EFFECTS OF ZINC, COPPER AND SELENIUM SUPPLEMENTATION ON THE HUMORAL IMMUNE SYSTEM OF WEANED STEERS.

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Abstract

The study was conducted to determine the effects of zinc (Zn), copper (Cu) and/or selenium (Se) on the humoral immune system of weaned steers fed diets deficient in these minerals at two Eastern Oregon sites. Steers were bled weekly for eight weeks to measure plasma mineral levels and antibody response to keyhole limpet hemocyanin (KLH). Zinc was studied at one site where 9 steers were randomly assigned to treatments of zinc supplementation(z), 3.25 ml injectable zinc-oxide suspension (100 mg/ml oil) or a control (c). Plasma Zn levels were maintained at higher ($p<.05$) levels in supplemented steers from week 3 to 8. Steers were injected with 0.5 ml KLH on weeks 2 and 5 of the study. Humoral immune response did not differ ($p>0.1$) between Zn and C treatments throughout the study period. Copper and Se were studied at a second site where 20 steers were stratified by 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.[1] Plasma Cu levels were higher ($p<.05$) in treatments 2 and 3 from week 2 through 8. Immune response was not different ($p>.05$) between Cu and other treatments. Selenium plasma levels were higher ($p<.05$) in both Se treatments on weeks 2 through 5. Immune response was greater ($p<.05$) in steers receiving only Se than in Cu only or control steers but was not different from steers receiving both Se and Cu. Zinc, Cu or Se+ Cu supplementation did not increase ($p>.05$) humoral immune response. Increased humoral immune response was seen in weaned steers treated with Se only and challenged with KLH.

(**Key Words:** Selenium, Copper, Zinc, Humoral Immunity.)

Introduction

The need for adequate trace minerals for efficient growth and reproduction of animals has been documented by many researchers (Mayland et al., 1980; Underwood, 1981). The aspect of health and immunity as affected by trace mineral nutrition has been studied for about 50 years, with most work being completed recently. Zinc (Zn), copper (Cu) and selenium (Se) have received much attention in immune response and disease studies.

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[1]Mu-Se and Moly-Cu were donated by Schering-Plough Corp. Union, NJ.

Zinc has been demonstrated to be helpful in treating parakeratosis in cattle (Price and Wood, 1982; Legg and Sears, 1960), and swine (Tucker and Salmon, 1955). Spear (1988) completed a study where zinc methionine in a feed mixture increased antibody titers in cattle challenged with parainfluenza[3] virus (PI-3). Reduced thymus weight and increased leukocyte counts were observed in cattle with Zn deficiency (Bull, 1988). Other immune system factors observed included lymphoid atrophy, reduced numbers of immunoglobulin mu (IgM) and gamma (IgG) plaque forming cells per spleen and reductions in the concentrations of thymic hormones.

Copper is an essential mineral for the formation of hemoglobin in rats (Hart el at al., 1928). Neonatal ataxia in sheep was found to be caused by inadequate formation of the myelin sheath that surrounds the central nervous system and supplementation of Cu reduced the incidence of the disease (Bennetts and Chapman, 1937). Evidence was found that superoxide dismutase activity declined in Cu deficient animals; however, more recent studies by Arthur and Boyne (1985) indicated microbicidal functions of neutrophils were reduced before the reduction in superoxide dismutase occurred.

Selenium toxicity has been a concern in animal production for over 55 years (Franke, 1934). In 1957, Schwarz and Folts determined that Se was essential in protecting against necrotic liver degeneration in the rat. The Se containing compound, Factor 3, was determined to afford the protection from degeneration. Nutritional muscular dystrophy (white muscle disease) has been prevented in sheep supplemented with Se (Muth et al., 1958). Recently, Se has received the attention of many researchers examining immunity. Smith et al. (1984) observed a negative correlation between mean herd Se blood levels and the prevalence of intramammary infections in 32 Pennsylvania dairy herds. The incidence and duration of mastitis infections have been decreased with Se and vitamin E supplementation during the nonlactating period in dairy herds (Smith et al, 1984 and 1988). The humoral immune response to hen egg lysozyme antigens was increased in beef cattle fed 80 or 120 mg/kg Se in trace minearalized salt (Swecker et al, 1989). Sheffy and Schultz (1979) observed a decreased stimulation of IgG forming cells in Se deficient animals. Higher IgG and IgM antibody titers were observed in cattle challenged with infectious bovine rhinotracheitis virus when supplemented with Se (Reffett et al., 1988).

Cattle are more susceptible to infection at times of stress such as weaning. In general this is also a time when animals are vaccinated. If their immune system has the added problem of mineral deficiency, vaccinations may be of reduced value. Therefore, this study was conducted to examine the humoral immune response to a foreign antigen of steer calves with supplementation or deficiency of Zn, Cu and Se.

Materials and Methods

Zinc Phase: The Zn phase of the trial was conducted at Burns, OR, on the Eastern Oregon Agricultural Research Center, where cattle have been determined to be deficient in Zn (Whanger et al., 1987). Nine steer calves were randomly assigned to either a Zn supplemented (3.25 ml. injectable Zn oxide suspension) (4 steers) or a control (3.25 ml injected corn oil) (5 steers) treatment. After weaning steers were fed a diet of corn and native hay deficient in Zn (3.9 ppm). Injections were administered on weeks 1 and 4 of the 8 week trial. Weekly blood samples were collected from all animals by jugular venipuncture in vacutainer tubes containing sodium heparin, centrifuged to obtain plasma and refrigerated until analyzed. Plasma Zn levels were measured by flame atomic absorption spectrophotometry. Baseline Zn plasma levels were obtained by sampling one week prior to weaning. The steers were vaccinated with 0.5 ml of keyhole limpet hemocyanin vaccine (KLH) which contained I mg/ml of actual KLH on weeks 2 and 5 of the trial. Steers were bled weekly by jugular venipuncture in normal clot tube vacutainers, allowed to clot, centrifuged to obtain serum and frozen until analyzed. Analysis was completed using an indirect enzyme-linked immunosorbent assay (ELISA) with KLH adsorbed to the solid phase (Voller et al., 1979) with modifications (Dr. Lynn Woodard, Wyoming State Veterinary Lab). Baseline antibody levels were obtained for a negative control and background correction factor by bleeding steers the week of vaccination with KLH. The ELISA procedure was designed to measure IgG levels which will be at measurable levels within 7 days and reach peak level in 10 to 14 days after initial inoculation (Tizard 1987). Peak IgG levels are reached in 7 days following secondary infections.

Copper and Selenium Phase: The Cu and Se phase of the trial was conducted at the Union Station of the Eastern Oregon Agricultural Research Center where cattle have been determined to be marginal in Cu and deficient in Se (Whanger et al., 1987). Twenty steer calves were stratified by weight and randomly assigned to treatments of 1) Se supplemented (1 ml Mu-Se/90.8 kg body weight), 2) Cu supplemented (2 ml Moly-Cu), 3) SE + Cu supplemented, or 4) normal saline solution. After weaning, steers were fed a diet of native hay with marginal Cu (5.3 ppm) and deficient levels of Se (0.02 ppm). Mineral injections were administered on week 1 of the 8 week trial. Weekly blood samples were collected as stated in the Zn phase. Copper plasma levels were measured using the same procedure as for Zinc. Plasma Se levels were determined by an automated fluorimetric method (Brown and Watkinson, 1977). Baseline Cu and Se levels were determined one week prior to weaning. Humoral immune response was measured using the same procedure as for the Zn phase with the exception of vaccination occurring on weeks 2 and 6 of the trial.

Five steers from the Oregon State University research herd at Corvallis were hyperimmunized with 4 injections of 0.5 ml KLH vaccine at 2-week intervals. Ten days after the final vaccination these steers were bled and the serum 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) readings were done when the positive control serum reached an OD of 1.0 when diluted to a 1:250 titer.

Statistical analysis was conducted on treatment means using contrasts in the General Linear Models procedures of the Statistical Analysis System (SAS, 1982). Comparisons were made between treatments within each sampling period.

Results and Discussion

Zinc Phase: Adequate plasma Zn levels (>0.8 ppm) were maintained in supplemented steers throughout the trial except weeks 4 and 8, while control steers were deficient (<0.8 ppm) from week 3 through 8 of the trial. No differences (p>.1) were observed at any time in the OD readings between treatments. Optical densities are listed in Table 1. There appeared to be a decreased initial and secondary immune response in Zn supplemented steers. This is in contrast to Spear's (1988) finding. The animals were fed zinc methionine and were deficient when initial vaccination occurred in his trial. Neither group of steers in this study were Zn deficient at the time of initial KLH vaccination and therefore, they may have been able to adequately produce an immune response. The initial immune response appears to have an effect on secondary response through the number of IgG producing daughter cells within the body. (Tizard, 1987)

Copper and Selenium Phase: Steers not supplemented with Cu were deficient (<0.6 ppm) only on weeks 2, 3 and 4 of the trial. Copper and Cu + Se supplemented steers had higher (p<.05) plasma Cu levels than control steers throughout the trial except weeks 1, 6 and 7. All steers had deficient (<0.03 ppm) Se plasma levels at the initiation of the trial. Plasma Se was adequate on weeks 2 and 3 in Cu + Se and weeks 2, 3 and 5 in Se supplemented steers. At all other sampling dates supplemented steers were deficient. Control steers remained deficient throughout the trial. Steers supplemented with Se or Cu + Se had higher (p <.05) plasma Se levels than control throughout the trial except weeks 1 and 6.

The initial KLH vaccination was administered on week 2. The mean OD readings of treatment groups are listed in Table 2.Optical density readings were higher (p <.1) for Se than for control or Cu supplemented steers, but not different from Cu + Se steers on week 7. There were no differences (p>.1) in OD readings at any other time throughout the study, however, there were numerical differences. The Se supplemented steers had OD values numerically higher than other treatments at all sampling dates which agrees with Swecker et al (1989) and Reffett et al. (1987). The Cu supplemented steers had OD readings essentially equal to control steers while the Cu + SE steers were intermediate between Cu and Se supplemented steers. There appears to be no benefit in the humoral immune response from Cu supplementation when weaned steers are marginal in Cu nutrition or in Zn supplementation. Selenium supplementation had a positive effect on the secondary immune response. Copper, when used in addition to Se supplementation, appeared to reduce the positive effect of Se in both initial and secondary immune response of weaned calves.

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Table 1. Optical densities of steers vaccinated with KLH - zinc study.

Treatment	Week						
	2	3	4	5	6	7	8
Zn	.05	.05	.20	.15	.66	.65	.52
Control	.05	.17	.10	.09	1.05	.86	.85

Table 2. Optical densities of steer vaccinated with KLH - copper and selenium study.

Treatment	Week						
	2	3	4	5	6	7	8
Cu	N*	N	.01	N	N	.31[b]	.26
Se	N	.06	.07	.08	.02	.71[a]	.46
Cu + Se	N	.02	N	N	N	.48[a]	.54
Control	N	N	N	N	N	.33[b]	.27

[a,b]Means with different superscripts are different ($p<.01$) from others in the same column.

N-Optical density = 0.