

Histological study of the caecal tonsil in the cecum of 4- 6 months old white leghorn chicks

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Cecum, the largest part of chicken's large intestine, has an important role in liquid absorption and cellulose digestion. It acts as a defensive organ as well due to the presence of large masses of diffuse and nodular lymphatic tissue in lamina propria and sub mucosa. It has been shown that the caecal tonsil activity depends on the activity of bursa of fabricious and thymus. As the bursa of fabricious and the thymus are exposed to involution through these ages, finding the changes (if any) occur in the caecal tonsil has been our aims.

By this purpose we selected 18 healthy white leghorn chickens of 16, 20, and 24 weeks old and fixed the samples of their caecal tonsils in 10% buffered formalin, immediately after death. Routine histological laboratory methods were used and 6 μ m sections were stained with hematoxylin- eosin staining method and studied under light microscope. The nodular units (ND) width and height, ND fossulae's lumen width, distribution areas of the lymphatic nodules and crypt of lieberkuhn /mm² in the caecal tonsils of all ages were measured, and then analyzed with ANOVA test.

Results show ND's wide and height increasing, highest rate of diffuse and nodular accumulation of lymphatic tissue at 5 months old chickens samples, while decreasing at 4 and 6 months ones. Instead, the mucosal crypts depth, and the ND fossulae's lumen width were decreased in caecal tonsil of 5 months old chicken, and increase at 4 and 6 months old.

In our study similar changes of caecal tonsil in these ages inferred that the caecal tonsil activity depends on the activity of the bursa of fabricious and the thymus.

Key words: Cecum; caecal tonsil; nodular unit; histology

Introduction:

Chicken's large intestine consists of paired ceca and a short straight rectum joined to ileum and cloaca.

Ceca are two, right and left elongated blind sacs, each consisting of 3 parts: proximal part or base, middle, and distal part or apex.

The short, proximal part or base has a narrow lumen and a relatively thick wall. The long middle part or the body is wider and with thinner wall. The short distal part or apex is extends to a pointed end. The mucous membrane is similar to that of the small intestine, with less goblet cell and fewer glands. The villi are well developed at the basilar part, shorter and wider in the middle part and either shorter or absent in the apex (3). Cecum wall, thinner than other parts of intestine, contains lymphatic tissues most in the basilar part forming cecal tonsil (3). Kajavara et al (7) studied caecal tonsil evolutionary growth process in birds' foetal stage by using immuno histo chemistry. Kato et al (8) observed M cell-like cells in caecal tonsil epithelia of the over 2 month's old birds. Studied continued for better recognition of these cells, Jeurissen et al in 1989 and 1999, found some distinct epithelial cells among regular epithelium, characterized with short and irregular microvilli on the apical surface and darker cytoplasm, and considered them as M cell (5, 6). Thereafter Kitagawa et al in 2000 characterized the ultra structure of the M cells in the caecal tonsils (13). Kitagawa et al in 1996 and 98 showed lymphatic nodules dispersion along the Cecum of the 6 months old birds (11, 12). Bifus et al (1) by injecting neurotestosterone to 5 days fetus could prevent bursa of fabricious forming (bursectomy) and finally proved that caecal tonsils didn't grow by the injection and its lymph nodules lacking

lymphocyte accumulation. Another study by Hooshi and Mori (4) showed caecal tonsil dependence on thymus.

Since caecal tonsil activity depends on the activity of bursa of fabricius and thymus (1, 4) and since bursa of fabricius and thymus are exposed to involution (2, 9) when coming of age, and since no study has been done on caecal structural changes at this age limit, we tried to observe whether there is any structural change in this organ or not.

Materials and methods:

To study histological changes in avian Cecum at the age limit of premature, mature, and post mature, 18 healthy white leghorn of 16, 20, and 24 weeks old, of similar environmental and nutritional conditions, were purchased and transferred to the anatomy division of vet medicine faculty. After registering specifications and examinations to make sure of their health, the birds were slaughtered, and both lefts and rights ceca were separated from the ileocecal valve and fixed in 10% buffered formalin and send to division of histology. We separated caecal tonsil of each Cecum, and fixed them separately in 10% formalin. Other routine histological laboratory methods (14) were used and 6 μ m sections stained with H&E and studied under light microscope.

Nodular units (ND) width and height, ND fossulae's lumen width, in the cecal tonsils of all ages were measured by linear graticule, and distributional areas of the lymphatic nodules and crypt of lieberkuhn /mm² were measured by area measuring graticule, and then analyzed with ANOVA test.

Results and discussion:

Part of cecum base containing caecal tonsil can be easily separated from other parts due to thickening and the wall diameter increasing (fig.1). Mucosa in caecal tonsil can be seen in 2 forms. Part of it, containing tonsil, is whether covered by rather short villi or without it, and, the adjoining part, where without tonsil, is covered by long mucosal villi, similar to those of small intestine. Villa's covering epithelium contains simple columnar cells with striated border (absorptive) and goblet cells. Though caecal tonsil wall thickens, its lumen is also almost completely closed by villi and mucosal-sub mucosal prominence of nodular units. Nodular units are tonsil like structures formed by a mucosal- sub mucosal prominence and surrounded by a delicate layer of smooth muscle branching out of an inner circular muscle layer and muscularis mucosa. Surface epithelium has made a deep crypt into the nodular unit mostly covered by rather short villi. These crypts are called fossula and continued by lieberkuhn gland. Diffuse and nodular lymphatic tissue fills the lamina propria. Histological study of caecal tonsil in 4, 5, and 6 months old chickens showed its structural changes at these ages, while nodular units are quite separated from each other in 4 months old chickens. Villi can be seen at the apical surface and nodular unit fossulae. Surface villa's covering epithelium and fossulae at the beginning part contain absorptive and goblet cells (fig 2). Though the goblet cells in epithelium of fossula suddenly decrease or never seen. In this part there are both epithelium of simple columnar with striated border and simple columnar epithelium with darker cytoplasm at the apical surface. Lymphocytes are seen in rows inside the both type of covering cells; rather penetrate up to the cells' apex (fig 3). Lamina propria is full of lymphocyte, macrophage, and mast cells. Lymph nodules at the base of lamina propria contain big germinal centers. Nodular units thicken at 5 months of age, so come closer to each other. Fossulae ramifications are rather lost and the lumen is narrower, while mucosa thickness here increases due to accumulation of diffuse lymphatic tissue and lymph nodules.

At 6 months of age nodular unit's thicknesses decrease, so they keep their distance. Lymph nodules dispersion rate in nodular units showed that the most increase belong to the age of 5 months and decrease at the time before and after that (fig 4). Statistical results showed that nodular units thickness and lymph nodules spreading area increase significantly at the age of 5 months and decrease at 4 and 6 months of age ($P < .001$). On the other hand fossulae lumen thickness and lieberkuhn glands spreading area decrease significantly at this age and increase at 4 and 6 months of age (diagrams 1- 5).

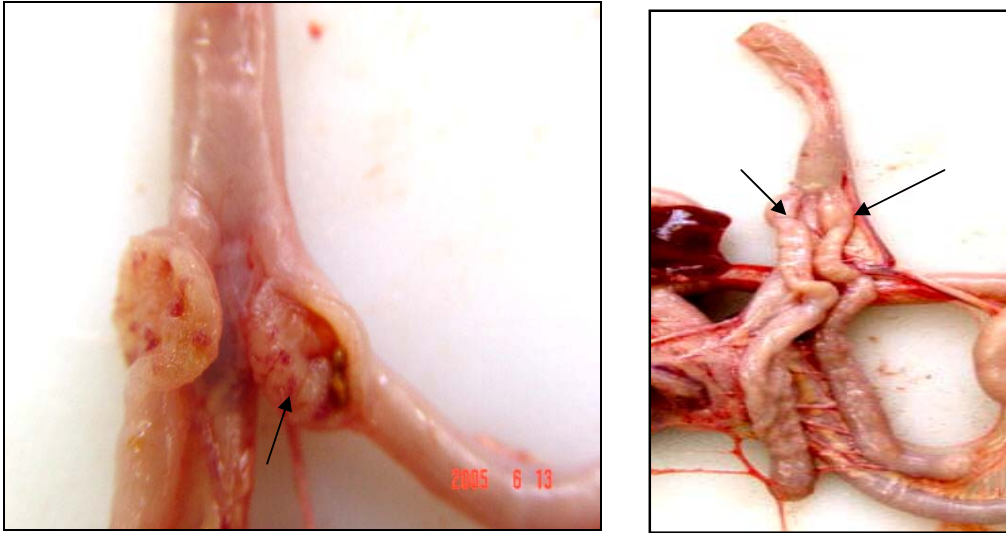


Fig 1: The right photograph shows the cecal tonsils (arrows) in 6 month old white leghorn chicken. Left photograph shows inside of the same cecal tonsil with 2 areas of tonsil (arrow) and without tonsil (opposite site).

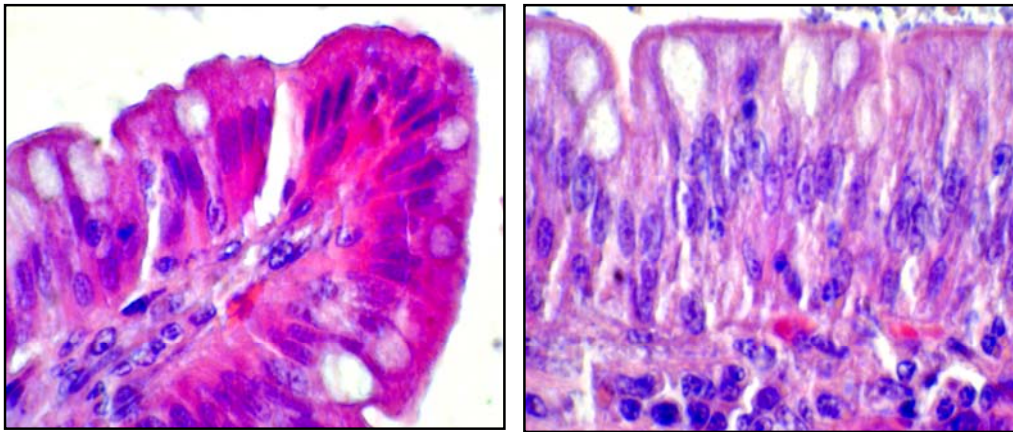


Fig 2: the right one is a photomicrograph of non tonsillar villus epithelium of caecal tonsil of 6 month old white leghorn chicken. Enterocytes and goblet cells are seen. The left shows villus apex epithelium of the same sample. As seen, here enterocytes are more accumulated with darker nucleus and cytoplasm and goblet cells rarely seen. H&E, 200x.

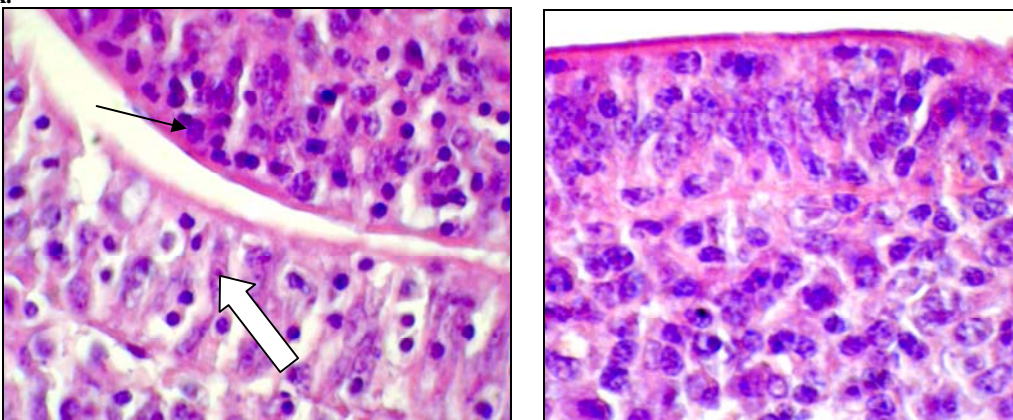


Fig 3: the right one is a photomicrograph of 4 month old white leghorn chicken epithelium of caecal tonsil villus. Enterocytes apical cytoplasm is accumulated and dark and the striated border seen on the surface. In the left 2 types of simple columnar epithelium is seen. As can be observed, apical cytoplasm of cells with thin arrow are darker and more accumulated than the others (thick arrow) and, are without goblet cells. Inside cytoplasm of both types lymphocytes are seen. H&E, 200x.

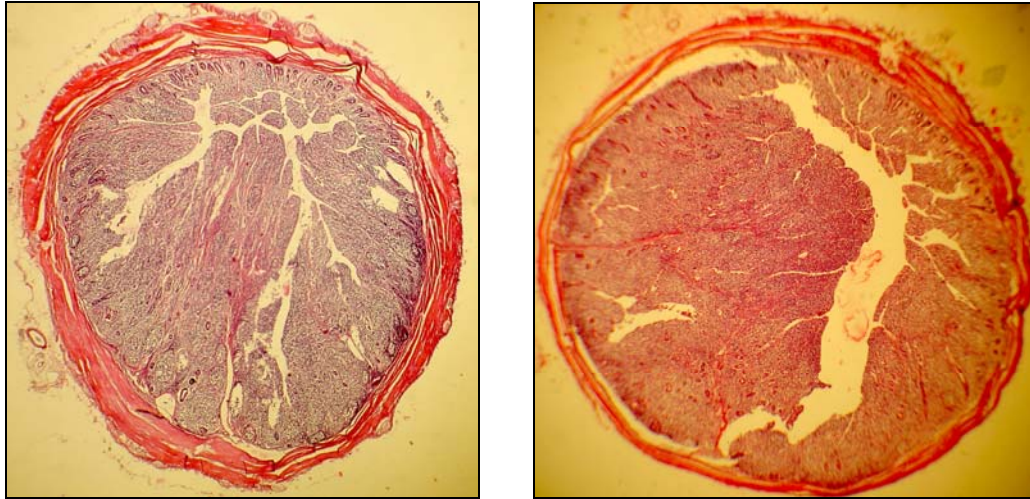


Fig 4: in the right, photomicrograph of caecal tonsil of 5 month old white leghorn chicken and in the left, the caecal tonsil of 6 month old white leghorn chicken are shown H&E, 200x..

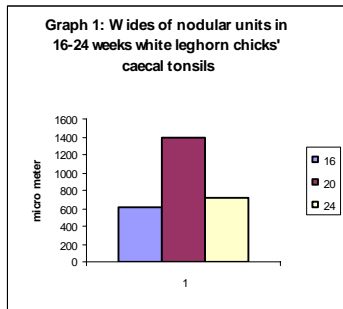


Diagram 1: composing nodular units width of caecal tonsil in 4- 6 months old white leghorn chicken .Differences between 1& 2 ($P < .001$), between 2&3 ($P < .001$), between 1 & 3 (NO).

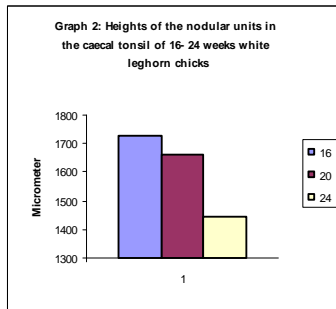


Diagram 2: composing nodular unit's height of caecal tonsil in 4- 6 months old white leghorn chicken .Differences between 1& 3 ($P = .043$), between 1&2 and between 2 & 3 (NO).

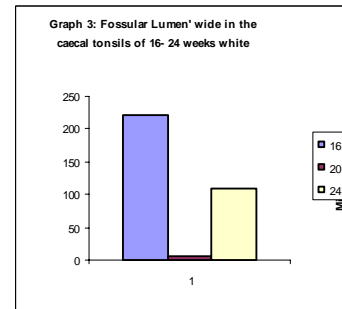


Diagram 3: composing nodular units fossulae lumen width of caecal tonsil in 4- 6 months old white leghorn chicken .Differences between 1& 2 ($P < .001$), between 1&3 ($P < .001$), between 2 & 3 ($P < .001$).

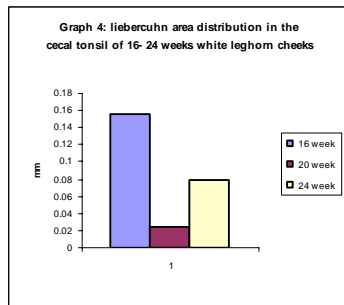


Diagram 4: composing lieberkuhn glands spreading area in nodular units of caecal tonsil in 4- 6 months old white leghorn chicken .Differences between 1& 2 ($P = .004$), between 2&3 and 1 & 3 (NO).

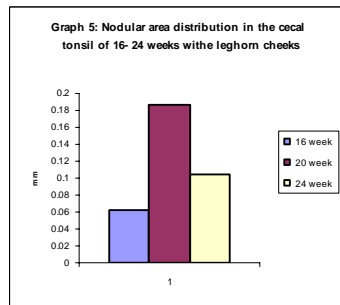


Diagram 5: composing lymph nodules spreading area in nodular units of caecal tonsil in 4- 6 months old white leghorn chicken .Differences between 1& 2 ($P = .018$), between 2&3 and 1 & 3 (NO).

Cecum, a part of large intestine, has structural and functional differences in mammals. Caecal tonsils, on which nearly half of the lymph nodules are accumulated, are major lymphoid tissue in the avian

cecum. Kitagawa et al, found out that 45.7 % of lymph nodules accumulated on caecal tonsils of 6 months old white leghorn chickens. Chickens' cecum is exposed to continual and constant invasion of bacterial or nonbacterial antigens of extracaecal origin, since it receives the back flowing urine from the urodeum of the cloaca through the rectum. Therefore, immunological surveillance against foreign microorganisms seemed necessary (11). Kitagawa et al, after examinations on 6 months old chicken's caecal tonsil on 1996, found it an accumulation of nodular unit where a fossula seen in the center (12). Kato et al, (1992) observed a set of morphologically similar cells, similar to M- like cells, on chickens' caecal tonsil epithelium. They concluded that these chickens' cells possess some histochemical and morphological qualifications of M cells, though absorption of foreign materials in them is not as progress as mammals' M cells (8). Kitagawa et al (2000 & 2003) observed the M cells and classified the follicle associated epithelium of the caecal tonsil in 4 groups: columnar cells, M cells, goblet cells, and, rare entero endocrine (10 & 13). Befus et al (1980) in their research on Peyer's patches concluded that: a day after being hatched, Peyer's patches in chicken's intestines are not observable with naked eyes. However, microscopic inspection of some infiltrated lymphoid cells Peyer's patches and caecal tonsils are observable in anticipated areas. Caecal tonsils and Peyer's patches are both easily observable in 10 days of age. Peyer patches increase in size up to about 10 weeks of age. It seems that intestinal lymphoid accumulation is exhausted by avian growing up. In some animals it develops as much as just a network of connective tissue remains. However, in some chicks, even after 1 year of age, part of intestinal lymphoid accumulations remains possessing the whole qualifications of those of younger birds. By age increasing, not only morphologic indications but also their abundance and diffusion are subject to change (1).

In this study, examinations showed that in caecal tonsils, nodular units width and lymph nodular area /mm² increase significantly in 5 months of age comparing to 4 and 6 months of age, and the extent of Lieberkuhn gland/mm² and the width of lumen fossula decrease significantly comparing to 4 and 6 months of age. The results inferred that caecal tonsil in 5 months of age has the most effect on digestive system immunity, and its decrease on 6 months of age simultaneous with bursa of Fabricius and thymus shows the start of exhausting of immunity.

There is no significant changes occur in the thickness of submucosa in the studied ages. Muscle thickness in this part of Cecum on 5 months of age is less than in other 2 ages. Considering the muscles role in transferring materials from Cecum, materials will be less transferred and more exposed to the caecal tonsils epithelia at this age, make better opportunity for lymphocytes to capture more antigens from the surface and improved immunity.

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