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Case Study: Concentrations of biotin in blood of Holstein bull calves supplemented with biotin and pantothenic acid

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ABSTRACT

Pantothenic acid interferes with biotin absorption in nonruminants. The objective of this study was to determine whether pantothenic acid affects the concentration of biotin in the blood of young calves. Weaned bull calves (n = 16) were fed ad libitum a pelleted starter including no supplemental B-vitamins and hay for 2 wk before the beginning and until the end of the experiment. Water was available ad libitum at all times. Two wk after weaning, calves were blocked by age and randomly assigned to 4 treatments according to a randomized complete block design. Treatments consisted of administering, for 14 d, a daily gelatin capsule containing no B-vitamins, 10 mg of biotin, 240 mg of pantothenic acid, and 10 mg of biotin + 240 mg of pantothenic acid. Expeller soybean meal was used as a carrier of the vitamins in the capsules. Blood samples were collected by venipuncture of the jugular vein at d 0 and 14. Concentrations of avidin-binding substances (ABS) in plasma were determined by a single-step competitive enzyme-binding assay. The concentrations of ABS in plasma were similar for all treatments ($P > 0.24$) and did not change after dosing vitamins over time (3.47 and 3.07 ng/mL for d 0 and 14, respectively; SEM = 1.5 ng/mL; $P > 0.63$). Plasma concentrations of ABS were substantially greater than those previously reported for lactating dairy cows (0.7–2.1 ng/mL). Based on these data, we cannot conclude whether or not an interaction exists between biotin and pantothenic acid in regards to biotin absorption.

Key words: B-vitamins, biotin, pantothenic acid, absorption, sodium-dependent multivitamin transporter

INTRODUCTION

Biotin is a water-soluble vitamin that is synthesized by plants and several microorganisms. Because they cannot synthesize biotin, mammals rely on intake through the diet and on microbial synthesis in the gastrointestinal tract to meet their biotin needs. A facilitated mechanism in the brush-border membrane of the enterocytes allows the absorption of biotin against a concentration gradient in the intestine of rats (Said and Redha, 1987), rabbits (Said and Derweesh, 1991), and humans (Said et al., 1987; Said et al., 1988). This transport is sodium dependent (Said and Redha, 1987; Said et al., 1987; Said et al., 1988; Said and Derweesh, 1991), and biotin absorption through this transporter can be decreased by pantothenic acid (Said, 1999), showing that this transporter is not specific for biotin. Prasad et al. (1998) named this transporter as sodium-dependent multivitamin transporter.

Studies addressing biotin absorption in ruminants are scarce (Ferreira et al., 2015). Even more, the presence of a biotin transporter similar to sodium-dependent multivitamin transporter in the gastrointestinal tract of ruminants has not been determined. Majee et al. (2003) observed that milk yield did not increase when biotin was supplemented along with a blend of other B-vitamins. The authors suggested that negative interactions between biotin and other B-vitamins might have occurred (Majee et al., 2003). Chatterjee et al. (1999) showed that the uptake of biotin was 65% less when cells were incubated with pantothenic acid (pantothenic acid–to–biotin molar ratio equal to 20) than when incubated without pantothenic acid.

Based on the assumption that biotin uptake in dairy cows occurs via a sodium-dependent multivitamin transporter system, a decrease in biotin uptake should occur when excessive pantothenic acid is fed simultaneously with biotin. In this study, we hypothesized that the concentration of avidin-binding substances (**ABS**; Ferreira et al., 2007) in blood diminishes when bull calves are challenged with a bolus containing high doses of pantothenic acid. Therefore, the objective of this study was to determine how the concentration of ABS in blood is affected by bolusing pantothenic acid to young Holstein bull calves.

MATERIALS AND METHODS

Calf Management

All procedures involving animals were approved by the Institutional Animal Care and Use Committee of Virginia Tech. Sixteen newborn bull calves were raised in individual hutches at the Virginia Tech Dairy Complex. Calves were fed twice daily with 2 L of a 12% (mass/vol) milk replacer (Centurion, Merrick's Inc., Middleton, WI). According to the manufacturer's specifications, the milk replacer contained supplemental biotin and pantothenic

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acid. Calves were also fed ad libitum a starter lacking supplemental B-vitamins, which was prepared by a commercial feed mill (Southern States Cooperative, Vinton, VA). According to the manufacturer's specifications, the starter contained 89.9% DM, 20.0% CP, 3.8% fat, 39.2% NDF, 1.0% calcium, and 0.6% total phosphorus (DM basis). Drinking water was available ad libitum. All calves were weaned at 56 d of age. After weaning, and throughout the experiment, calves were group housed and fed ad libitum the same starter and a mixed-grass hay.

Treatments and Sampling

Two wk after weaning of the last calf (d 0; Table 1), all calves were blocked by age and randomly assigned to 1 of 4 treatments according to a randomized complete block design. On d 0, blood samples (8 mL) were collected at 0600 h by venipuncture of the jugular vein. Blood was immediately transferred into heparinized tubes, stored in ice, and transferred to the laboratory. Plasma was obtained after centrifugation of blood at 3,000 × g (using rubber cushions) for 30 min at 4°C and stored at −20°C until ABS analysis.

For the following 14 d, calves received a gelatin capsule bolus with treatments. Boluses were dosed at 0600 h every day. Treatments consisted of (1) gelatin capsule containing no B-vitamins, (2) gelatin capsule containing 10 mg of biotin (Rovimix Biotin, DSM Nutritional Products Inc., Parsippany, NJ), (3) gelatin capsule containing 240 mg of pantothenic acid (Rovimix Calpan, DSM Nutritional Products Inc.), and (4) gelatin capsule containing 10 mg of biotin + 240 mg of pantothenic acid. Vitamins were mixed with expeller soybean meal as a carrier. On d 14, a second blood sample was collected from the jugular vein of all calves at 0600 h. Blood samples were processed as described above.

Concentrations of ABS in plasma (1:20 dilution) were determined by a single-step competitive enzyme-binding assay (Ridascreen Biotin kit; R-Biopharm GmbH, Darmstadt, Germany) as described by Ferreira et al. (2007).

Statistical Analysis

Statistical analysis was performed using Proc MIXED of SAS (SAS version 9.3, SAS Institute Inc., Cary, NC) as for a randomized complete block design with repeated measures, for which the calf was considered the subject (i.e., experimental unit). The statistical model included the effects of treatment (fixed, df = 3), block (random, df = 3), the treatment × block interaction as a whole-plot error (random, df = 9), time (fixed, df = 1), treatment × time interaction (fixed, df = 3), and the random residual error.

RESULTS AND DISCUSSION

The concentration of ABS in plasma did not differ among treatments ($P > 0.82$) and did not change after bolusing vitamins ($P > 0.34$, Figure 1). There are several potential explanations for these unexpected observations. Even though bolus administration could be questioned as an adequate means for dosing B-vitamins, lactating dairy cows dosed with gelatin capsules containing biotin showed elevated plasma concentrations of ABS within hours after challenge (G. Ferreira, unpublished data). Based on this information, bolusing was considered an adequate means for administering B-vitamins.

Table 1. Age and BW at the beginning of the experiment (d 0) of Holstein bull calves

Item	Average	Median	SD
Age at d 0, d	75.6	76.0	6.4
BW at d 0, kg	94.7	89.3	10.5

Concentrations of ABS in plasma (Figure 1) were substantially greater than those previously reported for lactating dairy cows (0.7 to 2.1 ng/mL; Zimmerly and Weiss, 2001; Rosendo et al., 2004; Ferreira et al., 2015). Unexpectedly, basal concentrations of ABS in plasma of calves were even greater than those typically observed for lactating dairy cows supplemented with biotin (Zimmerly and Weiss, 2001; Rosendo et al., 2004; Ferreira et al., 2015). Elevated concentrations of ABS in plasma of calves at 2 wk after weaning are difficult to explain. In this study, the pelleted starter contained no supplemental B-vitamins, so this was not a source of supplemental biotin. Even though the milk replacer contained supplemental B-vitamins, because biotin is a water-soluble vitamin with a short half-life (Frigg et al., 1993), most of the biotin from the milk replacer should have been cleared after 2 wk after weaning. Although not certain, the possibility of carrying over residual biotin from the milk replacer 2 wk after weaning was discarded. Knowledge of biotin synthesis within the rumen is quite limited, but we should not discard the possibility of ruminal synthesis as a source of biotin in young animals as the rumen develops.

The variation of concentrations of ABS in plasma (Figure 1) was >10-fold greater than previously reported (Zimmerly and Weiss, 2001; Rosendo et al., 2004; Ferreira et al., 2015). In this study calves were blocked by age, and the difference in age within block between the youngest and oldest calf was 13 d. It may be possible that age differences, and hence differences in rumen development (Lesmeister et al., 2004), contributed to this variation.

Another explanation for our unexpected results might be linked to the short half-life of water-soluble vitamins (Taylor et al., 1976; Frigg et al., 1993). The second blood sample, which was obtained on d 14, was collected 24 h after the last administered bolus. Based on the short half-life, and given the diet provided no supplemental B-vitamins, biotin could have been cleared by the time we

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