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Effects of Copper and Zinc Supplementation on Weight Gain and Hematological Parameters in Pre-weaning Calves

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Abstract

Cow-calf operations may be affected by trace mineral deficiencies, particularly copper (Cu) and zinc (Zn) deficiency, which may decrease the calf daily weight gain and alter hematological parameters. We evaluated the effect of Cu and Zn supplementation on pre-weaning calves ($n = 40$; 92 ± 6 kg initial body weight) from the Salado River basin, Buenos Aires, Argentina. Calves were divided into four groups ($n = 10$ each) and subcutaneously administered 0.3 mg/kg Cu (Cu group), 1 mg/kg Zn (Zn group), Cu and Zn together (Cu + Zn group), and sterile saline solution (control group) every 40 days for 120 days. Plasma Cu and Zn concentrations, hematological parameters, and weight were recorded every 40 days. A completely randomized 2 × 2 factorial treatment design was used and data were analyzed with a mixed model for repeated measures over time. Cu and Zn were detected in plasma after the second sampling. Cu × Zn interaction was significant ($p = 0.09$), being Cu concentration higher in the Cu + Zn than in the Cu group. Differences in weight gain (Zn × time interaction; $p < 0.01$) were observed in the Zn but not in the Cu group ($p > 0.1$). On the other hand, none of the treatments altered any of the hematological parameters assessed ($p > 0.1$). Our results show the risk of lower weight gain due to Zn deficiency in pre-weaning calves raised in the Salado River basin.

Keywords Copper · Zinc · Deficiency · Weight gain · Hematological parameters

Introduction

Trace minerals provide the essential nutrients animals need for physiological functions, such as growth and development, immunity, and reproduction. Consequently, their deficiency can negatively affect animal performance [1, 2]. Copper (Cu) deficiency is the second most frequent mineral deficiency in grazing cattle worldwide, causing considerable production losses in well-characterized areas [3]. On the other hand, zinc (Zn) deficiency is involved in health problems associated with the immune system and reproductive losses, as well as growth and integrity of the skin and hooves; however, the pathogenesis remains poorly understood [3].

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In the province of Buenos Aires, Argentina, beef cattle production represents the main economic activity of the Salado River basin (SRB). This area covers 5.5 million hectares and produces two million calves a year [4]. Animals are raised under an extensive system based on naturalized grass as the main source of nutrients. The economic benefit of the region resides on selling calves weaned at 6–7 months of age.

Different authors have reported Cu and Zn deficiency in the SRB [5–7], together with related effects such as decreased daily weight gain [3, 8, 9] and hematological changes [10]. Although the diagnosis of both deficiencies in the herd is based on the assessment of plasma Cu and Zn concentrations, there are discrepancies regarding data interpretation. In terms of Cu, concentrations > 57 μg/dL are considered adequate and < 57 μg/dL indicate a deficiency [3]. Nevertheless, these authors reported clinical symptoms of hypocuprosis with Cu levels < 30 μg/dL [3]. In contrast, Cu concentrations of 50–70, 20–50, and < 20 μg/dL are considered as marginal, deficient, and clinically evident disease, respectively [11]. Regarding Zn, concentrations > 90 μg/dL are considered to be adequate, 80–90 μg/dL as marginal, and < 80 μg/dL as deficient [2]. Both Cu and Zn deficiencies are associated with

hematological changes such as anemia, leukopenia, and altered tissue enzymes [10, 12].

Here we discuss whether plasma Cu and Zn concentrations are increased after parenteral Cu, Zn, and Cu + Zn supplementation of pre-weaning calves, thereby altering daily weight gain and hematological parameters.

Materials and Methods

All experimental procedures were approved by the Committee for the Care and Use of Laboratory Animals (CICUAL, for its Spanish acronym), School of Veterinary Sciences, La Plata National University, Argentina (Protocol no. 58-2-16P).

The trial was carried out on the experimental farm "Manantiales," located in Chascomús, Buenos Aires (35° 44′ 31.5" S 58° 06′ 11.7" W). The characteristics of the farm are comparable to those in the SRB, including poor drainage, floods, and higher quantity and quality grass production during spring.

Animals

A total of 40 clinically healthy Aberdeen Angus calves were used. They were kept as cow-calf pairs since time 0 of the trial (3 months of age) until weaning (7 months of age; time 120 of the trial). The vaccination program of calves included foot-and-mouth disease vaccine and two doses of clostridial vaccine before weaning. Gastrointestinal parasites were monthly examined through fecal egg counts.

The animals were fed on native and naturalized grass (*Chaetotropis elonga*, *Stenotaphrum secundatum*, *Paspalum dilatatum*, *Lolium perenne*, *Lotus tenuis*) with wide grass availability and ad libitum water consumption.

Groups and Treatments

Calves were assigned to one of four homogeneous groups according to weight, sex, and age ($n = 10$ each group), and treated as follows: Cu group, 0.3 mg/kg Cu edetate; Zn group, 1 mg/kg Zn edetate; Cu + Zn group, the same doses of Cu and Zn edetate (Suplenut®, Biogénesis Bagó); and control group, saline sterile solution. The animals were subcutaneously injected every 40 days from November 2015 to March 2016 on days 0, 40, 80, and 120 days.

Blood samples were obtained via jugular venipuncture and collected in Na_2EDTA-containing 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. Copper and Zn concentrations were measured in supernatants using atomic absorption spectrophotometry (AAS) (Perkin Elmer AAnalyst 200). Blood samples ($n = 5$ per group) were collected in tubes containing EDTA-K_3 and sent to a regional laboratory (Laboratorio Azul SA) to be processed for evaluation of hematological parameters (red cell count, hemoglobin concentration, hematocrit percentage, hematimetric indices, white blood cell count, absolute and relative leukocyte formula, and platelets count).

At the same time, grass was collected from three sites in the paddock according to animal behavior and forage intake. Samples were washed, dried, and exposed to acid digestion (3:1 nitric-perchloric acid mixture). The concentrations of Cu, Zn, and iron (Fe) were measured with AAS, whereas molybdenum (Mo) and sulfur (S) concentrations were measured using graphite furnace AAS and Arsenazo III titration (adapted from Hamm et al. [13]), respectively. In grass samples, Zn, Cu, Mo, and Fe concentrations were measured.

The quality of bovine drinking water was analyzed in a sample initially collected from water troughs, the only water source for the animals.

Individual animal weight was recorded early in the morning after the animals had fasted for 12 h.

Statistical Design and Analysis

We used a completely randomized statistical design. Data were analyzed using a mixed model of repeated measures over time and a 2 × 2 factorial arrangement using SAS statistical software 9.1. The main factor was parenteral supplementation with or without Zn or Cu. Supplementation with Zn and/or Cu, all their possible interactions and time were taken as fixed variables, whereas the animals represented the random variable. The SLICE option was used for mean separation if significant differences were reported for the main variables ($p < 0.05$), interactions ($p < 0.1$), or tendencies ($p < 0.15$). When only the treatment was significant, mean separation was done by a protected Fisher's test using the PDIFF-SAS option. Associations between plasma Cu and Zn concentrations and weight gain were assessed with correlation analysis using the same statistical software.

Results

In the Cu group, plasma Cu concentration increased after the second sampling (Cu × time interaction, $p < 0.01$; Table 1). We also found Cu × Zn interaction ($p = 0.09$; Table 1), being plasma Cu concentration higher in the Cu + Zn than in the Cu group (76.2 vs. 73.4 ± 2.1 μg/dL). In turn, plasma Cu concentration was lower in the Zn than in the control group (46.2 vs. 50.7 ± 2.1 μg/dL). In the case of plasma Zn concentration, it

Table 1 Copper (Cu) and zinc (Zn) concentration least square means and live weight of pre-weaning calves in the four study groups

Day	Groups				SEM	*p* value[1]				
	Control	Cu	Zn	Cu + Zn		Zn	Cu	Zn × Cu	Zn × T	Cu × T
Cu concentration (μg/dL)										
0	46	42	44	45	1.5	0.67	< 0.01	0.09	0.96	< 0.01
40	49	88	45	89						
80	53	87	49	89						
120	55	76	47	81						
Zn concentration (μg/dL)										
0	84	80	85	81	2.3	0.02	0.12	0.77	0.48	0.98
40	112	101	115	117						
80	108	102	115	109						
120	92	91	109	100						
Weight (kg)										
0	92	92	93	92	1.0	0.02	0.73	0.43	< 0.01	0.48
40	124[a]	124[a]	127[b]	126[b]						
80	145[a]	146[a]	148[b]	149[b]						
120	172[a]	173[a]	181[b]	177[b]						

SEM standard error of the mean, *T* time

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

[1] There was no Cu × Zn × time interaction for any of the variables

increased after Zn supplementation either alone or together with Cu ($p = 0.02$; Table 1).

In terms of weight, time differences were observed in the Zn-treated group, finding higher weight gain after the second sampling ($p < 0.02$; Table 1). Both Cu and Zn concentration correlated with weight (Cu: $r = 0.04$, $p = 0.53$; Zn: $r = 0.25$, $p < 0.01$).

Regarding hematological parameters (erythrocytes, leukocytes, and enzymes), no differences were detected in any of the four study groups (Table 2).

In grass samples, Zn, Cu, Mo, and Fe concentrations were 19 ± 5, 7.3 ± 1.2, 0.7 ± 0.4, 329 ± 80 ppm dry matter (DM), respectively, and S concentration was $0.13 \pm 0.06\%$ DM. No significant Cu and Zn contents were detected in drinking water.

Discussion

Cu deficiency may result in reduced daily weight gains in calves [3], particularly in cases of severe deficiency [11]. In the present study, marginal Cu deficiency did not affect either daily weight gain or hematological parameters. Nevertheless, our data correlated with previous results of Cu supplementation at the SRB reporting differences in weight gain in calves with Cu levels lower than 25 μg/dL [14], which are related to severe deficiency [15]. Likewise, decreased hemoglobin concentrations and low white cell counts were found in heifers only with Cu concentrations lower than 19 μg/dL [12]. The marginal Cu status reported in the present trial would be due to the Cu concentration in grass (7.3 ppm DM), which was lower than the required 10 ppm DM [16]. Moreover, other grass-related factors that may lead to Cu deficiency, such as Mo, Fe, and S, showed a moderate concentration, suggesting that they did not interfere with Cu absorption [17].

An interesting finding related to Cu behavior was the Cu × Zn interaction ($p = 0.09$) in the Cu + Zn group, since supplementation produced higher Cu concentrations as compared with the Cu group. Probably, Zn could have promoted metallothionein synthesis in the liver with the combined supplementation [18]. This protein enhances Cu capture and acts as a liver Cu storage through which ceruloplasmin is produced to constitute the main determinant of plasma Cu concentration [1, 19, 20]. Furthermore, Cu concentrations in the Zn group were lower than in the control group. Supplementation with Zn could have increased Cu requirements since some mechanisms depend on both elements. For example, Cu-Zn superoxide dismutase (Cu-Zn SOD) is an enzyme whose action is related to Zn concentration and also requires Cu for an adequate activity [21].

The study groups supplemented with Zn either alone or combined with Cu presented higher weight regardless of Cu supplementation, probably due to the altered intake and/or feed conversion produced by Zn deficiency in bovines [2, 8]. Studies on experimental animals indicate that Zn deficiency leads to lower water intake [22], altered thyroid function [23], IGF-1 signaling failure [24], and

Table 2 Least square means for hematological and serological parameters in pre-weaning calves from the four study groups

Day	Groups*			
	Control	Cu	Zn	Cu + Zn
Red cell/mm^3				
0	6442	6566	6438	6540
40	6538	6444	6540	6652
80	7618	7286	6988	7482
120	6978	6746	6880	6752
Hemoglobin (g/dL)				
0	13.08	13.32	13.00	13.22
40	13.18	13.26	13.14	13.68
80	13.90	13.36	13.46	14.08
120	13.18	13.46	13.66	13.28
Hematocrit (%)				
0	40	43	41	44
40	41	41	41	41
80	45	43	42	44
120	42	42	44	42
Leukocytes/mm^3				
0	6680	4645	5437	6108
40	5000	5820	5100	6200
80	5720	6320	7260	5920
120	5780	6040	5840	5780
ALP (U/L)				
0	159	165	154	146
40	165	165	159	200
80	178	180	190	174
120	201	172	205	172
AST (U/L)				
0	93	94	113	94
40	92	104	89	101
80	117	107	117	102
120	98	109	111	102
GGT (U/L)				
0	26	31	31	34
40	34	34	29	30
80	35	33	29	29
120	29	31	30	33

SEM standard error of the mean, *ALP* serum alkaline phosphatase (units per liter), *AST* aspartate aminotransferase (units per liter), *GGT* gamma glutamyl transpeptidase (units per liter)

* There was no Cu × Zn × time interaction for any of the variables

anorexia secondary to the suppression of hypothalamic neuropeptide Y [25].

The National Research Council recommends 30 ppm DM of Zn to reach the requirements [16] and suggests that lower average weight gain could occur with Zn dietary doses of 20 ppm DM [11, 26]. These data are in agreement with the 19 ppm DM Zn found in the present trial; Zn concentration was higher in the Zn groups and correlated with weight gain (r: 0.25; $p < 0.01$). Although it is agreed that Zn concentration should be taken as an indicator of Zn status in animals, most of the trials reporting differences in terms of weight gain in groups supplemented with or without Zn showed similar Zn concentrations [27, 28]. The time (weeks-months) required for the development of the deficiency as an indicator of Zn status might improve Zn concentration. In a previous trial, it was reported that 6 weeks were needed to distinguish the Zn-supplemented (40 ppm DM) from the control group (17 ppm) [29]. Other studies obtained similar results in 3 weeks, i.e., low daily weight gain but no differences in plasma Zn concentration [8]. In this trial, Zn supplementation every 40 days during 4 months was associated with higher weight gain. Further research showing the importance of herd risk diagnosis based on plasma Zn concentration could contribute to preventing Zn deficiency in calves.

Conclusions

Zinc parenteral supplementation every 40 days improved the daily weight gain of calves, indicating the risk for Zn deficiency in the SRB area. Marginal Cu concentration did not induce lower weight gain, and marginal Cu + Zn concentration did not alter hematological parameters.

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Compliance with Ethical Standards

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

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