Short communication: Effects of dietary 5,6-dimethylbenzimidazole supplementation on vitamin B₁₂ supply, lactation performance, and energy balance in dairy cows during the transition period and early lactation

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ABSTRACT

The current study was conducted to investigate the effects of 5,6-dimethylbenzimidazole (DMB) supplementation to the feed during the transition period and early lactation on the vitamin B₁₂ supply, lactation performance, and energy balance in postpartum cows. Twenty-four prepartum Holstein dairy cows were divided into 12 blocks based on their parity and milk yield at the last lactation and were then randomly allocated to 1 of 2 treatments: a basal diet without DMB (control) or a treatment diet that contained 1.5 g of DMB/d per cow. The study started at wk 3 before the expected calving day and ended at wk 8 postpartum. The feed intake and the lactation performance were measured weekly after calving. Blood parameters were measured on d -10, 0, 8, 15, 29, 43, and 57 relative to the calving day. Body weight was measured on the calving day and on d 57 after calving. The yields of milk, protein, and lactose in cows fed DMB were higher than in the control throughout the whole postpartum stage. On wk 8 postpartum, the vitamin B₁₂ content in the milk and sera was greater in cows fed DMB than in the control. The overall body weight loss from wk 1 to 8 postpartum was less in cows fed DMB than in the control. The plasma content of nonesterified fatty acids and β -hydroxybutyric acid was significantly lower in cows fed DMB than in the control throughout the whole experimental stage. In conclusion, dietary DMB fed during the transition period and early lactation improved the vitamin B₁₂ supply, milk production, and energy balance of postpartum dairy cows.

Key words: 5,6-dimethylbenzimidazole, vitamin B₁₂, transition, dairy cow

Short Communication

Vitamin B₁₂ (VB₁₂) is an essential component of methylmalonyl-CoA mutase, which catalyzes the isomerization of methylmalonyl-CoA to succinyl-CoA (an important step in hepatic gluconeogenesis; Padmakumar et al., 1997). In dairy cows, plasma VB₁₂ levels are lowest in 0 to 60 DIM during the lactation period (Girard and Matte, 1999; Girard and Matte, 2005), indicating that early postpartum is a VB₁₂ deficiency period for dairy cows. Because VB₁₂ can be degraded in the rumen (Girard et al., 2009), supplementation of VB₁₂ to cows can be carried out by intramuscular injection (Girard and Matte, 2005; Akins et al., 2013). A higher milk yield was produced with VB₁₂ injection than in the absence of VB₁₂ in early-lactating dairy cows (Girard and Matte, 2005) but not in mid-lactating dairy cows (Akins et al., 2013). However, intramuscular injection is not a practical approach in the modern dairy industry.

The substance 5,6-dimethylbenzimidazole (DMB) is a natural benzimidazole derivative and a component of VB₁₂ (Warren et al., 2002). It has been previously shown that dietary DMB increased the VB₁₂ concentration in the rumen fluid of sheep (Rickard et al., 1975) and lactating dairy cows (Brito et al., 2015), indicating that dietary DMB may be an alternative way to increase VB₁₂ in dairy cows. Thus, we hypothesized that the DMB addition during the transition period and early lactation may increase VB₁₂ supply and improve postpartum lactation performance. Thus, the current study was conducted to investigate the effects of dietary DMB addition, starting in the prepartum period, on the VB₁₂ supply feed intake, milk performance, and energy balance (EB) of postpartum dairy cows.

The animal study was approved by the Institutional Animal Care and Use Committee of Zhejiang University (Hangzhou, China). Twenty-four prepartum Holstein dairy cows (BW = 665 kg; SD = 8.7) were divided into 12 blocks based on parity (2.84; SD = 0.12) and milk yield at the last lactation (31.5 kg/d; SD = 0.49)

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Table 1. Ingredients and chemical composition of the diets for prepartum and postpartum dairy cows

Item	Prepartum	Postpartum
Ingredient, % of DM		
Corn grain, ground	12.2	24.2
Steam-flaked corn	—	7.33
Soybean meal	10.8	13.4
Barley	3.91	3.19
Cottonseed meal	3.20	—
Whole cottonseeds	—	5.07
Sugar beet pulp	4.17	5.35
Wheat bran	2.44	—
Corn silage	17.4	9.94
Alfalfa hay	7.95	22.9
Chinese wild ryegrass	24.1	—
Oat grass	11.5	4.91
Premix ¹	2.14	3.65
Chemical composition, % of DM unless noted		
OM	92.48	92.06
CP	11.8	16.3
NDF	42.3	32.3
ADF	23.3	18.5
Ca	0.83	0.88
P	0.38	0.42
Co, mg/kg	1.70	2.90
NE _L , Mcal/kg of DM	—	1.62

¹Formulated to contain (per kilogram of premix) 250 KIU of vitamin A, 50 KIU of vitamin D, 1,100 IU of vitamin E, 600 mg of Fe, 650 mg of Cu, 3,000 mg of Zn, 630 mg of Mn, 17 mg of Se, 36 mg of I, 8 mg of Co, 15 to 18% of NaCl, and ≤10% water.

and were randomly allocated within the blocks to 1 of 2 treatments: a basal TMR diet without (control) or with dietary DMB at 1.5 g/d per cow (treatment). The basal diets were formulated according to the dairy nutritional requirement suggested by NRC (2001). The treatment started at 3 wk before the expected calving day and ended at wk 8 postpartum. Cows in both groups were fed the same TMR rations. The ingredients and chemical composition of the pre- and postpartum TMR are shown in Table 1. The DMB was added to the feed 3 times a day by top-dressing during the experiment. The cows were housed in tiestall barns and had free access to fresh water.

During the postpartum period, the feed provided and refused was weighed for 3 consecutive days (d 3, 4, and 5) weekly to determine the DMI. Representative samples of the TMR and orts were collected weekly for a chemical composition analysis. All samples were dried at 65°C for 48 h and then ground through a 1-mm mesh screen using a high-speed grinder (Tecator 1093, Foss Tecator AB, Hoganas, Sweden) and stored at 4°C for later analysis. All samples were analyzed for DM (105°C for 5 h), CP (method 988.05; AOAC, 1990), ADF (method 973.18; AOAC, 1990), and NDF without the addition of sodium sulfite and amylase (Van Soest et al., 1991).

The individual milk yield was recorded on the same days (d 3, 4, and 5) for DMI determination with a milk-sampling device (Waikato Milking Systems NZ Ltd., Waikato, Hamilton, New Zealand). On the fourth experimental day of each postpartum week, a 50-mL aliquot of milk was collected from 3 milkings proportional to the yield (4:3:3, composite). Bronopol tablets (D&F Control Systems, San Ramon, CA) were added to the composite milk sample, which was then stored at 4°C for later analysis of protein, fat, lactose, and MUN (Laporte and Paquin, 1999). Another aliquot of the milk sample was collected at last milk sampling and stored at −20°C for determination of VB₁₂ (Girard and Matte, 2005).

Blood samples (5 mL) were obtained in evacuated tubes containing sodium heparin from the coccygeal vein 4 h after the morning feeding at −10, 0, 8, 15, 29, 43, and 57 d relative to calving. The plasma was harvested by centrifugation at 3,000 × *g* for 10 min at 4°C and stored at −20°C until analysis. Plasma samples were analyzed using an auto-analyzer 7020 (Hitachi High-Technologies Corp., Tokyo, Japan) for glucose, nonesterified fatty acids (NEFA), BHB, superoxide dismutase, BUN, and cholesterol by a previously described method (Wang et al., 2015). The plasma VB₁₂ content was determined by the method described by Girard and Matte (2005), with the plasma samples taken on 57 DIM.

The BW was estimated at d 0 and 57 after calving based on the method described by Yan et al. (2009). The EB at wk 1 and 8 postpartum was determined as follows (Spicer et al., 1990):

$$\text{EB (Mcal/d)} = (\text{DMI} \times \text{diet NE}_L) - [(0.08 \times \text{BW}^{0.75}) + (\text{milk NE}_L \times \text{milk yield})].$$

All data were analyzed using the PROC MIXED protocol of the SAS software system (SAS Institute, 2000). A randomized block design with repeated measurements was used with week, treatment, block, treatments nested in block, and the treatment × week interaction as fixed effects, and cow within treatment was used as a random effect. The statistical model was

$$Y_{ijk} = \mu + B_i + T_j + T(B)_{ij} + W_k + TW_{jk} + \varepsilon_{ijk},$$

where Y_{ijk} = dependent variable, μ = overall mean, B_i = block effect, T_j = treatment effect, W_k = week effect, and TW_{jk} = interaction of treatment and week. Two error terms are $T(B)_{ij}$ (treatment nested into block) and ε_{ijk} (the residual error). The results were reported as least squares means. The values of $P < 0.05$ were

Table 2. Effect of dietary 5,6-dimethylbenzimidazole (DMB) on feed intake, lactation performance, and BW change during the first 8 wk of lactation of the dairy cows¹

Item	DMB, g/d		SEM	P-value		
	0	1.5		Treatment	Week	Treatment × week
DMI, kg/d	21.4	23.2	0.53	0.04	<0.01	0.71
Milk yield, kg/d						
Raw	36.4	38.4	0.44	0.01	<0.01	0.53
Protein	1.13	1.21	0.017	<0.01	<0.01	0.88
Fat	1.45	1.51	0.050	0.26	0.16	0.22
Lactose	1.81	1.93	0.025	<0.01	<0.01	0.74
Milk content, g/100 g						
Protein	3.25	3.22	0.044	0.26	<0.01	0.86
Fat	4.08	3.93	0.157	0.24	<0.01	0.09
Lactose	4.94	4.99	0.035	0.11	<0.01	0.07
MUN	12.0	12.9	0.437	0.14	0.21	0.07
Vitamin B ₁₂ , ng/L ²	330	783	104.1	0.02	—	—
Feed efficiency ³	1.67	1.60	0.042	0.25	0.40	0.50
Nitrogen conversion ⁴	0.31	0.30	0.006	0.19	<0.01	0.45
BW change, kg	−42.9	−27.4	5.31	0.04	—	—
Energy balance, Mcal/d	−4.07	−2.02	0.67	0.04	<0.01	0.21

¹Sampling times: DMI and milk yield were recorded on d 3, 4, and 5 in each postpartum week, and milk components were determined on d 4 for each postpartum week.

²Vitamin B₁₂ concentrations were measured at wk 8 postpartum.

³Feed efficiency = milk yield/DMI.

⁴Nitrogen conversion = milk protein yield/CP intake.

used to define statistical significance, and the values $P < 0.10$ and $P > 0.05$ were defined as statistical trends.

The daily DMI was approximately 1.8 kg higher in cows fed DMB than in the control ($P = 0.04$; Table 2). The milk yield ($P = 0.01$), protein yield ($P < 0.01$), and lactose yield ($P < 0.01$) were higher in cows fed DMB than in the control. The VB₁₂ contents in the milk ($P = 0.02$) and plasma ($P = 0.04$) were greater in cows fed DMB than in the control. It was observed that milk yield was greater in DMB cows than in control cows throughout the whole postpartum period (Table 2). No interactive effects between sampling time and treatment were observed in the above-mentioned variables ($P > 0.05$). Our study was consistent with the observation by Brito et al. (2015) that dietary DMB (1.5 g/d per head) increased the synthetic efficiency of VB₁₂ in the rumen of late-lactating dairy cows, indicating a function of DMB in the stimulation of the ruminal VB₁₂ synthesis. The higher VB₁₂ content in the plasma and milk of cows fed DMB in the current study proved that dietary DMB is an efficient way to improve the VB₁₂ content in dairy cows. In addition, DMB had a beneficial effect on milk production, possibly due to an improvement of the DMI of these animals. However, the milk production increase in our study did not agree with the study of Brito et al. (2015), in which adding the same amount of DMB to the diet did not affect the milk production in dairy cows. This inconsistency was probably due to different stages of cows used in the 2 studies. Cows used in our study were in the transition

period and early lactation, whereas cows used in Brito et al. (2015) were in late lactation. The requirement of VB₁₂ was not consistency across the whole lactating period (Girard and Matte, 1999). The lowest VB₁₂ concentrations in plasma identified at 0 to 60 DIM suggested that dairy cows during this period had relatively greater requirements compared with animals in other stages. It is possible that the DMB effect may depend on lactation stage. Both dietary composition and physiological period of the 2 studies differed from each other. These 2 factors may affect the rumen populations and their ability to synthesize or use the VB₁₂.

Compared with the control, cows fed DMB had higher EB at both wk 1 and 8 postpartum ($P = 0.04$; Table 2). The BW loss was significantly greater in the control cows than in cows fed DMB ($P = 0.04$; Table 2). The plasma concentrations of NEFA ($P < 0.01$) and BHB ($P = 0.04$) were lower in cows fed DMB than in the control (Table 3). Concentrations of other plasma parameters, including glucose, BUN, cholesterol, and superoxide dismutase, in the plasma were not different between the treatments ($P > 0.05$). No interactive effects between sampling time and treatment were observed in plasma variables ($P > 0.05$). It has been reported that greater EB, less BW loss, and lower contents of NEFA and BHB in the plasma of early-lactating dairy cows can be partly due to their greater DMI (Schäff et al., 2013; Wang et al., 2013; Weber et al., 2016). In the current study, the improved EB in the DMB-added cows may be attributed to the greater DMI driven directly or

Table 3. Effect of dietary 5,6-dimethylbenzimidazole (DMB) on plasma variables of prepartum and postpartum dairy cows¹

Item	DMB, g/d			P-value		
	0	1.5	SEM	Treatment	Week	Treatment × week
Glucose, mmol/L	3.44	3.50	0.043	0.54	<0.01	0.85
BUN, mmol/L	4.58	4.89	0.147	0.14	<0.01	0.28
Cholesterol, mmol/L	3.52	3.88	0.236	0.51	<0.01	0.11
Nonesterified fatty acids, μ mol/L	323.7	219.2	19.93	<0.01	<0.01	0.26
BHB, μ mol/L	550.6	493.7	20.6	0.04	0.02	0.08
Superoxide dismutase, U/mL	110.4	111.4	1.42	0.63	<0.01	0.28
Vitamin B ₁₂ , ng/L ²	195	378	71.4	0.04	—	—

¹Sampling times: the blood was sampled at −10, 0, 8, 15, 29, 43, and 57 d relative to calving.

²Vitamin B₁₂ concentrations were measured at wk 8 postpartum.

indirectly by DMB addition. However, the mechanism of the effect of DMB on DMI regulation in dairy cows remains to be elucidated. In conclusion, dietary DMB from the prepartum stage improved the DMI, milk production, EB status, and VB₁₂ supply and reduced the BW loss of early-lactating dairy cows, suggesting that dietary DMB is a convenient vehicle to increase the VB₁₂ supply.

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