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Effect of Injectable Copper and Zinc Supplementation on Weight, Hematological Parameters, and Immune Response in Pre-weaning Beef Calves

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Abstract

Copper (Cu) and zinc (Zn) deficiency may cause poor weight gain, hematological changes, and immune failure in extensive beef cattle breeding systems. Diagnosis of the deficiency is based on plasma Cu and Zn concentrations; however, there are discrepancies regarding data interpretation. Here, plasma Cu and Zn concentrations are discussed as risk markers. We evaluated the effect of parenteral Cu and Zn supplementation on their plasma concentrations, weight gain, hematological parameters, and antibody titers to bovine herpes virus 1 (BoHV-1). Pre-weaning calves ($n = 40$; 99 ± 8 kg bw) from a typical breeding area of Argentina with background Cu and Zn deficiency were used. They were assigned to two homogeneous groups in a completely randomized design. Calves were subcutaneously injected with 0.3 mg/kg Cu and 1 mg/kg Zn (supplemented group), or saline solution (control), every 40 days during 120 days. Plasma Cu and Zn concentrations, hematological parameters, and weight were recorded. On days 40 and 80 of the trial, calves were vaccinated with inactivated BoHV-1. Antibody immune response was measured on days 80 and 120. Data were analyzed with a mixed model for repeated measures over time. Before treatment, plasma Cu was low and Zn was adequate in both groups. After treatment, plasma Cu increased and remained within a normal range, whereas plasma Zn remained constant. Supplemented animals had higher weight gain ($p < 0.01$); higher hematocrit, mean corpuscular volume, and mean corpuscular hemoglobin levels ($p < 0.05$); and higher immune response to BoHV-1 ($p < 0.05$). Our results suggest that Cu and Zn supplementation improved daily weight gain and the immune response of pre-weaning calves.

Keywords Beef cattle · Trace elements · Diagnosis · Immune response

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Introduction

The main beef cattle breeding area in Argentina is located in the Salado River basin (SRB), Province of Buenos Aires. The SRB produces around 2.8 million calves annually [1] and is affected by severe copper (Cu) and moderate zinc (Zn) deficiency [2, 3]. Diagnosis of Cu and Zn status in the herd is based on the analysis of their plasma concentrations. Unfortunately, the interpretation of results is controversial, particularly with regard to the productive response of calves to supplementation with both trace elements [4–7].

Extensive cattle production systems worldwide are affected by Cu deficiency [7], which can be either primary (low Cu content in the diet) or secondary (high levels of dietary antagonists such as molybdenum, sulfur, or iron) [8]. The clinical manifestations of Cu deficiency include hair depigmentation, alterations in connective and bone tissue, cardiovascular

disease, diarrhea, poor body condition, and even death [7]. On the other hand, Zn deficiency is among the most widespread mineral deficiencies because of low Zn concentrations in soil and plants [9]. Clinical signs of Zn deficiency include stiff gait and swelling of the hocks and knees, among others [10]. Although the clinical manifestations of both deficiencies are different, they are preceded by common subclinical consequences, some of which have economic impact [11–13]. In growing animals, Cu or Zn deficiency results in poor weight gain, altered hematological parameters, and immune failure [14–18]. Further, their coexistence could be even worse since both deficiencies share physiological roles [19–21].

Despite thresholds for risk of deficiency based on plasma Cu and Zn concentrations remain controversial [4, 12], supplementation trials have reported poor weight gain as a result of Cu and Zn deficiency [22–24]. Hematological changes associated with Cu and Zn deficiency have also been found, although there is no agreement on the parameters affected [16, 17, 24, 25]. Failures in the immune system have been extensively studied in Cu- and Zn-deficient bovines, showing that innate and acquired immunity, either humoral or cell-mediated, may be affected [14, 26, 27]. In this context, the immune response to bovine herpes virus 1 (BoHV-1) is a valuable indicator to assess immune capacity [28–31].

In this work, we evaluated the effect of parenteral Cu and Zn supplementation on plasma Cu and Zn concentrations, weight gain, hematological parameters, and immune response to BOHV-1 in calves. Plasma Cu and Zn concentrations are discussed here as risk markers.

Material and Methods

All experimental procedures were approved by the Committee for the Care and Use of Laboratory Animals (CICUAL, for its Spanish acronym), Facultad de Ciencias Veterinarias, Universidad Nacional de La Plata, Argentina (Protocol No. 58-2-16P).

The trial was carried out in the SRB area, on the experimental farm "Manantiales" located in Chascomús, Buenos Aires, Argentina (35° 44′ 31.5″ S, 58° 06′ 11.7″ W). There is no previous report of the use of vaccines containing BoHV-1 for any animal category.

Clinically healthy Aberdeen Angus female calves ($n = 40$; 3 months old, 99 ± 8 kg bw) were selected and kept as cow-calf pairs since the start of the trial at 3 months until weaning at 7 months of age. At the beginning of the experiment, no serum antibody titers to BoHV-1 were detected. The immunization program included foot-and-mouth disease vaccine and a multivalent clostridial vaccine. Gastrointestinal parasites were examined through fecal egg counts.

Calves were assigned to one of two homogeneous groups with respect to body weight and age ($n = 20$ each group). The supplemented group (SG) was subcutaneously injected with 0.3 mg/kg Cu edetate and 1 mg/kg Zn edetate (Suplenut® Biogénesis Bagó, Argentina), whereas the control group (CG) received saline sterile solution. Injections were administered every 40 ± 3 days from November 2016 to March 2017 on trial days 0 (3 months of age), 40, 80, and 120 (weaning). On days 40 and 80 of the trial, all calves were subcutaneously vaccinated with inactivated BoHV-1 (Bioqueratogen Air® Biogénesis Bagó, Argentina) according to label directions.

The animals were fed on widely available native and naturalized grass (*Chaetotropis elonga*, *Stenotaphrum secundatum*, *Paspalum dilatatum*, *Lolium perenne*, *Lotus tenuis*) and drank water ad libitum.

On day 0 of the trial, a sample of drinking water was collected from water troughs, which was the only water source of the animals, for quality analysis. Water was suitable for cattle consumption and did not have significant amounts of Cu or Zn. It had the following composition: total salts, 1056 mg/L; sulfates, 215 mg/L; hardness, 566 mg/L; Cu, 0.1 μg/L; Zn, 0.1 μg/L; pH, 7.2. At the same time, grass was collected from three sites in the paddock according to animal behavior intake. Samples were washed, dried, and exposed to acid digestion (3:1 nitric:perchloric acid mixture). The concentrations of Cu, Zn, and iron (Fe) in grass were measured with atomic absorption spectrometry (AAS) in an AAnalyst 200 spectrometer (Perkin Elmer, Buenos Aires, Argentina). Molybdenum (Mo) and sulfur (S) concentrations were measured using graphite furnace AAS and Arsenazo III titration, respectively (adapted from Hamm et al.) [32]. Mean Cu, Zn, Mo, and Fe concentrations in grass samples were 6 ± 0.72, 46 ± 3.60, 0.3 ± 0.05, and 415 ± 59 ppm dry matter (DM), respectively, whereas S concentration was 0.11 ± 0.03% DM.

Blood samples were obtained by jugular venipuncture and collected in Na_2-EDTA tubes previously washed with deionized water. They were kept at 4 °C until processing within 6 h after collection. Blood was centrifuged at 1500 rpm for 10 min and plasma was proportionally deproteinized with 10% trichloroacetic acid. Supernatant Cu and Zn concentrations were measured using AAS. Blood samples ($n = 10$ per group) were collected in K_3-EDTA tubes, sent to a regional laboratory (Laboratorio Azul SA), and processed for hematological parameter assessments. For BoHV-1 antibody titer determination, serum samples ($n = 10$) were obtained on days 40, 80, and 120 and analyzed with the serum neutralization (SN) assay [33, 34]. Titers were reported as $\log^{10}$ transformation of the reciprocal of the highest serum dilution in which there is complete neutralization of the cytopathic effect (CPE) [31].

Individual animal weight was recorded early in the morning ($n = 20$ per group) on days 0, 40, 80, and 120 of the trial.

Study Design and Statistical Analysis

A completely randomized design was used. Data were analyzed using a mixed model for repeated measures through time with SAS statistical software (9.1). Treatment (SG and CG), time (day), and their interaction were fixed variables, and animals were the random variable. The SLICE option of the program was used for mean separation if significant differences were reported for the treatment by time interaction. p values < 0.05 and < 0.1 were considered significant for the main effects and their interaction, respectively. p values < 0.1 and 0.15 were considered as a trend for the main variables and their interaction, respectively. Antibody titers were normalized using log transformation for their statistical analysis.

Results

Plasma Cu concentration in SG increased after Cu and Zn supplementation ($p < 0.01$). Plasma Zn concentration did not vary in both study groups ($p = 0.54$); however, a trend in the time by treatment interaction could be observed due to a decrease in Zn levels in SG on day 40 of the trial (Fig. 1).

Body weight gain showed an increasing trend in SG ($p = 0.08$), together with a significant time by treatment interaction ($p < 0.001$). Differences in weight between SG and CG were detected on days 80 and 120 (Table 1). Similarly, average daily gain (ADG) was different between groups ($p < 0.001$), but no time by treatment interaction was observed ($p = 0.19$) (Table 1).

Blood parameters showed a higher percentage of hematocrit in SG ($p < 0.05$). Mean corpuscular volume and mean corpuscular hemoglobin were also higher ($p < 0.05$). Hemoglobin concentration showed an increasing trend in SG ($p = 0.069$), whereas no differences were found in mean corpuscular hemoglobin concentration ($p = 0.15$), red blood cell count ($p = 0.9$), and leukocyte count ($p = 0.23$) (Table 2).

After vaccination, SN antibody titers against BoHV-1 increased in both groups, but they were even higher in SG on days 80 and 120 ($p < 0.05$) (Fig. 2).

Discussion

Measurement of plasma Cu concentration can help to establish animal Cu status and determine the risk of production losses [6]. In the present study, plasma Cu concentration was clearly different between groups; supplementation increased Cu concentration in SG, reaching the normal range proposed by different authors (> 57 μg/dL and > 70 μg/dL) [12, 35].

There is general agreement that values within the normal range indicate adequate liver Cu stores [7]. Supplementation with Cu at this stage increases hepatic stores, without significantly modifying plasma Cu concentration [36]. This would explain why Cu values remained within normal range (> 60 μg/dL) from day 40 of the present trial (Fig. 1). On the other hand, low plasma Cu concentrations < 30 μg/dL [37], < 25 μg/dL [23], or < 20 μg/dL [35] alert about the risk of possible productive losses. In our study, mean Cu values in CG (19.4 ± 1.39 μg/dL) were in agreement with the proposed risk values. Further, we found response to supplementation in

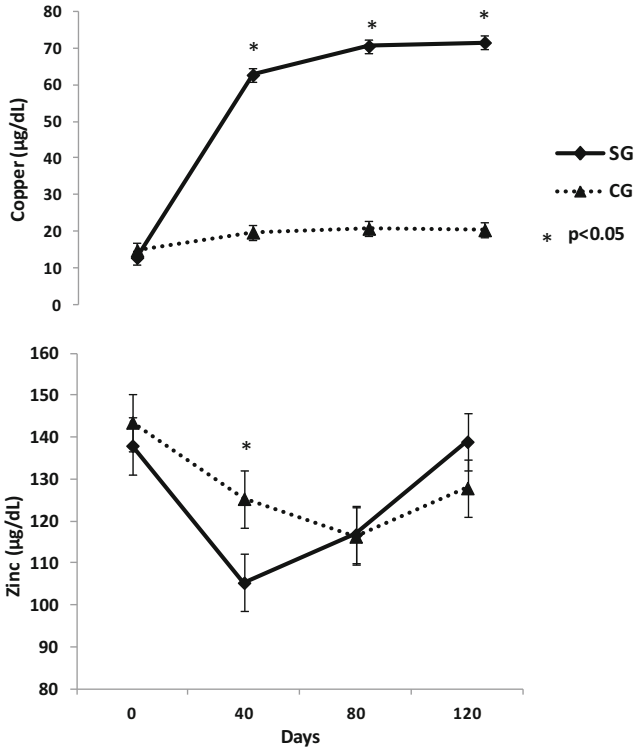

Fig. 1 Plasma Cu and Zn concentrations in pre-weaning calves ($n = 20$ in each group). SG, supplemented group (0.3 mg/kg Cu and 1 mg/kg Zn edetate on days 0, 40, and 80 of the trial); CG, control group (saline sterile solution on days 0, 40, and 80 of the trial). Data are expressed as least square means ± SEM

Table 1 Body weight and average daily gain in pre-weaning calves ($n = 20$ in each group)

	SG	CG	SEM	p value
Body weight (kg)				
Day 0	102.10	100.85	3.1843	0.7818
Day 40	133.20	126.10		0.1177
Day 80	165.55	155.85		0.0333
Day 120	183.90	171.75		0.0080
Average daily gain (g)				
Days 0–40	777.50	631.25	26.328	< 0.0001
Days 40–80	808.75	743.75		< 0.0001
Days 80–120	458.75	397.50		< 0.0001

SG, supplemented group (0.3 mg/kg Cu and 1 mg/kg Zn edetate on days 0, 40, and 80 of the trial); *CG*, control group (saline sterile solution on days 0, 40, and 80 of the trial); *SEM*, standard error of the mean

Table 2 Hematological parameters in pre-weaning calves ($n = 10$ in each group)

	Day	Group		SEM	p value	
		SG	CG		Group	Group × day
RBC count (× 10^6/μL)	0	9.09	9.69	0.4017	0.5492	0.6172
	40	9.97	9.49			
	80	8.81	8.51			
	120	9.58	9.67			
Hb (g%)	0	11.16	11.47	0.2914	0.0694	0.0297
	40	12.88[a]	11.94[b]			
	80	12.18	11.27			
	120	13.2[a]	12.53[b]			
PCV (%)	0	35.63	36.33	0.9064	0.0247	0.0220
	40	39.44[a]	36.11[b]			
	80	37.20[a]	33.78[b]			
	120	40.30	37.90			
MCV (fL)	0	39.25	37.63	0.7458	0.0159	0.3601
	40	39.22	38.22			
	80	42.37[a]	39.84[b]			
	120	42.09[a]	39.28[b]			
MCH (pg)	0	12.28	11.89	0.2214	0.0209	0.3593
	40	12.82	12.67			
	80	13.85	13.28			
	120	13.81[a]	12.98[b]			
MCHC (%)	0	31.35	31.58	0.3102	0.1523	0.8965
	40	32.73	33.08			
	80	32.74	33.37			
	120	32.80	33.06			
WBC count (×10^3/μL)	0	6.84	7.95	0.537	0.2366	0.5266
	40	7.06	7.29			
	80	7.36	8.48			
	120	7.23	7.65			

SG, supplemented group (0.3 mg/kg Cu and 1 mg/kg Zn edetate on days 0, 40, and 80 of the trial); *CG*, control group (saline sterile solution on days 0, 40, and 80 of the trial); *SEM*, standard error of the mean; *RBC*, red blood cell; *Hb*, hemoglobin; *PCV*, hematocrit; *MCV*, mean erythrocyte volume; *MCH*, mean corpuscular hemoglobin; *MCHC*, mean hemoglobin concentration; *WBC*, white blood cell

Different superscript letters in the same row indicate $p < 0.05$

SG, suggesting that supplementation was adequate to keep normal plasma Cu values.

Subcutaneous Cu injection allows the storage of Cu in the liver, which is then used by the animal according to its physiological needs [38]. Although 0.3–0.5 mg/kg Cu is adequate, doses close to 1 mg/kg body weight are toxic [39] due to the high Cu transfer rate from the application site to the liver when using soluble salts like edetate [40, 41]. Some salts are released slowly, thus minimizing death risk, but they can cause local reactions in the application site and are not efficient to generate liver Cu storage [42]. In this case, a higher frequency of supplementation would compensate for this situation. In the present study, parenteral Cu supplementation with edetate every 40 ± 3 days was adequate and sufficient to keep high plasma Cu concentrations and even to avoid the consequences of the deficiency.

The consequences of Zn deficiency are generally connected with plasma Zn concentrations < 80 μg/dL and low dietary Zn supply [12]. In the present study, diet covered Zn recommendations [43] and plasma Zn concentration was above the mentioned risk value. On the other hand, plasma Zn concentration was similar in SG and CG. Furthermore, it decreased in SG even below the values observed in CG on day 40 of the trial (Fig. 1), as already reported in other studies using parenteral Zn supplementation [28, 44, 45]. These results could be probably due to the complex transport system regulating cytoplasmic Zn concentrations [46]. In this sense, different factors may modify Zn transporter function and plasma Zn concentrations, such as inflammatory reactions, stressors, and hormonal concentrations [46–48]. Nevertheless, plasma Zn concentration is not a good indicator to evaluate the effect of subcutaneous Zn supplementation, at least when it is adequate before treatment.

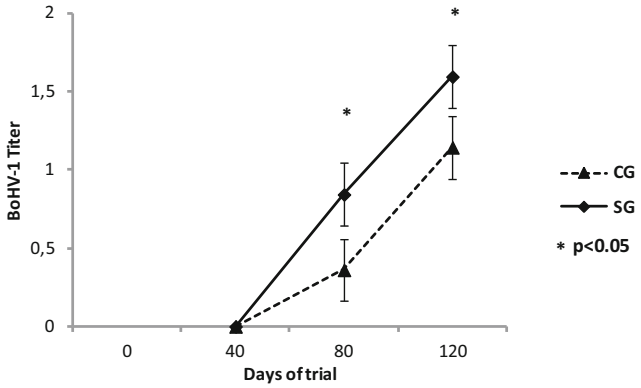

Fig. 2 Titer responses (log-transformed) for inactivated bovine herpes virus 1 (BoHV-1) vaccine in pre-weaning calves ($n = 10$ in each group). SG, supplemented group (0.3 mg/kg Cu and 1 mg/kg Zn edetate on days 0, 40, and 80 of the trial); CG, control group (saline sterile solution on days 0, 40, and 80 of the trial). SG and CG were vaccinated (BoHV-1) on days 40 and 80 of the trials

In our study, initial plasma Cu concentration indicated deficiency: it improved with supplementation and was associated with higher weight in SG on days 80 and 120. Similar results were obtained in CG when plasma Cu concentration remained < 25 μg/dL during 30 days [23]. The reasons for such lower ADG are controversial. A review of 23 dose-response trials suggests that there was response to Cu supplementation in cases of Cu deficiency by excess Mo [49]. However, calves with primary Cu deficiency also showed growth reduction [25]. A meta-analysis of 12 supplementation trials determined that ADG was directly associated with Cu supply in the diet and inversely associated with Mo and S concentrations in the diet [50]. In our study, Mo and S levels in food were not high and therefore did not affect dietary Cu availability [8, 51]. However, dietary Cu intake was below the recommended 10 ppm DM [43] and Fe levels were moderately high (> 250 ppm DM), but sufficient to interfere with intestinal Cu absorption [52].

The hematocrit, mean corpuscular volume, and mean corpuscular hemoglobin were lower in CG, but it is not considered anemia since the values were within normal ranges. Although hematological changes are associated with Cu deficiency in ruminants, there is no agreement on the possible causes of the deficiency and the parameters affected. In heifers and buffalos, Cu deficiency is associated with lower red blood cell count and hemoglobin concentrations [17, 53]. Probably, Fe deficiency is secondary to Cu deficiency, since intestinal Fe absorption and hepatic mobilization depend on Cu-dependent enzymes [54]. However, these changes would be present in cases of chronic deficiency [55]. A possible explanation for early hematological changes could be the reduction of erythrocyte antioxidant capacity [17, 56]. Decreased Cu-Zn SOD activity increases erythrocyte oxidative damage and reduces their half-life [57], which could in turn reduce phagocytic and lytic activity, and even the number of leukocytes [17, 58]. In the present trial, we did not observe differences in leukocyte count between groups.

In calves experimentally infected with BoHV-1, simple or conditioned Cu deficiency decreased the concentration of neutralizing antibodies [59–61]. The increase of BoHV-1 titers has been used as indicator of humoral immune response in bovines [31]. Studies evaluating the response to BoHV-1 immunization and its relationship with injectable mineral supplementation are scarce and have variable results. In this sense, calves supplemented with Cu, Zn, Se, and Mn presented higher BoHV-1 titers [28]. On the other hand, no significant differences were found using modified live vaccines (MLV) [30]. It should be borne in mind that we used inactivated BoHV-1 because MLV are forbidden in Argentina. Since the immune response is more difficult to obtain with inactivated BoHV-1 than with MLV, the use of adjuvants is required to increase such response [62, 63]. Despite neutralizing antibodies play a limited protective role in immunity against BoHV-1 infection [64, 65], they are an efficient tool for the evaluation of humoral immune response in animals [29]. Thus, inactivated BoHV-1 vaccination may be useful to detect lower antibody response in calves with Cu deficiency.

Conclusions

In pre-weaning calves, Cu supplementation resulted in higher weight gain and higher antibody titer to BoHV-1. It also avoided early erythrocyte changes. Plasma Cu concentration was a good indicator of such consequences. Thus, under these study conditions, the preventive effect of parenteral Cu supplementation every 40 ± 3 days was adequate.

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Compliance with Ethical Standards

All experimental procedures were approved by the Committee for the Care and Use of Laboratory Animals (CICUAL, for its Spanish acronym), Facultad de Ciencias Veterinarias, Universidad Nacional de La Plata, Argentina (Protocol No. 58-2-16P)

Conflict of Interest The authors declare that they have no competing interests.

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