

HUMORAL IMMUNE SYSTEM RESPONSE TO COPPER AND SELENIUM SUPPLEMENTATION
IN WEANED BEEF STEERS¹

T.O. Dill^{2*}, H.A. Turner², D.W. Weber², D.E. Mattson³, R.J. Baker³ and P.D. Whanger⁴

Oregon State University
Corvallis, Oregon 97331-6702

ABSTRACT

This study was conducted to determine the effects of copper (Cu) and/or selenium (Se) on the humoral immune system of weaned steers fed diets deficient or marginal in these minerals at two Eastern Oregon sites. Steers were bled weekly for 8 weeks to measure plasma mineral levels and antibody response to Keyhole limpet hemocyanin (KLH). Copper and Se were studied at the Eastern Oregon Agricultural Experiment Station near Union, Oregon. Forty-eight Hereford X Simmental steers were stratified by weaning weight and randomly assigned to treatments of injectable: 1) Se (1 ml Mu-Se/90.8 kg body weight), 2) Cu (2 ml Moly-cu), 3) both Se and Cu, or 4) normal saline solution. Plasma Cu levels were higher ($p < .05$) in treatments 2 and 3 on weeks 2 and 3. Immune response was different ($p < .05$) between Cu and control on week 7. Selenium plasma levels were higher ($p < .05$) in both Se treatments on weeks 2 and 3. Immune response was greater ($p < .05$) in steers receiving only Se than in Cu only or control steers on week 6 but, was not different ($p > .1$) from steers receiving both Se and Cu. Copper + Se steers had higher ($p < .05$) immune response than control on week 7 but, was not different ($p > .1$) on any other sampling date. Selenium either alone or in combination with Cu increased ($P < .05$) the humoral immune response of weaned steers. Cu when used with Se reduced ($P = .07$) the response of Se.

(KEY WORDS: Selenium, Copper, Humoral Immunity)

Introduction

Weaning is a time of stress on calves. Many producers wean and vaccinate at the same time to reduce the number of times calves are handled. It has been observed that calves exhibit morbidity shortly after weaning. This may be due to stress, vaccinations, improper nutrition or a combination of these factors. Plasma levels of selenium (Se) have been found to be deficient and plasma levels of copper (Cu) have been found to be marginal in weaned calves at the Eastern Oregon Agricultural Experiment Station near Union (Whanger et al., 1987). These trace minerals have been demonstrated to be involved with the normal health and immune system functions of animals (Underwood, 1980). The cost of vaccines and treatment of sick animals is one of the major factors in reduced profits for beef cattle operations. A secondary cost is the poor performance of these animals. If trace mineral deficiencies have an adverse affect on immune system response, these animals would be more susceptible to disease and vaccination when deficient may reduce the efficacy of vaccines.

This study was investigated the effects of adequate and deficient levels of Cu and/or Se nutrition on the humoral immune system of weaned beef steers.

Bennetts and Chapman (1937) demonstrated that neonatal ataxia could be prevented with Cu supplementation. A decrease in antibody forming cells has been reported by Chandra and Daton (1982) in Cu deficient animals. Increased phagocytosis of infectious agents by polymorphonuclear leukocytes (PMN) has been reported in humans after Cu supplementation (Heresi et al., 1985). Microbicidal activity of neutrophils was found to be lower in Cu deficient cattle when compared to those that were adequate (Boyne and Arthur, 1981).

In 1958, Muth et al., reported success in preventing white muscle disease in lambs when the ewes were supplemented with Se. Recently, Se has received the attention of researchers examining the immune response of animals. Smith et al. (1984) observed a negative correlation between herd Se blood level and the prevalence of intramammary infections in 32 Pennsylvania dairies. The incidence and duration of mastitis infections have been reduced with Se and vitamin E supplementation during the nonlactating period in dairy herds (Smith et al., 1988). Humoral immune response to hen egg lysozyme antigens was increased in beef cattle fed 80 or 120 mg/g Se in trace mineralized salt (Swecker et al., 1989). A decreased stimulation of immunoglobulin gamma (IgG) forming cells was observed in Se deficient animals (Sheffy and Schultz 1979). Reffett et al. (1988) observed higher IgG and immunoglobulin mu (IgM) antibody titers in cattle challenged with infectious bovine rhinotracheitis virus and supplemented with Se.

Materials and Methods

Forty-eight Hereford X Simmental steers approximately 10 months of age were stratified by weight and randomly assigned to one of four treatments in a randomized complete block design. Blocking criterion was sex location. Treatments were 1) control, 2) injection of normal saline solution; 2) Se supplemented, 1 ml Mu-Se 91 kg body weight and 1 injection of saline; 3) Cu supplemented, 2 ml Moly-Cu⁵ and 1 injection of saline; and 4) Se and Cu supplemented. Mineral injections were administered 1 week prior to weaning after blood was collected via jugular venipuncture to establish a baseline level and the steers were returned to their dams on pasture. At weaning, week 2, the steers were bled via jugular venipuncture, for a baseline immune response, then vaccinated with 5 ml Vira Shield-4⁶, 5 ml Fermicon-E and .5 ml Keyhole limpet hemocyanin vaccine (KLH). Vaccinations were repeated on week 6 at the same levels.

¹Oregon Ag. Exp. Sta. Tech. Paper No. 9614.

²Department of Animal Science.

³College of Veterinary Medicine.

⁴Department of Agricultural Chemistry.

⁵Schering-Plough Animal Health.

⁶Grand Laboratories.

⁷Bio-centric.

Weekly blood samples were collected to track the humoral immune response and plasma mineral levels for 7 more weeks. Following weaning steers were placed in pens with covered feed bunks and fed 8.2 kg per head per day native hay that was marginal (0.05 ppm) in Se and adequate in Cu (10.9 ppm). Steers were allowed access to a mineral free salt and water at all times.

Mineral blood samples were separated via centrifuge to obtain plasma which was then removed from the tube and frozen until analyzed. Plasma Cu samples were analyzed by atomic absorption spectrophotometry. Plasma Se levels were determined using an automated fluorimetric method after acid digestion (Brown and Watkinson, 1977). Humoral immune response to KLH was determined by an indirect enzyme linked immunosorbent assay (ELISA) with KLH adsorbed to the solid phase (Voller et al., 1979). Weekly blood samples were collected, serum was separated via centrifuge removed from the tube and frozen until analyzed. This procedure was designed to measure IgG levels.

Five fall born steers from another experimental herd were hyperimmunized with 4 injections of .5 ml KLH vaccine at 2 week intervals. Ten days after the final vaccination, blood was collected, the serum pooled and used as a positive control in the ELISA procedure. To eliminate day to day variation in the time required for color to develop, all optical densities (OD) were recorded when this positive control sample reached an OD of 1.0 at a dilution of 1:250 titer.

Statistical analysis was completed on treatment means using contrasts in the General Linear Models procedures of the Statistical Analysis System (SAS, 1982). Block and the treatment by time interaction were not significant ($P>.10$). Therefore, the model used was difference = treatment + error. Contrasts conducted were Se vs control, Cu vs control, Cu + Se vs control, Se vs Cu + Se and Cu vs Cu + Se. The contrasts tested were between treatment means within each sampling period.

Results and Discussion

Mean plasma Cu levels are presented in Table I. All treatment groups had adequate (>0.6 ppm) plasma Cu levels throughout the trial. On week 2, animals that received a cupric glycinate injection tended to have higher ($P<.10$) plasma levels of Cu, even though none of the groups were deficient. The following week, the steers in treatments 3 and 4 had higher ($P<.05$) plasma Cu levels than 1 and 2. Steers did not exhibit deficient (<0.6 ppm) plasma Cu levels due to an adequate level (10.9 ppm) of Cu contained in the hay. The requirement for Cu in growing cattle is 8 ppm (NRC, 1984) and therefore, the steers were consuming adequate Cu to meet their needs.

Mean plasma Se levels are reported in Table II. All treatment groups had plasma Se levels at or slightly above the deficient (0.03 ppm) level on week 1. On weeks 2 and 3 both groups receiving sodium selenite had higher ($P<.05$) plasma Se levels than treatments 1 or 3. Steers in treatment 2 maintained plasma Se levels at or above deficient levels for the entire trial period. However, steers in treatment 4 were deficient on weeks 7 and 8. Steers with no Se supplementation became deficient on week 4 (1) or week 5 (3). On week 4 the groups supplemented with Se had higher ($P<.05$) plasma Se levels than treatment 1, but only tended to be higher ($P<.10$) than 3. The suggested range of Se for growing cattle is 0.05 to 0.30 ppm (NRC, 1984). The 0.05 ppm in the hay was at the very bottom of this range and it may have been enough to maintain adequate levels of plasma Se.

Initial KLH vaccination was administered on week 2 of the trial. The means for all sample dates are presented in Figure I. Samples from week 2 were taken prior to vaccination and served as a negative control. Optical densities were not different ($P>.10$) between treatment groups until after the second KLH vaccination on week 6. On week 7 treatments 2 and 4 had higher ($P<.05$) OD readings compared to 1 or 3. The following week (week 8) treatment 2 steers had the highest ($P<.05$) OD readings. Treatment 4 steers had higher ($P<.05$) OD readings than 1 or 3. OD readings of treatment 4 was lower ($P=.07$) than 2. A trend following this same pattern was also observed in the ADG of the steers. However, the number of animals and the length of time are not adequate for these kind of data.

Two steers were afflicted with pneumonia during the trial. They were both mild cases and recovered after administration of Tetracycline. There was no difference ($P>.10$) in the total number of animals sick, number treated or in number of sick days between the treatment groups. However, numbers were too low to draw conclusions from these data. Droke and Loerch (1989) observed the same trend of higher antibody titers but no difference in morbidity among animals.

SUMMARY

Copper supplementation was effective in maintaining adequate plasma Cu levels. Copper was antagonistic to Se in the immune response. This phenomenon needs further research to determine the cause and establish the validity of these results.

Selenium supplementation was effective in maintaining higher plasma Se levels. The humoral immune response was improved with Se supplementation. However, when used in conjunction with Cu the increased response was not as great. The increased immune response should be conducive to fewer animals needing treatment for sicknesses. However, this was not observed in this trial. Since the additional cost of supplementation is not compensated for through reduced morbidity, it would be a questionable practice.

Literature Cited

- Bennetts, H. W. and F. E. Chapman. 1937. Copper deficiency in sheep in Western Australia: A preliminary account of the aetiology of enzootic ataxia of lambs and an anaemia of ewes. *Aust. Vet. J.* 13:138.
- Boyne, R. and J. R. Arthur. 1981. Effects of selenium and copper deficiencies on neutrophil function in cattle. *J. Comp. Pathol.* 91:271.
- Brown, M. W. and J. H. Watkinson. 1977. An automated fluorimetric method for the determination of nanogram quantities of selenium. *Analytica Chimica Acta.* 89:29.
- Chandra, R. K. and D. H. Daton. 1982. Trace element regulation of immunity and infection. *Nutr. Res.* 2:721.
- Droke, E. A. and S. C. Loerch. 1989. Effects of selenium and vitamin E on performance, health status and humoral immune response to Pasteurella haemolytica vaccination of feeder calves. *J. Anim. Sci.* 67:1350.
- Heresi, G., C. Castillo-Duran, C. Munoz, M. Arevalo and L. Schlesinger. 1985. Phagocytosis and

immunoglobulin levels in hypocupremic infants. Nutr. Res. 5:1327.

Muth, O. H., J. E. Oldfield, L. F. Remmert and J. R. Schubert. 1958. Effects of selenium and vitamin E on white muscle disease. Science. 128:1090.

National Research Council. 1984. Nutrient Requirements of Beef Cattle. Sixth Rev. Ed. Washington, D. C. National Academy of Science.

Reffett, J. K., J. W. Spear and T. T. Brown Jr. 1988. Effect of dietary selenium and vitamin E on the primary and secondary immune response in lambs challenged with Parainfluenza-3 virus. J. Anim. Sci. 66:1520.

S. A. S. 1982. SAS User's Guide: Statistics. SAS Inst. Inc. Cary, NC.

Sheffy, B. E. and R. D. Schultz. 1979. Influence of vitamin E and selenium on immune response mechanisms. Fed. Proc. 38:2139.

Smith, K. L., J. H. Harrison, D. D. Hancock, D. A. Todhunter and H. R. Conrad. 1984. Effect of vitamin E and selenium supplementation on incidence of clinical mastitis and duration of clinical symptoms. J. Dairy Sci. 67:1293.

Smith, K. L., J. S. Hogan and H. R. Conrad. 1988. Selenium in dairy cattle: it's role in disease resistance. Vet. Med. 83:72.

Swecker, W. S., D. E. Eversole, C. D. Thatcher, D. J. Blodgett, G. G. Schurig and J. B. Meldrum. 1989. Influence of supplemental selenium on humoral immune responses in weaned beef calves. Am. J. Vet. Res. 50:1760.

Underwood, E. J. 1980. The Mineral Nutrition of Livestock. Second Ed. Commonwealth Agric. Bureaux. London, England.

Voller, A., D. E. Bidwell and A. Bartlett. 1979. Enzyme Linked Immunosorbent Assay (ELISA). Dynatech Laboratories Inc. Alexandria, VA.

Whanger, P. D., I. Ryssen, H. A. Turner and I. J. Tinsley. 1987. Influence of routine management practices at Burns and Union, Oregon, on selenium, copper zinc and cobalt status of cattle. Special Rep. 801. Ag. Exp. Sta. Oregon State University. pp. 9-15.

FIGURE III. MEAN OPTICAL DENSITY FOR STEERS, CU AND SE PHASE (TRIAL II)

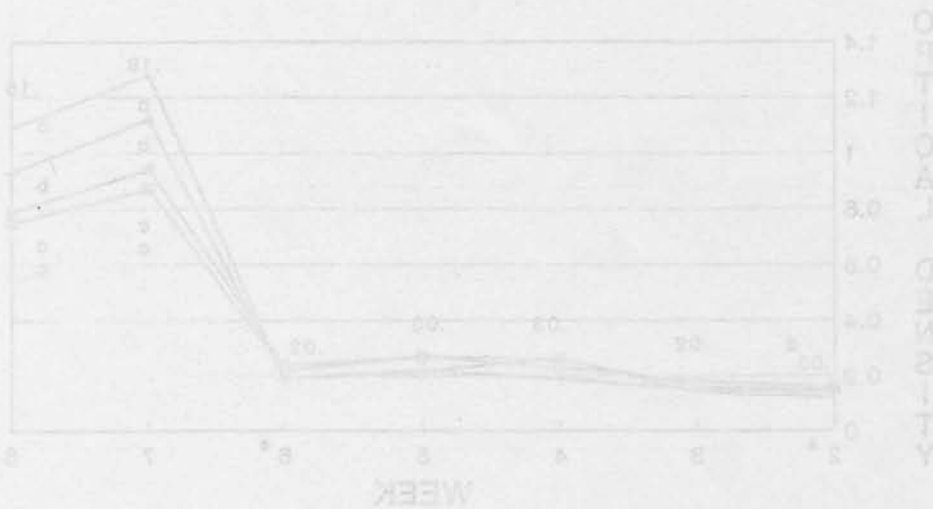


TABLE I. MEAN PLASMA COPPER LEVELS (ppm) OF STEERS

TREATMENT	WEEK							
	1 ^a	2	3	4	5	6	7	8
COPPER	.8	1.1	1.3 ^b	1.1	1.1	1.1	1.2	1.1
SELENIUM	1.0	.9	1.0 ^c	1.0	1.1	1.2	1.3	1.2
CONTROL	.9	.8	.9 ^c	1.0	1.0	1.1	1.1	1.1
COPPER+SELENIUM	.9	1.0	1.2 ^b	1.1	1.1	1.1	1.2	1.1
STD. ERROR	.08	.13	.18	.06	.05	.05	.08	.08

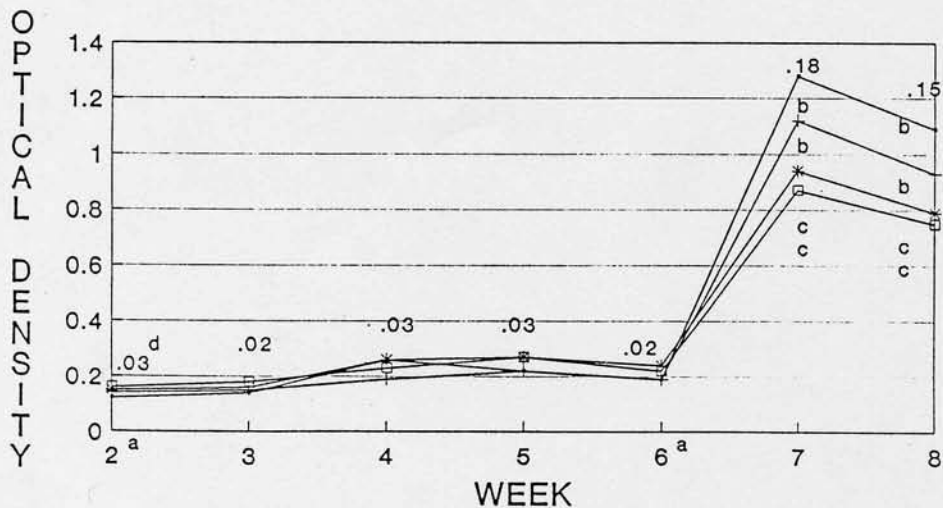
^aWeek of mineral injection.
^{b,c}Means within columns with different superscripts differ (P<.05).

TABLE II. MEAN PLASMA SELENIUM LEVELS (ppm) OF STEERS

TREATMENT	WEEK							
	1 ^a	2	3	4	5	6	7	8
COPPER	.04	.04 ^c	.03 ^c	.03 ^{cb}	.02	.02	.02	.02
SELENIUM	.03	.06 ^b	.05 ^b	.04 ^b	.03	.03	.03	.03
CONTROL	.03	.04 ^c	.03 ^c	.02 ^c	.02	.02	.02	.02
COPPER+SELENIUM	.03	.06 ^b	.04 ^b	.04 ^b	.03	.03	.02	.02
STD. ERROR	.005	.01	.009	.009	.005	.005	.005	.005

^aWeek of mineral injection.
^{b,c}Means within columns with different superscripts differ (P<.05).

FIGURE III. MEAN OPTICAL DENSITY FOR STEERS. CU AND SE PHASE (TRIAL II).



— Se + Cu plus Se * Cu □ Control
^aWeek of vaccination d-std. errors
^{b,c}Means with different letters differ (P<.05)