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ABSTRACT

With the aim to determine the bioavailability of Zn in three inorganic (oxide-ZnO; sulphate-$ZnSO_4$ and carbonate-$ZnCO_3$) and one organic Zn source (Zn-quelate at 10%), an experiment were conducted with 14 treatments. To obtain the standard curve of Zn deposition in the liver, a basal diet with added concentrations of 0, 50, 100, 150 e 200 mg/kg of Zn from oxide zinc was used. The 3 other Zn sources were added only at a dietary level of 100; 150 and 200 mg/kg of Zn. For each experimental diet, 5 New Zealand White and 5 Californian rabbits of 60 days old and both sexes were randomly assigned. All animals were sacrificed after 30 days to obtain a liver sample to verify the Zn deposition and the relative bioavailability. The results showed for ZnO source, an increase on Zn level decreased ($P<0.05$) the food intake of rabbits. Nevertheless, this decrease was not relate with a similar tendency in their weight gain, showing the higher ($P<0.05$) values for 50, 150 and 200 Zn mg/Kg. An increase of dietary Zn level for ZnO source was initially related to a higher ($P<0.05$) Zn deposition on the liver until 100 Zn mg/Kg. However, higher level of Zn provoked a decrease ($P<0.05$) on the liver Zn deposition. Respect to the effect of source, similar food intake and weight gain was observed in the different sources tested, but values for Zn deposition on liver were slightly lower ($P<0.05$) for $ZnSO_4$ and Zn-quelate sources. The New Zealand White rabbits presented higher ($P<0.05$) hepatic reservations of Zn than California. The sex did not affect significantly the studied variables. The bioavailability of Zn sources was 100% for ZnO, 102.43% for Zn - quelate, 102.66% for $ZnCO_3$ and 103.65% for $ZnSO_4$.

INTRODUCTION

According to Lang (1981), although minimum concentrations and toxic levels of most minerals have been greatly studied in rabbits, only a few of works have been developed in order to determinate their optimal inclusion for an adequate performance, and data in the literature concerning to the recommendations lead to discrepancies. Feeding standards published by institutions (N.R.C., 1977; I.N.R.A., 1989) are not definite respect to the adequate requirements of Zn, suggesting only that diets with less than 3 mg/Kg could cause deficiency signs.

On the other hand, there are several chemical factors, including potential redox, pH and especially the initial form (source) of the microelements, that could affect the digestion, solubility and readiness of these elements and consequently their bioavailbility (Madsen and Johnson, 1989; Bertechini *et al*., 1992; Valle and Falchuk, 1993; Vandergrift, 1993 and Edwards *et al*.,1998). The oxide of zinc is the more used source of Zn in the rabbit diets because oxide salts are less reactive, and they contain up to twice the cation concentration as sulphate salts. They, therefore, occupy less "space" in trace-mineral premixes (Edwards and Baker, 1999). However, in other species, specially in chicks, previous works had not found conclusive differences between the sulphate, oxides and carbonates bioavailability (Wedekind and Baker, 1990; Sandoval et al., 1997).

The present work has been developed with the aim of determining the bioavailability of four different sources of Zn (three inorganic and one organic) in rabbits under brazilian conditions.

MATERIAL AND METHODS

Four different sources of Zn were evaluated: oxide of zinc (ZnO) - OZ, sulphate of zinc ($ZnSO_4$) - SZ, carbonate of zinc ($ZnCO_3$) - CZ and a commercial quelate of Zn at 10% (Zn-quelate; Alltech Agroindustrial of Brazil Ltd.) - QZ.

Bioavailability of Zn was determined relative to the deposition of Zn in liver showed with the ZnO. So, to obtain the standard curve of feed intake and Zn deposition in the liver, a basal diet with added concentrations of 0, 50, 100, 150 e 200 mg/kg of Zn from oxide zinc (0Z, 50OZ, 100OZ, 150OZ and 200OZ diets, respectively) were used. Subsequently, determination of the deposition of Zn in liver for the other 3 sources was made adding to the basal diet concentrations of 100; 150 and 200 mg/kg of Zn: 100CZ, 150CZ and 200CZ diets for the $ZnCO_3$ source; 100SZ, 150SZ and 200SZ diets for the $ZnSO_4$ source; and 100QZ, 150QZ and 200QZ diets for the Zn-quelate at 10% source. Table 1 shows the main ingredients and the chemical composition of the basal diet, that was formulated in agree with the recommendation of De Blas (1989).

Table 1. Main ingredients (g/kg) and chemical composition (g/kg dry matter) of the basal diet

Ingredient	*Basal diet*
Corn	270
Alfalfa hay	460
Wheat bran	150
Soybean meal (44% CP)	100
Salt	5
Vitamin and mineral mixtures[1]	5
Complement[2]	10
Chemical composition	
Crude protein	167.1
Acid detergent fibre	185.7
Ca	6.3
P	5.3
Zn (mg/Kg)	48.8
Digestible Energy (kcal/kg)	2400

[1] free of Zn, especially formulated for the research.
[2] to be filled with the sources of Zn, kaolin and $CuSO_4$, to complete 1000 g.

Basal diet (whose vitamin-mineral mixture was free on Zn) was supplemented (Table 2) with the correspondent level of Zn and the amount of $CuSO_4$ necessary to supply the needs of the animals (in agreement with Smith *et al.*, 1997). All diets included kaolin as inert.

A total of 140 growing rabbits (5 New Zealand White and 5 California for each treatment) of both sexes, with 60 days of age and with an initial average weight of 1992.3 ± 213.3 g, were used. Animals were housed individually in cages of steel stainless. The diets were mixed pelleted and offered to the animals, take in to account an adaptation period of 7 days and during an experimental period of 30 days. During this period, the animals received daily 100 g of diet per animal and water comfortable deionized. The samples of the diets were ground in mill using a sieve of 1.0 mm and analysed by triplicate.

Table 2. Centesimal composition of complements (g/kg).

Source	*ZnO*					*$ZnCO_3$*			*$ZnSO_4$*			*Zn quelate*		
Level	*0*	*50*	*100*	*150*	*200*	*100*	*150*	*200*	*100*	*150*	*200*	*100*	*150*	*200*
Basal diet	99.0	99.0	99.0	99.0	99.0	99.0	99.0	99.0	99.0	99.0	99.0	99.0	99.0	99.0
ZnO	-	6.4	12.8	19.3	25.7	-	-	-	-	-	-	-	-	-
$ZnCO_3$	-	-	-	-	-	19.2	28.8	38.4	-	-	-	-	-	-
$ZnSO_4$	-	-	-	-	-	-	-	-	44.0	66.1	88.1	-	-	-
Quelate	-	-	-	-	-	-	-	-	-	-	-	100.0	150.0	200.0
Kaolin	968.5	962.1	955.7	949.2	942.8	949.3	939.7	930.1	924.5	902.4	924.5	868.5	818.5	768.5
$CuSO_4$	31.5	31.5	31.5	31.5	31.5	31.5	31.5	31.5	31.5	31.5	31.5	31.5	31.5	31.5

Weight gain (WG), food intake (FI) and feed conversion (FC) of rabbits were determinated. At the end of the experiment all the animals were slaughtered and the liver samples collected were conditioned properly in plastic sacks, being frozen for posterior reduction and maceration. Samples were weighed (5 g approximately) in porcelain crucible and 2.5 ml of $Mg(NO_3)_2.6\ H_2O$ at 66,6% was added with the objective of homogeinizate. Crucibles were heated in chapel with extractor at 100ºC until solution was completely evaporated and samples were incinerated in a muffle at 550° C for 16 hours. In cool samples 2 ml of HNO_3 at 50% was added to clean the ashes, being again the porcelain crucible taken in the heating foil for solution evaporation. After cooling, samples were incinerated again during 2 hours at 550° C, being dissolved the final ashes with 25 ml of HCl 1N. The Zn of solutions was determinated by spectrophotometer of atomic absorption (Perkin Elmer) at 403 to 213.9 nm. The values are expressed in the base of fresh matter, being the medium value of dry matter for the organ of 69.03%.

Statistical analysis of data was carried out according to the ANOVA and REG procedures of SAS (Statistical Analysis System Institute, 1985), and the test of Duncan at 5% for mean comparison was used. Through multiple lineal regression, using Zn concentration in the liver as dependent variable (Y) and Zn intake of the basal and added diets as independent variables (x_1, x_2, ...), it was obtained an equation:

$$\mathbf{Y_{Zn} = a + b_1x_1 + b_2x_2 + b_3x_3 + b_4x_4 + b_5x_5}$$

being, x_1 = the Zn intake with the basal diet; x_2 = Zn intake with ZnO; x_3 = Zn intake with $ZnSO_4$; x_4 = Zn intake with $ZnCO_3$; and x_5 = Zn intake with Zn-quelate.

The relative bioavailability was calculated for the slope ratio technique. Starting from the equation mentioned above, relative bioavailability of different sources of Zn was determined as the relation between the regression coefficient of each source (b_3, b_4 or b_5) and the regression coefficient obtained for the ZnO (b_2) considered as 100% available.

RESULTS AND DISCUSSION

Results of Table 3 show the effect of level and type of dietary Zn on the total FI, WG and Zn concentration on the liver. As could be show for ZnO source, an increase on Zn level decreased the FI of rabbits. Wedekind *et al.* (1992) also showed this tendency when dietary Zn level was increased. However, this decrease was not relate with a similar tendency in their WG, showing the higher values for 50, 150 and 200 Zn mg/Kg. Hossain *et al.* (1992), using several concentrations of Zn (0, 10, 30, 90 and 270 mg/Kg) from ZnO, did not find significant differences for WG, although FI and FC were affected. For chicks, Edwards *et al.* (1998) showed that the addition of the mineral didn't have any effect in the WG, because diet already provided enough Zn to cover its needs. Hossain and Bertechini (1993) showed that the better WG and FC were obtained with a diet containing 90 mg/Kg of Zn.

An increase of dietary Zn level was initially related to a higher Zn deposition on the liver until 100 Zn mg/Kg. However, contrary to that was expected a higher level of Zn provoked a decrease on the liver Zn concentration. Hossain *et al.* (1992) using ZnO in several concentrations (0, 10, 30, 90 and 270 mg/Kg) they found differences for the deposition of the mineral in several tissues of rabbits with 50 days of age, besides in the liver.

Table 3. Effect of diets on productive performance and Zn concentration on liver tissue

Diets	*Total intake (g)*[2]	*Weight gain (g)*[2]	*Conversion(g/g)*[2]	*Zn (mg/Kg)*[1]
0Z	3484.0 a	431.0 c	7.80 a	1.24 ab
50OZ	3476.0 a	676.0 a	5.28 bcd	1.16 b
100OZ	3470.3 a	480.0 bc	6.80 ab	1.40 a
150OZ	3379.2 ab	613.5 ab	5.97 bc	1.30 a
200OZ	3352.9 b	635.5 ab	5.81 bcd	1.17 ab
100CZ	3374.7 ab	706.0 a	5.06 cd	0.72 d
150CZ	3445.7 ab	607.5 ab	5.74 bcd	1.24 ab
200CZ	3389.6 ab	704.0 a	4.88 cd	0.77 d
100SZ	3338.7 b	666.0 a	5.17 cd	0.84 cd
150SZ	3420.4 ab	600.5 ab	6.00 bc	1.28 a
200SZ	3354.4 b	737.0 a	4.32 d	0.82 cd
100QZ	3380.9 ab	698.0 a	5.11 cd	0.80 cd
150QZ	3382.5 ab	632.0 ab	5.54 bcd	1.04 bc
200QZ	3354.4 b	726.0 a	4.69 cd	0.80 cd
Means	*3400.26*	*636.64*	*5.57*	*1.02*
CV (%)	*3.14*	*26.31*	*25.15*	*24.12*

[1] Averages followed by different letters, differ to each other for the test of Duncan ($P<0.01$)
[2] Averages followed by different letters, differ to each other for the test of Duncan ($P<0.05$)

Respect to the effect of source, similar FI and WG was observed in the different sources tested, but values for Zn concentration on liver were slightly lower for sulphate and quelate sources. The treatments 100OZ, 150OZ and 150SZ presented the largest results of hepatic deposition, and statistically similar to 150CZ and 200OZ values. Wedekind *et al.* (1992), fo diets with 0, 250, 500 and 750 mg/Kg of Zn coming from $ZnSO_4$, ZnO and Zn-Methionine, they didn't find lineal answers for any of the researched sources.

When the hepatic reservations of Zn were related to the breed (Table 4), it was observed that New Zealand White presented higher ($P<0.05$) Zn deposition than California. When was considered the sex, there was not significant difference for the studied variables ($P>0.05$). In agreement with the present work, Teixeira (1992) didn't find differences in the concentration of Zn in the liver of chicken fed different levels of ZnO and $ZnSO_4$, but they detected a influence of sex on the Zn deposition.

Table 4. Concentration of Zn (mg/Kg) according to breed

Breed	*Zn on liver*[1]
Californian	0.93 b
New Zealand White	1.09 a

[1] Averages followed by different letters differ to each other for the test of Duncan (P<0.05)

The multiple regression equation obtained for Zn deposition in function of dietary Zn intake with the different sources evaluated is to follow presented:

$$\mathbf{Y_{Zn} = 1.11 + 0.66\, x_1 + 0.55\, x_2 + 0.57\, x_3 + 0.56\, x_4 + 0.56\, x_5 \;\; (R^2 = 0.59;\ P<0.001)}$$

Starting from this equation, the relative bioavailabilities of the different sources studied are presented in Table 5.

Table 5. Bioavailability of the Zn sources (%)

Source	*Zn Bioavailability*
$ZnSO_4$	103.65
$ZnCO_3$	102.66
Zn-quelate	102.43
ZnO	100.00

As also have been show in other species the literature concerning Zn bioavailability is neither extensive nor totally conclusive (Wedekind and Baker, 1990; Aoyagi and Baker, 1993; Aoyagi *et al*., 1995; Edwards *et al*., 1998; Edwards and Baker, 1999). The results of the present work indicated that the type of source of Zn did not have any effect on the bioavailability of Zn in rabbits, showing a similar response with the different sources evaluated. In conclusion, also further research is needed to corroborate this results (other methodologies, statistical designs, ...), ZnO seems to be the most adequate sources for rabbits in function of its chemical and physical properties.

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