

## Effect of Copper and Zinc Supplementation on Growth, Blood Serum Copper and Zinc Levels, Scrotal Circumference and Semen Quality in Growing Male Boer × Nubian Bucks

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**Abstract:** The aim of the present study was to evaluate the effect of Cu and Zn supplementation on growth, blood serum Cu and Zn levels, scrotal circumference and semen quality in growing male Boer × Nubian bucks. Twenty eight male goats were fed twice daily at 07:00 and 19:00 h with a total mixed ration (TMR) containing 10.3 and 22.5 mg/kg DM of Cu and Zn, respectively. TMR was supplemented with four treatments being: control, Cu (21.7), Zn (5.6), and Cu-Zn (21.7 and 5.6, respectively) which was provided as a mineral premix. Live weight and Cu and Zn levels were analyzed under a completely randomized design with repeated measures. Scrotal circumference and semen quality were analyzed by analysis of variance using the general linear model. The comparison of differences between treatments was done using Tukey test. There were no differences in live weight, scrotal circumference, ejaculate volume and sperm concentration. Blood serum concentration of Cu increased ( $p < 0.0001$ ) with Cu treatment. Blood serum concentration of Zn increased ( $p < 0.05$ ) with Zn supplementation. The motility and viability of sperm in the ejaculate were affected by Cu and Zn supplementation treatments. Based on the above, in bucks fed with a TMR supplemented with Cu and Zn increase blood serum Cu and Zn levels and improved the percentage of sperm motility and viability from the ejaculate.

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### 1. Introduction

Metabolic and reproductive functions in goats are regulated by extrinsic factors, such as, seasonality, social and sexual interactions and nutritional status (Vázquez-Armijo *et al.*, 2011a). Since reproductive functions are highly demanding of nutrients, in quality and quantity, the nutritional status is an important modulator of reproduction in goats (Blache *et al.*, 2008). Trace mineral elements as copper (Cu) and zinc (Zn) are involved in many enzyme systems and may affect metabolic utilization of major dietary nutrients (Ramírez, 2007).

The need for Zn by most animals is based on its influence on enzymes and proteins and their activities; thus, the presence of Zn at cellular level is essential, for instance, in the gonads, where cell growth and division, occurs continuously (MacDonald, 2000). Zn deficiency could affect the spermatogenic process, as well, as primary and secondary sex organs development (Smith and Akinbamijo, 2000).

The information in mineral nutrition is limited and mineral-related nutritional imbalances could be frequent. Therefore, the reports on mineral supplementation of goats are scarce, and ignore changes in mineral status during different stages of growth, and their effects on sperm production.

In the present study, we investigate the effect of copper and zinc supplementation on growth, blood serum and seminal plasma Cu and Zn levels, scrotal circumference and semen quality in growing male Boer × Nubian bucks.

### 2. Material and Methods

The experiment was carried out at the experimental farm and laboratories of animal nutrition and reproduction of the Centro Universitario UAEM Temascaltepec, Universidad Autónoma del Estado de México at north latitude 19°02'04" and west longitude 100°02'14", 1300 m asl. Twenty eight Boer × Nubian male growing bucks of 6 to 7 months of age and 23.16 ± 2.16 kg body weight were

used in a completely randomized design. Bucks were housed individually in pens of 1.24 m × 0.82 m. After 2 weeks of adaptation of consuming a total mixed ration (TMR) composed of [g/kg dry matter (DM) basis]: oat hay, 46.06; alfalfa hay, 11.97; *Heliocarpus velutinus* leaves, 2.99; *Guazuma ulmifolia* leaves, 6.98; corn grain, 9.21; sorghum grain, 9.21; soybean meal, 5.03; molasses cane, 5.03; urea 46% N, 1.00; mineral premix, 2.52 (containing per kg of mineral premix: 19.60 g/kg calcium, 22.10 g/kg sulfur, 4 mg/kg cobalt, 15.93 mg/kg iodine, 15.49 mg/kg selenium). The TMR had the following nutritional composition [g/kg DM]: crude protein (CP), 147.4; neutral detergent fiber (NDF) 482.1; acid detergent fiber, 264.2; hemicellulose, 217.9; ash, 104.3; organic matter, 895.7; Cu, 10.30 mg/kg; Zn, 22.58 mg/kg. The TMR was the same as the one fed to bucks of the experiment previously done at the same farm by Vázquez-Armijo *et al.* (2011b). Each day, bucks were fed 1.1 kg/head of the diet, except for Cu and Zn, which were added in different concentrations from one of four mineral premixes formulated to meet the requirements for Cu (35 mg/d) and Zn (31 mg/d) (NRC, 2007). The concentration of trace mineral elements in the treatment premixes was: Cu-Zn treatment, 860.73 mg Cu/kg, 224.07 mg Zn/kg; Cu treatment, 860.73 mg Cu/kg, 0 mg Zn/kg; Zn treatment, 0 mg Cu/kg, 224.07 mg Zn/kg; and the control, 0 mg Cu/kg, 0 mg Zn/kg.

Animals body weight was recorded biweekly from start to end of the experiment. Blood samples were collected by duplicate on days 0, 1, 2, 8, 15, 30, 45, 60, 75 and 90 of the experiment, and were obtained by a jugular puncture. Blood samples were processed by centrifugation at 2,500 revolutions per minute for 10 min at 4 °C, and the serum was stored at -20 °C. The frozen serum was thawed, deproteinized with trichloroacetic acid (TCA) and stored at 4 °C for further analysis (Fick *et al.*, 1979). Seminal plasma was obtained and processed by the same way that blood serum was done, after quality valuation. Concentrations of Cu and Zn were measured by atomic absorption spectrophotometry at a wavelength of 324.8 nanometers for Cu and 213.9 nanometers for Zn (Fick *et al.*, 1979). The scrotal circumference (SC) was measured at the beginning and the end of the experiment. All bucks were trained for semen collection by an artificial vagina. At the end of experimental period one ejaculate from each buck was evaluate. The volume of semen was measured with graduated tubes. The sperm concentration was determined using a haemocytometer. Sperm motility assay was carried out as described by Pichardo *et al.* (2010); with a clear field microscope at ×100. Viability test was performed with trypan blue dye at ×200.

The live weight changes and Cu and Zn levels were analyzed under a completely randomized design with the PROC MIXED procedure (SAS, 2009). The SC and quality semen parameters were analyzed as a completely randomized design. In the case of significance ( $p < 0.05$ ) among treatments, Tukey's test was used to separate means (Steel and Torrie, 1989).

### 3. Results

No significant differences were observed for live weight (Figure 1).

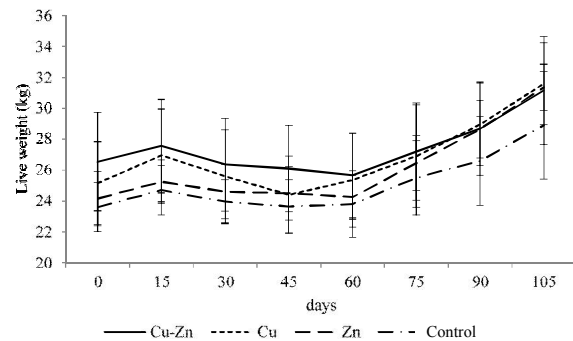


Figure 1. Live weight (mean ± SD) in growing bucks supplemented with Cu and Zn

The serum concentration of Cu was higher ( $p < 0.001$ ) (Figure 2) in bucks with Cu treatment since day 15; however, serum concentration of Cu in all bucks found was above the concentration that is considered deficient (< 0.6 µg/ml; Mills, 1987).

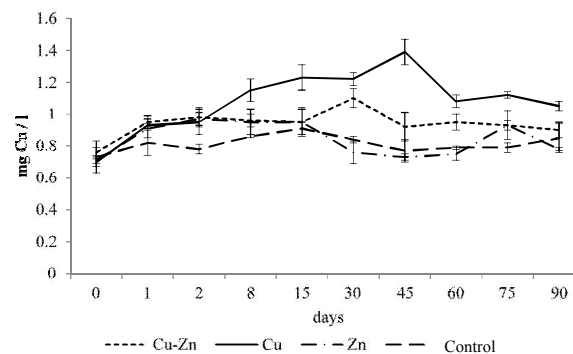


Figure 2. Effect of Cu and Zn supplementation on blood serum concentration of Cu (mean ± SD) in growing bucks

The effect of Cu and Zn supplementation on blood serum Zn levels is shown in Figure 3. No differences was observed between treatments at the beginning of the experimental period, however at 45, 60, 75 and 90 d of supplementation blood serum Zn concentration began to increase ( $p < 0.05$ ) for Zn treatment.

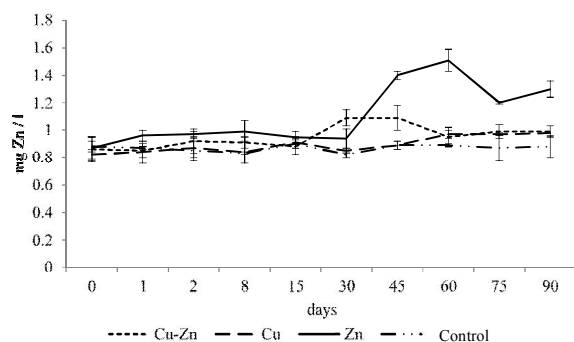


Figure 3. Effect of Cu and Zn supplementation on blood serum concentration of Zn (mean  $\pm$  SD) in growing bucks

No differences between treatments for scrotal circumference, volume of ejaculate and sperm concentration was observed. Sperm motility and viability from ejaculate were affected ( $p < 0.001$ ) by supplementation treatments (Table 1).

Table 1. Initial and final scrotal circumference, volume of ejaculate, sperm concentration, motility and viability of ejaculates from bucks supplemented with Cu and Zn

Parameter	Treatment				SEM
	Cu-Zn	Cu	Zn	Control	
Initial scrotal circumference (cm)	23.30	24.88	23.13	22.75	0.97
Final scrotal circumference (cm)	24.94	25.75	25.63	24.28	0.54
Volume of ejaculate (ml)	1.3	1.4	1.2	1.2	0.40
Sperm concentration ( $10^6/ml$ )	1732	1510	1885	1782	335.3
Motility (%)	43.18 <sup>a</sup>	46.24 <sup>a</sup>	47.54 <sup>a</sup>	40.15 <sup>b</sup>	3.12
Viability (%)	57.93 <sup>a</sup>	56.71 <sup>a</sup>	55.41 <sup>a</sup>	51.25 <sup>b</sup>	2.72

Different superscripts in the same row indicates difference ( $p < 0.05$ ).

#### 4. Discussions

In the present work live weight was unaffected by Cu and Zn supplementation, that are consistent with other studies (Jia *et al.*, 2008; Zhang *et al.*, 2008). Supplementation with 0, 100 and 200 mg Cu/d did not show differences in live weight from growing goats (Solaiman *et al.*, 2006). In addition, Neathery *et al.* (1973) had no differences in live weight changes of male goats consuming for six months a control diet and a Zn deficient diet. The reasons of this differences are still unknown; however, factors that could potentially affect this response are the mineral concentration of the basal diet, supplementation source (organic or inorganic), the absence or presence of antagonists, environmental factors, and healthy.

Solaiman *et al.* (2006) observed that Cu concentration in blood serum from goat kids supplemented with Cu (100 or 200 mg/d) remained within normal physiological ranges, besides that there was no differences in the control group (14.5 mg Cu/kg DM). In addition, Solaiman *et al.* (2001) reported no difference in plasma Cu concentration of Nubian goats supplemented with high doses of Cu (50-600 mg/d) for an experimental period of 31 weeks. It is worth mentioning that even though in this work variations are observed in concentration of Cu, these variations do not exceed those considered normal, which may be due to Cu supplementation should not necessarily increase the serum concentration of serum concentration in blood, due to the mechanisms of homeostasis (Ward *et al.*, 1993). In this work, the Cu serum concentration was lower in treatments containing Zn, since the major Cu antagonist is Zn (Jia *et al.*, 2009). In addition, Cu content in basal diet could be considered low (10.30 mg Cu/kg DM), which could increase the antagonistic effect of Zn (Jia *et al.*, 2009).

The mechanisms by which the mineral supplementation affects the blood mineral levels are not fully understood; however, based on the results, Zn supplementation affects blood serum Cu concentration, which can be due to the antagonistic effect between both microelements. The basal serum Zn level is found in range from 0.9 to 1.5 mg/l (Underwood and Suttle, 2003). The results obtained in the present study suggests that bucks with Cu and control treatment showed marginal deficiency of Zn during the first 30 d of the experimental period. Similar results were obtained by Jia *et al.* (2008), who found levels up to 0.84 mg/l in goats fed with a basal diet containing 22.3 mg Zn/kg DM. However, blood serum Zn levels is not increased with Zn supplementation by the mechanisms of homeostasis (Jia *et al.*, 2008).

Some studies indicated that the effects by Zn deficiency on testicular growth are irreversible; however, other studies mention that these effects may be reversible, and another works indicate that these effects depend on the length of the deficiency and the age at which was occur (Neathery *et al.*, 1973). The results obtained on scrotal circumference shown that even though at the beginning of the experimental period all bucks had marginal deficiency in blood serum Zn levels, Cu and Zn supplementation did not affect this variable, which could be explained by the age of bucks used in this experiment (shortly after weaning), suggesting that could be there a protein or energy deficit in the development of the reproductive characteristics (Almeida *et al.*, 2007).

Cu and Zn supplementation in bucks showed a significant increase in motility from ejaculate,

compared with the control treatment. These results are agree with those reported in supplemented bulls (Kumar *et al.*, 2006). The major motility could be due to that the primary energy donor necessary for the movement of sperm flagella is ATP, and the Zn controlled the sperm motility by the control of the use of energy through the ATP system, by means of regulation of the energy reserves of phospholipids and improved the oxygen consumption of the sperm (El-Masry *et al.*, 1994). Another explication to increasing sperm motility in bucks supplemented could be due to increased activity of the enzymes containing Zn, such as lactate dehydrogenase and sorbitol dehydrogenase, which play an important role in sperm motility (Nagamine *et al.*, 1990). Furthermore, Zn is a disposer of reactive oxygen species and protects the sperm from oxidative damage and lipid peroxidation by inhibiting of phospholipase (Eggert Kruss *et al.*, 2002). Therefore, the antioxidant action of Zn may be primarily responsible for the increase in sperm motility in bucks supplemented.

In the present study, we observed that Cu and Zn supplementation resulted in a significant increase in the percentage of viable sperm. Our results are agree with some works on rabbits (El-Masry *et al.*, 1994) and bulls supplemented (Kumar *et al.*, 2006), who reported an increase in the percentage of live sperm in animals with Zn supplementation. The increase in sperm viability may be due to the stabilization of the membrane by the action of Zn, by virtue of which, it prevents leakage of enzymes, proteins and other vital components of sperm, so what the functional life of sperm extends. Moreover, Zn also stabilizes ribosomes, lysosomes, DNA and RNA, which helps in the survival and normal functioning of sperm (Bettger and O'Dell, 1981). Bettger and O'Dell (1981) also reported that Zn protects the sperm from the damage induced by free radicals by "sweeping" excessive free radicals and thus improve sperm viability.

McCall *et al.* (2000) reported that Zn, as a constituent of numerous metalloenzymes, too is involved in enzymatic reactions related to carbohydrates, proteins, lipids and nucleic acid metabolism, which can helps to an increase in viability of sperm. Moreover, Zn has been reported as one of the primary factors behind for the production of an antibacterial substance released by the prostate in the ejaculate (McDonald, 2003) which may further help increase in the percentage of live sperm (Kumar *et al.*, 2006).

## 5. Conclusion

In general, reproductive characteristics studied in supplemented animals in this study can be

considered low when compared with normal values reported in Boer bucks (Greyling and Grobbelaar, 1983). However, the results were acceptable for animals at the age in which they were used for this study, which was 6 to 7 months old, in which are not fully developed (Almeida *et al.*, 2007). Based on the above, in bucks fed with a TMR supplemented with Cu and Zn, increase blood serum Cu and Zn levels and improvement the percentage of sperm motility and viability from the ejaculate.

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