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# ZINC MICROMINERAL IN PRODUCTION RABBITS WITH EIMERIOSIS

***Julia Ferreira Cury Silva***
FAZU – Faculdades Associadas de Uberaba
Uberaba – MG
http://lattes.cnpq.br/9159286328966091

***Bruno Paz Rosas***
FAZU – Faculdades Associadas de Uberaba
Uberaba – MG
http://lattes.cnpq.br/3855401469922859

***Gabriel Faria Pereira***
FAZU – Faculdades Associadas de Uberaba
Uberaba – MG
http://lattes.cnpq.br/6656108246728230

***Danielle Leal Matarim***
FAZU – Faculdades Associadas de Uberaba
Uberaba – MG
http://lattes.cnpq.br/5913111365847190

***Amanda Pifano Neto Quintal***
FAZU – Faculdades Associadas de Uberaba
Uberaba – MG
http://lattes.cnpq.br/0671357690549206

**Abstract**: The production of beef rabbits is growing in Brazil and efforts need to be applied in the feed industry to meet the nutritional demand of these animals. Among the nutrients, zinc stands out, since it acts as an enzymatic catalyst, it is related to the metabolism of nucleic acids, proteins and carbohydrates, helping in cellular metabolism and the immune system. Parasitic infections in rabbit breeding are common, especially by Eimeria sp., which leads to large production losses, morbidity and mortality. Thus, the objective was to evaluate the use of extra zinc supplementation in the feeding of naturally infected rabbits. The experiment was carried out in Santa Juliana - MG, using 20 rabbits, male and female crossbred New Zealand white x California, recently weaned, divided into two groups: control, without the extra supply of zinc and the treated group, with extra addition of zinc. zinc. The weight of the animals, total weight and average daily gain, dosage of serum plasma proteins, albumin, globulins, excretion of total minerals and zinc in the feces and parasite load in the feces were evaluated. The weight and average weight gain, total proteins, albumin and globulin did not change between the evaluated groups. There was a difference in mineral excretion between the groups, males in the treated group excreted less minerals than those in the control group, females in the supplemented group had higher excretion when compared to the control group. As for the excretion of only zinc in the feces, males in the control group had a much lower excretion when compared to the treated group, in females there was no difference in the excretion of zinc in the feces. The animals treated with extra supplementation had a lower parasite load when compared to the control group ($p<0.05$). Zinc mineral supplementation has the potential to be used as an interesting alternative in maintaining the health of production, increasing parasite control parameters.

**Keywords:** Rabbit farming, *Eimeria sp*., Mineral, Coccidiosis, Immune system.

## INTRODUCTION

The rabbit is a monogastric animal, which has rapid development and easy handling, raising its potential for better meat production (SILVA et al., 2017). For a good meat production, these animals need to receive an adequate diet, of great quality, with amounts of protein, carbohydrates and minerals that meet their nutritional requirements (MACHADO et al., 2011).

There are numerous researches on protein and energy requirements, but mineral requirements are also essential to complete this picture, as they perform several functions in the body, for performance, reproduction and immunity (KNOB et al., 2019).

The mineral zinc participates in important functions in the functioning of enzymes that are linked to the formation of proteins, in the metabolism of carbohydrates, for a good development of rabbits (NRC, 1997). Higher levels of zinc supplementation are used to raise rabbits intended for fur and fur production. For the production system intended for meat, the levels must be lower, not exceeding 150mg/kg (DE BLAS and WISEMAN, 2010).

Zinc is also linked to the immune activity of these animals, acting in the fight against foreign organisms and parasites, both in innate and adaptive defense (WESSELS; MAYWALD; PISTA, 2017).

From the point of view of health, coccidiosis remains one of the most important infectious causes of digestive disorders in fattening rabbits, being caused by an intercellular protozoan. Parasites of the genus Eimeria sp. result in morbidity, due to lower weight gain and diarrhea, but can cause significant mortality in domestic rabbits (ABDEL-AZEEM S. ABDEL-BAK, 2013).

There are few studies on eimeriosis in rabbits in Brazil. When analyzing breedings in northern Paraná, Cardoso and Guimarães Júnior (1993) identified eight species of Eimeria, through morphometric and morphological analysis of oocysts (*E. coecicola*, *E. flavescens*, *E. intestinalis*, *E. irresidua*, *E. magna*, E. *perforanse*, *E. stiedai*), and *E. perforans* and *E. coecicola* showed a higher occurrence, 36.8% and 79.8%, respectively.

Immunity to the parasite occurs when the animal becomes infected with a species and ends up being immune to that species, that is, if the animal is infected again by another species, it will go through the same process to become immune to another species of Eimeria sp. (PAKANDL et al., 2008).

As zinc acts directly as a cofactor of immune response, the objective of this work was to verify if the use of oral zinc as a food supplement increases the immune response, aiming at reducing the parasite load of Eimeria sp. in newly weaned rabbits to slaughter weight, being naturally infected.

## MATERIAL AND METHODS

### ANIMALS AND STUDY SITE

The experiment was carried out in the municipality of Santa Juliana - MG, at Fazenda Água Santa in the region of Lagoa Dourada, and this experiment was approved by the Ethics Committee in Animal Experimentation of Faculdades Associadas de Uberaba (Official nº 019/2021 CEUA/FAZU).

Twenty newly weaned crossbred male and female rabbits of the New Zealand White x California breed were used. The animals were kept in a system of creation of suspended metal cages, with a format of 1m x 0.6m x 0.4m, with two animals per cage.

The animals were divided by sex, males and females, and distributed in two treatments: (1) Control group (N=10, 5 males and 5 females), which consumed only the zinc present in the ration; (2) Group treated (N=10, 5 males and 5 females) with extra zinc supplementation.

The animals received water and food ad libitum, using Saborosa food for rabbits (Saborosa Rações®) and no vermifuges were administered from birth until the end of the experimental trial.

### ZINC AS A MINERAL SUPPLEMENT

The mineral was purchased from the pharmacy in the liquid form ZincoPed, at a concentration of 4mg/mL (Gallia®), and was provided orally using a 5mL syringe, with 2.4mL administered to the treated animals every two days, totaling 100mg total/animal, during the 30 days of the experiment, being the zinc requirement for rabbits between 30 to 60mg/kg in dry matter (DE BLAS and WISEMAN, 2010), The animals of the control group were also submitted to the administration of liquid ( water) via syringe, so that all animals were submitted to the same handling.

### ANIMAL WEIGHT

The animals were weighed, on a digital scale, weekly over the 30 days of treatment, for subsequent realization of weekly weight average, to verify the initial and final weight, and calculation of the total and average daily gain of the animals, to compare the treated and control groups.

### EGG COUNT PER GRAM OF STOOL (OPG)

Feces were collected from both groups at the end of the experiment, with plastic bags being placed under the cages. The analysis of feces was performed in the parasitology laboratory of FAZU, by the Centrifugal-Flotation Method with Zinc Sulfate (FAUST, 1938), comparing the parasite load between the experimental groups.

### MEASUREMENT OF SERUM PROTEINS, ALBUMINS AND GLOBULINS

Thirty days after the treatments, blood samples were collected from the animals, collected through the auricular vein, stored in vacuum tubes, and sent to the Uniube hematology laboratory for analysis of total proteins, globulins and albumins.

### EXCRETION OF TOTAL MINERALS AND ZINC IN THE FECES

Analyzes of mineral and zinc excretion in feces were performed at the Labfert laboratory, in Uberaba. For the analysis of total zinc, the method described by IN SDA 37 Cap III, E.10.2 was used, and for the analysis of mineral matter, the CBAA Method I, 05 was used. The collection was performed in the same way for the analysis of OPG, plastic bags are placed under the cages.

### STATISTICAL ANALYSIS

Descriptive statistical analysis was performed, considering the mean and standard deviation of the experimental groups. For statistical analysis, the GraphPad Prism 6.0 software was used to perform the Two-way ANOVA test, evaluating the experimental groups before and after treatment with zinc in blocks of males and females, considering the parameters: analysis of feces by OPG, weight, ADG, quantification of total proteins, globulins and albumins and excretion of total minerals and zinc in the feces, in a randomized block design, at a significance level of 5%.

## RESULTS AND DISCUSSION

The weights of the animals obtained weekly are shown in Table 1. Lower weight gain is observed at the beginning of the study in males and females that were submitted to zinc treatment; however, these values were equalized at the end of the study, with no difference in the final weight.

During the 30 days of treatment there was no significant difference, both in the total weight gain and in the average daily gain between the groups (Two-way Anova $p>0.05$). Total weight gain and mean daily gain averaged 937 and 34.9 for males with zinc; 870 and 31.2 for males without zinc; 962 and 32.1 for females with zinc and 940 and 31.3 for females without zinc (Figure 1).

Extra zinc supplementation, therefore, did not interfere with weight gain when comparing the control and treated groups; however, untreated males obtained at least 66-92g of weight less than the others, being approximately 5% less of the slaughter weight.

According to Freitas (2009), the spores of *Eimeria* in intestinal cells, causes these cells to malfunction and cause them to increase in size, and this will lead to their death, interfering with the absorption of nutrients, causing dehydration, weight loss and anemia.

In other monogastric species, differences in weight were observed when zinc supplementation was used. A study carried out by CARDOSO (2004) shows that extra zinc supplementation in broilers improved the average daily gain throughout the confinement period, and provided better results in the average live weight gain of these animals. FEDERIZZI (2014) also reports that Zn supplementation in the form of metal-amino acid complexes improves the ADG (average daily gain) of confined male pigs and the final weight of confined female pigs, in addition to altering the feed intake of these animals.

In the evaluation of the parasite load, oocytes of Eimeiria sp. were observed, being found in all the animals having a parasite load above 500 eggs per grams of feces at the beginning of the study.

However, when comparing the groups at the end of 30 days (Figure 2), it was evidenced

| DAYS | Zinc | | Control | |
|---|---|---|---|---|
| | Male | Female | Male | Female |
| Day 1 | 614 ± 112,76 | 603,7 ± 45,94 | 674 ± 45,7 | 675,3 ± 66,89 |
| Day 7 | 846 ±134,97 | 828,7 ± 62,52 | 891 ± 76,9 | 892,3 ± 72,11 |
| Day 14 | 1018 ± 109,67 | 1097 ± 66,01 | 1024 ± 169 | 1231,3 ± 43,01 |
| Day 21 | 1272 ± 90,70 | 1231,3 ± 80,93 | 1292 ± 268 | 1351 ± 58,51 |
| Day 30 | 1551 ± 65,60 | 1565,7 ± 69,59 | 1554 ± 270 | 1615,3 ± 94,74 |

Table 1. Average weight ± standard deviation in grams of male and female rabbits compared to the treated and control group over the days.

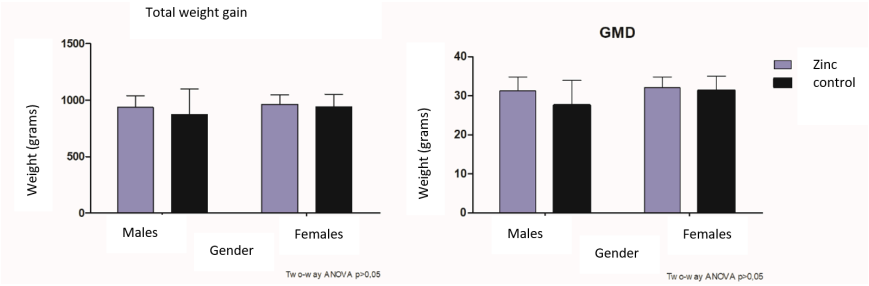


Figure 1. Total 30-day total weight gain and average daily gain in males and females, comparing zinc-treated and control groups.

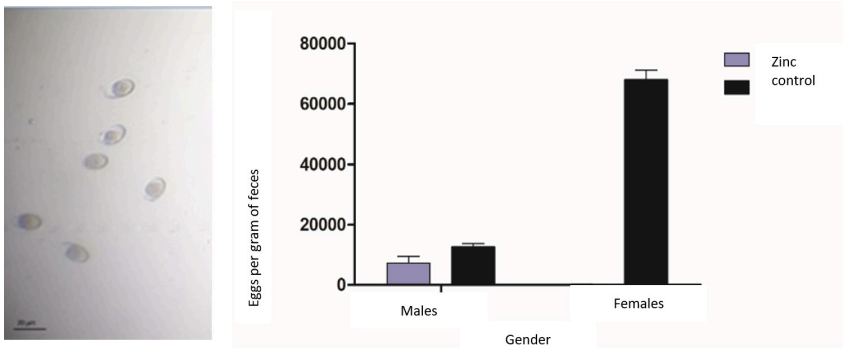


Figure 2. Egg count per gram of feces (OPG) in males and females, comparing zinc-treated and control groups, followed by representative coccidia imaging of:*Eimeria* sp.

| Gender | Total proteins | | Albumin | | Globulins | |
|---|---|---|---|---|---|---|
| | Zn | Control | Zn | Control | Zn | Control |
| Males | 5,5 ± 0,7 | 5,8 ± 0,4 | 3,8 ± 0,3 | 3,9 ± 0,05 | 1,7 ± 0,3 | 1,8 ± 0,3 |
| Females | 5,4 ± 0,2 | 5,6 ± 0,3 | 3,7 ± 0,2 | 3,9 ± 0,15 | 1,7 ± 0,2 | 1,7 ± 0,1 |

Table 2. Average and standard deviation of total proteins, globulin and albumin of the evaluated animals.

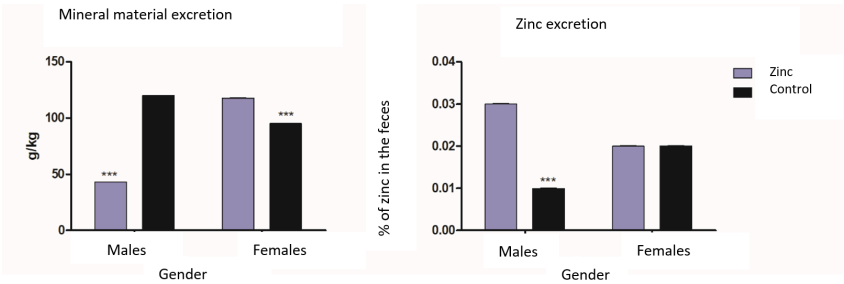


Figure 3. Excretion of Mineral Matter (g/kg) and Zinc (%) in the feces.

that the treated group had a lower parasite load when compared to the control group (Anova $p<0.05$). Both in males and females, highlighting the parasite load of the control females much higher than the treated group. It was evident that the supply of zinc as extra supplementation helped to reduce the parasite load in the animals, especially in females.

Coccidiosis is considered the major parasitic disease that causes severe economic losses in rabbit production (ABDEL-AZEEM S. ABDEL-BAK, 2013). Clinical disease in rabbits is characterized by intestinal villous atrophy, malabsorption of nutrients, electrolyte imbalance, diarrhea, loss of appetite, weight loss, anemia, hypoproteinemia, dehydration and, in some cases, high mortality (HEKER, 2016). Thus, it is expected that the greater the parasite load, the greater the clinical signs will be.

For analysis of total proteins, globulin and albumin, no changes were observed in concentrations, comparing the control group with the treated group (Table 2). All tabulated values are compatible with reference values for total proteins (5.4 to 7.5g/dL), albumin (2.5 to 4.5g/dL) and globulin (1.9 to 3.5g/dL) .

In a study by Freitas (2009), rabbits that were infected by sporulated oocysts of *Eimeria stiedae* showed an increase in total protein and globulin concentrations at 28 days, while albumin remained stable, even with the appearance of hepatic inflammation caused by parasitic reproduction, when compared to the control group.

To help interpret the amount of serum zinc, the method of binding zinc to plasma protein is suggested, which is a biomarker that shows the metabolic factors of this mineral (ARGEMI et al., 1988). Although our study did not observe differences in the amount of total proteins, Freitas (2009) reports that animals infected by endoparasites, the body's proteins are affected, directly interfering with the production of immunoglobulins.

Even though the animals in this study presented a high parasite load, this fact did not interfere in the concentrations of total proteins, albumin and globulin; and the immunity against the parasite Eimeria sp. may also be related to cellular immunity.

The lack of zinc causes numerous consequences in the body, interfering with the immune system. Therefore, the supplementation of zinc supply is an alternative to prevent diseases and high mortality rates (PRASAD, 2003).

When comparing the groups in relation to the excretion of mineral matter (Figure 3), there was a difference when comparing the treated and control males, since the treated males had a lower excretion when compared to the control group. In females, this relationship was inverted, where the treated group had greater excretion than the control. Regarding zinc mineral excretion, no difference was observed between females, however there was greater zinc excretion in males in the treated group ($p<0.05$), suggesting an activity of use and metabolism of this mineral with greater intensity in the group control.

Looking closely at the relationship between the excretion of mineral matter and zinc in the faeces, the group of males that were treated with the addition of zinc had a lower excretion of mineral matter and a greater excretion of zinc in the faeces, when compared to the other group ($0=p< 0.05$). Also, in the control treatment males, the inverse relationship was observed, they retained more zinc and maintained mineral matter at higher levels of excretion. In females, mineral matter excretion remained higher in the zinc-treated group, and there was no difference in zinc excretion between the two groups.

It was expected that the more zinc, the greater the excretion of this mineral would be observed in the feces, in order to

eliminate the surplus. However, zinc seems to act directly on the metabolism of other minerals, changing their proportions during excretion. Elements such as calcium can affect the absorption of zinc in the animal's body, but components such as magnesium can aid absorption (MCDOWELL, 1992). Van Soest (1994) reported that the presence of phytate in feed provided to animals decreases zinc absorption.

The difference in excretion metabolism of males and females, as additional data, can be explained by the observation of males in the group treated with extra zinc supplementation, which showed mounting behavior in the other mates; the males in the control group did not show any sign of sexual interest. Such an act may be related to the level of testosterone in animals. BALBINOT et al., (2019) describe that testosterone is directly related to spermatogenesis, and helps in the advancement of certain characteristics such as masculinity and libido, in which zinc directly participates in the production of this hormone. AZEVEDO (2005) reports the various effects of the mineral zinc in the production and secretion of hormones, mainly in the production of testosterone.

## CONCLUSION

Although mineral supplementation does not have a direct impact on animal performance, zinc supplementation can act as a potential regulator of parasite load in production rabbits. However, further studies are suggested to better understand the metabolism and excretion of minerals, especially in males. Furthermore, cellular and non-humoral immunity against coccidia must also be tested.

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