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Use of Glutamine in Post-Hatch Feed for Broiler Chicks

ABSTRACT

Post-hatch delayed placement damages, physical and physiological development of broiler chicks. The objective of this study was to ascertain adequate levels of glutamine inclusion in post-hatch and pre-starter feed, in order to minimize the negative effects of post-hatch delayed placement on broiler chicks. The birds were distributed in a completely randomized design, with four treatments and five replicates of ten birds each. Four levels of glutamine supplementation (0, 1, 2 and 3%) were used in the pre-starter feed, which was given to the chicks in the transportation box and during the pre-starter phase. After 24 hours of access to this feed, and at 7 days of age, the chicks' performance, yolk sac retraction, plasma glucose concentration, weight and small-intestine histomorphometry were evaluated. From seven to ten days of age, a metabolizability assay was performed. Glutamine supplementation tended to increase the use of the yolk sac, the concentration of plasma glucose ($p < 0.10$) and the depth of the crypt in the ileum after 24 hours of life ($p < 0.05$). There was no statistical difference between the glutamine supplementation levels, in relation to glucose, histomorphometry or metabolizability in the pre-starter phase. Glutamine concentrations did not differ in relation to performance, histomorphometry of the small intestine or metabolizability of nutrients in the pre-starter phase.

INTRODUCTION

The post-hatch period is considered to be a critical time for broiler chicks. It is a phase of adaptation to the environment external to the egg and specially to feed coming from exogeneous sources. What makes this period even more difficult is the fasting that these chicks undergo.

Because of standard incubator procedures, chicks do not have access to feed for a period of approximately 48 hours, from their hatching until their placement, which could mean they do not have access to feed for two or three days (Bhuiyan *et al.*, 2011). Delayed intake of feed can consequently hinder physiological and immunological development, thus reducing the overall productivity of chick-rearing (Van Den Brand *et al.*, 2010).

Specific formulations are used for feed in the post-hatch and pre-starter phases of broiler chicks. These include the presence of trophic agents to attenuate the undesirable effects of post-hatch fasting, with aim to achieve greater precocity in the physical and physiological development of broiler chicks.

Glutamine is among the possible trophic agents used. The importance of this substance is mainly in relation to the intestinal development of broiler chickens, such that it leads to greater use of nutrients from their diets, especially among birds that are exposed to



unfavorable conditions. Pelícia *et al.* (2015) observed a significant increase in the height of villi and depth of crypts through supplementation with glutamine in the diets of broiler chickens at the age of seven days.

Several studies on glutamine supplementation for broiler chickens have been conducted, but there is still little information regarding the possible use and benefits of this amino acid in the post-hatch and pre-starter phases, or regarding what the best supplementation to use during these phases might be.

In this light, the objective of the present study was to ascertain the appropriate levels of glutamine inclusion in post-hatch and pre-starter feed, from the

perspectives of minimizing the negative effects of post-hatch delayed placement and ensuring intestinal integrity and nutritional support for these chicks.

MATERIAL AND METHODS

The experiment was approved by the Ethics Committee on the Use of Animals (CEUA) under protocol no. 008/13.

The treatments consisted of four levels of supplementation (0, 1, 2 and 3%) of glutamine in the pre-starter feed. The experimental feeds were created from a baseline feed (Table 1), through adding doses of glutamine as a substitute for starch.

Table 1 – Composition and values calculated (%) for the baseline feed.

| Ingredients | Pre-starter feed 1 to 7 days | | | |
|---------------------------------|------------------------------|---------|---------|---------|
| | 0 | 1 | 2 | 3 |
| % of glutamine | | | | |
| Corn | 51.06 | 51.06 | 51.06 | 51.06 |
| Soybean meal | 39.50 | 39.50 | 39.50 | 39.50 |
| Soybean oil | 2.35 | 2.35 | 2.35 | 2.35 |
| Calcitic limestone | 0.88 | 0.88 | 0.88 | 0.88 |
| Dicalcium phosphate | 1.94 | 1.94 | 1.94 | 1.94 |
| L-lysine | 0.20 | 0.20 | 0.20 | 0.20 |
| DL-methionine | 0.25 | 0.25 | 0.25 | 0.25 |
| Threonine | 0.10 | 0.10 | 0.10 | 0.10 |
| Sodium bicarbonate | 0.19 | 0.19 | 0.19 | 0.19 |
| Table salt | 0.39 | 0.39 | 0.39 | 0.39 |
| Vitamin supplement ¹ | 0.10 | 0.10 | 0.10 | 0.10 |
| Mineral supplement ² | 0.05 | 0.05 | 0.05 | 0.05 |
| Glutamine | 0.00 | 1.00 | 2.00 | 3.00 |
| Starch | 3.00 | 2.00 | 1.00 | 0.00 |
| Total | 100.00 | 100.00 | 100.00 | 100.00 |
| Calculated composition | | | | |
| Metabolizable energy (kcal/kg) | 2951.00 | 2950.00 | 2949.00 | 2947.00 |
| Crude protein (%) | 22.280 | 23.478 | 24.676 | 25.870 |
| Calcium (%) | 0.922 | 0.922 | 0.922 | 0.922 |
| Available phosphorus (%) | 0.471 | 0.471 | 0.471 | 0.471 |
| Methionine (%) | 0.542 | 0.542 | 0.542 | 0.542 |
| Methionine + cystine (%) | 0.850 | 0.850 | 0.850 | 0.850 |
| Lysine (%) | 1.312 | 1.312 | 1.312 | 1.312 |
| Threonine (%) | 0.852 | 0.852 | 0.852 | 0.852 |
| Sodium (%) | 0.223 | 0.223 | 0.223 | 0.223 |
| Chlorine (%) | 0.283 | 0.283 | 0.283 | 0.283 |

¹ Vitamin supplement – guaranteed level per kilogram of product: 3,125,000 IU of vitamin A; 550,000 IU of vitamin D3; 3,750 mg of vitamin E; 625 mg of vitamin K3; 250 mg of vitamin B1; 1,125 mg of vitamin B2; 250 mg of vitamin B6; 3,750 mg of vitamin B12; 9,500 mg of niacin; 3,750 mg of calcium pantothenate; 125 mg of folic acid; 350,000 mg of DL-methionine; 150,000 mg of choline chloride 50%; 50 mg of selenium; 2,500 mg of antioxidant; and 1,000 g of vehicle q.s.p.

² Mineral supplement – guaranteed levels per kilogram of product: manganese, 150,000 mg; zinc, 100,000 mg; iron 1,00,000 mg; copper, 16,000 mg; and iodine, 1,500 mg.

The pre-starter feed (Table 1) was formulated using corn and soybean meal, following the recommendations for nutritional demands and feed composition proposed by Rostagno *et al.* (2005).

A total of 1,200 male chicks of the Cobb 500 strain were used. These chicks originated from eggs

produced by laying hens that were all of similar age, and their eggs were incubated in the same machine.

After a birth window of 12 hours, the chicks were removed from the hatchery. They were vaccinated and sexed, following the standard procedures of the hatchery, and were then transported to the aviary. All



procedures conducted with the chicks, from hatching until housing, lasted 24 hours.

The birds were distributed in the aviary following a completely randomized design. There were four treatments, with five replicates of 60 birds each, in the transportation boxes, which received 3 g/bird of pre-starter feed for consumption over 24 hours. During this 24-hour period, the chicks did not receive water, thus simulating what happens in the hatchery. After 24 hours of experimental feed consumption, 10 chicks per replicate, according to the average plot weight, were transferred to galvanized steel cages equipped with linear feeders and water dispensers. The chicks continued to receive the experimental feed in the cages until they reached seven days of age.

Water and feed were offered freely over the whole rearing period, with a 24-hour light cycle. The temperature of the environment was monitored twice a day (8:00 AM and 5:00 PM) using maximum and minimum thermometers within the shed. The mean maximum temperature during the experiment was 33.47 °C, while the mean minimum temperature was 25.48 °C.

To assess the effect of the experimental feed, the chicks underwent body weighing, weighing of the yolk sac and intestine, collection of fragments from the duodenum, jejunum and ileum, and measurement of glucose concentration over three stages of the experiment. The first sampling took place as soon as the 24-hour birth window closed and involved 15 chicks as a control group (mean body mass 44.54 g, mean yolk sac mass 5.87 g, mean intestine mass 1.87 g and blood glucose of 348.2); the second time was after the 24 hours within the transportation box, before the chicks were housed, with sampling of five birds per treatment; and the third sampling was conducted when the chicks completed seven days of life, again including five birds per treatment.

Weight gain was calculated as the difference between the final and initial weights. Feed consumption was calculated as the difference between the feed supplied and the leftovers at the end of each phase. Feed conversion was obtained through the ratio of feed consumption/weight gain. Viability was assessed through the formula $V (\%) = 100 - \text{mortality}$.

Glucose concentration was assessed through analysis within thirty seconds after drawing blood, using portable glucose metering equipment.

Five birds from each treatment were euthanized to evaluate intestinal development and yolk sac retraction.

The yolk sac and intestines (small and large) were then weighed on a scale with precision of 0.0001 g.

One chick per replicate was used for histomorphometric assessment of the small intestine. Two-centimeter fragments were collected from the duodenum, jejunum and ileum, and were fixed in buffered 10% formaldehyde for 24 hours. These samples were then subjected to standard histological procedures to produce histological slides. The sections were stained with hematoxylin and eosin (Hu *et al.*, 2012; Sousa *et al.*, 2015). The heights of 10 villi and the depths of 10 crypts were measured on each section.

A metabolic assay was conducted between the ages of seven and 10 days, using the total fecal collection method. Excreta were collected twice a day (8:00 AM and 5:00 PM), stored in plastic bags (which were identified according to the replicate) and kept in a freezer. At the end of the experimental period, the amount of feed consumed was determined, along with the total amount of fecal material produced. The quantities of excreta and experimental feed were used to determine the dry matter, nitrogen and ether extract content, as described by Silva & Queiroz (2002).

The results from bromatological analyses were used to calculate metabolizability coefficients (MC) for dry matter (MCDM), nitrogen (MCN) and ether extract (MCEE). Nitrogen balance (NB) and ether extract balance (EEB) were also calculated, using the formula:

$$MC (\%) = (\text{nutrient B} / \text{nutrient ingested DM (g)}) \times 100$$

Where nutrient B (g) = nutrient ingested – nutrient excreted

Variance analysis was performed to assess the significance of treatment effects using the SAEG statistical program (2007). Having significance, the polynomial regression analysis ($p < 0.05$) was applied, although p values between 0.05 and 0.10 are shown and described as trend.

RESULTS AND DISCUSSION

Descriptive presentation of the values of the live weights of the chicks before and 24 hours after the experimental ration, and the amounts of feed consumed for a better understanding of the subsequent results are shown in (Table 2). The values showed that the chicks consumed little amount of feed and, therefore, presented an average weight loss of 2.63 g during the period of 24 hours inside the transport boxes.



Table 2 – Mean weight loss and consumption of pre-starter feed supplemented with glutamine that was given to recently hatched chicks while they were in transportation boxes.

| Treatment | Weight before (g) | Weight after 24 hours (g) | Weight loss (g) | Feed consumption (g) |
|-----------|-------------------|---------------------------|-----------------|----------------------|
| Glutamine | | | | |
| 0% | 45.45 | 42.90 | 2.55 | 0.009 |
| 1% | 45.81 | 43.31 | 2.49 | 0.026 |
| 2% | 45.43 | 42.60 | 2.83 | 0.010 |
| 3% | 45.33 | 42.68 | 2.65 | 0.012 |
| Mean | 45.50 | 42.87 | 2.63 | 0.014 |

The fact that the chicks did not have access to water during this period may have been an aggravating factor for weight loss. The reduction in live weight of the chicks during this phase occurs due to higher use of energy reserves from the yolk sac, digestive and renal excretions, and dehydration (Leu *et al.*, 2002; Carvalho *et al.*, 2013).

Values for the live weight of the chicks, retraction of the yolk sac, small intestine weight and glucose levels (Table 3).

Although consumption of the experimental feed in the transportation boxes was low, there was a tendency to greater retraction of the yolk sac ($p=0.096$) with increasing glutamine supplementation ($Y = 3.94450 - 1.152850X + 0.384500X^2$; $R^2 = 0.99$, quadratic effect). There was improvement in the use of the yolk sac until reaching glutamine supplementation of 1.49%. Bhanja *et al.* (2009) observed greater reduction of yolk

sacs in birds that were fed immediately after hatching, thus suggesting that feed consumption might increase the mechanical activity of the intestine, such that the yolk sac would be absorbed more rapidly.

There was a tendency for blood glucose concentration to increase ($p=0.098$), with glutamine supplementation, up to 1.81% ($Y = 296.930 + 51.4300X - 14.1500X^2$; $R^2 = 0.99$, quadratic effect).

Fasting chicks go through a critical gluconeogenic metabolism period, with increases in ketosis and dehydration. Quick supplying of feed could minimize these effects, through altering the glycemic pattern of these birds (Vieira & Moran, 1999). Gluconeogenesis decreases as the plasma glucose concentrations increase through consumption of feed. Metabolizing of glutamine through the renal system may supply up to 25% of the glucose circulating in the plasma (Ribeiro Junior *et al.*, 2015).

Table 3 – Evaluation of yolk sac retraction, intestine weight and blood glucose of chicks fed with pre-starter feed supplemented with glutamine, after a 24-hour waiting period inside transportation boxes.

| Treatment | Weight (g) | Yolk sac (g) | Intestine (g) | Glucose |
|-----------------|------------|--------------|---------------|---------|
| Glutamine | | | | |
| 0% | 43.20 | 3.97 | 2.31 | 296.00 |
| 1% | 42.66 | 2.72 | 2.43 | 337.00 |
| 2% | 41.85 | 2.50 | 2.35 | 340.40 |
| 3% | 42.65 | 2.79 | 2.12 | 324.80 |
| *p-value | NS | NS | NS | NS |
| ¹ CV | 5.89 | 31.12 | 15.77 | 8.83 |

*Polynomial regression ($p<0.05$) ¹Coefficient of variation.

The results of the development of intestinal villi in chicks that consumed feed supplemented with glutamine (Table 4).

According to Maiorka *et al.* (2016), the heights of villi in the duodenum are most sensitive to glutamine. However, in the present study, supplementation

Table 4 – Evaluation of the development of villi (height of villi, depth of crypts and villus/crypt relationship) in the intestine of chicks fed with pre-starter feed supplemented with glutamine after a 24-hour waiting period in transportation boxes.

| Glutamine | Duodenum | | | Jejunum | | | Ileum | | |
|-----------------|----------|-------|-------|---------|-------|-------|--------|-------|-------|
| | Crypts | Villi | V/C | Crypts | Villi | V/C | Crypts | Villi | V/C |
| 0% | 35 | 256 | 7.19 | 37 | 179 | 4.86 | 33 | 164 | 4.95 |
| 1% | 38 | 241 | 6.33 | 36 | 147 | 4.13 | 48 | 185 | 3.85 |
| 2% | 40 | 261 | 6.52 | 34 | 170 | 5.04 | 36 | 176 | 4.94 |
| 3% | 37 | 208 | 5.58 | 37 | 148 | 4.04 | 36 | 160 | 4.46 |
| *p-value | NS | NS | NS | NS | NS | NS | <0,05* | NS | NS |
| ¹ CV | 12.43 | 25.73 | 26.52 | 18.07 | 12.29 | 16.67 | 7.53 | 9.99 | 11.95 |

*Polynomial regression ($p<0.05$) ¹Coefficient of variation.



with glutamine only influenced the depth of ileal crypts, presenting a quadratic effect ($p=0,001$) with maximum peak at 1.44% ($Y = 34.2667 + 10.600X - 3.66667X^2$; $R^2 = 0.42$). Increasing depth of crypts implies greater rates of cell proliferation and renovation of intestinal epithelium, thereby increasing the number of villi, since these structures originate from the lower part of the crypt (Wang *et al.*, 2008; Fernandes *et al.*, 2017).

Glutamine helps in the growth of intestinal villi, but the mechanism through which this stimulus occurs is not entirely clear. It has been suggested that glutamine might increase the exchange of Na^+ / H^+ in the cell membrane, or that it might increase the specific activity of the enzyme ornithine decarboxylase, which is essential for cell proliferation (Rhoads *et al.*, 1997). Moreover, glutamine is a precursor for synthesis of

amino acids, nucleotides and nucleic acids (Souba, 1993; Pelícia *et al.*, 2015). It has also been reported that glutamine increases gene transcription, thereby increasing mitogenic activity in the enzyme kinase (Blikslarger & Roberts, 1997).

Another study using a post-hatch delayed placement/feeding model for broiler chickens showed that the duodenum and jejunum presented complete and maximized recovery of intestinal morphology in chickens that were offered a diet with 1% glutamine for 48 hours, in comparison with chicken that were kept delayed placement for 48 hours (Yi *et al.*, 2005).

Performance results in the pre-initial phase (Table 5). In the present study, feed intake tended to decrease ($p=0.077$) with increasing glutamine supplementation ($Y = 168.099 - 3.47724X$; $R^2 = 0.87$, Linear effect), although there was no reduction in weight gain.

Table 5 – Performance in the pre-starter phase (1 to 7 days) among chicks that were given feed supplemented with glutamine.

| Treatment | Initial weight(g) | Final weight (g) | Weight gain (g) | Daily weight gain (g) | Consumption (g) | Feed conversion (g/g) |
|-----------------|-------------------|------------------|-----------------|-----------------------|-----------------|-----------------------|
| Glutamine | | | | | | |
| 0% | 43.02 | 187.07 | 144.05 | 20.57 | 168.14 | 1.07 |
| 1% | 43.32 | 180.83 | 137.51 | 19.64 | 165.80 | 1.08 |
| 2% | 42.64 | 182.02 | 139.26 | 19.06 | 158.62 | 1.11 |
| 3% | 42.80 | 180.96 | 138.06 | 19.24 | 158.94 | 1.10 |
| *p-value | NS | NS | NS | NS | NS | NS |
| ¹ CV | 1.51 | 4.70 | 6.04 | 8.23 | 3.99 | 4.87 |

*Polynomial regression ($p<0.05$) ¹Coefficient of variation.

Studies have shown that there is a positive correlation between the concentration of free glutamine and the rate of protein synthesis in skeletal muscle tissue (Souba, 1993). This means that the greater the concentration of circulating glutamine is, the higher the rate of protein synthesis will be and, consequently, the greater the weight gain will also be. However, this relationship was not observed in the present study. The concentrations at which glutamine is included in the diet are usually higher than those of other additives included in diets. The increases in this concentration to 2 and 3% may have inhibited the chicks' consumption, thereby

masking the metabolic effect of glutamine. Bartell & Batal (2007), observed that adding 1% glutamine increased weight gain, while adding 4% negatively influenced this variable.

Supplementation with glutamine did not influence the retraction of the yolk sac, the absolute intestine weight or the plasma glucose levels that were seen when the chicks reached seven days of age (Table 6). The yolk sac of a recently hatched chick originating from a young bird contains approximately 150 mg of glutamine (Vieira & Moran, 1999). However, the supplementation of glutamine in this experiment did not lead to better use of the yolk sac by the chicks.

Table 6 – Evaluation of yolk sac retraction and intestinal growth among chicks that were given pre-starter feed supplemented with glutamine, at the age of seven days.

| Treatment | Yolk sac (g) | ¹ Intestine weight (g) | Glucose (mg/dL) |
|-----------------|--------------|-----------------------------------|-----------------|
| Glutamine | | | |
| 0% | 0.45 | 15.68 | 338.20 |
| 1% | 0.18 | 17.23 | 350.20 |
| 2% | 0.76 | 14.98 | 320.80 |
| 3% | 0.22 | 14.77 | 345.20 |
| *p-value | NS | NS | NS |
| ² CV | 30.76 | 10.49 | 7.36 |

*Polynomial regression ($p<0.05$) ¹Weight of the small and large intestines; ²Coefficient of variation.



There was no statistical difference in glutamine concentration in relation to the variables of villus height, crypt depth and villus/crypt ratio when the chicks reached seven days of age (Table 7). Unlike the

results from the present study, Maiorka *et al.* (2000), found that there was an improvement in intestinal mucosal structure among broiler chickens that were fed with 1% glutamine during their first week of life.

Table 7 – Evaluation of the development of intestinal villi in chicks that were fed with pre-starter feed supplemented with glutamine, at the age of seven days.

| Treatment | Crypts ¹ | Duodenum | | | Jejunum | |
|-----------------|---------------------|--------------------|------------------|---------------------|--------------------|------------------|
| | | Villi ¹ | V/C ¹ | Crypts ¹ | Villi ¹ | V/C ¹ |
| Glutamine | | | | | | |
| 0% | 62 | 454 | 7.36 | 60 | 314 | 5.20 |
| 1% | 61 | 506 | 8.31 | 56 | 317 | 5.66 |
| 2% | 61 | 364 | 6.00 | 55 | 285 | 5.17 |
| 3% | 58 | 378 | 6.63 | 63 | 332 | 5.28 |
| *p-value | NS | NS | NS | NS | NS | NS |
| ² CV | 7.86 | 9.74 | 16.09 | 8.46 | 8.04 | 9.76 |

*Polynomial regression ($p < 0.05$) ¹Crypt depth (crypt, μm), villus height (villi, μm), villus/crypt ratio (V/C). ²Coefficient of variation.

The results from the metabolic assay demonstrated that there were no differences in glutamine concentrations in relation to the variables (Table 8).

The metabolizability coefficients of dry matter, nitrogen and ether extract were not influenced by the supplementation with glutamine ($p > 0.10$).

It had been assumed that adding glutamine to the diet would improve the digestibility of nutrients,

since addition of glutamine is related to increased amylase and trypsin activity, as reported by Tucci *et al.* (2011), and to increased digestibility of ether extract (Lima *et al.*, 2017). However, Martinez *et al.* (2012) also did not observe this effect on nutrient digestibility through supplementation with different levels of L-glutamine in the diet of broiler chickens.

Table 8 – Nitrogen balance (NB), ether extract balance (EEB), nitrogen metabolizability coefficient (NMC), ether extract metabolizability coefficient (EEMC) and dry matter metabolizability coefficient (DMMC) of chicks that were fed with pre-starter feed supplemented with glutamine, from seven to ten days of age.

| Glutamine | NB (g) | EEB (g) | NMC (%) | EEMC (%) | DMMC (%) |
|-----------------|--------|---------|---------|----------|----------|
| 0% | 18.47 | 54.67 | 62.42 | 86.86 | 73.60 |
| 1% | 13.74 | 53.75 | 51.60 | 88.64 | 72.04 |
| 2% | 15.96 | 51.89 | 54.05 | 87.79 | 70.54 |
| 3% | 18.40 | 58.19 | 52.58 | 91.74 | 70.54 |
| *p-value | NS | NS | NS | NS | NS |
| ¹ CV | 21.85 | 15.50 | 9.19 | 2.95 | 4.91 |

*Polynomial regression ($p < 0.05$) ¹Coefficient of variation.

CONCLUSIONS

Glutamine concentrations did not differ in relation to performance, histomorphometry of the small intestine or metabolizability of nutrients in the pre-starter phase.

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