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Performance, intestinal integrity and immune response in broilers fed diets supplemented with L- glutamine

Comportamiento productivo, integridad intestinal y respuesta inmune en pollos alimentados con dietas suplementadas con L-glutamina



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ABSTRACT

In two experiments, the addition of AminoGut (AG) was evaluated. In Experiment 1, 300 Ross 308 broilers were used in five treatments with three repetitions of 20 broilers each. In Experiment 2, 640 broilers were used in four treatments with five repetitions of 32 broilers each. Exp. 1 treatments: T1.- Control diet sorghum + soybean meal, T2.- As 1 + 700 ppm of the mixture of AG/ ton, T3.- As 1 + 1400 ppm of AG/ ton, T4.- As 1 + 2100 ppm of AG/ ton, T5.- As 1 + 2800 ppm of AG/ ton. In Exp. 2, treatment 5 was eliminated. In Experiment 1, there was an effect ($P<0.05$) on performance and carcass weight, with 700 ppm of AG. In Experiment 2, the height of intestinal villi and late hypersensitivity ($P<0.05$) improved with 700 ppm of AG, as well as carcass yield at 42 days. It can be concluded that the addition of 700 ppm of AminoGut in diets in broilers from 1 to 21 days of age, increased the performance, villus length, carcass yield and immune response.

Keywords: AminoGut, Glutamine, Glutamic Acid, performance, intestinal villi, immune response.

RESUMEN

En dos experimentos se evaluó la adición de AminoGut (AG). En el Experimento 1, se utilizaron 300 pollos Ross 308 en 5 tratamientos con 3 repeticiones de 20 pollos cada una. En el Experimento 2, se emplearon 640 pollos en 4 tratamientos con 5 repeticiones de 32 pollos cada una. Los tratamientos del Exp. 1: T1.- Dieta testigo sorgo + pasta de soya, T2.- Como 1 + 700 ppm de la mezcla de AG/ ton, T3.- Como 1 + 1400 ppm de AG/ ton, T4.- Como 1 + 2100 ppm de AG/ ton, T5.- Como 1 + 2800 ppm de AG/ ton. En el Experimento 2 se eliminó el tratamiento 5. En el Experimento 1, hubo efecto ($P<0.05$) en comportamiento



productivo y peso de la canal, con 700 ppm de AG. En el Experimento 2, mejoró la altura de vellosidades intestinales e hipersensibilidad tardía ($P < 0.05$) con 700 ppm de AG, así como el rendimiento en canal a los 42 días. Se puede concluir que la adición de 700 ppm de AminoGut en dietas para pollos de 1 a 21 días de edad, incrementó los parámetros productivos, longitud de las vellosidades, rendimiento en canal y respuesta inmune.

Palabras claves: AminoGut, parámetros productivos, vellosidades intestinales, respuesta inmune.

INTRODUCTION

Commercial broiler production is a dynamic activity in farm animals; this is due to genetic selection for rapid growth, good feed conversion and carcass yield (Cuca *et al.* 2009). Under commercial conditions, chicks can go 24 to 48 hours without consuming feed and water after leaving the emergence and transportation to production farms. This can cause a decrease in yolk reserves, stress and mortality in the newly hatched chick. Therefore, it is important that the chick have immediate access to feed and water for optimal intestinal development (Zulkifli *et al.* 2016).

Excess birds per square meter is a stress trigger, which can affect intestinal integrity and decreases the immune response in the intestinal epithelium (Wu *et al.* 2021). The use of glutamine in poultry diets may decrease such adverse effects on the intestinal epithelium, as glutamine is the main metabolic fuel for enterocytes, lymphocytes, macrophages, and fibroblasts in the small intestine (Bortoluzzi *et al.* 2018; Wu *et al.* 2018).

Glutamine is a free neutral amino acid in large amounts in muscle and plasma; its function is the synthesis of nonessential amino acids, nucleotides, nucleic acids, sugars, and proteins. Glutamine can be synthesized from ammonium and glutamate in skeletal muscle. Although glutamine is a nonessential amino acid, its requirements may not be met under stress conditions (Wu *et al.* 2021). Glutamine supplementation improves growth, gut development, meat quality and humoral immune response by supporting optimal lymphocyte proliferation and cytokine production by lymphocytes and macrophages (Bartell & Batal, 2007; Bortoluzzi *et al.* 2018; Wu *et al.* 2018; Jarred *et al.* 2019).

Aminogut commercial product, a commercial mixture with 10 % glutamine and 10 % glutamic acid to incorporate glutamine, has been shown to reduce mortality under heat stress conditions and in chickens reared at high stocking densities (Wu *et al.* 2018; Wu *et al.* 2021). The addition of glutamine improves weight gain, feed conversion and intestinal villus length (Olubodun *et al.* 2015; Zulkifli *et al.* 2016; Bortoluzzi *et al.* 2018; Xi *et al.* 2019).



The addition of glutamine, in addition to improving productive parameters, helps to decrease lesions in duodenum, jejunum and ileum, when chickens are challenged with the bacteria causing necrotic enteritis (Xue *et al.* 2018). Given the above, the inclusion of glutamine in broiler diets may also be an alternative to the use of antibiotics as growth promoters (Pelicia *et al.* 2013).

With this background, the objective of the present work was to evaluate the inclusion of various low levels of AminoGut in sorghum-soybean paste diets for broilers from 1 to 49 days of age and to measure its effect on productive behavior, carcass yield, intestinal integrity and cellular immunity.

MATERIAL AND METHODS

Two experiments were carried out at the Center for Education, Research and Extension in Poultry Production (CEIEPAv) from Faculty of Veterinary Medicine and Animal Husbandry of the National Autonomous University of Mexico. It is located at Manuel M. López street S/N, Santiago Zapotitlán Town, Delegation Tláhuac, Federal District, at an altitude of 2250 masl, at the parallel 19°17' north latitude and the meridian 99° 02' 30" west longitude. Under temperate sub-humid climate conditions (Cw). January is the coldest month and May is the warmest, the average annual temperature is 16°C and with an average annual rainfall of 747 mm (INEGI 2017).

Institutional Committee approved the experimental procedures for the Care and Use of Experimental Animals at the Faculty of Veterinary Medicine and Animal Husbandry of the National Autonomous University of Mexico with the official letter SICUAE MC-2023/2-2.

Experiment 1. Three hundred -1-day-old chicks of the Ross 308 strain, acquired in a commercial hatchery, were used. The birds were distributed in 15 flocks of 20 chicks each (half males and half females); housed in a conventional house in pens with cement floor, chip litter, without thermal insulation in the roof and side curtains.

In Experiment 2, 640 1-day-old chicks of the Ross 308 strain, obtained from a commercial hatchery, were used. Birds were distributed in 20 flocks of 32 chicks each (half males and half females) housed in a conventional house, pens with cement floor, chip litter, thermal insulation in the roof and side curtains. A stocking density of 12 birds/m² was managed in each pen. Birds were weighed one by one at the end of the experiment (49 days) to calculate flock uniformity.

In both experiments, birds were provided with heat during the first 4 weeks of life with infrared brooders (Quintana, 2011). In both experiments, birds were fed sorghum + soybean paste diets throughout the cycle; three feeding stages were managed, from 0-10 day's onset, 11 - 21 days growth and 22 - 49 days completion (Lesson & Summers 2005).



Glutamine 10 % + glutamic acid 10 % was added to the diets by means of a commercial AminoGut® mixture.

It was provided from 1-21 days of age (initiation and growth stages); subsequently, all treatments were provided with finishing diets from 22 to 49 days of age. Feed was provided free access throughout the production cycle. Diets used in both experiments are shown in Table 1.

Table 1. Composition of the experimental starter, grower and finisher diets used in Experiments 1 and 2 (kg)

| Ingredients | Initiator (0-10 days) | Growth (11-21 days) | Completion (22-49 days) |
|-------------------------|--------------------------|------------------------|----------------------------|
| Sorgum | 502.483 | 565.827 | 626.560 |
| Soybean Paste | 406.842 | 331.782 | 275.553 |
| Vegetable oil | 41.494 | 57.388 | 54.170 |
| Calcium Phosphate | 18.413 | 16.329 | 15.150 |
| Calcium carbonate | 15.267 | 13.897 | 13.388 |
| Salt | 3.814 | 3.837 | 3.848 |
| DL-Methionine | 3.471 | 2.929 | 2.048 |
| Choline Chloride 60% | 3.400 | 3.400 | 0.800 |
| L-Lysine HCl | 1.788 | 1.625 | 0.661 |
| Vitamin+Mineral Premix* | 1.500 | 1.500 | 1.500 |
| L- Threonine | 0.587 | 0.473 | 0.042 |
| Coccidiostat*** | 0.500 | 0.500 | 0.500 |
| Bacitracin MD 10% | 0.300 | 0.300 | 0.300 |
| Antioxidant | 0.150 | 0.150 | 0.150 |
| Piveg yellow (15g/kg) | 0.000 | 0.000 | 5.330 |
| Total | 1000 | 1000 | 1000 |
| Nutrient | Calculated analysis | | |
| ME (kcal/kg) | 3010 | 3175 | 3225 |
| Crude protein % | 24.00 | 21.00 | 19.00 |
| Met + Cis % | 1.059 | 0.925 | 0.785 |
| Lysine % | 1.435 | 1.217 | 0.998 |
| Calcium % | 1.000 | 0.900 | 0.850 |
| Non-phytic phosphorus % | 0.500 | 0.450 | 0.420 |
| Sodium % | 0.160 | 0.160 | 0.160 |

*Provides per Kg. Vitamin A 12, 000,000 IU; Vitamin D3 2, 500,00 IU; Vitamin E 15,000 IU; Vitamin K 2.0g; Thiamine 2.25g; Riboflavin 7.5g; Cyanocobalamin 0.010 g; Folic Acid 1.5g; Pyridoxine 1.5g; Calcium Pantothenate 10g; Niacin 45g. **Provides per kg Selenium 0.2g; Cobalt 0.2g; Iodine 0.3g; Copper 10g; Zinc 50g; Iron 100g; Manganese 110g; excipient cbp1000g.

*** Nicarbazin during initiation and growth; monensin during termination



In experiment 1, treatments were formed as follows. In experiment 2, only the first 4 treatments were used.

- T1= Sorghum diet + Soybean Paste.
- T2= As 1 + 700 ppm of AG for 21 days on Top (extra inclusion in the diet).
- T3= As 1 + 1400 ppm of AG for 21 days on Top.
- T4= As 1 + 2100 ppm of AG during 21 days on Top.
- T5= As 1 + 2800 ppm AG for 21 days on Top.

Water was offered in both investigations at free access during the 49 days of experimentation. Birds were vaccinated at 8 days of age against Newcastle disease (ND) via eye (one drop/chicken) and subcutaneously against Newcastle disease-Avian Influenza (ND/AI, 0.5 ml/bird). At 16 days of age, they were revaccinated only subcutaneously against ND/AI.

In both studies, weekly records of weight gain, feed consumption and feed conversion rate were kept.

In Experiment 2: at day 21 of age, the cellular immune response was evaluated by means of the basophilic cutaneous hypersensitivity test, by inoculation in the interdigital membrane of the lower limbs of the birds (10 chicks per treatment). The intradermal inoculation of Phytohemagglutinin (PHA-A Sigma-Aldrich, Inc.), was at a concentration of 0.1 mg/0.1 ml in the interdigital membrane of phalanges 3 and 4 of the right lower limb, using 2 chickens per replicate. The same procedure was performed on the interdigital membrane of the left leg, using sterile saline solution (0.1 ml) as a control. At 24 h post-inoculation, the thickness of the interdigital membrane was determined with a digital vernier, using the methodology described by [Gómez *et al.* \(2010\)](#).

At day 35 of age, 15 birds per treatment were slaughtered in order to determine the morphology of the intestinal villi of the duodenum. Duodenum portions taken were approximately 1 cm³ per sample. The processing of the samples was carried out in the tissue biology laboratory of the Department of Morphology of the Faculty of Veterinary Medicine and Animal Husbandry of the National Autonomous University of Mexico. The tissues were processed using the kerosene embedding technique; for this purpose, an automatic tissue processor (histokinete) was used to dehydrate, clarify and impregnate the samples. The reagents used were alcohol, xylol (Baker[®]) and kerosene (paraplast[®]). Using a Leica[®] model RM215RT microtome, 6 µm thick tissue sections were obtained from the kerosene blocks. The sections were placed on 25X75 mm slides and 0.8 - 1.1



mm Corning® brand coverslips; finally, they were stained with the Hematoxylin and Eosin technique (Morales, 2013).

Samples in which the length of intestinal villi was measured were obtained from 15 birds of each treatment at 35 days of age. Birds were humanely slaughtered as indicated in NOM-033-SAG/ZOO-2014. Access to the abdominal cavity was at 2 cm in caudal direction, tissues were obtained and a sample was taken from the duodenal loop with the use of scalpel, scissors and forceps. The tissue fragments obtained were approximately 1 cm³, to remove the intestinal content; sterile physiological saline solution at room temperature was used to clean the intestinal fragments. The fixation technique used was by luminal perfusion, and later by 10 % formalin immersion. The fixation time was 72 hours at room temperature (Estrada *et al.*, 1982).

At the end of the experiments, 15 birds per treatment were slaughtered at the CEIEPAv processing plant. Before slaughter, the birds were fasted for 8 hours and weighed individually to calculate carcass yield. On the other hand, the yellow pigmentation (b) of the skin was measured in hot (after slaughter), in the region of the breast fat, with a Minolta® CR-400 reflectance colorimeter.

The data of the variables under study were subjected to an analysis of variance, according to a completely randomized design, and if there was a statistical difference, a comparison of means test was performed using Tukey's test at a probability of 5% and 1%. For the mortality percentage variable before analysis, the arc-sine root transformation was performed before analysis with the SPSS version 17.0 statistical package.

RESULTS

Experiment 1

Table 2 shows the average data of the productive parameters at 49 days of age of the birds, with statistical difference ($P < 0.05$) between treatments, for weight gain, feed consumption, feed conversion and carcass yield; with better results in the treatment with 700 ppm of AminoGut, with respect to the other treatments. For the general mortality variable, no differences ($P > 0.05$) were found between treatments.



Table 2. Productive variables of chicks from 0-49 days (experiment 1)

| Treatment ppm AG | Weight gain (g) | Feed intake (g) | Feed conversion rate (g/g) | Carcass weight (g) |
|---------------------|-------------------|-------------------|-------------------------------|--------------------|
| 0 | 2903 ^b | 5684 ^b | 1.96 ^b | 2108 ^b |
| 700 | 3267 ^a | 5944 ^a | 1.82 ^a | 2375 ^a |
| 1400 | 3047 ^b | 5780 ^b | 1.90 ^{ab} | 2254 ^{ab} |
| 2100 | 3017 ^b | 5884 ^b | 1.95 ^{ab} | 2250 ^{ab} |
| 2800 | 2988 ^b | 5687 ^b | 1.90 ^{ab} | 2159 ^b |

Values with different letters for each variable are different ($P < 0.05$)

Experiment 2

Table 3 shows the results obtained at 49 days of age for weight gain, feed intake, feed conversion, carcass weight and skin yellowing. In the results obtained for weight gain, feed consumption and feed conversion were not significant differences ($P > 0.05$), among treatments.

It can be observed that in the carcass weight variable, there were differences ($P < 0.05$) ($P > 0.05$) between treatments, with greater carcass weight in the birds treated with AG. For breast skin yellowness, there were no differences between treatments ($P > 0.05$) (Table 3).

Table 3. Productive behavior of 0- 49 day-old chicks (experiment 2)

| Treatment ppm | Weight gain (g) | Feed intake (g) | Feed conversion (g/g) | Carcass weight (g) | Skin yellowing (b) |
|------------------|-------------------|-------------------|--------------------------|-----------------------|--------------------|
| 0 | 3026 ^a | 5908 ^a | 1.95 ^a | 2118 ^b | 46.0 ^a |
| 700 | 3043 ^a | 5889 | 1.93 ^a | 2191 ^{ab} | 47.0 ^a |
| 1400 | 3046 ^a | 5878 ^a | 1.93 ^a | 2223 ^a | 48.0 ^a |
| 2100 | 3046 ^a | 5831 ^a | 1.91 ^a | 2255 ^a | 48.0 ^a |

Values with the same letter are similar ($P > 0.05$)

Table 4 shows the average data obtained in the evaluation of the cellular immune response, by means of the late basophilic hypersensitivity test at 21 days of age. The results indicated an increase in the interdigital thickness after inoculation with phytohaemagglutinin with a significant effect ($P < 0.05$), where it can be seen that treatments 2, 3 and 4, which included AG, had a greater thickness of the interdigital



membrane, compared to the negative control treatment. In the case of inoculation with physiological saline solution, no differences were observed ($P>0.05$) between treatments.

The data of the measurement of the length of the villi in the duodenum at 35 days of age of the birds are also shown in table 4. It can be seen that there was a difference ($P<0.05$) between treatments; where treatment 2 with 700 ppm AG, showed the greatest height of the intestinal villi in the duodenum, with respect to the other treatments.

Table 4. Results of the late hypersensitivity test at 21 days of age and intestinal villi length in the duodenum of 35-day-old chicks in experiment 2

| Treatment ppm Gln/Glu | PHA day 21* mm | SS day 21** mm | Intestinal villi length (μm) |
|--------------------------|-------------------|-------------------|---|
| 0 | 0.48 ^b | 0.16 ^a | 2103 ^b |
| 700 | 0.58 ^a | 0.20 ^a | 2534 ^a |
| 1400 | 0.56 ^a | 0.15 ^a | 2070 ^b |
| 2100 | 0.56 ^a | 0.15 ^a | 2198 ^b |

Values with different letters for each variable are different ($P<0.05$). *PHA= Phytohaemagglutinin. **SS= Sterile saline solution

DISCUSSION

In the present study, it was observed that the addition of low doses of AminoGut (700 ppm) to broiler diets had an effect on weight gain, feed intake and feed conversion. In experiment 1 and experiment 2, there was a significant effect on carcass weight yield. These results coincide in part with those obtained by [Miguel *et al.* \(2009\)](#), who found improvements in weight gain, feed conversion ratio and carcass yield at 49 days of age when adding 200 to 800 ppm of AG to sorghum + soybean basal diets. On the other hand, [Bartell & Batal \(2007\)](#), added high doses of glutamine (1000 and 4000 ppm) in broiler diets, where they observed higher weight gain and feed conversion in broilers that received 10000 ppm glutamine; not so when 4 % was included in the diet, in which they found a toxic effect on the birds. As well as [Xi *et al.* \(2019\)](#) used different levels of glutamine (500, 1000 and 1500 ppm) in corn-soy diets for 0-14 day old Arbor Acres chickens, where they show that chickens fed glutamine (1000 and 1500 ppm) improved weight gain and feed conversion. Also [Wu *et al.* \(2019\)](#) added 500 and 1000-ppm glutamine to corn-soy diets for 1-21 day old broilers; where they found higher body weight in the glutamine treatments compared to the negative control diet. However, [Jarred *et al.* \(2019\)](#) included (500 and 1000 ppm) glutamine in the diet of 1- to 14-day-old chicks, and found no significant effect on body weight and feed conversion. Other researchers also



found no beneficial effect on performance when they added (500 ppm) AminoGut to diets for 1-42 day-old chicks under two stocking densities ([Shakeri et al. 2014](#)). These results partly coincided with those obtained in experiment 2, where there was no effect of glutamine and glutamic acid on performance parameters. Other authors found no beneficial effect of adding 1000-ppm glutamine, arginine and glycine to reduced protein diets for chickens subjected to a leaky gut model ([Barekatin et al. 2019](#)).

The results indicated higher carcass weight and breast weight in the treatments with these amino acids. Similarly, [Miguel et al. \(2009\)](#) observed that the use of AG improved broiler carcass yield. [Hu et al. \(2016\)](#) found that the inclusion of 1000-ppm glutamine in diets for Arbor Acres chickens from 1 to 35 days of age improved carcass quality (in pectoralis major muscle). On the other hand, [Kriseldi et al. \(2017\)](#), investigated in 1-41 day old broilers, including 1920 and 2240-ppm glutamine in reduced protein diets, where they found higher carcass and breast yield in broilers fed glutamine, compared to treatments without the amino acid.

In the present study, an increase in intestinal villus height was found with the inclusion of 700 ppm AG. These results were similar in part to those obtained by [Bartell & Batal \(2007\)](#), who added 1000 and 4000-ppm glutamine and found an increase in the length of intestinal villi in the duodenum compared to untreated birds. [Jarred et al. \(2019\)](#), included 500 and 1000-ppm glutamine in the diet of 21-day-old chicks, where they reported increased villus length in jejunum and decreased crypt depth compared to non-glutamine-treated chicks. However, other authors such as [Wu et al. \(2018\)](#) found no significant increase in jejunum and ileum intestinal villi length at 4, 7, 14 and 21 days of age when 500 and 1000 ppm glutamine was added to corn-soy diets. These authors did not report the length of villi in duodenum.

In the present investigation, the cellular immune response by means of the late basophilic hypersensitivity test evaluated at 21 days of age of chicks, was greater when AG was included, which indicates that these amino acids when consumed by the chicks promoted a greater cellular immune response as indicated by some studies such as that of [Bortoluzzi et al. 2018](#). [Liu et al. \(2020\)](#) indicate that the glutamine inclusion in the diet of 1-21 day old chickens increased the number of intraepithelial lymphocytes, number of goblet cells and decreased crypt depth in jejunum and ileum, compared to chickens infected with *Salmonella enteritidis*; but it wasn't better than the control group. In another study by [Wu et al. \(2021\)](#), they report that the inclusion of glutamine at 500 and 1000 ppm in the diet of growing chickens increased the number of intraepithelial lymphocytes in duodenum and jejunum, the phagocytic index of heterophils, number of goblet cells and the content of immunoglobulins (IgA, IgG and IgM) in the intestinal mucosa and serum. However, these studies did not evaluate the basophilic late hypersensitivity test in



interdigital subcutaneous tissue, in order to compare the results of this test performed in the present investigation.

Finally, most of the research conducted in broilers indicates that the addition of high doses (1000 to 4000 ppm) of AminoGut in the diet improves productive parameters and intestinal health by promoting greater length and integrity of intestinal villi.

In the present study, it was found that the inclusion of 700 ppm AminoGut in the diet of 1-21 day old broilers improved productive behavior and cellular and humoral immune response

CONCLUSIONS

From the results obtained and under the experimental conditions used in this study, it can be concluded that the use of 700 ppm AminoGut in sorghum + soybean diets during the first 21 days of age had a promoting effect, improving weight gain, carcass weight and feed conversion ratio in Ross 308 broilers at 49 days of age. Intestinal villi length at 35 days of age was greater, also with the inclusion of 700 ppm AminoGut in sorghum-soy diets for broilers. The inclusion of 700 ppm AminoGut increased the cellular immune response of the birds to the basophilic skin hypersensitivity challenge.

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