

## Histological Study of the Caecal Tonsil in the Cecum of 4- 6 Months of Age White Leghorn Chicks

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**Abstract:** 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. The caecal tonsil activity has been proved to be depended 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. Eighteen (18) healthy white leghorn chickens of 16, 20 and 24 weeks old were selected and fixed the samples of their caecal tonsils, thymuses and bursas of fabricious in 10% buffered formalin, immediately after slaughter. Routine histological laboratory methods were used and 6  $\mu\text{m}$  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 crypts of lieberkuhn / $\text{mm}^2$  in the caecal tonsils, cortices and medullas diameters of the thymuses and the cortices and medullas diameters of lymphatic nodules, heights of plicas and No of nodules/ plica in the bursas of fabricious of all ages were measured and then analyzed with one way ANOVA test. In the caecal tonsils, the most important changes regarding with involution process was significant decline of lymphatic nodules/  $\text{mm}^2$  of mucosal wall and nodular evacuation at 6 months ones, while thymuses decrease the cortical diameter at 5 months. No significant changes in regarding with involution process were shown in the bursas of fabricious through these ages and no changes in the sub epithelial lymphoid tissues of the caecal tonsils as well. By the results of this study, we come to know that the caecal tonsil growth can be depended on thymus and bursa of fabricious activity, since involution process is first begun in thymus at 5 months of age and then it occurs in thymic dependant areas of the caecal tonsil at 6 months of age. As no involution occurred in bursa of fabricious through these ages, caecal sub- epithelial lymphatic tissue dependant on bursa of fabricious remained unchanged.

**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<sup>[1]</sup>. Cecum wall, thinner than other parts of intestine, contains lymphatic tissues most in the basilar part forming caecal tonsil<sup>[1]</sup>. Kajavara *et al.*<sup>[2]</sup> studied caecal tonsil evolutionary growth process in birds' foetal stage by using immuno histo chemistry. Kato *et al.*<sup>[3]</sup> 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.*<sup>[4,5]</sup> 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. Thereafter Kitagawa *et al.*<sup>[6]</sup> characterized the ultra structure of the M cells in the caecal tonsils. Kitagawa *et al.*<sup>[7,8]</sup> showed lymphatic

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nodules dispersion along the Cecum of the 6 months old birds. Bifus *et al.*<sup>[9]</sup> 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<sup>[10]</sup> showed caecal tonsil dependence on thymus.

Since caecal tonsil activity depends on the activity of bursa of fabricious and thymus<sup>[9,10]</sup> and since bursa of fabricious and thymus are exposed to involution<sup>[11,12]</sup> 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, in relation with thymus and bursa of fabricious 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 from the ileocecal valve, both right and left thymic lobes and bursas of fabricious were separated 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<sup>[13]</sup> were used and 6 um sections stained with H&E and studied under light microscope. For caecal tonsils, Nodular units (ND) width and height, ND fossulae's lumen width, of all ages were measured by linear graticule and distribution areas of the lymphatic nodules and crypts of lieberkuhn /mm<sup>2</sup> were measured by area measuring graticule, for the thymuses of all ages, the cortical and medullar diameters of each lobules were measured by linear graticule, distribution area of reticular bodies/ mm<sup>2</sup> of medullas were measured by lattice graticule and for the bursas of fabricious, the plical heights and widths were measured by linear graticule, numbers of lymphatic follicles/plicas were measured in all plicas and then statistically analyzed with one way 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



Fig. 1: The photograph shows the caecal tonsils (arrows) in 6-month-old white leghorn chicken

Table 1: Histometrical analysis of the thymuses in 4- 6 months old white leghorn chicken Diameters are in mm

	Cortex diameter Mean± SD	Medulla diameter Mean± SD	Reticular St./ mm <sup>2</sup> Mean± SD
1-16 week	0.39±0.03	0.71± 0.09	0.003± 0.001
2-20 week	0.34± 0.02	0.90± 0.09	0.005± 0.0004
3-24 week	0.14± 0.01	0.99± 0.07	0.011± 0.002

Differences between 1& 2, 2& 3 and 1& 3 in all three columns are significant (P<0.001)

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. Villous covering epithelium contains simple columnar cells with striated border (absorptive) and goblet cells. In spite of 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 (Fig. 2). 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 sub epithelial fills the lamina propria and nodular lymphatic tissue stay deeper, at lamina propria. Histological study of caecal tonsil in 4, 5 and 6 months old chickens showed its structural changes through these ages, while nodular units are quite separated from each other in 4 months old chickens (Fig. 2). Villi can be seen at the apical surface and nodular unit fossulae. Surface villus covering epithelium and fossulae at the beginning part contain absorptive and goblet cells.

Table 2: Histometrical analysis of the cecal tonsils in 4- 6 months old white leghorn chicken

	ND wide/ $\mu$	ND height/ $\mu$	Fossulae lumen/ $\mu$	Nodules/ $\text{mm}^2$	Lieberkuhn/ $\text{mm}^2$
1-16 week	604 $\pm$ 59.61	1728.6 $\pm$ 135.5	220.2 $\pm$ 26.8	0.063 $\pm$ 0	0.175 $\pm$ 0.05
2-20 week	1393.4 $\pm$ 128	1660.6 $\pm$ 86.57	2.67 $\pm$ 0.2	0.172 $\pm$ 0.035	0.035 $\pm$ 0.01
3-24 week	722 $\pm$ 68.2	1445.6 $\pm$ 230.3	115.4 $\pm$ 4.03	0.088 $\pm$ 0.03	0.055 $\pm$ 0.01

For ND width, differences between 1& 2, 2& 3 with (P<0.001) and between 1& 3 with (P<0.003) are significant.

For ND height, differences between 1& 3, 2& 3 with (P<0.001) are significant, between 1&2 is NS (P= 0.488).

For Nodules/ $\text{mm}^2$ , differences between 1&2, 2& 3 with (P<0.001) and between 1& 3 with (P= 0.049) are significant.

For Lieberkuhn/ $\text{mm}^2$  differences between 1&2, 1& 3 with (P<0.001) and between 2& 3 with (P= 0.014) are significant.

Table 3: Histometrical analysis of the bursa of fabricius in 4- 6 months old white leghorn chicken, measurement units are all in mm

	Cortical diameter	Follicle diameter	Medulla diameter	Follicle no/ plica	Plica height
1-16 week	0.04 $\pm$ 0	0.36 $\pm$ 0	0.3 $\pm$ 0	6.18 $\pm$ 1	2.21 $\pm$ 0.1
2-20 week	0.03 $\pm$ 0	0.3 $\pm$ 0	0.23 $\pm$ 0	36.7 $\pm$ 2.5	3.67 $\pm$ 0.1
3-24 week	0.04 $\pm$ 0	0.3 $\pm$ 0	0.17 $\pm$ 0	37.8 $\pm$ 2.8	3.59 $\pm$ 0.2

For the cortical diameters, differences between 1& 2, 2 & 3 are significant (P<0.001) and between 1 & 3 is NS (P= 1)

For the follicle diameters, differences between 1 & 2, 1 & 3 are significant (P<0.001) and between 2 & 3 is NS (P= 1)

For the medulla diameters, differences between 1 & 2, 1& 3, 2 &3 are significant (P<0.001)

For follicle no/ plica, differences between 1& 2, 1& 3 are significant (P<0.001) and between 2 & 3 is NS (P= 0.380)

For the plica heights, differences between 1& 2, 1 & 3 are significant (P<0.001) and between 2 & 3 is NS (P= 0.279)

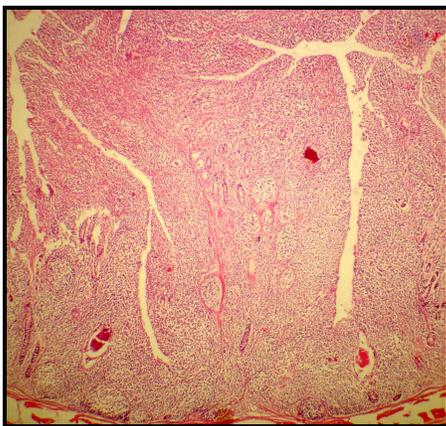
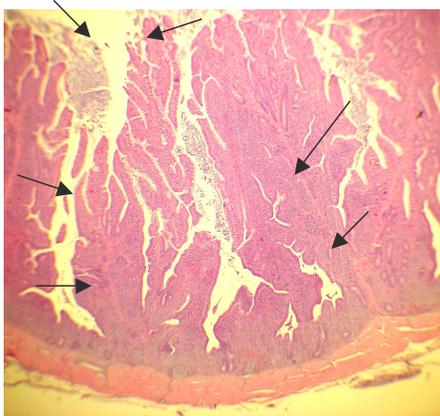


Fig. 2: The top, photomicrograph of one caecal tonsil of 4 month of age white leghorn chicken limited by arrows, and below, the caecal tonsil of 5 month of age white leghorn chicken are shown, H&E, 10x

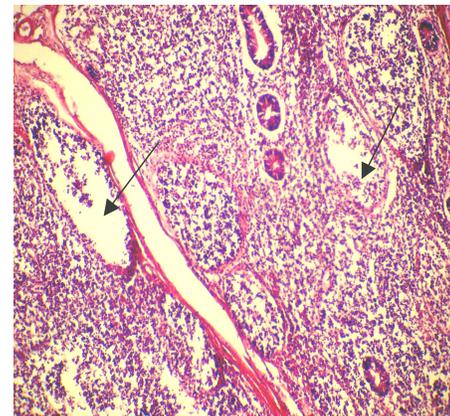
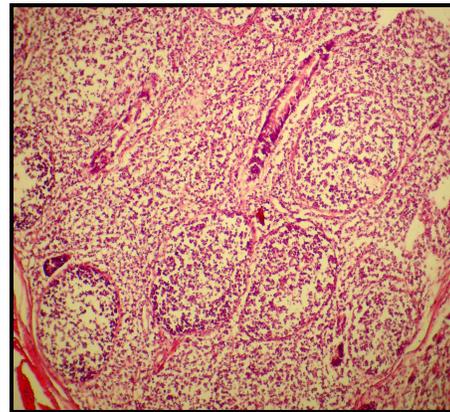


Fig. 3: The top, lymphatic nodules of cecal tonsils in 5 month of age white leghorn chicken compare with the nodules coming to evacuation (arrow) in 6 month of age white leghorn chicken (below), H&E, 30x

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 both types of covering cells; rather penetrate up to the cells' apex. 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 (Fig. 2). Fossulae ramifications are rather lost and the lumen is narrower, while mucosa thickness here increases due to accumulation of lymph nodules (Fig. 3). At 6 months of age nodular unit's thicknesses decrease, so they keep their distance. Evacuation of some nodules has been occurred in this age. Dispersion rate in nodular units showed that the most increasing belongs to the age of 5 months and decreases at the time before and after that (Fig. 3). Statistical results showed that nodular unit's width and lymph nodules spreading area increase significantly at the age of 5 months and decrease at 4 and 6 months of age ( $P < 0.001$ ). 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 (Table 2). Table 1 shows the histometrical changes in the thymuses and Table 3 shows histometrical changes in the bursas of fabricious of the same animals.

### CONCLUSION

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.*<sup>[7]</sup> 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. Kitagawa *et al.*<sup>[8]</sup> 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.

Kato *et al.*<sup>[3]</sup> 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<sup>[3]</sup>. Kitagawa *et al.*<sup>[6]</sup> 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 enteroendocrine<sup>[6,14]</sup>. Befus *et al.*<sup>[9]</sup> 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 avian growing up exhausts intestinal lymphoid accumulation. 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<sup>[9]</sup>.

Thymus cortex involution process, though significant in the interval of 4 to 5 months of age, is of very little changes; however the involution rate is highly increased between 5 to 6 months of age (Table 1). Thus we may suggest that the involution process has begun from around 5 months of age, which is synchronized with the highest growth rate and the highest increasing of lymphatic nodular distribution area in caecal tonsils (Table 2). The accumulation however decreases significantly in 6 months of age, so its dependence on thymus can be suggested. Table 3 showed that bursas of fabricious involution process have not begun until 6 months of age. Lack of change in sub-epithelial lymphatic tissue of caecal tonsils can be depended on lack of change in bursas of fabricious.

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, making better opportunity for lymphocytes to capture more antigens from the surface and improved immunity.

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