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EFFECT OF PREPARTUM DIETARY ENERGY DENSITY AND PROTEIN CONTENT ON PERIPARTUM BODY FAT MOBILIZATION, MILK YIELD, HEALTH PERFORMANCE AND PROTEIN TURNOVER IN HOLSTEIN COWS

By

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ABSTRACT

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Eighty Holstein cows (n=80; 40 primiparous and 40 multiparous) were used, in a complete randomized block design (20 blocks), where cows were blocked randomly by parity and expected calving date. Dry cows four weeks from their expected calving dates were fed one of the four experimental diets: 1) Low energy and low protein (LL). 2) Medium energy and medium protein (MM). 3) Medium energy and high protein (MH). 4) High energy and high protein (HH). After calving, all cows were fed the same diet according to NRC recommendations. Cows that were fed the HH diet had lower blood NEFA concentrations prepartum compared to the cows that were fed the LL diet ($P<0.1$). Treatment diets did not affect feed intake, body weight, body condition score, milk yield. Liver TG concentrations were affected by treatment diets. The results of this study suggest that

increased energy and protein for dry cows decreases plasma NEFA concentrations two weeks prepartum.

To my sweet little sister Assal Yousif.

and to my mother Awatif Sabra, her belief in family and in education provided me with the foundation necessary to engage in educational inquiry.

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INTRODUCTION

The dry period for dairy cows is a lactation preparation period rather than an insignificant rest period between lactations. During the dry period a cow not only rests but the body is preparing for important changes that will influence her next lactation. The mammary gland involutes, regenerates and produces high quality colostrum. Two-thirds of fatal growth occurs during the dry period and this growth takes priority over maintenance of the cows own body tissues.

Plasma nonesterified fatty acid (NEFA) concentrations increase prior to and at parturition, resulting in increased fatty acid uptake by the liver. The fatty acids are esterified and stored as triglycerides in the liver. Export of newly synthesized triglycerides from the liver as very low density lipoprotein occurs slowly in ruminants and is a major factor in the development of fatty liver. Deposition of triglycerides in the liver, seems to be a result of more uptake of NEFA than what can be oxidized and exported (Herdt, 1988). Accummulation of triglycerides in the liver infiltrate liver cells and interfere with its functions and causes some metabolic problems. Therefore, nutritional strategies to minimize the elevation in plasma NEFA

prior to calving is expected to lower liver triglycerides at calving and may decrease incidence of metabolic disorders (e.g., ketosis, displaced abomasum, retained placenta and milk fever). Research to determine methods to reduce fatty acid delivery to the liver or to enhance hepatic export of very low density lipoprotein (VLDL) near calving is warranted.

Objectives:

The general objectives of this study were:

1) To determine if a high energy and/or high protein diet prepartum increases peripartum nutrient intake, improves nutrient balance as indicated by plasma NEFA concentrations, affects protein turnover (creatinine and 3- methyl-histidine ratio) and increases milk yield.
2) To determine the interrelationships of serum NEFA and prepartum dietary energy density and protein content.

Review Of The Literature

Marginal quality of feed, imbalanced rations, and inadequate housing all characterize poor dry cow management practices. Deficient dry cow care may result in decreased milk yield, increased incidence of periparturient health disorders, and impaired fertility (Curtis et al., 1985). The result is reduced overall milk production efficiency (pounds milk per unit cost). Lactating cows need to be dried-off about 60 days before their expected date of calving. Metabolically, the dry period is when a dairy cow must alter her metabolic priorities for available nutrients from net tissue deposition (e.g., mid-gestation) to reserve tissue mobilization (e.g., early lactation) (Coppock, 1988). This metabolic transition does not occur abruptly, but gradually over about a three week period prepartum. Cessation of milking results in reabsorption of non-secreted milk, and rapid loss of mammary secretory epithelial cells. The highest time for susceptibility to new intramammary infections is the two weeks of involution (Cousins et al., 1980; Neave et al., 1950). Once the udder

reaches a stable non-secretory state, susceptibility to new infection is reduced (Nickerson, 1990). Susceptibility to new infections increases as the udder starts to produce secretions in preparation for lactation in early lactation (Cousins et al., 1980;Neave et al.,1950;Nickerson, 1990).

A short dry period (< 40 days) reduces subsequent milk yield (Coppock et al., 1974; Dias et al. 1982; Funk et al., 1987; Schaeffer et al., 1972). Also excessive days dry (> 70 days) have been shown to reduce milk yield, which would decrease profit (Coppock et al., 1974; Dias et al., 1982; Funk et al., 1987; Schaeffer et al; 1972). Factors such as age, milk yield and days open affect optimum length of dry periods.

Inadequate days dry or poor quality dry cow nutrition may adversely affect colostrum quality and quantity. Colostrum is a milk-like fluid secreted by mammary glands at the onset of lactation. Colostrum contains higher concentrations of minerals (except potassium), proteins, fat, and fat soluble vitamins (Naylor, 1986). It also contains high concentrations of maternal immunoglobulin essential for passive transfer of immunity to the neonate.

Lipogenesis (fat deposition) occurs in mid-gestation, and lipolysis (fat depletion) in late-gestation and early lactation (Bauman et al., 1980; Coppock, 1988; Emery, 1988; McNamara et al. 1986; Vernon, 1988). Fetal and placental maximum gain occurs in the last 11 weeks prepartum, which places great burden on the dry cow (Prior et al., 1979) . Loss of

maternal nutrient reserve for fetal development can occur if nutrients are inadequate. Presence of a second fetus increases pregnancy requirement, which may cause the increased incidence of health disorders associated with twin pregnancies (Koong et al., 1982).

Dairy cows can metabolically adapt to the increased demands of a given physiological state (i.e., pregnancy, lactation and growth) by homeorhetic regulation (Bauman et al., 1980; Emery; 1988). Increasing lipolytic activity is shown by the increased concentration of non-esterified fatty acids (NEFA) and B-hydroxybutyrate in the blood two to three weeks prepartum (Gerloff et al., 1986; Vernon, 1988). These metabolic changes are associated with decreased insulin (i.e., lipogenic hormone) activity (Gerloff et al, 1986; Vernon, 1988), and increased adipose tissue sensitivity to the β- adrenergic agonist (i.e., lipolytic hormone),e.g., epinephrine (Jaster et al., 1981; Vernon; 1988).

Lactogenesis is stimulated by the amount, and ratios of estrogen and progesterone during late gestation (Erb, 1977). Milk yield depends on secretory cell number (Knight et al., 1987; Tucker, 1987), and lactation increases lipolytic activity (Bauman et al., 1980; Emery, 1988; McNamara et al., 1986; Vernon, 1988). Incidence of clinical or subclinical metabolic disorders results from inability to adequately coordinate metabolism to meet production demands.

Nutrient Requirements:

Nutrient requirements for pregnancy represent nutrient amounts necessary to support both growth rate and maintenance of fetus, placenta, uterus and mammary gland. Conceptus maintenance expenditure is a significant portion of the total pregnancy requirement as evidenced by the low efficiency of utilization for metabolizable energy (Ferrel et al., 1976; Moe et al., 1972).

Energy required by pregnant cows at term is 175% of that required by an equal weight, of non-pregnant cows (Moe et al., 1972). Conceptus nutrient requirements depend upon fetal numbers, rate of growth and birth weight. Providing sufficient quantities of essential nutrients is just as critical for the dry cow as the lactating cow to maintain optimum performance. Dry cow feeding programs with nutrient deficiencies (e.g.- vitamin E, magnesium and selenium) have been associated with increased health disorders in the periparturient period (Eger et al., 1985; Hoffsis et al., 1989).

Dietary recommendations for dry cow, have been established by the National Research Council (NRC), for energy, protein, fiber, 14 minerals and three vitamins (NRC, 1989). National Research Council (NRC) has developed mathematical models of nutrient requirements for physiological functions (i.e., maintenance, growth, pregnancy, lactation, reserve deposition and weight loss). These models are summed over all appropriate

states for a particular animal to determine the total nutrient requirement. Increased energy above NRC recommendations during the dry period has been shown in some studies to decrease metabolic and reproductive disorders (Curtis et al. 1985).

Carbohydrate:

The amount of structural carbohydrates (i.e., cell wall fiber) and non-structural carbohydrates (i.e., cell contents) need to be balanced to meet dietary energy density and maintain maximum dry matter intake. Neutral detergent fiber (NDF), a measure of cell content, is negatively associated with dry matter intake. Acid detergent fiber (ADF) is more associated with feed digestibility than feed intake (Van et al., 1985). A sufficient amount of fiber needs to be maintained in the diet to optimize the balance of carbohydrates and achieve maximum intake . Non-structural carbohydrate levels need to be managed appropriately to obtain optimum body condition of the dry cow and prevent over conditioning, which adversely affects performance (Coppock, 1988; Smith et al., 1981).

Protein:

Dietary protein is partitioned in relation to its rumen degradability and solubility (NRC, 1989). Rumen-degradable and soluble protein support microbial protein production. Rumen undegradable protein and microbial

protein supply the amino acids that support the animal productive function.

A greater percentage of dietary protein should be fed as soluble protein, or non-protein nitrogen (NPN) if large amount of carbohydrates are fed to dry cows. These protein sources are rapidly converted to ammonia, an essential nutrient to cellulolytic bacteria (Sniffen et al., 1988).

Increased undegradable protein for dry cows has improved body condition at calving and subsequent lactation performance in first lactation heifers (Hook et al., 1989; Van et al., 1989). A higher protein (15%) diet, all from rumen-degradable sources, resulted in a 66% incidence of downer cow syndrome, compared to a lower protein (8%) diet (Julien et al., 1977). Other studies showed reduced metabolic and reproductive health disorders when feeding higher protein concentrations compared with NRC requirements three weeks prepartum (Curtis et al., 1985).

NRC Recommendations:

National Research Council (NRC) incorporates a 10% activity allowance of maintenance energy requirements for dairy cattle (NRC, 1989). This should be sufficient energy allotment to account for animal activity when housed in stanchions with limited exercise or free stall facilities. Additional increase in maintenance energy requirement of 10 to 20% for grazing quality or sparse pastures, respectively, are recommended (NRC, 1989). Nutrient requirements by the NRC are based on the animal being

exposed to stress free, thermoneutral environment (NRC, 1989). Climatic factors (e.g., ambient temperatures, humidity, air movement and precipitation), and animal factors (e.g., behavior, and hair length) all play a role in modifying the range of thermoneutrality (Fox et al., 1988; NRC, 1981). Heat stress during pregnancy reduced calf birth weight by 7 kg and altered placental and maternal hormonal concentrations (Collier et al., 1982).

More milk is produced in the subsequent lactation from cows under shade compared to ones without (Collier et al., 1982). Although this difference in milk yield was not statistically significant, these researchers conclude that, heat stress during pregnancy, may indirectly affect milk yield through alterations in endocrine profiles, and reduced calf birth weights.

Ambient temperature below the lower critical temperature (i.e., cold stress) results in increased maintenance energy to maintain body temperature (Young, 1983;). Increased dry matter intake usually compensates for this increased energy need in marginal cold stress situations (NRC, 1981). Environmental conditions need to be accounted for in dry cow diet formulation (NRC, 1981). Otherwise, extreme body condition loss will occur under conditions of severe cold without dietary adjustments, which will have a serious detrimental impact on subsequent performance.

Dietary nutrient densities for dry cows, based on NRC recommendations are determined on an assumed average dry matter intake of 1.6 to 2.0% of body weight (NRC, 1989).

If a cow consumes less dry matter than expected of diets containing these suggested nutrient densities, she will ingest inadequate nutrient amounts to meet her defined requirements. Over consumption of dry matter produces the opposite results.

Feed Intake:

Feed intake of dry cows has been reported (Van Saun et al., 1989; Zamet et al., 1979) to range from 7 to 15 kg/d, and it is affected by many factors (NRC, 1987) (e.g., environmental and physiological). Much of the intake variation is accounted for by animal parity and dietary forage: concentrate ratio.

Cows entering their first lactation consume less dry matter (mean, 7 to 12 kg / d) than older cows (Marquardt et al., 1977; Van Saun et al., 1989; Zamet et al., 1979) (mean 10 to 15 kg / d). Low forage diets are consumed in lesser amounts than high forage diets (Coppock et al., 1972).

Decline of dry matter intake for dry cows beginning two to three weeks prepartum, followed by a further drop 48 to 72 hours before calving, was reported (Coppock etal., 1972; Marquardt et al., 1977; Van et al., 1989).

Management goals for dry cows should focus on maximizing dry matter intake throughout the dry period to allow more rapid increases in intake postpartum. This may be accomplished by feeding ad libitum a total mixed ration appropriately formulated for energy and fiber levels.

Intake during the dry period (i.e., close-up period) is critical in preventing subsequent health disorders and increasing milk production (Curtis et al., 1984; Curtis et al., 1985; Erb et al., 1988). Bertics et. al. 1992 concluded that, liver triglyceride immediately after calving was negatively correlated with dry matter intake. Nutrient imbalance (nutrient deficiencies or excess) in the dry cow diet was reported to increase incidence of parturient hypocalcemia (Curtis et al., 1984; Erb et al., 1988), hypomagnesemic tetany (Hoffsis et al., 1989; Littledike et al., 1981), retained placenta (Eger et al., 1985) , mastitis (Erskine et al., 1987), udder edema (Emery et al., 1969; Littledike et al., 1981), ketosis (Foster, 1988; Gerloff, 1988; Littledike et al, 1981), and displaced abomasum (Coppock et al., 1972; Coppock, 1974). Epidemiologic studies have shown periparturient health disorders not to be totally independent events, but a complex of interrelated disorders (Curtis et al., 1983; Curtis et al., 1985; Erb et al., 1981; Erb et al., 1985; Erb et al., 1988).

Forages should make up most of the dry cow ration. High forage rations (>85%), have been thought to maintain maximal rumen fill(i.e., volume), stimulate rumen motility, and allow healing of rumen wall lesions

resulting from high-grain lactation rations. Many forage and roughage ingredients can be fed to the dry cow when rations are appropriately formulated for energy, protein, fiber, and mineral concentrations. Dry cows require long course fibrous material to stimulate rumination and saliva flow to promote maximal fiber fermentation.

Body condition score (degrees of body fatness) should be evaluated during the dry period. Body condition scoring grades cows by amount of subcutaneous fat stores over the loin, pelvis, and tailhead into five categories (Wildman et al., 1982), emaciated (1); thin (2); average (3); fat (4); and obese (5) . Body condition scoring is an excellent cow monitor that is easily quantified for evaluation of nutritional programs.

Body condition at calving plays an important role in determining subsequent health (Fronk et al., 1980;), productive (Garnsworthy et al., 1987), and reproductive (Ducker et al., 1984) performance. Moderate body condition score(3-3.5) is essential to support milk production in early lactation, and to initiate reproductive cycle. Under good management practices fewer than 10% of the dry cows should have condition scores over 4.0 or under 2.5 (Ferguson et al., 1988). Either extreme in body condition results in reduced milk yield (Garnsworthy et al., 1987), increased health disorders (Fronk et al., 1980), and impaired fertility (Butler et al., 1989).

Body reserves to be utilized in early lactation start to build in the late lactation when energy balance is positive. For this reason attention should be placed to ensure adequate reserve at calving.

High-producing dairy cows have a tremendous metabolic challenge during early lactation to provide adequate substrates for synthesis of large quantities of milk. Intake of energy-yielding nutrients from the diet usually is less than energy output in milk, resulting in mobilization of body tissue to supply the nutrient deficit. Most cows can adjust their metabolism to meet this challenge; some cows, however, are unable to make the necessary adjustments to maintain homeostasis. Metabolic diseases or disorders occur as a result.

Ketosis, Fatty Liver, And Fat Cow Syndrome

Lactation ketosis and fatty liver are two interrelated disorders of energy metabolism. Estimates of the incidence of ketosis range from 2 to 15% (Baird, 1982; Littledike et al., 1981). Subclinical ketosis may occur even more frequently. Cows with clinical ketosis usually have fatty liver (Foster 1988).

Ketosis results in decreased milk production, increased veterinary expenses, and possibly decreased reproductive life (Baird, 1982; Schults, 1988). Association has been noted between fatty liver and increased

susceptibility to disease and reproductive problems (Gerloff et al, 1986; Reid, 1982).

A protocol to induce ketosis and fatty liver experimentally has been developed (Mills et al., 1986; Veenhuizen et al., 1991). The protocol involves subjecting

cows in early lactation (10 to 14 d postpartum), to moderate feed restriction, and a dietary source of ketone bodies (i.e., 1,3-butanediol), fatty liver and ketosis develop gradually over a period of two to four weeks.

Lactation ketosis:

Ketosis (i.e., acetonemia) is a metabolic disease of dairy cows in early lactation, which is characterized by increased concentrations in blood of the ketone bodies, i.e., 3-hydroxybutyrate (BHBA), acetoacetate (AcAc) and acetone.

Cows usually are affected within the first three to four weeks postpartum, with older cows being more susceptible (Schultz, 1988). Economic losses occur through decreased milk production, treatment expenses, and possibly decreased productive lifetime (Baird, 1982). Signs of primary ketosis (i.e., that which occurs as a primary disease, not as a secondary complication to another disease or disorder) are somewhat nonspecific. Signs may include decreased rumen activity, decreased appetite, nervousness or deranged behavior, and decreased milk production.

Ketone bodies are present in urine and milk. Presence of fever is an indication of infection.

The two major changes in blood are increased concentration of ketone bodies and decreased concentration of glucose. Other biochemical changes in blood include increased concentrations of nonesterified fatty acids (NEFA) and acetate, and decreased concentrations of insulin, triglycerides, free and esterified cholesterol, and phospholipid. Cows with subclinical ketosis may show no outward signs, but have increased concentrations of NEFA, and BHBA, and decreased concentrations of glucose in blood; subclinical ketosis may either remit or progress into clinical ketosis.

The general biochemical progression toward ketosis is thought to be as follows; production of glucose by the liver is inadequate to meet demands of increasing milk synthesis, and concentration of glucose decreases in blood. Insulin, with concentration already being low during early lactation, may decrease further and allow increased mobilization of NEFA and glycerol from adipose tissue. Uptake of NEFA by liver increases production of ketone bodies and deposition of triglyceride in liver, and this sometimes leads to clinical ketosis. During early lactation, most high-producing cows will go through some degree of hypoglycemia, increased mobilization of NEFA and production of ketone bodies without progressing to ketosis. Baird (1977) stated that ketosis represents one extreme of the

"normal" continuum of glucose deficit and fatty acid mobilization in early lactation.

Fatty liver:

Fatty liver (i.e., hepatic lipidosis) is a general term for accumulation of lipid in the hepatocyte or parenchymal cells of the liver. It occurs in many species during various conditions. Reviews of fatty liver in dairy cows include those of(Reid, 1982; Roberts et al., 1981; Herdt, 1988; Herdt et al., 1982; Emery, 1979; Emery et al., 1992; and Vazquez-Anon et al., 1994).

Triglyceride is the primary type of lipid that accumulates in liver during the postpartum period in normal (Collins et al., 1980), or overconditioned cows (Fronk et al., 1980). Hepatic triglyceride also accumulates during fasting in lactating (Brumby et al., 1975; Herdt et al., 1983;) or non-lactating (Reid et al., 1977) cows, or during secondary disorders such as displaced abomasum (Herdt et al., 1983). Triglyceride also accumulates in liver of ewes with pregnancy toxemia, or a combination of fasting and administration of phlorizin and epinephrine (Herdt, 1988). Content of cholesterol esters increased postpartum in fatty liver (Collins et al., 1980), and during fasting in lactating cows (Brumby et al., 1975). No increase in cholesterol ester was observed in non-lactating cows (Reid et al.,

1977). Phospholipid content may be decreased (Herdt etal., 1983), unchanged (Brumby etal., 1975; Collins et al., 1980; Reid et al., 1977), or increased (Fronk et al., 1980). Cows obese at calving were subject to a complex of postpartum diseases known as fat cow syndrome or fatty liver syndrome, which include development of severe fatty infiltration of the liver (Henderson et al., 1982).

Degree of fatty liver has been measured in biopsy samples by using microscopic point-counting methods, chemical methods, or buoyant density (Herdt, 1988). According to Gaal et al., 1983; the three classifications of degree of fatty liver are mild (0-20% of cell volume or < 5% by weight as triglyceride), moderate (20-40% of volume or 5-10% of weight as triglyceride), or severe (>40% of volume or >10% of weight as triglyceride).

Occurrence of fatty liver is related to the degree of mobilization of body tissue after calving. Excessive tissue mobilization is indicated by increased NEFA's concentration in plasma and loss of body weight and condition score. Deposition of triglyceride in tissues such as liver, which normally takes up and utilizes NEFA, seems to be a result of more uptake of NEFA, than what can be oxidized or exported (Herdt, 1988).

Severe or clinical fatty liver is characterized by decreased responses to treatments for other diseases or disorders (Herdt, 1988). Fatty liver was reported to decrease reproductive efficiency in some studies (Reid, 1983;

Reid et al., 1979; Reid et al., 1983) but not in others (Gerloff et al., 1986). Cows with fatty liver had decreased white cell counts in blood (Reid et al., 1984), and a greater incidence of infectious diseases (Gerloff et al., 1986). Cows with fatty liver also retained bacteria in the mammary gland for longer periods after an experimental infection (Hill, 1985).

Study of the ultrastructure of hepatocytes from cows with severe fatty liver revealed increased cell volume, decreased volume of rough endoplasmic reticulum per cell, and evidence of mitochondrial damage (Reid et al., 1980). The latter two changes were reflected in vivo by decreased albumin concentration, and increased activities of mitochondrial enzymes in blood. Grohn and Lindberg(1985), observed a decrease in the amount of Golgi apparatus in fatty liver from ketotic cows, and an increase peroxisome in mildly ketotic cows, but a decrease in peroxisome in severely ketotic cows.

In ruminants, the liver is the major source of endogenous plasma triglyceride, with intestinal denovo synthesis being of little consequence (Pullen et al., 1988). Secretion of very-low-density lipoprotein (VLDL) by ruminant liver, however, is low when compared with that of non-ruminants (Kleppe et al., 1988). Pullen et al. (1988) found that the turnover rate of the stored triglyceride pool in sheep liver was decreased when the size of that pool increased, suggesting that fatty liver may further decrease the ability to secrete lipoprotein. Decreasing ability of hepatocytes to

synthesize apoprotein necessary for secretion of triglyceride rich lipoprotein was said to contribute to development of fatty liver (Herdt et al., 1983; Reid et al., 1980).

Some researchers have observed changes in concentrations of lipoprotein in blood from cows with fatty liver. Concentrations of dextran sulfate precipitable (DSP) lipoprotein decreased in cows that developed severe fatty liver in association with occurrence of displaced abomasum, but they increased in cows that had fatty liver induced by fasting (Herdt et al., 1983). In a field study, cows with naturally occurring fatty liver also had decreased DSP lipids (Gerloff et al., 1986). Rayssiguier et al. (1988), observed decreased concentrations of low-density lipoprotein (LDL) in blood of cows with postparturient fatty liver.

Many investigators have attempted to identify metabolites or enzymes in blood that would predict accurately the presence and degree of fatty liver in dairy cows. These attempts have, for the most part, been unsuccessful, and studies from different groups often provide conflicting results.

In general, changes in blood associated with fatty liver include increased concentrations of NEFA, BHBA, and bilirubin, and decreased concentrations of glucose, total cholesterol, albumin, magnesium and insulin (Reid et al., 1983). These changes are very similar to those that occur during ketosis. Other changes noted in some studies include increased

activities of the enzyme aspartate aminotransferase (glutamate-oxaloacetate aminotransferase) (Grohn etal., 1983; Herdt et al., 1982; Lotthammer, 1982; Schultz, 1988), ornithinecarbamoyltransferase (Grohn et al., 1983), acid and alkaline phosphatase (Bogin et al., 1988), and glutamate dehydrogenase (Bogin et al., 1988). Gerloff et al. (1986), noted a negative correlation between concentrations of the thyroid hormones and degree of fatty liver.

Now, liver biopsy remains a necessary procedure for accurately determining content of lipid in liver (Herdt, 1988). It is obvious that improved diagnostic techniques for fatty liver are needed.

Reid and Roberts (1982), and Gerloff et al. (1986 a, b, c) proposed that fatty liver may be more an indicator of negative energy balance during early lactation than a separate disease. Furthermore, negative energy balance may be the causative factor in increased susceptibility to other diseases and reproductive problems. Although, the general cause of fatty liver seems to be excessive uptake of NEFA by the liver, cellular and molecular mechanisms of development remain to be determined.

Fat Cow Syndrome:

Morrow (1976) and Morrow et al. (1979) describe fat cow syndrome as a complex of metabolic and infectious disorders, occurring during the postpartum period in cows that calve in an obese condition, Signs of the

syndrome include anorexia, weakness, and associated diseases that may include milk fever, ketosis, displaced abomasum, retained fetal membranes, metritis, or mastitis. Response to treatment for these diseases usually is poor; rates of mortality up to 25% have been observed in affected herds (Marrow et al., 1979).

Cows with fat cow syndrome have fatty livers. Obesity at the onset of lactation may lead to rapid mobilization of NEFA, which is worsened by the decreased appetite usually observed in such cows (Bines et al., 1983; Treacher et al., 1986). Biochemical changes in blood include decreased concentration of triglycerides and increase concentrations of NEFA, ketone bodies, urea, and bilirubin (Morrow, 1976). Concentration of glucose may be either decreased or increased.

Increased mobilization of body tissue by fat cows after parturition was observed to be a combination of adipose tissue and muscle mass (Reid et al., 1986). Reid et al.(1986) speculated that mobilized body protein was not adequate for synthesis of lipoprotein, contributing to development of fatty liver in the fat cows. Researchers should determine the role of quantity and quality of dietary protein in preventing and alleviating fatty liver and fat cow syndrome in early lactating dairy cows.

Metabolism of Glucose and lipids in the liver of Dairy Cattle

Gluconeogenesis:

Because of ruminal fermentation of dietary carbohydrates, little glucose is available for absorption from the small intestine of ruminants. Ruminants are dependent on high rates of gluconeogenesis to supply their needs for glucose. Use of large quantities of glucose by the mammary gland of high-producing cows creates a tremendous demand for gluconeogenesis most of which occurs in the liver. Glucose utilization and gluconeogenesis have been reviewed (Lindsay, 1979; Young, 1977) and will be discussed only briefly.

Precursors and Pathways:

Precursors for gluconeogenesis include propionate, lactate, glycerol and amino acids. Reynolds et al.(1988 a,b,) measured maximal contributions of propionate, lactate and amino acids to be 55, 18 and 16% of net hepatic glucose production. Pyruvate and glycerol supplies less than 2% of glucose production in another study (Lomax et al., 1983). Propionate is the predominant gluconeogenic precursor in fed ruminants, and gluconeogenesis increases with increased propionate supply.

About 50% of the lactate flux was derived from glucose in lactating cows, with the remainder presumably being absorbed from the gastrointestinal tract (Baird et al., 19833). Recycling of glucose through lactate accounted for only 2% of glucose flux in lactating cows (Baird et al., 1983). Reynolds et al. (1988), determined that the net amount of lactate taken up by the liver of early-lactating cows could provide a maximum of 18% of hepatic glucose production. Baird et al. (1983) concluded that, most lactate taken up by the liver of lactating cows is oxidized or used for synthesis of compounds other than glucose.

Glycerol may become quantitatively more important for gluconeogenesis in cows with severe negative energy balance during early lactation (Peel et al., 1987). The contribution of amino acids to gluconeogenesis in ruminants has been debated, with estimates ranging from 11 to 30% (Lindsay, 1978; Reynolds et al., 1988).

The pathways of glucose synthesis in ruminants are similar to those in non-ruminants. Subcellular distribution of enzymes may vary, however. Initial metabolism of propionate occurs in the mitochondria. Propionate is activated to propionyl-CoA, carboxylated to methylmalonyl-CoA, and converted to succinyl-CoA and then oxaloacetate (OAA). Phosphoenolpyruvate carboxykinase (PEPCK), the major enzyme controlling conversion of propionate to glucose, is found in both mitochondrial and cytosolic compartments in ruminants (Ballard et al., 1969). Thus, OAA

can be converted to phosphoenol pyruvate (PEP) in mitochondria, with PEP exiting the mitochondria for conversion to glucose, or alternatively, OAA can leave the mitochondria as malate, be reconverted to OAA in the cytosol, and then converted to glucose. Lactate, pyruvate and amino acids that are converted to pyruvate require pyruvate carboxylase (PC) to be converted to OAA. Ruminants have PC located in both cytosolic and mitochondrial compartments. Propionate, lactate, pyruvate and amino acids all require participation of the citric acid cycle or at least are metabolized through common intermediates.

Glycerol, on the other hand, enters the glyconeogenic pathway at the triose phosphate stage, and thus its metabolism is independent of the citric acid cycle.

Regulation:

Substrate availability is a primary regulator of gluconeogenesis in ruminants; gluconeogenesis increases after eating and decreases with fasting in ruminants, which are opposite the responses observed in non-ruminants (Young,1977). Hormonal controls of gluconeogenesis in ruminants have been summarized (Bassett, 1978; Trenkle; 1981). Glucagon stimulates glycogenolysis and promotes gluconeogenesis, whereas insulin largely exerts its effects on peripheral tissues to increase glucose utilization. Glucocorticoid increases gluconeogenesis by increasing

delivery of amino acids to liver from skeletal muscle and increasing their incorporation into glucose. Although growth hormone increases availability of glucose for milk synthesis (Peel et al., 1987), direct effects on gluconeogenesis have not been shown. Catecholamine and thyroxine evidently stimulate gluconeogenesis in dairy cows.

Fatty Acid Metabolism:

Lipid metabolism in the liver of ruminants has been reviewed (Bell, 1980; Emery et al., 1991). The liver of ruminants is a major organ for energy processing (Emery et al. 1992); which is similar to the situation in non-ruminants. In contrast to many non-ruminant species, however, the liver is quantitatively unimportant in fatty acid synthesis (Ballard et al., 1969). Most synthesis of fatty acids in ruminants occurs in adipose tissue (Vernon, 1980). Some synthesis of fatty acids from acetate occurs in ruminant liver. Constant demand for gluconeogenesis in ruminants places use of cytosolic OAA for lipogenesis at a disadvantage.

Fatty acids are taken up by liver in proportion to their blood flow and concentration reaching the liver (Bell, 1980; Emery et al 1992). Emery (1993) stated that, one-third of the circulating NEFA are received by the liver, which is considered very high relative to the proportion of body weight present as liver. Rate of uptake can be modified further by changes in the NEFA to albumin ratio, with high ratios favoring increased uptake

(Bell,1980). The fractional extraction of NEFA from blood by liver is about 10% in sheep (Katz et al., 1969) and early-lactating dairy cows (Reynolds et al., 1988). Major NEFA in ruminants are palmitic, stearic and oleic acids (Bell, 1980), with stearic acid being utilized more poorly by the liver than either palmitic or oleic acids. Fatty acids are toxic within cells and so are activated quickly to acetyl-CoA esters and either oxidized or esterified (Bell, 1980).

Oxidation of Fatty Acids:

About 10% of the NEFA taken up by ruminant liver are oxidized to CO_2 (Jesse et al., 1986 a, b; Lomax et al., 1983). Mitochondria from ruminant seems to oxidize NEFA at slower rates than mitochondria non-ruminants (Koundakjian et al., 1970).

A major fate of NEFA within the liver may be conversion to the ketone bodies, ACAA and BHBA. Hepatic ketogenesis in ruminants occurs in mitochondria via the 3-hydroxy-3-methylglutaryl- CoA (HMG-CoA) pathway, similar to non-ruminants (Bell, 1980). The limiting step for oxidation and ketogenesis from NEFA is catalyzed by carnitine palmitoyltransferase I (CPTI) (Butler et al., 1988), which regulates entry of NEFA into mitochondria. Ketogenesis thus depends to a great extent on delivery of NEFA to the liver, which increases during starvation and ketosis.

Alternate viewpoints describe increased ketogenesis within

mitochondria as either an inhibition of the citric acid cycle's activity, or as an over flow process in which citric acid cycle activity is unchanged (Ballard et al., 1969). Zammit (1984) suggests that citric acid cycle activity is regulated by NAD/NADH ratios so that total energy production is maintained.

Although most circulating acetate originates from fermentation in the reticulo-rumen and the lower gut, substantial production of endogenous acetate occurs in the liver of cows (Lomax et al., 1983; Reynolds et al., 1988; Snoswell et al., 1978)and sheep (Bergman et al., 1971). The liver may also utilize substantial quantities of acetate (Pethick et al., 1981).

Esterification of Fatty Acids:

Enzymes for esterification are located on the endoplasmic reticulum and the outer mitochondrial membrane. Alternate pathways besides esterification of glycerol-3-phosphate have been proposed (Benson et al., 1971). Herdt et al., (1988) reported large increases in activity of phosphatide phosphohydrolase early during development of induced fatty liver in sheep, whereas activities of glycerol-phosphate acyltransferase and diacylglycerol acyltransferase increased more slowly and to a lesser degree.

Export of Triglyceride from Liver:

In non-ruminant animals, NEFAs are esterified in the liver to form triglycerides, which then are packaged into VLDL and secreted (Havel,

1987). The capacity of ruminant liver to esterify fatty acids, however, evidently is greater than its capacity to export triglyceride as VLDL. Reasons for the apparently low capacity of ruminant liver to synthesize and/or secrete VLDL are not known. At least three hypotheses have been proposed. First, sheep hepatocyte have a continuous basal lamina that may physically limit secretion of large particles such as VLDL (Grub et al., 1971). Second, insufficient synthesis of apoprotien may limit rate of formation of VLDL (Herdt et al., 1983; Reid et al., 1980). Third, low rates of synthesis of phospholipid or cholesterol may limit formation of VLDL Brumby et al., 1975; Fronk et al., 1980; Herdt et al., 1983). The first hypothesis was rejected by Pullen et al. (1988), who directly measured secretion of labelled triglyceride from the liver after injection of a tracer fatty acid into a mesenteric vein.

The rate of synthesis of triglyceride in liver determines the rate of VLDL secretion in non-ruminants, and the supply of other components of VLDL is thought to be regulated by the rate of triglyceride synthesis. It seems that regulation of VLDL secretion in ruminant liver may be different from in non-ruminants.

Triglyceride synthesized in excess of the amount that can be exported as VLDL accumulates as lipid droplets and can results in fatty liver. Pullen et al.(1988) found that hepatic triglyceride in sheep exited in two pools: 1) A microsomal pool presumably associated with secretion of lipoprotein, and

2) a lipid droplets pool that is relatively inert. Turnover of the inert droplet pool was slower than the microsomal pool and 80 to 90% of the hepatic triglyceride was in the inert droplet pool. Pathways for removal of the accumulated lipid droplets are unknown in ruminants, but in non-ruminants very little transfer of droplet triglyceride to VLDL occurs; Rather, stored triglyceride in lipid droplets must first be hydrolyzed by lysosomal acid lipase (Debeer et al., 1982). The fatty acids that are liberated then can be oxidized or reesterified and secreted as VLDL.

Characteristics and metabolism of lipoprotein in ruminants have been reviewed (Emery, 1979; Grummer et al., 1988; Palmquist 1976). Bovine lipoprotein were isolated predominantly in the high-density lipoprotein (HDL) range (90%), with lesser amounts of LDL (10%) and VLDL or chylomicron (<1%). Apoprotein composition of bovine lipoprotein differs from that of non-ruminants (Grummer et al., 1987).

Metabolically, the VLDL and chylomicron fractions, are the most active in providing triglyceride-fatty acids to peripheral tissues, most of which will be taken up by the mammary gland in lactating cows (Palmquist et al., 1978).

With onset of lactation, metabolism of adipose tissue is redirected toward providing fatty acids to other organs of the body (Vernon, 1980). Lipolysis increases and lipogenesis ceases (McNamara et al., 1986; Pike et al., 1980; Smith et al., 1988). These changes were shown to begin during the last

requirements for the dry cow are the sum for maintenance, pregna month before parturition. The result of changes in adipose tissue metabolism during early lactation is a tremendous increase in the ability of adipose tissue to mobilize NEFA.

As discussed already, NEFAs are taken up in large quantities by liver, kidney, heart and skeletal muscle (Lindsay, 1975). It is likely that the mammary gland also takes up NEFA directly when concentrations of NEFA are increased in blood (Pullen et al 1984).

Peripheral utilization of ketone bodies by ruminants has been reviewed (Heitman et al., 1987). Although most production of ketone is by gut mucosa and liver as discussed already, there is evidence for production in skeletal muscle (Pethick et al., 1983). Unlike non-ruminants, brain and nervous tissue of ruminant animals do not use ketone bodies, even during starvation (Linsay et al., 1976). Ketone bodies are used, however, by heart, skeletal muscles, kidneys, and the lactating mammary gland (Heitman et al., 1987).

It has been assumed on the basis of early evidence (Bergman, 1971), that bovine ketosis is a problem of overproduction of ketone bodies, and that ketone body utilization is not limiting. Herdt (1988) suggested that utilization may be impaired at high concentrations. Utilization also may be decreased during deficiency of insulin (Balasse et al., 1971).

Ketone bodies exert several effects on metabolism and serve as

energy sources. Ketone bodies decrease proteolysis in skeletal muscle, and serve as primers for fatty acid synthesis.

In addition ketone bodies may decrease gluconeogenesis in ruminants (Radcliffe et al., 1983). Ketone bodies directly inhibit lipolysis in bovine adipose tissue (Metz et al., 1972), and stimulate secretion of insulin, which also acts to decrease lipolysis (Heitman et al., 1987; Gerloff et al., 1986).

SUMMARY

Nutrient ncy and reserve replenishment needs with additional requirements for growth during the first two pregnancies. Maintenance energy requirements can be dramatically increased by level of activity and adverse environmental conditions. A wide variety of feed ingredients can be successfully fed to dry cows as long as rations are appropriately formulated to meet energy, protein, minerals and vitamins requirements.

Dry matter intake declines as a cow approaches calving, therefore, dietary nutrient density needs to be adjusted to compensate. This suggests that a single dry period diet may be inappropriate to sufficiently meet requirements throughout the dry period. A close-up cow group may be important, especially for herds experiencing increased incidence of health disorders around calving.

This second dry cow group would also ease monitoring of cows for impending parturition, particularly those with uncertain breeding dates and metabolic disorders. The early dry cow ration is formulated for high fiber and low energy density while the close-up ration contains higher energy density

with less fiber. Both rations contain sufficient other nutrients based on expected intake. This two group system provides maximal flexibility in managing for optimum body condition at calving.

A good dry cow program should result in reduced incidence of metabolic diseases, complete pregnancy with available calf and maximize genetic potential for milk production. Overall a dry cow program is critical to performance.

MATERIALS AND METHODS

Eighty Holstein cows were used (n=80), forty primiparous (first lactation) and forty multiparous (had one lactation or more), in a complete randomized block design (CRBD). The cows were housed in MSU dairy barn and fed a diet(TMR) formulated according to NRC recommendations. Four weeks before the projected calving date, the experimental period began and continued until calving. Water was available 24 h a day. Cows entered the experiment in blocks of four, based on projected calving dates with primiparous and multiparous cows in separate blocks (20 blocks). Cows were fed ad libitum at about 1500 h, each day, one of four experimental diets, from four weeks prepartum until calving. Orts were measured at about 1300 h. The four diets contained:

1) Low energy and low protein (1.3 Mcal NEl/Kg and 12.2% crude protein (CP).
2) Medium energy and medium protein (1.49 Mcal NEl/Kg and 14.2% crude protein (CP).
3) Medium energy and high protein (undergradable intake protein added), (1.48 Mcal NEl/Kg and 16.2% CP).

4) High energy and high protein (1.61 Mcal NEl /Kg and 15.9% crude protein (CP).

* Diets and predicted intake are described in table 1 and 2.

After calving all cows were fed ad libitum according to NRC recommendations twice daily (0330 h and 1600 h), until week 10 postpartum.

Starting six weeks before the projected calving date, feed intake was recorded daily. Cows were weighed 2x/week (Thursday and Friday) at 0800 h every week until the fourth week postpartum, and then 2x / week every other week until the end of the experiment (10 weeks postpartum). Blood samples (for NEFA concentrations), as indicator for nutrient status, were collected via puncture of the tail vein 2x/week (Monday and Thursday) at about 1600 h, and daily starting 10 days before expected date of calving until 7 days postpartum. Milk yield was recorded daily (3x / d) and milk composition measured 2x / week(Mon. and Thr.). Fat, protein & lactose in milk were measured using an infrared analyzer (Multispec, Wheldrake, UK) at Michigan DHIA (East Lansing). Body condition score (1-5) was measured at two week intervals by three different people, who didn't know the treatments, and their scores were averaged. Urine samples for creatinine & 3-methyl-histidine concentrations as indicator of muscle mass and protein turnover, were collected 2x / week (Monday and Friday) at about 0700 h. until week 4 after calving & then every other week until the end of the

experiment.

A liver (25-50 mg) biopsy was taken at 24 h postpartum using a needle biopsy procedure. Health problems were recorded.

Feed Formulations:

Cows were housed in a free stall until 6 weeks prepartum & then moved to tie stalls until the end of the experiment. All cows were fed the low diet ad libitum(TMR) from 7 weeks prepartum until 4 weeks prepartum when the experimental diet started. Cows that were fed the high diet were fed the medium diet for two days before they started the experimental diet, in order to make a gradual shift from the low to the high energy diets. Total mixed ration (TMR) was fed throughout the trial (table 1 and 2). Silage dry matter was used to determine the amounts to be mixed to get the required energy & protein concentrations.

Feed samples for the experimental diets(samples of individual forages and other ingredients) were taken weekly for analysis of dry matter, CP, NDF, ADF, and Ash.

Blood Collection & NEFA Analysis:

Samples were collected in vacutainer tubes containing EDTA to prevent blood clotting. Samples were centrifuged the same day for 15 min. at 3000 rpm and 4° C, and plasma was then collected and frozen at -20° C until assayed.

A few months later 19 samples from, around day -35; -28; -21; -14; -10; -7; -4; -2; -1; -0; 1; 2; 4; 7; 14; 28; 42; 56; and 70, were used for NEFA analysis. Blood plasma samples were thawed overnight in the cold room (4° C), and 5 ul from each sample(triplicate) were pipetted into a 96-well microtiter plate. NEFA concentration was measured using NEFA-C Kit(Wako chemicals USA, Dallas, TX); as modified by McCutcheon and Bauman, 1986), in which 100 microliters from color reagent A were added and shaken in the biolog machine(Biolog, Inc., Hayward CA). After 30 minutes, 200 microliter of color reagent B were added and shaken in the biolog. Thirty minutes later optical density was read at 550 nm wave length.

Urine Collection & Analysis:

Samples were collected in 50 ml tubes, by rubbing the vulva until the cow urinate. About 5 ml from each sample was frozen (-20° C) for later analysis.

Urine samples from weeks -2; -1; +1; +2; and +3 weeks were thawed and centrifuged at 3000 rpm for 15 min. and 4° C to remove sediments. Five microliters were taken for creatinine analysis and the rest was refrozen for N-methyl histidine (N^{T}-MH) analysis. Raw urine samples (not deproteinized) were analyzed for creatinine using Sigma Kit number

555-A (Sigma chemical CO., St. Louis,Mo) based on the Jaffe reaction.

The procedure for N^T-MH analysis was reported by Simmons (1993). Samples for N^T-MH analysis were thawed and deproteinized with 50% sulfosalicylic acid (SSA); (0.9 ml urine & 0.1 ml 50% SSA). The samples were vortexed and centrifuged at 15000 rpm for 15 min. and 4° C. The supernatant was diluted 1:1 with sodium hexanesulfonate because this served as the mobile phase in the chromatograph. The amount of sodium hexane sulfonic acid needed for dilution was based on the size of the peaks that were eluted after different dilutions of the samples.

After dilution the mixture was vortexed and placed into a high-performance liquid chromatography (HPLC) vial. The samples were chilled at 4° C and then analyzed by reversed-phase HPLC separation using ion-pairing and post-column derivatization with o-phthaldehyde and fluorescence detection (Friedman and Smith, 1980), N^T-MH peaks in the samples were identified on the bases of retention time as compared with N^T-MH standards. Concentrations of N^T-MH in samples were calculated by comparing the peak area of N^T-MH in standards (50 mg/ml) and samples.

A stock solution of 5 mM sodium hexanesulfonate with a pH 3.2 was used as the mobile phase in the chromatograph. Sodium hexanesulfonate increases the retention of and allows for good resolution of N^T-MH. The solution consisted of 1 L of HPLC-quality water and 0.94 g 1-hexane sulfonic acid, and sodium salt (Aldrich, Milwaukee, WI; F.W. 188.22, 98%).

Approximately 1 ml glacial acetic acid was added dropwise to lower the pH to 3.2. The solution was stirred for 30 to 45 minutes and then it was allowed to stand for 30 to 45 minutes. Next, the solution was filtered through a 0.45 um aqueous filter (47 mm diameter; Millipore Corp., Bedford, MA) with a glass prefilter (Whatman GF/C, 4.25 cm; Whatman, Inc., Clifton, NJ) on top of a Millipore-type all glass vacuum filtration system with a 1 L flask. Lastly, a vacuum was applied for about one minute to degas the solution. The solution was stirred rapidly with a magnet while it was being degassed.

The o-phthaladehyde reagent was prepared by dissolving 30 g of boric acid in 1 L of HPLC-quality H20. To adjust pH, 20 g of potassium hydroxide was added to the solution first, then approximately 5 g of potassium hydroxide was added slowly until a pH of 10.4 was achieved. The solution was stirred for at least 30 minutes and then it was allowed to stand for at least 30 minutes. Next, the solution was filtered in the same manner as the mobile phase; however, the solution was not degassed. Separately, a solution which contained 600 mg fluoraldehyde (Pierce OPA crystals, catalog no. 26015; Pierce chemical CO., Rockford, IL), 10 ml ethanol, 200 ml of β-mercaptoethanol, and 1 ml of 30% (W/V) aqueous solution of Brij 35 (Pierce chemical CO., Rockford, IL) was prepared. The o-phthalaldehyde solution and the borate solution was mixed in a dark glass bottle just before it was used and stored under N2 gas. The borate solution

was prepared one day ahead of time, but the O-phthaldehyde solution was prepared just prior to use.

Injections of 25 ml of the samples were added to a 25cm by 4.6 mm internal diameter, Vydac C-18 peptide/protein column (Rainin Instrument Co. Inc., Wodurn, MA). The flow rate of the mobile phase was 0.8 ml/min and isocratic conditions were used to separate N^{T}-MH from other urinary components. The column was periodically washed with acetonitrile to remove non-polar contaminants which were retained on the column.

Calculations of NMH (nmoles/ml urine) concentrations:

1) nmoles NMH standard injected = ng NMH injected * 1 nmole/169.2 ng

2) nmoles NMH/injection = sample NMH area * nmoles NMH standard injected/standard NMH area

3) nmoles NMH/ml urine = nmoles NMH/injection * 1 injection/ul sample * 1000 ul/ml

Statistical Models

The variations between treatments among different parameters from the chosen independent variables was evaluated using the generalized linearmodel procedure of (SAS, 1995) in the following models:

Liver Fat

Y_{ijk} $= \mu + \alpha_i + B_{j(i)} + \gamma_k + \alpha\gamma_{ik} + E_{(ijk)}$

Y_{ijk} = Observed response for different liver fat parameters.

μ = Overall mean.

α_i = Fixed effect of ith parity level .

Bj(i) = Random effect of jth block level within ith parity level (to test parity).

γ_k = Fixed effect of kth treatment level .

$E_{(ijk)}$ = Random residual effect.

Body Weight:

Y_{ijkl} $= \mu + \alpha_i + B_{(i)j} + \gamma_k + \alpha\gamma_{ik} + \gamma B_{(i)jk} + \delta_l + \alpha\delta_{il} + \delta\gamma_{lk} + \alpha\gamma\delta_{ikl} + E_{(ijkl)}$

Y_{ijkl} = Observed response for body weight.

μ = Overall mean.

α_i = Fixed effect of jth parity level.

$B_{j(i)}$ = Random effect of jth block level within ith parity level (to test parity).

γ_k = Fixed effect of kth treatment level.

δ_l	= Fixed effect of lth week level.
$E_{(ijkl)}$	= Random residual effect.

Body Condition Score (BCS):

Y_{ijk}	$= \mu + \alpha_i + B_{j(i)} + \gamma_k + \alpha\gamma_{ik} + E_{(ijk)}$
Y_{ijk}	= Observed response for BCS.
μ	= Overall mean.
α_i	= Fixed effect of ith parity level .
Bj(i)	= Random effect of jth block level within ith parity level (to test parity).
γ_k	= Fixed effect of kth treatment level .
$E_{(ijk)}$	= Random residual effect.

Nonesterified fatty acids (NEFA):

Y_{ijkl}	$= \mu + \alpha_i + B_{(i)j} + \gamma_k + \alpha\gamma_{ik} + \gamma B_{k(i)j} + \delta_l + \alpha\delta_{il} + \delta\gamma_{lk} + \alpha\gamma\delta_{ikl} + E_{(ijkl)}$
Yijkl	= Observed response for NEFA.
μ	= Overall mean.
α_i	= Fixed effect of jth parity level.
$B_{j(i)}$	= Random effect of jth block level within ith parity level (to test parity).
γ_k	= Fixed effect of kth treatment level.

δ_l = Fixed effect of lth day level.

$E_{(ijkl)}$ = Random residual effec.

Milk and Milk Components:

Y_{ijkl} $= \mu + \alpha_i + B_{(i)j} + \gamma_k + \alpha\gamma_{ik} + \gamma B_{(i)jk} + \delta_l + \alpha\delta_{il} + \delta\gamma_{lk} + \alpha\gamma\delta_{ikl} + E_{(ijkl)}$

Y_{ijkl} = Observed response for milk yield and components.

μ = Overall mean.

α_i = Fixed effect of jth parity level.

$B_{j(i)}$ = Random effect of jth block level within ith parity level to test parity).

γ_k = Fixed effect of kth treatment level.

δ_l = Fixed effect of lth week level.

$E_{(ijkl)}$ = Random residual effec.

Dry Matter Intake:

Y_{ijkl} $= \mu + \alpha_i + B_{(i)j} + \gamma_k + \alpha\gamma_{ik} + \gamma B_{(i)jk} + \delta_l + \alpha\delta_{il} + \delta\gamma_{lk} + \alpha\gamma\delta_{ikl} + E_{(ijkl)}$

Y_{ijkl} = Observed response for dry matter intake.

μ = Overall mean.

α_i = Fixed effect of jth parity level.

$B_{j(i)}$ = Random effect of jth block level within ith

parity **level (to test parity).**

γ_k = **Fixed effect of kth treatment level.**

δ_l = **Fixed effect of lth week level.**

$E_{(ijkl)}$ = **Random residual effec.**

<u>Energy Balance:</u>

$$Y_{ijkl} = \mu + \alpha_i + \gamma_k + \alpha\gamma_{ik} + C_{j(ik)} + \delta_l + \alpha\delta_{il} + \delta\gamma_{lk} + \alpha\gamma\delta_{ikl} + E_{(ijkl)}$$

Y_{ijkl} = **Observed response for energy balance.**

μ = **Overall mean.**

α_i = **Fixed effect of jth parity level.**

$C_{j(ik)}$ = **Random effect of cows within ith parity and kth treatment.**

γ_k = **Fixed effect of kth treatment level.**

δ_l = **Fixed effect of lth week level.**

$E_{(ijkl)}$ = **Random residual effect.**

Formulas used for energy balance (NRC, 1989) (Mcal / lb):

Energy for milk production = Milk yield * 2.2 (41.63 * fat + 24.13 * protein + 21.6 * lactose - 11.72)/1000

Energy for maintenance :

Lactating cows = $0.08 * BW^{.0.75}$

Dry cows = $0.104 * BW^{.0.75}$

Results

Intake and Energy Balance:

No differences in DMI were observed among the different treatments prepartum ($P > 0.58$; Table 3) or postpartum ($P > 0.7$). However there were differences in intake of CP, NDF and NEl ($P < 0.05$) and that is because the diets were formulated to obtain such differences. Prepartum feed intake was higher for multiparous cows than for primiparous cows ($P < 0.01$). There was a less decline in the dry matter intake in the last three weeks before calving for primiparous cows(13%) than for multiparous cows(18%; $P<0.01$; Figure 1 and 2). Dry matter intake increased about 53% for primiparous cows from week 1 to week 9 postpartum, and about 72% for multiparous cows ($P<0.01$).

Data from week 4 prepartum and week 10 postpartum for energy balance were dropped because of missing values. Energy balance increased prepartum as dietary energy density increased ($P < 0.02$), and all cows were in positive energy balance prepartum except the primiparous cows fed the low diet. Multiparous cows had higher energy balance than primiparous cows prepartum ($P < 0.01$), but there was no difference postpartum ($P > 0.2$). All cows were in negative

energy balance in the first 10 weeks postpartum. Dietary treatments did not affect energy balance postpartum ($P > 0.4$). There was a tendency for the calculated energy balance of primiparous cows to drop more rapidly toward calving (83%) than it did for the multiparous cows (57%), i.e. parity*week interaction ($P < 0.1$).

Non-esterified Fatty Acids:

Prepartum NEFA concentrations were lower for multiparous cows than for primiparous cows, and the reverse is true postpartum ($P < 0.05$). The concentrations of NEFA increased from 90 μeq/L at 4 weeks prepartum to about 1000 μeq/L around calving for multiparous cows (Figure 4), and 800 μeq/L for primiparous cows (Figure 3; $P < 0.01$). The concentrations dropped after calving until they became stable at 4 weeks postpartum, which was the same as 4 weeks prepartum. Dietary treatments did not affect NEFA concentrations prepartum for primiparous cows ($P > 0.67$; Table 4), but there was a difference prepartum in NEFA concentration for multiparous cows due to treatments ($P < 0.01$). The cows that were fed the L diets had higher blood NEFA concentrations than that which were fed the H diets. Multiparous cows tend to mobilize more NEFA before calving than primiparous cows i.e. parity*day interaction ($P < 0.05$). Multiparous cows receiving different dietary treatments responded differently with respect to NEFA concentrations during prepartum period.

The cows fed L and M diets had more NEFA than the cows fed H and MH diets interaction ($P < 0.01$). Multiparous cows fed L diet prepartum had more NEFA than cows fed H diet ($P < 0.01$). Also multiparous cows fed M diet had higher NEFA than cows fed MH diets ($P < 0.05$).

Postpartum NEFA concentrations for primiparous cows were not affected by dietary treatments ($P > 0.62$), and the same results were obtained from the multiparous cows ($P > 0.43$).

Liver Fatty Acid:

Liver fatty acid results are shown in table 5. Liver fatty acid concentrations (mg/g wet liver) at calving were higher for multiparous than for primiparous cows ($P < 0.05$). Dietary treatments did not affect liver fatty acid concentrations for primiparous ($P > 0.45$) or multiparous cows ($P > 0.19$), also they did not affect triglyceride fatty acid per gram liver for primiparous cows ($P > 0.42$) or multiparous cows ($P > 0.16$).

There was a treatment effect ($P<0.07$) on liver fatty acid and liver TG when we combined the multiparous and primiparous cows, in which the (LL) diets were higher than the (HH) diets ($P<0.02$). Diets also had no effect on liver fatty acid percentages for primiparous ($P > 0.1$) or multiparous cows ($P > 0.91$). However, the numerical values for liver fatty acid contents tend to decrease with the increase of energy density.

<u>Body Condition Score (BCS) and Body Weight (BW):</u>

Body condition scores were adjusted using BCS 4 weeks prepartum as covariate, and the first block from each parity was taken away from the statistical analysis because of several missing values. Body condition score was higher prepartum for multiparous cows than primiparous cows ($P < 0.01$; Table 6), and there was no difference postpartum ($P > 0.34$). Body condition scores decreased from around 3.25 at 4 weeks prepartum to around 2.25 at 10 weeks postpartum (Figure 5 and 6). There were no differences among treatments prepartum ($P > 0.51$) or postpartum ($P > 0.71$).

Dietary treatments did not affect BW prepartum ($P > 0.12$) or postpartum ($P > 0.12$), but they affected changes in BW for multiparous cows. Multiparous cows had higher BW than primiparous cows ($P < 0.01$). Body weights increased about 7% from 4 weeks prepartum to calving ($P < 0.01$), and then dropped sharply after calving until about two weeks postpartum after which they remained fairly stable (Figure 7 and 8). Multiparous cows lost more weight postpartum than primiparous cows fed the same diets i.e. wk*Parity interaction ($P < 0.01$). As crude protein increased in the diet for multiparous cows before calving, they gain more weight before calving ($P<0.05$) and lost more weight after calving ($P < 0.05$).

Milk Yield and Milk Components:

There was no effect of different treatments on milk yield for primiparous cows ($P > 0.28$; Table 7) and multiparous cows ($P > 0.99$).

Milk yield was higher for multiparous cows than primiparous cows ($P < 0.01$). Milk yield increased with time after calving and reached the maximum in about nine weeks ($P<0.01$; Figure 9). Multiparous cows reached the maximum milk production (6 wk) before primiparous cows (10 wk) i.e. parity*week interaction ($P<0.01$). There was no difference between the different treatments on milk components.

Diseases

Incidence of milk fever, ketosis, displaced abomasum, metritis, clinical mastitis and udder edema has been summarized in Table 8. The prepartum diets did not significantly affect the incidence of peripartum diseases ($P > 0.2$).

Urinary N^{T}-MH and Creatinine:

The urinary concentration of creatinine for primiparous cows were higher than for multiparous cows (Table 9; $P<0.08$). The different treatment had no effect on urinary N^{T}-MH, creatinine, and their ratios ($P>0.1$), except for the ratios prepartum for primiparous cows ($P<0.01$) and multiparous cows ($P<0.06$). The H and MH diets had higher ratios than the L and M diets respectively. The decrease in urinary creatinine concentrations from 2 wks prepartum to 3 wks postpartum was about 47% for the primiparous cows. Urinary N^{T}-MH to creatinine ratios dropped about 4% for the primiparous cows), and 24% for multiparous cows (Fig. 11), from 2 wks to 1 wk prepartum, and then increased about 46% for primiparous and 73% for multiparous cows at 1 wk postpartum, and then dropped again about 21% for primiparous and 32% for multiparous cows at 3 wks postpartum.

APPENDICES

APPENDIX A

Table 1. Ingredients of The Diets

Treatment				
Ingredients %DM	LL	MM	MH	HH
Alfalfa Silage	31.4	32.9	32.2	33.2
Corn Silage	30.3	20.9	20.6	11.8
Cotton Seed Hulls	30.0	15.4	15.7	1.8
Corn Grain Cracked	0.0	0.0	0.0	18.0
Corn Grain Ground	5.4	23.4	20.0	23.2
Soybean Meal 44	0.9	5.5	1.4	10.0
Soyplus	0.0	0.0	7.4	0.0
Anion Salts	1.3	1.3	1.3	1.3
Ca17 P21	0.3	0.1	0.1	0.0
Lime	0.0	0.1	0.1	0.3
Blood Meal	0.0	0.0	0.8	0.0
Mineral/Vitamin	0.4	0.4	0.4	0.4

LL = Low energy, low protein diet.
MM = Medium energy, medium protein diet.
MH = Medium energy, high protein diet.
HH = High energy, high protein diet.

APPENDIX B

Table 2. Ls MEans For Prepartum Nurtrient Intake Of The Diets (Primiparous and Multiparous cows)

Treatment				
Nutrient Intake	LL	MM	MH	HH
DM, Kg/d	11.5	12.2	12.5	13.0
CP, Kg/d	1.4	1.7	2.0	2.1
NEL, Mcal/d	14.9	18.2	18.5	21.2
NDF, Kg/d	5.9	4.9	5.0	3.8
ASH, Kg/d	1.0	1.0	1.1	1.1

LL = Low energy, low protein diet
MM = Medium energy, medium protein diet.
MH = Medium energy, high protein diet.
HH = High energy, high protein diet.

APPENDIX C

Table 3. Dry matter and nutrient intake, and energy balance of cows. (Least square means)

	Treatments					
	LL	MM	MH	HH	SEM	P
No.of cows	10	10	10	10		
DM (Kg/d)						
Primiparous	9.2	10	10.5	10.3	1.0	0.67
Multiparous	13.8	13.8	14.4	15.7	1.2	0.58
CP (Kg/d)						
Primiparous	1.1	1.5	1.7	1.7	0.20	0.02
Multiparous	1.7	2.0	2.3	2.5	0.2	0.01
NDF (Kg/d)						
Primiparous	4.7	4.3	4.2	3.0	0.4	0.04
Multiparous	7.1	5.5	5.9	4.8	0.01	0.01
NEL (Mcal/d)						
Primiparous	11.9	15.9	15.7	16.3	1.5	0.09
Multiparous	18.0	20.5	21.3	25.2	1.8	0.02
Energy balance 3wksprepartum						
Primiparous	-1.3	2.9	2.1	4.4	1.4	0.02
Multiparous	2.7	4.8	6.1	10.3	1.4	0.01
Energy balance 9wks postpartum						
Primiparous	-2.5	-0.9	-0.4	-2.0	0.9	0.4
Multiparous	-1.3	-2.4	-4.4	-1.4	1.4	0.4

Energy balance units (Mcal NE_l/d)

APPENDIX D

Table 4. Least squares means (ueq/L)for nonesterified fatty acid (NEFA)

Treatments						
	LL	MM	MH	HH	SEM	P
No. of cows	10	10	10	10		
4 wks prepartum to calving						
Primparous	258	285	266	220	32.6	0.7
Multiparous[1]	202	221	166	134	14.9	0.01
2 wks prepartum to calving						
Primparous	307	349	322	266	45.1	0.73
Multiparous[1]	243	274	194	148	21.5	0.01
Calving to 10 wks postpartum						
Primparous	309	301	307	347	25.5	0.62
Multiparous	415	429	459	517	45.1	0.43
Calving to 2 wks pospartum						
Primparous	422	413	410	478	42.5	0.71
Multiparous	595	595	636	725	70.2	0.56

[1] Contrast LL vs HH($P < 0.01$), and MM vs MH ($P < 0.05$)

APPENDIX E

Table 5. Least squares means for liver fatty acids

Treatments						
	LL	MM	MH	HH	SEM	P
No. of cows	8	6	9	7		
mg of total fatty acids per g liver						
Primparous	26.63	18.61	20.12	18.91	4.2	0.48
Multiparous[1]	40.1	34.3	31.0	27.5	4.7	0.23
mg of triglyceride fatty acids per g liver						
Primparous	10.22	7.5	8.85	5.63	2.1	0.42
Multiparous[1]	20.15	22.7	14.4	13.4	3.8	0.17
% fatty acids in TG per total fatty acids in liver						
Primparous	33.76	39.0	41.22	27.29	4.3	0.1
Multiparous	52.3	50.4	46.3	46.0	4.9	0.90

[1] No. of cows for multiparous, LL = 9; MM = 6; MH = 9; HH = 7

APPENDIX F

Table 6. Least squares means for body condition score (BCS)[1]and body weight (BW); kg

	Treatments					
	LL	M M	M H	H	SEM	P
No. of cows	9	9	9	9		
BCS 1 wk prepartum (cov - 4 wk)						
Primparous	3.35	3.33	3.43	3.39	0.1	0.64
Multiparous[1]	3.49	3.50	3.50	3.63	0.1	0.51
BW last month prepartum						
Primparous	634	602	628	584	16.9	0.12
Multiparous[1]	755	756	750	726	19.7	0.62
BW first 10 wks postpartum						
Primparous	634	602	628	584	16.9	0.12
Multiparous[1]	648	649	644	635	17.4	0.94
BCS wk 5 to 10 postpartum (cov -4 wk)						
Primparous	2.46	2.49	2.61	2.50	0.1	0.73
Multiparous	2.30	2.32	2.36	2.43	0.2	0.71

[1] BCS is a five point scale (1 = extremely thin, to 5 = overconditionned)

APPENDIX G

Table 7. Least squares means for milk yield and composition

Treatments						
	L	M	MH	H	SEM	P
No. of cows	10	10	10	10		
Milk Yield (kg/d)						
Primparous	35.4	32.1	31.5	32.9	3.2	0.28
Multiparous[1]	45.4	45.5	45.0	44.8	3.4	0.99
SCM (Kg/d)						
Primparous	32.5	30.2	30.0	31.1	2.5	0.39
Multiparous[1]	42.1	43.5	42.6	43.3	2.8	0.88
Fat% Primparous	3.5	3.7	3.7	3.7	0.1	0.57
Fat% Multiparous[1]	3.6	3.8	3.7	3.8	0.1	0.40
Fat (Kg/d)						
Primparous	1.2	1.2	1.1	1.2	0.1	0.80
Multiparous	1.6	1.7	1.6	1.7	0.1	0.70
Protein %						
Primparous	3.0	3.0	3.1	3.1	0.1	0.62
Multiparous	3.1	3.1	3.0	3.2	0.1	0.08
Protein (Kg/d)						
Primparous	1.0	1.0	1.0	1.0	0.1	0.26
Multiparous	1.4	1.4	1.4	1.4	0.1	0.81
Primparous	5.0	5.0	4.9	4.9	0.04	0.82

Table 7. (Cont'd)

Lactose %						
Primiparous	5.0	5.0	4.9	4.9	0.04	0.82
Multiparous	4.9	4.9	5.0	5.0	0.1	0.51
Lactose (Kg/d)						
Primiparous	1.8	1.6	1.6	1.6	0.2	0.19
Multiparous	2.2	2.3	2.3	2.2	0.2	0.99
SCC Primiparous	123	112	186	204	40	0.31
SCC Multiparous	183	246	245	180	74	0.86

[1] Contrast L vs H, and M vs MH P< 0.07

APPENDIX H

Table 8. Numbers of selected diseases peripartum

	Treatments			
	LL	MM	MH	HH
Milk fever	2	2	2	2
Ketosis	2	3	5	5
Displaced abomasum	1	3	2	0
Metritis	0	2	0	3
Clinical mastitis	1	1	0	1
Udder edema	0	0	1	0

X^2 test was used, non is significant ($P>0.20$)

APPENDIX I

Table 9. Mean for Urine Creatinine (mg/dl) and N^{τ}Methylhistidine (nmols/ml)

Treatment						
Item	L	M	MH	H	SEM	P
Creatinine Prepartum						
Primiparous	195	192	186	198	27	0.9
Multiparous	171	169	169	166	16	0.9
N^{T}-MH Prepartum						
Primiparous	214	234	264	289	30	0.4
Multiparous	165	238	195	222	40	0.1
N^{T}-MH to Creatine ratio Prepartum						
Primiparous[1]	111	117	146	141	12	0.01
Multiparous	101	142	122	145	24	0.06
Creatine Postpartum						
Primiparous	109	108	124	120	11	0.4
Multiparous	103	85	104	97	9	0.4
N^{T}-MH Prepartum						
Primiparous	192	165	220	215	26	0.3
Multiparous	173	139	152	162	27	0.7
N^{T}-MH to Creatine ratio Postpartum						
Primiparous	170	153	174	176	11	0.3
Multiparous	159	156	145	165	12	0.8

[1] Contrast H vs L, and M vs MH ($P < 0.01$)

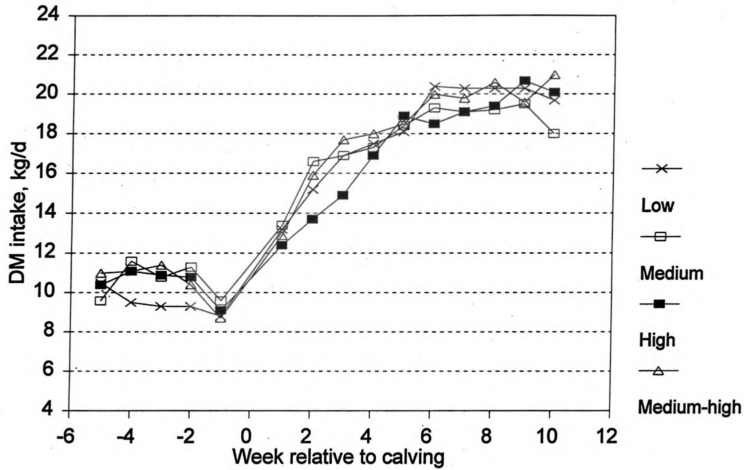

Figure 1. DMI, Primiparous Cows

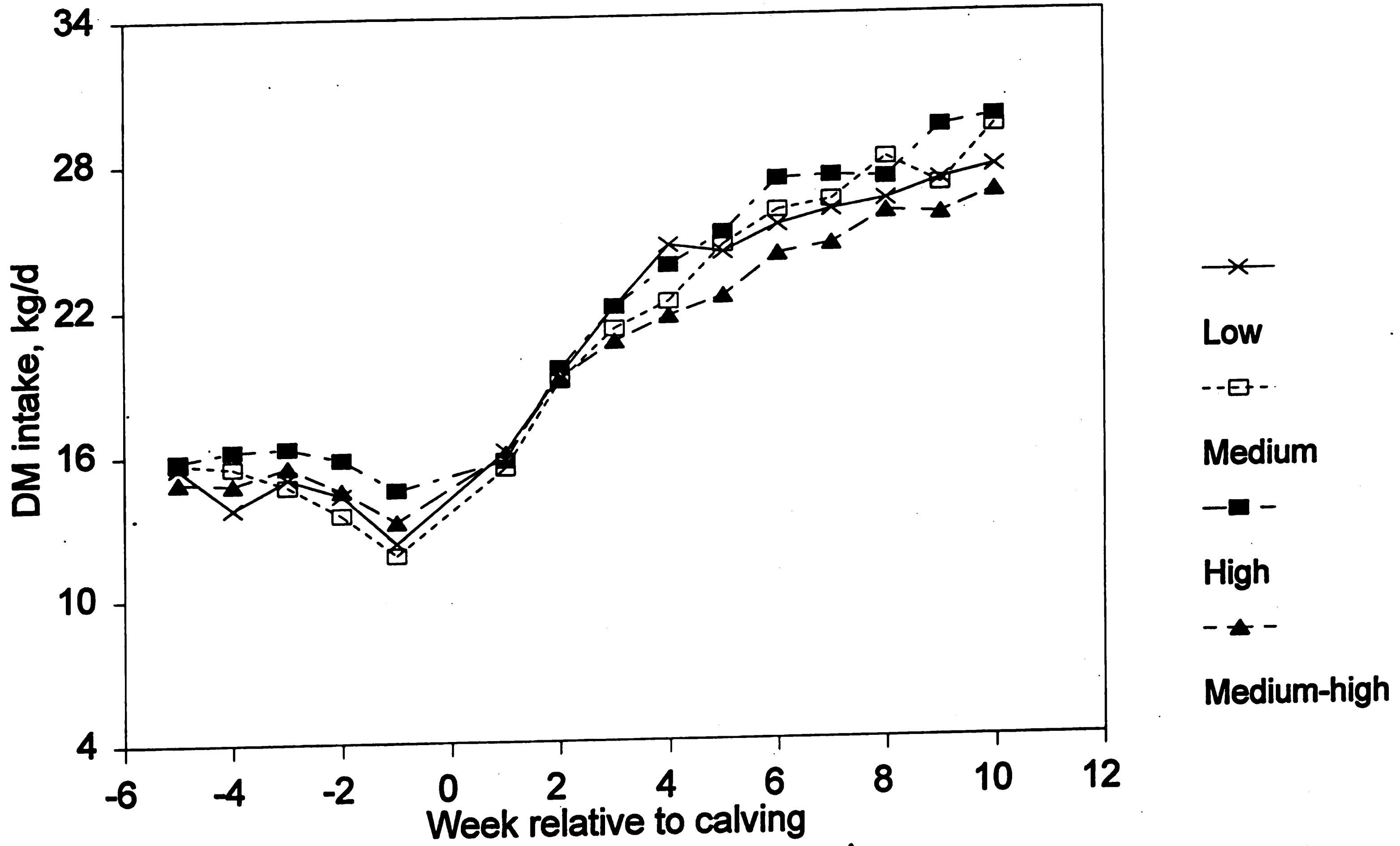

Figure 2. DMI, Multiparous Cows

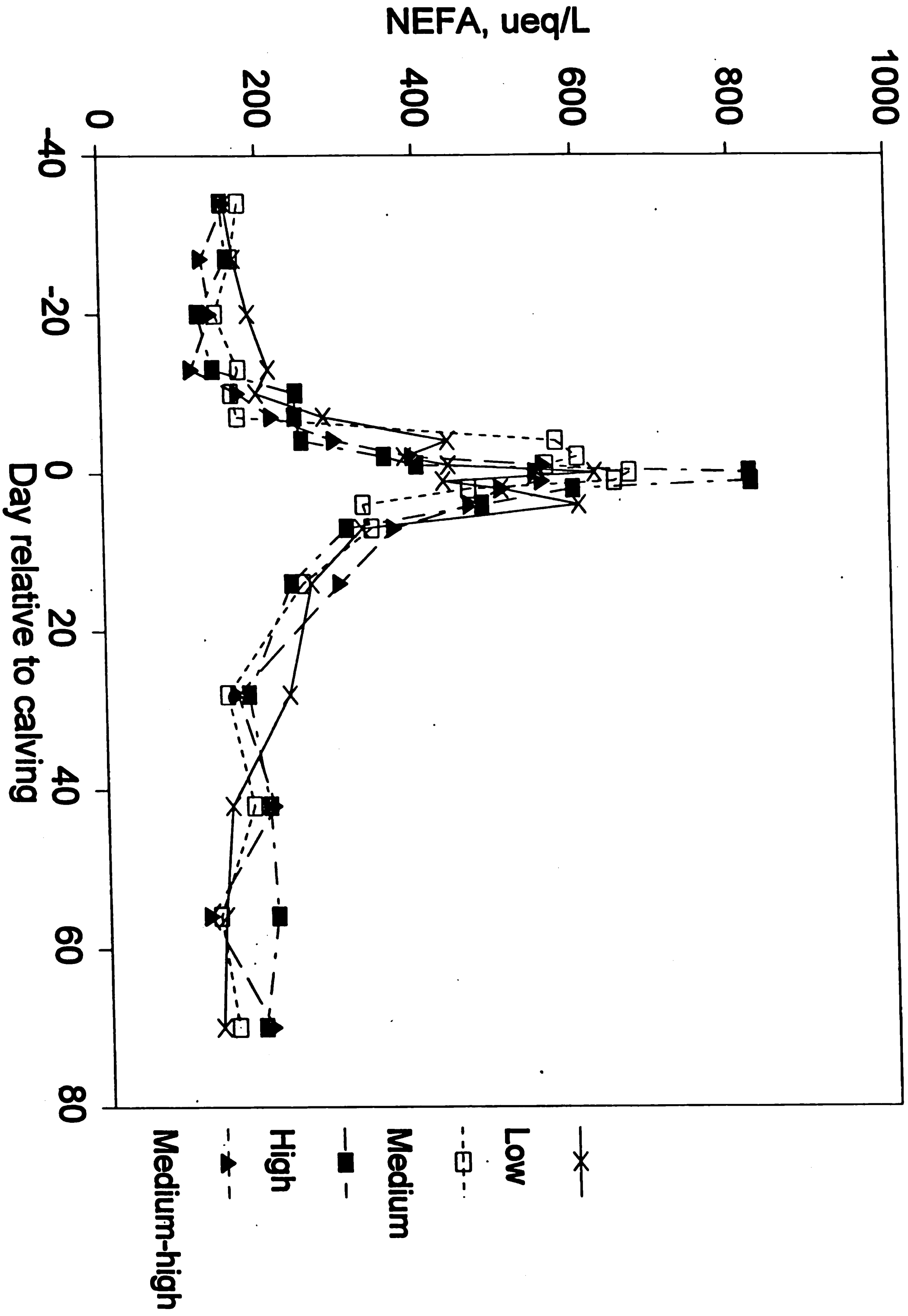

Figure 3. Plasma NEFA-Primiparous cows

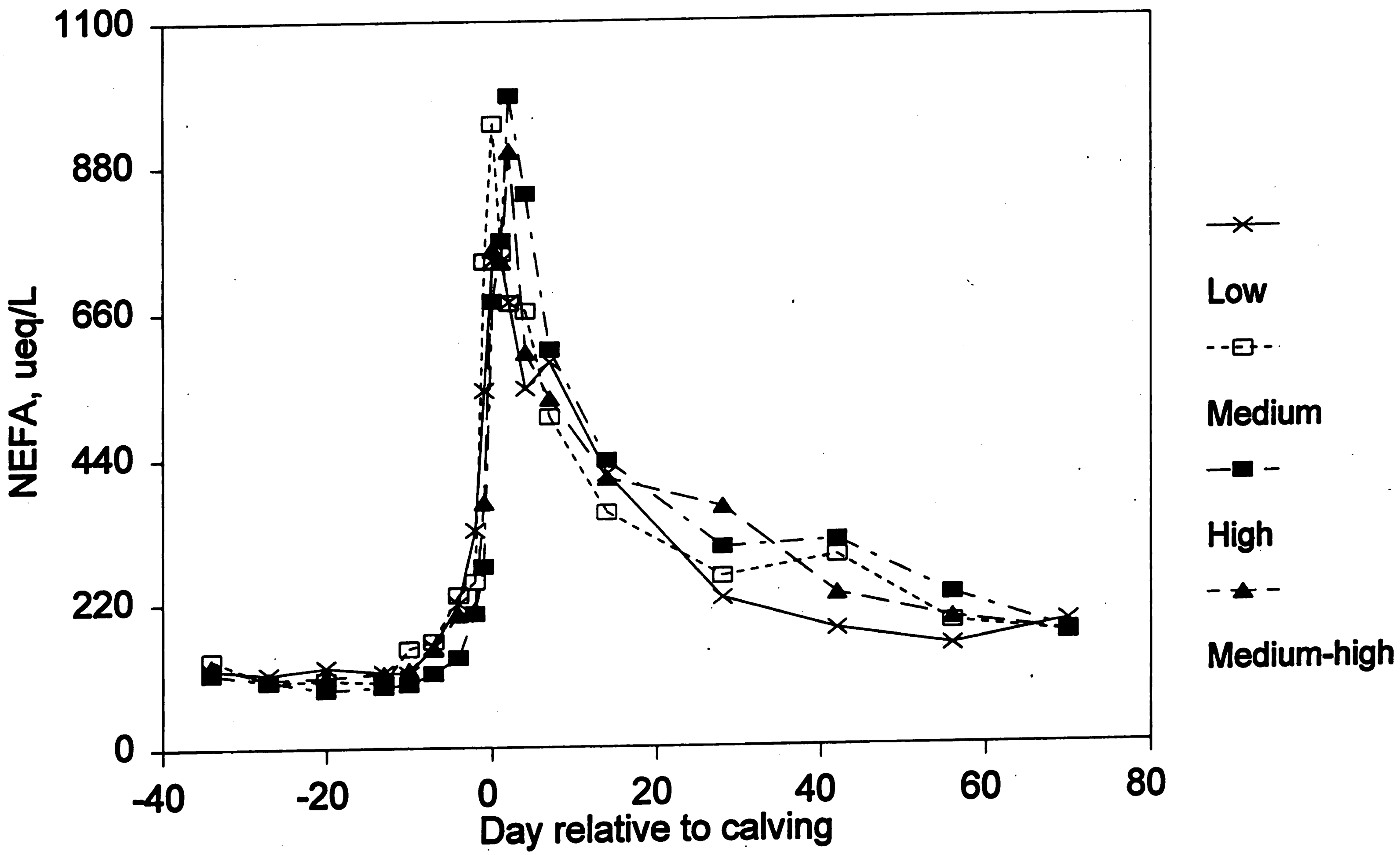

Figure 4. Plasma NEFA-Multiparous cows

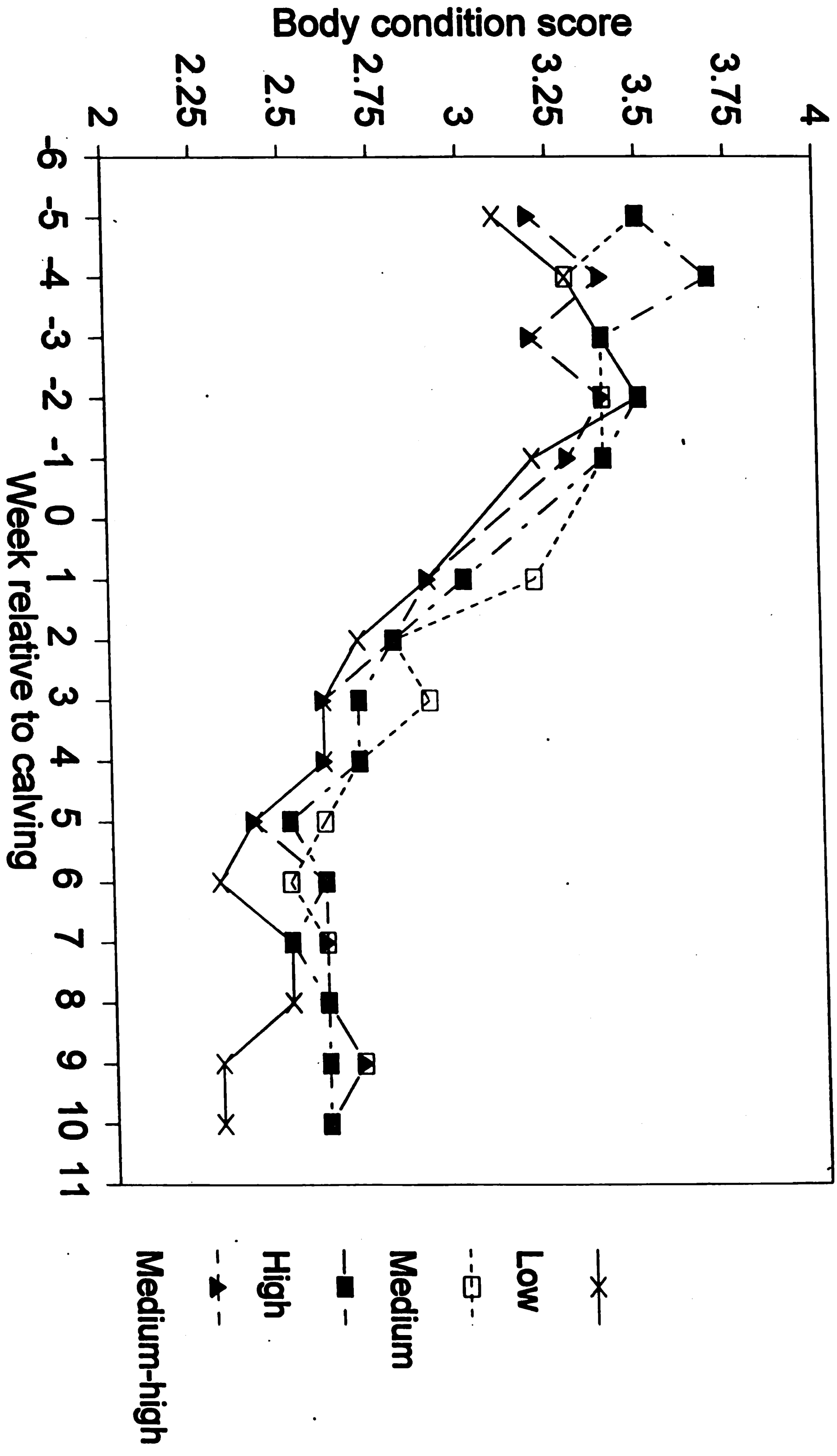

Figure 5. BCS, Primiparous cows

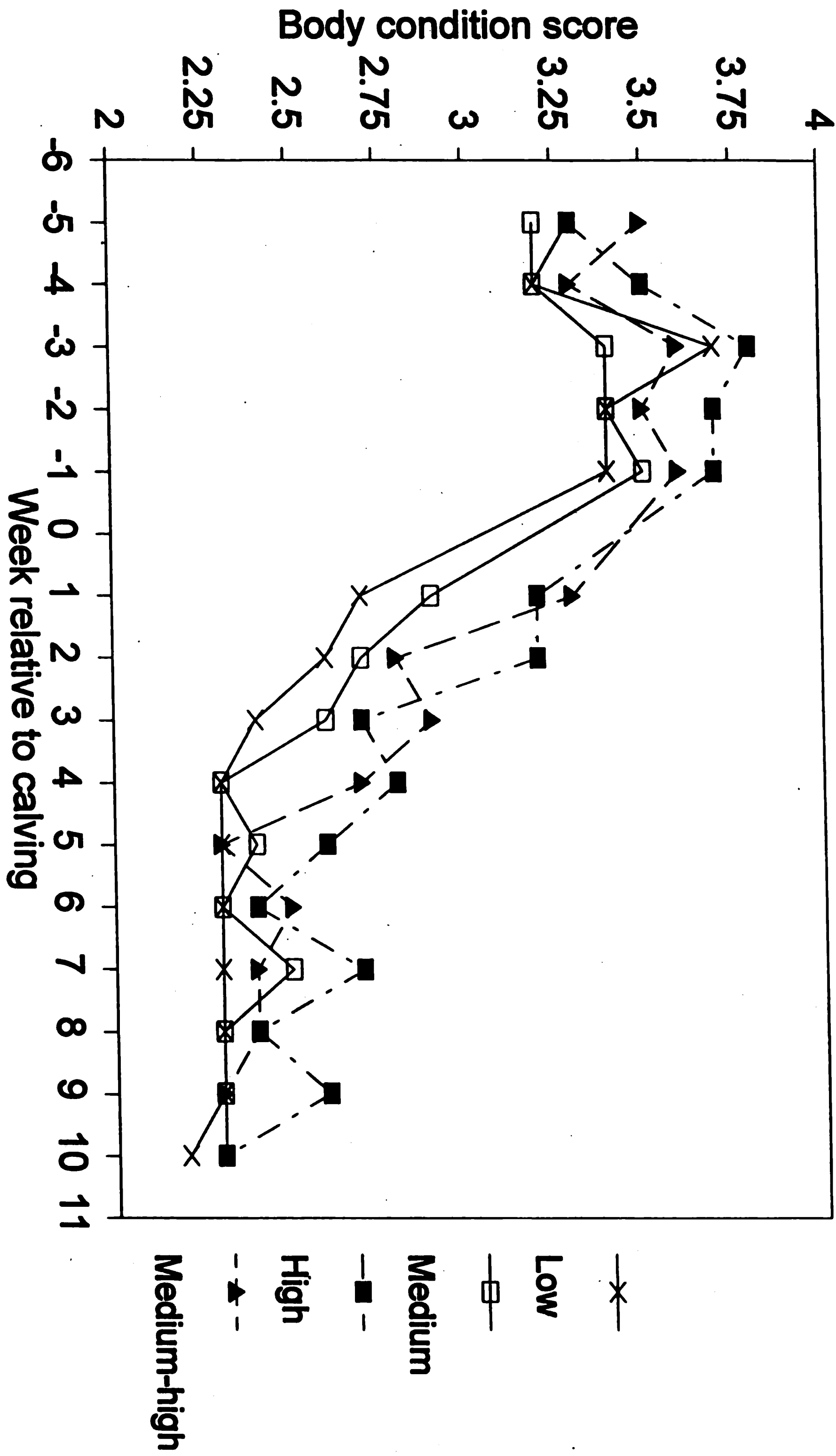

Figure 6. BCS, Multiparous cows

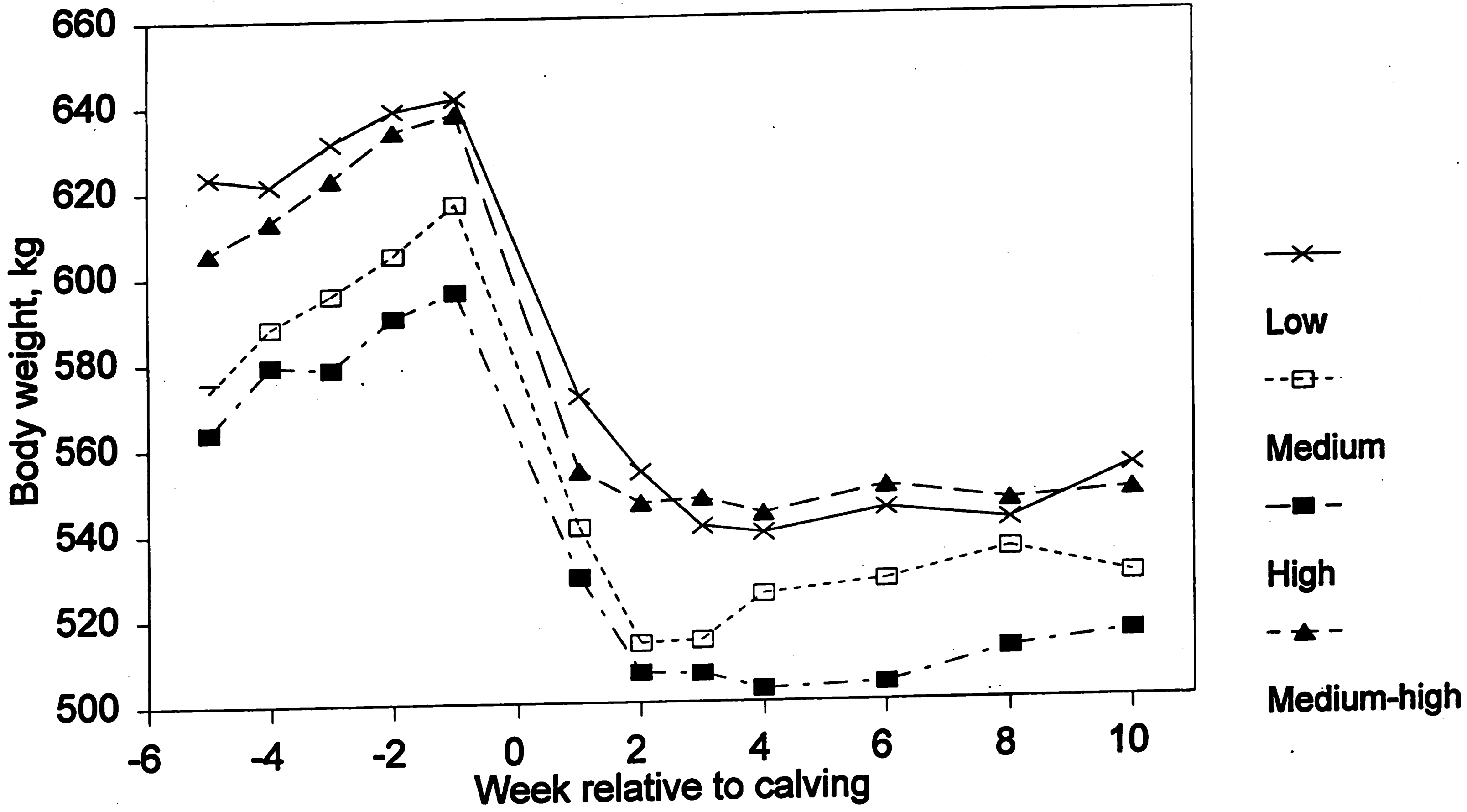

Figure 7. BW - Primiparous cows

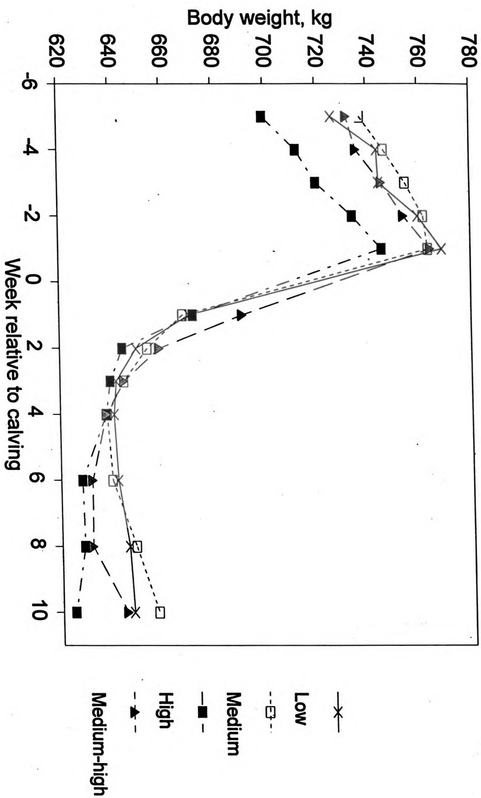

Figure 8: BW - Multiparous cows

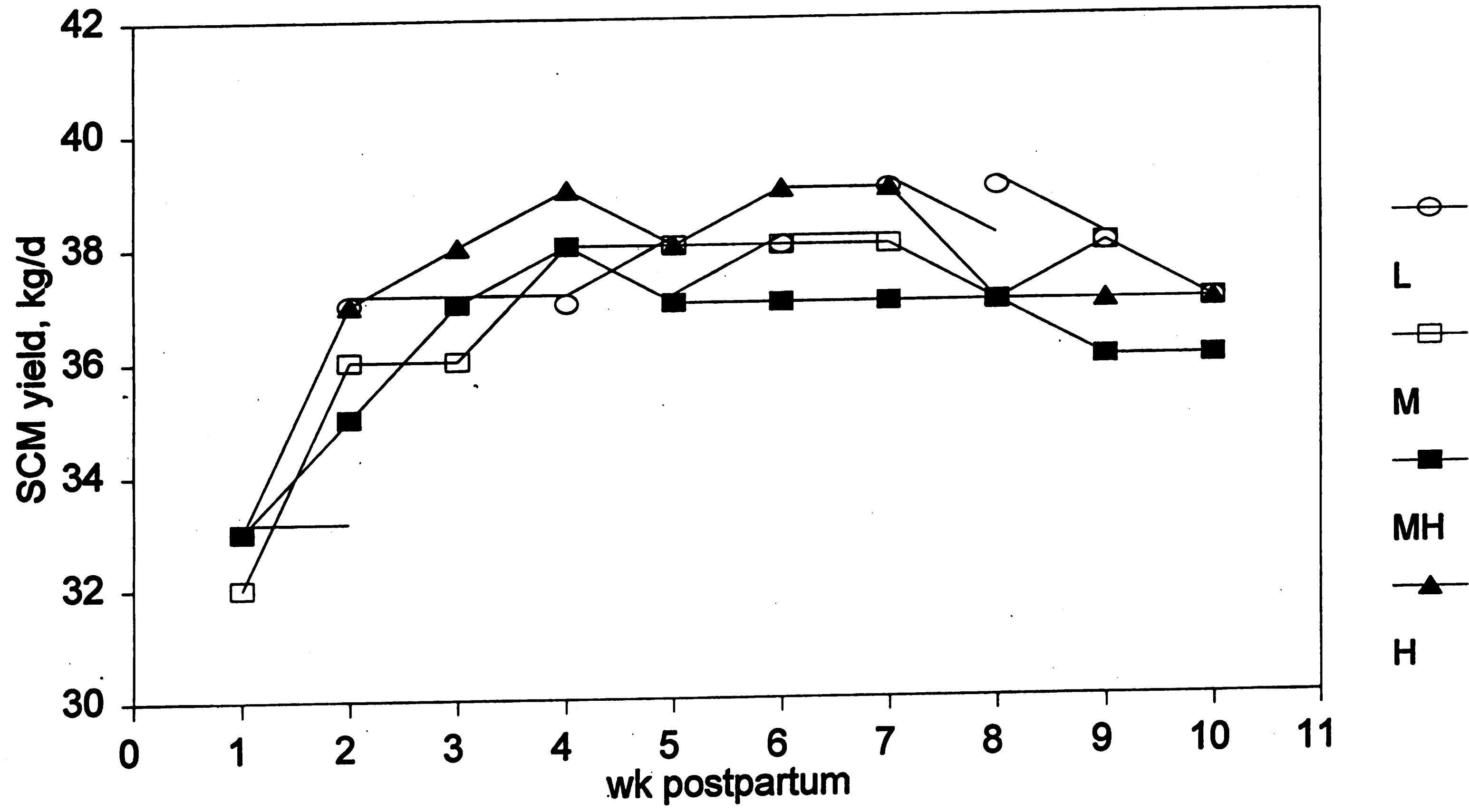

Figure 9. SCM Yield of Cows

Discussion

Several investigators (Marquardt et al., 1977; Van saun et al., 1989; Zamet et al., 1979; Johnson et al., 1981) have also shown increased DMI in multiparous compared to primiparous cows, and this is in agreement with the findings of this study. The increased DMI might be explained by the fact that multiparous cows have greater body size, bigger rumen capacity, and higher requirements. Also DMI as a percentage of body weight was different; 1.9% for multiparous vs 1.6% for primiparous. A previous study (Johnson et al., 1981) also showed no effect of dry period diet on DMI when different energy concentrations were fed. The largest decline in DMI in this study began two weeks prepartum; which is consistent with other studies (Bertics et al., 1992; Coppock et al., 1972; Marquardt et al., 1977; Grummer et al., 1995). This decline may be partly explained by space occupied by uterine contents (Forbes, 1987).

Pregnant primiparous cows need more energy per Kg of BW than multiparous cows. They use their energy for maintenance, pregnancy and growth, where as multiparous cows use the energy only for maintenance and pregnancy. Also because pepartum DMI for primiparous cows was lower than multiparous cows, therefore; calculated energy balance was

lower for primiparous cows prepartum than for multiparous cows. However; there were no differences due to parity in the calculated energy balance postpartum because the extra energy intake was used to produce more milk. All groups were in negative energy balance averaged 5 wk postpartum; due to the fact that intake was less than requirements for the high milk yield in early lactation, and this was consistent with previous studies (Lin et al., 1984).

Prepartum body fat mobilization as indicated by blood NEFA concentrations was different for multiparous cows reflecting differences in their energy intake. The more energy intake (HH diet) is expected to result in a less body fat mobilization and consequently lower blood NEFA concentration. However, it was not clear why we didn't obtain the same results for the primiparous cows. The prepartum energy balance for multiparous cows was higher than for primiparous cows, consistent with their lower NEFA concentrations because blood NEFA concentrations are inversely related to energy balance. The blood NEFA concentrations postpartum were higher for multiparous cows than for primiparous cows, because they mobilized more body fat to support higher milk production. Also NEFA concentrations postpartum for all cows were higher than prepartum; reflecting an increased demand for energy during early lactation. Lactating cows requirements are higher than that for dry cows as indicated by NRC (1989), so they mobilized more fat to meet the increased

requirements.

The increase in blood NEFA concentrations at calving time is due to the increase in adipose tissue mobilization to meet the high energy demand for the stressful physiological changes and for the high milk production.

Our results are in agreement with those reported in the literature (Johnson et al., 1981; Grummer et al., 1995)). The data indicate that lipolysis increases at parturition to provide energy for milk production, and that the cows continued to utilize body fat during early lactation. As in our companion field trial (Dyke et al., 1995), the sharp increase in blood NEFA concentration started seven days prepartum. This sharp increase happened regardless of the fact that most of the cows were in calculated positive energy balance. This means that there were other factors than DMI depression which contributed to the increase in blood NEFA concentration around calving time, as mentioned before by Vazquez-Anon et al., (1989) and Herdt (1988) when they found that increased NEFA concentration started prior to DMI depression. The drop in blood NEFA concentration postpartum reflects the end of the calving stress, recovery of DMI, and the improvement in energy balance status of the cow.

Multiparous cows tend to mobilize more fat, as indicated by increased blood NEFA concentration toward calving time than primiparous cows. Apparently this is to prepare for higher milk production. Their requirements for lactogenesis may be higher and their milk production peaks earlier during

the lactation period.

Liver TG and fatty acids at calving were higher for multiparous cows than for primiparous, which supports the idea that multiparous cows mobilized more fat towards calving time to meet the higher energy requirements for lactation. Liver TG and fatty acids were not measured prepartum, so multiparous cows possibly started higher. Liver fatty acids decreased with increased energy density of the diet prepartum. This means that the more energy available from the diet results in less NEFA mobilized from the tissue. The concentrations of TG in the liver were smaller, and also the percentages of fatty acids in the liver were much lower than in the case of fatty liver. Where the TG in fatty liver will be over 10%, which means that those cows were in better nutritional status than the normal cows that were analyzed by Herdt et al. (1988). They found 8% liver total lipid concentration as percentage of wet weight, and 4% liver TG concentration as percentage of wet weight.

The calculated energy balance prepartum was higher for multiparous cows than for primiparous cows , indicating that multiparous cows had accumulated more body reserves, and hence more BCS (a measurement of body fatness). Also multiparous cows mobilized less NEFA prepartum than primiparous cows as discussed before. There were no observed differences in BCS (adjusted by covarying on beginning of the experiment) due to parity postpartum, because multiparous cows mobilized more NEFA than

primiparous cows.

Primiparous cows had lower body weight than multiparous cows, because they are growing and they had not reached their mature size yet. Body weight (BW) increased toward calving because of the increase in conceptus growth, and may be body tissue growth. The drop in BW after calving is inversely related to milk production, because body tissues mobilized to produce energy for milk production. Multiparous cows lost more weight after calving than primiparous cows fed the same diet, may be because they mobilized more adipose tissue for milk production after calving, as shown by greater blood NEFA concentration and higher milk production.

In contrast to the report of Grummer et al. (1995), BW and BCS at calving were not affected by dietary energy level prepartum. This may be due to the fact that their treatment started 170 days prepartum, so the cows had enough time to make use of the excess energy. Body condition score data from 1 wk prepartum paralleled that of BW.

There was a positive relationship between increased dietary protein prepartum for multiparous cows, and increased weight loss postpartum. This may be explained by the observation that milk production peaked earlier (6 wk) in cows fed MH and H diets compared to the cows fed L and M diets (9 wk).

Milk yield was higher for multiparous cows, and their lactation milk

yield peaked earlier than primiparous cows. This is may be because they ate more, had larger body reserves, and mobilized more adipose tissue, which is consistent with the results of other studies (Grummer et al., 1995; Boisclair et al., 1989; Johnson et al., 1981). The increase in diet energy density prepartum did not affect milk yield or milk composition.

Our limited data suggests that the dry period diet with varying energy and protein content above that recommended by NRC had no effect on incidence of peripartum metabolic problems, and that may be due to the low numbers of cows that we used. This is in contrast to the results of other studies (Curtis et al., 1985) which suggested that; increasing energy and protein content of the diet in the last 3 wk prepartum above that of NRC requirements may reduce incidence of metabolic and reproductive disorders. The results from the field study (Dyke et al., 1995), which included >2000 cows from >90 farms, showed that; incidence of metabolic diseases was higher with cows that had higher blood NEFA concentration around calving time.

It is now acknowledged that the skeletal musculature is a major tissue in whole body protein metabolism (Young et al., 1979). Creatinine is a byproduct of muscle cell energy metabolism, and it is released from the muscle in amounts proportional to the muscle mass, so it is used as an indicator for muscle mass. During the breakdown of protein, 3-methylhistidine (NMH) is released and it cannot be used for synthesis of

protein, so it is used as an indicator of the in vivo rate of muscle protein breakdown in experimental animals and humans. The ratio of urinary NMH / creatinine is used as an estimate of myofibirillar protein degradation which is standardized for muscle mass and for urine volume.

It was found that the rate of protein synthesis in pregnant mice is about twice that of virgin ones, but most of the extra protein is deposited in the fetuses and their membranes (Millican et al., 1987). The same results were obtained in this study, where an increase in urine creatinine concentration and a decrease in NMH / creatinine ratios were observed. However, it is not clear why the high protein diets had higher NMH/creatinine ratios prepartum.

The increased in NMH / creatinine ratios observed immediately after calving can be explained as follows; body protein must be mobilized to meet the deficiency of dietary protein postpartum, so muscle breakdown to provide amino acids used for synthesis of milk protein and lactose is important during the first weeks of lactation. It is also possible that increased circulating NMH in early lactation is in part the consequence of NMH released particularly from uterine tissue during the involution process after parturition, and also turnover increase in gastrointestinal tract in early lactation. The was higher for primiparous cows than for multiparous cows, may be because they are growing and more protein turnover occurred.

Summary and Conclusion

The first objective of this study was to determine if a high energy and/or high protein diet prepartum increases peripartum nutrient intake, improves nutrient balance as indicated by serum NEFA, creatinine and NMH, and increases milk yield postpartum. It is observed in this experiment that, dry period diet with varying energy and protein levels above NRC recommendations had no effect on feed consumption, body condition score, and milk production. Energy intake in primiparous and multiparous cows increased, and prepartum plasma NEFA concentrations decreased as nutrient density increased.

The second objective was to determine the interrelationships of plasma NEFA and prepartum dietary energy density and protein content.

Increased dietary energy and protein for dry cows decreased plasma NEFA two weeks prepartum, which might have decreased liver fat. Further studies, using a larger number of cows are needed to test for the increase of energy and to maintain the same amount of protein as in the MM diet.

Also to see the effect of these diets on peripartum diseases.

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