

# PLASMA LEVELS OF WATER SOLUBLE VITAMINS IN VARIOUS CLASSES OF CATTLE

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## Story in Brief

Plasma samples from suckling beef calves, transport-stressed weaned calves, 500 kg feedlot cattle, and lactating dairy cows were analyzed for vitamin C, folic acid, pantothenic acid, and vitamin B<sub>12</sub>. Plasma from stressed calves also was analyzed for vitamin B<sub>6</sub>. Differences in plasma levels were detected between groups. Plasma vitamin C was highest in suckling calves, but similar for stressed calves, feedlot cattle and dairy cows. Folic acid levels were highest in feedlot cattle and lowest in suckling calves and stressed calves. Pantothenic acid was markedly lower in all cattle than in nonruminants; it was similar in suckling calves and feedlot cattle but lower in stressed calves and dairy cows. Vitamin B<sub>12</sub> was similar for suckling calves, stressed calves, and dairy cows, but low in feedlot cattle. Stressed calves had extremely low concentrations of plasma vitamin B<sub>6</sub>.

(Key Words: B-Vitamins, Stress, Vitamin C, Folic, Panthothenic, B<sub>12</sub>, B<sub>6</sub>.)

## Introduction

Requirements of cattle for water soluble vitamins have not been defined. The supply of B-vitamins to cattle depends upon a combination of factors - dietary supply, ruminal bypass of the dietary vitamins, and microbial synthesis. Ruminal action can result in either net synthesis or net loss of B-vitamins for the animal (Zinn et al., 1987).

Generally, microbial synthesis of B-vitamins has been considered sufficient to meet requirements for cattle. However, supplemental B-vitamins have increased production in various classes of cattle recently. For example, niacin supplementation to dairy cows in early lactation has decreased the incidence of ketosis and increased milk production. Niacin supplementation has improved performance of some cattle fed growing and finishing diets. Reproduction in dairy cows may be enhanced by folic acid injections. Data establishing normal plasma levels of B-vitamins in cattle are needed in order to

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detect responses to an increased supply and to determine when cattle may be deficient.

With the exception of vitamin B<sub>12</sub>, B-vitamins are not stored in the body. They are depleted rapidly during periods of increased demand or reduced supply. B-vitamin supply tends to be correlated with feed intake. Although they have not been studied in cattle, stress and illness in other species markedly increases requirements for vitamin B<sub>6</sub>, folic acid and pantothenic acid.

Dietary B-vitamin supplements for stressed calves have shown mixed results. Many B-vitamins are extensively degraded in the rumen. Stressed, sick or inappetent calves often are given injections of commercial B-vitamin mixtures, although this practice has declined due to concerns about injection-site lesions. Formulas for injectable B-vitamin complexes are not based on any research findings. Shipping stress reduces plasma vitamin C and vitamin B<sub>6</sub> levels but does not alter thiamin and riboflavin levels (Personal communication, C. Nockels, 1991). Because vitamin C, vitamin B<sub>6</sub>, folic acid and pantothenic acid deficiencies presumably can compromise the immune response, vitamin status may be critical to the health of stressed calves.

In this study, blood samples were obtained from 1) suckling beef calves, 2) three loads of transport-stressed calves, 3) feedlot cattle and 4) lactating dairy cows. We determined the concentrations of plasma B-vitamins in these different classes of cattle, focusing on B-vitamins critical to the immune response of stressed cattle: vitamin B<sub>6</sub>, folic acid, vitamin C and pantothenic acid. Vitamin B<sub>12</sub> also was measured. The objective of this study was to develop baseline data on normal concentrations and ranges of these B-vitamins in specific classes of cattle. By comparison with the nonruminant literature and by comparison with other classes of cattle, these values may provide useful for evaluating B-vitamin status.

## Materials and Methods

Blood was obtained from suckling calves from 2 commercial beef herds. The British X Limousin calves (average weight 500 lb) were 6 to 8 months old; the Angus/Hereford calves (average weight 420 lb) were 6 to 8 months old. Dams of both groups were grazing dormant winter range and had no access to green grass. Two groups of OSU dairy cows were sampled at different times of year. All cows were producing in excess of 50 lb milk daily; mean production was 78 lb. These cows were fed a total mixed ration based on corn and corn silage. The Angus X feedlot cattle (1100 lb) were being fed a high-concentrate corn-based diet. Three loads of stressed heifer calves (average weight 440 lb) were sampled within 1 day of arrival on trucks from Missouri, Arkansas and Georgia.

Blood was obtained by jugular venipuncture into tubes containing either sodium heparin or potassium EDTA as an anticoagulant. These blood samples



were immediately chilled on ice and centrifuged as quickly as possible after sampling. Multiple aliquots of 0.5 to 3.5 ml plasma from each animal were frozen separately in polypropylene tubes. Plasma for vitamin C analysis was diluted 1:1 with 10% (w/v) fresh metaphosphoric acid, vortexed and frozen immediately. All samples were prepared under subdued lighting because of the sensitivity of vitamin B<sub>6</sub> to light.

Vitamin C was analyzed by a standard colorimetric method. Folic acid and vitamin B<sub>12</sub> were analyzed by a radioassay method (Quantaphase Radioassay, Bio-Rad Clinical Division, Hercules, CA). Vitamin B<sub>6</sub> was analyzed by HPLC. Plasma pantothenic acid was measured using an indirect ELISA assay (Song et al., 1990).

Plasma vitamin data were analyzed by analysis of variance models including animal group as a class. Means were separated using Duncan's Multiple Range test.

## Results and Discussion

Plasma vitamin concentrations varied among groups of cattle (Table 1). Duodenal flow of many B-vitamins has been shown to vary markedly with energy content of the diet and feed intake. Therefore a low plasma concentration for a B-vitamin in one class of cattle compared to another does not necessarily indicate a deficiency. Limited information is available on adequate plasma B-vitamin concentrations in cattle or other livestock; therefore, normal and deficient levels in humans were used as a basis for comparison. Species differences in plasma concentrations may exist for some B-vitamins.

Cattle synthesize their own vitamin C. Yet, supplemental C has proven beneficial to stressed pigs, another species that synthesizes vitamin C. Differences among groups of cattle in plasma vitamin C were evident. Suckling calves had high vitamin C levels, similar to those of normal humans even though cow's milk contains very little vitamin C (1 to 2 mg per 100 g; G.M. Jaffe, 1980). Green forage supplies moderate amounts of vitamin C, but in our study both groups of calves had been raised on dormant winter range. Vitamin C is depleted during persistent stress, but sudden stress may elevate plasma levels. Whether blood sampling elevated plasma vitamin C in the calves in this study is not known. The difference in levels between the two groups of suckling calves (1.25 vs 0.68 mg/ml) may reflect several facts. The first group was more accustomed to handling; the second had been weaned overnight, and may have been stressed longer than the first group.

Plasma vitamin C levels were much lower in the shipping-stressed calves than in suckling calves, but even these values are not abnormally low when compared to plasma concentrations of dairy cows and finishing steers, neither of which had access to pasture. Transport-stressed calves, purchased from

Table 1. Plasma vitamin levels in cattle.

Vitamin	Item	Class of cattle				SE
		Suckling calf	Transport-stressed calf	Feedlot steer	Dairy cow	
Vitamin C, mg/dl	N	35	41	16	44	.09
	mean	1.04 <sup>a</sup>	.44 <sup>b</sup>	.49 <sup>b</sup>	.42 <sup>b</sup>	
	range	.54 - 2.12	.22 - .76	.34 - .83	.13 - .76	
Folic acid, ng/mL	n	35	13	14	31	1.7
	mean	6.8 <sup>d</sup>	9.7 <sup>c</sup>	26.0 <sup>a</sup>	13.1 <sup>b</sup>	
	range	3.2 - 11.0	6.4 - 14.6	14.2 - 35.2	7.1 - 25.4	
Vitamin B <sub>12</sub> , pg/mL	n	34	13	14	17	35
	mean	229 <sup>a</sup>	251 <sup>a</sup>	160 <sup>b</sup>	286 <sup>a</sup>	
	range	113 - 277	116 - 396	97 - 297	150 - 396	
Pantothenic acid, nM	n	31	25	16	14	.019
	mean	.128 <sup>a</sup>	.095 <sup>b</sup>	.143 <sup>a</sup>	.089 <sup>b</sup>	
	range	.039 - .195	.027 - .167	.062 - .201	.023 - .186	

abcd Means in the same row with different superscript differ ( $P < .05$ ).



auction barns in other states, recently had been weaned, deprived of feed, and transported for several days before arriving at the Pawhuska Research Station. Compared to normal human levels, some of the stressed calves and some of the dairy cows would be considered deficient in vitamin C (plasma concentrations under 0.3 mg/dL). Plasma levels of vitamin C may reflect recent dietary intake, while leukocyte concentrations, which are more difficult to measure, are considered to reflect body stores.

Feedlot cattle and dairy cows had high plasma folate concentrations compared to humans (minimum of 6 ng/mL), presumably due to abundant microbial production. Plasma levels in both suckling and stressed calves were similar to normal human plasma levels; however, values for suckling calves ranged from 3.2 to 11.0 ng/mL. Hence, some calves might be marginally deficient.

When dietary cobalt is adequate, ruminal B<sub>12</sub> synthesis should be adequate. Ruminant tissues are excellent dietary B<sub>12</sub> sources for man. Yet plasma B<sub>12</sub> levels in cattle were not high when compared to normal levels in humans (Bentley et al., 1954). B<sub>12</sub> status in cattle has been extensively studied; however, problems with both microbiological and radioassay methods have complicated interpretation of data. Differences between classes of cattle using a very selective analytical method have not been reported previously.

Vitamin B<sub>12</sub> concentrations in plasma were similar in suckling calves, stressed calves and dairy cows. However, plasma from feedlot cattle was low in vitamin B<sub>12</sub>. Based on this particular radioassay method, humans with plasma concentrations under 160 pg/mL are considered deficient. The low B<sub>12</sub> concentrations in plasma of feedlot cattle fed high concentrate diets may be related to production of ruminal B<sub>12</sub> analogs. For most diets, only 15 to 20% of the B<sub>12</sub>-related compounds produced in the rumen have B<sub>12</sub> activity for the animal (Dryden and Hartman, 1971). Feeding more grain increases analog production but decreases B<sub>12</sub> concentrations in serum and tissue of steers, dairy cows or sheep fed more grain. We observed the lowest plasma B<sub>12</sub> (97 pg/mL) in one feedlot steer. Considering published B<sub>12</sub> concentrations in cattle organs, B<sub>12</sub> deficiency may not be a problem in normal cattle, but B<sub>12</sub> should be investigated specifically in cattle fed high-grain rations with limited forage for a prolonged finishing period, e.g., calf-fed Holsteins. One of the early symptoms of B<sub>12</sub> deficiency is reduced feed intake.

Pantothenic acid in cattle plasma was markedly lower than the 1.0 nM expected in humans or rats. The mean plasma pantothenic acid for the 86 cattle was only .115 nM. Pantothenic acid status of cattle has not previously been studied, although a deficiency has been produced in calves fed a semi-purified diet; even in humans, deficiencies are rarely observed. The ELISA method can detect reduced plasma pantothenate in rats after only 10 days of feeding a deficient diet (Song et al., 1990).

Plasma pantothenic acid was highest in feedlot cattle and suckling calves (.143 and .128 nM), and lowest in dairy cows and stressed calves (.089 and

.095 nM). The high levels in feedlot cattle may be related to high production of ruminal pantothenic acid. Pantothenic acid concentration in rumen contents was nearly four times higher in sheep fed a diet containing 90% corn than in sheep fed prairie hay (Hollis et al., 1954).

The lowest individual pantothenic acid levels were found in certain dairy cows and stressed calves. In cattle, as in other species, ascorbic acid and pantothenic acid metabolism may be interrelated (Fox, 1991). Lower levels in dairy cows may reflect higher requirements due to pregnancy and production as well as lower synthesis from mixed diets, possibly due to ruminal pH. Cows in this study were fed a total mixed diet containing buffers.

Low levels of plasma pantothenic acid in stressed calves may be due to low dietary intake, impaired ruminal function, and increased requirements due to stress. Stress increases production of corticosteroids, which can increase destruction of pantothenic acid (Fox, 1991).

The concentration of vitamin B<sub>6</sub>, particularly pyridoxal phosphate, in plasma is used as an indicator of vitamin B<sub>6</sub> status. There is little variation in vitamin B<sub>6</sub> requirements across species, although there is considerable variation within a species in the concentration and distribution of the B<sub>6</sub> vitamers in plasma (Coburn et al., 1984).

Mean concentrations of total B<sub>6</sub>, pyridoxal phosphate, pyridoxal, 4-pyridoxic acid, and pyridoxine in plasma from 9 stressed calves averaged 159, 111, 7.8, 34.0 and 7 nM, respectively. Concentrations in suckling calves from another study were five to 8 times these values (686, 402, 96, 91, and 50; Coburn et al., 1984). This suggests that vitamin B<sub>6</sub> status was severely compromised in our transport-stressed calves. More information on B<sub>6</sub> vitamer concentrations and distribution in plasma from various classes of cattle, especially stressed calves, is needed. Due to its high ruminal survival, B<sub>6</sub> might be supplemented in the diets for ruminants.

## Conclusions

Plasma concentrations of vitamin C, folic acid, pantothenic acid and vitamin B<sub>12</sub> vary among classes of cattle. Some of this variation can be attributed to the type of diet (grain vs hay, degree of grain processing) and feed intake. Copious production of folic acid in the rumen is reflected in high plasma folic acid concentrations compared to nonruminants. However, ruminal degradation, ruminal analog production, poor absorption, and increased production or stress may be responsible for abnormally low plasma concentrations of some B-vitamins. High milk production may be inversely correlated with some vitamins (vitamin C and pantothenic acid). The extremely low levels of vitamin B<sub>6</sub> in plasma from stressed calves suggest that stress depletes vitamin B<sub>6</sub> in calves, and may contribute to their depressed



immunocompetence. Dietary supplementation alone may not solve these potential deficiencies because vitamin C, pantothenic acid and vitamin B<sub>12</sub> are extensively (78 to 97%) destroyed in the rumen.

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