Effect of Dietary Thiamin Supplementation on Milk Production by Dairy Cows

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ABSTRACT

We conducted three experiments to determine the effects of dietary thiamin supplementation on milk production by dairy cows. In trial 1, 28 Holstein cows were blocked by parity and assigned randomly to either placebo or thiamin top-dress for the 8-wk experiment to provide a supplemental thiamin intake of 0 or 150 mg/d per cow. Within each of these groups, cows were further assigned randomly to two total mixed rations (TMR) for 4 wk, with the TMR treatments then reversed for a second 4-wk experimental period. Milk yield was 2.7 kg/d higher for thiamin-supplemented cows. Yields of milk fat and protein were increased 0.13 and 0.10 kg/d, respectively, by dietary thiamin supplementation. In trial 2, 20 multiparous Holstein cows were used in a crossover design with 4-wk periods. Placebo or thiamin premixes were added to TMR to provide an approximate daily supplemental thiamin intake of 0 or 300 mg/cow. Milk and protein yields tended to be 0.7 and 0.04 kg/d higher, respectively, for thiamin-supplemented cows. In trial 3, 16 multiparous Holstein cows were used in a replicated 4×4 Latin square with 21-d periods. Placebo or thiamin premixes were added to TMR to provide an approximate daily supplemental thiamin intake of 0 or 300 mg/cow. Dry matter intake tended to be 0.8 kg/d lower for thiamin-supplemented cows. Milk fat percentage tended to be 0.18 percentage units lower and fat yield was 0.08 kg/d lower for thiamin-supplemented cows. Thiamin supplementation tended to increase milk and component production when dietary concentrations of neutral and acid detergent fiber were lower and nonfiber carbohydrate was higher than recommended.
(**Key words:** thiamin, milk production, intake)

Abbreviation key: C = control diet, **CBP** = corn byproduct diet, **CS** = corn-soybean meal diet, **NFC** = nonfiber carbohydrate, **PEM** = polioencephalomalacia, **TH** = thiamin supplemented diet, **TLC** = theoretical length of cut.

INTRODUCTION

Most of the study of thiamin for ruminants has been in relation to a clinical central nervous system condition observed in beef cattle called polioencephalomalacia (**PEM**). Cattle with clinical signs of PEM, which include circling behavior, rigid stance, and convulsions, respond dramatically to large intravenous doses of thiamin (3, 6). Incidence of PEM appears to be related to ruminal destruction of thiamin by thiaminase enzymes produced by ruminal bacteria (3, 6). Cattle fed high concentrate or feedlot diets are most susceptible to PEM, but it has also been observed in grazing animals (3, 6).

In lactating dairy cows, we are unaware of any reports of PEM or performance response to dietary thiamin supplementation. Without extensive ruminal thiamin destruction, a thiamin deficiency in lactating dairy cows seems unlikely, since Erdman (7) estimated a fivefold higher small intestinal flow of thiamin (ruminal escape + production) relative to requirements extrapolated with data from lactating sows. Some practical feeding guides recommend dietary thiamin supplementation when high levels of corn gluten feed are used in beef cattle diets (2).

The main objective of our first trial was to evaluate intake and milk production by dairy cows being fed a corn byproduct-based diet (**CBP**) versus a corn-soybean meal-based diet (**CS**). Because a corn byproduct-based on corn gluten feed was under evaluation in trial 1, a secondary objective was to evaluate the effect of dietary thiamin supplementation on lactation performance. Based on the results of trial 1, our objective in trials 2 and 3 was to further evaluate the effect of dietary thiamin supplementation on intake and milk production by dairy cows. Corn silage was the main forage used in trial 2, because it was available after a forage feeding trial conducted by our laboratory. Alfalfa silage was the sole forage used in trial 3, because our main objective was to evaluate the effect of processing alfalfa silage

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on intake, digestion, and milk production by dairy cows. Although there is no proposed relationship between these factors, dietary thiamin supplementation was included in the factorial design used in trial 3 to gain more data on its effects. Only effects related to dietary thiamin supplementation are presented and discussed in this paper.

MATERIALS AND METHODS

Trial 1

Twenty-eight Holstein cows (16 multiparous and 12 primiparous) averaging 142 DIM (SD = 41) and 605 kg of BW (SD = 82) at trial initiation were blocked by parity and assigned randomly to either placebo or thiamin top-dress for the 8-wk experiment. Within each of these groups, cows were further assigned randomly to TMR containing either CS or CBP for 4 wk with the TMR treatments then reversed for a second 4-wk experimental period. Before the start of the experiment, all cows were fed diet CS during a 2-wk preliminary period. Cows were housed and fed individually in tie stalls. The top-dress—composed of either wheat middlings or a thiamin mononitrate—wheat middlings mixture was fed at the rate of 57 g/d per cow to individual cows once daily to provide a supplemental thiamin intake of 0 (**C**) or 150 (**TH**) mg/d per cow.

The ingredient composition of CS and CBP diets is presented in Table 1. Both diets contained 55% alfalfa silage and 45% concentrate (DM basis). Corn byproduct was included in the CBP diet as replacement for two-thirds of the ground, shelled corn found in the CS diet. The dried, pelleted corn byproduct (Koch Feed Products, Wichita, KS) was composed of wet corn gluten feed and starch sludge, starch, and syrup. Starch sludge is composed of the settlings from starch cookers used in the production of corn syrup. The higher CP content of corn byproduct than shelled corn allowed for elimination of soybean meal from the CBP diet and dictated that diet CS also be formulated for 20% CP (DM basis). Diets were formulated to meet or exceed NRC (11) requirements for minerals and vitamins and were fed as TMR once daily. All cows were injected with bST (Posilac, Monsanto Company, St. Louis, MO) every 14 d starting on d 1 of the experiment.

Dry matter content of alfalfa silage was determined weekly with a 60°C forced-air oven to adjust as-fed ratios of diet ingredients. The amounts of TMR offered and refused were recorded daily during the experimental period. The alfalfa silage and concentrate mixtures were sampled on d 15 and 22 of each period and each was composited by period for nutrient analysis. Composite feed samples were dried for 48 h in a 60°C forced-air oven and ground to pass a 1-mm Wiley mill screen (Arthur H. Thomas, Philadelphia, PA). Samples were analyzed for DM, OM, and CP (1), NDF using α-amylase (Sigma no. A3306; Sigma Chemical Co., St. Louis, MO) and sodium sulfite (15), and ADF (8). Nonfiber carbohydrate (**NFC**) content was calculated as the difference between 100 and the sum of (NDF + CP + ash + fat) with NDF, CP, and ash determined analytically and fat with NRC (11) tabular values.

Cows were milked twice daily and production was recorded at each milking during the preliminary and experimental periods. Milk samples taken from a.m. and p.m. milkings on d 20 and 21 and d 27 and 28 of each period were analyzed for fat, CP, and urea-nitrogen by infrared analysis (Ag Source Milk Analysis Laboratory, Menomonie, WI). Milk composition was calculated as an average of a.m. and p.m. samples with the proportion of daily milk production at that milking as a weighting factor.

Data from wk 3 and 4 and wk 7 and 8 of the experiment were analyzed as a split-plot design using the general linear models procedure of SAS (13). Milk production data from the second week of the preliminary period were used as a covariate. The model included covariate, parity (primiparous vs. multiparous cows), diet (CS vs. CBP), top-dress (C vs. TH), cow, and two-way interaction terms. Cow effects were used as the error term for testing top-dress effects and residual error was used to test diet and top-dress by diet interactions.

Trial 2

Twenty multiparous Holstein cows averaging 195 DIM (SD = 33) and 657 kg of BW (SD = 52) at trial initiation were used in a crossover design with 4-wk periods. Wheat middlings or thiamin mononitrate-wheat middlings mixture were added to TMR to provide an approximate daily supplemental thiamin intake of 0 or 300 mg/cow.

Dietary ingredient composition is presented in Table 1. Diets contained 50% forage (two-thirds corn silage and one-third alfalfa silage) and 50% concentrate (DM basis). Placebo or thiamin supplements were added to respective TMR at 0.5% of DM. Diets were formulated for 17.5% CP (DM basis) and to meet or exceed NRC (11) requirements for minerals and vitamins. Diets were fed as TMR once daily. All cows were injected with Posilac every 14 d starting on d 1 of the experiment.

Dry matter content of corn silage and alfalfa silage was determined weekly with 60°C forced-air oven for adjustment of as-fed ratios of dietary ingredients. Cows were housed and fed individually in tie stalls. The amounts of TMR offered and refused were recorded daily. The corn silage, alfalfa silage, and concentrate

Table 1. Diet ingredient and nutrient composition for trials 1, 2, and 3.

	Trial 1		Trial 2		Trial 3	
Item	CS[1]	CBP[2]	C[3]	TH[4]	C	TH
Ingredient, % of DM						
Alfalfa silage[5]	55.0	55.0	16.5	16.5	60.0	60.0
Corn silage[6]	...	...	33.5	33.5	...	...
Ground shelled corn	36.4	11.4	27.9	27.9	26.6	26.6
Corn byproduct[7]	...	33.0	...	...	...	...
Animal fat	...	...	...	...	0.9	0.9
Soybean meal, solvent	6.9	...	8.9	8.9	...	...
Soybean meal, expeller[8]	...	...	9.0	9.0	9.8	9.8
Urea	...	...	0.3	0.3	...	...
Calcium carbonate	...	...	1.2	1.2	...	...
Dicalcium phosphate	0.9	...	0.7	0.7	0.7	0.7
Magnesium oxide	0.2	...	0.2	0.2	0.2	0.2
TM salt[9]	0.4	0.4	0.3	0.3	0.4	0.4
Sodium bicarbonate	...	...	0.8	0.8	0.7	0.7
Vitamin supplement[10]	0.2	0.2	0.2	0.2	0.2	0.2
Thiamin supplement[11]	top-dress	top-dress	0.5	0.5	0.5	0.5
Nutrient						
DM, %	62.5	62.1	63.6	63.6	58.2	58.2
CP, % of DM	19.4	20.7	17.0	17.0	18.7	18.7
NDF, % of DM	24.6	30.2	24.3	24.3	32.3	32.3
ADF, % of DM	18.3	17.7	15.6	15.6	22.2	22.2
NFC[12], % of DM	44.8	38.3	46.7	46.7	36.0	36.0

[1]CS = Corn-soybean meal diet.

[2]CBP = Corn byproduct diet.

[3]C = Control diet.

[4]TH = Thiamin-supplemented diet.

[5]Trial 1: Contained 22.8% CP, 36.8% NDF, and 28.4% ADF (DM basis). Trial 2: Contained 25.2% CP, 36.7% NDF, and 32.9% ADF (DM basis). Trial 3: Contained 20.3% CP, 44.1% NDF, and 32.8% ADF (DM basis).

[6]Contained 35.6% DM and 7.3% CP, 40.6% NDF, and 23.9% ADF (DM basis).

[7]Contained 88% DM and 21.4% CP, 25.6% NDF, 13.6% ADF, and 3.4% ether extract (DM basis).

[8]SoyPlus, West Central Cooperative, Ralston, IA.

[9]Trace-mineralized salt: NaCl, 92.5 to 95.5%; not less than 0.55% Zn, 0.55% Mn, 0.35% Fe, 0.14% Cu, 0.008% I, 0.006% Se, and 0.002% Co.

[10]Vitamin supplement was added to provide vitamins A, D, and E at the rate of 150,000, 50,000, and 500 IU/d per cow.

[11]Trial 1: Wheat middlings or thiamin mononitrate-wheat middlings mixture top-dressed at the rate of 57 g/d per cow to individual cows once daily to provide 0 or 150 mg of thiamin/d per cow. Trials 2 and 3: Wheat middlings or thiamin mononitrate-wheat middlings mixture added to TMR to provide an approximate daily thiamin intake of 0 or 300 mg/cow.

[12]NFC = Nonfiber carbohydrate = 100 − (NDF + CP + ether extract + ash).

mixture were sampled on d 15 and 22 of each period and each was composited by period for nutrient analysis. Composite feed samples were dried for 48 h in a 60°C forced-air oven and ground to pass a 1-mm Wiley mill screen (Arthur H. Thomas, Philadelphia, PA). Samples were analyzed for DM, OM, CP, NDF, and ADF, and NFC was calculated as described for trial 1.

Cows were milked twice daily, and production was recorded at each milking. Milk samples taken from a.m. and p.m. milkings on d 20 and 21 and d 27 and 28 of each period were analyzed for fat, CP, and urea-nitrogen by infrared analysis (Ag Source Milk Analysis Laboratory, Menomonie, WI).

Dry matter intake and milk production data from wk 3 and 4 and wk 7 and 8 were analyzed as a crossover design using the general linear models procedure of SAS (13).

Trial 3

Sixteen multiparous Holstein cows (eight ruminally cannulated and eight intact) averaging 120 DIM (SD = 36) and 600 kg of BW (SD = 39) at trial initiation were used in a replicated 4 × 4 Latin square design with 21-d periods, 14 d for dietary adaptation. Thiamin supplementation and alfalfa silage processing were main effects in the 2 × 2 factorial arrangement of treatments. Wheat middlings or thiamin mononitrate-wheat middlings mixture were added to TMR to provide an approximate daily supplemental thiamin intake of 0 or

300 mg/cow. Alfalfa silage was either harvested at 0.95 cm theoretical length of cut (**TLC**) without rolling or 1.90 cm TLC with a 1-mm roll clearance with an experimental pull-type chopper fitted with an on-board roller mill.

Dietary ingredient composition is presented in Table 1. Diets contained 60% alfalfa silage and 40% concentrate (DM basis). Placebo or thiamin supplements were added to respective TMR at 0.5% of DM. Diets were formulated for 18.5% CP (DM basis) and to meet or exceed NRC (11) requirements for minerals and vitamins. Diets were fed as TMR once daily. All cows were injected with Posilac every 10 d starting on d 1 of the experiment.

Dry matter content of alfalfa silage was determined weekly with a 60°C forced-air oven for adjustment of as-fed ratios of diet ingredients. Cows were housed and fed individually in tie stalls. The amounts of feed offered and refused were recorded daily. The alfalfa silage treatments and concentrate mixtures were sampled on d 15 of each period for nutrient analysis. Feed samples were dried for 48 h in a 60°C forced-air oven and ground to pass a 1-mm Wiley mill screen (Arthur H. Thomas, Philadelphia, PA). Samples were analyzed for DM, OM, CP, NDF, and ADF, and NFC was calculated as described for trial 1.

Cows were milked twice daily and production was recorded at each milking. Milk samples taken from a.m. and p.m. milkings on d 17, 18, and 19 of each period were analyzed for fat, CP, and urea-nitrogen by infrared analysis (Ag Source Milk Analysis Laboratory, Menomonie, WI).

Ruminal fluid from the eight ruminally cannulated cows was sampled immediately before feeding and at 4, 8, and 12 h postfeeding on d 19 and 20 of each period. Samples were taken from five different locations in the rumen via the cannula using a custom-made metal filter probe and pH was determined (Twin pH meter Model B-213, Spectrum Technologies, Inc., Plainfield, IL).

The 48-h ruminal in situ DM degradation of the alfalfa silage treatments was determined in ruminally cannulated cows on d 19 of each period. In situ bags (25×35 cm, 52-μ pore size) were made of Dacron polyester cloth (R102 Marvelaire White, N. Erlanger, Blumgardt and Co., Inc., New York). The alfalfa silage treatments were incubated with triplicate bags per cow and matching incubation alfalfa silage with diet alfalfa silage by cow and period. Twenty-five grams of DM was weighed into each bag (30 mg/cm^2 sample size to surface area ratio) and incubated without drying or grinding at 2 h postfeeding. In situ bags were placed in a nylon laundry bag and positioned in the ventral rumen. Duplicate blank bags were incubated in each laundry bag to correct for influx of DM into the sample bags. In situ bags were washed in a commercial washing machine with cold water for two cycles of 12 min each (4). Bags and residue were then dried at 60°C for 48 h to determine DM disappearance.

Data from wk 3 of each period were analyzed as a replicated Latin square using the general linear models procedure of SAS (13). Ruminal pH data were analyzed using PROC MIXED of SAS (10) for repeated measures.

RESULTS AND DISCUSSION

Dietary nutrient composition and DMI and milk production data from the three experiments are presented in Tables 1 and 2, respectively.

Trial 1

Diets contained 19.4 to 20.7% CP, which exceeds NRC (11) guidelines. This was related to the high CP content of the alfalfa silage and its dietary inclusion rate and the higher CP content of corn byproduct and its dietary substitution rate for ground, shelled corn. Although not measured, high ruminal degradability of dietary CP was likely because high CP degradability has been reported for alfalfa silage, solvent-extracted soybean meal, and corn gluten feed (14). The concentration of dietary NDF (27.4% of DM on average) was above the NRC (11) minimum recommended allowance. However, the concentration of NDF in diet CS was slightly below the NRC (11) minimum recommended allowance, and diet CS contained 5.6 percentage units less NDF than diet CBP. This was related to the higher NDF content of corn byproduct and its substitution rate for corn grain. Concentration of dietary NFC (41.5% of DM on average) was slightly above the optimum concentration of 40% (DM basis) suggested by Nocek and Russell (12), but diet CS contained 6.5 percentage units more NFC than diet CBP.

Milk yield was 2.7 kg/d ($P = 0.01$) higher for cows fed TH. Milk fat and protein percentages were unaffected by dietary thiamin supplementation, but yields of fat and protein were increased 0.13 and 0.10 kg/d ($P = 0.01$), respectively, for TH. Grigat and Mathison (9) reported that dietary thiamin supplementation increased average daily gain in feedlot steers fed all-concentrate diets. Positive effects of dietary thiamin supplementation on milk and component yields were evident on both CS and CBP diets, and no thiamin supplementation × diet interaction was observed. This suggests that effects of dietary thiamin supplementation observed in this trial were unrelated to the use of corn byproduct vs. corn grain plus soybean meal. This finding contradicts Berger et al. (2) who recommended dietary thiamin supplementation only when feeding high levels of corn gluten feed.

Table 2. Effect of dietary thiamin supplementation on DMI, milk production, and milk composition by dairy cows in trials 1, 2, and 3.

	Trial 1[1]				Trial 2				Trial 3			
Item	C[2]	TH[3]	SEM	*P* =	C	TH	SEM	*P* =	C	TH	SEM	*P* =
DMI, kg/d	23.1	24.0	0.7	NS	24.9	24.8	0.2	NS	26.3	25.5	0.3	0.15
Milk yield, kg/d	32.1	34.8	0.5	0.01	38.8	39.5	0.3	0.15	40.8	40.6	0.4	NS
Fat, %	3.39	3.50	0.06	NS[4]	3.40	3.41	0.01	NS	3.74	3.56	0.05	0.06
Fat yield, kg/d	1.07	1.20	0.02	0.01	1.30	1.32	0.02	NS	1.51	1.43	0.02	0.05
Protein, %	3.28	3.34	0.04	NS	3.24	3.24	0.01	NS	2.91	2.83	0.05	NS
Protein yield, kg/d	1.05	1.15	0.02	0.01	1.23	1.27	0.01	0.09	1.18	1.15	0.02	NS
Urea-nitrogen, mg/dl	14.5	14.4	0.4	NS	20.6	20.3	0.4	NS	17.9	17.8	0.5	NS

[1]Means are covariate-adjusted least square means.

[2]C = Control diet.

[3]TH = Thiamin-supplemented diet.

[4]NS = Not significant ($P > 0.15$).

Trial 2

The concentrations of dietary NDF and ADF were below the NRC (11) minimum recommended allowance. The concentration of dietary NFC (46.7% of DM) was well above the optimum concentration of 40% (DM basis) suggested by Nocek and Russell (12). Dietary CP concentration (17.0% of DM) was lower than for trial 1 (20.1% of DM on average). Although not measured in either trial, lower ruminal degradability of dietary CP for this trial versus trial 1 was likely because of the use of corn silage and expeller-extracted soybean meal (14).

Milk and protein yields tended to be 0.7 ($P = 0.15$) and 0.04 kg/d ($P = 0.09$) higher, respectively, for cows fed TH. Dietary thiamin supplementation did not affect DMI or milk fat in this trial.

Trial 3

Dietary concentrations of NDF (32.3% vs. 24.3 to 30.2% of DM) and ADF (22.2% vs. 15.6 to 18.3% of DM) were higher and NFC (36.0% vs. 38.3 to 46.7% of DM) was lower for this trial than for trials 1 and 2. Also, dietary NDF from forage was higher for this trial than for trials 1 and 2 (26.5% vs. 19.7 to 20.2% of DM). The concentration of CP in the diet for this trial (18.7% of DM) was intermediate between diets for trials 1 (20.1% of DM on average) and 2 (17.0% of DM).

Intakes of DM tended to be 0.8 kg/d lower ($P = 0.15$) for cows fed TH. Milk fat percentage tended to be 0.18 percentage units lower ($P = 0.06$) and fat yield was 0.08 kg/d lower ($P = 0.05$) for cows fed TH. Dietary thiamin supplementation did not affect milk yield or protein in this trial. No interactions of thiamin supplementation by alfalfa silage processing were observed.

Ruminal fluid was sampled as part of our evaluation of alfalfa silage processing effects on digestion. Thus, ruminal data were also collected regarding the effects of dietary thiamin supplementation. Ruminal fluid pH was unaffected by dietary thiamin supplementation and there were no treatment × sampling time interactions; pH averaged 6.49 for C versus 6.52 for TH and 6.26 for C versus 6.27 for TH at 4 h and 8 h postfeeding, respectively (data not presented in table). In situ DM degradation of alfalfa silage was also unaffected by dietary thiamin supplementation, averaging 68.8% and 69.0% for C and TH, respectively (data not presented in table).

CONCLUSIONS

Supplementing dietary thiamin at the rate of 150 to 300 mg/d per cow increased or tended to increase milk and component production in trials 1 and 2, respectively. In trial 1, increased milk, fat, and protein yields in response to dietary thiamin supplementation were observed in cows fed TMR containing either corn-by-product or corn-soybean meal-based concentrates. Differences in response to dietary thiamin supplementation between trials cannot be clearly explained, but diets fed in trials 1 and 2 contained lower concentrations of NDF from forage and total NDF and higher concentrations of NFC than the diet fed in trial 3. Also, the CP concentration of diets fed in trial 1 greatly exceeded NRC (11) guidelines and of diets fed in trials 2 and 3. These differences in dietary nutrient concentrations between trials may have influenced ruminal thiaminase production by rumen bacteria and hence the observed response to dietary thiamin supplementation (3, 6). Another possible explanation for the observed variation in response to dietary thiamin supplementation between trials could be the presence or nonpresence of an antithiamin factor produced by *Fusarium* molds (5). However, we collected no thiaminase or mycotoxin data in these trials to support or refute either explanation. It is unlikely that response differences among trials were related to problems with thiamin stability, because the expected loss of potency is only 5% over 8- to 12-wk of storage after mixing in a premix and negligible over 24 h after mixing in TMR (M. B. Coelho, BASF Corp., Mount Olive, NJ, personal communication). The trend for reduced DMI and milk fat in response to dietary thiamin supplementation noted in trial 3 was unexpected, and we offer no explanation for this observation. Results of these trials suggest a possible role for thiamin supplementation when dietary concentrations of NDF and ADF are lower and NFC is higher than recommended. More research with lactating dairy cows on the response to dietary thiamin supplementation and on nutritional factors affecting this response is warranted.

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