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# Effects of B Vitamin Injections on Plasma B Vitamin Concentrations of Feed-Restricted Beef Calves Infected with Bovine Herpesvirus-1<sup>1</sup>

P. L. Dubeski<sup>2</sup>, F. N. Owens<sup>3</sup>, W. O. Song<sup>4</sup>, S. P. Coburn<sup>5</sup>, and J. D. Mahuren<sup>5</sup>

Department of Animal Science, Oklahoma State University, Stillwater 74078-0425

**ABSTRACT:** For nonruminants, stress and disease greatly increase requirements for vitamin B<sub>6</sub>, folic acid, pantothenic acid, and ascorbate. The effects of feed restriction, virus infection, and vitamin injections on plasma concentrations of B vitamins critical to the immune response were evaluated. Twelve beef steer calves, 6 to 8 mo of age, were fed below maintenance for 17 d and deprived of food for 3 d during a 20-d period after weaning. They then were inoculated intranasally with live attenuated bovine herpesvirus-1 (BHV-1). Six calves received saline injections and six received injections of a B vitamin mixture and ascorbate every 48 h for 14 d before and 14 d after inoculation. A mild respiratory infection developed in

all calves 4 to 5 d after inoculation. In control calves, restricted intake and food deprivation decreased plasma vitamin B<sub>6</sub> and pantothenate and increased vitamin B<sub>12</sub> but did not affect folic acid and ascorbate concentrations. Vitamin injections increased plasma concentrations of vitamin B<sub>6</sub>, folic acid, vitamin B<sub>12</sub>, pantothenic acid, and ascorbate ( $P < .002$ ). Plasma concentrations of vitamin B<sub>6</sub>, vitamin B<sub>12</sub>, pantothenic acid, and ascorbate, but not folic acid, were markedly reduced in all calves during the BHV-1 infection ( $P = .001$ ). The vitamin B<sub>6</sub>, pantothenic acid, vitamin B<sub>12</sub>, and ascorbate status of stressed calves may affect their immune response to vaccination or infection.

Key Words: Immunity, Bovine Herpesvirus, Stress, Pyridoxal, Vitamins

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## Introduction

Stress during transport and marketing can compromise the immune system and predisposes cattle to develop certain infectious diseases, including the bovine respiratory disease complex. Transport is an acute stressor for cattle and can elevate serum cortisol concentrations for 4 to 7 d (Von Tungeln, 1986). Cortisol and other glucocorticoids suppress the immune response in cattle and other species (Golub and Gershwin, 1985; Roth, 1985).

Feed and water intake often are decreased during marketing and transport of cattle, reducing the supply of energy and of nutrients synthesized by ruminal microbes, including the B vitamins. At the same time,

requirements for water-soluble vitamins critical to the immune response, particularly vitamin B<sub>6</sub>, folic acid, pantothenic acid, and ascorbate, may be increased substantially by stress. In nonruminants, pantothenic acid and ascorbate are required for glucocorticoid synthesis (Goodman, 1960; Dvorak, 1984) and vitamin B<sub>6</sub> is important in regulation of glucocorticoid function (Allgood et al., 1990; Maksymowych, 1990). Mueller and Thomas (1975) estimated that stress or "moderate" injury may increase requirements of vitamin B<sub>6</sub> and folic acid by 8 to 15 times, respectively, and may double or quadruple requirements for vitamin B<sub>12</sub> and pantothenic acid, respectively. Supplementation with ascorbate and vitamin B<sub>6</sub> have helped to overcome cortisol-induced immunosuppression in both humans and experimental animals.

This study was designed to investigate short-term changes in plasma concentrations of several B vitamins that are critical to the immune response and that may be depleted during stress and disease. With other species, plasma vitamin concentrations are used routinely to assess B vitamin deficiencies and status (Jaffe, 1984; Sauberlich, 1984b). Although plasma B vitamin concentrations of ruminants have rarely been assessed, one might expect plasma changes to reflect alterations in vitamin status. Plasma concentrations of folic acid, vitamin B<sub>12</sub>, pantothenic acid, vitamin

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<sup>2</sup>Present address: Agriculture and Agri-Food Canada, Lacombe Research Centre, Lacombe, Alberta, Canada T4L 1W1.

<sup>3</sup>To whom correspondence should be addressed.

<sup>4</sup>Dept. of Food Sci. and Human Nutr., Michigan State Univ., East Lansing 48824.

<sup>5</sup>Biochem. Dept., Fort Wayne Hospital and Training Center, Fort Wayne, IN 46815.

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B<sub>6</sub>, and ascorbate were measured throughout the study to investigate the effects of vitamin injections, restricted feeding and food deprivation, and BHV-1 infection.

### Materials and Methods

**Animal Care.** The experiment protocol was reviewed and approved by the Oklahoma State University Animal Care and Use Committee.

**Experimental Procedure.** Twelve Hereford × Angus calves 6 to 8 mo of age were used in the study. Each day of the study will be designated relative to the day that calves were inoculated with bovine herpesvirus-1. The study began when calves were weaned on April 23, 1991 (d -21). During the 20 d following weaning, the calves were adapted to their surroundings and to B vitamin injections. During the trial, each calf received prairie grass hay at 1% of its initial BW daily on an as-fed basis until d 2, except during a 3-d period of food deprivation (d -8 to d -6) to simulate conditions during marketing and transport. The feeding rate was increased to 1.5% of initial BW on d 3 until the end of the trial so that calves would maintain BW. The calves were inoculated intranasally with an attenuated vaccine strain of bovine herpesvirus-1 on d 0. Calves were stressed by weaning, the prolonged period of restricted feeding including 3 d of food deprivation, and infection with bovine herpesvirus-1 (BHV-1). Bovine herpesvirus-1 was used as a model for the bovine respiratory disease complex because it can cause a primary respiratory tract infection and predisposes cattle to *Pasteurella* infection. However, use of an attenuated vaccine strain of BHV-1 in this study was equivalent to a vaccination stress rather than acute disease stress. The detailed experimental procedures were described (Dubeski et al., 1996).

**Vitamin Treatments.** The calves were assigned randomly to two treatment groups (control, +Vit) with six calves per treatment. Each calf was injected i.m. with B vitamins and ascorbate (+Vit) or sterile saline (controls) every 48 h from d -11 to d 14 of the experiment. The B vitamin and ascorbate solutions were custom-formulated (American Veterinary Products, Fort Collins, CO). Vitamin concentrations, dosages given, and estimated requirements extrapolated from requirements of growing swine on a metabolic body weight basis are presented in Table 1.

**Blood Samples.** Blood samples (50 mL) were collected from each animal in 10-mL evacuated glass tubes (Vacutainer, Sherwood Medical, St. Louis, MO), 30 mL with potassium EDTA and 20 mL with sodium heparin as the anticoagulant. As presented in Table 2, blood samples were collected immediately before feeding at seven different times from each animal. Although vitamin injections were discontinued on d

Table 1. B vitamin requirements and amounts supplied by injection

Vitamin	Estimated requirement <sup>a</sup>	Stress factor <sup>b</sup>	Dosage <sup>c</sup>
Thiamin	6.76 mg	—	13.5 mg
Riboflavin	16.91 mg	—	33.8 mg
Niacin	67.64 mg	—	135.0 mg
Folic acid	2.09 mg	15	60.0 mg
Pantothenic acid	54.1 mg	2	216.0 mg
Vitamin B <sub>6</sub>	6.76 mg	8	108.0 mg
Vitamin B <sub>12</sub>	67.64 µg	2	270.0 µg
Sodium ascorbate	Unknown	—	1,000.0 mg

<sup>a</sup>Daily B vitamin requirements for a 190.5-kg calf were estimated on a metabolic body weight basis as equivalent to 3.56 times the requirements for a 34-kg pig (NRC, 1988).

<sup>b</sup>The estimated daily requirement was multiplied by factors of 2 for pantothenic acid and vitamin B<sub>12</sub>, 15 for folic acid, and 8 for vitamin B<sub>6</sub> to account for the increased requirements of these specific B vitamins during stress or "moderate" injury (Mueller and Thomas, 1975).

<sup>c</sup>Twice the daily estimated requirement was supplied by injection every 2 d, with one 3-mL dose containing the B vitamins and one 4-mL dose containing sodium ascorbate. Controls received identical doses of sterile saline solution.

14, additional blood samples were collected on d 16 and d 18 from +Vit calves to monitor withdrawal effects. When blood samples for B vitamin analysis were taken on the same day that B vitamins were injected, blood samples were obtained < 1 h before vitamins were injected.

Plasma was separated from blood after centrifugation at 1,500 × g and was frozen (-20°C) in small aliquots for subsequent analysis of the vitamins. Samples were processed under subdued lighting to minimize photodegradation of the B<sub>6</sub> vitamins and ascorbate. For ascorbate analysis, heparinized plasma was mixed with fresh 10% (wt/vol) metaphosphoric acid (1:1) and frozen immediately.

**Vitamin Analysis.** Ascorbate in heparinized plasma was measured colorimetrically after derivitization with 2,4-dinitrophenylhydrazine (McCormick, 1986).

Table 2. Plasma sampling dates, number of vitamin injections, and time since last injection

Experimental day	Number of previous vitamin injections	Days since last vitamin injection
-45 (before weaning)	0	—
-15 (after weaning) <sup>a</sup>	0	—
-8 (feed deprivation began)	2	2
-5 (feed deprivation ended)	4	1
0 (BHV-1 inoculation)	6	2
5 (BHV-1 infection peak)	9	1
14 (final injection)	13	2
16 (after injections)	13	4
18 (after injections)	13	6

<sup>a</sup>Calves were weaned on d -21.

Plasma concentrations of both folate and vitamin B<sub>12</sub> were measured in plasma (containing potassium EDTA) using a radioassay method (Quantaphase Folate and Vitamin B<sub>12</sub> Radioassay, Bio-Rad Clinical Division, Hercules, CA). Pantothenic acid in heparinized plasma was measured using a radioimmunoassay (Wyse et al., 1979). B<sub>6</sub> vitamers in heparinized plasma were analyzed using a cation-exchange HPLC procedure (Coburn and Mahuren, 1983).

**Statistical Analysis.** Plasma vitamin data were analyzed by ANOVA using the GLM procedure of SAS (1985) for the completely randomized design (all animals were inoculated but only half received vitamin injections) for the main effects of vitamin treatment. Because some of the samples were obtained before and some after inoculation, averages across collection dates would not be meaningful. Consequently, effects of vitamin injection were tested within each individual collection date. Changes from one date to another also were examined. In each case, the effect of vitamin injection was tested within each sample period using animal within treatment as the error term.

## Results and Discussion

### Animal Health and Stress

Details of animal feed intake, weight loss, immune measurements, and clinical signs are reported separately (Dubeski et al., 1996). The stress model used in this study (weaning, a long-term period of moderate feed restriction, 3 d of food deprivation after adaptation, no long-distance shipping) probably

resulted in less extreme physical and nutritional changes than occur in many shipping-stressed calves. Calves lost 7.7% of BW gradually during the first 20 d of the study. This is similar to the weight loss for many cattle during a single haul, but considerably less severe than a BW loss of 11% or more observed in highly stressed cattle (Griffin, 1983). Furthermore, these cattle were not stressed by transport, one of the most severe stressors for cattle, which elevates plasma cortisol for 4 to 7 d (Crookshank et al., 1979). Even after prolonged feed restriction, calves in this study had higher concentrations of plasma folic acid, vitamin B<sub>12</sub>, pantothenic acid, vitamin B<sub>6</sub>, and ascorbate than plasma concentrations of shipping-stressed calves in a previous survey (Dubeski, 1992). This might suggest that stress has a larger impact than restricted feed intake on plasma vitamin concentrations and perhaps on vitamin status.

Bovine herpesvirus-1 infection caused mild respiratory disease in all calves, apparently limited to the upper respiratory tract. Clinical symptoms peaked at 4 to 6 d after infection. The infection was equivalent to vaccination with a live attenuated virus strain, and the immune response was comparable to a successful vaccination except that humoral immunity (mean IgG titer to BHV-1 at 14-d after infection) seemed to be greater in vitamin-injected calves (1,120 vs 550,  $P = .115$ ).

### Folic Acid

Plasma concentrations are presented in Table 3. Plasma folate tended to be greater in calves after than before weaning (26.7 vs 15.4 nM). Plasma folate concentrations in stressed calves, feedlot steers, and lactating dairy cows averaged 22.0, 58.9, and 29.7 nM,

Table 3. Folic acid concentration in plasma (nM)

Day of experiment	Treatment mean <sup>a</sup>		SEM	Prob.
	Control	+Vit		
-45 Before weaning	15.4	15.4	3.8	—
-15 After weaning	26.7	26.7	2.9	.97
-8 Feed restriction began	29.4	64.1	5.4	.002
-5 Feed restriction ended	29.7	74.3	4.5	.001
0 BHV-1 inoculation	22.9	930.5	145.6	.002
5 Infection peak	24.7	66.6	4.8	.001
14 Final vitamin injection	27.6	63.9	5.9	.002
16 After end of injections	NA <sup>b</sup>	86.0	—	—
18 After end of injections	NA <sup>b</sup>	55.9	—	—
Contrast or change				
B vitamins (d -8 vs d -15)	5.6	37.4	5.2	.003
Feed deprivation (d -5 vs d -8)	-2.2	10.2	7.0	.32
Refeeding (d 0 vs d -5)	-6.8	856.2	147.2	.002
BHV-1 (d 5 vs d 0)	1.8	-864.1	149.0	.002
Recovery (d 14 vs d 5)	2.7	-2.7	5.0	.45

<sup>a</sup>n = 6.

<sup>b</sup>Not analyzed.

respectively, in samples obtained from various classes of cattle (Dubeski, 1992). Plasma folic acid concentration in the control calves did not decrease during food deprivation.

B vitamin injections increased ( $P = .02$ ) mean plasma folic acid concentration on d -8 and thereafter. The folic acid dose was equivalent to 60 mg/d, 15 times the dietary requirement (Table 1). Mean plasma folate in +Vit calves ranged from 55.9 to 86.0 nM (except pre-injection and on d 0), which is similar to the range for plasma folate concentrations of feedlot steers (32.2 to 79.7, mean 58.9 nM; Dubeski, 1992). Girard et al. (1989) injected 2-wk-old heifers with 2.5, 5.0, 10, and 20 of mg folic acid and successfully increased serum folate from an initial concentration of 18.6 to 33.5 nM, the amount found in 4-mo-old heifers. However, these relatively low dosages of folic acid did not increase serum folate concentrations in 4-mo-old heifers.

The extremely high plasma folic acid concentration (930.5 nM) in +Vit calves on d 0 is difficult to explain. These samples were taken 2 d after the previous B vitamin injection and immediately before BHV-1 inoculation. Extremely high concentrations of other vitamins (vitamin B<sub>12</sub>, pantothenic acid, vitamin B<sub>6</sub>, but not ascorbate) were observed on d 0. These samples were analyzed several times and at different dilutions in order to rule out experimental error.

Plasma folic acid was not decreased by the mild BHV-1 infection. Concentrations were slightly higher in controls on d 5 vs d 0, whereas in calves receiving vitamin injections, concentrations dropped drastically from d 0 to d 5 (interaction of vitamin injection with plasma concentration change of  $P = .002$ ). If BHV-1 increases folic acid requirements, a decrease in plasma folic acid would be expected in the control calves. Folic

acid concentrations on d 5 for both treatment groups were very similar to their respective concentrations on d -8, d -5, and d 14. Relatively high plasma folic acid concentrations (14.5 to 33.1 nM) in shipping-stressed calves (Dubeski, 1992) also suggest that folic acid status, as appraised by plasma folate concentration, was not seriously decreased by stress and reduced feed intake.

### Vitamin B<sub>12</sub>

Plasma vitamin B<sub>12</sub> concentrations are presented in Table 4. Plasma concentrations of vitamin B<sub>12</sub> tended to be greater after than before weaning. Generally, values during the trial were higher than means from stressed calves, feedlot steers, and lactating dairy cows (185, 118, and 211 pM) from the survey conducted by Dubeski (1992).

Short-term changes in plasma B<sub>12</sub> have not been investigated except in response to injection. Plasma B<sub>12</sub> concentration in control calves increased during the period of injections (d -8 and after) and reached a maximum concentration on d 0. Plasma concentrations tended to be increased with food deprivation and during infection, probably due to release of vitamin B<sub>12</sub> from liver reserves. The liver contains high concentrations of vitamin B<sub>12</sub> (Saubertlich, 1990). Liver tissue is catabolized during food or water deprivation (Shorthose and Wythes, 1988).

An increased vitamin B<sub>12</sub> requirement during BHV-1 infection may have been responsible for the decreases in vitamin B<sub>12</sub> concentration between d 0 to d 5. Plasma B<sub>12</sub> dropped 31% in controls. The reasons for the decrease in plasma B<sub>12</sub> following BHV-1 administration are not known, but they could reflect the interrelationships between B<sub>12</sub> and folate, methionine and choline metabolism (Nauss and Newberne,

Table 4. Vitamin B<sub>12</sub> concentration in plasma (pM)

Day of experiment	Treatment mean <sup>a</sup>		SEM	Prob.
	Control	+Vit		
-45 Before weaning	169	169	26	
-15 After weaning	220	214	24	.86
-8 Feed restriction began	222	326	36	.07
-5 Feed restriction ended	275	526	36	.001
0 BHV-1 inoculation	301	1,597	149	.001
5 Peak BHV-1 infection	207	456	24	.001
14 Final vitamin injection	277	541	30	.001
16 After end of injections	NA <sup>b</sup>	491	—	—
18 After end of injections	NA <sup>b</sup>	418	—	—
Contrast or change				
B vitamins (d -8 vs d -15)	11	112	33	.06
Feed deprivation (d -5 vs d -8)	42	199	17	.001
Refeeding (d 0 vs d -5)	26	1,071	117	.001
BHV-1 (d 5 vs d 0)	-94	-1,141	142	.001
Recovery (d 14 vs d 5)	70	85	17	.54

<sup>a</sup>n = 6.

<sup>b</sup>Not analyzed.

1981), and an increased B<sub>12</sub> requirement for cellular proliferation in the immune response. Plasma B<sub>12</sub> concentration had increased to 277 pM at d 14, which may reflect a decreased requirement, an increase in ruminal synthesis related to the higher feed intake on d 3 to d 14 (Zinn et al., 1987), or changes in liver storage or release of the vitamin.

The role of vitamin B<sub>12</sub> status in resistance to disease is not clear. Research in humans has centered on the autoimmune phenomena in pernicious anemia. Animal studies are limited because of difficulties in producing a B<sub>12</sub> deficiency in experimental animals. Because of the extensive ruminal production of B<sub>12</sub> and its analogs when cattle are fed Co-deficient diets and(or) high-concentrate diets, vitamin B<sub>12</sub> deficiencies may develop more quickly in ruminants than in humans or laboratory animals. Consequently, the ruminant could be a useful model for B<sub>12</sub> metabolism.

In +Vit calves, plasma B<sub>12</sub> tended to be greater than in controls at d -8 ( $P = .07$ ) and was markedly elevated ( $P < .01$ ) in +Vit calves on subsequent days. As occurred for plasma folate, B<sub>12</sub> concentrations were extremely high on d 0 (1,597 vs 301 pM in +Vit vs control calves, respectively). Plasma B<sub>12</sub> concentration tended to be slightly higher on d 14 than on d 5 but similar to the means on earlier days.

### Pantothenic Acid

Plasma concentrations of pantothenic acid tended to be greater after than before weaning (Table 5). Plasma values in this study were greater than mean values for transport-stressed calves and lactating dairy cows (.095 and .089  $\mu\text{M}$ ) but more similar to those of feedlot steers (.143  $\mu\text{M}$ ) from the survey of Dubeski (1992). The low concentrations in stressed

calves may reflect the role of pantothenic acid in adrenal function and corticosteroid synthesis (Goodman, 1960; Fidanza et al., 1978). A stress model similar to that used in this experiment did not induce the high, sustained serum cortisol concentrations found in shipping-stressed calves (d'Offay and Rosenquist, 1988); the added stress of transport may be responsible for more extreme decreases in plasma pantothenate.

In contrast to folate and vitamin B<sub>12</sub>, plasma pantothenic acid concentrations in control calves tended to be decreased during food deprivation. Plasma concentrations were consistently but not significantly greater in calves receiving vitamin injections.

Plasma pantothenate may be more sensitive to stress factors such as BHV-1 infection than to the supply of pantothenic acid. In unstressed rats, a dietary pantothenate deficiency may take 2 to 3 wk to affect plasma pantothenate concentrations (Song et al., 1990). However, in the current study, the mild BHV-1 infection markedly reduced plasma pantothenate concentrations within 5 d.

Bovine herpesvirus-1 infection resulted in lower plasma pantothenate concentrations on d 5 than at other times for both treatment groups ( $P < .001$ ). In both groups, plasma pantothenate increased on d 14, possibly due partly to increased feed intake as well as recovery from BHV-1 infection.

### Vitamin B<sub>6</sub>

Due to the complexity of analysis, B<sub>6</sub> vitamers were measured only in plasma samples taken on d -15, 0, and 5 (Table 6). Total B<sub>6</sub> was similar in both treatment groups before B vitamin treatments began

Table 5. Pantothenic acid concentration in plasma ( $\mu\text{M}$ )

Day of experiment	Treatment mean <sup>a</sup>		SEM	Prob.
	Control	+Vit		
-45 Before weaning	.128	.128	.019	—
-15 After weaning	.196	.183	.017	.60
-8 Feed deprivation began	.137	.143	.015	.79
-5 Feed deprivation ended	.122	.156	.013	.11
0 BHV-1 inoculation	.159	.771	.079	.001
5 Peak BHV-1 infection	.100	.113	.037	.75
14 Final vitamin injection	.164	.179	.013	.42
16 After vitamin injection	NA <sup>b</sup>	.19	—	—
18 After vitamin injection	NA <sup>b</sup>	.20	—	—
Contrast or change				
B vitamins (d -8 vs d -15)	-.059	-.040	.021	.53
Feed deprivation (d -5 vs d -8)	-.014	.013	.014	.20
Refeeding (d 0 vs d -5)	.030	.611	.074	.001
BHV-1 (d 5 vs d 0)	-.029	-.676	.090	.001
Recovery (d 14 vs d -5)	.033	.066	.043	.60

<sup>a</sup>n = 6.

<sup>b</sup>Not analyzed.

Table 6. B<sub>6</sub> vitamer concentration in plasma (nM)

Day of experiment	B <sub>6</sub> vitamer	Treatment mean <sup>a</sup>		SEM	Prob.	
		Control	+Vit			
-15 (before vitamin treatments)	Pyridoxal phosphate (PLP)	65.3	-35.2	10.6	.07	
	Pyridoxal (PL)	124.7	133.2	20.4	.78	
	Pyridoxine (PN)	0.0	0.0	0.0	1.00	
	Total B <sub>6</sub>	190.0	168.4	20.6	.53	
	4-Pyridoxic acid (4-PA)	25.0	24.8	2.2	.96	
0 (BHV-1 Inoculation)	Pyridoxal phosphate	56.8	55.7	10.7	.94	
	Pyridoxal	93.2	645.2	42.4	.001	
	Pyridoxine	0.0	1,822.3	305.4	.002	
	Total B <sub>6</sub>	150.0	2,523.2	180.2	.001	
	4-Pyridoxic acid	32.7	2,060.0	133.5	.001	
5 (Peak BHV-1)	Pyridoxal phosphate	68.8	91.5	22.2	.49	
	Pyridoxal	63.0	95.0	4.9	.001	
	Pyridoxine	0.0	9.7	6.8	.34	
	Total B <sub>6</sub>	131.8	196.2	21.5	.04	
	4-Pyridoxic acid	23.2	51.2	10.7	.10	
Contrasts and interactions		PLP	PL	PN	B <sub>6</sub>	4-PA
B vitamins (d -15 vs d 0 and d 5)		.18	.001	.006	.001	.001
BHV-1 (d 0 vs d 5)		.13	.001	.001	.001	.001
BHV-1 × B vitamin interaction		.35	.001	.003	.001	.001

<sup>a</sup>n = 6.

(d -15), although pyridoxal phosphate (**PLP**) tended to be higher in control calves. This may be due to variable hydrolysis of PLP to pyridoxal during prolonged storage before analysis (11 mo). Data are not available concerning B<sub>6</sub> vitamer stability in bovine plasma. Freezing rat plasma at -20°C for 55 wk did not affect B<sub>6</sub> content; however, these workers hydrolyzed the phosphate esters of the B<sub>6</sub> vitamers using a potato acid phosphatase before chromatography, so hydrolysis of PLP to pyridoxal was not assessed (Hefferan et al., 1986).

Coburn et al. (1984) previously measured much higher plasma PLP concentrations in three calves (402 ± 131 nM) than were detected in our calves using a very similar HPLC procedure. Pyridoxal phosphate was the primary B<sub>6</sub> vitamer in plasma, whereas pyridoxal was the primary vitamer in the current study. Nine samples from transport-stressed calves were analyzed simultaneously with samples from the current study. Samples from the stressed calves contained 126 ± 22 nM vitamin B<sub>6</sub>, primarily in the form of PLP (111 ± 18 nM), whereas pyridoxal, pyridoxine, and the vitamin B<sub>6</sub> excretory product, 4-pyridoxic acid, averaged 8 ± 5 nM, 7 ± 7 nM, and 34 ± 2 nM, respectively (Dubeski, 1992). Compared to the transport-stressed calves, all samples from the current study were higher in total B<sub>6</sub> but lower in PLP and higher in pyridoxal. The samples from the transport-stressed calves were stored for only 6 mo. These data could be interpreted to suggest that longer storage causes hydrolysis of PLP to pyridoxal without affecting total B<sub>6</sub>.

The effect of B vitamin injection was analyzed by contrasting values from d -15 vs d 0 plus d 5. B

vitamin treatment increased pyridoxal, 4-pyridoxic acid (**PA**), total B<sub>6</sub> (*P* < .001), and pyridoxine (*P* < .006). The interaction between BHV-1 infection and B vitamin treatment also was significant for these vitamers. Consequently, effects of B vitamin treatment will be discussed separately for d 0 and d 5.

On d 0, pyridoxal, pyridoxine, 4-pyridoxic acid, and total B<sub>6</sub> all were higher (*P* < .002) in plasma from +Vit than in plasma from control calves. Pyridoxal phosphate was not different (*P* = .94). Plasma from injected calves contained high concentrations of pyridoxine because pyridoxine hydrochloride was the B<sub>6</sub> source injected. The pyridoxine hydrochloride is converted gradually to the other B<sub>6</sub> vitamers. Plasma from +Vit calves also contained extremely high amounts of 4-pyridoxic acid, a major B<sub>6</sub> metabolite that in many species is excreted in the urine.

Compared to d -15, total B<sub>6</sub> on d 0 had decreased in control calves but increased in +Vit calves (interaction *P* < .001). Total B<sub>6</sub> may have fallen in controls because of a diminished supply with restricted feeding because duodenal B<sub>6</sub> supply is correlated with intake (Zinn et al., 1987). Conversely, +Vit calves received injections equal to eight times the estimated requirements. Because these estimates were designed to meet elevated dietary requirements associated with stress and disease, they probably exceeded requirements for the relatively unstressed calves in this study.

Plasma and tissue concentrations of B<sub>6</sub> vitamers are highly sensitive to changes in supply in non-ruminants. Blood and tissue concentrations were reduced 10 to 90% in rats by feeding a B<sub>6</sub>-deficient diet for only 2 wk (Sampson and O'Connor, 1989). In

contrast, depriving food from healthy male dogs for 40 h decreased plasma PLP by 15% and increased pyridoxal by 20%; baseline concentrations again were achieved by 48 h after refeeding (Barnard et al., 1986). Similar comparisons are not available for ruminants but continued ruminal digesta outflow would be expected to attenuate fluctuations in supply and in plasma concentrations.

Various studies indicate that plasma PLP is derived primarily from recent dietary vitamin B<sub>6</sub> intake, particularly pyridoxine, and little pyridoxal is recycled through the liver into PLP in plasma (Coburn et al., 1992). Similar PLP concentrations in the two treatment groups on d 0 and higher concentrations of pyridoxal in +Vit calves on d 0 (645 vs 93 nM,  $P < .001$ ) could indicate that PLP in plasma was degraded to pyridoxal during sample storage.

Total B<sub>6</sub> and all vitamers were lower in both control and +Vit calves on d 5 than on d 0. The most striking observation was the almost complete absence of pyridoxine from plasma of +Vit calves; only one calf had a detectable content of pyridoxine (57 nM). Similarly, both 4-pyridoxic acid and total B<sub>6</sub> were extremely decreased by BHV-1 infection in +Vit calves compared with control calves. Pyridoxal was decreased in both treatment groups as well.

Changes in concentrations of B<sub>6</sub> vitamers in this study indicate that B<sub>6</sub> metabolism was markedly affected by infection. Similarly, plasma PLP was sensitive to infection in hospital patients and was highly related to survival of critically ill surgical patients (Keniston et al., 1990). Changes in plasma concentrations during disease may reflect alterations in vitamin B<sub>6</sub> metabolism and increased requirements (Reynolds and Leklem, 1985; Merrill and Henderson, 1987). Pyridoxal phosphate is an essential cofactor for

numerous reactions in DNA, RNA, and protein synthesis that are crucial for the cell transformation and proliferation during the immune response, and furthermore, infection induces many catabolic reactions that require PLP (Beisel, 1977). Vitamin B<sub>6</sub> status in this study was very sensitive to a challenge to the immune system such as vaccination with live attenuated BHV-1. Increased susceptibility of stressed calves to disease and the frequent failure of stressed calves to respond appropriately to vaccination might be related to vitamin B<sub>6</sub> status.

### Ascorbate

Plasma ascorbate tended to be decreased during the trial compared with before weaning (Table 7). Generally, plasma ascorbate concentrations were higher in these calves before weaning than in stressed calves, feedlot steers, and dairy cows (25.0, 27.8, and 23.8  $\mu\text{M}$ , respectively) in a previous survey (Dubeski, 1992).

Plasma ascorbate concentrations were similar for the two treatment groups on d -15, before B vitamin injections began. Plasma ascorbate tended to decline in control and vitamin-injected calves between d -15 and d -8. This may be related to the negative energy balance; however, plasma ascorbate concentration did not decrease in control calves during the 3 d of food deprivation.

In humans, the major excretory route for ascorbate is the urine. When plasma concentration exceeds about 79.5  $\mu\text{M}$ , substantial amounts of ascorbate spill over into urine (Jaffe, 1984). Although plasma concentrations in humans can be maintained as high as 227  $\mu\text{M}$  with frequent and high ascorbate intakes, plasma ascorbate normally ranges from 45.4 to 79.5

Table 7. Ascorbic acid concentration in plasma ( $\mu\text{M}$ )

Day of experiment	Treatment mean <sup>a</sup>		SEM	Prob.
	Control	+Vit		
-45 Before weaning	59.2	59.2	2.3	—
-15 After weaning	55.1	50.5	2.8	.26
-8 Feed deprivation began	39.7	39.7	1.7	.94
-5 Feed deprivation ended	39.7	46.5	2.3	.09
0 BHV-1 inoculation	42.0	47.7	5.7	.001
5 Peak BHV-1 infection	21.6	32.4	3.4	.07
14 Final vitamin injection	36.9	42.0	4.0	.31
16 After vitamin injection	NA <sup>b</sup>	30.1	—	—
18 After vitamin injection	NA <sup>b</sup>	29.0	—	—
Contrast or change				
B vitamins (d -8 vs d -15)	-11.4	-5.7	3.4	.39
Feed deprivation (d -5 vs d -8)	0.0	5.7	2.8	.11
Refeeding (d 0 vs d -5)	2.8	58.4	4.5	.001
BHV-1 (d 5 vs d 0)	-20.4	-71.5	5.1	.001
Recovery (d 14 vs d 5)	14.8	7.9	3.4	.14

<sup>a</sup>n = 6.

<sup>b</sup>Not analyzed.

$\mu\text{M}$  (Jaffe, 1984); concentrations below  $17.0 \mu\text{M}$  indicate a deficiency (Sauberlich, 1984a). Control calves in the current study had plasma ascorbate concentrations below the range expected for humans receiving adequate intakes of ascorbate.

Plasma ascorbate in +Vit calves was not markedly increased by the injection of 1,000 mg of sodium ascorbate every 48 h. Even though by d -8 the +Vit calves had received two ascorbate injections, plasma concentrations remained the same as in control calves. Carried nonspecifically in blood, ascorbate is taken up by organs using specific transport mechanisms that accumulate ascorbate (Jaffe, 1984). Ascorbate concentrations several hundred times those in plasma are found in the pituitary, adrenal, and thymus; concentrations are 10 to 100 times higher in small intestinal mucosa, lymph glands, lung, liver, spleen, and white blood cells (Sauberlich, 1984a). Hence, repletion of ascorbate by tissues might explain a lag before injections increased plasma ascorbate.

At the end of the food deprivation period (d -5), plasma ascorbate tended ( $P < .09$ ) to be higher in the +Vit calves than in control calves. By this time, the +Vit calves had received four injections.

On d 0, plasma ascorbate was higher ( $P < .001$ ) in +Vit than in control calves for the first time during the study. In both groups of calves, plasma ascorbate was lower ( $P < .001$ ) at the time of peak BHV-1 infection (d 5) than on d 0, although concentrations still tended to be higher in the +Vit calves ( $P = .07$ ). Jagos et al. (1977) reported that with calves from 2 to 3 mo of age, plasma concentrations were  $28.4 \pm 10.2$ ,  $10.2 \pm 6.2$ , and  $17.0 \pm 7.9 \mu\text{M}$  for healthy calves, calves with acute bronchopneumonia, and calves 3 wk after acute bronchopneumonia, respectively. Leukocytes contain very high concentrations of ascorbate compared with most tissues and plasma, and leukocytes can actively take up ascorbate during viral infection. Viral infections such as the common cold rapidly deplete the ascorbate content of leukocytes (Hume and Weyers, 1973).

With recovery from BHV-1 infection by d 14, plasma ascorbate concentrations in plasma increased in both groups of calves ( $P < .001$ ). However, concentrations remained lower in controls than at any time prior to BHV-1 infection and in +Vit calves compared with concentrations on d -5 and d 0, suggesting that requirements as reflected by plasma ascorbate concentration may not have been fully satisfied. Perhaps either ascorbate requirements continued to be elevated or repletion of tissues or leukocytes was continuing at the expense of plasma.

After cessation of ascorbate injections, plasma ascorbate in +Vit calves dropped to concentrations lower than previously observed in control calves. Perhaps catabolism of ascorbate remains elevated when macrodoses of ascorbate are terminated, but whether scurvy results from such a withdrawal

remains controversial (Hornig and Moser, 1981). Long-term administration of ascorbate at high concentrations should be used with caution.

## Implications

A mild respiratory (BHV-1) infection in steer calves markedly decreased plasma concentrations of vitamin B<sub>6</sub>, vitamin B<sub>12</sub>, pantothenic acid, and ascorbate but not folic acid. Restricting feed intake and depriving food for 3 d decreased plasma concentrations of vitamin B<sub>6</sub> and pantothenate, and increased plasma B<sub>12</sub>, but did not markedly affect blood plasma folic acid and ascorbate concentrations. Like nonruminants, ruminants may be depleted in certain water-soluble vitamins when stress or immune challenge increase physiological requirements, especially when ruminal production is limited, conditions that occur often during the shipping and marketing process. Depletion of B vitamin and ascorbate status during shipping and marketing may contribute to the enhanced susceptibility of cattle to infectious disease during the first few weeks after arrival at feedlots. Based on plasma concentrations, status for certain vitamins critical to the immune response in cattle is decreased by an immune challenge, making supplementation desirable to counteract stress and disease.

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