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## Effect of dietary supplements of biotin, intramuscular injections of vitamin B<sub>12</sub>, or both on postpartum lactation performance in multiparous dairy cows

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### ABSTRACT

The current study was conducted to investigate the effects of dietary supplementation of biotin, intramuscular injections of vitamin B<sub>12</sub> (VB<sub>12</sub>), or both beginning at the prepartum period on feed intake and lactation performance in postpartum dairy cows. Forty-eight dairy cows were allocated into 12 blocks, based on parity and milk yield of the previous lactation cycle, and randomly assigned to 1 of 4 treatments. Supplementation of VB<sub>12</sub> (weekly intramuscular injections of 0 or 10 mg) and biotin (dietary supplements of 0 or 30 mg/d) were used in a 2 × 2 factorial arrangement in a randomized complete block design of 12 blocks with repeated measures. The study started at 3 wk before the expected calving date and ended at 8 wk after calving. Feed intake and lactation performance (milk yield and composition) were recorded weekly after calving. Blood variables were measured on d -10, 0, 8, 15, 29, 43, and 57 relative to calving. When VB<sub>12</sub> was given, the cows had greater feed intake, better lactation performance and lower body weight loss in the postpartum period compared with animals without injection of VB<sub>12</sub>. The VB<sub>12</sub>-injected cows had lower plasma nonesterified fatty acids and β-hydroxybutyrate concentrations but higher plasma superoxide dismutase activity compared with cows without VB<sub>12</sub>. Cows fed a biotin supplement had higher milk protein yield (6 and 8 wk) and lactose yield (6–8 wk), compared with animals without biotin. However, under the present experimental conditions, we found no additive effect of a combined supplement of biotin and vitamin B<sub>12</sub> on lactation performance of dairy cows.

**Key words:** vitamin B<sub>12</sub>, biotin, dairy cows, lactation performance

### INTRODUCTION

Hepatic gluconeogenesis plays an important role in net hepatic glucose generation (Reynolds, 2006), and increasing hepatic gluconeogenesis is an efficient way to increase the milk yield in lactating dairy cows (Rukkwamsuk et al., 1999; Karcher et al., 2007). In gluconeogenesis, the propionate absorbed by hepatic cells is transformed to methylmalonyl-CoA by propionyl-CoA carboxylase, a biotin-dependent enzyme (Scott, 1999; Hügler et al., 2003), and further isomerized to succinyl-CoA by methylmalonyl-CoA mutase, a vitamin B<sub>12</sub> (VB<sub>12</sub>)-dependent enzyme (Padmakumar et al., 1997). Thus, a beneficial effect would be expected when additional VB<sub>12</sub> or biotin is provided to the dairy cows with an insufficient supply in VB<sub>12</sub> or biotin during early lactation.

The effect of biotin or vitamin B<sub>12</sub> on milk production in lactating cows has been addressed in some studies. In early-lactation dairy cows, supplementary biotin could improve DMI and milk yield (Zimmerly and Weiss, 2001); however, this was not found in studies conducted by Fitzgerald et al. (2000) and Ganjkhanelou et al. (2007). In terms of VB<sub>12</sub>, Girard and Matte (2005) found that intramuscular injection of VB<sub>12</sub> alone tended to increase milk total solid contents in cows fed dietary supplements of folic acid, but in the report of Akins et al. (2013) intramuscular injection of VB<sub>12</sub> did not improve lactation performance of dairy cows.

It was noted that plasma biotin concentration of postpartum dairy cows was 50% of that in prepartum dairy cows (Rosendo et al., 2004). Similarly, plasma VB<sub>12</sub> concentration was at the lowest level during 0 to 60 DIM (Girard and Matte, 1999); these data suggest a shortage of VB<sub>12</sub> and biotin in transition dairy cows. Although the independent addition of VB<sub>12</sub> or biotin has been proven to be beneficial to early-lactating dairy cows (Zimmerly and Weiss, 2001; Girard and Matte, 2005), it is not clear whether a combined (VB<sub>12</sub> plus biotin) treatment beginning at the precalving stage would have an interactive effect on the lactation performance of transition cows. Thus, the current study was conducted to investigate the effects of dietary biotin/

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intramuscular VB<sub>12</sub> injection, starting at the prepartum stage, on feed intake, milk performance, BW change, and plasma variables in the postpartum dairy cows.

## MATERIALS AND METHODS

### *Animals, Diets, and Experimental Design*

This experiment was carried out at the Hangjiang Dairy Farm (Hangzhou, China). Animal use was approved by the Animal Care Committee of Zhejiang University. Forty-eight multiparous Holstein dairy cows (BW = 691 kg, SD = 9.9; parity = 2.75, SD = 0.10; milk yield of last lactation period = 31.2 kg/d, SD = 0.55) were allocated into 12 blocks based on the parity and milk yield of previous lactation period. The cows within each block were randomly assigned to 1 of 4 treatments—control, biotin, VB<sub>12</sub>, and biotin plus VB<sub>12</sub>—in addition to the basal TMR. Biotin (30 mg/d) was added to the cow ration 3 times daily during the feeding period. The VB<sub>12</sub> (10 mg/wk) was administered by intramuscular injections (Duplessis et al., 2017). The ingredients and chemical composition of pre- and postpartum TMR are presented in Table 1. Cows were housed in tiestall barns and had free access to fresh water during the experimental period. The TMR was provided to the cows daily at 0630, 1400, and 1900. The cows were milked 3 times daily during the feeding period. The experiment was conducted from 3 wk before expected calving to 8 wk after calving.

The experiment was carried out along with another study (Wang et al., 2018), and both experiments shared control animals. Therefore, the data for the control group were similar to Wang et al. (2018).

### *DMI, Milk Production, and Milk Composition*

To calculate DMI, the feed offered and refused were weighed weekly for 3 consecutive days during the postpartum period. The representative samples of the TMR and orts were collected weekly for analysis of chemical composition. All samples were dried at 65°C for 48 h, ground through a 1-mm screen mesh using a high-speed grinder (Tecator 1093, Hogan, Sweden), and stored in plastic bottles at 4°C to analyze the contents of DM (method no. 934.01), CP (method no. 955.04), ether extract (method no. 920.39), ash (method no. 942.05), and ADF (method no. 973.18) according to the AOAC (1990). The NDF was analyzed by the method of Van Soest et al. (1991).

The individual milk yield was recorded on the same days as DMI determination using a milk-sampling device (Waikato Milking Systems NZ Ltd., Waikato, Hamilton, New Zealand). Milk samples collected from

each cow on d 4 of each week were used to determine milk composition by using a Foss FT+ instrument (Foss Electric, Hillerød, Denmark). The milk samples collected at wk 8 were used to determine VB<sub>12</sub> and biotin concentration. The collected milk samples were prepared according to the method described by Indyk et al. (2014). In brief, 10.0 mL of milk was diluted with 40 mL of water and ultrasonicated. A 5-mL aliquot was placed in a 100°C water bath for 15 min and then centrifuged (4,500 × *g*, 15 min, 4°C; Indyk et al., 2014). Afterward, the supernatant was filtered through combined 0.45- and 0.22-μm filter paper for determination of VB<sub>12</sub> by radioassay using a commercial kit (SimulTRAC B12/FOLATE-S, MP Biomedicals, Santa Ana, CA; Duplessis et al., 2017); and biotin concentration by using another commercial kit (art. no. H1601, R-Biopharm GmbH, Darmstadt, Germany; Chen et al., 2012).

### *Blood Sampling*

Blood samples (5 mL) were obtained from the coccygeal vein into evacuated tubes containing sodium heparin 4 h after the morning feeding on d -10, 0, 8, 15, 29, 43, and 57 relative to calving. Plasma was then harvested by centrifugation at 3,000 × *g* for 10 min at 4°C and stored at -20°C until analysis. Plasma

**Table 1.** Dietary ingredients and chemical composition of pre- 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 <sup>1</sup>	2.14	3.65
Composition, % of DM		
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, <sup>2</sup> mg/kg	1.70	2.90
Ash	7.52	7.94
NE <sub>L</sub> , Mcal/kg of DM	—	1.62

<sup>1</sup>Contained (per kilogram of premix): 250 KIU of vitamin A, 50 KIU of vitamin D, 2,300 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, 15–18% of NaCl, water ≤10%.

<sup>2</sup>Co content in the premix.

samples were analyzed using an auto-analyzer 7020 (Hitachi High-Technologies Corp., Tokyo, Japan) for glucose, nonesterified fatty acids, BHB, BUN, and cholesterol, according to the method described in the previous study (Wang et al., 2013). The assay for plasma superoxide dismutase (SOD) activity was conducted using commercial kit (Nanjing Jiancheng Bioengineering Institute, Nanjing, China; A001-1, Xiong et al., 2015). Plasma sampled at d 57 were used to determine VB<sub>12</sub> and biotin, with the same methods used for milk samples.

### **BW and Energy Balance Status**

Animal BW was measured on d 0 and 57, relative to calving day, based on the measurement of body length and heart girth using the equation  $BW \text{ (kg)} = \text{heart girth}^2 \text{ (m)} \times \text{body length (m)} \times 90$  (Yan et al., 2009). The energy balance (EB) status on wk 1 and 8 postpartum was determined as  $EB \text{ (Mcal/d)} = (\text{DMI} \times \text{diet NE}_L) - [(0.08 \times \text{BW}^{0.75}) + (\text{milk NE}_L \times \text{milk yield})]$  (Spicer et al., 1990).

### **Statistical Analysis**

Data were analyzed using the MIXED procedure (SAS Institute, 2000). Supplementation of VB<sub>12</sub> (weekly intramuscular injections of 0 or 10 mg) and biotin (dietary supplements of 0 or 30 mg/d) were used in a 2 × 2 factorial arrangement in a 12 randomized complete block design with repeated measures. For milk production variables (DMI, yield and composition), covariance structure type AR(1) was used to fit equally spaced repeated measures. For plasma variables, covariance structure spatial power was applied to fit unequally spaced repeated measures (Littell et al., 1996; Chung et al., 2009). The model included block, VB<sub>12</sub>, biotin, week, and the interactions of VB<sub>12</sub> × biotin, VB<sub>12</sub> × week, biotin × week, and VB<sub>12</sub> × biotin × week as fixed effects. Cows within treatments were subjected as random variables. For all analyses, least squares means were calculated and differences between treatments were separated with a PDIFF option. Significant differences were established at  $P < 0.05$  and trends at  $P \leq 0.10$  and  $P > 0.05$ .

## **RESULTS**

### **Feed Intake, Lactation Performance, and BW Change**

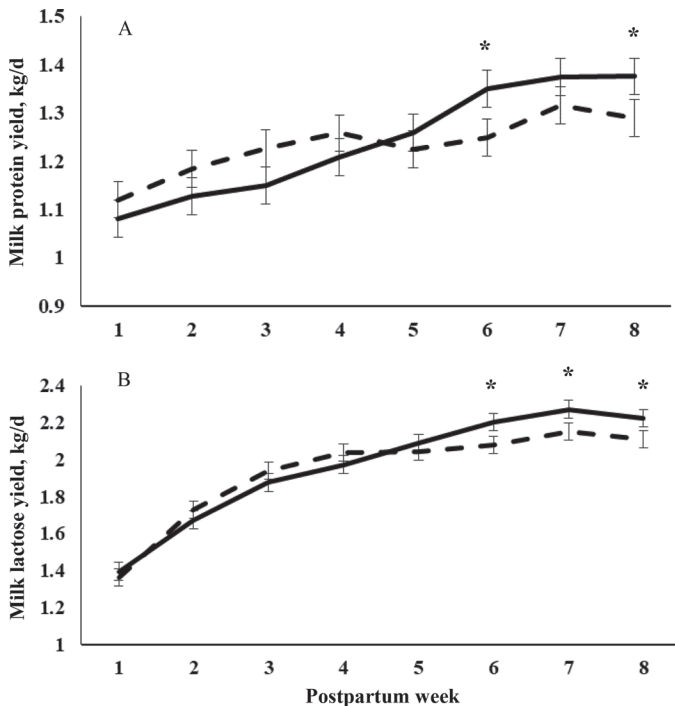
Postpartum DMI was higher in cows with VB<sub>12</sub> injection, compared with those not injected with VB<sub>12</sub> (Table 2;  $P < 0.01$ ). Compared with cows that were not treated with VB<sub>12</sub>, raw ( $P < 0.01$ ) and energy-

corrected ( $P = 0.05$ ) milk yields were greater in cows with VB<sub>12</sub> injection (Table 2). Overall, yields of protein and lactose were higher in VB<sub>12</sub>-injected cows compared with cows without injection of VB<sub>12</sub> ( $P < 0.01$ ; Table 2). Lactose content was higher in VB<sub>12</sub> supplemented cows compared with cows that did not treated with VB<sub>12</sub> ( $P < 0.01$ ; Table 2). The VB<sub>12</sub> content in the milk was greater in VB<sub>12</sub>-supplemented cows than in milk of animals without VB<sub>12</sub>-injection ( $P < 0.01$ ; Table 2). No VB<sub>12</sub> × week effect was observed on lactation performance of transition cows ( $P > 0.05$ ). No effect of biotin on overall lactation performance was observed in postpartum dairy cows ( $P > 0.05$ ). However, milk protein yield was higher in cows supplemented with biotin, compared with that of animals without biotin in wk 6 and 8 postpartum (biotin × week effect,  $P = 0.02$ ; Figure 1A). Higher milk lactose yield was observed in cows fed biotin during wk 6 to 8 postpartum compared with the animals without no biotin supplemented (biotin × week,  $P = 0.05$ ; Figure 1B). The milk biotin content was greater in biotin-supplemented cows than that of animals without biotin supplementation ( $P < 0.01$ , Table 2). The N conversion in the biotin-supplemented cows tended to be higher than the animals without biotin supplementation ( $P = 0.08$ ). Supplementation of both VB<sub>12</sub> and biotin had an interactive effect on improving milk protein content ( $P = 0.09$ ).

The BW loss was lower in cows with VB<sub>12</sub> injection ( $30.8 \pm 2.92$  kg), compared with animals without VB<sub>12</sub> injection ( $40.2 \pm 3.13$  kg,  $P = 0.03$ ; Table 2), with no interaction of VB<sub>12</sub> and biotin on BW change ( $P > 0.05$ ). We found no effect of VB<sub>12</sub>, biotin, and their interaction on the EB and feed efficiency ( $P > 0.05$ ).

### **Plasma Variables**

The effects of biotin and VB<sub>12</sub> on plasma variables are listed in Table 3. When cows were injected with VB<sub>12</sub>, plasma nonesterified fatty acids concentration was lower throughout the entire experimental period compared with that of cows not treated with VB<sub>12</sub> ( $P < 0.01$ ). Reduced plasma BHB was observed in cows treated with VB<sub>12</sub>, compared with that of cows without VB<sub>12</sub> injection ( $P = 0.04$ ). The SOD concentration in the plasma was significantly greater in VB<sub>12</sub>-treated cows compared with that of animals not supplemented with VB<sub>12</sub> ( $P = 0.05$ ). Supplementation of both VB<sub>12</sub> and biotin tended to have an interactive effect on improving plasma SOD concentration ( $P = 0.08$ ). Plasma biotin was greater in cows given biotin compared with animals without biotin supplementation ( $P = 0.04$ ). Plasma VB<sub>12</sub> was greater in cows injected with VB<sub>12</sub> compared with that of animals without VB<sub>12</sub>. Moreover, no significant effects of VB<sub>12</sub> × week or biotin ×



**Figure 1.** Effects of dietary biotin [0 (dashed line) or 30 mg/d (solid line)] given from 3 wk before the expected calving date to 8 wk postpartum on milk protein yield (A,  $P = 0.02$ ) and milk lactose yield (B,  $P = 0.05$ ). Bars indicate the SEM; an asterisk (\*) indicates a significant difference ( $P < 0.05$ ).

week on plasma variables was observed ( $P > 0.05$ , data not shown).

## DISCUSSION

In the current study, DMI was approximately 1.7 kg/d higher in  $\text{VB}_{12}$ -injected cows compared with cows without injection. This result is inconsistent with the studies of Girard and Matte (2005) and Duplessis et al. (2017), in which  $\text{VB}_{12}$  injection had no effects in DMI and milk production in dairy cows. Girard and Matte (2005) conducted their study in primiparous lactating cows (starting at 4 wk postpartum), different from our study starting at 3 wk prepartum with multiparous dairy cows. It was reported that when plasma  $\text{VB}_{12}$  concentration is greater than 200 pg/mL in multiparous cows, additional folates increased milk protein content, milk yield, and milk protein yield, but no improved performance was observed in primiparous cows whose plasma  $\text{VB}_{12}$  concentrations were lower (Girard et al., 1995; Girard and Matte, 1998). Akins et al. (2013) observed no effect of  $\text{VB}_{12}$  supplementation in cows with a low folate status (lower than 20 ng/mL). In the current study, plasma  $\text{VB}_{12}$  concentration of animals in control group was lower than 200 pg/mL (about 170 pg/mL), whereas  $\text{VB}_{12}$  level was over 400 pg/mL after  $\text{VB}_{12}$  injection. Thus, it is possible that folate status of cows in the current study (not measured) was high

**Table 2.** Effect of dietary biotin (Bio) and intramuscular injection of vitamin  $\text{B}_{12}$  ( $\text{VB}_{12}$ ) on DMI, lactation performance, and BW change during first 8 wk of lactation in dairy cows<sup>1</sup>

Item	Treatment				SEM	P-value		
	Control	Bio	$\text{VB}_{12}$	Bio + $\text{VB}_{12}$		Bio	$\text{VB}_{12}$	Biotin + $\text{VB}_{12}$
DMI, kg/d	21.4	21.1	23.1	22.8	0.58	0.29	<0.01	0.75
Milk yield, kg/d								
Raw	36.4	37.2	39.5	39.7	0.99	0.66	<0.01	0.82
ECM <sup>2</sup>	36.5	38.6	37.3	39.3	0.788	0.50	0.05	0.95
Fat	1.45	1.51	1.48	1.49	0.041	0.83	0.61	0.61
Protein <sup>3</sup>	1.13	1.17	1.26	1.30	0.025	0.88	<0.01	0.47
Lactose <sup>3</sup>	1.81	1.83	1.99	2.03	0.065	0.30	<0.01	0.74
Content, g/100 g								
Fat	4.08	4.18	3.84	3.97	0.141	0.67	0.13	0.83
Protein	3.25	3.19	3.18	3.31	0.038	0.56	0.61	0.09
Lactose	4.94	4.99	5.02	5.05	0.025	0.30	0.04	0.15
Biotin, ng/mL	0.094	0.28	0.113	0.319	0.0372	<0.01	0.44	0.79
$\text{VB}_{12}$ , ng/mL	0.330	0.405	1.543	2.438	0.390	0.23	<0.01	0.31
BW change, kg	-42.9	-37.5	-32.0	-29.6	4.24	0.37	0.03	0.73
Energy balance, Mcal/d	-4.07	-3.75	-2.28	-2.01	1.16	0.29	0.84	0.99
Feed efficiency <sup>4</sup>	1.67	1.68	1.66	1.67	0.043	0.97	0.99	0.86
Nitrogen conversion <sup>5</sup>	0.31	0.31	0.30	0.32	0.09	0.08	0.51	0.40

<sup>1</sup>Sampling 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. Biotin and  $\text{VB}_{12}$  concentrations were measured in the 8th week postpartum.

<sup>2</sup>ECM =  $12.55 \times \text{fat yield (kg/d)} + 7.39 \times \text{protein yield (kg/d)} + 5.34 \times \text{lactose yield (kg/d)}$ , from Orth (1992).

<sup>3</sup>Biotin  $\times$  week interaction,  $P \leq 0.05$ .

<sup>4</sup>Feed efficiency = milk yield/DMI.

<sup>5</sup>Nitrogen conversion = milk protein yield/CP intake.

enough to allow a detectable effect on DMI and milk yield. We inferred that effect of VB<sub>12</sub> injection on DMI and lactation performance of dairy cows depended on parity and availability of VB<sub>12</sub> and folate in the blood.

Biotin supplementation (30 mg/d) did not affect DMI, which is in agreement with previous studies [20 mg/d for prepartum and 30 mg/d for postpartum in Rosendo et al. (2004); 10 or 20 mg/d in Chen et al. (2012)], but is not consistent with Zimmerly and Weiss (2001) and Chen et al. (2012), who observed higher milk yield after biotin supplementation. Chen et al. (2012) started their work at the early lactating period (DIM = 40), whereas our experiment began at precalving. Although they started their study at prepartum stage, Zimmerly and Weiss (2001) included both primiparous and multiparous cows. It is not clear whether the response is different between the cows of different parities. Therefore, the different responses to biotin addition among different studies could be attributed to different lactation stages and parity of the cows. However, we observed that biotin addition increased protein (6 and 8 wk) and lactose yield (from 6 to 8 wk) in a time-dependent manner compared with cows without biotin. The improved lactose yield could be associated with the positive effect of biotin on hepatic gluconeogenesis in dairy cows (Majee et al., 2003) through increasing propionyl-CoA carboxylase decarboxylase activity (Ouattara et al., 2013). Moreover, the enhanced hepatic gluconeogenesis is beneficial to reduce AA deamination in the liver and to increase AA utilization efficiency in the mammary gland (Seymour et al., 1990), resulting in more AA flowing to mammary gland for milk protein synthesis. Under the experimental conditions of our study, the positive effects of a biotin supplementation on protein and lactose yields were visible only after 6 wk postpartum.

An interaction on lactation performance between biotin and VB<sub>12</sub> could be expected based on their respective roles in propionate utilization for gluconeogenesis (Padmakumar et al., 1997; Scott, 1999; Hügler et al., 2003). However, under the conditions of our experiment, the biotin × VB<sub>12</sub> interactions were not significant for plasma glucose concentration or milk lactose yield and lactation performance.

Compared with animals without VB<sub>12</sub> injection, the reduced BW loss, lower body fat mobilization (lower nonesterified fatty acids and BHB in the plasma) and lower oxidative stress in VB<sub>12</sub>-injected cows may partly attributed to the increased DMI (Wang et al., 2013; Konvičná et al., 2015; Kuhla et al., 2016). In contrast, biotin had limited effect on BW change due to its limited effect on DMI in the current study.

## CONCLUSIONS

Weekly injection of VB<sub>12</sub>, starting at the prepartum stage, increased postpartum DMI and lactation performance and reduced BW loss of dairy cows. Biotin had a limited effect on overall lactation performance but did show a time-dependent effect on protein and lactose yield. Under the present experimental conditions, we found no interactive effect of a combined supplement of biotin and vitamin B<sub>12</sub> on lactation performance of dairy cows.

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**Table 3.** Effects of dietary biotin (Bio) and intramuscular injection of vitamin B<sub>12</sub> (VB<sub>12</sub>) on plasma variables in prepartum and postpartum dairy cows<sup>1</sup>

Item	Treatment				SEM	P-value		
	Control	Bio	VB <sub>12</sub>	Bio + VB <sub>12</sub>		Bio	VB <sub>12</sub>	Biotin + VB <sub>12</sub>
Glucose, mmol/L	3.44	3.44	3.43	3.54	0.037	0.32	0.33	0.33
Cholesterol, mmol/L	3.55	3.10	3.62	3.66	0.224	0.36	0.16	0.27
BUN, mmol/L	4.58	4.41	4.74	4.81	0.118	0.81	0.11	0.50
Nonesterified fatty acids, μmol/L	323.9	303.8	216.2	246.3	15.04	0.82	<0.01	0.24
BHB, μmol/L	550.5	522.0	471.1	499.2	24.42	0.93	0.04	0.21
SOD, <sup>2</sup> U/mL	110.4	105.9	111.1	114.5	1.60	0.82	0.04	0.08
Biotin, <sup>3</sup> ng/L	732	1,258	751	1,214	22.5	0.04	0.95	0.89
VB <sub>12</sub> , <sup>3</sup> ng/L	195	162	418	441	52.2	0.93	<0.01	0.59

<sup>1</sup>Sampling times: the blood was sampled at -10, 0, 8, 15, 29, 43 and 57 d relative to calving.

<sup>2</sup>SOD = superoxide dismutase.

<sup>3</sup>Sampling time: the blood was sampled at 57 d relative to calving.

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