

HISTOLOGICAL FINDINGS OF THE BURSA OF FABRICIUS IN BROILER CHICKS SUPPLIED WITH THE METHIONINE SUPPLEMENT METHIO GROW

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ABSTRACT

The study aimed to determine the effects of higher levels of methionine supplementation than the recommended NRC level in the diets on the weight and histomorphology of the bursa of Fabricius as indicators for the immune status of broiler chickens. A total of 60, one-day-old, broiler chicks were divided into 4 groups (Control, T1, T2, and T3 groups) and accommodated in separate pens at the College of Agricultural Engineering Sciences, University of Sulaimani. The chicks were fed on a starter diet for the first 11, a grower diet on days 12-25 and a finisher diet on days 26-42. The methionine supplement MethioGrow was added daily to the drinking water of the treatment groups T1,

T2, and T3 by the levels 0.25, 0.50, 0.75 ml per liter respectively for 42 days, whereas those of the control group were provided with additives-free drinking water. On day 42, the chicks were euthanized and the bursa of Fabricius was excised, weighed by a sensitive balance and 5µm thick tissue sections were obtained, stained by Hematoxylin and Eosin stains and examined by a light microscope to examine the histological findings. The weights' mean of the bursa of Fabricius on day 42 was significantly higher in groups T2 and T3 than in the control group and the microscopic examination of the bursa of Fabricius revealed slight increase in numbers of lymphocytes in the medullary region of the lymphoid follicles in chicks of the treatment groups 1 and 2 (T1 and T2) and slight increase in lymphocytes in both the cortical and medullary regions of the lymphoid follicles in chicks of the treatment group 3 (T3) in comparison with chicks of the control group. In conclusion, the obtained results revealed that supplementation of higher levels of methionine in the broiler diet than the level recommended by the NRC has positive effects on the immune status of the broilers and they support the suggestions of previous reports that referred to the insufficiency of methionine requirements of broiler based on recommendations of NRC to meet the real requirements of the commercial poultry farms.

INTRODUCTION

Methionine, the first limiting essential amino acid in most commercial feeds, is able to improve the growth performance and carcass quality of poultry ⁽¹⁻⁵⁾. In addition, it is also enhanced the immune functions including antibody production and cell-mediated immune responses in broilers ⁽⁶⁾. Methionine deficiency may result in diminished humoral immunity and non-specific immunocompetence of broilers ⁽⁷⁾, and it can lead to restrain the development of the bursa of Fabricius ⁽⁸⁾.

The minimum requirements for a given nutrient for optimal production were established by the National Research Council (NRC) ⁽⁹⁾. However, the recommended minimum requirements for the different nutrients of the NRC are usually based on the needs of healthy birds under ideal management, whereas birds in commercial systems are normally exposed to different kinds of stresses therefore, there is an important necessitate for multiple focus and attention to the actual broiler requirements. Furthermore, it is not known whether

the nutrient requirement values that improve productivity in healthy, unchallenged birds are optimal for immune competence and disease resistance ⁽¹⁰⁾.

There are some evidences that higher levels of essential amino acids in the feed than the recommended levels of NRC may be required to achieve optimal growth performance, immunocompetence and disease resistance ^(11,12). The bursa of Fabricius has an essential role in the maturation and differentiation of the B-lymphocytes and thus is closely related to normal humoral immune function in chickens ^(13,14). So, the structure of the bursa can be used as a good indicator for studying the effects of many factors of B-cell function. Therefore, the present study was conducted to determine the effects of higher level of methionine supplementation than the recommended NRC level in the diets on the weight and histomorphology of the bursa of Fabricius as indicators for the immune status of broiler chickens.

MATERIAL AND METHODS

Experimental Design

A total of 60, one-day-old, un-sexed broiler chicks (Rose 308) were obtained from Taqtaq hatchery of Kosar Company for Agriculture and Poultry, and were randomly divided into 4 groups named Control, T1, T2 and T3 groups, the chicks of each group were further divided into 3 replicates (5 chicks each) named R1, R2, and R3. The chicks were housed in separate, temperature, ventilation & light-controlled pens at the College of Agricultural Engineering Sciences, University of Sulaimani and were fed on a starter diet for the first 11 days of their life and then replaced by a grower diet on days 12-25 days and a finisher diet on days 26-42. The diets were set up to carry out the standard requirements of broiler chicks with standard protein, energy and methionine composition (Table 1) with no antibiotics or growth promoter supplementations.

Table 1. Protein, energy and methionine composition* of the diet supplied to the experimental broiler chicks during their different growing periods

		Ingredients		
		Protein (%)	Energy (Kcal/Kg)	Methionine %
Diet	Starter	22.8	3079	0.48
	Grower	21	3139	0.37
	Finisher	19.1	3212	0.32

* The ingredients compositions in the diets were determined according to NRC (9).

Chicks of the treatment groups T1, T2, and T3 were respectively supplemented (daily for 42 days, via drinking water) with 0.25 ml, 0.50 ml, 0.75 ml per liter of the methionine supplement MethioGrow (Miavet, Germany), whereas those in the control group were provided with additives-free drinking water (0.00 gm methionine per liter).

Weight measurements of the bursa of Fabricius and histopathological examination

At the end of the experimental period on day 42, the chicks were euthanized by cervical dislocation, dissected and the bursa of Fabricius was excised, weighed by a sensitive balance, fixed in 10% formalin and undergone a series of histological preparations, sectioned using a rotary microtome into 5µm thick tissue sections, stained by Hematoxylin and Eosin stains ⁽¹⁵⁾, and then examined by the different magnification powers of a light microscope to examine and describe the histopathological findings.

Statistical Analysis

The obtained data were analyzed using the SPSS program version 21.0 (IBM Crop, 2016). The significant of variance between weight means of the bursa of Fabricius was determined using Duncan's multiple range test under the probability (p<0.05).

RESULTS

Weight of the bursa of Fabricius

The mean of weights of the bursa of Fabricius on day 42 was significantly higher at $p < 0.05$ in groups T2 and T3 than that of the control group and T1 (Table 2).

Table 2: Means \pm SE of the bursa of Fabricius of the broiler chicks in the different groups' replicates of the present study

Group *	Control	T1	T2	T3
Replicate 1**	2.77	3.31	5.40	5.33
Replicate 2	3.31	3.80	4.92	4.67
Replicate 3	3.06	4.71	4.12	4.48
Mean \pm S.E. ***	3.05 \pm 0.43 ^b	3.94 \pm 0.96 ^{ab}	4.81 \pm 0.76 ^a	4.83 \pm 0.71 ^a

* The levels of methionine supplementation in drinking water of the chicks were 0.25, 0.5 and 0.75 ml / liter for the groups T1, T2 and T3 respectively whereas the control group's chicks receive additive-free drinking water.

** Each group contains 15 broiler chicks divided randomly into 3 replicates, 5 chicks each.

*** Within the last row, weight means values with different small alphabetical superscripts differ from each other significantly ($p < 0.05$).

Histopathological findings of the bursa of Fabricius

The microscopic examination of the tissue sections obtained from the bursa of Fabricius on day 42 of the chicks revealed slight increase in numbers of lymphocytes in the medullary region of the lymphoid follicles in chicks of the treatment groups 1 and 2 (T1 and T2) and slight increase in numbers of lymphocytes in both the cortical and medullary regions of the lymphoid follicles in chicks of the treatment group 3 (T3) in comparison with chicks of the control group (Figures 1-5).

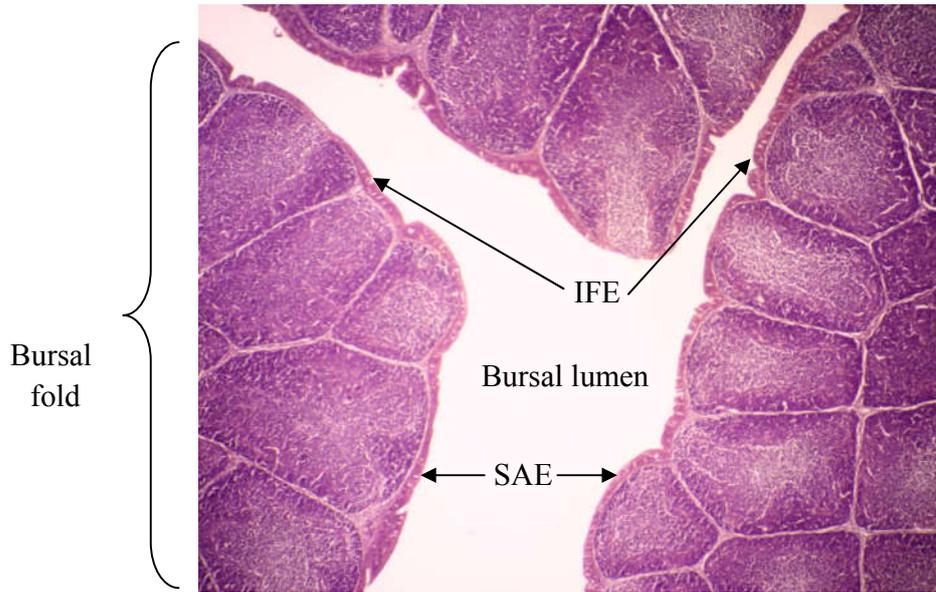


Figure 1: Microscopic view of a tissue section obtained from the Bursa of Fabricius of a chick in the control group. It shows normal-looking lymphoid follicles within three bursal folds covered by normal surface associated epithelium (SAE) and interfollicular epithelium (IFE), H and E, X 100.

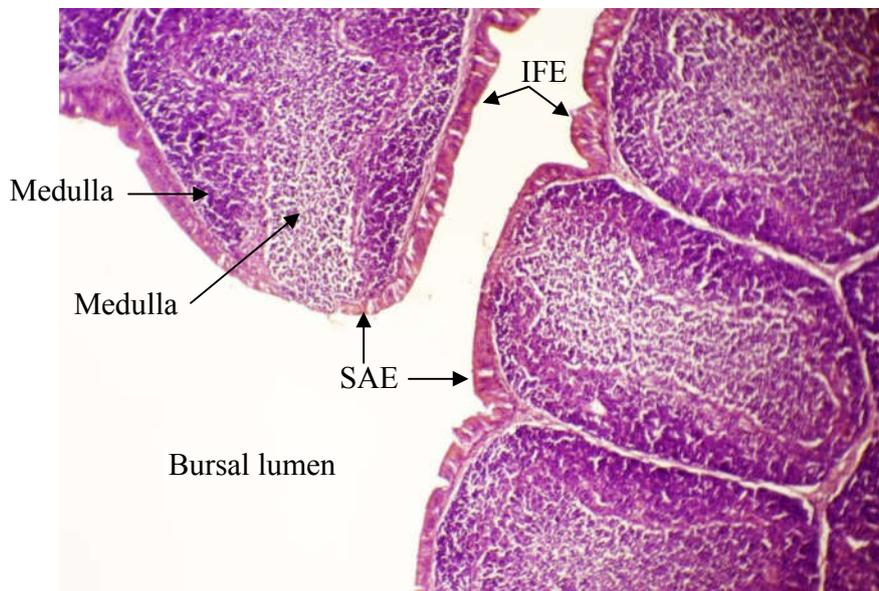


Figure 2: Higher magnification view of the previous figure. It shows the cortex and medulla within normal-looking lymphoid follicles covered by normal surface associated epithelium (SAE) and interfollicular epithelium (IFE). H and E, X200.

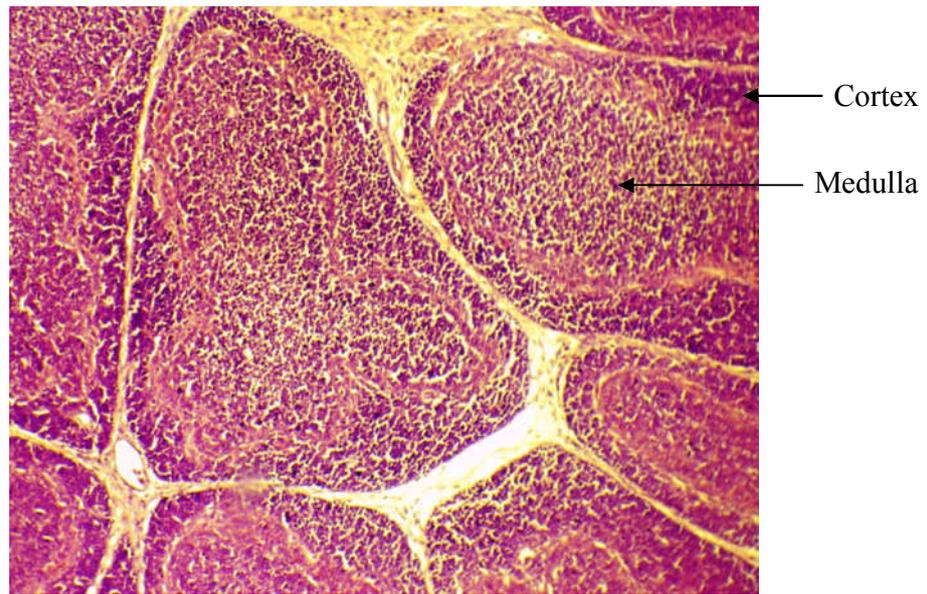


Figure 3: Microscopic view of some lymphoid follicles in the Bursa of Fabricius of a chick in the treatment group 1 (T1). It shows slight increase in numbers of lymphocytes in the medullary region. H and E, X200.

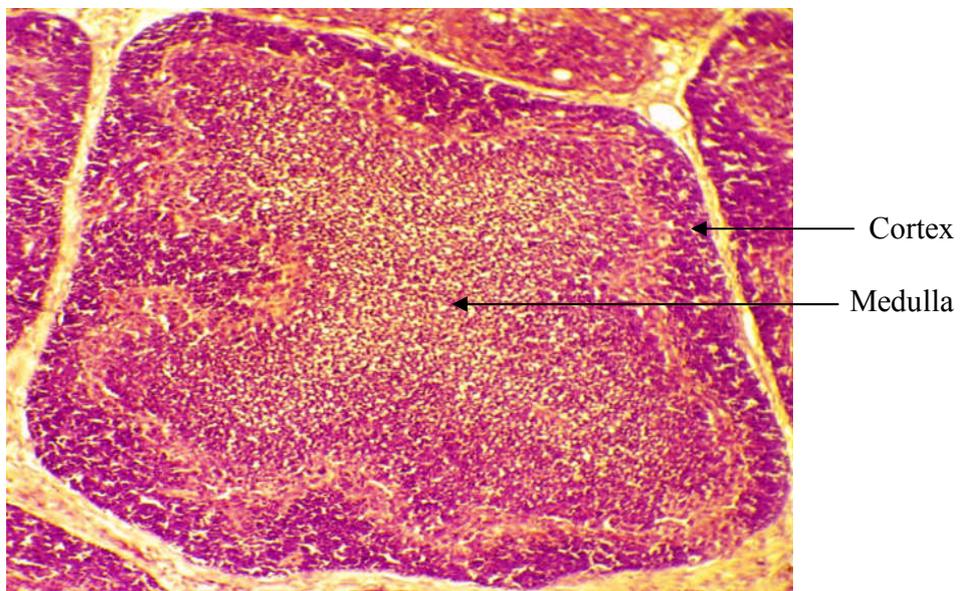


Figure 4: Microscopic view of a lymphoid follicle in the Bursa of Fabricius of a chick in the treatment group 2 (T2). It shows slight increase in numbers of lymphocytes in the medullary region. H and E, X400.

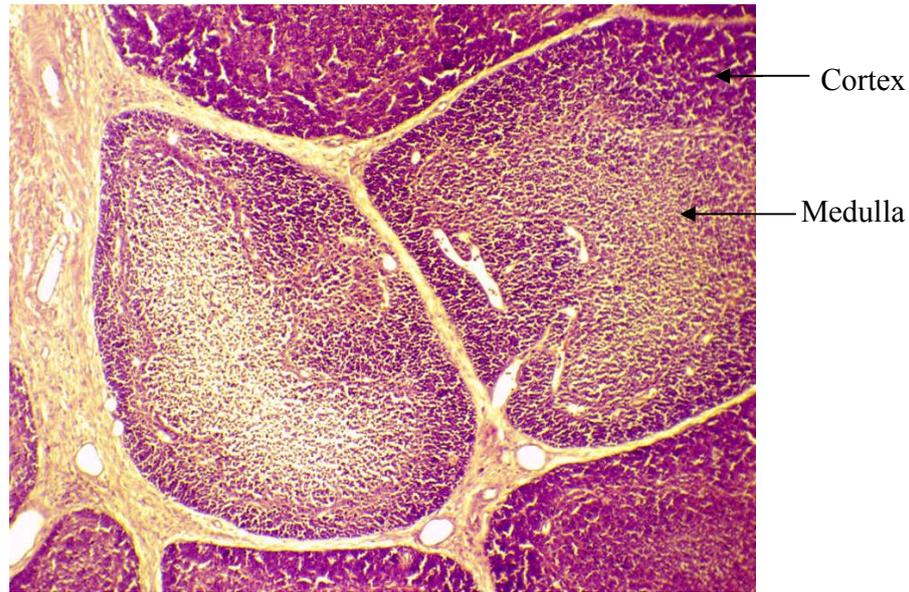


Figure 5: Microscopic view of a lymphoid follicle in the Bursa of Fabricius of a chick in the treatment group 3 (T3). It shows slight increase in numbers of lymphocytes in both the cortical and medullary regions. H and E, X200.

DISCUSSION

The mean of weights of the bursa of Fabricius on day 42 was significantly higher in groups T2 and T3 than that of the control group. This finding which reflects the importance of the amino acid methionine in development of the bursa of Fabricius and in the immune function, particularly humoral immunity^(8,16) is in agreement with the results of other related researches^(10, 17) which reported a significant increase in weights of the lymphoid organs (bursa of Fabricius and spleen) and better levels of humoral immunity following exposing the broiler chickens to a higher level of methionine than the recommended level of the NRC⁽⁹⁾ and it is also in agreement with the results of Fasuyi and Alertor⁽¹⁸⁾ who reported that a better performance of the broilers can be obtained with adequate supplementation of essential amino acids especially methionine, which is present in marginal quantities in most commercial poultry feeds.

The microscopic examination of the tissue sections obtained from the bursa of Fabricius on day 42 of the chicks age revealed slight increase in in numbers lymphocytes in the medullary region of the lymphoid follicles in chicks of the treatment groups 1 and 2 (T1 and T2) and slight increase lymphocytes in both the cortical and medullary regions of the

lymphoid follicles in chicks of the treatment group 3 (T3) in comparison with chicks of the control group. This finding is in constant with the findings of some related researches ^(8, 16, 17) which indicated that the higher level of methionine supplementation improve the immune status of the broiler chicks by promoting the proliferation and maturation of lymphocytes in the Bursa of Fabricius.

The results obtained from the current study revealed that supplementation of higher concentrations of methionine in the broiler diet than the concentration recommended by the NRC has positive effects on the immune status of the broilers and they support the suggestions of previous reports ^(8, 10, 18) referred to the insufficiency of methionine requirements of broiler based on recommendations of NRC ⁽⁹⁾ to meet the real requirements of the new commercial poultry and commercial broiler companies. In conclusion, the results of the present study revealed that supplementation of the broiler diet with of higher levels of the methionine supplement MethioGrow has positive effects on the immune status of the broilers.

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النتائج النسيجية لغدة فابريشيا في أفراخ اللحم المجهزة بمدعم الميثايونين ميثايوگرو

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الخلاصة

كان الهدف من هذه الدراسة هو تحديد التأثيرات الناتجة عن تجهيز عليقة الدواجن بمستويات من الميثايونين أعلى من تلك التي يوصي بها المجلس البحثي الدولي (NRC) على الوزن والشكل النسيجي لغدة فابريشيا كدلائل للحالة المناعية لدجاج اللحم. قسمت ٤٠ فرخة لحم بعمر يوم واحد الى ٤ مجاميع (مجموعة سيطرة ومجموعات معاملة ١ و ٢ و ٣) و ربيت في أقفاص منفصلة في كلية العلوم الهندسية الزراعية في جامعة السليمانية. غذيت الافراخ بعليقة ابتدائية في الايام ١١ الاولى وعليقة نمو في الايام ١٢-٢٥ وعليقة انتهائية في الايام ٢٦-٤٢. أضيف مكمّل الميثايونين ميثايوگرو MethioGrow يوميًا إلى مياه الشرب لمجموعات العلاج T1 و T2 و T3 بالمستويات ٠.٢٥ و ٠.٥٠ و ٠.٧٥ مل لكل لتر على التوالي لمدة ٤٢ يومًا فيما جهزت أفراخ مجموعة السيطرة بماء شرب خال من الإضافات. في اليوم ٤٢ تم التضحية بالافراخ وتم إستئصال غدة فابريشيا وتحديد وزنها بواسطة ميزان حساس ومن ثم أخذت منها مقاطع نسيجية بسمك ٥ مايكرومتر ولونت بصيغتي الهيماتوكسلين والايوسين وفحصت بالمجهر الضوئي لأجل تحديد النتائج النسيجية. أظهر معدل وزن غدة فابريشيا في اليوم ٤٢ زيادة معنوية في مجموعات المعاملة ٢ و ٣ بالمقارنة مع مجموعة السيطرة وأظهر الفحص النسيجي لغدة فابريشيا زيادة طفيفة في عدد الخلايا اللمفية في منطقة لب الجريبات اللمفية في أفراخ مجموعات المعاملة ١ و ٢ وزيادة طفيفة في عدد الخلايا اللمفية في منطقتي قشرة ولب الجريبات اللمفية في أفراخ مجموعة المعاملة ٣ بالمقارنة مع أفراخ مجموعة السيطرة. تدل نتائج هذه التجربة على أن تجهيز عليقة الدواجن بمستويات من الميثايونين أعلى من تلك التي يوصي بها المجلس البحثي الدولي (NRC) له تأثيرات إيجابية على الحالة المناعية لافراخ اللحم وهي تدعم إقتراحات لتقارير علمية سابقة كانت قد أشارت الى عدم كفاية مستويات الميثايونين لافراخ اللحم المعتمدة من قبل المجلس البحثي الدولي (NRC) لتلبية الاحتياجات الحقيقية لحقول تربية الدواجن التجارية.

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