EFFECTS OF CLOSE-UP DIETARY ENERGY STRATEGY AND PREPARTAL DIETARY MONENSIN ON PRODUCTION AND METABOLISM IN HOLSTEIN COWS

BY

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THESIS

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ABSTRACT

Effects of close-up dietary energy strategy and prepartal dietary monensin on production and metabolism in Holstein cows

The objective of this study was to evaluate the inclusion of 22 g/ton of monensin (M) in a single group diet (controlled energy-high fiber, CE) and a two-group diet (CE during the far-off and a high energy diet during close-up, CU) during the dry period (DP) on production and metabolism in the first 84 days postpartum. The CE diet was formulated for a dietary energy concentration of 1.30 Mcal NE_L /kg DM and the CU diet for 1.49 Mcal NE_L /kg DM. A total of 102 cows (70 multiparous and 32 primiparous) were assigned randomly to 1 of the 4 treatments in a 2 (CE or CE/CU DP feeding strategy) × 2 (inclusion of 0 or 22 g/ton of M) factorial arrangement. After calving, all cows received a lactation diet formulated for a dietary energy concentration of 1.70 Mcal NE_L /kg DM and 14 g/ton of M. Liver samples were obtained of a subset of mature cows at -10 and 7 d relative to calving. Dry matter intake (DMI) was not affected by the inclusion of M or diet fed during the far-off period, but was 1.7 kg greater during the close-up period for cows that were fed the CU diet than cows fed CE ($P < 0.001$). None of the treatments affected DMI, body weight, or body condition score during the 84 d of lactation. Neither of the feeding strategies affected milk yield, milk component yields, percentage of milk protein, or fat-corrected milk except that percentage of milk fat was higher for CU than for CE ($P = 0.03$). The inclusion of M increased lactose content ($P < 0.01$) and yields of lactose ($P = 0.03$), fat ($P = 0.01$), total solids ($P = 0.03$), and FCM ($P = 0.01$), and tended to increase milk yield ($P = 0.06$). Fat, protein, and total solids contents were not affected by the inclusion of M. Cows that were fed CE had greater NEFA concentrations prepartum ($P < 0.01$) but lower NEFA postpartum ($P = 0.05$). The inclusion of M during the DP did not affect prepartum or postpartum NEFA and BHBA concentrations. Concentrations of total lipid, triglyceride, and glycogen in liver of mature cows were not affected by treatments. . Feeding a diet of higher dietary energy density for 21 d before calving did not benefit production or metabolism. The CU treatment increased milk fat content, but it was probably due to the higher NEFA concentration. The inclusion of M in the DP increased yields of milk fat, lactose, total solids, and FCM.

Key words: monensin, prepartum dietary energy, metabolism

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LIST OF ABBREVIATIONS

BCS	Body condition score
BHBA	Beta-hydroxybutyrate
BW	Body weight
CE	Controlled energy
CP	Crude protein
CRC	Controlled release capsule
CU	Close-up
d	Day(s)
DCAD	Dietary cation-anion difference
DIM	Days in milk
DM	Dry matter
DMI	Dry matter intake
EB	Energy Balance
DP	Dry period
FCM	Fat-corrected milk
IgG	Immunoglobulin G
kg	Kilogram(s)
M	Monensin
N	Nitrogen
NDF	Neutral detergent fiber
NEFA	Nonesterified fatty acids
NE_L	Net energy of lactation
NFC	Non-fiber carbohydrates
NRC	National Research Council
NSC	Non-structural carbohydrates
RDP	Rumen degradable protein
SCC	Somatic cell count
SCM	Solids-corrected milk
SEM	Standard error of means
TDN	Total digestible nutrients
TG	Triglycerides
TMR	Total mixed ration
VFA	Volatile fatty acid
wk	Week(s)

CHAPTER I:

LITERATURE REVIEW

INTRODUCTION

According to Grummer (1995), it has been a misconception for many years, even for researchers, that feeding dairy cows during the dry period (DP) is not as important as other periods within the productive life of dairy cows. The DP is important, but does not stand alone because in order to have an optimal dry cow management and maximize lactation results, one must take in consideration the fresh period also. The 3 wk prepartum and 3 wk postpartum are considered the transition period in dairy cows (Drackley, 1999, Grummer, 1995) or the periparturient period (Drackley, 1999). During this period cows have a greater demand for energy, but may have decreased dry matter intake (DMI) (Petersson-Wolfe et al., 2007).

The increase in energy requirement is due to the changes in the endocrine system (Rabelo et al., 2005), metabolism, and physiology (Mashek and Beede, 2000) related to the final stages of gestation, calving, and the upcoming lactation (DeGaris et al., 2010b). All of these changes are interrelated, which makes the transition period harder to study (DeGaris and Lean, 2008). Drackley et al. (2001) stated that the glucose and metabolizable energy requirements can increase from two to three times during the transition period. During the DP is when the fetus grows the most, requiring a much greater quantity of nutrients (Bell, 1995). Also, during this period the udder is prepared for milk production (Davis et al., 1979). There is less energy available due to the decrease of DMI, which may be as much as 30% (Bertics et al., 1992, Grummer, 1995).The cow can be in a state of negative energy balance (EB) during the late DP (Dann et al., 1999), and will be in even greater negative EB after calving (Mashek and Beede, 2000).

The decrease in DMI cannot be attributed to a single factor, and only a few responsible factors have been identified (Grummer, 1995). The decrease in DMI in the last 2 wk of gestation may be due in part to various endocrine changes (Ingvartsen and Andersen, 2000). Body condition score (BCS) can affect DMI, as well as milk production and reproductive performance (Waltner et al., 1993). This is the case for both under- and over-conditioning (Douglas et al., 2006) and not just prepartum, but postpartum too (Drackley, 1999).

Grummer (1995) suggested that high producing dairy cows with an increase in energy intake during the transition period have improved health, milk production, and reproductive performance. Therefore, in order to have benefits on production and fewer health issues, an adequate nutrition and management program should implemented during the transition period (Dann et al., 1999, Drackley, 1999). On the other hand, an inadequate transition period can increase the risks of metabolic disorders and other health issues, decrease production, and decrease reproductive performance, consequently decreasing profits (Drackley, 1999, Rabelo et al., 2003).

Loor et al. (2006) stated that "many of the common periparturient health disorders are strongly linked to energy balance." When the energy requirement increases and cannot be satisfied by nutrient intake, cows are at greater risk of milk fever, ketosis, and fatty liver (Grummer, 1993), retained placenta, metritis, and displaced abomasum (Drackley, 1999). Energy deficiency can be indicated by an increase in the concentrations of nonesterified fatty acids (NEFA) and beta-hydroxybutyrate (BHBA) in blood (Grummer, 1993, Ospina et al., 2010).

Drackley (1999) mentioned that the requirements of energy for lactation (net energy for lactation, NE_L) in one study exceeded intake by 26% at 4 d after calving. Additionally, he calculated that the mammary gland uses about 97% of the NE_L intake for milk production was. With these numbers in mind, it is clear that there is limited amount of energy available for maintenance.

Cows eventually satisfy their energy needs by increased DMI, but meanwhile body fat is used for energy (Ospina et al., 2010). Energy from fat is mobilized as NEFA, some which is later oxidized or re-esterified into triglycerides (TG) by the liver. When the liver cannot keep up with the production of TG from NEFA that are being mobilized, TG accumulate in the liver (Drackley et al., 2001).

One way suggested to prevent decreased production and reproductive performance is to increase the energy content of the ration by increasing the amount non-fiber carbohydrates (NFC) during the transition period (Grummer, 1995; Minor et al., 1998; Vandehaar et al., 1999). If energy intake is maintained or increased, this strategy could avoid negative EB before calving, promote papillae growth, and favor adaptation of ruminal microorganisms to a new diet balanced for milk production, which generally is higher in grains (Rabelo et al., 2003). These diets must be balanced for nutrient requirements for the cow and fetus, and the effect of these on ruminal health and nutrient availability must be considered (Grummer, 1995). But, Rabelo et al (2003) stated that

there have been a number of studies where an increase in the energy density of a transition diet was associated with an even greater decrease of DMI, and hence decreased energy intake (Minor et al., 1998).

TWO-STAGE DRY PERIOD FEEDING PROGRAM

Because of increased energy requirements the transition period is a critical time in which a dairy cow needs to be prepared nutritionally and metabolically for calving and the lactation period (Minor et al., 1998), especially to reduce the risk of metabolic disorders . As mentioned earlier, fatty liver and ketosis are two of the most common metabolic issues due to insufficient energy intake (Grummer, 1995). Various investigators (Grummer, 1995; Minor et al., 1998; Vandehaar et al., 1999) have suggested that the use of supplemental NFC during this time is beneficial for the cow to minimize trouble after calving. However, in some cases the increased energy density in diets has resulted in a decrease of total energy intake due to "increased feed inappetance" or decreased DMI (Pushpakumara et al., 2003).

For many years, the importance of studying and understanding how to feed dry cows has grown among investigators (Dann et al., 2006). Most of the research has focused on dividing the DP into two stages: stage 1, "far-off," which starts when the cow has been dried off and continues to about 21 d before calving, and stage 2, "close-up," which is usually the last 3 wk of gestation. Grummer (1995), considered as one of the pioneers of transition cow feeding studies, suggested that DMI prepartum was directly related to DMI postpartum. Many researchers soon began to question his statement and started studying how to maximize DMI and energy intake during the early transition period (close-up) or the whole DP, or alternatively limiting energy intake during close-up or the whole DP (Minor et al., 1998; Dann et al. 1999; Vandehaar et al. 1999; Mashek and Beede, 2000, 2001; McNamaraet al., 2003; Rabelo et al., 2003; Dann et al., 2006; Douglas et al., 2006; Fairfield et al., 2007; Degaris et al. 2008; Winkelman et al., 2008; and Janovick and Drackley, 2010). Not many of the studies using transition cows explain how cows were fed during the far-off stage, which limits the interpretation of results (Dann et al., 2006). In some cases, where the diets were formulated to limit energy intake and not DMI, multiparous cows would still overconsume energy relative to their requirements, and heifers probably would do the same too (Janovick and Drackley, 2010)

In order to prevent consuming more energy than it is required for dairy cows (NRC 2001), limiting DMI and energy intake prepartum may improve general health, and especially the liver's health, of cows postpartum (Dann et al., 2006, Douglas et al., 2006). However, it is not as easy to restrict how much an individual cow eats, or determine the exact nutrient intake, in commercial settings (Janovick and Drackley, 2010). Dann et al. (2006) suggested that the use of chopped wheat straw in transition diets can be used to limit the energy consumed prepartum. In spite of the studies done on dry cows, there have not been many controlled studies in which wheat straw was evaluated as a "physical restriction" to ad libitum DMI (Janovick and Drackley, 2010).

Diets should be balanced for DMI, allowing cows not to over consume energy or other nutrients at that level of DMI (Janovick and Drackley, 2010). But, according to Winkelman et al. (2008), in order to have adequate energy consumption in the early transition period, the diets should be high in energy. Postpartum, ruminal carbohydrate availability should be increased in order to enhance milk production (Dann et al., 1999).

Mashek and Beede (2000) evaluated the use of supplemental corn grain in 189 cows during the last 3 wk of gestation. Cows that were fed supplemental corn had reduced BHBA, but had no difference in NEFA, other blood variables, or incidences of health issues compared to non-supplemented cows. They found that heifers that were fed supplemental corn had higher somatic cells, greater days open, and lower milk protein compared to heifers that were not fed supplemental corn. Cows with more than two parturitions that were fed supplemental corn produced milk with higher protein content, and had lower somatic cell count and days open. In summary, supplementing with corn prepartum was beneficial with cows with more than 2 parturitions.

Mashek and Beede (2001) stated that cows fed an energy-dense diet for 6 wk instead of 3 wk had improved energy status in the first 2 wk of treatment. Also, these cows gained the most BCS and lost the least weight after calving compared to the 3-wk treatment. Concerning milk production, Mashek and Beede (2001) reported that the cows in the 6-wk treatment had higher milk protein content through the first 60 d. The authors concluded that the cows on the long treatment may have a better energy status than cows on the short treatment, but that the long-term effect could not be determined due to the variability in the experiment (Mashek and Beede, 2001).

Agenas et al. (2003) evaluated the responses in DMI, BW, and milk yield when cows were fed the same diet (low, medium, or high energy density) for ad libitum intake throughout the DP. Cows that were fed the high-

energy diet had a prolonged negative EB and lower DMI from wk 6 to wk 12 of lactation (Agenas et al., 2003). They also reported that there was no difference in the energy-corrected milk yield during the first 4 wk of lactation. Also, during the first 4 wk of lactation, the cows fed the high energy diet lost the most weight and body condition, which was reflected in greater milk fat content.

Janovick and Drackley (2010) evaluated three DP diets that supplied different energy intakes (80, 100, and 150% of the NE_L required according to the NRC 2001) on postpartum performance (8 wk). Cows and heifers that were supplied with 150% of their energy requirement gained BCS during the DP, but not enough to be considered over-conditioned. Multiparous cows that were fed 150% of their energy requirement lost the most BCS postpartum compared with cows on the other treatments. There was no significant difference among treatments for BCS with the heifers. Multiparous cows fed the high energy diet had lower DMI compared to cows fed the low energy diet during in the first 3 wk postpartum (Janovick and Drackley, 2010). Cows that were fed the high energy diets prepartum had greater fat content, and therefore greater 3.5% fat-corrected milk (FCM), during their first 3 wk of lactation. These cows contributed the most from their body (energy reserves to supply energy for milk production, and had the greatest negative EB among all treatments. Overall, Janovick and Drackley (2010) concluded that there was no difference among treatments in variables measured prepartum and postpartum for primiparous cows; in multiparous cows, a large change of DMI and EB prepartum affected postpartum DMI and BCS negatively; and that straw was effective in controlling energy intake prepartum on all cows.

Mashek and Beede (2001) stated that a method to increase energy content of a diet is to partially replace forages with grains and that this may allow a better control of the energy balance variation in the transition period, allowing the cow to moderately mobilize body fat to compensate for the difference in energy intake, and reducing the risk for health disorders.

Loor et al. (2006) presented results of a dairy cow study in which the main purpose was to measure hepatic gene expression in response to restricted and non-restricted energy intakes. Concentrations of NEFA and BHBA increased gradually for both treatments, but both concentrations from the non-restricted treatment were numerically lower during the DP and significantly higher after calving (Loor et al., 2006). French (2006) conducted a trial where he measured DMI and blood parameters in dry cows, and concluded that the increase in serum NEFA coincides with the decrease in DMI prepartum.

Dann et al. (2006) evaluated the effect of far-off diets relative to close-up diets in regard to transition metabolism and performance. The three far-off diets they evaluated were formulated to satisfy 80, 100, or 150% of the NE_L requirements for this period, according to the NRC (2001). The two close-up diets (fed to all cows 24 d before estimated calving) were balanced to meet the NRC requirements for this period, but one group was fed ad libitum to ensure at least 140% of the NE_L the other was limit-fed to provide 80% of the NE_L required. After calving, all cows were fed the same lactation diet to d 56 after calving. For the first 10 d of lactation, far-off dietary treatments had effects on DMI, EB, and concentrations of NEFA and BHBA (Dann et al., 2006). Cows that were fed 150% of their required NE_L had lower DMI and EB, but higher NEFA and BHBA concentrations during the first 10 d of lactation. Overall, cows fed 150% of the required NE_L had the greatest NEFA concentration and those fed 80% of the required NE_L had the lowest. Regarding the close-up diets and their interaction with the far-off diets, no significant difference was found. The treatment effects decreased as the days in milk increased. Dann et al. (2006) concluded that feeding over the NRC NE_L recommendation during the far-off period has a greater negative effect than the differences in the close-up diets on transition period metabolism.

Contreras et al. (2004) sought to determine if feeding a close-up diet for the entire DP compared to a two-stage program would have any effects on performance, health, and reproduction. They found no difference in milk yield between feeding programs. However, cows enrolled in the two-stage feeding program had higher milk fat, 3.5% FCM, and protein during their first 5 mo of lactation, and also gained less BCS during the DP than cows fed the close-up diet for the entire DP.

It is common for commercial farms to feed close-up diets 3 wk prior to calving. The rationale for this feeding period can be explained at least partially by the research done by Robinson et al. (2001) and Mashek and Beede (2001). Robinson and collaborators retrospectively evaluated various actual close-up period lengths; 1 to 4, 5 to 8, 9 to 12, and 13 to 19 d before estimated calving. They as concluded that the best close-up period length was 9 to 12 days for heifers, but that the best close-up period for multiparous cows could not be determined with the data obtained (Robinson et al., 2001). On the other hand, Mashek and Beede (2001) evaluated a close-up diet for a 3 wk or 6 wk period before the estimated calving date. Cows in the longer period group had a better EB during the first 2 wk of lactation. No difference was found in BHBA concentrations. Overall, data collected varied from farm to farm, preventing the determination of long-term effects of close-up period length (Mashek and

Beede, 2001). Assuming that close-up diets are more expensive than far off diets, it is best to utilize them in the shortest period possible to save cost (Contreras et al., 2004). According to Hutjens (2003), and having in mind that cows should consume a close-up diet for at least 9 to 12 days, 18% of the cows would calve early and would only receive the close-up diet for 5 days, and 4% of dairy cows would calve early enough to miss the entire close-up period. Therefore, it is recommended for the close-up period be at least 3 wk long for all cows to consume this diet for at least 10 d (Hutjens, 2003).

Changes in the endocrine system and the decrease of DMI during the transition period affects metabolism, leading to an increase of fat mobilization from adipose tissue and glucose mobilization from glycogen in the liver (Grummer, 1995). The decrease of 30% in DMI would be considered normal in cows and heifers (Bertics et al., 1992, Grummer, 1995), although this decrease in DMI can be attributed to the changes in the endocrine system. Likewise, NEFA can double in concentration in the last 2 to 3 wk of gestation (Grummer, 1995). Force feeding cows during the DP reduced the total concentration of NEFA (Bertics et al., 1992). On the contrary, Vazquez-Anon et al. (1994) reported an increase in NEFA prepartum, without a decrease of DMI – indicating that an increase of prepartum NEFA may be attributed to endocrine changes. Consequently, the rapid increase in NEFA can be attributed to the stress of parturition, which diminishes after calving but does not decrease to its prepartum levels (Grummer, 1995). By the first day of lactation liver triglycerides (TG) has increased markedly, but the TG concentration in the liver is maintained or continues to increase until the third wk (Bertics et al., 1992, Vazquez-Anon et al., 1994). Heifers seem to be less susceptible to high liver TG concentration (Grummer, 1995). The postpartum concentrations of NEFA and BHBA decreased as the days in lactation increased, but increased with the days exposed to prepartum treatment, age, and BCS, the latter effect being greater (DeGaris et al., 2010b). When evaluating the length of time in which a cow is fed a late gestation diet, there may be effects on metabolites associated with minerals, protein, and energy and a positive effect on production and reproduction as length of feeding increases (DeGaris et al., 2010b).

Many unknowns concerning the transition period remain to be addressed. However, doing research in this period can be costly due to the number of cows that must be enrolled to compensate for the high variability within treatments on variables such as metabolic disorders (Grummer, 1995).

POSTPARTUM TRANSITION PERIOD

The transition period is an important period nutritionally. Most cows that pass through this period have a negative EB postpartum, which can last up to 15 wk (Pushpakumara et al., 2003). Energy can be obtained from dietary energy intake or from energy stored in tissues as fat or glycogen, and is used for maintenance or production. In general the deficit of energy during negative EB is compensated by energy from adipose tissue and muscle protein, which is mobilized by metabolic and endocrine processes. Lipolysis in adipose tissue increases the concentration of NEFA in blood. When NEFA concentration in the blood exceeds the ability of the liver to process the NEFA, fatty liver occurs.

Approximately 80 % of the diseases or disorders in adults cows occur during the first 100 d of lactation (DeGaris et al., 2010a). Ospina et al. (2010) presented the results of a prospective cohort study of 100 northeastern United States herds in which they attempted to establish critical thresholds in NEFA and BHBA concentrations to predict periparturient diseases such as displaced abomasum, clinical ketosis, and metritis and/or retained placenta, and to determine the magnitude of association of the metabolites with diseases in the first 30 d of lactation. They found that the NEFA concentration that predicted any of the diseases mentioned earlier was 0.29 mEq/L prepartum and 0.57 mEq/L postpartum. Concerning BHBA, the threshold value for predicting any of the diseases was 10 mg/dL postpartum. Even though both metabolites are associated with displaced abomasum, clinical ketosis, metritis, and/or retained placenta, postpartum NEFA was most associated with the risk of developing any of the diseases within the first 30 d of lactation (Ospina et al., 2010).

Postpartum EB was increased and NEFA and liver TG concentrations were decreased when the DP was shorter (Rastani et al., 2005). It is known that negative EB is associated with health disorders such as fatty liver and ketosis (Bertics et al., 1992)

The amount of adipose tissue in a cow has effects on milk yield and health (Waltner et al., 1993). Usually, extremes of BCS have a negative effect. Waltner et al. (1993) studied the BCS of 350 cows and heifers during their entire lactations. They concluded that the BCS decreased as parity increased and that BCS loss varied quadratically with the DIM. Also, Waltner et al. (1993) concluded that parity had a greater relationship with milk fat and milk yield than did BCS. On the other hand, BCS at calving and the loss of BCS related quadratically to milk yield within

lactation. No relationships were found between BCS and the incidence of pyometra, metritis, or retained placenta (Waltner et al., 1993).

MONENSIN

As discussed already, energy balance is an important factor for milk production and health, especially during the transition period when high energy demands coincide with a decrease of DMI. As the demand for energy increases and the energy intake does not satisfy the requirements, fat mobilization occurs (Melendez et al., 2004). Fat mobilization results in an increase of NEFA and BHBA concentrations in the blood, and an increase of TG in the liver (Vazquez-Anon et al., 1994). These changes in metabolites may lead to various diseases, such as ketosis, milk fever, fatty liver, retained placenta, metritis, and displaced abomasum (Bertics et al., 1992, Drackley, 1999, Grummer, 1995, Melendez et al., 2004, Ospina et al., 2010). Adding ionophores to transition cow diets may help to modulate effects of NEB by increasing glucose availability (Petersson-Wolfe et al., 2007).

Ionophores are feed additives that have been used since 1977 in the beef industry to improve feed efficiency and prevent coccidiosis (Petersson-Wolfe et al., 2007). Monensin (M), an ionophore, is a carboxylic polyether that is produced naturally from a *Streptomyces cinnamonensis* strain. Monensin alters the rumen micro-flora by selectively inhibiting the growth of gram-positive bacteria (acetate and hydrogen producers), resulting in an increase of the rumen bacteria that will increase the production of propionate, which is the primary glucose precursor (Dubuc et al., 2009). In addition, by increasing propionate production monensin reduces methane production in the rumen, and decreases the risk of ketosis, displaced abomasum, and related disorders (Duffield and Bagg, 2000, Duffield et al., 2008b, Duffield et al., 1998b).

According to Duffield et al. (2003), supplementation of M at 9 to 23 mg/kg of dietary DM reduced milk fat in cows that receive diets low in non-structural carbohydrates (NSC), less than 40.2% (DM basis), but not in cows that receive diets with higher NSC content. They later tried to prove the effects of the interaction between diet composition and M on milk fat.

Duffield et al. published the results of a meta-analysis on the impact of M on metabolism (2008a), production (2008b), and health and reproduction (2008c). Data were obtained from a total of 80 papers that

reported on the use of M. Originally they located a total of 161 papers; after screening 80 papers were selected, but only 59 had appropriate and usable data for the variables of interest (Duffield et al., 2008a) .

Out of the 59 papers, Duffield et al. (2008a) used 30 papers for the meta-analysis to determine the effect of M on cow metabolism. Results obtained from this meta-analysis showed that M reduced the blood concentrations of NEFA and BHBA by 7 and 13%, increased glucose and urea by 3 and 6%, and had no significant effect on insulin and milk urea. Furthermore, the diet, method of M administration, and stage of lactation affected the response of metabolites to M (Duffield et al., 2008a). Top-dressing with M or using controlled-release capsules (CRC) reduced the effect on BHBA, but top-dressing had the most positive effect on glucose compared to CRC or supplementing in the TMR. Also, using M in early lactation had a greater effect on BHBA, but using it during the dry and lactation period amplified the effect on NEFA concentrations. Duffield et al. (2008a) concluded from the findings resulting from this analysis indicated that the use of M improves energy metabolism in dairy cows.

Thirty six of the 59 papers, a total of 9,677 cows studied, had acceptable data for the meta-analysis of the effects of M on production (Duffield et al., 2008b). Some of the results reported by Duffield et al. (2008b) were that the use of M decreased DMI by 0.3 kg/d, increased milk production by 0.7 kg/d, and increased milk production efficiency by 2.5%. Monensin also decreased fat content by 0.13% but did not decrease milk fat yield; decreased protein content by 0.003% but increased protein yield by 0.016 kg/d; and had no effect on lactose content. Monensin increased BCS by 0.03 units (on a 5-unit scale) and BW by 0.06 kg/d. Monensin was associated with a decrease of short-chain fatty acid contents in milk fat. They also reported that there was a difference in milk yield and milk components depending on how the M was fed, the dose fed, diet, and stage of lactation. In particular the diet affected the responses of milk fat and yield to M supplementation. Overall, Duffield et al. (2008b) concluded from the findings of this meta-analysis that BCS can be conserved and milk efficiency improved with the use of M. Duffield et al. (2008b) reported that the effect of M on percentage and yield of milk fat was dependent on the diet.

For the meta-analysis of the effects of M on health and reproduction of dairy cows, Duffield et al. (2008b) used 16 papers out of the 59 available. These 16 papers resulted in data from approximately 9,500 cows. Monensin supplementation had no significant effects on occurrence of milk fever, lameness, dystocia, retained placenta, or metritis, nor on first-service conception risk or days to pregnancy. Favorable effects were found regarding the risk of ketosis, displaced abomasum, and mastitis. The delivery method of M affected its efficacy in

decreasing the incidence of metritis and retained placenta (Duffield et al., 2008c), being more effective when offered in a CRC than when offered in the TMR or top-dressed. Duffield et al. (2008b) concluded that the use of M decreases the risk of ketosis, displaced abomasum, and mastitis, but may increase the risk of dystocia, which may lead to greater risk for incidence of retained placenta and, in turn, metritis if offered for a prolonged DP.

OBJECTIVES

Based on the literature to date, there is a continued need to evaluate a single-group DP diet based on the controlled energy principles with a conventional two-group (far-off and close-up) system. Furthermore, effects of M supplementation in such prepartum diets are unknown. Therefore, the purpose of this experiment was to evaluate the inclusion of M in two different DP feeding programs (one group controlled-energy diet versus a two-group far-off plus close-up diet). Response variables included effects on DMI, blood metabolites (BHBA and NEFA), liver metabolites (triglycerides, glycogen, and total lipids), BW, BCS, calving (difficulty score, and cow and calf BW), colostrum (first milking yield and immunoglobulin content), production (milk and components), feed efficiency, and health to 84 d after calving.

CHAPTER II:

EFFECTS OF CLOSE-UP DIETARY ENERGY STRATEGY AND PREPARTAL DIETARY MONENSIN ON PRODUCTION AND METABOLISM IN HOLSTEIN COWS

INTRODUCTION

Use of "controlled-energy diets" during the DP has become popular in recent years and may be the most defensible concept in dry cow nutrition from a scientific basis (Drackley and Dann, 2008). These diets are formulated with large amounts of bulky, high-NDF forage such as cereal straw to dilute energy content and limit energy intake to near the cow's requirements (Janovick and Drackley, 2010). Controlled-energy diets maintain more constant DMI before calving, decrease NEFA and BHBA concentrations after calving, and result in less lipid accumulation in liver postpartum (Janovick et al., 2011). In contrast, overfeeding energy during the DP is clearly detrimental, resulting in steep decreases in DMI before calving, prolonged elevations in NEFA and BHBA postpartum, and increased hepatic lipid infiltration (Dann et al., 2006; Douglas et al., 2006; Janovick and Drackley, 2010; Janovick et al., 2011). Field observations indicate that controlled-energy diets may decrease metabolic disorders and improve subsequent reproductive success (Beever, 2006). With this strategy, the idea that a "close-up" (steam-up" or "pre-fresh") diet is needed before calving has been called into question (Beever, 2006; Drackley and Dann, 2008). Indeed, a recent experiment in our group demonstrated that there was no advantage to a two-group (far-off plus close-up) strategy over a single-group controlled-energy diet (Richards et al., 2009).

Administration of M at 15 or 30 g/ton DM beginning 1 wk prepartum decreased concentrations of BHBA postpartum (Sauer et al., 1989). Similarly, R administered as CRC during the last 3 wk prepartum decreased BHBA postpartum (Duffield et al., 1998a) and also significantly decreased energy-related disorders postpartum (Duffield et al., 2002). Introduction of a M CRC increased total tract apparent digestibilities of NDF and energy (Plaizier et al., 2000) but did not affect ruminal pH or subsequent milk production (Fairfield et al., 2007). Less information is available on effects of dietary supplementation of M before parturition. Direct comparison of CRC vs. dietary administration prepartum showed that both decreased BHBA postpartum relative to an unsupplemented control, but only the CRC decreased loss of BCS postpartum (Petersson-Wolfe et al., 2007). Dietary supplementation of M (300 mg as top-dress) increased glucose supply to the cow, probably by increasing ruminal propionate production

(Arieli et al., 2001), and decreased NEFA concentration 1 wk postpartum but did not improve milk production or composition (Vallimont et al., 2001).

Little research information is available in which M administration has been compared in different diets within the same study. Chung et al. (2008) supplemented M as a top-dress (330 mg/d) for the last 28 d prepartum to either a high-forage diet or a diet with large amounts of non-forage fiber added. Monensin had little effect on DMI, milk production, or metabolic indicators, although tendencies for interactions of M and diet were detected for milk yield and milk fat content (Chung et al., 2008). No research is available on use of M in controlled-energy diets. Its use on one hand seems paradoxical if the goal is to control energy intake; on the other hand its effects to increase efficiency of nutrient use should be beneficial no matter what diet is fed. Furthermore, M use in lactation diets has become widespread in the United States and many other countries. Debate has ensued whether supplementation should be continued during the DP or whether a "rest period" for the rumen without M during the DP might improve its efficacy during the ensuing lactation.

Consequently, the objectives of this study were to determine the effects of M supplementation prepartum in single-group high-straw, low-energy dry cow diets, in comparison with the traditional two-group approach with a higher-energy close-up diet.

MATERIALS AND METHODS

The study was conducted at the University of Illinois Dairy Research and Teaching Unit. All procedures were approved by the University of Illinois Institutional Animal Care and Use Committee (protocols number 08179 and 08225).

Design, cows, and management

The trial was a randomized design with a 2 × 2 factorial arrangement of treatments. The four DP treatments consisted of a single controlled-energy feeding program or a two-stage DP feeding program, each without or with inclusion of M (Table 1). After calving, all cows were offered the same lactation ration, containing M.

Both first-calving heifers and older cows were used in the experiment. Cows were blocked by parity, BCS, and expected calving date and then randomly assigned to treatments. Lactating cows were dried off at least 50 d

prior to expected parturition and moved to the experimental freestall barn. Cows entering their first lactation were moved into the experimental freestall barn at least 50 d prior to expected parturition. After parturition, all cows were moved into individual tiestalls.

Throughout the DP, all cows were housed in a barn with four pens of 10 sand-bedded free stalls. Cows and heifers were grouped together (up to 10 cows/pen). The day cows were dried off, they were moved to the freestall pens and assigned a Calan feed gate (American Calan, Northwood, NH). All cows began receiving the experimental diets immediately and data were collected, but only the last 21 d of the far-off period were used for statistical analysis due to the variability in length of this period. For the close-up period, only the last 14 d before calving were analyzed for the same reason. Three days before the estimated calving date or as calving signs appeared, cows were moved to individual concrete-floored calving pens (bedded with straw) located within the freestall pens. Immediately after calving, cows were moved to a tie-stall barn and were assigned an individual stall for the duration of the study.

Diets and feeding

Diets were balanced according to NRC (2001) recommendations. The controlled-energy diet was formulated for a DMI of 12.2 kg/d (for mature cows) with an energy density of 1.30 Mcal NE_L/kg DM (Table 3). For the two-stage program, the same diet was fed for the far-off stage (from the day the cow was dried to 21 d before the estimated day of calving) and a close-up diet was fed during the close-up stage (from 21 d before estimated day of calving to actual day of calving). This diet (Tables 1 and 2) was designed to be intermediate in ingredients and nutrient profile to far-off and lactation diets. The close-up diet was balanced for DMI of 10.5 kg/d (for mature cows) and an energy density of 1.49 Mcal NE_L/kg DM. Each of the two DP diets was formulated both with and without M at a target of 22 g/ton (24.2 g/metric ton) of total dietary DM. After calving, all cows were fed the same lactation diet (Table 1) that was formulated for a DMI of 22.7 kg/d for mature cows and an energy density of 1.70 Mcal NE_L/kg DM (Table 2). The lactation diet contained M at 14 g/ton (15.4 g/metric ton) of dietary DM.

All diets were mixed in a Keenan 140 TMR mixer wagon using PACE software and Mech-Fiber technology (Richard Keenan & Co., Borris, Co. Carlow, Ireland). All ingredients were sampled weekly for determination of DM content to adjust ration formulation. After weekly adjustment of the rations to maintain the desired DM ratio of

ingredients, the ration DM content was adjusted to 46% for all DP diets by addition of water daily when diets were mixed. No water was added to the lactation diet.

Cows were fed once daily at 0600 h in amounts to provide a target of 2 to 3 kg (wet weight) of refusal. Feed was pushed up to the cows at least three times daily. Refusals were removed daily before feeding and weighed.

Feed analysis and determination of DMI

Samples of all TMR were obtained weekly and composited by month for analysis. Samples were analyzed for chemical composition using wet chemistry techniques at a commercial laboratory (Cumberland Valley Analytical Services, Hagerstown, MD). Three to five samples of individual ingredients obtained throughout the experiment also were analyzed by the same techniques.

To calculate DMI during the DP, samples of TMR and pooled refusal for each diet were obtained weekly and dried in an oven to determine DM content. For the lactation phase, the calculation of DMI was different due to the splashing of water on the feed by some cows from their drinking cups. The amounts of feed offered and refusal were weighed daily for each cow. The DM content of the refusals was measured subjectively using a calibrated 1 to 4 score. If the water content of the refusal was “normal” (similar to feed offered), the refusal was scored 1, and if the refusal was “watery”, it was scored 4. Intermediate scores of 2 and 3 were assigned according to whether they were closer to normal or watery, respectively. Samples of the refusals of different scores were obtained weekly and the DM content determined. At the end of the experiment all measured refusal DM contents for each score were averaged as a difference from the DM content of the offered TMR. These mean values were then used as constants for the refusal DM of each score to estimate DM content of the refusals, and daily DMI was calculated.

BW and BCS

All dry cows were weighed once weekly on Thursday and lactating cows were weighed weekly on Wednesday. Each cow and her calf were weighed within 12 h of calving. Body condition scores were assigned for all cows weekly by at least 2 technicians. The scores were averaged before statistical analysis.

Calving measurements

Dry cows were observed at least four times daily for calving and possible calving difficulties. Calving difficulty was scored on a scale of 1 to 5, where 1 was when a cow calved between observations without showing any signs, 2 was when a cow showed signs and calved without difficulty in less than 6 hours, 3 was when a cow was observed for 6 hours and the calving progressed naturally, 4 was when the calving lasted more than 6 hours and the cow needed some assistance for calving, and 5 was when the cow could not calve on her own and required assistance because of position or size of calf.

All cows were first milked within 8 h of calving in the milking parlor and the total colostrum was stored in a refrigerator. The stored colostrum was weighed within the first 12 h after calving and sampled for later IgG analysis. Samples were stored in a freezer at -20°C until analysis by radial-immunodiffusion techniques by the University of Illinois Veterinary Diagnostic Laboratory.

Milk yield and composition

All cows were milked three times daily for the 84 d of the lactation phase of the trial. Daily milk yield was measured electronically (DairyPlan, Westfalia Surge Inc., Naperville, IL). Daily milk yield was the sum of the three milkings, and weekly means of daily production were calculated. Milk was sampled once weekly for the milkings within a 24-h period. Within 12 h after the last samples were collected, the samples were composited by equal volumes for each cow, and composite samples were shipped to a commercial laboratory (Dairy Lab Services, Dubuque, IA) for analysis of milk composition. Samples were analyzed for contents of fat, true protein, lactose, total solids, urea nitrogen, and somatic cells. Weekly yields of milk solids and SCM, 3.5% FCM, and 4% FCM were calculated.

Concentrations of NEFA and BHBA in blood

Blood samples were obtained by puncture of a tail vein or artery three times weekly (Monday, Wednesday, and Friday) from all cows before the morning feeding. Serum aliquots were obtained within 4 h from the time the last sample was collected. Serum was stored in a freezer at -20°C. At the end of the trial, samples were identified based on time relative to actual calving that corresponded to one sample per week during the far-off period (up to 4 wk, sample from mid-point of week used) and samples that corresponded to -13 d (-14 to -12 d), -10 d (-11 to -9 d), -7 d (-8 to -6 d), -4 d (-5 to -3 d), and -1 d (-2 or -1 d) relative to calving during the close-up

period. Postpartum, samples were identified that corresponded to +1 d (+1 or +2 d), +4 d (+3 to +5 d), +7 d (+6 to +8 d), +10 d (9 to +11 d), +13 d (+12 to +14 d), +16 d (+15 to +17 d), and +19 (+18 to +20 d) relative to calving, and then one sample per week from wk 4 through wk 12. These samples were analyzed for concentrations of BHBA and NEFA by autoanalyzer methods at the UIUC Veterinary Diagnostic Laboratory.

Liver biopsy and analysis of liver tissue composition

Liver was sampled via puncture biopsy (Hughes, 1962; Veenhuizen et al., 1991) from cows under local anesthesia at approximately 0900 h. Biopsies were obtained from at least 10 second or greater lactation cows selected randomly from each treatment. Liver tissue samples were frozen immediately in liquid N. A portion of the tissue samples was later analyzed for concentrations of glycogen (Lo et al., 1970), total lipid (Drackley et al, 1991), and triglyceride (Foster and Dunn, 1973).

Statistical analysis

All data were analyzed with mixed models using procedures in SAS. To avoid problems with fitting covariance structure, pre- and postpartum variables were analyzed separately; far-off and close-up period variables were also analyzed separately. For variables with repeated measures (week or day) the MIXED procedure with the REPEATED statement was used. The following model was used:

$$y_{ijklm} = \mu + D_i + R_j + P_k + DR_{ij} + DP_{ik} + RP_{jk} + DRP_{ijk} + C_{l(ijk)} + T_m + TD_{im} + TR_{jm} + TP_{km} + TDR_{ijm} + TDP_{ikm} + TRP_{ikm} + TDRP_{ijkm} + e_{ijklm}$$

where y_{ijkl} = an observation from the ith diet, jth M treatment, kth parity, lth cow, and mth wk relative to calving; μ = the grand mean; D_i = effect of the ith diet; R_j = effect of the jth M treatment; P_k = effect of the kth parity; DR_{ij} = effect of the diet by M interaction; DP_{ik} = effect of the diet by parity interaction; RP_{jk} = effect of the M by parity interation; DRP_{ijk} = effect of the diet by M by parity interaction; $C_{l(ijk)}$ = random experimental error from the lth cow nested within the ith diet, jth M treatment, and kth parity; T_m = effect of the mth time (day or wk); TD_{im} = effect of the time by diet interaction; TR_{jm} = effect of the time by M interaction; TP_{km} = effect of the time by parity interaction; TDR_{ijm} = effect of the time by diet by M interaction; TDP_{ikm} = effect of the time by diet by parity interaction; TRP_{ikm} = effect of the time by M by parity interaction; $TDRP_{ijkm}$ = effect of time by diet by M by parity interaction; and e_{ijklm} = random residual error associated with the ith diet, jth M treatment, kth parity, lth cow, and mth time, assumed to be random and normally distributed.

For variables without repeated measurements, the MIXED procedure was used with a model similar to that above, but containing only diet, M, parity, and all interactions. Degrees of freedom were estimated by using the Kenward-Roger option in the model statement. Least squares means were generated and were separated by the PDIFF option when protected by a significant F-test. Mean differences were considered significant when $P < 0.05$, and tendencies or trends were declared at $0.05 < P \leq 0.10$.

RESULTS AND DISCUSSION

Diet formulation and nutrient composition

Diets were formulated on the basis of a lactation diet in which corn silage was the predominant forage, with smaller amounts of alfalfa silage and alfalfa hay (Table 2). Such diets and ingredients would be typical over much of the Midwestern and Northeastern US. The strategy in formulating the DP diets was to keep the proportion of corn silage similar to the lactation diet, with smaller amounts of alfalfa silage and a large amount of wheat straw to dilute the energy content and control intake. For the close-up diet used in the two-group strategy, the goal was to add or delete ingredients such that the diet represented a "half-way" diet in terms of forages and major concentrate ingredients. It can be said that this diet represented well a "transition diet," but perhaps may be less typical of what had come to be a conventional pre-fresh or close-up diet because of its large amount of straw, high-RUP protein sources, and wet brewers' grains. Because the CE diet was formulated to serve both as the single-group DP diet and the far-off diet in the two-group system, blood meal, a small amount of SoyChlor®, magnesium sulfate, and Vitamin E were included.

The strategy in diet formulation is shown by the as-formulated nutrient profiles (Table 3). Several key concepts were incorporated in formulation, based on this lab's previous research and on feedback from the field. For the CE diet, NE_L density was set at approximately 1.30 Mcal/kg DM, which for a mature cow at ad libitum intake would be predicted to supply approximately 15 Mcal of NE_L daily. The goal was to achieve a metabolizable protein intake of at least 1,000 g/d. Starch content was to be about 14% of DM, and NFC content between 26 and 28% of DM. Based on field experiences, calcium was set to between 0.9 and 1.0% of DM, magnesium content to approximately 0.5%, phosphorus to 0.35%, sulfur to 0.35%, and potassium content was minimized. The DCAD was adjusted to less than 40 mequiv/kg DM based on field experiences. For the close-up diet, NE_L density was adjusted

to be intermediate to the CE and lactation diets at approximately 1.49 Mcal/kg, predicted to supply 15 to 16 Mcal of NE_L daily. Contents of fat, starch, and NFC were also set to be intermediate. In management of mineral specifications, this diet aimed to achieve a negative DCAD of between -10 and -20 mequiv/kg DM by addition of greater amounts of SoyChlor®, although urine pH was not monitored. Vitamin E was supplemented to both diets to achieve intakes of >2,000 IU daily.

Analyzed composition of forages and ingredients used in the diets is presented in Tables 4 and 5. Because fewer samples were available for the individual ingredients, these values are provided as references only, and diet composition was based on composite samples of the TMR, which where sampled weekly throughout the experiment. Mean composition (with standard deviations) of the TMR analyses is presented in Table 6.

The analyzed composition of the diets (Table 6) demonstrates that the desired approach used in diet formulation was largely achieved. This is true both in comparing the differences between the CE and CU diets relative to the lactation diet as well as the similar composition of each diet without or with M. Based on continuing evolution of knowledge and experience with these diets, a few dietary characteristics are worth mentioning. The content of sugars was low across all diets; performance of the lactating cows might be improved by increasing the sugar content to >4% of DM (Broderick et al., 2008). Whether sugar content should be increased in the DP diets also is not clear. Starch content of the CE diets was slightly lower than the 14% target, and this could be adjusted in future experiments or practice. The analyzed concentrations of minerals varied somewhat from formulated values although the difference was not deemed to be critical.

The concentration of M in the lactation and DP concentrate mixes was verified by independent analysis. No M was detected in the CE and CU concentrate mixes (assayed concentration <0.9 g/T, the level of detection, in 4 samples of each). Expected concentrations of M in the concentrate mixes were 32.7, 113.5, and 68.8 g/T for the lactation, CE+R, and CU+R grain mixes, respectively. Mean assayed concentrations (± SD) of CE were 28.5 ± 4.0 (n = 5), 117.5 ± 15.7 (n = 4), and 61.8 ± 4.7 (n = 4) for lactation, CE +R, and CU+R concentrate mixes, respectively. Mean analyzed concentrations were 87.2, 103.5, and 89.8% of expected, respectively, and therefore all diets were similar to the formulated concentrations.

Prepartum measurements

DMI

No difference was found within diets and the use or M for DMI during the far-off period (Table 7). Differences were found within parity, days, and the interaction of these. As expected, multiparous cows had higher DMI (10.1 kg) than primiparous cows (8.5 kg), even though multiparous cows started eating less during the far-off period. The reasons that might justify for cows eating less than heifers during the first days of the far-off period were: heifers adapted to the Calan gates faster than the cows due to similar previous feeding systems, and that heifers were ingesting a similar diet to the CE diet, which was very different compared to the late lactation diet. In regards to days before the transition period (21 d before calving), DMI started at the lowest point (5.7 kg) on day -28. From day – 24 through the day before the transition period started (21 days before estimated calving), DMI increased slightly with minor differences within days which can be explained by adaptation to current feeding system. Considering the interaction of parity and weeks in lactation, cows had higher DMI than heifers during all days with exception of d -28 to -26, but were not statistically different from heifer intakes in the same days.

During the 14 d analyzed from the close-up period, significant differences were found due to diet and days (Table 7). Cows that were fed the CU diet had a higher DMI (10.4 kg) compared to the cows that were fed CE diet (8.7 kg), as expected. Also as expected, there were differences among days until calving, with DMI decreasing as the calving date came closer. There was a tendency (P = 0.07) that multiparous cows had a higher DMI (9.9 kg) compared to primiparous cows (9.2 kg). There was also a tendency (P = 0.07) for cows that ingested the diets with M to consume 0.75 kg less DM than the cows fed the control diets.

NE_L intake

Differences in NE_L intake during the far-off dry period were found within parity, days in period, and the interaction of these, as would be expected from the differences found in DMI. No differences were found due to diets or M inclusion (Table 7). Cows ingested 18% more NE_L than heifers (12.76 Mcal vs. 10.79Mcal). Both increased their NE_L intake from the day they started up to their last day in the far-off period. With the interaction of parity and days in the far-off period, cows had the greatest NE_L intake by the end of this period. On the contrary, there were no differences in NE_L intake between cows and heifers in the first four days in this period (-28: 6.62, 7.96; -27: 8.20, 8.64; -26: 9.55, 9.90; and on day -25: 10.67 kg, 10.31kg; consecutively).

Different from the CU DMI, significant differences were also found between parity groups for NE_L intake. All cows in the high energy CU diet consumed 31.7% more NE_L (14.87 kg) during this period than did cows fed the low energy diet (11.29 kg), which would be expected by the composition of the diets (Table 7). As could be predicted by considering rumen capacity, multiparous cows (13.70 Mcal) consumed 10% more NE_L than primiparous cows (12.33 Mcal). Considering the NE_L intake in regards to the days relative to calving, there was no difference within the first 9 d in the close-up period, a slight difference up to the 13th day, and significant decreases on the day before calving, as expected.

BW and BCS

As expected, significant differences were found in the effects of:

- parity (cows weighed almost 21% more than heifers) ,
- week (the first week had the lowest BW (690 kg) and the last week had the highest (726)kg, but not significantly different from the week before last (721 kg)), and
- the interaction of parity x week (heifers in general had the lowest BW, with the first week being the lowest, 631.37 kg, and cows on the last week had the highest, 798kg).

Unexpectedly, differences were found as the interaction of diet x parity x week during the far-off period. Even though there was no difference due to diet, BW was numerically different (719 kg for the CE versus 705.kg for the high energy CU diet). This can only be explained that the heaviest cows (primi- and multiparous) were randomly assigned to the one-diet DP feeding system.

Differences in the close-up period difference were only found between parity groups. Cows had a BW average of 797kg and heifers 661 kg. No differences in BCS were detected either in the far-off or close-up periods (Table 7).

Calving measurements

No significant differences due to diet or M supplementation were found for cow BW, calf BW, calving difficulty, colostrum weight, colostrum IgG concentration, or total colostrum IgG (Table 8).

Cow BW

When analyzing the cow's body weight at calving, a significant difference was only found for the effect of parity. As expected, multiparous cows (723 kg) weighed more than primiparous cows (593 kg) when weighed within the first 12 h after calving.

Calf BW

After taking into consideration the sex of the calf, no other effects significantly influenced calf BW. Sex of calf was the only effect that affected the calf body weight. Males (45.6 kg) weighed approximately 8% more than females (42.2 kg).

Calving difficulty

Cows that were supplemented with M had a slightly higher difficulty calving score in comparison to the cows that were not supplemented with M (1.6 and 1.2, consequently). No other significant difference was found for calving difficulty. No difference was found due to sex of the calf.

Colostrum weight, colostrum IgG concentration, and total colostrum IgG

The only difference found in regards to colostrum weight was in the effect of parity, in which colostrum weight was 48% higher in multiparous cows (6.8 kg) than in primiparous cows (4.8 kg). This was expected since multiparous cows tend to have a greater milk yield than primiparous cows.

Even though there was no difference found in regards to the colostrum IgG concentration, there were differences in total colostrum IgG. The total amount of IgG present on the first colostrum milking was more than 55% greater for multiparous cows (464.4 g) of than for primiparous cows (298.8 g).

Postpartum measurements

The results after analyzing all the data collected after calving in regards to DMI, NE_L intake, body weight, body condition score, and milk yield and its composition are summarized below.

DMI and NE_L intake

Even though M has been known to decrease DMI, it did not have that effect during this trial (Table 9)., nor did the DP feeding system or the interaction of these. Statistical differences ($P < 0.05$) were found between DMI of primiparous (16.8 kg) and multiparous (21 kg) cows, and to week in lactation, which increased throughout the 12 wks of the trial as expected due to body size and appetite after calving.

As it would be expected given the results for DMI after calving, differences were found between parity groups and days in lactation for NE_L intake. No significant differences were found between diets or M supplementation (Table 9).

BW

Diet fed and inclusion of M did not affect BW of multiparous or primiparous cows (Table 9). There difference between cows and heifers approached significance ($P = 0.052$). Significant differences where found within weeks, independent of treatments. Considering the interaction of parity × week, cows overall had greater BW than heifers.

BCS

The BCS was only affected by parity (means of 3.2 and 3.0 for multiparous and primiparous, respectively) and week after calving. No significant differences were found between diets or M supplementation (Table 9). Independent of parity, the BCS decreased from wk 1 to wk 7 and plateaued through wk 12 as it could have been foreseen due to the loss of BCS after calving and the increase after reaching nadir. Body condition score likely reached a plateau after the cows had achieved a balance of energy between intake and requirements.

Milk yield and composition

A summary of the results of milk yield and components for cows fed prepartum diets with or without M in the lactation experiment can be found in Table 10.

As expected due to mammary gland size and milk production curve, differences were found between cows and heifers (47.1 kg and 37.5 kg) and weeks in lactation, but no other differences were found. Production increased significantly from wk 1 (29.9 kg) to wk 5 (44.3 kg). No significant difference was found between weeks 5 to 12. There was a tendency ($P = 0.07$) for cows that were fed M during the DP to have higher milk yields (43.6 kg) than cows that were not supplemented with M (41.0 kg), independent of parity.

Cows fed the CU diet had a statistically higher milk fat percentage (3.77%) than cows fed the CE diet (3.54%) regardless of parity. Milk fat percentages statistically decreased from wk 1 to wk 5, plateaued from wk 5 through 7, decreased from 7 to 8, and plateaued again from wk 8 through 12. As expected, the highest milk fat content was during the first week postpartum and milk fat decreased thereafter.

Milk fat yield (kg/d) produced per cow was affected by M during the DP, and between cows and heifers. The use of M during the DP increased daily fat production by 8.36%, from 1.44 to 1.56 kg/d. Cows produced 25.5% more fat than heifers.

Considering the results of milk yield and fat content, it could have been predicted that differences would be found between the supplementation or not of M, parity, and wk in lactation, in regards to 3.5% FCM (Figure 1). In general, adding M to the transition diet increased 3.5% FCM 7.6% (41.2 to 44.3 kg/d). Overall, heifers produced 20.4% less 3.5% FCM than cows (47.6 kg). Also, the first week of lactation resulted in the lowest 3.5% FCM and the wk 6 (resulted in the greatest, although not statistically different from wk 4, 5, 7, 8, 9, and 10. Results were similar for 4.0% fat FCM (Figure 2).

The percentage of protein in milk was affected by the week in lactation. Week one was the highest (3.86%), followed by wk 2 (3.02%) and wk 3 (2.75%). Cows produced 24.8% more protein than heifers (1.28 and 1.03 kg/d, respectively). In addition, Protein yield was lower during the first 4 wk than the later8 wk.

The percentage of lactose in the milk was affected by differences between diets, the use of M, parity, and week. There was a tendency for lactose content to vary with the interaction of diet, the inclusion of M, and week ($P = 0.06$). In general, cows (primiparous and multiparous) that were fed the CU diet had higher lactose content (4.93%) than cows fed the CE diet (4.87%). Heifers had 1.55 % higher lactose content (4.94 %) than cows (4.87%). The addition of M to the diets increased lactose percentage by 1.55% (from 4.87% to 4.94%). Furthermore, cows irrespective of treatment) had the lowest lactose content in wk 1, 2, and 3 with wk 1 being the lowest and wk 3 the highest). After wk 4, lactose content did not statistically increase through wk 12.

Differences in lactose yield were only found between the use of M (= 2.17 kg/d vs. 2.00 kg /d without M), parity (cows = 2.31 kg/d vs. H = 1.86 kg/d), and week in lactation (the first four weeks being the lowest and increasing weekly thereafter).

After calving, milk urea N decreased from wk 1 (13.5 mg/dL) to wk 2 (12.3 mg/dL) but increased from there on. Effects of diet and M supplementation were not significant. Differences for somatic cell count were only found within weeks in lactation. Week 1 had the highest count (391) followed by wk 12 (215), both statistically similar. Week 4 had the lowest SCC (52), which was not significantly different from wk 2 and 3, and 5 through 12.Other solids in the milk were affected by diet fed, the use of M, parity, and week in lactation. Overall, cows

(independent of parity) fed the CU diet or that ingested M during the DP had the highest other solids percentage. Multiparous cows had a lower percentage of other solids compared to primiparous cows. In addition, independent of treatments, other solids content increased from wk 1 to wk 3 and stabilized from then on.

The percentage of total solids in milk was affected by diet fed during the DP, week of lactation, and the interaction of diet, the inclusion of M, and week after calving. Cows, independent of parity, that were fed the CU diet had 2.9% more solids than cows that were fed the CE diet. Total solids (%) decreased significantly from wk 1 (14.65%) through wk 5 (11.90%). There were minimal differences from wk 5 through 12.

The yield of solids-corrected milk was increased by feeding M during the dry period, was lower for primiparous than for multiparous cows, and increased with week in lactation. The highest SCM yields resulted from using M (Figure 3) in multiparous cows

Blood and liver metabolites

Concentrations of NEFA and BHBA were measured in blood serum to assess energy status. During the far-off DP (Tables 12 and 13) neither BHBA nor NEFA concentrations were affected by diet or M supplementation. The concentration of NEFA was greater (P = 0.02) for primiparous heifers (0.654 ± 0.030 mM) than for multiparous cows (0.570 ± 0.022 mM; Figure 4), possibly reflecting the greater social adaptations required of the heifers as they were placed into groups with the older cows. In addition, an interaction of M x week (P = 0.046) showed that M supplementation decreased the NEFA concentration in the first week after dry-off (Figure 4). Parity and treatment effects were not significant for BHBA during the far-off DP (Tables 12 and 13; Figure 5).

The concentration of NEFA during the close-up period was greater for cows fed CE than for cows fed CU, but was not affected by M (Tables 12 and 13). Moderate increases in NEFA concentration prepartum have been observed previously in cows fed CE diets relative to those fed higher-energy diets (Richards et al., 2009; Janovick et al., 2011). Concentrations of NEFA were not different between parities, but increased as parturition approached (Figure 6). Neither diet nor M supplementation affected BHBA concentration during the close-up period (Tables 12 and 13). A tendency (P = 0.068) for parity x day interaction showed that BHBA concentrations increased more prepartum for multiparous cows than for primiparous heifers (Figure 7). However, interactions of parity and day with diet and M were not significant (Figure 8).

The concentration of NEFA during the first 21 d postpartum was lower for cows fed CE during the DP than for those fed CU (Tables 12 and 13), in agreement with previous studies (Richards et al., 2010; Janovick et al., 2011). Effects of parity and interactions of treatments with parity and day postpartum were not significant. Postpartal BHBA concentrations were not affected by diet or M supplementation (Tables 12 and 13), in contrast to previous studies with similar diets (Richards et al., 2009; Janovick et al., 2011) and M supplementation (Duffield et al., 2008a). However, overall concentrations were not particularly high, which might explain the difference among studies. Concentrations of BHBA were greater for multiparous cows than for primiparous cows and increased with day postpartum.

During the remainder of the lactation period, NEFA concentration was not affected by parity (Figure 9). A tendency for interaction of M and wk (Figure 9) showed that NEFA concentration was greater for R supplemented cows early after calving, possibly because of the greater milk production for those cows. Concentrations of BHBA tended ($P < 0.10$) to be greater in multiparous cows than in primiparous cows during wk 4 to 12.

Overall concentrations of NEFA were greater in this study than in our previous studies. The reason for this is not clear; whether differences reflect an analytical peculiarity or dietary adequacy cannot be determined. Nevertheless, relative differences between diets are consistent with previous data.

Concentrations of total lipid, TG, and glycogen in liver were not affected by diet or M supplementation (Tables 11 and 12). Total lipid and TG were increased postpartum, whereas glycogen was decreased.

CONCLUSIONS

Supplementation of M (22 g/ton) during the entire DP increased production of SCM, FCM, milk lactose, milk fat, and milk total solids, while tending to increase milk yield. This effect was consistent whether M was supplemented to a two-stage DP feeding strategy or to a single-group controlled-energy high-fiber (CE) diet. Whether the effect of M to increase yields of milk and milk solids should be interpreted as stimulation by supplementation or prevention of a decrease by removing M during the DP cannot be entirely elucidated from these data. Both multiparous and primiparous cows received M in the previous lactation or growing rations, respectively, and all cows received M postpartum. Thus, it may be that continued feeding of M during the DP prevented losses in milk and milk solids yields due to adaptation to M postpartum, rather than a stimulation of production due to M addition. Regardless, it appears that M should be supplemented throughout the DP and lactation to provide maximal productive benefit.

Feeding a one-group CE diet or a two stage DP feeding strategy, made little difference with respect to production or metabolic variables - with the exception of milk fat and lactose concentrations. The lower milk fat for cows fed one-group CE likely was attributable to less NEFA from adipose tissue being incorporated into milk fat.

Metabolic variables in blood and liver were not affected greatly by the inclusion of M or diet fed (Table 11), which was somewhat surprising. Perhaps this is attributable to the generally healthy and highly productive nature of cows on each dietary treatment.

CHAPTER III:

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CHAPTER IV: TABLES AND FIGURES

Table 1: Treatments used on the "Effects of close-up dietary energy strategy and prepartal dietary monensin (M) on production and metabolism in Holstein cows" Trial.

Treatment*	Description	M (g/ton)
1	single controlled-energy feeding program	0
2	single controlled-energy feeding program	22
3	two-stage DP feeding program	0
4	two-stage DP feeding program	22
-	Lactation diet	14

* After treatment, all cows were fed the same lactation diet.

Table 2. Ingredient composition of diets (% of DM)[1].

Ingredient	CE	CU	Lactation
Alfalfa silage	12.00	8.20	5.000
Corn silage	33.00	35.90	33.000
Alfalfa hay	0.00	3.50	5.000
Wheat straw	36.00	15.40	2.000
Cottonseed	0.00	0.00	3.600
Ground shelled corn	0.00	9.00	17.555
Soybean hulls	2.00	4.00	5.000
Soybean meal	7.94	5.50	4.600
Soyplus	0.00	2.00	5.900
SoyChlor 16-7	0.15	1.45	0.000
Wet brewers grains	0.00	6.00	10.000
Blood meal 85%	1.00	1.00	1.000
Biotin	0.00	0.35	0.350
Calcium sulfate	0.00	0.00	0.100
DCAD-Plus	0.00	0.00	0.400
Elanco experimental feed[2]	4.00	4.000	2.545
Energy Booster	0.00	0.00	0.900
Urea	0.45	0.30	0.150
Limestone	1.30	1.30	1.250
Dicalcium phosphate	0.12	0.18	0.250
Magnesium oxide	0.21	0.20	0.100
Sodium bicarbonate	0.00	0.00	0.750
Magnesium sulfate 7H20	0.91	0.78	0.000
UI Dairy mineral/vitamin premix[3]	0.20	0.22	0.200
Salt (plain)	0.32	0.30	0.350
Vitamin A[4]	0.015	0.015	0.000
Vitamin D[5]	0.025	0.025	0.000
Vitamin E premix[6]	0.38	0.38	0.000

[1] CE = controlled energy prepartum diet; CU = close-up prepartum diet; Lactation = diet fed postpartum.

[2] Contained 516 g/ton monensin in carrier of ground corn and mineral oil.

[3] Contained a minimum of 5.0% Mg, 10.0% S, 7.5% K, 2.0% Fe, 3.0% Zn, 3.0% Mn, 5,000 mg/kg Cu, 250 mg/kg I, 40 mg/kg Co, 150 mg/kg Se, 2,200,000 IU/kg Vitamin A, 660,000 IU/kg Vitamin D_3, and 22,000 IU/kg Vitamin E.

[4] Contained 30,000 kIU/kg.

[5] Contained 5,009 kIU/kg.

[6] Contained 44,000 IU/kg.

Table 3. Composition of total diets as formulated.

Component	CE	CU	Lactation
DM, %	51.02	46.49	45.14
NE_L [1], Mcal/kg	1.30	1.49	1.70
CP, % of DM	13.85	15.02	17.00
Metabolizable protein[1], g/d	1096	1080	---
ADF, % of DM	33.81	26.88	21.58
NDF, % of DM	47.35	39.44	33.31
NDF from forage, % of DM	44.95	31.74	20.36
Fat, % of DM	2.25	3.00	4.94
Ash, % of DM	9.09	8.58	8.08
NFC, % of DM	27.86	34.08	36.95
Ca, % of DM	0.97	0.99	0.89
P, % of DM	0.35	0.35	0.38
Mg, % of DM	0.50	0.50	0.28
K, % of DM	1.44	1.26	1.35
S, % of DM	0.35	0.35	0.22
Na, % of DM	0.14	0.14	0.36
Cl, % of DM	0.61	0.63	0.44
Fe, mg/kg	636	540	446
Zn, mg/kg	83	95	96
Cu, mg/kg	16	17	17
Mn, mg/kg	108	107	94
Co, mg/kg	0.2	0.2	0.2
I, mg/kg	0.60	0.6	0.5
Se, mg/kg	0.33	0.38	0
Vit A, 1000 IU/kg	14,880	15,932	10,541
Vit D, 1000 IU/kg	2,605	2,740	1,353
Vit E, IU/kg	133.2	219	49
Suppl Vit A, 1000 IU/kg	8,900	9,340	4,400
Suppl Vit D, 1000 IU/kg	2,572	2,704	1,320
Suppl Vit E, IU/kg	211	216	44
DCAD (mequiv/kg)	39	-11.5	244

[1] As calculated from NRC (2001) model, based on expected DMI.

Table 4. Analyzed composition of forages used in the experiment (mean ± SD).

Component	Alfalfa hay	Alfalfa silage	Corn silage	Wheat straw
DM, % as fed	83.01 ± 3.84	53.80 ± 10.47	31.98 ± 2.39	86.31 ± 2.87
TDN, % of DM	55.17 ± 1.70	54.75 ± 1.48	69.45 ± 0.40	47.13 ± 2.80
NE_L, Mcal/kg DM	1.23 ± 0.04	1.23 ± 0.03	1.59 ± 0.01	1.04 ± 0.06
CP, % of DM	19.00 ± 3.68	22.15 ± 2.10	7.18 ± 0.26	4.07 ± 0.45
Adjusted protein, % of DM	18.83 ± 3.94	21.05 ± 1.74	6.58 ± 0.19	3.17 ± 0.42
Soluble protein, % of CP	33.2 ± 3.80	49.25 ± 3.36	49.10 ± 6.11	47.13 ± 1.80
ADF protein, % of DM	1.84 ± 0.15	3.30 ± 0.75	1.31 ± 0.07	1.29 ± 0.12
NDF protein, % of DM	3.68 ± 1.69	6.08 ± 1.55	1.52 ± 0.16	1.41 ± 0.05
RDP, % of CP	66.60 ± 1.90	74.62 ± 1.71	74.58 ± 3.07	73.57 ± 0.85
NDF, % of DM	49.87 ± 9.54	43.92 ± 1.99	42.08 ± 0.82	81.03 ± 2.26
ADF, % of DM	39.33 ± 6.38	37.70 ± 1.23	26.68 ± 0.65	57.27 ± 0.84
Lignin, % of DM	9.25 ± 1.70	9.93 ± 0.65	3.77 ± 0.18	8.40 ± 1.61
Lignin, % of NDF	18.57 ± 0.40	22.60 ± 1.49	8.98 ± 0.26	10.37 ± 1.99
NFC, % of DM	23.67 ± 2.14	24.98 ± 2.31	45.30 ± 0.70	6.53 ± 0.84
Sugars, % of DM	5.00 ± 0.35	1.55 ± 0.49	1.05 ± 0.10	0.83 ± 0.40
Starch, % of DM	1.30 ± 0.10	1.28 ± 0.30	32.02 ± 0.66	1.20 ± 0.36
Crude fat, % of DM	1.73 ± 0.45	2.62 ± 0.29	2.68 ± 0.10	0.87 ± 0.38
Ash, % of DM	9.43 ± 1.99	12.45 ± 2.38	4.30 ± 0.24	8.90 ± 1.02
Ca, % of DM	1.12 ± 0.21	1.66 ± 0.05	0.23 ± 0.02	0.38 ± 0.31
P, % of DM	0.31 ± 0.06	0.35 ± 0.03	0.23 ± 0.02	0.08 ± 0.006
Mg, % of DM	0.21 ± 0.06	0.33 ± 0.03	0.17 ± 0.02	0.19 ± 0.11
K, % of DM	3.19 ± 0.97	3.05 ± 0.86	1.02 ± 0.07	1.63 ± 0.08
S, % of DM	0.23 ± 0.07	0.31 ± 0.03	0.10 ± 0.008	0.06 ± 0.01
Na, % of DM	0.040 ± 0.015	0.050 ± 0.080	0.018 ± 0.009	0.012 ± 0.002
Cl, % of DM	0.44 ± 0.21	0.64 ± 0.06	0.17 ± 0.05	0.44 ± 0.10
Fe, mg/kg DM	145 ± 34	1218 ± 344	262 ± 324	184 ± 102
Mn, mg/kg DM	30 ± 7.6	60 ± 13.0	15 ± 4.6	62 ± 19.2
Zn, mg/kg DM	23 ± 3.0	31 ± 1.5	26 ± 3.4	14 ± 0
Cu, mg/kg DM	9.0 ± 1.0	12.5 ± 0.6	4.8 ± 1.0	3.3 ± 0.6

Table 5. Analyzed composition of byproduct ingredients and concentrate mixes used in the experiment (mean ± SD).

Component	Wet brewers grains	Whole cottonseed	CE concentrate	CE +R concentrate	CU concentrate	CU+R concentrate	Lactation concentrate
DM, % as fed	27.75 ± 1.80	85.00 ± 1.00	87.67 ± 0.60	87.87 ± 0.45	87.23 ± 0.92	87.00 ± 0.61	86.94 ± 1.26
TDN, % of DM	73.10 ± 6.59	91.60 ± 0.42	67.72 ± 0.57	68.68 ± 2.10	72.98 ± 1.80	72.42 ± 2.28	76.48 ±0.80
NE_L, Mcal/kg DM	1.71 ± 0.16	2.22 ± 0	1.54 ± 0.02	1.56v± 0.05	1.67 ± 0.05	1.66 ± 0.06	1.76 ± 0.03
CP, % of DM	31.17 ± 4.91	20.65 ± 0.07	36.98 ± 1.38	39.72 ± 1.06	25.1 ± 0.86	25.2 ± 1.01	21.64 ± 0.81
Adjusted protein, % of DM	28.43 ± 4.62	20.65 ± 0.07	36.98 ± 1.38	39.72 ± 1.06	25.1 ± 0.86	25.2 ± 1.01	21.64 ± 0.81
Soluble protein, % of CP	14.27 ± 4.60	24.15 ± 4.31	25.75 ± 2.62	29.80 ± 3.80	22.25 ± 1.51	23.28 ± 3.38	20.30 ± 2.60
ADF protein, % of DM	4.48 ± 0.41	1.58 ± 0.28	0.76 ± 0.11	0.80 ± 0.13	1.09 ± 0.17	1.09 ± 0.22	1.01 ± 0.25
NDF protein, % of DM	5.65 ± 0.19	1.89 ± 0.28	1.51 ± 0.81	1.33 ± 0.39	2.04 ± 0.59	2.15 ± 0.64	2.57 ± 0.82
NDF, % of DM	52.57 ± 2.27	43.10 ± 1.56	10.42 ± 1.66	12.18 ±0.83	16.40 ± 2.19	17.50 ± 1.90	16.46 ± 1.25
ADF, % of DM	27.47 ± 4.04	33.25 ± 2.33	6.78 ± 1.29	7.75 ± 0.95	9.52 ± 1.49	9.65 ± 1.83	7.94 ±1.06
Lignin, % of DM	6.38 ± 1.43	7.89 ± 0.37	0.90 ± 0.32	1.09 ± 0.22	1.23 ± 0.12	1.49 ± 0.17	1.36 ± 0.10
Lignin, % of NDF	12.10 ± 2.49	18.30 ± 1.56	8.52 ± 2.53	8.98 ± 1.78	7.55 ± 1.16	8.50 ± 0.29	8.30 ± 0.76
NFC, % of DM	8.53 ± 4.55	13.35 ±1.77	32.82 ± 1.45	30.55 ± 1.34	45.68 ±1.15	43.92 ± 1.54	51.00 ± 1.79
Sugars, % of DM	1.43 ± 0.15	6.60 ± 0.28	7.08 ± 0.45	7.35 ± 0.42	5.28 ± 0.13	4.82 ± 0.26	4.86 ± 0.29
Starch, % of DM	1.90 ± 0.90	2.15 ± 0.64	20.60 ± 2.33	19.00 ± 0.71	32.58 ± 0.28	31.22 ±1.91	36.22 ± 1.64
Crude fat, % of DM	8.83 ± 2.85	20.65 ± 0.35	1.65 ± 0.10	1.78 ± 0.10	2.50 ± 0.55	2.80 ± 0.73	3.42 ± 0.54
Ash, % of DM	4.60 ± 1.18	4.15 ± 0.07	19.62 ± 1.13	17.08 ± 2.04	12.38 ±1.31	12.70 ± 1.98	10.08 ± 0.48
Ca, % of DM	0.39 ± 0.07	0.18 ± 0.02	4.76 ± 0.50	4.09 ± 0.02	3.21 ± 0.40	3.26 ± 0.50	2.08 ± 0.24
P, % of DM	0.66 ± 0.18	0.66 ± 0.08	0.55 ± 0.04	0.55 ± 0.2	0.48 ± 0.01	0.46 ± 0.02	0.47 ± 0.04
Mg, % of DM	0.26 ± 0.06	0.41 ± 0.04	1.54 ± 0.05	1.45 ± 0.07	0.96 ± 0.07	1.04 ± 0.06	0.35 ± 0.03
K, % of DM	0.06 ± 0.02	1.34 ± 0.09	1.80 ± 0.19	1.76 ± 0.10	1.22 ± 0.08	1.40 ± 0.17	1.64 ± 0.08
S, % of DM	0.50 ± 0.08	0.27 ± 0.03	1.02 ± 0.06	0.95 ± 0.06	0.63 ± 0.05	0.63 ± 0.05	0.29 ± 0.01
Na, % of DM	0.026 ± 0.011	0.022 ± 0.006	0.841 ± 0.049	0.734 ± 0.034	0.630 ± 0.047	0.449 ± 0.038	0.900 ± 0.096
Cl, % of DM	0.03 ± 0.006	0.06 ± 0	1.36 ± 0.10	1.24 ± 0.04	1.15 ± 0.13	1.10 ± 0.07	0.60 ± 0.03
Fe, mg/kg DM	213 ± 21.6	57 ± 5.6	758 ±70.4	751 ± 91.1	525 ± 37.2	498 ± 26.0	428 ± 43.3
Mn, mg/kg DM	67 ± 17.9	17 ± 2.8	406 ± 46.5	378 ± 55.1	244 ± 18.0	238 ± 17.1	154 ± 19.4
Zn, mg/kg DM	127 ± 33.5	40 ± 9.2	427 ± 61.8	372 ± 43.4	257 ± 26.3	237 ± 15.4	189 ± 34.6
Cu, mg/kg DM	6.7 ± 3.8	8.5 ± 0.7	67.0 ± 3.6	67.5 ± 5.0	42.8 ± 7.2	43.2 ± 8.9	32.4 ± 3.9

Table 6. Analyzed composition of TMR in the experiment (mean ± SD).

Component	CE	CE +R	CU	CU+R	Lactation
DM, % as fed	46.31±	47.12 ± 2.33	46.49 ± 3.18	46.53 ± 2.67	47.26 ± 2.41
TDN, % of DM	56.28 ± 2.13	57.07 ± 2.33	61.76 ± 2.12	61.32 ±2.80	69.88 ± 2.24
NE_L, Mcal/kg DM	1.26 ± 0.06	1.28 ± 0.06	1.40 ± 0.06	1.39 ± 0.07	1.61 ± 0.06
NE_M, Mcal/kg DM	1.18 ± 0.07	1.21 ± 0..08	1.36 ± 0.07	1.35 ± 0.10	---
CP, % of DM	13.04 ± 1.06	13.47 ± 0.98	16.07 ± 1.02	16.04 ± 1.53	18.55 ± 0.73
Adjusted protein, % of DM	12.22 ± 1.16	12.69 ± 1.26	14.83 ± 1.34	15.36 ± 1.86	17.65 ± 1.15
Soluble protein, % of CP	36.45 ± 2.46	32.78 ± 2.14	29.02 ± 3.28	31.91 ± 1.23	21.02 ± 2.32
ADF protein, % of DM	1.62 ± 0.32	1.56 ± 0.27	2.23 ± 0.44	1.86 ± 0.45	2.21 ± 0.58
NDF protein, % of DM	2.66 ± 0.84	2.80 ± 0.61	3.65 ± 0.98	3.88 ± 0.90	4.46 ± 0.77
RDP, % of CP	68.22 ± 1.23	66.40 ± 1.07	64.54 ±1.64	65.97 ± 0.60	60.50 ± 1.15
NDF, % of DM	53.68 ± 3.38	52.61 ± 3.42	44.37 ± 3.39	44.72 ± 3.38	37.76 ± 2.92
ADF, % of DM	36.68 ± 2.34	35.87 ± 2.22	28.92 ± 1.74	29.32 ±2.44	22.52 ± 1.82
Lignin, % of DM	5.87 ± 0.62	5.50 ± 0.57	5.09 ± 0.49	5.27 ± 0.76	4.63 ± 0.70
Lignin, % of NDF	10.91 ± 0.75	10.44 ± 0.79	11.46 ± 0.60	11.73 ± 0.96	12.21 ± 1.05
NFC, % of DM	23.71 ± 2.14	24.28 ± 2.87	31.42 ±2.84	30.96 ± 3.09	36.17 ± 2.10
Sugars, % of DM	1.23 ± 0.40	1.64 ± 0.44	1.74 ± 0.34	1.70 ± 0.77	1.83 ± 0.44
Starch, % of DM	12.45 ± 1.44	13.68 ± 1.93	18.91 ± 1.79	18.72 ± 2.82	24.62 ± 1.50
Crude fat, % of DM	1.63 ± 0.26	1.67 ± 0.22	2.38 ± 0.48	2.45 ± 0.46	4.48 ± 0.42
Ash, % of DM	10.58 ± 1.39	10.76 ± 1.04	9.42 ± 0.73	9.68 ±1.14	7.51 ± 0.36
Ca, % of DM	1.36 ± 0.40	1.39 ± 0.22	1.52 ± 0.24	1.55 ± 0.32	1.22 ± 0.12
P, % of DM	0.26 ± 0.02	0.26 ± 0.02	0.34 ± 0.03	0.33 ± 0.04	0.41 ± 0.02
Mg, % of DM	0.58 ± 0.19	0.54 ± 0.09	0.55 ± 0.07	0.55 ± 0.10	0.30 ± 0.02
K, % of DM	1.62 ± 0.14	1.64 ± 0.16	1.50 ± 0.10	1.47 ± 0.14	1.55 ± 0.07
S, % of DM	0.27 ± 0.04	0.29 ± 0.04	0.31 ± 0.06	0.31 ± 0.07	0.24 ± 0.01
Na, % of DM	0.18 ± 0.03	0.19 ± 0.03	0.18 ± 0.04	0.17 ± 0.04	0.39 ± 0.03
Cl, % of DM	0.54 ± 0.09	0.55 ± 0.09	0.61 ± 0.12	0.60 ± 0.11	0.40 ± 0.02
Fe, mg/kg DM	752 ± 152.3	768 ± 182.2	719 ± 115.9	945 ± 646.6	419 ± 42.6
Mn, mg/kg DM	123 ± 9.2	117 ± 13.8	124 ± 17.0	120 ± 19.8	101 ± 8.6
Zn, mg/kg DM	130 ± 63.7	102 ± 16.7	128 ± 23.6	122 ± 27.6	115 ± 10.5
Cu, mg/kg DM	18 ± 2.0	18 ± 2.2	20 ± 2.2	19 ± 4.2	21 ± 1.6

Table 7. Main effects of prepartum diet and monensin (M) supplementation on prepartum DMI, NE_L intake, BW, and BCS for cows in the lactation experiment.

	Diet			M			*P*		
Period and Variable	CE	CU	SE	0g/ton	22g/ton	SE	Diet	R	Diet x R
Far-off period									
DMI, kg/d	9.1	9.4	0.21	9.4	9.1	0.21	0.21	0.29	0.12
NE_L intake, Mcal/d	11.55	12.00	0.26	11.89	11.66	0.26	0.22	0.54	0.12
BW, kg	719	705	12.8	706	718	12.8	0.43	0.49	0.38
BCS	3.34	3.34	0.04	3.33	3.34	0.04	0.98	0.80	0.67
Close-up period									
DMI, kg/d	8.7	10.4	0.29	9.9	9.2	0.29	<0.0001	0.14	0.99
NE_L intake, Mcal/d	11.29	14.87	0.41	13.48	12.68	0.41	<0.0001	0.17	0.84
BW, kg	728	729	12.7	719	738	12.7	0.93	0.31	0.24
BCS	3.32	3.41	0.04	3.36	3.37	0.04	0.18	0.99	0.60

Table 8. Main effects of prepartum diet and monensin (M) supplementation on calving data for cows in the lactation experiment.

	Diet			M			*P*		
Variable	CE	CU	SE	0g/ton	22g/ton	SE	Diet	R	Diet x R
BW, kg	665	652	11.8	668	649	11.8	0.42	0.25	0.69
Calf BW, kg	42.7	45.1	1.0	43.8	44.1	0.9	0.090	0.82	0.68
Calving difficulty score	1.31	1.51	0.12	1.24	1.58	0.12	0.25	0.039	0.98
First milking colostrum, kg	5.51	6.12	0.52	5.70	5.94	0.52	0.41	0.75	0.51
Colostral IgG, g/L	76.1	63.1	7.2	76.4	62.8	7.2	0.21	0.19	0.74
Total IgG secreted in first colostrum, g	390	373	43	421	342	43	0.79	0.20	0.72

Table 9. Postpartum DMI, NE_L intake, BW, BCS, and efficiencies for cows fed prepartum diets without or with monensin (M) in the lactation experiment.

	Prepartum diet					*P*		
Variable	CE	CE+M	CU	CU+M	SE	Diet	M	Diet x R
DMI, kg/d	19.0	19.0	18.2	19.2	0.65	0.64	0.40	0.41
NE_L intake, Mcal/d	30.6	30.6	29.3	31.0	1.05	0.65	0.40	0.42
BW, kg	598	593	575	590	14.8	0.37	0.70	0.50
BCS	3.09	3.09	3.05	3.11	0.04	0.74	0.50	0.54

Table 10. Milk yield and milk components for cows fed prepartum diets without or with monensin (M) in the lactation experiment.

	Prepartum diet					*P*		
Variable	CE	CE+M	CU	CU+M	SE	Diet	M	Diet x R
Milk, kg/d	41.4	44.7	40.5	42.4	1.4	0.24	0.064	0.61
Peak milk, kg/d	48.6	51.3	48.0	48.9	1.6	0.38	0.25	0.55
Fat, %	3.51	3.56	3.77	3.75	0.10	0.026	0.86	0.69
True protein, %	2.75	2.70	2.82	2.75	0.06	0.30	0.34	0.86
Lactose, %	4.82	4.92	4.90	4.96	0.028	0.042	0.006	0.56
Total solids, %	11.98	12.07	12.38	12.25	0.13	0.010	0.81	0.61
Fat, kg	1.41	1.55	1.47	1.57	0.048	0.39	0.011	0.59
True protein, kg/d	1.12	1.19	1.12	1.17	0.048	0.80	0.22	0.80
Lactose, kg/d	2.01	2.21	1.99	2.12	0.068	0.45	0.021	0.60
Total solids, kg/d	4.91	5.35	4.96	5.24	0.169	0.84	0.028	0.63
SCC (x 1000)	145	151	102	171	77	0.87	0.62	0.68
Milk urea N, mg/dL	13.9	14.1	13.8	14.1	0.25	0.90	0.30	0.96
Solids-corrected milk[1], kg/d	36.5	40.0	37.2	39.5	1.2	0.91	0.018	0.61
3.5% Fat-corrected milk[2], kg/d	40.8	44.6	41.4	43.9	1.2	0.99	0.013	0.59
4.0% Fat-corrected milk[3], kg/d	37.7	41.2	38.3	40.6	1.2	0.99	0.013	0.59

[1] SCM = Milk (kg) * ((12.24*fat %*0.01) + (7.10*protein % *0.01) + (6.35 *lactose % * 0.01) − 0.0345).

[2] 3.5% FCM = Milk (kg) * 0.4324 + Milk fat (kg) * 16.216.

[3] 4.0% FCM = Milk (kg) * (0.4 + 0.15 * Milk fat %).

Table 11. Main effects of prepartum diet and monensin (M) supplementation on composition of liver tissue from cows in the lactation experiment.

	Diet			M			*P*			
Variable	CE	CU	SE	0g/ton	22g/ton	SE	Diet	M	Diet x R	Day
Total lipid, % wet wt.										
d −10	4.59	4.16	1.00	4.67	4.08	1.00	0.42	0.55	0.89	<0.0001
d 7	11.63	10.29	1.19	10.00	11.92	1.16				
Triacylglycerol, % wet wt.										
d −10	1.15	0.47	0.43	0.99	0.63	0.43	0.33	0.91	0.73	<0.0001
d 7	5.95	5.66	0.52	5.56	6.05	0.56				
Glycogen, % wet wt.										
d −10	2.88	3.19	0.44	2.96	3.12	0.44	0.40	0.83	0.22	0.0005
d 7	0.76	1.28	0.49	0.99	1.05	0.49				

Table 12. Main effects of prepartum diet and monensin (M) supplementation on concentrations of BHBA and NEFA in blood from cows in the lactation experiment.

	Diet			M			*P*		
Period and Variable	CE	CU	SE	0g/ton	22g/ton	SE	Diet	M	Diet x R
Far-off period									
BHBA, m*M*	0.438	0.427	0.026	0.435	0.434	0.026	0.78	0.90	0.72
NEFA, m*M*	0.606	0.618	0.026	0.620	0.604	0.026	0.73	0.65	0.93
Close-up period									
BHBA, m*M*	0.526	0.486	0.051	0.554	0.493	0.051	0.29	0.41	0.69
NEFA, m*M*	0.678	0.544	0.033	0.594	0.625	0.033	0.005	0.51	0.50
Postpartum, d 1 to 21									
BHBA, m*M*	0.809	0.849	0.066	0.820	0.839	0.066	0.67	0.83	0.34
NEFA, m*M*	0.823	0.905	0.029	0.840	0.889	0.029	0.05	0.24	0.17
Postpartum, d 22 to 84									
BHBA, m*M*	0.580	0.624	0.045	0.565	0.639	0.045	0.50	0.25	0.23
NEFA, m*M*	0.511	0.514	0.023	0.489	0.536	0.023	0.91	0.15	0.75

Table 13. Concentrations of BHBA and NEFA in blood for cows fed prepartum diets without or with monensin (M) in the lactation experiment.

	Prepartum diet					*P*		
Period and Variable	CE	CE +M	CU	CU+M	SE	Diet	M	Diet x R
Far-off period								
BHBA, m*M*	0.433	0.442	0.436	0.418	0.038	0.78	0.90	0.72
NEFA, m*M*	0.596	0.616	0.625	0.611	0.038	0.73	0.65	0.93
Close-up period								
BHBA, m*M*	0.578	0.547	0.530	0.441	0.072	0.29	0.41	0.69
NEFA, m*M*	0.644	0.707	0.544	0.543	0.047	0.005	0.51	0.50
Postpartum, d 1 to 21								
BHBA, m*M*	0.754	0.864	0.885	0.814	0.095	0.66	0.83	0.34
NEFA, m*M*	0.769	0.877	0.910	0.901	0.043	0.050	0.24	0.17
Postpartum, d 22 to 84								
BHBA, m*M*	0.504	0.655	0.625	0.622	0.066	0.50	0.25	0.23
NEFA, m*M*	0.482	0.539	0.496	0.533	0.033	0.91	0.15	0.75

Figure 1. Mean daily yield of 3.5% fat-corrected milk (FCM) by week for multiparous (solid symbols) and primiparous (open symbols) cows. **A.** Main effect of diet for cows fed CE (circles) or CU (squares) diets. Effects in model: diet ($P = 0.99$), parity ($P < 0.0001$), diet x parity ($P = 0.16$), week ($P < 0.0001$), diet x week ($P = 0.94$), diet x parity x week ($P = 0.99$). Average SEM = 1.6 kg/d for multiparous, 2.1 kg/d for primiparous cows. **B.** Main effect of monensin (M) for cows not supplemented (upward triangles) or supplemented with M (downward triangles). Effects in model: M ($P = 0.013$), parity ($P < 0.0001$), M x parity ($P = 0.42$), week ($P < 0.0001$), M x week ($P = 0.42$), M x parity x week ($P = 0.44$). Average SEM = 1.6 kg/d for multiparous, 2.1 kg/d for primiparous cows.

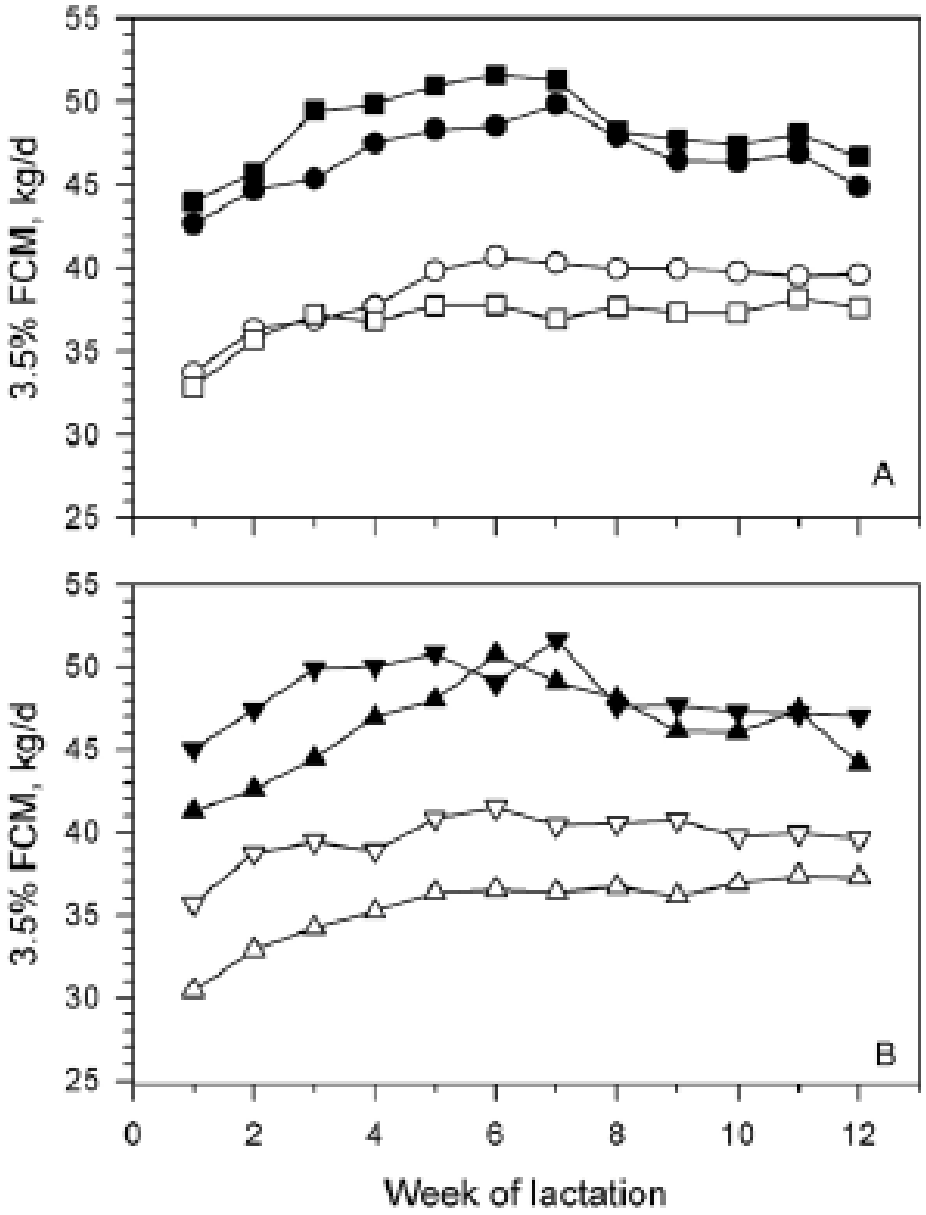

Figure 2. Mean daily yield of 4.0% fat-corrected milk (FCM) by week for multiparous (solid symbols) and primiparous (open symbols) cows. **A.** Main effect of diet for cows fed CE (circles) or CU (squares) diets. Effects in model: diet ($P = 0.99$), parity ($P < 0.0001$), diet x parity ($P = 0.16$), week ($P < 0.0001$), diet x week ($P = 0.94$), diet x parity x week ($P = 0.99$). Average SEM = 1.5 kg/d for multiparous, 1.9 kg/d for primiparous cows. **B.** Main effect of monensin (M) for cows not supplemented (upward triangles) or supplemented with M (downward triangles). Effects in model: M ($P = 0.013$), parity ($P < 0.0001$), M x parity ($P = 0.42$), week ($P < 0.0001$), M x week ($P = 0.42$), M x parity x week ($P = 0.44$). Average SEM = 1.5 kg/d for multiparous, 1.9 kg/d for primiparous cows.

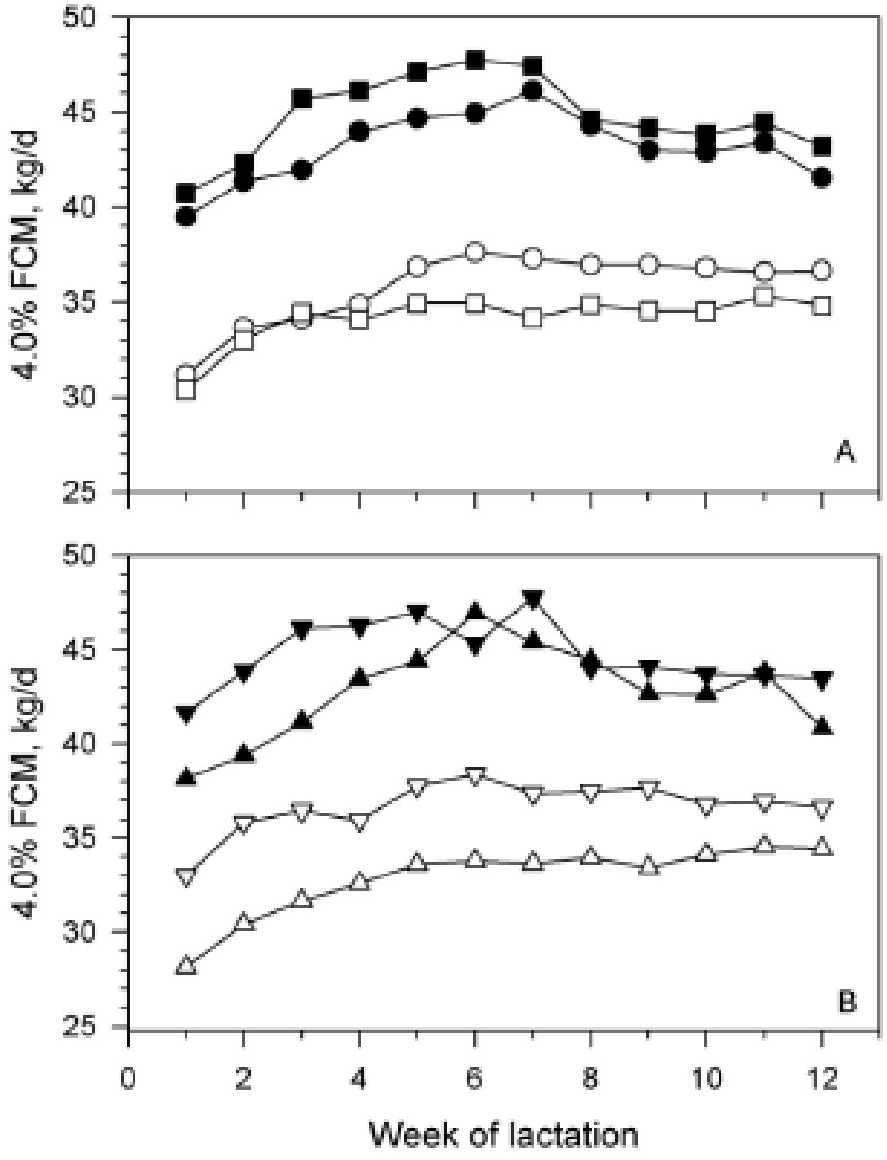

Figure 3. Mean daily yield of solids-corrected milk (SCM) by week for multiparous (solid symbols) and primiparous (open symbols) cows. **A.** Main effect of diet for cows fed CE (circles) or CU (squares) diets. Effects in model: diet ($P = 0.91$), parity ($P < 0.0001$), diet x parity ($P = 0.24$), week ($P < 0.0001$), diet x week ($P = 0.97$), diet x parity x week ($P = 0.99$). Average SEM = 1.4 kg/d for multiparous, 1.9 kg/d for primiparous cows. **B.** Main effect of monensin (M) for cows not supplemented (upward triangles) or supplemented with M (downward triangles). Effects in model: M ($P = 0.018$), parity ($P < 0.0001$), M x parity ($P = 0.50$), week ($P < 0.0001$), M x week ($P = 0.38$), M x parity x week ($P = 0.39$). Average SEM = 1.4 kg/d for multiparous, 1.9 kg/d for primiparous cows.

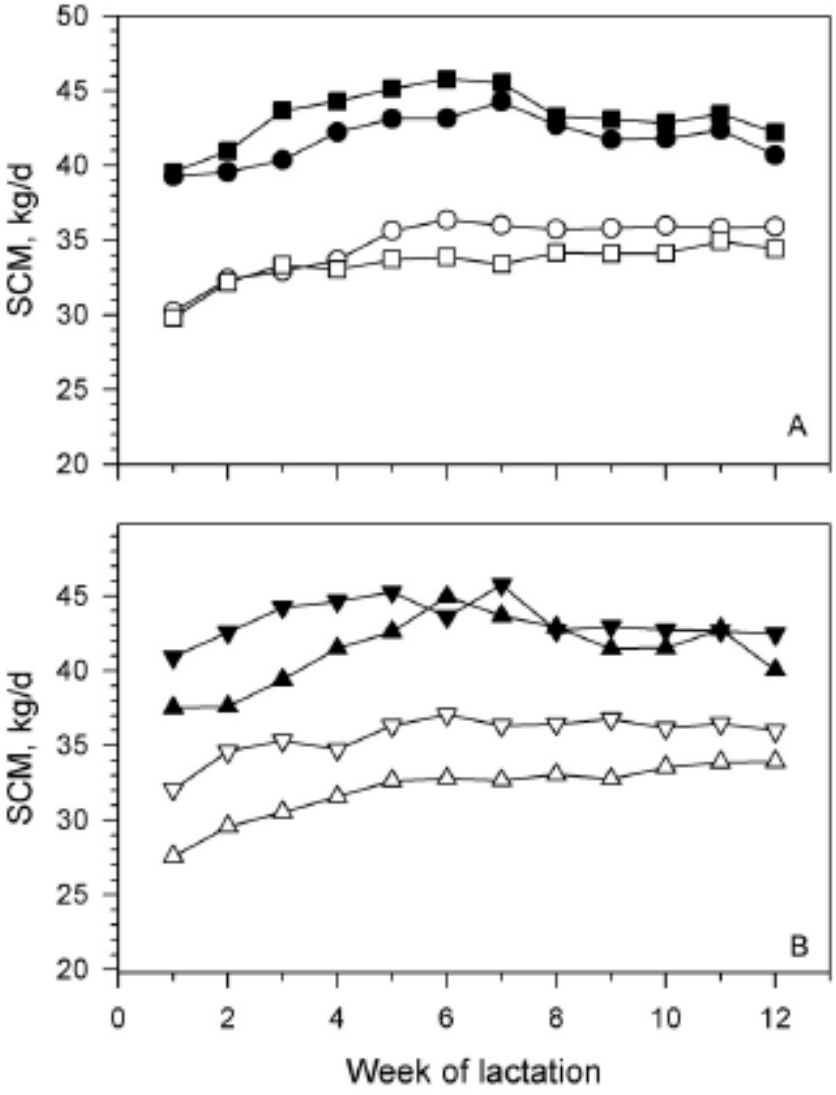

Figure 4. Mean weekly concentrations of NEFA in serum for multiparous (solid symbols and lines) and primiparous (open symbols and dotted lines) cows during the far-off DP. **A.** Main effect of diet for cows to be fed CE (circles) or CU (squares) diets in the close-up period. Effects in model: diet (*P* = 0.73), parity (*P* = 0.028), diet x parity (*P* = 0.70), week (*P* < 0.0001), diet x week (*P* = 0.24), diet x parity x week (*P* = 0.35). Average SEM = 0.05 m*M* for multiparous, 0.07 m*M* for primiparous cows. **B.** Main effect of monensin (M) for cows fed diets not supplemented (upward triangles) or supplemented with M (downward triangles) during the far-off DP. Effects in model: M (*P* = 0.65), parity (*P* = 0.028), M x parity (*P* = 0.21), week (*P* < 0.0001), M x week (*P* = 0.046), M x parity x week (*P* = 0.61). Average SEM = 0.05 m*M* for multiparous, 0.07 m*M* for primiparous cows.

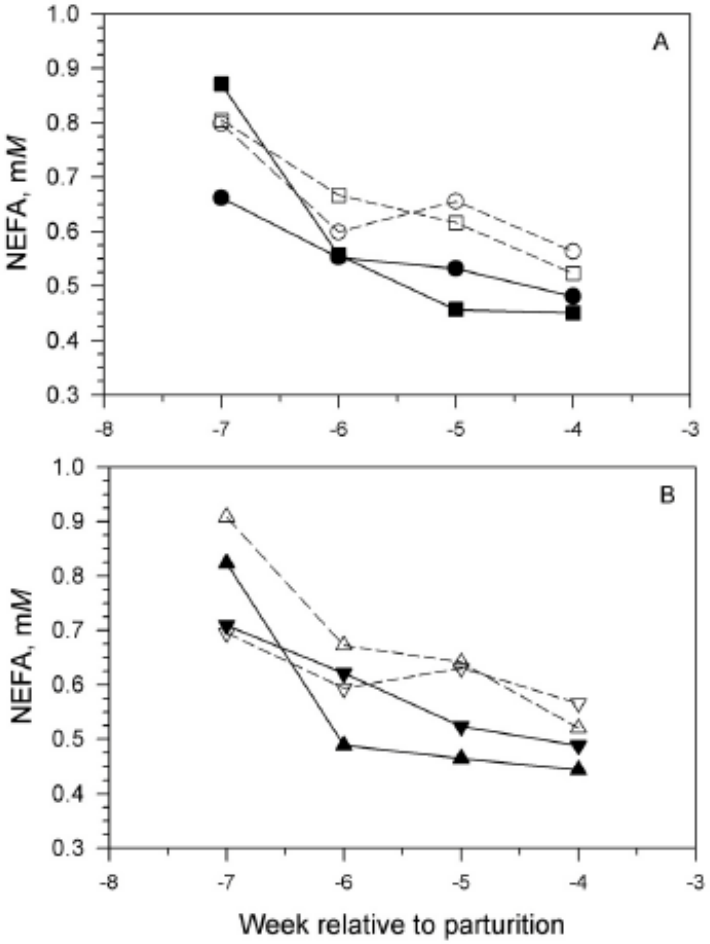

Figure 5. Mean weekly concentrations of BHBA in serum for multiparous (solid symbols and lines) and primiparous (open symbols and dotted lines) cows during the far-off DP. **A.** Main effect of diet for cows to be fed CE (circles) or CU (squares) diets in the close-up period. Effects in model: diet (*P* = 0.78), parity (*P* = 0.31), diet x parity (*P* = 0.33), week (*P* = 0.76), diet x week (*P* = 0.23), diet x parity x week (*P* = 0.20). Average SEM = 0.04 m*M* for multiparous, 0.06 m*M* for primiparous cows. **B.** Main effect of monensin (M) for cows fed diets not supplemented (upward triangles) or supplemented with M (downward triangles) during the far-off DP. Effects in model: M (*P* = 0.90), parity (*P* = 0.31), M x parity (*P* = 0.31), week (*P* = 0.76), M x week (*P* = 0.40), M x parity x week (*P* = 0.33). Average SEM = 0.04 m*M* for multiparous, 0.06 m*M* for primiparous cows.

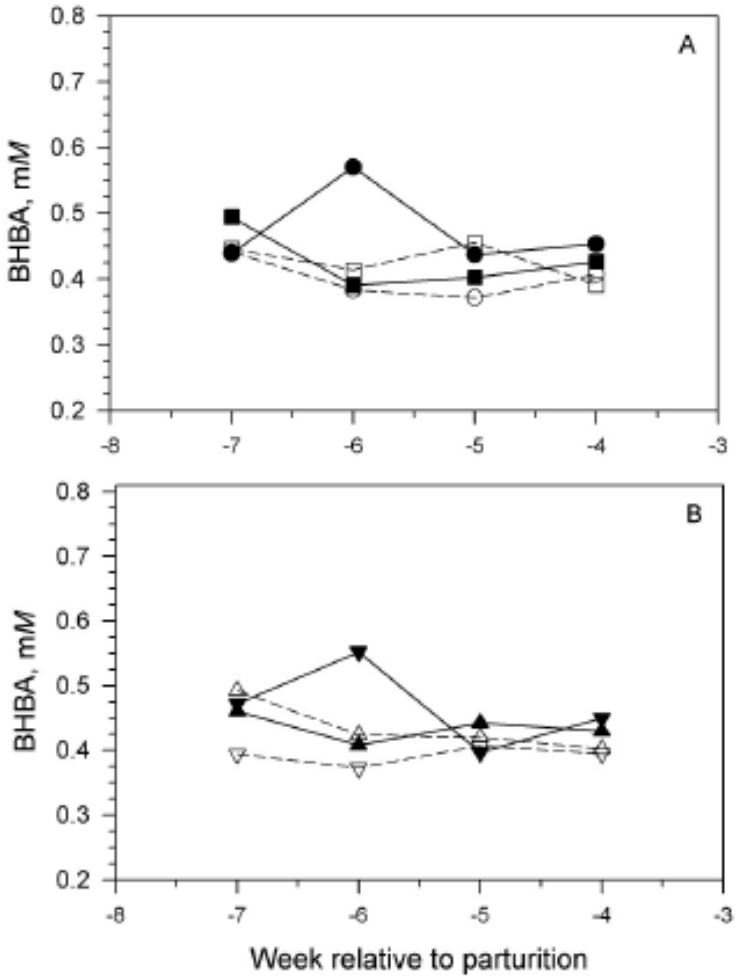

Figure 6. Mean daily concentrations of NEFA in serum for multiparous (solid symbols and lines) and primiparous (open symbols and dotted lines) cows during the close-up DP. **A.** Main effect of diet for fed CE (circles) or CU (squares) diets. Effects in model: diet ($P = 0.005$), parity ($P = 0.24$), diet x parity ($P = 0.32$), day ($P < 0.0001$), diet x day ($P = 0.31$), diet x parity x day ($P = 0.80$). Average SEM = 0.05 m*M* for multiparous, 0.07 m*M* for primiparous cows. **B.** Main effect of monensin (M) for cows fed diets not supplemented (upward triangles) or supplemented with M (downward triangles). Effects in model: M ($P = 0.51$), parity ($P = 0.24$), M x parity ($P = 0.76$), day ($P < 0.0001$), M x day ($P = 0.81$), M x parity x day ($P = 0.90$). Average SEM = 0.05 m*M* for multiparous, 0.07 m*M* for primiparous cows.

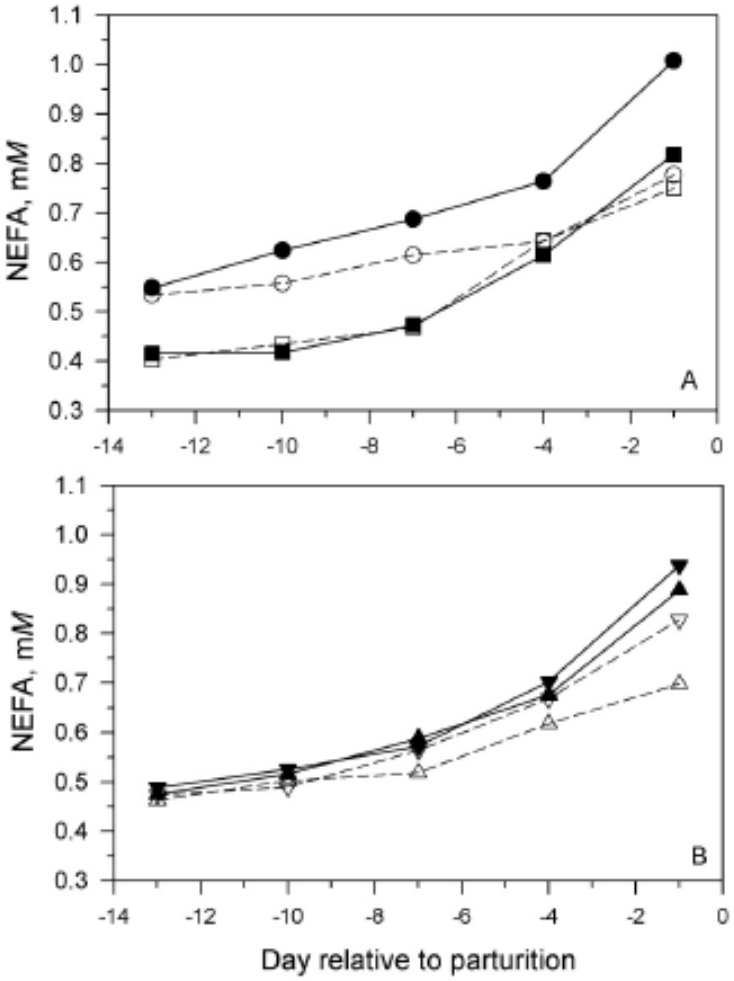

Figure 7. Mean daily concentrations of BHBA in serum for multiparous (circles) and primiparous (squares) cows during the close-up DP. Effects in model: parity ($P = 0.019$), day ($P < 0.0001$), parity x day ($P = 0.068$). Average SEM = 0.05 m*M* for multiparous, 0.07 m*M* for primiparous cows.

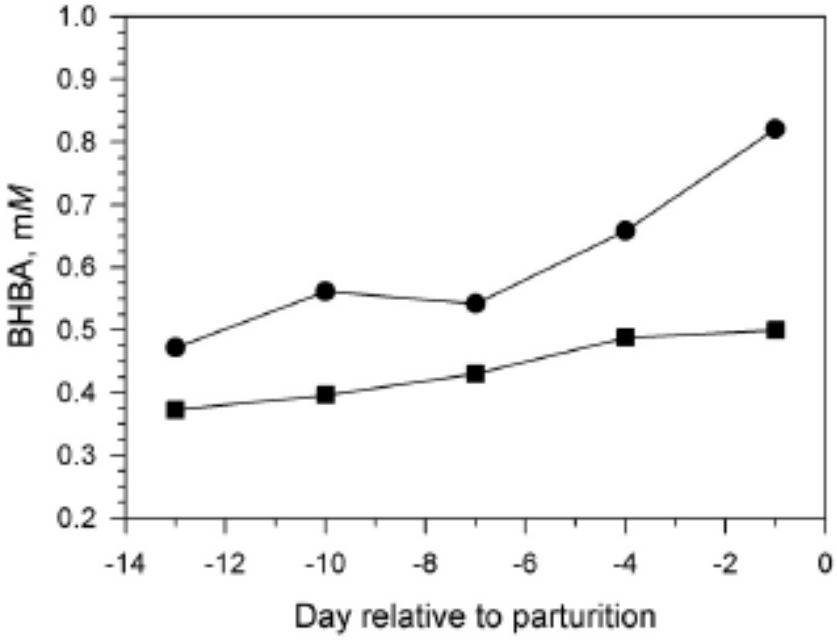

Figure 8. Mean daily concentrations of BHBA in serum for multiparous (solid symbols and lines) and primiparous (open symbols and dotted lines) cows during the close-up DP. **A.** Main effect of diet for fed CE (circles) or CU (squares) diets. Effects in model: diet ($P = 0.29$), parity ($P = 0.019$), diet x parity ($P = 0.39$), day ($P < 0.0001$), diet x day ($P = 0.81$), diet x parity x day ($P = 0.85$). Average SEM = 0.07 m*M* for multiparous, 0.10 m*M* for primiparous cows. **B.** Main effect of monensin (M) for cows fed diets not supplemented (upward triangles) or supplemented with M (downward triangles). Effects in model: M ($P = 0.41$), parity ($P = 0.019$), M x parity ($P = 0.71$), day ($P < 0.0001$), M x day ($P = 0.97$), M x parity x day ($P = 0.85$). Average SEM = 0.07 m*M* for multiparous, 0.10 m*M* for primiparous cows.

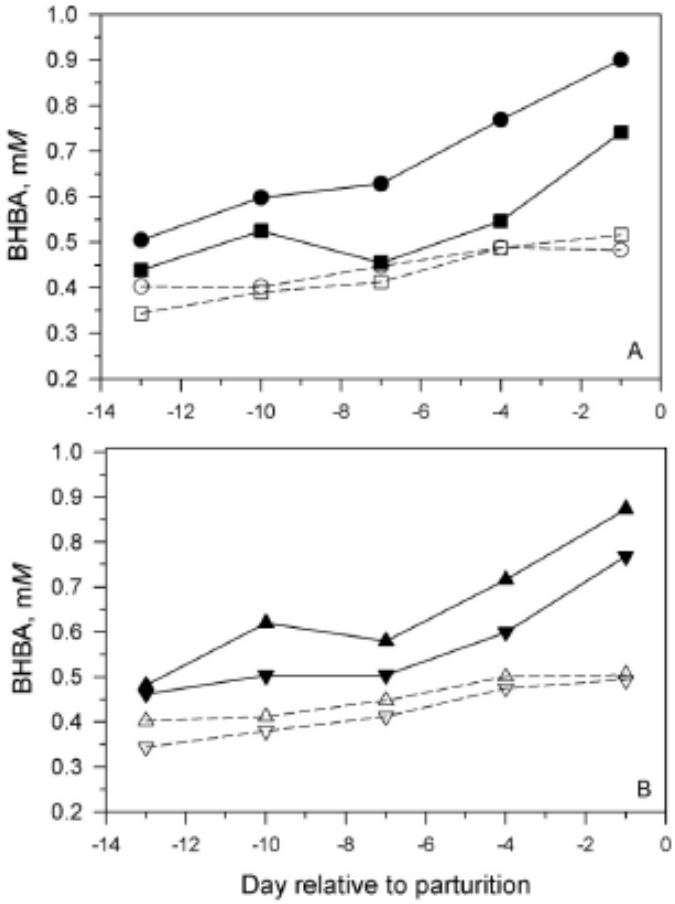

Figure 9. Mean weekly concentrations of NEFA in serum for multiparous (solid symbols and lines) and primiparous (open symbols and dotted lines) cows during the lactation period. **A.** Main effect of diet for fed CE (circles) or CU (squares) diets. Effects in model: diet ($P = 0.91$), parity ($P = 0.25$), diet x parity ($P = 0.72$), week ($P < 0.0001$), diet x week ($P = 0.96$), diet x parity x week ($P = 0.79$). Average SEM = 0.05 m*M* for multiparous, 0.07 m*M* for primiparous cows. **B.** Main effect of monensin (M) for cows fed diets not supplemented (upward triangles) or supplemented with M (downward triangles). Effects in model: M ($P = 0.15$), parity ($P = 0.25$), M x parity ($P = 0.75$), week ($P < 0.0001$), M x week ($P = 0.078$), M x parity x week ($P = 0.34$). Average SEM = 0.05 m*M* for multiparous, 0.07 m*M* for primiparous cows.