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Post-hatching Development of Ventriculus in Muscovy Duck: Light and Electron Microscopic Study

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ABSTRACT

The current study described the developmental sequence of the ventriculus of the post-hatching Muscovy ducks of both sexes ranging from 1-60 days old, by using gross-histomorphometic measurements and by using light microscope, scanning electron microscope and transmission electron microscope. The ventriculus was extended from the level of the 4th intercostal space to terminate behind the last rib at variable distances dependent on the age of the duck. The statistical analysis revealed that the length of the ventriculus from that of the stomach was decreased by the advancement of the age, while the weight was increased. At all developmental age-stages, the cuticula gastrica was composed of two layers; vertical rods and horizontal matrix. The vertical rods projected slightly as dentate processes beyond the surface of the mucosa at 30-60 days old. The type of the gizzard gland was different according to the age; it was simple tubular type lining by one type of cells (chief cells) at 1-15 days old, but were compound-branched type lining by two types of cells; chief and basal cells at 30-60 days old. By semithin sections, the secretory basophilic granules within the cells lining of the tubular glands were increased by ageing. Transmission electron microscopy exhibited that the chief cells had numerous large sizes electron dense and electron lucent secretory granules. In conclusion, there are wide variations in the morphometrical analysis and the structure of the ventriculus at the developmental age-stages of the duck.

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Introduction

The avian gastrointestinal tract of the post-hatching stages undergoes the morphologic and physiologic changes, which increase the surface area for digestion and absorption (Overton and Shoup, 1964). The stomach of the poultry is located in the vertical plane in the left ventral part of the body cavity (Ishida & Mochizuki, 1976). The avian stomach consists of three compartments; proventricular, ventricular and pyloric parts (Hodges, 1974). The ventriculus is also termed (Pars muscularis or gizzard) (Baumel *et al.*, 1993). The ventriculus of the current studied birds is separated from the proventriculus by a well distinct isthmus (intermediate zone) (Madkour *et al.*, 2018). It is characterized by a great morphological and functional variability between and within the avian species (Działa-Szczepańczyk, 2005). Klasing (1999) reported that the size of the gizzard is large in grains-eating birds and small in soft-eating birds. Moreover, Hassouna (2001) added that the role of the gizzard is the storage of food in soft eating birds, mechanical treatment of the food in hard diet eating birds while in the intermediate diet eating birds, the function of the gizzard is storage and physical digestion. The avian ventriculus with its cuticle is corresponding to the function of teeth in mammals (Heath and Olusanya, 1985).

The pseudostratified columnar lining epithelium of the gizzard is exhibited at 5th day of incubation (Pinheiro *et al.*, 1989). Several published articles in variable species of birds stated that the surface epithelium of the gizzard mucosa is invaginated at regular intervals into the glandular layer to form shallow crypts or gizzard pits into which the gizzard tubular glands are opened (Eglitis and Knouff, 1962; Bezuidenhout and Van Aswegen, 1990; Olsen *et al.*, 2002; Mahdy, 2009; Zaher *et al.*, 2012).

This study provides sufficient information about the developmental stages of the ventriculus of the post-hatching Muscovy ducks by using the histo-morphometric measurements, light and electron microscope and compare the obtained findings with the previous data in other bird species.

Materials and methods

Birds and samples collection

Post-hatching Muscovy ducks (*Cairina moschata*) (n=55) of both sexes (1-60 days old) were divided into 5 groups (1, 7, 15, 30, and 60 days old), which were collected from local farms

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in Assiut Governorate, Egypt. The slaughtering of the birds was performed following the animal ethical committee of the Faculty of Veterinary Medicine, SVU, Qena, Egypt.

Gross anatomical manifestation

The position and relation of the ventriculus with the other viscera were inspected for each group, then the stomach was separated from the body cavity and fixed in 10% neutral buffered formalin (NBF). After description of the external morphology of the gizzards, they were sectioned in three planes to show the different views of their muscles and lumen (Fig. 1c). The terms were used in the current study, adopting to Nomina Anatomica Avium (Baumel *et al*., 1993).

Light microscopy

The samples were washed then fixed in 10% neutral buffer formalin (NBF). After proper fixation, the specimens were dehydrated in ascending grades of alcohol then cleared in methyl benzoate and embedded in paraffin wax. Serial sections (3.5-4 µm in thickness) were cut by Microtome (Leica RM2235, Leica Biosystems) and stained by the following stains; Haematoxylin and eosin (Harris, 1900), Masson's trichrome (Goldner, 1938), combined Periodic Acid Shiffs-Haematoxylin (PAS/HX) (McManus, 1946), PAS-Alcian blue technique (Mowry, 1956) and Grimelius silver nitrate impregnation (Grimelius, 1968).

Morphometric measurements

The different measurements of the ventriculus (Fig.1a and b) were taken for each bird by using a digital Vernier caliper. The measurements of the thickness of the thick and thin muscles of the gizzard were taken from a midway section through the thick muscles in a dorsoventral plane parallel to the tendinous centers (Fig. 1d). On the other hand, the histomorphometric measurements of the ventriculus layers (Fig. 1e) were taken using Image J software (http://Fiji.sc/Fiji).

Scanning electron microscopy (SEM)

According to Madkour and Mohamed (2019b), in briefly, the specimens of the ventriculus were prepared, washed and fixed in 4% gluteraldehyde solution, then post fixed in 2% buffered osmium tetraoxide. After fixation, the specimens were dehydrated then coated with gold. Finally, using (JSM-4500 LV) SEM at 10 kV for examination of the samples.

Preparations of semithin sections

Preparations of the resin embedding samples were previously performed by Soliman and Madkour (2017). The specimens were fixed in 2.5% glutaraldehyde and 4% paraformaldehyde, postfixed in 1% osmium tetroxide, dehydrated in acetone and then embedded the specimens in spur's resin. Semithin sections (0.5um thickness) were stained with toluidine blue (Spurr, 1969), finally examined by light microscope.

Transmission electron microscopy (TEM)

Ultra-thin sections (60-80 nm) of the ventriculus were taken by using a Reichert ultra-microtome. The stained grids by uranyl acetate and lead citrate were examined by using a (JEOL100CX II) TEM.

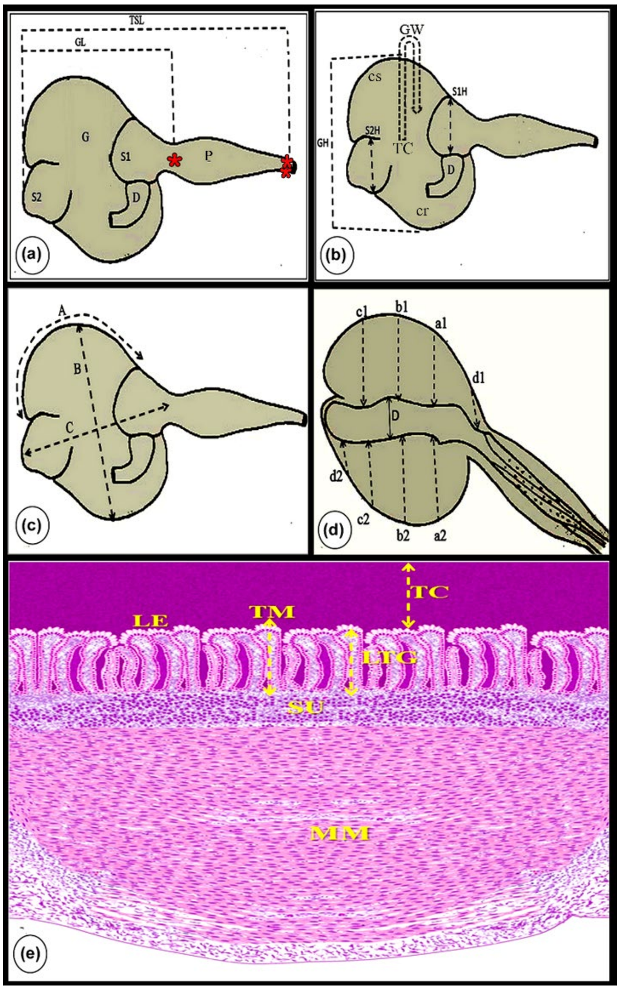

Fig. 1. Diagrams of the stomach; (a, b) Illustrated the different gross measurements: Total stomach length (TSL): Distance between the esophageo-proventricular junction (double stars) to the caudal end of caudoventral blind sac (S2). Gizzard length (GL): Distance between the proventricular-gizzard junction (star) and the caudal end of caudoventral blind sac (S2). Gizzard width (GW): Distance between the tendinous centers (TC) of either side of the gizzard. Gizzard height (GH): Distance between the caudodorsal (cs) and the cranioventral (cr) thick muscles. Craniodorsal (S1H) and caudoventral (S2H) blind sacs height: Distance from the highest point to the lowest point (double head arrow), duodenum (D). (c)Three planes showing different views of the gizzard muscles and lumen. Note, plane A- The midway section through the thick muscles in a dorsoventral plane parallel to the tendinous centers, plane B- The midway section through the tendinous centers and the thick muscles in a dorsoventral plane and perpendicular to section A, and plane C-The midway section through the thin muscles in a craniocaudal plane. (d) Measurements of the thickness of the thick and thin muscles of the gizzard at plane A. Note, the thickness of the caudodorsal thick muscle at the cranial (a1), middle (b1) and caudal (c1) levels, the thickness of the cranioventral thick muscle at cranial (a2), middle (b2) and caudal (c2) levels, the thickness of the craniodorsal thin muscle (d1), the thickness of the caudoventral thin muscle (d2) and the diameter of the lumen of the gizzard at the widest part (D). (e) Illustrated the different histological measurements of the ventriculus. Note, thickness of the cuticle layer (TC), the thickness of the mucosa (TM), the length of the tubular glands (LTG), lamina epithelialis (LE), submucosa (SU), muscular layer (MM).

Digital coloring of scanning and transmission electron microscopic images

The scanning and transmission electron microscopic images had been colored by using the Photo Filter 6.3.2 program to clarify the structure. The method was used by several authors (Madkour and Mohammed, 2021; Soliman and Madkour, 2021; Madkour *et al*., 2021a; Madkour *et al*., 2021b).

Statistical analysis

All data in Table 1and 2 were calculated and expressed as the mean value ± standard error (SE) using the Statistical Package for Social Science (SPSS) software program, version 17.0.

Results

Gross anatomical and morphometrical studies

The ventriculus was a spheroid organ. It was lied in the left caudal half of the body cavity; its ventral part crossed the midline to the right side. It was extended from the level of 4th intercostal space to terminate14.31, 20.05 and 27.46 mm behind the last rib and 10.73, 14.43 and 18.16 mm in front of the vent at 1, 7, 15 days old, respectively. While in older ages; 30, 60 days old, the ventriculus was shaped like a biconvex lens which was firm to touch and of red color; it appeared tendinous in its center. It occupied the most part of the left side of the caudal half of the body cavity, terminated behind the last rib by 44.04, 43.17 mm and cranial to the vent by 38.38 and 55.34 mm at 30 and 60 days old respectively (Figs. 2-4). The morphometrical measurements of the ventriculus in all studied age-stages were summarized in Table 1.

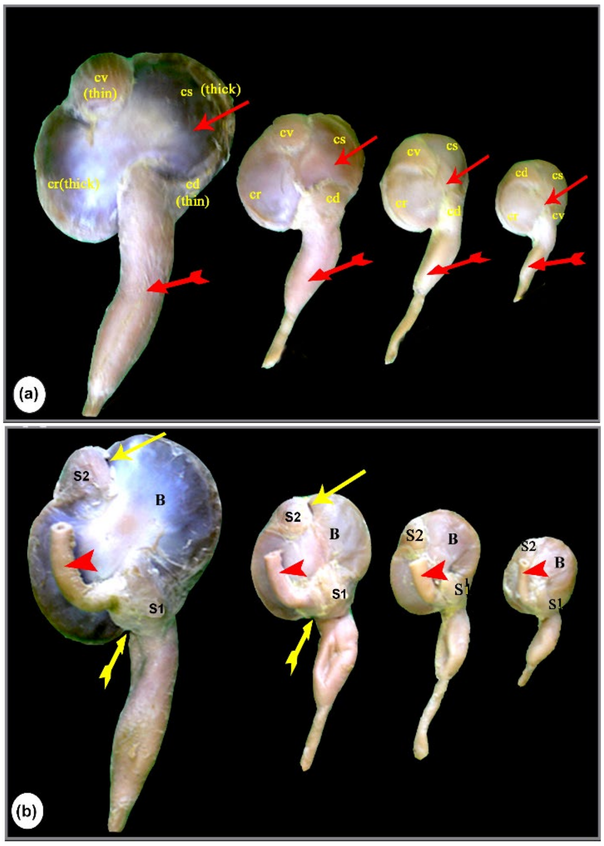

Fig. 2. Photographs of the left (a) and of the right (b) surfaces of the stomach of 1, 7, 15 and 60 days old from the right to left. Note, gizzard (red arrow) consisted of body (B) and craniodorsal (S1), caudoventral blind (S2) blind sacs, caudodorsal thick muscle (cs), cranioventral thick muscle (cr), craniodorsal thin muscle (cd) and caudoventral thin muscle (cv), proventriculus (red barbed arrow), duodenum (arrow head), caudal groove (yellow arrow) and cranial groove (yellow barbed arrow).

The right surface of the ventriculus at 1-15 days old age was related to the limbs of the duodenum, pancreas, ceca, and ilium, in addition to the jejunal segments. These structures separated the right surface of the ventriculus from the right body wall (Fig. 3a, b, e, f). A small cranioventral area of this surface at older ages was related to the visceral surface of the right lobe of the liver (Fig.4a, b). The left surface of the ventriculus was mostly related to the left abdominal air sac through and small part of the remnant of the vitelline diverticulum (Figs. 3c, g). The dorsal surface of the ventriculus at 1-15 days old was mainly related to the jejunal segments and the initial part of the cecum in addition to the distal part of the cecum, ilium, distal parts of the descending and ascending duodenum and pancreas at 30, 60 days old (Figs. 3d, h and 4c, d). Moreover, the ventral contour of the ventriculus reached the floor of the body cavity but was related cranially to the dorsal aspect of the carina of the sternum at older ages.

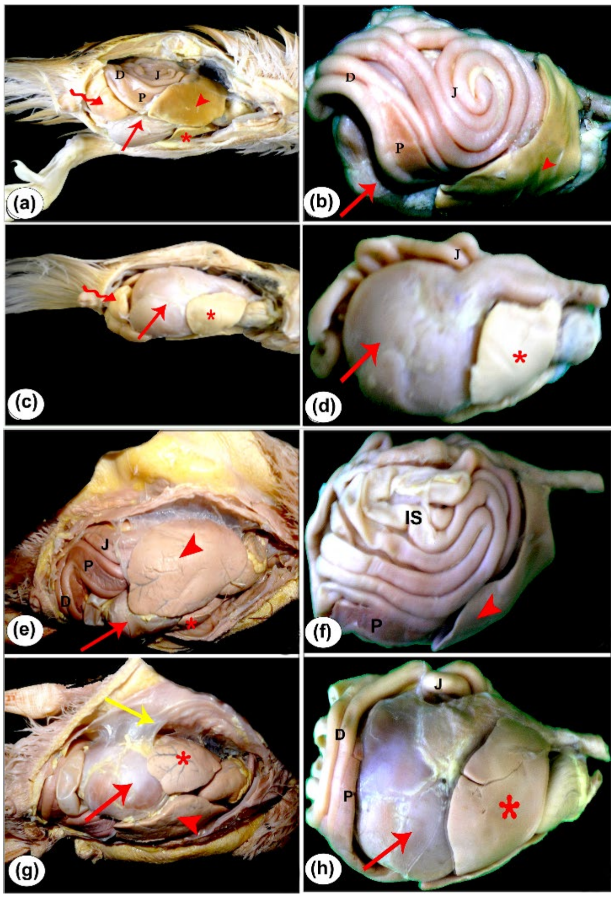

Fig. 3. Photographs of the right side (a, b), (e, f) and of the left side (c, d), (g, h) of the gizzard of one day and 15 days old inside and outside the body cavity respectively, showing its position and relation. Note, gizzard (red arrow), right lobe of the liver (arrow head), left lobe of the liver (star), remnant of vitelline diverticulum (twisted arrow), jejunal loops (J), duodenum (D) and pancreas (P), intestinal segments (IS) and left abdominal air sac (yellow arrow).

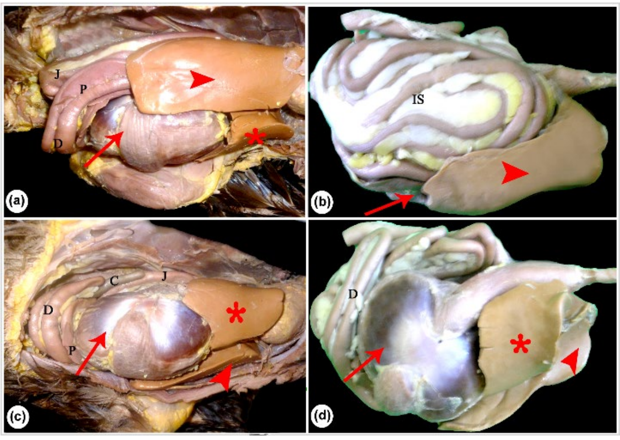

Fig. 4. Photographs of the ventrolateral side (a), of the right side (b) and of the left side (c, d) of the gizzard of 60 days old inside and outside body cavity showing its position and relation. Note, gizzard (arrow), right lobe of the liver (arrow head), left lobe of the liver (star), jejunal loops (J), duodenum (D) and pancreas (P), cecum (C) and intestinal segments (IS).

The length of the ventriculus was increased by one and a half fold at 15 days, 3 folds at 60 days old compared with that of one day old. Its percentage from the total length of stomach and the body cavity was decreased by the advancement of the age. This percentage was 57.22%, 55.75%,54.88%, 54.82%, 52.28% from the total length of stomach and 35.62%, 35.32%, 35.30%, 29.50%, 26.15% from the total length of the body cavity at 1, 7, 15, 30, 60 days old, respectively. However, the weight of the ventriculus was increased by nearly four and a half folds at 15 days, 30 folds at 60 days old compared with that of one day old. Its percentage from the weight of the stomach was increased by ageing, constituted 86.66%, 87.97%, 88.65%, 88.93% and 91.44% at 1, 7, 15, 30, 60 days old, respectively.

In all studied ages, the ventriculus of the duck consisted of a body and two blind sacs: craniodorsal and caudoventral blind sacs (Fig. 2b). The percentage of the craniodorsal and caudoventral blind sacs height from that of the ventriculus was decreased by the development. It formed 40.96%, 38.73%, 37.36%, 35.87%, 32.24% for the craniodorsal blind sac and 46.17%, 40.09%, 38.32%, 36.31%, 31.21% for the caudoventral blind sac at 1, 7, 15, 30, 60 days old, respectively.

The ventricular wall in all studied ages was made up mostly of the muscles which consisted of two pairs, two lateral thick muscles (Mm laterales) and two intermediate thin muscles (Mm intermedii). The lateral thick muscles of the body were the caudodorsal (M. crassus caudodorsalis) and cranioventral (M. crassus cranioventralis) muscles. The intermediate thin muscles of the blind sacs were craniodorsal (M. tenuis craniodorsalis) and caudoventral (M. tenius caudoventralis) muscles.

The junction between the caudodorsal thick muscle and the caudoventral thin muscles was marked by a caudal groove, whereas the junction between the cranioventral thick muscle and the craniodorsal thin muscle was marked by a cranial groove. These grooves were faint at young ages and became distinct with the advancement of the age (Fig. 2b).

The midway section through the thick muscles in the dorsoventral plane parallel to the tendinous center showed that the caudodorsal thick muscle merged cranially with the craniodorsal thin muscle and the cranioventral thick muscle merged caudally with the caudoventral thin muscle (Fig. 5a). The measurements of the thickness of the thick muscles as well as the thickness of the thin muscles at the cranial, middle and caudal levels differed basing on the age of the bird. The thickness of the thick muscles was extremely increased by nearly 2.5 and 4.5 folds at 15 and 60 days old when compared with those at one day old. Concerning to the thin muscles of the ventriculus, it was noticed that the thickness of the craniodorsal muscle was thicker than the caudoventral thin muscle in all developmental studied stages. The midway section through the thick muscles in the dorsoventral plane parallel to the tendinous center showed the difference in the size of the two thick muscles, their asymmetric shape and their associated with the thin muscles. In this section, the ventriculus lumen appeared nearly S-shaped in all examined ages (Fig. 5a). The length and diameter of the ventriculus lumen at 60 days old was nearly 3.5 and 4 folds respectively, compared with those at one day old.

The second midway section through the thick muscles and tendinous center in the dorsoventral plane showed that the ventriculus lumen in all studied ages was nearly rectangular, surrounded in both sides by semi ellipsoid thick muscles. The margin of this lumen was slightly wrinkled toward thick muscles and smooth toward tendinous center at one day old, while at 7days old, it was slightly wrinkled toward both sides. Up to 15 days old, the margin of the lumen was more wrinkled than the previous ages (Fig. 5b). The third midway section through the thin muscles in the craniocaudal plane showed that the dorsal and ventral walls of the ventriculus lumen in all studied ages formed two discs like horny plates called the dorsal and ventral grinding horny plates. These horny plates

Table 1. The gross morphological measurements of the ventriculus (gizzard) (mm and g)

Age	1 day	7 days	15 days	30 days	60 days
Body cavity length (mm)	53.36±0.11	66.07±0.52	90.38±2.47	183.16±3.92	231.48±2.58
Stomach length (mm)	33.22±0.47	41.86±0.61	58.14±1.45	98.59±0.87	115.79±1.91
Body weight (g)	53.14±1.5	79.64±1.1	247.00±1.64	915.82±4.61	1753.18±3.50
Stomach weight (g)	2.55±0.14	4.99±0.49	11.28±0.53	48.79±1	74.31±2.97
Muscular stomach					
Length (mm)	19.01±0.26	23.34±0.37	31.91±0.73	54.05±0.48	60.54±0.37
Width (mm)	11.90±0.22	15.07±0.20	19.52±0.23	32.96±0.36	38.24±0.65
Height (mm)	19.21±0.29	26.49±0.21	35.30±0.23	58.54±0.40	71.79±1.14
Weight (mm)	2.21±0.13	4.39±0.49	10.00±0.57	43.39±1	67.95±2.85
Distance bet. last rib and caudal end of gizzard (mm)	14.31±0.58	20.05±0.5	27.46±1.21	44.04±0.22	43.17±1.79
Distance bet. caudal end of gizzard and vent (mm)	10.73±0.39	14.43±0.76	18.16±1.29	38.38±0.52	55..34±0.94
Height of craniodorsal blind sac (mm)	7.87±0.15	10.26±0.03	13.19±0.16	21.00±0.43	23.15±1.02
Height of caudoventral blind sac (mm)	8.87±0.31	10.62±0.14	13.53±0.11	21.26±0.40	22.41±0.41
Thickness of cranioventral thick Muscle					
at cranial level (mm)	5.56±0.16	9.61±0.30	13.30±0.46	18.82±1.01	24.48±1.01
at middle level (mm)	5.15±0.14	9.33±0.32	12.39±0.42	18.52±1.02	23.39±1.40
at caudal level (mm)	3.01±0.30	6.73±0.66	8.76±0.15	12.50±1.01	18.37±1.29
Thickness of caudodorsal thick Muscle					
at cranial level (mm)	3.40±0.42	6.00±0.46	9.46±0.18	15.36±0.32	17.47±0.88
at middle level (mm)	5.03±0.30	8.11±0.36	12.02±0.62	21.76±0.41	24.70±0.51
at caudal level (mm)	5.36±0.29	8.42±0.26	12.56±0.66	22.18±0.31	26.06±0.54
Thickness of thin Muscles					
Craniodorsal thin M. (mm)	0.62±0.04	1.45±0.03	1.88±0.02	2.55±0.21	4.67±0.25
Caudoventral thin M. (mm)	0.45±0.05	1.24±0.05	1.67±0.08	1.96±0.04	3.52±0.14
Gizzard lumen					
Length (mm)	17.40±0.42	20.38±0.67	31.38±0.42	55.99±0.35	59.45±2.07
Diameter (mm)	5.70±0.44	6.16±0.74	8.62±0.40	11.65±0.98	22.22±1.36

were faint at one day old and became distinct gradually with the advancement of the age (Fig. 6a-c).

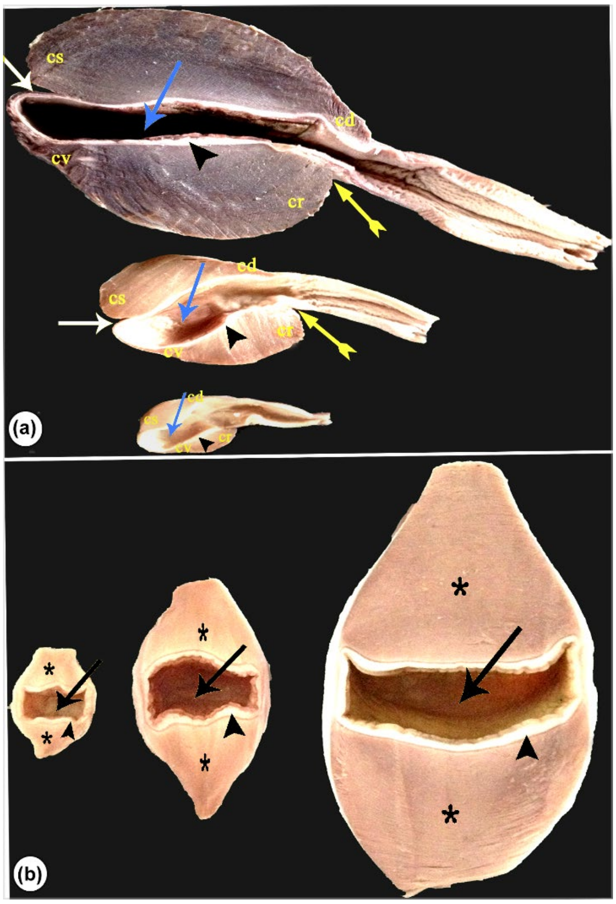

Fig. 5. Photographs of the internal morphology of the gizzard (a, b) after sectioning through the muscles showing (a) Midway section through the thick muscles in dorsoventral plane parallel to the tendinous center of 1, 15 and 60 days old from lower to upper. Note, caudodorsal thick muscle (cs) and cranioventral thick muscle (cr), craniodorsal thin muscle (cd) and caudoventral thin muscle (cv), caudal groove (white arrow), cranial groove (barded arrow), S shaped gizzard lumen (blue arrow) and margin of lumen (arrow head). (b) Midway section through the thick muscles and tendinous center in dorsoventral plane of 1, 15 and 30 days old from left to right. Note, rectangular shaped gizzard lumen (arrow), margin of lumen (arrow head) and semi ellipsoid thick muscles in both sides (stars).

The cuticle lining the gizzard lumen appeared nearly smooth at one day old. While at 7-15 days old, low gastric longitudinal parallel folds (Plicae ventriculares) were observed in the body of the ventriculus. With the advancement of the age, these folds became well distinct and separated by narrow grooves (Fig. 6a-c).

Microscopical and micrometrical findings

The ventriculus in all age-stages consisted of mucosa, submucosa, musculo-tendinous and serosa layers. The histo-morphometric measurements of the ventricular layers were summarized in Table 2.

The mucosa was folded into crypts or pits, consisted of lamina epithelialis and lamina propria. The lamina epithelialis invaginated toward the lamina propria forming the ventricular tubular glands. The lining epithelium was columnar cells at the top of the ventricular glands and decreased in height toward the base of the glands to be cuboidal cells. The epithelial cells had oval or rounded nucleus surrounded by basophilic cytoplasm (Fig. 7a-f). At one day old, the most superficial epithelial cells were undergoing degeneration contained darkly stained nucleus likely to be apoptosis which became extensive at 7, 15days old (Fig. 7a-c, e). The degenerative cells were sloughing off into the lumen of the ventriculus to be added to the secretions of the glandular tubules forming the cuticle (cuticula gastrica). The cuticula gastrica composed of two layers; the vertical rods and the horizontal matrix. The vertical rods were secreted by the mucosal glands; the secretion hardening within the lumen of the tubular glands. The horizontal matrix between the rods was a secretion of the surface epithelium. The desquamated cells of the surface epithelium were trapped within the horizontal matrix (Fig. 7b). At 30-60 days old, the sloughed epithelial degenerative cells at the mucosal surface were more distinct and the vertical rods of the cuticle projected slightly as dentate processes beyond the surface of the mucosa (Fig 8a).

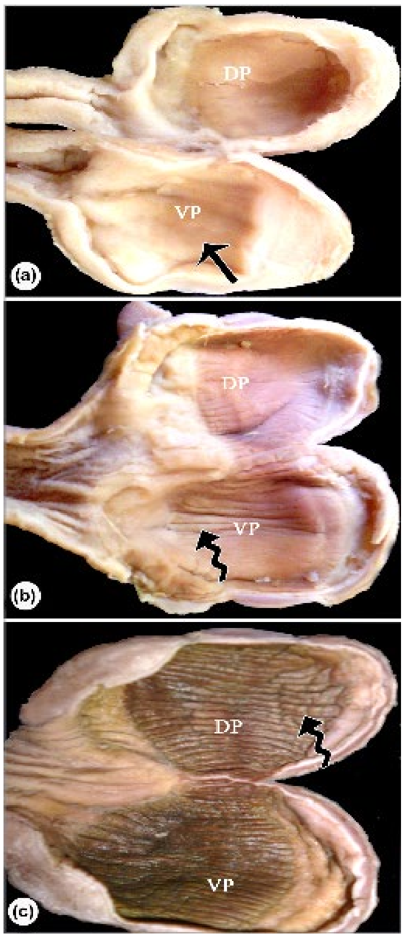

Fig. 6. Photographs of the internal morphology of the gizzard (a-c) after the midway section through the thin muscles in craniocaudal plane of 1, 15 and 60 days old from upper to lower. Showing the smooth cuticle (arrow), gastric longitudinal folds (twisted arrow), dorsal (Dp) and ventral (Vp) grinding horny plates.

The lamina propria consisted of loose connective tissue slightly infiltrated by lymphocytes, which increased by the advancement of the studied stages to be a great proportion of the lymphatic infiltrations around the ventricular glands (Fig. 8b, c). At 1-15days old, the ventricular glands were simple tubular type occupied the majority of the lamina propria till the submucosa (Fig. 7a, c, e). However, at 30, 60 days old, the ventricular glands were compound-branched type and less condensation within lamina propria than the previous ages (Fig. 8a). The length of the tubular glands was increased by nearly one and a half fold at 15 days and two folds at 60 days when compared with that at one day old.

The lumens of the ventricular glands contained lightly stained homogenous acidophilic secretions. The surface epithelium of the mucosa of the ventriculus and apical cells of the tubular glands showed positive alcian blue and PAS reactions, while the deep cells of the tubular glands showed neg ative reactions. The secretion within the lumen of the glands showed intensive reactions for PAS only (Fig. 8d). The cuticula

Table 2. The histomorphometric measurements of the verntriculus (gizzard) layers (μm)

Items	1day	7days	15 days	30 days	60 days
Thickness of the cuticula gastrica (μm)	237.92±3.17	245.51±10.09	316.91±15.14	375.98±15.72	426.34±17.06
Thickness of the mucosa (μm)	406.78±5.48	427.40±4.76	583.38±7.52	629.33±14.06	745.16±18.60
Length of the tubular glands (μm)	329.58±13.26	349.48±8.19	494.86±13.98	531.15±13.05	629.79±12.41

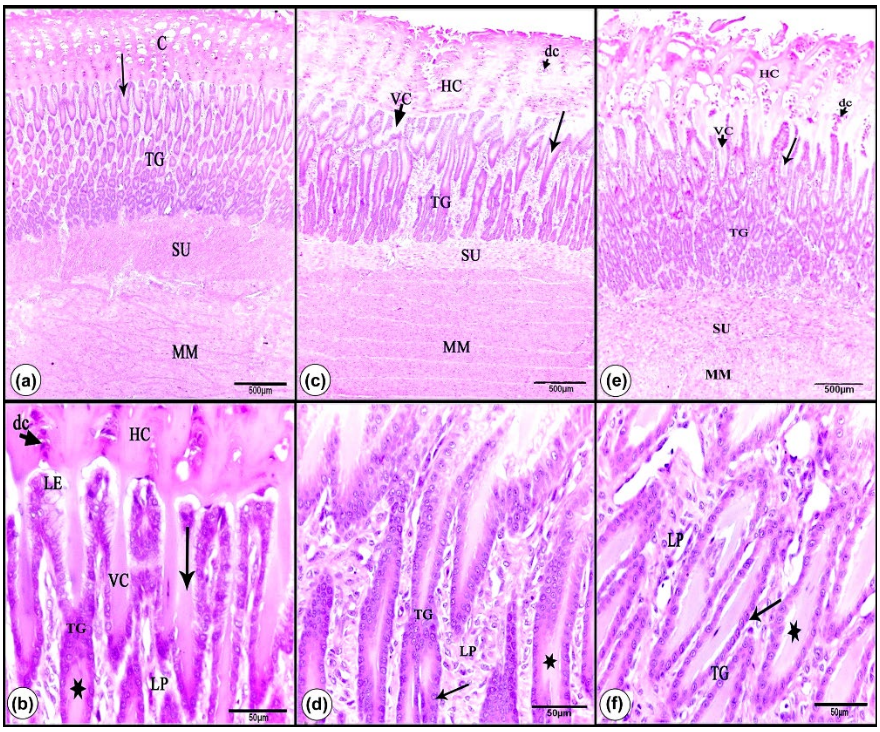

Fig. 7. Photomicrographs of the gizzard (a, b) of one day, (c, d) of 7 days, (e & f) of 15days old showing mucosa folded into crypts or pits (arrow) covering by cuticula gastrica (C) composed of vertical rods (VC) and horizontal matrix (HC), lamina epithelialis (LE) lining by columnar cells to cuboidal cells, desquamated cells of the surface epithelium contained darkly stained nucleus (dc), secretion (star) within the lumen of the gland, the simple tubular glands (TG) occupied the lamina propria (LP) till submucosa (SU), muscular layer (MM). Paraffin sections stained with H&E (a-f).

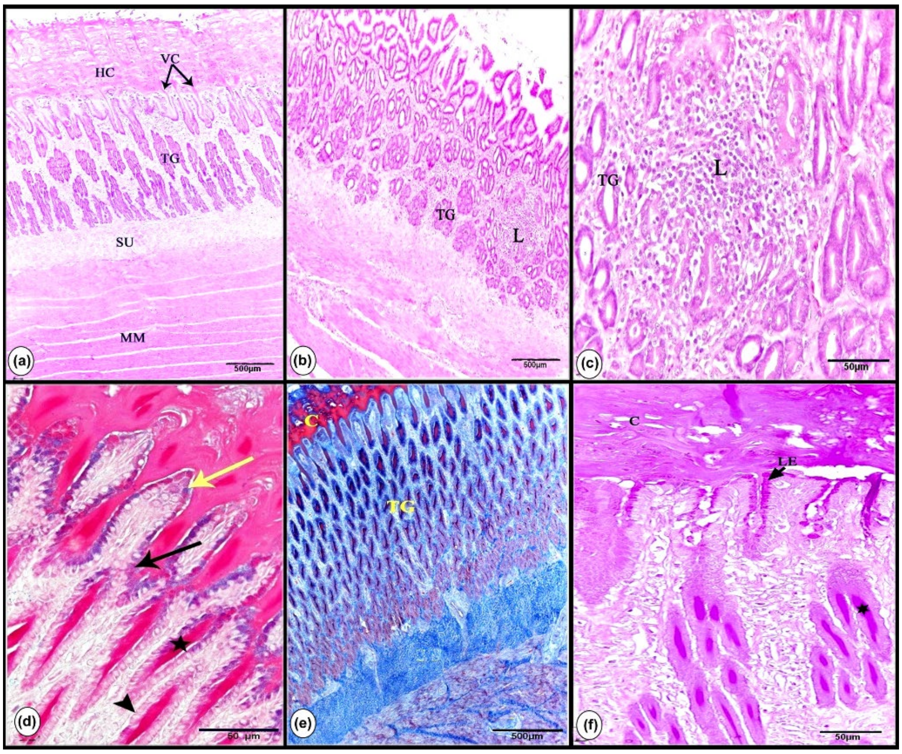

Fig. 8. Photomicrographs of the gizzard (a-c, f) of 30 days, (d, e) of one day old showing (a) Vertical rods (VC) projected as dentate processes, horizontal matrix (HC) of the cuticle, compound-branched tubular glands (TG), submucosa (SU) and muscular layer (MM). (b, c) Tubular glands (TG) surrounding by a great proportion of lymphatic infiltration (L). (d) Positive PAS and Alcian blue reactions for the surface epithelium (yellow arrow) and apical cells of the tubular glands (black arrow), negative reactions for deep cells of the tubular glands (arrow head) and intensive PAS reaction for the secretion within the lumen of the glands (star). (e) Cuticle (C) and secretion within the lumen of the tubular glands (TG) stained red with Masson's trichrome stain. (f) Stronger PAS reaction for the cuticle (C), the lamina epithelialis (LE) and the secretion within the lumen of the glands (star). Paraffin sections stained with H&E (a-c), PAS-Alcian blue (d), Masson's Trichrome (e) and PAS-Haematoxylin (f).

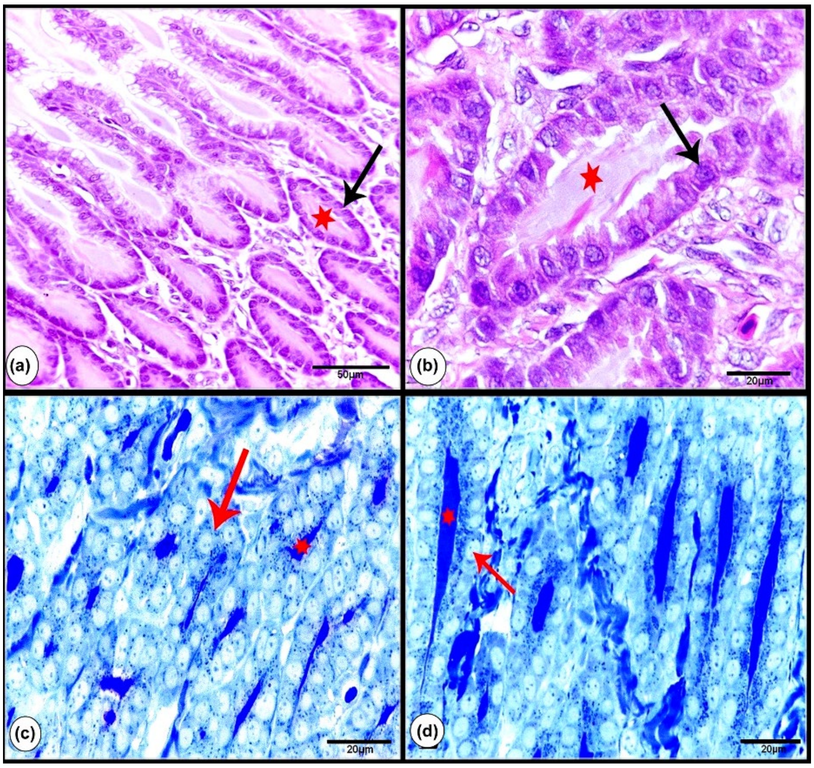

Fig. 9. Photomicrographs of the tubular glands of the gizzard (a-c) of one day, (d) of 15 days old showing (a, b) Tubular glands lining with low columnar or cuboidal cells with large rounded or oval nuclei (arrow) and the lumen of the glands fill with secretion (star). (c, d) Cells lining of the tubular glands (arrow) contained secretory basophilic granules, the secretion within the lumen (star). Paraffin sections stained with H&E (a, b), semithin sections stained with toluidine blue (c,d).

gastrica and the secretion within the lumen of the tubular glands stained red with Masson's trichrome technique (Fig. 8e). The cuticula gastrica, cells of the lamina epithelialis of the ventriculus mucosa, and the secretory materials within the lumen of the glandular tubules showed stronger reactions for PAS technique (Fig. 8f).

At 1-15 days old, the epithelial lining of the simple tubular glands was low columnar or cuboidal cells with large rounded or oval nuclei and deeply stained basophilic cytoplasm (Figs. 7b, d, f and 9a, b). At one day old, the semithin sections showed the cells lining of the superficial and the basal parts of the tubular glands contained fewer amounts of secretory basophilic granules, distributed at the apical parts of cytoplasm (Fig. 9c) which slightly increased at 15days old (Fig. 9d).

At 30 and 60 days old, the cells lining of the compound tubular glands of the ventriculus were similar to those seen at one day old. While, at the deep part of the ventricular glands appeared flattened cells with oval shaped nuclei and deeply stained cytoplasm (Fig. 10a). At the latter ages, the semithin sections claimed two types of the cells lining of the tubular glands; chief and basal cells. The chief cells were the main cells of the glands contained excessive amount of secretory basophilic granules distributed throughout apical part of cytoplasm, these granules decreased gradually toward superficial parts of the glands (Fig.10b). Few numbers of the basal cells were found at the deep part of the ventricular glands. The basal cells were flattened with oval shaped nuclei and pale cytoplasm without any secretory granules (Fig. 10c). The most superficial epithelial cells contained small metachromatic granules stained purplish in color with toluidine blue (Fig. 10d).

By TEM, the surface epithelial cells were low columnar and contained oval basal nuclei with several nucleoli. The surface epithelial cells contained apical situated small sizes granules (Fig. 10e). The chief cells of the tubular glands were low columnar with large irregular rounded nuclei and cytoplasm contained numerous large sizes electron dense and electron lucent secretory granules (Fig. 10f).

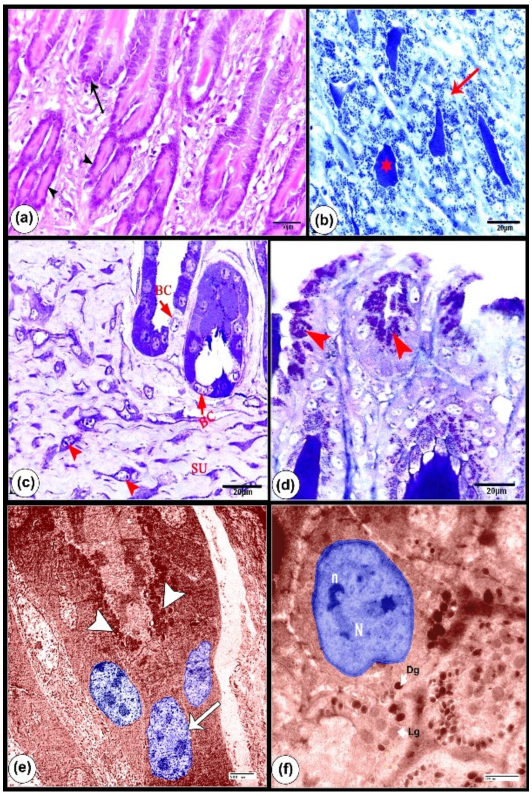

Fig. 10. Photomicrographs of the tubular glands of the gizzard (a) of 30 days and (b-f) of 60 days old, showing (a) Chief cells lining of the tubular glands were low columnar or cuboidal cells with rounded to oval nuclei (arrow) and flattened with oval shaped nuclei (arrow heads) at the deep part of the glands. (b) Chief cells contained excessive amount of secretory basophilic granules (arrow), secretion within lumen of glands (star). (c) Basal cells (BC) at the deep part of the gizzard glands, active fibroblasts (arrow heads) and submucosa (SU). (d) Superficial epithelial cells contained small metachromatic granules (arrow heads). (e) Surface epithelial cells were low columnar contained oval basal nuclei with several nucleoli (arrow) and small sizes granules (arrow heads). (f) Cell lining of the glands contained irregular rounded nucleus (N), 2 nucleoli (n) and 2types of secretory granules; electron dense (Dg) and electron lucent (Lg). Paraffin sections stained with H&E (a), semithin sections stained with toluidine blue (b-d), ultrathin sections (e, f).

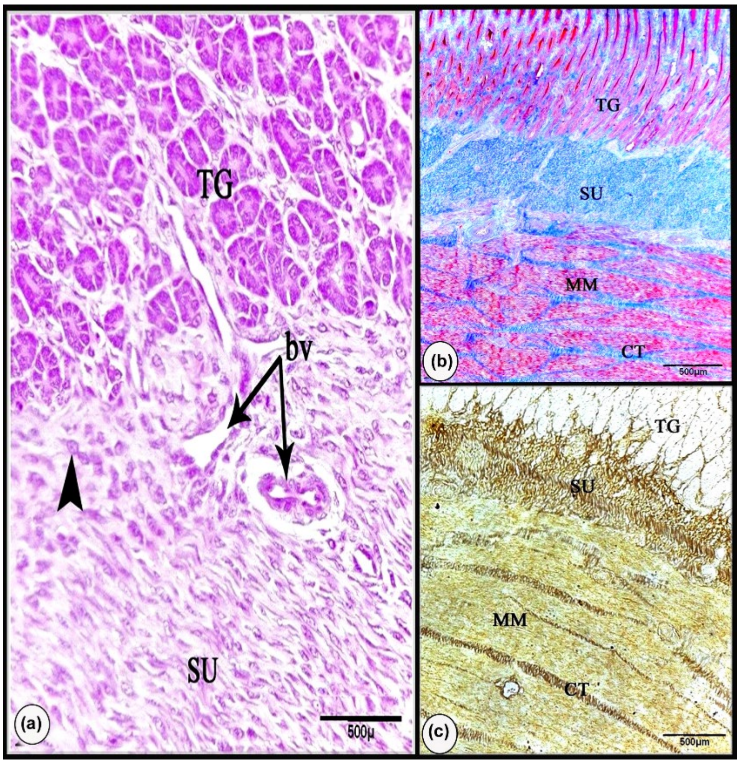

Fig. 11. Photomicrographs of the gizzard of one day old, showing tubular glands (TG), submucosa (SU) rich in blood vessels (bv) and ganglionic cells (arrow head), connective tissue septa (CT) separated the muscular layer (MM) and the submucosa were stained blue by Masson's Trichrome and dark brown by Grimelius silver nitrate stains. Paraffin sections stained with H&E (a), Masson's Trichrome (b) and Grimelius silver nitrate stains (c).

The submucosa was well-developed and represented by a layer of connective tissue rich in blood vessels, ganglionic cells and nerve fibers extended between the tubular glands and penetrated deeply to the muscular layer (Fig. 11a-c). The submucosa connective tissue layer showed positive reactions for Masson's trichrome technique (Fig. 11b). With Grimelius silver nitrate impregnation technique, the ganglionic cells and nerve fibers in the layer of submucosa connective tissue stained dark brown (Fig. 11c).

The muscular layer of the ventriculus was well-developed and represented the main bulk of the ventriculus wall. Most of the muscular bands were circulatory arranged smooth muscle fibers. The muscular bundles at one day old were separated by a thin sheath of the connective tissue septa intersecting with each other and some these septa contained blood vessels, nerve fibers, intramural ganglia and merged with the submucosa of the ventriculus (Figs. 11b and 12a, b). At 7-15 days old, these connective tissue septa that surrounding the muscular bundles ran in various directions and intersecting with each other form "a Trawl-like pattern" (Fig. 12c, d). At 30 and 60 days old, "Trawl-like pattern" became more obvious and the muscles bundles had polygonal outline (Fig. 12e, f). The connective tissue septa stained brown with Grimelius silver nitrate impregnation (Fig. 11c).

The muscular wall was covered by a thick tendinous layer of parallel reticular collagenous bundles (Fig. 13a-c) which stained blue with Masson's trichrome technique (Fig. 13b) and stained brown with Grimelius silver nitrate impregnation indicating the presence of the reticular fibers (Fig. 13c). The wall of the ventriculus was covered externally by the serosa which consisted of connective tissue containing ganglionic cells of the nervous plexus and was covered by mesothelium from most outside.

By SEM, the cuticle covering the tubular glands of the blind sac increased in the thickness toward the body (Fig. 14a). At higher magnification, the two layers of the cuticle (vertical rods and horizontal matrix) and the tubular glands became well distinct by the advancement of the age. In addition to the vertical rods appeared as the radiations within the horizontal matrix (Fig. 14b, c). The glands were lined by columnar epithelium (Fig. 14d).

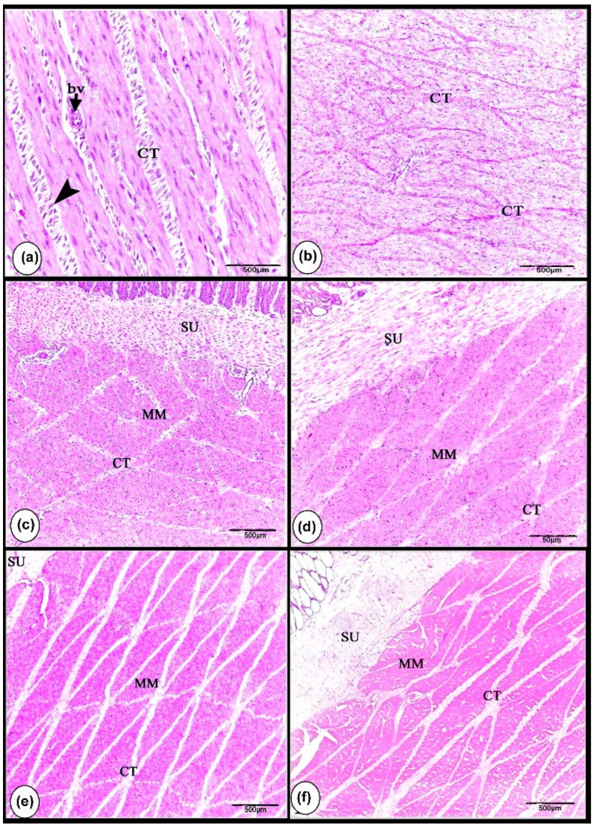

Fig. 12. Photomicrographs of the muscular layer of the gizzard (a, b) of one day, (c) of 7 days, (d) of 15 days, (e) of 30 days and (f) of 60 days old, showing (a) The muscles bundles separated by connective tissue septa (CT) containing blood vessels (bv) and ganglionic cells (arrow head). (b) Thin connective tissue septa intersecting with each other (CT). (c-f) Submucosa (SU), muscular layer (MM) separated by connective tissue septa (CT) with each other form "a Trawl-like pattern". Paraffin sections stained with H&E (a-f).

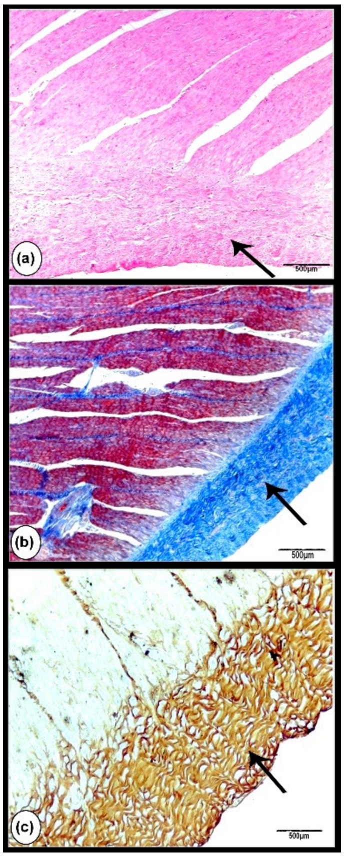

Fig. 13. Photomicrographs of the musculo-tendinous layer of the gizzard (a-c) of one day old, showing (a) Tendinous layer (arrow) stained with H&E. (b) Tendinous layer stained blue with Masson's trichrome (arrow). (c) Tendinous layer stained dark brown with Grimelius silver nitrate (arrow). Paraffin sections stained with H&E (a), Masson's trichrome (b), Grimelius silver nitrate (c).

Discussion

The current results revealed that, the ventriculus was spheroid shaped at younger ages, and became biconvex lens, firm to touch and tendinous in its center. The same organ shape of the old ages was reported by Ibrahim (1992) regarding duck, fowl, quail and pigeon, Hassouna (2001) regarding goose and turkey and Mot (2010) regarding dove and owl whereas that of the young ages reported by Ahmed *et al*. (2002) in quail and Kirk *et al*. (1993) in kakapo, kea and kaka. The ventriculus is sott-walled and bag like in heron (Ibrahim, 1992), kidney shaped in falcon (Abumandour, 2014). Furthermore, this organ is a thick-walled rounded organ and smaller than the proventriculus in the ostrich (Mahdy, 2009). The variation in the shape of the gizzard between the different species of the birds due to the diversity of their diets, food habits to adaption the function of digestion.

Concerning the position of the ventriculus, it lied in the left caudal half of body cavity; its ventral part crossed the midline to the right side and reached the floor of the body cavity. It extended from the level of 4th intercostal space to terminate behind the last rib and in front of the vent at variable distances dependent on the age of the duck. This result nearly agrees with the findings of Nishida *et al*. (1976) in goose and Ibrahim (1992) in duck, fowl, pigeon and heron. But disagrees with that of the latter author in dove, quail and jackdaw who mentioned that gizzard lay in the middle part of the body cavity; its ventral contour did not reach the ventral abdominal wall. Moreover, Hassouna (2001) in goose and turkey reported that the gizzard extended from the level of the 5th rib to about 0.3 and 0.5 cm caudal to the caudal border of the last one respectively. Madkour and Mohamed (2019a) stated the gizzard of the laughing dove and rock pigeon extended from the level of the 5th intercostal space to terminat12.25±1.18, 17.27±0.88 mm behind the last rib and 10.90±0.70, 19.48±0.49 mm infront of the vent respectively. In ostrich, this organ occupies the cranioventral part of the middle third of the body cavity from the 4th to7th vertebral ribs (Mahdy, 2009). On the other hand, the ventriculus of the carnivores' birds like falcon lay in the left dorsal and ventral regions of the thoraco-abdominal cavity (Abumandour, 2014). The afore mentioned findings confirmed

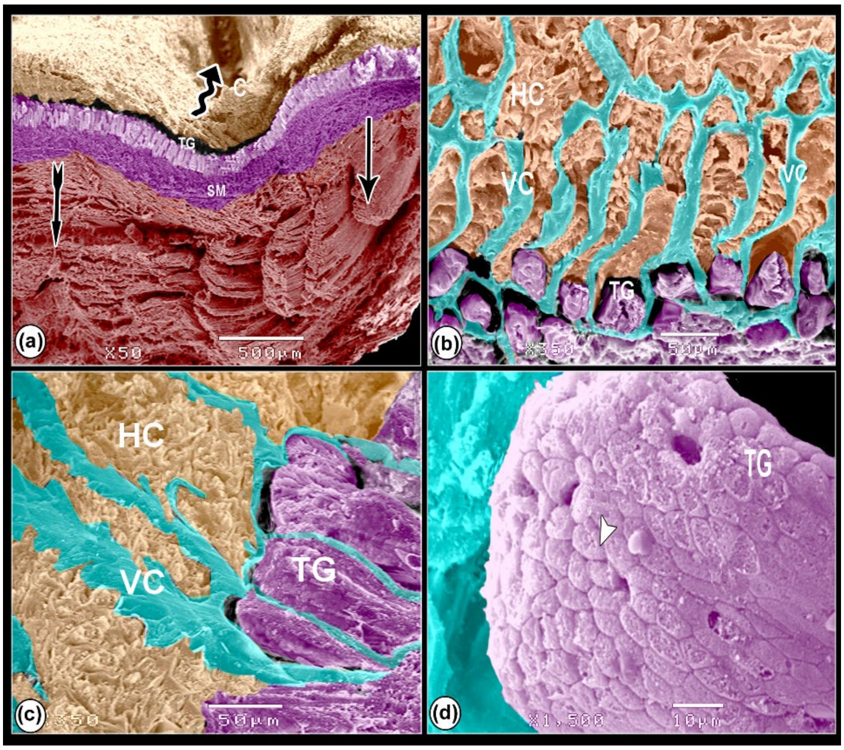

Fig. 14. Colored scanning electron micrographs of the gizzard (a, b) of one day and (c, d) of 60 days old. Note, the thin muscle of the blind sac (arrow), the thick muscle of the gizzard body (barbed arrow), caudal groove (twisted arrow), submucosa (SM), vertical rods (VC) and horizontal matrix (HC) of the cuticle (C) and polyhedral shaped cells (arrow head) of the tubular glands (TG).

by Nishida *et al*. (1976) who stated that the situation of the stomach and its morphology are a variable to a great extent according to the difference in the food habits.

The morphometrical measurements of the gizzard reported in Table 1, explained in the all studied age-stages that the length of the gizzard was one and a half fold the width. While the length was less than the height. The same results were registered by Hassan and Moussa (2012) in duck and pigeon and Madkour and Mohamed (2019a) in laughing dove and rock pigeon. As well as, Mahdy (2009) found that the craniocaudally diameter of the ventriculus is greater than the dorsoventrally diameter in ostrich. In contrast to findings from the present study, Abumandour (2014) in falcon recorded that the gizzard width is greater than the length by two and a half folds.

The obtained data indicated that the ratio of the gizzard length to that of the body cavity constituted about 35% at 1-15 days, then decreased with the advancement of the age to form 29% at 30 days old and 26% at 60 days old. Ibrahim (1992) recorded this ratio as 40% in duck and decreases to become10% in heron and Jackdaw. Regarding the gizzard weight, it represented in all studied ages 3.8-5.5% of the body weight and 86.6-91.4% of the stomach weight. In this respect, the ratio between the gizzard weight and the body weight is 2.2% in duck and 2.6% in pigeon (Hassan and Moussa, 2012), 1.91 to 2.4% in the domestic turkey (Latimer and Rosenbaum, 1926), 1.49 to 2.18% in fowl (Hafez, 1955), 1.1 to 1.36% in houbara and kori bustards and 2.21% in rufous crested bustards (Bailey *et al*., 1997).

In agreement with the findings obtained by Moore (1998) in goose and Madkour and Mohamed (2019a) in laughing dove and rock pigeon, the caudodorsal thick muscle of the gizzard was continuous with the craniodorsal thin muscle, whereas the cranioventral thick muscle was continuous with the caudoventral thin muscle. Functionally, the thin and thick muscles contract alternately; first the thin muscles contracts to push ingesta into the center of the muscular stomach, then the thick muscles contracts to grind the ingesta.

In the gained results, the cuticle layer lining the gizzard lumen was nearly smooth at one day and appearance the longitudinal parallel folds by the advancement of the ages. Abumandour (2014) clarified that the cuticle layer of the gizzard of falcon has low fine folds concentrated around the pyloric opening only while the rest of the cavity without the cuticle layer. In the domestic birds, Koch (1973) described the cuticle as a leather-like, yellow-brown coating. However, Hassouna (2001) and Ibrahim (1992) described the cuticle in the meat eating birds (kestrel, heron, jackdaw, and owl) as a jell-like membrane. In contrary, Hassouna (2001) and Al-Saffar and Eyhab (2014) observed in owl that the gizzard hasn't the cuticle layer. On the other hand, Deka *et al*. (2017) demonstrated hair like projections within the cuticle layer of Greater Adjutant stork's gizzard. The difference in the description of the cuticle layer is highly correlated with the age and the food consumed of the avian species. These support the results of King *et al*. (2000) who reported that the cuticle is thick in granivorous and herbivorous species, e.g. the domestic fowl, pigeon, ducks, and geese but thin and soft in frugivorous and nectarivorous. Langlois (2003) stated that the cuticle acts as a grinding surface and protects the underlying mucosa from digestion by the acid and pepsin secreted by the glandular stomach and mechanical process of food particles. Our results added that the power of the grinding process of the gizzard was increased by the dorsal and ventral disc like grinding horny plates of the cuticle which facilitate the generation of high intra-lumen pressures, since contractile forces in the thick muscles will be directly transferred to the lumen contents.

In all developmental stages of the post hatching ducks, the wall of the gizzard consisted of four layers; mucosa, submucosa, musculo-tendinous and serosa which coincides with the observation of the most authors (Eidaroos *et al*., 2008) in duck, geese, chicken, quail and ostrich, (El-Mansi *et al*., 2021) in Eurasian collared dove, (Prasad and Kakade, 1992) in duck, (Madkour and Mohamed, 2019a) in laughing dove and rock pigeon, (Martínez *et al*., 2000; Selvan *et al*., 2008) in chicken (Bailey *et al*., 1997) in captive bustards, (Ahmed *et al*., 2011) in quail and (Kadhim *et al*., 2011) in red jungle fowl. While, Catroxo *et al*. (1997) in red-capped cardinal, Hanafy *et al*. (2020) in Garganey and Rocha and De Lima (1998) in burrowing owl discovered the absence of the submucosa layer of the gizzard. These results contrast with findings at the current study at all studied developmental ag-stages, the submucosa was well-developed rich in blood vessels, ganglionic cells and nerve fibers to hold the muscular layer tightly to the mucous membrane and provides a firm basis for the grinding action of the cuticula gastrica.

Histological investigations in this study revealed that the cuticula gastrica of the gizzard formed by sloughing off the degenerative cells of the surface epithelium into the lumen of the gizzard with secretions of the glandular tubules. It composed of vertical rods and horizontal matrix. These data are in agreement with those of Das and Biswal (1967) regarding duck, (Kadhim *et al*., 2011) regarding red jungle fowl and Aitken (1958) regarding chicken. The current result added, the vertical rods of the cuticle appeared as dentate processes beyond the surface of the mucosa at the older ages. These vertical rods give a great mechanical strength on the cuticle, and the rods act to increase the abrasiveness of the cuticle (Klasing, 1999). The cuticula gastrica of the ventriculus, and particularly the vertical rods, was PAS-positive in all studied age-stages, which was in agreement with Kadhim *et al*. (2011) in red jungle fowl, Sinha and Mrigesh (2018) in Uttara fowl and Zhu (2015) in Black-tailed crake. In contrary to Selvan *et al*. (2008) who stated that the cuticle showed positive reactions for both neutral and acid mucin.

On the other side, by SEM view, the surface cuticle layer of the gizzard appeared as bundles of the rods in laughing dove (Madkour and Mohamed, 2019a), as multiple parallel plates separated by deep grooves in Eurasian collared dove (El-Mansi *et al*., 2021), as finger-like projections in Garganey (Hanafy *et al*., 2020) and composes of raised depositions in kadaknath fowl (Das *et al*., 2013). While, our SEM view has been described the vertical rods of the cuticle as the radiations within the horizontal matrix.

In the present study, the mucosa of the ventriculus in all age-stages was lined by simple columnar epithelium at the top of the ventricular glands, these results agree with that reported by Hanafy *et al*. (2020) and Zhu *et al*. (2013) in Garganey. While it was lined by cuboidal epithelium at the base of the glands like that obtained by Hassan and Moussa (2012) in pigeon. Moreover, the mucosa of the ventriculus was folded as the reports of Jain (1976) in P. krameri and Abumandour (2014) in falcon. This observation contrasts with the findings of Akester (1986) in Galuus and Vittoria and Richetti (1975) in carnivorous and granivorous birds, who found that the mucosa isn't folded.

The gizzard glands were simple tubular glands at the young ages, this finding come in alignment with that reported by Prasad and Kakade (1992) and Hassan and Moussa (2012) in duck, Eidaroos *et al*. (2008) in geese, Bezuidenhout and Van Aswegen (1990) in ostrich, AL-Taai and Hasan (2020) in sturnus vulgaris and Eglitis and Knouff (1962) in chicken. Our results added the type of these glands was compound-branched at the older ages. On the contrary, there are no special glands within the gizzard mucosa in penguins (Olsen *et al*., 2002). The epithelial lining of the simple tubular glands (observed at the young ages) was one type cells (chief cells) low columnar or cuboidal cells while of the compound tubular glands of the ventriculus (observed at the older ages) two type of cells; (chief cells) similar to those seen at the simple tubular glands and (basal cells) flattened cells with oval shaped nuclei. The findings reported at the older ages are somewhat similar to the statement of Hassan and Moussa (2012) in duck and pigeon, Ahmed *et al*. (2011) in quail and Kadhim *et al*. (2011) in red jungle fowl. The latter authors found third type of the cells within the tubular glands combined the structural features of both chief and basal cells. While Madkour and Mohamed (2019a) found only one type of cells (chief cells) in laughing dove and rock pigeon. Some authors observed the endocrine cells within the gizzard (Toner, 1964b; Turk, 1982; Wang *et al*., 2010). The chief cells of the gizzard glands play role in producing a protein rich secretion (King and Mclelland, 1984) while basal cells might supply the carbohydrate component of the secretion in the gizzard glands, and they could be a stem cell of the chief cell (Toner, 1964a).

In the present study as well as Prasad and Kakade (1992) in duck, Zaher *et al*. (2012) in quail, the muscular layer of the ventriculus was represented by thick circularly oriented smooth muscle fibers. Conversely, El-Mansi *et al*. (2021) in Eurasian collared dove, Deka *et al*. (2017) in greater adjutant stork, El Nahla *et al*. (2011) in cattle egret, Batah and Selman (2012) in Fulica atra and Mahdy (2009) in ostrich, stated that this layer arranged in two layers of smooth muscle fibers; inner circular and longitudinal layers. Furthermore, Kadhim *et al*. (2011) in red jungle fowl and Hodges (1974) in fowl, observed three layers of the tunica musculosa; internal oblique, a thick middle circular and longitudinal layers. The current work described the connective tissue septa that surrounding the muscular bundles as "Trawl-like pattern" which become distinct by ageing to increase the power of the muscular contraction during grinding the food. In this concern, fibrocartilagenous plates has been noticed between the muscular bundles of the tunica muscularis of fowl (Verma *et al*., 1999), moreover, Malewitz and Calhoun (1958) demonstrated in turkey cartilaginous tissue at the junction of the smooth muscle and fibrous aponeuroses. Like our findings, Prasad and Kakade (1992) in duck, Gabella (1985) in chicken, Ahmed *et al*. (2011) in quail, the collagenous tendinous layer arranged parallel to each other external to the muscular layer. The collagenous tendinous layer of the studied gizzard formed of the reticular fibers stained brown by Grimelius silver nitrate impregnation, these reticular fibers act as supporting mesh in the ventriculus.

Conclusion

This work highlighted the development of the ventriculus of the post-hatching Muscovy ducks for the first time by gross, morphometry, scanning and transmitting electron microscopy which that reflected with the feeding behavior at the different ages. The weight of the ventriculus is greatly increased by 30 folds at 60days. The type of the gizzard tubular glands and their cells lining is different from the young and older ages.

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Conflict of interest

The authors declare no competing interests

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