2008

Effect of pre-calving diet energy content on immunologic and metabolic parameters in the transition cow

Hayley Ruth Springer
Iowa State University

Effect of pre-calving diet energy content on immunologic and metabolic parameters in the transition dairy cow

by

Hayley Ruth Springer

A thesis submitted to the graduate faculty

in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

Major: Biomedical Sciences (Physiology)

Program of Study Committee:
Jesse P. Goff, Major Professor
Dean H. Riedesel
James A. Roth

Iowa State University

Ames, Iowa

2008

TABLE OF CONTENTS

ABSTRACT

Over the last 30 years the trend on dairies has been to increase the energy content of the pre-partum ration to enhance dry matter intake (DMI) during the final weeks of gestation and prepare the rumen for the higher energy diets of lactation. In the last few years a number of dairies have shifted to a completely different dietary strategy; feeding a lower, but adequate, energy diet, which utilizes straw as a major component of the diet. The objective of this study was to determine if the institution of a lower energy pre-partum diet had any negative effects on metabolic or immunologic status of cows when compared to cows fed a higher energy pre-partum diet. Primiparous heifers were assigned to either a HIGH E diet (1.56 Mcal NE(L) / kg) or a LOW E diet treatment (1.35 Mcal NE(L)/kg diet) about five wks before calving. DMI was 1.2 kg/d greater in the HIGH E cows in the three weeks prior to calving. The LOW E diet did reduce the magnitude of DMI depression just before calving, but apart from a small reduction in plasma non-esterified fatty acid concentraion (NEFA) the day of calving, few major benefits of this reduction were seen. DMI after calving was similar in the two groups of cows. Body condition scores of the cows just before calving and at 21 and 44 days in milk (DIM) were statistically similar across the dietary treatments. A glucose tolerance test was performed on a subset of animals in each dietary treatment about one week prior to calving. No statistically significant difference was observed in glucose clearance from the blood or insulin secretion pattern suggesting no difference in tissue sensitivity to insulin was induced by the dietary treatments. Milk production during the first 45 days of lactation was statistically similar in HIGH E (27.1 kg/d) and LOW E cows (28.2 kg/d). Dietary treatment had no significant effect on liver triglyceride content on day 1 after calving, days to first ovulation, or plasma NEFA profile after calving. In vitro immune

function was assessed by neutrophil iodination, differential white blood cell counts, and whole blood interferon-γ production in response to both recall antigens and non-specific stimulation. There was no evidence that prepartal dietary energy impacted the degree to which these in vitro tests of immune function were suppressed in the transition cow. It is suspected that prepartum energy would have an effect on colostrum quality, but in this study, prepartum dietary energy had no effect on protein and IgG content of colostrum. The competency of the immune system as a whole was assessed by response to intramammary challenge with a mildly pathogenic strain of *Escherichia coli*. Throughout the course of the challenge, quantitative milk bacterial culture, milk somatic cell count, rectal temperature, differential WBC counts, serum minerals, and acute phase response were assessed and no differences were noted between dietary treatments. All animals were similarly able to successfully recover from the experimentally induced E. coli mastitis. Feeding a high energy diet prior to calving offers no advantages to health or production over a lower energy diet. The utilization of a low energy density diet to limit energy intake pre-partum is not detrimental to the dairy heifer.

INTRODUCTION

A pivotal time in the life of a dairy cow occurs during a period three weeks prior to through three weeks after calving, denoted as the transition period, when the dairy cow faces tremendous metabolic and physiologic changes that challenge the homeostatic mechanisms of the body (Grummer, 1995). As a result of these challenges, the transition cow is at a much greater risk of metabolic and infectious disease than cows in other stages of lactation. Managing the transition cow to minimize health problems provides vital economic benefit for dairy producers, as the impact of diseases of the transition period can affect the productivity of the cow for the upcoming as well as subsequent lactations.

One of the greatest challenges during the transition period is meeting the nutrient demands of the cow. In late gestation, the demands of the pregnant uterus are high, but the demands associated with early lactation are much greater. These changes are accentuated by the decreased dry matter intake (DMI) seen in almost all cows around the time of calving (Bertics et al., 1992, Grummer, 1995). The combination of reduced DMI and increased energy demand results in a period of negative energy balance.

In addition to the metabolic changes seen in the transition cow, both neutrophil and lymphocyte function are impaired (Kehrli et al., 1989a, Kehrli et al., 1989b). This immunosuppression results in a higher risk of infectious disease, such as mastitis and metritis. Reduced neutrophil function has also been associated with the occurrence of retained placenta (Kimura et al., 2002b). Changes in immune function have, in part, been related to the onset of lactation. Kimura et al. demonstrated that some aspects of immunosuppression were alleviated by mastectomy (Kimura et al., 1999a). This suggests

that these changes may be related to the negative energy balance associated with the onset of lactation.

Much of the recent research in transition cow nutrition has focused on maximizing energy intake during the close-up period, but more recent research has suggested that the degree of decline in DMI at the time of calving may actually have a greater impact on the health of the dairy cow than the level of energy intake at that time (Grummer et al., 2004). Further evidence has suggested that those cows suffering the greatest decline in feed intake just before calving are the most immune suppressed, resulting in a higher incidence of metritis and endometritis in these cows (Hammon et al., 2006, Urton et al., 2005). The purpose of this study is to test the hypothesis that metabolic profiles, milk production, and immune function would be improved by feeding heifers a diet that met, but did not exceed their energy requirements during the last five weeks before calving, as opposed to a diet that exceeded their energy requirement.

Thesis Organization

This thesis begins with a review of pertinent literature, followed by two journal articles. The first journal article addresses the metabolic effects of varying pre-calving dietary energy while the second paper addresses the immunologic parameters. Hayley Springer is the primary researcher and author on both papers. Jesse Goff is the corresponding author of both papers. Doug Bannerman and Brian Nonnecke served as corresponding authors for the immune function paper. The papers are followed by a comprehensive discussion and a general conclusion. This is followed by appendices containing protocols utilized during the study and a comprehensive reference list.

LITERATURE REVIEW

Managing the transition cow is one of the greatest challenges of the dairy industry. Metabolic problems, immunosuppression, and infectious disease are common in these cows as they face great nutritional, social, and hormonal changes around the time of calving. Much research has been done on these changes, yet methods to alleviate the problems are still elusive. The following section presents information on the transition period, the immune system, immunosuppression during the transition period, metabolic diseases, infectious diseases, and a possible dietary change to ease the transition from gestation to lactation: feeding a diet that meets but does not exceed the energy requirements of the cow. The goal of this diet is to prevent the drop in DMI at calving and to stimulate postpartum intake. The increased fiber content also seems to be able to reduce the incidence of displaced abomasum following calving.

During the transition period, the dairy cow must undergo immense metabolic and physiologic adaptation due to the high demands of lactation. In late gestation the dairy cow requires 0.82 Mcal of energy, 117g protein, 10.3 g Ca, 5.4 g P, and 0.2 g Mg beyond daily maintenance needs to support fetal growth. Following parturition, the production of 10 kg of colostrum requires 11 Mcal of energy, 140g protein, 23 g Ca, 9 g P, and 2 g Mg above maintenance (Goff and Horst, 1997a). This jump in nutrient requirements comes at a time when dry matter intake (DMI) is depressed (Bertics et al., 1992, Grummer, 1995), likely due to a variety of factors including increased concentration of sex hormones, mobilization of lipid stores, and possibly decreased rumen capacity due to the increasing size of the fetus and associated tissues (Ingvartsen and Andersen, 2000). Hormone levels fluctuate greatly around the time of calving. At parturition, plasma levels of glucocorticoid, estradiol, and estrone

peak (Smith and Schanbacher, 1973). Failure to accommodate the metabolic demands of the onset of lactation results in a variety of metabolic diseases in the transition cow. The elevated incidence of infectious disease is likely due to a period of immunosuppression associated with calving.

Immune System

The immune system can be divided into two major branches: innate and adaptive. The innate immune system provides the first line of defense while the adaptive immune system has a slower, but more specific response to pathogens. Proper function of both of these branches is vital to disease prevention.

The innate immune system is the body's first line of defense against invading pathogens and has both cellular and non-cellular components. Physical barriers, such as skin and the muco-cilliary ladder in the respiratory tract, as well as chemical barriers, including lactoferrin and lysozyme, comprise the non-cellular defenses of the innate immune system. The two primary cells of the innate immune system are neutrophils and macrophages.

Neutrophils, or polymorphonuclear leukocytes (PMN), are the first cellular responders to a site of infection. These cells circulate throughout the body patrolling for signs of infection, loosely tethered to the endothelium by adhesion molecules such as L-selectin (CD62L). Upon recognition of a chemotactic signal, the neutrophil leaves the blood vessel by a process of adherence and extravasation facilitated by β2-integrin (CD11/CD18). The selectin family is characterized by a lectin-like domain that allows binding to carbohydrate groups. The interaction between L-selectin on neutrophils and carbohydrate groups on the endothelial cell allows the neutrophil to roll along the endothelial wall where it can interact with inflammatory mediators at sites of inflammation and become activated.

Activation of the neutrophil increases the affinity of β2-integrin for intercellular adhesion molecules (ICAM) on the endothelial cell, allowing tight adhesion and extravasation of the neutrophil. Upon extravasation, the neutrophil follows chemotactic signals to the site of infection where it can phagocytize, then kill the pathogen by releasing oxygen radicals into the phagocytic vacuole. These steps are collectively known as the oxidative burst.

The macrophage is a phagocytic cell of the innate immune system which, in addition to ingesting and eliminating foreign material, is an antigen presenting cell. Macrophages break down foreign proteins to peptides that are presented on class II major histocompatability (MHC) molecules on the surface of the cell. These peptides are then recognized by B cells and T cells in the adaptive immune system. Macrophages also communicate with cells of the adaptive immune system by secretion of growth factors, called cytokines, which modulate the immune response, thus the macrophage is an important mediator between the innate and adaptive branches of the immune system.

There are primarily two types of cells in the adaptive immune system, B cells and T cells, both of which have antigen receptors that are specific to a certain antigen. B cells recognize antigen via surface bound immunoglobulins (Ig). Upon antigen recognition, the B cell proliferates, producing plasma cells and memory B cells. The plasma cells secrete immunoglobulin that binds to the pathogen and facilitates its destruction or removal from the body. During the proliferation of naïve B cells following activation, a process of refining the antigenic specificity of the immunoglobulins occurs, producing memory cells that have a higher affinity for the antigen. This affinity maturation, paired with the greater number of antigen-specific memory cells compared to naïve cells, allows a stronger, faster response should the antigen be encountered a second time. Activated B cells also have surface MHC

II, along with the necessary costimulatory molecules, allowing presentation of antigen to T cells.

There are two major types of T cells, helper T cells (T_H), and cytotoxic T cells (T_C). A third subset, the γδ T cell, are often considered to have a role in the innate immune system, or as a link between the innate and adaptive immune systems (Born et al., 2006). The two major subsets of T cells are physically distinguished by surface molecules. All T cells have CD3, T cell receptors, on their surface. In addition to CD3, T_H cells express CD4 co-stimulatory molecule and T_C cells express CD8. Unlike the B cell, which can recognize antigen directly, T_H and T_C cells can only recognize antigen that has been processed and presented on MHC molecules. T_H cells recognize antigen in the context of MHC class II on professional antigen presenting cells such as B cells and macrophages. T_H cells can be further divided into T_H -1 and T_H-2 cells based on the cytokines secreted. T_H-1 cells primarily secrete cytokines, including IFN-γ, IL-2, and TNF-β, that support inflammation and stimulate macrophages and cytotoxic T lymphocytes (CTL). T_H-2 cells, on the other hand, activate B cells and antibody dependent immune responses by secreting cytokines including IL-4, IL-5, IL-6, and IL-10. Tc cells recognize antigen presented on MHC class I molecules, which are expressed by all nucleated cells of the body. Unlike the peptides presented on MHC II, which are primarily derived from extracellular pathogens, MHC I presents intracellular peptides that, under normal circumstances, are self peptides. In the case of a viral infection or a neoplastic cell, the peptides presented in association with MHC I molecules are recognized by T_C cells as non-self. After antigen recognition and stimulation by cytokines secreted by T_H cells, T_C cells differentiate into CTL, which identify and eliminate cells presenting non-self peptides on MHC I.

Although B and T cells require specific antigens for stimulation, some plant proteins can non-specifically stimulate these cells, as well as other immune cells. These proteins are known as mitogens and are often used in research to nonspecifically induce immune cell proliferation. These assays can be utilized for *in vitro* assessment of immune function. In this study, mitogens are employed in demonstrating immune function in the transition dairy cow.

Transition Cow Immunology

The exact cause of impaired immune function in the transition cow is unknown, but is likely the consequence of many interrelated factors. Analysis of immune function in mastectomized cows during the transition period has demonstrated some of the impacts of lactational demands on the immune system. Intact, milk producing, cows show a drop in lymphocyte function and changes in leukocyte subsets that were not evident in mastectomized cows (Kimura et al., 2002a, Nonnecke et al., 2003). Neutrophil function was suppressed in both intact and mastectomized cows at the time of parturition, but functionality was restored more quickly following parturition in mastectomized cows (Kimura et al., 1999a). Although these studies reveal that the mammary gland does have an effect on immune function in the periparturient cow, other factors must be involved, as not all signs of immunosuppression were eliminated by mastectomy. The hormonal changes at calving may also play a role in periparturient immunosuppression. Glucocorticoids, which are elevated at calving, have been shown to reduce both neutrophil and lymphocyte function (Roth and Kaeberle, 1981, Roth and Kaeberle, 1982). Sex hormones, such as estradiol, which are elevated at calving, have also been shown to inhibit immune function (Lamote et al., 2004). Although hormonal and nutrient changes are likely key players in immune suppression in the

transition dairy cow, other factors, such as management, and social stresses probably also contribute.

The neutrophil plays such an essential role as a first responder in host defense that it is often studied when assessing immune function. In the transition cow, a number of studies have found impaired oxidative burst using a variety of measures, including myeloperoxidase activity, cytochrome C reduction, and chemiluminescence (Cai et al., 1994, Detilleux et al., 1995, Kehrli et al., 1989b, Kimura et al., 1999a, Mateus et al., 2002, Mehrzad et al., 2002). Random migration of neutrophils has also been found to be impaired in the transition cow (Detilleux et al., 1995, Kehrli et al., 1989b). Parturition has also been associated with a reduced proportion of cells expressing the adhesion molecule L-selectin (CD62L), as has been demonstrated in some (Kimura et al., 1999b, Meglia et al., 2001), but not all studies (Harp et al., 2005). This response may be largely attributed to the effects of glucocorticoids on L-selectin expression (Burton et al., 1995). A study of changes in mRNA in neutrophils around calving revealed reduced expression of some genes related to basic life functions, such as the citric acid cycle and DNA binding proteins (Madsen et al., 2002). In addition to impairment in neutrophil function, the transition period also has negative impacts on the adaptive immune system.

Several studies have found reduced lymphocyte proliferation in response to mitogen during the transition period (Kehrli et al., 1989a, Nonnecke et al., 2003, Shafer-Weaver et al., 1996). Changes in lymphocyte populations have also been noted. Reduced percentages of cells positive for CD3, CD4, γδTCR, and CD8 have been found (Kimura et al., 1999b, Kimura et al., 2002a). These changes were not seen in mastectomized cattle (Kimura et al., 2002a). Nonnecke et al. demonstrated reduced secretion of IgM and IFN-γ in the dairy cow

during the first two weeks of lactation (Nonnecke et al., 2003). There is also evidence of changes in T_H subtypes in the transition cow, with T_H-2 predominating during this period (Shafer-Weaver et al., 1996). These changes in immune function are likely the reason for the high incidence of infectious disease in the transition cow, but they may also contribute to other transition cow diseases, such as retained placenta.

Metabolic Disease in the Transition Cow

Increased energy needs paired with reduced energy intake results in a negative energy balance during the transition period. To provide energy for the developing fetus and the onset of lactation, lipids are mobilized from adipose tissue, as seen by the rise in non-esterified fatty acids (NEFA) in plasma at parturition (Emery et al., 1992). The NEFA are taken up by the liver and can be either completely oxidized, or can undergo incomplete oxidation, yielding ketone bodies such as β-hydroxybutyrate (BHBA), acetoacetate, and acetone. Ketone bodies are an alternative to glucose for a fuel source in many tissues. Although ketones serve as an important energy source, high levels of BHBA and acetoacetate can reduce the functionality of organs due to cytotoxic effects (Bobe et al., 2004). In particular, these ketones can inhibit β-oxidation, the citric acid cycle, and gluconeogenesis (Bobe et al., 2004, Veenhuizen et al., 1991). Cows with clinical ketosis show reduced appetite and milk yield, rapid body weight loss, and are usually listless. Subclinical ketosis, affecting up to 34% of dairy cattle, has a significant economic impact due to reduced milk production and increased risk of other metabolic diseases in the periparturient period (Duffield, 2000).

In addition to complete and incomplete oxidation of NEFA taken up by the liver, fatty acids can also be esterified to triglycerides. In ruminants, the ability to form triglycerides

within the liver is similar to that of other species, but the rate of export from the liver is quite low (Ingvartsen and Andersen, 2000). The level of lipid mobilization in the transition cow often results in triglyceride formation at a rate that exceeds the ability of the liver to export it, leading to accumulation of lipids in the liver (Goff and Horst, 1997b). Most dairy cattle have some degree of lipid accumulation in the liver at the time of calving, but excessive lipid accumulation can impair the metabolic activity of the hepatocyte (Bobe et al., 2004). Hormone sensitivity, as well as hormone levels are also affected by fatty liver. Hormone levels are altered due to reduced clearance by the liver, and reduced steroid synthesis due to impaired cholesteryl ester production (Bobe et al., 2004). Clinically, fatty liver reduces health status, productivity, and fertility, resulting in economic losses due to increased veterinary bills, longer calving intervals, and reduced milk yield (Bobe et al., 2004).

In addition to elevated energy demands, the onset of lactation brings with it a great increase in demand for minerals, particularly calcium. During early lactation, the dairy cow requires an extra 20-30g of Ca per day, which is largely met by calcium mobilization from bone (Goff, 2000). In addition to its utilization in milk, calcium is also essential for many other life processes, particularly muscle contraction. Thus, failures of the mechanisms of calcium homeostasis result in a disease state known as milk fever or hypocalcemia. When blood calcium falls, symptoms of hypocalcemia, including unsteadiness, cold ears, normal to low temperature, increased pulse rate, and in more advanced cases, inability to rise, begin to show. In severe cases, hypocalcemia can result in coma and death.

Homeostatic mechanisms to maintain blood calcium primarily revolve around parathyroid hormone (PTH) secretion from the parathyroid gland in response to low blood calcium. PTH acts on the bone and kidneys. In bone, it induces increased calcium

mobilization. In the kidney, renal tubule reabsorption of calcium is increased, but more importantly, 25-hydroxyvitamin D is converted to 1,25-dihydroxyvitamin D, which acts on the intestine to increase dietary absorption. Several factors can contribute to the failure of these homeostatic mechanisms. Hypomagnesemia can contribute to the development of milk fever by inhibiting PTH signaling pathways. Several of the enzymes in the PTH signaling pathway require Mg^{2+} for full function, thus, low blood magnesium can impair PTH signaling (Rude et al., 1998). Metabolic alkalosis has also been shown to impair calcium homeostasis by reducing tissue sensitivity to PTH. Alkalosis has been shown to reduce calcium mobilization from the bone due to increased osteoblastic activity and reduced osteoclastic activity (Bushinsky, 1996). Reduced responsiveness of the kidney to PTH has been evidenced by reduced 1,25-dihydroxyvitamin D production relative to increases in PTH in cattle on a cationic diet. These diets increase the risk of milk fever due to induction of metabolic alkalosis (Goff et al., 1991). It has been found that feeding anionic diets that induce a slight acidosis reduce the incidence of milk fever (Gaynor et al., 1989). Anionic diets have proven to be an effective preventative for hypocalcemia, and are associated with significantly higher serum calcium at calving (Goff et al., 1991, Phillippo et al., 1994).

Displaced abomasum (DA) is another high priced metabolic disease of the transition period. Cost per case is estimated at $250-400 depending on the procedure used to correct the problem (Bartlett et al., 1995). In a normal cow, the abomasum sits on the ventral floor of the abdomen, to the right of the rumen. Displacement of the abomasum occurs when gas collects in the abomasum, causing it to shift either to the left (LDA) or to the right (RDA) of the abdomen, with LDA being more common, particularly in the transition period (Constable et al., 1992). The gas that accumulates in the abomasum is due to the reaction of bicarbonate

with HCl to produce CO2, as well as gas production from rumen contents entering the abomasum (Sarashina et al., 1990).

There are a variety of reasons that DA are associated with the transition period. Ketosis, also common during the transition period, is a risk factor for DA (Curtis et al., 1985). There is also suggestion that upon delivery of the calf, a void is left in the abdomen, which may allow the abomasum to more easily become displaced (Constable et al., 1992, Ingvartsen and Andersen, 2000). Although this may have some effect, it is not required for a DA, as DA can occur in young cattle and males. Abomasal atony has also been associated with increased risk of DA (Constable et al., 1992). High levels of VFA in the abomasum have been found to reduce abomasal motility in sheep (Gregory, 1987). High grain or finely chopped diets may elevate levels of VFA entering the abomasum by reducing the rumen fiber mat, allowing grain to fall to the bottom of the rumen, where fermentation occurs and digesta can pass out of the rumen prior to VFA absorption (Ingvartsen and Andersen, 2000) A higher energy close-up diet has been shown to both increase (Correa et al., 1990), and decrease (Curtis et al., 1985) the incidence of LDA. This discrepancy may be due, in part, to higher than recommended levels of calcium and energy in close-up rations on farms studied by Correa et al. (Correa et al., 1990). High forage dry cow diets have been shown to reduce the incidence of LDA (Coppock et al., 1972). Reduced contractility of both the rumen and abomasum has been demonstrated in experimentally induced hypocalcemia (Daniel, 1983). Massey et al. found that cows with hypocalcemia are 4.8 times more likely to develop LDA than cows without hypocalcemia (Massey et al., 1993). Administration of calcium chloride gels around the time of calving has been shown to both reduce (Oetzel, 1996) or not change (Hernandez et al., 1999) the incidence of LDA. In the study by Hernandez et al. the calcium

gels did not affect serum calcium, whereas those used by Oetzel did (Hernandez et al., 1999, Oetzel, 1996).

Retained placenta is diagnosed when the placenta is not expelled within 12-24 hours after calving. The cause of retained placenta is currently unknown. It is estimated that retained placenta occurs in 8.6 percent of lactations (Kelton et al., 1998), with older cows at a greater risk (Curtis et al., 1985, Markusfeld, 1987). The reduced ability of the uterus to contract during hypocalcemia may also be a factor in retained placenta, as cow with milk fever have been shown to have greater incidence of retained placenta (Curtis et al., 1985, Erb et al., 1985). Immune function and its suppression at the time of calving may also contribute to retained placenta. Gunnick suggested a theory that the fetal membranes must be recognized as "foreign" by the immune system in order to be expelled. This theory was backed by demonstration of reduced leukocytic activity and reduced chemotaxis toward a cotyledon preparation in cows that developed retained placenta (Gunnink, 1984a, Gunnink, 1984b, Gunnink, 1984c). In addition, Kimura et al. demonstrated reduced neutrophil function both pre- and postpartum in cows that developed retained placenta (Kimura et al., 2002b). Retained placenta has been shown to be a risk factor for metritis, an infectious disease common during the transition period (Coleman et al., 1985, Emanuelson et al., 1993).

Infectious Disease in the Transition Cow

In addition to the metabolic diseases that occur during the transition period, the immunosuppression experienced during this time period increases the incidence of infectious diseases such as metritis and mastitis. Metritis is an inflammation of all layers of the uterus often due to bacterial infection. Many factors predispose the cow to metritis including, dystocia and retained placenta (Bruun et al., 2002), induced parturition, stillbirth, and

multiple births (Markusfeld, 1984). Uterine infection can occur due to a wide range of bacteria (Sheldon et al., 2002). Many cows have bacterial contamination of the uterus postpartum, but the spike in progesterone at the first ovulation can suppress the immune system enough to allow contaminating bacteria to establish infection (Lewis, 2003, Olsson et al., 1998). Uterine infection can damage uterine tissue, causing reduced reproductive efficiency both during and after infection (Bonnett et al., 1991). In addition, uterine infection can further affect fertility by altering function of the ovary and endocrine system (Sheldon, 2004).

Mastitis, an inflammation of the udder, accounts for huge economic losses to the dairy industry. Wells et al. reported the cost of clinical mastitis to be $1.5-2 billion annually, with an additional loss of $960 million due to subclinical infection (Wells et al., 1998). Mastitis is caused by a wide variety of organisms, including both contagious and environmental pathogens. Some common mastitis pathogens include *Escherichia coli*, *Staphylococcus aureus*, and *Streptococcus uberis*, as well as coagulase-negative S*taphylococcus*, *Mycoplasma*, *Serratia*, *Klebsiella*, and *Pseudomonas* species to name just a few. In order to cause mastitis, a pathogen must be able to enter and colonize the mammary gland. The dairy cow has an intricate defense system in the udder that works well under most conditions to prevent this. The first line of defense for the udder is at the teat canal, which is the primary site of entrance for mastitis pathogens. Between milkings, the teat sphincter is closed with a keratin plug in place. Milking removes the keratin plug and relaxes the sphincter muscle. This relaxation can last for some time after milking, allowing a period of greater susceptibility to infection immediately after milking. Within the gland, several non-cellular defenses are present, including lactoferrin, lactoperoxidase, and complement

components. In addition, there is cellular surveillance of the mammary gland, primarily by macrophages (Paape et al., 2000). Upon ingestion of a pathogen, the macrophage releases cytokines that recruit PMN and initiate an acute inflammatory response.

Infection of the mammary gland with *E.coli* is usually cleared quite quickly, but in some cases, it can escalate into severe disease. *E. coli* carries an endotoxin called lipopolysaccharide (LPS). This LPS induces an acute inflammatory response that is responsible for many of the systemic symptoms of *E. coli* mastitis, including fever, lack of appetite, and dehydration. These systemic symptoms are in addition to local signs of infection, including swelling of the udder, abnormal milk, and hardness of the udder. *E. coli* infection is particularly prominent around the time of calving and its severity is thought to be primarily influenced by host factors (Burvenich et al., 2003). One of the main characteristics of the cow that affects severity of infection is neutrophil recruitment and function. Other immune factors also play a large role in severity. It is for this reason that experimental *E. coli* infection can be utilized as a measure of host defense mechanisms as a whole.

Transition Cow Nutrition

The optimal energy content of the diet fed to the cow prior to calving is a controversial topic. The trend over the last forty years has been to feed the cow a diet with a higher energy concentration than she requires, if she is consuming the expected amount of diet DM. DMI is approximately 1.7% of heifer body weight and 1.9% of cow body weight (Hayirli et al., 2003). The rationale for this strategy includes: 1. Adaptation of rumen microbes (Huntington et al., 1981) and rumen papillae (Dirksen and Liebich, 1985) to the type of high-energy diet the cow will receive in lactation; 2. Maximizing dry matter intake prior to calving as this may be correlated to dry matter intake in early lactation (Grummer,

1995); and, 3. A diet with higher energy concentration may prevent the cow from going into negative energy balance just before calving when it is common to observe a marked decline in DM intake. This rationale is based on the premise that the dry matter intake depression just before calving would be similar in cows fed low and high energy diets during the final weeks of gestation, which may not be true (Beever, 2006, Grummer, 2008).

Increasing the non-fiber carbohydrate content of a pre-partum ration will often increase dry matter intake across the entire three weeks of the close-up period (Emery et al., 1969, Holcomb et al., 2001, Minor et al., 1998). Increasing the energy content of the pre-partum diet has also been reported in some studies to result in lower blood non-esterified fatty acid (NEFA) or B-hydroxybutyrate (BHBA) concentrations and lower liver triglyceride accumulation (Doepel et al., 2002, Mashek and Beede, 2000, Vandehaar et al., 1999). However, this approach does not generally increase DM intake after calving or increase milk production. There is also indirect evidence that supplying energy to the cow in excess of her needs may render her tissues less sensitive to insulin (Dann et al., 2006). Displacement of the abomasum is also promoted whenever the NFC content of the diet is increased, unless great care is taken to maintain physically effective fiber content of the ration (Coppock, 1974). When higher energy diets are fed for a period exceeding 5-6 weeks prior to calving the cows may also gain excess body condition. Data also suggest increasing energy density of pre-partum diets may lead to a greater decline of DM and energy intake in the 2-4 days before calving, i.e. the number of kg/d by which DM intake is depressed just before calving may be greater in cows on the higher energy diets (Hayirli et al., 2002, Ingvartsen and Andersen, 2000, Minor et al., 1998, Olsson et al., 1998). Retrospective evidence suggests those cows suffering a bigger change (# kg) in DM intake just before calving will be at greater risk of

energy related disease (Grummer et al., 2004). Studies on periparturient immune function also suggest those cows suffering the greatest decline in feed intake just before calving are most immune suppressed, leading to increased incidence of metritis and endometritis (Hammon et al., 2006, Urton et al., 2005). If intake during the week before and after calving is critical to avoidance of fatty liver-ketosis and improved immune function-how do we best feed the cow to achieve this?

The purpose of this study was to test the hypothesis that metabolic profiles, milk production, and immune function would be improved by feeding heifers a diet that met but did not exceed their energy requirements during the last 5 weeks before calving, as opposed to a diet that exceeded their energy requirement. Heifers sometimes have larger problems with energy balance than do older cows, especially if their body condition is excessive (Melendez et al., 2006). They eat less on a body weight basis (Hayirli et al., 2002) and, because they are still growing, they have higher energy requirements / kg body weight (NRC, 2001). They have minimal problems with hypocalcemia at calving, a factor which can reduce DM intake in older cows around the time of calving confounding interpretation of DM intake depression in older animals (Marquardt et al., 1977).

EFFECT OF ENERGY CONTENT OF PRE-PARTUM DIET ON DRY MATTER INTAKE AND METABOLIC PARAMETERS OF PERIPARTURIENT DAIRY COWS

A paper to be submitted to the *Journal of Dairy Science*

H. R. Springer and J. P. Goff

INTERPRETIVE SUMMARY

Pre-partum diet energy and metabolic status of heifers. Heifers were fed a HIGH E (1.56 Mcal NE(L) /kg) or LOW E energy (1.35 Mcal NE(L)/kg) diet the last five wk of gestation. Dry matter intake the last three wk of gestation was 1.2 kg / d greater in the cows fed HIGH E diet but this did not result in higher DMI after calving. Milk production, liver triglyceride content, and days to first ovulation were similar in both groups of cows. Feeding a high energy diet in late gestation does not offer any benefits over feeding a lower, but adequate energy diet to heifers.

ABSTRACT

Over the last 40 years the trend on dairies has been to increase the energy content of the pre-partum ration to enhance DMI during the final weeks of gestation and prepare the rumen for the higher energy diets of lactation. In the last few years a number of dairies have shifted to a completely different dietary strategy; feeding a lower energy diet, which utilizes straw as a major component of the diet. The objective of this study was to determine if the institution of a lower energy pre-partum diet had any negative effects on metabolic status of cows when compared to cows fed a higher energy pre-partum diet. Primiparous heifers were assigned to either a HIGH E diet (1.56 Mcal NE(L) / kg) or a LOW E diet treatment (1.35 Mcal NE(L)/kg diet) about five wks before calving. DMI was 1.2 kg/d greater in the HIGH E

cows in the three weeks prior to calving. The LOW E diet did reduce the magnitude of DMI depression just before calving, but apart from a small reduction in plasma non-esterified fatty acid concentraion (NEFA) the day of calving, few major benefits of this reduction were seen. DMI after calving was similar in the two groups of cows. Body condition scores of the cows just before calving and at 21 and 44 DIM were statistically similar across the dietary treatments. A glucose tolerance test was performed on a subset of animals in each dietary treatment about one week prior to calving. No statistically significant difference was observed in glucose clearance from the blood or insulin secretion pattern suggesting no difference in tissue sensitivity to insulin was induced by the dietary treatments. Milk production during the first 45 days of lactation was statistically similar in HIGH E (27.1 kg/d) and LOW E cows (28.2 kg/d). Dietary treatment had no significant effect on liver triglyceride content on day 1 after calving, days to first ovulation, or plasma NEFA profile after calving. Feeding a high energy diet prior to calving offers no advantages to health or production over a lower energy diet. The utilization of a low energy density diet to limit energy intake pre-partum is not detrimental to the dairy heifer.

Key Words: straw diet • pre-partum diet • transition cow, NEFA

INTRODUCTION

The optimal energy content of the diet fed to the cow prior to calving is a controversial topic. The trend over the last forty years has been to feed the cow a diet with a higher energy concentration than she requires, if she is consuming the expected amount of diet DM. DMI is approximately 1.7% of heifer body weight and 1.9% of cow body weight (Hayirli et al., 2003). The rationale for this strategy includes: 1. Adaptation of rumen microbes (Huntington et al., 1981) and rumen papillae (Dirksen and Liebich, 1985) to the

type of high-energy diet the cow will receive in lactation; 2. Maximizing dry matter intake prior to calving as this may be correlated to dry matter intake in early lactation (Grummer, 1995); and, 3. A diet with higher energy concentration may prevent the cow from going into negative energy balance just before calving when it is common to observe a marked decline in DM intake. This rationale is based on the premise that the dry matter intake depression just before calving would be similar in cows fed low and high energy diets during the final weeks of gestation, which may not be true (Beever, 2006, Grummer, 2008).

Increasing the non-fiber carbohydrate content of a pre-partum ration will often increase dry matter intake across the entire three weeks of the close-up period (Emery et al., 1969, Holcomb et al., 2001, Minor et al., 1998). Increasing the energy content of the pre-partum diet has also been reported in some studies to result in lower blood non-esterified fatty acid (NEFA) or β-hydroxybutyrate (BHBA) concentrations and lower liver triglyceride accumulation (Doepel et al., 2002, Mashek and Beede, 2000, Vandehaar et al., 1999). However, this approach does not generally increase DM intake after calving or increase milk production. There is also indirect evidence that supplying energy to the cow in excess of her needs may render her tissues less sensitive to insulin (Dann et al., 2006). Displacement of the abomasum is also promoted whenever the NFC content of the diet is increased, unless great care is taken to maintain physically effective fiber content of the ration (Coppock, 1974). When higher energy diets are fed for a period exceeding 5-6 weeks prior to calving the cows may also gain excess body condition. Data also suggest increasing energy density of pre-partum diets may lead to a greater decline of DM and energy intake in the 2-4 days before calving, i.e. the number of kg/d by which DM intake is depressed just before calving may be greater in cows on the higher energy diets (Hayirli et al., 2002, Ingvartsen and Andersen,

2000, Minor et al., 1998, Olsson et al., 1998). Retrospective evidence suggests those cows suffering a bigger change (# kg) in DM intake just before calving will be at greater risk of energy related disease (Grummer et al., 2004). Studies on periparturient immune function also suggest those cows suffering the greatest decline in feed intake just before calving are most immune suppressed, leading to increased incidence of metritis and endometritis (Hammon et al., 2006, Urton et al., 2005). If intake during the week before and after calving is critical to avoidance of fatty liver-ketosis and improved immune function-how do we best feed the cow to achieve this?

The purpose of this study was to test the hypothesis that metabolic profiles and milk production would be improved by feeding heifers a diet that met but did not exceed their energy requirements during the last 5 weeks before calving, as opposed to a diet that exceeded their energy requirement. In a companion paper, the effect of these diets on periparturient immune function and disease resistance will be examined. Heifers sometimes have larger problems with energy balance than do older cows, especially if their body condition is excessive (Melendez et al., 2006). They eat less on a body weight basis (Hayirli et al., 2002) and, because they are still growing, they have higher energy requirements / kg body weight (2001). They have minimal problems with hypocalcemia at calving, a factor which can reduce DM intake in older cows around the time of calving confounding interpretation of DM intake depression in older animals (Marquardt et al., 1977).

MATERIALS AND METHODS

Animals and Dietary Treatments

All procedures performed on the animals were approved by the Animal Care and Use Committee of the USDA National Animal Disease Center. Twenty-Four heifers in their 3rd-

5th month of pregnancy were purchased from a single source and maintained on 15% crude protein, hay-corn silage growing heifer diet in an open lot. At 9-15 weeks prior to calving cows were brought into a free stall barn equipped with fans over the feed lane and over free stalls that were bedded with sand. Cows were fed behind Calan gates (American Calan, Northwood, NH) to which they were trained and well acclimated by at least 9 wk prior to calving. At 6 wk prior to expected median date of calving, all heifers were immunized with 10^7 colony forming units of attenuated *Mycobacterium bovis BCG strain Pasteur* subcutaneously in the mid-cervical region of the neck to allow later assessment of in vitro recall response of peripheral blood mononuclear cells to *M. bovis* purified protein derivative (Pfizer, Kalamazoo, MI) (Foote et al., 2007) (see companion paper). Actual calving date ranged from 5 to 12 wks after vaccination. One heifer aborted the day after vaccination and was removed from the study. The remaining heifers were split into two groups taking care to have an even distribution of BCS across the two groups. Cows were scored for body condition using a 5-point scale (Ferguson et al., 1994). Three observers scored each cow to within 0.25 of a body condition score and the average score from these observations is reported. Body condition score (BCS) was assessed at time of blocking into treatment groups and frequently during the experimental period. Results are reported for the last week before calving and at 21 $\pm$ 2 and 44 $\pm$ 3 days in lactation. At 5 wk before expected calving date (based on palpation of the reproductive tract) until calving the cows were fed ad libitum either a low energy, high straw (LOW E) diet with net energy for lactation (NE(L)) of 1.35 Mcal/kg DM or a high energy, corn silage-grass hay (HIGH E) diet with NE(L) of 1.56 Mcal/kg DM (Table 1). Both groups were fed the low energy diet from 9-15 wk prior to calving until about 5 wk prior to calving. After calving, all cows received the same lactation

ration balanced to support 34 kg daily milk production (NRC, 2001). Milk production and feed intake were recorded daily. Cows were milked twice a day and were fed behind the same Calan gate during the pre-partum and lactating periods. They were moved to maternity pens bedded with straw adjacent to the free stall area when calving was imminent (within 12 hrs) and were offered their assigned pre-partum diet while in the maternity pens. Cows were moved back to the free stall and their Calan gate within 12 hrs after calving after their first milking.

Jugular vein blood samples were collected from all cows on a daily basis beginning about 3 weeks prior to calving and extending into the first 8 weeks after calving to develop a metabolic profile of the cows, determine date of first ovulation, and for assessment of immune system status (described in a companion paper).

On day 5 after calving all cows were experimentally infected in one quarter of the mammary gland with *Escherichia coli* as a means of assessing immune competence. The details of this bacterial challenge and the results of the study are reported in the companion paper.

Glucose Tolerance Test

A subset of cows on each dietary treatment was subjected to an intravenous glucose tolerance test. Our intention was to test glucose disappearance and insulin secretion response in cows that had been fed the experimental diets for at least four wks but not to test any cow that appeared to be within 3 d of calving. Six cows from the HIGH E and nine cows from the LOW E diets fit these parameters and underwent a glucose tolerance test. The cows were placed into headlocks prior to the morning feeding and body weight estimated based on heart girth. Blood samples were taken at 0, 15, 30, and 75 min prior to administration of glucose.

An indwelling catheter was placed into the left jugular vein and glucose was infused so that each cow received 50 mg glucose / kg BW over a ten minute period. Blood samples were then obtained from the contralateral jugular vein at 5, 10, 15, 20, 30, 40, 50, 60, 80, 100, 120, 150 and 180 min after infusion for determination of blood glucose and insulin concentrations. Blood glucose concentrations were determined by a colorimetric method based on glucose oxidase (Trinder, 1969) and adapted to microtiter plates. Insulin was determined by radioimmunoassay (Coat-A-Count Insulin, Diagnostic Products Corp., Los Angeles, CA).

Liver Biopsy

Between 12 and 24 hrs after calving approximately 120 mg of liver tissue was harvested by percutaneous biopsy of the liver. Each cow was sedated with 20 mg xylazine and anesthetized by local infiltration of 2% lidocaine into the skin and subcutaneous tissues of the 10th intercostal space on the right side of the cow. A stab incision was made through the skin and intercostal muscles. The liver was biopsied 6-9 times with a six inch 14 G Tru-Cut biopsy needle (Allegiance Healthcare Corp, McGaw Park, IL). Each needle insertion yielded 15-20 mg liver tissue. The incision was closed with a single suture. No post biopsy complications (fever, abnormal swelling etc.) were observed in any of the cows. Liver samples were rinsed by dipping in saline, blotted dry and placed into pre-weighed polyethylene cryotubes and kept on ice until transport to the lab where they were maintained at -80 C until analysis. Liver sample triglyceride (TG) and DNA content were determined and the results are expressed as μg TG / μg DNA. Briefly, each liver sample was homogenized in 2ml phosphate buffered saline. A 100 ul aliquot of this homogenate was removed for determination of sample DNA content. TG was extracted from the remaining homogenate with 2 ml chloroform:methanol (2:1). The chloroform phase was isolated and

placed into a second tube. This step was performed two more times and the chloroform extracts combined into a single test tube and dried to completion under nitrogen in a vacuum hood. The triglyceride content was determined by enzymatic lipolysis of the triglycerides to glycerol and fatty acids, followed by colorimetric determination of glycerol concentration which involves a series of reactions between glycerol, ATP, 4-aminoantipyrine and sodium N-ethyl-N-(3-sulfopropyl) m-anisidine to form quinonimine dye which absorbs light maximally at 540 nm (Sigma kit TR0100, Sigma Chemical Co, St Louis, MO). DNA concentration in the homogenate was determined using Hoechst 33258 (Sigma Chemical Co., St Louis, MO), a fluorochrome that intercalates with DNA specifically to produce enhancement of fluorescence, providing a method to quantify DNA in the presence of RNA (Labarca and Paigen, 1980).

Plasma Analytical Methods

Plasma calcium and magnesium concentrations were determined by atomic absorption spectrophotometry (Perkin Elmer, 1965). Plasma NEFA (NEFA-C; Wako Pure Chemical Industries, Osaka, Japan) (Johnson and Peters, 1993), B-hydroxybutyrate (BHBA) (BHBA dehydrogenase, kit 310-UV; Sigma Chemical, St Louis, MO) (Williamson et al., 1962) and blood urea nitrogen (BUN) (Sigma kit 640, Sigma Chemical, St Louis, MO) concentrations were determined by colorimetric assays adapted to microtiter plates. Plasma progesterone was determined by radioimmunoassay (Coat-a-Count Progesterone, Diagnostic Products Corp., Los Angeles, CA). A cow was considered to have ovulated the day before plasma progesterone exceeded 1 ng/ml. Plasma α-tocopherol, retinol, and β-carotene concentrations were determined by high performance liquid chromatography (Catignani and Bieri, 1983).

Statistical Analysis

Data were analyzed using a repeated measures analysis of variance (PROC MIXED)(SAS, 1999, Littell et al., 1998). The model included the fixed effects of treatment (HIGH E vs. LOW E Diet), day relative to parturition, and treatment x day interaction, the random effect of cows nested within treatment, and the residual error. For each variable analyzed, cow nested within treatment was subjected to three covariance structures: compound symmetry, autoregressive order 1, and unstructured covariance. The covariance that resulted in the Akaike's information criterion closest to zero was used. Means and SEM are reported for all data. When significant effects ($P < 0.05$) due to treatment, day, or treatment x day interactions were detected, means separation was conducted by the Tukey-Kramer option in SAS. Data were also split into two periods: before calving (days equal to or less than 1) and after calving (days greater than 0). DM intake data was also subjected to a separate analysis consisting of just the immediate pre-calving period – comprised of the last 3 days of gestation to assess the extent of the DM intake depression in each treatment group. Data analyses were conducted as described above for the two periods to determine significant effects. Data obtained during the glucose tolerance test were analyzed in a separate but similar model, with time relative to glucose infusion utilized in place of day relative to parturition.

RESULTS AND DISCUSSION

General Health

Three animals placed in the HIGH E group failed to complete the study. One heifer assigned to the HIGH E group delivered a stillborn, small calf the day after BCG vaccination, prior to the initiation of diet treatment. The smallest heifer in the HIGH E group could not

deliver a calf without a Caesarian section and was removed entirely from the study. A second heifer on the HIGH E diet developed severe udder edema on the second day after calving, which eventually led to mastitis. Her data obtained prior to the day of calving remain in the data set but upon calving she was removed from the study as she was treated for udder edema with furosamide. One heifer on the HIGH E diet was given a glucose infusion on day 10 after calving for treatment of mild ketosis and she recovered uneventfully and was left in the data set. All the other cows had no demonstrable health issues prior to infection of the mammary gland or any disorders following recovery from the experimental mastitis induction. The size of the data set precludes any inference about the effects of the diet on overall health of the animals.

Effects of Diet on DMI and Metabolic Parameters from Three Wks Pre-Partum to Four Days Pre-Partum

Increasing the energy density of the pre-partum diets will generally increase DMI across the last three wks of gestation (Emery et al., 1969, Holcomb et al., 2001, Minor et al., 1998, Rabelo et al., 2003, Vandehaar et al., 1999). Our results were consistent with these studies. The HIGH E group consumed 12.2 kg DM /d during this period, which was 1.2 kg/d greater than cows fed the LOW E diet who consumed 11.0 kg DM /d (Fig 1.). Therefore, the HIGH E cows consumed 19.0 Mcal NE(L)/ day while cows fed the LOW E diet consumed 14.9 Mcal NE(L) / d. The energy requirement for these cows is approximately 12 Mcal NE(L) / d (2001), so cows on the LOW E diet still received energy beyond their requirement.

Elevated blood NEFA concentrations during this period may be predictive of cows with increased risk of developing displaced abomasum (Cameron et al., 1998, LeBlanc et al., 2005) and retained placenta (Conner et al., 2001), and increased risk of being culled in early

lactation (Duffield, 2007). In the Minor et al., (Minor et al., 1998), Rabelo et al., (Rabelo et al., 2003) and VandeHaar et al., (Vandehaar et al., 1999) studies, increasing the energy content of the pre-partum diet reduced blood NEFA concentrations prior to calving. Plasma NEFA concentrations of the HIGH E cows between -22 d and -4 d (0.15 + 0.02 M) were lower ($P < 0.05$) than in LOW E cows during this period (0.21 + 0.02 M) ($P < 0.02$) (Fig. 2), but in both groups of cows the NEFA concentrations were well below the suggested close-up cow critical NEFA level of 0.4 -0.5 M that would predict cows "at-risk" for metabolic disorders (Duffield, 2007, Van Saun, 2006).

The glucose tolerance test results show a similar zenith in blood glucose concentration was achieved in both groups of cows at the cessation of glucose infusion. The rate at which blood glucose concentration returned to normal was similar in both groups of cows (Fig 3). Though there is a trend toward increased insulin secretion in cows fed the HIGH E diet, plasma insulin secretion was stimulated by the glucose infusion to similar degrees across the dietary treatments ($P = 0.28$). Reduced tissue sensitivity to insulin has been demonstrated in cows with high BCS and is suspected to play a role in susceptibility of the transition cow to metabolic disease (Holtenius and Holtenius, 2007, Rukkwamsuk et al., 1998). Overfeeding dairy cows during the pre-partum period may accentuate insulin resistance in adipose tissue, leading to increased NEFA mobilization, lower DMI, and greater risk for lipid-related metabolic disorders (Holtenius et al., 2003). Our data do not support the theory that dietary energy fed to the cow can affect insulin sensitivity of the tissues, perhaps because BCS was similar in the two groups of cows at calving or perhaps we did not have enough animals in the study. Approximately one week before calving, the BCS of cows fed the HIGH E diet averaged 3.65 $\pm$ 0.15 and 3.56 $\pm$ 0.08 in the LOW E cows.

Plasma BUN concentration was significantly higher in LOW E cows prior to calving than in HIGH E cows ($P<0.05$) (Fig. 4). Since dietary protein concentrations were comparable and DMI was higher in the HIGH E cows, the LOW E cows might be expected to have lower BUN concentration as the total nitrogen entering the rumen should have been lower. However, a likely explanation is that the rumen degradable protein nitrogen entering the rumen of the LOW E cows is not incorporated into microbial protein very well because of the lack of starch or other readily fermentable carbon sources in the rumen. This nitrogen enters the blood as ammonia and is converted to urea in the liver, raising BUN. The NRC (2001) model predicted that these diets would supply similar amounts of metabolizable protein to the cows, assuming similar DMI (Table 1). However, since BUN levels are higher in the LOW E cows these data suggest the NRC model overestimates the contribution of the LOW E diets to metabolizable protein. It may be necessary to provide more dietary rumen undegradable protein to meet the metabolizable protein requirements of the dry cow fed a LOW E diet. There were no apparent ill-effects from this reduced availability of metabolizable protein on the LOW E diet.

Effects of Diet on DMI and Metabolic Parameters during the Immediate Close-Up Period (Three d Pre-Partum to Calving)

DMI decreased in both groups of cows during the last week of gestation. However, there was a significant day X treatment interaction in DMI during the last 3 days of gestation. The HIGH E cows were consuming 12.1 $\pm$ 0.6 kg DM on Day -3 and just 9.9 $\pm$ 0.6 kg DM the day before calving. The LOW E cows were consuming 10.4 $\pm$ 0.6 kg DM on Day -3 and 10.3 $\pm$ 0.6 kg DM the day before calving. The cows fed the HIGH E diet had a significant reduction of 2.2 kg DMI/ d or 18% in the last 3 days of gestation, while LOW E cows

maintained their intake through this period. Total DMI the last three days of gestation was 33.8 kg in HIGH E cows and 31.5 kg in LOW E cows.

NEFA concentrations rose rapidly the day of calving in both groups and were 0.45 M in HIGH E cows and 0.36 M in LOW E cows. This represented a 3-fold NEFA concentration increase in HIGH E cows and a 1.7-fold increase in LOW E cows from the average value observed across the period from 3 wks to 3 days prior to calving.

The depressed DMI during the final days of gestation observed in the cows fed the HIGH E diet parallels the pattern of DMI seen in many previous studies (Bertics et al., 1992, Grummer et al., 2004, Hayirli et al., 2002, Marquardt et al., 1977). Grummer et al., (2004) suggested that the degree of depression in DMI at the time of calving may have a more significant effect on postpartum performance than the level of DMI itself. The same authors went on to suggest that higher pre-partum DMI may actually promote a greater magnitude of DMI depression when compared to cows with lower pre-partum DMI. They also suggest the delta change in DMI during the final days of gestation may act as a stimulus for the mobilization of fat from adipose tissue. Our data support this argument. The HIGH E group had both higher pre-partum DMI and a greater degree of DMI depression at the time of calving, even though they were consuming more energy the final day of gestation than the LOW E cows on that day (15.4 Mcal vs 13.9 Mcal NE(L)). And, at least on the day of calving, the cows fed the HIGH E diet had higher NEFA concentrations in their blood than the LOW E cows. It appears that the trigger for body fat mobilization has less to do with absolute energy intake than it does with intake relative to the previous day or days intake of energy.

Effects of Diet on DMI and Metabolic Parameters Post-Calving

DMI increased after calving in cows in both diet treatments at a similar rate. The intramammary *E.coli* challenge affected both groups of cows to a similar degree and the rate of recovery of DMI after challenge was similar. Other aspects of the immune response to the *E. coli* challenge are described in a companion paper. Despite the *E.coli* challenge, DMI across the first 40 d of lactation was 16.0 kg /d in the HIGH E cows and 15.75 kg /d in LOW E cows.

Plasma NEFA concentration increased slightly above calving levels in both groups the first two days of lactation but were on the decline at the time of the *E.coli* intramammary challenge. There was no statistically significant difference across the dietary treatments. Because the mastitis challenge was expected to depress DMI no further determinations of NEFA were performed. The combination of movement in and out of the maternity pen, lactation diet, liver biopsy, and initiation of milk production likely contributed to the rise in NEFA in both groups after calving, though the average remained well below levels that would be considered indicative of severe negative energy balance in both groups of cows. The triglyceride content of the liver, determined in the biopsies obtained the day after calving, was similar in both groups of cows. In some studies, increasing the energy content of the pre-partum diet also increased glycogen and/or reduced the triglyceride levels in the liver around the time of calving, factors which should have reduced the risk of development of the fatty liver ketosis complex (Doepel et al., 2002, Minor et al., 1998, Vandehaar et al., 1999). In our study, liver triglycerides at calving were relatively low in all cows (0.57 and 0.58 µg triglyceride/ µg DNA for the HIGH E and LOW E cows respectively) and there was no effect of diet, consistent with the findings of Rabelo et al., (Rabelo et al., 2005). Our two internal

lab standard livers, obtained from cows diagnosed with clinical fatty liver, were found to have 1.77 and 1.95 µg triglyceride/ µg DNA. Plasma BHBA concentrations at 4 DIM were similar across dietary treatments (HIGH E – 8.1 + 0.6 mg/dl and LOW E – 8.6 + 0.5 mg/dl). Examining BHBA at time points between 7 and 14 DIM is generally felt to be more informative of the degree of negative energy balance experienced by the cow; however the intra-mammary challenge with *E.coli* was felt to be a factor that would render interpretation of BHBA at these later DIM meaningless. Plasma BUN concentrations were similar in cows fed the HIGH E and LOW E diets during the lactation phase of the study, when they were fed the same diets.

Milk production was similar in both groups of cows (Fig. 5). There was no effect of diet on days to first ovulation, which was 24 and 30 DIM for the HIGH E and LOW E cows respectively (P= 0.27) (Fig. 5).

Other Dietary Effects across the Entire Experimental Period

Plasma concentrations of α-tocopherol, retinol, and β-carotene were not statistically different across the dietary treatments. All three compounds exhibited a significant decline around the time of calving followed by a recovery over the first two weeks of lactation, as reported in other studies (Goff and Stabel, 1990, Weiss et al., 1990). Similarly, plasma Ca and Mg concentrations did not differ across dietary treatments though there was a significant sub-clinical decline in plasma Ca concentration in the first few days of lactation in both groups (Fig. 4). Plasma P concentration also declined in both groups of cows on the first few days of lactation, and the decline was greater in cows fed the HIGH E diet ($P < 0.05$) (Fig. 4). The mineral levels were not low enough to cause noticeable clinical symptoms.

Several research groups report maximizing DMI or energy intake during the close-up period improves post-partum intake and performance (Dann et al., 1999, Doepel et al., 2002, Mashek and Beede, 2000, Minor et al., 1998, Rabelo et al., 2003, Vandehaar et al., 1999). In contrast, several studies provide evidence that restricting energy intake during either the entire dry period (Agenas et al., 2003, Douglas et al., 2006, Holtenius et al., 2003, Tesfa et al., 1999) or the close-up period (Holcomb et al., 2001) to levels at or even below NRC requirements (2001) has no negative impact on postpartum DMI. Our results also suggest that higher DMI pre-partum, which was associated with the higher energy diet, does not lead to greater DMI in early lactation.

The two diets had similarly successful outcomes postpartum. The management conditions utilized in this trial may have contributed. In this study, heifers were housed in an un-crowded barn (about 1.2 free stalls per heifer), and were given sole access to their respective diets via Calan gates which provided 0.82 M bunk space / cow. On commercial dairies the cows rarely have this much space. Increased stocking density is associated with an increased number of displacements from the feed bunk (Huzzey et al., 2006), reduced feed intake (Grant, 2007), and reduced ability to rest comfortably (Krawczel et al., 2008). Age, height, and weight have been positively correlated with increasing social standing, thus, heifers are more likely to be of lower social standing in mixed parity groups (Arave and Albright, 1976). Lower social standing has been associated with more frequent displacement from post-and-rail feed bunks (Huzzey et al., 2006). In this study the heifers were housed away from cows on the dairy, avoiding another common stressor on commercial dairies. Movement of dairy cattle into different pens or groups of cows during the transition period is a common practice. Altered social group has been associated with an increased number of

displacements from the feed bunk and decreased feeding time (von Keyserlingk et al., 2008). Throughout the duration of this study, there were no changes in social groups. The management conditions of this study eliminated many of the social and behavioral challenges the heifer can face under typical management conditions.

Both low and high energy density diets were successful under the conditions of this study. The decision to implement a low or high energy pre-partum diet must take into account the specific management practices of the dairy and how these will affect feed availability for heifers. It is something of a misnomer to refer to the straw based diet utilized in this study as a low energy diet. It did provide adequate energy to the cows. Providing adequate, but not excessive energy, in late gestation has been instituted successfully on dairies and has been reported to improve health of the cows when compared to the higher energy pre-partum diets (Beever, 2006, Drackley and Janovick Guretzky, 2007). Further studies should focus on the interaction between diet energy and health in older cows and under typical commercial conditions, where over-crowding and cow movement in and out of pens are common stressors that might further impact feed intake around the time of calving. The use of a low potassium straw to achieve a LOW E pre-partum diet does offer an opportunity to reduce dietary cation-anion difference to a greater extent than is possible with most other forages. However, it does require reduction of the straw to a small average particle size, approximately 6 cm, to effectively prevent the cows from sorting the ration.

CONCLUSIONS

Over the last 40 years the trend on dairies had been to increase the energy content of the pre-partum ration to enhance DMI during the final weeks of gestation. In the last few years a number of dairies have shifted to a completely different dietary strategy, which

utilizes straw as a major component of the diet to limit total energy content consumed by the cow. In this study, the LOW E and the HIGH E diets had similar outcomes in terms of milk production and time to first ovulation and were successful in limiting health problems through the transition period. The LOW diet did reduce the magnitude of DMI depression just before calving, but apart from a small reduction in NEFA the day of calving, few major benefits of this reduction were seen. With optimal management, the utilization of a low energy density diet to limit energy intake pre-partum is not detrimental to the dairy heifer despite her relatively greater energy needs compared to the multiparous cow.

ACKNOWLEDGMENTS

The authors wish to thank the animal caretakers who provided the daily care and feeding of the experimental animals: Norman Tjelmeland, Creig Caruth, Jerry Grove, and Andrew Mosier. Expert technical assistance in performing the assays of the metabolic parameters was provided by Amanda Hall, Duane Zimmerman and Derrel Hoy. Adam Ward, Billy Brown, and Lindsey Zaebst assisted throughout the glucose tolerance testing, milking of the cows, monitoring of cows during experimental challenge, blood sampling and conduct of laboratory assays.

Table 1. Composition of experimental HIGH and LOW Energy diets fed prior to calving and the lactation diet fed to all cows after calving.

	HIGH Energy	LOW Energy	Lactation
Ingredient (% DM)			
Corn silage	40.3	9.6	24.8
Grass hay, late mat.	34.5	19	
Wheat straw		43	2.6
Soybean meal, solv. 44% CP	17.2	20.4	6.9
Legume forage hay, mid-mat.			21.7
Corn grain, ground, dry			20.5
Expellers soybean meal[1]			9.3
Soybean hulls			9.4
Chloride anion supplement[2]	3.4	3.4	
Molasses, sugarcane	2.1	2	1.9
Yeast[3]	0.71	0.52	0.43
Calcium sulfate (2 H_2O, 74% $CaSo_4$)	0.41	0.69	
Calcium phosphate (Di-)	0.52	0.74	0.28
Calcium carbonate			0.35
Potassium carbonate			0.35
Sodium bicarbonate			0.43
Magnesium oxide	0.09	0.26	0.32
Magnesium sulfate (7 H2O, Epsom salts)	0.36		
Salt	0.3	0.28	0.35
Dry cow vitamin-trace mineral[4]	0.11	0.11	
Lactating cow vitamin-trace mineral[5]			0.39
Nutrient profile (% DM)			
Crude protein	14.9	15	17.9
NDF	43.5	56.1	32.9
ADF	28.1	34.6	22
Fat	2.4	1.8	3.2
NFC	34.8	20.7	40.7
Ca	0.71	0.8	0.85
Phos	0.44	0.38	0.4
Magnesium	0.38	0.4	0.43
Chloride	0.9	0.62	0.51
Potassium	1.52	1.1	1.74
Sodium	0.15	0.15	0.33
Sulfur	0.34	0.31	0.26
DCAD (mEQ/kg DM)[6]	-12	-23	286
NRC 2001 protein and energy[7]			
Metabolizable protein (g/d)	1018	1011	2080
NEl (Mcal/kg)	1.56	1.35	1.66

1. SoyPLUS, West Central, Ralston, IA
2. SoyChlor, West Central, Ralston, IA
3. Diamond V XP, Cedar Rapids, IA
4. Supplied trace minerals in amounts that met or exceeded NRC absorbed requirement plus 80,000 IU vitamin A (retinyl acetate), 20,000 IU vitamin D_3, and 1500 IU vitamin E (tocopheryl acetate)
5. Supplied trace minerals in amounts that met or exceeded NRC absorbed requirement plus 120,000 IU vitamin A (retinyl acetate), 30,000 IU vitamin D_3, and 1800 IU vitamin E (tocopheryl acetate)
6. DCAD = (Na + K) – (Cl + S)
7. As predicted from the NRC 2001 model utilizing a 544 kg heifer in late gestation consuming 10.6 kg DM/d or an early lactation cow consuming 17.7 kg DM/d.

Table 2. Effect of Pre-partum diet energy concentration on body condition score, days to first ovulation, and liver triglyceride content of primiparous cows.

	HIGH Energy (1.56 Mcal/kg DM)	LOW Energy (1.35 Mcal/kg DM)
Body condition score		
Last wk gestation	3.65 ± 0.15	3.56 ± 0.08
~21 days in milk	3.33 ± 0.11	3.12 ± 0.08
~44 days in milk	3.44 ± 0.17	3.14 ± 0.12
Days to first ovulation	24.6 ± 3.1	30.0 ± 3.4
Liver Triglyceride content (µg triglyceride/ µg DNA)	0.57 ± 0.08	0.58 ± 0.11

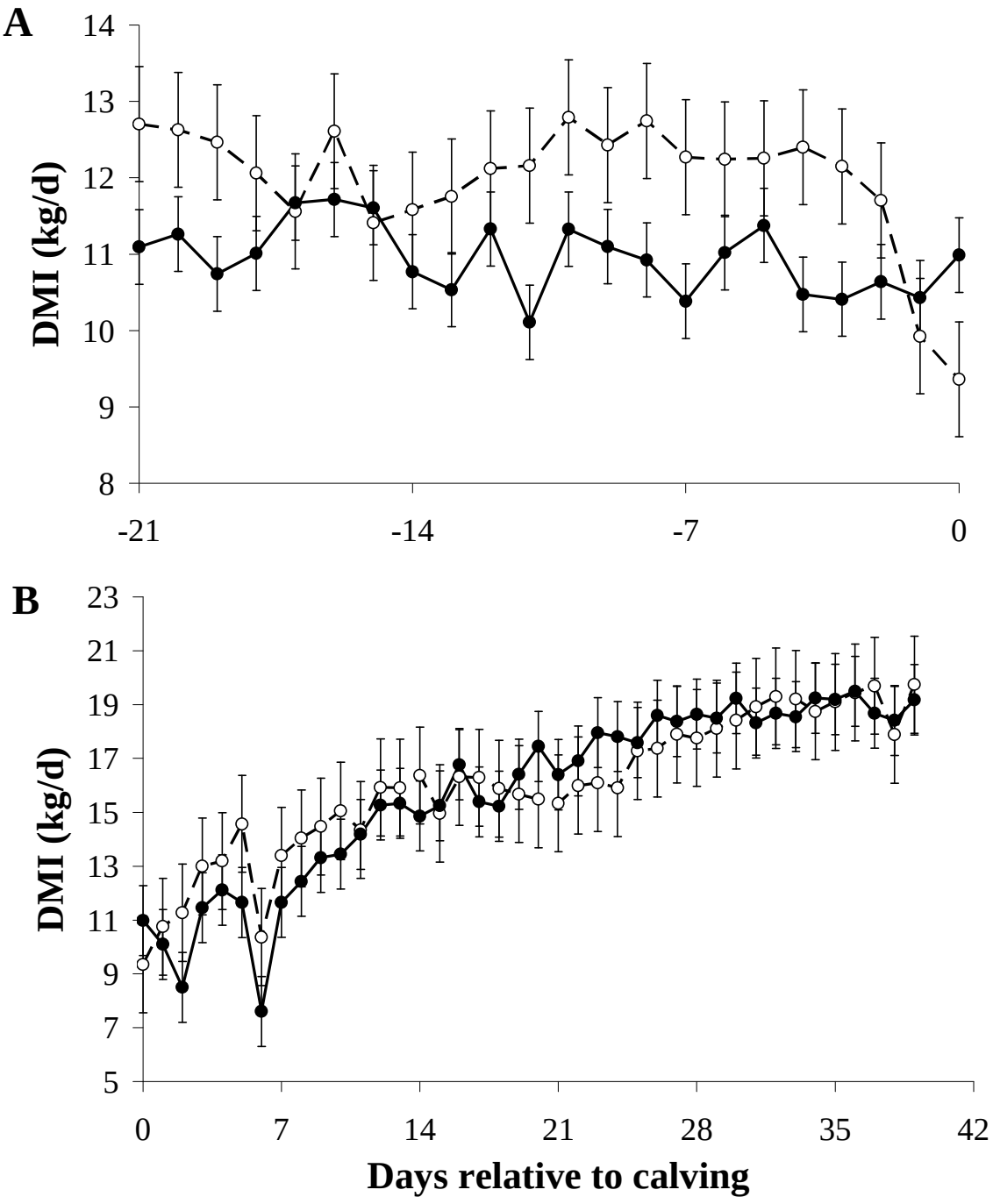

Figure 1 – Mean and SEM prepartum (A) and postpartum (B) dry matter intake in LOW E (●) and HIGH E (○) groups.

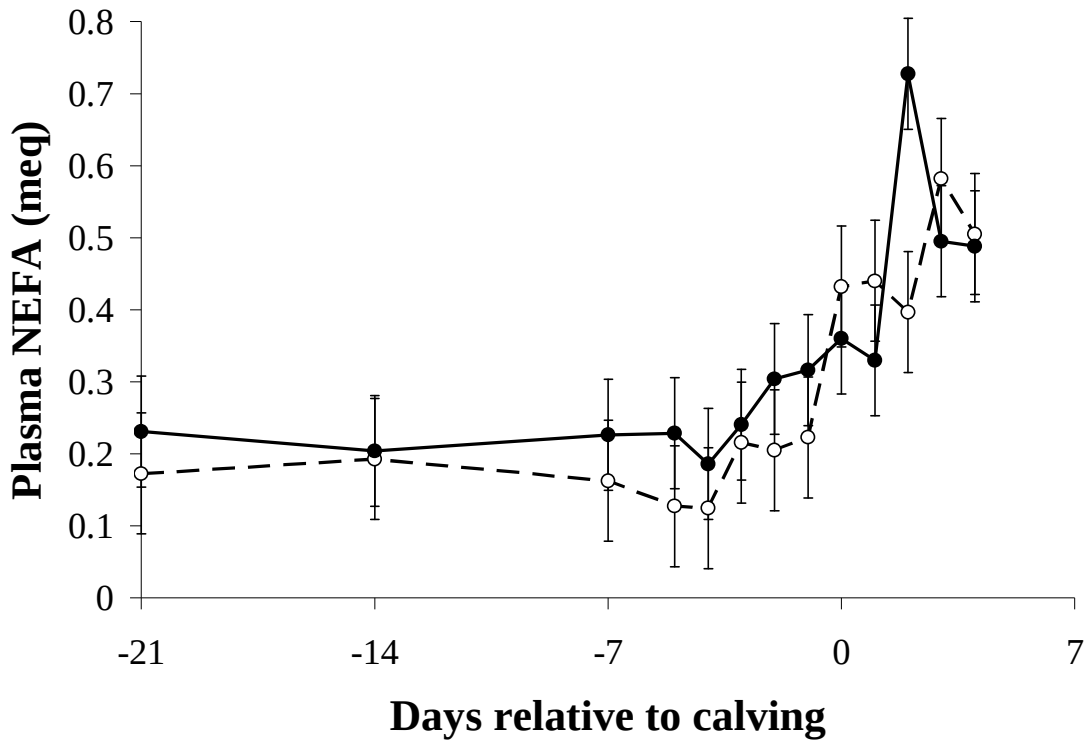

Figure 2 – Mean and SEM concentrations of plasma non-esterified fatty acids in LOW E (●) and HIGH E (○) groups as measured by colorimetric assay.

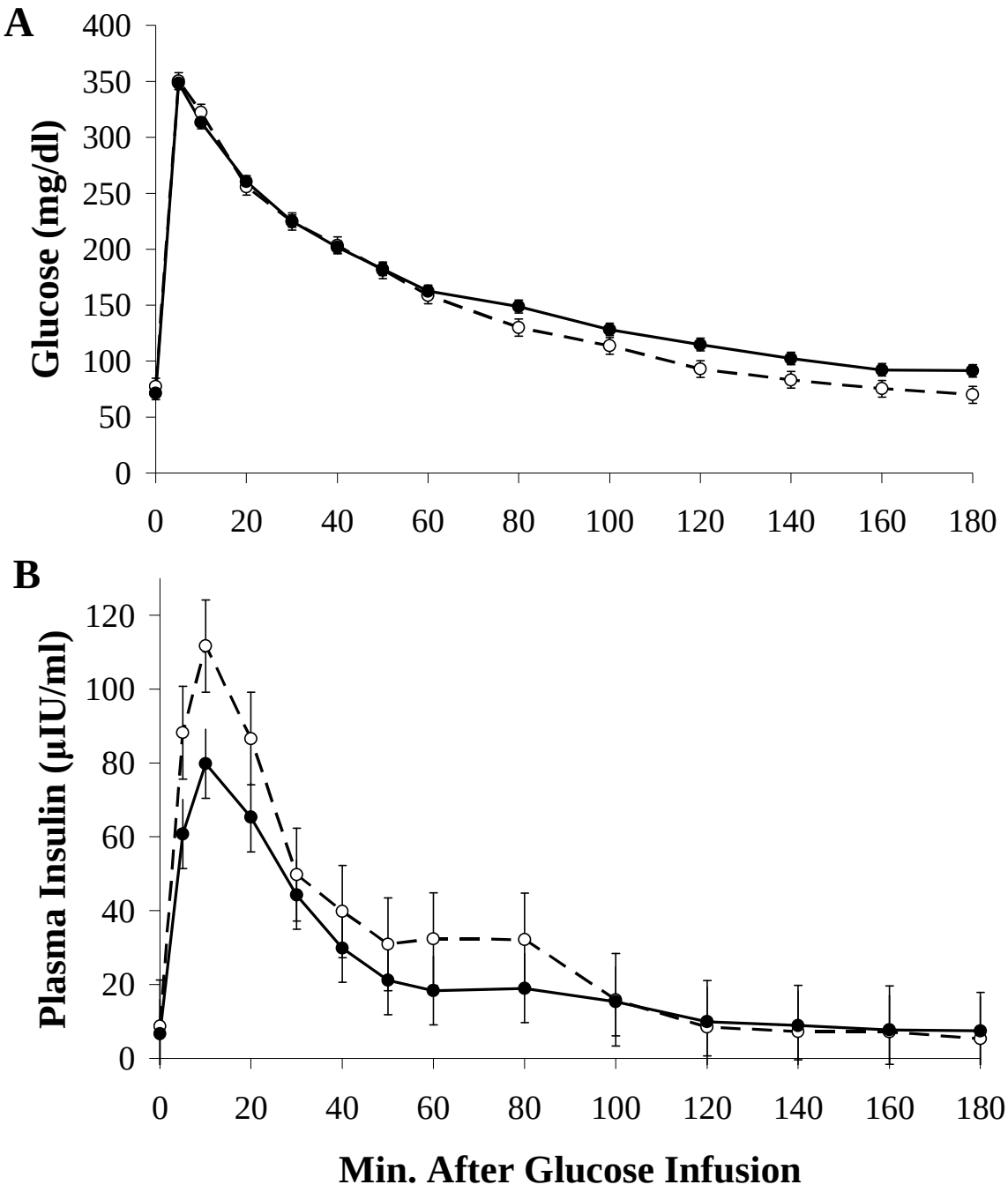

Figure 3 – Mean and SEM concentrations of (a) plasma glucose as measured by colorimetric assay and (b) plasma insulin as measured by radioimmunoassay in LOW E (●) and HIGH E (○) groups following a 50 mg/kg glucose infusion.

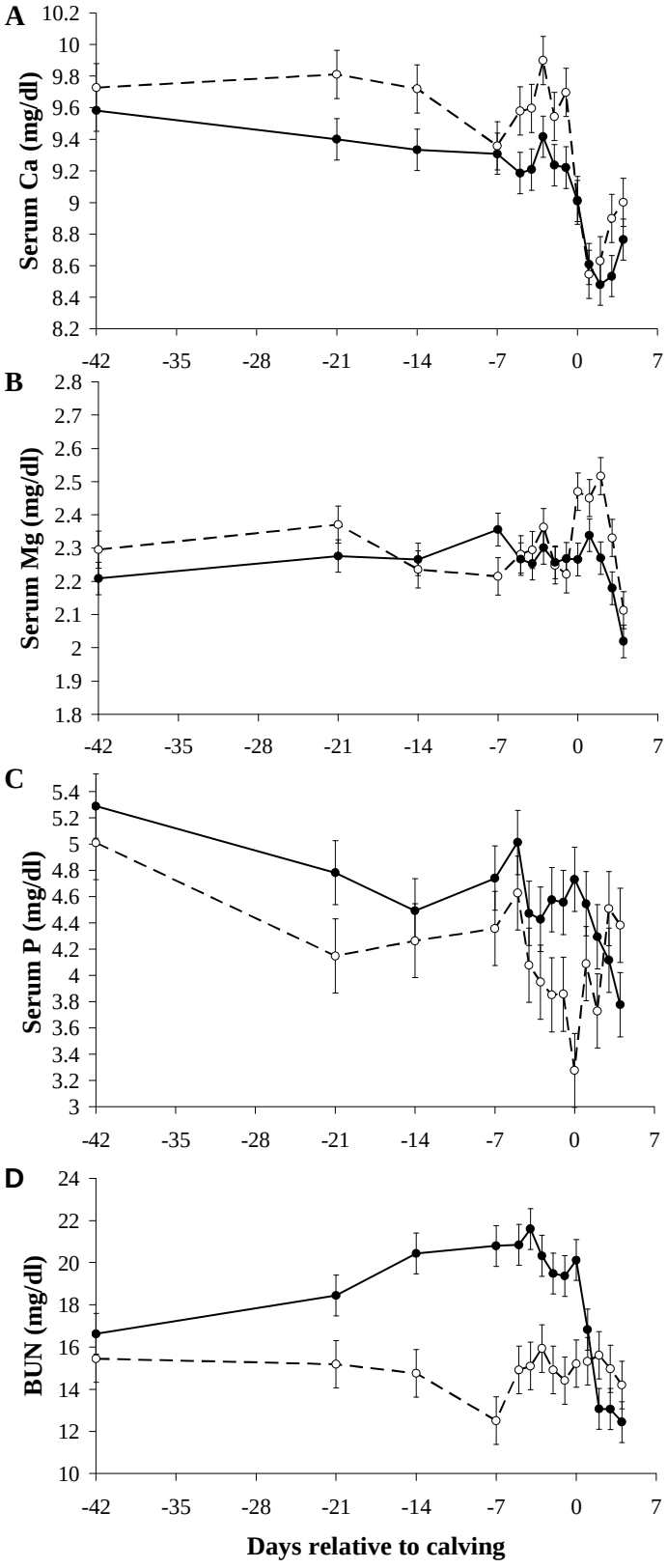

Figure 4 – Mean and SEM concentrations of (A) serum calcium, (B) serum magnesium, and (C) serum phosphorus as determined by atomic absorption, and (D) blood urea nitrogen as determined by colorimetric assay in LOW E (●) and HIGH E (○) groups during the periparturient period.

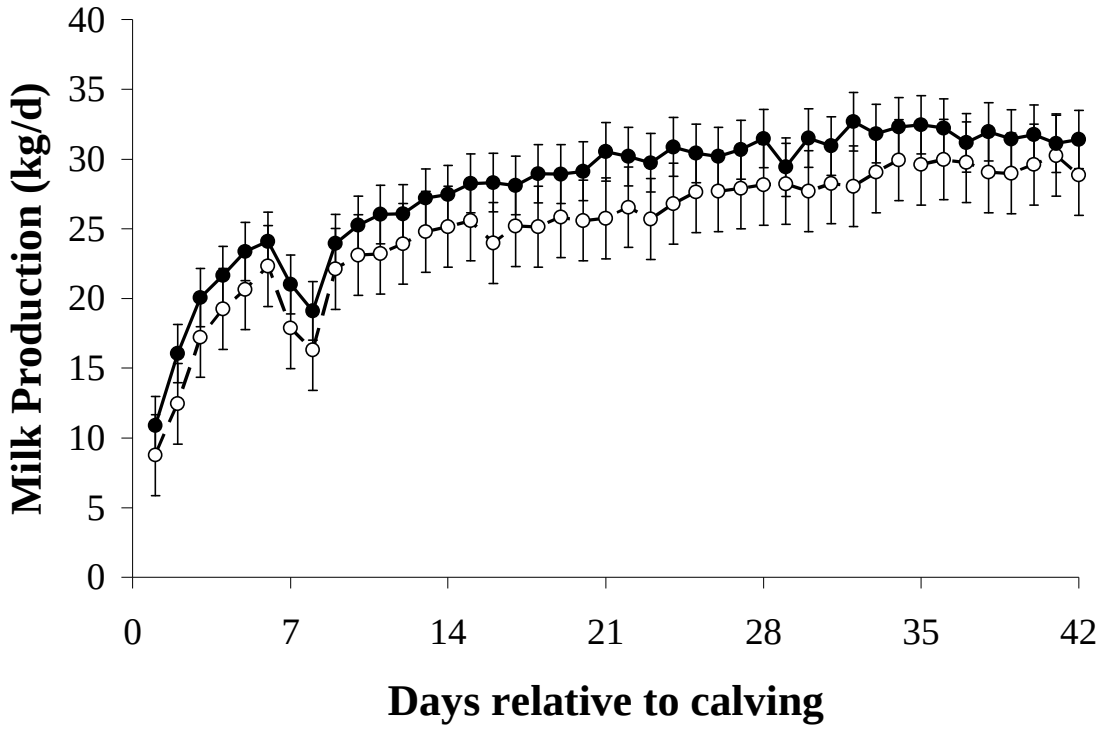

Figure 5 – Mean and SEM of milk production in LOW E (●) and HIGH E (○) groups.

EFFECT OF ENERGY CONTENT OF PRE-PARTUM DIET ON IMMUNE FUNCTION OF PERIPARTURIENT DAIRY COWS AND RESPONSE TO INTRAMAMMARY BACTERIAL CHALLENGE

A paper to be submitted to the *Journal of Dairy Science*

H. R. Springer, D.D. Bannerman, B.J. Nonnecke, and J. P. Goff

INTERPRETIVE SUMMARY

Pre-partum diet energy and metabolic status of heifers. Heifers were fed a HIGH E (1.56 Mcal NE(L) /kg) or LOW E energy (1.35 Mcal NE(L)/kg) diet the last five wk of gestation. Function of neutrophils and peripheral blood mononuclear cells assessed in vitro were not affected by diet. Response to intramammary challenge with a mildly pathogenic Escherichia coli after calving was also not influenced by pre-calving diet. Feeding diets providing more energy than the cow requires for the final weeks of gestation provides no benefit to the immune system of heifers.

ABSTRACT

Nutritional management during the transition period plays an important role in postpartum immune function. Recently, some dairies have moved away from feeding high energy dry cow diets toward feeding a lower, but adequate energy diet. In this study, heifers were fed a HIGH E (1.56 Mcal NE(L) /kg) or LOW E (1.35 Mcal NE(L) /kg) diet the last five weeks of gestation. The objective of this study was to determine if dietary energy differences in the prepartum dairy heifer had an effect on peripartum immune function. In vitro immune function was assessed by neutrophil iodination, differential white blood cell counts, and whole blood interferon-γ production in response to both recall antigens and non-specific stimulation. There was no evidence that prepartal dietary energy impacted the degree

to which these in vitro tests of immune function were suppressed in the transition diary heifer. It is suspected that prepartum energy would have an effect on colostrum quality, but in this study, prepartum dietary energy had no effect on protein and IgG content of colostrum. The competency of the immune system as a whole was assessed by response to intramammary challenge with a mildly pathogenic strain of Escherichia coli. Throughout the course of the challenge, quantitative milk bacterial culture, milk somatic cell count, rectal temperature, differential WBC counts, serum minerals, and acute phase response were assessed and no differences were noted between dietary treatments. All animals were similarly able to recover from the experimentally induced E. coli mastitis. Feeding diets providing more energy than the dairy heifer requires for the final weeks of gestation provides no benefit to the immune system.

INTRODUCTION

Effective management of the transition dairy cow is one of the greatest challenges in the dairy industry. The transition cow faces tremendous metabolic changes as well as impaired immune function, resulting in an increased incidence of both metabolic and infectious disease. Immunosuppression in the transition cow involves impairment of both the innate and adaptive branches of the immune system, with the function of both neutrophils and lymphocytes shown to be impaired during this time (Kehrli et al., 1989a, Kehrli et al., 1989b). Immunosuppression begins prior to calving, as cows developing metritis postpartum have been shown to have reduced immune function prepartum compared to their healthy counterparts (Cai et al., 1994, Hammon et al., 2006, Kim et al., 2005). In addition to increased incidence of infectious disease, immunosuppression, particularly impaired neutrophil and lymphocyte function, have been associated with retained placenta (Gunnink,

1984b, Kimura et al., 2002b). Nutritional management during the transition period likely plays an important role in preventing postpartum infectious disease. Prepartum changes in energy balance and amount of time spent feeding have been associated with an increased incidence of uterine health problems, suggesting a link between nutrition and immune function in the transition cow (Hammon et al., 2006, Urton et al., 2005).

Most recent studies on nutrition in the transition cow have focused on maximizing energy intake by utilizing high energy diets, but retrospective evidence suggests that the degree of depression in DMI at the time of calving, not the absolute energy intake, may play a larger role in improving energy balance in the early postpartum period (Grummer et al., 2004). In this study, we utilize a dry cow diet that meets, but does not exceed the energy needs of the dairy heifer, compared with one that exceeds energy requirements. The goal of the lower energy diet was to minimize DMI depression at the time of calving. The metabolic, production, and reproductive parameters associated with the experimental diets are discussed in a companion paper.

The purpose of this study was to determine if dietary energy differences during the dry period could affect immune function in the transition cow. Immune function was assessed in a variety of ways. The innate branch of the immune system was tested *in vitro* via neutrophil iodination. Impairment of any of a number of steps leading up to neutrophil degranulation results in reduced iodination, allowing this to be an effective screening method for neutrophil dysfunction (Roth and Kaeberle, 1981). Peripheral blood mononuclear cell (PBMC) function was assessed *in vitro* by IFN-γ production in response to recall and non-recall stimulation. In addition to *in vitro* tests of immune function, an *in vivo* challenge model was used to assess the ability of the immune system as a whole to eliminate a bacterial

pathogen. The *in vivo* challenge consisted of the intramammary inoculation of *Escherichia coli* in the early postpartum period. Immune function and time to recovery from infection were assessed throughout the challenge.

MATERIALS AND METHODS

Animals and Experimental Design

Twenty-four Holstein heifers were used to evaluate the effect of two different close-up diets on metabolic and immunologic parameters during the prepartum and early post-fresh periods. Heifers were fed either a low energy density straw based dry cow diet (LOW E) or a traditional, higher energy density dry diet (HIGH E) for the five wks prior to calving (see companion paper). The LOW E diet was designed to meet the energy requirements of the heifers (NRC 2001). The HIGH E diet exceeded the requirements considerably (see companion paper). Three cows on the HIGH E energy diet were removed from the experiment. One aborted prior to the start of the study, one had dystocia and required a Caesarian section, and one had severe udder edema causing severe inflammation of the udder necessitating treatment. All procedures were approved by the Animal Care and Use Committee of the USDA National Animal Disease Center, Ames, IA.

Periparturient Immune Function

Blood samples for immune function were collected weekly from 5-7 weeks prepartum until approximately 2 weeks prepartum, when sampling was increased to twice per week. Following parturition, blood samples were collected on 0 or 1, 4, 14, 21, 28, and 42 DIM. Blood used for determination of neutrophil function was collected by jugular venipuncture into 10% (vol/vol) 2x acid citrate dextrose (77μM sodium citrate, 38μM citric acid, 122μM dextrose). Neutrophil function was assessed by neutrophil iodination, an assay

which quantifies the conversion of inorganic iodide to a trichloracetic acid precipitable, protein bound form following the interaction of hydrogen peroxide produced within the neutrophil phagocytic vacuoles with myeloperoxidase present in primary granules. The iodination reaction occurs primarily within the phagocytic vacuole, but can also occur extracellularly upon release of myeloperoxidase and hydrogen peroxide to the extracellular environment. Neutrophil iodination is assessed in isolated neutrophils following exposure to pre-opsonized zymosan, which consists zymosan that has been pre-treated with bovine serum. In order to successfully iodinate tyrosine and other residues on proteins within the phagocytic vacuole or extracellularly multiple steps within the pathway need to be functioning properly, including phagocytosis of the opsonized zymosan particle, transportation of the phagocytic vacuole within the cell, degranulation of primary granule, normal oxidative metabolism within the neutrophil, and sufficient levels of active myeloperoxidase enzyme. If any of these steps is impaired, it will be reflected by a reduced value for neutrophil iodination; thus this assay is an effective screening method for neutrophil dysfunction (Roth and Kaeberle, 1981).

At 6 wk prior to expected median date of calving, all heifers were immunized with 10^7 colony forming units of attenuated *Mycobacterium bovis* bacillus Calmette-Guerin (BCG) strain Pasteur subcutaneously in the mid-cervical region of the neck to allow later assessment of *in vitro* recall response of peripheral blood mononuclear cells to *M. bovis* purified protein derivative (Foote et al., 2007). A whole blood assay was used to assess the in vitro recall response to BCG vaccination, using purified protein derivative from *M. bovis* (PPDb) (CSL Ltd. Parkville, Victoria, Australia). Pokeweed mitogen (PWM) was also used to assess ability of both B and T –lymphocytes to respond to non-specific stimulation. Blood

samples for these assays were collected into Vacutainer tubes containing sodium heparin (Becton Dickinson, Franklin Lakes, NJ) at -21, -14, -7, 0, 4, 14, 21, 28, and 42 days relative to calving. One ml of whole blood was transferred to each of 4 wells of a 24 well microtiter plate. Each of the following was incubated in duplicate with whole blood: 66 µl of phosphate buffered saline (PBS) to determine baseline IFN-γ production, 66 µl PPDb (20 µg) to determine recall response to an antigen the animals had been previously sensitized to. Plates were gently shaken after addition of PPD, and PBS, and were incubated at 37˚C with 5% CO_2 for 24 hr. A second plate was prepared in a similar manner with each of the following incubated in duplicate with whole blood: 20 µl PBS to determine baseline IFN-γ production or 20µl pokeweed mitogen (1µg/ml) to examine response to a non-specific stimulus. This plate was gently shaken and incubated at 37˚C with 5% CO_2 for 6 hr. After incubation, plates were centrifuged at 1170xg for 20min at 21˚C. Plasma was removed and stored at –20˚C until analysis for IFN-γ production. Plasma IFN-γ was determined using the Bovine IFN gamma Screening Set (Pierce Biotechnology, Rockford, IL). IFN-γ production was determined by subtracting the baseline IFN-γ production (PBS wells) from IFN-γ production in stimulated wells for each respective assay.

At first milking a sample of colostrum was collected and frozen at -20 C until analysis. Volume of first milk was also recorded. Colostrum protein concentration (g / 100 ml) was estimated using a clinical refractometer (American Optical, Corporation, Buffalo, NY) with the colostrum thawed to room temperature, mixed well, and diluted 1:3 in phosphate buffered saline prior to refractometry. Colostrum immunoglobulin G

concentration was estimated by radial immunodiffusion (Bovine IgG RID kit, VMRD, Inc., Pullman, WA), following dilution of the colostrum 1:3 in phosphate buffered saline.

Mastitis Challenge

Mastitis challenge was initiated at the evening milking the fifth day after calving. *Escherichia coli* strain P4, a strain derived from a clinical case of mastitis, was used in the challenge model (Bramley et al., 1976). Bacteria were prepared as previously described (Bannerman et al., 2004) , with the following alterations. Plate counts were determined using spread-plates on blood agar, rather than pour plates using trypticase soy broth. On the day of inoculation, the culture was diluted to 150 cfu/ml based on the plate counts. At the time of infection, 3cc of the bacterial preparation described above was infused into the right front quarter. Cows were infected on different days, so the inoculation dose of *E. coli* was not the same for all cows. The actual infection dose was determined by culturing the inoculant upon return from the barn. The average infectious dose was 746 cfu with a range of 24 to 4100. The wide range of doses was due to laboratory error, but the infused doses were all sufficient to establish infection and even the highest dose is lower than that often used in mastitis challenges (Lohuis et al., 1990, Rambeaud et al., 2003).

Blood and milk samples were collected at the following time points relative to infection: 0h, 12h, 18h, 24h, and every 12 hrs thereafter. Rectal temperatures were also taken on the same schedule. Milk samples for culture were serially diluted and 100µl of each dilution was plated onto blood agar plates (Becton Dickenson, Franklin Lakes, NJ) and were incubated aerobically at 39˚C for 24 hours. When milk cultures in an individual cow failed to detect any *E. coli* at 2 consecutive time points, blood samples and temperature were no longer collected. All sampling stopped after 4 more time points. Blood samples were taken

by jugular venipuncture into serum separator Vacutainers for serum samples, sodium heparin Vacutainers to assess whole blood IFN-γ response and haptoglobin analysis, and into EDTA Vacutainers for white blood cell (WBC) counts (Becton Dickinson). Differential WBC counts were determined using a Hemavet 1500 multi-species hematology system (CDC Technologies/Drew Scientific Inc., Oxford, CT). Milk samples were preserved using bronopol and natamycin (Broad Spectrum Microtabs , D & F Control Systems, Inc., Dublin, CA) and were stored at 4˚C until analysis by an automated cell counter (Bentley Somacount 150, Bentley Instruments, Inc., Chaska, MN).

Serum iron was determined using Iron/TIBC Reagent Set (Pointe Scientific, Inc., Canton, MI). This kit determines total serum iron concentration by the ferrozine reaction after release of Fe from transferrin by acidic pH. Samples were run in triplicate and all volumes were scaled for use in 96 well microtitre plates. Briefly, 100 µl of iron buffer reagent was added to each well, followed by 20 µl of sample. A baseline absorbance was taken at 560 nm to zero the spectrophotometer prior to addition of 20 µl of a 1:8 dilution of acidic color reagent. The plate was incubated at 37˚C for 15 minutes prior to the final absorbance reading at 560nm. Values were determined based on a standard curve run on each plate. Plasma haptoglobin was determined using the Bovine Haptoglobin ELISA Kit (Immunology Consultants Laboratory, Inc., Newberg, OR). Serum calcium and magnesium were determined by atomic absorption spectrophotometry (Perkin-Elmer, 1965)and phosphorus by colorimetric spectrophotometry (Parekh and Jung, 1970).

Statistics

Data were analyzed using a repeated measures ANOVA (PROC MIXED, SAS, NC.) (Littell et al., 1998). The model included the fixed effects of treatment (HIGH E vs. LOW E

Diet), day relative to parturition, and treatment x day interaction, the random effect of cow nested within treatment, and the residual error. For each variable analyzed, cow nested within treatment was subjected to three covariance structures: compound symmetry, autoregressive order 1, and unstructured covariance. The covariance that resulted in the Akaike's information criterion closest to zero was used. Means and SEM are reported for all data. When significant effects ($P < 0.05$) due to treatment, day, or treatment x day interactions were detected, means separation was conducted by the Tukey-Kramer option in SAS. Data obtained during the E. coli mammary gland challenge were analyzed in a separate but similar model, with time relative to E. coli infusion utilized in place of day relative to parturition.

RESULTS AND DISCUSSION

Peripartum Immune Function

Dietary treatment had no significant effects on neutrophil function as assessed by neutrophil iodination (Fig. 1). There was a significant time effect ($p<0.05$), with iodination values at calving falling 45% and 32% for HIGH E and LOW E respectively from a baseline average determined from assays performed during the period from 3 to 6 weeks prepartum. This is a similar decline to that reported in a study by Kimura et al., in which there was approximately a 30% decline in neutrophil iodination over the same time period (Kimura et al., 1999a).

WBC numbers/µl of blood were similar between treatments, with WBC count markedly elevated on the day of calving. Both neutrophil and lymphocyte numbers/ µl were similar between treatments (Fig. 2). Neutrophil numbers/µl showed a dramatic increase on the day of calving, rapidly returning to baseline levels after calving. The pattern of increase in blood WBC and neutrophil numbers / µl is similar to that reported by Kimura, but in that

study, WBC number dropped below baseline immediately postpartum, which was not seen in this study (Kimura et al., 2002a). In addition, the absolute numbers of both WBC and neutrophils were higher in this study than was reported by Kimura (Kimura et al., 2002a). Blood samples from cows in both treatments showed a gradual reduction in lymphocyte counts as parturition approached. There were no significant differences in blood platelet numbers / µl between treatments. Blood platelet numbers postpartum were higher than prepartum.

IFN-γ production in response to PPDb was similar between treatments, with significantly reduced IFN-γ production in response to recall antigens as parturition approached in both groups. PPDb stimulated PBMC production of IFN-γ showed similar declines at the time of calving with a reduction of 71% and 62% for HIGH E and LOW E respectively from the average of values observed during the period from 2 to 3 weeks prepartum (Fig. 3). IFN-γ production by mononuclear cells in response to non-specific PWM stimulation was relatively constant across the peripartum period (Fig. 3). Lymphocyte function has been demonstrated to be impaired during the first week after calving, and based on other studies we had expected IFN-γ production in response to PWM would decline during the transition period (Kehrli et al., 1989a, Nonnecke et al., 2003). This was not noted in this study and may suggest differences in results obtained from whole blood preparations versus isolated lymphocyte culture. It may also reflect inappropriate dosing or time of exposure to PWM in that cells were producing IFN-γ at high levels even with just 6 h incubation with the PWM. There was no evidence that pre-partal dietary energy impacted

the degree to which any of these *in vitro* estimates of PBMC function were suppressed in the transition dairy cow

Nutrition during late gestation is often suspected to determine colostrum quality. Surprisingly, there is little work in dairy cattle upon which to base an opinion. In ewes whose diet intake was restricted to 60% of their requirement or those allowed to consume 140% above their requirements, the total amount of IgG secreted in colostrum was reduced compared to that of ewes fed at their requirements (Swanson et al., 2008). In this study both total energy and protein consumed were affected. In another study of ewes it was found that excessive protein fed in late gestation reduced total IgG secreted in colostrum (Wallace et al., 2006). Other studies have found no effect of dietary protein on colostrum quality (Blecha et al., 1981, Olson et al., 1981). Feeding less energy than required to sows in late gestation reduces protein and IgG concentration of colostrum (Goransson, 1990). In our study the dietary energy content fed to dairy cows in late gestation had no significant effects on protein or IgG concentration of colostrum or the total amounts of protein or IgG secreted in the colostrum produced at first milking (Table 1). In beef cows supplementing the late gestation diet with energy above that of controls also has been observed to have no effect on colostrum IgG content (Dietz et al., 2003, Halliday et al., 1978, Hough et al., 1990). Feeding heifers more energy than they require in late gestation does not confer any advantages in terms of colostrum quality or quantity.

Mastitis Challenge

While *in vitro* tests of immune function have advantages in terms of cost and being non-invasive, ultimately it does not resolve the question of whether the actual resistance to infection was affected by the diet. In this study cows were challenged in one quarter of the

mammary gland with a low number of an E. coli strain that cows can clear without any pharmacologic intervention (Bannerman et al., 2008). There was some variability in the number of bacteria infused in quarters to induce mastitis, but the bacteria colony forming units (CFU) in milk of the infected quarters were not different between treatments, with peak counts at 12h for both treatments (HIGH E= 2.01 X 10^5 and LOW E = 3.26 x 10^5 CFU / ml milk) (Fig. 4). Bacteria CFU in the milk declined from that point on. The time it took to clear the mammary gland of the E. coli, defined as the first time point when two consecutive time points exhibited zero CFU / 100 µl milk, was similar in both groups of cows (HIGH E = 105 $\pm$ 14 h; LOW E = 130 $\pm$ 20 h) (P=0.36) (Table 2). The log SCC of the quarter to be infected was 4.71 ± 0.13 and 4.73 ± 0.14 in the HIGH E and LOW E cows prior to E.coli challenge. SCC increased dramatically in infected quarters of all cows, with log SCC peaking at 24h in the infected quarter of HIGH E cows at 7.88 ± 0.14 SCC/ml and 7.41 ± 0.24 SCC/ ml in LOW E cows (P=0.14) (Fig. 4). The SCC did not return to baseline levels during the 240 hrs that milk SCC was monitored following infection in either group. SCC in the contralateral control quarter was consistently at baseline levels between treatments and across time points after infection. Rectal temperatures were similar between treatments; peaking at 12h post-infection, and returning to normal, defined as the first time point when rectal temp fell below 39˚C following infection, by 38 $\pm$ 4.5 h in HIGH E cows and 48 $\pm$ 7.8 h in LOW E cows (P=0.32) (Fig. 5) (Table 2).

WBC counts were similar between treatments, with counts initially decreasing to a nadir at 18h, followed by a rebound above baseline by 36h (Fig. 6). Neutrophil counts were not different between treatments and followed a pattern similar to WBC counts, decreasing through 18h, then increasing above starting counts by 36h with a gradual return to normal by

120h (Fig. 6). The initial decline in neutrophil counts is likely associated with a massive recruitment of neutrophils to the mammary gland, followed by a rise above baseline as the body produced additional neutrophils in response to the infection. Lymphocyte counts dropped after infection, reaching their lowest value at 18h for both treatments (Fig. 6). Platelet counts were not different between treatments and did not vary across time points after infection.

The acute phase response is mediated by the liver, resulting in increased or decreased production and release of a variety of proteins that are important in the innate immune response. Following an inflammatory stimulus, pro-inflammatory cytokines, particularly TNF-α, IL-1, and IL-6, promote or inhibit production of acute phase proteins by the liver (Jiang et al., 2008). These proteins play various roles in the innate immune system. Serum haptoglobin, represents a positive acute phase protein whose concentration in serum typically increases during inflammation. Haptoglobin binds and sequesters heme iron in the circulation. Increasing circulating haptoglobin concentrations during a bacterial infection may prevent heme iron from becoming available to bacteria. Hepcidin, another important protein in iron homeostasis, has a wider range of actions on iron metabolism during infection. Hepcidin production is upregulated by IL-6 and results in reduced iron absorption from the intestine, as well as internalization of ferroportin, a major iron transport protein, into macrophages, which results in a drop in serum iron concentration (Hugman, 2006). (Wrighting and Andrews, 2006). Iron is transported within the serum bound primarily to transferrin. One mole of transferrin has the ability to bind 2 moles of iron, though typically only 15-30% of iron binding capacity of transferrin is actually utilized. Transferrin is considered a negative acute phase protein- its serum concentration typically decreases during

inflammation. One might suspect that increasing transferrin during times of inflammation would be beneficial as the ability to sequester more free iron might be increased. However, the reduction in transferrin in blood may have the greater role of reducing the total amount of serum iron that might be available for bacterial growth (Bertoni et al., 2008). Serum iron concentration was similar between treatments, with a significant drop in serum iron after infection, reaching a nadir at 36h post-infection (Fig. 7). There were no statistically significant differences in total iron binding capacity of the serum as a result of prepartum diet. Unsaturated iron binding capacity (UIBC) was different between treatments, with HIGHE cows having a higher UIBC. This difference began prior to *E. coli* inoculation, so it may be related to and effect of calving, rather than the infection itself. Haptoglobin began to rise 18h post-infection, and peaked at 60 and 72 hours post infection in HIGH E and LOW E diets respectively (Fig. 7). There were no significant diet effects in the response of haptoglobin concentrations to infection. These results are consistent with previous reports of serum haptoglobin following experimental *E. coli* mastitis (Suojala et al., 2008). The serum level of haptoglobin at the time of the infection was similar in both groups of cows. This also suggests that the acute phase response of the cows to the act of parturition and onset of lactation was not affected by diet. High and sustained levels of haptoglobin following parturition have been found to be indicative of animals that are at risk of increased health problems (Bertoni et al., 2008).

Serum calcium concentration was similar across dietary treatments, with Ca decreasing after infection in both groups, reaching its lowest point at 18h post-infection (Fig. 8). Serum magnesium was similar between treatments and showed a slight decrease from 12-36 h post-infection (Fig. 8). Serum phosphorus decreased shortly after infection, reaching

a nadir at 12 h post-infection. Overall, there was a trend for the serum phosphorus concentration to decrease to a greater extent in the LOW E group. (Fig. 8). Ca levels in serum have been shown to decline during infusion of lipopolysaccharide in cattle (Waldron et al., 2003). Interleukin 1 and perhaps other cytokines elicited by endotoxemia likely play a role as plasma calcium and phosphorus concentrations decrease rapidly in cows following administration of interleukin 1 (Goff et al., 1992).

CONCLUSIONS

Feeding primiparous cows the HIGH E E (1.56 Mcal/kg), providing excess energy or the LOW E providing adequate energy (1.35 Mcal/kg), diets during the last 5 wk of gestation had no effects on in vitro tests of neutophil or peripheral blood mononuclear cell function as calving approached or in the first days of lactation. Neutrophils and PBMC from animals on both diets followed typical patterns of immunosuppression associated with the time of calving. Cows were subjected to an intramammary E.coli challenge on day 5 of lactation and all animals were similarly able to successfully recover from the experimentally induced *E. coli* mastitis. Under the management conditions of this study, dietary treatment did not affect the disease fighting ability of the immune system during the transition period. The movement of the dairy industry away from excessive energy diets during the prepartum period toward the use of lower, but adequate, energy, high straw diets does not appear to have any detrimental effects on immune competence of the primiparous dairy cow.

ACKNOWLEDGMENTS

The authors wish to thank the animal caretakers who provided the daily care and feeding of the experimental animals: Norman Tjelmeland, Creig Caruth, Jerry Grove, and Andrew Mosier. Expert technical assistance in performing the assays of the metabolic

parameters was provided by Amanda Hall, Duane Zimmerman and Derrel Hoy. Adam Ward, Billy Brown, and Lindsey Zaebst assisted throughout the glucose tolerance testing, milking of the cows, monitoring of cows during experimental challenge, blood sampling and conduct of laboratory assays.

Table 1 - Colostrum (1st milk) quality as affected by prepartal diet energy concentration (Mean ± SEM).

	Diet	
	HIGH E	LOW E
Protein concentration (g/100 ml)	14.85 ± 0.33	15.41 ± 0.56
IgG concentration (mg/100 ml)	7250 ± 420	7050 ± 412
Volume first milk (L)	6.31 ± 0.911	7.71 ± 0.64
Total Protein in 1st milk (g)	941 ± 141	1175 ± 89
Total IgG in 1st milk (g)	442 ± 59	543 ± 54

Table 2 – Time following infection of the mammary gland for rectal temperature to return to 102.8 C or less and time following infection when milk samples became free of bacteria, defined as the first time point when two consecutive time points exhibited zero CFU / 100 µl milk

	Diet		
	HIGH E	LOW E	P value
Time to baseline temp. (hrs ± SEM)	38 ± 4.5	48 ± 7.8	0.32
Time to bacterial clearance (hrs ± SEM)	105 ± 14	130 ± 20	0.36

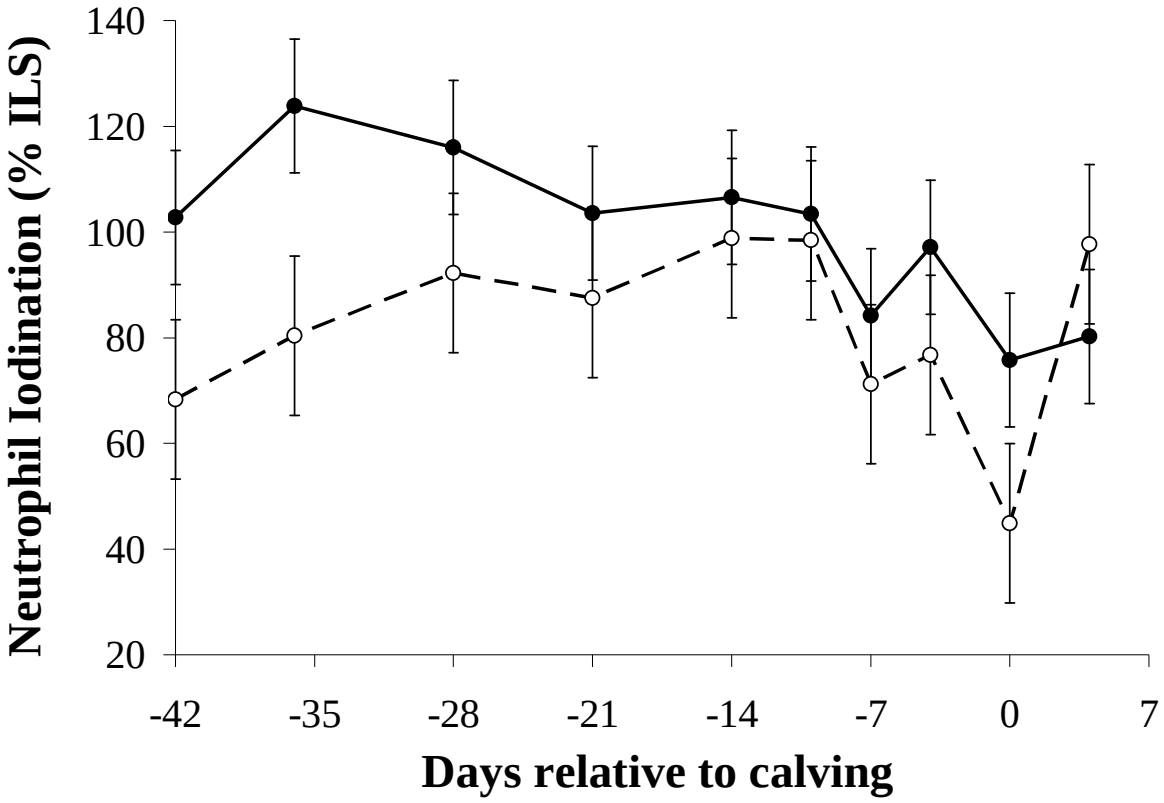

Figure 1 – Mean and SEM neutrophil myeloperoxidase activity in LOW E (●) and HIGH E (○) groups during the periparturient period as determined from incorporation of radioactive inorganic iodide in trichloroacetic acid precipitable protein by neutrophils. Results are expressed as percentage of myeloperoxidase activity in three non-pregnant, non-lactating cows and one steer that served as internal laboratory controls.

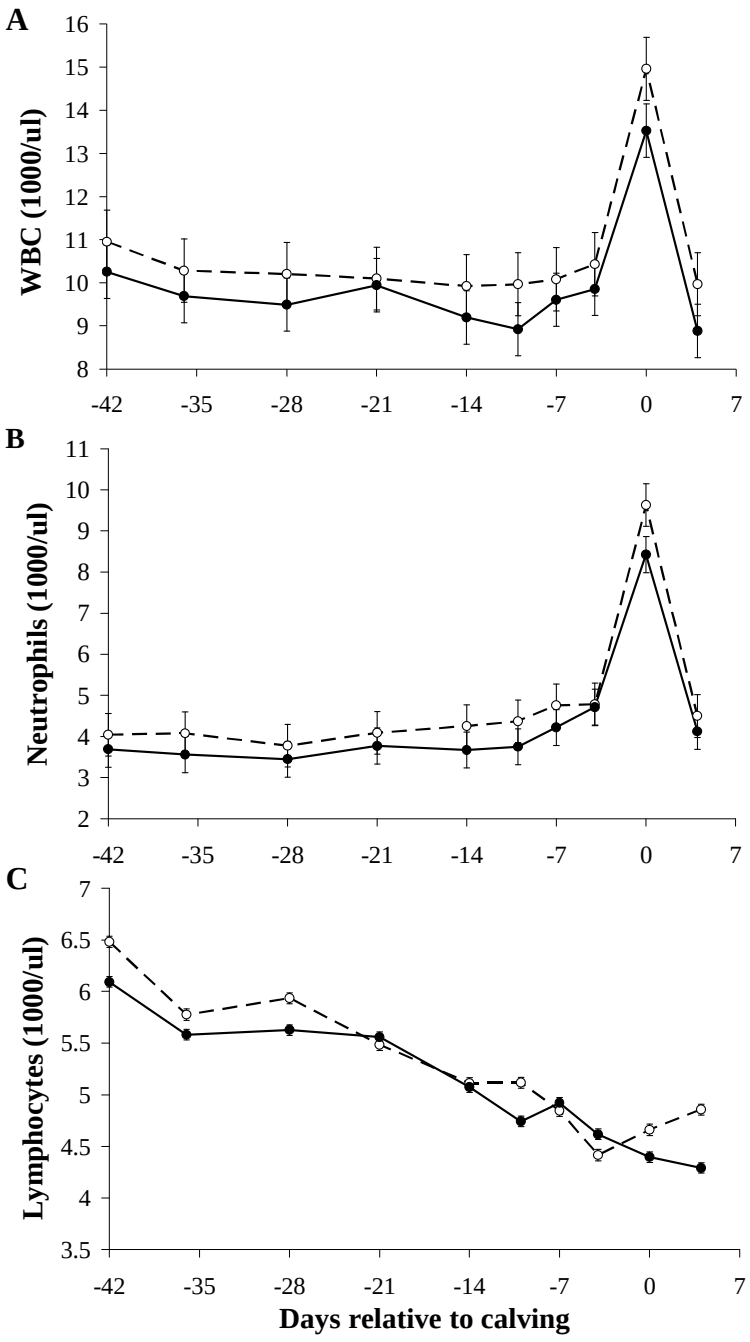

Figure 2 – Mean and SEM (A) white blood cell, (B) neutrophil, and (C) lymphocyte number / µl blood, in LOW E (●) and HIGH E (○) groups during the periparturient period as measured by an automated hematology system.

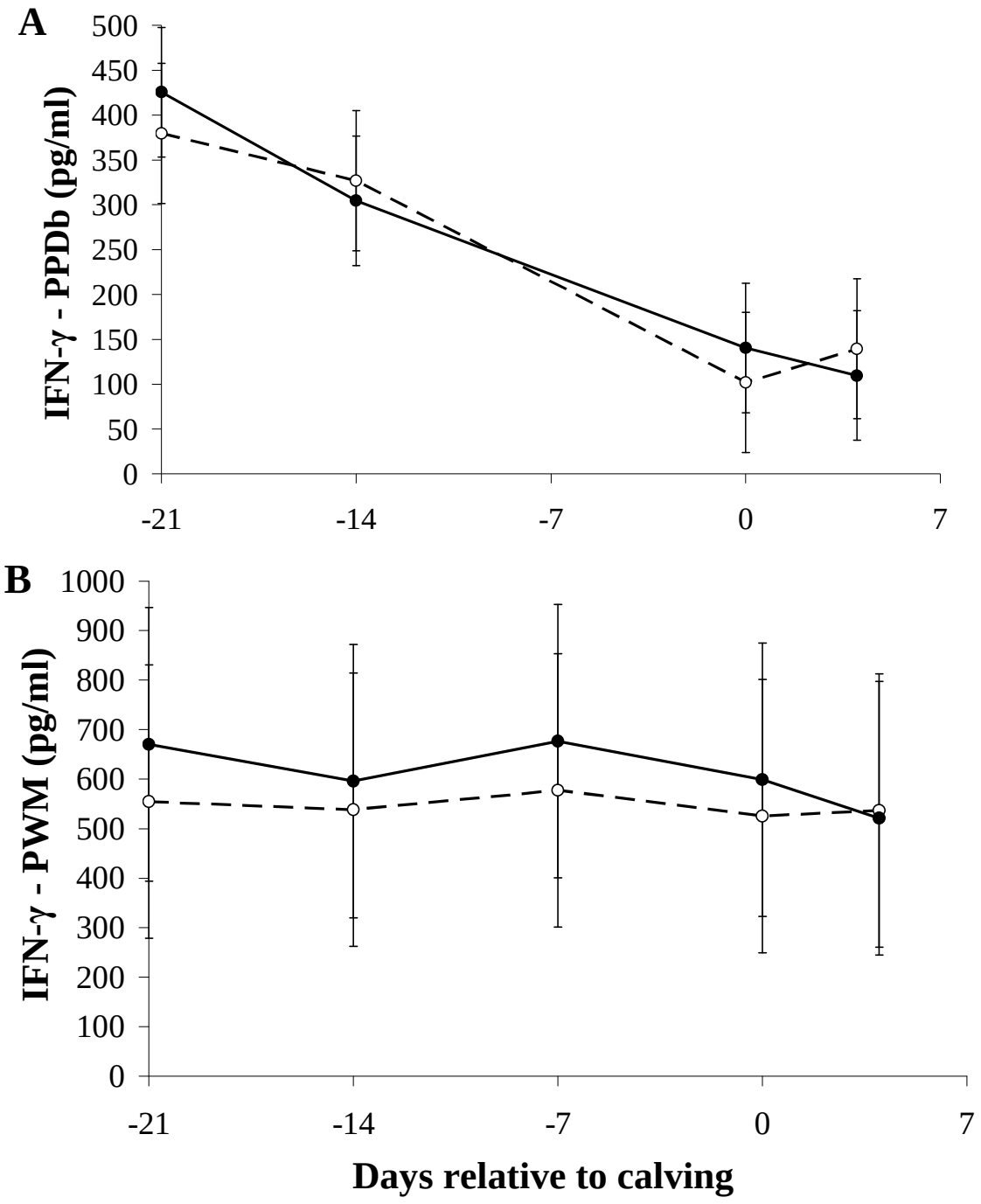

Figure 3 – Mean and SEM whole blood IFN-γ production as measured by ELISA in response to stimulation by (A) Mycobacterium bovis PPD or (B) pokeweed mitogen during the periparturient period in LOW E (●) and HIGH E (○) groups.

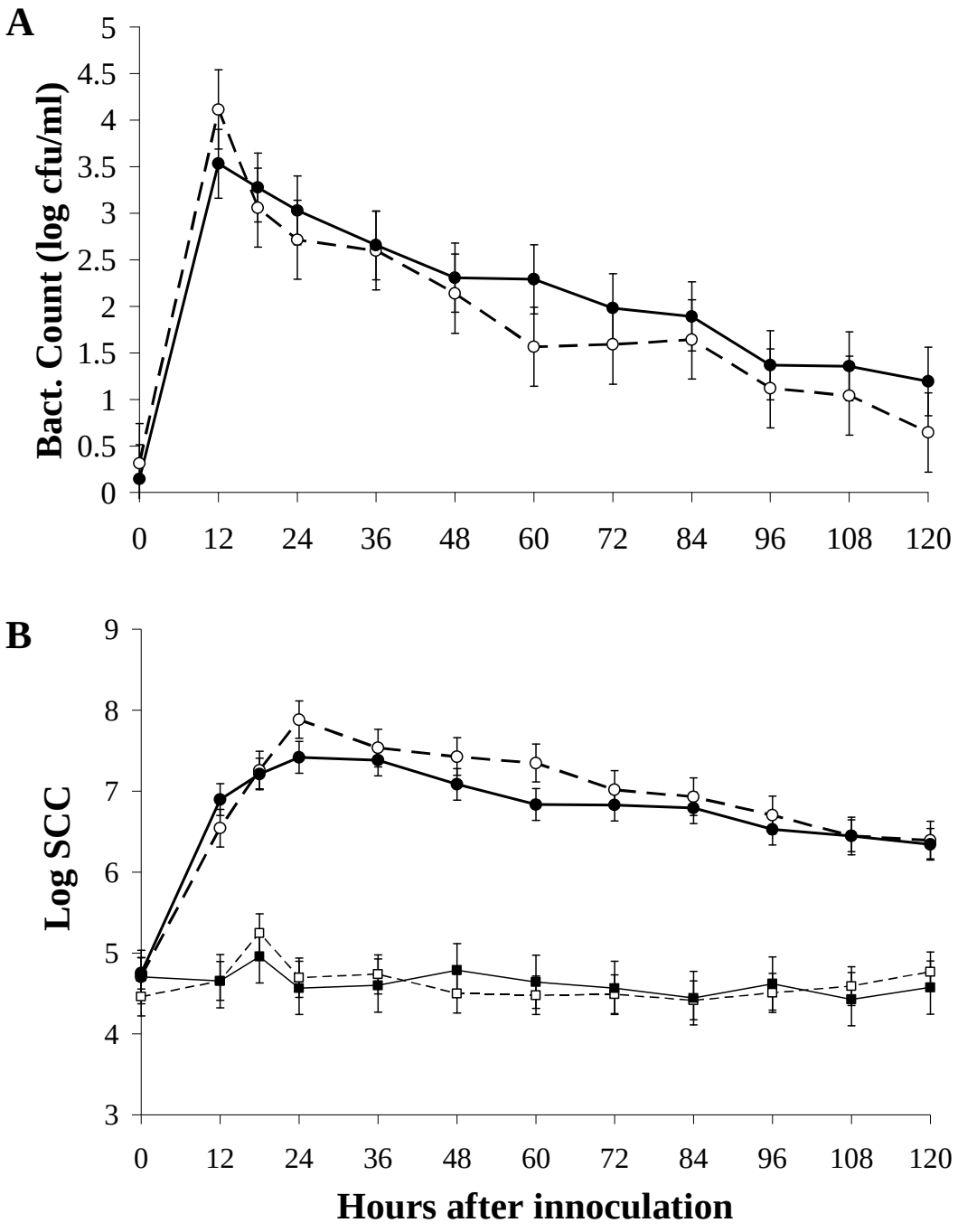

Figure 4 – Mean and SEM (A) log of milk bacterial numbers as determined by quantitative plate count and (B) log of somatic cell count of the front right quarter as determined by an automated cell counter for LOW E (●) and HIGH E (○) groups during the intramammary E. coli challenge. Mean and SEM log of somatic cell counts for the front left quarter is displayed as LOW E (■) and HIGH E (□) groups.

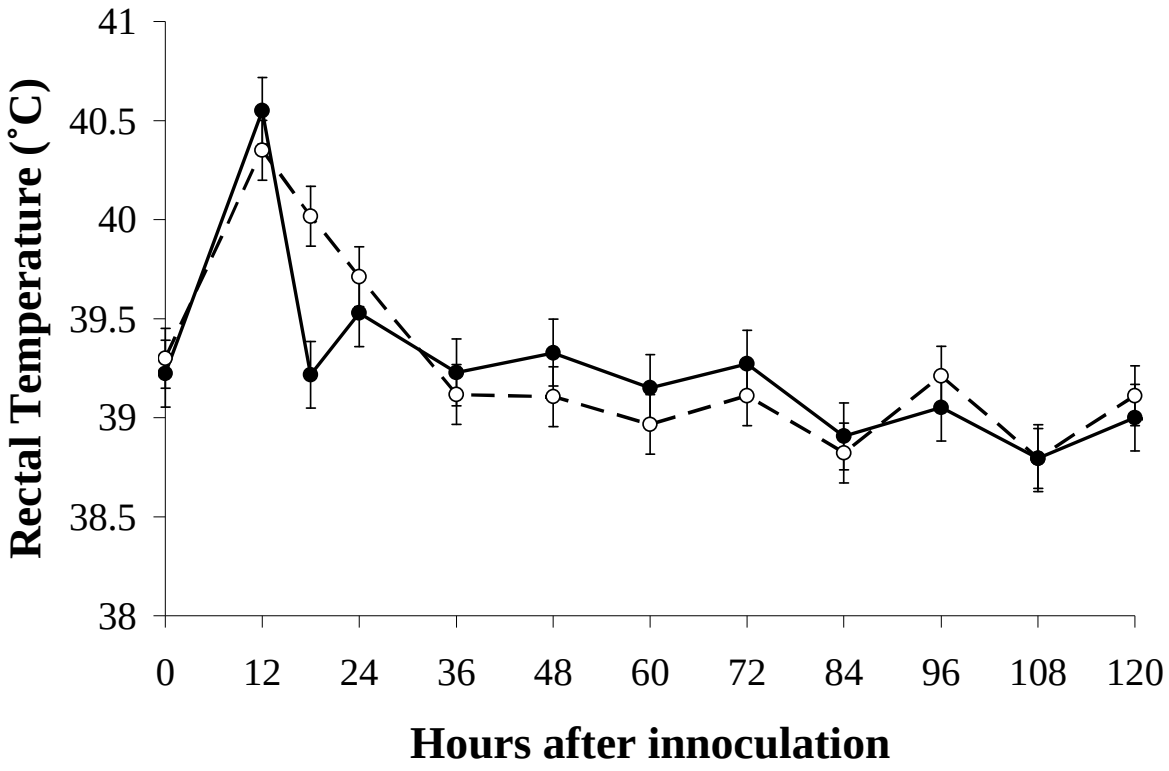

Figure 5 – Mean and SEM rectal temperatures in LOW E (●) and HIGH E (○) groups during the hours following intramammary E. coli challenge.

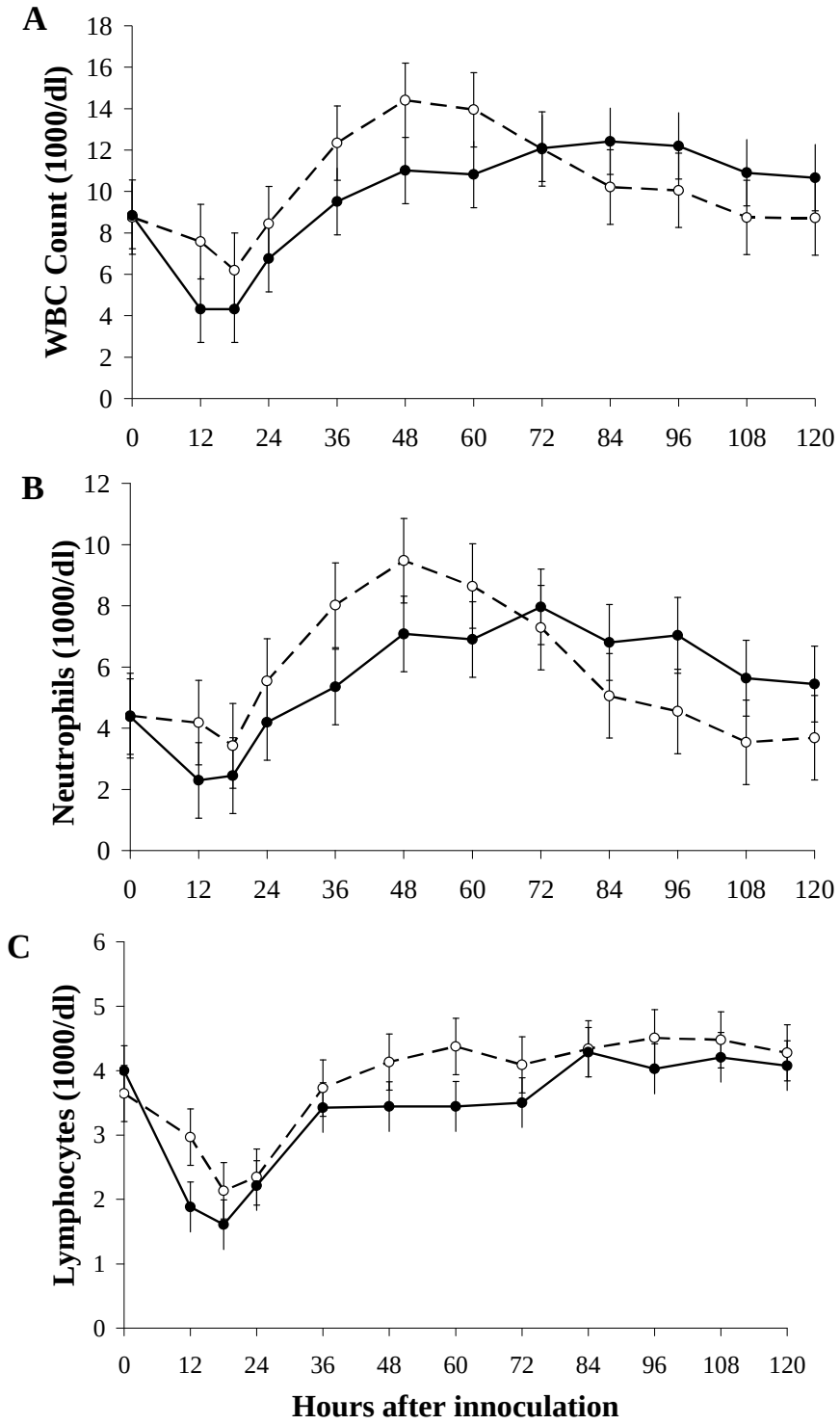

Figure 6 – Mean and SEM (A) white blood cell counts, (B) neutrophil count, and (C) lymphocyte count in LOW E (●) and HIGH E (○) groups during the hours following intramammary E. coli challenge as measured by an automated hematology system.

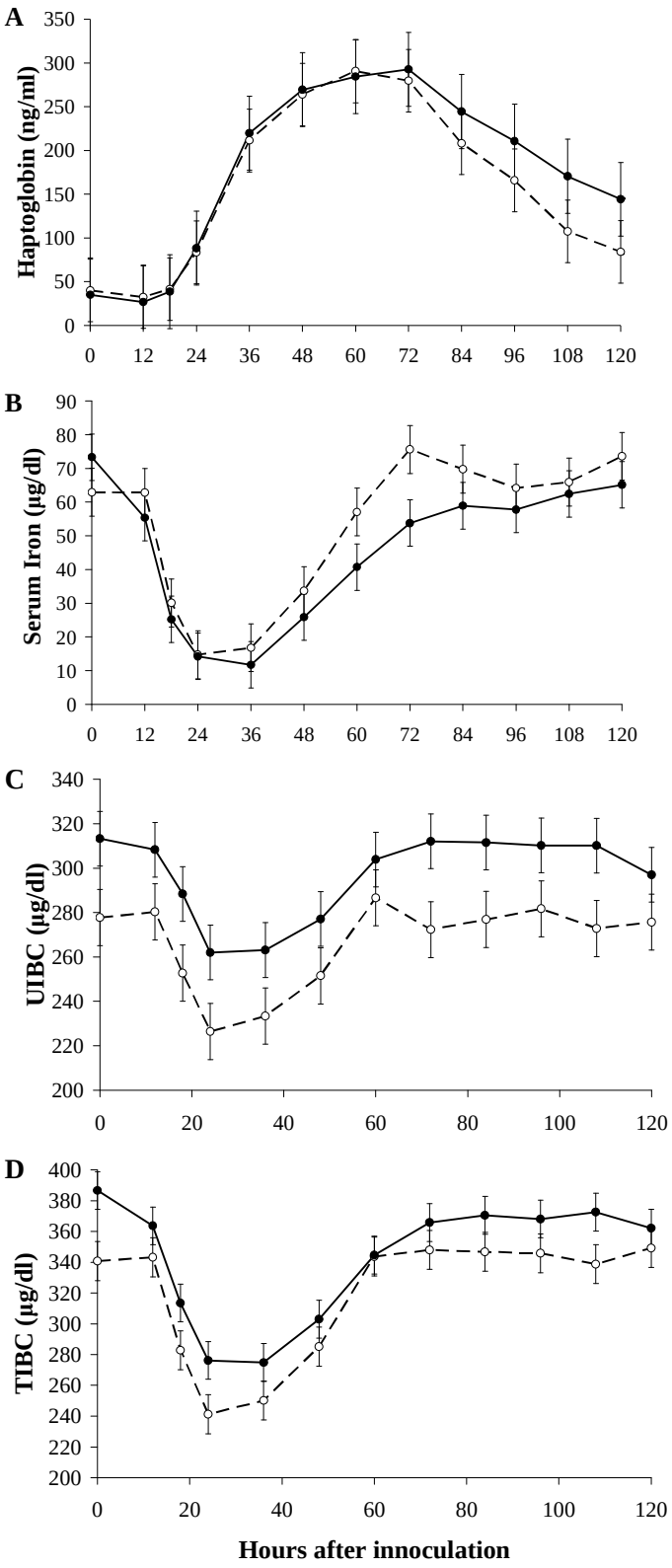

Figure 7 – Mean and SEM (A) plasma haptoglobin concentration as measured by ELISA, (B) serum total Fe concentration, (C) unsaturated iron binding capacity, and (D) total iron binding capacity as measured by colorimetric ferrozine assay, in LOW E (●) and HIGH E (○) groups during the hours following intramammary E. coli challenge.

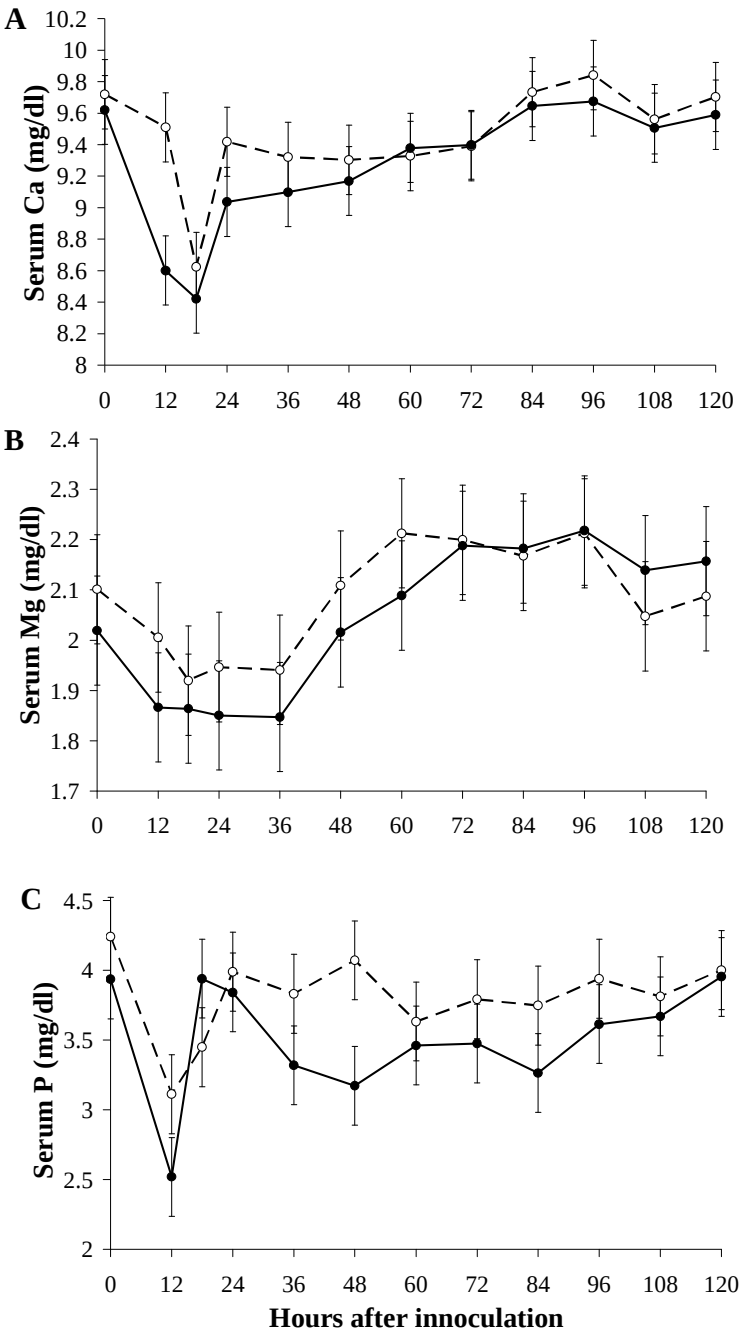

Figure 8 – Mean and SEM (A) serum calcium, (B) serum magnesium, and (C) serum phosphorus concentrations in LOW E (●) and HIGH E (○) during the hours following intramammary E. coli challenge as measured by atomic absorption.

GENERAL DISCUSSION

Effects of Diet on DMI and Metabolic Parameters Pre-Partum

Increasing the energy density of the pre-partum diets will generally increase DMI across the last three wks of gestation (Emery et al., 1969, Holcomb et al., 2001, Minor et al., 1998, Rabelo et al., 2003, Vandehaar et al., 1999). Our results were consistent with these studies. The HIGH E cows consumed 19.0 Mcal NE(L)/ day while cows fed the LOW E diet consumed 14.9 Mcal NE(L) / d. The energy requirement for these cows is approximately 12 Mcal NE(L) / d (2001), so cows on the LOW E diet still received energy beyond their requirement.

DMI decreased in both groups of cows during the last week of gestation. However, the cows fed the HIGH E diet had a significant reduction of 2.2 kg DMI/ d or 18% in the last 3 days of gestation, while LOW E cows maintained their intake through this period. The depressed DMI during the final days of gestation observed in the cows fed the HIGH E diet parallels the pattern of DMI seen in many previous studies (Bertics et al., 1992, Grummer et al., 2004, Hayirli et al., 2002, Marquardt et al., 1977). Grummer et al., (Grummer et al., 2004) suggested that the degree of depression in DMI at the time of calving may have a more significant effect on postpartum performance than the level of DMI itself. The same authors went on to suggest that higher pre-partum DMI may actually promote a greater magnitude of DMI depression when compared to cows with lower pre-partum DMI. They also suggest the delta change in DMI during the final days of gestation may act as a stimulus for the mobilization of fat from adipose tissue. Our data support this argument. The HIGH E group had both higher pre-partum DMI and a greater degree of DMI depression at the time of calving, even though they were consuming more energy the final day of gestation than the

LOW E cows on that day (15.4 Mcal vs 13.9 Mcal NE(L)). And, at least on the day of calving, the cows fed the HIGH E diet had higher NEFA concentrations in their blood than the LOW E cows. It appears that the trigger for body fat mobilization has less to do with absolute energy intake than it does with intake relative to the previous day or days intake of energy.

Elevated blood NEFA concentrations during this period may be predictive of cows with increased risk of developing displaced abomasum (Cameron et al., 1998, LeBlanc et al., 2005) and retained placenta (Conner et al., 2001), and increased risk of being culled in early lactation (Duffield, 2007). In the Minor et al., (Minor et al., 1998), Rabelo et al., (Rabelo et al., 2003) and VandeHaar et al., (Vandehaar et al., 1999) studies, increasing the energy content of the pre-partum diet reduced blood NEFA concentrations prior to calving. Plasma NEFA concentrations of the HIGH E cows between -22 d and -4 d (0.15 + 0.02 M) were lower ($P < 0.05$) than in LOW E cows during this period (0.21 + 0.02 M) ($P < 0.02$), but in both groups of cows the NEFA concentrations were well below the suggested close-up cow critical NEFA level of 0.4 -0.5 M that would predict cows “at-risk” for metabolic disorders (Duffield, 2007, Van Saun, 2006). NEFA concentrations rose rapidly the day of calving in both groups. This represented a 3-fold NEFA concentration increase in HIGH E cows and a 1.7-fold increase in LOW E cows from the average value observed across the period from 3 wks to 3 days prior to calving.

The glucose tolerance test results show a similar zenith in blood glucose concentration was achieved in both groups of cows at the cessation of glucose infusion. Reduced tissue sensitivity to insulin has been demonstrated in cows with high BCS and is suspected to play a role in susceptibility of the transition cow to metabolic disease (Holtenius

and Holtenius, 2007, Rukkwamsuk et al., 1998). Overfeeding dairy cows during the pre-partum period may accentuate insulin resistance in adipose tissue, leading to increased NEFA mobilization, lower DMI, and greater risk for lipid-related metabolic disorders (Holtenius et al., 2003). Our data do not support the theory that dietary energy fed to the cow can affect insulin sensitivity of the tissues, perhaps because BCS was similar in the two groups of cows at calving or perhaps we did not have enough animals in the study.

Plasma BUN concentration was significantly higher in LOW E cows prior to calving than in HIGH E cows ($P<0.05$). Since dietary protein concentrations were comparable and DMI was higher in the HIGH E cows, the LOW E cows might be expected to have lower BUN concentration as the total nitrogen entering the rumen should have been lower. However, a likely explanation is that the rumen degradable protein nitrogen entering the rumen of the LOW E cows is not incorporated into microbial protein very well because of the lack of starch or other readily fermentable carbon sources in the rumen. This nitrogen enters the blood as ammonia and is converted to urea in the liver, raising BUN. The NRC (2001) model predicted that these diets would supply similar amounts of metabolizable protein to the cows, assuming similar DMI. However, since BUN levels are higher in the LOW E cows these data suggest the NRC model overestimates the contribution of the LOW E diets to metabolizable protein. It may be necessary to provide more dietary rumen undegradable protein to meet the metabolizable protein requirements of the dry cow fed a LOW E diet. There were no apparent ill-effects from this reduced availability of metabolizable protein on the LOW E diet.

Effects of Diet on DMI and Metabolic Parameters Post-Calving

DMI increased after calving in cows in both diet treatments at a similar rate. The intramammary *E.coli* challenge affected both groups of cows to a similar degree and the rate of recovery of DMI after challenge was similar. Plasma NEFA concentration increased slightly above calving levels in both groups the first two days of lactation but were on the decline at the time of the *E.coli* intramammary challenge. The combination of transition to a new diet, liver biopsy, and initiation of milk production likely contributed to the rise in NEFA in both groups after calving, though the average remained well below levels that would be considered indicative of severe negative energy balance in both groups of cows.

The triglyceride content of the liver, determined in the biopsies obtained the day after calving, was similar in both groups of cows. In some studies, increasing the energy content of the pre-partum diet also increased glycogen and/or reduced the triglyceride levels in the liver around the time of calving, factors which should have reduced the risk of development of the fatty liver ketosis complex (Doepel et al., 2002, Minor et al., 1998, Vandehaar et al., 1999). In our study, liver triglycerides at calving were relatively low in all cows and there was no effect of diet, consistent with the findings of Rabelo et al., (Rabelo et al., 2005). Plasma BHBA concentrations at 4 DIM were similar across dietary treatments. Examining BHBA at time points between 7 and 14 DIM is generally felt to be more informative of the degree of negative energy balance experienced by the cow; however the intra-mammary challenge with *E.coli* was felt to be a factor that would render interpretation of BHBA at these later DIM meaningless. Plasma BUN concentrations were similar in cows fed the HIGH E and LOW E diets during the lactation phase of the study, when they were fed the same diets.

Milk production was similar in both groups of cows (Figure 6). There was no effect of diet on days to first ovulation, which was 24 and 30 DIM for the HIGH E and LOW E cows respectively (P= 0.27).

Plasma concentrations of α-tocopherol, retinol, and β-carotene were not statistically different across the dietary treatments. All three compounds exhibited a significant decline around the time of calving followed by a recovery over the first two weeks of lactation, as reported in other studies (Goff and Stabel, 1990, Weiss et al., 1990). Similarly, plasma Ca and Mg concentrations did not differ across dietary treatments though there was a significant sub-clinical decline in plasma Ca concentration in the first few days of lactation in both groups. Plasma P concentration also declined in both groups of cows on the first few days of lactation, and the decline was greater in cows fed the LOW E diet ($P < 0.05$). The mineral levels were not low enough to cause noticeable clinical symptoms.

Several research groups report maximizing DMI or energy intake during the close-up period improves post-partum intake and performance (Dann et al., 1999, Doepel et al., 2002, Mashek and Beede, 2000, Minor et al., 1998, Rabelo et al., 2003, Vandehaar et al., 1999). In contrast, several studies provide evidence that restricting energy intake during either the entire dry period (Agenas et al., 2003, Douglas et al., 2006, Holtenius et al., 2003, Tesfa et al., 1999) or the close-up period (Holcomb et al., 2001) to levels at or even below NRC requirements (2001) has no negative impact on postpartum DMI. Our results also suggest that higher DMI pre-partum, which was associated with the higher energy diet, does not lead to greater DMI in early lactation.

Effect of Diet on Peripartum Immune Function

Dietary treatment had no significant effects on neutrophil function as assessed by neutrophil iodination. There was a significant time effect ($p<0.05$), with iodination values falling 45% and 32% for HIGH E and LOW E respectively between the prepartum period (an average over the period from 3 to 6 weeks prepartum) and the day of calving. This is a similar decline to that reported in a study by Kimura et al., in which there was approximately a 30% decline in neutrophil iodination over the same time period (1999a).

WBC counts were similar between treatments, with counts markedly elevated on the day of calving. Both neutrophil and lymphocyte counts were similar between treatments. Neutrophil counts showed a dramatic increase on the day of calving, rapidly returning to baseline levels after calving. The pattern of increase in WBC and neutrophil counts is similar to that reported by Kimura, but in that study, WBC counts dropped below baseline immediately postpartum, which was not seen in this study (2002a). In addition, the absolute numbers of both WBC and neutrophils were higher in this study than was reported by Kimura (2002a). Both treatments showed a gradual reduction in lymphocyte counts as parturition approached. There were no significant differences in platelet count between treatments, with higher platelet counts postpartum compared to prepartum values.

IFN-γ production in response to PPDb was similar between treatments, with significantly reduced IFN-γ production in response to recall antigens as parturition approached in both groups. PPDb stimulated PBMC production of IFN-γ showed similar declines at the time of calving with a reduction of 71% and 62% for HIGH E and LOW E respectively between 2 to 3 weeks prepartum and the day of calving. IFN-γ production by mononuclear cells in response to non-specific PWM stimulation showed no change across

the peripartum period. Lymphocyte function has been demonstrated to be impaired during the first week after calving, thus it would be expected that IFN-γ production in response to PWM would decline during the transition period (Kehrli et al., 1989a). This was not noted in this study and may suggest an improvement in immune function in periparturient heifers over the older cows studied by Kehrli (Kehrli et al., 1989a). There was no evidence that pre-partal dietary energy impacted the degree to which these *in vitro* tests of immune function were suppressed in the transition dairy cow

Nutrition during late gestation is often suspected to determine colostrum quality. Surprisingly, there is little work in dairy cattle upon which to base an opinion. In ewes whose diet intake was restricted to 60% of their requirement or those allowed to consume 140% above their requirements, the total amount of IgG secreted in colostrum was reduced compared to that of ewes fed at their requirements (Swanson et al., 2008). In this study both total energy and protein consumed were affected. In another study of ewes it was found that excessive protein fed in late gestation reduced total IgG secreted in colostrum (Wallace et al., 2006). Other studies have found no effect of dietary protein on colostrum quality (Blecha et al., 1981, Olson et al., 1981). Feeding less energy than required to sows in late gestation reduces protein and IgG concentration of colostrum (Goransson, 1990). In our study the dietary energy content fed to dairy cows in late gestation had no significant effects on protein or IgG concentration of colostrum or the total amounts of protein or IgG secreted in the colostrum produced at first milking. In beef cows supplementing the late gestation diet with energy above that of controls also has been observed to have no effect on colostrum IgG content (Dietz et al., 2003, Halliday et al., 1978, Hough et al., 1990). Feeding heifers more

energy than they require in late gestation does not confer any advantages in terms of colostrum quality or quantity.

Effect of Diet on Mastitis Challenge

While *in vitro* tests of immune function have advantages in terms of cost and being non-invasive, ultimately it does not resolve the question of whether the actual resistance to infection was affected by the diet. In this study cows were challenged in one quarter of the mammary gland with a low number of an E. coli strain that cows can clear without any pharmacologic intervention (Bannerman et al., 2008). The time it took to clear the mammary gland of the E. coli, defined as the first time point when two consecutive time points exhibited zero CFU / 100 µl milk, was similar in both groups of cows. SCC increased dramatically in infected quarters of all cows, with log SCC peaking at 24h in the infected quarter. The SCC did not return to baseline levels during the 240 hrs that milk SCC was monitored following infection in either group. SCC in the contralateral control quarter was consistently at baseline levels between treatments and across time points after infection. Rectal temperatures were similar between treatments.

WBC counts were similar between treatments, with counts initially decreasing to a nadir at 18h, followed by a rebound above baseline by 36h. Neutrophil counts were not different between treatments and followed a pattern similar to WBC counts, decreasing through 18h, then increasing above starting counts by 36h with a gradual return to normal by 120h. The initial decline in neutrophil counts is likely associated with a massive recruitment of neutrophils to the mammary gland, followed by a rise above baseline as the body produced additional neutrophils in response to the infection. Lymphocyte counts dropped after infection, reaching their lowest value at 18h for both treatments. Platelet counts were not

different between treatments and did not vary across time points after infection. The acute phase response is mediated by the liver, resulting in production of a variety of proteins that are important in the innate immune response. Serum haptoglobin, an acute phase protein, is assessed as a direct measurement of the acute phase response. Serum Fe was measured as an indirect indicator of acute phase response. Following an inflammatory stimulus, pro-inflammatory cytokines, particularly IL-6, promote production of acute phase proteins by the liver. These proteins play various roles in the innate immune system. The function of both haptoglobin and hepcidin are to limit iron availability to microorganisms. Haptoglobin does so by directly binding and sequestering free iron in circulation. Hepcidin, an important protein in iron homeostasis, has a wider range of actions on iron metabolism during infection. Hepcidin production is upregulated by IL-6, which results in reduced iron absorption from the intestine, as well as internalization of ferroportin, a major iron transport protein, into macrophages, which results in a drop in serum iron concentration (Hugman, 2006). Hepcidin production has been linked to the anemia of inflammation associated with chronic inflammatory diseases (Wrighting and Andrews, 2006).

Serum iron concentration was similar between treatments, with a significant drop in serum iron after infection, reaching a nadir at 36h post-infection. Haptoglobin began to rise 18h post-infection, and peaked at 60 and 72 hours post infection in HIGH E and LOW E diets respectively. There were no significant differences in haptoglobin levels between treatments. These results are consistent with previous reports of serum haptoglobin following experimental *E. coli* mastitis (Suojala et al., 2008).

Serum calcium concentration was similar across dietary treatments, with Ca decreasing after infection in both groups, reaching its lowest point at 18h post-infection.

Serum magnesium was similar between treatments and showed a slight decrease from 12-36 h post-infection. Serum phosphorus decreased shortly after infection, reaching a nadir at 12 h post-infection. Overall, there was a trend for the serum phosphorus concentration to decrease to a greater extent in the LOW E group. Ca levels in serum have been shown to decline during infusion of lipopolysaccharide in cattle (Waldron et al., 2003). Interleukin 1 and perhaps other cytokines elicited by endotoxemia likely play a role as plasma calcium and phosphorus concentrations decrease rapidly in cows following administration of interleukin 1 (Goff et al., 1992).

Management Factors

The two diets had similarly successful outcomes postpartum. The management conditions utilized in this trial may have contributed. In this study, heifers were housed in an un-crowded barn (about 1.2 free stalls per heifer), and were given sole access to their respective diets via Calan gates which provided 0.82 M bunk space / cow. On commercial dairies the cows rarely have this much space. Increased stocking density is associated with an increased number of displacements from the feed bunk (Huzzey et al., 2006), reduced feed intake (Grant, 2007), and reduced ability to rest comfortably (Krawczel et al., 2008). Age, height, and weight have been positively correlated with increasing social standing, thus, heifers are more likely to be of lower social standing in mixed parity groups (Arave and Albright, 1976). Lower social standing has been associated with more frequent displacement from post-and-rail feed bunks (Huzzey et al., 2006). In this study the heifers were housed away from cows on the dairy, avoiding another common stressor on commercial dairies. Movement of dairy cattle into different pens or groups of cows during the transition period is a common practice. Altered social group has been associated with an increased number of

displacements from the feed bunk and decreased feeding time (von Keyserlingk et al., 2008). Throughout the duration of this study, there were no changes in social groups. The management conditions of this study eliminated many of the social and behavioral challenges the heifer can face under typical management conditions.

Both low and high energy density diets were successful under the conditions of this study. The decision to implement a low or high energy pre-partum diet must take into account the specific management practices of the dairy and how these will affect feed availability for heifers. It is something of a misnomer to refer to the straw based diet utilized in this study as a low energy diet. It did provide adequate energy to the cows. Providing adequate, but not excessive energy, in late gestation has been instituted successfully on dairies and has been reported to improve health of the cows when compared to the higher energy pre-partum diets (Beever, 2006, Drackley and Janovick Guretzky, 2007). Further studies should focus on the interaction between diet energy and health in older cows and under typical commercial conditions, where over-crowding and cow movement in and out of pens are common stressors that might further impact feed intake around the time of calving. The use of a low potassium straw to achieve a LOW E pre-partum diet does offer an opportunity to reduce dietary cation-anion difference to a greater extent than is possible with most other forages. However, it does require reduction of the straw to a small average particle size, approximately 6 cm, to effectively prevent the cows from sorting the ration.

GENERAL CONCLUSIONS

Over the last 30 years the trend on dairies had been to increase the energy content of the pre-partum ration to enhance DMI during the final weeks of gestation. In the last few years a number of dairies have shifted to a completely different dietary strategy, which utilizes straw as a major component of the diet to limit total energy content consumed by the cow. In this study, the LOW E and the HIGH E diets had similar outcomes in terms of milk production and time to first ovulation and were successful in limiting health problems through the transition period. The LOW diet did reduce the magnitude of DMI depression just before calving, but apart from a small reduction in NEFA the day of calving, few major benefits of this reduction were seen. The immunologic profile of both the LOW E and HIGH E diets was similar and followed the typical patterns of immunosuppression associated with the time of calving. All cows were similarly able to recover uneventfully from an intramammary E. coli challenge at day 5 of lactation. With optimal management, the utilization of a diet that meets, but does not exceed the energy requirements of the pre-calving heifer, is not detrimental to the dairy heifer despite her relatively greater energy needs compared to the multiparous cow.

REFERENCES

Agenas, S., E. Burstedt, and K. Holtenius. 2003. Effects of feeding intensity during the dry period. 1. Feed intake, body weight, and milk production. J Dairy Sci. 86(3):870-882.

Arave, C. W. and J. L. Albright. 1976. Social rank and physiological traits of dairy cows as influenced by changing group membership. J Dairy Sci. 59(5):974-981.

Bannerman, D. D., A. C. Kauf, M. J. Paape, H. R. Springer, and J. P. Goff. 2008. Comparison of Holstein and Jersey innate immune responses to Escherichia coli intramammary infection. J Dairy Sci. 91(6):2225-2235.

Bannerman, D. D., M. J. Paape, J. W. Lee, X. Zhao, J. C. Hope, and P. Rainard. 2004. Escherichia coli and Staphylococcus aureus elicit differential innate immune responses following intramammary infection. Clin Diagn Lab Immunol. 11(3):463-472.

Bartlett, P. C., M. Kopcha, P. H. Coe, N. K. Ames, P. L. Ruegg, and R. J. Erskine. 1995. Economic comparison of the pyloro-omentopexy vs the roll-and-toggle procedure for treatment of left displacement of the abomasum in dairy cattle. J Am Vet Med Assoc. 206(8):1156-1162.

Beever, D. E. 2006. The impact of controlled nutrition during the dry period on dairy cow health, fertility and performance. Anim Reprod Sci. 96(3-4):212-226.

Bertics, S. J., R. R. Grummer, C. Cadorniga-Valino, and E. E. Stoddard. 1992. Effect of prepartum dry matter intake on liver triglyceride concentration and early lactation. J Dairy Sci. 75(7):1914-1922.

Bertoni, G., E. Trevisi, X. Han, and M. Bionaz. 2008. Effects of inflammatory conditions on liver activity in puerperium period and consequences for performance in dairy cows. J Dairy Sci. 91(9):3300-3310.

Blecha, F., R. C. Bull, D. P. Olson, R. H. Ross, and S. Curtis. 1981. Effects of prepartum protein restriction in the beef cow on immunoglobin content in blood and colostral whey and subsequent immunoglobin absorption by the neonatal calf. J Anim Sci. 53(5):1174-1180.

Bobe, G., J. W. Young, and D. C. Beitz. 2004. Invited review: pathology, etiology, prevention, and treatment of fatty liver in dairy cows. J Dairy Sci. 87(10):3105-3124.

Bonnett, B. N., R. B. Miller, S. W. Martin, W. G. Etherington, and B. C. Buckrell. 1991. Endometrial biopsy in Holstein-Friesian dairy cows. II. Correlations between histological criteria. Can J Vet Res. 55(2):162-167.

Born, W. K., C. L. Reardon, and R. L. O'Brien. 2006. The function of gammadelta T cells in innate immunity. Curr Opin Immunol. 18(1):31-38.

Bramley, A. J., W. B. Faull, J. L. Young, and J. R. Walton. 1976. Correspondence: Controlling coliform mastitis. Vet Rec. 98(12):244.

Bruun, J., A. K. Ersboll, and L. Alban. 2002. Risk factors for metritis in Danish dairy cows. Prev Vet Med. 54(2):179-190.

Burton, J. L., M. E. Kehrli, Jr., S. Kapil, and R. L. Horst. 1995. Regulation of L-selectin and CD18 on bovine neutrophils by glucocorticoids: effects of cortisol and dexamethasone. J Leukoc Biol. 57(2):317-325.

Burvenich, C., V. Van Merris, J. Mehrzad, A. Diez-Fraile, and L. Duchateau. 2003. Severity of E. coli mastitis is mainly determined by cow factors. Vet Res. 34(5):521-564.

Bushinsky, D. A. 1996. Metabolic alkalosis decreases bone calcium efflux by suppressing osteoclasts and stimulating osteoblasts. Am J Physiol. 271(1 Pt 2):F216-222.

Cai, T. Q., P. G. Weston, L. A. Lund, B. Brodie, D. J. McKenna, and W. C. Wagner. 1994. Association between neutrophil functions and periparturient disorders in cows. Am J Vet Res. 55(7):934-943.

Cameron, R. E., P. B. Dyk, T. H. Herdt, J. B. Kaneene, R. Miller, H. F. Bucholtz, J. S. Liesman, M. J. Vandehaar, and R. S. Emery. 1998. Dry cow diet, management, and energy balance as risk factors for displaced abomasum in high producing dairy herds. J Dairy Sci. 81(1):132-139.

Catignani, G. L. and J. G. Bieri. 1983. Simultaneous determination of retinol and alpha-tocopherol in serum or plasma by liquid chromatography. Clin Chem. 29(4):708-712.

Coleman, D. A., W. V. Thayne, and R. A. Dailey. 1985. Factors affecting reproductive performance of dairy cows. J Dairy Sci. 68(7):1793-1803.

Conner, T., T. H. Herdt, and L. Neuder. 2001. Association of Prepartum Plasma Non-esterified Fatty Acid Concentration with Retained Placenta in Multiparous Holstein Cows. Annual Convention of the American Association of Bovine Practitioners:140-141.

Constable, P. D., G. Y. Miller, G. F. Hoffsis, B. L. Hull, and D. M. Rings. 1992. Risk factors for abomasal volvulus and left abomasal displacement in cattle. Am J Vet Res. 53(7):1184-1192.

Coppock, C. E. 1974. Displaced abomasum in dairy cattle: etiological factors. J Dairy Sci. 57(8):926-933.

Coppock, C. E., C. H. Noller, S. A. Wolfe, C. J. Callahan, and J. S. Baker. 1972. Effect of forage-concentrate ratio in complete feeds fed ad libitum on feed intake prepartum and the occurrence of abomasal displacement in dairy cows. J Dairy Sci. 55(6):783-789.

Correa, M. T., C. R. Curtis, H. N. Erb, J. M. Scarlett, and R. D. Smith. 1990. An ecological analysis of risk factors for postpartum disorders of Holstein-Friesian cows from thirty-two New York farms. J Dairy Sci. 73(6):1515-1524.

Curtis, C. R., H. N. Erb, C. J. Sniffen, R. D. Smith, and D. S. Kronfeld. 1985. Path analysis of dry period nutrition, postpartum metabolic and reproductive disorders, and mastitis in Holstein cows. J Dairy Sci. 68(9):2347-2360.

Daniel, R. C. 1983. Motility of the rumen and abomasum during hypocalcaemia. Can J Comp Med. 47(3):276-280.

Dann, H. M., N. B. Litherland, J. P. Underwood, M. Bionaz, A. D'Angelo, J. W. McFadden, and J. K. Drackley. 2006. Diets during far-off and close-up dry periods affect periparturient metabolism and lactation in multiparous cows. J Dairy Sci. 89(9):3563-3577.

Dann, H. M., G. A. Varga, and D. E. Putnam. 1999. Improving energy supply to late gestation and early postpartum dairy cows. J Dairy Sci. 82(8):1765-1778.

Detilleux, J. C., M. E. Kehrli, Jr., J. R. Stabel, A. E. Freeman, and D. H. Kelley. 1995. Study of immunological dysfunction in periparturient Holstein cattle selected for high and average milk production. Vet Immunol Immunopathol. 44(3-4):251-267.

Dietz, R. E., J. B. Hall, W. D. Whittier, F. Elvinger, and D. E. Eversole. 2003. Effects of feeding supplemental fat to beef cows on cold tolerance in newborn calves. J Anim Sci. 81(4):885-894.

Dirksen, G. U. and H. G. Liebich. 1985. Adaptive changes of the ruminal mucosa and their functional and clinical significance. Page 116 *in* Bovine Practitioner. Vol. 20.

Doepel, L., H. Lapierre, and J. J. Kennelly. 2002. Peripartum performance and metabolism of dairy cows in response to prepartum energy and protein intake. J Dairy Sci. 85(9):2315-2334.

Douglas, G. N., T. R. Overton, H. G. Bateman, 2nd, H. M. Dann, and J. K. Drackley. 2006. Prepartal plane of nutrition, regardless of dietary energy source, affects periparturient metabolism and dry matter intake in Holstein cows. J Dairy Sci. 89(6):2141-2157.

Drackley, J. K. and N. A. Janovick Guretzky. 2007. Controlled energy diets for dry cows. Western Dairy Management Conference, Reno, NV.

Duffield, T. 2000. Subclinical ketosis in lactating dairy cattle. Vet Clin North Am Food Anim Pract. 16(2):231-253, v.

Duffield, T. F. 2007. Prepartum Metabolic Monitoring. Annual convention of the American Association of Bovine Practitioners, Vancouver, BC.

Emanuelson, U., P. A. Oltenacu, and Y. T. Grohn. 1993. Nonlinear mixed model analyses of five production disorders of dairy cattle. J Dairy Sci. 76(9):2765-2772.

Emery, R. S., H. D. Hafs, D. Armstrong, and W. W. Snyder. 1969. Prepartum grain feeding effects on milk production, mammary edema, and incidence of diseases. J Dairy Sci. 52(3):345-351.

Emery, R. S., J. S. Liesman, and T. H. Herdt. 1992. Metabolism of long chain fatty acids by ruminant liver. J Nutr. 122(3 Suppl):832-837.

Erb, H. N., R. D. Smith, P. A. Oltenacu, C. L. Guard, R. B. Hillman, P. A. Powers, M. C. Smith, and M. E. White. 1985. Path model of reproductive disorders and performance, milk fever, mastitis, milk yield, and culling in Holstein cows. J Dairy Sci. 68(12):3337-3349.

Ferguson, J. D., D. T. Galligan, and N. Thomsen. 1994. Principal descriptors of body condition score in Holstein cows. J Dairy Sci. 77(9):2695-2703.

Foote, M. R., B. J. Nonnecke, D. C. Beitz, and W. R. Waters. 2007. Antigen-specific B-cell responses by neonatal calves after early vaccination. J Dairy Sci. 90(11):5208-5217.

Gaynor, P. J., F. J. Mueller, J. K. Miller, N. Ramsey, J. P. Goff, and R. L. Horst. 1989. Parturient hypocalcemia in jersey cows fed alfalfa haylage-based diets with different cation to anion ratios. J Dairy Sci. 72(10):2525-2531.

Goff, J. P. 2000. Pathophysiology of calcium and phosphorus disorders. Vet Clin North Am Food Anim Pract. 16(2):319-337, vii.

Goff, J. P. and R. L. Horst. 1997a. Effects of the addition of potassium or sodium, but not calcium, to prepartum ratios on milk fever in dairy cows. J Dairy Sci. 80(1):176-186.

Goff, J. P. and R. L. Horst. 1997b. Physiological changes at parturition and their relationship to metabolic disorders. J Dairy Sci. 80(7):1260-1268.

Goff, J. P., R. L. Horst, F. J. Mueller, J. K. Miller, G. A. Kiess, and H. H. Dowlen. 1991. Addition of chloride to a prepartal diet high in cations increases 1,25-

dihydroxyvitamin D response to hypocalcemia preventing milk fever. J Dairy Sci. 74(11):3863-3871.

Goff, J. P., Y. Naito, M. E. Kehrli, Jr., P. Hayes, and M. Daley. 1992. Physiologic effects of administration of interleukin 1 beta in cows. Am J Vet Res. 53(11):1983-1987.

Goff, J. P. and J. R. Stabel. 1990. Decreased plasma retinol, alpha-tocopherol, and zinc concentration during the periparturient period: effect of milk fever. J Dairy Sci. 73(11):3195-3199.

Goransson, L. 1990. The effect of late pregnancy feed allowance on the milk composition of the sow's colostrum and milk. Acta Vet Scand. 31(1):109-115.

Grant, R. J. 2007. Taking Advantage of Natural Behavior Improves Dairy Cow Performance. Western Dairy Management Conference, Reno, NV.

Gregory, P. C. 1987. Inhibition of reticulo-ruminal motility by volatile fatty acids and lactic acid in sheep. J Physiol. 382:355-371.

Grummer, R. R. 1995. Impact of changes in organic nutrient metabolism on feeding the transition dairy cow. J Anim Sci. 73(9):2820-2833.

Grummer, R. R. 2008. Nutritional and management strategies for the prevention of fatty liver in dairy cattle. Vet J. 176(1):10-20.

Grummer, R. R., D. G. Mashek, and A. Hayirli. 2004. Dry matter intake and energy balance in the transition period. Vet Clin North Am Food Anim Pract. 20(3):447-470.

Gunnink, J. W. 1984a. Post-partum leucocytic activity and its relationship to caesarian section and retained placenta. Vet Q. 6(2):55-57.

Gunnink, J. W. 1984b. Pre-partum leucocytic activity and retained placenta. Vet Q. 6(2):52-54.

Gunnink, J. W. 1984c. Retained placenta and leucocytic activity. Vet Q. 6(2):49-51.

Halliday, R., A. J. Russel, M. R. Williams, and J. N. Peart. 1978. Effects of energy intake during late pregnancy and of genotype on immunoglobulin transfer to calves in suckler herds. Res Vet Sci. 24(1):26-31.

Hammon, D. S., I. M. Evjen, T. R. Dhiman, J. P. Goff, and J. L. Walters. 2006. Neutrophil function and energy status in Holstein cows with uterine health disorders. Vet Immunol Immunopathol. 113(1-2):21-29.

Harp, J. A., T. E. Waters, and J. P. Goff. 2005. Adhesion molecule and homing receptor expression on blood and milk polymorphonuclear leukocytes during the periparturient period of dairy cattle. Vet Immunol Immunopathol. 104(1-2):99-103.

Hayirli, A., R. R. Grummer, E. V. Nordheim, and P. M. Crump. 2002. Animal and dietary factors affecting feed intake during the prefresh transition period in Holsteins. J Dairy Sci. 85(12):3430-3443.

Hayirli, A., R. R. Grummer, E. V. Nordheim, and P. M. Crump. 2003. Models for predicting dry matter intake of Holsteins during the prefresh transition period. J Dairy Sci. 86(5):1771-1779.

Hernandez, J., C. A. Risco, and J. B. Elliott. 1999. Effect of oral administration of a calcium chloride gel on blood mineral concentrations, parturient disorders, reproductive performance, and milk production of dairy cows with retained fetal membranes. J Am Vet Med Assoc. 215(1):72-76.

Holcomb, C. S., H. H. Van Horn, H. H. Head, M. B. Hall, and C. J. Wilcox. 2001. Effects of prepartum dry matter intake and forage percentage on postpartum performance of lactating dairy cows. J Dairy Sci. 84(9):2051-2058.

Holtenius, K., S. Agenas, C. Delavaud, and Y. Chilliard. 2003. Effects of feeding intensity during the dry period. 2. Metabolic and hormonal responses. J Dairy Sci. 86(3):883-891.

Holtenius, P. and K. Holtenius. 2007. A model to estimate insulin sensitivity in dairy cows. Acta Vet Scand. 49:29.

Hough, R. L., F. D. McCarthy, H. D. Kent, D. E. Eversole, and M. L. Wahlberg. 1990. Influence of nutritional restriction during late gestation on production measures and passive immunity in beef cattle. J Anim Sci. 68(9):2622-2627.

Hugman, A. 2006. Hepcidin: an important new regulator of iron homeostasis. Clin Lab Haematol. 28(2):75-83.

Huntington, G. B., R. A. Britton, and R. L. Prior. 1981. Feed intake, rumen fluid volume and turnover, nitrogen and mineral balance and acid-base status of wethers changed from low to high concentrate diets. J Anim Sci. 52(6):1376-1387.

Huzzey, J. M., T. J. DeVries, P. Valois, and M. A. von Keyserlingk. 2006. Stocking density and feed barrier design affect the feeding and social behavior of dairy cattle. J Dairy Sci. 89(1):126-133.

Ingvartsen, K. L. and J. B. Andersen. 2000. Integration of metabolism and intake regulation: a review focusing on periparturient animals. J Dairy Sci. 83(7):1573-1597.

Jiang, L., P. Sorensen, C. Rontved, L. Vels, and K. L. Ingvartsen. 2008. Gene expression profiling of liver from dairy cows treated intra-mammary with lipopolysaccharide. BMC Genomics. 9:443.

Johnson, M. M. and J. P. Peters. 1993. Technical note: an improved method to quantify nonesterified fatty acids in bovine plasma. J Anim Sci. 71(3):753-756.

Kehrli, M. E., Jr., B. J. Nonnecke, and J. A. Roth. 1989a. Alterations in bovine lymphocyte function during the periparturient period. Am J Vet Res. 50(2):215-220.

Kehrli, M. E., Jr., B. J. Nonnecke, and J. A. Roth. 1989b. Alterations in bovine neutrophil function during the periparturient period. Am J Vet Res. 50(2):207-214.

Kelton, D. F., K. D. Lissemore, and R. E. Martin. 1998. Recommendations for recording and calculating the incidence of selected clinical diseases of dairy cattle. J Dairy Sci. 81(9):2502-2509.

Kim, I. H., K. J. Na, and M. P. Yang. 2005. Immune responses during the peripartum period in dairy cows with postpartum endometritis. J Reprod Dev. 51(6):757-764.

Kimura, K., J. P. Goff, and M. E. Kehrli, Jr. 1999a. Effects of the presence of the mammary gland on expression of neutrophil adhesion molecules and myeloperoxidase activity in periparturient dairy cows. J Dairy Sci. 82(11):2385-2392.

Kimura, K., J. P. Goff, M. E. Kehrli, Jr., and J. A. Harp. 1999b. Phenotype analysis of peripheral blood mononuclear cells in periparturient dairy cows. J Dairy Sci. 82(2):315-319.

Kimura, K., J. P. Goff, M. E. Kehrli, Jr., J. A. Harp, and B. J. Nonnecke. 2002a. Effects of mastectomy on composition of peripheral blood mononuclear cell populations in periparturient dairy cows. J Dairy Sci. 85(6):1437-1444.

Kimura, K., J. P. Goff, M. E. Kehrli, Jr., and T. A. Reinhardt. 2002b. Decreased neutrophil function as a cause of retained placenta in dairy cattle. J Dairy Sci. 85(3):544-550.

Krawczel, P. D., C. T. Hill, H. M. Dann, and R. J. Grant. 2008. Short communication: effect of stocking density on indices of cow comfort. J Dairy Sci. 91(5):1903-1907.

Labarca, C. and K. Paigen. 1980. A simple, rapid, and sensitive DNA assay procedure. Anal Biochem. 102(2):344-352.

Lamote, I., E. Meyer, L. Duchateau, and C. Burvenich. 2004. Influence of 17beta-estradiol, progesterone, and dexamethasone on diapedesis and viability of bovine blood polymorphonuclear leukocytes. J Dairy Sci. 87(10):3340-3349.

LeBlanc, S. J., K. E. Leslie, and T. F. Duffield. 2005. Metabolic predictors of displaced abomasum in dairy cattle. J Dairy Sci. 88(1):159-170.

Lewis, G. S. 2003. Steroidal regulation of uterine resistance to bacterial infection in livestock. Reprod Biol Endocrinol. 1:117.

Littell, R. C., P. R. Henry, and C. B. Ammerman. 1998. Statistical analysis of repeated measures data using SAS procedures. J Anim Sci. 76(4):1216-1231.

Lohuis, J. A., Y. H. Schukken, J. H. Verheijden, A. Brand, and A. S. Van Miert. 1990. Effect of severity of systemic signs during the acute phase of experimentally induced Escherichia coli mastitis on milk production losses. J Dairy Sci. 73(2):333-341.

Madsen, S. A., P. S. Weber, and J. L. Burton. 2002. Altered expression of cellular genes in neutrophils of periparturient dairy cows. Vet Immunol Immunopathol. 86(3-4):159-175.

Markusfeld, O. 1984. Factors responsible for post parturient metritis in dairy cattle. Vet Rec. 114(22):539-542.

Markusfeld, O. 1987. Periparturient traits in seven high dairy herds. Incidence rates, association with parity, and interrelationships among traits. J Dairy Sci. 70(1):158-166.

Marquardt, J. P., R. L. Horst, and N. A. Jorgensen. 1977. Effect of parity on dry matter intake at parturition in dairy cattle. J Dairy Sci. 60(6):929-934.

Mashek, D. G. and D. K. Beede. 2000. Peripartum responses of dairy cows to partial substitution of corn silage with corn grain in diets fed during the late dry period. J Dairy Sci. 83(10):2310-2318.

Massey, C. D., C. Wang, G. A. Donovan, and D. K. Beede. 1993. Hypocalcemia at parturition as a risk factor for left displacement of the abomasum in dairy cows. J Am Vet Med Assoc. 203(6):852-853.

Mateus, L., L. Lopes da Costa, H. Carvalho, P. Serra, and J. Robalo Silva. 2002. Blood and intrauterine leukocyte profile and function in dairy cows that spontaneously recovered from postpartum endometritis. Reprod Domest Anim. 37(3):176-180.

Meglia, G. E., A. Johannisson, L. Petersson, and K. P. Waller. 2001. Changes in some blood micronutrients, leukocytes and neutrophil expression of adhesion molecules in periparturient dairy cows. Acta Vet Scand. 42(1):139-150.

Mehrzad, J., L. Duchateau, S. Pyorala, and C. Burvenich. 2002. Blood and milk neutrophil chemiluminescence and viability in primiparous and pluriparous dairy cows during late pregnancy, around parturition and early lactation. J Dairy Sci. 85(12):3268-3276.

Melendez, P., J. P. Goff, C. A. Risco, L. F. Archbald, R. Littell, and G. A. Donovan. 2006. Incidence of subclinical ketosis in cows supplemented with a monensin controlled-release capsule in Holstein cattle, Florida, USA. Prev Vet Med. 73(1):33-42.

Minor, D. J., S. L. Trower, B. D. Strang, R. D. Shaver, and R. R. Grummer. 1998. Effects of nonfiber carbohydrate and niacin on periparturient metabolic status and lactation of dairy cows. J Dairy Sci. 81(1):189-200.

Nonnecke, B. J., K. Kimura, J. P. Goff, and M. E. Kehrli, Jr. 2003. Effects of the mammary gland on functional capacities of blood mononuclear leukocyte populations from periparturient cows. J Dairy Sci. 86(7):2359-2368.

NRC. 2001. Nutrient Requirements of Dairy Cattle. 7 ed. National Academy Press, Washington, DC.

Oetzel, G. R. 1996. Effect of calcium chloride gel treatment in dairy cows on incidence of periparturient diseases. J Am Vet Med Assoc. 209(5):958-961.

Olson, D. P., L. F. Woodard, R. C. Bull, and D. O. Everson. 1981. Immunoglobulin levels in serum and colostral whey or protein-metabolisable energy restricted beef cows. Res Vet Sci. 30(1):49-52.

Olsson, G., M. Emannuelson, and H. Wiktorsson. 1998. Effects of different nutritional levels prepartum on the subsequent performance of dairy cows. Livestock Production Science. 53:279-290.

Paape, M. J., K. Shafer-Weaver, A. V. Capuco, K. Van Oostveldt, and C. Burvenich. 2000. Immune surveillance of mammary tissue by phagocytic cells. Adv Exp Med Biol. 480:259-277.

Parekh, A. C. and D. H. Jung. 1970. Serum inorganic phosphorus determination using p-phenylenediamine as a reducing agent. Clin Chim Acta. 27(3):373-377.

Perkin-Elmer. 1965. Analytical methods for atomic absorption spectrophotometry. Perkin-Elmer Corp., Norwalk, CT.

Phillippo, M., G. W. Reid, and I. M. Nevison. 1994. Parturient hypocalcaemia in dairy cows: effects of dietary acidity on plasma minerals and calciotrophic hormones. Res Vet Sci. 56(3):303-309.

Rabelo, E., R. L. Rezende, S. J. Bertics, and R. R. Grummer. 2003. Effects of transition diets varying in dietary energy density on lactation performance and ruminal parameters of dairy cows. J Dairy Sci. 86(3):916-925.

Rabelo, E., R. L. Rezende, S. J. Bertics, and R. R. Grummer. 2005. Effects of pre- and postfresh transition diets varying in dietary energy density on metabolic status of periparturient dairy cows. J Dairy Sci. 88(12):4375-4383.

Rambeaud, M., R. A. Almeida, G. M. Pighetti, and S. P. Oliver. 2003. Dynamics of leukocytes and cytokines during experimentally induced Streptococcus uberis mastitis. Vet Immunol Immunopathol. 96(3-4):193-205.

Roth, J. A. and M. L. Kaeberle. 1981. Evaluation of bovine polymorphonuclear leukocyte function. Vet Immunol Immunopathol. 2(2):157-174.

Roth, J. A. and M. L. Kaeberle. 1982. Effect of glucocorticoids on the bovine immune system. J Am Vet Med Assoc. 180(8):894-901.

Rude, R. K., M. E. Kirchen, H. E. Gruber, A. A. Stasky, and M. H. Meyer. 1998. Magnesium deficiency induces bone loss in the rat. Miner Electrolyte Metab. 24(5):314-320.

Rukkwamsuk, T., T. Wensing, and M. J. Geelen. 1998. Effect of overfeeding during the dry period on regulation of adipose tissue metabolism in dairy cows during the periparturient period. J Dairy Sci. 81(11):2904-2911.

Sarashina, T., S. Ichijo, J. Takahashi, and S. Osame. 1990. Origin of abomasum gas in the cows with displaced abomasum. Nippon Juigaku Zasshi. 52(2):371-378.

SAS. 1999. SAS User's Guide Statistics, Version 8.0 Edition. SAS Institute, Cary, NC.

Shafer-Weaver, K. A., G. M. Pighetti, and L. M. Sordillo. 1996. Diminished mammary gland lymphocyte functions parallel shifts in trafficking patterns during the postpartum period. Proc Soc Exp Biol Med. 212(3):271-280.

Sheldon, I. M. 2004. The postpartum uterus. Vet Clin North Am Food Anim Pract. 20(3):569-591.

Sheldon, I. M., D. E. Noakes, A. N. Rycroft, D. U. Pfeiffer, and H. Dobson. 2002. Influence of uterine bacterial contamination after parturition on ovarian dominant follicle selection and follicle growth and function in cattle. Reproduction. 123(6):837-845.

Smith, K. L. and F. L. Schanbacher. 1973. Hormone induced lactation in the bovine. I. Lactational performance following injections of 17 -estradiol and progesterone. J Dairy Sci. 56(6):738-743.

Suojala, L., T. Orro, H. Jarvinen, J. Saatsi, and S. Pyorala. 2008. Acute phase response in two consecutive experimentally induced E. coli intramammary infections in dairy cows. Acta Vet Scand. 50:18.

Swanson, T. J., C. J. Hammer, J. S. Luther, D. B. Carlson, J. B. Taylor, D. A. Redmer, T. L. Neville, J. J. Reed, L. P. Reynolds, J. S. Caton, and K. A. Vonnahme. 2008. Effects of gestational plane of nutrition and selenium supplementation on mammary development and colostrum quality in pregnant ewe lambs. J Anim Sci. 86(9):2415-2423.

Tesfa, A. T., M. Tuori, L. Syrjala-Qvist, R. Poso, H. Saloniemi, K. Heinonen, K. Kivilahti, T. Saukko, and L. A. Lindberg. 1999. The influence of dry period feeding on liver fat and postpartum performance of dairy cows. Animal Feed Science and Technology. 76:275-295.

Trinder, P. 1969. Determination of blood glucose using 4-amino phenazone as oxygen acceptor. J Clin Pathol. 22(2):246.

Urton, G., M. A. von Keyserlingk, and D. M. Weary. 2005. Feeding behavior identifies dairy cows at risk for metritis. J Dairy Sci. 88(8):2843-2849.

Van Saun, R. J. 2006. Metabolic Profiles for Evaluation of the Transition Period. Annual Convention of the American Association of Bovine Practitioners, St. Paul, MN.

Vandehaar, M. J., G. Yousif, B. K. Sharma, T. H. Herdt, R. S. Emery, M. S. Allen, and J. S. Liesman. 1999. Effect of energy and protein density of prepartum diets on fat and protein metabolism of dairy cattle in the periparturient period. J Dairy Sci. 82(6):1282-1295.

Veenhuizen, J. J., J. K. Drackley, M. J. Richard, T. P. Sanderson, L. D. Miller, and J. W. Young. 1991. Metabolic changes in blood and liver during development and early treatment of experimental fatty liver and ketosis in cows. J Dairy Sci. 74(12):4238-4253.

von Keyserlingk, M. A., D. Olenick, and D. M. Weary. 2008. Acute behavioral effects of regrouping dairy cows. J Dairy Sci. 91(3):1011-1016.

Waldron, M. R., B. J. Nonnecke, T. Nishida, R. L. Horst, and T. R. Overton. 2003. Effect of lipopolysaccharide infusion on serum macromineral and vitamin D concentrations in dairy cows. J Dairy Sci. 86(11):3440-3446.

Wallace, J. M., J. S. Milne, D. A. Redmer, and R. P. Aitken. 2006. Effect of diet composition on pregnancy outcome in overnourished rapidly growing adolescent sheep. Br J Nutr. 96(6):1060-1068.

Weiss, W. P., D. A. Todhunter, J. S. Hogan, and K. L. Smith. 1990. Effect of duration of supplementation of selenium and vitamin E on periparturient dairy cows. J Dairy Sci. 73(11):3187-3194.

Wells, S. J., S. L. Ott, and A. H. Seitzinger. 1998. Key health issues for dairy cattle--new and old. J Dairy Sci. 81(11):3029-3035.

Williamson, D. H., J. Mellanby, and H. A. Krebs. 1962. Enzymic determination of D(-)-beta-hydroxybutyric acid and acetoacetic acid in blood. Biochem J. 82:90-96.

Wrighting, D. M. and N. C. Andrews. 2006. Interleukin-6 induces hepcidin expression through STAT3. Blood. 108(9):3204-3209.

APPENDIX 1. NEUTROPHIL IODINATION ASSAY

The standard reaction mixture for the determination of stimulated iodination contains 2.5 x 10^6 PMNs, ~100 nCi ^{125}I, 40 nmoles NaI and 0.5 mg of OZ in 0.5 ml of EBSS. This assay is performed essentially as described by Roth JA, Kaeberle ML. 1981. Evaluation of bovine polymorphonuclear leukocyte function. *Vet Immunol Immunopathol* 2:157-174. The only exception is that we run it at the body temp of the cow 39 C. Twenty minutes after the PMNs are added the reaction was stopped by addition of 3.0 ml of cold 10% (w/v) trichloroacetic acid (TCA). The resulting precipitate is washed two additional times in TCA and the amount of radioactivity in the precipitate is determined in a gamma counter. The results are expressed as nmole of NaI incorporated/ 10^7 PMNs/hr as follows:

$$\text{nmole NaI} = \frac{(\text{Animal cpm} - \text{Bkg cpm})}{(\text{Std cpm} - \text{Bkg cpm})} * (40 \text{ nMoles}) \frac{60 \text{ min}}{20 \text{ min}} * \frac{10^7 \text{ PMNs}}{2.5 \times 10^6 \text{ PMNs}} = \text{nMoles}/10^7 \text{ PMN/hr}$$

The primary source of Na^{125}I is opened and diluted in a charcoal-filtered hood placed inside a chemical hood. A 1.0 mCi aliquot is diluted in 2 ml of a 0.05 N NaOH solution and stored in a 1-inch lead container in another hood. From this stock solution (0.5 mCi/ml), a working solution of Na^{125}I will be made as needed. The working solution is 30 ml of a 1.5 to 2.0 □Ci/ml solution. A 50 □l aliquot (50 - 100 nCi) of this material is used in a typical reaction, with a maximal daily usage of less than 5 □Ci. Neutrophil myeloperoxidase assays are done in capped tubes in a closed tumbler in a walk-in incubator held at 39°C. The tubes are then transferred into a hood where the caps are immediately removed and discarded into radioactive waste. The MPO assays are stopped with 2-3 ml of cold (4° C) 10% (w/v) trichloroacetic acid (TCA). The tubes are centrifuged at 1171 X's g for 5 minutes at 4° C to collect the precipitate and are washed twice more with 3 ml of cold TCA. The supernatants and washings are collected and held for half-life decay and liquid disposal inside a hood. The cpm of radioactivity remaining in the precipitate is determined in a gamma counter. The Total (Standard) CPM tubes typically have between 30,000 - 60,000 cpm in a gamma counter.

The most hazardous part of the assay is the preparation of the dilute Na^{125}I solution from the commercially supplied radioiodine (Amersham is good for us but any will work).

Neutrophil Iodination Assay

Tubes	Cold NaI in EBSS	Opsonized Zymosan (10 mg/100 ml)	Hot ^{125}I in EBSS (~50,000 cpm)	EBSS	PMNs
Standards	50 µl	50 µl	50 µl	300 µl	
Background	50 µl	50 µl	50 µl	300 µl	
Animals	50 µl	50 µl	50 µl	300 µl	50 µl

A. Set-up and label 11 X 75 mm snap cap plastic tubes in duplicate in special tumbler racks for all animals, Standard and Background tubes.

B. Load in order:

1. Cold NaI
2. Opsonized zymosan (*after grinding in clean sterile tissue grinder*)
3. EBSS
4. Hot Na^{125}I {With appropriate safety ventilation}
5. Cap Standard and Background tubes.
6. Pre-heat the tubes and media for ~ 5 min by placing the entire rack in the walk-in incubator and cover the tubes.
7. Start assay in the hood by adding PMNs to the corresponding animal tubes.
8. Cap the animal tubes and carefully load the rack into the iodination tumbler in the walk-in incubator.
9. Turn on the tumbler and start a timer for 20 minutes. Tumbler rotates ~ 60 times/min.
10. Stop the assay by removing the caps from all but the Standard tubes, and adding 3 ml of cold 10% (w/v) trichloroacetic acid (TCA).
11. Centrifuge in Sorvall RC3C on Program #5 (1171 X g, for 5 minutes @ 0 C), aspirate supernatant in hood. Repeat steps 10 & 11 twice more, then count the TCA-precipitable protein in the gamma counter. Load the Standard tubes first, followed by the background tubes and then the animal tubes in the proper order.

APPENDIX 2. INF-γ BLOOD STIMULATION

Pokeweed Whole Blood Stimulation

1. Collect blood in heparinized green-top vacutainers.
2. Place 1 ml whole blood from each sample into 4 wells of a 24-well microtiter plate
3. Two wells from each sample are incubated with each of the following
    a. 20 µl phosphate buffered saline (negative control – background IFN-γ)
    b. 20 µl PWM (1µg/ml)
4. Plates were gently shaken and incubated at 37˚C with 5% CO2 for 6 hours
5. After incubations, plates were spun for 20 minutes at 2000 RPM
6. Plasma was collected into 1.5ml cryotubes and stored at -20˚C until IFN-γ analysis.

M. bovis/M. avium PPD Whole Blood Stimulation.

1. Collect blood in heparinized green-top vacutainers.
2. Place 1 ml whole blood from each sample into 6 wells of a 24-well microtiter plate
3. Two wells from each sample are incubated with each of the following
    a. 66 µl phosphate buffered saline (negative control – background IFN-γ)
    b. 66 µl M. bovis PPD (20 µg)
    c. 66 µl M. avium PPD (20 µg)
4. Plates were gently shaken and incubated at 37˚C with 5% CO2 for 24 hours
5. After incubations, plates were spun for 20 minutes at 2000 RPM
6. Plasma was collected into 1.5ml cryotubes and stored at -20˚C until IFN-γ analysis.

Plasma samples were evaluated for IFN-γ using the Bovine IFN gamma Screening Set (Pierce Biotechnology, Rockford, IL).

APPENDIX 3. SERUM IRON DETERMINATION

All reagents are from Iron/TIBC Reagent Set (Pointe Scientific, Inc., Canton, MI)

Standard Curve:

250 µg/dl, 125 µg/dl, 62.5 µg/dl, 31.25 µg/dl, 7.8 µg/dl

Protocol:

1. Add 100 µl of iron buffer reagent to each well of a 96 well microtiter plate.
2. Add 20 µl of deionized water to the blank well and 20 µl of the appropriate standard to each of the standard wells, and 20 µl of the appropriate serum sample to each sample well. All samples and standards were run in duplicate.
3. A background spectrophotometric reading was taken (Molecular Devices Thermomax Microplate Reader, Sunnyvale, CA) at 560nm with a 3 second shake prior to reading.
4. 20 µl of 1:8 dilution of color reagent was added to each well.
5. Plates were incubated at 37˚C for 15 minutes.
6. Samples were again read on the spectrophotometer at 560nm, and the initial reading was subtracted from the final reading.
7. Fe values were calculated based on the standard curve for each plate.

ACKNOWLEDGMENTS

The authors wish to thank the animal caretakers who provided the daily care and feeding of the experimental animals: Norman Tjelmeland, Creig Caruth, Jerry Grove, and Andrew Mosier. Expert technical assistance in performing the assays of the metabolic parameters was provided by Amanda Hall, Duane Zimmerman and Derrel Hoy. Adam Ward, Billy Brown, and Lindsey Zaebst assisted throughout the glucose tolerance testing, milking of the cows, monitoring of cows during experimental challenge, blood sampling and conduct of laboratory assays.