# EFFECT OF B-VITAMIN INJECTIONS ON VIRUS INFECTION AND IMMUNITY IN FEED-RESTRICTED WEANED BEEF CALVES

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## Story in Brief

Following weaning, beef steer calves were nutritionally stressed by fasting and limit-feeding in metabolism stalls in order to test the effect of vitamin B injections on the immune response of stressed calves. The calves lost 13.3% of initial weight on this program. Calves were then inoculated intranasally with live attentuated infectious bovine rhinotracheitis virus (IBRV), a common pathogen implicated in the bovine respiratory disease complex of shipping-stressed calves. This stress/IBRV model resulted in a mild respiratory infection in all calves, compared to that occurring in shipping-stressed calves. Extensive measurements of the immune response (neutralizing antibody titer, virus titration, interferon production and lymphocyte blastogenesis) suggest no benefit from injection of B-vitamins and Vitamin C under these conditions.

Key Words: B-Vitamins, Stress, Cattle, Disease, IBR

## Introduction

Bovine Respiratory Disease complex (BRD) and other stress-related diseases of shipped stocker and feeder calves cost Oklahoma cattlemen between 37 and 100 million dollars annually. This economic loss includes expenses for 1) medications and veterinary costs, 2) reduced rate and efficiency of gain, and 3) death loss (about 63,000 cattle per year).

Stress is a major component in the development of BRD. This can explain the high incidence of the disease within the first 3 weeks after arrival. Before arrival, calves typically are weaned, transported to markets, and undergo feed and water deprivation and diet changes, before being sold and shipped again. On arrival calves often are dehydrated and rumen function is impaired; many calves will not eat for 3 or more days after arrival.

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Requirements for some B-vitamins are increased by stress or disease, particularly certain B-vitamins (vitamin $B_6$, folic acid, pantothenic acid) critical to the immune response. Requirements for these three vitamins are increased by up to 20-fold by stress or injury in humans (Mueller and Thomas, 1975). Survival of seriously ill patients has been correlated with plasma concentration of $B_6$, and improved by $B_6$ supplementation (Enriquez et al., 1988).

Vitamin $B_6$ status may be very low in sick or shipping-stressed cattle (C. F. Nockels, Private Communication, 1991). We attempted to measure B-vitamin status and effect of B-vitamin injections on B-vitamin status, viral infection and immune response. Infection with the infectious bovine rhinotracheitis virus (IBRV), also known as bovine herpesvirus I, was used as a model for BRD, because IBRV infection often predisposes cattle to bacterial pneumonia. However, IBRV usually produces only mild infection under laboratory conditions and is unlikely to cause mortality. All calves selected were initially seronegative (negative for antibody) to IBRV, in order to avoid variability due to previous exposure. The calves were deliberately stressed by weaning, injections, restricted feeding and a 3-day fast, stresses similar to those calves normally undergo following weaning but less in magnitude than the stress of transport, before inoculation with IBRV.

## Materials and Methods

Calves from several OSU beef herds were tested for presence of antibodies to IBRV which would indicate prior exposure or maternal antibodies. All calves tested were seronegative to IBRV. Twelve 6 to 8 month old steer calves (Hereford X Angus crosses) from one herd which had been wintered on dormant native range were weaned on April 23, 1991. They were trucked 10 miles to the Veterinary Medicine building and weighed on arrival. They were housed individually in metabolism stalls. The calves were assigned randomly to two treatments (Control, +Vit), with 6 calves per treatment. The control calves received sterile saline injections while the +Vit calves received injections of B-vitamins and vitamin C (see Table 1). The injection treatments occurred every 2nd day from 10 days after arrival on May 3 until the end of the experiment 31 days later.

On arrival, each calf was fed 2 lbs. of native prairie grass hay (4% crude protein). From April 24 to May 5, and May 9 to May 16, calves were offered native prairie grass hay once daily at approx. 1% of body weight. Because of the very low protein concentration in prairie hay, alfalfa hay (21% crude protein) was substituted for 50% of the prairie hay intermittently and for the final week of the study. Calves were fasted May 6, 7, and 8. On May 17,

**Table 1. B-vitamin requirements and levels supplied by injection.**

| Vitamin | Estimated Requirement[a] | X | Stress factor[b] | Dosage[c] |
|---|---|---|---|---|
| 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 $B_6$ | 6.76 mg | | 8 | 108.0 mg |
| Vitamin B12 | 67.64 µg | | 2 | 270.0 µg |
| Vitamin C | Unknown | | | 1000.0 mg |

[a] Daily B-vitamin requirements for a 420 lb calf were estimated on a metabolic body weight basis as equivalent to 3.56 times the requirements for a 75 lb pig (NRC, 1990).

[b] The estimated daily requirement was multiplied by factors of 2 for pantothenic acid and vitamin $B_{12}$, 15 for folic acid, and 8 for vitamin $B_6$ to account for the increased requirements of these specific B-vitamins during stress or "moderate" injury (Mueller and Thomas, 1975).

[c] Twice the daily estimated requirement was supplied by injection every 2 days, with one 3 ml dose containing the B-vitamins and one 4 ml dose containing the vitamin C.

feeding rate was adjusted to approx. 1 1/2% of initial body weight so that calves would maintain body weight.

Rectal temperature was measured every morning at 7 a.m. before feeding. Body weight was measured in the morning before feeding.

On May 14 (Day 0), the 12 calves were each inoculated with $10^7$ $TCID_{50}$ IBR virus (vaccine strain) in 1 ml intranasally with a syringe. The calves had received 6 injections of B-vitamins or sterile saline by Day 0.

Blood samples were obtained from the calves by jugular venipuncture. On sampling days coinciding with B-vitamin injections, samples were taken before injections were given. Nasal secretions were sampled on Day 0, 2, 4, 5, 7, and 9 by inserting tampons intranasally for 15 minutes. Nasal swabs for virus isolation were taken on the same days. Nasal secretions, serum, and plasma were frozen at -20C. Nasal swab samples for virus titration were frozen at -70C.

Lymphocyte blastogenesis tests were used to assess the mitogenic response of mixed leukocytes to pokeweed antigen on arrival and on days 0, 5, 7, 9 and 14. Four concentrations of pokeweed mitogen (0, 0.25, 0.50, 1.0 ug/ml) were tested. Humoral immunity was assessed by measuring serum neutralizing antibodies on Days 0, 14, and 28. IBRV was titrated by plaque assay in MDBK cells. Interferon in nasal secretions was measured by plaque reduction assays.

Blood samples were analyzed for vitamins C, $B_6$, $B_{12}$, folic acid, pantothenic acid and niacin.

## Results and Discussion

Weaning and handling did not greatly stress these calves. They readily adjusted to the metabolism crates, and within 1 to 2 days of arrival often remained at rest despite the presence of humans, eventually even when rectal temperature was measured. During the fast period, activity was reduced and they drank very little water. B-vitamin and saline injections appeared to cause little discomfort, although vitamin C injections may have caused temporary discomfort. The lack of discomfort of the B-vitamin injection, in contrast to some commercial B-vitamin injections, may be related to the absence of the common antibacterial preservative, benzyl alcohol, in the formulation we used.

Feed intake influenced body temperature. Body temperature averaged 102.2F during the first 3 days of the study. During the 3 mornings of the fast period, and the following morning before re-feeding, average body temperature was 101.6, 101.1, 100.9 and 100.6 F. The day following re-feeding, temperature averaged 101.5F.

On arrival, calf weight ranged from 288 to 424 lb., with a mean weight of 364 lb. Average weight declined with restricted feeding to 345 lb. one week later, and 339 lb. the following week. Following the 3-day fast, average weight was 315.4 lb. Four days later, weight was 336 lbs, and 337 and 339 lb. the following weeks (on the maintenance feeding schedule).

The restricted feeding/fasting regimen resulted in an average shrink of 13.3% over a period of 16 days. Shrink in shipping-stressed calves is correlated with morbidity during the receiving phase. Transport, the most serious stress factor, alone can account for most of the shrink, with length of transport correlated with total shrink. Shrink results in loss of both gastrointestinal tract contents and wet tissue. Associated impairment of ruminal function may cause inappetence leading to continued nutritional deficiencies. The stress model used in this study, with a relatively long-term period of feed restriction and a 3-day fast after adaptation, may not have

resulted in as extreme physiological and nutritional changes as occur in shipping-stressed calves acutely stressed by one or more long hauls.

Feed consumption was consistent throughout the study. All feed was consumed within 2 hours of feeding every morning, except on day 1 when 3 calves did not clean up their feed.

IBRV infection caused mild respiratory disease in the calves, limited to the upper respiratory tract. Clinical symptoms peaked at 4 to 6 days post-infection. Temperature was elevated in one +Vit calf on Day 4, in 5 calves on Day 5, in 6 calves on Day 6, and remained elevated in 2 of these calves on Day 7. No elevation in body temperature was detected in 3 of the calves on any day after infection.

By Day 4 and Day 5, all calves developed a serous nasal discharge progressing to a mild mucopurulent discharge. The nasal mucosa became inflamed and typical IBR plaques were observed in several calves. Little coughing occurred.

B-vitamin treatment did not significantly affect immune parameters. Neutralizing antibody titer was not significantly different on days 14 and 28 (8.0 and 22.0 for controls vs. 10 and 27.3 for +Vits). Similarly, virus levels in nasal mucosa were not different (Table 2). Production of interferon, an important broad spectrum antiviral compound initiated by infected cells, also was not altered significantly by B-vitamin injection (Table 2).

The lymphocyte blastogenesis test (LBT) is often performed in survey experiments such as this as an indicator of cell-mediated immunity (CMI). CMI appears important in recovery from viral infections, so may influence susceptibility of calves to secondary bacterial infections in BRD. (Our use of the IBR model for BRD will be improved in future by combining initial IBR infection with subsequent Pasteurella haemolytica infection to better assess such interactions).

Variation within animals and sample periods was extremely high for the LBT, and no clear trends were evident (Table 2). The stimulation index was significantly higher in the control calves in the initial sample taken 3 days after arrival and on day 7 postinfection. The LBT involves the in vitro culture of white blood cells in a medium containing fetal calf serum and mitogen for 5 days. The ability of the cells to respond to the mitogen by proliferation is measured. However, the medium and fetal calf serum may provide sufficient B-vitamins (example $B_6$) to standardize the in vitro response of cells and therefore the LBT might not relate to the in vivo response. This should be investigated by modifying the medium and/or replacing fetal calf serum with autologous serum whenever nutritional status is affected by the experimental treatment.

Results show no evidence that immune responsiveness was enhanced by injections of B-vitamins and vitamin C. Previous nutritional history, health status and degree of stress may be important in determining vitamin status of calves and response to supplementation.

**Table 2. Effect of B-vitamin injections on response to IBRV injection and lymphoocyte blastogenesis.**

| Item | Treatment | |
|---|---|---|
| | Control | + B-vit |
| No. of animals | 6 | 6 |
| Neutralizing antibody titers | | |
| Day 0 | 0 | 0 |
| Day 14 | 8.0 | 10.0 |
| Day 28 | 22.7 | 27.3 |
| Average IBRV titers ($log_{10}$ PFU/ml) | | |
| Day 0 | 0 | 0 |
| Day 2 | 4.58 | 4.92 |
| Day 4 | 6.15 | 6.05 |
| Day 5 | 5.50 | 5.17 |
| Day 7 | 5.78 | 5.62 |
| Day 9 | 3.32 | 3.15 |
| Average interferon titers ($PR_{50}$ units/ml) | | |
| Day 0 | 0 | 0 |
| Day 2 | 23.3 | 18.3 |
| Day 4 | 3540 | 1160 |
| Day 5 | 2540 | 2950 |
| Day 7 | 5250 | 6100 |
| Day 9 | 165 | 138 |
| Average temperature (F) at peak temperature response (Day 5) | 103.5 | 103.2 |
| Average stimulation index (LBT) | | |
| 3 days after arrival | 440[a] | 210[b] |
| Day 0 | 180 | 380 |
| Day 5 | 1640[a] | 250[b] |
| Day 7 | 230 | 550 |
| Day 14 | 500 | 240 |

[a,b] Means in the same row with different superscripts are different ($P<0.05$).

## Literature Cited

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