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Short communication: Effects of dietary 5,6-dimethylbenzimidazole supplementation on vitamin B_{12} 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 B_{12} 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 B_{12} 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 B_{12} supply, milk production, and energy balance of postpartum dairy cows.

Key words: 5,6-dimethylbenzimidazole, vitamin B_{12} , transition, dairy cow

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Short Communication

Vitamin B_{12} (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 VB_{12} 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 VB_{12} to cows can be carried out by intramuscular injection (Girard and Matte, 2005; Akins et al., 2013). A higher milk yield was produced with VB_{12} injection than in the absence of VB_{12} 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 |

 $^1\mathrm{Formulated}$ 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 (Day and 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 $-20^{\circ}\mathrm{C}$ 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 $3,000 \times g$ 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):

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

$$Y_{ijk} = \mu + B_i + T_j + T(B)_{ij} + W_k + TW_{jk} + \epsilon_{ijk}, \label{eq:equation:$$

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

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