



## Short Communication

### Supplementation of diets for Santa Ines sheep with organic and inorganic zinc sources

Flávio Garcia Vilela<sup>1</sup>, Marcus Antonio Zanetti<sup>2</sup>, Arlindo Saran Netto<sup>2</sup>, José Esler de Freitas Júnior<sup>3</sup>, Francisco Palma Rennó<sup>1</sup>, Beatriz Conte Venturelli<sup>1</sup>, Taissa de Souza Canaes<sup>1</sup>

<sup>1</sup> Departamento de Nutrição e Produção da Faculdade de Medicina Veterinária e Zootecnia – USP, Av. Duque de Caxias Norte, Nº 225, Bairro Jardim Elite, Pirassununga – SP, CEP: 13635-900.

<sup>2</sup> Departamento de Zootecnia da Faculdade de Engenharia de Alimentos e Zootecnia - USP.

<sup>3</sup> Departamento de Zootecnia da Universidade Estadual Paulista Julio de Mesquita - UNESP.

**ABSTRACT** - This research was conducted with objective to evaluate the effect of different zinc (Zn) sources and doses in the diet for Santa Ines sheep. Forty lambs at weaning, with 18.4 kg of body weight were supplemented with three different sources of zinc (zinc oxide (ZnO), zinc amino acid and zinc proteinate) and three doses of zinc (200, 400 and 600 mg/kg DM) added to the basal diet. At every 28 days, animals were weighted and blood samples were collected for analyses of zinc (Zn), alkaline phosphatase and immunoglobulin G (IgG) and M (IgM). At the end of experiment, liver samples were collected for determination of the hepatic zinc levels. Zinc was analyzed with atomic absorption spectrophotometer, while phosphatase alkaline and immunoglobulins G and M were analyzed using Laborlab and Bioclin kits, respectively. There was no effect of diets on phosphatase alkaline levels and hepatic zinc, but there was difference in the plasmatic zinc levels and IgG and IgM levels. Based on the accumulation of hepatic zinc, the estimate of the zinc bioavailability, through the regression equation, showed that supplementation with organic and inorganic sources of zinc did not differ in the diet of Santa Ines sheep.

Key Words: bioavailability, immunoglobulin, lambs, minerals

## Introduction

Minerals are present at variable amounts in feeds commonly offered to ruminants (roughage and concentrates). However, the concentration of one or more minerals in feeds or in the diet often does not meet the nutritional requirements of animals for a specific productive purpose, which makes supplementation of the diet with sources of minerals necessary. Among the most common forms of supplementation is the ionic (chlorides, sulfates, oxides, hydroxides, carbonates). However, some of these sources of minerals, especially trace elements, have low absorption in the animal organism or low bioavailability (Spears, 1996).

The effect of zinc on markers of immunity has been studied for several decades (Pedersen & Toft, 2000; Cordova & Alvares-Mon, 1995). Zinc is an essential trace element for normal development and function of immune cells such as neutrophils and natural killer cells, for the functions of T lymphocytes and cytokine production (Shankar & Prasad, 1998). Zinc deficiency can be demonstrated by decrease in the production of immunoglobulins (G and M).

The absorption of zinc occurs mainly in the small intestine, especially in the duodenum, where this absorption

is more pronounced. Deficient animals have a greater capacity to absorb zinc than those with normal levels of this element in their organism (Mertz, 1988). In ruminants, the liver has higher zinc levels than bone. Therefore, the tissue should be preferred for accumulation studies, according to Fick et al. (1979).

To carry out studies of bioavailability with levels of Zn in the diets close to the recommended, 30 mg/kg DM (NRC, 2007), a purified basal diet without the presence of zinc or with very low levels is necessary. Due to the difficulty to formulate a purified diet, taking into account that the feeds used to sheep have zinc, in this experiment, diets with high levels of zinc were utilized.

The objective of this research was to evaluate the effect of the supplementation with different sources and levels of zinc for lambs at weaning on the bioavailability of organic Zn in comparison with inorganic Zn.

## Material and Methods

This research was conducted at the Faculty of Animal Science and Food Engineering, Universidade de São Paulo, Pirassununga, São Paulo, Brazil during 114 days (between

November 17, 2008 and March 11, 2009); the first 30 days were named depletion period, in which the animals received only the basal diet without additional supplementation of zinc. A total of 40 Santa Inês lambs at weaning at four months of age and 18.4 kg on average were used.

Animals were supplemented with three different sources of zinc (zinc oxide (ZnO), zinc amino acid and zinc proteinate) and three doses of zinc (200, 400 and 600 mg/kg DM) added to the basal diet. After, animals were randomly assigned the following treatments: basal diet without supplementation of zinc (Zn); basal diet + 200, 400, and 600 mg Zn/kg DM in the form of ZnO; basal diet + 200, 400 and 600 mg Zn/kg DM in the form of Zn amino acid; basal diet + 200, 400 or 600 mg Zn/kg DM in the form of Zn proteinate. Ammerman et al. (1995) recommend diets with high levels of this mineral with the purpose of obtaining greater differences in the parameters studied.

Animals were kept individually in cages constructed of plastic material to avoid possible contamination with minerals, and each cage had a trough for feed supplementation and a reservoir to supply water *ad libitum*. To facilitate the mixture and hamper the selection by the animal, complete diets with fiber incorporated by the cottonseed hulls were used (Table 1). In the period of depletion and at the beginning of the experiment (0 to 28 days), 800 g of dry matter intake of feed were supplied per animal; 900 g halfway through the experiment (28 to 56 days); and 1 kg at the end of the experimental period (56 to 84 days). The quantities of feed provided were calculated so as to meet the nutritional requirements of growing lambs in early maturation for a body weight gain of 200 g/d (NRC, 2007) and so that there were no orsts, thus ensuring the accurate intake of different sources and levels of dietary zinc. The only variation in the diet provided to the animals was the addition of different sources and doses of zinc to the basal diet.

Table 1 - Composition of basal diet

Ingredients <sup>3</sup>	g/kg DM	Zn <sup>2</sup>
Ground corn	553.0	27.0
Wheat meal	10.0	85.0
Cottonseed hulls	250.0	17.0
Soybean meal	125.0	58.0
Soybean oil	10.0	-
Limestone	12.0	-
Dicalcium phosphate	20.0	-
Urea	10.0	-
Mineral mixture <sup>1</sup>	10.0	-
Total	-	27.28

<sup>1</sup> Composition per kg of mineral mixture: Na - 145.00 g; Mn - 1200.00 mg; Co - 35.00 mg; Fe - 1600.00 mg; Cu - 500.00 mg; S - 15.00 g; I - 75.00 mg; Cr - 15.00 mg; Ca - 110.00 g; P - 80.00 g; Se - 12.00 mg; Mo - 250 mg.

<sup>2</sup> mg/kg DM.

<sup>3</sup> Bromatological composition, in percentage of DM: dry matter - 88%; crude protein - 12.2%; ether extract - 3.18%; ash - 3.06%; neutral detergent fiber - 26.07%; acid detergent fiber - 18.5%.

Animals were weighed every 28 days and had their blood collected by the jugular vein using vacutainer tube with anticoagulant (EDTA). These blood samples were used for analyses of plasma zinc, alkaline phosphatase and immunoglobulins G and M. Analyses of zinc were performed on atomic absorption spectrophotometer (Miles et al., 2001), while the analyses of phosphatase were performed by kinetic method (Bowers & McComb, 1966) with the aid of Laborlab kit (CAT N° 09800). Analysis of immunoglobulin G and M were performed by immunoturbidimetric test with the aid of Bioclin kits K062 and K063, respectively.

At the end of the experiment, animals were slaughtered, and samples of the liver were collected for study of hepatic levels of Zn. These samples were also analyzed in atomic absorption spectrophotometer (Miles et al., 2001).

The data of alkaline phosphatase, Zn, IgG and IgM were analyzed as repeated measures in time using PROC MIXED of SAS (Statistical Analysis System, version 9.1). For variables measured along time, the model included diet (source), time (collection days), and the interaction between time and source as fixed effects. Random effect of animal within diet was used. The method of Kenward-Rogers was used to calculate the degrees of freedom of the denominator of F-tests. The G matrix of variances and covariances with Toeplitz-type structure with one band was the one which best described the data. According to Wolfinger (1993), one of the procedures for selection of covariance structure is using the AIC (Akaike's Information Criterion), in which higher values suggest a better structure. Diet effects on specific collection days were determined by the option PDIF with means obtained through LSMEANS. Significance level of 0.05 was adopted.

## Results and Discussion

There was no difference ( $P > 0.05$ ; Table 2) in the alkaline phosphatase levels in function of the different sources of Zn. Despite the higher plasma levels of zinc when supplementation with ZnO 600, ZnO 400 and Zn proteinate 600 was used (Figure 1), these levels did not reflect in increased activity of alkaline phosphatase. However, alkaline phosphatase may be influenced by other factors and, considering the high levels of zinc used, they may have caused a similar response among the sources.

Engle et al. (1997) reported that zinc sources (organic and inorganic) did not affect the plasma zinc concentrations in their study. However, when calves were supplemented with 500 mg zinc/kg, the organic zinc had better absorption than inorganic zinc, based, especially, on the higher

concentrations of zinc in the plasma (Wright & Spears 2004). Ward et al (2002) observed greater weight gains for animals that ingested zinc in the organic form. Spears & Kegley (2002) also reported higher weaning weights for calves supplemented with organic zinc when compared with control groups that received zinc oxides or sulfates.

There was difference ( $P<0.05$ ; Table 2) in the plasma levels of Zn in function of the different sources and doses of Zn. For McDowell (2003), serum levels from 0.60 to 0.80 mg/dL are considered normal (Figure 1). All diets, except for Zn proteinate 200 and the control, had levels above the normal, indicating that the supplementation used in others diets were superior in relation to the absorption of zinc. The supplementations with 400 and 600 mg of ZnO and 600 mg of Zn proteinate/kg DM were superior ( $P<0.05$ ) to the supplementation in the control diet. This result, considering the plasma levels of zinc as an indicator of bioavailability, indicates that, regardless of the dose used, the organic source of Zn amino acid resulted in lower absorption of Zn. On the other hand, Kincaid et al. (1997), determining the effects of Zn used as source, oxides and amino acids for Holstein steers in diets with 60, 150 and 300 mg of Zn/kg DM, had higher concentrations when supplemented with Zn methionine and Zn lysine, but not for ZnO. This difference found in relation to the lower plasma concentration of Zn amino acid in this research can possibly be attributed to many variables in the digestive

tract that interfere in the absorption of minerals such as pH and especially the presence of other minerals and their antagonistic interactions.

There was difference ( $P<0.05$ ) between the sources (Table 2) and doses (Figure 2) of Zn tested in relation to the levels of immunoglobulin G. The curve of ZnO 600 was above the others from the collection at 28 days, and this supplementation was statistically superior to control supplementation (Figure 2). There was significant time  $\times$  source interaction ( $P=0.004$ ) for the IgG variable (Table 2). This interaction can be explained by increased plasma zinc concentration when the zinc oxide 600 was used as source of supplementation (Figure 1).

There was difference ( $P<0.05$ ; Table 2) in the levels of immunoglobulin M in function of different sources and doses of zinc. The immunoglobulin M levels increased in the period between 0 and 28 days of collection and then remained high both for zinc amino acid 200 and zinc proteinate 400, while the other levels of immunoglobulin M decreased or remained stable (Figure 3).

The specific cellular immune response includes the system T and B lymphocytes. These cells are responsible for the synthesis of antibodies, the establishment of resistance to the invading microorganism and death of microorganisms (Bonham et al., 2002). Prasad (1998) demonstrated that T cell function was affected in humans with moderate deficiency of zinc. The functions of T lymphocytes, such as delayed

Table 2 - Plasma concentrations in response to zinc supplementation

Item	Source				CV (%)	P value <sup>1</sup>		
	Control diet	Zn amino acid	Zn proteinate	Zn oxide		Time	Source	T $\times$ S
Alkaline phosphatase (U/L)	668.25a	537.12a	572.00a	621.00a	25.17	0.506	0.070	0.998
Zinc, mg/dL	0.70b	0.85b	0.78b	1.03a	37.76	<0.001	<0.001	0.003
Immunoglobulin G, g/dL	1.77b	1.87b	1.84b	1.99a	14.57	<0.001	<0.001	0.004
Immunoglobulin M, g/dL	0.17b	0.21a	0.20ab	0.17b	13.12	<0.001	<0.001	0.013

Means followed by different letters in the same row, differ by the Tukey test ( $P<0.05$ ).

<sup>1</sup> Probability of fixed effect of time, source and interaction time vs. source (T  $\times$  S) adjusted by command PROC MIXED of software SAS.

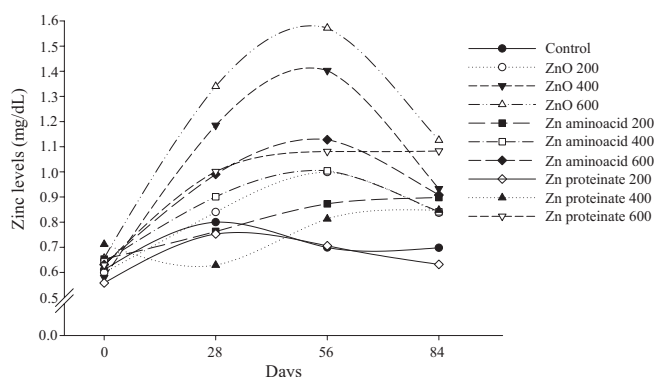


Figure 1 - Plasma zinc levels, in relation to collection days.

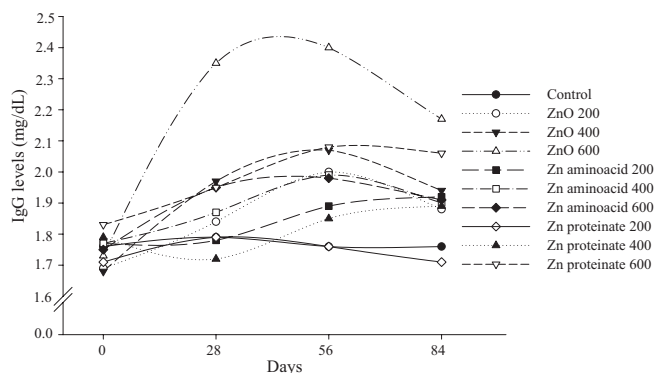


Figure 2 - Immunoglobulin G (IgG) levels, in relation to collection days.

hypersensitivity and cytotoxic activity are suppressed during the Zn deficiency, but restored by supplementation (Chandra, 1990). The participation of Zn in the specific cellular immune response is performed through its role in the clonal expansion of lymphocytes (Shankar & Prasad, 1998), by inhibition of apoptosis (Zalewski, 1996) and by maintaining the integrity of the cell membrane through binding of Zn at the thiol grouping (Shils et al., 2002).

There was no difference between diets ( $P>0.05$ ; Figure 4) in relation to the levels of hepatic Zn. The level of Zn in the liver of sheep receiving control diet was of 137 mg/kg DM, while in animals supplemented with ZnO, Zn amino acid and Zn proteinate were 136.47 and 255.45 mg/kg DM. The hepatic zinc levels obtained for ZnO (200, 400 and 600), zinc amino acid (200, 400 and 600) and zinc proteinate (200, 400 and 600) were 155.64, 189.08, 187.64, 235.41, 167.27, 255.45, 136.47, 239.04 and 209.12 mg/kg DM, respectively. According to Puls (1994), normal levels of Zn in the liver are between 75 and 300 mg/kg DM, and the hepatic Zn values are within this interval (Figure 4).

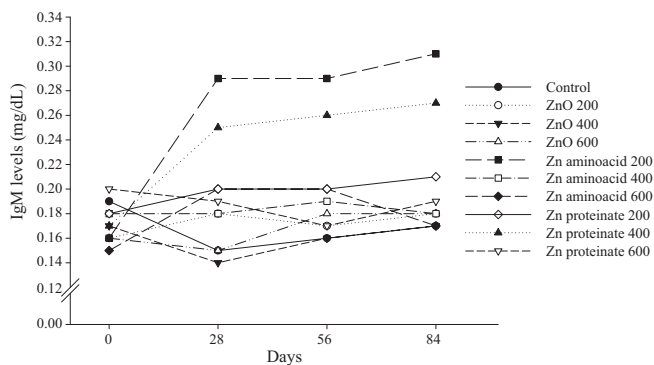


Figure 3 - Immunoglobulin M (IgM) levels, in relation to collection days.

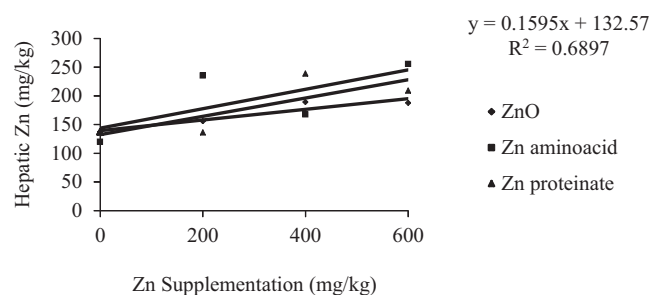


Figure 4 - Hepatic zinc levels, in relation to levels of supplementation.

## Conclusions

Based only on hepatic accumulation, the Zn bioavailability, estimated through regression equations, shows that the organic and inorganic sources do not differ statistically. In relation to the variables measured along time, the diet that had ZnO (inorganic zinc) shows higher levels in half of the parameters analyzed, which may imply that the advantage of utilizing inorganic over organic sources is evident.

## References

- AMMERMAN, C.B.; BAKER, D.H.; LEWIS, A.J. **Bioavailability of nutrients for animals**. San Diego: Academic Press, 1995. 441p.
- BONHAM, M.; O'CONNOR, J.M.; HANNIGAM, B.M. et al. The immune system as a physiological indicator of marginal copper status? **British Journal of Nutrition**, v.87, p.393-403, 2002.
- BOWERS, G.N.; McCOMB, R.B. A continuous spectrophotometric method for measuring the activity of serum alkaline phosphatase. **Clinical Chemistry**, v.12, p.70-89, 1966.
- CHANDRA, R.K. Micronutrients and immune function. An overview. **Journal of the American Medical Association**, v.587, p.9-16, 1990.
- CORDOVA, A.; ALVAREZ-MON, M. Behavior of zinc in physical exercise: A special reference to immunity and fatigue. **Neuroscience and Biobehavioral Reviews**, v.19, p.439-445, 1995.
- FICK, K.R.; McDOWELL, L.R.; MILES, P.H. et al. **Methods of mineral analysis for plant and animal tissues**. 2.ed. Gainesville: University of Florida, 1979. 90p.
- ENGLE, T.E.; NOCKELS, C.F.; KIMBERLING, C.V. et al. Zinc repletion with organic and inorganic forms of zinc and protein turnover in marginally zinc deficient calves. **Journal Animal Science**, v.75, p.3074-3081, 1997.
- KINCAID, L.R.; CHEW, B.P.; CRONRATH, J.D. Oxide and amino acids as sources of dietary zinc for calves: effects on uptake and immunity. **Journal Animal Science**, v.80, p.1381-1388, 1997.
- McDOWELL, L.R. **Minerals in animal and human nutrition**. New York: Academic Press, 2003. 625p.
- MERTZ, W. **Trace elements in human and animal nutrition**. 5.ed. New York: Academic, 1988. 489p.
- MILES, P.H.; WILKINSON, N.S.; McDOWELL, L.R. **Analysis of minerals for animal nutrition research**. 3.ed. Gainesville: University of Florida, 2001. 118p.
- NATIONAL RESEARCH COUNCIL - NRC. **Nutrient requirements of small ruminants**. 7.ed. Washington, D.C.: National Academic Press, 2007. 384p.
- PEDERSEN, B.K.; TOFT, A.D. Effects of exercise on lymphocytes and cytokines. **British Journal of Sports Medicine**, v.34, p.246-251, 2000.
- PRASAD, A.S. Zinc and Immunity. **Molecular and Cellular Biochemistry**, v.188, p.63-69, 1998.
- PULS, R. **Mineral levels in animal health**. 2.ed. Clearbrook: Sherpa International, 1994. p.83-109.
- SHANKAR, A.H.; PRASAD, A.S. Zinc and immune function: The biological basis of altered resistance to infection. **American Journal of Clinical Nutrition**, v.68, suppl. 2, p.4475-4635, 1998.
- SHILS, M.E.; OLSON, J.A.; SHIKE, M.; ROSS, A.C. **Tratado de nutrição moderna na saúde e na doença**. 9.ed. São Paulo: Manole, 2002. 2122p.

- SPEARS, J.W. Organic trace minerals in ruminant nutrition. **Animal Feed Science Technology**, v.58, p.151-163, 1996.
- SPEARS, J.W.; KEGLEY, E.B. Effect of zinc source (zinc oxide vs. zinc proteinate) and level of performance, carcass characteristics and immune response of growing and finishing steers. **Journal Animal Science**, v.80, p.2747-2752, 2002.
- WARD, J.D.; SPEARS, J.W.; KEGLEY, E.B. Effect of trace animal source on mineral metabolism, performance, and immune response in stressed cattle. **Journal Animal Science**, v.80, p.300, Suppl.1, 2002.
- WOLFINGER, R. Covariance structure selection in general mixed models. **Communications in Statistics Simulation and Computation**, v.22, p.1079-1106, 1993.
- WRIGHT, C.L.; SPEARS, J.W. Effect of zinc source and dietary level a zinc metabolism in Holstein calves. **Journal Animal Science**, v.87, p.1085-1091, 2004.
- ZALEWSKI, P.D. Zinc and immunity: implications for growth, survival and function of lymphoid cells. **Journal of Nutrition Immunology**, v.4, p.39-80, 1996.