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Effect Of Inoculation Local and Commercial Chicken Eggs with Arginine Amino Acid on the Development of Bursa of Fabricius

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Abstract

The present study emphasizes the importance of *Ovo* feeding strategies, particularly the supplementation of fertilized eggs with amino acids such as L-arginine to enhance the Bursa of Fabricius development. Targeted nutritional interventions during embryogenesis offer a promising approach to improving the overall health, productivity, and market value of poultry. The experiment was done in the College of Agriculture - University of Sulaimani to study the effects of arginine on the bursal tissue of commercial and local Kurdish chickens. One hundred fertile eggs involved from the Ross-308 broiler breeder strain and one hundred fertile eggs from a local breed. The in ova inoculation treated included a control group without inoculation and three groups of inoculation with 1%, 2%, and 3% Arginine solutions. The inoculations were carried out twice, on the 14th and 18th days of incubation. Four chicks at one-day age from each Treated group were euthanized, and their bursa of Fabricius was preserved for histological slide preparation. The study found that bursal follicular proliferation in Kurdish breeds showed significant improvements in lymphoid follicles and number compared to commercial breeds. The scoring system for bursal follicular proliferation was higher in the G4 (3% Arginine) Treated group. However, bursal sections from Fabricius bursa showed significant aggravation in follicle number, with G3 and G4 Treated groups scoring higher. Eggs inoculated with arginine showed significant follicular growth in both breeds.

Keywords: Arginine, Chicken, Inoculation, and Bursa of Fabricius

Introduction

Due to the selection of *Gallus gallus* chickens for meat production, inbred strains with accelerated growth performance particularly with respect to improved muscle growth, which primarily takes place during embryogenesis have been produced (1,2). The yolk, which mostly consists of lipids and has minimal amounts of carbohydrates, is where most nutrients and energy are obtained throughout embryogenesis (3). Consequently, gluconeogenesis from essential amino acids is necessary for the health of the embryo and post-hatch chicken (4,5). Researchers have discovered that adding amino acids to fertilized broiler eggs a process known as "ova feeding" may provide poultry producers a different way to improve the hatchability and muscular growth weight of newly hatched chicks (6,7).

Bioactive compounds, such as polyphenols, bioactive amino acids, and prebiotics, when nourished and supplemented, can improve immune function, reduce osteoporosis, and lower the risk of heart disease (8). Likewise, prior research has shown that the application of amino acids, carbohydrates, and vitamins to eggs via in ova feeding can enhance the rate of hatching, body weight, rate of survival, growth performance, and size for marketing (9). Furthermore, hatchability might be impacted by the in Ova feeding place and duration, as shown by a previous study (10).

L-arginine is a semi-essential amino acid in poultry diets. Where the bird cannot be configured in the body, unlike the mammals (11). It is abundantly available in soybean plants and is the main source for preparing birds' diets. Several researchers used the L-arginine amino acid to study their effect on egg productivity (12), egg quality traits (13), Carcass traits (14), and body weight (15). The aim of the current study is to evaluate the effect of inoculation of the Arginine amino acid into the egg sac with different doses on the bursa of Fabricius in both commercial and local chicken.

Materials and Methods

The experimental was done at the University of Sulaimani - College of Agricultural Engineering Science - department of Animal Science from (12/5 to 20/7/2024), A total of 100 fertile eggs from the Ross-308 broiler breeder strain were procured from a commercial company, while an additional 100 fertile eggs from a local breed were sourced from the Kurdish local chicken from the directorate of agricultural research of Selmani. The eggs were randomly assigned to four distinct Treated groups, each Treated with three replicates with 4 eggs per replicate. The Treated groups included a negative control (no inoculation). On the 7th day of incubation, the eggs were candled, and any infertile eggs or those containing dead embryos were excluded from the study.

The in ova inoculation Treated included a control group with no inoculation and three groups inoculation with 1%, 2%, and 3% Arginine solutions, prepared by dissolving 1g, 2g, and 3g of Arginine in 100 ml of

distilled water, respectively. The inoculations were carried out twice, on the 14th and 18th days of incubation inside the egg sac. Post-inoculation, the inoculation sites were sealed with adhesive tape, and incubation was resumed. Following hatching, the chicks were weighed using a precision balance with 0.1 g accuracy. Subsequently, four chicks from each Treated group were euthanized, and their bursa of Fabricius was excised, weighed using a balance with 0.01 g precision, and preserved in 40% formalin for histological slide preparation.

Histological technique:

The histological protocol was performed at the end point of the experiment; chicks were sacrificed and then euthanized in a humane practice. Consecutively, after scarification collecting tissue samples for histological preparation started necropsy findings. Briefly, samples from bursal tissue were collected and fixed into tissue cassettes then dipped into a 10% buffered formaldehyde solution for about 48 hours. Thereafter, sections were dehydrated by passing through a series of ascending ethanol alcohol, followed by three repetitive steps of xylene clearance. Next, the processed sections were infiltrated and embedded in melted paraffin blocks using an automated wax embedder at (60 -70^{o}C). Paraffinized blocked tissues were sectioned to 5 µm, after that, tissue sections were placed on glass slides and dried using a hot plate holder. Later on, tissue sections were deparaffinized and cleaned with xylene solution for 30 minutes then dried for 5 minutes. Finally, tissue sections were stained with Harris's hematoxylin and eosin solution, cleaned with xylene and the cover slipped (16, 17).

Semi quantitative lesion scoring

Lesion scoring was estimated semi-quantitatively via image analyzer software (AmScope, 3.7) using a microscope eye-piece camera (MD500, 2019), and tissue samples were analyzed under the light microscope (NOVEL XSZ-N107T, China). Within the bursal tissue follicular proliferation and hyperplasia were estimated and measured in percentage of calculated cell numbers from randomly selected different fields, whereas the number of growing follicles was counted in randomly chosen four fields in each section then the mean average was calculated statistically in percentage. On the other hand, infiltration of inflammatory cells as well as vascular congestions was statistically not significant to measure. The mean percentage of all calculated values were expressed as following score system (score 0-25% as grade one plus; score 25-50% as grade two pluses; score 50-75% as grade three pluses; score 75-100% as grade four pluses) (18, 19).

Results

Local chicks: At first, figure 1demonstrate microscopically examined Bursal sections from different Treated groups after day 14 of inoculation. The control negative group, which received no treatment, showed typical histological architecture of standard juvenile Bursal tissue, which continued, unchanged and exhibited normal arrangement of few growing Bursal follicles. In contrast, Bursal sections supplemented with Arginine at different doses reveal the presence of significant follicular proliferation evident by a considerable degree of cellular hyperplasia.

On the other hand, Bursal sections from eggs inoculated with Arginine at day 18 exhibit even more significant follicular proliferation together with cellular hyperplasia in a dose dependent manner since the morphological regulating were more significant P<0.05 in G3 and G4 2% and 3% Arginine respectively, apparent by the uprising of the scoring system as shown in table 1. Generally, all Treated groups with Arginine in Kurdish breed demonstrate significant Bursal proliferation with notable increase in the number of the follicles.

Table 1: Micro-morphological quantitative assay of Bursal sections from Kurdish breed at 14 and 18 days

Experimental Groups N=5	Follicular Proliferation* (Mean %)**	Follicular Hyperplasia* (Mean %)**	Number of Follicles * (Mean %)**	Scoring System (0 -100%)	Grading System (+ - ++++)
Kurdish Breed					
14 Days					
(G1) CNG†	21.37 % ^{D}#	24.81 % ^{D}	18.64 % ^{C}	0-25 %	+
(G2) AG (ARG) 1%	34.61 % ^{C}	39.48 % ^{C}	27.31 % ^{B}	25-50 %	++
(G3) AG (ARG) 2%	59.34 % ^{B}	65.79 % ^{B}	58.17 % ^{A}	50-75 %	+++
(G4) AG (ARG) 3%	64.71 % ^{A}	69.04 % ^{A}	59.45 % ^{A}	50-75 %	+++
Kurdish breed					
18 Days					
(G1) CNG†	22.19 % ^{D}#	25.36 % ^{D}	22.16 % ^{D}	0-25 %	+
(G2) AG (ARG) 1%	38.27 % ^{C}	43.89 % ^{C}	32.67 % ^{C}	25-50 %	++
(G3) AG (ARG) 2%	65.18 % ^{B}	72.06 % ^{B}	61.85 % ^{B}	50-75 %	+++
(G4) AG (ARG) 3%	76.14 % ^{A}	79.23 % ^{A}	74.96 % ^{A}	75-100 %	++++

Notes: *Bursal follicular hyperplasia, follicular proliferation and the number of the growing follicles were estimated in (%) of cell and follicular numbers. **Each value represents mean percentage (n=5). #Statistical comparison among groups: Mean values with different capital letters have significant differences at (P < 0.05). †G1: Control negative group (CNG), Distilled water; G2: Arginine group (AG 1%); G3: Arginine group (AG 2%); G4: Arginine group (AG 3%).

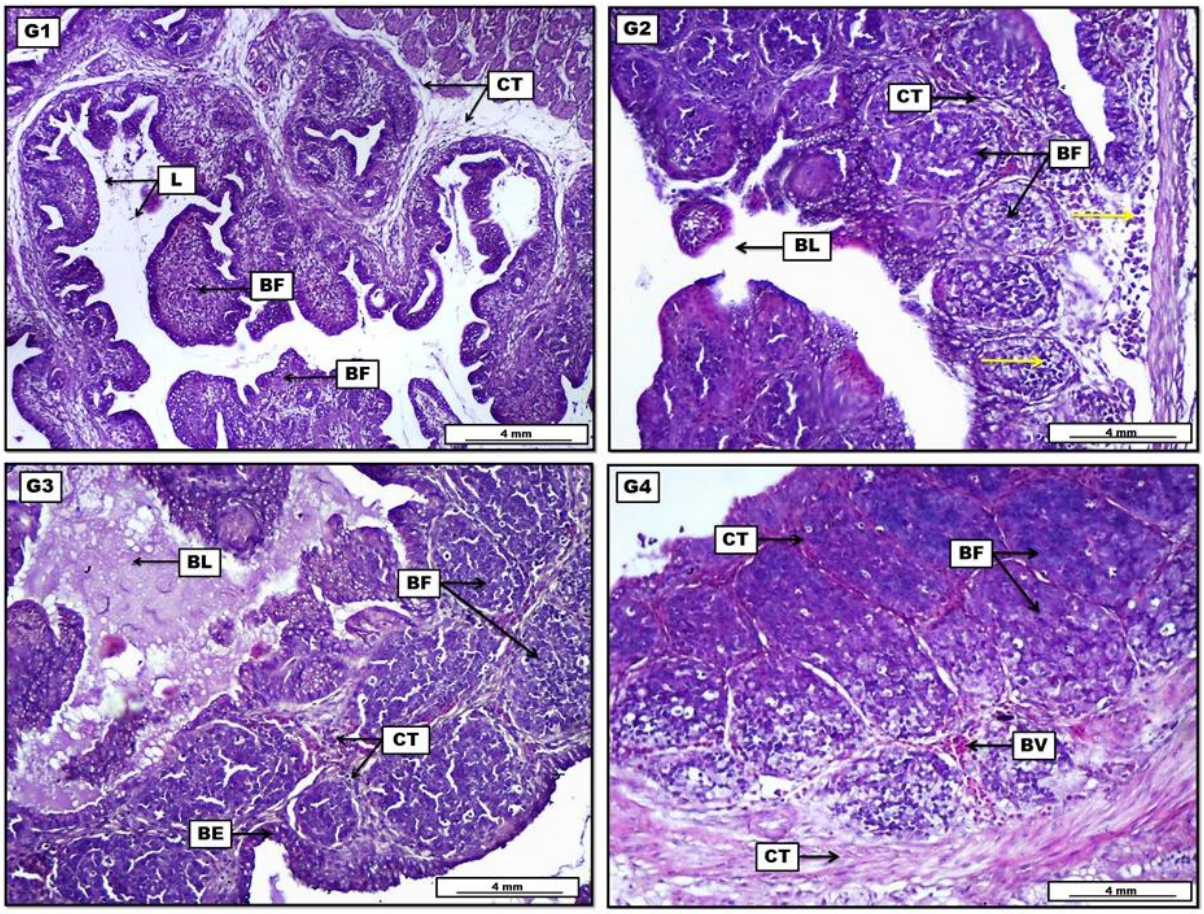

Figure 1: Photomicrograph of Bursa of Fabricius from Kurdish breed groups at day 14; (G1): Shows normal and primitive growing follicles (BF), represented by typically arranged papillary-like projection of the Bursal folds with their extended connective tissue (CT). The section also shows typical Bursal lumen (L). (G2): Received 1% Arginine at day 14, demonstrates obvious growing and proliferation of Bursal follicles (BF), distributed diffusely throughout the Bursal tissue separated by connective tissue septum (CT), along with significant distribution of mononuclear inflammatory cells mainly lymphocytes (yellow arrows). The section also shows distinct Bursal lumen (BL). (G3): Received 2% Arginine at day 14, elucidate the presence of significant follicular regeneration and hyperplasia (BF) lined by clear Bursal epithelial layer (BE) with obvious connective tissue septum separated the Bursal follicles (CT). In addition to the presence of light purple proteinaceous fluid within the Bursal lumen (BL). (G4): Received 3% Arginine at day 14, show significant Bursal follicular proliferation (BF) with high grade of capsular and septal connective tissue proliferation (CT). The section also reveals some normally appeared blood vessels (BV) congested with clear nucleated RBCs. H&E. Scale bar: 4 mm.

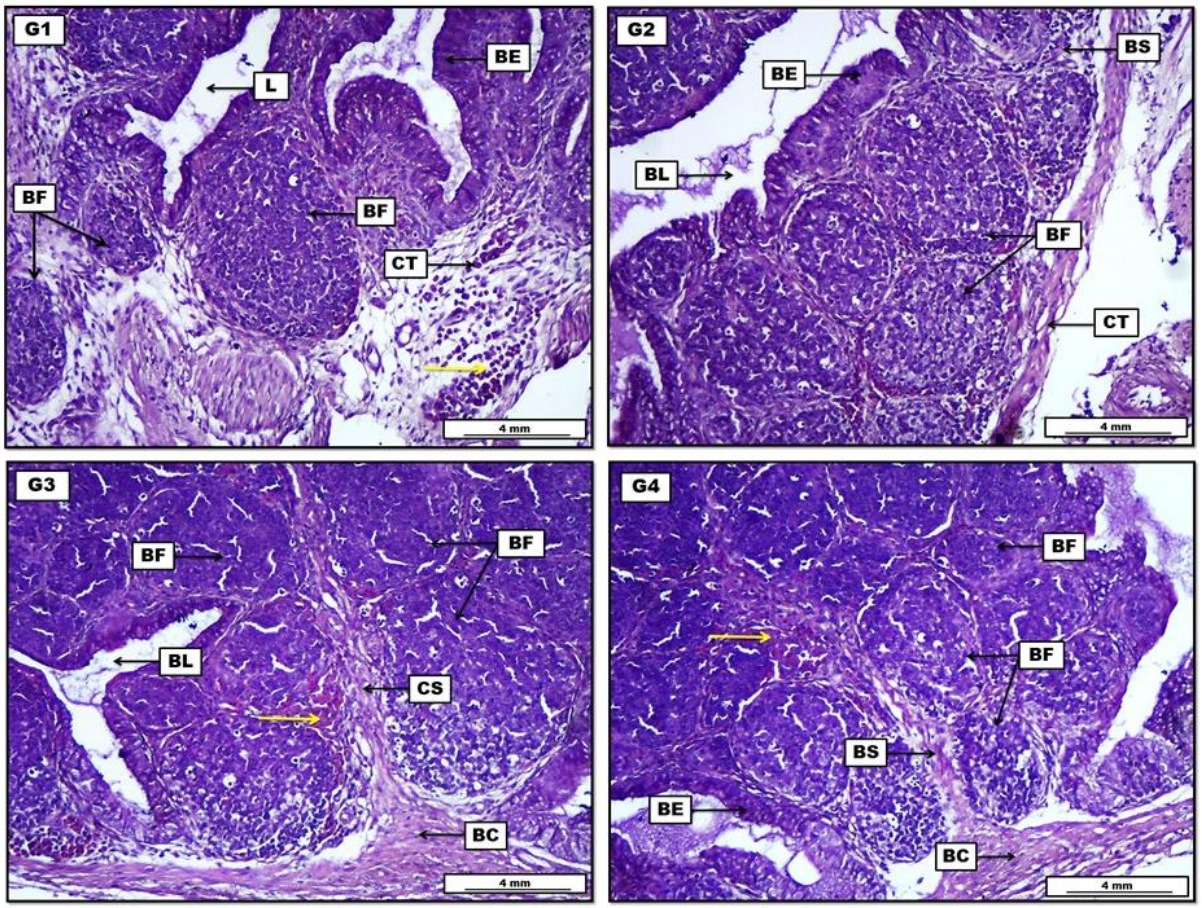

Figure 2: Photomicrograph of Bursa of Fabricius from Kurdish breed groups at day 18; (G1): Show significant Bursal follicular proliferation (BF) lined with clear Bursal epithelium (BE). Yellow arrows designate for diffuse infiltration of inflammatory cells within the submucosal connective tissue (CT). The section also reveals clear lumen with mild purplish fluid (L), with slightly vascular congestion and dilation. (G2): Received 1% Arginine at day 18, reveals significant follicular proliferation and hyperplasia (BF), separated and lined with distinct Bursal septum (BS) and Bursal epithelium (BE), respectively. Bursal tissue is surrounded with visible collagenous capsule (CT), in addition to low-grade proteinaceous fluid within the lumen Bursal (BL). (G3): Received 2% Arginine at day 18, show high grade of Bursal follicular hyperplasia (BF). The section also demonstrates a very distinct connective tissue septum separate the cortical tissue (CS), with obvious and thick Bursal capsule (BC). Moreover, the section demonstrates vascular congestion (Yellow arrows) together with slight proteinaceous fluid within Bursal lumen (BL). (G4): Received 3% Arginine at day 18, displays a very significant follicular proliferation (BF) with clear and intelligible cortical-Bursal septum (BS). Bursal tissues show moderate vascular congestion (yellow arrow), and typically appearing Bursal capsule (BC). Bursal lumen is lined with obvious and intact Bursal epithelium. H&E. Scale bar: 4 mm.

Commercial Breed; Initially, table 2 illustrate in details the scoring system of Bursal follicular proliferation, to begin with the sections taken at day 14 after inoculation, most of the histological changes including follicular proliferation and hyperplasia together with the number of growing follicles were significantly increased in all Treated groups in compare with control one, however the scoring was much higher in G4 (3% Arginine) in compare to other Treated groups as shown in figure 3. On the other hand, sections from bursa of fabricius taken at day 18, reveal significant aggravation in the number of follicles manifested by increased in follicular proliferation and enlargement of their size, the result was much scored in G3 (2% Arginine) and G4 (3% Arginine) respectively in a dose dependent manner in compare to Treated group G2 (1% Arginine). In conclusion, Bursal sections in Kurdish breed in compare to commercial breed show more significant improvement in the lymphoid follicles as increase in follicular proliferation and number. Furthermore, egg inoculated with Arginine at doses 2% and 3% demonstrate much significant follicular growth in both breeds, yet it was much scored and more significant in Kurdish breed than in commercial one.

Discussion

Evaluating l-arginine as an immunomodulator of the Bursa of Fabricius that can promote growth increase in pre-hatched chicks was the major goal of the study. As described by (20), the peak value of the Bursa of Fabricius at the embryonic day 17, which the research attributed to intense growth of the organ, corresponds to the evaluation phase of growth development of the Bursa of Fabricius in chicken, the same finding was observed by (21) who found in his study the Bursa of Fabricius weight was at the peak in the embryonic age 18 days. That's why we can notice the studied traits of Bursa of Fabricius in 14 days differ slightly from the Bursa of Fabricius traits in 18 days. Moreover (22) observed in his study significant high proliferative activity at the embryonic day 17. Injecting the egg with 3% of L-arginine increases Follicular proliferation, follicular hyperphasia, and the number of follicles significantly in both the local chicken and the commercial breed. (23) found in his study deficiency in L-arginine reduces the immune organs weight (Thymus, spleen, and Bursa of Fabricius).

Creatine has a greater ability to spare glycine and arginine in chicks, while creatinine and glycocyamine have less of an effect. Creatine levels and Arginine consumption are correlated, and when Arginine is inadequate, there may be a greater need for glysine (24). It was formerly believed that the advantage of combining glycine and Arginine stemmed from glycine's function in excreting excess nitrogen as uric acid. (25) discovered that supplementing chicks fed Arginine -limiting diets with glysine, α-aminoisobutyric acid, and threonine reduced excretion, leading to an increase in body weight gain and feed utilization efficiency. It was also shown that α-aminoinoisobutyric acid significantly decreased urea and inhibited renal arginase activity.

Table 2: Micro-morphological quantitative assay of Bursal sections from Commercial breed at 14 and 18 days

Experimental Groups N=5	Follicular Proliferation* (Mean %)**	Follicular Hyperplasia* (Mean %)**	Number of Follicles * (Mean %)**	Scoring System (0 -100%)	Grading System (+ - ++++)
Commercial Breed					
14 Days					
(G1) CNG†	17.34 % ^{D}#	21.52 % ^{D}	15.87 % ^{D}	0-25 %	+
(G2) AG (ARG) 1%	29.36 % ^{C}	34.11 % ^{C}	24.62 % ^{C}	25-50 %	++
(G3) AG (ARG) 2%	35.77 % ^{B}	44.56 % ^{B}	32.61 % ^{B}	25-50 %	++
(G4) AG (ARG) 3%	57.32 % ^{A}	64.41 % ^{A}	54.36 % ^{A}	50-75 %	+++
Commercial breed					
18 Days					
(G1) CNG†	21.44 % ^{D}#	24.98 % ^{D}	19.76 % ^{D}	0-25 %	+
(G2) AG (ARG) 1%	35.41 % ^{C}	38.62 % ^{C}	31.52 % ^{C}	25-50 %	++
(G3) AG (ARG) 2%	56.27 % ^{B}	61.74 % ^{B}	55.38 % ^{B}	50-75 %	+++
(G4) AG (ARG) 3%	65.81 % ^{A}	71.46 % ^{A}	62.84 % ^{A}	50-75 %	+++

Notes: *Bursal follicular hyperplasia, follicular proliferation and the number of the growing follicles were estimated in (%) of cell and follicular numbers. **Each value represents mean percentage (n=5). #Statistical comparison among groups: Mean values with different capital letters have significant differences at ($P < 0.05$). †G1: Control negative group (CNG), Distilled water; G2: Arginine group (AG 1%); G3: Arginine group (AG 2%); G4: Arginine group (AG 3%).

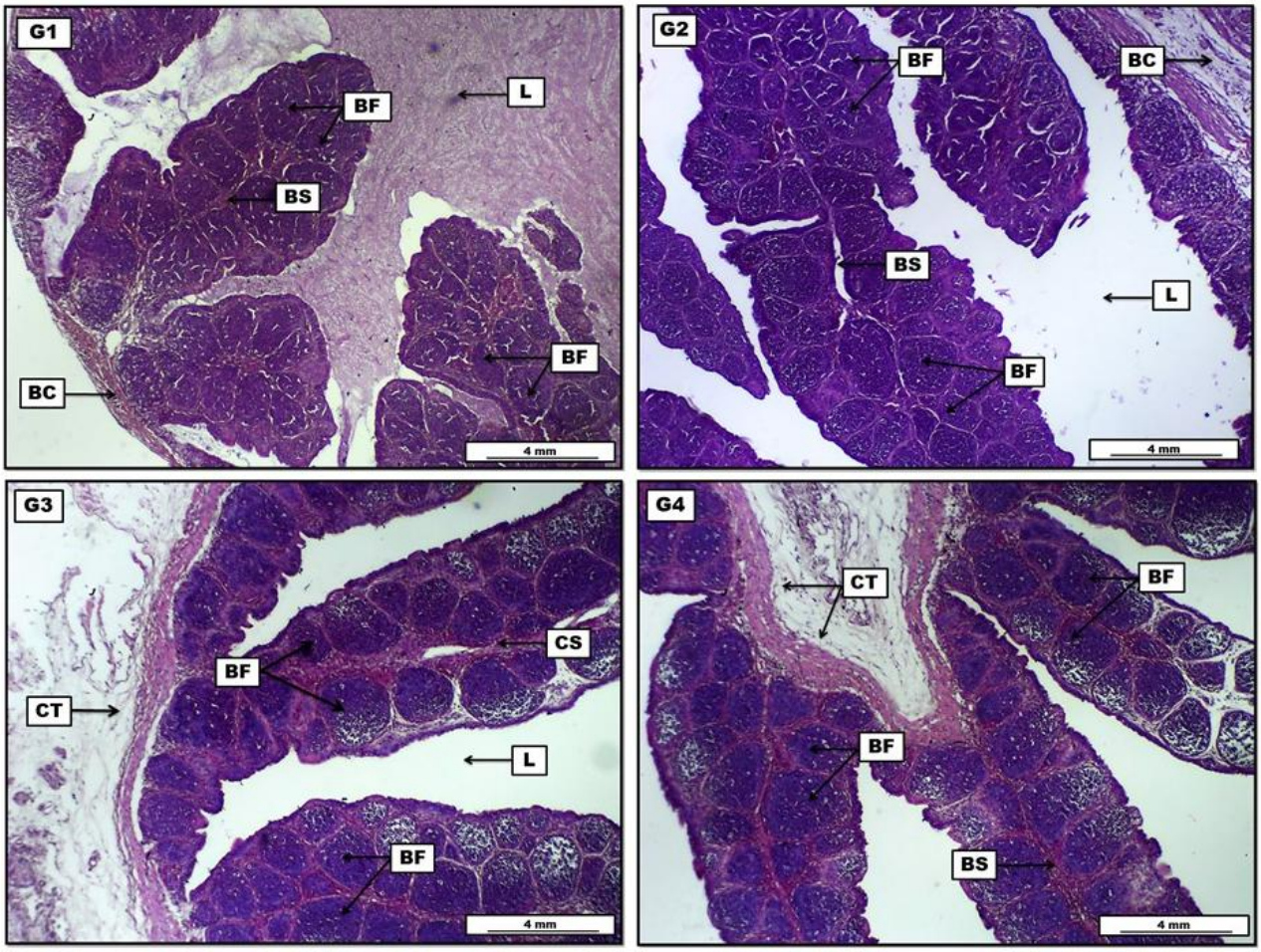

Figure 3: Photomicrograph of Bursa of Fabricius from Commercial breed groups at day 14; (G1): Display slight to moderate Bursal follicular growth (BF), with apparent Bursal septum separate the follicles (BS). The section also reveals thin Bursal capsule (BC), together with the presence of light pink acidophilic fluid within the lumen (L). (G2): received 1% Arginine at day 14, show visible growing of Bursal follicles (BF), evident by clear increase in their numbers separated with obvious Bursal septal tissue (BS), together with significant proliferation of the Bursal capsule (BC). The lumen is clear and empty form accumulated fluid. (G3): Received 2% Arginine at day 14, reveals prominent follicular proliferation (BF) within the cortex, evident by typically appeared finger like projection of the Bursal tissue. Presence of clear proliferated capsular (CT) and septal (CS) connective tissue among the follicles, the lumen is clear and empty (L). (G4): Received 3% Arginine at day 14, show significant follicular proliferation (BF) manifested by significant increase their numbers within the papillary-like projected follicular folds, together with the presence of clear Bursal septum between the follicles (BS) along with notable submucosal connective tissue (CT). H&E. Scale bar: 4 mm.

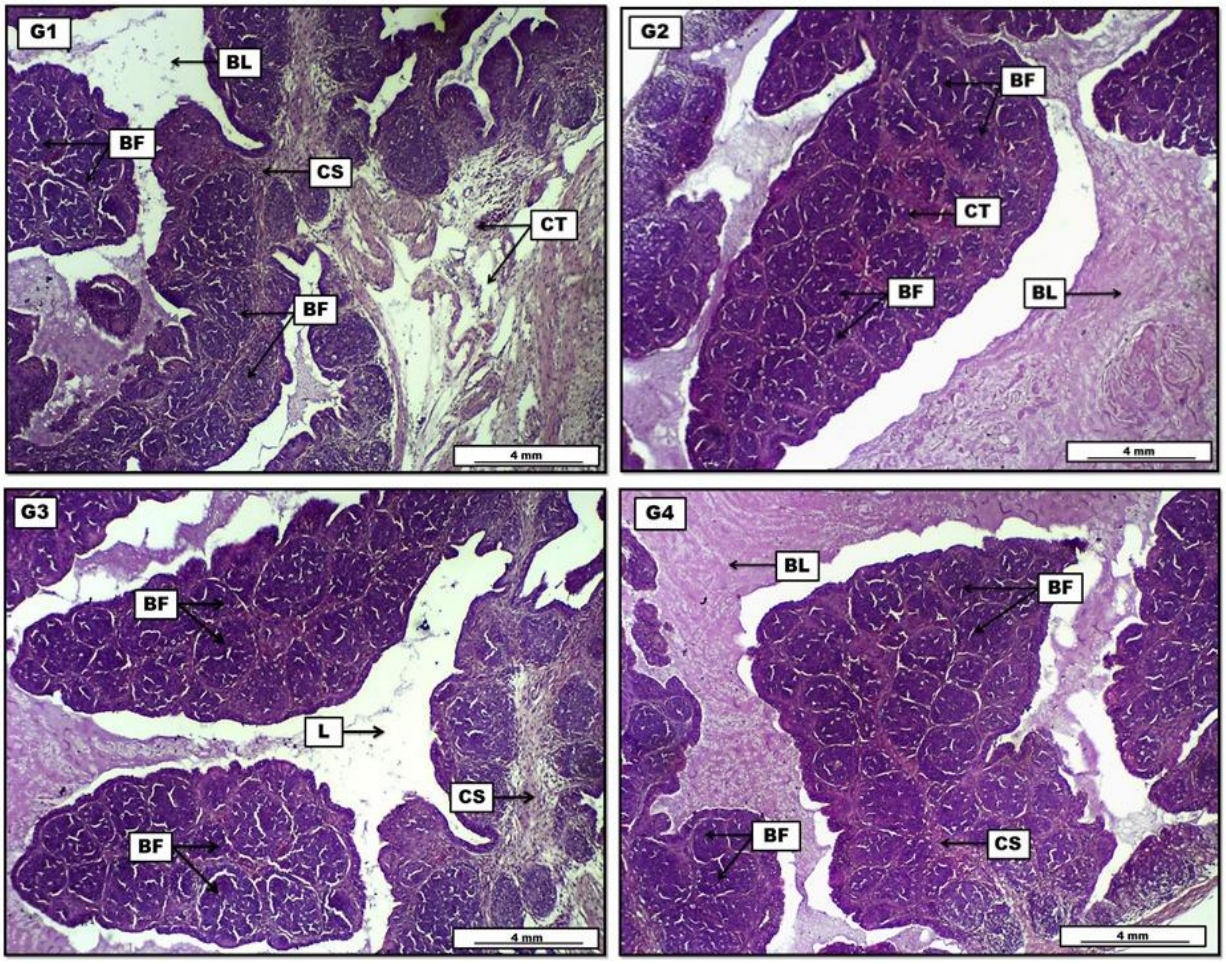

Figure 4: Photomicrograph of Bursa of Fabricius from Commercial breed groups at day 18; (G1): Demonstrate classically arranged and low grade Bursal follicular tissue proliferation within the cortex (BF), separated with connective tissue stromal tissue (CS), the section also reveals apparent turbid purplish fluid within the Bursal lumen (BL) and thick capsular tissue (CT). (G2): Received 1% Arginine at day 18, show notable distribution of moderately proliferated Bursal follicles (BF) within the stromal connective tissue (CT), together with diffuse accumulation of amorphous slightly pinkish proteinaceous fluid within Bursal lumen (BL). (G3): Received 2% Arginine at day 18, reveal moderately significant proliferation of Bursal follicles within the cortical tissue (BF), together with dense capsular connective tissue cover the organ (CS), furthermore, the section shows area of acidophilic amorphous fluid stuffed the slightly empty lumen (L). (G4): Received 3% Arginine at day 18, demonstrate moderately graded Bursal follicular hyperplasia (BF), which bordered and separated longitudinally by connective tissue septa (CS). Bursal lumen (BL) displays the presence of acidophilic slightly pinkish turbid proteinaceous fluid, which occupied most of the luminal area. H&E. Scale bars: 4 mm.

Supplementing with metyl-arginine can mitigate growth depression in hens caused by an excess of dietary Arginine. It is confirmed that there is a link between Arginine and Methionine, and one of the mechanisms involved in creatine biosynthesis. Met acts as a donor of methyl for the synthesis of creatine. In protein-free chickens, supplemented Methionine and Arginine have a nitrogen-sparing effect (26).

Accompanying mitochondria to myosin filaments to transfer high-energy phosphate and serve as a store for ATP regeneration, creatine, a precursor to creatine phosphate, is essential for proper muscle activity (27). It can be argued that high- Arginine meals promote creatine production, while factors affecting creatine synthesis or excretion decrease the availability of Arginine for protein synthesis and growth (28).

Conclusion

In conclusion, our result indicates the bursal sections from Fabricius bursa showed significant aggravation in follicle number, with G3 and G4 Treated groups scoring higher. Eggs inoculated with arginine showed significant follicular growth in both breeds.

Conflicts of interest

The authors declare that there is no conflict of interest.

Ethical Clearance

This work is approved by The Research Ethical Committee

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تأثير تطعيم بيض الدجاج المحلي والتجاري بحمض الأرجينين الأميني على تطور جراب فابريشيوس

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الخلاصة

تركّز هذه الدراسة على الأهمية الاستراتيجيات للتغذية داخل البيضة، لا سيما التزويد بالأحماض الأمينية مثل الأرجينين لتعزيز نمو جراب فابريشيوس. إن التدخلات الغذائية الموجهة أثناء مرحلة التخلق الجنيني تمثل نهجًا واعدًا لتحسين الصحة العامة والإنتاجية والقيمة السوقية للدواجن.تم تنفيذ التجربة في كلية الزراعة – جامعة السليمانية لدراسة تأثير الأرجينين على نسيج الجراب في سلالتين من الدجاج :الدجاج التجاري روز 308، والدجاج الكردي المحلي. شملت الدراسة مائة بيضة مخصبة من سلالة روز 308 ومائة بيضة مخصبة من السلالة المحلية. شملت المعاملات داخل البيضة مجموعة سيطرة (دون تطعيم) وثلاث مجموعات تطعيم بمحاليل الأرجينين بتركيزات 1%، 2%، و3%. وقد أُجري التطعيم داخل البيضة مرتين: في اليومين الرابع عشر والثامن عشر من الحضانة. بعد الفقس، تم قتل أربعة أفراخ بعمر يوم واحد من كل مجموعة معالجة، وتم حفظ جراب فابريشيوس الخاص بها لتحضير المقاطع النسيجية. أظهرت الدراسة أن التحسّن في تكاثر الجريبات اللمفاوية في سلالة الدجاج الكردي كان ملحوظًا مقارنةً بالدجاج التجاري، سواء من حيث عدد الجريبات أو تطورها. كما أن نظام التقييم الخاص بتكاثر الجريبات في جراب فابريشيوس سجل أعلى القيم في المجموعة المعالجة الرابعة أرجينين، ومع ذلك، فإن بعض المقاطع النسيجية أظهرت زيادة مفرطة في عدد الجريبات، وخاصة في المجموعتين الثالثة والرابعة. بشكل عام، بيّن التطعيم بالأرجينين داخل البيضة نموًا ملحوظًا للجريبات اللمفاوية في كلا السلالتين.

الكلمات المفتاحية :الأرجينين، الدجاج، التطعيم داخل البيضة، جراب فابريشيوس.