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A preliminary study on the effect of cobalt supplementation on RB51 *Brucella abortus* antibody response in weaned beef calves

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Abstract

Cobalt (Co) is utilized by rumen microbial organisms for synthesis of vitamin B_{12}, a necessary cofactor for vital metabolic pathways in lipid and carbohydrate energy metabolism. Cobalt supplementation has recently attracted attention because of increased carcass weight in feedlot cattle, and increased milk production in dairy cattle. The objective of this study was to evaluate the effect of different levels of Co supplementation on humoral immune response to *Brucella abortus* vaccine in weaned beef calves. Twenty-seven beef steers weighing 450 lb ± 50 lb (204 kg ± 22.7 kg) were utilized in the study. Calves were randomly assigned to be fed supplemental Co at 0.1, 0.4, or 1.0 mg/kg of body weight in their diet, corresponding to the National Research Council (NRC) recommended level, 4 times the NRC recommended level, and 10 times the NRC recommended level. Higher levels of Co supplementation resulted in increased antibody response ($P \leq 0.004$) to RB51 *Brucella abortus* vaccine.

Key words: bovine, humoral immune function, *Brucella abortus,* cobalt, weaned beef calves

Résumé

Le cobalt (Co) est utilisé par les organismes microbiens du rumen pour la synthèse de la vitamine B_{12}, un co-facteur essentiel impliqué dans des voies métaboliques vitales du métabolisme énergétique des lipides et des carbohydrates. Les suppléments en cobalt ont suscité de l'intérêt récemment en raison de l'augmentation de la masse des carcasses chez les bovins en parc d'engraissement et de l'augmentation de la production de lait chez les vaches laitières. L'objectif de cette étude était d'évaluer l'effet de suppléments en cobalt sur la réponse immunitaire humorale à un vaccin préparé avec une souche de *Brucella abortus* chez des veaux de boucherie sevrés. Des bouvillons de boucherie ($N = 27$) pesant 450 lb ± 50 lb (204 kg ± 22.7 kg) ont été utilisés dans l'étude. Les veaux ont été alloués aléatoirement à des groupes recevant des doses de 0.100 ppm, 0.400 ppm ou de 1.00 mg/kg de poids corporel dans leur régime alimentaire, ce qui correspond, respectivement, à la dose recommandée par le *National Research Council* (NRC), à 4 fois leur dose recommandée ou à 10 fois leur dose recommandée. Un apport avec un plus haut niveau de cobalt a causé une augmentation de la production d'anticorps ($P \leq 0.004$) en réponse à un vaccin préparé avec la souche RB51 de *Brucella abortus*.

Introduction

All animals require cobalt (Co) as an integral component in vitamin B_{12}. In ruminants Co is utilized by rumen microbes for vitamin B_{12} production. Vitamin B_{12}, which is absorbed in the ileum, is a vital cofactor in specific metabolic enzymes in lipid and carbohydrate energy metabolism. Cattle obtain Co from forages according to availability of Co from the soil;[20] a deficiency is strongly geographically and geologically dependent on soil and water concentrations and availability of the element.[15] Iron in soil or water reduces Co absorption in cattle, thereby predisposing to Co deficiency; manganese, zinc, and iodine are also antagonists.[11]

Early studies of Co were focused on prevention of wasting disease syndromes, clinically observed as ill-thrift, weight loss, anorexia, and poor production in ruminants in certain areas of the world.[15,19] Ovine white liver disease (OWLD) in sheep, common in Co deficient areas, is a result of Co deficiency limiting synthesis of vitamin B_{12}, which in turn results in reduced β oxidation of free fatty acid (FFA) and accumulation of FFA deposits that displace normal liver tissue.[15,19] Normal mobilization of FFA into triglycerides for liver export as very low density lipoproteins requires 2 vitamin B_{12} derived cofactors. Vitamin B_{12} deficiency causes accu-

mulation of triglycerides, which gives the characteristic white appearance to liver tissue.

Ruminant production was restricted in certain areas in the world until the early 1900s because of "bush sickness" and "wasting disease". New Zealand scientists initially considered these diseases to result from iron deficiency, but later Underwood and Filmer discovered the syndrome was due to cobalt deficiency.[18] Cobalt treatments with as little as 1 ppm per day resulted in reversal of the disease syndrome.[7] This discovery allowed grazing of previously unusable large coastal areas of Australia and other Co deficient areas of the world.[15,19]

Interactions between the immune system and micro-minerals in beef cattle are extremely complex, but necessary for normal immune response to infection and disease. Concentrations of micro-minerals should be maintained within optimal limits to preserve the functional integrity of cells and tissues vital to normal immune function.[2,7,19] Deficiencies require the animal to metabolically compensate for the nutrient deviation, resulting in depressed immune function, and diminished animal health and performance.[2] Deficiency of certain minerals may impair normal phagocytic function and other components of innate immunity, such as cytokine production, with irregular function of humoral and cell mediated immunity in the adaptive immune system.[3]

Differences in micro-mineral intake, absorption, and metabolism in beef cattle often determine response to infection and disease, and are influenced by age, breed, physiological status (pregnancy and lactation), and stress (scarcity of feed or water, climatic, physiological, and environmental factors).[2,19] Stress stimulates cortisol production which negatively affects immune function, and gestation and lactation decrease Co concentration levels in the liver.[5,19,21] There are also breed differences in Co requirements as Continental breeds have up to twice the mineral requirement than British breeds.[11] Studies have shown that feeding Co at 9.1 mg/lb (20 mg/kg) of dry matter intake resulted in increased growth,[13,14] increased milk production in dairy cattle,[5] and increased hot carcass weight in feedlot steers.[17]

Cobalt is extremely safe as a supplement fed orally. Risk of toxicity has been described by Puls.[11] Signs of toxicity and deficiency are almost identical. Animals with Co toxicity often show reduced growth rate, rough and dull hair coat, muscular incoordination, and increased PCV and hemoglobin. The most pronounced toxic sign is reduced weight gain. Sheep have a higher Co requirement than cattle, therefore signs of toxicity are more likely in cattle than sheep. Deficient sheep fed Co at 1000 times the recommended level did not exhibit signs of toxicity until after 8 weeks, whereas toxicity in cattle can occur when cobalt is fed orally at 300 times the level recommended by the National Research Council (NRC). Iron is the most common antagonist, and can be fed to ruminants with Co toxicity as Fe competes for intestinal absorption sites in the intestine.[11]

Extensive information on the role of cobalt on health and production of ruminants has been published;[7,8,11,15] however, there is limited information on the effect of supplemental cobalt on response to various vaccines. The objective of this study was to evaluate the effect of different levels of Co supplementation on humoral immune response to *Brucella abortus* vaccine in weaned beef calves.

Materials and Methods

Animal History

The protocol was approved by the Montana State University Animal Care Committee. Twenty-seven spring-born steer calves (Angus x Hereford x Red Angus x Lowline) with an average body weight of 450 ± 50 lb (204 ± 22.7 kg) were selected for the study from a group of 80 animals raised on a ranch in southwestern Montana. Every third calf processed through the chute was selected and match-grouped accordingly by body weight and genetics. The calves had not received antimicrobials, ionophores, or growth promoting implants. Calves were processed in October of 2010 with a combination 7-way clostridial bacterin-toxoid and *Histophilus somni* bacterin,[a] a combination infectious bovine rhinotracheitis, bovine viral diarrhea virus types 1 and 2, parainfluenza-3, and bovine respiratory syncytial virus vaccine and *Mannheimia haemolytica-Pasteurella multocida* bacterin,[b] and topical doramectin.[c] Twenty days later calves were weaned and acclimated for 20 days prior to the start of the study.

The study was conducted on a ranch in southwestern Montana. Steers were housed in 3 open pens of 9 head each without shelter for the duration of the study. On day 1 of the study, calves were given an individual electronic identification ear tag, weighed individually, blood was collected via the tail vein, and liver biopsy specimens were collected.

Steers were randomly assigned (per passage through the chute at processing) to 1 of 3 Co levels in the mineral supplement to provide: 1) 0.1 mg/kg of body weight (BW) cobalt proteinate,[d] the level recommended by the NRC (1X group);[9,10] 0.4 mg/kg BW cobalt proteinate, 4 times the recommended NRC level (4X group); and 1.0 mg/kg BW cobalt proteinate, approximately 10 times the recommended NRC level (10X group), in a commercial mineral mix (Table 1). Mineral supplements were fed *ad libitum* for the duration of the trial, and were routinely monitored for consumption and wastage twice weekly. Mixed grass-alfalfa hay (88.32% dry matter, 14% crude protein)[e] was fed *ad libitum* to meet requirements to achieve weight gains between 1.25 and 2.0 lb (0.57

Table 1. Composition analysis of base mineral.*

Ingredient	Content
Salt (NaCl)	17.5% (min) 21.0% (max)
Copper	2200 mg/kg
Selenium	35 mg/kg
Zinc	7500 mg/kg

*Mineral mix manufactured by CHS Nutrition, Sioux Falls, SD.

and 0.91 kg)/day. Hay was fed once daily, and refusals were weighed every 14 days.

On day 60, all steers were vaccinated with *Brucella abortus* RB51[f] with the approval of the United States Department of Agriculture Animal Plant and Health Inspection Service and the Montana State Veterinarian. On day 90, steers were individually weighed, blood was collected via the tail vein, and liver biopsies were collected.

Liver Biopsy Procedures

Biopsy sites were clipped of hair, scrubbed with povidone iodine, flushed with chlorhexadine, and anesthetized with 2% lidocaine HCL. A core liver sample was obtained using the Shackleford-Courtney Liver Biopsy Instrument,[g] via a true-cut technique as previously described.[h,4,12] Liver biopsy samples were rinsed using 0.10 M physiological buffered saline solution (pH 7.4), and then placed in aluminum foil, folded in, and frozen in liquid nitrogen until they were analyzed.

Analytical Procedures

Mineral supplement was analyzed for cobalt proteinate content.[d] Serum antibody titers to RB51 *Brucella abortus* were determined using enzyme linked immunosorbent assay.[i] Liver biopsy specimens were analyzed for Co (wet matter basis, evaluating 13 different minerals) using inductively-coupled plasma mass spectroscopy[j] at the US Fish Technology Center, Bozeman, MT.

Statistical Analysis

Statistical analysis of antibody titers was completed using one-way ANOVA means (Fisher's Least Significant Difference Test). Differences were considered significant at $P<0.05$. Prior to statistical analysis, assumptions were made that normality of the calves does exist, and the titer measurements are independently distributed with the mean and the standard deviation (mean, and SD). Constant variance was assumed and independence is made as each pen of 9 calves was used as an experimental unit for both the antibody response to *Brucella abortus* and for the liver Co levels. Paired Co levels from liver biopsies (first- and last-day liver Co biopsy results) were compared. *Brucella abortus* ELISA titers were evaluated by the same ANOVA method. Because of data obtained from animal repeatability in this study, the evaluation of *Brucella abortus* titers was considered the highest priority statistically.

Results and Discussion

The mineral supplement was formulated to provide Co at 0.1 (1X), 0.4 (4X), and 1.0 (10X) mg/kg of body weight; however, Co intake measured during the 90-day trial was 0.139, 0.489, and 0.898 mg/kg body weight; slightly more than targeted in the 1X and 4X groups. Cattle were fed a low energy, mixed alfalfa-grass hay diet, thus weight gain was minimal during the 90 day study period. Total weight gain during this period was 101, 106, and 116 lb (45.8, 48.1, and 52.6 kg) for steers in the 1X, 4X, and 10X treatment groups, respectively; weight gains did not differ between treatment groups ($P>0.05$). There was no morbidity or mortality in the calves during the study period.

Antibody response to *Brucella abortus* RB51 vaccination was higher in the 4X and 10X treatment groups ($P<0.004$) compared to steers in the 1X group (Figure 1); however, antibody responses in the 4X and 10X treatment groups did not differ ($P<0.05$). These results suggest that NRC recommendations for Co levels in the diet could be increased to enhance the immune system response to vaccine in recently weaned beef calves.

Improved antibody production in weaned beef calves fed increased dietary Co is most probable a direct result of increased vitamin B_{12} production by rumen microbial synthesis. Vitamin B_{12} plays an important role in immune system regulation with increases in NK

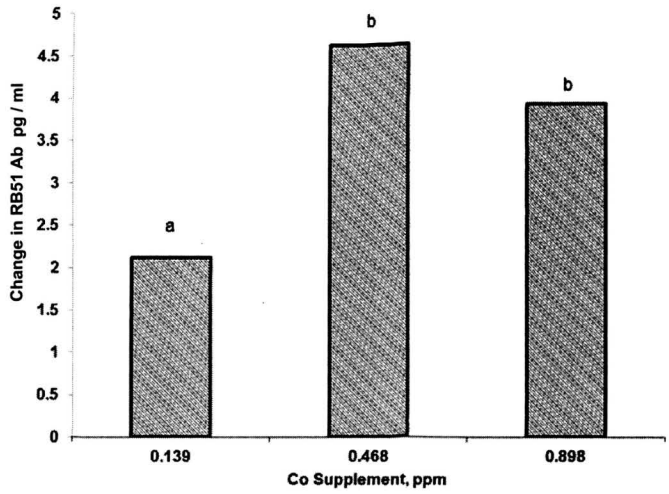

Figure 1. Antibody response of beef steers fed varying levels of cobalt and vaccinated with RB51 *Brucella abortus* vaccine. Antibody levels are reported as units per mL, measured as picograms per mL.

(natural killer) cell activity and CD8+ cells affecting cytotoxic cells, therefore acting as an immunomodulator.[16] Vitamin B_{12} is critical for folate metabolism in DNA synthesis required in B cell proliferation and plasma cell production for antibody response.[16] Vitamin B_{12} is a compromising factor in cell mediated immunity by altering antibody response to antigens.[3]

Ruminal microbial vitamin B_{12} derived cobalamins (Cbl) are essential micronutrients for synthesis of methylcobalamin (MeCbl) and adenosylcobalamin (AdoCbl), the respective cofactors in methionine synthase (MS) and mitochondrial L-methylmalonyl-CoA mutase vital to cystol metabolism.[19] Methionine is required for production and mobilization of immune cells to combat invading pathogens.[3]

Liver Co levels (Figure 2) at time 0 did not differ significantly between treatment groups, but did vary between animals. At the end of the 90-day study period, liver Co concentrations had decreased, but were similar between treatments ($P<0.05$). In contrast, other researchers did not find a decrease in liver Co levels following supplementation of mature dairy cows[21] or steers fed growing and finishing diets.[17] Steers in the present study were fed a low energy hay diet, with weight gains of 1.12 to 1.29 lb (0.51 to 0.59 kg)/day, and Co was provided in the mineral mix rather than inclusion in a total mixed ration. It is unclear why liver Co levels decreased in the present study, but may have been related to ration differences, winter stress, or stage of growth.

Cobalt is also necessary for storage of copper (Cu) in bovine liver tissue.[6] Proper Cu levels are necessary for immune system function in beef cattle, therefore Co may play an indirect role in immune function by altering Cu metabolism.[5] One study showed a linear correlation between liver Co and Cu levels, suggesting that Co is needed for Cu storage in liver tissue.[5] Mineral concentrations in liver biopsies in the present trial showed similar results (Figure 3).

During the past 50 years, beef cattle production in the United States has increased nearly 50% due to improved genetics, advances in nutrition, biotechnology, advances in animal health, and value added management. The National Research Council recommends that Co be included in the diet at 0.1 mg/kg (dry matter intake);[9] this has been the recommendation since the 1950s when production performance of beef cattle was two-thirds of present day production expectations.[j] The recommended Co levels were derived from experiments during the 1950s using cattle that were genetically different, raised with a different production focus, and fed different rations. Today typical beef cattle are 35 to 40% larger anatomically, grow at much faster rates, and are developed with an economic focus on muscle growth with efficient gain that was not considered possible 50 years ago.[k] Other research has demonstrated that increased Co levels are needed to minimize metabolites of propionate metabolism that negatively effect intake and growth.[14] Because of these dramatic changes in the beef industry, the dietary requirement for Co is likely higher today than it was in the 1950s.

This pilot study tested the hypothesis that Co supplementation in excess of NRC recommendations positively improved the humoral immune response in beef steers using RB51 *Brucella abortus* vaccine as the proxy. Individual animal Co intake levels were not measured in this study as pen was the experimental unit. Plans for a future study includes individual feeding of calves, monitoring post-weaning health in the feedlot, collecting carcass information at harvest, and perhaps,

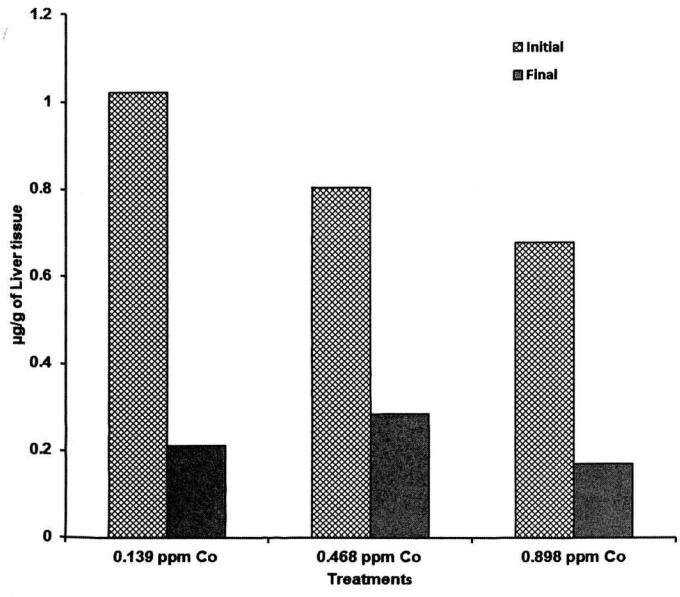

Figure 2. Liver cobalt content in steers fed varying levels of Co in mineral supplement during a 90-day trial period.

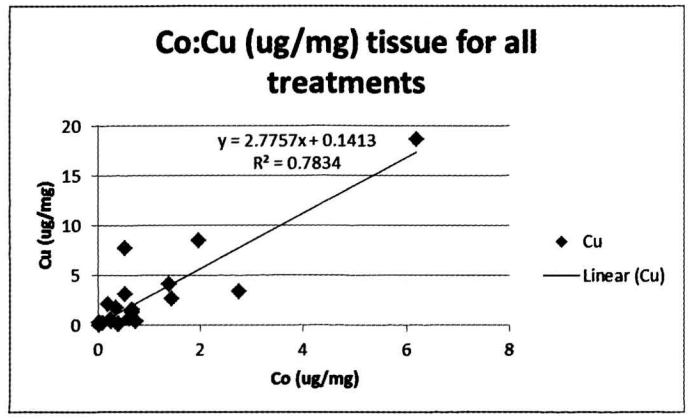

Figure 3. Relationship of liver biopsy cobalt concentration to liver copper concentration in calves fed supplemented cobalt during a 90-day trial period.

to evaluate Co fed pre-weaning in a more challenging environment to determine effects on BRD.

Conclusions

Results of this study suggest that NRC recommended levels of dietary Co should be increased to provide improved antibody response in beef calves at weaning. Treatment levels 4X and 10X times the NRC recommendations resulted in increased antibody response to RB51 *Brucella abortus* vaccine in weaned beef calves. It is yet unknown whether increased Co supplementation will reduce risk of BRD through enhanced immune response.

If future research shows that Co supplementation results in improved immune function, this could lead to improved welfare and health in beef calves at weaning. Increased supplementation of Co post-weaning could improve immune function by increasing antibody production, reducing sickness at weaning, and improve beef calf production with potential increased return on investment to production units.

Endnotes

[a]Vision 7+ Somnus,® Merck Animal Health, Summit, NJ
[b]Express 5 +PMH,® Boehringer Ingelheim Vetmedica, St. Joseph, MO
[c]Dectomax Pour On,® Pfizer Animal Health, Exton, PA
[d]Balchem Corp, Salt Lake City, UT
[e]Midwest Laboratories, Omaha, NE
[f]Professional Biological Company, Denver, CO
[g]Sontec Instruments, Centennial, CO
[h]Sager RB. Cobalt supplement affecting immune function in beef calves. Master of Science Thesis, Montana State University, Bozeman, MT, 2011.
[i]Department of Immunology and Infectious Diseases, Montana State University, Bozeman, MT
[j]ICP-MS, Perkin Elmer, Optima 5300 DV, Optical Emission Spectrometer, Waltham, MA
[k]Dr. J.A. Paterson, Professor Emeritus, Montana State University, personal communication, ARNR 570, November 12, 2009

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The author declares no conflict of interest.

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