

## EMBRYONIC AND POST-EMBRYONIC MORPHOLOGICAL EVALUATION OF THE DEVELOPING CAECAL TONSIL OF THE NIGERIAN INDEGINOUS DOMESTIC FOWL (*GALLUS GALLUS DOMESTICUS*)

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### ABSTRACT

The pre- and post-hatch development of the caecal tonsil was studied in Nigerian indigenous domestic fowl from embryonic incubation day (EID) 10 till day (D) 42 post hatch. The embryos were harvested from the gravid eggs for the pre-hatch studies. The caeca were exteriorized through a ventral abdominal incision in both embryos and post-hatch chicks. Samples of the caeca were collected and fixed in Bouin's fluid. Fixed tissue were routinely processed and stained with H&E for histological studies. At EID 10, the caeca had differentiated on both sides of the terminal ileum, followed by a marked increase in length and luminal capacity by EID 14 and EID 18 respectively. At hatch (D 0), the base, body and apex of the caeca were recognizable. By D 14, the caeca developed superficial nodular structures that became more conspicuous at D 28 and D 42. Histologically, by EID 10, the caecal wall had differentiated into four distinct layers and the mucosa of pseudostratified columnar epithelium was thrown into prominent longitudinal folds, but the submucosal cells appeared relatively undifferentiated. By EID 14, distinct lymphocytes appeared in the submucosa. EID 18 was marked by high concentration of fibroblasts within the cores of the epithelial fold. At hatch (D 0), more lymphocytes infiltrated into the tonsil and numerous Lieberkuhn glands developed in the submucosa. Between D 14 and D 28, there was marked increase in lymphocyte density and commencement of lymphatic nodule formation. At D 42, numerous encapsulated lymphatic nodules filled with lymphocytes developed in the tonsil.

Keywords: caecal tonsil, indigenous domestic fowl, pre-hatch, post-hatch, gross, histology, development

### INTRODUCTION

The importance of indigenous domestic fowl as a source of animal protein and emergency cash income cannot be overemphasized (Mtileni *et al.*, 2016). The indigenous domestic fowls in Nigeria are characterized along genetic lines of feather type such as normal or frizzled feathered, body structure such as naked neck, and colour variants such as black, white, brown, mottled. Most of the classification by the different agro-ecological zones considered mainly the normal feathered indigenous domestic fowl because they are the most prominent, whereas the naked neck and frizzled feathered are rare and almost becoming endangered (Ajayi, 2010). However, these strains of indigenous domestic fowls have varied degrees of immune competence.

The predominant indigenous breeds of domestic fowl kept by rural communities are selected mainly for their adaptive traits rather than their production performances (Moreki *et al.*, 2010; Moges *et al.*, 2010; Negassa *et al.*, 2014). The indigenous domestic fowls have been shown to be more resistant to some diseases as well as excellent gene reservoirs, particularly for those genes that have adaptive values (Minga *et al.*, 2004; Egahi *et al.*, 2010; Melesse *et al.*, 2011).

The ability of organisms to resist disease is a fundamental responsibility of a defense mechanism controlled by the lymphoid system. This system is composed of several lines of defense to prevent pathogen entry and subsequent infection (Erf, 2004, 2007).

Structurally, the avian lymphoid system is divided into two distinct components namely the primary and the secondary components (Nnadozie *et al.*, 2019). The secondary component consists of all regions of normal lymphocytic aggregation or proliferation outside the primary component (bursa of Fabricius and thymus). This includes among others the spleen and the mucosa associated lymphoid tissues (MALT) such as the caecal tonsil (Davison *et al.*, 2008; Islam *et al.*, 2012). In galliformes, the large intestine consists of paired caeca and a short straight rectum joined to ileum and cloaca. Caecum plays important role in liquid absorption and cellulose digestion, but also acts as a defensive organ due to the presence of large masses of diffuse and nodular lymphatic tissue in the lamina propria and submucosa that formed the caecal tonsils (Rezaian and Hamedi, 2007). The morphological properties and developmental processes of the caecal tonsil have been widely studied in various avian species, but a knowledge gap still exists on the morphological features of the developing caecal tonsil of Nigeria indigenous domestic fowl.

The aim of this study is to evaluate the age related morphological changes in the caecal tonsil of Nigerian indigenous domestic fowl at embryonic and early post-hatch periods of development.

## MATERIALS AND METHODS

A total of fifteen chick embryos and twenty five post-hatch chicks were used for the study. One hundred and twenty eggs of indigenous domestic fowls were acquired from apparently healthy laying domestic fowls raised by free range back yard method in Ovim community, Isuikwuato Local Government Area of Abia State, in the South-Eastern region of Nigeria. The eggs were incubated in an electric egg incubator at 37°C and 55 – 60% relative humidity (Yoshimura *et al.*, 2009; Oznurlu *et al.*, 2010). Five chick embryos were harvested on each of Embryonic Incubation Day (EID) 10, 14 and 18 by cracking the gravid egg shell around the vertical midline using a small knife. The entire transverse diameter of the shell was cut using Surgical Scissors to expose the embryo. The foetal membrane and the yolk sac were detached from the embryo and the embryo washed to remove foetal debris.

Following hatching, the chicks were housed in deep-liter pen in the poultry unit of the College of Veterinary Medicine, Michael Okpara University of Agriculture, Umudike, Abia State, Nigeria. The chicks were fed commercially compounded feed (Topfeed<sup>®</sup>) broiler starter and water was given *ad libitum*. No medications including vaccination were given throughout the period of study.

Five randomly selected chicks were sacrificed at hatch or day zero (D 0) and each of D 7, D 14, D 28 and D 42 post-hatch by inhalation anaesthesia using chloroform. The caeca of both embryos and post-hatch chicks were collected by

ventral abdominal dissection (Alboghobeish and Mayahi, 2003), and observed for gross features.

## HISTOLOGICAL INVESTIGATION

Samples of the caecal base were fixed in Bouin's fluid and transferred to 70% ethanol after 24 hours. The specimens were processed by placing them in ascending grades of ethanol in the following order, first 95% ethanol for 1 hour and second 95% ethanol for 1¼ hours, first absolute ethanol for 1½ hours and second absolute ethanol for 2 hours to ensure proper dehydration of the tissues. It was then transferred to mixture of equal volumes of ethanol and xylene where it was left overnight. It was later cleared in two changes of xylene for 1 hour each. It was then infiltrated for 1 hour with molten paraffin wax in the oven at 60°C.

The tissues were embedded in paraffin wax, and the blocks were trimmed and mounted on wooden chuck, and then taken to the microtome for sectioning at 5µm thickness. The sections were floated in floating-out bath from where it was picked with clean albumenized slides. The slides were placed in a staining dish and excess wax was removed by two changes of xylene, hydrated by descending grades of ethanol in the following order- absolute ethanol, 95% ethanol and 70% ethanol for 2 minutes each. The slides were taken to water and then stained with Ehrlich hematoxylin for 15 minutes, and then washed in water for 5 minutes, differentiated in 1% acid ethanol for 3 seconds, and blued in running tap water for 10 minutes. It was then counter stained with eosine for 2 minutes.

Excess eosine was removed in ascending grades of ethanol in the following order- 75% ethanol, 95% ethanol and absolute ethanol for 2 minutes each. It was then cleared in two changes of xylene and cover slipped with Depex mountant. The slides were viewed under a light microscope and selected images were captured using moticam 2.0 digital camera attached to a computer.

## RESULT

### GROSS MORPHOLOGY

At EID 10, the embryonic intestine with its associated loops had been established, and the thin tubular caeca that tightly adhered to the distal ileum had differentiated, but relatively indistinguishable as it blends in colour with the bulk of the embryonic intestine. By EID 14, the caeca increased remarkably in length with more pronounced features, but still delicate (Figure I). At EID 18, there was further increase in length and luminal capacity of the caeca, and the descriptive parts of the organ such as the base, body and apex became more pronounced (Figure II).

The caeca at hatch (D 0), resembled those of EID 18 except for a slight variation in size of the organ. By D 7 and D 14 post-hatch, the caeca increased more in length and luminal

capacity. Between D 28 and D 42 post-hatch, superficial ampullaceous structures indicating locations of the tonsils became more prominent (Figure III).

### HISTOLOGICAL OBSERVATIONS

At EID 10, the caecal walls had differentiated into four distinct layers; the mucosa, submucosa, muscularis and serosa; and the mucosa consisting of pseudostratified columnar epithelium had formed a number of longitudinal folds (Figure IV). The submucosa apparently consisted of mesenchymal tissue with diffused accumulations of immature cells suspected to be predominantly juvenile lymphocytes and some reticular cells (Figure V). By EID 14, there was a general increase in thickness of the caecal wall especially the muscularis and submucosal regions, with a corresponding increase in submucosal cell density. The mucosal epithelium remained pseudostratified columnar epithelium and the circularly oriented smooth muscle cells of the tunica muscularis further differentiated, showing the typical spindle shape of each fibre (Figure VI). By EID 18, the cross sectional area of the submucosa remarkably increased with fibroblasts and Lieberkuhn glands appearing in numbers in the submucosa. There was slight increase in density of lymphocytes which dominated the subepithelial cell population (Figure VII).

At hatch or D 0, there was marked increase in number of epithelial folds and the tunica muscularis distinctly increased in thickness. The epithelium remained pseudostratified columnar epithelium, although the apical region of some plicae appeared to possess simple columnar cells. Numerous Lieberkuhn glands developed in the submucosa, and more lymphocytes appeared to have infiltrated into the tonsil (Figure VIII).

At D14 post-hatch, the tonsil increased markedly in cross sectional area with a corresponding increase in tonsil cell density. Lieberkuhn glands became more numerous in number in the tunica submucosa (Figure IX).

At D 28 post-hatch, the tonsil was characterized by the appearance of developing lymphatic nodules and increased lymphocyte density. By D 42 post-hatch, numerous encapsulated lymphatic nodules had established in the tonsils. The intra- and extra-nodular cells appeared morphologically similar and consisted predominantly of lymphocytes (Figure X).

### DISCUSSION

Development of the caeca into blind-end sacs was very prominent at hatch, although the growth had progressed reasonably before embryonic incubation day 14. The superficial ampullaceous structure often found at the base of the caecum which usually indicates the location of tonsil was only conspicuous in the indigenous domestic fowls at post-hatch periods. Such ampullae were equally observed in post-

hatch turkeys (Nnadozie *et al.*, 2019). However, Kitamura *et al.* (1976) stated that no such structures exist on the surface of the caecal base in duck.

The wall of the caecum consisting of four histological layers as observed in Nigerian indigenous domestic fowl agrees with reports of Rezaian and Hamed (2007) and Majeed *et al.* (2009) in domestic domestic fowls. However, remarkable variations exist among avian species in the nature of mucosal epithelium. In Nigerian indigenous domestic fowl, the mucosa is composed of pseudostratified columnar epithelium in both embryonic and post-hatch chicks; but Nnadozie *et al.* (2019) observed simple columnar epithelium with goblet cells in the turkey. Variation in nature of epithelial lining maybe more of digestive related, and probably depends on the level of absorption required in the caecum. The distribution of diffuse lymphatic tissues in Nigerian indigenous domestic fowl occurs in the lamina propria-submucosa, while the lymphatic nodules are domicile in the submucosa. Kitamura *et al.* (1976) made similar observation in the duck, and stated that the diffuse lymphatic tissue and the lymphatic nodule with germinal centers are found to be separately located in the lamina propria and submucosa respectively. However, Nnadozie *et al.* (2019) noted that both diffused lymphatic tissue and lymphatic nodules occur in the lamina propria-submucosa in turkey as equally observed in chicken (Akter *et al.*; 2006; Rezaian and Hamed, 2007; Majeed *et al.*; 2009). In contrast, Hoffmann-Fezer (1973) noted that the diffuse lymphatic tissue with germinal centers occupy only the lamina propria in the domestic fowl. The pattern of distribution of lymphatic tissue structures in the caecal tonsil maybe a mere structural design as no known immunological significance has been attributed to it.

The circular orientation of smooth muscle fibres of the tunica muscularis as seen in Nigerian indigenous domestic fowl is similar to the orientation of muscle tissues in the caecal wall of turkey (Nnadozie *et al.*, 2019). The significance of muscle orientation in the caecal wall can be more of digestive than immunological related to enhance movement of caecal contents within the lumen.

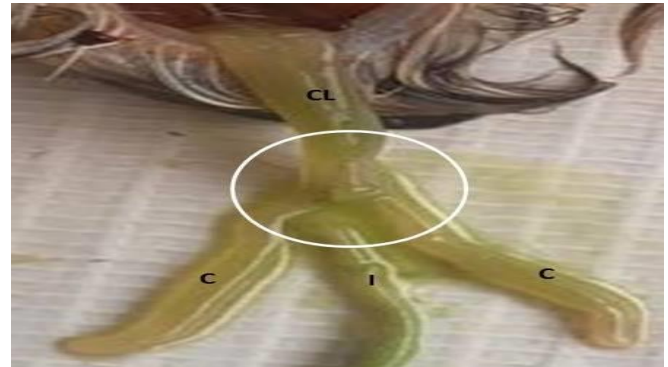
At EID 10, there was no obvious indication of tonsil establishment in the caeca, although several euchromatic epithelial reticular cells have occupied the submucosa, probably to establish enabling microenvironment for the yet to arrive immune-associated lymphocytes. Payne (1971) reported that caecal tonsil primordium appears about day 10 of incubation in domestic fowl, while Moral *et al.* (1998) and Islam *et al.* (2012) demonstrated none existence of significant development of the embryonic caecal tonsil before EID 14 in White Leghorns and native domestic fowl of Bangladesh respectively.

EID 14 was marked by a tremendous improvement in development of the tonsil as the lamina propria-submucosa was massively infiltrated by lymphocytes. The gastrointestinal tract is a vulnerable system to microbes due to the anatomical design of its extremities associating with the external environment. Therefore, the early establishment of mucosa-associated lymphoid tissue as observed in the caeca of Nigerian indigenous domestic fowl through accelerated migration of lymphocytes may be an immunological strategy to provide adequate protection to the chicks prior to hatching. Islam *et al.* (2012) stated that the lumen of the caeca at EID 14 is filled by mucoid substance with the submucosa consisting of small amount of scattered lymphocytes. Nnadozie *et al.* (2019) also reported such minute aggregates of lymphocytes in day-old turkey poults and suggest that this lymphocyte population could be the basal lymphocyte accumulation of the caecal tonsil. According to Dunon *et al.* (1997), these basal levels may coincide with the early waves of T-cell emigration from the thymus. The growth of the tonsil at EID 18 was marked by increase in connective tissue proliferation with increased density of fibroblasts, and development of substantial number of Lieberkuhn glands in the submucosa. However, the functional significance of fibroblasts in the caecal tonsil at late incubation period when an up-shoot of lymphocyte is expected is not understood, but it may be associated with reinforcement of the stroma to provide adequate support to the immune cells. The preponderance of Lieberkuhn glands can be related to digestive needs as the embryos will soon hatch and start independent existence which involves feeding and digestion. These Lieberkuhn glands may be the source of the mucoid accumulation observed in the lumen of the caeca at EID 14 (Islam *et al.*, 2012).



**Figure I:** Gross photograph of a dissected embryo at incubation day 14 showing some abdominal viscera. Note

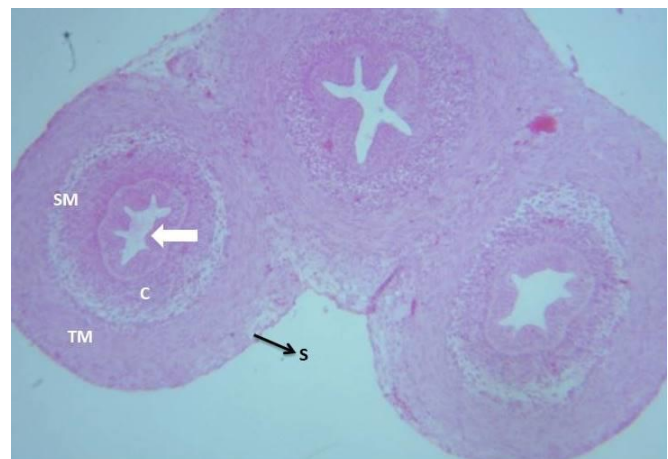
the caecum, C. BF: bursa of Fabricius; V: ventriculus; PV: Proventriculus



**Figure II:** Gross photograph of a dissected embryo at incubation day 18 showing part of exteriorized intestine. Note the caeca, C with the base in the circle. I: ileum; CL: colon

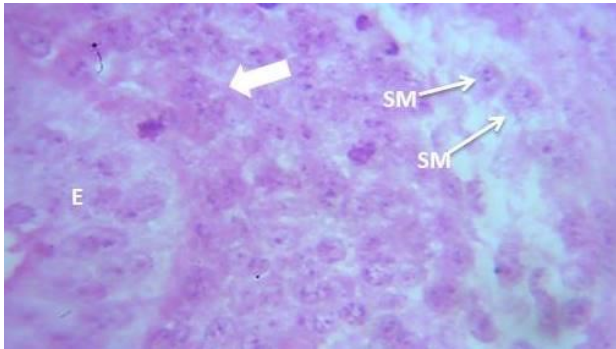


**Figure III:** Gross photograph of a dissected chick at day 42 post hatch showing exteriorized intestines. Note the caecum, C and the slightly enlarged part, (arrow) on the base of the caeca. CL: colon

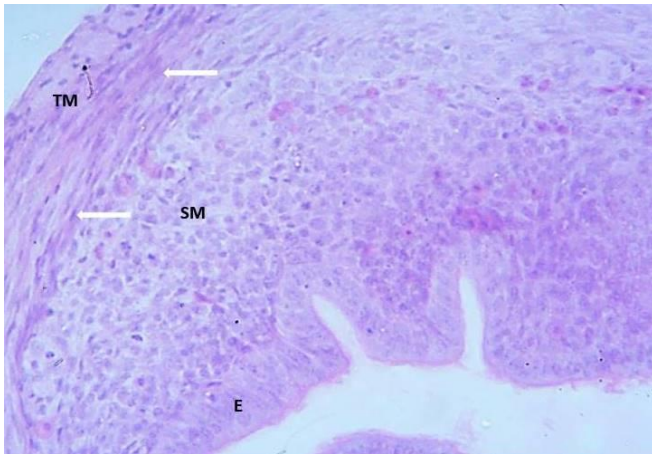


**Figure IV:** Photomicrograph of a transverse section of the caeca, C, at incubation day 10 showing the wall of the caecal

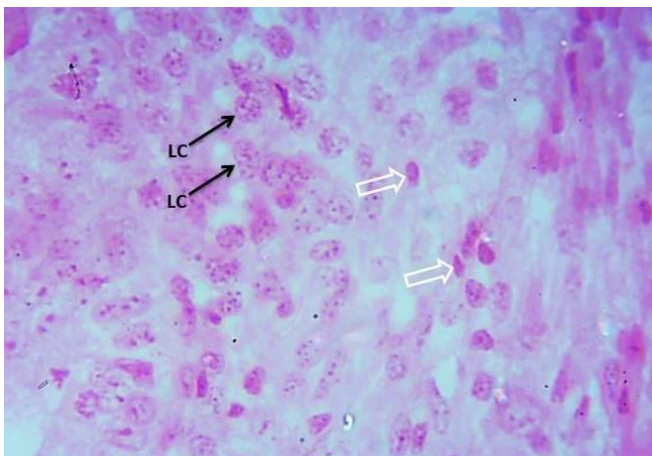
base. Note the mucosal folds (arrow); submucosa, SM; tunica muscularis, TM and serosa, S. I: ileum (Haematoxylin and Eosin stain; X100).



**Figure V:** Photomicrograph of a transverse section of the caecum at incubation day 10 showing the embryonic caecal tonsil. Note the primitive submucosal cells (arrow) suspected to be immature lymphocytes in mesenchymal connective tissue. RC: reticular cell; E: epithelium (Haematoxylin and Eosin stain; X1000)

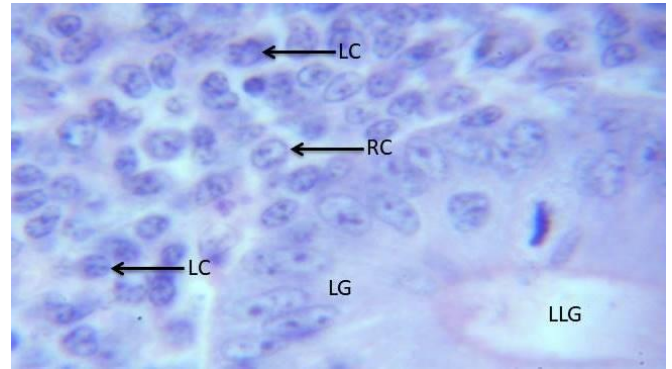


**Figure VI:** Photomicrograph of a transverse section of the caecal tonsil at incubation day 14. Note the submucosal, SM cell density and the spindle shaped smooth muscle cells (arrow) of the tunica muscularis, TM. E: pseudostratified columnar epithelium (Haematoxylin and Eosin stain; X400)

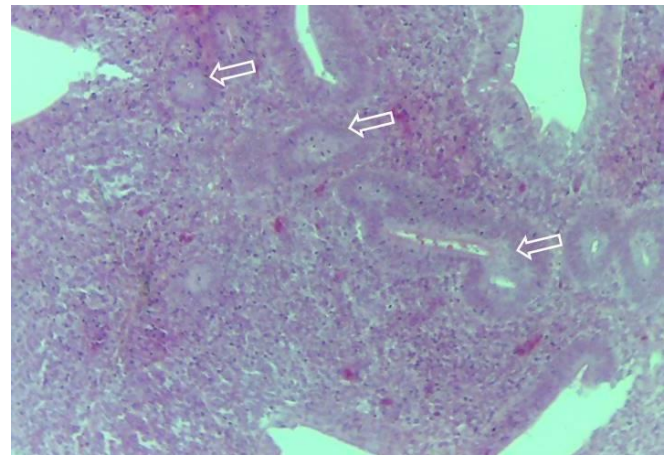


**Figure VII:** Photomicrograph of a transverse section of the

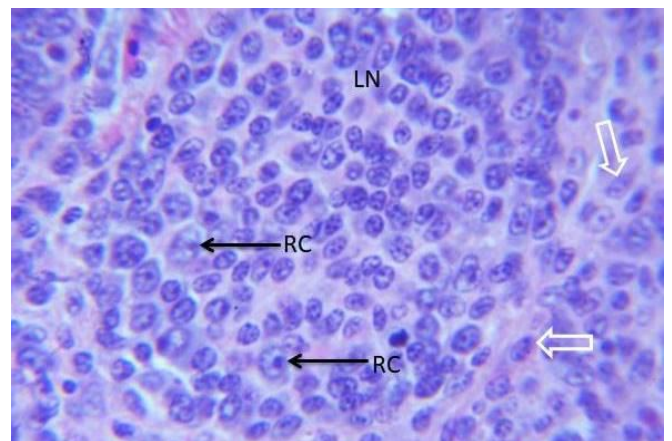
caecal tonsil at incubation day 18 showing the parenchyma of the tonsil. Note the fibroblasts (arrow) and the numerous lymphocytes, LC in the subepithelial region (Haematoxylin and Eosin stain; X1000).



**Figure VIII:** Photomicrograph of a transverse section of the caecal tonsil at hatch showing the parenchyma. Note the Lieberkuhn gland; LG and the cell density of the tonsil. LLG: lumen of Lieberkuhn gland; LC: lymphocyte; RC: reticular cell (Haematoxylin and Eosin stain; X1000)



**Figure IX:** Photomicrograph of a transverse section of the caecal tonsil at day 14 post-hatch showing the parenchyma. Note the Lieberkuhn glands, (arrow) surrounded by tonsillar cells in the submucosa (Haematoxylin and Eosin stain; X100).



**Figure X:** Photomicrograph of a transverse section of the

caecal tonsil at day 42 post-hatch showing an encapsulated lymphatic nodule, LN. Note the intra- and extra-nodular cell populations. ARROW: lymphocyte; RC: reticular cell (Haematoxylin and Eosin stain; X1000).

At hatch and during the rest of the post-hatch periods under investigation, the caecal tonsil was marked by progressive increase in lymphocyte densities. This increase in density of lymphoid cells may be due to exposure of the birds to antigenic substances in the environment, since birds start to peck and learn to associate pecking with ingestion and feeding soon after clearing the shell (Hogan, 1984). This therefore implies that after hatching the development of the caecal tonsils is concomitant with increased lymphoid function in the chick intestines which is dependent on the presence of enteric bacteria and coincides with enterocyte and villus development (Bar-shira et al., 2005). Moreover, the caeca are exposed to continual and constant invasion of microbial and non-microbial antigens of extra-caecal origin, since it receives the back flowing urine from the urodeum of cloaca through the rectum (Rezaian and Hamed, 2007).

It is reported that in domestic fowl and quail reared in a germ-free environment no germinal centers are evident in the caecal tonsils and the lymphoid tissue is remarkably reduced suggesting that gut flora is essential in stimulating full tonsillar development (Hedge et al., 1982). Befus et al. (1980) equally demonstrated accelerated proliferation of lymphocytes in the Peyer's patches and caecal tonsil in domestic fowl at early post-embryonic periods.

The early development of lymphatic nodules in the caecal tonsil as observed from this study points at the importance of a functional immune mechanism in the gut system since it is highly prone to both internal and environmental assaults. Bar-shira and Friedman (1999) described the lymphoid follicle (nodule) as the hallmark of developing adaptive immune response.

According to these authors, the nodules contain naive T and B cells undergoing differentiation and division in the process of generating effectors and memory cells, both of which may migrate to the tissues.

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