Revista Brasileira de Ciência Avícola
ISSN: 1516-635X
revista@facta.org.br
Fundação APINCO de Ciência e Tecnologia Avícolas
Brasil

Fischer da Silva, AV; Borges, SA; Maiorka, A; Givisiez, PEN; Rocha, C; Macari, M
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Revista Brasileira de Ciência Avícola, vol. 9, núm. 2, abril-junio, 2007, pp. 111-115
Fundação APINCO de Ciência e Tecnologia Avícolas
Campinas, SP, Brasil

Brazilian Journal of Poultry Science
Revista Brasileira de Ciência Avícola

ISSN 1516-635X Apr - Jun 2007 / v.9 / n.2 / 111 - 115

Ornithine Decarboxylase Expression in the Small Intestine of Broilers Submitted to Feed Restriction and Glutamine Supplementation

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■ Keywords

Feed nutrition, glutamine, intestinal mucosa, mucosa growth, ornitine decarboxylase.

Arrived: August / 2006
Approved: July / 2007

ABSTRACT

Six hundred and forty one-day-old Cobb male broilers were used to evaluate ornithine decarboxylase (ODC) expression in the mucosa of the small intestine. Birds were submitted to early feed restriction from 7 to 14 days of age. The provided feed was supplemented with glutamine. A completely randomized design with a 2 x 2 factorial arrangement was used (with or without glutamine, with or without feed restriction). Restricted-fed birds were fed at 30% the amount of the *ad libitum* fed group from 7 to 14 days of age. Glutamine was added at the level of 1% in the diet supplied from 1 to 28 days of age. Protein concentration in the small intestine mucosa was determined, and ODC expression at 7, 14, 21, and 28 days of age was evaluated by dot blotting. ODC was present in the mucosa of broilers, and the presence of glutamine in the diet increased ODC activation. Glutamine prevented mucosa atrophy by stimulating protein synthesis, and was effective against the effects of feed restriction. Dot blotting can be used to quantify ODC expression in the intestinal mucosa of broilers.

INTRODUCTION

Functional development of the intestines is essential for successful poultry production. It has been shown that mammals and birds present intestinal mucosa atrophy when submitted to feed restriction (Palo *et al.,* 1995; Fischer da Silva, 2001). Fasting, even for a small period of time, may interfere with cell synthesis, and negatively affect bird growth. Feed ingredients contain compounds that directly stimulate the growth of the intestinal mucosa. These can also be converted into trophic factors after digestion (Uni & Sklan, 1998, Tarachai & Yamauchi, 2000).

Biogenic amines, especially polyamines, are among growth factors present in the intestinal mucosa (Sipponen *et al.*,1976). According to Janne *et al.* (1991), these are essential components for cellular growth and differentiation. The polyamines spermine, spermidine, and their diamine precursor, putrescine, are aliphatic cations, which are essential for normal physiology and growth of virtually every cell (Yang, 1984). Putrescine formation depends on the decarboxylation of ornithine by the action of the enzyme ornithine decarboxylase (ODC) (Luck and Baylin, 1980). This enzyme is found in all eucaryotic cells, and the levels of its products are highly regulated, having an important role in the initiation of cell proliferation and mitochondrial function (Tabor and Tabor, 1984). The increase in ODC activity is one of the first biochemical events associated to the induction of proliferative cells (Wang *et al.*, 1996).

Increased ODC activity after trophic stimulation or during tissue regeneration has been observed in different types of tissues (Tabor

and Tabor, 1984). Indeed, it was reported that rats submitted to fasting had increased ODC activity in the mucosa of the small intestine and colon during re-feeding (Madsen *et al.,* 1996).

Simple amines and amino acids may stimulate ODC activity in the mucosa. Evidences show that asparagine, glutamine, serine, and glycine, present in the lumen, stimulate mucosal growth by inducing the expression of the ODC gene (Wang *et al.*,1996).

The present study evaluated the effects of diets supplemented with glutamine on the expression of intestinal mucosa ornithine decarboxylase in the of broiler chickens submitted to early feed restriction (7 to 14 days of age).

MATERIAL AND METHODS

Day-old male Cobb chicks (n=640) were randomly distributed in floor pens (1.50 x 3.50m). Birds were fed isocaloric diets containing corn, soybean meal, soybean oil, dicalcium phosphate, limestone, sodium chloride, DL-Methionine, and mineral and vitamin supplements, according to the guidelines and nutritional requirements published by the NRC (1994). Water was provided *ad libitum*. From one to six days of age, birds were fed *ad libitum* diets containing either no glutamine or 1% glutamine. Quantitative feed restriction at 30% as compared to the *ad libitum* fed group was applied from seven to 14 days of age. During this period, birds were divided into four treatments: glutamine supplementation and *ad libitum* feeding, glutamine supplementation and feed restriction, no glutamine supplementation and *ad libitum* feeding, and finally no glutamine supplementation and feed restriction. Birds were fed *ad libitum* thereafter.

Four birds per treatment were submitted to a twelve-hour-fasting, and then sacrificed by cervical dislocation at 7, 14, 21, and 28 days of age. Samples of different segments of the small intestine were collected and identified, fast-frozen in liquid nitrogen, and stored at -70°C. Intestinal segments were longitudinally opened and the mucosa was scraped. A pool (mixture) was prepared with four samples from each treatment. Scrapings of each treatment were mixed, weighed ,and homogenized, and a mixture was prepared for each intestinal segment (duodenum, jejunum, ileum) at each age (7, 14, 21, and 28 days). Samples were placed in a 50-mL polypropylene tube, and diluted with 20 mL lysis buffer (20 mM Tris; 0.05 mM EDTA; 0.05 mM pyridoxal phosphate; 5 mM DTT, pH 7.4). Homogenization was performed three times (30 s) using an ultra-turrax homogenizer at 20,000rpm and ice-bath intervals of 30 s. After homogenization, the lysate was sonicated (Sonicador Branson Sonifier 250) for 5 min and then centrifuged at 30,000 g for 30 min at 4° C using a Himac CR 20b2 centrifuge and rotor RPR 20-2 (Hitachi Corp.).The supernatant was transferred to a 15-mL polypropylene tube, and homogenized 10 times with a Potter-Elvehjem homogenizer and ice-bath intervals. Two 300-mL aliquots were taken for protein determination and dot blotting .

Total protein determination was performed according to the microplate method described on the BioRad Protein Dye Assay (BioRad, Hercules CA, USA), based on Bradford (1976). Samples were evaluated in duplicate, and readings were performed at 595 nm.

ODC expression was evaluated by dot blotting using nitrocellulose filters. Membranes were humidified with water for 5 min, and water excess was drained before placing the filters on the BioDot Apparatus (BioRad, Hercules, CA, USA). Samples were prepared as described below and volumes were adjusted to 20 mL, using double-distilled water before being loaded into the BioDot apparatus and vaccum-filtered.

As pure ODC was not available, a reference sample (a pool of duodenum samples collected at 7, 14, 21, and 28 days from birds that were not submitted to feed restriction or were received glutamine in the diet) was used to produce a standard curve of ODC expression. The standard curve was prepared using 10, 20, 30, 40, 50, and 60mg of total protein loaded to the filter.Densitometer detection limit was also evaluated using increasing amounts of one sample with strong color signal, which was loaded at 10, 20, 30, 40, 50, and 60mg of total protein. A jejunum sample from 28-day-old birds submitted to feed restriction and with feed supplemented with glutamine was used for that purpose. Pools of remaining samples were loaded at 15, 20, and 30mg of total protein. After the samples were loaded and vacuum-filtered, the membrane was dried and washed with double-distilled water. Blocking was performed with 20 mL TBS (10mM Tris, pH 8.0; 0.15 M NaCl) containing 5% skimmed milk and 0.02% Tween 20 (blocking buffer) for one hour at room temperature and continuous shaking.

The membrane was incubated with anti-ODC mouse monoclonal antibody (Sigma, Saint Louis, MO, USA) diluted 1:500 in 20 mL blocking buffer for 1 h at room temperature. Four washings of 5 min each were performed with TBS-T (TBS added with 0.05% Tween-

20), and one washing of 10 min was performed with TBS. The membrane was incubated for one hour in blocking buffer with alkaline phosphatase-labeled anti-mouse IgG (01136 Sigma) diluted at 1:5000. Washings were performed as previously described, and the signal was developed by the addition of 20 mL AP buffer (100 mM Tris-HCl pH 9.5; 100 mM NaCl; 5 Mm $MgCl_2$), 132 mL nitro-blue tetrazolium chloride (NBT, 50mg/mL in dimethylformamide), and 66 mL 5-bromo-4-chloro-3-indolylphosphate p-toluidine (BClP, 50mg/mL in 70% dimethyl formamide. The reaction was stopped with 3% TCA (trichloroacetic acid). The membrane was washed with double-distilled water, and dried at room temperature protected from light. The color signal of the membrane was read at 525 nm using a densitometer (Shimadzu CS-9301, Shimadzu Corporation, Tokyo, Japan), and the results of the ornithine decarboxylase expression in the small intestine of broilers were expressed in area (mm^2).

RESULTS AND DISCUSSION

The area of ODC expression in the jejunum, ileum, and duodenum of chicks at 7 days of age is shown in Figure 1. Birds fed glutamine showed higher ODC expression in the duodenum and jejunum at seven days of age, although expression in the ileum was equivalent between treatments with glutamine. In mammals, Yang *et al.* (1984) reported an increase in ODC activity of the small intestine mucosa in the first 7 days of lactation, and the authors also observed significant increases in the intestinal weight and spermidine levels in the mucosa during the same period. Maiorka *et al.* (2000) reported that glutamine supplementation to a corn-soybean meal based diet increased villi size in the duodenum and ileum of broilers during the first seven days of age. However, the mechanism by which glutamine stimulates the proliferation of enterocytes is unclear. Nevertheless, Rhoads *et al.* (1997) suggested that glutamine is related to two events associated to cell proliferation in the jejunum of swine: increase in Na^+/H^+ exchange in the plasmatic membrane, and increase of the specific activity of ODC. It also has been suggested that glutamine activates mitogenesis (Blikslager & Roberts 1997).

At 14 days, higher ODC expression was observed in the duodenum and the ileum of feed-restricted birds receiving diets supplemented with glutamine (Figure 2). During the first weeks of age, glutamine had a higher impact on intestinal mucosa growth , increasing villi height in the duodenum and the ileum (Fischer da Silva 2001). During this period, birds need more available energy and protein for cell multiplication and production of digestive enzymes.

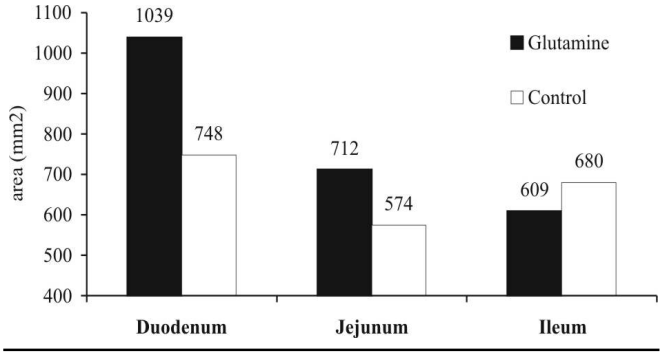

Figure 1 – ODC expression in the small intestine of broilers at 7 days of age. The area readings are indicated.

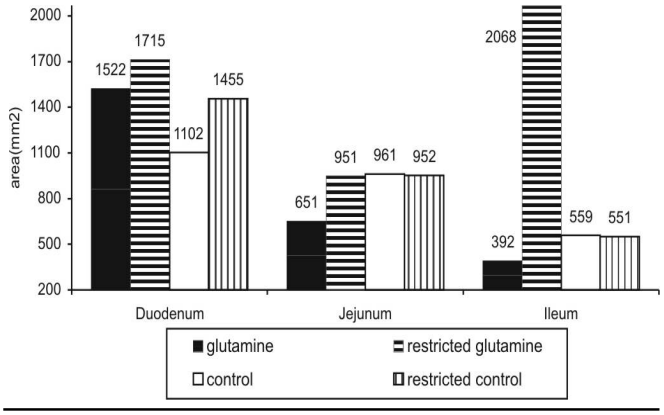

Figure 2 – ODC expression in the small intestine of broilers at 14 days of age. The area readings are indicated.

The gastrointestinal tract grows rapidly after hatching, mainly during the first two weeks of life. A significant increase in intestinal mucosa growth indicates intestinal maturation (Senkoyiu & Janssen, 1988). A marked increase in ODC activity and polyamine biosynthesis was observed in the intestinal mucosa of newly born rats up to the third week of life. In that period, the intestinal mucosa cells changed rapidly, from fetal to adult animal cells (Luck *et al.*, 1980). According to this author, the increase in ODC activity is the result of increased biosynthesis of polyamines, which can effect intestinal mucosa maturation and regeneration. Similarly, Yang *et al.* (1984) observed that, during injury and sloughing of the intestinal mucosa, there was fast regeneration and proliferation of the intestinal mucosa following increased ODC activity.

The amount of ingested food influences greatly the

development of mucosa and other structures of the gastrointestinal tract (Steiner *et al.*, 1968). Rats submitted to a six–day fasting presented 53% decrease in small intestine weight as compared to 32% decrease in total body weight. Moreover, cell numbers in the intestinal mucosa were reduced, as well as RNA, protein and water levels in the intestinal mucosa cells as compared to these levels in other mucosa cells. Shamoto *et al.* (1999) reported decreased villi height and cell proliferation in the crypts when broilers were submitted to fasting, although birds recovered during re-feeding. Recovery after fasting is highly dependent on the presence of nutrients in the intestinal lumen (Tarachai & Yamauchi, 2000).

At 21 days of age, the mucosa of feed-restricted birds supplemented with glutamine showed higher ODC expression in the duodenum, followed by the jejunum, and lastly the ileum (Figure 3). At 28 days, higher ODC expression was observed in the duodenum of non-feed restricted birds fed the diet supplemented with glutamine (Figure 4). In the jejunum and the ileum, higher ODC expression was verified in the feed-restricted birds not supplemented with glutamine.

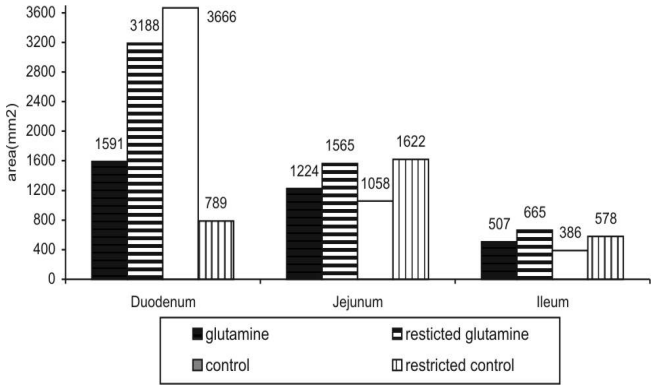

Figure 3 – ODC expression in the small intestine of broilers at 21 days of age. The area readings are indicated.

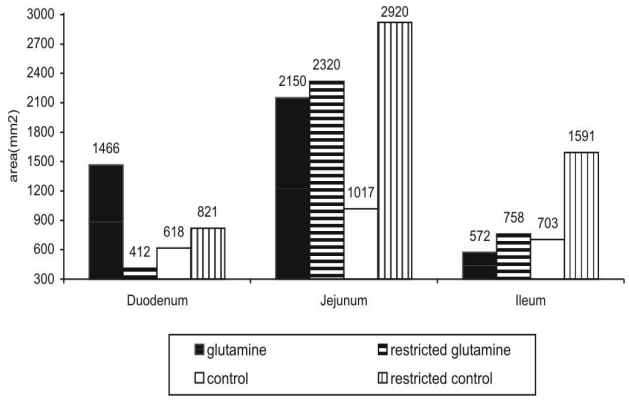

Figure 4 – ODC expression in the small intestine of broilers at 28 days of age. The area readings are indicated.

CONCLUSIONS

Glutamine supplemented diets increased the expression of ornithine decarboxylase in the intestinal mucosa of broilers.

Glutamine supplementation prevented deleterious reduced intestinal mucosa atrophy in feed-restricted birds through the stimulation of protein synthesis.

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