EFFECTS OF FLUNIXIN MEGLUMINE ON PYREXIA, PRODUCTION AND BIOENERGETIC VARIABLES IN POSTPARTURIENT DAIRY COWS

By

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STATEMENT BY AUTHOR

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APPROVAL BY THESIS DIRECTOR

This thesis has been approved on the date shown below:

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Lance H. Baumgard  
Associate Professor of Animal Sciences  
Date
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<td>BCS</td>
<td>Body condition score</td>
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<tr>
<td>BHB</td>
<td>β-hydroxybutyrate</td>
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<td>BW</td>
<td>Body weight</td>
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<tr>
<td>Co-A</td>
<td>Coenzyme-A</td>
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<tr>
<td>COX</td>
<td>Cyclooxygenase</td>
</tr>
<tr>
<td>CPT-I</td>
<td>Carnitine palmitoyltransferase-I</td>
</tr>
<tr>
<td>CTL</td>
<td>Cytolytic T helper</td>
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<td>DC</td>
<td>Dendritic cell</td>
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<td>DIM</td>
<td>Days in milk</td>
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<td>DMI</td>
<td>Dry matter intake</td>
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<td>EBAL</td>
<td>Energy balance</td>
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<td>FADH</td>
<td>Flavin adenine dinucleotide H</td>
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<td>FM</td>
<td>Flunixin meglumine</td>
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<td>FSH</td>
<td>Follicle stimulating hormone</td>
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<tr>
<td>IGF-I</td>
<td>Insulin-like growth factor-I</td>
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<tr>
<td>IL</td>
<td>Interleukin</td>
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<tr>
<td>IMI</td>
<td>Intramammary infection</td>
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<td>INF</td>
<td>Interferon</td>
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<tr>
<td>LCFA</td>
<td>Long chain fatty acids</td>
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<td>LH</td>
<td>Luteinizing hormone</td>
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<tr>
<td>LPL</td>
<td>Lipoprotein lipase</td>
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<tr>
<td>ME</td>
<td>Metabolized energy</td>
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<tr>
<td>MHC</td>
<td>Major histocompatibility complex</td>
</tr>
<tr>
<td>mRNA</td>
<td>Messenger ribonucleic acid</td>
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<tr>
<td>NADH</td>
<td>Nicotinamide adenine dinucleotide H</td>
</tr>
<tr>
<td>NEFA</td>
<td>Non-esterified fatty acids</td>
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<tr>
<td>NE_L</td>
<td>Net energy of lactation</td>
</tr>
<tr>
<td>NK</td>
<td>Natural killer</td>
</tr>
<tr>
<td>NRC</td>
<td>National research council</td>
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<tr>
<td>NSAID</td>
<td>Non-steroidal anti-inflammatory drug</td>
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<td>PEBAL</td>
<td>Positive energy balance</td>
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<tr>
<td>PG</td>
<td>Prostaglandin</td>
</tr>
<tr>
<td>PGE₂</td>
<td>Prostaglandin E2</td>
</tr>
<tr>
<td>PGF₂α</td>
<td>Prostaglandin F2α</td>
</tr>
<tr>
<td>PUN</td>
<td>Plasma urea nitrogen</td>
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<tr>
<th>Abbreviation</th>
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<tr>
<td>SCFA</td>
<td>Short chain fatty acids</td>
</tr>
<tr>
<td>TG</td>
<td>Triglyceride</td>
</tr>
<tr>
<td>Th</td>
<td>T helper</td>
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<td>TNFα</td>
<td>Tumor necrosis factor alpha</td>
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<tr>
<td>VLDL</td>
<td>Very low density lipoprotein</td>
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ABSTRACT

During early lactation dairy cows often experience health disorders, which are usually associated with decreased production and reproduction variables. Following parturition, cows use more energy for maintenance and milk production than they consume and enter into a state of negative energy balance. Negative energy balance in early lactation is thought to contribute to decreased milk production, reduced reproductive performance, and increased health disorders. Flunixin meglumine (FM) is an anti-pyretic (fever reducing) and anti-inflammatory drug that is commonly used in the dairy industry. This study evaluated the effect of FM on pyrexia, production and bioenergetic variables in postparturient dairy cows.
CHAPTER 1

LITERATURE REVIEW

Periparturient Nutrient Demand

The Periparturient Period

During the periparturient period (defined as 3 wks prepartum until 3 wks post partum; Grummer, 1995), the dairy cow faces many challenges/changes that may impair its ability to maximize performance during the rest of the lactation. These changes include many pen transfers and the challenges associated with a new group hierarchy and new diets. In addition, periparturient cows undergo physiological changes associated with late gestation, parturition, and lactation initiation. Goff and Horst (1997) summarized that the majority of metabolic diseases such as milk fever, ketosis, retained placenta, and displaced abomasums usually occur during the first 2 wks post-partum. Due to the above challenges the transition cow experiences and the economic implications farmers may deal with during this period, close attention to transition cow management may pay long-term dividends.

Gravid Uterus Metabolism

Bell (1995) suggested the best approach to describe the metabolic impact of the conceptus on the dam is to investigate the requirements of the fetus, uterine tissues, placenta, and the fetal membranes. This review also stated the fetus receives most of the nitrogen and carbon for growth from glucose and amino acid uptake. Bell and coworkers (1995) recorded an increase of the fetal relative size from 45% of the uterine dry weight
on 190 d to nearly 80% on 270 d. Hay et al. (1983) suggested glucose and lactate oxidation by the fetus in ewes accounts for 50 to 60% of fetal oxidation, while Faicheny and White (1987) indicated amino acid oxidation accounts for 30 to 40% of the fetal respiration. Comline and Silver (1976) indicated that the remaining 10 to 15% of substrate for fetal oxidation was contributed by maternal acetate in late gestation cows. Bell (1993) suggested the limited amount of fat oxidized by the fetus is due to the restricted transport of long and short fatty acids through the placenta. Similarly, Elphick et al. (1979) found that fetal fat deposition in sheep was also limited due to placental lipid impermeability.

Although the uteroplacental tissues account for < 20% of the gravid uterus during late gestation (Bell, 1995), the tissue consumes 35 to 50% of maternal oxygen and > 65% of the uterine glucose uptake (Reynolds et al., 1986). In a study conducted by Freetly and Ferrel (1998) on late-gestation ewes, the uteroplacental tissues used 71% of the glucose taken up by the gravid uterus, while the fetus utilized the remaining 29%. Bell and colleagues (1995) indicated that the whole gravid uterus mean accretion rates were 138 g/d and that the non-fetal tissue of the conceptus accounted for only 18 g/d between d 190 and 270 of gestation in Holstein cows.

**Dam Metabolism**

Late gestation cows undergo metabolic changes to accommodate fetal nutritional needs. These include changes in glucose, protein, and fatty acid oxidation. Although the
fetus cannot take direct advantage of lipid oxidation, the dam increases its utilization of adipose tissue to spare glucose and possibly amino acids for fetal utilization (Bell, 1995). Freetly and Ferrel (1998) demonstrated increased hepatic lactate uptake by pregnant ewes followed by enhanced glucose release from the liver, suggesting lactate is in important gluconeogenetic precursor. In this study there was greater hepatic lactate uptake in twin pregnancies compared to single ones. This may imply the liver responds to energy requirements of the gravid uterus (Reynolds et al., 2003). Reynolds and coworkers (2003) also witnessed increased liver lactate removal and glucose release from the liver on the day of parturition, suggesting cycling of lactate and glucose carbon between the uterus and liver to provide energy for uterine contractions.

An altered dry matter intake (DMI) and endocrine status are typical phenomena in periparturient cows. Petterson and coworkers (1993) recorded diminished peripheral insulin (a strong lipogenic signal) sensitivity in underfed pregnant ewes. The transition period is associated with a metabolic shift from an overall state of adipose lipogenesis to lipolysis resulting in increased circulating non-esterified fatty acid (NEFA) concentrations (Bell, 1995). Dry matter intake in gestating cows decreases 3 wk prepartum with the most significant decrease (up to 30%) occurring during the last week of gestation (Bertics et al., 1992; Grummer, 1995). Grummer (1995) concluded that plasma NEFA concentration almost double between 17 d and 2 d prepartum. Elevated NEFA and ketone levels also occur in force-fed pregnant ewes to meet their predicted energy requirement for conceptus growth and dam maintenance (Petterson et al., 1994), suggesting that NEFA levels increase is partly independent of energetic state (Grummer,
This hypothesis is in agreement with the findings of Reynolds et al. (2003) who demonstrated decreased arterial concentration of insulin and elevated NEFA levels at 9 d prepartum compared to 19 d prepartum in cows. Elevated NEFA levels were also recorded in mastectomized cows on the day of calving, suggesting that the small increase in adipose tissue mobilization is independent of milk production (Goff et al., 2002). The authors hypothesized that increased cortisol levels during parturition caused adipose tissue mobilization. Samara and colleagues (1996) summarized that increased cortisol concentrations elevated NEFA release from adipose tissue in humans. In summary, increased lipid mobilization and fatty acid oxidation by the dam can spare glucose and amino acids for fetal utilization.

Amino acids, although not the primary source, significantly contribute to gluconeogenesis (Drackley et al., 2001), and alanine and glutamine are the primary gluconeogenic amino acids (Nelson and Cox, 2003). Danfaer and colleagues (1995) infused amino acids into the mesenteric vein of goats and found that amino acids contributed 24% to glucose synthesis. They also observed that increased amino acid availability elevated its contribution to gluconeogenesis. This may suggest that amino acid contribution is, at least, partly dependent on its supply (Drackley et al., 2001).

**Lactation Metabolism**

Immediately after parturition the mammary gland nutrient requirement for glucose, amino acids, and fatty acids is approximately 2.7, 2.0, and 4.5 times more than the gravid uterus, respectively (Bell, 1995). Since DMI following calving increases at a
lower rate than milk production (Bauman and Currie, 1980), a variety of physiological changes occur in order to meet the enhanced nutrient demand. These changes include alterations in carbohydrate, lipid, and protein metabolism.

Adaptation in carbohydrate metabolism during lactation is primarily characterized by the increase in hepatic gluconeogenesis. Early lactation is characterized by a massive increase in glucose requirement, mainly for lactogenesis (Bell, 1995). Bell (1995) also noted that glucose uptake by the mammary gland 2 d postpartum is five times greater than on d 7 to 9 prepartum. Reynolds and coworkers (2003) reported that liver glucose production nearly doubled at 11 days in milk (DIM) compared to 9 d prepartum and is probably the result of increased feed intake and milk production.

The major substrates for hepatic gluconeogenesis in ruminants are propionate from microbial fermentation, lactate from the Cori cycle, amino acids from proteolysis or net portal- drained visceral absorption, and glycerol from triglyceride breakdown during lipolysis (Seal and Reynolds, 1993). Propionate contribution to hepatic gluconeogenesis was found to be approximately 50-60% during the transition period (Reynolds et al., 2003). In addition, this study demonstrated that lactate contributed to hepatic gluconeogenesis approximately 15-20%, glycerol approximately 1-4%, and amino acids minimum of 20-30%.

The role of lipid mobilized from fat storage in transition lactating cows is to meet energy requirements during a period when the cow is in a state of negative energy balance (NEBAL). Gibb et al. (1992) demonstrated that fat accounts for 69% of the total energy content stored in the body of lactating cows in their first week of lactation. The
researchers also found that during the first 8 wk of lactation the cows lost 34% of their body fat. Bell (1995) describes the massive mobilization of NEFA from adipose tissue during the transition period as the, “metabolic hallmark of the transition from pregnancy to lactation”. During the final stages of gestation, adipose tissue lipogenesis is suppressed and lipolysis rates increase (McNamara and Hillers, 1986). Adipose tissue lipolysis increases even further during the first few weeks of lactation to meet daily energy requirements for maintenance and lactogenesis as exogenous energy is limited due to decreased DMI. Plasma NEFA concentrations are good indicator for the extent of adipose mobilization (Bell, 1995). Overton and Waldron (2004) noted that during a period of negative energy balance, skeletal muscle utilizes NEFA as an energy source and reduces its dependence on glucose. Additionally, Pullen and coworkers (1989) suggested that adipose-derived NEFA contributes approximately 10% to the total milk fatty acid pool.

The periparturient lactating cow undergoes changes in protein metabolism in addition to carbohydrate and lipid alterations. These adaptations occur in order to satisfy protein requirements both in non-mammary and mammary gland tissues. Milk protein production in high yielding lactating dairy cows accounts for as much as of 82% of digestible protein intake (Clark et al., 1978). This high amino acid demand for gluconeogenesis and milk synthesis drives the postpartum lactating cow to a negative nitrogen balance for the first couple of weeks of lactation (Bauman and Elliot, 1983; Bell, 1995). Negative nitrogen balance during early lactation is satisfied by mobilizing muscle or whole body protein (Bell, 1995; Bequette et al., 1998). Baracos et al. (1991)
recorded decreased rates of muscle and skin protein synthesis in early lactating goats, allowing milk protein synthesis to take place at the expense of body reserves.

**Energy Balance**

Energy balance (EBAL) is the difference between energy consumed by the animal and the energy used for maintenance and/or production (milk, wool, etc.). An animal in positive energy balance (PEBAL) increases body tissue mass while an animal in NEBAL mobilizes tissue for energy and therefore loses body weight. Direct or indirect calorimetry is used to evaluate EBAL; although accurate, this method is not always feasible because it is expensive and labor intensive. Alternative methods, to evaluate EBAL include changes in body weight (BW), changes in body condition score (BCS), circulating blood metabolites, and energy calculations (see preceding by Moore et al., 2005). The energy calculation is based on a set of formulas, which attempts to estimate EBAL of an animal in a specific physiological state (growth, lactation, etc.). The accuracy of this method depends on the measurement precision of feed intake and milk production and their respective energy values (NRC, 2001).

The NRC (2001) presented the following formula to calculate EBAL for lactating cows: \( \text{EBAL} = \text{net energy intake} - (\text{maintenance requirements} + \text{milk energy}) \). Net energy consumed can be determined by the product of feed intake and feed energy content. The use of Calan gates or individual stalls are common techniques to measure feed intake for individual animals however, this technique is primarily used in research farms and smaller herds.
Energy required for maintenance is calculated using the following equation: net energy for maintenance = 0.08 x BW^{0.75} (NRC, 2001).

Net energy for lactation is calculated according to the NRC (2001) by the following equation: net energy for lactation = [(0.0929 x fat %) + (0.0547 x crude protein %) + (0.0395 x lactose %)] x milk yield.

**Negative Energy Balance**

Dairy cows usually experience a severe energy deficit in early lactation due to an inability to meet their energy requirements for maintenance and copious milk production and as a result, enter a state of NEBAL. In fact, gestating cows can enter a state of NEBAL as early as 10 d prepartum (Allen and Bradford, 2007), and lactating dairy cows reach NEBAL nadir approximately 4-9 d postpartum (Baumgard at el., 2007a). The exact amount of time cows remain in NEBAL is controversial but estimates are from 4 to 12 wks (Moallem et al., 2000; Block et al., 2001; Gummer and Rastani, 2003). The variability in the return to PEBAL and possible explanations behind the inconsistencies have recently been explored (Moore et al., 2005).

**Metabolic Disorders Associated with NEBAL**

Cows are at an increased risk of health disorders during the first 2 wk of lactation (Goff and Horst, 1997). These disorders include, hepatic lipidosis, which is strongly related to the occurrence of ketosis, hypocalcaemia, and milk fever.
**Hepatic Lipidosis.** The condition of NEBAL results in enhanced adipose mobilization and elevated hepatic lipid concentrations. Rapid rates of fat entry coupled with low rates of hepatic lipid oxidation can lead to triglyceride (TG) surplus, resulting in hepatic lipidosis.

A portion of the NEFA enters the liver and undergoes oxidation (Figure 1) which is regulated by the formation of malonyl coenzyme A (CoA) and carnitine palmitoyltransferase I (CPT-I, Drackley, 1999). Malonyl-CoA, an important regulator of mitochondrial fatty acid translocation, is produced from acetyl-CoA via acetyl-CoA carboxylase. Increased malonyl-CoA concentrations inhibit CPT-I (Grummer, 1993). Zammit (1996) noted that in laboratory rodents, CPT-I is less sensitive to malonyl-CoA inhibition when insulin levels are reduced. This may explain why higher levels of NEFA enter the mitochondria during periods of hepatic TG accumulation since mitochondrial NEFA entry is regulated via CPT-I (Drackley, 1999).
Figure 1. Lipid metabolism in adipose tissue, liver and mammary gland in the dairy cow. Adapted from Drackley (1999).

Peroxisomal β-oxidation has been suggested by Drackley (1999) to play an increasing role during extensive NEFA mobilization and acts as an “overflow” pathway to fatty acid oxidation. Long chain fatty acids (LCFA) enter the peroxisome and are broken down to short chain fatty acids (SCFA) and acetyl-CoA. Peroxisomal β-oxidation is considered half as efficient as mitochondrial oxidation, since energy in the form of FADH₂ is lost as heat, while NADH can be utilized within the mitochondria, while the fate of acetyl-CoA is unknown (Drackley, 1999).

Compared to monogastrics, minimal amounts of hepatic NEFA are packaged and exported as very low density lipoprotein (VLDL) in the bovine liver (Church, 1988). Apolipoprotein B mRNA, a VLDL constituent, has been shown to be lower in cows with fatty liver (Mazur et al., 1992). Grummer (1993) observed decreased VLDL secretion as
a direct result of lower apolipoprotein B synthesis. Methionine (a limiting amino acid in dairy cattle) is a key amino acid in the formation of apolipoprotein B (Bauchart, 1993), and therefore dairy cows consuming a methionine deficient diet during the transition period, may be prone to hepatic lipidosis. Slow TG export from the liver as VLDL in relation to NEFA entry, results in hepatic TG accumulation (Drackley, 1999). Increased fatty acid mobilization in conjunction with an elevated glucose requirement can lead to hepatic acetyl-CoA accumulation (Van Soest, 1994). Carbons of acetyl-CoA and oxaloacetate are necessary to synthesize citrate in the Kreb cycle (Nelson and Cox, 2003). When oxaloacetate is directed toward gluconeogenesis, citrate formation is inhibited due to insufficient carbon quantity in the reaction and consequently acetyl-CoA accumulates in the mitochondria (Nelson and Cox, 2003). Acetyl-CoA will then serve as substance for ketogenesis.

**Ketosis.** Dairy cows can begin to show clinical signs of ketosis 10 d to 3 wk postpartum; ketone bodies (β-hydroxybutyrate (BHB) and acetoacetate) are ketosis indicators found in milk, and urine (Goff and Horst, 1997). Kronfeld (1982) proposed four types of ketosis; primary ketosis occurs when the cow is not offered sufficient nutrients. Secondary ketosis develops when DMI is reduced as a result of another disease. Alimentary ketosis is a result of digesting high amounts of ketogenic precursors. Finally, spontaneous ketosis occurs when elevated blood ketones are present despite a nutrient sufficient diet.

Similar to fatty liver, ketosis occurs when circulating NEFA levels are elevated. In addition, the low rate of glycogen synthesis in the liver along with high TG accumulation can induce ketosis (Drackley et al., 1992). Drackley and coworkers (1992)
suggested that a high TG:glycogen ratio in hepatic tissue may induce ketosis, and concluded cows with a TG:glycogen ratio greater than 1.5 during early lactation have an increased susceptibility to ketosis and fatty liver. In an earlier study by Veenhuizen et al. (1991), where a ketosis induction protocol was used, hepatic glycogen levels were significantly decreased 28 d after calving. In support of this data, Bertics et al. (1992) demonstrated a correlation between TG:glycogen and BHB.

**Hypocalcemia and Milk Fever.** As lactation begins cows have an increased demand for calcium, and a majority of lactating dairy cows experience some degree of hypocalcemia during early lactation, while some are affected with milk fever; a severe case of hypocalcemia (Horst et al., 1994). Hypocalcemic cows may lose muscle tone in the uterus and teat sphincters, which can potentially lead to retained placenta and mastitis (Goff and Horst, 1997). When milk fever occurs, blood calcium levels become too low to support normal nerve and muscle function. As a result, cows can stagger, fall down, and in severe cases are unable to get up (Church, 1988), and in rare events, untreated cows with milk fever may die (Hutjens, 2003).

**Reproduction Problems Associated with NEBAL**

Negative energy balance during the periparturient period is associated with low fertility in lactating dairy cows. Many studies have evaluated the relationship of NEBAL and production variables (Table 1). The relationship between DMI prior to parturition and DMI postpartum is well established (Butler, 2006). Grummer (1995) summarized data from several studies and noted a high correlation ($r = 0.53$) between DMI 1 d
prepartum and DMI 21 d post partum. Butler (2006) noted that certain organs and tissues that are associated with pregnancy such as ovaries, hypothalamus/pituitary gland, and the liver must recover from pregnancy and parturition in order for the cow to become pregnant again. The author also summarized that recovery of these organs and tissues is characterized by a complete follicular development that leads to the first ovulation.

<table>
<thead>
<tr>
<th>EBAL variable</th>
<th>Correlation</th>
<th>Reproduction variable</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Days to nadir</td>
<td>0.31</td>
<td>Days to first ovulation</td>
<td>Beam and Butler (1998)</td>
</tr>
<tr>
<td>Days to nadir</td>
<td>0.55</td>
<td>Days to first ovulation</td>
<td>Beam and Butler (1997)</td>
</tr>
<tr>
<td>Days to nadir Value at ovulation</td>
<td>0.61</td>
<td>Days to first ovulation</td>
<td>Zurek et al. (1995)</td>
</tr>
<tr>
<td>Days to nadir Days to first</td>
<td>0.49</td>
<td>Days to first ovulation</td>
<td></td>
</tr>
<tr>
<td>Cumulative EBAL from parturition to</td>
<td>0.85</td>
<td>Days to first ovulation</td>
<td>Canfield and Butler</td>
</tr>
<tr>
<td>Nadir</td>
<td>0.46</td>
<td>Days to first ovulation</td>
<td>(1991)</td>
</tr>
<tr>
<td>Average daily EBAL (1-14 DIM)</td>
<td>Not significant</td>
<td>Days to first ovulation</td>
<td>Canfield et al. (1990)</td>
</tr>
</tbody>
</table>

The recovery of the above organs and tissues is adversely associated with NEBAL. Beam and Butler (1999) concluded that improvement in NEBAL nadir has been correlated with enhanced follicular function and a shorter interval to first ovulation. Pulsatile luteinizing hormone secretion causes the ovulation of the first follicle via preovulatory follicular growth and oestradiol production (Butler, 2001). Negative energy balance during the periparturient period impairs luteinizing hormone and follicle
stimulating hormone pulsatility from the anterior pituitary (Jolly et al., 1995; Butler, 2006). There is an association between increased concentration of hepatic TG postpartum and longer intervals to first ovulation and decreased fertility (Rukkwamsuk et al., 1999; Jorritsma et al., 2000). In addition, decreased plasma insulin and IGF-I during this time inhibits ovarian responsiveness to gonadotropins (Butler, 2006).

Butler (2006) noted that cows with a higher efficiency ratio (milk production/DMI) are in greater NEBAL as more of their energy requirement for milk is supplied from body reserves rather than from feed intake. These cows have a longer interval to first ovulation than cows with a lower efficiency ratio. Thus, transition cows that increase their DMI at a faster rate will have an improved energy balance and will probably have increased fertility rates.

**Milk Yield in NEBAL**

As summarized above, the lactation onset is characterized by a dramatic increase in nutrient demand of the transition cow, which does not consume sufficient amount of energy and enters a state of NEBAL. de Vries and Veerkamp (2000) conducted a study with 470 first lactation heifers and recorded an energy deficit average of 185.7 Mcal of net energy of lactation (NE\textsubscript{L}) during the first 180 DIM. Furthermore, periparturient cows 4 d postpartum have tripled their demand for glucose, doubled the demand for amino acids, and increased their demand for fatty acids approximately five fold when their nutrient demand is compared to 250 d of gestation (Bell, 1995).
Dairy cows remain in a state of NEBAL approximately 6-7 weeks postpartum (de Vries and Veerkamp, 2000; Gummer and Rastani, 2003). Drackley (1999) noted that mammary gland nutrient demand in high producing dairy cows 4 DIM accounted for 97% of their metabolized energy. Brown (1969) concluded that due to the extreme nutrient demand by the mammary gland in a high yielding cow relative to the total metabolism, the cow should be considered an appendage to the mammary gland compared to vise versa.

During early lactation, when the cow is in a state of NEBAL, it uses its body reserves to aid in milk production and milk yield constantly increases until it reaches its peak at 40-70 DIM and the cow progresses toward PEBAL (Figure 2). It was established that animals eat to meet their requirements (Church and Pond, 1988). Therefore, additional metabolized energy (ME) during a state of NEBAL, should increase milk production without decreasing feed intake, (Baumgard et al., 2007a). It is interesting to note that in mid-late lactation, when the cow is in a state of PEBAL, milk production diminishes as lactation progresses. It is thought that additional metabolized energy in this state would improve feed efficiency rather than increase yield (Baumgard et al., 2007a). Although it is well documented that excessive fat oxidation during periparturient period leads to metabolic disorders such as fatty liver and ketosis (Grummer, 1993; Goff and Horst, 1997; Drackley, 1999), it is not clear that NEBAL limits peak milk yield per se (Baumgard et al., 2007a).

The success of the periparturient period has a significant economic effect and may determine the profitability of a cow throughout the lactation cycle. Peak milk yield
during early lactation is thought to “prime” the mammary gland for the rest of the lactation period so that every additional kg of milk yield at peak production represents an increase of as much as 127-200 kg in total yield (Drackley, 2001; Baumgard et al., 2007a).

Animals Eat to Meet Their Energy Requirement:

During NEBAL: ↑ metabolizable energy = ↑ milk yield

During PEBAL: ↑ metabolizable energy = ↑ efficiency

![Diagram showing theoretical lactation and energy balance curves. Bioenergetics would predict that increasing metabolizable energy will have different effects on production parameters depending upon calculated energy balance status. Adapted from Baumgard et al. (2007a).]

**Figure 2.** Theoretical lactation and energy balance curves. Bioenergetics would predict that increasing metabolizable energy will have different effects on production parameters depending upon calculated energy balance status. Adapted from Baumgard et al. (2007a).

**Strategies to Alleviate NEBAL**

As discussed above, the transition period has a substantial affect on milk production and reproductive performance, and is therefore considered a very critical state
in the lactation cycle. Various strategies to alleviate NEBAL during the periparturient period have been suggested. These include increased energy density, fat supplementation, reduced milking frequency (1x/d), and use of rumen inert conjugated linoleic acid to induce milk fat depression (Moore et al., 2005). While some of these methods may benefit the transition dairy cow, others present challenges such as rumen acidosis and reduced DMI (Palmquist and Jenkins, 1980). Additional research should be conducted to investigate how to alleviate the state of NEBAL in periparturient cows.

**Immune Response**

The immune response system is made of many different cells-types, tissues, and organs, which coordinate a response to foreign antigens. Although immune system cells are not physically connected, they maintain communication via the nervous system, and molecule secretion (Lydyard et al., 2004). The immune system is made up of two lines of defense: the first is the innate immune response, which undergoes very little changes throughout the animal’s life. The second is the adaptive immune system, which is much more specific than the innate system and gets activated simultaneously with the latter, especially when the innate immune system is unable to cope with the antigen (Lydyard et al., 2004).

The innate immune system is composed from several cell types including phagocytes, natural killer (NK) cells, mast cells and basophils, and dendritic cells. The two main types of phagocytes are neutrophils and macrophages. Neutrophils are produced in the bone marrow, while macrophages are derived from monocytes in the
circulatory system (Prescott et al., 2002; Lydyard et al., 2004). Macrophages dispose of
dead body cells and microbes and can also coat microorganisms or foreign material and
enhance opsonization by antibodies (Prescott et al., 2002). Thus, macrophages play an
important role in bridging the innate immune system with the adaptive system.

Natural killer cells are produced in the bone marrow and found throughout the
different body tissues, mostly in the circulation (Lydyard et al., 2004). These cells
recognize tumor cells and virus infected cells and destroy them (Janeway et al., 2001).
Natural killer cells release a granule substance, specifically perforins and granzymes,
which attach to the surface of infected cell and creates pores in the cell membrane and
this ultimately induces apoptosis (Lydyard et al., 2004).

Mast cells and basophils have very similar morphology; while mast cells are
found in connective tissues throughout the body, basophils are present in the circulation
in very low numbers (Lydyard et al., 2004). Basophils and probably mast cells, are
produced in the bone marrow and mast cells secrete a variety of cytokines including:
interleukin (IL)-1, IL-2, IL-3, IL-6, and Tumor necrosis factor (TNF)-α (Kuby, 1994).

Dendritic cells (DC) utilize pattern recognition receptors to identify specific
pathogen-associated molecular pattern (Prescott, 2002), and are the most potent
stimulator of T cells (Janeway et al., 2001). Their primary function is to bind and carry
antigens to the peripheral lymphoid organs and present them to T lymphocytes (which
destroy them), but DC can also phagocyte pathogens (Janeway et al., 2001).

Molecules of the innate immune system mediate its action with the adaptive
immune system. Though these molecules react to specific structures on antigens, they
still have the ability to recognize many different types of pathogens that express these structures (Lydyard et al., 2004). The main molecules are of the complement system, acute-phase proteins, and cytokines.

The complement system is made of plasma proteins that are produced mainly by hepatocytes and monocytes and are constantly found in the blood and other body fluids (Lydyard et al., 2004). The complement system has three activation pathways: the classical pathway, which is triggered by antibody complexes, the MB-lectin pathway, triggered by mannan-binding lectin (a serum constituent that binds to encapsulated bacteria), and the alternative pathway, which is triggered by pathogen surfaces (Janeway et al., 2001). This system protects the body against pathogens in three ways. First, it produces proteins that bind to antigens and opsonise them for engulfment by phagocytes. Second, some of the proteins that bind the pathogen attract more phagocytes to the infected area. Finally, coating proteins create pores in certain bacteria membranes and damage it.

Acute-phase proteins are an additional type of molecule that are a part of the innate immune defense and protect the body against bacteria and protozoa. Upon activation, macrophages and NK cells release IL-1, IL-6, TNFα, and interferon (INF)-γ, which signals hepatocytes to produce acute-phase proteins (Lydyard et al., 2004). The main functions of acute-phase proteins are to maximize complement system activation, microbe opsonization, and limit microbial induced tissue damage. A primary protein is the C-reactive protein, which binds to the phosphorylcholine portion of some bacteria and
fungal-wall lipopolysaccharides (Janeway et al., 2001). The C-reactive protein, both opsonises bacteria and activates the complement system through the classical pathway.

Cytokines are also molecules of the innate immune system, which act in an autocrine/paracrine, and an endocrine fashion (Janeway et al., 2001). Different cytokines may be released as a result of a stimulus, and they might have similar or opposing activities, thus their biological impact is thought to be the sum of all of their effects (Lydyard et al., 2004). Cytokines can be grouped by their cells of origin, however, in some cases more than one cell type can produce different cytokines (Lydyard et al., 2004). These cytokines include interferons, lymphokines, monokines, and chemokines. Interferons are pro-inflammatory cytokines that reduce infection as specific humoral and cellular immunity develop from a viral infection. They are divided to two groups; type-I INF include INFα and INFβ, and INFγ make up type-II interferons.

Interferon-α is produced mainly by infected leucocytes, epithelial cells, and fibroblasts, and INFβ is produced primarily by fibroblasts and epithelial cells (Lydyard et al., 2004). These two kinds of cytokines are primarily induced by IL-1 and TNFα. Type-I INF’s are secreted by infected cells and bind to both the infected cells and nearby cells (Janeway et al., 2001). Once they bind to cells they inhibit both viral replication and cell protein synthesis, thus limiting viral infection. Interferons also enhance NK cells activity and induce antigen-specific cytolytic T lymphocytes responses (CTL, Lydyard et al., 2004).

Interferon-γ are much more specific than type-I interferon as they are cytokines of the adaptive immune system (Lydyard et al., 2004). These interferons are produced
primarily by T helper-1 (Th) cells and NK cells and play an antiviral role, as well as a regulatory role of the specific immunity. Additionally, INFγ are involved in CTL development and its response to immunoglobulin-G. T helper-1 cells and CTL react to the major histocompatibility complex to produce INFγ, which activate monocytes both locally and systemically. Finally, INFγ enhance macrophage killing of bacteria.

Lymphokines are cytokines that are produced primarily by the lymph system and act as growth factors for lymphocytes and/or affect the immune response (Lydyard et al., 2004). Lymphokines such as IL-2, aid in T cells immune response and IL-4 is involved in B cell growth.

Monokines are proinflammatory cytokines, which have a variety of local and systemic actions and are also key mediators of inflammation and fever (Kuby, 1994; Lydyard et al., 2004). Upon ingestion of Gram-negative bacteria, monocytes secrete IL-1, IL-6, IL-8, IL-12, and TNFα (produced also by T cells, Lydyard et al., 2004). The monokines IL-1, IL-6, and TNFα have a few key roles; a) they induce an elevated body temperature along with lymphocyte activation to increase the specific immune response and reduce pathogen replication, b) they mobilize neutrophils for phagocytosis, and c) they induce acute-phase protein production. Additionally, IL-1 and TNFα induce vascular endothelium for preparation of neutrophil chemotaxis and increase vascular permeability (Lydyard et al., 2004).

Chemokines include approximately 50 cytokines, which are involved in chemoattraction of lymphocytes, monocytes, and neutrophils and are produced by monocytes or macrophages (Lydyard et al., 2004). Chemokines, which are made in
response to a physical damage or an infection, direct other cells to attack the pathogen and improve their ability to cope with it.

**Innate Immunity and Inflammation**

Inflammation is a local, non-specific defense response by the tissue to a microbial invasion or injury. This fine tuned, instantaneous mechanism is characterized by redness, warmth, pain, and swelling in the infected area (Prescott et al., 2002). There are two types of inflammation: acute and chronic inflammation. The type of inflammation is determined based on the response duration and on the major inflammatory cell type involved in the inflammatory response (Lydyard et al., 2004). Acute inflammation, which lasts from only minutes to a few days, is an initial response primarily by neutrophils to bacteria. Macrophages and lymphocytes are the primary cells that are involved with chronic inflammation, which can last as long as several months to years (Lydyard et al., 2004).

The release of inflammatory mediators from the infecting microbes, infected cells, and other cells such as mast cells and macrophages, induce an acute inflammatory response (Lydyard et al., 2004). Mast cells and macrophages secrete IL-1, TNFα (inducing PG synthesis), histamine and nitric oxide, causing vasodilation and increased vascular permeability (Lydyard et al., 2004). Interleukin-1 and TNFα causes increased expression of intracellular adhesion molecule-1 and vascular cells adhesion molecule-1, permitting neutrophil entry to the inflammation site via tight junctions (Lydyard et al., 2004). This allows fluid containing antibacterial proteins, clotting factors, antibodies,
and granulocytes to enter the infected site and release inflammatory mediators in order to phagocytose microbes and repair damage tissue (Lydyard et al., 2004).

**Fever**

Fever is directly related to the immune response as inflammatory stimuli provoke the synthesis of the propyretic message (Kluger et al., 1998; Aronoff and Nielsen, 2001). Body thermoregulation is a process that involves several organs including the hypothalamus, limbic system, lower brainstem, the reticular formation, spinal cord, and the sympathetic ganglia (Boulant, 1997). The region of the “preoptic area” in the hypothalamus includes the preoptic nuclei of the anterior hypothalamus, which maintains body temperature around the set point (Aronoff and Neilson, 2001). The set point temperature is regulated by balanced temperature-sensitive neuron activity, which incorporates messages regarding core and peripheral body temperature and evoke behavioral and physiologic responses to control heat production or dissipation (Boulant, 1997).

Saper and Breder (1994) described fever as a regulated body temperature increase due to an elevated hypothalamic set point. Fever is thought to improve the inflammatory response to infection as it regulates inflammatory cytokine expression and enhances leukocyte function (Aronoff and Neilson, 2001). During a febrile (fever) response the body undergoes physiological changes as described below.

Microbial surface components evoke a febrile response via stimulation of pyrogenic cytokines such as IL-1β, TNFα, and IL-6, which act directly on the
hypothalamus (Luheshi, 1998; Aronoff and Neilson, 2001). Interlukin-6 concentration has been shown to have the greatest correlation with pyrexia (LeMay et al., 1990; Roth et al., 1993). The enzyme cyclooxygenase (COX)-2 is induced by these inflammatory cytokines and also by bacterial lipopolysaccharides (Simon, 1999). It catalyzes prostaglandin (PG) synthesis from arachidonic acid including PGE₂ (Saper and Breder, 1994). This PG is thought to be the proximal mediator of a febrile response, which alters the firing rate of neurons within the hypothalamus resulting in an increased thermoregulatory set point (Aronoff and Neilson, 2001).

**Metabolism During Inflammation and Fever**

Microbial or viral infection, tissue injury, and the inflammatory process are characterized by an altered metabolism (Dinarello, 1984). During an immune response, which is characterized by decreased insulin sensitivity, (Grunfeld and Feingold, 1991; Grimble, 2002), animals become anorexic and the glucose availability as an energy source is reduced, resulting in increased muscle proteolysis and adipose lipolysis in order to meet energy demands (Saper and Breder, 1994; Kotler, 2000). Additionally, the acute-phase response is an energy-intensive process that requires large quantities of amino acids (derived from skeletal muscle break-down; Kotler, 2000). Thus, the immune response to inflammation and fever is characterized by metabolic shifts in protein, lipid, and glucose physiology.

Cytokines are involved with the immune response and play an important role in altering metabolism. Multiple studies indicate that increased IL-1 and TNFα
concentrations in rodents induce anorexia (Moldawer et al., 1988; Tracy et al., 1988; Morosovsky et al., 1989). These cytokines also induce skeletal muscle proteolysis (Kotler, 2000), a process that is stimulated by glucocorticoids and thyroid hormone (Mitch and Goldberg, 1996). Baracos and coworkers (1983) indicate increased IL-1 levels promote protein catabolism via PGE$_2$ accumulation in the muscle. Additionally, increased PGE$_2$ in febrile rats enhanced muscle breakdown (Rodemann and Goldberg, 1981). Glutamine is a major end product of muscle break-down and is known to be a primary fuel for immune system cells (Newsholme et al., 1990; Hill and Hill, 1998). Baracos et al. (1983) proposed that COX inhibitors may reduce proteolysis as they inhibit PGE$_2$ production. This is supported by studies including rats infected with bacteria and treated with a COX inhibitor demonstrating reduced proteolysis compared to controls (Goldberg et al., 1984).

During the acute-phase response lipogenesis rates are reduced, while lipolysis rates increase (Eckersall, 2000). Tumor necrosis factor decreases lipoprotein lipase (LPL) activity, thus inhibiting triglyceride synthesis in adipose tissue, while stimulating lipolysis (Grunfeld and Feingold, 1991). Additionally, INF and IL-1 decreased LPL activity in cultured mice adipocytes (Beutler et al., 1985; Patton et al., 1986).

Infection alters glucose metabolism via accelerated glycogenolysis, decreased glycogen synthesis, increased peripheral glucose utilization, and increased hepatic gluconeogenesis (Grunfeld and Feingold, 1991). Increased peripheral glucose utilization despite decreased insulin sensitivity is mediated by cytokine activity on glucose transporters, as described by Cornelius and coworkers (1989). They reported a 150%
increase of glucose transporters in monokine induced L6 myotubes compared to controls. Carey and coworkers (2006) hypothesized IL-6 elevates glucose up-take via increased translocation of glucose transporter-4 (GLUT-4). This conclusion however, is contradicted by the finding of Rotter and coworkers (2003), who demonstrated IL-6 and TNFα induces insulin resistance in human adipocytes. During early stages of infection glucose levels increase to support the immune response (Grunfeld and Feingold, 1991). However, during severe infection glucose utilization may exceed glucose production and the host may become hypoglycemic (Grunfeld and Feingold, 1991). During sepsis, blood insulin concentration increases, but hyperinsulemia is attenuated as the host becomes insulin resistant (Grunfeld and Feingold, 1991). Kettelhut and coworkers (1987) indicated that COX inhibitors eliminated hyperglycemia and hypoglycemia in rats treated with high dose of TNF.

**Postpartum Inflammation**

Inflammation is the specific or nonspecific immune response of higher organisms to tissue injury or to foreign (or perceived as foreign) organisms (Tizard, 1996). Following parturition the uterus undergoes changes that allow it to return to its prepartum size by approximately 30 d postpartum (Bondurant, 1999). These changes in cattle include release of the placenta, ischemic necrosis and sloughing of the caruncular epithelium, replacement of damaged tissue with new epithelium, and shortening of the muscle fibers of the myometrium. In addition to the uterine recovery to its prepartum size, the postpartum period is often associated with uterine infection, as approximately
90% of postpartum uteri are contaminated with bacteria for the first 15 d but only 9% of them remain contaminated by 45 d postpartum (Paisley et al., 1986; Dohmen et al., 1995; Stevenson, 1997).

In some herds as much as 40% of postpartum cows undergo a uterine infection treatment (Lewis, 1997). Uterine infection may delay involution and reduce ovarian activity and fertility (Hussain and Daniel, 1991; Amiridis et al., 2001). Clinical signs of uterine infection include fever, anorexia, depression, reduced milk production, abdominal pain, and uterine pus (Zhou et al., 2001; Sheldon et al., 2004). Noakes (1996) summarized that in order for a successful pregnancy to take place, bacterial contamination should be eliminated from the uterus, uterine involution should occur, and the cow should cycle normally.

Sheldon and coworkers (2004) evaluated the association between postpartum pyrexia and uterine bacterial infection in dairy cows. In this study, normal rectal temperature was defined as 38.5 °C and febrile cows as a temperature > 39.4°C as it was suggested by Radostits and coworkers (2000). In this study, although the rectal temperature was frequently above 39.4°C, the mean rectal temperature during 10 d postpartum was 38.6°C. The authors indicated that both mean and maximum rectal temperature in cows with retained placenta was not different from cows that did not retain their placenta. They also reported that milk yield was not affected by pyrexia. Thus, they concluded that rectal temperature alone was not a good indicator for the number of uterine bacteria or of the presence of specific uterine pathogens. An earlier
study by Smith et al. (1998) showed no correlation between haptoglobin (an acute phase protein) concentration and rectal temperature in dairy cows during puerperal metritis.

**Non-steroidal Anti-inflammatory Drugs – Mechanism of Action**

Non-steroidal anti-inflammatory drugs (NSAID) have antipyretic and anti-inflammatory properties and are commonly used in food production animals (Abramson, 1991). Although different forms of NSAID’s are widely used, the mechanism of actions of the drugs is not completely understood (Warner and Mitchell, 2002). Non-steroidal anti-inflammatory drugs inhibit COX activity and block PG synthesis, which is known to mediate symptoms of inflammation including fever, hyperalgesia, increased vascular permeability and edema (Morteau, 2000).

The cascade of events for PG production is shown in figure 3 (Morteau, 2000). Arachidonic acid is separated by phospholipases from the cell membrane and is the substrate for COX, which catalyze PG production through a two step process (Morteau, 2000). Interleukin-1, TNFα and, INFγ trigger PG formation, including PGE₂, which is associated with pain and fever (Saper and Breder, 1994; Dinarello, 2000). There are three known isoforms of COX; 1, 2 and 3. COX-1 is expressed in most tissues and is thought to maintain basal PG production in different tissues and organs, including the gastrointestinal tract and the kidneys (Wolfe et al., 1999; Morteau, 2000). Cyclooxygenase-2 is nearly undetectable in most tissues under normal conditions, however, it is upregulated by inflammation (Wolfe et al., 1999). Cyclooxygenase-2 expression is induced in human and murine fibroblasts by different cells including IL-1β.
and TNFα, and by INFγ and IL-1β in macrophages (Morteau, 2000). Cyclooxygenase-3, a recent discovery, which is found in the cerebral cortex and heart, does not seem to be associated with fever (Tomlinson et al., 2004), and its function still being investigated (Chandrasekharan et al., 2002). A widely used NSAID in the animal health industry is flunixin meglumine (FM), which is thought to block the action of all COX isoforms, thus, markedly reducing PG levels (Tomlinson et al., 2004), resulting in reduced febrile response and inflammation. Flunixin meglumine is an NSAID that has recently been approved for use in lactating dairy cows for control of pyrexia associated with bovine respiratory disease and endotoxemia and for the control of inflammation in endotoxemia (FDA, 2004).

**Figure 3.** Pathways of inflammation (Morteau, 2000).
Flunixin Meglumine Use in the Dairy Industry

As discussed above, upon parturition the transition cow faces challenges that may impair its ability to produce and reproduce. The effect of FM on postpartum reproductive health has been investigated. Flunixin meglumine administration is associated with a decreased prostaglandin synthesis via COX inhibition (Brander et al., 1991). This NSAID is used in veterinary medicine as an anti-pyretic, analgesic (pain killer), and anti-inflammatory agent (Snow, 1983; Hardee et al., 1985). Amiridis et al. (2001) indicated that cows treated with FM had a faster uterine involution and earlier first estrus despite PGF$_{2\alpha}$ inhibition. Prostaglandin F$_{2\alpha}$ treatment in cows has been shown to increase fetal membrane passage (Stocker and Waelchli, 1993). Amiridis and coworkers (2001) suggested that the FM dose used in their study (2.2 mg/kg of BW twice daily for the first 2 DIM and once daily during the following 2 d) was too low to eliminate PGF$_{2\alpha}$ effect on involution. Thun and coworkers (1993) reported that high FM dose (2.2 mg/kg of BW twice daily, for the first 10 d postpartum) administered to cows significantly inhibited PG production but did not inhibit uterine involution, return to cyclicity, and first postpartal cycle length.

Odensvik and Fredriksson (1993) treated dairy cows with 56 or 28 doses of FM (2.2 mg/kg of BW four times or twice daily during first 14 DIM) and reported that FM did not alter uterine involution or time before onset of ovarian activity. Additionally, PGF$_{2\alpha}$ synthesis was not entirely suppressed during the study. Guilbault et al. (1987) summarized that FM (1 g twice daily for first 6 DIM) did not affect uterine involution rate but decreased ovarian activity. In a later study, Königsson and coworkers (2001)
indicated that FM (2.2 mk/kg of BW twice daily, 3-6 DIM) did not alter uterine involution and DMI in cows with induced retained placenta. The authors also reported that FM treated cows experienced rectal bleeding. Flunixin meglumine’s effect on reproduction variables is presented in Table 2.

<table>
<thead>
<tr>
<th>Reference</th>
<th>Time to uterine involution</th>
<th>Time to first estrus</th>
<th>Effect on ovarian activity</th>
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<tr>
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<td>Königsson et al. (2001)</td>
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<td>Odensvik and Fredriksson (1993)</td>
<td>←</td>
<td>⇔</td>
<td>←</td>
</tr>
<tr>
<td>Thun et al. (1993)</td>
<td>←</td>
<td>←</td>
<td></td>
</tr>
<tr>
<td>Guilbault et al. (1987)</td>
<td>←</td>
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</tr>
</tbody>
</table>

Throughout the dry period and the early stages of lactation bacterial population may increase in the udder and cause clinical mastitis (Goff and Horst, 1997). This occurs partly due to the immune system impaired activity during the last week of gestation through the first week of lactation (Goff and Horst, 1997). This allows a possible intramammary infection (IMI) that was suppressed during the dry period to gain strength (Goff and Horst, 1997). Gram-negative bacteria utilize iron for growth (Todhunter et al., 1990), which can be obtained from the increased citrate (an important contributor to iron transport, Graham et al., 1998) concentration in bovine colostrum (Oliver and Bushe, 1987). Additionally, the combination of keratin plug loss on teat-ends (7-10 d prepartum; Smith et al., 1985), and less effective teat-end sealing may increase susceptibility to clinical mastitis (Goff and Horst, 1997). Decreased plasma calcium concentration taking
place with the onset of lactation is associated with reduced smooth muscle contraction needed for teat sphincter closure after milking (Goff and Horst, 1997).

Clinical signs of mastitis include general depression, fever, increased heart rate, inflamed and warm udder, and anorexia (Dascanio et al., 1995). Non-steroidal anti-inflammatory drugs reduce inflammation, pain, and body temperature and are used to reduce systemic signs of severe mastitis cases (Fitzpatrick et al., 1998). Flunixin meglumine’s effect on mastitis has been evaluated by several studies (Table 3).

**Table 3.** Effects on FM on mastitis.

<table>
<thead>
<tr>
<th>Reference</th>
<th>Flunixin meglumine treatment</th>
<th>Effect on rectal temperature</th>
<th>Effect on milk production recovery</th>
<th>Effect on appetite</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wagner et al. (2004)</td>
<td>1x 2.2 mg/kg of BW</td>
<td>↓</td>
<td>↓</td>
<td>↔</td>
</tr>
<tr>
<td>Green et al. (1997)</td>
<td>2x 1 g</td>
<td>↔</td>
<td>↔</td>
<td>↔</td>
</tr>
<tr>
<td>Dascanio et al. (1995)</td>
<td>1x 1 g</td>
<td>↔</td>
<td>↔</td>
<td>↔</td>
</tr>
<tr>
<td>Anderson and Hunt (1989)</td>
<td>2x 1.1mg/kg of BW</td>
<td>↓</td>
<td>↓</td>
<td>↔</td>
</tr>
<tr>
<td>Lohuis et al. (1989)</td>
<td>2 or 3x 1.1 mg/kg of BW</td>
<td>↓ (after 2nd dose)</td>
<td>↓</td>
<td>↔</td>
</tr>
<tr>
<td></td>
<td>2 or 3x 2.2 mg/kg of BW</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anderson et al. (1986)</td>
<td>7x 1.1mg/kg of BW</td>
<td>↓</td>
<td>↔</td>
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</tr>
</tbody>
</table>
CHAPTER 2
EFFECTS OF FLUNIXIN MEGLUMINE ON PYREXIA, PRODUCTION AND BIOENERGETIC VARIABLES IN POSTPARTURIENT DAIRY COWS

 Partially funded by Schering Plough- Animal Health

Abstract

Multiparous cows (n=26) were randomly assigned to one of two treatments beginning at parturition. Treatments were flunixin meglumine (FM; Banamine®, 50 mg/mL, Schering Plough Animal Health, Kenilworth, NJ) at a dose of 2.2 mg/kg of BW and control (saline) at 2.2 mg/kg of BW. All treatments were administrated I.V. via a jugular catheter daily for the first 3 DIM. Individual milk yield (MY) and DMI were recorded daily for the first 35 DIM. Body temperature (BT) was measured daily at 0700 and 1600 h for the first 7 DIM. Milk composition was determined on 2, 7, 14, 21, 28, and 35 DIM and blood plasma was harvested on 1, 2, 3, 4, 7, 14, 21, 28, and 35 DIM. BW and BCS were determined on -7, 1, 7, 14, 21, 28, and 35 DIM. Flunixin meglumine tended to increase BT during 1-3 DIM (38.89 vs. 38.61°C, \( P = 0.07 \)) and tended to increase BT during the first 7 DIM (38.99 vs. 38.76°C, \( P = 0.06 \)), and there was no treatment differences in overall MY (35.2 kg/d), 3.5% FCM (37.6 kg/d), or ECM (37.7 kg/d). Compared to controls, FM had no effect on DMI (2.97% of BW), or overall energy balance (-2.32 Mcal/d). There were no treatment differences in milk fat (3.91%), milk protein (3.32%), milk lactose (4.57%), and milk SCC (532 x 1000/mL). Treatment had no effect on plasma glucose (66.5 mg/dL) or plasma NEFA (553 µEq/L), but
circulating PUN tended to be lower in FM treated cows (16.4 vs. 14.5 mg/dL).

Irrespective of treatment, when separated into a BT hierarchy (warmest 50% vs. coolest 50%; 39.21 vs. 38.64°C), cows with a higher body temperature during the first 7 DIM had an overall lower PUN (13.8 vs. 16.6 mg/dL), higher plasma NEFA (642 vs. 493 μEq/L), and tended to have a lower energy balance (-4.09 vs. -1.19 Mcal/d). Warmer cows also had increased milk SCC (955 vs. 200 x 1000/mL), but BT had little or no effect on other milk components and production parameters. Daily FM administration for the first 3 d after parturition had little or no effect on production or energetic variables, but tended to decrease PUN levels in transition dairy cows.

Introduction

During the periparturient period (~3 wk prepartum to ~3 wk postpartum; Grummer, 1995), dairy cows face many challenges/changes that may impair their ability to maximize performance during the rest of the lactation (Grummer, 1995; Goff and Horst, 1997; Overton and Waldron, 2004). Energy balance (EBAL) is the difference between energy consumed and the energy used for maintenance and/or production. During early lactation cows typically cannot consume enough calories to meet their requirements for maintenance and copious milk yield and enter into a state of negative energy balance (NEBAL), which has been linked to metabolic disorders and reproductive failures (Drackley, 1999; Butler, 2006).

Inflammation is the specific or nonspecific immune response to tissue injury or to foreign (or perceived as foreign) organisms (Tizard, 1996). Following parturition the
uterus quickly experiences a verity of structural and immunological events including fetal membrane release, ischemic necrosis and sloughing of the caruncular epithelium, healing of damaged tissue and growth of new epithelium, and shortening of the muscle fibers of the myometrium that allow it to return to its prepartum size (Bondurant, 1999). In addition to uterine recovery, the postpartum period is often associated with a severe uterine bacterial contamination (Bondurant, 1999). In some herds as much as 40% of postpartum cows are treated for uterine infection (Lewis, 1997), which may delay involution and reduce ovarian activity and fertility (Hussain and Daniel, 1991; Amiridis et al., 2001). Clinical signs to uterine infection include fever, anorexia, depression, reduced milk production, abdominal pain, and uterine discharge (Zhou et al., 2001; Sheldon et al., 2004).

Non-steroidal anti-inflammatory drugs (NSAID) have antipyretic and anti-inflammatory properties and are commonly and safely used in food production animals (Abramson, 1991). Non-steroidal anti-inflammatory drugs inhibit cyclooxygenase (COX) activity and block prostaglandin (PG) synthesis, which is known to mediate inflammation symptoms including fever, hyperalgesia, increased vascular permeability and edema (Morteau, 2000). Flunixin meglumine (FM) is an NSAID that has recently been approved (2004) by the Food and Drug Administration for use in lactating dairy cows for control of pyrexia associated with bovine respiratory disease and endotoxemia and for controlling of inflammation during endotoxemia. We hypothesized that FM treatment for the first 3 DIM would reduce parturition induced inflammation and
fever, which could result in improved appetite and consequently enhance production and bioenergetic variables.

**Materials and Methods**

**Design, Animals, and Treatments**

The University of Arizona Institutional Animal Care and Use Committee approved all procedures involving animals. Multiparous cows (n=32; 30 Holsteins, one Brown Swiss and one mixed breed), were housed together in a single pen at The University of Arizona Dairy. Six cows were removed from the study; three due to severe dystocia (two of these also had retained placenta), one due to lameness and mastitis, one due to uterine infection and DMI decrease, and one due to metabolic problems.

Upon arrival (April 4, 2006) approximately 32 d prior to parturition, cows where assigned randomly to one of two treatments; FM (n=14, Banamine®, 50 mg/mL, Schering-Plough Animal Health, Kenilworth, NJ) or control (n=12). The groups had a similar 305ME (11,865 and 11,795 kg, respectively), parity (2.07 and 2.25, respectively), and calving dates (248 days pregnant). Treatments were administrated I.V. via a jugular catheter daily for the first 3 DIM and within 5 hr postpartum using a 12- gauge x 2” Midicut Intravenous Cannula (MWI Veterinary Supply Co., Glendale, AZ) to insert and guide flexible Tygon Microbore Tubing (ID=1.02 mm, OD=1.78 mm; VWR Intl., Brisbane, CA). Treatments were administered as followed: after calving, cows were weighed and the respective treatment (FM or saline) was calculated and prepared at 2.2
mg/kg of BW. The jugular vein area was surgically prepared with Povidine (0.75%, Grapevine, TX) and 75% alcohol.

After treatment administration, two identification systems were utilized to prevent dosing errors. A plastic band was put on a hind leg (a pink leg band identified FM treatment and a blue leg band marked controls). In addition, a chalk stick was used to mark treated cows with the following method: one line was marked on each hip after a treatment so that each cow had 3 chalk lines on both hips after the 3rd day of treatment. Flunixin meglumine treated cows were marked with horizontal lines and saline treated cows were marked with vertical lines.

Cows were fed a close-up total mixed ration (TMR) formulated by Dairy Nutrition Services (Chandler, AZ) to meet or exceed the predicted energy, protein, mineral, and vitamin requirements (NRC, 2001). Alfalfa hay was the main forage source, and steam-flaked corn was the primary concentrate (Table 4). Feed was sampled every other day and dry matter content was determined (100°C for a minimum of 7 d). The TMR was sampled weekly and analyzed by wet chemistry methods (Dairy One Inc., Ithaca, NY).

Cows were trained to eat from Calan gates (Calan Broadbent feeding system; American Calan, Northwood, NH) and the training procedure lasted approximately 14 d. Following the training period, individual feed intake was recorded approximately 14 d prior to expected parturition day. Cows were fed twice daily *ad libitum* portions of fresh feed at 0700 and 1700 h; and feed weigh backs were measured and recorded daily at 0700 h. After parturition cows were fed a lactating TMR recommended to meet or exceed the
predicted energy, protein, mineral, and vitamin requirements (NRC, 2001; Table 4).

Shade and fresh water was available to cows at all times.

Cows were milked at 0900 and 2100 h and yield was recorded daily. Daily milk yield was averaged weekly for statistical analysis. Milk samples were obtained from each cow on 2, 7, 14, 21, 28 and 35 DIM and were stored at 4°C (bronopol tablet; D&F Control System, San Ramon, CA). Milk composition was analyzed at the Arizona DHIA (Tempe, AZ) using AOAC (2000) approved infrared analysis equipment and procedures. Blood samples were obtained from the coccygeal vein from each cow on -7, 1, 2, 3, 4, 7, 14, 21, 28 and 35 DIM. Samples were kept on ice until centrifuged at 3000 x g for 15 min. Plasma was split into two aliquots and immediately frozen at -20°C and later analyzed for NEFA, PUN, and glucose concentrations. Plasma NEFA, glucose, and PUN concentrations were determined enzymatically using commercially available kits validated for use in our laboratory (NEFA C kit, Wako Chemicals USA Inc., Richmond VA; Lot #37E5; Urea Nitrogen (BUN) Reagent, Anaheim, CA; Lot#08976, 08436, 07785, 6612; Autokit Glucose, Wako Chemicals USA, Inc., Lot #EF971). These procedures were scaled down and conducted in 96-well microplates (Rainin Instrument, LLC, Oakland, CA) and read using a microplate photometer (Multiskan Ascent, Thermo Electron Corporation, Vantaa, Finland). The inter- and intra-assay coefficients for the NEFA, PUN, and glucose assays were 3.9, 5.1, 2.5, 3.9, 5.3, and 2.6%, respectively.

Body weights were recorded on -7, 1, 7, 14, 21, 28, and 35 DIM before the morning milking. Body condition scores (BCS) were estimated independently by two trained individuals on -7, 1, 7, 14, 21, 28, and 35 DIM using a five-point system.
Body temperatures (BT) were measured by a rectal thermometer (GLA M700 Digital Thermometer, San Luis Obispo, CA) at 0700 and 1600 h for the first 7 DIM. Cows were observed daily for general health status.

Calculations

Prepartum energy balance was calculated using the following equation (NRC, 2001): energy balance = net energy of intake – (net energy of maintenance + net energy of pregnancy). Net energy intake was calculated by multiplying the daily DMI by the calculated net energy value of the diet. Energy requirement for maintenance was computed using the following equation (NRC, 2001): net energy of maintenance = 0.08 x BW^{0.75}. Pregnancy requirements were estimated using the following equation (NRC, 2001): net energy of pregnancy = [(0.00318 x days pregnant − 0.0352) x (calf BW/45)]/0.218. Because calves were not weighed after parturition, an average birth weight of 45 kg was used to equate net energy of pregnancy. Postpartum energy balance was estimated using the following equation (NRC, 2001): energy balance = net energy of intake – (net energy of maintenance + net energy of lactation). Net energy of lactation was estimated by the following equation: [(0.0929 x fat %) + (0.0547 x crude protein %) + (0.0395 x lactose %)] x milk production. Daily EBAL values were subjected to a third-order polynomial regression analysis to minimize variation, and predicted daily energy values from these equations were used in the statistical analysis as previously described (Lucy et al., 1991; Moore et al., 2004; Odens et al., 2007). Third-order polynomial regression analysis was conducted using scatter plot in Microsoft® Office Excel 2003.
Days to EBAL nadir were identified as the day at which the lowest predicted net EBAL value occurred. 3.5% fat corrected milk (FCM) and energy corrected milk (ECM) were calculated (NRC, 2001) using the following equations: 3.5% FCM = (0.432 x milk yield) + (16.23 x milk fat yield); ECM = (0.327 x milk yield) + (12.95 x milk fat yield) + (7.2 x milk protein yield). Feed efficiency was calculated using the following equation: feed efficiency = 3.5% FCM/DMI

**Statistical Analysis**

Milk yield, MY as % of BW, DMI, DMI and % of BW, plasma NEFA and glucose, PUN, EBAL, feed efficiency, and body temperature were analyzed by repeated measures using the PROC MIXED procedure of SAS (2005) with an autoregressive covariance structure and day or week of lactation as the repeated affect. The previous 305ME was used as a covariate on all measurements. Dry matter intake from the week prior to calving was also used as a covariate on production variables but the interpretation did not differ, therefore the 305ME was used on all measured parameters. The model contained day or week of lactation, treatment and day or week of lactation x treatment interactions. Cows were the random effect, and day or week of lactation, treatment and DIM or week of lactation x treatment interaction were the fixed effects. Dry matter intake and MY were also analyzed as a percentage of BW (PBW). Milk components were analyzed by repeated measures using the PROC MIXED procedure of SAS (2005) with an autoregressive covariance structure and day of lactation as the repeated affect. The model contained day of lactation, treatment and day x treatment interactions. Cows
were the random effect, and day of lactation, treatment and day of lactation x treatment interaction were the fixed effects. Energy balance nadir was analyzed using the PROC MIXED procedure of SAS (2005), with treatment as the dependent variable and did not contain a time or repeated component.

Additional analyses were conducted for cows separated into a BT hierarchy irrespective of treatment; 50% of the cows with the lower BT (n=5 FM treated cows and n=8 controls) and 50% of the cows with the higher BT (n=9 FM treated cows and n=4 controls). Production and plasma metabolites variables were then analyzed accordingly. Standard errors of the mean are reported and differences considered significant when \( P < 0.05 \) unless otherwise stated.
**Table 4. Ingredients and chemical composition of diets**

<table>
<thead>
<tr>
<th>Composition</th>
<th>Prepartum</th>
<th>Postpartum</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ingredient, % of DM</td>
<td>Prepartum</td>
<td>Postpartum</td>
</tr>
<tr>
<td>Alfalfa hay</td>
<td>68.08</td>
<td>65.88</td>
</tr>
<tr>
<td>Whole cotton seed</td>
<td>3.25</td>
<td>8.76</td>
</tr>
<tr>
<td>Barley</td>
<td>12.64</td>
<td>9.84</td>
</tr>
<tr>
<td>Steam flaked corn</td>
<td>12.64</td>
<td>11.33</td>
</tr>
<tr>
<td>Supplement</td>
<td>3.39</td>
<td>2.5</td>
</tr>
<tr>
<td>Maxxer</td>
<td>.</td>
<td>1.68</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Chemical analysis, % of DM</th>
<th>Prepartum</th>
<th>Postpartum</th>
</tr>
</thead>
<tbody>
<tr>
<td>CP</td>
<td>21.45</td>
<td>19.02</td>
</tr>
<tr>
<td>NDF</td>
<td>29.58</td>
<td>27.02</td>
</tr>
<tr>
<td>ADF</td>
<td>23.57</td>
<td>20.96</td>
</tr>
</tbody>
</table>

\[\text{NE}_{\text{L}} \text{ Mcal/kg DM} \]

<table>
<thead>
<tr>
<th>(\text{NE}_{\text{L}} \text{ Mcal/kg DM} )</th>
<th>Prepartum</th>
<th>Postpartum</th>
</tr>
</thead>
<tbody>
<tr>
<td>(\text{NE}_{\text{L}} \text{ Mcal/kg DM} )</td>
<td>1.63</td>
<td>1.76</td>
</tr>
</tbody>
</table>

1Values represent an average of samples collected and composited throughout the trial. Dry matter averaged 50% and 52% for the prepartum and postpartum diets, respectively.

2The prepartum supplement contained 2.10% fat, 7.16% Ca, 0.14% P, 4.00% Mg, 3.50% S, 0.17% K, 0.06% Na, 34.67% Cl, 1628.8 mg/kg of Zn, 933.6 mg/kg of Mn, 403.3 mg/kg of Fe, 497.1 mg/kg of Cu, 54.0 mg/kg of Co, 11.0 mg/kg Se, 0.9 mg/kg of Mo, 54.1 mg/kg of I, 537.5 of IU/g of vitamin A, 65.9 IU/g of vitamin D, and 7.1 IU/g of vitamin E.

3The postpartum supplement contained 1.47% fat, 5.74% Ca, 6.47% P, 4.06% Mg, 0.50% S, 0.23% K, 15.25% Na, 2.33% Cl, 1776.4 mg/kg of Zn, 1832.6 mg/kg of Mn, 1102.9 mg/kg of Fe, 77.7 mg/kg of Cu, 15.7 mg/kg Se, 7.0 mg/kg of Mo, 44.3 mg/kg of I, 309.2 of IU/g of vitamin A, 30.6 IU/g of vitamin D, and 1.0 IU/g of vitamin E.

4Calcium salts of palm oil (Tarome Inc., Eloy, AZ)

**Results**

There were obvious time effects on most production and metabolic variables as would be expected during the transition period (Tables 5-11). Flunixin meglumine treated cows had increased daily BT (38.99 vs. 38.76°C; \(P = 0.03\); Table 5; Figure 4) during the first 7 DIM and tended to be increased when evaluated on a weekly basis (38.99 vs. 38.76°C; \(P = 0.06\); Table 6). There was a treatment x time interaction as both
groups had similar body temperatures at 1 DIM but cows treated with FM had increased BT at 2 and 3 DIM ($P = 0.03$; Table 5; Figure 11, appendix A).

Dry matter intake was coincidentally reduced in cows destined for FM treatment (14.90 vs. 13.26 kg/d; $P = 0.03$; Table 6) during the week prior to calving. Controls had increased DMI (18.83 vs. 14.80 kg/d; $P < 0.01$; Table 5; Figure 5A) and DMI as a PBW (2.52 vs. 2.10%; $P < 0.01$; Table 5; Figure 5B) for the first 7 DIM. Additionally, there was a treatment x time interaction for DMI and DMI as a PBW during the first 7 DIM as there were no difference on the first day of lactation, but FM treated cows had decreased DMI during the following 6 d. Overall DMI was reduced in FM treated cows (22.04 vs. 19.48 kg/d; $P < 0.01$; Table 6; Figure 6A) for the duration of the study, but there was no overall effect on DMI when evaluated on a PBW (2.98%; Table 6; Figure 6B).

Overall MY was reduced in FM treated cows during the first 7 DIM (29.04 vs. 25.54 kg/d; $P = 0.04$; Table 5; Figure 12, appendix A), but treatment had no effect on MY as a PBW (3.73%; Table 5; Figure 13, appendix A) during this time. Treatment did not effect overall MY (35.18 kg/d), MY as a PBW (5.02%; Table 6; Figures 7A and 7B), 3.5% FCM (37.64 kg/d) and ECM (37.67 kg/d; Table 6; Figures 14 and 15, appendix A) during the first 5 WOL. Milk fat (3.91%), protein (3.32%), lactose (4.57%) and somatic cell count (SCC, 532 x 1000/mL; Table 7; Figures 16, 17, 18, and 19, appendix A) were similar among treatments throughout the study.

Flunixin meglumine treated cows had similar BW (776 kg) and BCS (3.41; Table 6) the week prior to calving. Body weight (705 kg) and BCS (3.45; Table 6, Figures 20 and 21, appendix A) were not affected by treatment throughout the first 5 WOL.
Calculated EBAL was increased (3.5 vs. 0.8 Mcal/d; \( P < 0.05 \); Table 6) for controls prepartum, tended to be higher (-2.1 vs. -5.3 Mcal/d; \( P = 0.09 \); Table 5; Figure 8A) during the first 7 DIM, but was similar (-2.4 Mcal/d; Table 6; Figure 8B) to FM treated cows during the first 5 WOL. Energy balance nadir (-6.4 Mcal) and days to EBAL nadir (12.8 d; Table 5) were unaffected by treatment. Feed efficiency tended to be increased for FM treated cows (2.2 vs. 1.7; \( P = 0.08 \); Table 5) for the first 7 DIM, but was similar (1.9; Table 6; Figures 22 and 23, appendix A) during the first 5 WOL. Treatment had no effect on plasma glucose (66.5 mg/mL) or plasma NEFA (553 \( \mu \)Eq/L; Table 8; Figures 9A and 9B) throughout the study, but circulating PUN tended to be reduced in FM treated cows (16.4 vs. 14.5 mg/dL; \( P = 0.08 \); Table 8; Figure 9C).

When cows were separated into a BT hierarchy (irrespective of FM treatment; 50% of the cows with the lower BT and 50% of the cows with higher BT; 38.6 vs. 39.2°C; \( P < 0.01 \); Table 9) DMI as a PBW tended to be increased for cows with a lower BT for the first 5 WOL (3.09 vs. 2.81%; \( P = 0.06 \); Table 9; Figure 24, appendix A). There were no BT differences in MY as a PBW (5.00%), 3.5% FCM as % of BW (5.41%), and ECM as a PBW (5.39%; Table 9; Figures 25, 26 and 27, appendix A) during the study. There was a treatment x time interaction (\( P = <0.05 \); Table 10; ; Figure 28, appendix A) effect on milk fat content as cows having a higher BT had increased values in early lactation but similar values as time progressed. Milk SCC was increased (955 vs. 200 x 1000/mL; \( P = 0.03 \); Table 10; Figure 29, appendix A) in cows having a higher BT. Milk protein and lactose (Table 10; Figures 30 and 31, appendix A) were not affected by BT. During the first 5 WOL, EBAL tended to be increased for cows with
lower BT (-1.3 vs. -4.1 Mcal; \( P = 0.08 \); Table 9; Figure 32, appendix A). There was a treatment x time interaction for increased efficiency as cows with a higher BT cows had increased efficiency immediately after calving but similar values as lactation progressed \((P = 0.04; \text{Table 9; Figure 33, appendix A})\). Cows having a higher BT had decreased PUN (16.6 vs. 13.8 mg/dL; \( P < 0.01 \); Figure 10A) and increased NEFA (642 vs. 493 \( \mu \text{Eq/L}; P < 0.01 \); Table 11; Figure 10B), but circulating plasma glucose was similar between groups (66.5 mg/mL; Figure 34, appendix A).
Table 5. Effects of flunixin meglumine (FM) treatment (during 1-3 DIM) on postpartum (1-5 WOL) production variables in transition dairy cows.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control</th>
<th>FM</th>
<th>SEM</th>
<th>TRT</th>
<th>DIM2</th>
<th>TRT x DIM</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>1-3 DIM</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BT, °C</td>
<td>38.61</td>
<td>38.89</td>
<td>0.11</td>
<td>0.07</td>
<td>0.12</td>
<td>0.03</td>
</tr>
<tr>
<td>DMI, kg/d</td>
<td>17.82</td>
<td>14.99</td>
<td>0.82</td>
<td>0.02</td>
<td>0.34</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>DMI as % of BW</td>
<td>2.37</td>
<td>2.10</td>
<td>0.13</td>
<td>0.16</td>
<td>0.29</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>MY, kg/d</td>
<td>23.52</td>
<td>21.34</td>
<td>1.40</td>
<td>0.28</td>
<td>&lt;0.01</td>
<td>0.43</td>
</tr>
<tr>
<td>MY as % of BW</td>
<td>3.12</td>
<td>2.94</td>
<td>0.17</td>
<td>0.47</td>
<td>&lt;0.01</td>
<td>0.61</td>
</tr>
<tr>
<td>EBAL, Mcal</td>
<td>-1.27</td>
<td>-4.39</td>
<td>1.28</td>
<td>0.10</td>
<td>&lt;0.01</td>
<td>0.48</td>
</tr>
<tr>
<td>Feed efficiency</td>
<td>1.39</td>
<td>1.77</td>
<td>0.18</td>
<td>0.15</td>
<td>&lt;0.01</td>
<td>0.26</td>
</tr>
<tr>
<td><strong>1-7 DIM</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BT, °C</td>
<td>38.76</td>
<td>38.99</td>
<td>0.08</td>
<td>0.03</td>
<td>0.11</td>
<td>0.07</td>
</tr>
<tr>
<td>DMI, kg/d</td>
<td>18.83</td>
<td>14.80</td>
<td>0.60</td>
<td>&lt;0.01</td>
<td>0.28</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>DMI as % of BW</td>
<td>2.52</td>
<td>2.10</td>
<td>0.10</td>
<td>&lt;0.01</td>
<td>0.08</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>MY, kg/d</td>
<td>29.04</td>
<td>25.54</td>
<td>1.18</td>
<td>0.04</td>
<td>&lt;0.01</td>
<td>0.46</td>
</tr>
<tr>
<td>MY as % of BW</td>
<td>3.86</td>
<td>3.59</td>
<td>0.16</td>
<td>0.24</td>
<td>&lt;0.01</td>
<td>0.71</td>
</tr>
<tr>
<td>EBAL, Mcal</td>
<td>-2.1</td>
<td>-5.3</td>
<td>1.3</td>
<td>0.09</td>
<td>&lt;0.01</td>
<td>0.92</td>
</tr>
<tr>
<td>Feed efficiency</td>
<td>1.7</td>
<td>2.2</td>
<td>0.2</td>
<td>0.08</td>
<td>&lt;0.01</td>
<td>0.29</td>
</tr>
<tr>
<td>EBAL nadir, Mcal</td>
<td>-5.0</td>
<td>-7.8</td>
<td>1.3</td>
<td>0.13</td>
<td></td>
<td></td>
</tr>
<tr>
<td>EBAL nadir, d</td>
<td>14.0</td>
<td>11.5</td>
<td>1.6</td>
<td>0.29</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

1* Treatments were 2.2 mg/kg of BW saline (control) or 2.2 mg/kg of BW FM for the first 3 DIM.

2Days in milk.

3Body temperature.

4Dry matter intake.

5Dry matter intake as a percentage of body weight.

6Milk yield.

7Milk yield as a percentage of body weight.

8Energy balance.
Table 6. Effects of flunixin meglumine (FM) treatment (during 1-3 DIM) on production variables (1-5 WOL) in transition dairy cows during 1 week prior to parturition and the first 5 WOL.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Treatments&lt;sup&gt;1&lt;/sup&gt;</th>
<th></th>
<th>SEM</th>
<th></th>
<th>TRT</th>
<th>WOL&lt;sup&gt;2&lt;/sup&gt;</th>
<th>TRT x WOL</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>FM</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Prepartum</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DMI&lt;sup&gt;3&lt;/sup&gt;, kg/d</td>
<td>14.90</td>
<td>13.26</td>
<td>0.50</td>
<td>0.03</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BW&lt;sup&gt;4&lt;/sup&gt;, kg</td>
<td>804</td>
<td>748</td>
<td>24</td>
<td>0.11</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BCS&lt;sup&gt;5&lt;/sup&gt;</td>
<td>3.47</td>
<td>3.34</td>
<td>0.17</td>
<td>0.59</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>EBAL&lt;sup&gt;6&lt;/sup&gt;, Mcal</td>
<td>3.5</td>
<td>0.8</td>
<td>0.9</td>
<td>&lt;0.05</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Postpartum</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BT&lt;sup&gt;7&lt;/sup&gt;, °C</td>
<td>38.76</td>
<td>38.99</td>
<td>0.08</td>
<td>0.06</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DMI, kg/d</td>
<td>22.04</td>
<td>19.48</td>
<td>0.49</td>
<td>&lt;0.01</td>
<td>&lt;0.01</td>
<td>0.35</td>
<td></td>
</tr>
<tr>
<td>DMI as % of BW&lt;sup&gt;8&lt;/sup&gt;, %</td>
<td>3.07</td>
<td>2.89</td>
<td>0.10</td>
<td>0.24</td>
<td>&lt;0.01</td>
<td>&lt;0.01</td>
<td>0.35</td>
</tr>
<tr>
<td>MY&lt;sup&gt;9&lt;/sup&gt;, kg/d</td>
<td>36.13</td>
<td>34.23</td>
<td>1.34</td>
<td>0.33</td>
<td>&lt;0.01</td>
<td>0.30</td>
<td></td>
</tr>
<tr>
<td>MY as % of BW&lt;sup&gt;10&lt;/sup&gt;, %</td>
<td>4.99</td>
<td>5.04</td>
<td>0.17</td>
<td>0.83</td>
<td>&lt;0.01</td>
<td>0.14</td>
<td></td>
</tr>
<tr>
<td>3.5 % FCM&lt;sup&gt;11&lt;/sup&gt;, kg/d</td>
<td>38.33</td>
<td>36.95</td>
<td>1.64</td>
<td>0.56</td>
<td>&lt;0.01</td>
<td>0.54</td>
<td></td>
</tr>
<tr>
<td>ECM&lt;sup&gt;12&lt;/sup&gt;, kg/d</td>
<td>38.44</td>
<td>36.90</td>
<td>1.55</td>
<td>0.49</td>
<td>&lt;0.01</td>
<td>0.59</td>
<td></td>
</tr>
<tr>
<td>BW, kg</td>
<td>724</td>
<td>686</td>
<td>21</td>
<td>0.20</td>
<td>&lt;0.01</td>
<td>0.44</td>
<td></td>
</tr>
<tr>
<td>BCS</td>
<td>3.56</td>
<td>3.34</td>
<td>0.10</td>
<td>0.15</td>
<td>0.55</td>
<td>0.75</td>
<td></td>
</tr>
<tr>
<td>EBAL, Mcal</td>
<td>-1.2</td>
<td>-3.6</td>
<td>1.1</td>
<td>0.15</td>
<td>&lt;0.01</td>
<td>0.84</td>
<td></td>
</tr>
<tr>
<td>Feed efficiency</td>
<td>1.8</td>
<td>2.0</td>
<td>0.1</td>
<td>0.15</td>
<td>0.12</td>
<td>0.54</td>
<td></td>
</tr>
</tbody>
</table>

<sup>1</sup>Treatments were 2.2 mg/kg of BW saline (control) or 2.2 mg/kg of BW FM for the first 3 DIM.

<sup>2</sup>Week of lactation.

<sup>3</sup>Dry matter intake.

<sup>4</sup>Body weight.

<sup>5</sup>Body condition score.

<sup>6</sup>Energy balance.

<sup>7</sup>Body temperature.

<sup>8</sup>Dry matter intake as a percentage of body weight.

<sup>9</sup>Milk yield.

<sup>10</sup>Milk yield as a percentage of body weight.

<sup>11</sup>3.5% fat corrected milk.

<sup>12</sup>Energy corrected milk.
<table>
<thead>
<tr>
<th>Milk Parameter</th>
<th>Control</th>
<th>FM</th>
<th>SEM</th>
<th>TRT</th>
<th>DIM²</th>
<th>TRT x DIM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fat, %</td>
<td>3.88</td>
<td>3.94</td>
<td>0.16</td>
<td>0.79</td>
<td>0.06</td>
<td>0.96</td>
</tr>
<tr>
<td>Protein, %</td>
<td>3.31</td>
<td>3.33</td>
<td>0.10</td>
<td>0.90</td>
<td>&lt;0.01</td>
<td>0.97</td>
</tr>
<tr>
<td>Lactose, %</td>
<td>4.58</td>
<td>4.57</td>
<td>0.04</td>
<td>0.90</td>
<td>&lt;0.01</td>
<td>0.28</td>
</tr>
<tr>
<td>SCC³, x 1000/mL</td>
<td>673</td>
<td>391</td>
<td>254</td>
<td>0.44</td>
<td>0.30</td>
<td>0.48</td>
</tr>
</tbody>
</table>

³Somatic cell count.

Table 7. Effects of flunixin meglumine (FM) treatment (during 1-3 DIM) on milk composition (1-5 WOL) in transition dairy cows.

¹Treatments were 2.2 mg/kg of BW saline (control) or 2.2 mg/kg of BW FM for the first 3 DIM.
²Days in milk.
³Somatic cell count.
Table 8. Effects of flunixin meglumine (FM) treatment (during 1-3 DIM) on postpartum (1-5 WOL) plasma energetic metabolites in transition dairy cows.

<table>
<thead>
<tr>
<th>Plasma Metabolite</th>
<th>Treatments&lt;sup&gt;1&lt;/sup&gt;</th>
<th>SEM</th>
<th>TRT</th>
<th>DIM&lt;sup&gt;2&lt;/sup&gt;</th>
<th>TRT x DIM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose, mg/dL</td>
<td>Control 66.3</td>
<td>1.7</td>
<td>0.86</td>
<td>&lt;0.01</td>
<td>0.98</td>
</tr>
<tr>
<td></td>
<td>FM 66.7</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NEFA&lt;sup&gt;3&lt;/sup&gt;</td>
<td>Control 524</td>
<td>38</td>
<td>0.29</td>
<td>&lt;0.01</td>
<td>0.37</td>
</tr>
<tr>
<td></td>
<td>FM 582</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PUN&lt;sup&gt;4&lt;/sup&gt;</td>
<td>Control 16.4</td>
<td>0.7</td>
<td>0.08</td>
<td>&lt;0.01</td>
<td>0.53</td>
</tr>
<tr>
<td></td>
<td>FM 14.5</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<sup>1</sup>Treatments were 2.2 mg/kg of BW saline (control) or 2.2 mg/kg of BW FM for the first 3 DIM.
<sup>2</sup>Days in milk.
<sup>3</sup>Non-esterified fatty acids.
<sup>4</sup>Plasma urea nitrogen.
Table 9. Effects of body temperature during the first 7 DIM on production variables (1-5 WOL) in transition dairy cows.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Body Temperature</th>
<th></th>
<th></th>
<th></th>
<th>TRT</th>
<th>WOL²</th>
<th>TRT x WOL</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Bottom 50%</td>
<td>Top 50%</td>
<td>SEM</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BT³, °C</td>
<td>38.6</td>
<td>39.2</td>
<td>0.04</td>
<td></td>
<td>&lt;0.01</td>
<td></td>
<td></td>
</tr>
<tr>
<td>DMI as % of BW⁴</td>
<td>3.09</td>
<td>2.81</td>
<td>0.10</td>
<td>0.06</td>
<td>&lt;0.01</td>
<td>0.32</td>
<td></td>
</tr>
<tr>
<td>MY as % of BW⁵</td>
<td>5.10</td>
<td>4.91</td>
<td>0.17</td>
<td>0.42</td>
<td>&lt;0.01</td>
<td>0.93</td>
<td></td>
</tr>
<tr>
<td>3.5% FCM as % of BW⁶</td>
<td>5.46</td>
<td>5.35</td>
<td>0.26</td>
<td>0.78</td>
<td>&lt;0.01</td>
<td>0.72</td>
<td></td>
</tr>
<tr>
<td>ECM as % of BW⁷</td>
<td>5.44</td>
<td>5.34</td>
<td>0.24</td>
<td>0.76</td>
<td>&lt;0.01</td>
<td>0.76</td>
<td></td>
</tr>
<tr>
<td>EBAL⁸, Mcal</td>
<td>-1.3</td>
<td>-4.1</td>
<td>1.1</td>
<td>0.08</td>
<td>&lt;0.01</td>
<td>0.10</td>
<td></td>
</tr>
<tr>
<td>Feed efficiency</td>
<td>1.8</td>
<td>2.0</td>
<td>0.1</td>
<td>0.15</td>
<td>0.08</td>
<td>0.04</td>
<td></td>
</tr>
</tbody>
</table>

¹ Separated into a body temperature hierarchy; 50% of the cows (n=13) with the lowest (bottom) BT and 50% of the cows (n=13) with the highest (top) BT.
²Week of lactation.
³Body temperature.
⁴Dry matter intake as a percentage of body weight.
⁵Milk yield as a percentage of body weight.
⁶3.5% fat corrected milk as a % of body weight.
⁷Energy corrected milk as a % of body weight.
⁸Energy balance.
Table 10. Effects of body temperature during the first 7 DIM on milk composition (1-5 WOL) in transition dairy cows.

<table>
<thead>
<tr>
<th>Milk Parameter</th>
<th>Body Temperature&lt;sup&gt;1&lt;/sup&gt;</th>
<th>SEM</th>
<th>TRT</th>
<th>DIM&lt;sup&gt;2&lt;/sup&gt;</th>
<th>TRT x DIM</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Bottom 50%</td>
<td>Top 50%</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fat, %</td>
<td>3.81</td>
<td>4.03</td>
<td>0.15</td>
<td>0.34</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Protein, %</td>
<td>3.29</td>
<td>3.37</td>
<td>0.10</td>
<td>0.58</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Lactose, %</td>
<td>4.58</td>
<td>4.57</td>
<td>0.04</td>
<td>0.90</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>SCC&lt;sup&gt;3&lt;/sup&gt;, x 1000/mL</td>
<td>200</td>
<td>955</td>
<td>233</td>
<td>0.03</td>
<td>0.21</td>
</tr>
</tbody>
</table>

<sup>1</sup> Separated into a body temperature hierarchy; 50% of the cows (n=13) with the lowest (bottom) BT and 50% of the cows (n=13) with the highest (top) BT.

<sup>2</sup> Days in milk.

<sup>3</sup>Somatic cell count.
Table 11. Effects of body temperature during the first 7 DIM on plasma energetic metabolites (1-5 WOL) in transition dairy cows.

<table>
<thead>
<tr>
<th>Plasma Metabolite</th>
<th>Body Temperature$^1$</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th>TRT</th>
<th>DIM$^2$</th>
<th>TRT x DIM</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Bottom 50%</td>
<td>Top 50%</td>
<td>SEM</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glucose, mg/dL</td>
<td>66.9</td>
<td>66.0</td>
<td>1.7</td>
<td>0.72</td>
<td>&lt;0.01</td>
<td>0.75</td>
<td></td>
<td></td>
</tr>
<tr>
<td>NEFA$^3$, µEq/L</td>
<td>493</td>
<td>642</td>
<td>34.19</td>
<td>&lt;0.01</td>
<td>&lt;0.01</td>
<td>0.21</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PUN$^4$, mg/dL</td>
<td>16.6</td>
<td>13.8</td>
<td>0.7</td>
<td>&lt;0.01</td>
<td>&lt;0.01</td>
<td>0.13</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

$^1$ Separated into a body temperature hierarchy; 50% of the cows (n=13) with the lowest (bottom) BT and 50% of the cows (n=13) with the highest (top) BT.

$^2$ Days in milk.

$^3$ Non-esterified fatty acids.

$^4$ Plasma urea nitrogen.
Figure 4. Effects of flunixin meglumine (FM) on body temperature. Treatments were 2.2 mg/kg of BW saline (control) or 2.2 mg/kg of BW FM for the first 3 DIM. Values represent least square means (n=12 or 14) for the control and FM respectively; SEM of body temperature for the first 7 DIM averaged 0.08 and ranged from 0.07 to 0.08 °C/d. Number sign (#) indicates treatment x time interaction with $0.05 < P < 0.1$. 
Figure 5. Effects of flunixin meglumine (FM) on DMI (A) and DMI as a percentage of BW (B). Treatments were 2.2 mg/kg of BW saline (control) or 2.2 mg/kg of BW FM for the first 3 DIM. Values represent least square means (n=12 or 14) for the control and FM respectively; SEM of DMI for the first 7 DIM averaged 0.60 and ranged from 0.58 to 0.63 kg/d; SEM of DMI % of BW for the first 7 DIM averaged 0.10 and ranged from 0.097 to 0.100% BW/d. Asterisk sign (*) indicates treatment x time interaction with $P < 0.05$ and number sign (#) indicates treatment x time interaction with $0.05 < P < 0.1$. 
Figure 6. Effects of flunixin meglumine (FM) on DMI (A) and DMI as a percentage of BW (B). Treatments were 2.2 mg/kg of BW saline (control) or 2.2 mg/kg of BW FM for the first 3 DIM. Values represent least square means (n=12 or 14) for the control and FM respectively; SEM of DMI for the first 5 WOL averaged 0.49 and ranged from 0.47 to 0.51 kg/wk; SEM of DMI % of BW for the first 5 WOL averaged 0.10 and ranged from 0.099 to 0.108% BW/d.
Figure 7. Effects of flunixin meglumine (FM) on MY (A) and MY as a percentage of BW (B). Treatments were 2.2 mg/kg of BW saline (control) or 2.2 mg/kg of BW FM for the first 3 DIM. Values represent least square means (n=12 or 14) for the control and FM respectively; SEM of MY for the first 5 WOL averaged 1.34 and ranged from 1.29 to 1.39 kg/wk; SEM of MY % of BW for the first 5 WOL averaged 0.17 and ranged from 0.16 to 0.17%/d.
Figure 8. Effects of flunixin meglumine (FM) on energy balance (EBAL) during the first 7 DIM (A) and the first 5 WOL (B). Treatments were 2.2 mg/kg of BW saline (control) or 2.2 mg/kg of BW of FM for the first 3 DIM. Values represent least square means (n=12 or 14) for the control and FM respectively; SEM of EBAL for the first 7 DIM averaged 1.28 and ranged from 1.23 to 1.33 Mcal/d; SEM of EBAL for the first 5 WOL averaged 1.13 and ranged from 1.09 to 1.17 Mcal/d.
Figure 9. Effects of flunixin meglumine (FM) on plasma glucose (A), NEFA (B), and PUN (C). Treatments were 2.2 mg/kg of BW saline (control) or 2.2 mg/kg of BW FM for the first 3 DIM. Values represent least square means (n=12 or 14) for the control and FM respectively; SEM of plasma glucose on 1-4, 7, 14, 21, 28, and 35 DIM averaged 1.7 and ranged from 1.6 to 1.8 mg/dL/d; SEM of plasma NEFA on 1-4, 7, 14, 21, 28, and 35 DIM averaged 38 and ranged from 36 to 39 µEq/L/d; SEM of PUN on 1-4, 7, 14, 21, 28, and 35 DIM averaged 0.7 and ranged from 0.7 to 0.8 mg/dL/d.
Figure 10. Effects of body temperature on PUN (A) and plasma NEFA (B) on 1-4, 7, 14, 21, 28, and 35 DIM. Cows were separated into a BT hierarchy (50% of the cows with the lowest (bottom) BT; 50% of the cows with the highest (top) BT, irrespective of FM and saline treatments). Values represent least square means (n=13) for the two treatment groups; SEM of plasma NEFA on 1-4, 7, 14, 21, 28, and 35 DIM averaged 34 and ranged from 32 to 37 µEq/L/d; SEM of PUN on 1-4, 7, 14, 21, 28, and 35 DIM averaged 0.7 and ranged from 0.6 to 0.7 mg/dL/d.
Discussion

The success of the periparturient period has a significant impact on cow performance and ultimately may determine farm profitability. Dairy cows usually experience a severe energy deficit in early lactation due to an inability to meet their energy requirements for maintenance and copious milk production and as a result enter a state of NEBAL (Baumgard et al., 2007a). Negative energy balance during the periparturient period is associated with increased metabolic and reproductive disorders (Drackley, 1999). In addition, immune function in periparturient cows is reduced and they become more susceptible to infection (Mallard et al., 1998). The uterine tissue trauma and subsequent inflammation and potential fever associated with parturition maybe one reason appetite is reduced postcalving. To evaluate this we administered FM to cows immediately after parturition and for the first 3 DIM in an attempt to alleviate fever and inflammation and improve bioenergetic variables.

In contrast to our hypothesis, FM administration increased BT during 2-4 DIM and tended to increase BT during the first 7 DIM (Table 5; Figure 4). Although increased compared to controls, BT in FM treated cows remained below the febrile critical point (39.5°C, Radostits et al., 2000). The increase in BT following FM treatment in our study is not consistent with previous research and the reason is currently unknown. Although FM treatment did not reduce BT in transition cows with retained placenta (Königsson et al., 2001), mastitis (Dascanio et al., 1995; Green et al., 1997), or in heat-stressed lactating cows (Soto et al., 2003), it was effective at decreasing BT in cows with uterine metritis (Amiridis et al., 2001), mastitis (Anderson et al., 1986; Anderson and Hunt, 1989; Lohuis
et al., 1989; Wagner et al., 2001), and Escherichia coli exposed heifers (Odensvik et al., 1996).

We evaluated DMI on absolute values and DMI as a PBW (due to numerical difference in BW between the two treatments prior to calving). Regardless of evaluation method, FM treated cows had reduced DMI immediately postpartum (Table 5; Figure 5). Although not completely understood, reduced DMI might have been caused by increased BT as previous studies observed decreased DMI in febrile heifers (Steiger et al., 1999) and goats (Takeuchi et al., 1995). However, overall DMI as a PBW was similar between treatments (Table 6) and this agrees with previous research by Königsson and coworkers (2001) who demonstrated that FM treatment in transition cows did not affect DMI.

Milk production was analyzed both as absolute values and MY as a PBW as there is a clear positive correlation between BW and MY (Gains et al., 1947; Erb and Ashworth, 1961; Clark and Touchberry, 1962; Hooven et al., 1968). Milk yield decreased in FM treated cows during the first 7 DIM (Table 5), but was overall not different throughout the study (1-5 WOL, Table 6). Although not well understood, it appears the reduced MY immediately postpartum is probably due to decreased nutrient intake as the temporal pattern of DMI (Figure 6) closely parallels the temporal MY pattern (Figure 7). Further evidence suggesting that inadequate DMI was responsible for the decreased MY in the current study is that FM treatment did not reduce DMI and thus MY in transition cows (Königsson et al., 2001) or in cows with mastitis (Anderson et al., 1986; Anderson and Hunt, 1989; Dascanio et al., 1995; Green et al., 1997; Wagner et al., 2004).
Flunixin meglumine treated cows tended to have a decreased energy balance and improved feed efficiency during the first 7 DIM (Table 5; Figure 8A), but both variables were similar during the first 5 WOL (Table 6; Figure 8B). To the best of our knowledge this is the first trial to evaluate FM effect on EBAL and feed efficiency in transition dairy cows. Reduced DMI for FM treated cows during the first 7 DIM probably caused the decreased EBAL and increased feed efficiency.

Flunixin meglumine treated cows had similar circulating glucose and NEFA (Figures 9A and 9B) but tended to have lower PUN levels compared to controls (Table 8; Figure 9C). Previous research on neonatal calves exposed to *Escherichia coli* and treated with FM (Semrad, 1993a; 1993b) demonstrated no effect of FM on glucose variables. To the best of our knowledge no other study has evaluated FM effects on plasma NEFA and PUN during the transition period. Nonesterified fatty acids are a product of adipose mobilization and increased NEFA in early lactation is thought to predispose cows to fatty liver and ketosis (Drackley, 1999; Drackley et al., 2001). Plasma urea nitrogen can originate from a verity of sources, but because DMI was actually lower in FM treated cows, we believe the reduced PUN is a result of decreased skeletal muscle proteolysis. Cows in early lactation are in a negative protein balance (Bell, 1995), and muscle catabolism is the primary method of supplying additional amino acids during this period.

Cytokine secretion during inflammation is associated with skeletal muscle proteolysis (Kotler, 2000). Baracos and coworkers (1983) indicated that increased interleukin-1 levels promoted protein catabolism via PGE₂ accumulation in the muscle. Additionally, increased PGE₂ in febrile rats enhanced muscle breakdown (Rodemann and
Goldberg, 1981). Non-steroidal anti-inflammatory drugs, such as FM, reduce PG synthesis via cyclooxygenase (COX) inhibition (Morteau, 2000), and consequently reduce muscle proteolysis (Baracos et al., 1983; Goldberg et al., 1984). Therefore, the reduced PUN in FM treated cows is probably due to a decreased PG induced muscle proteolysis. The reduced PUN may be of interest from a reproduction view point as increased PUN decreases a variety of reproduction parameters (Rhoads et al., 2004; 2006).

As stated earlier, the increased BT in FM treated cows during the current study was not expected, but might have resulted from vasodilation inhibition and thus impaired heat dissipation. Vasodilation is a key mechanism cows use to dissipate heat (Neuwirth et al., 1979; West, 1999), and PG play a role in vasodilation (Neisius et al., 2002; Lydyard et al., 2004). Prostaglandins are produced from long-chain polyunsaturated fatty acid via COX-1 and COX-2 (Dinarello, 2000; Morteau, 2000), and reducing COX action reduces vasodilation in humans (Kellogg et al., 2005; McCord et al., 2006). In addition, FM administration decreases PG synthesis via COX inhibition (Brander et al., 1991). Therefore, FM treatment may have impaired cutaneous vasodilation and this might have prevented cows from effectively dissipating heat, which would consequently increase BT.

When cows were separated into a BT hierarchy, DMI as a PBW tended to be higher in cows with decreased BT (Table 9). This is surprising as increased DMI should theoretically be associated with an increase in the heat increment of feeding. However, previous research demonstrated that animals with increased BT had decreased DMI (Takeuchi et al., 1995; Steiger et al., 1999). There was no effect of BT hierarchy on MY
and because of the reduced DMI warm cows were in a more severe NEBAL. Cows with a lower BT had reduced plasma NEFA (Table 11; Figure 10A) and this is interesting as we recently hypothesized that oxidizing NEFA generates more heat compared to burning glucose (Baumgard et al., 2007b). The increase PUN in cool cows (Table 11; 10B) is consistent with this theory as mobilizing more skeletal muscle (for glucose production) would be necessary to meet the energy requirements if NEFA delivery was reduced.

An alternative and just as likely explanation for the decreased NEFA in cooler cows is that these cows also had increased DMI and thus an improved EBAL during the transition period (Table 9). Energy balance and NEFA are highly correlated (Bauman et al., 1988; Dunshea et al., 1989) and this enhanced EBAL was probably associated with increased circulating insulin levels (not measured in the current study). Insulin is a potent anti-lipolytic signal and reduces adipose mobilization and therefore this may explain the reduced NEFA. However, insulin is also a potent inhibitor of muscle proteolysis and therefore why the cooler BT cows had an increase in PUN is not well understood. Another possible explanation is the there was an increased incidence of subtherapeutic mastitis and this is supported by the increased milk SCC in warmer cows (Table 10).

**Conclusion**

The present study demonstrated that FM treatment immediately postpartum for the first 3 DIM did not alter overall production variables in periparturient dairy cows. Identifying why FM treated cows had increased BT during the first WOL is of interest.
Furthermore, it will be of interest to identify why FM decreased PUN and if this reduction is of benefit to the transition cow.
APPENDIX A
ADDITIONAL GRAPHS

EFFECTS OF FLUNIXIN MEGLUMINE ON PYREXIA, PRODUCTION AND BIOENERGETIC VARIABLES IN POSTPARTURIENT DAIRY COWS

Figure 11. Effects of flunixin meglumine (FM) on body temperature. Treatments were 2.2 mg/kg of BW saline (control) or 2.2 mg/kg of BW FM for the first 3 DIM. Values represent least square means (n=12 or 14) for the control and FM respectively; SEM of body temperature for the first 3 DIM averaged 0.11 and ranged from 0.10 to 0.11 °C/d. Asterisk sign (*) indicates treatment x time interaction with $P < 0.05$. 
Figure 12. Effects of flunixin meglumine (FM) on MY. Treatments were 2.2 mg/kg of BW saline (control) or 2.2 mg/kg of BW FM for the first 3 DIM. Values represent least square means (n=12 or 14) for the control and FM respectively; SEM of MY for the first 7 DIM averaged 1.18 and ranged from 1.13 to 1.22 kg/d.

Figure 13. Effects of flunixin meglumine (FM) on MY as a percentage of BW. Treatments were 2.2 mg/kg of BW saline (control) or 2.2 mg/kg of BW FM for the first 3 DIM. Values represent least square means (n=12 or 14) for the control and FM respectively; SEM of MY % of BW for the first 7 DIM averaged 0.16 and ranged from 0.15 to 0.16% /d.
**Figure 14.** Effects of flunixin meglumine (FM) on 3.5% fat corrected milk (FCM). Treatments were 2.2 mg/kg of BW saline (control) or 2.2 mg/kg of BW FM for the first 3 DIM. Values represent least square means (n=12 or 14) for the control and FM respectively; SEM of 3.5% FCM for the first 5 WOL averaged 1.64 and ranged from 1.59 to 1.70 kg/d.

**Figure 15.** Effects of flunixin meglumine (FM) on energy corrected milk (ECM). Treatments were 2.2 mg/kg of BW saline (control) or 2.2 mg/kg of BW FM for the first 3 DIM. Values represent least square means (n=12 or 14) for the control and FM respectively; SEM of ECM for the first 5 WOL averaged 1.55 and ranged from 1.49 to 1.61 kg/d.
Figure 16. Effects of flunixin meglumine (FM) on milk fat content. Treatments were 2.2 mg/kg of BW saline (control) or 2.2 mg/kg of BW FM for the first 3 DIM. Values represent least square means (n=12 or 14) for the control and FM respectively; SEM of milk fat content on 2, 7, 14, 21, 28, and 35 DIM averaged 0.16 and ranged from 0.15 to 0.16%/d.

Figure 17. Effects of flunixin meglumine (FM) on milk protein content. Treatments were 2.2 mg/kg of BW saline (control) or 2.2 mg/kg of BW FM for the first 3 DIM. Values represent least square means (n=12 or 14) for the control and FM respectively; SEM of milk protein content on 2, 7, 14, 21, 28, and 35 DIM averaged 0.100 and ranged from 0.096 to 0.100%/d.
Figure 18. Effects of flunixin meglumine (FM) on milk lactose content. Treatments were 2.2 mg/kg of BW saline (control) or 2.2 mg/kg of BW FM for the first 3 DIM. Values represent least square means (n=12 or 14) for the control and FM respectively; SEM of milk lactose content on 2, 7, 14, 21, 28, and 35 DIM averaged 0.040 and ranged from 0.042 to 0.045% /d.

Figure 19. Effects of flunixin meglumine (FM) on somatic cell count (SCC) concentration. Treatments were 2.2 mg/kg of BW saline (control) or 2.2 mg/kg of BW FM for the first 3 DIM. Values represent least square means (n=12 or 14) for the control and FM respectively; SEM of SCC concentration on 2, 7, 14, 21, 28, and 35 DIM averaged 254 and ranged from 244 to 264 x 1000/mL/d.
Figure 20. Effects of flunixin meglumine (FM) on body weight (BW). Treatments were 2.2 mg/kg of BW saline (control) or 2.2 mg/kg of BW FM for the first 3 DIM. Values represent least square means (n=12 or 14) for the control and FM respectively; SEM of BW for the first 5 WOL averaged 21 and ranged from 20 to 22 kg/d.

Figure 21. Effects of flunixin meglumine (FM) on body condition score (BCS). Treatments were 2.2 mg/kg of BW saline (control) or 2.2 mg/kg of BW FM for the first 3 DIM. Values represent least square means (n=12 or 14) for the control and FM respectively; SEM of BCS for the first 5 WOL averaged 0.10 and ranged from 0.10 to 0.11/d.
Figure 22. Effects of flunixin meglumine (FM) on feed efficiency. Treatments were 2.2 mg/kg of BW saline (control) or 2.2 mg/kg of BW FM for the first 3 DIM. Values represent least square means (n=12 or 14) for the control and FM respectively; SEM of feed efficiency for the first 7 DIM averaged 0.19 and ranged from 0.18 to 0.20 /d.

Figure 23. Effects of flunixin meglumine (FM) on feed efficiency. Treatments were 2.2 mg/kg of BW saline (control) or 2.2 mg/kg of BW FM for the first 3 DIM. Values represent least square means (n=12 or 14) for the control and FM respectively; SEM of feed efficiency for the first 5 WOL averaged 0.080 and ranged from 0.081 to 0.087 /d.
**Figure 24.** Effects of body temperature on DMI as a percentage of BW during the first 5 WOL. Cows were separated into a BT hierarchy (50% of the cows with the lowest (bottom) BT; 50% of the cows with the highest (top) BT, irrespective of FM and saline treatments). Values represent least square means (n=13) for the two treatment groups; SEM of DMI % of BW for the first 5 WOL averaged 0.10 and ranged from 0.09 to 0.11% BW/d.

**Figure 25.** Effects of body temperature on MY as a percentage of BW during the first 5 WOL. Cows were separated into a BT hierarchy (50% of the cows with the lowest (bottom) BT; 50% of the cows with the highest (top) BT, irrespective of FM and saline treatments). Values represent least square means (n=13) for the two treatment groups; SEM of MY % of BW for the first 5 WOL averaged 0.17 and ranged from 0.16 to 0.18% BW/d.
Figure 26. Effects of body temperature on 3.5% fat corrected milk (FCM) as a percentage of BW during the first 5 WOL. Cows were separated into a BT hierarchy (50% of the cows with the lowest (bottom) BT; 50% of the cows with the highest (top) BT, irrespective of FM and saline treatments). Values represent least square means (n=13) for the two treatment groups; SEM of 3.5% FCM of BW for the first 5 WOL averaged 0.26 and ranged from 0.24 to 0.28% BW/d.

Figure 27. Effects of body temperature on energy corrected milk (ECM) as a percentage of BW during the first 5 WOL. Cows were separated into a BT hierarchy (50% of the cows with the lowest (bottom) BT; 50% of the cows with the highest (top) BT, irrespective of FM and saline treatments). Values represent least square means (n=13) for the two treatment groups; SEM of 3.5% FCM of BW for the first 5 WOL averaged 0.24 and ranged from 0.22 to 0.25% BW/d.
Figure 28. Effects of body temperature on milk fat content on 2, 7, 14, 21, 28, and 35 DIM. Cows were separated into a BT hierarchy (50% of the cows with the lowest (bottom) BT; 50% of the cows with the highest (top) BT, irrespective of FM and saline treatments). Values represent least square means (n=13) for the two treatment groups; SEM of milk fat content for 7, 14, 21, 28, and 35 DIM averaged 0.15 and ranged from 0.14 to 0.17% /d.

Figure 29. Effects of body temperature on somatic cell count (SCC) on 2, 7, 14, 21, 28, and 35 DIM. Cows were separated into a BT hierarchy (50% of the cows with the lowest (bottom) BT; 50% of the cows with the highest (top) BT, irrespective of FM and saline treatments). Values represent least square means (n=13) for the two treatment groups; SEM of SCC for 7, 14, 21, 28, and 35 DIM averaged 233 and ranged from 214 to 251 x 1000/mL/d.
Figure 30. Effects of body temperature on milk protein on 2, 7, 14, 21, 28, and 35 DIM. Cows were separated into a BT hierarchy (50% of the cows with the lowest (bottom) BT; 50% of the cows with the highest (top) BT, irrespective of FM and saline treatments). Values represent least square means (n=13) for the two treatment groups; SEM of milk protein content for 7, 14, 21, 28, and 35 DIM averaged 0.10 and ranged from 0.09 to 0.11% /d.

Figure 31. Effects of body temperature on milk lactose on 2, 7, 14, 21, 28, and 35 DIM. Cows were separated into a BT hierarchy (50% of the cows with the lowest (bottom) BT; 50% of the cows with the highest (top) BT, irrespective of FM and saline treatments). Values represent least square means (n=13) for the two treatment groups; SEM of milk lactose content for 7, 14, 21, 28, and 35 DIM averaged 0.04 and ranged from 0.04 to 0.05% /d.
Figure 32. Effects of body temperature on energy balance (EBAL) during the first 5 WOL. Cows were separated into a BT hierarchy (50% of the cows with the lowest (bottom) BT; 50% of the cows with the highest (top) BT, irrespective of FM and saline treatments). Values represent least square means (n=13) for the two treatment groups; SEM of EBAL for the first 5 WOL averaged 1.10 and ranged from 1.01 to 1.18 Mcal/d.

Figure 33. Effects of body temperature on feed efficiency during the first 5 WOL. Cows were separated into a BT hierarchy (50% of the cows with the lowest (bottom) BT; 50% of the cows with the highest (top) BT, irrespective of FM and saline treatments). Values represent least square means (n=13) for the two treatment groups; SEM of feed efficiency for the first 5 WOL
averaged 0.08 and ranged from 0.08 to 0.09 /d. Asterisk sign (*) indicates treatment x time interaction with \( P < 0.05 \).

Figure 34. Effects of body temperature on plasma glucose on 1-4, 7, 14, 21, 28, and 35 DIM. Cows were separated into a BT hierarchy (50% of the cows with the lowest (bottom) BT; 50% of the cows with the highest (top) BT, irrespective of FM and saline treatments). Values represent least square means (n=13) for the two treatment groups; SEM of plasma glucose on 1-4, 7, 14, 21, 28, and 35 DIM averaged 1.7 and ranged from 1.6 to 1.9 mg/dL/d.
APPENDIX B

THE EFFECT OF YEA-SACC HEAT STRESS SUPPLEMENTATION ON PRODUCTION AND BIOENERGETIC VARIABLES IN HEAT STRESSED LACTATING DAIRY COWS

Materials and Methods

Twenty-three multiparous, lactating Holstein cows (120 ± 30 DIM, 690 ± 67 kg BW) were randomly assigned to one of two dietary treatments: Yea-Sacc Heat Stress (YSHS; n=12, Alltech, Lexington, KY) or control (n=11). Cows were fed either 10 g YSHS top-dressed on a total mixed ration (TMR) twice daily or a TMR without YSHS supplement (controls). The total length of the trial was 28 days with two experimental periods consisting of: 1) 7 d of thermoneutral acclimation conditions, and 2) 21 d of heat-stress (HS). All cows were not receiving rbST throughout the study. Animals were maintained in tie-stall stanchions at the University of Arizona’s Agricultural Research Complex and all procedures were approved by the University of Arizona Institutional Animal Care and Use Committee.

During the acclimation period, all animals were housed in constant thermoneutral conditions (18°C, 20% humidity with a 12 and 12 h light and dark cycle). Starting on the first day of HS and continuing until the last day of the study, cows experienced cyclical daily temperatures (in an attempt to mimic daily variation) ranging from 29.4°C to 37.8°C (20% humidity and a 12 and 12 hour light and dark cycle). Body temperature indices were obtained three times daily (0600, 1200, and 1800 h) and included respiration rates (breaths/min), surface temperature (shoulder, rump and tail-head) recorded with an
infrared temperature gun (Raynger®MX™ model RayMX4PU Raytek C, Santa Cruz, CA), core body temperatures obtained with a rectal thermometer (GLA M700 Digital Thermometer, San Luis Obispo, CA, three times daily), and sweating rate measured at the rump from a clean shaved area using a VapoMeter (Delfin Technologies Ltd., Kuopio, Finland, three times a day, once weekly). All cows with a rectal temperature of 40.5°C or greater were removed from the climate chambers and cooled with cold water until the rectal temperature dropped below 40.0°F.

All cows were individually fed a TMR twice daily (0500 and 1700 hr) and orts were recorded prior to the AM feeding. During both experimental periods cows were allowed to eat ad libitum. The TMR was formulated by Dairy Nutrition Services (Chandler, AZ) to meet or exceed the predicted requirements (NRC, 2001) of energy, protein, minerals and vitamins. Alfalfa hay was the primary forage with steam flaked corn as the primary concentrate (Table 1). The TMR was sampled weekly and analyzed by wet chemistry methods (Chandler, AZ).

Cows were milked twice daily (0500 and 1700 h) with yields recorded at each milking. Samples from each cow were collected on d 7, 14, 21, and 28. One aliquot, stored at 4°C with a preservative (bronopol tablet; D&F Control System, San Ramon, CA), was analyzed by Arizona DHIA (Tempe, AZ) using AOAC (2000) approved infrared analysis equipment and procedures for milk components. The second aliquot was stored at -20°C until analyzed for fatty acid composition. Body weights (BW) and body condition scores (BCS) were obtained on all animals shortly after morning milking and prior to feeding on d 1, 7, 14, 21, and 28.
Weekly blood samples were collected via coccygeal venipuncture on d 7, 14, 21, and 28, following the morning milking and the plasma was harvested and stored at -20°C until analyzed for plasma urea nitrogen (PUN), glucose, and NEFA concentrations. All plasma NEFA, glucose, and PUN concentrations were measured enzymatically using commercially available kits validated in our laboratory (NEFA C kit; Wako Chemicals USA, Richmond, VA; Autokit Glucose C2; Wako Chemicals USA, Richmond, VA). PUN concentrations were measured via the colorimetric method (Urea Nitrogen Reagent Set; TECO Diagnostics, Anaheim, CA). The inter- and intra-assay coefficients for the NEFA, PUN, and glucose assays were 3.6, 4.7, 3.2, 3.5, 4.7, and 3.1%, respectively.

Calculations

Energy balance was calculated using the following equation: energy balance = net energy of intake – (net energy of maintenance + net energy of lactation). Net energy of intake was calculated by multiplying the daily DMI by the calculated net energy value of the diet. Energy requirement for maintenance was computed using the following equation (NRC, 2001): net energy of maintenance = 0.08 x BW^{0.75}. Maintenance costs were increased by 25% during the HS conditions as recommended by the NRC (2001). Net energy of lactation was estimated by the following equation: [(0.0929 x fat %) + (0.0547 x crude protein %) + (0.0395 x lactose %)] x milk production. Daily EBAL values were subjected to a third-order polynomial regression analysis to minimize variation, and predicted daily energy values from these equations were used in the statistical analysis as previously described (Lucy et al., 1991; Moore et al., 2004; Odens
93
et al., 2007). 3.5% fat corrected milk (FCM) and energy corrected milk (ECM) were calculated (NRC, 2001) using the following equations: 

\[ 3.5\% \text{ FCM} = (0.432 \times \text{milk yield}) + (16.23 \times \text{milk fat yield}) \]

\[ \text{ECM} = (0.327 \times \text{milk yield}) + (12.95 \times \text{milk fat yield}) + (7.2 \times \text{milk protein yield}) \]

Feed efficiency was calculated using the following equation: 

\[ \text{feed efficiency} = 3.5\% \text{ FCM}/\text{DMI}. \]

**Statistical Analysis**

Daily milk yield (MY), MY as a percentage of BW, dry matter intake (DMI), DMI as a percentage of BW, energy balance (EBAL), and feed efficiency were condensed into weekly values and analyzed by repeated measures using the PROC MIXED procedure of SAS (2005) with an autoregressive covariance structure and week of lactation as the repeated affect. The previous 305ME was used as a covariate on all measurements. Plasma NEFA, glucose, and PUN were analyzed by repeated measures using the PROC MIXED procedure of SAS (2005) with an autoregressive covariance structure and day of experiment as the repeated affect. The model contained day or week of experiment, treatment and day or week of experiment x treatment interactions. Cows were the random effect, and day or week of experiment, treatment and day or week of experiment x treatment interaction were the fixed effects. Milk components were analyzed by repeated measures using the PROC MIXED procedure of SAS (2005) with an autoregressive covariance structure and day of experiment as the repeated affect. The model contained day of experiment, treatment and day x treatment interactions. Cows
were the random effect, and day of experiment, treatment and day of experiment x treatment interaction were the fixed effects.

Table 12. Ingredients and chemical composition of diets\(^1\).

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>% of DM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alfalfa hay</td>
<td>52.03</td>
</tr>
<tr>
<td>Whole cotton seed</td>
<td>8.43</td>
</tr>
<tr>
<td>Steam flaked corn</td>
<td>29.12</td>
</tr>
<tr>
<td>Supplement(^2)</td>
<td>2.69</td>
</tr>
<tr>
<td>Maxxer(^3)</td>
<td>1.78</td>
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<tr>
<td>Amino plus(^4)</td>
<td>3.19</td>
</tr>
<tr>
<td>Molasses</td>
<td>2.78</td>
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<table>
<thead>
<tr>
<th>Chemical analysis, % of DM</th>
</tr>
</thead>
<tbody>
<tr>
<td>CP</td>
</tr>
<tr>
<td>NDF</td>
</tr>
<tr>
<td>ADF</td>
</tr>
</tbody>
</table>

\(^1\)Values represent an average of samples collected and composited throughout the trial. Dry matter averaged 50% for the diet.

\(^2\)The supplement contained 1.14% Fat, 10.42% Ca, 4.49% P, 3.80% Mg, 0.49% S, 0.19% K, 15.83% Na, 7.52% Cl, 2029.06 mg/kg of Zn, 1991.82 mg/kg of Mn, 974.24 mg/kg of Fe, 583.45 mg/kg of Cu, 67.86 mg/kg of Co, 12.28 mg/kg Se, 6.81 mg/kg of Mo, 43.68 mg/kg of I, 304.9 of IU/g of vitamin A, 30.2 IU/g of vitamin D, and 1.0 IU/g of vitamin E.

\(^3\)Calcium salts of palm oil (Tarome Inc., Eloy, AZ).

\(^4\)Soybean based supplement; 51.7% CP (Hastings, NB)
Results

Dry matter intake tended to be lower for YSHS treated cows (16.51 vs. 17.68 kg/d, \( P = 0.07 \), Figure 1A) but treatment had not effect on DMI as a percentage of BW (PBW, 2.61% /d, Table 2, Figure 1B). Treatment had no effect on MY (28.16 kg, Figure 2A), MY as a PBW (4.30% /d, Figure 2B), 3.5% FCM (29.05 kg, Figure 3A) and ECM (28.14 kg, Table 2, Figure 3B). Milk fat (3.69%, Figure 7), protein (2.69%, Figure 8), lactose (4.60%, Figure 9), and SCC (250 x 1000/mL, Table 3, Figure 10) were similar throughout the study.

Body condition score (2.75 vs. 2.45, \( P = 0.10 \), Figure 6A) tended to be higher for controls, but BW remained unchanged (652 kg, Table 2, Figure 6B). Yea-Sacc HS had no effect on EBAL (-1.09 Mcal/d, Figure 4) and feed efficiency were not effected by treatment (1.71, Table 2, Figure 5). Plasma glucose (64.5 mg/dL, Figure 11A) and NEFA (146 µEq/L, Figure 11B) were similar, but PUN tended to be lower (13.5 vs. 14.6 mg/dL, Table 4, Figure 11C) in YSHS treated cows.
Table 13. Effects of Yea-Sacc Heat Stress (YSHS) supplementation on production variables in heat stressed lactating Holstein cows.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control</th>
<th>YSHS</th>
<th>SEM</th>
<th>TRT</th>
<th>WOL</th>
<th>TRT x WOL</th>
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</thead>
<tbody>
<tr>
<td>DMI³, kg/d</td>
<td>17.68</td>
<td>16.51</td>
<td>0.43</td>
<td>0.07</td>
<td>&lt;0.01</td>
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<tr>
<td>DMI % of BW⁴</td>
<td>2.64</td>
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<td>0.07</td>
<td>0.48</td>
<td>&lt;0.01</td>
<td>0.38</td>
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<td>MY⁵, kg/d</td>
<td>29.64</td>
<td>26.68</td>
<td>1.33</td>
<td>0.13</td>
<td>&lt;0.01</td>
<td>0.56</td>
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<td>MY % of BW⁶</td>
<td>4.44</td>
<td>4.15</td>
<td>0.22</td>
<td>0.36</td>
<td>&lt;0.01</td>
<td>0.49</td>
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<td>3.5 % FCM⁷, kg/d</td>
<td>30.43</td>
<td>27.67</td>
<td>1.42</td>
<td>0.18</td>
<td>&lt;0.01</td>
<td>0.40</td>
</tr>
<tr>
<td>ECM⁸, kg/d</td>
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<td>26.87</td>
<td>1.29</td>
<td>0.18</td>
<td>&lt;0.01</td>
<td>0.43</td>
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<td>-1.13</td>
<td>0.70</td>
<td>0.94</td>
<td>&lt;0.01</td>
<td>0.59</td>
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<tr>
<td>Feed efficiency</td>
<td>1.72</td>
<td>1.69</td>
<td>0.06</td>
<td>0.77</td>
<td>&lt;0.01</td>
<td>0.59</td>
</tr>
<tr>
<td>BW¹⁰, kg</td>
<td>669</td>
<td>634</td>
<td>19</td>
<td>0.21</td>
<td>&lt;0.01</td>
<td>0.74</td>
</tr>
<tr>
<td>BCS¹¹</td>
<td>2.75</td>
<td>2.45</td>
<td>0.11</td>
<td>0.06</td>
<td>&lt;0.01</td>
<td>0.59</td>
</tr>
</tbody>
</table>

¹Treatments were YSHS free TMR (control) or 10 g/d of YSHS top dressed twice daily on the TMR.
²Week of lactation.
³Dry matter intake.
⁴Dry matter intake as a percentage of body weight.
⁵Milk yield.
⁶Milk yield as a percentage of body weight.
⁷3.5% Fat corrected milk.
⁸Energy corrected milk.
⁹Energy balance
¹⁰Body weight
¹¹Body condition score
Table 14. Effects of Yea-Sac Heat Stress (YSHS) supplementation on milk composition variables in heat stressed lactating Holstein cows.

<table>
<thead>
<tr>
<th>Milk Parameter</th>
<th>Treatments</th>
<th>SEM</th>
<th>TRT</th>
<th>DIM²</th>
<th>TRT x DIM</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>YSHS</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fat, %</td>
<td>3.66</td>
<td>3.71</td>
<td>0.11</td>
<td>0.79</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Protein, %</td>
<td>2.64</td>
<td>2.73</td>
<td>0.06</td>
<td>0.32</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Lactose, %</td>
<td>4.62</td>
<td>4.57</td>
<td>0.07</td>
<td>0.66</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>SCC³ x 1000/mL</td>
<td>94</td>
<td>406</td>
<td>169</td>
<td>0.21</td>
<td>0.33</td>
</tr>
</tbody>
</table>

¹Treatments were YSHS free TMR (control) or 10 g/d of YSHS top dressed twice daily on the TMR.
²Days in milk.
³Somatic cell count.
Table 15. Effects of Yea-Sac Heat Stress (YSHS) supplementation on plasma metabolites in heat stressed lactating Holstein cows.

<table>
<thead>
<tr>
<th>Plasma Metabolite</th>
<th>Treatments (^1)</th>
<th>SEM</th>
<th>TRT</th>
<th>DIM (^2)</th>
<th>TRT x DIM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose, mg/dL</td>
<td>Control</td>
<td>64.4</td>
<td>0.89</td>
<td>&lt;0.01</td>
<td>0.41</td>
</tr>
<tr>
<td></td>
<td>YSHS</td>
<td>64.6</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NEFA(^3), µEq/L</td>
<td>Control</td>
<td>142</td>
<td>0.74</td>
<td>0.64</td>
<td>0.11</td>
</tr>
<tr>
<td></td>
<td>YSHS</td>
<td>149</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PUN(^4), mg/dL</td>
<td>Control</td>
<td>14.6</td>
<td>0.10</td>
<td>&lt;0.01</td>
<td>0.22</td>
</tr>
<tr>
<td></td>
<td>YSHS</td>
<td>13.5</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

\(^1\)Treatments were YSHS free TMR (control) or 10 g/d of YSHS top dressed twice daily on the TMR.

\(^2\)Days in milk.

\(^3\)Non-esterified fatty acids.

\(^4\)Plasma urea nitrogen.
Figure 35. Effects of Yea-Sacc Heat Stress (YSHS) on DMI (A) and DMI as a percentage of BW (B). Treatments were TMR (control) or 10 g of YSHS top dressed twice daily on the TMR. Values represent least square means (n=12 or 11) for YSHS and control respectively; SEM of DMI averaged 0.43 and ranged from 0.42 to 0.43 kg/d; SEM of DMI % of BW averaged 0.07 and ranged from 0.066 to 0.069%/d.
Figure 36. Effects of Yea-Sacc Heat Stress (YSHS) on MY (A) and MY as a percentage BW (B). Treatments were TMR (control) or 10 g of YSHS top dressed twice daily on the TMR. Values represent least square means (n=12 or 11) for YSHS and control respectively; SEM of MY % of BW averaged 1.33 and ranged from 1.30 to 1.36 kg/d; SEM of MY % of BW averaged 0.22 and ranged from 0.21 to 0.22 kg/d.
Figure 37. Effects of Yea-Sacc Heat Stress (YSHS) on 3.5% fat corrected milk (FCM; A) and energy corrected milk (ECM; B). Treatments were TMR (control) or 10 g of YSHS top dressed twice daily on the TMR. Values represent least square means (n=12 or 11) for YSHS and control respectively; SEM of 3.5 FCM averaged 1.42 and ranged from 1.39 to 1.44 kg/d; SEM of ECM averaged 1.29 and ranged from 1.26 to 1.31 kg/d.
Figure 38. Effects of Yea-Sacc Heat Stress (YSHS) on energy balance (EBAL). Treatments were TMR (control) or 10 g of YSHS top dressed twice daily on the TMR. Values represent least square means (n=12 or 11) for YSHS and control respectively; SEM of EBAL averaged 0.70 and ranged from 0.69 to 0.72 Mcal/d.

Figure 39. Effects of Yea-Sacc Heat Stress (YSHS) on feed efficiency. Treatments were TMR (control) or 10 g of YSHS top dressed twice daily on the TMR. Values represent least square means (n=12 or 11) for YSHS and control respectively; SEM of feed efficiency averaged 0.06 and ranged from 0.062 to 0.065 /d.
Figure 40. Effects of Yea-Sacc Heat Stress (YSHS) on body condition score (BCS, A) and body weight (BW, B). Treatments were TMR (control) or 10 g of YSHS top dressed twice daily on the TMR. Values represent least square means (n=12 or 11) for YSHS and control respectively; SEM of BCS averaged 0.11 and ranged from 0.106 to 0.111 kg/d; SEM of BW averaged 19 and ranged from 18.8 to 19.9 kg/d.
Figure 41. Effects of Yea-Sacc Heat Stress (YSHS) on milk fat content. Treatments were TMR (control) or 10 g of YSHS top dressed twice daily on the TMR. Values represent least square means (n=12 or 11) for YSHS and control respectively; SEM of milk fat content averaged 0.11 and ranged from 0.1096 to 0.1134 %/d.

Figure 42. Effects of Yea-Sacc Heat Stress (YSHS) on milk protein content. Treatments were TMR (control) or 10 g of YSHS top dressed twice daily on the TMR. Values represent least square means (n=12 or 11) for YSHS and control respectively; SEM of milk protein content averaged 0.06 and ranged from 0.061 to 0.064 %/d.
**Figure 43.** Effects of Yea-Sacc Heat Stress (YSHS) on milk lactose content. Treatments were TMR (control) or 10 g of YSHS top dressed twice daily on the TMR. Values represent least square means (n=12 or 11) for YSHS and control respectively; SEM of milk lactose content averaged 0.07 and ranged from 0.066 to 0.068 %/d.

**Figure 44.** Effects of Yea-Sacc Heat Stress (YSHS) on milk somatic cell count (SCC). Treatments were TMR (control) or 10 g of YSHS top dressed twice daily on the TMR. Values represent least square means (n=12 or 11) for YSHS and control respectively; SEM of milk SCC content averaged 169 and ranged from 166 to 173 x 1000/mL/d.
Figure 45. Effects of Yea-Sacc Heat Stress (YSHS) on plasma glucose (A), NEFA (B), and PUN (C). Treatments were TMR (control) or 10 g of YSHS top dressed twice daily on the TMR. Values represent least square means (n=12 or 11) for YSHS and control respectively; SEM of plasma glucose averaged 1.0 and ranged from 0.98 to 1.02 mg/dL/d; SEM of plasma NEFA averaged 16 and ranged from 16.0 to 15.3 µEq/L/d; SEM of PUN averaged 0.5 and ranged from 0.45 to 0.46 mg/dL/d.
APPENDIX C

DOES NEGATIVE ENERGY BALANCE (NEBAL) LIMIT MILK SYNTHESIS IN EARLY LACTATION?*

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*Adapted from a paper first published by the authors in the Proceedings of the 2006 Southwest Nutrition Conference

Introduction

Energy balance (EBAL) is the difference between energy consumed and energy used for both maintenance and production (milk, meat, reproduction, etc.). For a detailed description of the different methods of calculating EBAL see our recent review (Moore et al., 2005). Frequently in a cow's life cycle, there are instances when energy availability, or more specifically a lack of available energy, may limit milk or milk component synthesis, reduce reproductive performance and prevent body condition replacement. Examples include the transition period in both TMR and pasture-based systems, periods of poor feed quality and adverse environmental situations such as heat stress and drought. Incidentally this bioenergetic phenomenon is not exclusive to dairy cows; as most female mammals experience a similar nutrient imbalance after parturition and in fact, the severity of this nutrient inequality is quite minor in cows compared to a large number of other species (see our recent review, Collier et al., 2005).

Cows in early lactation typically cannot consume enough calories to meet the energetic requirements of maintenance and copious milk secretion, and consequently enter into a state of negative energy balance (NEBAL). In fact, reduced feed intake and
NEBAL can be observed 7-10 days prior to calving. Post-calving the severity, magnitude and day of NEBAL nadir (~4-9 days in milk) are closely associated with metabolic disorders and reproductive failures (Butler, 2000; Drackley, 1999; Buckley et al., 2003; Rhoads et al., 2005). The impact of NEBAL on reproductive parameters is even more critical in strict pasture-based systems as pasture allowance is restricted and calving patterns must coincide with forage availability to maintain farm sustainability (Rhodes et al., 2003). Attempts to improve or alleviate NEBAL traditionally involve increasing dietary energy density via the addition of concentrates or fats (Schingoethe & Casper, 1991; Hayirli & Grummer, 2004). However, the effectiveness of these dietary strategies is frequently inconsistent and is associated with potential drawbacks (i.e. acidosis and reduced DMI; Hayirli & Grummer, 2004). There are a number of reviews concentrating on the benefits and limitations of increasing the dietary content of grains and fats with regards to EBAL and they will not be discussed further in this paper.

Interestingly and contrary to what is often reported (Broom, 1995; Veerkamp, 1998; Veerkamp et al., 2000; Heuer, 2004; Oltenacu & Algers, 2005), genetically superior or higher producing cows have similar calculated NEBAL parameters (severity, magnitude etc.) and blood energetic variables when compared to their lesser producing herd mates (Vicini et al., 2002; Crooker et al., 2006). The increased milk yield associated with genetic progress is accompanied by homeorhetic mechanisms that favor increased feed intake during early lactation (Crooker et al., 2001; Crooker et al., 2006). It is logical to predict that selecting animals for increased milk production simultaneously selects animals capable of coordinating metabolism to sustain evolutionary advantages.
Furthermore, the fact that genetic selection for milk yield doesn’t intensify NEBAL parameters, jeopardize health or cause cow “burn out” is due to natural coordinated homeorhetic mechanisms as we recently described (Collier et al., 2005).

**Metabolic Adaptations to Reduced Nutrient Intake**

The early lactation cow is a classic example of lactation-induced NEBAL resulting from an inability of the cow to consume enough feed to meet the energy demands of lactation and maintenance requirements (Moore et al., 2005). Negative energy balance is associated with a variety of metabolic changes that are implemented to support the dominant physiological condition of lactation (Bauman and Currie, 1980). Marked alterations in both carbohydrate and lipid metabolism ensure partitioning of dietary derived and tissue originating nutrients towards the mammary gland, and not surprisingly many of these changes are mediated by endogenous somatotropin which is naturally increased during periods of NEBAL (Bauman and Currie, 1980). One characteristic response is a reduction in circulating insulin coupled with a reduction in systemic insulin sensitivity. Compared to a well-fed cow in positive energy balance, the reduction in insulin action allows for adipose lipolysis and mobilization of non-esterified fatty acids (NEFA; Bauman and Currie, 1980). Increased circulating NEFA are typical in “transitioning” cows and represent a significant source of energy (and precursor for milk fat synthesis) for cows in NEBAL. Post-absorptive carbohydrate metabolism is also altered by the reduced insulin action during NEBAL with the net effect of reduced glucose uptake by systemic tissues (i.e. muscle and adipose). The reduced nutrient
uptake coupled with the net release of nutrients (i.e. amino acids and NEFA) by systemic tissues are key homeorhetic (an acclimated response vs. an acute/homeostatic response) mechanisms implemented by cows in NEBAL to support lactation (Bauman and Currie, 1980).

**Bioenegetics of Production**

It is well known that animals primarily eat to meet their energy requirements (Church and Pond, 1988), but this is slightly complicated in ruminants due to the effects of forage quality and gut fill (Van Soest, 1982) and the hepatic oxidation hypothesis of feed intake regulation (Allen et al., 2005). Nonetheless, if an animal is in positive EBAL (PEBAL), providing additional metabolizable energy (ME) should not theoretically increase milk yield, but rather decrease feed intake and thus improve efficiency. In contrast, if an animal is in NEBAL, adding additional ME would logically increase milk production without altering feed intake. Both of the above scenarios assumes that calculated net whole animal EBAL is tightly linked with the mammary glands energetic and nutrient requirements to synthesize milk. Adding additional energy (or any nutrient for that matter), if milk synthesis wasn’t limited by nutrient availability, can not “push” milk as milk synthesis itself “drives/pulls” nutrient and energy intake (i.e. DMI; Bauman & Currie, 1980; Collier et al., 2005). As demonstrated in Figure 1, predicting the effects (milk yield, DMI and feed efficiency) of enhanced ME probably depends on whether or not the animal is in NEBAL or PEBAL.
During established lactation, decreased energy and nutrient availability (either experimentally induced or due to poor feed quality [drought, heat stress, spoiled feed etc.]) is closely matched by a coinciding decrease in milk yield. As a consequence of the reduction in milk synthesis, actual calculated net EBAL remains near zero. When nutrient supply or the level of nutrition increases, milk yield parallels the enhanced nutrient state. Therefore, clearly in mid to late lactation, nutrient/energy availability can limit or restrict milk synthesis.

During early lactation the connection between nutrient supply and milk production appears uncoupled. This is especially obvious during the first 10 days in milk (DIM) where milk yield is increasing at a steep slope while calculated EBAL is simultaneously decreasing towards its nadir (see theoretical diagram in Figure 1). Milk yield continues to increase until peak (~40-70 DIM) while cows are still in calculated NEBAL (albeit progressing towards PEBAL; Figure 1). Obviously tissue mobilization accounts for the energy deficit in early lactation, but it’s interesting that there is a stark contrast between dietary energy/nutrient supply and milk production during early vs. later lactation. Why doesn’t tissue mobilization compensate for the decrease in nutrient supply and thus maintain production in later lactation, even temporally?

Although early lactation NEBAL is frequently blamed for a variety of metabolic and reproductive disorders (Drackley, 1999; Butler, 2000), whether or not it limits or prevents maximum milk yield is not clear. Attaining a high milk yield in early lactation and specifically peak milk yield, is thought to “prime” the gland for the entire lactation, and retrospective statistical analysis indicates that for every one unit (kg or lb etc.)
increase at peak lactation equates to a 127 unit increase in total lactation yield (Dr. Bob Everett, Cornell University; Personal Communication). A variety of different approaches have attempted to alter or improve EBAL and they include 1) supplemental fats, 2) additional concentrates, 3) reduced milking frequency (i.e. 1x/d), 4) propylene glycol, 5) monensin and 6) conjugated linoleic acid induced milk fat depression (CLA-MFD). The first four approaches have limitations (i.e. palatability, acidosis, mammary function etc.) that create difficulties when evaluating their effect on EBAL.

**Figure 1.** Theoretical lactation and energy balance curves. Bioenergetics would predict that increasing metabolizable energy will have different effects on production parameters depending upon calculated energy balance status.
Conjugated Linoleic Acid Transition Trials

A unique approach to improve transition period EBAL is to decrease the milk energy content, thus manipulating the energy expenditure side of the EBAL equation, rather than the energy intake portion. Fat is the most energetically expensive milk component to synthesize (50% of total milk energy; Tyrell & Reid, 1965) and the milk parameter most easily manipulated by management (Bauman & Davis, 1974; Bauman et al., 2001). Therefore, governing milk fat via controlled MFD offers a novel technique/opportunity to improve EBAL through the transition period.

We’ve conducted three CLA-MFD trials during the transition period (Moore et al., 2004; Kay et al., 2004; Odens et al., 2006), with the two later trials designed to evaluate the effects of CLA-MFD on EBAL parameters and production variables. As we predicted (Baumgard et al., 2002), both trials indicate that when EBAL is improved due to CLA-MFD, milk yield is enhanced (Figures 2 and 3). Furthermore, during experimentally induced NEBAL in established lactating cows, CLA-MFD increases both milk yield and milk protein synthesis (DeVeth et al., 2006; Kay et al., 2007). As would bioenergetically be predicted by Figure 1, CLA-MFD does not increase milk yield during established lactation when cows are in PEBAL (Geisy et al., 2002; Perfield et al., 2002). Our studies demonstrate that a dietary supplement of CLA reduces milk fat synthesis immediately postpartum and may be useful as a management tool to alleviate NEBAL and improve milk production in TMR and pasture-fed dairy cows.
**Monensin Transition Trials:**

Another dietary approach to improve transition EBAL that has recently become available to dairy producers is monensin (Rumensin, Elanco Animal Health, Greenfield, IN). Feeding ionophores, specifically monensin, alters rumen metabolism/physiology to favor a more energetic fermentation pathway (see reviews by Schelling, 1983; Ipharraguerre & Clark, 2003). A number of papers demonstrate an improved energy status (NEFA, ketones, glucose etc.) with monensin (Ipharraguerre & Clark 2003) and this is especially apparent in early lactation (Green et al., 1999; Duffield et al., 2003; Melendez et al., 2004; Gallardo et al., 2005; Zahra et al., 2006). As would bioenergetically be predicted by Figure 1, monensin feeding typically increases milk yield with no effect on feed intake during early lactation (Hays et al., 1996; Beckett et al., 1998; Gallardo et al., 2005). In contrast, during later lactation, monensin improves feed efficiency (little or no change in milk yield coinciding with small reductions in feed intake; see reviews by Ipharraguerre & Clark, 2003; McGuffey et al., 2003).
Figure 2. Effects of pasture fed cows (PAS) supplemented with rumen inert palm oil (HYPRO) or CLA on milk yield in transitioning lactating dairy cows. Adapted from Kay et al. 2006.

Figure 3. Effects of rumen inert CLA on milk yield compared to cows fed a rumen inert palm oil. Adapted from Odens et al., 2007
Summary

Based on evidence from transition period CLA-MFD and monensin trials, it appears that milk yield in early lactation is limited by a lack of energy intake. Obviously anything that increases ME during this stage of lactation would potentially benefit milk production, whereas increasing ME during mid to late lactation, a period when cows would presumably be in PEBAL, wouldn’t logically increase milk yield but probably increase feed efficiency.
REFERENCES


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