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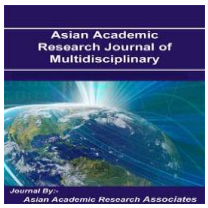


# IMMUNE RESPONSE AND INTESTINAL MORPHOMETRY OF BROILERS SUPPLEMENTED WITH L-GLUTAMINE AND ZINC

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**Abstract**

The objective of this study was to evaluate the effect of the addition of L-glutamine and zinc on morphometry of the cloacal pouch and small intestine and weight of the lymphoid organs. Birds were distributed in a completely randomized design, in factorial scheme 2 (1 and 2% L-glutamine) and 3 (0, 90 and 120 mg of Zn / kg of feed) were used, and a control, with five replications and 18 birds each. The supplementation of 1% of L-glutamine combined or not with zinc provided an reduction of crypt depth and increase of wall thickness of the jejunum. The inclusion of 2% de L-glutamine associated with all levels of the mineral test caused an increase in the total and medullary area of the cloacal pouch and reduction of the ratio cortical / medullary and percentage of the cortical area . The addition of L-glutamine and zinc in diets of the broilers in the period from 1 to 21 days of age influences the total and medullary area of the cloacal pouch, the depth of crypts of the duodenum and ileum, and thickness of the jejunum wall.

**Keywords**: aminoacids; cloacal pouch; crypts; mineral.

## 1. Introduction

The associated supplementation of L-glutamine and zinc has shown positive results in research on human nutrition [1; 2]. However, there are no reports of this association in the nutrition of broiler chickens, there are only works in the literature highlighting the beneficial effects of both nutrients in isolation.

The glutamine is considered a non-essential amino acid, and is the main source of energy for enterocytes and cells of the immune system [3; 4]. However, in stress situations, the use of glutamine by intestinal cells increases significantly, due to oxidative stress and depression of immune defenses [5]. Thus, under these conditions, supplementation of L-glutamine is justified, since tissue synthesis will not be able to supply the demand for this amino acid [6].

The zinc is a micromineral that is involved in several functions such as carbohydrate, protein and nucleic acid metabolism [7]. It is also present in the enzyme superoxide dismutase and plays an important role in the immune system [8]. It has also provided satisfactory results regarding the development of intestinal structures, allowing an increase in the area of intestinal absorption and better utilization of dietary nutrients [9].

In the absence of information on the effects of the addition of zinc-associated L-glutamine on broilers diets, this study was conducted to evaluate the effect of this combination on cloacal pouch histomorphometry, absolute lymphoid organ weight and intestinal morphometry of the duodenum, jejunum and ileum of broiler chickens from 1 to 21 days of age.

## II. Materials and Method

The study was carried out in the poultry sector of the Department of Animal Science of the Agricultural Sciences Center of the Federal University of Piauí, Teresina, Piauí, Brazil, in June and July 2015, whose geographical coordinates are: latitude of 5 5 'south, longitude 42 °

48' west and humid tropical climate, with annual rainfall around 1337.7 mm [10]. The procedures were approved by the Ethics Committee on Animal Experimentation/EAEC/UFPI, under protocol number 087/12.

A total of 630 male broilers from the Ross lineage were used during the period from 1 to 21 days of age with a mean initial weight of 38,17±0,77g. The birds were distributed in a completely randomized design, in a 2x3 + 1 factorial scheme, with two levels of L-glutamine (1 and 2%) associated to three levels of zinc (0.90 and 120mg / kg of feed) in the organic form , a control diet and five replicates. These animals were housed in boxes of 2.7m2, equipped with tubular feeders and pendulum drinkers, located in masonry sheds covered with ceramic tiles and cemented floor.

The animals received a pre-start diet up to 7 days (Table 1) and a diet for the initial phase (Table 2), from 8 to 21 days of age. The diets were formulated to meet the nutritional requirements recommended by [11]. Zinc was supplemented in the diets to replace the inert material and L-glutamine was introduced into the diet formulation. The birds received feed and water at will.

Table 1. Percentage and calculated composition of the experimental diets for broilers in the pre-initial phase (1 to 7 days of age)

| Ingredients | Levels of glutamine (%)/ zinc (mg/kg) | | | | | | |
|---|---|---|---|---|---|---|---|
| (%) | 0/0 | 1 | 1/90 | 1/120 | 2 | 2/90 | 2/120 |
| Corn | 52,000 | 54,500 | 54,500 | 54,500 | 57,400 | 57,400 | 57,400 |
| Soybean | 33,430 | 30,170 | 30,170 | 30,170 | 26,810 | 26,810 | 26,810 |
| Vegetable | 4,827 | 4,322 | 4,322 | 4,322 | 3,678 | 3,678 | 3,678 |
| Dicalcium | 1,770 | 1,805 | 1,805 | 1,805 | 1,842 | 1,842 | 1,842 |
| Calcitic | 0,975 | 0,975 | 0,975 | 0,975 | 0,980 | 0,980 | 0,980 |
| NaCl | 0,508 | 0,508 | 0,508 | 0,508 | 0,510 | 0,510 | 0,510 |
| L-Lysine- | 0,063 | 0,168 | 0,168 | 0,168 | 0,278 | 0,278 | 0,278 |
| L- | 0,000 | 0,000 | 0,000 | 0,000 | 0,011 | 0,011 | 0,011 |
| Valine | 0,155 | 0,215 | 0,215 | 0,215 | 0,277 | 0,277 | 0,277 |
| Threonine | 0,007 | 0,055 | 0,055 | 0,055 | 0,104 | 0,104 | 0,104 |
| Nucleus[a] | 6,000 | 6,000 | 6,000 | 6,000 | 6,000 | 6,000 | 6,000 |
| Zinc[b] | 0,000 | 0,000 | 0,090 | 0,120 | 0,000 | 0,090 | 0,120 |
| Glutamine[c] | 0,000 | 1,000 | 1,000 | 1,000 | 2,000 | 2,000 | 2,000 |
| Kaolin | 0,264 | 0,282 | 0,192 | 0,162 | 0,109 | 0,019 | 0,000 |
| TOTAL | 100,000 | 100,000 | 100,000 | 100,000 | 100,000 | 100,000 | 100,000 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| Calculated Composition | | | | | | | |
| Crude | 22,399 | 22,401 | 22,401 | 22,401 | 22,402 | 22,402 | 22,402 |
| ME | 2959,99 | 2959,99 | 2959,99 | 2959,99 | 2960,00 | 2960,00 | 2960,00 |
| Lysine dig. | 1,324 | 1,324 | 1,324 | 1,324 | 1,325 | 1,325 | 1,325 |
| Methionine | 0,669 | 0,653 | 0,653 | 0,653 | 0,637 | 0,637 | 0,637 |
| Threonine | 0,861 | 0,861 | 0,861 | 0,861 | 0,862 | 0,862 | 0,862 |
| Tryptophan | 0,253 | 0,234 | 0,234 | 0,234 | 0,226 | 0,226 | 0,226 |
| Valine (%) | 1,020 | 1,019 | 1,019 | 1,019 | 1,020 | 1,020 | 1,020 |
| Calcium (%) | 0,921 | 0,920 | 0,920 | 0,920 | 0,921 | 0,921 | 0,921 |
| Match | 0,470 | 0,470 | 0,470 | 0,470 | 0,471 | 0,471 | 0,471 |
| Sodium (%) | 0,219 | 0,219 | 0,219 | 0,219 | 0,219 | 0,219 | 0,219 |
| Zinc (mg/kg) | 146,869 | 145,961 | 235,961 | 265,961 | 145,094 | 235,094 | 265,094 |
| Glutamine | 0,000 | 1,007 | 1,007 | 1,007 | 2,014 | 2,014 | 2,014 |

[a]Guarantee levels per kg of product: moisture (max.) 120g / kg; crude protein (min.) 340g / kg; ethereal extract 45g; crude fiber 10g; mineral matter 300g; calcium 22g; calcium 28g; phosphorus 5,200mg; methionine 65g; lysine 45gg; threonine 27g; tryptophan 3,780mg; vitamin A 250,000UI; vitamin D3 60,000 IU; vitamin E 833UI; vitamin K3 50mg; vitamin B1 50mg; vitamin B2 133mg; vitamin B6 83mg; vitamin B12 333mg; niacin 100mgg; pantothenic acid 233mg; folic acid 25mg; biotin 0.66mg; biotin 0.66mg; choline 5,900mg; manganese 1,666mg; zinc 1600mg; chelated zinc 400mg; iron 837mg; copper 1,667mg; iodine 21mg; selenium 6mg; BHT 1764 mg; 8.335U phytase; protease 2.500UN; amylase 2.500UN; β-glucanase 2.083UN; xylanase 4.165UN; cellulase 3.750UN; senduramycin + nicarbazine 1.100mg.
[b]Availa®Zn 100.000mg/kg
[c] Metabolizable energy based [7] and crude protein analyzed (119,74).

Table 2. Percentage and calculated composition of the experimental diets for broilers in the initial phase (8 to 21 days of age)

| Ingredients | Levels of glutamine (%)/ zinc (mg/kg) | | | | | | |
|---|---|---|---|---|---|---|---|
| (%) | 0/0 | 1 | 1/90 | 1/120 | 2 | 2/90 | 2/120 |
| Corn | 58,300 | 60,500 | 60,500 | 60,500 | 62,600 | 62,600 | 62,600 |
| Soybean | 34,414 | 31,400 | 31,400 | 31,400 | 28,246 | 28,246 | 28,246 |
| Vegetable | 3,259 | 2,854 | 2,854 | 2,854 | 2,484 | 2,484 | 2,484 |
| Dicalcium | 1,530 | 1,560 | 1,560 | 1,560 | 1,600 | 1,600 | 1,600 |
| Calcitic | 0,907 | 0,907 | 0,907 | 0,907 | 0,907 | 0,907 | 0,907 |
| NaCl | 0,482 | 0,482 | 0,482 | 0,482 | 0,482 | 0,482 | 0,482 |
| L-Lysine- | 0,000 | 0,000 | 0,000 | 0,000 | 0,082 | 0,082 | 0,082 |
| L- | 0,000 | 0,000 | 0,000 | 0,000 | 0,002 | 0,002 | 0,002 |
| Valine | 0,029 | 0,085 | 0,085 | 0,085 | 0,145 | 0,145 | 0,145 |
| Threonine | 0,038 | 0,080 | 0,080 | 0,080 | 0,127 | 0,127 | 0,127 |
| Premix min | 1,000 | 1,000 | 1,000 | 1,000 | 1,000 | 1,000 | 1,000 |
| Zinc[b] | 0,000 | 0,000 | 0,090 | 0,120 | 0,000 | 0,090 | 0,120 |
| Glutamine[c] | 0,000 | 1,000 | 1,000 | 1,000 | 2,000 | 2,000 | 2,000 |
| Kaolin | 0,041 | 0,132 | 0,042 | 0,012 | 0,325 | 0,235 | 0,205 |
| Total | 100,000 | 100,000 | 100,000 | 100,000 | 100,000 | 100,000 | 100,000 |

| Calculated Composition | | | | | | | |
|---|---|---|---|---|---|---|---|
| Crude Protein | 21,200 | 21,198 | 21,198 | 21,198 | 21,201 | 21,201 | 21,201 |
| ME (kcal/kg) | 3050,0 | 3049,99 | 3049,99 | 3049,99 | 3050,00 | 3050,00 | 3050,00 |
| Lysine dig. | 1,211 | 1,134 | 1,134 | 1,134 | 1,117 | 1,117 | 1,117 |
| Methionine | 0,608 | 0,593 | 0,593 | 0,593 | 0,577 | 0,577 | 0,577 |
| Threonine dig | 0,792 | 0,790 | 0,790 | 0,790 | 0,790 | 0,790 | 0,790 |
| Tryptophan | 0,239 | 0,222 | 0,222 | 0,222 | 0,206 | 0,206 | 0,206 |
| Valine (%) | 0,937 | 0,936 | 0,936 | 0,936 | 0,937 | 0,937 | 0,937 |
| Calcium (%) | 0,841 | 0,840 | 0,840 | 0,840 | 0,840 | 0,840 | 0,840 |
| Match | 0,401 | 0,400 | 0,400 | 0,400 | 0,401 | 0,401 | 0,401 |
| Sodium (%) | 0,210 | 0,210 | 0,210 | 0,210 | 0,210 | 0,210 | 0,210 |
| Zinc (mg/kg) | 88,568 | 87,703 | 177,70 | 207,703 | 86,758 | 176,758 | 206,758 |
| Glutamine | 0,000 | 1,007 | 1,007 | 1,007 | 2,014 | 2,014 | 2,014 |

[a] Guarantee levels per kg of product: methionine 313,60g; lysine 168g; threonine 29.40g; vitamin A 1,200,000IU; vitamin D3 265,000; vitamin E 2,000UI; vitamin K3 260mg; vitamin B1 191mg; vitamin B2 630mg; vitamin B6 290mg; vitamin B12 1,700mg; niacin 4.200mg; pantothenic acid 1,300mg; folic acid 100mg; biotin 7mg; choline 26g; manganese 7,000mg; zinc 6,000mg; iron 5,000mg; copper 900mg; iodine 100mg; selenium 30mg; phytase 50,000 U; 30,000U amylase; β-glucanase 25,000 U; xylanase 50,000 U; 45,000 U cellulase; 30,000 U protease; ethoxyquin 6.666mg; Bacillus licheniformis 2x10E11UFC; Bacillus subtilis 1x10E11UFC; virginiamycin 1,650mg; maduramicin 500mg.
[b] Availa®Zn 100.000mg/kg
[c] Metabolizable energy based on [7] and crude protein analyzed (119,74).

The monitoring of the temperature and relative humidity of the sheds was carried out by means of maximum and minimum thermometers and thermohygrometer of dry and humid bulb kept in the center of the shed. The readings of the thermometers were performed three times a day (8, 13 and 16 hours), except for maximum and minimum, which were read only in the morning. These data were later converted to Globe and Humidity Temperature Index (ITGU), as proposed by [12]. The light program adopted was the continuous (24 hours of natural + artificial light) using 60W incandescent lamps.

On the 21 st day two birds were weighed and identified, and later slaughtered, as recommended by the Regulation of Industrial and Sanitary Inspection of Animal Products [13], to evaluate the absolute weight of lymphoid organs (thymus, spleen and cloacal pouch), calculated on the live weight of the fowl [organ weight / live weight] * 100] and collections

of cloacal pouch and small intestine portions for morphometric analysis, according to [14] and [15], respectively.

The percentage of the bursal lymphoid follicle cortex and the morphometric variables of the intestine were determined according to methods described by [16] and [14], respectively.

The data on environmental variables were submitted to mean and standard deviation calculations. The other parameters were subjected to analysis of variance, and when significant, L-glutamine levels were compared by Duncan's test and zinc levels by regression analysis. In the comparison of each treatment with the control diet, the Dunnett test was applied, according to PROC GLM procedures of the SAS software (2002). With the exception of the histomorphometry of the cloacal pouch, which violated the principles of normality and homoscedasticity and, for this reason, the test used was Kruskal-Wallis. A = 0.05 was used.

## III. Results and Discussion

Considering the values of the environmental variables obtained during the experimental trial (Table 3), the animals were raised under heat stress conditions, and during the first and second week, they were exposed to temperatures above and below the thermal comfort range.

Table 3. Environmental conditions observed during the experimental period

| Age (week) | Humidity (%) | Temperatures (°C) | | | ITGU[1] |
|---|---|---|---|---|---|
| | | Maximum | Minimum | Average | |
| 1º | 67,61±12,84 | 32,27±1,06 | 22,68±0,95 | 27,60±0,49 | 80,13±2,48 |
| 2º | 63,71±15,35 | 32,69±0,48 | 22,44±1,06 | 27,56±0,54 | 80,11±2,55 |
| 3º | 69,82±18,63 | 32,20±1,05 | 23,60±0,83 | 27,90±0,64 | 79,18±4,89 |

[1]ITGU- Globe and Humidity Temperature Index

The environment in which the research was carried out showed that the birds were raised under conditions of heat stress mainly during the third week of life, during the first and

second week temperatures were below and above the thermal comfort range, since according to [17] comfortable temperatures for birds are 31.3 ° C; 26.3 - 23.2 ° C and 22.5-23.2 ° C respectively in the first, second and third week of life. And the temperature of the globe and humidity in the first week goes from 77 to 81 and in the second and third week of life the interval is 74.5 to 77 [18].

The weights of the lymphoid organs did not differ from the control treatment (p> 0.05) and also there was no interaction between L-glutamine and zinc for these variables (p> 0.05) (Table 4).

Table 4. Absolute weight of the thymus, spleen and cloacal pouch of broilers fed diferente levels of L-glutamine and zinc in the phase of 1 to 21 days of age

| Parameters | Control | L-Glutamine (%) | Zinc (mg/kg) | | | Average | CV (%) | Valeu P | |
|---|---|---|---|---|---|---|---|---|---|
| | | | 0 | 90 | 120 | | | L | Q |
| Absolute weight (g) | | | | | | | | | |
| Thymus | 4,687 | 1 | 4,864 | 4,254 | 4,403 | 4,507 | 20,4 | 0,66 | 0,78 |
| | | 2 | 3,826 | 4,077 | 3,944 | 3,949 | | | |
| Average | | | 4,345 | 4,166 | 4,173 | | | | |
| Spleen | 0,912 | 1 | 0,904 | 0,818 | 0,797 | 0,840 | 19,8 | 0,73 | 0,23 |
| | | 2 | 0,779 | 0,999 | 0,834 | 0,871 | | | |
| Average | | | 0,841 | 0,908 | 0,816 | | | | |
| Cloacal pouch | 1,649 | 1 | 2,017 | 1,932 | 1,949 | 1,966 | 27,6 | 0,43 | 0,59 |
| | | 2 | 2,193 | 1,842 | 1,857 | 1,964 | | | |
| Average | | | 2,105 | 1,887 | 1,903 | | | | |

L, Q: probability of linear and quadratic order regarding the inclusion of zinc in the diet.

The highest scores for total and medullary area, and the lowest for the cortical / medullary ratio and percentage of cortical area for the cloacal pouch in the period from 1 to 21 days, were verified in the treatments with the 1% L-glutamine combinations without the addition of supplemental zinc and 2% of this amino acid with all zinc levels studied when compared to the control treatment (p <0.05) (Table 5). For the cortical area, no difference was found between treatments (p> 0.05).

Table 5. Mean of cloacal pouch histomorphometry scores as a function of L-glutamine and zinc levels added to diets of broiler chickens in the 1 to 21 days old phas

| Parameters | Levels of L-glutamine (%) and zinc (mg / kg) | | | | | | |
|---|---|---|---|---|---|---|---|
| | 0/0 | 1/0 | 1/90 | 1/120 | 2/0 | 2/90 | 2/120 |
| Total area | 8,80B | 18,40A | 14,60B | 14,80B | 26,40A | 23,20A | 19,80A |
| Medullary area | 8,40B | 18,80A | 14,20B | 15,00B | 26,20A | 23,60A | 19,80A |
| Cortical area | 18,80 | 16,80 | 19,80 | 19,80 | 17,60 | 17,20 | 16,00 |
| Cortical / medullary | 28,20A | 15,20B | 21,60AB | 18,80AB | 13,20B | 12,80B | 16,20B |
| % cortical area | 27,80A | 15,40B | 21,80AB | 18,00AB | 13,20B | 13,40B | 16,40B |

Averages followed by the same letter on the same line do not differ by the Kruskal-Wallis test at 5% probability.

The main objective of the research with functional nutrients, regarding the histomorphometric parameters of the cloacal pouch, is to provide the increase of the cortical area [19; 20], since it is in this region that differentiation and maturation occurs of B lymphocytes responsible for antibody production [21], allowing birds to respond promptly when exposed to an adverse situation. However, in this research, the addition of 1% of L-glutamine and 2% associated with all zinc levels studied, unlike other functional nutrients, increased the marrow area, in this way a more detailed study including research of the effect of this supplementation on the structure and cellular components of cloacal pouch become important to understand the behavior of these nutrients in relation to comfort conditions and in acute and chronic thermal stress.

Considering the above, it can be inferred that the cortical area scores and increase of the medullary area in response to L-glutamine and zinc supplementation may indicate lower antibody production, and, therefore, the birds were not in a position to respond promptly to adverse conditions. However, the increase of the marrow region can also be seen as a positive result of the addition of these nutrients in the diet, since this region is mainly composed of lymphoblasts [22], which are younger cells and which after maturation will form the lymphocytes.

Based on these studies, it was observed that the effects of adding L-glutamine and zinc on lymphoid organ weight and cloacal pouch histomorphometry seem to depend on factors such as age and the stimulus of stressors. Thus, it is assumed that the temperature oscillations above and below the values of the thermal comfort range verified in this research during the three weeks of life of the birds (Table 3) may not have been sufficient to trigger an immune response, since the The degree of immunosuppression depends on the duration and intensity of stress, animal genetics and individual physiology [23].

In relation to the intestinal morphometry of the duodenum,it was observed when comparing with control group that treatments with the addition of 1 and 2% of L-glutamine, both combined with 90mg of zinc / kg of feed presented lower crypt depth and lower height of vilo, respectively, ($p < 0.05$) (Table 6).

Table 6. Intestinal morphometry of the duodenum of broilers fed different levels of L-glutamine and zinc in the phase 1 to 21 days of age

| Control | L-Glutamine | Zinc (mg/kg) | | | Average | CV (%) | Value P | |
|---|---|---|---|---|---|---|---|---|
| | | 0 | 90 | 120 | | | L | Q |
| Crypt Depth (µm) | | | | | | | | |
| 172,427 | 1 | 151,952 | 110,695 * | 120,929 | 128,350 | 24,2 | 0,09 | 0,35 |
| | 2 | 133,102 | 124,622 | 112,602 | 123,440 | | | |
| Average | | 143,570 | 116,880 | 116,770 | | | | |
| Height of villi (µm) | | | | | | | | |
| 1169,220 | 1 | 1027,231 | 1025,27 | 993,062a | 1016,77 | 11,3 | 0,67 | 0,82 |
| | 2 | 1230,318 | 920,757 | 1001,052 | 1050,71 | | 0,01 | 0,02 |
| Average | | 1117,490 | 997,060 | 978,820 | | | | |
| Height of villi: Crypt Depth (µm) | | | | | | | | |
| 6,900 | 1 | 7,027b | 9,971a | 8,208a | 8,416 | 20,2 | 0,38 | 0,05 |
| | 2 | 9,500a | 7,743a | 8,922a | 8,722 | | 0,53 | 0,13 |
| Average | | 8,126 | 8,981 | 8,565 | | | | |
| Wall thickness (µm) | | | | | | | | |
| 1352,79 | 1 | 1234,168 | 1237,175 | 1187,560 | 1221,930 | 9,98 | 0,22 | 0,23 |
| | 2 | 1313,734 | 1113,84 | 1210,878 | 1212,82 | | | |
| Average | | 1269,530 | 1182,36 | 1199,220 | | | | |

Means followed by asterisks differ from the control treatment by Dunnett's test ($P < 0.05$).
Means followed by the same lowercase letter in the column for the same variable do not differ by Duncan's test ($P > 0.05$).
L, Q: probability of linear and quadratic order regarding the inclusion of zinc in the diet.

The lower crypt depth found for the duodenum with the combination of 1% L-glutamine and 90 mg zinc / kg, and in the ileum, with the addition of 1% L-glutamine combined with 120 mg of zinc, indicated a lower energy expenditure with cell renewal, because according to [24] high values of crypt depth indicate greater cell proliferative activity, aiming to guarantee adequate rate of epithelial renewal and to compensate for loss of villi heights, however, less deep crypts may indicate a better state of intestinal health [25].

As for the association of L-glutamine and zinc, it was observed that there was no influence on the depth of crypt and thickness of the duodenum wall ($p > 0.05$).

The values of zinc, within the levels 2 and 1% of L-glutamine, exerted a quadratic effect represented by the following equations: $\hat{Y} = 0.051x^2 - 8.0266x + 1230$, ($P < 0.05$, $R^2 = 1$) and $\hat{Y} = -0{,}0008x^2 + 0.1013x + 7.027$ ($P < 0.05$; $R^2 = 1$), with minimum and maximum points of 78.70 and 63.31 mg of zinc / kg of feed. In the unfolding of the interaction, it was observed that for the treatment without the addition of supplemental zinc, the inclusion of 2% of L-glutamine provided higher villi height and higher villi / crypt ratio ($p < 0.05$) (Table 6).

Regarding the jejunum morphometry, effect was found only for wall thickness for treatments with 1% L-glutamine without the addition of supplemental zinc and 2% of L-glutamine associated with 120mg of zinc / kg of feed when compared to the control ($p < 0.05$) (Table 7). These results indicate that nutrients have altered negatively the structure of the muscular wall of the segments, since these alterations are used as criteria for the identification of inflammatory bowel disease or antinutritional substances in the diets [14].

Table 7. Intestinal morphometry of the jejunum of broilers fed different levels of L-glutamine and zinc in the phase 1 to 21 days of age

| Control | L-Glutamin | Zn (mg/kg) | | | Average | CV (%) | Value P | |
|---|---|---|---|---|---|---|---|---|
| | | 0 | 90 | 120 | | | L | Q |
| Crypt Depth (µm) | | | | | | | | |
| 100,746 | 1 | 126,369 | 134,336 | 140,640 | 132,727 | 18,77 | 0,81 | 0,55 |
| | 2 | 140,736 | 123,576 | 132,073 | 132,129 | | | |
| | Average | 133,550 | 128,960 | 135,290 | | | | |
| Height of villi (µm) | | | | | | | | |
| 871,846 | 1 | 808,653 | 768,720 | 824,912 | 797,050 | 14,81 | 0,23 | 0,95 |
| | 2 | 761,615 | 869,598 | 891,541 | 837,30 | | | |
| | Average | 785,130 | 819,160 | 862,990 | | | | |
| Height of villi: Crypt Depth (µm) | | | | | | | | |
| 7,327 | 1 | 6,442 | 5,782 | 6,044 | 6,096 | 23,15 | 0,44 | 0,87 |
| | 2 | 5,756 | 7,185 | 7,185 | 6,710 | | | |
| | Average | 6,099 | 6,483 | 6,767 | | | | |
| Wall thickness (µm) | | | | | | | | |
| 836,187 | 1 | 1101,148* | 1009,028 | 1058,773 | 1055,940 | 10,51 | 0,30 | 0,48 |
| | 2 | 988,808 | 1074,790 | 1146,891* | 1064,680 | | | |
| | Average | 1044,980 | 1041,900 | 1109,130 | | | | |

Means followed by asterisks differ from the control treatment by Dunnett's test ($P < 0.05$).
Means followed by the same lowercase letter in the column for the same variable do not differ by Duncan's test ($P > 0.05$).
L, Q: probability of linear and quadratic order regarding the inclusion of zinc in the diet.

For the intestinal morphometry of the ileum, the Dunnett's test ($p < 0.05$) showed that birds receiving 1% L-glutamine combined with 120 mg of supplementary zinc / kg of feed presented lower crypt depth when compared to control birds (Table 8). However, the same effect was not observed for the other variables ($p > 0.05$).

Tabela 8. Morfometria intestinal do íleo de frangos de corte alimentados com diferentes níveis de L-glutamina e zinco na fase de 1 aos 21 dias de idade

| Control | L-Glutamine | Zn (mg/kg) | | | Average | CV (%) | Value P | |
|---|---|---|---|---|---|---|---|---|
| | | 0 | 90 | 120 | | | L | 0 |
| Crypt Depth (µm) | | | | | | | | |
| 114,706* | 1 | 81,197 | 104,238 | 66,829* | 84,089 | 22,17 | 0,203 | 0,041 |
| | 2 | 106,216 | 105,673 | 95,646 | 103,002 | | | |
| | Average | 93,707 | 104,956 | 79,637 | | | | |
| Height of villi (µm) | | | | | | | | |
| 610,388 | 1 | 584,177 | 574,410 | 501,294 | 553,294 | 18,62 | 0,139 | 0,512 |
| | 2 | 647,514 | 637,067 | 576,204 | 623,410 | | | |
| | Average | 615,850 | 665,740 | 534,590 | | | | |
| Height of villi: Crypt Depth (µm) | | | | | | | | |
| 5,762 | 1 | 7,176 | 5,629 | 7,541 | 6,782 | 18,31 | 0,998 | 0,041 |
| | 2 | 6,478 | 6,010 | 6,111 | 6,206 | | | |
| | Average | 6,827 | 5,820 | 6,905 | | | | |
| Wall thickness (µm) | | | | | | | | |
| 815,604 | 1 | 729,208 | 788,296 | 692,328 | 736,610 | 15,57 | 0,337 | 0,175 |
| | 2 | 808,727 | 826,170 | 738,075 | 794,770 | | | |
| | Average | 768,970 | 807,230 | 712,660 | | | | |

Means followed by asterisks differ from the control treatment by Dunnett's test ($P < 0.05$).
L, Q: probability of linear and quadratic order regarding the inclusion of zinc in the diet.

The combined supplementation of L-glutamine and zinc did not influence the variables analyzed for ileal morphometry ($p > 0.05$). However, in an isolated way, the zinc levels exerted a quadratic effect on the depth of crypt and the relation of height of villi: depth of crypt, respectively, according to the following equations: $\hat{Y} = -0{,}0081x^2 + 0{,}8517x + 93{,}707$ ($P < 0$) ($P < 0.05$, $R\ 2 = 1$), reaching the maximum and minimum values with 52.57 and 58.37 mg of zinc / kg diet.

Supplementation of L-glutamine and zinc suggests positive effects on the development of intestinal structures [26; 27; 28]. However, in this study addition of this amino acid isolated or combined with supplemental zinc did not provide the expected effect on the absorptive surface of the duodenum, jejunum and ileum, which would be lower crypt

depth and higher villi height, which would indicate better absorption of nutrient and lower energy losses with cell turnover [29].

According to [28] the zinc acts by repairing the intestinal lesions, reducing the apoptosis index and increasing the proliferation rate of epithelial cells, villus height and villus: crypt ratio. In this way, it can be inferred that in this research the absence of effects of zinc supplementation on these variables may be related, in part, to the possible difference in the immunological status of the birds.

The absence of effects of L-glutamine and zinc supplementation on small intestinal morphometry may be related to the immunological status of the birds and also to intestinal maturation, since the complete development of the duodenum villi of broilers occurs until the seven [30] and of the jejunum and ileum continue until 14 days of age [31]. That is, to the 21 days of age the birds already presented complete development of the intestinal structures. Some authors [3; 27] confirmed this proposition, because when investigating the effect of the addition of L-glutamine in the pre-initial phase of broilers, observed a better development of the intestinal mucosa, showing that this amino acid plays an important role in the maturation of the intestines of the chicks, which occurs in the first days of life of the birds [30].

## IV Conclusions

The addition of L-glutamine and zinc in broilers diets in the period from 1 to 21 days of age, although not influencing the weight of the lymphoid organs, provides an increase in the total and medullary area of the cloacal pouch, of jejunal wall thickness and lower depth of crypts of the duodenum and ileum.

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