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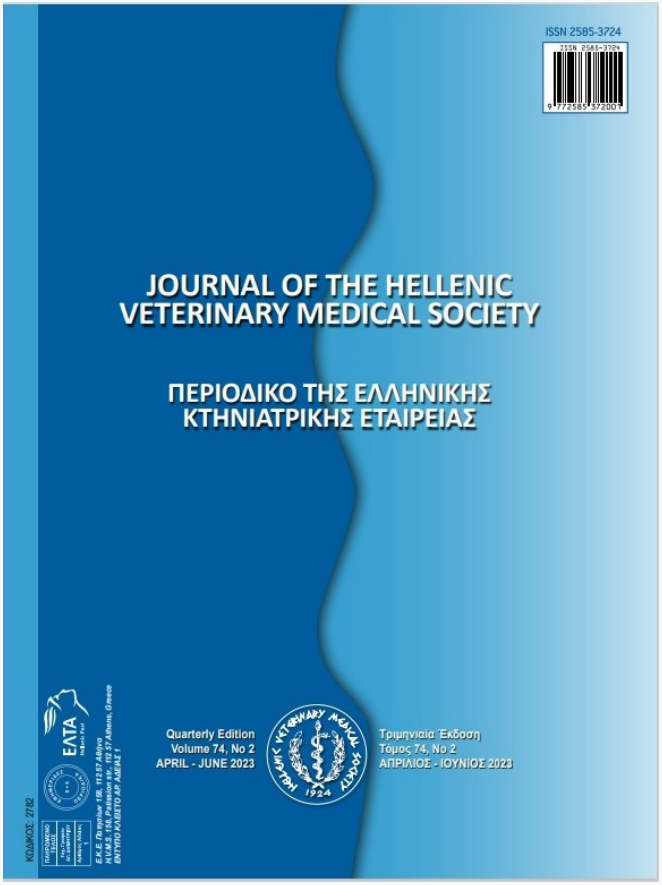

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Research article
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Evaluation of Bursal Index and Bursal Lesion Scores in Broiler Flocks

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Background and Aims: The bursa of Fabricius (BF) is recognized as a vital organ that plays a crucial role in birds immunity. In order to determine the immunological status of the birds, this study was conducted to evaluate the bursal index, and bursal lesion scores in broilers.

Materials and Methods: Therefore, a total of 200 male broilers of Ross 308 strain, 35-45 days of age, from 10 broiler flocks were randomly selected, weighed, then sacrificed. At necropsy, the bursa of Fabricius were obtained, examined, and weighed. Bursal index (BI) was calculated. Representative pieces of tissues from bursa of Fabricius were preserved in 10% neutral buffer formaldehyde for further histopathological evaluation.

Results: The results obtained from (BI) calculated during the study period for all 22 broiler farms, revealed that more than 81% of the birds were included in bad group (BI<0.15%) and less than 19% in mediocre group (0.18%<BI<0.20%). Degree of microscopic lesions between different groups and even within one group varied from 0 (normal) to 5 (atrophy of BF) and included lymphoid necrosis in follicles, hyperplasia and folding of bursal inner epithelium, and formation of fibrous connective tissue within the follicles. Average bursal lesion scores for all groups under study was recorded as 2.09%.

Conclusions: On the basis of all results including (BI) and bursal lesion scores it is concluded that bursal lymphoid structures suffer various degree of pathological lesions indicating suppressed immunity in broiler farms along with poor performance.

***Keywords*:** Broiler chicks, Bursa of fabricius, Bursal body index, Bursal lesion scoring.

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INTRODUCTION

The immune system of birds comprises principally of the bursa of Fabricius, and thymus. Other than that spleen, mucosa-associated lymphoid tissues, bone marrow, and blood also plays a vital role in bird's immunity (1). The bursa of Fabricius, is a central lymphoid organ, chestnut in shape, present dorsally to the cloaca (2). It is a site of B lymphocyte repertoire differentiation and maturation, located in the dorsal terminal part of the cloacae of the birds that plays a primordial role in the poultry immunity (3, 4). Young birds have larger and more active bursa. It reduces in size at the age of six months, then increases at the age of 8-10months afterwards undergoes shrinkage like thymus (5). Former studies suggest that bursa is a major organ in evoking the immune system and therefore formation of antibodies (6).

It is according to its physiological state that will especially depend on the immune status of poultries at the beginning of broiler chickens weight development. The different aggressions of the environment (stress, bad hygiene, vaccination, pathologies…) undergone by birds, influential on the anatomical and physiological development of the bursa of Fabricius. It can, therefore, lead to an immunosuppression at certain birds. The use of vaccines called virulent "warm" can sometimes compromise immunity at poultries while causing some lesions of this organ (7, 8). The quantitative development of industry and the expansion of breeding farms on one hand and the high sensitivity of the modified strains on the other hand, by providing sensitive hosts for pathogenic microorganisms on a wide scale, have increased the possibility of disease occurrence. The occurrence of different diseases, some of which are due to the irregular structure of the farms and their management problems, and some others due to unfavorable weather conditions such as high fluctuations in temperature and serious lack of air humidity along with the need to improve the quality of all production-related aspects indicates the need for the full health of the bird's immunity system to deal with the adverse conditions (8).

Since the bursa of Fabricius, as one of the most important members of the primary immune system, plays an important role in the occurrence of favorable immune responses to the given vaccines, it is expected that any serious damage to the lymphatic structures of the bursa will weaken the function of the humoral branch of the immune system. Considering the abundance of environmental and infectious factors in broiler farms, it is expected that the health and efficiency of the Bursa of Fabricius have a significant impact on vaccination performance, improving growth performance and strengthening the immune system. Therefore, the present study was carried out with histological and morphometric examination (BBR) of the bursa of Fabricius of broiler poultry under common breeding conditions in order to evaluate the immunity status of the birds.

MATERIALS AND METHODS

Animals

To carry out this project, 20 broiler farms with 15,000 broilers of ROSS 308 strain were selected around Rasht city, Iran, all of which were male and aged between 35 and 45 days. The chickens of these farms were reared in groups and under suitable conditions in terms of light and ventilation, and all rearing conditions were on the bedding and almost the same in all the farms. The duration of the rearing period was 7 weeks and the diets were adjusted according to the guidelines for rearing and feeding the ROSS 308 broiler strain and free access to feed was prepared.

All birds in selected farms had received vaccines, recommended for broiler farms in the study area, as given in Table 1.

Sampling

The experimental protocol was ratified by the Animal Ethic Committee of the Islamic Azad University, Rasht, Iran, and the experiment was performed with

Table 1: Vaccination program for broiler farms recommended in the study area

Vaccines	Approximate age	Vaccination rout
Infectious bronchitis H120	One day old	Spray
AI + ND	first week	Injection
Hotchner B1	first week	Eye drop
Infectious bronchitis H120	second week	drinking water
Gumboro	14 days old	drinking water
Newcastle	third week	drinking water

respect to the International Guidelines for research involving animals (Directive 2019/63/EU).

The birds were killed by cervical subluxation method and bursa of Fabricius, were collected through ventral abdominal dissection.

Preparation of tissues for histological study

The tissues obtained from the chickens were fixed in 10% Neutreal Buffered Formaldehyde for 72 hours and were dehydrated in the series of ascending grade of alcohol followed by clearing in three changes in xylene, and the tissues then infiltrated with different grades of melted paraffin in the oven. The tissues were then embedded in paraffin and finally the sections were cut at4 μ thickness using Rotary microtome (MIC 509, Euromex, Japan). After cutting, the sections were floated on luke-warm water in a floatation bath at 37 0C for stretching and then the sections were mounted on clean slides using an adhesive (Egg albumins) and dried on a slide warmer at 37 0C. The sections were stained using Mayer's Hematoxylin and Eosin (Bancroft 2004).

The histological structures of the lymphoid tissues were observed using light microscope under low (×10) and high (×40) magnification. Photographs from the selected specimens were prepared for better illustration of the results.

Scoring

Two hundred bursa collected at 35-45 days of age, showed bursal lesion scores varying from 0 to 5. Bursal lesion score was performed as per the method of Farzana Aktar et al. (2020) on a 0 - 5 scale (9).

Bursal score 1: The bursa histologically revealed scattered lymphoid necrosis in a few follicles. The cortex and medulla were indistinct. There was mild increase in interfollicular stromal tissue.

Bursal score 2: The bursal plica revealed moderate to severe lymphoid depletion in most of the bursal follicles. The lining epithelium was corrugated.

Bursal score 3: The bursa revealed severe lymphoid depletion in almost all the follicles, which appeared pale and vacuolated. There was infiltration of heterophils in the bursal follicle and inter-follicular stroma. The lining epithelium was denuded in a few plicae. In a few follicles there were cellular debris and cystic cavities. The lining epithelium was corrugated.

Bursal score 4: The bursa revealed loss of lymphoid follicles glandular transformation and increase in inter follicular connective tissue. Cystic cavities were observed in some follicles. Infiltration of macrophages, plasma cells and lymphoid cells were observed in the connective tissue stroma. The lining epithelium was corrugated.

Bursal score 5: Bursa microscopically revealed complete loss of architecture. There was no intact lymphoid follicle and the entire area was filled up by fibrous tissue. The lining epithelium was highly corrugated.

Bursal Index (BI)

Based on body weights and bursal weights recorded, BI was calculated on the basis of the following formula: BI = (Bursal weight / body weight) × 100

Add statistical analysis and give details

RESULTS

Bursal parameters which considered in this study included: bursa weight / body weight ratio (BI) and histopathologic findings. The bursal index (BI) calculated during the study period for all 22flocks of broiler chickens demonstrates that 81% belonged to the bad class [(bad class (BI< 0.15%] and less than 19% of the birds are located in mediocre class [mediocre class (0.15% <BI<0.18%)] (Table 3). BI in non of these farms were belonged to medium class (0.18% < BI< 0.20%) and / or excellent class (BI> 0.20%). Bursal lesional scoresurvey demonstrated that 100% of the examined organs revealed lesions of different degrees.

Distribution of bursal microscopic lesions among different flocks and even within a particular flock varied from 0to5 (bursal atrophy and fibrosis). These lesions were characterized by varying degrees of lymphoid necrosis within the follicles, hyperplasia and folding of bursal inner epithelium, and replacing of follicles by fibrous connective tissue formation.

Mean pathological lesion scoring of BF in each flock varied from 0.25 to 4.5 and mean total scoring for all flocks under investigation was recorded as 2.09.

Finally, it is concluded that parameters of bursal evaluation including BI and histopathology of Bursa, are practical characteristics and low cost devices in field studies in evaluation of broiler immunity status. Results are given in brief in Tables 2 and 3.

Table 2: score average of histopathologic lesions and BI (g)

Farm No	Mean pathological lesions in each farm	Mean body weight(kg) in each farm	Mean bursal weigh (gr) in each farm	Bursal / Body weight ratio in each farm
1	0.25	1.665	1.56	0.092%
2	2	1.967	0.88	0.043%
3	2.25	2.131	1.27	0.057%
4	1	1.702	0.9	0.052%
5	2.75	2.169	1.04	0.049%
6	0.5	2.177	1.03	0.047%
7	3	2.340	1.53	0.064%
8	2.75	1.891	1.05	0.054%
9	1.25	1.900	3.09	0.15%
10	0.5	2.171	0.95	0.043%
11	1	2.062	0.99	0.047%
12	2.25	1.815	2.89	0.15%
13	3	2.054	2.04	0.097%
14	2.5	2.001	1.06	0.054%
15	4.5	2.295	1.43	0.062%
16	0.75	2.656	0.50	0.018%
17	1.5	1.338	0.87	0.066%
18	0.75	2.362	1.69	0.07%
19	2.6	1.958	3.57	0.17%
20	0.5	1.602	2.25	0.13%
Total Average	**1.78**	**2.0128**	**1.5295**	**0.07575**

Table 3: Mean BI (%) and determining immune status of the broilers

Farm No.	**BI < 0.15%**	**0.15% < BI < 0.18%**	**0.18% < BI < 0.20%**	**BI > 0.20%**
	Excellent	**Medium**	**Mediocre**	**Bad**
1	-	-	-	0.092%
2	-	-	-	0.043%
3	-	-	-	0.057%
4	-	-	-	0.052%
5	-	-	-	0.049%
6	-	-	-	0.047%
7	-	-	-	0.064%
8	-	-	-	0.054%
9	-	-	0.15%	-
10	-	-	-	0.043%
11	-	-	-	0.047%
12	-	-	0.15%	-
13	-	-	-	0.097%
14	-	-	-	0.054%
15	-	-	-	0.062%
16	-	-	-	0.018%
17	-	-	-	0.066%
18	-	-	-	0.07%
19	-	-	0.17%	-
20	-	-	-	0.13%
Total			18.18%	81.81%

DISCUSSION

Bursal parameters which considered in this study included bursa weight / body weight ratio (BI) and histopathologic findings.

The bursal index (BI) calculated during the study period for all 22 flocks of broiler chickens demonstrates that 81% belonged to the bad class [bad class (BI< 0.15%)] and less than 19% of the birds are located in mediocre class [mediocre class (0.15% <BI<0.18%)] (Table 3). BI in non of these farms were belonged to medium class (0.18% < BI< 0.20%) and / or excellent class (BI> 0.20%). Bursal lesional score-survey demonstrated that 100% of the examined organs revealed lesions of different degrees.

Bursal index (BI) is one of the most important parameters to evaluate the immunosuppression caused by IBD virus and by IBD vaccine (10, 11, 12).

The results obtained from this study showed that the weight of the bursa of Fabricius is not proportional to the age and weight of the bird. Alloui et al. (2012) also pointed out in their studies the disproportion between the weight of the bursa and the age and body weight of the bird (13).

The infectious bursal disease is an important viral disease in young chickens, which is caused by a double-stranded RNA virus belonging to the Birnaviridae family. This disease is of major importance due to the high rate of spreading and mortality during the acute phase of the disease or by weakening immunity during a chronic period (14).

There are many other reasons, including non-infectious factors, which have similar effects on the weakening of the bursa of Fabricius. The association of an injury from the bursa with the use of some infectious bursal disease vaccines in some conditions and as a result weakening of immune function has been recorded (15).

Immune suppression, which occurs as a result of the lesions created in this vital organ, in many cases hinders the optimal performance of vaccination and creates complex and serious conditions such as poor absorption, decreasing weight gain, opportunistic infections, and finally various diseases in herds (13).

As seen in the results of the present experiment, the distribution of microscopic lesions observed among different herds and even within a herd was very diverse and varied from zero (normal) to five (bursal atrophy and fibrosis). These lesions were seen especially with varying degrees of lymphoid necrosis in the follicles, hyperplasia and folding of the bursal epithelium, and the formation of fibrous connective tissue in the follicles.

Mean pathological lesion scoring of BF in each flock varied from 0.25 to 4.5 and mean total scoring for all flocks under investigation was recorded as 2.09.

In the results of previous tests, lymphocyte necrosis, depletion of lymphocyte population, atrophy and apoptosis of lymphoid follicles in relation to the histopathological observations of the samples taken from the bursa of Fabricius organ of vaccinated chickens at the age of 35 days have been reported, which are consistent with the results of the present experiment (16).

The previous experiments have shown that obtaining the desired result from repeated vaccinations, which are impossible to avoid in many cases due to environmental pollution, is an essential factor in the full health of the birds' immune system but several factors can be listed among them that have inhibitory and destructive effects on the performance of the immune system (17, 18, 19).

Management factors such as stresses, nutritional factors such as mycotoxins, and biological factors such as bacteria, viruses and parasites are among the most important factors that can weaken the immune system of birds. The effect of some of these factors indirectly reduces the performance of the immune system, and some directly destroy the immune structures. The abundance of these factors at the level of broiler farms raises the expectation that the health and consequently the efficiency of the bursa of Fabricius should be far from what is considered normal (20, 21).

CONCLUSIONS

It was concluded that parameters of bursal evaluation including BI and histopathology of Bursa, are practical characteristics and low cost devices in field studies in the evaluation of broiler immunity status.

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