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## Age related histological and histochemical studies on hard palate of broiler chicks

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### Abstract

A study was conducted on histological and histochemical feature of the hard palate of 32 broiler chicks at 7,11,18,25 and 32days of age. The tissues of hard palate containing rostral part of median palatine ridge, region of anterior maxillary gland, rostral region of choanal cleft and region having palatine papillae were collected. The results illustrated that the median palatine ridge presented a blunt papilla like appearance which sloped downward and backward was covered by stratified squamous keratinized epithelium. The median palatine ridge presented a blunt papilla like appearance which sloped downward and backward was covered by stratified squamous keratinized epithelium. The thickness of maxillary ramphotheca was increased with the advancement of age of birds. The epithelium showed the presence of keratin layer in the areas where the blunt papillae were present. Some of these papillae have the lamellated arrangement of the cells. At rostral region of choanal cleft a strong Alcianophilic reaction indicated the presence of the weakly acidic sulfated mucopolysaccharides, hyaluronic acid and sialomucins and glycogen. The respiratory epithelium was modified into simple columnar type of follicle associated epithelium (FAE) where the cells similar to the M-cells were also present.

**Keywords:** Maxillary ramphotheca, choanal cleft, median palatine ridge

### Introduction

The fowl is an omnivorous bird and variation in food resources has resulted in its adaption to different environments leading to difference in shape and structure of the palate and oropharynx (King and McLelland, 1984). The palate and oropharynx is the first area for food selection and intake and plays a very important role in maintenance of food in oral cavity, movement and in swallowing of bolus as a reflection of the different lifestyle of birds (Dehkordi *et al.*, 2010) [3]. The macroscopic and microscopic structure of palate differs greatly with the feeding habits (Hodges, 1974) [4]. However, specific information on the microscopic anatomy of the hard palate of the fowl is scanty. Keeping in view the importance of histology of the hard palate, the present study will be a boon to the avian histologists and pathologists.

### Materials and Methods

The present study was conducted on 30 broiler chicks of one week to one month (7-32 days) of age. These birds were divided into 5 groups having 6 birds in each group. The head of dead birds were collected after post-mortem examination at 7, 11, 18, 25 and 32 days of age. The tissues of hard palate containing rostral part of median palatine ridge, region of anterior maxillary gland, rostral region of choanal cleft and region having palatine papillae were collected and fixed in 10% neutral buffered formalin solution for 48 hours. The tissues were processed for routine paraffin technique and paraffin sections of 5-6  $\mu$  were cut and stained with routine Harris' hematoxylin and eosin stain for general architecture, Gomori's method for reticular fibres, Weigert's method for elastic fibres, Alcian blue method for muco-substances (pH 2.5), McManus' method for glycogen (PAS), PAS-Alcian blue method for acidic and neutral mucosubstances (pH 2.5), Meyer's mucicarmine method for mucin, colloidal iron method for acid mucopolysaccharides, Ayoub-Shklar method for keratin and pre-keratin (Luna, 1968) [5], mercury bromphenol blue method for protein (Pearse, 1968) [6] and Crossman's trichrome stain for collagen fibres (Crossman, 1937) [2].

### Results and Discussion

The hard palate was studied at rostral part of the median palatine ridge, region of anterior maxillary gland, rostral region of choanal cleft and palatine papillae.

**1. Rostral part of median palatine ridge:** The median palatine ridge presented a blunt papilla like appearance which sloped downward and backward was covered by stratified squamous keratinized epithelium (Fig. 1) as observed in the emu (Crole and Soley, 2011)<sup>[1]</sup> and ostrich (Tivane, 2008)<sup>[11]</sup>. However, the epithelium became non-keratinized in its portion extending to the choana in ostrich (Tivane, 2008)<sup>[11]</sup>. The epithelium was comprised of strata basal, spinosum, and corneum with varying number of rows in different strata. The basal surface of the epithelium constituted by stratum basale was undulating leading to the formation of papillary pegs which were narrow, elongated and vertically oriented. The chromatin material of basal cells' nuclei was dense and deeply basophilic whereas; the cytoplasm was eosinophilic and finely granular. The cells of the stratum spinosum contained irregular nuclei which were larger in size and vertically oriented. Their chromatin material was dense and deeply basophilic and uniformly distributed. The nucleoli were not distinct in these nuclei. The nuclei of the superficial layers were comparatively smaller in size and their nucleoli were much distinct. The cytoplasm of all these cell types was finely granular and eosinophilic in nature. These cells presented the processes which gave the appearance of spine or spicules like arrangement. The stratum granulosum cell layers were not distinct. The stratum corneum was comprised of round to oval nuclei with their longitudinal axis parallel to the epithelium. These nuclei were very less basophilic with very fine distribution of chromatin material and contained one centric/eccentric nucleolus. The size of the nuclei was drastically reduced towards the surface of the epithelium and some of the nuclei showed the degenerative changes. The cytoplasm of all these cell types was finely granular and slightly eosinophilic with a basophilic tinge. The outermost keratinized layer demonstrated by Ayoub-Shklar method was almost regular except at some places where it showed desquamation. The epithelium was devoid of the PAS activity however small concentration of neutral mucopolysaccharides was observed in the stratum corneum and in the keratinized layer.

Laterally the median palatine ridge presented a blunt papilla like appearance which sloped downward and backward was covered by stratified squamous keratinized epithelium. In this region, the basal surface of the epithelium did not present the pegs however the cell layers and the thickness were almost similar to that of the median palatine ridge. A few structures similar to that of Herbst corpuscles were also observed. The thickness of maxillary ramphotheca was increased with the advancement of age of birds.

The dermis was having dense and irregular connective tissue comprising of connective tissue cells, fibers, small sized blood vessels, and fine blood capillaries (Fig. 2). A dense arrangement of the collagen bundles was also present. The deeper portion was having loose irregular connective tissue, bone, cartilage, nerve bundles and fatty tissue which were surrounded by the dense connective tissue. The elastic fibers and the collagen fibers were also present in the connective tissue separating the lobes and lobules of the fatty tissue and formed the septae like arrangement. A higher concentration of fibers was also observed around the hyaline to elastic type of cartilage (Fig. 3). A weak to moderate positive activity for acidic mucopolysaccharides was observed in the septae by colloidal iron method. In the deepest part, bone was present which was surrounded by the periosteum having dense arrangement of collagen fibers.

**2. Region of anterior maxillary gland:** This region was present where the median palatine ridge bifurcated into the lateral palatine ridges and was lined by stratified squamous keratinized epithelium having a few surface papillae. These papillae were mostly blunt in shape and some had laminated arrangement in birds of 25-32 days. The size of the papillae increased with advancement of age.

The deeper portion of the epithelium was having large number of finger like papillary- projections separated by inter papillary pegs. The epithelium was comprised of stratum basale, stratum spinosum and stratum corneum. The vertically oriented nuclei of the stratum basale cells were narrow, and elongated with strongly basophilic chromatin material. The stratum spinosum contained the varying number of cell rows. The histological features of the nuclei of the cells present in the region of pegs were similar to those of stratum basale cells. The size of the nuclei increased as moved towards the superficial layers. These nuclei were less basophilic because of fine dusting of chromatin material however, their nucleoli were strongly basophilic. These cells presented the cytoplasmic processes which led to give the spiny appearance. The nuclei of further superficial layers were very small and round in shape. The stratum corneum also possessed the different number of rows. Their rod shaped nuclei were strongly basophilic and showed pyknotic appearance. The cytoplasm of all the cell layers was finely granular and strongly eosinophilic in nature. The epithelium showed the presence of keratin layer in the areas where the blunt papillae were present. Some of these papillae have the lamellated arrangement of the cells.

The propria submucosa was having dense regular connective tissue comprising of connective tissue cells, fibers, fine blood capillaries and a few fibroblasts. It was having the dense arrangement of collagen bundles. A dense arrangement of elastic fibers present towards the deep portion sent a few isolated elastic fibers towards the interpapillary pegs.

Large clusters of tubulo-alveolar mucous type of glands present in the deeper part were anterior maxillary glands (Fig. 4) localized only towards the mid portion and not observed towards the lateral sides. The glandular tissue was surrounded by a dense connective tissue having collagen bundles (Fig. 5) and elastic fibers. The alveoli were lined by pyramidal shaped cells. Their nuclei were round to oval in shape and were present towards the basal portion of the cells. The cytoplasm of these cells was finely granular and eosinophilic in nature. The alveoli and glandular ducts were strongly positive for acidic mucopolysaccharides, glycogen, weakly sulfated acidic muco-substances, hyaluronic acid and sialomucins mucins. In the birds of 11 days onwards, large number of corpuscles was present at the junction of the connective tissue and the glandular alveoli. The propria submucosa was having less connective tissue, however it was denser in nature. The glands were categorized in to two sub-types, the superficially placed glands contained the large number of mucous cells with more concentration of acidic mucopolysaccharides, whereas those of the deeper layer had comparatively less number of mucous cells.

The connective tissue of deeper portion was loose, irregular having the fatty tissue and the corpuscles like structures. In the deepest part, the fragments of the bone, the dense connective tissue and the hyaline-elastic type of cartilage were observed. The connective tissue septae, fatty tissue and cartilage showed positive activity of acidic mucopolysaccharides.

Just lateral to the median palatine ridge on either side, the epithelium was of similar nature but it was devoid of the papillae and the glands. The propria submucosa was having mainly loose and irregular connective tissue with more concentration of fine blood capillaries, fatty tissue, and bones with dense arrangement of connective tissue followed by few muscles, medium sized blood vessels, nerve bundles and the hyaline-elastic type of cartilage.

**3. Rostral region of choanal cleft:** The portion of the hard palate at the level of the narrow part of the choanal cleft was lined by stratified squamous keratinized to non-keratinized epithelium as demonstrated by special stain. The deeper surface of the epithelium presented the pegs or the papillae whereas the superficial surface was smooth except the lateral portion where papillae were present (Fig. 6). The cells of stratum basale were having round to oval nuclei with less basophilic chromatin material. The stratum spinosum was constituted by varying number of rows of cells. The nuclei were larger in size and generally round in shape with very less basophilic chromatin material towards the superficial layers. The cytoplasm of all these cell types was finely granular and eosinophilic in nature. The spicule like arrangement was not visible. The more superficially placed nuclei were smaller in size and started showing vacuolated appearance. The nuclei of the stratum corneum were elongated rod shaped with pyknotic changes having dense chromatin material. The nuclei were visible only in the few cells. The cytoplasm of these cell types was finely granular and more eosinophilic than those of other cell layers. The more superficially placed cells exhibited the characteristics of keratinization only at few places. The epithelial height was drastically reduced towards the deeper part of the choanal cleft.

The epithelium towards the cleft was modified from stratified squamous to the respiratory epithelium in the birds of 11 days onwards. The pseudo-stratified columnar ciliated having few goblet cells showed very strong reaction for the acidic mucopolysaccharides as reported in chhukar partridge (Sagsoz *et al.*, 2013). A strong Alcianophilic reaction indicated the presence of the weakly acidic sulfated mucopolysaccharides, hyaluronic acid and sialomucins and glycogen. The respiratory epithelium was modified into simple columnar type of follicle associated epithelium (FAE) where the cells similar to the M-cells were also present.

The propria-submucosa was having dense irregular connective tissue though its thickness was comparatively lesser than that of the earlier tissues. It was comprised of connective tissue cells and different types of fibers especially the predominance of collagen fibers. Just deep to the connective tissue cluster of glandular alveoli were lined by pyramidal shaped cells as reported in other birds (Samar *et al.*, 1999; Crole and Soley, 2011)<sup>[9, 1]</sup>. These glands have been reported as simple branched tubular glands producing mucous secretion (Tivane 2008; Crole and Soley, 2011)<sup>[10, 11, 1]</sup>. The glands were classified into median and lateral palatine glands as reported earlier in chicken (Samar *et al.*, 2002)<sup>[10]</sup> were surrounded by connective tissue fibres. The glands and the glandular ducts were showing the strong positive reaction for the acidic mucopolysaccharides. The activity was more in the superficially placed lateral palatine glands. Strong Alcianophilic reaction in the median and lateral palatine glands and their ducts indicated the presence of the weakly acidic sulfated mucopolysaccharides, hyaluronic acid and sialomucins. Mayer's mucicarmine method demonstrated that

the activity for mucins was moderate in the median palatine glands and was more in the lateral palatine glands. Strong activity for the glycogen and acidic mucopolysaccharides was observed in both types of the glands. A negative reaction for the proteins was observed by bromphenol blue method. Deep to the glandular tissue, different sized blood vessels, nerve bundles, fine blood capillaries were present in the loose irregular connective tissue. The specific feature was the presence of the muscles on either side just deep to the connective tissue of the choanal cleft. These muscle bundles present on either side of the cleft merged with each other in the median position and merged with the dense connective tissue present adjacent to the bone. Deep to the bone, the fatty tissue was present. The collagen fibers were densely arranged around the bone.

On the lateral side, the surface epithelium presented the papillae which were having the characteristics of the surface epithelium as described earlier. The lateral palatine glands were present which were again similar to those of median palatine glands though their number was comparatively lesser. The superficially placed glandular clusters having more number of the mucous cells than those placed into the deeper part. These glands exhibited various PAS reaction as described earlier. Few blood capillaries, small blood vessels; nerve bundles were present in between the clusters of the glands. The muscles were absent in this region.

In the birds of 11 days onwards, the propria-submucosa had small aggregations of the lymphoid tissue which led to the infiltration into the surface epithelium also. In the partridge, solitary and aggregated lymphoid follicles were noted in the connective tissue and around the glands along the palate and at the periphery of the choanal cleft (Samar *et al.*, 2002; Tivane, 2008; Crole and Soley, 2011)<sup>[10, 11, 1]</sup>.

**4. Region of palatine papillae:** The region of the hard palate having the palatine papillae was lined by stratified squamous non-keratinized epithelium. The basal surface was having the papillary pegs which were fewer and wider in nature. The nuclei of stratum basale cells were comparatively less basophilic in nature. The cells of the stratum spinosum were distributed in the different rows and were having almost similar histological features. The stratum corneum was present as a distinct layer and it was having a lighter cytoplasm. Towards the free surface, the palatine papillae having blunt, round tips were directed backward. Towards the cleft, the epithelium was modified and it was drastically reduced in terms of the cell layers. This portion was lacking the presence of papillae. This epithelium was further modified in the deeper portion of the choanal cleft into the respiratory type i.e. the pseudostratified columnar ciliated epithelium. It was associated with underlying glands having larger number of the goblet cells and at places the lymphoid tissue was also present.

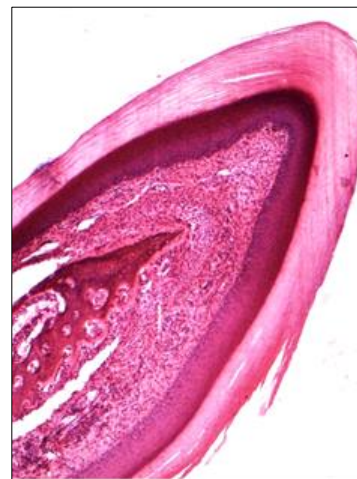
The propria submucosa was having dense regular connective tissue comprising of connective cells, fibers, fine blood capillaries and a few fibroblast. It was having a dense arrangement of collagen bundles and few elastic fibres. The propria-submucosa towards the medial portion in the region of transition into the respiratory type was devoid of the presence of the striated muscles. However, large number of the muscle fasciculi was present towards the lateral portion.

The median palatine glands were surrounded by the connective tissue layer forming a capsule like structure. At places, the lymphoid tissue was present in between the

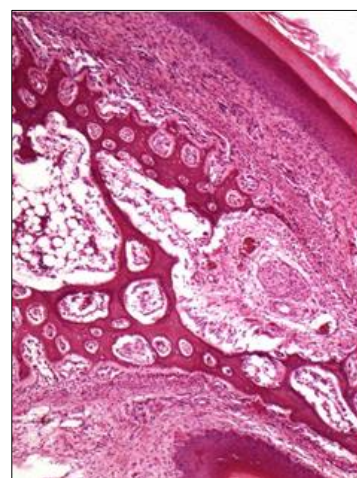


clusters of the glandular alveoli. Some of the glandular alveoli were associated with the lymphoid aggregations. At the junction of the glandular tissue and the connective tissue, the elastic fibers were observed. The lateral palatine glands were comparatively lesser in number and extended up to the last papilla towards the lateral side. The lateral palatine glands showed the presence of the acidic mucopolysaccharides whereas; neutral mucopolysaccharides were observed in a few cells of the median palatine glands as reported earlier in chicken (Samar *et al.*, 2002)<sup>[10]</sup>. The authors revealed that the cells and lumen of the lateral palatine glands gave strong PAS reaction, whereas; the apical cytoplasm of the cells of the median palatine glands produced PAS reactions of varying intensity. Samar *et al.* (1999)<sup>[9]</sup> also reported that all mucous cells of the palatine glands gave PAS-positive activity in the penguins. It was also observed that the rostral salivary glands and their excretory ducts gave a stronger PAS-positive reaction compared with the caudal salivary glands, and it was evident in the caudal salivary glands, the reaction was centered in the apical cytoplasm of the mucous cells. The rostral and caudal palatine glands of the other birds species also contained neutral mucins (Samar *et al.*, 1995, 1999, 2002)<sup>[8, 9, 10]</sup>. However, the findings obtained in the present study showed that, similar to the findings in the penguin (Samar *et al.*, 1995, 1999)<sup>[8, 9]</sup> and the seagull (Samar *et al.*, 1995)<sup>[8]</sup>, where cells producing a mucous secretion, gave a stronger reaction with PAS which indicated the presence of greater amount of acidic mucins. In chicken, Samar *et al.* (2002)<sup>[10]</sup> reported that the lateral glands were made of mucous secretory units, whilst the medial glands were made of serous secretory units. During the present study only mucous secretory units were present. The study was in agreement with the presence of alveolar glandular structures producing mucous secretion has been demonstrated in the penguin and seagull (Samar *et al.*, 1995, 1999)<sup>[8, 9]</sup>.

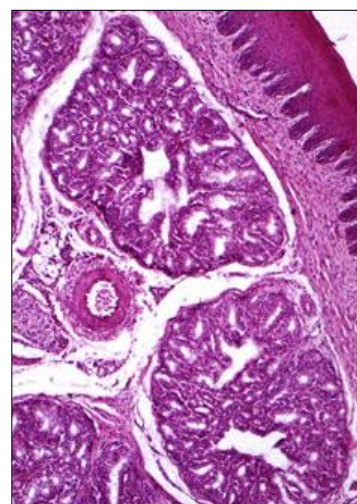
The medial and lateral palatine glands and the respiratory epithelium showed very strong Alcianophilic reaction indicating the presence of the weakly acidic sulfated mucopolysaccharides, hyaluronic acid and sialomucins which was in agreement with the observations found in chicken (Samar *et al.*, 2002)<sup>[10]</sup>. Furthermore, it was observed that the secretion of the palatine glands contained glycoproteins, carboxylated proteoglycans, weakly and strongly sulphated mucins, sialic acid and hyaluronic acid, but lacked glycogen in chhukar partridge (Sagsoz *et al.*, 2013). Mayer's mucicarmine method showed that the glandular acini/alveoli were positive for acidic mucopolysaccharides but glandular ducts showed comparatively weak activity. Very strong reaction for the glycogen was observed in the medial and lateral palatine glands as demonstrated by McManus' PAS method which was contradictory to the presence of glycogen resulted in a negative reaction in both the rostral and caudal glands, which demonstrated that the palatine glands of the partridge (Sagsoz *et al.*, 2013) were lacking glycogen. The main function of the salivary glands was to secrete lubricating molecules which helped to protect the oral mucosa from desiccation, mechanical damage, external toxic substances and microbial toxins by constituting a barrier (Samar *et al.*, 1995; Crole and Soley, 2011; Sagsoz *et al.*, 2013)<sup>[8, 11]</sup>.



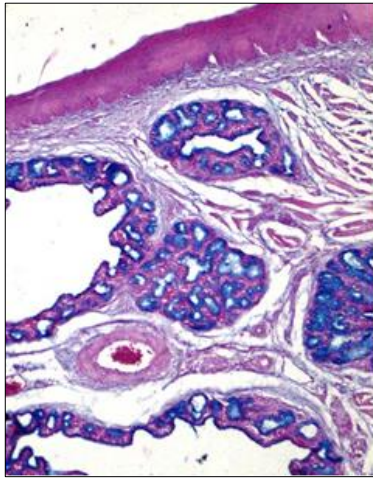
**Fig 1:** Photograph showing the median palatine ridge presented a blunt papilla like appearance covered by stratified squamous keratinized epithelium



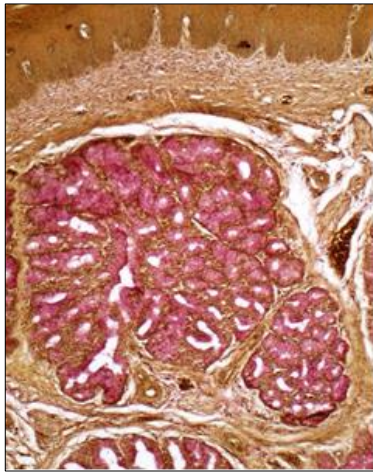
**Fig 2:** The dermis was having dense and irregular connective tissue comprising of connective tissue cells, fibers, small sized blood vessels, and fine blood capillaries



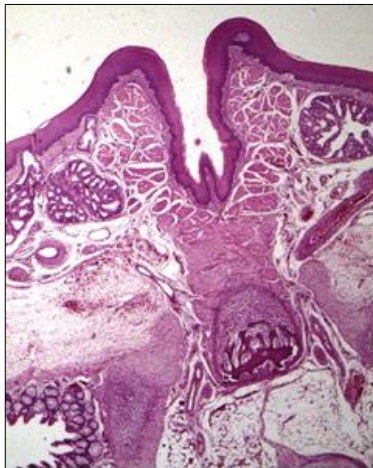
**Fig 3:** A higher concentration of fibers was also observed around the hyaline to elastic type of cartilage (Fig. 3)



**Fig 4:** Large clusters of tubulo-alveolar mucous type of glands present in the deeper part were anterior maxillary glands localized only towards the mid portion and not observed towards the lateral sides



**Fig 5:** The glandular tissue was surrounded by a dense connective tissue having collagen bundles and elastic fibers



**Fig 6:** The deeper surface of the epithelium presented the pegs or the papillae whereas the superficial surface was smooth except the lateral portion where papillae were present

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