Introduction
The B-vitamins include thiamin (B1), riboflavin (B2), niacin (B3), pantothenic acid (B5), the B6 complex (pyridoxal, pyridoxamine, pyridoxine), biotin (B8 or Vitamin H), folic acid (B9), and B12. Each of the B-vitamins plays a key role as either an enzymatic cofactor or metabolic constituent in many facets of intermediary metabolism, e.g. about half of the propionate that reaches the ruminant liver is converted to glucose by a series of enzymatic reactions (Brockman, 1993) which require biotin, B12, niacin, pantothenic acid, and riboflavin. These B-vitamins, along with the others, also play other key roles in the metabolism of carbohydrates, fats, and proteins.

Ruminant B-vitamin research was quite prevalent during the mid 1940’s to late 1950’s, with the majority of this research being conducted in sheep, calves, and steers. This work clearly showed that even when relatively vitamin-free diets were fed the microbial population of the rumen synthesized B-vitamins; thus, making determination of B-vitamin requirements for ruminants difficult. Later work by Miller et al. (1986) and Zinn et al. (1987) showed that B-vitamin appearance at the small intestine exceeded that provided by feedstuffs and that when B-vitamins were supplemented (Zinn et al., 1987) many were degraded or metabolized by the rumen microbial population. The current dogma (NRC, 2001) is that since the ruminant microbial population synthesizes B-vitamins, additional supplementation to dairy cows is unnecessary. While clinical B-vitamin deficiencies are rarely observed in common feeding situations with ruminants, recent research with several B-vitamins has shown that supplementation may improve dairy cow performance (Schwab and Shaver, 2004).

Aspects of this topic have been extensively reviewed and discussed by the authors for the 2004 California Animal Nutrition Conference DSM Nutritional Products Technical Symposium and 2005 Cornell Nutrition Balchem Encapsulates Technical Symposium, and therefore much of the information from those reviews will not be repeated herein. Readers are referred to Schwab and Shaver (2004 and 2005) for that background.

Responses to Dietary B-Vitamin Supplementation
Biotin
Hoof health. Tomlinson et al. (2004) reviewed the role of biotin in hoof health. Mülling et al. (1999) likened the zinc-dependent keratinization process in the hoof to that of building a brick wall. Keratin (the “bricks”) is a major structural component of the hoof epidermis, providing structural integrity to the hoof. The intercellular cementing substance (the “mortar”) acts to glue the keratin-matrix together. This intercellular cementing substance is a complex of lipid molecules, the formation of which requires biotin (Mülling et al., 1999). Zinc and biotin together allow keratinizing squamous cells (the “mason”) to build a hoof wall with a greater ability to resist environmental stresses (Tomlinson et al., 2004). Higuchi and Nagahata (2001) reported that serum biotin concentration decreased as sole horn moisture increased. Due to the rate of hoof growth, several months must pass before benefits of biotin are seen on hoof parameters (Fitzgerald et al., 2000, Lischer et al., 2002). Several research studies investigating the effects of biotin on hoof health have been conducted in recent years.

Two experiments reported that 20 mg/d biotin supplementation reduced (P < 0.05) white-line separation (WLS). After 100 d of biotin supplementation prevalence of WLS on the right hind limb was reduced by 76.4%, but there was no difference between control and biotin supplemented cows after 293 d (Midla et al., 1998). The incidences of heel erosion, sole hemorrhage, toe length, or hoof angle were not different between control and supplemented cows. Hedges et al. (2001) reported an overall 35% reduction in the incidence of WLS in...
Two of three experiments initially designed to test the effects of biotin supplementation on hoof health also reported increases ($P < 0.05$) in milk production. Midla et al. (1998) reported that 305-d mature equivalent milk production of first lactation animals was 320 kg higher ($P < 0.05$) for biotin-supplemented animals relative to controls (39.7 vs. 38.7 kg/d). Plasma biotin concentrations were also increased. In the experiment of Bergsten et al. (2003), biotin supplementation increased ($P < 0.01$) DHIA-estimated, PTA-adjusted milk production by 878 kg or 2.9 kg/d over control cows; herd average milk production was 32.2 kg/d. Interestingly, the authors also reported that biotin-supplemented primiparous cows had 61 fewer ($P = 0.02$) days to conception and 1.5 fewer ($P = 0.004$) breedings per conception than control animals. Fitzgerald et al. (2000) reported numerical 1.2 kg/d increase in milk production (18.5 kg/d biotin vs. 17.3 kg/d control) from 20 mg/d supplemental biotin.

Although lameness is a multi-factorial disease, these results show that biotin supplementation can aid in reducing various disorders of the hoof.

Milk production. Initial research on biotin supplementation to dairy cattle was focused on hoof health. However, there is a growing body of research showing that biotin supplementation can improve lactation performance.

Experiments designed specifically to test the effect of biotin supplementation on production and metabolic parameters show similar results as those above. Zimmerly and Weiss (2001) fed incremental amounts of biotin (0, 10, or 20 mg/d) to eighteen primiparous and 27 multiparous dairy cows from 14 d before expected calving to 100 DIM. Cows were blocked by parity and expected calving date, and multiparous cows were also blocked by previous lactation milk production. There was no effect on DMI but milk production increased ($P = 0.05$) linearly with increasing biotin supplementation (36.9, 37.8, 39.7 kg/d for 0, 10, and 20 mg/d biotin, respectively). Plasma and milk biotin concentrations increased linearly ($P < 0.01$) by 67 and 68%, respectively. Milk true protein production also increased ($P = 0.05$) linearly. There was no effect of biotin supplementation on milk fat percentage or production, milk true protein percent, body condition score (BCS), or BW. Other measured parameters that were not influenced by biotin supplementation were serum glucose, insulin, and NEFA and molar percentages of ruminal VFA.

Margerison et al. (2002) reported on 36 multiparous Holstein cows that were allocated by calving date and blocked by parity, previous milk production, BW, and BCS. Eighteen cows served as controls and 18 were supplemented with 22 ± 2 mg/d biotin over 120 d post calving. Milk production was 2.0 kg/d higher ($P < 0.001$) for biotin supplemented than control cows (39.2 vs. 37.2 kg/d, respectively). There was no influence of biotin supplementation on DMI, milk composition, BCS, or BW.

Rosendo et al. (2004) evaluated the effects of 20 mg/d dietary biotin on performance, liver metabolism, and blood parameters in multiparous early-lactation dairy cows. The biotin-treatment diet (20 cows) or the control basal diet (18 cows) were fed from -10 to 70 DIM, and cows were blocked by expected calving date, previous lactation milk production, and BCS. Intake of DM, BW, milk production, milk components and component production, and days from calving to first estrus and from calving to conception were not influenced by biotin supplementation. Biotin supplementation increased ($P < 0.01$) plasma (54%) and milk (64%) biotin concentrations and plasma glucose (5%) concentrations over controls. Plasma NEFA, BHBA, and urea-N and total liver lipids, triacylglycerols, protein and glycogen were not influenced by biotin supplementation. Zimmerly and Weiss (2001) fed incremental amounts of biotin (0, 10, or 20 mg/d) to eighteen primiparous and 27 multiparous dairy cows from 14 d before expected calving to 100 DIM. Cows were blocked by parity and expected calving date, and multiparous cows were also blocked by previous lactation milk production. There was no effect on DMI but milk production increased ($P = 0.05$) linearly with increasing biotin supplementation (36.9, 37.8, 39.7 kg/d for 0, 10, and 20 mg/d biotin, respectively). Plasma and milk biotin concentrations increased linearly ($P < 0.01$) by 67 and 68%, respectively. Milk true protein production also increased ($P = 0.05$) linearly. There was no effect of biotin supplementation on milk fat percentage or production, milk true protein percent, body condition score (BCS), or BW. Other measured parameters that were not influenced by biotin supplementation were serum glucose, insulin, and NEFA and molar percentages of ruminal VFA.
Effects of supplemental biotin and a B-vitamin blend on lactation performance of dairy cows were investigated in two trials by Majee et al. (2003). In trial one, 24 multiparous Holstein cows (46 ± 8 DIM) were assigned to four treatments in a 4 × 4 Latin square design with 28-d periods. The four treatments were: 1) control diet (C), 2) 20 mg/d biotin (B), 3) 20 mg/d biotin plus a B-vitamin blend (BBVIT1X), and 4) 40 mg/d biotin plus the B-vitamin blend at twice the level in treatment three (BBVIT2X). The B-vitamin blend used in treatment three consisted of thiamin (150 mg/d), riboflavin (150 mg/d), pyridoxine (120 mg/d), B<sub>12</sub> (0.5 mg/d), niacin (3000 mg/d), pantothenic acid (475 mg/d), and folic acid (100 mg/d). Trial two was conducted to test the effects of three treatments: 20 mg/d biotin (B1X), 40 mg/d biotin (B2X), and treatment three used in experiment 1 (BBVIT1X). In this experiment, 24 multiparous cows (84 ± 15 DIM) were allocated to a 3 × 3 Latin square design with 28-d periods. In trial one, DMI was increased (P < 0.05) 0.7 kg/d for B vs. C and BBVIT1X (25.7 versus 25.0 and 25.0 kg/d, respectively) and 1.3 kg/d for B vs. BBVIT2X (25.7 versus 24.4 kg/d). Milk production increased (P < 0.05) 1.7 kg/d for B versus C (38.9 vs. 37.2 kg/d) and 1.5 kg/d for B versus BBVIT2X (38.9 versus 37.5 kg/d). Production of protein and lactose were higher (P < 0.05) for B versus C and BBVIT2X, and fat production was higher (P < 0.05) for B versus BBVIT2X. Milk composition was largely unaffected by treatments except for lactose percent where B and BBVIT1X were higher (P < 0.05) than C and BBVIT2X. Body weight, apparent total-tract DM, OM, and NDF digestibilities, and plasma concentrations of glucose, NEFA, and BHBA were not influenced by treatments. No treatment differences were observed in the second trial.

In the experiment of Richards et al. (2002), 40 non-lactating, pregnant dairy cows were assigned to one of two treatments from -21 to 90 DIM to either a basal diet or a diet containing a ruminally-protected combination of choline, niacin, vitamin B<sub>12</sub>, biotin, folic acid, and thiamin. The exact proportions of the vitamins in the supplement were not provided because a proprietary product was being tested. Milk production was increased by 3.7 kg/d for the treatment vs. control (35.4 vs. 31.7 kg/d). Milk component production and serum NEFA, BHBA, triacylglycerides, glucose, and urea were unaffected by treatment.

In experiments where differences (P < 0.05) in milk production were observed, supplementation of 20 mg/d dietary biotin increased milk production 2.1 kg/d on average. In the three experiments designed primarily to measure production responses (Zimmerly and Weiss, 2001; Majee et al., 2003; and Rosendo et al., 2004), two reported milk production increases (P < 0.05) averaging 2.3 kg/d. In the experiment of Majee et al. (2003), it is unknown whether the increase in milk production was due to the increase in DM intake or vice versa. Overall, biotin supplementation had little effect on milk component percentages.

Blood metabolites were largely unaffected by biotin supplementation. Rosendo et al. (2004) observed a 3.2 mg/dl or 4.8% increase (P < 0.05) in blood glucose concentration with biotin supplementation. However, Zimmerly and Weiss (2001) found lower blood glucose concentrations in biotin supplemented cows (4.5 mg/dl or 7.1% below controls; P > 0.10) with a concomitant increase in milk production. Majee et al. (2003) reported no differences in blood glucose concentrations between control and biotin-supplemented cows, but milk production increased for biotin-supplemented cows. Thus, it is questionable whether the difference reported by Rosendo et al. (2004) is of biological significance. The general lack of response to biotin on blood NEFA, BHBA, BW and BCS, and liver parameters suggests that energy balance of cows was similar across treatments and that additional milk production was not due to adipose tissue mobilization.

Observed increases in milk and plasma biotin are significant in that they suggest that biotin supplemented in an unprotected form is not completely degraded or utilized by ruminal microbes. This is supported by Zinn et al. (1997) and Santschi et al. (2005), who measured duodenal flows of B-vitamins and estimated that only supplemental biotin and vitamin B<sub>6</sub> appreciably escaped ruminal degradation. Frigg et al. (1993) and Santschi et al. (2005) estimated that bioavailability of oral biotin was 48 and 55%, respectively.

It is well established that cellulolytic microbes require biotin for growth (Baldwin and Allison, 1983; Milligan et al., 1967). However, in the experiment of Zimmerly and Weiss (2001) biotin supplementation elicited no changes in molar percentages of ruminal VFA, and there were no effects of biotin on total tract NDF digestibility (Majee et al., 2003). It has been
shown in vitro that as the amount of concentrate in the digestion media increases, biotin synthesis decreases (Abel et al., 2001). This leads to the theory that dairy cows fed higher concentrate diets may be more likely to respond to supplemental biotin. Though the results shown here are far from conclusive, they seem to argue against this premise. The diets fed by Midla et al. (1998), Zimmerly and Weiss (2001), Majee et al. (2003), and Rosendo et al. (2004) had respective dietary forage and total NDF percentages of 42% and 34%, 50% and 32%, 50% and 32%, and 37% and 34%. Of these experiments, only that of Rosendo et al. (2004) failed to show an increase in milk production due to biotin supplementation. The results of Majee et al. (2003) also suggest that there is no advantage to supplementing 40 mg/d of biotin over 20 mg/d. Clearly there are a myriad of factors that may influence biotin synthesis and possibly the response to supplementation.

Negative responses to feeding the B-vitamin blend at the high dosage reported by Majee et al. (2003) are difficult to interpret. B-vitamin requirements for dairy cows are largely undefined and a blend of B-vitamins with varying relative supplementation rates may elicit a different response. The authors speculated that palatability issues could have existed and that there may have been negative interactions between the vitamins at the higher dosage. The experiment of Richards et al. (2002) showed a positive milk production response when a mix of B-vitamins was provided in an encapsulated form. Clearly there is opportunity for further research on supplementation of individual B-vitamins or mixtures.

The short-term (100 d) continuous lactation study of Zimmerly and Weiss (2001) and the short-term (28 d periods) Latin square experiment of Majee et al. (2003), along with the relatively slow growth of hoof horn (Fitzgerald et al., 2000, Lischer et al., 2002), suggest that increases in milk production were not due entirely to improvements in hoof health. The research summarized herein, though not conclusive, also suggests that increases in milk production due to biotin are not due to improvements in nutrient digestibility (and therefore diet energy value) or changes in rumen function. A possible explanation for observed milk production increases lies in the biochemical role of biotin. Biotin is essential for the proper functioning of four carboxylase enzymes: acetyl-CoA carboxylase, pyruvate carboxylase (PC), propionyl-CoA carboxylase (PPC), and methylcrotonyl-CoA carboxylase (MCC). Holocarboxylase synthetase is required to attach biotin to these four carboxylase enzymes, thereby activating them. It is unlikely that any changes in MCC would influence milk production as this enzyme is involved solely in leucine metabolism. Acetyl-CoA carboxylase is involved in fat metabolism and is also tightly regulated, so it is an unlikely candidate for influencing milk production. Recent research in biotin-deficient rats (Rodriguez-Melendez et al., 2001) has shown that when biotin is supplemented there were increases in hepatic and renal levels of holocarboxylase synthetase mRNA and PC and PCC+MCC protein. As well, activities of PC and PCC were increased both in liver and kidney. Both PC and PCC are enzymes involved in gluconeogenesis. Pyruvate is an important intermediate in the conversion of lactate and many amino acids into glucose; propionate is one of, if not the most important glucose precursor in ruminants. The liver is the major glucose-producing organ in the dairy cow. Therefore, in theory, if lactating dairy cows are marginally biotin deficient, and if the amounts and activities of PC and PCC can be increased, then possibly hepatic glucose production, and therefore lactose synthesis and milk production, may be increased as well. Research is needed to elucidate biotin’s mode of action for increasing milk production.

Supplementing biotin to lactating dairy cows should prove economically advantageous. Using the 3.7 and 1.5 lb/cow/d increases in milk yield and DM intake, respectively, reported by Majee et al. (2003), and assuming a milk price of $12/cwt, $0.05 cost for 20 mg/d biotin, and a $0.07 per lb DM TMR cost, income minus feed cost is expected to increase about 30 cents per cow per d or about $90 per lactation from dietary biotin supplementation. Additional economic benefits could also be realized if reductions in culling due to lameness occur.

Folic acid
Investigations into the role of folic acid in dairy cattle have been pioneered by C. L. Girard and colleagues in Lennoxville, Quebec. The objective of an early trial (Girard et al., 1989) was to investigate the variation in serum folates during gestation. Seventy multiparous dairy cows were assigned to five groups, each of which represented their physiological stage: parturition (0 to 1 DIM), 2 mo postpartum, 3 and 6 mo of gestation, and dry cows (2 mo before...
parturition. Plasma volume averaged 31.3 L, and was not influenced by physiological stage. Total serum folates were lowest at parturition (503.6 μg), highest at 2 mo postpartum, and then decreased as gestation progressed. Total serum folates decreased by 40% from around breeding (2 mo postpartum) to parturition. The drop in total serum folates as gestation progressed may indicate an increased folic acid requirement by the fetus, or may be due to a drop in DMI as lactation progresses.

The objective of a later experiment (Girard et al., 1995) was to investigate the effects of an injection of folic acid on the performance of dairy cows. Twenty-four multiparous and 16 primiparous cows were injected weekly with 0 (sterile saline) or 160 mg of folic acid from the day they were confirmed pregnant (about 45 d post-breeding) to six weeks after the subsequent calving. The weekly 160 mg injections of folic acid increased serum folates by 20% (P = 0.001) during gestation and by 17% (P = 0.03) after calving; serum folates were lower for primiparous cows than multiparous cows both before (P = 0.005) and after (P = 0.03) parturition. Milk folates during the 45 d after breeding to dry-off and colostral folates were numerically higher for treated than control cows (53.9 vs. 44.0 ng/ml and 312.3 vs. 287.0 ng/ml, respectively); however, there was no effect of supplemental folic acid during the first six weeks after parturition. Folic acid supplementation did not affect DMI or BW. From 45 d after breeding to dry off, supplementation of folic acid tended (P = 0.09) to increase milk production by 1.5 kg/d. After calving, folic acid supplementation did not affect milk production, nor was there a treatment by parity interaction. In multiparous cows, milk protein percentage was numerically increased from 3.2% to 3.5% by folic acid supplementation.

Girard and Matte (1998) supplemented diets of 63 cows (32 primiparous and 31 multiparous) with 0, 2, or 4 mg/d of folic acid per kg BW from one mo before expected calving through a full lactation. These treatments equate to approximately 0, 1.5, and 3.0 g/d of supplemental folic acid. Concentrations of folates increased linearly in serum (P = 0.0001) and quadratically (P = 0.03) in milk as supplemental folic acid increased. Primiparous cows fed folic acid at 0, 2 or 4 mg/kg BW per d produced 8195 ± 202, 7654 ± 236, and 7699 ± 280 kg of milk, respectively, whereas multiparous cows fed these levels produced 8284 ± 560, 8548 ± 380, and 8953 ± 191 kg of milk, respectively. For multiparous cows, supplementation with 4 mg/kg BW per day increased milk production over controls by 2.2 kg/d (P = 0.06) during the first 100 days of lactation and by 3 kg/d (P = 0.05) from 100 to 200 days of lactation. Milk production from 200 to 300 DIM was not influenced by folic acid supplementation. Dry matter intake, BW, and concentrations of milk fat, total solids, lactose, casein-N, or whey-N were unaffected by folic acid supplementation. A subsequent lactation study from the same laboratory (Girard et al., 2005) utilized 54 multiparous cows fed folic acid at 0, 3, and 6 mg/kg BW with and without rumen protected methionine. In this study folic acid supplementation did not improve lactation performance.

These experiments suggest that folic acid supplementation may increase milk production in multiparous dairy cows, though results are inconsistent. Although the ingredient composition of the diets fed in Girard and Matte (1998) and Girard et al. (2005) were presented, dietary nutrient composition was not provided for either study. Therefore, potential response differences between the two studies due to possible dietary nutrient differences can not be inferred. Folate status of cows appears similar between studies as serum folate concentrations of control group cows were similar (approximately 16.0 ng/ml). Since folic acid was supplemented in a dietary form in both studies, it is uncertain whether the positive effects (or lack of effects) reported in multiparous cows were due to changes in metabolic parameters or were a result of changes in the rumen environment. However, Chiquette et al. (1993) found that folic acid supplementation at 2 mg/kg BW per d via the diet did not affect total tract ADF or NDF digestibilities in growing steers fed either high or low forage diets. Likewise, folic acid supplementation did not affect ruminal pH or molar concentrations of acetate and propionate. Therefore, it is more likely that the milk production increases observed by Girard and Matte (1998) were due to an improvement in metabolic status, though the exact mechanisms through which this occurs remain unknown.

**Niacin**

Niacin is the most comprehensively researched B-vitamin in dairy cows. Initial studies conducted by Schultz and colleagues in 1972 (Waterman and Schultz, 1972a,b; Waterman et al., 1972) showed that niacin supplementation (160 g/d) decreased plasma...
concentrations of non-esterified fatty acids (NEFA) and ketone bodies in cows with clinical or subclinical ketosis. This lead to the theory that niacin reduced adipose tissue mobilization, thereby reducing hepatic uptake of fatty acids and the incidence of fatty liver. Indeed, niacin has been shown to be antilipolytic (DiPalma and Thayer, 1991), binding to inhibitory G-protein-coupled receptors and thereby reducing adipocyte cyclic-AMP concentrations and inhibiting lipolysis. Since the early reports of Schultz (Waterman and Schultz, 1972a,b; Waterman et al., 1972), dozens of research articles have since been published covering all stages of lactation, various feeding and management situations, and many combinations of dietary ingredients. Five recent, comprehensive reviews summarizing niacin research are available: Drackley (1992), Erdman (1992), Girard (1998), NRC (2001), and Schwab et al. (2005). In the studies summarized in these reviews, niacin was primarily supplemented at 6 or 12 mg/d.

Drackley (1992) summarized 24 reports with responses from 40 treatment comparisons; no consideration was given to statistical significance. Across all studies and relative to controls, niacin supplementation had minimal effects on DMI (-0.12 kg/d), milk production (+0.36 kg/d), or milk component percentages and production. On average, 51% of the comparisons showed positive numerical responses to supplemental niacin; milk production was increased in 65% of the studies. Averaged responses to niacin were all positive when only early lactation (less than 15 weeks postpartum) studies were considered. Average DMI and milk production increased by 0.04 and 0.57 kg/d, respectively; milk components were increased slightly. Several of the reports summarized by Drackley (1992) included supplementation of dietary fat to the rations. In this situation niacin responses were largely negative, and relative to controls, DMI, milk production, and 4% fat-corrected milk production were reduced by 0.04 and 0.42 and 0.84 kg/d, respectively. Milk protein content was increased 0.09% units by niacin supplementation. Across all variables summarized, 31% showed a positive response and 63% (five of eight) showed a positive milk protein percentage response. When diets without fat addition were considered, the niacin responses were largely positive. Dry matter intake decreased by 0.16 kg/d and milk production increased by 0.62 kg/d, on average. Positive responses were observed for fat content (0.03% units) and 4% fat-corrected milk production (0.77 kg/d). Milk protein content and production were increased minimally. Drackley (1992) also summarized results of niacin supplementation on blood NEFA, â-hydroxybutyrate (BHBA), and glucose concentrations. When cows were fed niacin at 3 to 12 g/d in early lactation, niacin had no consistent effects on blood NEFA concentrations but BHBA concentrations were decreased by varying degrees in 11 of 14 (79%) comparisons. Niacin increased blood glucose concentrations relative to controls in 10 of 16 comparisons.

Erdman (1992) summarized data from 29 experimental comparisons where 3, 5-6, or 12 grams of niacin were supplemented daily; as in Drackley (1992) no consideration was given to statistical significance. When averaged across all levels of supplementation, niacin increased milk and 3.5% fat-corrected milk production, both by 0.3 kg/d per day. There was no advantage to supplementing more than 3 g/d of niacin. When only early-lactation (less than 15 weeks postpartum) studies where considered, niacin supplementation increased milk production by 0.40 kg/d. Effects of niacin supplementation on milk fat or protein percentages—whether averaged across levels of supplementation or subdivided into early and mid-lactation—were minimal. When diets with supplemental fat were considered, niacin supplementation reduced milk and 3.5% fat corrected milk production by 1.1 and 1.3 kg/d, respectively; milk fat and protein content were largely unaffected.

Girard (1998) summarized 22 experiments and categorized them into groups according to the cows’ stage of lactation. In the 12 studies where niacin was supplemented in early lactation, milk production was increased significantly (5.4% on average) in four studies, and another four studies reported a tendency for increased milk production (6.3% on average). Dry matter intake was increased by 5% in two (one P < 0.05 and one P < 0.10) of the 12 early lactation studies. In nine reports where mid-lactation cows were supplemented with niacin, three reported a milk production increase (P < 0.05) which averaged 3.9%. Dry matter intake was increased (P < 0.05) in two studies by an average of 7.5%. There was no influence of niacin supplementation in late-lactation cows.

The NRC (2001) summarized 30 treatment comparisons examining the effects of niacin on milk production. One comparison reported an increase (P
Results of niacin supplementation trials are consistently inconsistent. Significant improvements in milk production or in blood parameters occur infrequently and stage of lactation and changes in feeding practices rarely increase the likelihood of observing a benefit from niacin supplementation. Few studies included in the aforementioned reviews supplemented more than 12 g/d niacin or adequately investigated effects in transition cows. Given the benefits observed by French (2004), the molecular action of niacin on adipose tissue, and challenges with transition cows in regards to adipose tissue mobilization and concomitant hepatic lipidosis (Grummer, 1993), high-dose niacin supplementation may be beneficial for transition cows. Presently however, there seems to be little value in supplementing niacin to lactating dairy cattle, especially when response variability and economic returns are considered. Due to the almost complete ruminal destruction of niacin (Zinn et al., 1987; Santschi et al., 2005), use of rumen-protected niacin supplements may be useful in future experiments.

**Thiamin**

Thiamin has been extensively studied in relation to polioencephalomalacia (PEM) in beef cattle. PEM is a clinical condition characterized by unusual behavior, such as circling, excitability, muscular tremors and convulsions, and in severe cases, coma. This disease has been attributed to low thiamin-containing diets or to the presence of thiamin-degrading enzymes, thiaminases, which eliminate thiamin activity by altering the structure of the vitamin. Thiaminase production has been associated with rumen bacteria, but no specific microbe or groups of microbes responsible for thiamin degradation have been identified (Dawson et al., 1997). In vitro (Sapienza, 1981) and in vivo (Brent and Bartley, 1984) studies suggest that thiaminase activity is higher in animals fed high-concentrate diets with low ruminal pH. Soita and Brent (1993) reported that thiaminase I activity increases in dairy cattle feces when switched from low- to high-concentrate diets. Brent (1976) and DeOliveira et al. (1997) suggested that lactic acidosis may be a precursor for spontaneous development of PEM.
High dietary sulfide concentrations (greater than 0.4%) have also been implicated in the development of PEM (Gould et al, 2002). High sulfur intake can be detrimental to ruminants as ruminal reduction of sulfur produces hydrogen sulfide (Gould, 1998), the production of which has been linked to PEM (Gould, 1998). Hydrogen sulfide and its ionic forms are highly toxic substances which have been shown to interfere with cell respiration (Beauchamp et al., 1984). Ward and Patterson (2004) evaluated the effects of dietary thiamin supplementation in steers supplied water with high sulfur content. Sixty-three steers were provided water which contained either low (393 ppm) or high (3,800 ppm) concentrations of sulfates; high-sulfate water was achieved via sodium sulfate addition. A sub-group of the steers provided with high-sulfate water were supplemented with 1 g/ hd per d of dietary thiamin. Clinical signs of PEM occurred in only one steer fed high-sulfate water, but thiamin supplementation increased average daily gain ($P < 0.05$) and tended to increase gain to feed ratio ($P < 0.10$).

Although there have not been any reports of PEM occurring in lactating dairy cattle, Erdman (1992), in a review, suggested that blood concentrations of the thiamin-dependent enzyme transketolase are lower in high producing cows. To our knowledge, the report of Shaver and Bal (2000) is the only published work investigating the effects of dietary supplemental thiamin on milk production by dairy cows. In the first trial, 28 Holstein cows were randomly assigned to receive either a placebo or thiamin-containing topdress for eight weeks. Within each of these groups the cows were further assigned randomly to total mixed rations containing either a corn-soybean meal based diet or a corn byproduct-based diet for four weeks, then reversed for a second four-week period. Both diets utilized alfalfa silage (55% of diet DM) as the sole forage source. The thiamin-containing top-dress was designed to provide for supplemental thiamin intake of 150 mg/d per cow. Data was collected from the last week of each period. Milk production was 2.7 kg/d higher ($P = 0.01$) for cows fed the thiamin top-dress than the placebo. Dry matter intake was unaffected by treatment but milk fat and protein production were increased 0.13 and 0.10 kg/d ($P = 0.01$), respectively, for the thiamin-supplemented cows without effects on milk fat and protein concentrations. There was no thiamin supplement by diet interaction, suggesting that the effects of supplemental thiamin were unrelated to the feeding of the corn byproduct-based diet.

In trial two, twenty multiparous cows were used in a crossover design with four-week periods. Wheat middlings or a thiamin mononitrate-wheat middlings mixture was added to the ration to provide for an approximate supplemental thiamin intake of 0 or 300 mg/d per cow. Diets contained 50% forage (67% corn silage, 33% alfalfa silage) and 50% concentrate (DM basis). Milk and protein production tended to be 0.7 ($P = 0.15$) and 0.04 ($P = 0.09$) kg/d higher, respectively, for cows supplemented with thiamin. Trial three was of similar design to trial two (0 or 300 mg/d supplemental thiamin), however alfalfa silage was the sole forage source (60% of ration DM), and the alfalfa silage was harvested at either 0.95 or 1.90 cm theoretical length of cut. Eight of the 16 multiparous cows were fitted with ruminal cannulae to facilitate rumen fluid sampling and determination of 48-hr in situ degradability of the alfalfa silage. There were tendencies for lower DMI ($P = 0.15$) and milk fat percentage ($P = 0.06$), and fat production was lower ($P = 0.05$) for thiamin supplemented versus unsupplemented cows. Ruminal pH or in situ DM degradation of alfalfa silage were not influenced by treatment.

Differences in response to thiamin supplementation in these three trials are difficult to explain as no measurements of thiamin status, the presence of thiaminase enzymes, or thiaminase activity were collected. The authors theorized that due to higher concentrations of non-fiber carbohydrates and lower forage NDF concentrations in diets fed in Trials 1 and 2 relative to Trial 3, that ruminal thiamin synthesis may have been reduced or thiamin may have been degraded by thiaminases. Clearly, more conclusive research needs to be conducted to determine whether thiamin supplementation is beneficial for lactating dairy cows.

References


