FEEDING BEHAVIOR AND METABOLISM OF TRANSITION DAIRY COWS SUPPLEMENTED WITH MONENSIN

by

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B. S., University of Nebraska – Lincoln, 2007
M. S., Kansas State University, 2009

AN ABSTRACT OF A DISSERTATION

submitted in partial fulfillment of the requirements for the degree

DOCTOR OF PHILOSOPHY

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Abstract

The mechanisms behind the metabolic changes observed when transition cows are administered monensin, as well as the effects of supplementing mid-lactation cows with two commercial amino acid products were investigated. Traditionally, the effects of monensin are attributed to increased gluconeogenic precursor supply, but recent research indicated that the effects of monensin extend beyond gluconeogenic flux. Thus, the primary objectives of Experiment 1 were to determine if monensin modulates transition cow feeding behavior, ruminal pH, and/or expression of key metabolic genes. Overall, monensin decreased time between meals prepartum (126 vs. 143 ± 5.0 min; $P < 0.03$) with a trend appearing postpartum (81.4 vs. 88.8 ± 2.9 min; $P < 0.08$), which could be related to the smaller ruminal pH standard deviation during the first day cows received the lactation ration (0.31 vs. 0.26 ± 0.015; $P < 0.02$). Monensin also increased liver mRNA abundance of carnitine palmitoyltransferase 1a (0.15 vs. 0.10 ± 0.002 arbitrary units; $P < 0.04$), which corresponded to a slower rate of liver triglyceride (TG) accumulation from 7 days before calving through 7 days post calving (412 vs. 128 ± 83 mg TG/g protein over this time period; $P = 0.03$). No significant effects of monensin supplementation were observed on other metabolic parameters or milk production. Overall, these results confirm that the effects of monensin on transition cows extend beyond altered propionate flux. In Experiment 2, mid-lactation cows consuming a control diet containing 26% wet corn gluten feed (dry matter basis) were compared to cows consuming the same diet supplemented with lysine embedded within Ca salts of fatty acids and the isopropyl ester of 2-hydroxy-4-(methylthio) butanoic acid, a methionine precursor. This trial was conducted because the NRC (2001) model indicated a lysine deficiency prior to supplementation; however amino acid supplementation had no effects. This trial was then extended to decrease dietary CP from 17.9% to 17.1%, and further increase lysine and methionine supply in the treatment diet. No production or intake effects were observed during this period, but MUN was decreased in the treated group (10.8 vs. 12.5 ± 0.2 mg/dL; $P < 0.001$).

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Preface - Importance of Research Focusing on the Transition Period

The 21 days before and after calving has become an accepted definition for the transition period in dairy cattle nutrition. During this period many physiological changes occur, predisposing the dairy cow to an array of disorders. Field surveys indicate that more than 50% of cows will experience some degree of at least one metabolic or infectious disease post-calving (Van Saun, 2010). Metabolic disorders can severely harm cow performance. Peak milk will be lower, reducing the cow's production over that entire lactation. Some diseases make rebreeding challenging, which reduces pregnancy rate. Cows experiencing disorders are at increased risk for culling, which can squander a producer's investment in breeding, vaccinations, and a dry period. The economic impact of transition disorders, combined with animal welfare concerns, make it a necessity to improve transition cow nutrition programs and management strategies.

The most common disorders associated with the transition period are ketosis, milk fever, displaced abomasum, retained fetal membranes, metritis, and mastitis. The etiology of each disease differs slightly; still, epidemiological studies show a strong relationship between each disease and the risk for contracting other diseases. This puts an enormous emphasis on prevention. Table 1.1 briefly summarizes the known causative factors, effects, treatment, and preventative measures for these common diseases. Although this table mentions the direct causes, most of the time the cause of a transition disorder in any given cow is multi-factorial (Duffield, 2011). Therefore, conducting research focused on alleviating transition-related disorders requires systematic knowledge of transition cow metabolism. The last 30 years have brought noteworthy progress to this field. This 2 chapter literature review will provide an overview of transition cow metabolism and present available data on the effects of monensin on the metabolic parameters of transition dairy cows.

Chapter 1 - Literature Review: The Transition Dairy Cow

Transition Cow Metabolism

Feed Intake

Dry matter intake can fluctuate dramatically during the transition period. Decreases in dry matter intake (DMI) have been well documented as parturition approaches (Grummer, 1995; Ingvartsen and Andersen, 2000; NRC, 2001), conversely, nutrient demands for fetal growth and initiation of lactation increase. Some of the earliest researchers in this area reported that DMI decreased 28% during the final 17 d prior to calving (Bertics et al., 1992). A more recent report including data from 699 Holsteins, offered 1 of 49 diets ad libitum, showed DMI decreased 32.2% on average in the final 3 wk of gestation (Hayirli et al., 2002). The magnitude of the depression observed varied depending on cow BCS and dietary RUP, NDF, and EE concentrations.

Cows that experience greater declines in DMI around parturition appear to experience greater incidence of periparturient disease (Mashek and Grummer, 2003). Zamet et al. (1979) were one of the earliest groups to examine the relationship between voluntary intake and periparturient abnormalities. Data obtained from their 89 transition cows showed that animals diagnosed with some type of abnormality consumed 18% less feed between 1 d prepartum and 3 d postpartum; then from d 4 to 30 postpartum, intake of these cows was 20% lower compared to healthy cows. Other scientific investigators have reported a similar relationship between intake and disease incidence (Lean et al., 1994; Hammon et al., 2006; Huzzey et al., 2007). With this data alone it is difficult to differentiate cause from effect, but it is known that early lactation cows consuming less feed will experience a more dramatic negative energy balance (Grummer and Rastani, 2003).

Energetic status can play a key role in immunity, influencing susceptibility to a host of disorders (LeBlanc, 2010); therefore, improving energy status should be a central focus of transition cow programs. Data reviewed by Grummer and Rastani (2003) indicate that feed energy intake has the greatest influence on energy status when compared to milk production variables, which contrasts with implications of the NRC model (2001). Thus researchers should pursue all avenues that could improve feed energy consumption, without compromising rumen health.

Cows that are over-conditioned appear to experience greater intake depression during transition (Garnsworthy and Topps, 1982; Roseler et al., 1997; Hayirli et al., 2002). This can be explained by the hepatic oxidation theory (Allen et al., 2009). In brief, the hepatic oxidation theory proposes that intake regulation in ruminants is based on hepatic energy status. When fuels such as propionate or NEFA are delivered to the liver, signals sent to the brain via the vagus nerve decrease, causing satiety. During periods of negative energy balance, the mobilization of energy reserves causes the release of NEFA from adipose tissue. This elevates the circulating plasma NEFA concentration and uptake of NEFA by the liver is directly related to plasma concentration (Bell, 1980; Pethick et al., 1984). During such a scenario, hunger is likely suppressed from elevated liver energy status driven by increased fatty acid oxidation (Allen et al., 2009). Because excessively conditioned cows are predisposed to mobilize greater quantities of adipose tissue (Garnsworthy and Topps, 1982), they generally have greater circulating NEFA concentrations (Drackley, 1999) that will induce satiety, escalating the level of intake depression experienced.

An array of transition cow feed intake data was not discussed. Because of the multifactorial nature of transition disorders, a more complete assessment of intake data is outside

the scope of this literature review. Instead, other aspects of cow metabolism must be covered. The remaining sections of this chapter will provide a brief overview of the metabolic changes experienced during the dairy cow's transition period.

Gluconeogenesis

Prior to the initiation of lactation, circulating glucose is relatively stable and typically at a concentration of ≈3.5mM. Then at parturition, a transient increase is sometimes observed followed by an immediate decrease of up to 1 mM (Studer et al., 1993; Vazquez-Aon et al., 1994). This transient increase could be the result of increased glucagon and glucocorticoid concentrations causing glycogen depletion; Vazquez-Anon et al. (1994) reported 70% of glycogen was depleted prior to calving. It is also conceivable that the early spike in glucose is related to up-regulation of the gluconeogenic mechanisms needed to support lactation, because around calving researchers have observed a substantial increase in hepatic release of glucose simultaneous to increased hepatic removal of the glucogenic precursors propionate, lactate, and glycerol (Reynolds et al., 2003; Doepel et al., 2009).

Blood glucose concentrations decline rapidly during the first week postpartum, and concentrations generally level out between ≈2.5 to 3 mM (Studer et al., 1993; Vazquez-Aon et al., 1994; Stephenson et al., 1997; Reynolds et al., 2003). The reason blood glucose remains low for the first several days of lactation could be related to demands associated with lactogenesis. To put this in perspective, the mammary gland requires 2.7 times more glucose on d 4 of lactation than what was being supplied to the gravid uterus at 250 d of gestation (Bell, 1995). In addition to lactose synthesis, glucose is required to replenish the energy reserves such as glycogen. To further emphasize the quantity of glucose demanded by lactating dairy cows, the total glucose turnover for a high producing cow can exceed 3 kg/d (Bauman and Elliot, 1983).

To compensate, homeorhetic mechanisms increase glucose production and decrease glucose uptake by peripheral tissues (Bauman and Currie, 1980; Doepel et al., 2009). Still, glucose availability does not keep pace with the demands of the mammary gland. To compensate, body tissue is mobilized.

Tissue mobilization

Adipose tissue becomes a major source of energy for early lactation dairy cows (Andrew et al., 1995). The adipose tissue triglyceride is converted to glycerol and NEFA; NEFA are bound to the transport protein albumin in the blood. The liver receives some of these molecules and removes approximately 16–23% of NEFA (Pullen et al., 1989; Reynolds et al., 2003), but the albumin bound NEFA and glycerol can also provide energy to the mammary gland, spleen, and muscle. The NEFA taken up by the hepatocytes undergo beta-oxidation or become re-esterified to triglycerides and exported in very low density lipoproteins (Emery et al., 1992; Van den Top et al., 2005). These processes collectively have inadequate capacity to match the influx of fatty acids throughout early lactation, thus fat accumulates in the liver and can impact hepatic function, particularly gluconeogenic (Cadrniga-Valio et al., 1997) and ureagenic capabilities (Strang et al., 1998). To compensate, the liver increases peroxisomal oxidative capacity (Grum et al., 1996). Unfortunately, the first step of this pathway promotes free radical production causing oxidative stress. Such conditions are referred to as fatty liver syndrome (or hepatic lipidosis). Fatty liver indirectly promotes the array of metabolic disorders discussed in Table 1.1.

One of the most prevalent diseases resulting from mobilization of large amounts of adipose tissue is ketosis. This is because the liver's ability to completely oxidize fatty acids is exceeded by the supply of NEFA, resulting in ketone body production. Liver ketogenesis can drive plasma acetone concentrations to above 0.80 mM, and plasma BHBA concentrations above

3.5 mM (Reist et al., 2003), which can cause diverse effects that are discussed elsewhere in this review. An abundance of epidemiological studies have clearly demonstrated that cows experiencing ketosis are at a much greater risk for developing a second metabolic disorder (Goff, 2006).

Mobilization of adipose tissue alone does not eliminate the nutrient deficit faced in early lactation. Consequently, cows also catabolize muscle protein (Motyl and Barej, 1986; Plaizier et al., 2000). The magnitude of this proteolysis depends on diet, DMI, and level of production.

Researchers have reported losses ranging from 12 kg (Komaragiri et al., 1998) to 21 kg (Komaragiri and Erdman, 1997) between 14 d prepartum to 35 d postpartum, and a loss of 14 kg from 14 d prepartum to 38 d postpartum (Chibisa et al., 2008). The amino acids supplied from catabolism can be used to support hepatic gluconeogenesis (Bauman and Currie, 1980), but priority is given to milk protein production (Doepel et al., 2009).

One of the reasons body tissues are easily mobilized is related to insulin concentration and sensitivity of the animal. Even if insulin remains high, sensitivity is decreased around parturition (Petterson et al., 1993; Petterson et al., 1994) allowing nutrients to be directed towards the fetus or used to support lactation. This homeorhetic adaptation is employed by all mammals to some degree surrounding parturition (Bauman and Elliot, 1983) and also inhibits the body's ability to take up glucose within adipose and muscle tissue, sparing glucose for use by the mammary gland (Bell and Bauman, 1997). Insulin resistance is also related to the amount of adipose tissue present, and adipose tissue derived hormones and inflammatory cytokines (Ruan and Lodish, 2004).Thus, cows with excessive body condition will be less responsive to insulin, allowing for greater release of NEFA.

It is clear that dairy cows experience a range of metabolic adaptations as they transition into lactation. This makes understanding metabolic regulation of these animals complex, leaving scientific investigators ample opportunity to expand our knowledge base in order to improve farm management capabilities.

Improving Metabolic Adaptation during the Transition Period

Scientific investigators have directed a tremendous amount of effort toward uncovering details about metabolism and immune function during the transition period. The knowledge acquired has helped nutritionists devise strategies to mitigate the issues faced. This section will briefly summarize hallmarks of a successful transition program.

As mentioned previously in this review, the severity of negative energy balance experienced in early lactation is tightly linked to feed intake (Grummer and Rastani, 2003). This makes DMI a major determinant of transition cow success. Therefore, managers should formulate diets to contain optimal NDF concentrations to promote adequate DMI, without allowing cows to over-consume energy prepartum. Achieving this can be difficult, but Overton (2011) published recommendations to feed diets composed of 1.30 to 1.39 Mcal/kg of NEL during the far-off period, and 1.41 to 1.46 Mcal/kg of NEL during the close-up period. Allowing cows to consume diets of greater energy density puts cows at an increased risk for contracting some type of metabolic disease, but providing inadequate energy is linked to lower milk yield postpartum (Overton, 2011).

Proper diet formulation is a vital component, but it is not a panacea for transition cow DMI programs. Many times extraneous factors can limit DMI. Sometimes the diet is formulated correctly, but not offered in great enough quantities. This can be related to bunk space or feed push-up. During late gestation and early lactation, cows require greater bunk space compared to

what is required for late lactation animals (Cameron et al., 1998; Cook and Nordlund, 2004). An adequate supply of water is also necessary to ensure cows are consuming maximum amounts of dry matter (DM; NRC, 2001).

Body condition score also plays a pivotal role in the success of transition cows. Cows need to have enough body condition to compensate for the decrease in DMI, but they should not be over conditioned, as excessive condition can increase risk of metabolic disease (Dyk, 1995; Contreras et al., 2004). Dyk (1995) classified cows into one of three BCS classes: low (2.75 to 3.25), medium (3.25 to 4.00), and high (≥ 4.00), and reported ketosis incidence rates of 8.9, 11.5, and 15.7%, respectively. Garnsworthy (2010) suggests that optimal BCS at calving is 2.5 to 3.0 (1-5 scale). Targeting such a BCS will allow for herd variation and a reasonable safety margin; this will provide cows enough energy reserves so that if they lose 0.4 to 0.9 units of BCS they will remain above dangerously low thresholds, yet it keeps them below the problematic 4.0 range.

Stress can also have an adverse impact on transition cow success. Among the array of negative impacts, stress increases circulating cortisol levels which can suppress the immune system (Roth et al., 1982). A major inducer of stress for a cow can be changing pens (Cook and Nordlund, 2004; Gupta et al., 2005). Regrouping also causes stress because of the social trauma associated with cows learning where they rank in the pen hierarchy, and this stress can reduce feed intake. Schirmann et al. (2011) observed that feeding rate decreased by 10% during the 2 days following regrouping; cows remaining in their home pen did not show a DMI depression, but cows that changed pens decreased DMI by approximately 9% on the day of the move. In contrast, Coonen et al. (2011) did not see a difference in DMI, plasma NEFA concentrations, or milk production when comparing close-up cows housed in the same pen to close-up cows from a

conventional dynamic housing system in which cows were added to the pen up to 2 times weekly. Overall, the majority of data indicates that transition programs should target a minimal number of pen moves and regroupings, but this strategy might not be as important as previously thought.

Proper function of the calcium homeostatic mechanisms is also important. Almost all transition related disorders can result from hypocalcemia (Goff, 2006). This is because calcium is needed by peripheral blood mononuclear cells to mount an immune response (Kimura et al., 2006), and more importantly, hypocalcemia results in milk fever. Typically as blood calcium concentrations decline cows secrete parathyroid hormone. Under physiologically normal conditions parathyroid hormone will stimulate bone calcium mobilization and intestinal calcium absorption via 1α,25-dihydroxycholecalciferol (vitamin D3). This mechanism becomes impaired under metabolic alkalosis conditions; presumably because the confirmation of the parathyroid hormone receptor becomes distorted, decreasing tissue sensitivity (Goff and Horst, 2003). The understanding of this led to the development of dietary cation anion difference (DCAD) equations. Balancing diets for a low (+5 meq/100 g) or negative DCAD reduces blood pH (blood pH $\leq$ 7.35) and appears to produce successful results. Dietary cation-anion difference is typically based on dietary concentration of only 2 cations [potassium (K) and sodium (Na)] and 2 anions [chlorine (Cl) and sulfur (S)]. The NRC (2001) reports 3 equations for calculating DCAD: (Na + K – Cl – S); (Na + K – Cl); [(Na + K + 0.15 Ca + 0.15 Mg) – (Cl + 0.6 S + 0.5 P)]. Low DCAD is often achieved through ingredient manipulation, but the Na and K concentrations of available ingredients could dictate supplementation of an anion source.

Another area beyond diet formulation and good management that has helped ease the transition into lactation is the use of dietary feed additives (i.e. propylene glycol, monensin,

yeasts, choline, niacin, etc.). A plethora of research has been conducted to test the effects of feed additives on transition cow metabolism and performance. Results have been mixed, but available data appears promising for the feed additive monensin. The remaining portion of this review will discuss the available knowledge regarding the effects of monensin on transition cows.

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Table 1.1 Summary of common transition cow disorders.[1]

Disease	Cause	Effects	Treatment	Preventative Measures
Ketosis[2]	Negative energy balance leads to a glucose deficiency (type 1). Can also be a result of insulin resistance (type 2).	Elevated levels of circulating ketone bodies. High urinary potassium excretion.	Increase feed intake. Increase diet energy density, >1.77 Mcal/kg is infeasible. Provide glucose precursors: propylene glycol,[3, 4] glycerol,[5] or calcium propionate[6]. Administer B-vitamin complexes[7]; particularly B12 because of its role in energy metabolism.[8,9] Intravenous administration of dextrose.[10]	Supply adequate quality feed. Maximize cow comfort to encourage intake. Eliminate anything that is limiting DMI (Availability of feed and water; a minimum of 30 inches/cow of bunk space is recommended during the transition period). Monitor fresh cows to prevent subclinical cases from escalating. Some feed additives, such as monensin[11] and niacin,[12, 13] could also help.
Milk fever	Hypocalcemia = low blood calcium. Possibly caused by inadequate binding of parathyroid hormone to its receptor.	Decreases muscle contraction. Cow has trouble walking or standing. Compromises ability to expel placenta increasing susceptibility to metritis. Teat sphincter might not close, increasing the risk of mastitis.	Elevate blood calcium back near 8.5–10 mg/dL, typically with intravenous administration of calcium borogluconate[14]. Adding calcium to the diet helps, but meeting demands in an off-feed cow is difficult (fresh cows require ≈0.8% dietary calcium).[15]	Adjust dietary cation/anion difference to < 0 mEq/100 g DM; also, reduce calcium levels in prepartum diets.[16] Feeding sodium aluminium silicate prepartum also works because it binds dietary calcium.[17]
Displaced abomasum	Lack of gut muscle contraction related to hypocalcemia, or a lack of mechanical stimulation because of inadequate feed intake or dietary physically effective fiber.	Stops feed intake. Can block blood circulation. Can be fatal.	Roll and tack technique, or surgical correction.	Stimulate feed intake.[18] Provide effective fiber. Maintain blood calcium concentrations of ≈10 mg/dL.
Retained placenta	Lack of muscle contractions, or host does not recognize placenta as a foreign object so immune system does not attempt to expel it.[19]	Increases susceptibility to infections (particularly metritis),[20] which can lead to other diseases.[18,21]	Increase blood calcium levels. Possibly manually remove placenta.[22]	Employ strategies to prevent milk fever.[21] Vitamin E supplementation and/or injections before calving are effective.[23] Supply adequate selenium levels.[24]
Mastitis	Microorganisms invade the teat and produce toxins, injuring the mammary gland. Early lactation cows are at greater risk because of a compromised immune system.[25]	Decreased milk quality. Decreased saleable milk. Can cause a decrease DMI.[26] Increases risk for other diseases.[21, 25]	Use of antibiotics[27] and anti-inflammatory agents.[28]	Proper nutrition, clean cows and environment, and properly trained employees.[27]
Metritis	Microorganisms invade the uterus causing an infection. Risk is increased when calving in unsterile environments, and for cows that have a retained placenta.	Elevated body temperature. Intake can decline[29] exacerbating negative energy balance, increasing risk of other diseases.[18, 21, 25] Makes rebreeding challenging.[30]	Treat the associated inflammation, which can increase feed intake.[31] Also, there are a variety of antibiotics that can be used to treat infection.	Calve in a clean, dry environment. Prevent retained placentas. Provide support for a strong immune system. Minimize stress because stress increases cortisol levels suppressing the immune system.[32]

[1]Other proposed transition related disorders include lameness, acidosis, and udder edema.
[2]Baird, 1982. [3]Schultz, 1952. [4]Hamada et al., 1982. [5]Gruchy et al., 1968. [6]Goff et al., 1996. [7]Girard, 1997. [8]Herbert and Das, 1976. [9]Kennedy et al., 1990. [10]Fox, 1971. [11]Duffield et al., 2008. [12]Fronk and Schultz. 1979. [13]Ghorbaniet al., 2008. [14]Robertson, 1949. [15]NRC, 2001. [16]Lean et al., 2006. [17]Anonymous. 2004. [18]Ingvartsen, 2006. [19]Gunnink, 1984. [20]Drillich et al., 2006. [21]Goff, 2006. [22]Laven, 1995. [23]LeBlanc et al., 2002. [24]Eger et al., 1985. [25]Overton and Waldron, 2004. [26]Lukas et al., 2008. [27]Bradley, 2002. [28]McDougall et al., 2009. [29]Urton et al., 2005. [30]Fourichon et al., 2000. [31]Bradford and Farney, 2010. [32]Roth et al., 1982.

Chapter 2 - Literature Review: Effects of Monensin on Feed Intake and Metabolism Relevant to Transition Dairy Cattle

Overview of Sodium Monensin

Monensin is a carboxylic polyether ionophore isolated from culture filtrates of *Streptomyces cinnamonensis*. The structure of this compound was uncovered in the late 1960's (Agtarap et al., 1967). This compound has been used extensively in feeding beef cattle, poultry, and most recently dairy cattle. In the United States, monensin is commercially available as a granulated sodium salt marketed under the trade name Rumensin® 90 (Elanco Animal Health, Greenfield, IN). This salt must be added to diets and orally consumed by animals. Additionally, in some countries monensin can be obtained within a controlled release capsule (CRC), which is an oral bolus that must be endogenously administered to the rumen. The primary known benefits from administering monensin include improved feed efficiency, control of coccidiosis, and reduced metabolic disorders.

Mode of Action

Many biological responses have been observed when feeding monensin, but Schelling (1984) consolidated these responses into a list of 7 modes of action that have been summarized for this review (Table 2.1). Briefly, monensin is bacteriostatic against gram positive bacteria, which alters acetate to propionate ratio, changes ruminal gas production, spares ruminal protein, and modifies digestibilities. These effects lead to improvements in feed efficiency. There is also evidence in feedlot cattle that monensin modulates intake, possibly improving overall animal health and performance through reduced ruminal pH variance. Several other studies provide evidence that monensin affects more than ruminal parameters, and explaining everything monensin does physiologically would take a more extensive review of literature; thus, in a broad sense monensin is a drug, and depending on the circumstance drugs can produce various animal responses.

Beyond Schelling's (1984) list, the dynamicity of associative effects makes it conceivable that some of monensin's modes of action have not yet been discovered. The development and application of tools that quantify gene expression will aid such discoveries. Preliminary information, discussed later in this chapter, indicates that monensin does alter expression of some metabolic genes (Karcher et al., 2007).

Role of Monensin in Transition Cow Health

A plethora of researchers have documented the effects of monensin on dairy cows. Duffield et al. summarized information gathered from available reports and published it in 3 meta-analyses (Duffield et al., 2008a, 2008b, 2008c). These meta-analyses included data from 59 separate studies in which monensin was fed to dairy cattle, and many of these studies involved transition cows. Their analysis (Duffield et al. 2008a) indicated that monensin significantly decreased blood concentration of ketones (BHBA by 13% and acetoacetate by 14%) and NEFA (7%), and increased blood glucose (3%) and urea (6%). This same data set was used to show that the relative risk (RR) of ketosis (RR = 0.75), displaced abomasum (RR = 0.75), and mastitis (RR = 0.91) were significantly decreased when monensin was administered. These meta-analyses (Duffield et al., 2008a, 2008b, 2008c) provide convincing evidence that monensin helps dairy cows overcome challenges presented during early lactation. The following sections will highlight key points from available data in an attempt to develop a mechanistic understanding of the specific changes promoted by monensin treatment, and how these changes can impact transition cows.

Effects of Monensin on Feed Intake

As discussed previously, transition cow health is directly linked to feed intake. If cows continue eating, the negative energy balance does not escalate and many metabolic disorders can be overcome or avoided. Voluntary intake is linked to rumen pH (Fulton et al., 1979), suggesting transition cow feeding strategies should promote a desirable mean rumen pH (≥ 5.8; Dohme et al., 2008) and minimize fluctuations.

There is a theory that monensin supplementation results in more consistent feed intake, potentially improving overall health of cattle. An abundance of studies done with feedlot animals support this theory (Burrin et al., 1988; Stock et al., 1995; Erickson et al., 2003). It is important to acknowledge that the intake modulation has typically been associated with an overall decrease in DMI (Anonymous, 1995). Published intake prediction equations suggest a consistent 4 to 10% decrease in intake when feedlot cattle are administered monensin (Fox et al., 1988; NRC, 2000). Such a DMI depression could be detrimental to a transition dairy cow, negating benefits of more consistent intake patterns. Interestingly, more recent studies did not show an effect of monensin on total DMI in feedlot cattle (Erickson et al., 2003; Depenbusch et al., 2008). It is difficult to speculate on what this means for dairy cattle, but it indicates that it is possible to feed monensin without decreasing intake.

Data specific to the effects of monensin on DMI in dairy cattle is limited and inconsistent. Sauer et al. (1989) reported 1.2 kg/d lower DMI of cows fed 30 g monensin/ton of DM compared to cows not receiving monensin from 1 wk prepartum through 3 wk postpartum, while others observed no differences (Van der Werf et al., 1998; Phipps et al., 2000; Odongo et al., 2007). One reason for this discrepancy may be that Sauer et al. (1989) used early lactation cows. Additional literature from early lactation cows is limited. An abstract reporting postpartum

DMI of 966 cows in response to monensin fed at 0, 7, 15, or 22 g/ton of DM showed a linear reduction in DMI (20.4, 20.4, 19.8, and 19.6 ± 0.17 kg/d; Shah et al., 2008). Interestingly, weekly DMI data showed the rate of change in DMI linearly increased as monensin dose increased during the first 12 weeks of milk (0.036, 0.047, 0.045, and 0.051 ±0.004 % per d; Shah et al., 2008). These investigators did a follow-up trial (Schroeder et al., 2009) with 44 transition cows and observed increased postpartum DMI in monensin-treated cows, but neither treatment means nor rate of change in DMI were reported in the abstract. Both of these abstracts (Shah et al., 2008; Schroeder et al., 2009) indicate monensin modulates intake, and possibly in a manner that benefits early lactation cows. It is plausible that monensin altered feeding behavior in these experiments (Shah et al., 2008; Schroeder et al., 2009), which could have had favorable effects on the rumen ecosystem impacting total DMI. This makes the feeding behavior of transition dairy cows administered monensin of particular interest, but this has not been investigated.

The majority of data indicates monensin affects intake in some manner, and there are several theories that have been proposed to explain this response. One of the more widely accepted theories is that monensin decreases diet palatability. Erickson et al. (2004) observed an aversion against diets containing monensin and a preference for diets containing lasalocid or no ionophore in a free-choice study using Holstein heifers.

Other evidence suggests intake modulation can be explained through a ruminal mechanism. Baile et al. (1979) observed a tendency for monensin to decrease DMI during a 6-hour test period when it was offered in feed (1.19 vs. 3.19 ± 0.11 kg), or infused directly into the rumen (2.68 vs. 3.19 ± 0.11 kg) of 12 cattle fed high roughage diets. These same researchers repeated the trial with 12 different steers fed a high concentrate diet, and reported that monensin significantly decreased DMI, whether it was offered in feed (0.51 vs. 3.39 ± 0.30 kg) or directly

infused into the rumen (2.00 vs. 3.39 ± 0.30 kg). Both times, the magnitude of intake depression was most severe when monensin was offered in feed, but this could be a behavioral adaptation; cattle may have learned they would receive monensin-free feed after the 6 hour test period, so they chose to minimize consumption of monensin-treated feed during the test. Nonetheless, infusing monensin directly into the rumen decreased intake in both trials, implying that effects on intake extend beyond palatability. Because ruminants regurgitate rumen digesta for chewing, palatability aversion is still plausible if monensin was attached to any regurgitated rumen particles.

Because monensin in the rumen stimulates greater propionate production, it is possible that reduced intake is a chemostatic response (Allen, 2000). Propionate resulted in satiety when infused into the portal vein of sheep (Anil and Forbes, 1980; Farningham and Whyte, 1993), and mesenteric vein of steers (Elliot et al., 1985). More relevant, infusing buffered propionate into the rumen of sheep and goats (Baile, 1971) and lactating cows (Sheperd and Combs, 1998) significantly depressed intake. These findings can be explained through the hepatic oxidation theory (Allen et al., 2009), which is based heavily on oxidation of propionate.

It has also been observed that monensin reaching post-ruminal organs can decrease voluntary intake. Bierman (2001) conducted a study using 4 crossbred steers in a 4 × 4 Latin square design. Treatments were 250 mg of monensin infused directly into the rumen, 125 mg of monensin into the portal vein, 3 mg into the hepatic vein, or a control, which meant no monensin or placebo was administered. The rumen dose was chosen because that dose was reported to cause a depression in intake (Parrot, 1992). The portal dose was based on monensin radio-tracer studies, which were used to estimate that 50-60% of monensin is absorbed and transported to liver. The authors then speculated that the majority of monensin is metabolized by the liver, so

the mesenteric treatment was 2.5% of the portal dose. All 4 steers were fed the same basal diet; the Latin square had 7 d periods with 11 d breaks between periods, and monensin was dosed on d 4. Cumulative changes in 12 h DMI compared to pre-dose DMI were -27.5, -14.7, +3.2, and +17.9 g/kg metabolic body size for rumen, portal, mesenteric, and control treatments, respectively. Contrasting the control vs. the monensin treatments showed a significant effect ($P < 0.04$), and contrasting the rumen infusion vs. the portal and mesenteric infusions showed a tendency for an effect ($P < 0.09$). Overall, this research indicates that effects on intake are not exclusive to the rumen.

It has also been proposed that monensin increases the metabolizable energy value of feed by decreasing energy lost as gas, thus the animal will be more efficient with the feed consumed, requiring less DM to supply maintenance and productive energy. The major gas believed to be altered is methane, although the duration of decreased methanogenesis has been debated (Johnson and Johnson, 1995). For the most part, it is accepted that monensin is inhibitory to methanogens. The inhibition is likely mediated through hydrogen sink activities (Van Nevel and Demeyer, 1977; Chen and Wolin, 1979), so less hydrogen is available for methanogenesis. As a consequence of reducing methane production, some researchers suggest H_2S production will increase from 3.54 to 6.70 μmol/g of fermentable DM (Kung et al., 2000) and high levels of H_2S could depress intake (Kandylis, 1984). Interestingly, more recent research suggests monensin does not impact H_2S production if dietary sulfur concentrations are within the range of what is typically observed in feedlot diets (Quinn et al., 2009). The discrepancy between these reports is probably because Kung et al. (2000) obtained samples from donor animals fed a 50:50 blend of alfalfa hay and corn silage, while Quinn et al. (2009) obtained samples from steers fed a 75% concentrate diet. Conventional transition cow diets contain concentrate levels that fall

somewhere in the middle of the levels used in these studies, so it does not appear that monensin inclusion will elevate H_2S production enough to impact transition cow DMI.

Effects of Monensin on Rumen pH

As mentioned previously, modulated intake could impact rumen pH, but data from dairy cattle is limited and inconsistent. Monensin administered through a CRC increased transition cow rumen pH (Green et al., 1999), but this data must be interpreted with caution because ruminal samples were collected using an esophageal tube, making salivary contamination a concern (Duffield et al., 2004). Instead, indwelling pH probes present a more accurate technique to quantify rumen pH conditions. To my knowledge, only two published studies have used this type of probe to measure differences in ruminal pH between monensin supplemented and control cows (Mutsvangwa et al., 2002; Fairfield et al., 2007), and saw no evidence that monensin affects rumen pH. Further examination is however warranted because Mutsvangwa et al. (2002) only used 6 cows which might not have provided adequate statistical power to detect differences, and both studies used a CRC, which might not modulate intake to the magnitude that dietary monensin does. Furthermore, neither of these studies examined effects on rumen pH variance.

Monensin has been reported to attenuate rumen pH declines even without directly modulating intake. Nagaraja et al. (1981) administered monensin intraruminally (1.3 mg/kg BW) to rumen-fistulated steers maintained on an alfalfa hay diet and then experimentally induced acidosis using finely ground corn or solubilized glucose. Monensin-treated cattle maintained higher rumen pH values, greater propionate and total VFA concentrations, with lower lactate concentrations compared to cattle not receiving antibiotics. Burrin and Britton (1986) conducted a similar acidosis challenge and reported similar results, but in their study steers were given dietary monensin.

Reported increases of ruminal pH from monensin treatment typically seem to be observed in diets with greater NFC. In the study by Burrin and Britton (1986), high-moisture corn made up 75.5% of DM in the acidosis challenge diet. Similarly, during the challenge conducted by Nagaraja et al. (1981), glucose was administered at a rate of 12.5 g/kg of BW and ground corn was given at 27.5 g/kg of BW. These levels of carbohydrates undoubtedly elevate lactic acid concentrations, and monensin can hinder lactate production via bacteriostatically eliminating lactate-producing bacteria (Russell and Strobel, 1989). Some researchers claim this as the primary mechanism through which monensin alters pH, and that lactate needs to exceed ≈5 mM for monensin to have an effect, explaining why a pH effect was observed in the feedlot studies and not in the dairy cattle studies. Simply put, fresh cow diets likely do not contain enough rapidly-fermentable carbohydrates to promote excessive levels of lactic acid production.

Data obtained from non-periparturient dairy cows supports this theory. Osborne et al. (2004) induced subacute ruminal acidosis in mid-lactation cows receiving a conventional TMR and did not see an effect of monensin on pH, but the grain challenge model (Keunen et al., 2002) used in their trial does not promote ruminal lactate concentrations that exceed 1 mM. Additionally, Ruiz et al. (2001) did not see an effect of monensin in mid-lactation cows (DIM = 126) fed fresh forage and a corn meal concentrate mix. Likewise, researchers reported no effect of monensin on rumen pH in mid-lactation cows (DIM = 194) fed a conventional TMR (Mathew et al., 2011). In contrast, Gehman et al. (2008) reported a tendency for monensin to increase rumen pH (5.89 vs. 5.79 ± 0.07) in mid-lactation cows (DIM =101) during a brown midrib corn silage study. However, one must keep in mind brown midrib corn silage is more easily fermented compared to conventional corn silage (Oba and Allen, 2000) so lactic acid production could have been elevated, although it is unlikely that concentrations exceeded 5 mM.

Glucogenic Effects of Monensin

As discussed in the metabolism section of this review, the onset of lactation nearly triples the demand for glucose (Bell, 1995). The dairy cow is capable of synthesizing glucose from a variety of precursors to meet this demand, but the majority of gluconeogenesis occurs in the liver, from the precursor propionate (50-60%; Reynolds et al., 2003). Monensin selectively modifies ruminal flora in a manner that promotes production and increases the pool size of propionate (Van Maanen et al., 1978). Greater propionate supply increases hepatic gluconeogenesis (Aiello and Armentano, 1987). This could help explain why meta-analysis of 34 trials shows that monensin increased plasma, serum, or blood glucose by 3.2% (Duffield et al., 2008a). Thus, in transition cows, an increased supply of glucose is often assumed to be the primary benefit of monensin supplementation.

In contrast, when propionate kinetics were measured during the periparturient period, using isotopic tracers, monensin did not affect ruminal propionate production (Markantonatos et al., 2009). This helps explain why some studies have observed monensin to have beneficial effects on plasma lipids or ketones with no effect on plasma glucose concentration (Sauer et al., 1989; Petersson-Wolfe et al., 2007); in fact, some researchers even reported a tendency for decreased glucose concentration (Stephenson et al., 1997). This evidence suggests that the beneficial effects of monensin on transition cows extend beyond gluconeogenic flux.

Arieli et al. (2001) did not observe changes in blood glucose concentration, but observed an increase in distribution space and glucose pool size when feeding monensin to prepartum cows, suggesting increased uptake of glucose by peripheral tissues in response to monensin. If monensin does, in fact, alter clearance of circulating glucose, then blood/plasma/serum glucose concentration is a poor proxy for gluconeogenic flux and glucose turnover data are required to

evaluate the effects of monensin on this pathway accurately. Data in this area are limited, but in a heat stress study, rate of appearance of glucose was 10% higher for monensin supplemented cows on an equivalent DMI basis (Wheelock et al., 2009).

Monensin also impacts the minor glucogenic precursors. Monensin decreases ruminal lactate production (Callaway and Martin, 1997). Lactate has been measured to supply about 8% of precursors for hepatic gluconeogenesis from late gestation through early gestation (Reynolds et al., 2003). However, it must be noted that in transition cows lactate can be a product of the Cori Cycle, and not entirely derived in the rumen. Available evidence indicates approximately 5 to 10% of glucose is derived from lactate in the Cori Cycle (Nocek, 1997). Monensin also spares amino acids from ruminal degradation (Schelling, 1984), and the theoretical contribution of amino acids to liver glucose production could be as large as 15-20% (Reynolds et al., 1988; Reynolds et al., 2003).

Monensin in a broad sense is a drug, and not all of its effects have been uncovered. For instance, monensin has been observed to increase gene expression of cytosolic phosphoenolpyruvate carboxykinase (Karcher et al., 2007). Phosphoenolpyruvate carboxykinase is known to be a rate-determining gluconeogenic enzyme (Greenfield et al., 2000; Agca et al., 2002). Although interesting, it must be remembered that a change in expression is not a direct measure of altered activity or glucogenic flux. Still, increased expression of phosphoenolpyruvate carboxykinase during monensin treatment presents many possibilities. The simplest explanation is that hepatic gluconeogenesis regulation is linked to a feed-forward mechanism through which ruminal production of glucogenic precursors (e.g. propionate) up-regulates phosphoenolpyruvate carboxykinase (Donkin et al., 2009); but if precursor production is not altered, monensin could be having a direct effect on the enzyme. Even independent of

monensin's effects, more research is warranted to obtain a clear understanding of links between glucogenic gene expression and other metabolic parameters.

Lipid Metabolism and Monensin

The effect of monensin on lipid metabolism has not been researched to the extent that glucose has. This is an oversight by the scientific community because transition cows mobilize large amounts of adipose tissue during periods of negative energy balance. This causes the release of NEFA, which get delivered to the liver and metabolized through 1 of 3 pathways. They can be oxidized (generating either reducing equivalents or ketone bodies), exported as triglycerides in lipoproteins, or accumulate within the liver as triglycerides. This section will highlight the known information regarding how monensin affects this area of metabolism.

Duffield et al. (2008a) summarized data from 24 monensin trials that reported NEFA response and concluded that monensin decreases circulating NEFA concentrations by 7.1%. Across the trials summarized, effects were typically only seen if monensin was administered during the transition period, which could be expected because this is the time period of elevated NEFA concentrations (Grummer, 1995). This effect on NEFA is likely related to the improved energy status associated with the greater propionate supply from monensin treatments. Alternatively, propionate is an insulin secretagogue (Koppel et al., 1988) which could suppress lipolysis, inhibiting NEFA release within monensin-treated cows. Meta-analysis did not indicate a statistical affect of monensin on insulin concentrations, but showed a numerical increase of 17.3%. It is surprising that a difference of that magnitude was not statistically significant, but the number of cows used for the insulin data set was relatively small (n = 510).

Theoretically, because monensin decreases circulating NEFA concentrations, cows treated with monensin should maintain BCS better than control cows. This theory was confirmed

in the meta-analysis (Duffield et al., 2008b). However, such an effect on BCS could lower milk fat production. Duffield et al. (2008b) concluded that monensin decreases milk fat concentration 0.13%, but monensin increases milk production enough that milk fat yield was not different between monensin and control cows (raw weighted mean difference = -0.002 kg/d; $P = 0.16$). It must be noted that these numbers were obtained from cows in all stages of lactation so the magnitude of the effect is likely different during early lactation. Duffield et al. (2008b) did not report means, but did mention that during early lactation the effect of monensin on milk fat concentration and yield was less.

Also noteworthy is the difficulty related to quantifying treatment effects on β-oxidation of fatty acids. Thus, researchers rely on indirect measurements such as mRNA expression of major control-point enzymes, circulating lipoprotein concentrations, and liver triglyceride content. Carnitine palmitoyl transferase-1a (CPT1a) is one of the enzymes receiving an increasing amount of attention. This enzyme is important for translocating fatty acids from the cytosol into the mitochondria, making it a central component for determining oxidative flux of fatty acids within the liver (Drackley, 1999). Currently no reports exist examining the effects of on monensin on CPT1a; thus an examination of such effects is warranted.

The effects of monensin on total circulating cholesterol concentrations were assessed in the same meta-analysis (Duffield et al., 2008b) and no difference was detected. However, only 6 studies reported cholesterol concentrations. A study designed to look at the effects of monensin on specific lipoproteins during the transition period showed that monensin-treated cows had greater serum VLDL and LDL concentrations postpartum compared to control cows (Mohebbi-Fani et al., 2006), which suggests monensin increases the capacity of the liver to export triglycerides. Because it has also been shown that cows with fatty liver have reduced circulating

VLDL and LDL (Herdt et al., 1983; Katoh, 2002), anything that increases lipoprotein secretion should help to limit accumulation of liver lipids. Such effects should improve the liver's glucogenic and ureagenic capabilities (Overton and Waldron, 2004).

The best way to determine the degree to which lipids have infiltrated the liver involves conducting liver biopsies. This is probably why relatively little data exists documenting the effects of monensin on liver triglyceride content. The only document that I am aware of showing liver triglyceride content of periparturient dairy cows fed monensin reports a tendency for monensin to lower liver triglyceride percentages during the third week of lactation (≈17.5 vs. ≈12.5% of liver wet weight; Zahra et al., 2006).

Antiketogenic Effect of Monensin

Typically, ketosis is a result of an imbalance between glucose supply and demand (Herdt, 2000). During the weeks immediately following parturition, glucose supply falls short of lactation requirements, thus homeorhetic changes promote the oxidation of fatty acids. Insulin resistance can also promote fatty acid oxidation. Therefore, some information on lipid metabolism can be extrapolated based on ketone production.

Ketone bodies are intermediate metabolites of fatty acid oxidation and can be used as a fuel source by many organs. The presence of high levels of circulating ketone bodies is referred to as ketosis and has many negative effects on metabolism. The most noticeable effect is decreased milk production (Lucey et al., 1986; Rajala-Schultz et al., 1999), but elevated ketone concentrations have been reported concurrent to decreased immunocompetency (Hammon et al., 2006; Galvo et al., 2010).

Sauer et al. (1989) conducted the first controlled experiment reporting antiketogenic effects of monensin. For their study, monensin was added to diets at least 1 wk prior to

parturition at two doses, 15 g/ton of DM or 30 g/ton of DM, and data collected from these cows was compared to a group not receiving monensin. Thus, there were 3 treatment groups and each group contained 12 cows. Treatment administration continued through 3 weeks postpartum. Sauer et al. (1989) reported that 50% of the cows in the control group had clinical or subclinical ketosis, whereas the incidence was 33 and 8% in the 15 and 30 g/ton groups, respectively. Furthermore, the group fed the high dose of monensin had significantly lower blood BHBA compared to control cows (3.9 vs. 7.2 mg/dL, respectively).

Since the late 1980's many other trials have been conducted making it clear that monensin significantly decreases blood/plasma/serum ketone concentrations. The most convincing evidence was compiled by Duffield et al. (2008a) from 33 trials showing reductions of 13.4% for BHBA and 14.4% for acetoacetate. These findings can possibly be explained by an increased energy balance mediated through greater provisions of propionate (Drackley et al., 1991), but not entirely. If propionate is not altered, as was discussed previously in the Markantonatos et al. (2009) data, monensin must be improving energy metabolism through some other means that has not yet been explained. It must also be noted that epidemiology researchers have observed that cows diagnosed with other clinical diseases are at a much greater risk for contracting ketosis (Ingvartsen, 2006). So a decreased incidence rate of ketosis would be expected if monensin improves other health parameters of dairy cows during the transition period.

Monensin and Cow Health

In the United States, monensin is not currently labeled to improve cow health, but researchers have speculated that monensin has positive implications for reducing certain diseases. Determining the impact of monensin on the incidence of any disease is challenging

because most studies lack appreciable statistical power. Research discussed in previous sections of this review indicates that monensin attenuates rumen acidosis and ketosis, but the effects of monensin on other disorders were not discussed.

Monensin is useful for reducing incidence of bloat in pasture fed dairy cattle. Lowe et al. (1991) clearly showed this by pooling data from 6 experiments involving 368 cows. Cameron and Malmo (1993) observed similar results in a much larger study involving 5,102 cows. This discussion is relevant to cow health, but bloat is not typically an issue in transition cows.

The disorders that are more relevant to transition cows have been discussed in Table 1.1. Duffield et al. (2002) examined the effects of monensin on incidence rate of these diseases during the first 90 d postpartum across 45 farms in Canada. The study consisted of 1,317 Holstein cows that were randomized to receive either a monensin CRC or serve as a negative control. Data indicated tendencies for monensin to reduce occurrence of retained placenta, displaced abomasum, and other digestive disorders. These same researchers added an additional data set obtained from 1,010 cows across 25 farms (Duffield et al., 1999). The pooled data indicated a 40% decrease in ketosis and displaced abomasum, and a 25% reduction in incidence of retained placenta, in response to monensin treatment. Monensin also decreased the number of cows diagnosed with more than one illness by 26%. Effects on other periparturient diseases were not observed. Similarly, a Dutch study was carried out across 8 research farms and showed that monensin reduced the rate of clinical mastitis from 19% in the controls to 11% in the treated group (Heuer et al., 2001). Clinical mastitis was determined by using SCC, and 250,000 cells/mL was used as the threshold. No effects were observed concerning duration of infection. In this same trial (Heuer et al., 2001) monensin reduced the risk for non-infectious lameness which matches the expected effects on ruminal acidosis discussed previously. Effects on other

periparturient diseases were not detected, but it is worth noting that monensin reduced the time from calving to conception in cows diagnosed with endometritis (Heuer et al., 2001). In contrast to Duffield et al. (2002) and Heuer (2001), Beckett et al. (1998) reported no difference in risk for any periparturient disease in similarly-designed study using 686 cows across 12 Australian dairy herds.

Perhaps the most accurate depiction of the effects of monensin on lactating cow health is the meta-analysis conducted by Duffield et al. (2008c). These researchers combined data from 16 papers, including the studies discussed in the previous paragraph. Overall, monensin significantly decreased the relative risk (RR) of ketosis (RR = 0.75), displaced abomasum (RR = 0.75), and mastitis (RR = 0.91). Monensin did not affect risk of retained placenta, milk fever, metritis, endometritis, or lameness. Interestingly, method of monensin administration influenced the risk of retained placenta and metritis, with risk being significantly lower for the CRC treatment compared to feeding monensin.

It is not surprising that monensin lowers the risk of diseases such as ketosis and displaced abomasum, because these diseases are likely related to energy status, which can be improved through greater provision of propionate. It is however surprising that monensin has been observed to decrease incidence of an infectious disease (e.g. mastitis). Examining mechanistic immune effects suggests this is possible because monensin influences the cow's ability to respond to disease, partially through the antiketogenic effects discussed previously, and through direct effects on the immune system as shown through acute phase proteins (Stephenson et al., 1997; Crawford et al., 2005).

Acute phase proteins typically increase in concentration during times of inflammation (Bionaz et al., 2007). Numerous studies in the past decade have demonstrated that inflammation

is common in the periparturient period (Bradford and Farney, 2010); therefore, we expected acute phase protein concentrations to increase around calving. Interestingly, Crawford et al. (2005) observed that transition heifers with a clinical disorder (n = 54) had higher serum haptoglobin levels during the first week after calving if they had been administered monensin beginning 21 d before calving compared to cows not receiving monensin (1.53 vs. 0.83 ± 0.33 g/L). Likewise, a different research group reported increased ceruloplasmin (≈15 vs. ≈19 μM) postcalving when monensin was administered to 24 Holstein cows beginning 50 d prepartum (Stephenson et al., 1997). These findings suggest that monensin-treated cows have improved capacity to mobilize acute phase proteins, which could mean the immune system has greater ability to fight off infections. This response helps explain why cows given monensin have a lower relative risk of getting mastitis. This hypothesis is supported by evidence from the Stephenson et al. (1997) study that reported the monensin-treated cows had significantly higher chemotaxis of neutrophils (Stephenson et al., 1996). It is possible that improved neutrophil function was an indirect result of antiketogenesis because ketone bodies impair the chemotactic response of leukocytes (Suriyasathaporn et al., 2000). However, these researchers (Stephenson et al., 1996) also speculated that results could be related to altered mineral metabolism.

Immune response can be directly linked to mineral metabolism, but little work has been done to examine the effects of monensin on this area. Work done in feedlot cattle showed that monensin increased apparent absorption (13.1 vs. 8.1 g/d) and retention (12.4 vs. 7.7 g/d) of calcium (Spears et al., 1989), which aligns with results obtained from lambs fed monensin (Greene et al., 1986). These results imply that monensin could reduce milk fever. However, other investigators conducting similar trials did not observe affects on calcium absorption or retention in lambs (Kirk et al., 1985) or growing steers (Starnes et al., 1984). To my knowledge, only one

study has reported plasma calcium concentrations of transition cows in response to monensin and they observed no effects (Stephenson et al., 1997). The reason for discrepancy among these ruminant studies is unclear, but meta-analysis did not detect an effect of monensin on incidence of milk fever (Duffield et al., 2008c). Beyond preventing milk fever, peripheral blood mononuclear cells need calcium to mount an immune response (Kimura et al., 2006). Periparturient cows are typically deficient in calcium (Goff and Horst, 1997); therefore if monensin can increase calcium absorption it should improve the transition cow's ability to mount an immune response.

There are several other minerals that could be of interest, but copper and selenium are of particular interest because they play important roles in reducing oxidative stress. Biopsies indicated higher copper content (196 vs. 159 mg) and retention (5.9 vs. 4.7%) in the livers of sheep fed monensin compared to controls (Van Ryssen, 1991), but saw no differences for iron, zinc, or manganese. Costa et al. (1985) observed improved retention of radio labeled selenium (23.2 vs. 13.4 ±1.3%) and zinc (9.0 vs. 6.3 ± 0.3%) in dairy breed steers fed monensin. These authors speculated that increased retention was mediated through increased absorption from the gut.

To my knowledge, the only comprehensive review covering the effects of monensin on mineral metabolism in ruminants pre-dates some key studies (Spears, 1990). This review highlights some inconsistencies between studies, but also indicates monensin has a variety of effects on other minerals depending on diet composition and physiological state of the animals. Combined, this selection of mineral-related literature indirectly suggests that monensin could improve transition cow health through enhancing the cow's immune system.

Conclusion

The transition period refers to the weeks surrounding calving. During this time a cow's health is compromised because she has to recover from stresses associated with calving. These stresses consist of providing adequate nourishment to the fetus during its final phase of gestational development, parturition, initiation of lactation, going into a negative energy balance, and adapting to different diets. All of these stresses make dairy cows prone to a plethora of metabolic disorders. The economic impact of these disorders creates an unprecedented need to devise management tools and strategies that will help dairy cows survive the transition period unscathed. The addition of sodium monensin to the diets of transition dairy cows appears to improve the metabolic status of cows through this critical time period, but we do not have solid explanations for certain effects. The objective of the research reported in this dissertation was to get a more mechanistic understanding of how monensin alters transition cow metabolism by examining parameters that have not previously been investigated, such as feeding behavior and gene expression in the liver.

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Table 2.1 Summarized modes of action of monensin[1]

- Alters VFA profile
- Modulates feed intake
- Alters ruminal gas production
- Modifies digestibilities
- Protein sparing effect
- Modified level of gut fill, altering rate of passage
- Other (indirect effects of the first 6 mechanisms)

[1]Adapted from Schelling (1984).

Chapter 3 - Effects of Monensin on Metabolic Parameters, Feeding Behavior, and Productivity of Transition Dairy Cows[1]

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Abstract

The effects of monensin on transition cow metabolism may be dependent on modulation of feeding behavior, rumen pH, and/or expression of key metabolic genes. Multiparous Holstein cows were used to determine the effects of monensin (400 mg/cow daily) on these variables. Cows were randomly assigned, based on calving date, to control or monensin treatments (n = 16 per treatment) 21 d before their expected calving date, and cows remained on treatments through 21 d postpartum. Feeding behavior and water intake data were collected daily. Liver biopsies were conducted after assessing BCS and BW on d -21, -7, 1, 7, and 21 relative to calving for analysis of triglyceride (TG) content as well as mRNA abundance of cytosolic phosphoenolpyruvate carboxykinase (cPEPCK), carnitine palmitoyltransferase 1a (CPT1a), and apolipoprotein B (Apo B). Blood samples were collected 21, 7, and 4 d before expected calving and 1 (day of calving), 4, 7, 14, and 21 d postpartum for nonesterified fatty acid (NEFA), β-hydroxybutyrate (BHBA), glucose, insulin, and haptoglobin analyses. Ruminal pH was collected every 5 min on d 1 through 6 postpartum via a wireless indwelling probe. On d 7 postpartum, a caffeine clearance test was performed to assess liver function. Data were analyzed using mixed models with repeated measures over time. Monensin decreased mean plasma BHBA (734 vs. 616 ± 41 μM; $P < 0.05$) and peak concentrations (1076 vs. 777 ± 70 μM on d 4 postpartum; $P < 0.01$). Monensin also decreased time between meals prepartum (143 vs. 126 ± 5.0 min; $P < 0.03$) and postpartum (88.8 vs. 81.4 ± 2.9 min; $P < 0.08$), which could be related to a smaller ruminal pH standard deviation in the first day after cows changed to a lactation ration (0.31 vs. 0.26 ± 0.015; $P < 0.02$). Monensin also increased liver mRNA abundance of CPT1a (0.10 vs. 0.15 ± 0.002 arbitrary units; $P < 0.04$), which corresponded to a slower rate of liver TG accumulation from d -7 to +7 (412 vs. 128 ± 83 mg TG/g protein over this time period, $P = 0.03$). No significant effects of monensin supplementation were observed on milk production, liver

cPEPCK, Apo B, plasma NEFA, glucose, insulin, or haptoglobin. No effects on disease incidence were detected, but sample size was small for detecting such effects. Overall, results confirm that the effects of monensin on transition cows extend beyond altered propionate flux.

Key words: Monensin, transition cow, liver function, rumen pH

Introduction

The weeks surrounding parturition are a critical time in the life cycle of a high-producing dairy cow. During this period, cows make many metabolic adjustments to support the transition from pregnancy to lactation. Furthermore, dairy cows produce milk in excess of their ability to consume energy, resulting in a period of negative energy balance in early lactation (Grummer, 1995). In recent years, monensin has been used to help mitigate the effects of negative energy balance, presumably by promoting ruminal production of glucogenic precursors (Duffield et al., 2008a,c).

Under normal physiological conditions, monensin alters ruminal digestion in a manner that augments propionate production rate and concentration in the rumen (Van Maanen et al., 1978). Greater propionate supply leads to increased hepatic gluconeogenesis (Aiello and Armentano, 1987), which could improve the overall energetic balance of the transition cow. Thus, an increased supply of glucose is often assumed to be the primary benefit of monensin supplementation. Observations of Sauer et al. (1989) support the hypothesis that monensin decreases the acetate:propionate ratio in transition cows. In contrast, when propionate kinetics were measured during the periparturient period, monensin did not affect ruminal propionate production (Markantonatos et al., 2009). Some studies have observed monensin to have beneficial effects on plasma lipids or ketones with no effect on plasma glucose concentration (Sauer et al., 1989; Petersson-Wolfe et al., 2007); in fact, one group even reported a tendency for decreased glucose concentration (Stephenson et al., 1997). This evidence suggests that the beneficial effects of monensin likely extend beyond gluconeogenic flux, and may even be independent of changes in gluconeogenesis.

Transition cow health is directly linked to DMI, partly because negative energy balance is not as severe in early lactation cows with higher intakes (Bertics et al., 1992). Recent transition

cow research indicates a beneficial effect of monensin on the postpartum DMI curve (Shah et al., 2008) or overall DMI (Schroeder et al., 2009), but feeding behavior was not measured in either study. Research from feedlot cattle suggests dietary monensin could modulate intake patterns, thus reducing dramatic changes in rumen pH while cattle are adapting to a high-energy diet (Stock et al., 1995; Nagaraja et al., 1997). Furthermore, data obtained from mid-lactation cows induced with SARA indicates that administering monensin in feed increases meal frequency during the challenge and recovery periods (Lunn et al., 2005). The hypothesis that monensin affects transition cow rumen pH has not been extensively investigated. Monensin administered through a controlled-release capsule increased transition cow rumen pH (Green et al., 1999), but these data must be interpreted with caution because ruminal samples were collected using an esophageal tube. Research conducted using indwelling probes to measure pH showed no difference between monensin supplemented cows and control cows (Mutsvangwa et al., 2002; Fairfield et al., 2007), but Mutsvangwa et al. (2002) only had 3 animals per treatment and Fairfield et al. (2007) used a controlled-release capsule, which may not modulate intake as much as dietary monensin. Furthermore, neither of these studies examined differences in variance of rumen pH.

The primary objectives of this study were to determine the effects of monensin on transition cow feeding behavior and metabolic parameters. The secondary objectives were to assess the effects of monensin on ruminal pH and productivity of transition cows.

Materials and Methods

All experimental procedures were approved by the Institutional Animal Care and Use Committee at Kansas State University.

Design and Treatments

Thirty-two multiparous Holstein transition cows from the Kansas State University Dairy Cattle Teaching and Research Facility were utilized in a randomized complete block design. Cows were blocked by expected calving date (16 blocks) and randomly assigned within block to 1 of 2 treatments (n = 16 cows per treatment) 21 d before their expected calving date. Cows remained on their respective treatments through 21 d postpartum. The treatment group received monensin (Rumensin®, Elanco Animal Health, Greenfield, IN) as a top-dress at a rate of 400 mg/cow per d, and the control group received no monensin for the duration of the study. Cows were dried off an estimated 45 d before calving. Monensin was excluded from the far-off dry cow ration to help ensure that no cows entering the study were influenced by prior monensin exposure. Cows entered the study from January to June 2010.

Monensin was premixed into a ground corn carrier and 0.91 kg of the premix was offered daily to each cow in the treatment group. Monensin treatments were administered by top-dressing and manually mixing the premix into the upper 33% of each TMR once per day. The ground corn carrier was top-dressed to the control cows at the same rate in the same manner. The monensin dose was selected based on previous research (Schroeder et al., 2009) and approached the maximum FDA-approved label dose for dry cows of 410 mg/cow per d. Diets were formulated to meet or exceed NRC (2001) requirements (Table 1). Samples of all dietary ingredients were collected weekly and stored at -20°C. Upon study completion, feed ingredients were composited into bimonthly samples for wet chemistry analysis of CP, ADF, NDF, ether extract, and ash by Dairy One Forage Laboratory (Ithaca, NY).

Management of Cows and Data Collection

Cows were dried off and moved into a free-stall pen approximately 45 d prepartum where cows received a low energy diet (≈1.35 Mcal/kg) containing no monensin. Dry cows were moved into the maternity barn approximately 1 wk before starting the study. Cows were allowed ad libitum access to the designated treatment rations by an electronic gating system (Roughage Intake System, Insentec B.V., Marknesse, The Netherlands), one cow assigned per gate. After parturition, cows were moved into a tie-stall facility where they remained through 21 d postpartum. Individual feed bunks in the tie-stall facility were suspended from load cells and bunk weight was monitored continuously by computer. Feed weights and times were stored prior to and immediately after any deviation in bunk weight. Dry cows were fed twice daily (0800 and 1530 h) to accommodate the capacity of the feeding system used prepartum, and lactating cows were fed once daily (1500 h) to minimize the time during which feeding behavior could not be recorded. All feeding activity, including meal length and size, were recorded electronically. As-fed feed intake of each cow was recorded on a daily basis. As-fed ration consumption was adjusted for DM content for determination of meal and daily DMI. Dry matter percentage was determined weekly for the corn silage and bimonthly for each concentrate ingredient; these values were used to determine ration DM for each week. Water was offered ad libitum, and individual water consumption was also recorded daily throughout the study. During summer months, the maternity barn and tie-stall facility were both cooled using evaporative pads.

Cows were milked 3 times daily in a milking parlor, and milk yields were recorded at each milking. Milk samples were collected from each milking beginning at 4 DIM and continuing through 21 DIM. Samples were analyzed for concentrations of fat, true protein, lactose (B-2000 Infrared Analyzer; Bentley Instruments, Chaska, MN), urea nitrogen (MUN spectrophotometer, Bentley Instruments), and somatic cells (SCC 500, Bentley Instruments;

Heart of America DHIA, Manhattan, KS). Data from individual milkings were averaged by cow-day, using a statistical model to account for the random effect of milking. Energy-corrected milk yield was calculated as: 0.327 × milk yield + 12.86 × fat yield + 7.65 × protein yield (Dairy Record Management Systems, 2010). Solids-corrected milk production was calculated as: 12.3 × fat yield + 6.56 × SNF yield - 0.0752 × milk yield (Tyrrell and Reid, 1965).

Body weight was measured 2 h prior to feeding on d -21 and -7 relative to expected calving, and on d 1, 7, and 21 postpartum. Immediately after BW was obtained, liver samples were collected via needle biopsy. For collection of liver tissue, an area spanning the 10th and 11th ribs was shaved, aseptically prepared, and anesthetized with 3.5 mL of subcutaneous lidocaine hydrochloride (Agri Laboratories, St. Joseph, MO). After 5 min, a #10 blade (Feather Safety Razor, Kita-Ku, Osaka, Japan) was used to make a stab incision into the body wall. A 14-gauge × 15 cm biopsy needle (SABD-1415-15-T, US Biopsy, Franklin, IN) was inserted through the incision and 200 mg of tissue was collected (a total of 10 biopsies). Liver samples were snap-frozen in liquid nitrogen immediately after collection, then stored at -80°C until subsequent analysis. Blood samples were collected from the coccygeal vessels after each biopsy and also 2 h prior to feeding on d -4 relative to expected calving date and on d 4 and 14 postpartum. Approximately 14 mL of blood was collected into 2 tubes, one containing potassium EDTA and the other containing potassium oxalate with sodium fluoride as a glycolytic inhibitor (Vacutainer, Becton Dickinson, Franklin Lakes, NJ).Blood samples were centrifuged at 2000 x g for 10 min immediately after sample collection, and plasma was harvested and frozen at -20°C until subsequent analysis of glucose, NEFA, BHBA, insulin, and haptoglobin concentrations. Body condition score (BCS) was evaluated by 3 trained investigators independently on the same day (+/- 1 d) as BW and liver sample collections.

Indwelling ruminal pH probes (Rumen Sensors, Kahne Limited, Auckland, New Zealand) were delivered to the rumen as an oral bolus after liver biopsy on d 1 postpartum. These probes measured rumen pH every 5 min and used a radio frequency to transmit this data to a computer. Electronic data were collected in real time and were also stored on the probe. Stored data were downloaded during the biopsy on d 7 to ensure collection of all recorded data. Measurement of rumen pH was limited to the first 7 DIM because of concern about drift in pH measurements from probes remaining in situ for more than 7 d. Probes removed from 2 cows (cannulated for previous studies) on d 7 postpartum generated pH values of 6.94 and 6.96 in pH 7.0 buffer and 4.01 and 3.97 in pH 4.0 buffer, suggesting that data analyzed here were valid.

Liver function was assessed using an in vivo caffeine metabolism test on d 7 postpartum (Lakritz et al., 2006). To conduct this test, jugular catheters (#1411, Mila International, Erlanger, KY) were inserted and caffeine was administered as caffeine-sodium benzoate (C4144, Sigma-Aldrich Co., St. Louis, MO) in a sterile pyrogen-free normal saline solution (25 mg of caffeine/mL of solution). Caffeine was infused intravenously in a bolus dose at the rate of 1 mg caffeine/kg BW, and the d 7 postpartum BW was used to calculate the amount of caffeine to infuse. Caffeine infusions were initiated at feeding time. Blood samples were collected immediately prior to infusion and at 30-min intervals for 180 min following infusion using disposable 5-mL syringes. Blood was immediately transferred to a tube containing potassium EDTA (Vacutainer, Becton Dickinson, Franklin Lakes, NJ). Plasma was harvested as described above and frozen at -20°C until analysis. Catheter patency was maintained by flushing with 6 mL of sterile 3.5% sodium citrate solution following each collection.

Facilities and equipment were observed daily for abnormalities. Postpartum cows (and prepartum cows with abnormalities) underwent a health inspection daily, including monitoring

for urine ketones (ReliOn ketone test strips, Bayer Healthcare LLC., Mishawaka, IN) and rectal temperature. Health records were kept throughout the study to register the incidence of ketosis, left displaced abomasum, retained placenta, metritis, milk fever, lameness, and other abnormalities. Ketosis was defined as a urine ketone concentration > 80 mg/dL, or urine ketone concentrations > 40 mg/dL for more than 2 consecutive days. Mastitis was defined as a SCC greater than 200,000 at any milking after d 3 (Dohoo et al., 2011). Other diseases were diagnosed according to definitions established by Kelton et al. (1998). If cows displayed signs of any disorder described they were treated according to on-site standard operating procedures. Cows were removed from the study if they were diagnosed with a displaced abomasum (n = 3) or severe lameness (n = 1). Data obtained from these cows prior to removal from the study were included in all analyses. Cows diagnosed with a displaced abomasum were removed on d 7 (control), 8 (monensin), and 13 (control) postpartum, respectively. The cow removed for lameness issues was removed on d 7 (control) postpartum.

Liver and Plasma Analyses

Approximately 20 mg of liver was placed in 500 µL of chilled phosphate-buffered saline (pH 7.4) and homogenized. The homogenate was centrifuged at 2000 x g for 10 min at 4°C and 100 µL of the supernatant was then removed for free glycerol and total protein analysis. Triglyceride (TG) content was measured using a method adapted from Starke et al. (2010). The remaining liver homogenate was incubated with 100 µL of lipase (porcine pancreatic lipase, MP Biomedicals) for 16 h at 37ºC, and glycerol content was then determined by an enzymatic glycerol phosphate oxidase method (#F6428, Sigma-Aldrich Co.). Triglyceride content was calculated based on the difference between glycerol concentrations before and after lipase

digestion. Total protein content of the original homogenate was analyzed by a Coomassie blue (Bradford, 1976) colorimetric method (kit #23236, Thermo Scientific, Pierce, Rockford, IL). To avoid potential bias introduced by differences in moisture content of liver samples, liver TG concentration was normalized by protein concentration, which is unaltered in fatty liver (Fronk et al, 1980).

The mRNA abundance of cytosolic phosphoenolpyruvate carboxykinase (cPEPCK), carnitine palmitoyltransferase 1a (CPT1a), apolipoprotein B (Apo B), and ribosomal protein subunit 9 (RPS9) in liver tissue was determined by real-time PCR. Total RNA was isolated from liver samples using a commercial kit (RNeasy Lipid Tissue Mini Kit, Qiagen, Valencia, CA), and spectroscopy was used to quantify RNA (Nanodrop-1000, Nanodrop Technologies Inc., Wilmington, DE). Coding DNA was then synthesized from 2 µg of total RNA using a commercial kit (High-Capacity cDNA Reverse Transcription kit, Applied Biosystems, Foster City, CA). Quantitative real-time PCR was performed in triplicate with 1/20 of the cDNA product in the presence of 200 nM gene-specific forward and reverse primers (Table 2) using SYBR green fluorescent detection (ABI 7500 Fast, Applied Biosystems). Messenger RNA abundance was quantified using the delta delta Ct method, with RSP9 used to normalize values. Abundance of RPS9 mRNA within liver tissue did not differ in response to treatment or across days in the study (all $P > 0.50$), making it a valid reference gene.

Plasma was analyzed for NEFA using an enzymatic colorimetric procedure (NEFA-HR, Wako Chemicals USA, Richmond, VA), glucose by a colorimetric kit (kit #439-90901, Wako Chemicals USA), insulin by a bovine-specific sandwich ELISA (#10-1201-01, Mercodia AB, Uppsala, Sweden), haptoglobin by a bovine-specific ELISA (kit #2410-7 using 3000-fold dilution, Life Diagnostics, West Chester, PA), and BHBA using an enzymatic reaction (kit

#H7587-58, Pointe Scientific, Inc.). Absorbance was read on a spectrophotometer (Powerwave XS, Biotek Instruments, Winooski, VT) and calculations were performed using Gen5 software (Biotek Instruments).

High-performance liquid chromatography was used to quantify plasma caffeine following the procedures of Lakritz et al. (2006). Briefly, 500 µL of plasma was mixed with 500 µL of 0.8 M perchloric acid. The mixture was centrifuged at 14,000 × g for 20 min at 21°C, and 400 µL of the clarified supernatant was transferred to an autosampler vial containing 20 µL of 4 M NaOH. Vials were capped and 50 µL were injected into a Discovery BIO Wide Pore C18 guard column (2 cm x 4 mm, 5 µm particle size; Supelco #568272-U, Sigma-Aldrich) and a Discovery BIO Wide Pore C18 column (25 cm x 4.6 mm, 5 µm particle size; Supelco # 568222-U, Sigma-Aldrich). The photochemical reaction was carried out in a mobile phase consisting of 20 mM phosphate buffer (pH 3.0)-acetonitrile, 85:15 (v/v), at a flow rate of 1 mL/min. Absorbance was read at 273 nm using an Acutect 500 UV/Vis detector (#06-653-5, Thermo Fisher Scientific).

Data and Statistical analysis

Feeding behavior variables were calculated from logged data that included the start and end weights as well as start and end times of meals. To generate meaningful meal pattern data, feeding bouts are grouped into meals, but there is no broadly-accepted definition of what constitutes a meal. Previous reports have used minimum thresholds for intermeal intervals ranging from 2 to more than 40 min (Tolkamp et al., 2000). Selecting a threshold was complicated in this study because cows started in a pen setting and then moved into tie-stalls. Therefore, approaches to data analysis were examined from 4 studies conducted by different laboratories. In these studies, minimum intermeal intervals were defined as 7.5 (Dado and Allen,

1993), 8 (Heinrichs and Conrad, 1987), 20 (Vasilatos and Wangsness, 1980), and 27.74 min (DeVries et al., 2003). For our feeding systems and housing situations, a 12-min intermeal interval was determined to be appropriate, because it is within the range of previously-used thresholds and because it generated meal frequencies similar to those reported in the studies above. Therefore, meals were combined if the intermeal interval was less than 12 min. After combining meals accordingly, any meal < 0.2 kg DM was excluded from behavior analysis. Ruminal pH data were analyzed to determine mean pH, standard deviation, amount of time spent below pH 5.8, and area (pH × min) under pH 5.8 for each cow each day. The threshold of 5.8 is representative of subacute ruminal acidosis according to previously established standards (Dohme et al., 2008). Caffeine elimination half-lives were determined by plotting post-infusion caffeine concentrations over time for each animal, performing an exponential regression on these values ($y = a \times e\text{-}bx$, where a = intercept and b = slope), and using the equation: half-life = ln 2/slope (Lakritz et al., 2006). Distribution volume was determined as the dose of caffeine infused divided by the intercept.

Differences in caffeine elimination were determined using the REML procedure of JMP (version 8.0, SAS Institute, Cary, NC) with the fixed effect of treatment and the random effect of cow. Other data were analyzed using mixed models with repeated measures over time (SAS 9.1, SAS Institute). Spatial power covariance structures were used to model repeated measures over time within cow for data with time points that were not equally spaced (BW, BCS, and plasma and liver variables). For data collected daily (DMI, pH, feeding behavior, and production variables), autoregressive (AR[1]) covariance structures were used. Fixed effects were treatment, day relative to parturition, and treatment × day. Individual cows were treated as a random effect. Values were deemed outliers and omitted from analysis when Studentized residuals were greater

than > |3.0|. After initial outlier removal, the model was repeated and Studentized residuals greater than > |3.5| were deemed outliers. No more than 3% of data were removed from any single analysis. Plasma haptoglobin, insulin, liver TG, and CPT1a and Apo B mRNA data were log-transformed prior to analysis to achieve normal residual distributions, and the reported means and standard errors were back-transformed (Bland and Altman, 1996). Milk SCC data were transformed to somatic cell linear score (Schukken et al., 2003). Prepartum and postpartum measures were analyzed separately for DMI, water intake, and feeding behavior. Effects were declared significant at $P < 0.05$, and trends are discussed at $P < 0.10$.

Results and Discussion

No significant difference occurred between treatments in deviation from expected calving date (-2.0 vs. -0.8 ± 1.0 d for control and monensin, respectively; $P = 0.40$). Actual calving dates ranged from 10 d prior to expected calving to 7 d after expected calving.

Feed Intake, Rumen pH, Body Weight, Body Condition, and Milk Production

Daily DMI is shown in Figure 1A. Intake of DM decreased prior to parturition and increased after parturition in both groups ($P < 0.001$), which resembles intake patterns observed in many transition cow studies (Ingvartsen and Andersen, 2000). Dry matter intake was not affected by treatment pre- or postpartum. This was surprising because other transition cow research has shown monensin to have an effect. Sauer et al. (1989) reported 1.2 kg/d lower DMI of cows fed 30 g monensin/ton of DM compared to cows receiving no monensin from 1 wk prepartum through 3 wk postpartum. Shah et al. (2008) observed a linear decrease in DMI over a complete lactation when monensin was fed at 0, 7, 15, or 22 g/ton of DM. The reports of Sauer et

al. (1989) and Shah et al. (2008) align with the 2.3% decrease in DMI concluded by meta-analysis using data from 4,445 dairy cows in all stages of lactation (Duffield et al., 2008b). In contrast, researchers found that monensin accelerated the rate of DMI increase in early lactation (Shah et al., 2008) and increased postpartum DMI in monensin-supplemented cows (Schroeder et al., 2009), although treatment means were not reported in these abstracts.

Although intakes were similar, monensin supplementation tended to decrease time between meals (Table 3 and Figure 1B; $P < 0.08$), which is consistent with reports showing that inclusion of monensin in feedlot diets results in more consistent feed intake patterns throughout the day (Burrin et al., 1988; Erickson et al., 2003). Even though the intermeal interval was shorter with monensin supplementation, the number of meals consumed per day and average meal duration did not differ between treatments. Although meal sizes were similar overall (Table 3), a treatment × day interaction was observed for postpartum meal size (effects on d 8, 15, 20, and 21, Figure 1C). The small increase in meal frequency, coupled with similar to larger meal sizes, resulted in small, non-significant increases in DMI for monensin-supplemented cows during the postpartum period. Lunn et al. (2005) observed similar meal pattern results during a SARA challenge with mid-lactation cows, and concluded that monensin impacts feeding behavior during times when rumen pH is low. In their study, mean ruminal pH was greater than the 5.9 observed in our study (Lunn et al., 2005). This implies that the cows in our study were experiencing SARA to a greater degree, and that decreased intermeal interval in fresh cows on monensin treatment may have been related to the low ruminal pH observed. However, monensin's effects in this study were not dependent on the presence of SARA. Cows received a low energy diet prepartum that should not have caused SARA, yet we still observed a shorter

intermeal interval prepartum, suggesting that another mechanism must be involved in this response to monensin.

As expected, DMI was noticeably different pre- and postpartum; however, the dramatic decrease in meal length (Table 3) for postpartum cows compared to prepartum cows likely reflects differences in feeding behavior of cows in tie-stall vs. pen housing rather than a true stage of production effect. Devries et al. (2003) indirectly examined this hypothesis and found that cows housed in a free-stall barn consumed dramatically fewer and larger meals than cows housed in tie-stall facilities (Vasilatos and Wangsness, 1980; Dado and Allen, 1995). These 3 studies all investigated early lactation cows, and it was suggested that social interactions result in less frequent access to feed in a free-stall situation, and that when cows gain access to the feedbunk, they remain there even when not eating (DeVries et al., 2003).

Daily water consumption did not differ between treatments throughout the experimental period (Table 3). Water intake of both groups was steady throughout the 21-d prepartum period. Postpartum water intake started around 80 L/d, and, as expected, increased ($P < 0.001$) to approximately 115 L/d by d 21.

Mean ruminal pH and the total time/d ruminal pH was below 5.8 were not affected by treatment or day ($P > 0.28$; Table 4) during the first 6 d postpartum. Some researchers suggest that a key role of monensin is to alter ruminal pH, but that lactate needs to exceed ≈5 mM for monensin to have such an effect (Osborne et al., 2004). Thus, fresh cow diets likely do not contain enough rapidly fermentable carbohydrates to promote excessive levels of lactic acid. If this is true, it would explain why monensin typically increases pH in feedlot studies (Nagaraja et al., 1981; Burrin and Britton, 1986) but few effects have been observed in dairy cattle. The lack of an effect on mean ruminal pH and time below 5.8 coincides with results from both transition

(Mutsvangwa et al., 2002; Fairfield et al., 2007) and mid-lactation cow studies (Ruiz et al., 2001; Osborne et al., 2004; Mathew et al., 2011); however, these studies did not examine differences in variance of ruminal pH. In our study, cows supplemented with monensin had a smaller standard deviation of ruminal pH (Figure 2; $P < 0.02$) on d 1 postpartum, but no differences were detected beyond d 1. The more stable ruminal pH in monensin-supplemented cows could have facilitated quicker adaptation of ruminal microflora to the lactation diet, and may be related to the shorter intermeal interval in this group. Notably, an effect of day was detected for area under pH 5.8 ($P < 0.03$), with a relatively steady increase from 113 min × pH units on d 1 to 169 ± 18 min × pH units on d 6 postpartum (data not shown).

Cows receiving monensin tended to have a lower body weight on d 1 postpartum ($P < 0.09$), but no other differences were observed between treatments for BCS or BW (data not shown). On average, cows lost 0.6 BCS units (3.3 to 2.7) and 110 kg BW (767 to 657 kg) during the experiment. Milk production (39.0 vs. 39.3 ± 1.7 kg/d for control and monensin, respectively; $P = 0.92$) and concentrations of fat, protein, lactose, and SNF did not differ ($P > 0.18$) between dietary treatments, but MUN was higher for monensin-supplemented cows (11.8 vs. 10.4 ± 0.42 mg/dL; $P < 0.02$). There is no a clear explanation for the observed effect on MUN. It has been shown that impaired liver function associated with lipid accumulation results in decreased ureagenic capability (Strang et al., 1998), but neither liver TG content nor the caffeine clearance test demonstrated dramatic effects of monensin on liver health. The MUN response could also be a result of monensin's ruminal protein sparing effect, allowing more escape protein to reach the small intestine. Duffield et al. (1998) observed a rise in blood urea nitrogen in early postpartum cows given monensin controlled-release capsules and suggested it could be related to gluconeogenesis from nonessential amino acids, because the prerequisite deamination results in

increased urea production. Blood urea nitrogen distributes freely into body fluids, including milk, so this proposed mechanism could help explain why MUN was higher in cows fed monensin. This is something of a paradox, however, because if monensin increases propionate supply, it may decrease the need to use amino acids for glucose production, potentially limiting ureagenesis. A simpler explanation is to consider that monensin-supplemented cows consumed, on average, an additional 0.23 kg/d of CP, with no increase in milk protein yield. If metabolizable protein supply did not limit milk protein yield in either group, then the increase in MUN for the monensin treatment was an expected response to increased CP intake.

Metabolic and Endocrine Changes

Plasma NEFA, BHBA, glucose, and insulin concentrations are displayed in Figure 3. As expected, plasma NEFA concentrations increased dramatically from 222 ± 80 µM 21 d before expected calving to a peak of 878 ± 80 µM on d 1 postpartum (Figure 3A; $P < 0.001$). Monensin supplementation did not significantly alter NEFA concentrations throughout the study. This was somewhat unexpected, because a meta-analysis including 24 studies with plasma NEFA data demonstrated that monensin could decrease NEFA concentration (Duffield et al., 2008a). However, the small mean response to monensin (36.6 µEq/L) reported in the meta-analysis was similar to the numerical difference between treatments in this study, suggesting that the current study was simply underpowered to detect such an effect.

Monensin treatment decreased plasma BHBA over the course of the entire study (734 vs. 616 ± 40.9 µM; $P < 0.05$) with a significant effect of day ($P < 0.001$) and a treatment × day interaction ($P < 0.01$; Figure 3B). Most notably, monensin significantly decreased plasma BHBA on d 4 postpartum (777 vs. 1077 ± 71 µM; $P < 0.01$). The effect on BHBA is not surprising

given that almost all relevant publications have reported similar decreases in BHBA (Duffield et al, 2008a). Lower BHBA concentrations are likely a result of more complete fatty acid oxidation in the liver. Monensin can increase the supply of propionate to the liver (Van Maanen et al., 1978), which could redirect acetyl-CoA toward oxidation in the TCA cycle and away from ketone production (Allen et al., 2009).

Plasma glucose concentrations decreased after parturition in both groups ($P < 0.001$), but monensin did not affect plasma glucose concentrations pre- or postpartum. Meta-analysis indicated that monensin can increase plasma glucose concentration of transition cows, but increases were not consistently reported (Duffield et al., 2008a). Furthermore, because monensin does not always affect ruminal propionate production in transition cows (Markantonatos et al., 2009), we would not necessarily expect substrate-driven changes in gluconeogenic flux, although monensin could also alter gluconeogenic enzyme capacity (Karcher et al., 2007). Arieli et al. (2001) did not observe changes in blood glucose concentration, but observed an increase in distribution space and glucose pool size when feeding monensin to prepartum cows, suggesting increased uptake of glucose by peripheral tissues in response to monensin. If monensin does, in fact, alter clearance of plasma glucose, then plasma glucose concentration is a poor proxy for gluconeogenic flux and glucose turnover data are required to evaluate the effects of monensin on this pathway accurately. Data in this area are limited, but in one study, rate of appearance of glucose was 10% higher for monensin supplemented cows on an equivalent DMI basis (Wheelock et al., 2009).

Overall, monensin treatment did not affect plasma insulin concentration. However, effects of day ($P < 0.001$) and treatment × day interaction ($P < 0.05$) were significant. The treatment × day interaction showed a tendency for higher plasma insulin concentration in

monensin-fed cows on d 7 postpartum ($P < 0.08$). In the meta-analysis by Duffield et al. (2008a), data from 5 relevant reports in which plasma insulin was measured did not demonstrate an effect of monensin on insulin concentration in transition cows. Increased insulin concentration appears unlikely to be a primary mechanism by which monensin alters periparturient metabolism.

A day effect ($P < 0.001$) was detected for plasma haptoglobin concentration (Figure 4) as a result of an increase in the early postpartum period. Haptoglobin is an acute phase protein that increases in concentration during times of inflammation (Bionaz et al., 2007); therefore, we expected the effect of day during the transition period. Monensin did not significantly alter haptoglobin concentrations. The numerical difference between treatments on d 1 postpartum, however, seemed to correspond with the findings of Crawford et al. (2005), who reported elevated haptoglobin in diseased transition heifers given monensin. We attempted to assess whether a similar differential response could be found in our data, but were unable to conduct a valid analysis because only 1 cow on the monensin treatment had an observed health disorder before d 4 postpartum.

Liver Indices

Many metabolic fuels are oxidized or synthesized by the liver, making the health and function of this organ extremely important to early lactation dairy cows. In the present study, TG content, mRNA abundance of key genes, and caffeine clearance were used as measures of the liver's metabolic function.

Liver TG content throughout the experiment is shown in Figure 5A. All cows experienced an increase in liver TG following parturition ($P < 0.001$). A trend for a treatment × day interaction was detected ($P < 0.09$), driven primarily by a tendency for increased liver TG

content in monensin-treated cows on d -7 ($P < 0.09$) and the numerical difference in the opposite direction observed on d 7 postpartum. Little data exists reporting transition cow liver TG content in response to monensin, but one group reported a tendency for animals administered monensin to have lower liver TG content ($P = 0.12$) and higher glycogen content ($P = 0.02$) 3 wk into lactation (Zahra et al., 2006).

A major source of metabolic fuel and substrate for liver TG synthesis in transition cows are NEFA. Carnitine palmitoyl transferase 1a is important for translocating FA from the cytosol into the mitochondria, making it a central component for determining oxidative flux of FA within the liver (Drackley, 1999). If a cow fed monensin is more efficient at oxidizing FA, less liver TG accumulates, which has positive implications for transition cow health (Herdt, 1988). Results from this study indicated that liver CPT1a mRNA was greater in cows fed monensin (0.10 vs. 0.15 ± 0.002 arbitrary units; $P < 0.04$, Figure 5B). The difference was driven mainly by effects on d -7 and 1 (both $P < 0.05$). These results suggest a novel mechanism underlying the role of monensin in improving the overall health of transition cows. This response led us to evaluate whether treatment altered the rate of liver TG accumulation during this time period. A contrast statement was used to determine whether the increase in liver TG from d -7 to d 7 differed by treatment. The increase in liver TG concentration was significantly greater for the control compared to monensin (412 vs. 128 ± 83 mg TG/g protein over this period, $P = 0.03$). This differential rate of TG accumulation coincides with the treatment effects on liver CPT1a mRNA abundance. Previous findings also have suggested that monensin increases the liver's capacity to export VLDL (Mohebbi-Fani et al. 2006). This stimulated us to measure expression of Apo B in liver tissue from d -7 to 7, but treatment had no effect on Apo B mRNA abundance during this period ($P = 0.42$). Expression of Apo B was upregulated over this time period (0.18,

0.35, and 1.13 ± 0.28 arbitrary units for d -7, 1, and 7, respectively). Expression of Apo B appeared to adapt to the increased TG synthesis in the liver of these transition cows, but monensin did not regulate lipoprotein secretion through Apo B transcription in this experiment.

The evidence that monensin lowers transition cow plasma ketone concentrations is substantial (Duffield et al. 2008a), but most reports referenced in the meta-analysis of Duffield et al. (2008a) explain the effect on ketones through increased supply of propionate for hepatic gluconeogenesis. To our knowledge, our report is the first to consider the effects of monensin on abundance of CPT1a in dairy cows, and suggests that monensin has a positive effect on lipid metabolism in the liver. Although beta-oxidation of FA can result in either complete oxidation of acetyl-CoA or to ketone production, increased CPT1a abundance could benefit liver function because increased mitochondrial oxidation could 1) limit TG synthesis, 2) decrease reliance on peroxisomal oxidation with its subsequent production of reactive oxygen species, and 3) prevent accumulation of lipid metabolites that may impair metabolic function (FA-CoA, ceramides, peroxides, etc.). Nevertheless, these findings should not be over-interpreted because our results are limited to the transcript level, and we did not observe obvious corresponding decreases in liver TG content or hepatic inflammation (as measured by plasma haptoglobin concentration).

The liver is the primary site for gluconeogenesis, and cPEPCK is thought to be a rate-determining enzyme for hepatic gluconeogenesis from propionate, lactate, and amino acids (Greenfield et al., 2000). The relative abundance of liver cPEPCK mRNA differed across day of study ($P < 0.02$), but was not different between treatments. The slight change through the transition period (an 85% increase from d 1 to d 21 postpartum) is consistent with previous reports (Greenfield et al., 2000; Hartwell et al., 2001); however, when monensin was fed to transition cows, cPEPCK expression increased (Karcher et al., 2007), so it was surprising that no

differences were detected in this study. Because cPEPCK is one of several rate-determining gluconeogenic enzymes, this is consistent with the lack of treatment effect on plasma glucose concentration, although mRNA abundance is not a measure of enzyme activity and plasma glucose concentration is not a measure of gluconeogenic flux.

Liver diseases, including fatty liver, decrease activity of the cytochrome P450 (CYP450) enzyme complex (Frye et al., 2006), and activity of CYP450 can serve as an index of normal liver function. We assessed metabolism of caffeine because it is metabolized by CYP450 and has few side effects when administered at low doses (Lakritz et al., 2006). Results of the caffeine challenge tests are shown in Table 4. Caffeine elimination half-life was 226 and 208 ± 13.5 min for control and monensin cows, respectively, and no differences were detected ($P = 0.34$). The volume of distribution of caffeine also was calculated, and similar results across treatments suggest no bias in administering the caffeine dose; thus, our results do not indicate that CYP450 activity was enhanced or impaired by monensin treatment. Reports of caffeine clearance in dairy cows are limited. DeGraves et al. (1995) examined hepatic function of later lactation dairy cows and reported an average elimination half-life of 228 min (range was 156 to 414 min), which was remarkably similar to our results. Caffeine elimination half-life also was correlated with log-transformed plasma haptoglobin concentration ($P = 0.03$, $r = 0.41$), similar to results from a separate study conducted in our laboratory (Morey et al., 2011). These positive correlations between markers of impaired liver function and liver inflammation are consistent with their utility as gauges of metabolic health in transition cows.

Management and Health

Incidence of health disorders are shown in Table 5. No differences were found between treatment groups. Because only 32 experimental units were used for this study, detecting differences in disease incidence would have been difficult. A more powerful assessment of the effects of monensin on cow health is the meta-analysis conducted by Duffield et al. (2008c). These researchers combined data from 16 papers; overall, monensin significantly decreased the relative risk of ketosis, displaced abomasum, and mastitis. The BHBA response observed in our study is consistent with the means reported in the meta-analysis (Duffield et al., 2008c). Therefore, monensin likely lowers the risk of diseases such as ketosis and displaced abomasa because these diseases are related to energy status, although our study lacked statistical power to detect these differences.

Conclusions

In this first report of monensin's effects on feeding behavior combined with ruminal pH dynamics in transition cows, monensin increased meal frequency and minimized ruminal pH variance in the first day after cows changed to a lactation ration. Monensin supplementation also significantly increased liver mRNA abundance of CPT1a, a key mediator of liver mitochondrial FA oxidation, and decreased the rate of liver TG accumulation in the 2 wk around parturition. Consistent with previous results, monensin significantly decreased peak plasma BHBA concentrations in postpartum cows, but did not alter concentrations of plasma NEFA, glucose, or insulin in the postpartum period. Despite the observed beneficial effects on metabolism, no significant effects on milk production or disease incidence were detected.

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Figure 3.1 Dry matter intake (A), intermeal interval (B), and meal size (C) during the experimental period. A. There was an effect of day pre- and postpartum ($P < 0.001$); prepartum SEM = 0.88, postpartum SEM = 0.87. **B.** Monensin shortened intermeal interval prepartum ($P < 0.03$) and tended to shorten intermeal interval postpartum ($P < 0.08$). There was an effect of day postpartum ($P < 0.001$); prepartum SEM = 10.6, postpartum SEM = 4.77 († denotes $P < 0.10$; * denotes $P < 0.05$). **C.** There was an effect of day pre- and postpartum on meal size ($P < 0.001$), and a treatment × day interaction was detected postpartum ($P < 0.02$); prepartum SEM = 0.18, postpartum SEM = 0.09.

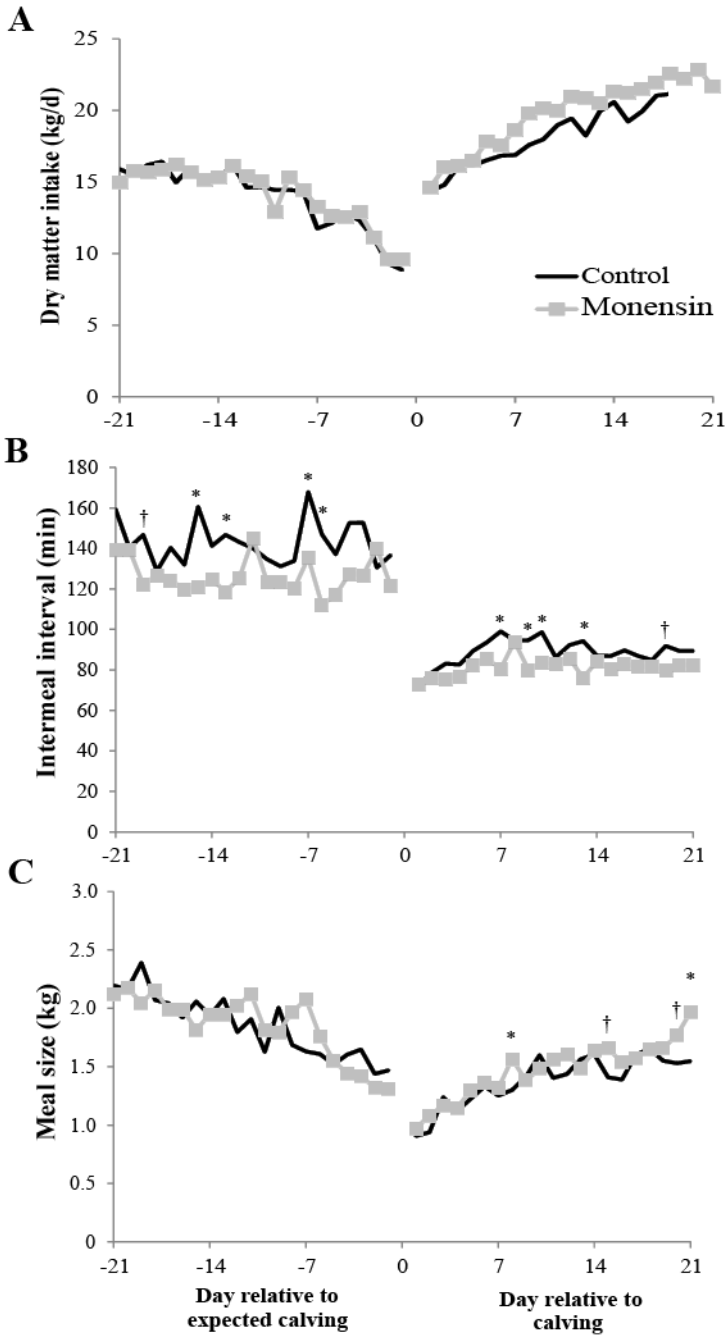

Figure 3.2 Standard deviation of rumen pH until d 7 postpartum. Monensin decreased standard deviation of ruminal pH for the first day after calving ($P < 0.02$). SEM = 0.02 (*denotes $P < 0.05$).

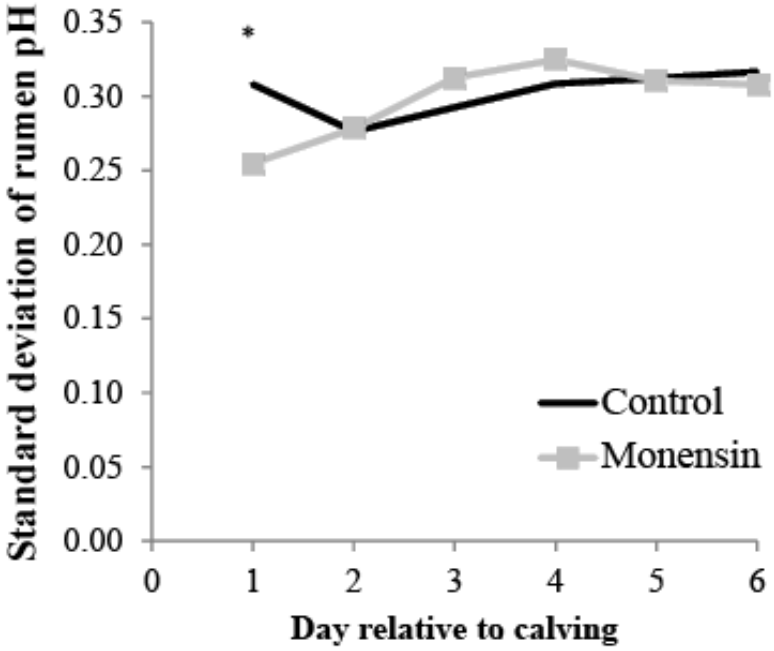

Figure 3.3 Plasma concentrations of nonesterified fatty acids (A), beta-hydroxybutyrate (B), glucose (C), and insulin (D) during the experimental period. A. No treatment effects were detected, but there was a day effect ($P < 0.001$); SEM = 84.9. **B.** Effects of treatment ($P < 0.05$), day ($P < 0.001$), and treatment × day interaction ($P < 0.01$) were detected; SEM = 73.6 (*denotes $P < 0.05$). **C.** No treatment effects were detected, but a day effect ($P < 0.001$); SEM = 2.38. **D.** Significant effects of day ($P < 0.001$) and day × treatment interaction ($P < 0.05$) were detected. Cows receiving monensin tended to have higher plasma insulin concentrations on d 7 postpartum. SEM are shown on D († denotes $P < 0.10$).

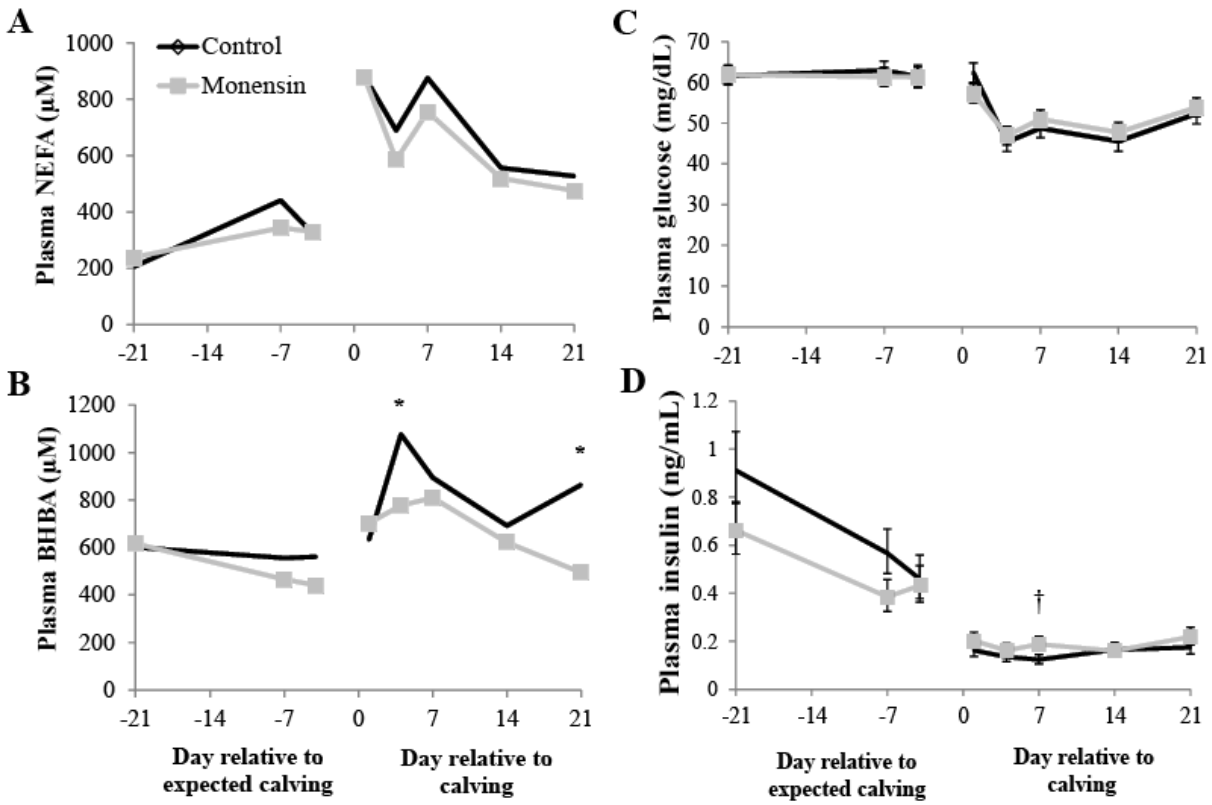

Figure 3.4 Plasma concentrations of haptoglobin during the experimental period. No treatment differences were detected; however, an effect of day was detected ($P < 0.001$). SEM are shown on the graph.

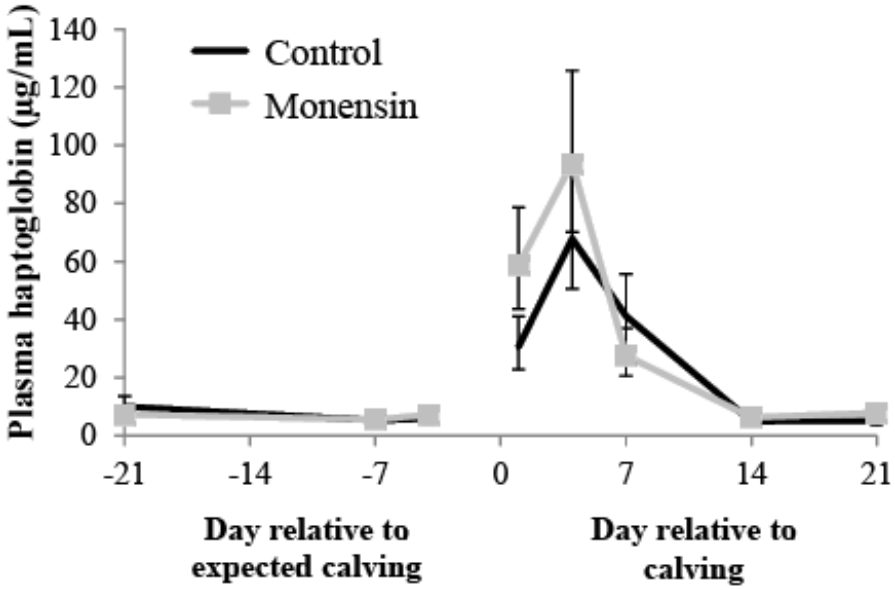

Figure 3.5 Triglyceride content (A) and messenger RNA abundance of carnitine palmitoyl transferase 1a (B) in liver tissue during the experimental period. A. A tendency for a treatment × day effect was detected ($P < 0.09$). Liver triglyceride (TG) content increased in both groups during the study period ($P < 0.001$); SEM are shown on the graph (†denotes $P < 0.10$). **B.** Monensin significantly increased abundance of carnitine palmitoyl transferase 1a (CPT1a) relative to the control gene ribosomal protein subunit 9 (RPS9; $P < 0.04$); SEM are shown on the graph (*denotes $P < 0.05$).

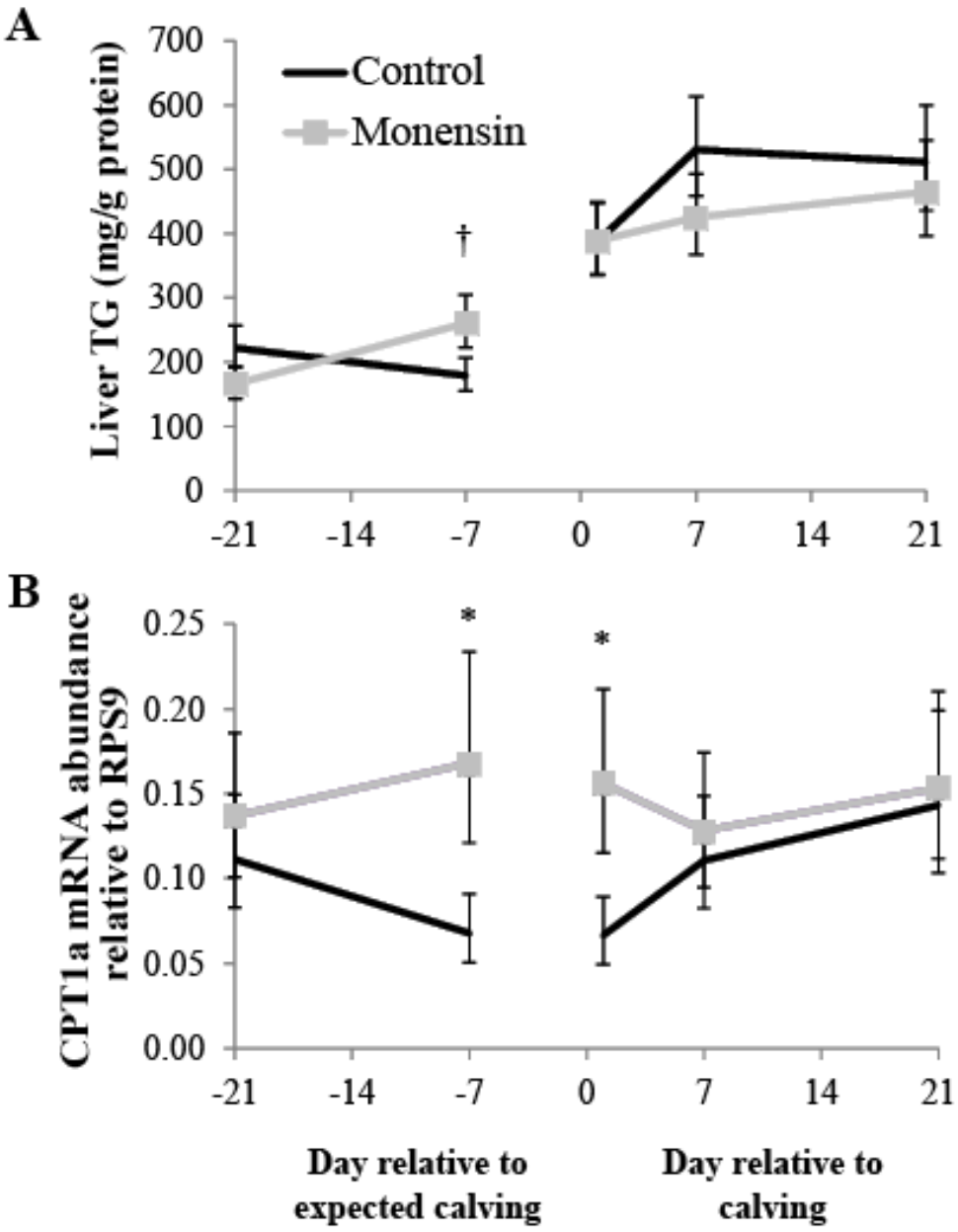

Table 3.1 Ingredient and nutrient composition of diets

	Prepartum	Postpartum
Ingredient, % of DM		
Corn silage	30.3	34.0
WCGF[1]	19.6	21.5
Prairie hay	39.7	-
Alfalfa hay	-	16.6
Cottonseed	-	6.7
Corn grain[2]	6.5	12.4
Soybean meal 48	4.1	-
Expeller soybean meal	-	7.1
Micronutrient premix[3,4]	0.3	2.8
Nutrient, % of DM		
DM, % as-fed	57.2	54.2
CP	13.1	17.3
ADF	28.4	19.7
NDF	49.9	36.0
NFC	35.5	38.0
Ether extract	3.4	5.0
Ash	6.9	8.8
NE_L, Mcal/kg[5]	1.58	1.68

[1]Wet corn gluten feed; Sweet Bran, Cargill, Inc.
[2]A portion of the corn grain (0.91 kg/cow daily) served as the top-dress carrier for 400 mg monensin (Rumensin®, Elanco Animal Health, Greenfield, IN) for supplemented cows. The same amount of corn alone was top-dressed for control cows.
[3]Prepartum premix consisted of 42.6% vitamin E premix, 11.9% Se premix, 11.6% trace mineral salt, 10.8% limestone, 9.71% vitamin A premix, 6.47% 4-plex, 4.31% vitamin D premix, 2.17% magnesium oxide, and 0.53% ethylenediamine dihydroiodide.
[4]Postpartum premix consisted of 48.4% limestone, 27.3% sodium bicarbonate, 12.6% trace mineral salt, 6.04% magnesium oxide, 2.33% 4-plex, 1.51% Se premix, 1.16% vitamin E premix, 0.46% vitamin A premix, 0.21% vitamin D premix, and 0.03% ethylenediamine dihydroiodide.
[5]Estimated according to NRC (2001).

Table 3.2 Primers used for RT-PCR detection of transcripts in liver tissue

Gene	Accession number[1]	Forward primer Reverse primer	Region amplified[2]
CPT1a	DV820520.1	CTTCCCATTCCGCACTTTC CCATGTCCTTGTAATGAGCCA	616 – 719
cPEPCK	NM_174737.2	CGAGAGCAAAGAGATACGGTGC TGACATACATGGTGCGACCCT	427 – 562
ApoB	XM_583270.3	TCCTTGATTCCACATGCAGCT GGTGTGCAAAGGATGCGTTAG	8610 – 8720
RPS9	DT860044.1	GAACAAACGTGAGGTCTGGAGG ATTACCTTCGAACAGACGCCG	233 – 344

[1]From NCBI Entrez Nucleotide Database (http://www.ncbi.nlm.nih.gov/sites/entrez?db=nucleotide).
[2]Amplicons span an exon-exon boundary, as predicted by aligning the specified sequence to the bovine genome using Splign (http://www.ncbi.nlm.nih.gov/sutils/splign).

Table 3.3 Mean feed and water intake and feeding behavior during the transition period

	Control	Monensin	SEM	*P*-value
Prepartum water intake, L/d	20.6	19.1	1.6	0.48
Prepartum DMI, kg/d	13.9	14.1	0.6	0.83
Intermeal interval, min	143	126	5.0	0.03
Meal frequency, d^{-1}	7.57	8.05	0.36	0.35
Meal size, kg DM	1.85	1.85	0.12	0.99
Meal length, min	43.4	42.9	2.3	0.88
Postpartum water intake, L/d	101.6	101.6	2.7	0.99
Postpartum DMI, kg/d	18.4	19.8	0.6	0.14
Intermeal interval, min	88.8	81.4	2.9	0.08
Meal frequency, d^{-1}	13.7	14.8	0.5	0.12
Meal size, kg DM	1.38	1.47	0.06	0.29*
Meal length, min	14.1	14.5	0.6	0.65

*Treatment × day interaction detected ($P < 0.02$)

Table 3.4 Rumen pH parameters from days 1 through 7 and results of day 7 caffeine clearance test

	Control	Monensin	SEM	*P*-value
Ruminal pH measures				
Mean pH	5.90	5.89	0.04	0.84
Standard deviation of ruminal pH	0.302	0.298	0.012	0.80*
Time under pH 5.8, min/d	569.3	583.5	62.1	0.87
Area under pH 5.8, pH × min/d	140.1	143.2	20.2	0.91
Caffeine clearance measures				
Half-life, min	226.3	207.9	13.5	0.34
Distribution volume, L	444.0	445.7	15.0	0.94

*Treatment × day interaction detected ($P < 0.05$)

Table 3.5 Incidence of health disorders during the experimental period

Disorder[1]	Control	Monensin
RP	0	1
Fever > 39.4°C	5	7
Ketosis	5	3
Hypocalcemia	2	0
Metritis	1	1
Mastitis	7	5
Displaced abomasum	2	1
Other digestive disorder	4	3
One or more disorders	12	11
Dystocia[2]	3	5

[1]No differences were detected between treatment groups ($P > 0.10$) using Fisher's exact test.

[2]Dystocia was defined as a calving difficulty score > 1; calving scores were defined according to Dairy Record Management Systems (2010).

Chapter 4 - Supplementing lysine and methionine in a lactation diet containing high concentrations of wet corn gluten feed did not alter milk protein yield: analysis of model predictions

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Abstract

Primiparous (n = 33) and multiparous (n = 63) lactating Holstein cows (186 ± 51 days in milk) were used to evaluate the effects of supplementing metabolizable AA using lysine in a matrix of Ca salts of fatty acids (Megamine-L, Arm & Hammer Animal Nutrition) and the isopropyl ester of 2-hydroxy-4-(methylthio) butanoic acid (HMBi; MetaSmart, Adisseo Inc.) in diets containing > 26% wet corn gluten feed (dry matter basis). Cows were blocked by production level, parity, and pregnancy status, then randomly assigned to 1 of 8 pens and allowed a 7 d adaption period before receiving treatments. The study consisted of two 28-d treatment periods, in which DMI and production were monitored daily and milk components analyzed 3 d/wk. Period (P) 1 and 2 data were analyzed separately using mixed models with repeated measures. During P1, pens were assigned randomly to either of 2 diets formulated to differ by metabolizable AA supply. Model predictions from the NRC (2001) indicated negative lysine (-8.1 g/d) and methionine (-1 g/d) balances for the control cows, while the Cornell Net Carbohydrate and Protein System 5.0 and 6.1 models indicated positive balances for these AA (+25.9 and 21.8 g/d for lysine; +14.7 and 18.9 g/d for methionine, respectively). Supplementing 30 g/d metabolizable lysine in a Ca soap matrix and 2.4 g/d metabolizable methionine as HMBi led to positive predicted lysine and methionine balances by all 3 models, and predicted metabolizable lysine:methionine ratios ranging from 2.9 to 3.1. No treatment effects were observed for dry matter intake or performance (mean milk production: 40.1 kg/d). Results provided no evidence of a lysine deficiency in the control diet, despite the negative lysine balance and low lysine:methionine ratio predicted by the NRC model. For P2, the treatment diet was modified to replace some wet corn gluten feed with corn silage, decrease dietary CP from 17.9% to 17.1% by removing expeller soybean meal, and further increase lysine and methionine supply. In P2, no treatment effects were observed for dry matter intake or milk production, but

MUN was decreased in the treated group (10.8 vs. 12.5 ± 0.2 mg/dL). Given the results from both periods, it is likely that the diet fed to control cows was not truly deficient in metabolizable lysine or methionine and that the NRC model underpredicted amino acid delivery to the small intestine, or supplemental AA products did not sufficiently escape ruminal degradation.

Keywords: dairy, by-product, model prediction

Introduction

Formulation of diets to meet the protein requirements of dairy cattle has evolved from a focus simply on CP to estimating MP supply, which attempts to account for ruminal protein degradation, microbial yield, and ruminal bypass protein. Formulating for MP has been shown to improve the efficiency of dietary nitrogen utilization (Leonardi et al., 2003; Noftsger and St Pierre, 2003; Chen et al., 2011), while possibly improving production (Schwab, 2010). However, formulating for an adequate MP supply may still fail to meet the requirements of the cow if AA profile is not considered. This concept has been widely recognized by the U.S. dairy industry; a survey conducted in 2010 indicated that 81% of nutritionists and veterinarians that formulate diets balance for AA (Dairy, 2010). Enhanced knowledge of protein nutrition has been directly responsible for this progression, but within our industry today the cost of protein sources, combined with environmental regulations, demand even more efficient use of dietary protein.

By-products from corn biofuel production are often used to provide protein and energy in lactation diets. In 2010, of the nutritionists and veterinarians who formulate rations and completed the survey, 92% used distillers grains or considered using them (Dairy, 2010). Many other by-products of corn milling are also fed to dairy cattle including corn germ meal, corn bran, corn meal, and corn gluten feed. Like corn grain, the predominant protein in these by-products is zein, which is known to be low in lysine (Coleman and Larkins, 1999). Kelzer et al. (2010) characterized the AA profile of 7 corn by-products and observed that RUP lysine was below 3.5% in all 7 feedstuffs. Therefore, it is not surprising when nutrition models predict that diets containing large concentrations of corn by-products do not supply enough lysine to high producing cows. It is also recognized that many lactation diets do not supply adequate methionine; thus, lysine and methionine have often been deemed first-limiting AA in lactation diets (Schwab et al., 1976; Schwab et al., 1992; NRC, 2001).

The objective of this study was to evaluate the effects of supplementing commercial rumen escape AA products in a diet that the NRC model (2001) predicted to be deficient in lysine and methionine supply. The products used to provide the additional AA contained lysine embedded within Ca salts of fatty acids (Megamine-L®, Arm & Hammer Animal Nutrition, Princeton, NJ) and the methionine precursor isopropyl ester of 2-hydroxy-4-(methylthio) butanoic acid (HMBi; MetaSmart®, Adisseo Inc., Antony, France). A secondary objective of this study was to compare current models with respect to predicting metabolizable AA supply in diets that contain large concentrations of the byproduct wet corn gluten feed.

Materials and Methods

Experimental procedures were approved by the Institutional Animal Care and Use Committee at Kansas State University.

Primiparous (n = 33) and multiparous (n = 63) Holstein cows (186 ± 51 DIM; mean ± SD) from the Kansas State University Dairy Cattle Teaching and Research Facility were used in this production study. Cows were blocked by milk production, parity, and pregnancy status. Cows within each block were then randomly assigned to 1 of 8 identical pens with 12 free stalls in each pen. Cows were moved into pens on May 3, 2010 and the study began on May 10, 2010, allowing for a 1-week adaptation period. During the adaption period, all pens received a common diet.

The study consisted of two 28-d treatment periods (P). During P1 pens were offered 1 of 2 rations that were formulated to differ in metabolizable AA supply (Table 1). During P2 the treatment diet was modified to decrease dietary CP and further increase lysine and methionine supply. Treatment diets were randomly assigned to pen. Cows were fed once daily at 110% of the expected intake. The amounts of feed delivered and refused were recorded on d 19, 20, 21,

26, 27, and 28 of each period. The TMR were analyzed for DM on those days. Samples of all dietary ingredients were collected on d 19, 21, 26, and 28 and composited into 1 sample per period for wet chemistry analysis by Dairy One Forage Laboratory (Ithaca, NY).

Cows were milked 3 times daily in a milking parlor, and milk yields were recorded at each milking. Milk samples were collected from every milking on each Monday, Wednesday, and Friday throughout the experiment. Samples were analyzed for concentrations of fat, true protein, lactose (B-2000 Infrared Analyzer; Bentley Instruments, Chaska, MN), and urea nitrogen (MUN spectrophotometer, Bentley Instruments; Heart of America DHIA, Manhattan, KS). Energy-corrected milk yield was calculated as: 0.327 × milk yield + 12.86 × fat yield + 7.65 × protein yield (Dairy Record Management Systems, 2010).

The chemical composition of the feed ingredients, intake data, production data, and animal descriptors were all entered into three computer programs/models: Spartan Dairy Ration Evaluator 3.0 (Michigan State University, East Lansing, MI) which is based on the NRC model (2001) with some additional improvements developed at Michigan State University; CPM-Dairy 3.0.10 from Cornell University, University of Pennsylvania, and Miner Institute, which uses the Cornell Net Carbohydrate and Protein System (CNCPS) 5.0 (Fox et al., 2004); and Nutritional Dynamic System v3 (RUM&M, Emilia, Italy) which uses the CNCPS 6.1 model. Models were used to determine the predicted supply of AA against model-specific requirements. For all 3 programs, a feed library was created so the nutrient profile of each dietary ingredient matched laboratory analyses. Megamine-L was assumed to be 16% digestible rumen bypass lysine on a DM basis, and HMBi was assumed to be 17% digestible rumen bypass methionine on a DM basis.

A total of 95 of the 96 cows completed the study; one cow was removed from the study due to mastitis. Intake data for each pen was divided by the number of cows present in that pen on that day. All data were analyzed using the mixed procedure of SAS (version 9.2, SAS Institute Inc., Cary, NC). All models included the fixed effects of day, treatment, and day × treatment interaction and the random effect of pen. Repeated measures over time were modeled with a first-order autoregressive covariance structure; the subject was pen for intake data and cow within pen for milk production and component data. Denominator degrees of freedom were estimated by the Kenward-Roger method. Significance was declared at $P < 0.05$ and tendencies at $0.05 \leq P < 0.10$.

Results and Discussion

In formulating experimental diets, the strategy was to maintain large concentrations of corn byproducts within diets. Therefore, the control diet (Table 1) contained 26.7% wet corn gluten feed (WCGF) on a DM basis. Wet corn gluten feed has been reported to contain as little as 6.5% RUP (% of CP; Kononoff et al., 2007), so a diet containing such a great quantity of WCGF would exacerbate any MP deficiency. The P1 treatment diet was similar to the control, with the primary differences being replacement of 190 g/cow of the Ca salts of fatty acids (Megalac-R, Arm & Hammer Animal Nutrition, Princeton, NJ) with a source of Ca salts of fatty acids that was embedded with lysine (Megamine-L), and addition of 14 g/cow of HMBi. As expected, the predicted supplies of metabolizable lysine and methionine were slightly elevated with AA supplementation (Figure 1), while other nutrient concentrations remained similar across treatments (Table 1).

Model predictions obtained through the NRC (2001) indicated negative lysine (-8.1 g) and methionine (-1 g) balances for the control cows, however CNCPS 5.0 and 6.1 predicted

positive balances for these AA (25.9 and 21.8 g for lysine; 14.7 and 18.9 g for methionine, respectively; Figure 1). Amino acid supplementation using the commercial products led to a predicted positive lysine and methionine balance by all 3 models in both periods.

Period 1 intake and production means for both groups are shown in Table 2. Mean DMI was 26.6 kg/d and mean milk yield was 40.1 kg/d, with means of 3.10% fat and 3.06% protein. No treatment effects were observed for any of the parameters measured. Milk protein yield, the variable of greatest interest, was numerically lower for the AA-supplemented diet compared to control (1.21 vs. 1.23 ± 0.02 kg/d, $P = 0.54$).

In P2, the treatment diet was modified such that 3.6% WCGF was replaced with corn silage, expeller soybean meal was decreased from 4.9 to 2.2% of diet DM, and Ca salt-lysine matrix and HMBi concentrations were increased by 0.5 and 0.14%, respectively. These changes resulted in a decrease in dietary CP from 17.9% to 17.1% with similar predicted lysine and methionine supply compared to the P1 treatment diet (Figure 1).

The performance of both groups of cows during P2 is shown in Table 3. Consistent with the decrease in dietary CP, MUN was decreased in the AA-supplemented group (10.8 vs. 12.5 ± 0.2 mg/dL, $P < 0.001$) without impacting milk production ($P = 0.51$). For this period, mean DMI was 24.4 kg/d and mean milk yield was 35.8 kg/d, with means of 3.18% fat and 3.04% protein. Beyond treatment effects on MUN, no effects were observed for any production parameters measured. As in P1, milk protein yield was numerically lower for the AA-supplemented diet compared to the control (0.99 vs. 1.02 ± 0.02 kg/d, $P = 0.20$).

Results from P1 do not support the hypothesis that increasing lysine and methionine supply would increase production of cows fed a corn byproduct based diet. There are a number of possible explanations for the lack of response. One possibility is that the products used to

provide supplemental methionine and lysine did not increase the metabolizable supply of these AA. However, past research has indicated that the lysine source used in this study provides 70% protection from ruminal degradation at 3 h and 47% protection at 12 h, suggesting effective post-ruminal delivery of lysine (Block et al., 2010; Zuccarello et al., 2011). For the HMBi, a calibration curve established by modeling the area under the curve response to increasing doses of methionine indicates that approximately 50% of HMBi is absorbed through the rumen wall, and thus escapes ruminal degradation (Graulet et al., 2005). The efficacy of HMBi is further supported by production responses showing that HMBi supplementation increases milk protein when production appears to be limited by metabolizable methionine supply (St Pierre and Sylvester, 2005; Phipps et al., 2008; Chen et al., 2011).

Another possible reason why lysine and methionine supplementation did not increase production in P1 is that something else was first-limiting in this scenario. Our study narrowly focused on lysine and methionine because substantial research has supported the focus on a lysine and methionine deficiency in diets similar to those fed in our study (Nichols et al., 1998; Wickersham et al., 2004; Vyas and Erdman, 2009; Patton, 2010), and infusion of lysine and/or methionine has increased production in several dairy nutrition studies (Schwab et al., 1976; Pisulewski et al., 1996; Varvikko et al., 1999). Histidine has often been observed as a limiting AA in dairy cattle diets, but the controlled research showing this was conducted with diets containing large proportions of grass silages, barley, oats, and canola, instead of corn sources (Schwab et al., 2005). Corn grain contains almost no tryptophan (Coleman and Larkins, 1999), so diets with large amounts of corn silage and corn by-products, like this one, could possibly be deficient in tryptophan. The NRC (2001) also suggests that phenylalanine and isoleucine are

frequently limiting, behind lysine and methionine, in corn-based diets. Energy intake could have also been limiting, but all model predictions indicated a positive energy balance.

Hormonal signaling (Burgos et al., 2010) and energy status (Proud, 2007) of the mammary cells will also influence protein synthesis. It is possible that the fermentability of the carbohydrates in these diets triggered an insulin response (Grant et al., 1990), which led to greater efficiency of milk protein production, compensating for any marginal AA deficiency. We hypothesize this based on the low milk fat concentration observed (≈3.1%). Given the low dietary fat concentrations (< 5%) the milk fat depression was likely related to excessive carbohydrate fermentation. In such a situation, a large proportion of the dietary NFC is fermented to produce propionate, which is a secretagogue of glucose and insulin (Harmon, 1992). An abundance of research has been published linking elevated insulin concentration to improved milk protein production (McGuire et al., 1995; Mackle et al., 1999; Molento et al., 2002). Blood samples were not collected in our study so this hypothesis cannot be evaluated. We also recognize that the effects of AA supply on mammary intracellular signals promoting translation can be independent of insulin (Appuhamy et al., 2011), but this was observed in a media that was considerably deficient in AA supply (5% of normal AA supply). If AA were supplied at levels near the requirements, such as in our study, the response to elevated insulin and essential AA could be less independent; therefore milk protein production does not increase with greater supply of metabolizable AA.

It is also possible that the model prediction, which was used to suggest a lysine limitation in the control diet, was wrong. Such an error could be attributed to inaccurate predictions of metabolizable lysine supply and/or lysine requirements. Schingoethe et al. (2009) pointed out that lysine supply from distillers grains of the current market is actually greater than the NRC

(2001) suggests (3.15 vs. 2.24% of CP), which is likely due to improvements in the dry corn milling process that promotes minimal heat damage. It is likely that similar progress has been made with wet corn milling, although surveys have not been conducted to validate this. Regardless, database values should be used cautiously.

Amino acid balancing using models

The rumen is a dynamic biological system, and this introduces many complexities when trying to supply specific AA to the small intestine. While models can be useful tools for such complex problems, it is important to remember that they have been developed based on empirical relationships, often with little regard to mechanisms (Allen, 2011). Given the differences in biology across the ruminant models evaluated in the present study, it is not surprising that they predict different requirements (Figure 1).

Specifics related to differences in the models used for this project have been recognized elsewhere (Whitehouse, 2009; Schwab, 2010). In brief, the NRC (2001) and CNCPS 5.0 contain known errors related to pool sizes, digestion rates, and passage rates, whereas the CNCPS 6.1 model is an updated version of CNCPS 5.0 with modifications in those areas. To date, we are not aware of any published evaluation of CNCPS 6.1 against a data set independent of the one used to derive equations; thus, we do not know if these modifications are actually improvements. Nonetheless, for the types of diets fed in this study, CNCPS 6.1 predicts lower lysine, methionine, and microbial CP flow to the small intestine, but higher overall rumen escape of dietary protein compared to the previous models.

No model provides perfectly accurate predictions of AA supply or requirements, but there is still ample economic pressure to balance for AA. There are 3 common methods that nutritionists use to assess the adequacy of lysine and methionine supply: 1) the predicted total g

of each AA supplied, 2) the predicted AA supply as a percentage of MP, and 3) the predicted ratio of lysine to methionine supply. The utility and/or rank in importance of each measure can be debated, but too often, dairy nutrition publications narrowly focus on one measurement and overlook the other two.

Theoretically, nutrient requirements should be expressed by mass because cows require amounts of nutrients, not percentages. However, estimating actual MCP, endogenous CP, and RUP is complicated so AA requirements are often given as a ratios. This seems logical because many other nutrient requirements are expressed as ratios. Furthermore, providing AA proportionate to other nutrients does present an advantage if intake is sporadic or unknown. Controlled studies have indicated that supplementing methionine (Leonardi et al., 2003; Cho et al., 2007) and/or lysine (Wang et al., 2010) based on targeted percentages of MP can increase milk protein production or allow dietary CP to be reduced. However, it is interesting that some research did not show a production effect when supplying greater lysine and methionine as a % of MP, and suggested that this is related to preset homeostatic regulation (Buchholtz et al., 2008). Additionally, a meta-analysis examining 281 published diets indicated that neither methionine nor lysine as a percent of MP were correlated with milk protein content (Patton et al., 2003). This may be because ratios can be misleading and potentially underemphasize MP supply. For example, if MP is supplied exactly at the required amount, 6.8% lysine may be necessary to meet a cow's requirement, but if MP is oversupplied by 10%, then 6.1% lysine would provide the same grams of lysine and similarly meet the requirement. Likewise, if MP supply is very deficient, lysine at 6.8% of MP will be far short of production requirements.

The other ratio used in AA balancing is lysine to methionine. This ratio is not always discussed in AA research, yet the proper ratio is important for ensuring efficient use of these two

AA. This ratio can, however, be misleading when one of these AA is supplied at a rate dramatically different from requirements. Nonetheless, both of the ratios discussed should be considered concurrent to quantity of AA supplied. These ratios will also provide utility when comparing numbers across prediction models. The dramatic differences across these models is represented in Figure 1 and demonstrates that comparing absolute quantities of AA supply across models is meaningless.

Part of the reason this study was undertaken was because the NRC model predicted an inadequate ratio of lysine to methionine (2.92), and these AA as a percentage of MP were less than NRC (2001) recommendations for maintenance and milk protein production (7.2 and 2.4% of MP for lysine and methionine, respectively). However, given the results of P1, it does not appear that metabolizable lysine or methionine supply was limiting production.

One reason that models may have had limited precision in this study is because most of the diets within the databases used to create these models did not contain high inclusion rates of non-forage fiber sources, and non-forage fiber sources alter the kinetics of digestion and passage rate (Firkins, 1997). Another concern is that models do not account for associative effects within diets, which is likely to be a major factor when substantial amount of NFC are replaced by non-forage NDF (Beckman and Weiss, 2005). For the time being, improved prediction of MP will be a major emphasis of some models, but improvements in accuracy will likely be limited until models are more precise in predicting diet effects on passage rates and digestion kinetics.

Many dairy nutritionists overfeed N in an attempt to ensure that AA supplies are not limiting (Broderick, 2006). This approach generally allows for maximal milk yield, but does not optimize the use of dietary N. The results in period 2 demonstrate the utility of rumen escape AA because we were able to decrease dietary CP without sacrificing production. However, we must

acknowledge that this is speculation because our study lacked a low CP control diet to prove that the rumen escape AA allowed this to happen. Regardless, given the cost of protein sources and the increasing scrutiny on the environmental impact of overfeeding N, nutritionists must start formulating for improved N efficiency. Based on available information, nutritionists can use protein supply ratios to help focus attention on potential dietary limitations, but accounting for total grams of each AA will likely be more useful for lowering total CP. Decreasing dietary CP does present the risk of decreasing milk production; therefore, responses must be closely monitored.

Conclusion

Results from this study demonstrated few responses from supplementing rumen-protected lysine and methionine. This study also confirms the substantial variability among current models with regard to predicted metabolizable AA supply. If the AA deficiencies predicted by the NRC (2001) model were correct, treatment cows should have produced more milk or milk components than control cows. However, given the results, it is likely that the diet fed to control cows was either not deficient in these AA or the supplemental AA products used did not efficiently escape ruminal degradation. Regardless, with current models it is difficult to determine AA balance, and a single expression of AA requirements that consistently correlates with production performance remains elusive.

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Figure 4.1 Prediction of metabolizable lysine and methionine requirements and supply by three different models. Bars represent supply of the respective amino acid relative to its requirement as specified by each model. Values shown above the bars present the predicted supply of that amino acid (g/d) according to each model. Model predictions were generated using actual data measured during the study.

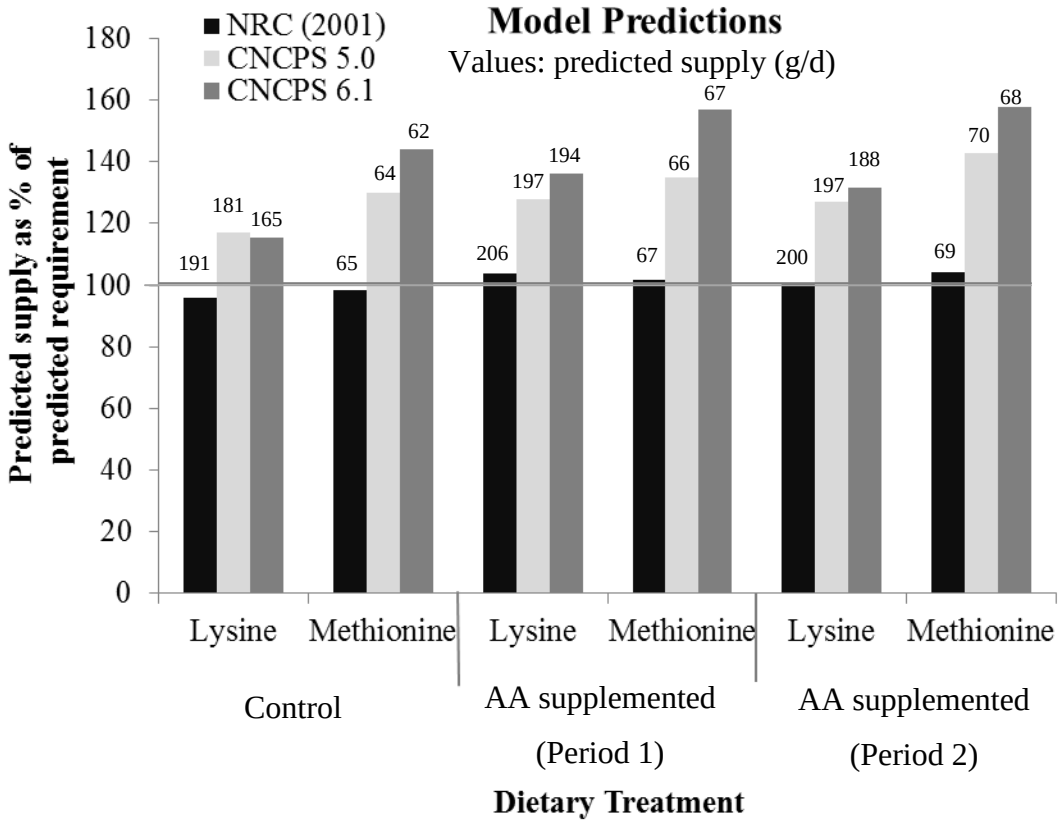

Table 4.1 Ingredient and nutrient compositions (% of DM) of diets fed to lactating Holstein cows

Item	Dietary treatment		
	Control	P1 AA supplemented	P2 AA supplemented
Ingredient			
Corn silage	23.4	23.4	27.8
WCGF[1]	26.7	26.6	23.0
Alfalfa hay	18.5	18.7	19.0
Cottonseed	5.4	5.3	5.6
Ground corn grain	14.2	14.2	14.7
Ground milo	2.7	2.7	2.8
Expeller soybean meal[2]	4.9	4.9	2.2
Fish meal, Menhaden	0.3	0.3	0.3
Ca salts of long chain fatty acids[3]	0.7	…	…
Ca salts of fatty acid-lysine matrix[4]	…	0.7	1.2
HMBi[5]	…	0.04	0.18
Micronutrient premix[6]	3.3	3.3	3.4
Nutrient			
DM (% as-fed)	55.6	55.6	52.9
CP	17.8	17.9	17.1
NDF	33.3	32.9	30.6
Crude fat	4.7	4.5	4.7
Starch	24.0	23.5	25.6
NFC[7]	37.6	37.9	41.1
Ash	6.9	6.8	6.7
NE_L[8] (Mcal/kg)	1.68	1.67	1.74
Calcium	0.83	0.79	0.94
Phosphorus	0.54	0.53	0.50
Magnesium	0.35	0.33	0.33
Potassium	1.41	1.42	1.30
Sodium	0.42	0.44	0.44

[1]Wet corn gluten feed, Sweet Bran (Cargill, Inc., Blair, NE).
[2]Soybest® (Grain States Soya, Inc., West Point, NE).
[3]Megalac®-R (Arm & Hammer Animal Nutrition, Princeton, NJ).
[4]Ca salts of fatty acids + lysine monohydrochloride, Megamine®-L (Arm & Hammer Animal Nutrition).
[5]Isopropyl ester of 2-hydroxy-4-(methylthio) butanoic acid, MetaSmart (Adisseo Inc., Antony, France).
[6]The premix consisted of 41.6% limestone, 32.5% sodium bicarbonate, 6.50% Diamond V XP, 5.40% trace mineral salt, 5.40% Magnesium oxide, 5.20% vitamin E premix, 1.66% 4-plex, 0.93% Se premix, 0.36% vitamin A premix, 0.16% vitamin D premix, 0.05% EDDI, and 0.21% Rumensin 80.
[7]Calculated as DM − (CP + NDF + ether extract + ash).
[8]Estimated according to NRC (2001).

Table 4.2 Effects of supplementing lysine embedded within Ca salts of fatty acids[1] and HMBi[2] on performance of lactating Holstein cows in Period 1

	Treatment			
Item	Control	P1 AA supplemented	SEM	*P*-value
DMI (kg/d)	26.4	26.7	0.5	0.65
Yield (kg/d)				
Milk	40.1	40.1	0.7	0.98
Milk fat	1.22	1.25	0.03	0.59
Milk protein	1.23	1.21	0.02	0.54
Milk lactose	1.95	1.96	0.04	0.96
ECM[3]	38.2	38.5	0.7	0.81
Milk fat (%)	3.07	3.12	0.06	0.59
Milk protein (%)	3.08	3.04	0.04	0.51
Milk lactose (%)	4.88	4.88	0.03	0.97
MUN (mg/dL)	13.9	14.3	0.2	0.25

[1]Megamine®-L, Arm & Hammer Animal Nutrition, Princeton, NJ
[2]The methionine precursor isopropyl ester of 2-hydroxy-4-(methylthio) butanoic acid (MetaSmart®, Adisseo Inc., Antony, France)
[3]ECM = (0.327 × milk yield) + (12.86 × fat yield) + (7.65 × protein yield); (Dairy Record Management Systems, 2010)

Table 4.3 Effects of supplementing lysine embedded within Ca salts of fatty acids[1] and HMBi[2] on performance of lactating Holstein cows in Period 2

	Treatment			
Item	Control	P2 AA supplemented	SEM	*P*-value
DMI (kg/d)	24.5	24.3	0.4	0.91
Yield (kg/d)				
Milk	36.0	35.5	0.6	0.51
Milk fat	1.04	1.06	0.03	0.65
Milk protein	1.02	0.99	0.02	0.20
Milk lactose	1.63	1.60	0.03	0.36
ECM[3]	38.2	38.5	0.7	0.81
Milk fat (%)	3.12	3.23	0.06	0.59
Milk protein (%)	3.05	3.02	0.04	0.59
Milk lactose (%)	4.85	4.84	0.04	0.87
MUN (mg/dL)	12.5	10.8	0.2	<0.001

[1]Megamine-L, Arm & Hammer Animal Nutrition, Princeton, NJ
[2]The methionine precursor isopropyl ester of 2-hydroxy-4-(methylthio) butanoic acid (MetaSmart, Adisseo Inc., Antony, France)
[3]ECM = (0.327 × milk yield) + (12.86 × fat yield) + (7.65 × protein yield); (Dairy Record Management Systems, 2010)

Table 4.4 Predicted metabolizable lysine and methionine supplies as a percentage of predicted metabolizable protein supply by 3 different models, and predicted metabolizable lysine:methionine ratio

	Control		P1 AA supplemented		P2 AA supplemented	
	Lysine	Methionine	Lysine	Methionine	Lysine	Methionine
% of MP						
NRC (2001)	6.3	2.2	6.8	2.2	7.2	2.5
CNCPS 5.0	6.1	2.1	6.6	2.2	7.1	2.5
CNCPS 6.1	5.3	2.0	6.0	2.1	6.5	2.3
Lysine:methionine[1]						
NRC (2001)	2.92		3.07		2.90	
CNCPS 5.0	2.84		2.99		2.81	
CNCPS 6.1	2.66		2.89		2.77	

[1]Calculated based on g of predicted AA supply. Model predictions were generated using actual data measured during the study.