547</td>
<td>0.84981</td>
<td>-0.66505</td>
</tr>
<tr>
<td>P116</td>
<td>-0.85524</td>
<td>0.95731</td>
<td>-0.81273</td>
</tr>
<tr>
<td>P125</td>
<td>-0.42323</td>
<td>0.95865</td>
<td>-0.47103</td>
</tr>
<tr>
<td>P126</td>
<td>0.12112</td>
<td>0.96851</td>
<td>0.00400</td>
</tr>
<tr>
<td>P130</td>
<td>-0.24751</td>
<td>0.95676</td>
<td>-0.31039</td>
</tr>
<tr>
<td>P136</td>
<td>0.08632</td>
<td>0.64279</td>
<td>-0.64358</td>
</tr>
<tr>
<td>P140</td>
<td>-0.59907</td>
<td>0.81049</td>
<td>-0.81025</td>
</tr>
<tr>
<td>P142</td>
<td>0.24795</td>
<td>0.62154</td>
<td>0.02188</td>
</tr>
<tr>
<td>P143</td>
<td>-0.96334</td>
<td>0.94633</td>
<td>-0.76390</td>
</tr>
<tr>
<td>P147</td>
<td>0.08761</td>
<td>NA</td>
<td>NA</td>
</tr>
</tbody>
</table>

^1NA - Not available
VITA

Amy Elizabeth Beem was born on August 26, 1978, in Sioux City, Iowa. Amy moved with her mother to Baton Rouge, Louisiana, in April 1988. After graduating from Bishop Sullivan High School, Baton Rouge, Louisiana, in 1996 Amy began an undergraduate program at the University of Southwestern Louisiana, Lafayette, Louisiana, in accounting. After only one semester she decided to pursue her dream of becoming a veterinarian and continued in the Pre-Veterinary curriculum at USL for the following three semesters. In the fall of 1998 Amy entered Louisiana State University and graduated in December of 2000 with a bachelor of science degree in animal dairy and poultry science. Beginning in the spring of 2001, Amy started a master's program in the Department of Dairy Science at LSU under the direction and guidance of Dr. H. Gale Bateman, II. During her first year of graduate studies, Amy was admitted into the Louisiana State University School of Veterinary Medicine to which she deferred acceptance until the following year to continue her master's program. In the fall of 2003, Amy entered her first year of Veterinary School and then returned to the LSU Department of Dairy Science the following summer to complete her master's program. Amy was a research assistant under Dr. Bateman in the nutrition group at the LSU Department of Dairy Science during her entire graduate program. In August of 2003, Amy will receive her degree of Master of Science and will immediately continue her veterinary education for her 2006 scheduled graduation date from Louisiana State University School of Veterinary Medicine.