THE ROLE OF CALCIUM ON NEUROENDOCRINE REGULATION OF ENERGY BALANCE IN PERIPARTURIENT DAIRY CATTLE

by

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(Under the Direction of Mark A. Froetschel)

ABSTRACT

Improved calcium status may facilitate the transition dairy cow to more effectively use supplemental fat and reduce health problems associated with negative energy balance. This research tested the effect of enhancing calcium mobilization pre-partum by feeding anionic salts on the ability of cows to consume and metabolize relatively high levels of supplemental fat postpartum. Diurnal circulating concentrations of hormones and metabolites (insulin, glucose, blood urea nitrogen (BUN), non-esterified fatty acids (NEFA), and plasma calcium) were measured in early lactation. Cows fed anionic salts pre-partum and supplemental fat post-partum produced as much as 28% more milk in early lactation (week 8 through 12) than the control-fed counterparts. Endocrine and metabolic parameters indicate feeding an anionic salt prepartum diet enhanced calcium status in early lactation and improved insulin responsiveness, dietary fat utilization, and adipose tissue mobilization in a manner consistent with greater animal health and productivity.

INDEX WORDS: Dietary Cation-Anion Difference (DCAD), Anionic Salts, Calcium status, Ruminally Inert Fat (RIF), Transition Cow

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DEDICATION

To my mom, who means the world to me and to Alex, who is my peace.

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CHAPTER 1

LITERATURE REVIEW

The transition dairy cow

The modern cow in U.S. dairy herds is a product of intensive genetic selection for milk production and increasingly requires proper nutrition and management to meet her production potential (Vandehaar and St.-Pierre, 2006). The milk production per cow in the U.S. has increased approximately 1% per year over the last 50 years. There still is substantial opportunity to make further progress with genetic selection and improve milk production. High producing cows produce as much as 40 to 50 kg of milk per day in early lactation and world record cows produce as much as 90 kg/d for an entire lactation (Vandehaar and St.-Pierre, 2006). However, a consequence of selecting dairy cattle for even higher levels of milk production is the increased incidence of health and management problems associated with the animal's ability to cope with the demands of high production (Goff, 2006). Some of the health problems of high producing dairy cattle are related to the difficulty the cow has adjusting to up-regulating intake and metabolism to meet their nutrient requirements in early lactation. This duration of adjustment is known as the transition period. The transition period is generally defined as the two weeks prior-to parturition and early lactation (Bell, 1995). During transition, nutrient requirements change initially due to fetal and mammary growth in late gestation and even more dramatically due to increasing milk production in early lactation. The transition cow in early lactation must increase voluntary intake, hepatic gluconeogenesis, decrease peripheral tissue glucose utilization, increase

fatty acid mobilization from adipose tissue, amino acid mobilization from muscle tissue and mineral absorption from intestinal tissue and resorption from skeletal tissue (Bauman and Currie, 1980; Bell, 1995). The high-producing dairy cow is most susceptible to metabolic disorders and related health problems in early lactation as a result of changing metabolic demands during the transition period between pregnancy, parturition and particularly during early lactation (Goff, 2006). Bauman and Currie (1980) define the changes that occur in transition dairy cattle as homeorhesis, a coordination of metabolism in various tissues to support a physiological state (intake energy of lactation). Substantial endocrine involvement is required for the dairy cow to change from homeostatic to homeoretic metabolism (Lucy et al., 2001). In addition, there is considerable economic incentive to minimize metabolic disorders such as hypocalcemia or milk fever (Horst et al., 1997), ketosis, displaced abomasums, metritis, retained placenta and mastitis in high producing dairy cattle (Teter, 2005). Furthermore, subclinical impacts of transition that can result in lowered milk production and poorer feed conversion may have even greater negative economic consequences than the clinical manifestations that result in acute morbidity.

Nutritional status and metabolic disorders

Most clinical and subclinical problems that occur in high producing dairy cattle in early lactation involve either a negative calcium and/or energy status related to lactogenesis exceeding up- regulation of intake (Goff, 2006). A considerable amount of research has been directed towards nutrition of transition dairy cattle. It is becoming well-recognized that proper feeding and nutrition of transition cows especially during late gestation can promote productivity by improving calcium status and stimulating caloric intake in early lactation. Nutritional intervention can minimize or eliminate the incidence of metabolic disorders and related health problems that occur in transition and result in greater milk production for the entire lactation.

Nutritional intervention to aid the transition cow is focused on helping the cow in the close-up prepartum period (2-4 weeks), adjust to mobilizing body stores of calcium and energy as well as maximizing the stimulus and gastrointestinal capacity for greater levels of nutrient intake.

Energy balance

Dairy cattle are in negative energy balance in early lactation because they do not initially up- regulate their caloric intake to meet the greater nutritional demands that occur for increasing milk production. Typically dairy cattle are unable to consume enough calories to meet the requirement for milk production until at least several weeks into lactation and will lose 50 to 100 kg of BW. Energy balance is defined as the difference between intake energy and the sum of required energy for heat production and lactation. Negative energy balance increased adipose tissue lipolysis, mobilization and coupled with insufficient gluconeogenesis results in incomplete metabolism of fatty acids and ketone production (Van Knegsel et al., 2007). Ketosis, characterized by increased circulating ketones, is a metabolic disease condition often linked to hypocalcemia and present in 3.3 percent of cows in high producing herds (Jordan and Fourdraine, 1993). The incidence of ketosis can be increased by over-feeding cows during the dry-period. Cows that are fed higher energy diets in excess of maintenance requirements during the dry period tend to have less appetence and more metabolic related disorders in early lactation than those that were fed to gain back body reserves in late lactation and fed at maintenance during the dry period. Overfeeding calories during the dry-period can result in a “fatty liver” condition and a greater incidence of ketosis in early lactation. Dairy cattle fed to attain excessive body condition in the dry period have suppressed intake post partum, lowered hepatic function, gluconeogenesis and higher concentrations of circulating ketone bodies and non esterified fatty acids. It is best to replenish body condition in late lactation and maintain the cow during the dry-

period. Some researchers are even proposing using more physical NDF in prepartum diets to control energy intake and improve health and performance of transition dairy cattle. (Douglas et al., 2006; Drackley and Guretzky, 2007).

Calcium balance

After parturition, plasma calcium concentrations that are usually very constant and regulated can be greatly reduced due to the demands associated with colostrum and milk production (Goff and Horst, 1997). Hypocalcemia results in metabolic and physiological conditions postpartum that decrease both intake and milk yield. Calcium is an intracellular "second messenger" and is involved in the bioactivity of key hormones such as insulin and cholecystokinin that regulate satiety and intake. The role of calcium on the activity of these hormones may be linked to a transition cow having difficulty up-regulating intake to meet its milk production requirements.

Dietary calcium has been implicated as important in adipose tissue regulation of energy balance in rodent models used in human obesity research applications. Intracellular Ca^{++} concentrations in adipose tissue have been linked to enhanced lipolysis and mobilization of fatty acids to preserve thermogenesis during caloric restriction and attenuate lipogenesis during periods of calorie over-consumption (Zemel et al., 2000; Zemel, 2003). Consumption of calcium rich dairy foods in human populations has been observed in demographic studies to reduce all of the components of the insulin resistance syndrome including body mass index (Zemel, 2003). Elevated concentrations of circulating NEFA in transition dairy cows would indicate that lipolysis and mobilization of adipose tissue is not being limited by hypocalcemia. However, based on comparative animal studies, the relationship between circulating and intracellular

calcium on adipose tissue metabolism and insulin resistance is deserving of more research attention in transition dairy cattle.

Intracellular calcium is required for the active contraction of muscle fibers. Some skeletal muscle involvement is a symptom of acute periparturient paresis or hypocalcemia in lactating cows. The impact of hypocalemia on smooth muscle contractility may predispose the transition cow to a number of health conditions including dystocia, prolapsed uterus, retained placenta and early metritis as quantitated in an epidemiological study from Finland (Grohn et al., 1989). Also, gastrointestinal motility and specifically reticulo-ruminal motility has been implicated as being negatively affected by hypocalcemia (Froetschel et al., 2004). Schonewille et al. (1999) induced hypocalcemia in nonpregnant, nonlactating and multiparous cows with Na-EDTA infusion and demonstrated that cattle fed a negative DCAD diet (-230 meq/kg DM) were more capable of mobilizing calcium as compared to those that were fed a positive DCAD diet (+332 meq/kg DM). Froetschel et al. (2004) used this technique to induce hypocalcemia and demonstrated that calcium status was linked to the amplitude of reticulo-ruminal contractions in rumen fistulated steers. Reticular-ruminal motility is critical for rumen microbial digestive function as it is needed for microbial inoculation, volatile fatty acid (VFA) absorption, and liquid and particulate passage rate. Lowered gastrointestinal fill and hypomotilty due to lower intracellular calcium concentrations in reticulo-rumen muscle tissue may lead to a greater incidence of displaced abomasum that occurs more frequently in transition dairy cattle with hypocalcemia and ketosis.

Hypocalcemia is also linked to a suppressed immune response and greater incidence of infectious disorders such as retained placenta, metritis and mastitis. Immune suppression may be related to elevated circulating concentrations of cortisol, a natural immune suppressant hormone that is higher in cows that are predisposed to hypocalcemia (Goff and Horst, 1997). Increased

circulating concentrations of cortisol in cows with hypocalcemia may block the immune system activity that is involved in breaking placental attachments at calving and increase the incidence of retained placenta.

Endocrine and metabolite regulation: Insulin and other regulators

Insulin is a peptide hormone with a molecular weight of 5080 Daltons, composed of 51 amino acid residues and is produced in the pancreas of mammals from the beta cells in the Islets of Langerhans. The amino acid structure of insulin was determined by Sanger and associates in the mid 1940's (Sanger, 1959). Insulin largely coordinates post absorptive metabolism. Its primary function is to control blood glucose levels by regulating its uptake by peripheral tissues, most notably muscle and adipose, and thereby impacts centrally mediated control of appetite and energy balance. Circulating levels of blood glucose affect centrally mediated/hypothalamic or glucostatic control of intake (Mayer, 1952). As related to its effects on glucose one could characterize insulin as an appetite hormone. Exogenous administration of insulin induces hypoglycemia, decreases hypothalamic concentrations of glucose and indirectly stimulates appetite.However, insulin has been recently characterized as a satiety hormone because it will down regulate intake with exogenous administration while maintaining blood glucose levels experimentally using a clamp technique (Woods et al., 2000). Insulin is believed to be transported directly into the brain where it binds to receptors and induces a general neuroendocrine effect consistent with a satiety response.

Insulin is involved in direct control of cellular uptake of glucose and certain amino acids and indirect uptake of fatty acids (Emery et al., 1992). Insulin directly regulates transporter proteins (Glut-1 and Glut-4 proteins) in the cell membrane responsible for active transport of glucose. Insulin indirectly influences circulating levels of glucose by its effects on glycogen

synthesis, fatty acids synthesis and esterification of tryglicerides, proteolysis, lipolysis, gluconeogenesis, and amino acid utilization (Cheatham and Kahn, 1995). Insulin secretion is mediated by incretin hormones, secreted by the small intestine in response to luminal stimuli during the digestive process in response to digestive and absorptive metabolism, and mediate satiety through the effects on hypothalamic receptors and gut motility (Reilling and Reynolds, 2007). Insulin thereby stimulates peripheral tissues, adipose and muscle, to enhance utilization of absorbed nutrients.

In nonruminants, insulin secretion in increased by glu-dependent insulinotropic polypeptide (GIP) and glucagon-like peptide 1-(7-36) amide (GLP-1), which are gut hormones secreted by the small intestine in response to luminal nutrient concentrations. These hormones are classified as incretins in non-ruminants (Fehmann et al., 1995). The role of insulin and related incretin pathways is less understood in ruminants. Ruminants do not typically absorb appreciable glucose from their diet and are more in a constant state of gluconeogenesis. Circulating levels of glucose in ruminants are relatively lower and more constant than those found in monogastrics (60 - 80 vs 80 - 120 mg/dL: Goff, 2004). The ruminant liver is specialized in that its enzyme activities direct its metabolism of propionate and glucogenic amino acids towards gluconeogenesis. Ruminants differ from monogastric species in that their metabolic regulation for gluconeogenesis is located specifically in hepatic tissue and separated from lipogenesis that occurs specifically in adipose tissue. Hepatic lipogenesis indicates a pathological condition in a ruminant. Ruminant adipose tissue uses primarily acetate, butyrate and pre-formed lipids as substrates for lipogenesis. This coordination of metabolic regulation allows for ruminants to conserve glucose for vital functions such as brain metabolism, fetal metabolism and mammary tissue lactose synthesis and not as a substrate for fatty acid synthesis.

Based on the unique metabolism to conserve glucose for vital functions, the importance of the role of insulin in satiety as discovered in rodents as animal models for humans may not be the same in ruminants. The post absorptive state of the ruminant is uniquely different. Blood glucose concentrations are low and do not vary as much in ruminants. Ruminants are using mainly propionate and glucogenic amino acids as substrates for hepatic gluconeogenesis. Feeding higher levels of readily fermentable carbohydrate in transition cow rations increases plasma insulin concentrations and was related to an intake depression; however, greater insulin secretion in response to a glucose load was associated with minimal effects on intake depression (Bradford and Allen, 2007). Since ruminant hepatic metabolism is different, the impact of insulin on satiety may depend on its hepatic metabolism as it passes first into the portal vein feeding the liver after being secreted from the pancreas. High fat diets and obesity can reduce insulin sensitivity in monogastric species causing these animals to secrete more insulin in order to control post absorptive levels of blood glucose. Insulin insensitivity related to fat intake and obesity predisposes humans to a maturity-onset type of diabetes. Mammals in general become insulin resistant or insensitive during late pregnancy resulting in decreased ability of insulin to promote lipogenesis and oppose lipolysis (Bell, 1995). Generally, insulin is increased in late gestation and then declines after parturition and then increases during late lactation.

Inducing hyperlipdemia by intravenous infusion of tallow emulsion to non-lactating Holstein cows caused insulin resistance by impairing both sensitivity and maximum responsiveness to insulin (Pires et al. 2007). The induction of insulin resistance may serve to increase availability of glucogenic nutrients to the periparturient cow but over-stimulation may provide an excess of triglycerides and NEFA and further predispose adipose tissue to become more insulin resistant which further increases NEFA and increases the risk of metabolic

disorders. Early lactation is also characterized by a decrease in plasma insulin that accompanies a decrease in energy balance in early lactation cows (Ingvartsen & Andersen, 2000). Conversely, greater insulin secretion in response to a glucose load was associated with a minimal intake depression in early lactation of transition dairy cows (Bradford and Allen, 2007).

Only recently have researchers quantify circulating concentrations of incretin hormones in high producing transition dairy cattle. Plasma concentrations of gut peptides (insulinotropic polypeptide amide, and cholecystokinin) were found to increase linearly 5-19 d after calving reflecting changes in intake and lactogenesis (Reilling and Reynolds, 2007). It is believed that the satiety effect of these incretin hormones may not be realized in the transition cow because it is insulin dependent and both insulin and glucose were lower in early lactation.

The commercialization of bovine somatotropin (BST) or growth hormone (GH) to enhance the productivity of lactating dairy cattle indicates that this hormone is an important regulator of lacotogenesis. However, it is also recognized that its effect on nutrient partitioning is realized only after peak lactation. The impact of GH mainly on mobilization of adipose tissue does not benefit the early lactation cow that is already in negative energy balance. Growth hormone plays a central role in the change in nutrient metabolism required to support lactation in high producing dairy cattle in that it coordinates stimulated mobilization of fatty acids from adipose tissue and hepatic gluconeogenesis (Lucy et al., 2001). These researchers modeled GH expression in transition dairy cattle and its impact on early lactation. The decrease in feed intake and coordinated hormonal events at parturition such as decreasing progesterone, increasing estradiol and glucocorticoids inhibit hepatic GH receptor expression. The resulting loss in hepatic GH activity leads to a decrease in circulating insulin like growth factor (IGF-1) and its negative feedback on pituitary GH secretion. The resulting increase in GH in early lactation

signals adipose tissue fatty acid mobilization and hepatic gluconeogenesis. The increase in feed intake during early lactation increases hepatic GH receptors that allow the cow to respond to GH mediated nutrient partitioning. The consequence of inadequate GH receptor expression in early lactation can predispose the cow to fatty liver and ketosis.

Leptin a relatively new hormone discovered in the mid 90's is produced from adipose tissue and may be responsible for the inappetance of over-conditioned fresh cows. Leptin reduces intake and heat production of laboratory animals and has been implicated as being related to depressed intake of dairy cattle in early lactation. Lactating dairy cattle lose body condition before reaching peak milk production and intake in early lactation. Leptin increases the animal's sensitivity to cholecystokinin (Matson and Ritter, 1999) and influences many of the other neuron-hormonal regulators involved in intake regulation. reported that maternal plasma leptin concentration increased from 5.3 to 9.5 ng/ml in sheep between prebreeding and mid pregnancy and then declined through late pregnancy and early lactation. Block et al. (2001) also reported that plasma leptin concentrations in periparturient cows decreased by 50% after parturition and remained depressed during early lactation. Block et al. (2003) mentioned that insulin and leptin are positively related and insulin and GH are inversely related. Supplemental fat decreases caloric intake in early lactation cows via an unknown mechanism. Leptin induced sensitivity to CCK may be partly responsible for the negative influence of dietary fat on intake and milk production of cows in early lactation. Circulating concentrations of leptin increased ($P < 0.01$) 7.2 % (4.54 vs. 4.87 ± 0.07 ng/ml) in cows post-calving as compared to pre-calving and decreased approximately 15% due to higher levels of fat in the diet and were more than 50% greater in cows fed DCAD (2.6 vs 1.2 ng/ml) (Kumar et al.,2003). Although changes in leptin as reported by Kumar et al. (2003) did not appear to occur in a manner consistent with previous

reports, 3-6% fat supplementation did not suppress intake in fresh cows which was also atypical implicating that both of these effects may have been the consequence of an improved calcium status of the cows fed anionic salts or DCAD prepartum.

Nutritional intervention to improve transition cow health and productivity

Even though cattle can initially mobilize nutrients from body reserves to meet needs for milk production this can not sustain high productivity and will eventually result in lowered production as the animal depletes more available adipose and muscle tissue reserves. Higher producing multiparous cows are most susceptible to health problems related to hypocalcemia and ketosis. It is well recognized that it is critically important to manage fresh cows in a manner that allows them to maximize their intake in early lactation. It is important that fresh cows are fed rations correctly formulated for all required nutrients and are fed an optimal concentration of high quality forage that will effectively stimulate rumination and support rumen function, gastrointestinal health, and intake. Also, it is important to house fresh cows in groups such that they are less subject to competitive feeding behavior from other higher producing cows that are past transition.

In regards to feed energetics of prepartum cows, it appears that it is important to group feed dry cows according to their closeness to parturition. Overton and Waldren (2004) recommended minimizing overfeeding of nutrients during the early dry period but increasing nutrient supply to facilitate metabolic adaptation to lactation during the late dry period. However, more recently Drackley & Guretzky (2007), reviewed a series of their experiments, suggesting that increasing the energy density of prepartum rations does not necessarily improve caloric or nutrient intake postpartum. Dry matter intake decreases as much as 32% during the final three weeks of gestation and 89% of that decline occurs during the final week of gestation (Hayirli et

al., 2002). These researchers related intake depression during late gestation to parity, body condition score and specific dietary macronutrients such as fat, fiber, and protein. It appears that more research is needed to better understand the proper feeding regimen and nutritional requirements of the prepartum dairy cow.

Since high producing cows will not consume enough feed to meet their requirements for milk production in early lactation, a substantial amount of nutrition research has been conducted to develop hyperalimentation strategies to improve nutrient balance of transition dairy cattle especially with respect to energetics. Increased supplementation strategies with both glucogenic and lipogenic precursors have had mixed results (van Knegsel et al., 2007; Bradford and Allen, 2007). Unfortunately, increasing energy density of postpartum diets does not effectively counter-act negative effects of reduced intake in early lactation (Bradford and Allen, 2007). Furthermore, feeding higher levels of concentrate can cause acidosis related health problems and shorten lifetime productivity and profitability. The second highest incidental health problem in dairy cattle is lameness (Goff, 2006). Laminitis is often caused by founder or induced by overfeeding readily fermentable carbohydrate.

The use of supplemental dietary fat in lactation rations has generally been increased in recent years but it is well recognized that its use in early lactation rations must be done carefully. Early lactation cows appear to be more sensitive to dietary fat with regard to its effects on caloric intake (Choi and Palmquist, 1996). Generally appreciable amounts of supplemental fat (3-5%) are not recommended until cows are post peak lactation. Recommended levels for transition cows are set at less than 1% of the ration DM mainly to support reproductive function. Although it has yet to be realized, developing nutritional strategies to enhance fat supplementation of

transition cows may improve their health and productivity especially in warmer climates with heat stress and higher fiber forages.

Feeding anionic salts or DCAD prepartum

A major breakthrough in feeding transition cows involves feeding anionic salts prepartum to alter their electrolyte balance by inducing greater populations of parathyroid receptor populations and their activity needed for mobilization of skeletal reserves of calcium and phosphorus to maintain blood pH. Up-reglation of bone demineralization pathways prepares the close-up prepartum cow for calcium mobilization needed for early lactation and minimizes the risk of hypocalcemia and periparturient paresis in early lactation. Initial research to suggest that manipulating Dietary Cation-Anion Difference (DCAD) prepartum would enable transition cows to achieve maximal intake and milk production in early lactation was conducted by Tucker et al. (1988). Other studies (West et al., 1991, 1992; Delaquis and Block, 1995a,b) also suggested that DCAD had a significant influence on acid-base status and lactation performance of dairy cows. Schonewille et al. (1999) induced hypocalcemia in nonpregnant, nonlactating and multiparous cows and demonstrated that cattle fed a negative DCAD diet (-230 meq/kg DM) were more capable to mobilize calcium as compared to when they were fed a positive DCAD diet (+332 meq/kg DM). Although the benefits of prepartum DCAD are readily apparent, specific recommendations on optimal feeding levels of DCAD for prepartum dairy cows are influenced by numerous factors including feed intake, acid-producing potential of the diet, and concentrations of other fixed ions, and has led to different formulation equation.

Equations:

$(Na^+ + K^+) – (Cl^- + S^{-2})$ (Ender et al., 1971)

$(Na^+ + K^+ - Cl^-)$ (Mongin, 1980)

$(Na^{+} + K^{+} + .15Ca^{+2} + .15Mg^{+2}) – (Cl^{-} + .6S^{-2} + .5P^{-3})$ (Goff and Horst, 1997)

Chloride salts are more acidogenic than sulfate salts (Goff and Horst, 1997; Oetzel, 1991; Tucker et al., 1991). Effectiveness of DCAD treatment prepartum is verified by reducing urine pH from approximately 7.5 to between 6.2 and 6.8 (Oetzel and Goff, 1998).

There are at least three commercial products being marketed as anionic salts that are proven effective at preventing milk fever. These products include Animate®, an ammonium choride salt product (International Mineral Corporation Toronto, Canada.), Biochlor® a hydrochloric acid treated fermentation byproduct (Church and Dwight Co., Inc. Princeton, NJ) and Soychlor®, a hydrochloric acid treated soybean meal (West Central Soy, Ralston, IA). Previous research has demonstrated that proper levels of anionic salts fed daily two weeks before calving will cause a mild metabolic acidosis Goff, 2006). This effect is best verified by a lowered urine pH (5.5 to 6.5) (Tucker 1988). The supplementation of anionic salts to achieve a mathematical negative DCAD stimulates mobilization of skeletal calcium reserves to maintain blood pH and prepares multiparous high producing cow for a better transition into lactation preventing milk fever (clinical and subclinical) and related conditions.

Feeding supplemental fat to transition cows

The feeding application of using supplemental fat to offset negative energy balance and associated metabolic disorders in transition cows is limited. Supplemental fat, by itself, can not correct negative energy balance and related problems in transition cows. Providing dietary levels of supplemental fat to meet the caloric difference between intake and milk production can actually acerbate problems associated with transition cows. Appreciable levels of dietary fat supplementation will reduce caloric intake indicating it is having a physiological effect in addition to affecting intake by substituting more calories in a lesser quantity of feed. It seems

that the beneficial effects of dietary fat on milk yield are not realized in early lactation. Schingoethe and Casper (1991) reported that 71% of the increase in milk yield by feeding a high fat diet during week 4 to week 16 postpartum was observed after week 16. They suggested that there is a slow or delayed response and a substantial long term response to dietary fat and that the entire lactational response to fat supplementation may be a better evaluation measure than its effect on milk production observed more immediately after its initial feeding. Glucogenic diets are more effective than lipogenic diets at improving energy balance of transition multiparous cows and resulted in decreased blood ketones and liver triacylglycerides indicating a lower risk of metabolic disorders (van Knegsel et al., 2007). Negative consequences of overfeeding supplemental dietary fat to transition dairy cattle may also depend on both the type and level of supplementation. In early lactation cows, lower levels of supplementation (< 1%) have improved energy balance and improved conception rate; whereas, higher levels of supplementation could be detrimental because of reduced caloric intake. Higher levels of fat supplementation are usually not recommended until after peak of lactation.

Ruminally inert (RI) fat in the form of calcium salts of fatty acids (~1 to 3%) is proven effective at improving caloric intake as required for milk production of high producing cows (Garcia-Bojalil et al., 1998b). An RI fat product containing more unsaturated fatty acids (18:1 and 18:2, Megalac-R, Church and Dwight Co., Inc. Princeton, NJ), has application for alleviating the impact of negative energy balance on reproduction efficiency (Garcia-Bojalil et al. 1998a) and providing specific unsaturated fatty acids that improve fertility (Staples et al., 1998). Specific fatty acids in transition rations are being proven to enhance metabolic health and reducing economic losses due to milk fever, dystocia, retained placenta, ketosis and displaced abomasums, lowered production and reproductive efficiency (Teter, 2005). The RI fat products

have a greater application for dairies located in warmer climates as lactating dairy cattle fed diets that contain RI fat will benefit from both a lower heat increment and the cattle should be more able to meet their caloric requirements despite the negative impact of higher-fiber warm season forages. However, there is concern that at higher levels of RI fat supplementation (>3%) an associated depression in intake may compromise the effectiveness of the RI fat products to improve energy balance (Choi and Palmquist, 1996).

A number of interacting physiological factors have been implicated as responsible for supplemental fat (>3%) depressing caloric intake. Unsaturated fatty acids appear to have a greater inhibitory effect on DMI (Allen 2000, Harvatine and Allen, 2005). The mechanism responsible for the animal's greater sensitivity to unsaturated fatty acids is not clear. It could be related to rumen hydrogenation of unsaturated fatty acids and associated changes in rumen fermentation patterns. Feeding unsaturated fats tends to reduce the acetate to propionate ratio in rumen fluid. It could also be related to the physical nature of unsaturated fatty acids making these more available to gastrointestional receptors involved in the satiety response.

Improving intake of transition cows has become focused upon alleviating negative energy balance during early lactation to improve the health and well being of the cow and milk production for the entire lactation. During transition, digestive and metabolic capacity increase substantially as related to increases in the mass of the gastrointestinal tract and the liver. In addition there is substantial mobilization of energy and protein reserves in adipose and muscle tissue. This physiological state is characterized by higher circulating levels of non-esterified fatty acids, ketone bodies (aceto-acetate, acetone and β-hydroxy butyrate) and blood urea nitrogen and lower levels of glucose and amino acids. Mobilization of endogenous adipose tissue and its demand on hepatic gluconeogenesis may limit the cow from using additional fatty acids supplied

in the diet. In addition, heightened sensitivity to supplemental fat with regard to caloric intake depression by cows in early lactation may be related to the endocrine regulation involving lipostatic and gastric controls. The adipose tissue hormone leptin increases the animal's sensitivity to the gastric hormone cholecystokinin. Greater CCK activity should result in dietary fats and proteins having an inhibitory action on gastrointestinal motility and magnifying the negative impact of dietary fiber on intake because of greater gastrointestinal distension. At present, supplemental fat (<1 to 3%) has to be precisely allocated in early lactation to allow the cow to cope with negative energy balance and not overwhelm its metabolic and digestive capacity to effectively utilize lipid as a source of metabolic energy.

Calcium status may improve supplemental fat use in transition cows

Recent evidence from our laboratory suggests that calcium status is crucial for the transition cow to effectively utilize higher levels of supplemental rumminally inert fat (Kumar et al., 2003). In a preliminary study, twelve cows, four per treatment group, were fed either 0%, 3% or 6% Megalac-R® (% DM) after being fed Biochlor® for two weeks prior to calving. An additional treatment group was fed 6% Megalac-R without prepartum DCAD. Results averaged from observations made during the first 8 weeks of lactation demonstrated that DMI of cows fed anionic salts for 2 weeks prepartum was not depressed by feeding supplemental fat at 3 to 6%. Although intake was not reduced by feeding supplemental fat, milk production was lower in cows fed 6% fat, and although not measured, presumably these cows gained more body weight. Cattle fed 6% fat supplemented diets without DCAD prepartum had lower intake and milk production. Changes in blood metabolites (glucose, calcium. blood urea nitrogen) and hormones (insulin and leptin) imply that anionic salts prepartum influence calcium and metabolic energy regulation postpartum. Based on these preliminary results another trial is warranted to help

confirm these results that DCAD allows transition cows to utilize higher levels of supplemental fat.

Objective and Hypothesis

The objective of this research was to quantify the effects of DCAD fed prepartum on intake and utilization of supplemental fat fed post partum in early lactation. Although a major objective based on previous research findings was to measure the impact of prepartum calcium status on postpartum energy balance, special emphasis in this thesis will be placed upon measuring diurnal (hourly) concentrations of circulating hormones and metabolites such as insulin, BUN, NEFA, glucose, calcium, and intake that occur in the first eight weeks of lactation. We believe that calcium status in periparturient dairy cows may enhance the use of high fat diets in early lactation.

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CHAPTER 2

THE ROLE OF CALCIUM ON NEUROENDOCRINE REGULATION OF ENERGY BALANCE PERIPARTURIENT DAIRY CATTLE

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Abstract

Thirteen periparturient multiparous Holstein cows were used in a randomized complete block designed experiment to determine the effect of anionic salts (Biochlor®) in prepartum diets on intake and utilization of diets containing ruminally inert fat (Megalac-R ®) post-partum. Cows were initially blocked by their mature equivalent milk production into three groups and then one cow from each group was randomly assigned to one of four treatments. Prepartum rations containing 75% wheat silage were mixed with different concentrates that varied only in DCAD supplementation to provide wide range of DCAD based on the simple equation (milliequvalents/100g DM = (Na+K)-(Cl) (Mongin, 1981). The prepartum DCAD treatment diet contained 9.3 % Biochor® estimated to provide dietary cation-anion difference of -14 milliequivalents/100g DM); whereas, the prepartum control diet (NDCAD) contained a blend of soybean meal and corn gluten feed and was estimated to provide dietary cation-anion difference of + 13.8 milliequivalents/100g DM. Postpartum total mixed rations contained 42 to 46% wheat silage and 0 or 5.3% (DM) fat supplementation in the concentrate from Megalac-R® (LF & HF, respectively). Daily intake and milk production of cattle were measured for 2 weeks prepartum and 12 weeks postpartum with cattle fed behind Calan® gates. Hourly feed consumption was measured and jugular blood samples collected over three 24 hour periods during week one, four and eight of lactation. Serum levels of insulin, glucose, blood urea nitrogen (BUN), non-esterified fatty acids (NEFA) and plasma calcium were determined. An interaction between DCAD treatment and week of lactation ($P < 0.01$) was observed with cows fed DCAD having 13.8 to 15.2% lower DMI in the first two week of lactation. However, DCAD treatment increased intake from week seven to eleven. An interaction between fat supplementation and week of lactation was observed for DMI ($P < 0.01$) with cattle fed HF

consuming 9.8-16.3% less dry matter than those fed LF during week two through eleven of early lactation. There was an interaction between DCAD and fat supplementation over time for milk production ($P < 0.01$). Cows fed NDCAD-HF produced as much as 18% less milk from week 1 to 9, than the other treatment groups; whereas, cattle fed DCAD-HF ration produced as much as 28.6% more milk apparent from week eight to twelve in early lactation than any other treatment. The HF diets increased circulating concentrations of insulin by 24.8 to 32% ($P < 0.01$) at 4 and 8 weeks postpartum. Feeding DCAD prepartum decreased insulin postpartum and feeding fat post partum increased insulin postpartum. Assuming that elevated insulin is due to insensitivity, DCAD improved insulin sensitivity, but did not counteract the effects of fat on this parameter. Insulin was negatively correlated to hourly DM intake. As the smaller meals were excluded from the data set this negative regression coefficient became stronger. Circulating concentrations of NEFA were higher in samples taken earlier in lactation, decreased by prepartum DCAD feeding and increased by supplementing fat postpartum. Cows fed control diets prepartum and fat post partum had the higher concentrations of NEFA at week eight postpartum. It appears that enhanced mobilization of calcium during transition by feeding DCAD improves both insulin sensitivity and adipose mobilization in positive manners. These results indicate that DCAD supplementation facilitates the transition cow's ability to utilize higher levels of supplemental fat in early lactation.

Introduction

The transition period is generally defined as two weeks prior-to parturition and early lactation (Bell, 1995). During transition, nutrient requirements change abruptly due to fetal and mammary growth in late gestation and then even more dramatically due to increasing milk

production in early lactation. The transition cow in early lactation must increase voluntary intake, hepatic gluconeogenesis, decrease peripheral tissue glucose utilization, and increase fatty acid mobilization from adipose tissue, and amino acid mobilization from muscle tissue (Bell, 1995). The transition cow is most susceptible to metabolic disorders and related health problems in early lactation as a result of changing metabolic demands during the transition period between pregnancy, parturition and especially during early lactation (Goff, 2006). There is considerable economic incentive to minimize metabolic disorders such as hypocalcemia or milk fever, ketosis, displaced abomasums, metritis, retained placenta and mastitis in high producing dairy cattle (Teter, 2005). Furthermore, subclinical effects of transition that lower milk production and reduce feed conversion and reproductive efficiency have substantial economic consequences that may supersede those resulting from clinical manifestations. Most maladies of transition dairy cattle are related to either a negative calcium and/or energy status related to lactogenesis exceeding up- regulation of intake (Goff 2006). Nutritional intervention is needed to minimize or eliminate the incidence of metabolic disorders and related health problems that occur during the transition period, and result in greater milk production for the entire lactation.

Ruminally inert (RI) fat in the form of calcium salts of fatty acids (~1 to 3% DM) is proven effective at improving caloric intake as required for milk production of high producing cows (Garcia-Bojalil et al. 1998a). However, there is concern that at higher levels of RI fat supplementation (>3% DM) an associated depression in caloric intake may compromise the effectiveness of the RI fat products to improve energy balance (Choi and Palmquist, 1996).

Recent evidence in our laboratory suggests that calcium status is crucial for the transition cow to effectively utilize higher levels of supplemental RI fat. In a preliminary experiment designed with a different objective in mind, cattle fed anionic salts prepartum were able to

consume 3% to 6% fat supplemented diets without compromising their intake. Although differences in milk production were not observed between prepartum anionic salt treatment(s) and post partum fat supplementation, fed at 6 % of ration DM, the lack of a negative effect of high levels of fat supplementation on intake are unprecedented in transition cows. Levels of fat supplementation greater than 3% are considerably above manufacturer's recommendations who recommend feeding .340 to .454 kg/cow/day from calving to confirmed pregnancy (Church and Dwight Co., Inc. Princeton, N.J. www.ahdairy.com).This level of supplementation should provide 1.3 to 1.7 % fat supplementation based on DM intakes of fresh cows projected to be 20 to 35 kg in early lactation.

As a result of this preliminary research, a working hypothesis was developed that stated that improved calcium status of transition dairy cows fed anionic salts may facilitate them to more effectively use greater levels supplemental-fat and thereby reduce their health problems and productivity constraints associated with negative energy balance. Furthermore, using supplemental fat to improve energy balance of transition cows may have even greater application for dairy cattle fed in warmer climates that are more exposed to heat stress and fed higher fiber forages. Thus, an experiment was planned to further test the ability of transition cows to consume and metabolize relatively high levels of supplemental fat postpartum due to their enhanced capability for calcium mobilization prepartum by feeding anionic salts. Although the major application of this research is to improve energy intake and energy balance special emphasis in this thesis is directed towards determining the relationship between diurnal circulating of insulin on concentrations of metabolites (glucose, blood urea nitrogen (BUN) non-esterified fatty acids (NEFA) and plasma calcium) and their potential involvement in underlying

mechanisms involved in intake regulation of transition cows of varying calcium status fed high levels of supplemental fat.

Materials and Methods

Animals were handled and managed under guidelines approved by The University of Georgia Animal Care and Use Committee. Twenty-four multiparous, Holstein dairy cows from UGA herd records were initially identified for use as experimental animals; however, due to failed conceptions and assorted health problems in early lactation (lameness, mastitis and displaced abomasums) complete data from only thirteen cows was obtained. The experiment was conducted as a randomized complete block design and a 2 X 2 factorial arrangement of treatments to determine the effect of anionic salts (Biochlor®: Church and Dwight Co. Inc., Princeton, NJ) in prepartum diets on intake and utilization of diets containing ruminally inert fat (Megalac-R ®) post- partum on intake, productivity and circulating concentrations of hormones and metabolites during early lactation (12 weeks). Cows were initially blocked by their mature equivalent milk production into three groups and then one cow from each group was randomly assigned to one of four treatment groups (prepartum DCAD without fat supplementation postpartum (DCAD-LF), prepartum DCAD with fat supplementation postpartum (DCAD-HF), prepartum control without fat supplementation postpartum (NDCAD-LF) and prepartum control with fat supplementation post-partum (NDCAD-HF) .

Prepartum rations containing 75% wheat silage, as a source of roughage, were mixed with different concentrates that varied only in their level of anionic salts to provide a wide range of DCAD based on the more empirical DCAD equation (Mongin, 1981) (milliequvalents/100g DM = (Na+K)-(Cl)). Prepartum DCAD treatment diet contained 9.3 % Biochlor® and was

estimated to provide dietary cation-anion difference of -14 milliequivalents/100g DM; whereas, prepartum control diet (NDCAD) contained a blend of soybean meal and corn gluten feed and was estimated to provide dietary cation-anion difference of + 13.8 milliequivalents/100g DM (Tables 1 & 2).

Postpartum total mixed rations contained 42 to 46% wheat silage with either 0 or 5.3% fat supplementation (LF & HF, respectively). The supplemental fat included in the concentrate was in the form of calcium salts of long chain fatty acids from the commercial product Megalac-R® (Church and Dwight Co. Inc., Princeton, NJ – www.ahdairy.com) (Tables 3 & 4).

Late gestation multiparous cows were managed on dry cow pasture (mixed cool season grasses) with minimal supplementation (75% wheat silage based total mixed ration to provide provide approximately 2.2 kg of concentrate/head). Close-up cows were moved to confinement free-stall housing approximately 2 weeks before parturition. Cows were trained to consume the total mixed ration from individually assigned Calan gates® (American Calan Inc., Northwood, NH) during this period. Urine samples were collected from cows fed prepartum experimental diets on day -10, -5 and -1 before their expected calving date and immediately analyzed for pH.

Following parturition, cows were fed experimental diets that differed in supplemental fat to supplement high fat diets. All other nutrients were fed according to National Research Council (2001) requirements. Individual feeding of cows was continued throughout the first 12 weeks of lactation using Calan gates®. Cows were fed a TMR diet twice daily, consisting of the 54 to 58% concentrate and 42 to 46% wheat silage depending on fat supplementation treatment. Silage and concentrate rations were mixed individually and fed manually. Daily feed refusals were measured and daily feed intakes for individual cows were measured for the entire length of the

experiment. Orts were measured before the afternoon feeding each day and daily intake was used to allocate the next days feeding based on 115% of the previous day intake.

In order to determine the relation between circulating concentrations of insulin and metabolites with intake and adipose tissue mobilization cows were fit with indwelling jugular catheters on one day during the first, fourth and eighth week of lactation and were bled every hour for a 24 hour period. Twenty four blood samples were taken at hourly intervals and were divided into two 10 ml tubes, for preparation of serum and plasma. Serum tubes were allowed to stand prior to refrigeration (4.4 ^{0}C) for blood clotting. Blood samples were then stored under refrigeration until processed using clinical centrifugation (2,000 rpm/ 10min.) within 24 h. Serum and plasma were then harvested from blood tubes and stored at –20 ^{0}C for subsequently analysis of glucose (Trinder, 1969), insulin (ImmuChemTM125I RIA kit, MP Biomedicals, LLC), non-esterified fatty acids (Wako NEFA-HR(2) Manual Procedure, Wako Pure Chemical Industries, Ltd.), blood urea nitrogen (Chaney and Marbach, 1962; Modified according to Sigma reagent – U-3383 urea buffer reagent) and calcium (Gindler and King, 1972). In addition, during the 24 hour blood collection periods, hourly intakes and cattle behaviors were observed and recorded. The behaviors were categorized as eating, laying, laying while ruminating, standing, standing while ruminating.

Daily milk production of the cows was monitored throughout the experiment. Weekly composite samples of silage and concentrate were collected and assayed for nutritional composition including CP (Leco FP 528 N analyzer), NDF (Ankom200, Fairport, NY), DM, and total fat. One cow was dropped due to feet problems, one due to low milk production and mastitis and one died due to an idiopathic gastrointestinal problem. Two cows were surgically corrected for displaced abomasum (both NDCAD, one LF and one HF respectively) and were

kept in the study and two cows (both NDCAD-LF diets) had displaced abomasums surgery but were removed from the study due to poor recovery.

Statistical analysis was performed for a randomized complete block designed experiment with a 2 X 2 factorial arrangement of treatments using the SAS GLM procedure (SAS User's guide, 1985). DMI, milk production, and hormone and metabolite concentrations were analyzed as a split-plot over time using a repeated measures analysis. The statistical model included the main effects (prepartum DCAD, postpartum fat and the prepartum DCAD by postpartum fat interaction), cow with interaction of prepartum DCAD by postpartum fat nested, time (either week and/or hour) and the main effect interactions with time. The cow term with the interaction of prepartum DCAD by postpartum fat nested was used as an error term to test main effects because of repeated measures in the trial. Repeated parameters in time were tested using the residual error term. When significant F values were determined ($P<.05$) differences were among treatment means were assessed using a least significant difference procedure of SAS. The relationship between hourly intake measurements and blood hormones and metabolites were assessed by SAS regression procedures.

Results and Discussion

Laboratory analysis of composite samples of concentrate and silage fed during the entire feeding trial are reported in Tables 5 and 6. Predictions of total mixed ration CP and NDF based on feed analysis data from silage and concentrate samples and the roughage concentrate coefficients for the prepartum and postpartum diet were similar to estimated values based on NRC, 2001 and a forage analysis taken and analyzed prior to the trial. The control and DCAD prepartum TMR were analyzed to contain 14.4 and 15.3 % CP and 58.5 and 57.5 % NDF as

compared to 15.1 and 15.0 % CP and 56.7 and 55.9 % NDF as estimated in the TMR. The control and fat supplemented postpartum TMR were analyzed to contain 17.4 and 17.8 % CP and 41.8 and 43.3 % NDF as compared to 18.3 and 18.6 % CP and 37.9 and 39.9 % NDF as estimated in the TMR. The relatively high NDF content of the TMR diets was a consequence of feeding substantial amounts of concentrate sources of NDF such as soyhulls, corn gluten feed and citrus pulp. The adjusted NDF (Mertens, 1992) values for the postpartum diets were estimated to be 34.8 to 37.2% for control and fat supplemented diets. The similarity between nutrient content predicted based on laboratory analysis with that from estimated values indicates that diets were formulated and mixed appropriately. Urine pH and ionizable calcium are reliable indicators of DCAD status with feeding anionic salt supplements to prepartum cows. Although the data is not reported, urine samples were taken from all cows prepartum after feeding and its pH was altered generally from 8 to below 6.5 in control and treated cows. This change in urine pH was anticipated for close-up cows fed the proper level anionic salts to achieve a negative DCAD.

Initially twenty-four multiparous, Holstein dairy cows were identified according to UGA records to be used as experimental animals; however, due to failed conceptions, and assorted health problems in early lactation (lameness, mastitis and displaced abomasums) only complete data was observed from thirteen cows were used. Five out of eighteen cows started on the prepartum diets were removed in early lactation due to clinical problems diagnosed as potentially associated with ketosis or periparturient paresis. All of removed cows were on the control prepartum diet without anionic salt supplementation. In addition two cows both fed NDCAD prepartum that recovered from surgery to correct displaced abomasums in early lactation were kept in the study.

The main effects of feeding DCAD treatments prepartum and fat treatments postpartum on milk production, DMI and circulating concentrations of hormones and metabolites are shown in Tables 7-9. There were very few statistical differences between prepartum and postpartum treatments for the main effects. The only significant main effect for treatment was observed for circulating concentration of NEFA that were increased by 17% in cattle fed supplemental fat. Drackley (1999) related plasma NEFA with fatty acid intake from several published experiments with lactating cows. They reported that for every kg of fatty acid consumed by post-transition cows there was a corresponding 81μM increase in plasma NEFA with concentrations ranging from 100 to 300 μM . However, Drackley (1999) stated that the magnitude of NEFA concentrations may increase by 1000 μM or more in transition cows. In this experiment, the average magnitude of of NEFA concentrations increased by 700 μM. Furthermore, the NEFA increased an additional 171 μM per kg of fat intake and NEFA concentrations ranged from 557 to 706 μM with increased fat supplementation. In this study, transition cows had comparatively higher circulting concentrations of NEFA and these increased at approximately twice the rate associated with supplementing fat as occurred in previous research (Drackley 1999) with transition cows fed lower levels of dietary fat.

Treatment effects were more statistically evident when data was analyzed as an interaction by week of lactation. This is logical as implied by the term transition as used to describe these cows and their dramatic changes in intake, performance, energy balance, blood metabolites and hormones that occur from late gestation and throughout early lactation.

There was an interaction between prepartum DCAD feeding and postpartum week of lactation ($P < 0.01$) (Figure 1). Lactating cows fed DCAD prepartum had 3.8 to 15.2% lower DMI in the first two week of lactation as compared to those fed the control diet prepartum.

However, intake of cattle fed DCAD prepartum, increased approximately 4 to 8% during week seven to eleven of lactation as compared to cattle fed the control diet prepartum. It is recognized that the recommended feeding levels anionic salts to adjust DCAD status of prepartum cows will suppress intake as compared to control diets. This effect is presumably related to the effect of metabolic acidosis. It appears that this negative effect of anionic salts on intake remains for two weeks into lactation even though the cows have been changed to a positive DCAD diet in early lactation. The positive effect of the prepartum DCAD treatment on intake during week 7-11 may have resulted as a consequence of these cows improved calcium status. There was a supplemental fat by week interaction for DMI ($P < 0.01$) (Figure 2). Lactating dairy cattle fed supplemental fat consumed 9.8-16.3% less dry matter than cattle fed the control diet during week two through eleven of lactation.

There was an interaction between prepartum feeding of DCAD and fat supplementation postpartum over week of early lactation for milk production (Figure 3). Cattle fed fat supplemented diets without DCAD, produced less milk from week 1 to 9, as much as 18% in week 3, than the other treatment groups; whereas, cattle fed fat supplemented diets with DCAD prepartum produced more milk from week eight to twelve and as much as 28.6% more milk than cattle fed other treatments. This result supports the hypothesis that prepartum cattle that have been fed anionic salts can more effectively utilize fat supplemented diets (Kumar, 2003).

Although the number of cows used in the present study were not sufficient to adequately test diet effects on intake and production the results do agree with the preliminary findings. The combined results of the present and past findings (Kumar, 2003) together do add to the suggestion that prepartum DCAD facilitates utilization of higher levels of supplemental fat in early lactation cows. More importantly the intake and performance results provide a meaningful

data set to compare changes in daily meal feeding patterns in early lactation with circulating concentrations of insulin and metabolites.

There were separate interactions between both individual main effects, prepartum DCAD and fat supplementation, and week of lactation observed to influence circulating concentrations of insulin. High fat diets increased the diurnal circulating concentrations of insulin by 24.8 to 32% ($P < 0.01$) in serum of early lactation cows at 4 and 8 weeks postpartum (Figure 4). These results may suggest that the cows fed supplemental fat became more insulin resistant as early lactation progressed. Infusion of a tallow emulsion in non-lactating cows to induce hyperlipdemia, as evidenced by increasing circulating concentrations of NEFA (79 to 347 u*M*),caused insulin resistance as evidenced by impairing glucose tolerance during an insulin challenge (Pires et al 2006). In late gestation insulin decreases and is thought to down regulate lipogenesis and upregulate lipolysis in adipose tissue (Bauman and Currie, 1980, Bell, 1995). Circulating concentrations of insulin were 11, 10 and 14 % lower in jugular serum of cows fed prepartum diets with DCAD during weeks 1, 4 and 8, postpartum, respectively (Figure 5). These results reflect that cows fed DCAD prepartum may have been more insulin sensitive and did not require as much insulin secretion to maintain blood glucose levels in early lactation. It appears that mobilization of calcium during transition may be influencing insulin sensitivity in a positive manner. Furthermore, insulin levels of cattle fed the DCAD diet prepartum and low fat postpartum had circulating concentration of blood insulin that were as much as 36% lower than the cattle fed other experimental diets (Figure 6). It appears that mobilization of calcium by feeding DCAD may be improving the sensitivity of insulin response, but does not counteract the decreased insulin sensitivity in animals fed supplemental fat. It is assumed that since insulin is a

satiety hormone as observed in other species then dietary treatments that lower its concentration in lactating cows could benefit productivity.

Circulating concentrations of NEFA were highest in cows during the first week of lacation and decreased 39% (791 to 480 μM, $P < 0.01$) during the subsequent 8 weeks of lactation. Circulating concentrations of NEFA were 10, 43 and 37% higher in jugular serum of cows fed supplemental fat week, 1, 4 and 8, postpartum, respectively (Figure 7). As expected, these results reflect that more adipose tissue mobilization of NEFA occurred in early lactation. The elevation in NEFA observed in this experiment was comparable to that reported by Douglas et al (2007) who reported NEFA in this same range during this stage of early lactation (0 to 20 days). In addition, since NEFA reflects mobilization of lipids from adipose tissue, these results show that cows fed fat had a greater capacity to mobilize lipids in early lactation. Feeding fat may result in increased hepatic desterification of absorbed lipid that exceeds its oxidation rate resulting in greater circulating NEFA (Reynolds et al 2003).

There were no differences in circulating NEFA due to prepartum feeding of DCAD in the first weeks of lactation (Figure 8). However, at eight weeks post partum, circulating NEFA were 15.4% lower in cows fed DCAD prepartum. There was a treatment by week interaction for NEFA in early lactation, suggesting that feeding DCAD prepartum will lower NEFA but this effect is lessened and delayed when cows are supplemented with fat (Figure 9). Cows fed control diets prepartum and fat diets post partum had elevated NEFA indicating mobilization of lipids from adipose tissue at week eight. Likely the increase in NEFA reflects a more negative energy balance associated with increasing caloric requirements for milk production coupled with insufficient caloric intake.

Circulating concentrations of blood glucose in the first eight weeks of lactation, increased 20% (35.8 to 43.1 mg/dl, $P < 0.01$), almost reaching the low end of the range specified for normal levels of blood glucose in cattle, 42-75 mg/dl (Merck 9th Ed.). Circulating concentrations of blood glucose were 2 to 15% lower in jugular serum of cows fed supplemental fat in early lactation (Figure 10). The difference in concentrations of circulating blood glucose was greatest in samples taken earlier into lactation. It appears that in the first week post partum, lactating cows fed supplemental fat had decreased gluconeogenesis. This may reflect the increase need of fat supplemented cows to utilize glucose in order to completely metabolize dietary fat as a source of energy. As weeks progressed in early lactation it appears that glucogenic capacity in cows fed supplemented cows increased. There was an interaction between feeding DCAD prepartum and week of lactation for circulating levels of blood glucose (Figure 11). Although there was no difference in the first week of lactation, blood glucose was 15% lower for cows fed DCAD at week four and 12% higher for cows fed DCAD at week eight as compared to cows fed control diets prepartum. It appears that cattle fed DCAD prepartum have a lower glucogenic capacity initially at week four, but recover by week eight. There were variable effects of feeding DCAD prepartum on blood glucose during week four and eight of lactation and these do not appear to relate to insulin which was consistently lower in cows feed DCAD prepartum (Figure12).

Circulating concentration of blood urea nitrogen (mg/dl) increased 17% (17 to 21 mg/dl; $P < 0.01$), during the first eight weeks of lactation. Supplemental fat resulted in variable effects over time on circulating concentrations of blood urea nitrogen (mg/dl) as it increased BUN by 12% on week one and 22 % on week eight but decreased 22% it in week four (Figure 13). The inconsistency of these results with BUN is difficult to explain. There was a time by treatment interaction of on circulating BUN caused by feeding DCAD pre- partum (Figure 14). In week

one cattle fed DCAD had 9.4% lower BUN, whereas as it was increased 7.6 -12.9 % in cattle fed DCAD prepartum at week four and eight in early lactation. There was an interaction observed between prepartum DCAD and postpartum fat supplementation shown in Figure 15. Elevated BUN concentrations may reflect greater intakes because it usually is correlated with the adequacy of protein supplementation. This BUN data does not follow a clear pattern as related to treatment or week of lactation.

When cows were approached to be bled at hourly intervals their behavior with respect to eating, ruminating, standing and lying were observed and recorded. The cows were considered as expressing idling behavior when they were either standing or lying without eating or ruminanting. Cows fed fat were observed to spend less time expressing eating and non-idling behavior than cows fed control postpartum diets. Researchers have reported that cows that have elevated NEFA exhibit less walking activity (Adewuyl et al 2006).

Feed consumption was measured each hour at the same time that hourly blood samples were taken during the 24 h blood sampling periods. Pearson correlation coefficients were determined between circulating concentrations of insulin and metabolites and observations of hourly feed consumption (Table 11). In order to determine the relationship between the extent of meal size and circulating concentrations of insulin and metabolites, separate correlations were determined with the feed intake data set sorted by meal size. Even though the cows ate only three or four larger meals per day the average feed intake per hour measured during the hourly feeding intervals was ~ 1.5 kg ±1.5 kg. Regressions of insulin and metabolites were initially conducted with all the hourly intake data and then with data sets that were adjusted into meal size increments. The hourly feed intake data sets were adjusted to exclude meal sizes less than one, two and three standard deviations of the mean. As the smaller meal data was excluded the

negative regression coefficient became stronger between insulin and intake. Furthermore, when the data set was arranged so that metabolite and insulin data occurred temporally after the blood sample the negative regression coefficient between insulin and intake became slightly stronger (Table 12). These data further support that insulin may be playing an important role in satiety and intake regulation of dairy cattle fed high fat diets.

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Table 1. Feed composition of prepartum total mixed rations fed as experimental diets with varying DCAD[a] content.

Feed	Prepartum diets	
	NDCAD % DM	**DCAD % DM**
Wheat Silage	72.5	71.5
Corn	7.9	9.3
Soy Hulls	4.7	3.1
Cane Molasses	1.3	1.2
Corn Gluten Feed	4.7	3.1
SBM 49%	7.9	1.6
Biochlor[a]	0	9.3
Dicalcium Phosphate	0.39	0.1
Limestone	0.21	0.34
Salt	0.16	0.16
Vitamin ADE [b]	0.06	0.06
Zinpro [c]	0.06	0.06
T Min [d]	0.06	0.06
Dynamate [e]	0.06	0.06

[a] Biochlor® Church & Dwight Co., Inc. Princeton, NJ.
[b] Vitamin ADE (IU/kg):Contains A 3.6×10^{10}, D3 4.5×10^{5}, E 6.8×10^{4}
[c] Zinpro Corp. Eden Prairie, Mn. Contains (%) Zn 4.0, Methionine 8.0, Protein 5.4, Fat 0.0, Fiber 13.9, Ash 51.5, Calcium 8.5, Salt 2.8.
[d] T min: Contains: Calcium min. 10.35% max. 12.4%, All others min. Cu 5%, Fe 5%, Mn 12, Zn 12, Co 600 ppm, I 2500 ppm, Se 600 ppm.
[e] Dynamate: Trademark by IMC, Inc. Mendilene, IL. Contains (%) Potassium 18, Magnesium 11, Sulfur 22.
Based on NRC 2001 values.
Abbreviation key: DM = Dry Matter, SBM = Soy bean meal, DCAD Dietary Cation Anion Difference, NDCAD = No Dietary Cation Anion Difference.

Table 2. Nutrient composition of prepartum total mixed rations fed as experimental diets with varying DCAD content[a].

Nutrient	Prepartum diets	
	NDCAD % DM	**DCAD[c] % DM**
Crude Protein	15.06	14.99
NEL (Mcal/kg)	1.57	1.53
Crude Fiber	25.60	24.83
NDF	56.72	55.90
Adjusted NDF[b]	53.04	52.05
Fat	3.03	3.07
UIP	34.80	36.73
Starch	16.95	17.47
Calcium	0.43	0.42
Phosphorus	0.39	0.39
Sodium	0.11	0.11
Potassium	1.66	1.67
Magnesium	0.18	0.18
Chloride	0.73	1.39
Sulfur	0.21	0.36
DCAD [d]	13.82	-14.31
DCAD II [e]	20.32	-4.09

[a] Calculated Based on NRC 2001 values.
[b] Mertens (1992)
[c] Biochlor® Church & Dwight Co., Inc. Princeton, NJ.
[d] Ender et al, 1971
[e] Goff et al, 1997
All nutrients calculated on a DM bases.
Abbreviation key: DM = Dry Matter, NEL = Net energy of lactation, NDF = Neutral detergent fiber, UIP = Undegradable intake protein, DCAD = Dietary Cation Anion Difference, NDCAD = No Dietary Cation Anion Difference.

Table 3. Feed composition of experimental diets fed to lactating dairy cows postpartum varying fat content[a].

Feed	Postpartum diets	
	Low Fat % DM	**High Fat % DM**
Wheat Silage	42.2	46.8
Corn	22.0	15.6
Citrus pulp	9.2	5.9
Cane Molasses	1.8	1.95
SBM %49	12.9	13.7
Megalac-R®	0	5.3
Dried distillers grains	3.1	3.3
SoyPlus	6.2	6.6
Dicalcium Phosphate	0.40	0.41
Limestone	1.60	0
Salt	0.09	0.10
Vitamin ADE [b]	0.04	0.04
Zin pro [c]	0.04	0.04
T MIN [d]	0.04	0.04
Magnesium oxide	0.18	0.20
Dynamate [e]	0.04	0.04

[a] Megalac-R® Church & Dwight Co., Inc. Princeton, NJ.
[b] Vitamin ADE (IU/kg):Contains A 3.6×10^{10}, D3 4.5×10^{5}, E 6.8×10^{4}
[c] Zinpro Corp. Eden Prairie, Mn. Contains (%) Zn 4.0, Methionine 8.0, Protein 5.4, Fat 0.0, Fiber 13.9, Ash 51.5, Calcium 8.5, Salt 2.8.
[d] T min: Contains: Calcium min. 10.35% max. 12.4%, All others min. Cu 5%, Fe 5%, Mn 12, Zn 12, Co 600 ppm, I 2500 ppm, Se 600 ppm.
[e] Dynamate: Trademark by IMC, Inc. Mendilene, IL. Contains (%) Potassium 18, Magnesium 11, Sulfur 22.
Based on NRC 2001 values.
Abbreviation key: DM = Dry Matter, SBM = Soy bean meal.

Table 4. Nutrient composition of experimental diets fed to lactating dairy cows postpartum varying fat content[a].

Nutrient	Postpartum diets	
	Low Fat % DM	**High Fat % DM**
Crude Protein	18.31	18.57
NEL (Mcal/kg)	1.65	1.90
Crude Fiber	13.71	14.35
NDF	37.9	39.9
Adjusted NDF[b]	34.8	37.2
Fat	3.47	7.58
UIP	44.45	44.11
Starch	22.60	18.44
Calcium	0.86	0.81
Phosphorus	0.40	0.39
Sodium	2.06	2.19
Potassium	1.75	1.81
Magnesium	0.29	0.30
Chloride	0.46	0.50
Sulfur	0.16	0.15
DCAD [c]	111.54	117.75
DCADII [d]	127.30	132.77

[a] Megalac-R® Church & Dwight Co., Inc. Princeton, NJ.
[b] Mertens (1992)
[c] Ender et al,. 1971
[d] Goff et al,. 1997
Based on NRC 2001 values.
All nutrients calculated on a DM bases.
Abbreviation key: DM = Dry Matter, NEL = Net energy of lactation, NDF = Neutral detergent fiber, UIP = Undegradable intake protein, DCAD = Dietary Cation Anion Difference.

Table 5. Analysis of experiment concentrate diets fed to cows prepartum and postpartum.

	Prepartum			Postpartum		
Item [a]	**NDCAD**	**DCAD**	**SE±**	**LF**	**HF**	**SE±** [b]
DM [b]	90.1	89.6	0.2	89.6	90.5	0.5
NDF [b]	28.2	26.5	0.9	21.1	19.8	0.6
CP [b]	23.4	26.1	0.4	22.1	23.8	0.5
Ash	8.0	8.0	0.2	8.2	7.5	0.2

[a] All values, except DM, are expressed as a percentage of dry matter.
[b] Abbreviation Key: NDCAD = No Dietary Cation-Anion Difference, DCAD = Dietary Cation-Anion Difference; HF = High Fat, LF = Low Fat, DM = Dry Matter, NDF = Neutral Digestible Fiber, CP = Crude Protein, SE = Standard error.

Table 6. Analysis of wheat silage during the feeding experiment.

	Month of feeding experiment							
Item [a]	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**SE±** [b]
DM [b]	23.6	22.5	24.6	26.4	24.6	25.5	24.3	1.31
NDF [b]	70.3	74.4	72.9	69.0	70.6	69.1	68.2	1.70
CP [b]	12.4	10.7	9.4	11.0	11.2	12.1	14.8	1.93
Ash	5.2	6.7	6.6	6.3	6.2	5.4	5.1	0.48

[a] All values, except DM, are expressed as a percentage % of dry matter.
[b] Abbreviation Key: DM = Dry Matter, NDF = Neutral Digestible Fiber;, CP = Crude Protein, SE = Standard error.

Table 7. Main effects of supplementation of DCAD in prepartum diets in dairy cows.

Item	NDCAD	DCAD	SE±
Milk (kg)	31.0	34.6	1.72
DMI (kg)	25.8	25.8	1.72
Insulin (μIU/ml)	40.0	34.8	4.3
Glucose (mg/dl)	39.3	38.8	2.4
NEFA (μM)	645	618	44
BUN (mg/dl)	17.8	18.4	1.1
Ca^{++} (mg/ml)	N/E	N/D	N/D

Abbreviation key: NDCAD = No Dietary Cation-Anion Difference, DCAD = Dietary Cation-Anion Difference, DMI = Dry Matter Intake, NEFA = Non-Esterified Fatty Acids, BUN = Blood Urea Nitrogen, SE = Standard error, N/D = Not enough data.

Table 8. Main effects of supplementation of fat in postpartum diets in lactating transition cows.

Item	LF	HF	SE±
Milk (kg)	32.8	33.8	1.72
DMI (kg)	27.5	24.0	1.72
Insulin (µIU/ml)	34.4	40.4	4.4
Glucose (mg/dl)	40.8	37.3	2.4
NEFA (μM)[a]	557	706	44
BUN (mg/dl)	17.8	18.4	1.1
Ca^{++} (mg/ml)	10.6	N/D	N/D

[a] $P < 0.05$

Abbreviation key: HF = High Fat, LF = Low Fat; DMI = Dry Matter Intake, NEFA = Non-Esterified Fatty Acids, BUN = Blood Urea Nitrogen, SE = Standard error; N/D = Not enough data.

Table 9. Interaction effects of DCAD supplementation in prepartum diets and supplemental fat in diets postpartum.

Item	NDCAD-LF	DCAD-LF	SE±	NDCAD-HF	DCAD- HF	SE±
Milk (kg)	33.6	34.0	2.5	30.4	35.2	2.4
DMI (kg)	28.6	26.5	2.5	23.0	25.1	2.3
Insulin (μIU/ml)	40.3	28.6	6.1	39.7	41.0	6.3
Glucose (mg/dl)	40.8	40.8	3.4	37.7	36.8	3.4
NEFA (μM)	600	514	62	689	722	64
BUN (mg/dl)	17.3	18.3	1.6	18.3	18.6	1.7
Ca^{++} (mg/ml)	11.7	8.6	N/E	N/E	N/E	N/E

Abbreviation key: NDCAD = No Dietary Cation-Anion Difference, DCAD = Dietary Cation-Anion Difference, HF = High Fat, LF = Low Fat, DMI = Dry Matter Intake, NEFA = Non-Esterified Fatty Acids, BUN = Blood Urea Nitrogen, SE = Standard error, N/E= Not established.

Table 10. Observations of animal behavior made at hourly intervals of a24 h period during week one, four and eight of lactation in cattle fed prepartum DCAD and post partum fat supplementation treatments. Data is expressed as the percentage occurrence of the individual behaviors expressed over the total 24 individual observations.

	Treatment					
Behavior	**DCAD**	**NDCAD**	**SE±**	**HF**	**LF**	**SE±**
Eating	11.5	13.7	2.8	8.9[a]	16.3[b]	2.3
Laying	20.8	21.4	4.1	21.9	20.4	4.0
Laying + Ruminating	26.5	28.4	3.3	25.4	29.5	3.5
Standing	23.9	24.9	3.8	28.5	20.6	3.8
Standing + Ruminating	20.1	10.3	3.4	14.3	16.0	3.4
Ruminating	46.6	38.6	3.7	39.7	45.5	3.5
Idle	44.8	46.4	3.5	50.1	41.0	3.5
Non Idle	58.1	52.3	2.8	48.6[a]	61.8[b]	2.8

[a,b] LS Means in the same row without a common superscript differ ($P < 0.05$).
Values are a percentage of one observation per hour divided by a total of 24 hours.
Abbreviation key: DCAD = Dietary Cation-Anion Difference, NDCAD = No Dietary Cation-Anion Difference, HF = High Fat, LF = Low Fat, SE = Standard error, Idle = lying + standing, Non Idle = ruminating + eating (Beauchemin et al., 1989).

Table 11. Pearson correlation coefficients of hourly DM intake with hormone and metabolites with data set adjustments for incremental meal size. Blood sample was taken immediately after the hourly feed intake observation.

	All data	Delete data < 3	Delete data < 6	Delete < 9
Number	819	282	104	24
Glucose	NS	NS	NS	NS
Insulin	NS	-.135	-.267	-.50
NEFA	.14	NS	NS	NS
BUN	NS	NS	NS	-.45
Ca	NS	NS	NS	NS

Table 12. Pearson correlation coefficients of hourly DM intake with hormone and metabolites with data set adjustments for incremental meal size. Blood sample was taken immediately before the hourly feed intake observation.

	All data	Delete data < 3	Delete data < 6	Delete < 9
Number	520	259	90	24
Glucose	NS	NS	NS	NS
Insulin	NS	-.211	-458	-.539
NEFA	NS	NS	NS	NS
BUN	NS	NS	NS	-.521
Ca	NS	NS	NS	NS

Figure 1. Effect of prepartum DCAD on postpartum dry matter intake during the first 12 weeks of lactation. Each least square means represent six observations Each point represents least square means from 6 animal observations and an average of seven daily observations for each week.

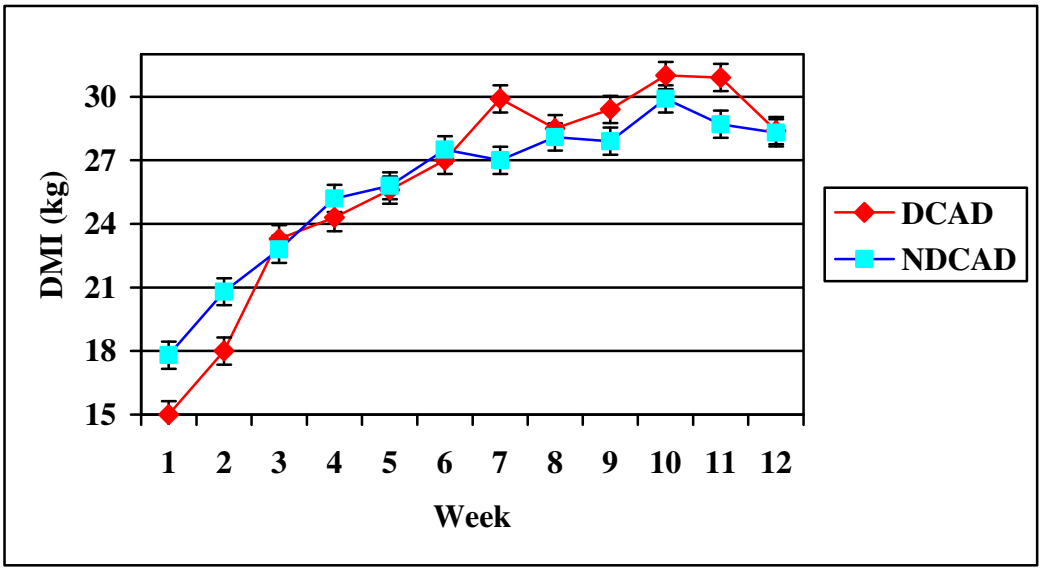


Figure 2. Effects of supplemental fat (0% VS 5.3% Megalac- R) on dry matter intake during the first 12 weeks of lactation. Each least square means represent six observations. Each point represents least square means from 6 animal observations and an average of seven daily observations for each week.

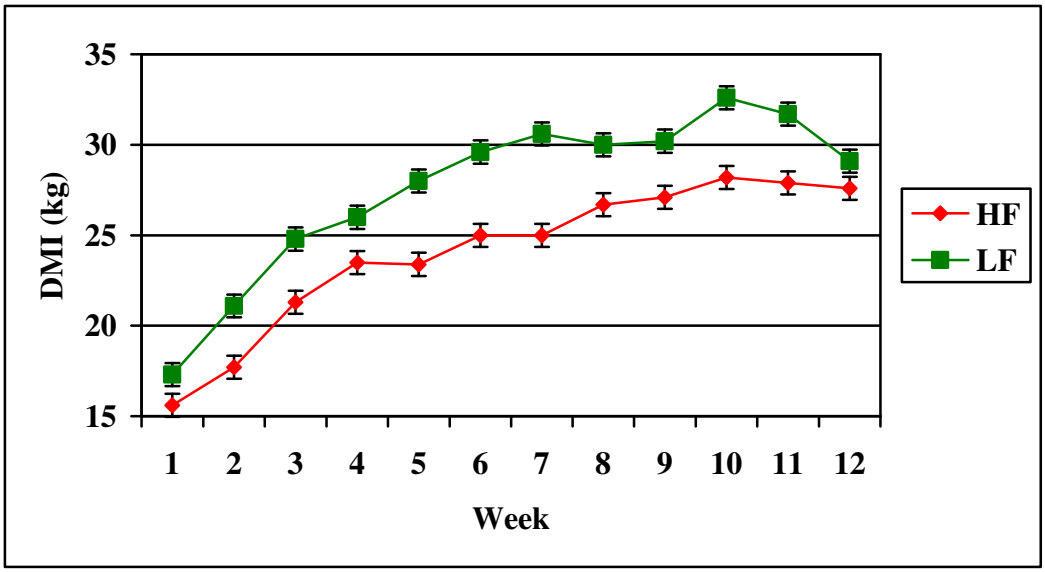


Figure 3. Effect of DCAD prepartum and fat feeding postpartum on milk production of lactating dairy cows. Each point represents least square means from intake of three cows average over a seven day period.

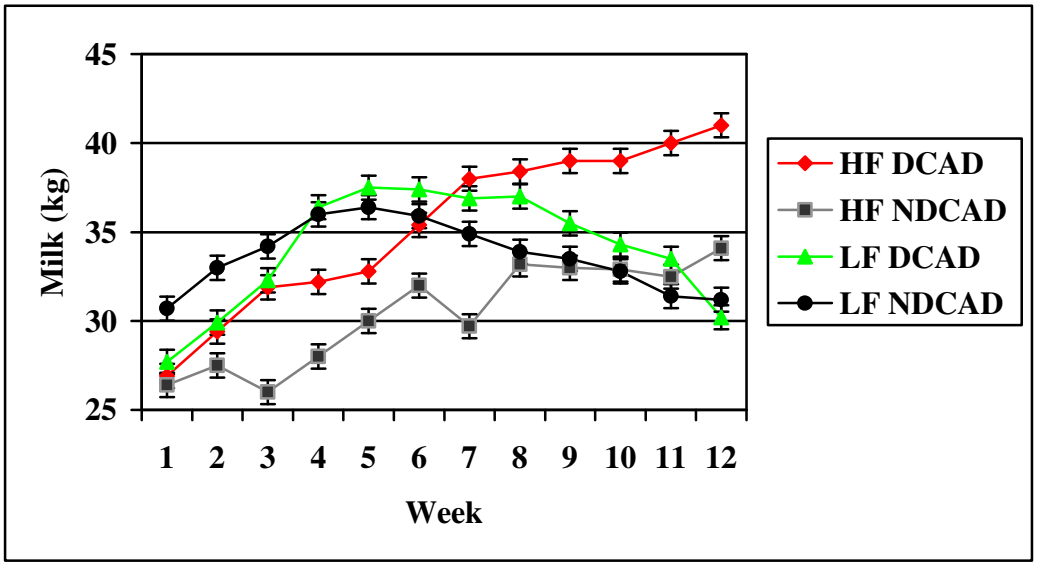


Figure 4. Effects of supplemental fat (0% VS 5.3% Megalac- R) on circulating concentrations of insulin in serum of dairy cows in early lactation. Each bar represents least square means from 6 animal observations and an average of samples taken at hourly intervals over a 24 hour period at three progressive weeks into lactation.

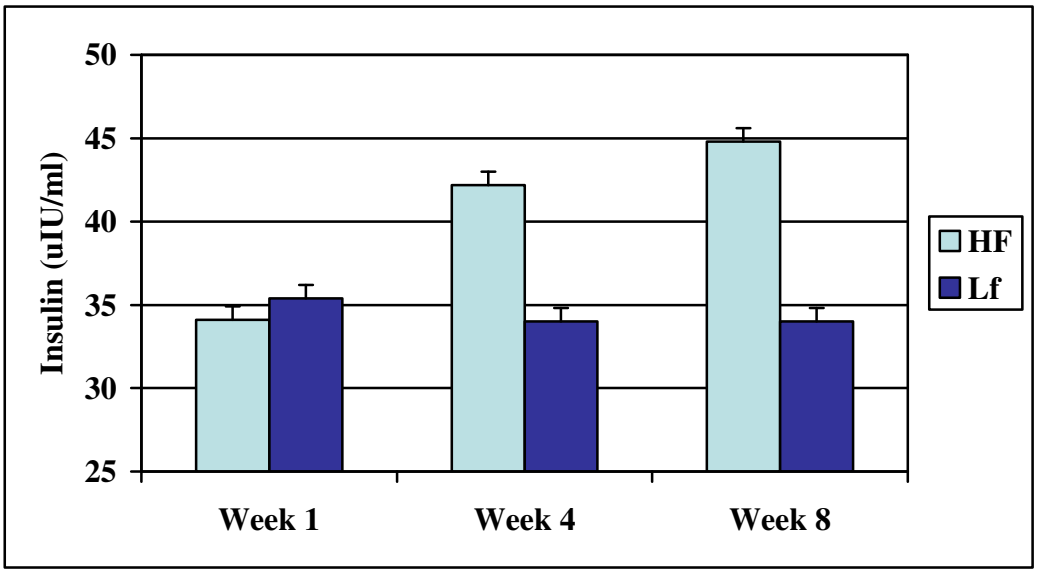


Figure 5. Effects of supplemental DCAD (Biochlor®) fed prepartum on circulating concentrations of insulin (uIU/ml) in serum of dairy cows in early lactation. Each bar represents least square means from 6 animal observations and an average hourly samples taken over a 24 hour period at three progressive weeks into lactation.

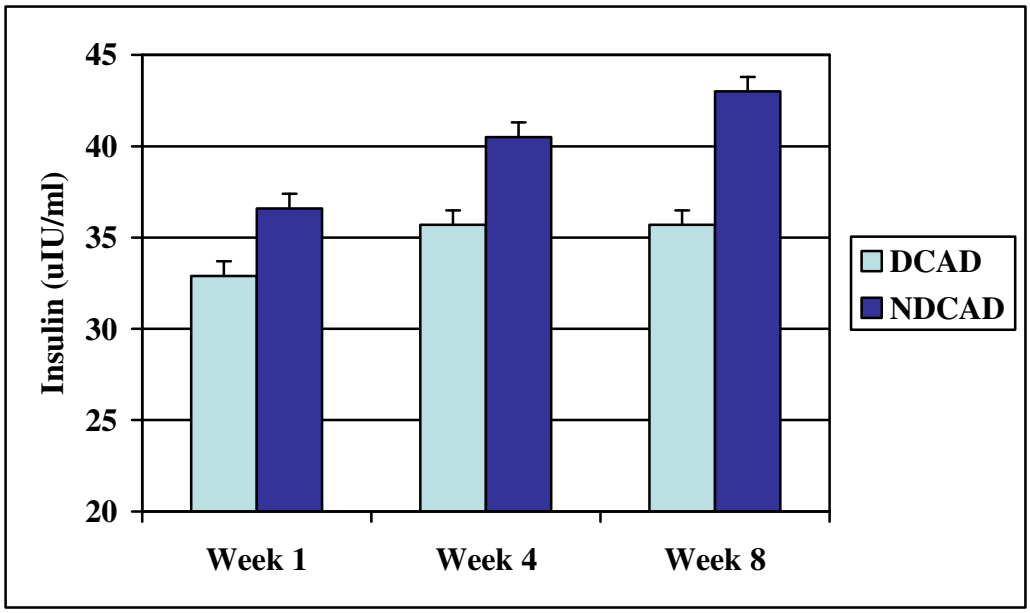


Figure 6. Effects of supplemental DCAD (Biochlor®) prepartum and fat supplemented postpartum fed on circulating concentrations of insulin (uIU/ml) in serum of dairy cows in early lactation. Each bar represents least square means from 3 animal observations and an average of hourly samples taken over a 24 hour period at three progressive weeks into lactation.

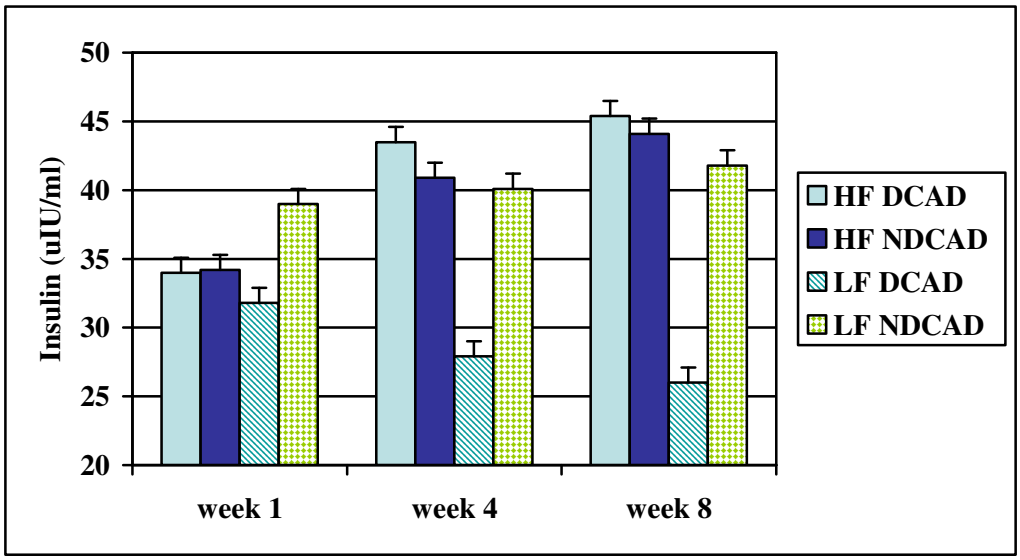


Figure 7. Effects of supplemental fat (0% VS 5.3% Megalac- R) on circulating concentrations of NEFA (*μM*) in serum of dairy cows in early lactation. Each bar represents least square means from 6 animal observations and an average of samples taken at hourly intervals over a 24 hour period at three progressive weeks into lactation.

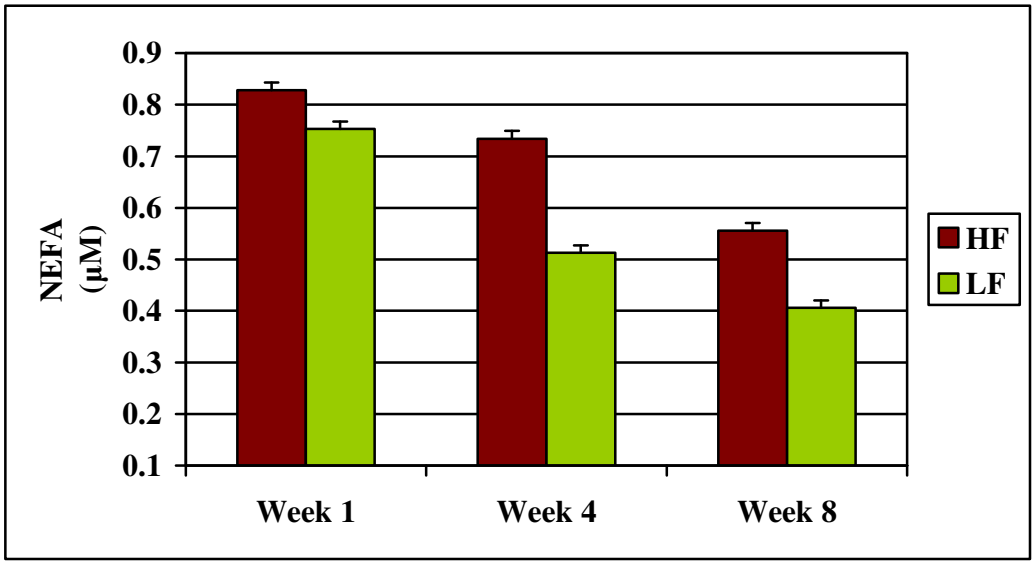


Figure 8. Effects of supplemental DCAD (Biochlor®) prepartum on circulating concentrations of NEFA (*μM*) in serum of dairy cows in early lactation. Each bar represents least square means from 6 animal observations and an average of samples taken at hourly intervals over a 24 hour period at three progressive weeks into lactation.

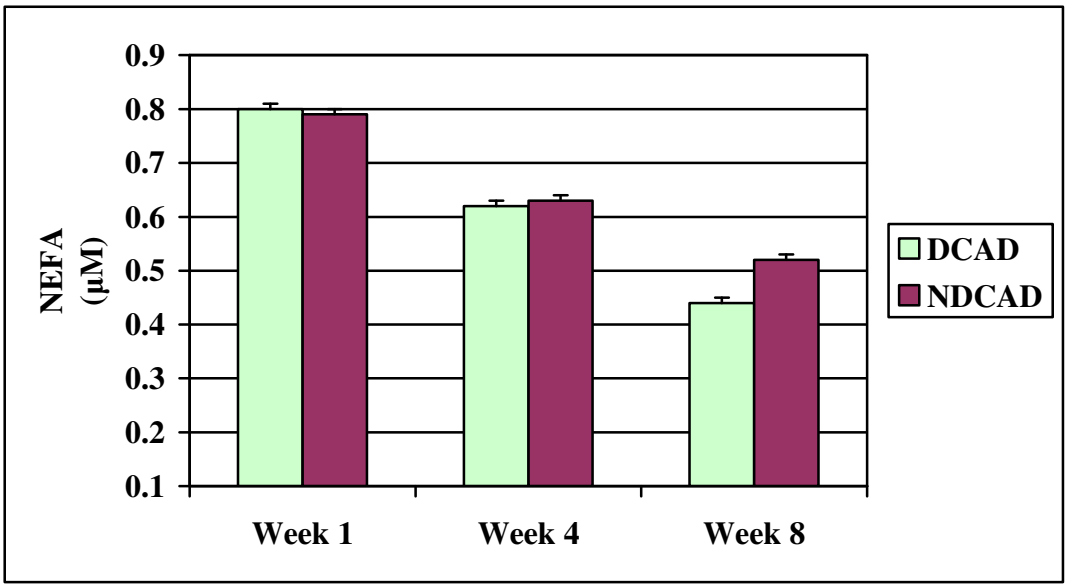


Figure 9. Effects of supplemental DCAD (Biochlor®) prepartum and fat supplemented postpartum fed on circulating concentrations of NEFA (μM) in serum of dairy cows in early lactation. Each bar represents least square means from 3 animal observations and an average of hourly samples taken over a 24 hour period at three progressive weeks into lactation.

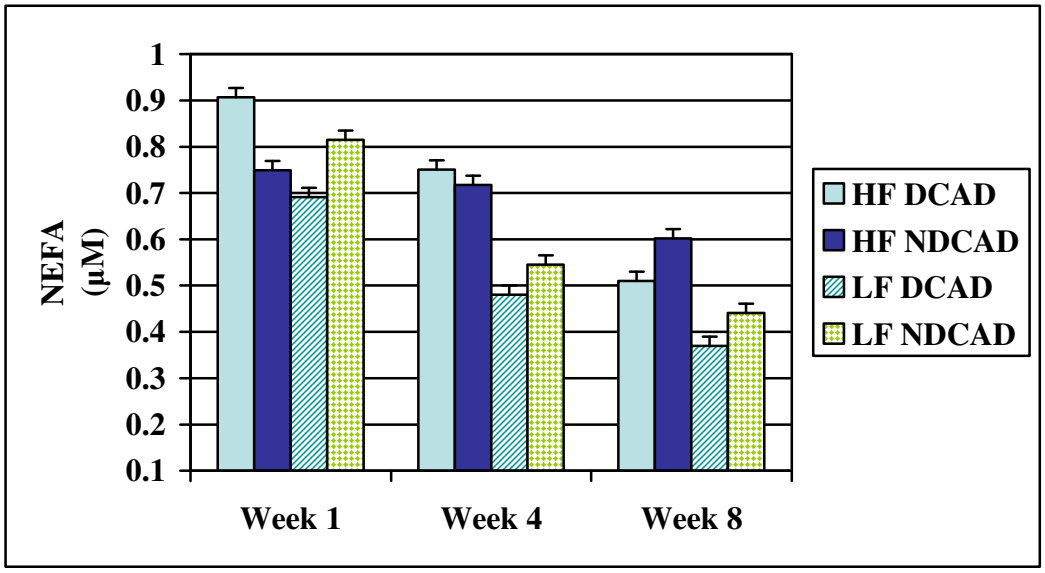


Figure 10. Effects of supplemental fat (0% VS 5.3% Megalac- R) on circulating concentrations of blood glucose (mg/dl) in serum of dairy cows in early lactation. Each bar represents least square means from 6 animal observations and an average of samples taken at hourly intervals over a 24 hour period at three progressive weeks into lactation.

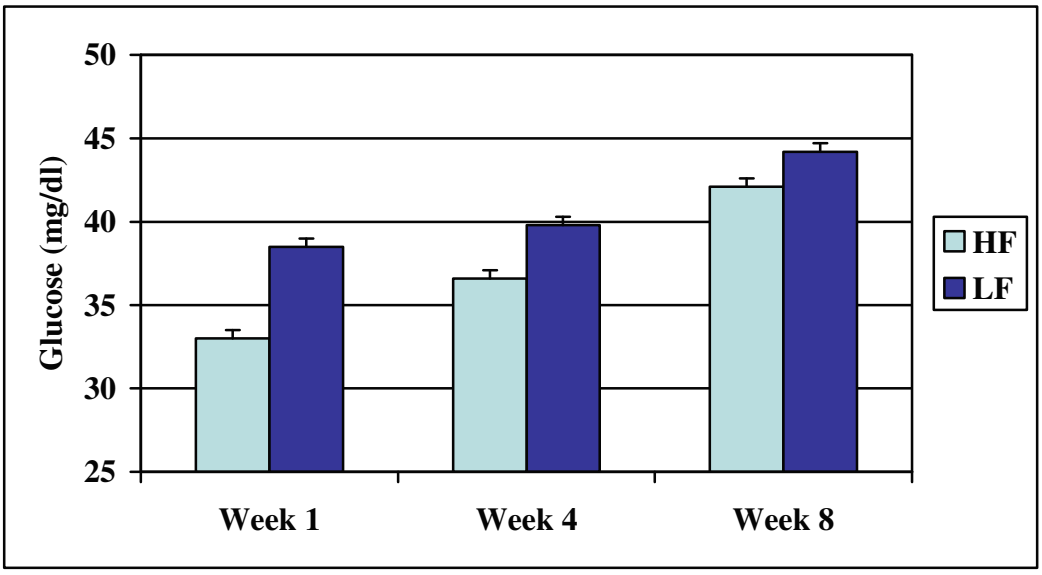


Figure 11. Effects of supplemental DCAD (Biochlor®) prepartum on circulating concentrations of blood glucose (mg/dl) in serum of dairy cows in early lactation. Each bar represents least square means from 6 animal observations and an average of samples taken at hourly intervals over a 24 hour period at three progressive weeks into lactation.

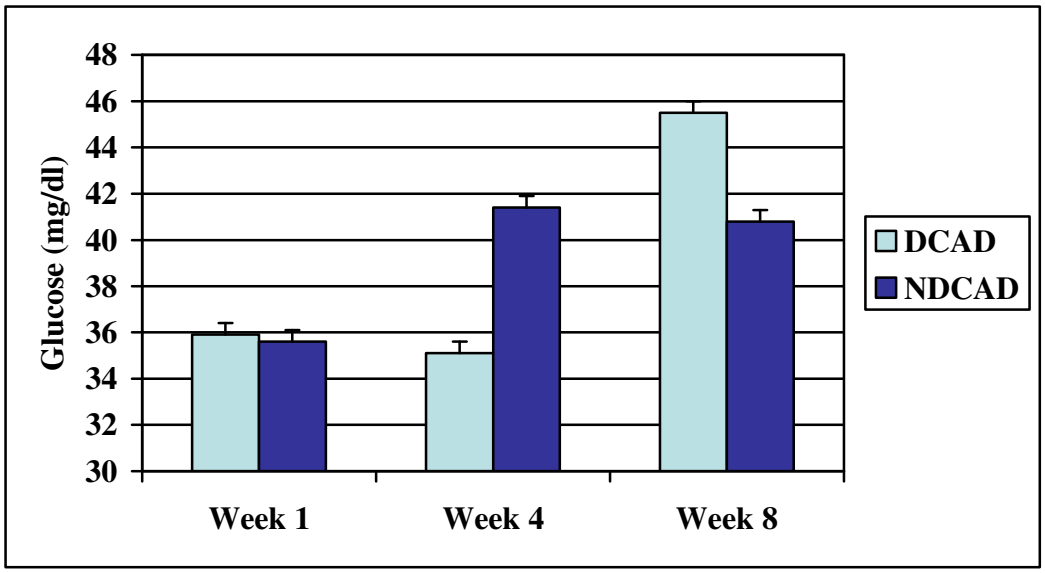


Figure 12. Effects of supplemental DCAD (Biochlor®) prepartum and fat supplemented postpartum fed on circulating concentrations of blood glucose (mg/dl) in serum of dairy cows in early lactation. Each bar represents least square means from 3 animal observations and an average of hourly samples taken over a 24 hour period at three progressive weeks into lactation.

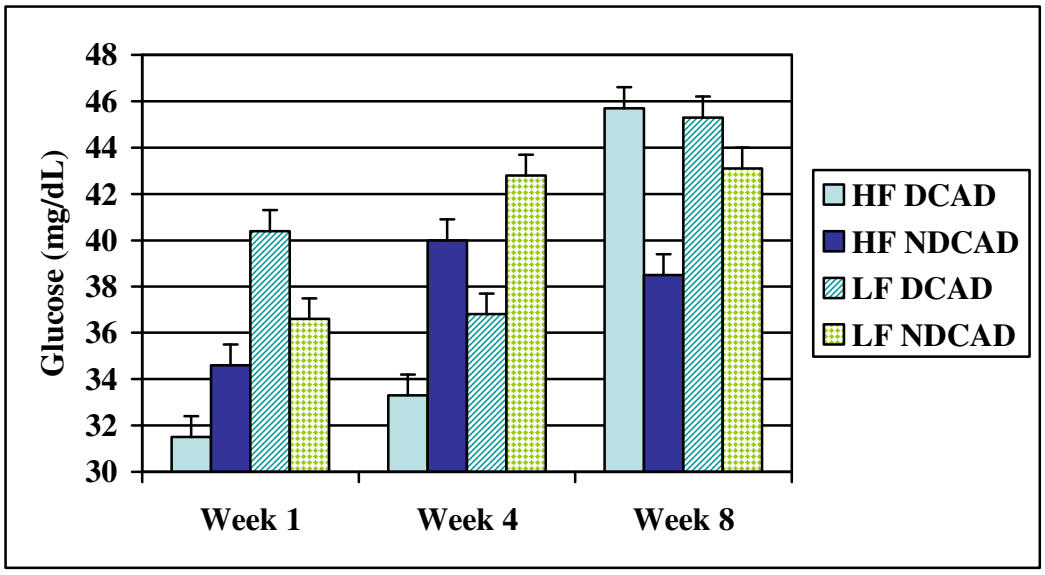


Figure 13. Effects of supplemental fat (0% VS 5.3% Megalac- R) on circulating concentrations of blood urea nitrogen (mg/dl) in serum of dairy cows in early lactation. Each bar represents least square means from 6 animal observations and an average of samples taken at hourly intervals over a 24 hour period at three progressive weeks into lactation.

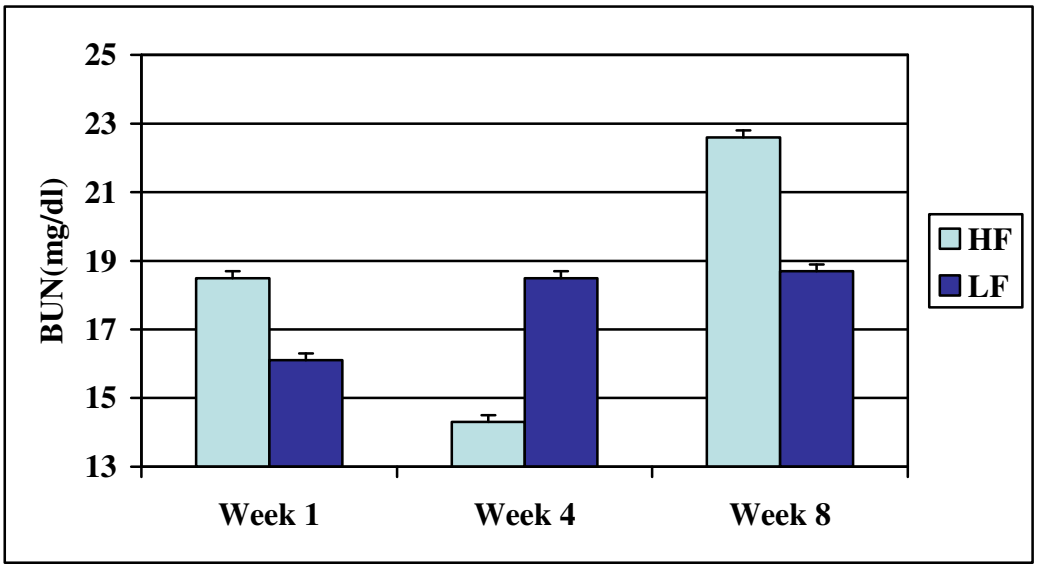


Figure 14. Effects of feeding supplemental DCAD (Biochlor®) prepartum on circulating concentrations of blood urea nitrogen (mg/dl) in serum of dairy cows in early lactation. Each bar represents least square means from 6 animal observations and an average of samples taken at hourly intervals over a 24 hour period at three progressive weeks into lactation.

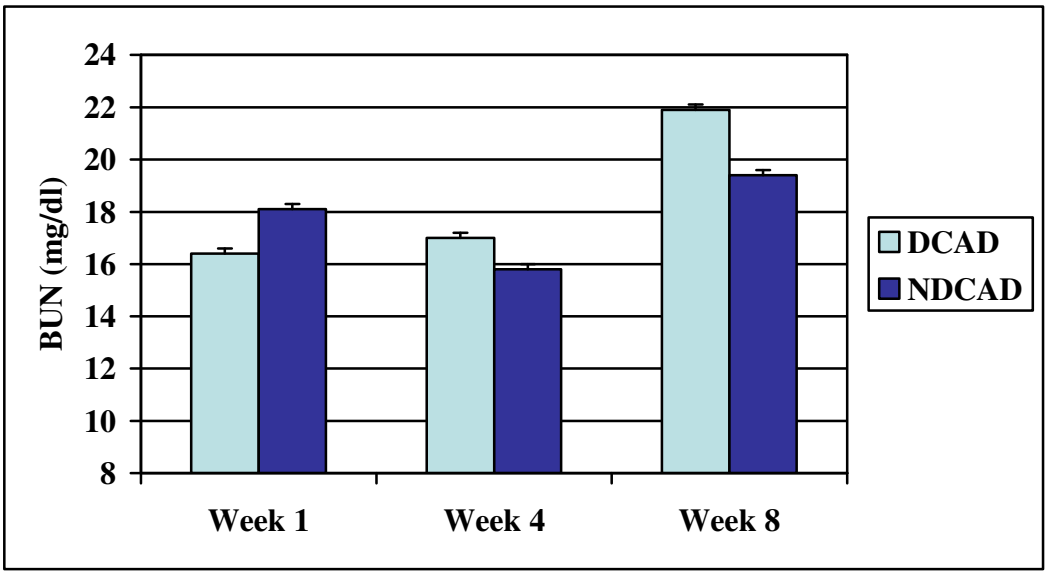
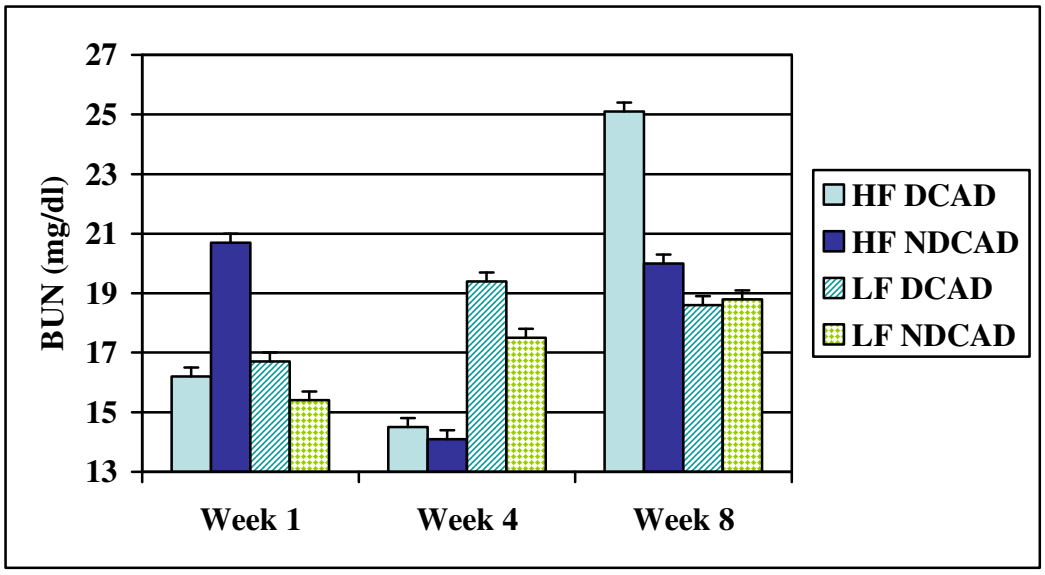


Figure 15. Effects of supplemental DCAD (Biochlor®) prepartum and fat supplemented postpartum fed on circulating concentrations of BUN (mg/dl) in serum of dairy cows in early lactation. Each bar represents least square means from 3 animal observations and an average of hourly samples taken over a 24 hour period at three progressive weeks into lactation.



CHAPTER 3

CONCLUSIONS

Improved calcium status may facilitate the transition dairy cow to more effectively use supplemental-fat and reduce health problems associated with negative energy balance. This research tested the effect of enhancing calcium mobilization prepartum by feeding anionic salts on the ability of cows to consume and metabolize relatively high levels of supplemental fat postpartum. Although the number of cows used in the present study were not sufficient to adequately test diet effects on intake and production, results agree with the preliminary findings, the combined results of the present and past findings together do suggest that prepartum DCAD facilitates utilization of higher levels of supplemental fat in early lactation cows. Intake and performance results provide a meaningful data set to compare changes in insulin and metabolites with daily meal feeding patterns in early lactation**.** Insulin was more negatively related to DMI. Also, DCAD seems to improve insulin sensitivity, but does not counteract the effects of fat**.** Using DCAD to enhance fat utilization could facilitate dairy production under heat stress conditions and feeding warm season forages.