

Histomorphology of the Lymphoid Tissues of Broiler Chickens in Kelantan, Malaysia

(Histomorfologi Tisu Limfa Ayam Pedaging di Kelantan, Malaysia)

MOHD ZAHIRUL ISLAM KHAN*, MOHD MASUM, MD. ZUBAYER IBNA KHAN, ABDUR RAHMAN BIN AZIZ, MORSHEDA NASRIN, MOHD NAZMUL HASAN SIDDIQUE & MOHD MOKHTAR BIN ARSHAD

ABSTRACT

The present research has been designed to understand the histomorphological development of lymphatic tissues of Cobb 500 strains of postnatal broiler chickens, aged between day old and D₁₄ of Kelantan, Malaysia by gross and histological study. In the present study, it was found that the gross weight, length and width of the thymus, bursa of Fabricius and spleen were increased with the advancement of ages of the broiler chickens and was found higher from D14 to D28. Fine septa of connective tissue divide the thymus into lobes and lobules. The cortex of lobule is densely populated with lymphocytes. The bursal follicles were composed of a peripheral cortex which was rich in bursal cells and centrally depopulated medulla. The mucosal folds of the bursa were lined by pseudostratified columnar epithelium. The spleen has two compartments, the red and white pulp. The red pulp consisted principally of red blood cells, while the majority of the populations of white pulp were the lymphocytes. The histological mean length and width of the thymic lobules, bursal follicles and white pulp of the spleen were grown faster with the advancement of ages at D14 and D28. In conclusion, the increment of gross and histological parameters of lymphoid organs of broilers in the present study was due to aging of broilers.

Keywords: Broiler; bursa of Fabricius; histomorphology; spleen; thymus

ABSTRAK

Kajian dasar dan histologi ini telah direka khusus untuk memahami pembangunan histomorfologi tisu limfa ayam pedaging baka Cobb 500 selepas beranak, berumur antara 1 hari hingga 14 hari di Kelantan, Malaysia. Kajian ini mendapati bahawa berat kasar, panjang dan lebar timus, bursa fabricius serta limpa telah bertambah dengan peningkatan umur ayam tersebut dari hari ke 14 ke hari ke 28. Septa halus tisu perantara membahagikan timus kepada cuping dan lobul. Korteks lobul jelas dipenuhi limfosit. Folikel bursal pula terdiri daripada korteks periferal yang kaya dengan sel-sel bursal dan mengosongi tengah medula. Lipatan mukosa bursa dibarisi oleh sel epitelium pseudostratified kolumnar. Limpa mempunyai dua petak, iaitu pulpa merah dan pulpa putih. Pulpa merah terdiri terutamanya daripada sel darah merah, manakala majoriti pulpa putih dipenuhi limfosit. Purata panjang dan lebar lobul thimik, folikel bursal dan pulpa putih limpa berkembang dengan lebih cepat mengikut pertambahan umur. Kesimpulannya, pertambahan parameter kasar dan histologi organ limfa pada ayam pedaging dalam kajian ini adalah disebabkan oleh proses penuaan.

Kata kunci: Ayam pedaging; bursa Fabricius; histomorfologi; limpa; timus

INTRODUCTION

Most living beings manage not only to survive but indeed thrive in potentially hostile milieu, without seeming effort. This freedom from disease is dependent on the existence of a complex and highly sophisticated defense system, called lymphoid system (Cortan 1988). The lymphoid system of fowl is consisting of unique organs and divided into two morphologically and functionally distinct components: The thymus, which produces T lymphocytes and responsible for cellular immunity, in contrast, bursa of Fabricius responsible for the production of B lymphocytes which causes humoral immunity (Cooper et al. 1965). The thymus and bursa of chicken is considered to be a 'central or primary' lymphoid tissue, whereas spleen, cecal tonsil and mucosa-associated lymphatic tissues (MALT) are considered

as 'peripheral or secondary' lymphoid tissues (Bach 1978; Getty 1975). The primary site for the development of lymphocytes is central one and the peripheral or secondary lymphoid tissues issues apparently depend on the central lymphoid tissue for their origin, development and function. The gross, histology, immunohistochemistry of the lymphatic tissues and its cellular component has been analyzed in the native and hybrid chickens of Bangladesh during their postnatal stages of growth (Karim et al. 2005; Khalil et al. 2003, 2002; Khan et al. 1996; Nasrin et al. 2013; Rahman et al. 2003). Review of literatures shows no information in this field in Malaysia. Therefore, the present research was designed to understand the morphometry and histology of the lymphatic tissues of broilers of Kelantan, Malaysia during their postnatal stages of growth.

MATERIALS AND METHODS

CHICKENS

The study was carried out on fifteen 'Cobb 500 strains' of broilers of both sexes to study the histomorphology of the lymphoid tissues (thymus, bursa and spleen). All the broilers purchased from Ladang Ternakan Ayam Daging, Kampong Beris Lalang, Bachok, Kelantan, Malaysia were grouped into D₁, D₁₄ and D₂₈ (5 broiler in each group). The birds has no developmental disorders and detectable disease that may cause any problem in the histological architecture of lymphoid tissues.

TISSUES USED FOR THE STUDY

The birds were killed by cervical subluxation method and the thymus was collected by ventral neck dissection and bursa of Fabricius and spleen were collected through ventral abdominal dissection, which were free from pathological lesions.

MORPHOMETRICAL STUDY

The thymus, bursa and spleen of broiler chickens were placed immediately in 10% neural buffered formalin and their weight, length and width were calculated out by conventional measuring methods. The mean histological length (µm) and width (µm) of white pulp of spleen, bursal follicles and thymic lobules were measured by ocular micrometer.

PREPARATION OF TISSUES FOR HISTOLOGICAL STUDY

The tissues obtained from the chickens were fixed in the 'Bouins fluid' (Gridley 1960) for 24 h and 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 at 6 µm thickness using sliding microtome (MIC 509, Euromex, The Netherlands). After cutting, the sections were floated on luke-warm water in a floatation bath at 37°C for stretching and then the sections were mounted on clean slides using an adhesive (Egg albumin) and dried on a slide warmer at 37°C. The sections were stained using Mayer's Hematoxylin and Eosin (H & E). 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.

STATISTICAL ANALYSIS

All values were expressed as mean ± SE. Statistical significance of difference was evaluated by using paired sample *t*-test. The statistical analyses were performed by using the SPSS windows package (SPSS Inc., Chicago, IL, USA).

RESULTS AND DISCUSSION

The thymus of all groups of chickens was a paired gland, one half of which was located on either side of the neck. Each half consisted of six to eight flattened yellowish white lobes of varying size of lymphoid tissue lying in the sub-dermal connective tissue of the neck region. These findings of the present study were similar to the report of Bach (1978) and Hodges (1974) in the hybrid chickens. In the present study it was shown that the lobes of the thymus were slightly elongated in D₂₈ which was possibly due to the postnatal enlargement of the thymus. This finding was similar to that of the hybrid chicken (Hodges 1974). The weight of the thymus in D₁, D₁₄ and in D₂₈ was 0.068±0.0427 g, 0.153±0.0013 g and 0.434±0.0231 g, respectively (Figure 1). The mean weight of the thymus was highest in D₂₈ ages of broiler followed by D₁₄ and D₁, respectively (Figure 1). The length of the thymus in D₁, D₁₄ and in D₂₈ was 2.80±0.108 mm, 10.75±0.629 mm and 13.30±0.576 mm, respectively (Figure 2). The mean length of the thymus was highest in D₂₈ ages of broiler and followed by D₁₄ and D₁, respectively (Figure 2). The mean width of the thymus was higher in D₂₈ than other age groups of chicken (Figure 3). Histologically, the thymus was enclosed by a thin connective tissue capsule. Numerous fine septa of connective tissue originated from the capsule and divided the organ into incompletely separated lobules. Each lobule organized into a peripheral cortex and a central medulla which was found to increase in size in D₁₄ and D₂₈ ages of broiler chickens (Figure 6). The cortex stained more deeply basophilic than that of medulla due to high concentration of cortical cells. The medulla was pale and diffuse Hassall's corpuscles were found, which were arranged in a concentric formation. Using high power objective, the presence of cords of lymphocytes was observed both in cortex and medulla. The histological architecture of the thymus in the present study is similar to the previous findings in White Leghorn chickens (Khan et al. 1988) and in Vencobb chickens (Karim et al. 2005). In the present study, it was showed that the bursa was globular in shape with slight anterior posterior compression at the different groups of broiler chicken. It was attached to the dorsal aspect of the proctodeum. The finding was similar to the study of Hodges (1974). The weight of the bursa of Fabricius in D₁, D₁₄ and in D₂₈ was 0.078±0.0027 g, 0.573±0.0578 g and 0.988±0.0047 g, respectively (Figure 1). The mean weight of the bursa of Fabricius was higher in D₂₈ than the other age groups of chicken (Figure 1). In the present study it was showed that the length of the bursa of Fabricius in D₁, D₁₄ and in D₂₈ was 5.50±0.600 mm, 14.50±0.605 mm and 11.50±0.750 mm, respectively (Figure 2). The mean length of the bursa of Fabricius was more in D₂₈ followed by D₁₄ and D₁ ages of broilers, respectively (Figure 2). Histological observation showed that the bursa was consisted of long thick mucosal folds which were projected into the lumen of the bursa. The middle region of the plica was thicker than the base and apical part. Numerous follicles filled the lamina propria

of each fold. All the follicles had clear margin and they were separated from the adjacent lymphoid tissue by connective tissue fibers, cells and intercellular space. Each bursal follicle composed a peripheral cortex and a central medulla which was found to increase in size in D_{14} and D_{28} ages of broiler chickens (Figure 7). A layer of undifferentiated epithelial cells occupied the periphery of the medulla, which was separated from the cortex by a capillary layer. The darkly stained cortex was composed of many closely packed small lymphocytes. The paler medulla contained immature lymphocytes of various sizes. The mucosal fold of the bursa was lined by pseudostratified columnar epithelium, except at the apex of each follicle, which was covered by a simple columnar epithelium. The populations of lymphocytes were uniformly distributed and the periphery of the medulla was smooth and regular in appearance in the follicles of chickens. The findings were similar to the germ free White Leghorn chickens (Honjo & Hirota 1993) and in Dekalb strain of White Leghorn chickens (Khan et al. 1998).

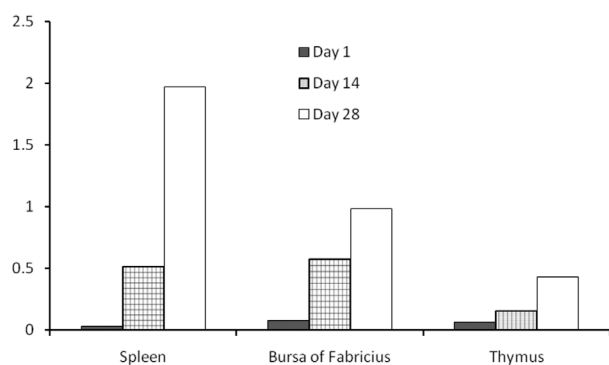


FIGURE 1. Comparison of weight (g) of spleen, bursa of Fabricius and thymus of chickens from D_1 to D_{28} . The weight of spleen, bursa of Fabricius and thymus of chickens are found significantly ($p < 0.01$) more in D_{14} and D_{28} of age. The values are given as the mean \pm standard error ($n=5$)

The shape of the spleen was rounded in different groups of broiler chicken; the findings were inconsistent to the study of Hodges (1974). It was found that the weight of the spleen in D_1 , D_{14} and D_{28} was 0.030 ± 0.0033 g, 0.517 ± 0.0097 g and 1.974 ± 0.0497 g, respectively (Figure 1). The mean weight of the spleen was higher in D_{28} than D_{14} and D_1 ages of chickens (Figure 1). The length of the spleen in D_1 , D_{14} and in D_{28} was 3.88 ± 0.315 mm, 13.58 ± 0.217 mm and 18.93 ± 0.394 mm, respectively (Figure 2). The mean length of the spleen in the present study was more in D_{28} followed by D_{14} and D_1 ages of broilers, respectively (Figure 2). The mean width of the spleen was higher in D_{28} than other groups of chicken (Figure 3).

Histologically, the spleen of the broiler was surrounded by a thick splenic capsule and a small number of trabeculi. The red pulp was less distinct and these were scatteredly distributed within the white pulp, composed mostly of

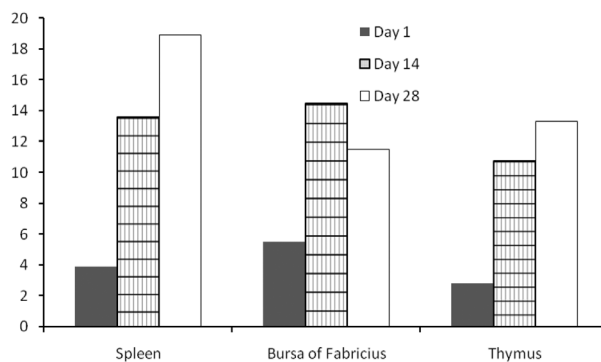


FIGURE 2. Length (mm) of spleen, bursa of Fabricius and thymus of chickens at D_1 , D_{14} and D_{28} . The length of the spleen and thymus of chickens are found significantly more ($p < 0.01$) in D_{14} and D_{28} ages of broilers. The values are given as the mean \pm standard error ($n=5$)

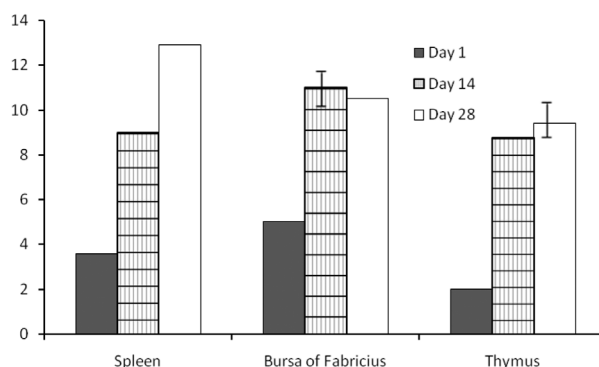


FIGURE 3. Width (mm) of spleen, bursa of Fabricius and thymus of chickens at D_1 , D_{14} and D_{28} . Width of spleen bursa of Fabricius and thymus of chickens are found significantly ($p < 0.01$) more in D_{14} and D_{28} of age. The values are given as the mean \pm standard error ($n=5$)

red blood cells. The white pulp was composed of network of reticular cells and reticular fibers within which small, medium and large sized lymphocytes and plasma cells were diffusely distributed. It contained sheathed arteries and lymphatic nodules. The red pulp of the spleen was formed from venous sinuses and anastomosing cord of reticular cells, macrophages, lymphocytes and blood cells. The immunocompetent cells were found both in the red and white pulps. Central arteriole was found in the white pulp. The network of the splenic tissue was consisted of a network of reticular cells and fibers. Histological structure of spleen of the present study in broiler is similar to the previous study (Bach 1978; Hodges 1974; Khan et al. 1998).

In the present study it was found that, the histological length and width of thymic lobule, bursal follicle and white pulp of spleen were significantly more in D_{14} and D_{28} than the broilers of day old (Figures 4 & 5). Previously we also reported similar fashion of growth in White leghorn Dekalb strain of chickens (Khan et al. 1998).

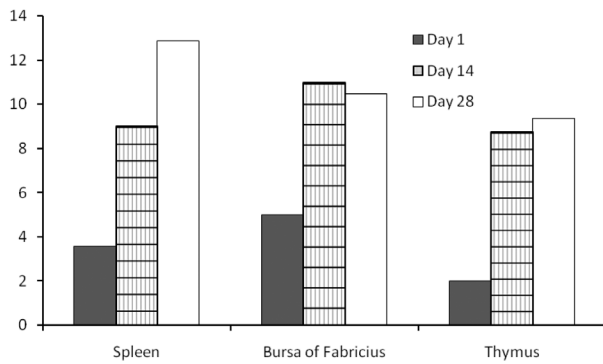


FIGURE 4. Length (μm) of the white pulp of spleen, bursal follicles and thymic lobules. The length of the white pulp of spleen, bursal follicles and thymic lobules is significantly ($p < 0.01$) higher in D_{14} D_{28} age of broilers than D_1 . The values are given as the mean \pm standard error ($n=5$)

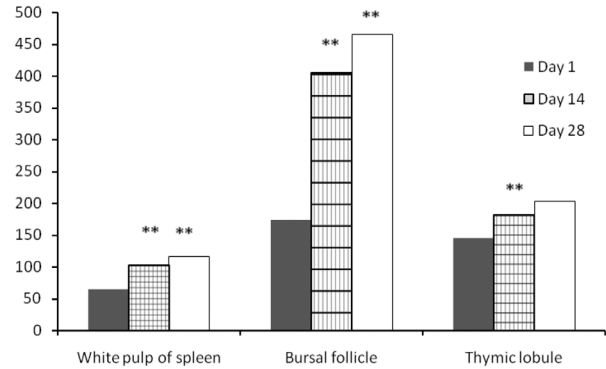


FIGURE 5. Width (μm) of white pulp of spleen, bursal follicles and thymic lobules. With the advancement of age, width of white pulp of spleen, bursal follicles and thymic lobules of chickens are found significantly ($p < 0.01$) more at D_{14} and D_{28} ages of broilers. The values are given as the mean \pm standard error ($n=5$)

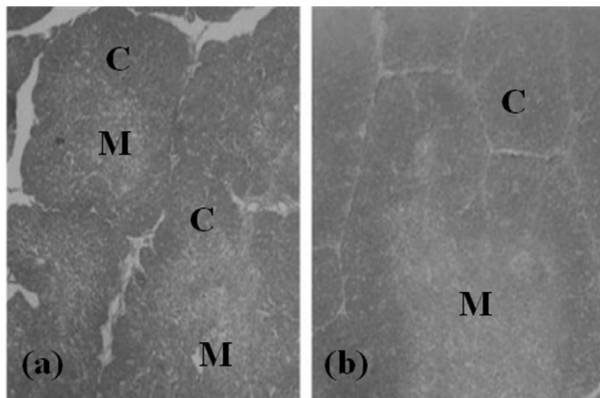


FIGURE 6. Histological sections from the thymus of D_1 (a) and D_{28} (b) postnatal broiler chickens showing thymic lobules. The thymic lobules are smaller in size in D_1 than D_{28} ages of broiler chickens. The cortex (C) and medulla (M) of the thymic lobules increased in size comparatively in D_{28} ages of chicken. X400. H & E stain

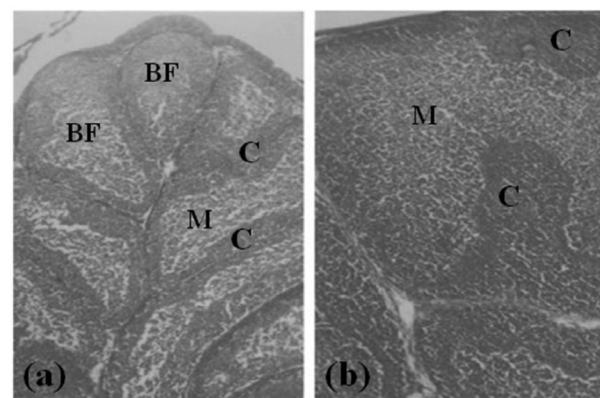


FIGURE 7. Histological sections from the bursa of Fabricius of D_1 (a) and D_{28} (b) postnatal broiler chickens showing bursal follicle (BF). The bursal follicles are smaller in size in D_1 than D_{28} ages of broiler chickens. The cortex C and medulla M of the follicles increased in size in D_{28} ages of chicken. X400. H & E stain

In conclusion, we suggest that the development of the lymphoid tissues of the postnatal broilers of Kelantan, Malaysia is age dependent.

ACKNOWLEDGEMENTS

We are grateful to Prof. Dr. Wan Zahari bin Mohamed from the Faculty of Veterinary Medicine, Universiti Malaysia Kelantan, Malaysia for translating the English abstract into bahasa Melayu.

REFERENCES

- Bach, J.F. 1978. *Immunology*. 5th ed., New York: John Wiley and Sons. pp. 15-35.
- Cooper, M.D., Peterson, R.D.A. & Good, R.A. 1965. Delineation of the thymic and bursal lymphoid systems in the chicken. *Nature* 205: 116-143.
- Cortan, R.S., Kumar, V. & Robbin, S.L. 1998. *Robbin's Pathogenic Basis of Disease*. 4th ed. Philadelphia: W.B. Saunders pp. 163-164.
- Getty, R. 1975. *Sission and Grossman's The Anatomy of the Domestic Animal*. 5th ed. Vol. 2. Philadelphia: W.B. Saunders Co. pp. 2010-2018.
- Gridley, M.F. 1960. *Manual of Histologic and Special Staining Technique*. New York: McGraw-Hill Book Company, Inc. pp. 28-29, 82-83.
- Hodges, R.D. 1974. *The Histology of the Fowl*. New York: Academic Press. pp. 35-108.
- Honjo, K. & Hirota, Y. 1993. Immunohistochemical investigations of lymphocytes in the lymphoid organs of cyclophosphamide treated chickens. *Journal of Veterinary Medical Science* 55: 895-897.
- Karim, M.R., Khan, M.Z.I. & Haque, Z. 2005. The dynamics of immunocompetent cells in the major lymphoid organs and mucosa associated lymphoid tissues of chemotherapy treated chickens. *Journal of the Bangladesh Society for Agricultural Science and Technology* 2: 5-8.
- Khalil, Mo., Khan, M.Z.I., Khalil, Ma. & Islam, M.R. 2003. A prospective study of prenatal and postnatal development of thymus of deshi chicken. *Mymensingh Medical Journal* 12: 20-24.

- Khalil, Mo., Khan, M.Z.I., Khalil, Ma. & Islam, M.R. 2002. Effects of age in the structure of the bursa of Fabricius of deshi chicken of Bangladesh. *Mymensingh Medical Journal* 11: 91-93.
- Khan, M.Z.I., Hashimoto, Y. & Asaduzzaman, M. 1998. Development of T-cell sub-populations in postnatal chicken lymphoid organs. *Vetrinarski Arhiv* 68: 183-189.
- Khan, M.Z.I., Hashimoto, Y., Konno, A., Kon, Y. & Iwanaga, T. 1996. Development of T-lymphocyte subpopulations in the postnatal chicken oviduct. *Cell and Tissue Research* 284: 317-325.
- Nasrin, M., Khan, M.Z.I., Siddiqi, M.N.H. & Masum, M.A. 2013. Mobilization of immunoglobulin (Ig)- containing plasma cells in Harderian gland, cecal tonsil and trachea of broilers vaccinated with newcastle disease vaccine. *Tissue and Cell* 45: 191-197.
- Rahman, M.L., Islam, M.R., Asaduzzaman, M. & Khan, M.Z.I. 2003. Lymphoid tissues in the digestive tract of Deshi Chicken (*Gallus domesticus*) in Bangladesh. *Pakistan Journal of Biological Science* 6: 1145-1150.
- Mohd Masum, Morsheda Nasrin & Mohd Nazmul Hasan Siddique
Department of Anatomy and Histology
Faculty of Veterinary Science
Bangladesh Agricultural University
Mymensingh-2202
Bangladesh
- Md. Zubayer Ibna Khan
Dhaka Medical College, Dhaka
Bangladesh
- *Corresponding author; email: zahirul@umk.edu.my
- Received: 4 March 2013
Accepted: 22 October 2013

Mohd Zahirul Islam Khan*, Abdur Rahman Bin Aziz & Mohd Mokhtar Bin Arshad
Faculty of Veterinary Medicine
University Malaysia Kelantan
Locked Bag 36, Pengkalan Chepa
16100 Kota Bharu, Kelantan
Malaysia