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## **Research Article**

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# Developmental Studies of the Gastric Junctions of the Post-hatching Muscovy Duck (*Cairina moschata*)

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# Abstract

The current study was carried out on 24 apparently healthy post-hatching Muscovy ducks of both sexes ranging from 1-60 days old. They were divided into 5 age groups to investigate the development of the junctions of the stomach; esophago-proventricular junction, proventriculogizzard junction (Isthmus gastris) and ventriculo-duodenal junction by using light and scanning electron microscopy. The longitudinal folds of the esophago-proventricular junction were separated by longitudinal grooves and they were crossed by thin transverse ridges; some of the longitudinal folds were branched toward the proventriculus at 60 days old. At one day old, the lymphoid infiltrations were observed in the lamina propria at the esophago-proventricular junction. With the advancement of the age, abundant diffused lymphoid infiltrations and esophageal tonsil were observed. In all age groups, the cuticle covering of the mucosa at proventriculus-gizzard junction increased in thickness toward the gizzard. Moreover, in one day old duckling, the proventricular glands were observed underneath the gizzard tubular glands, these glands were become well developed at 60 days old. The lamina propria of the gizzardduodenal junction showed slight lymphatic infiltrations at one day old age. Lymphatic infiltrations and more lymphatic aggregations were abundant at 7-15 days. While between 30-60 days age, lymph nodules were distinct. Numerous endocrine cells were also observed in mucosa of the gizzard-duodenal junction.

Keywords: Gizzard junction, Muscovy duck, Proventriculus.

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Competing interest: The authors have declared that no competing interest exists.

## Introduction

The wild form of the Muscovy duck is a forest duck, which is indigenous to Central South America and had and been domesticated before the arrival of Columbus. Its name may have been brought to England around 1580 by the Muscovite Trading Company (Appleby et al., 2004). The terminology relating to the anatomy of the avian stomach, the gastric apparatus consisted basically of two chambers, the proventriculus and gizzard. The isthmus (intermediate zone) is a structure between the proventriculus and the gizzard (Farner, 1960; McLelland, 1979, 1993 and Oliveira et al., 2008). In bird, there is no distinct esophageal sphincter and the loss of longitudinal folds is the only differing feature between esophagus and stomach (O'Malley, 2005). In domestic birds, the thinwalled isthmus (intermediate zone) has a and glandless mostly smooth mucous membrane and contains glands only in the duck (Koch, 1973). At the intermediate zone between proventriculus and gizzard, there is an internal lining, secreted by the inner tubular glands, which resemble that of the gizzard in being thicker and firmer than the mucous secretion of the proventriculus although its internal organization is usually less developed than in the gizzard secretion (McLelland, 1979). The pylorus arises from the right face of the ventriculus and connects the ventriculus to the duodenum. It is poorly developed in some species, such as domestic fowl, while and it forms a distinct chamber in aquatic species, such as great cormorant (Langlois, 2003). The ventriculo-duodenal junction forms a well-demarcated zone, easily recognizable because of the light colour of its wall (Larsson et al., 1974). The pylorus is a fold forming a valve between the gizzard and the duodenum, this fold prevents food from gizzard (Harrison re-entering the and Harrison, 1986). The available literature revealed that there is paucity of data on the anatomical details of the gastrointestinal tract especially gastric junctions of Muscovy ducks. Therefore, present investigation was planned to note light and electron microscopic details on post hatching development of various junctions of stomach in Muscovy ducks.

#### Material and methods

Twentv four healthy post-hatching Muscovy ducks (Cairina moschata) of both sexes ranging from 1-60 days old were obtained from local farms in Assiut Governorate were used for this study. They were divided into 5 age groups (viz. 1, 7, 15, 30 and 60 days old). The birds were sacrificed and bled completely, after incising body cavity, the gastrointestinal tract was separated from the body cavity and then stomach was dissected with part of esophagus and duodenum. For SEM, 3 birds of each 1, 15 and 60 days old groups were used. Esophagoproventricular, proventricular-gizzard and gizzard-duodenal junctions were washed for several times in normal saline and acetic acid 2%, then fixed in 4% glutaraldehyde solution for 24 hours, then post fixed in 2% buffered osmium tetraoxide. The fixed samples were washed in 0.1 M cacodylate buffer at pH 7.3, then dehydrated in ascending grades of ethanol, critical point-dried in liquid carbon dioxide, and mounted on metal stubs then coated with gold palladium in sputtering device. Specimens were then examined and photographed using JSM-4500 LV scanning electron microscope operated at 20 KV. For light microscopy, 3 birds of each group were used. The longitudinal sections of the junctions were taken just after sacrificing. washed with saline and then fixed in 10% neutral buffered formalin. After proper fixation, the tissue samples were dehydrated in ascending grades of alcohol, cleared in methyl benzoate and embedded in paraffin wax. Sections of 5µm thickness were taken and stained with Harri's Haematoxylin and Eosin (H&E), Periodic Acid Shiffs technique

(PAS) stains. The sections were examined with a light microscope. All stain techniques were adopted after (Bancroft and Gamble, 2008).

#### Result

The stomach (gaster) in all groups was consisted of two anatomically distinct parts; a small cranial part proventriculus (pars glandularis) and a large caudal part; gizzard (pars muscularis or ventriculus). The junction between two parts of the stomach is called proventricular-gizzard junction (isthmus gastris). The proventriculus was separated from esophagus by esophago-proventricular junction. The ventriculo-duodenal junction (pylorus) was arouse from the right face of the gizzard and connects the gizzard to the duodenum.

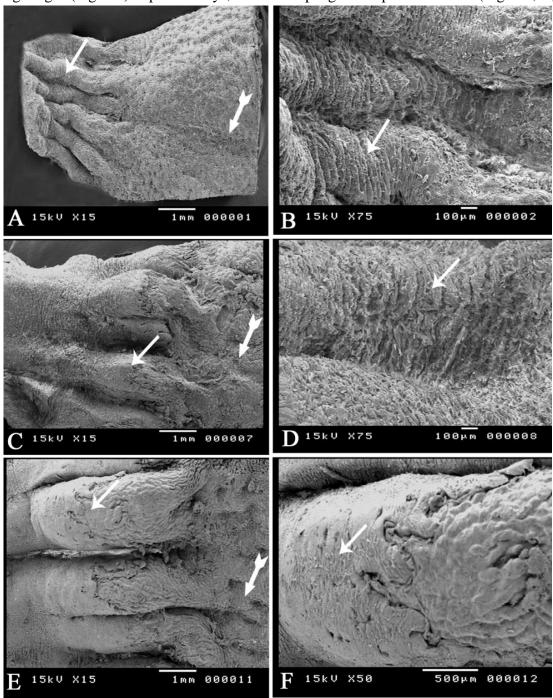
## I- Esophago-proventricular junction:

The scanning electron microscopic examination indicated that at one day, the longitudinal folds of the esophagoproventricular junction were separated by longitudinal grooves and thin transverse ridges, which were disappeared gradually toward the proventriculus, crossed them. With the advancement of the age, these folds became well developed; some of them were branched toward the proventriculus at 60 days old. Moreover, the thickness of the transverse ridges increased in size and they were separated by narrow transverse grooves (Fig. demarcation 1A-F). The between the esophagus and proventriculus could be recognized at one day old, which became clear at 15 days and clearly distinct at 60 days old. In addition, at the latter age, the openings of the esophageal glands were observed at the esophago-proventricular junction (Fig. 2A-C).

By light microscope, many developmental changes could be observed at the esophago-proventricular junction. At one

stratified squamous epithelium day. of esophagus had abruptly transformed to simple columnar epithelium of the proventriculus (Fig. 3A-C). In lamina propria, the number of adenomeres of the proventricular glands was decreased toward the esophago-proventricular junction, and the mucous esophageal glands gradually disappeared (Fig. 3A). In addition, the lymphatic infiltrations were observed in the lamina propria at this junction, they were penetrated by deep invaginations of simple columnar epithelium of the proventriculus wall. These lymphatic infiltrations were extended to the invaginated epithelium, which known as intraepithelial lymphoid was infiltrations (Fig. 3A, B). The muscularis mucosa, at the junction had bundles of circulatory arranged smooth muscle fibers located around the superficial lobules of the proventricular glands (Fig. 3A). The tunica muscularis of the proventriculus continued in the junction and markedly increased in thickness toward the esophageal region (Fig. 3A). At 7 days, the muscularis mucosa of the esophago-proventricular junction continued to grow deeper and occupied the upper portion of the lamina propria, and it was interspersed between the lobules of proventricular glands. At 15 days, abundant diffused lymphoid infiltrations and indistinct lymph nodules or tonsil-like aggregates (termed as esophageal tonsil) were seen in lamina propria, which were indistinctly encapsulated with connective tissue (Fig. 4A, **B**). The continuation of the muscularis mucosa and muscular layer of the proventriculus with the corresponding layers of the esophagus was more distinct at this age (Fig 4A). At 30 days, the diffuse lymphoid infiltrations were increased, and the esophageal tonsils were variable in size and their encapsulated connective tissue encapsulation became more distinct than earlier stages (Fig. 4C). At 60 days, the esophageal mucous glands were observed at the junction between esophagus and proventriculus. Lymphatic nodules above

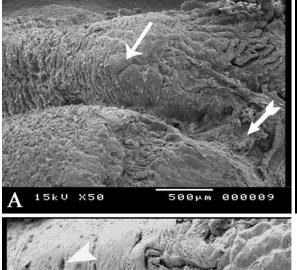
and underneath these glands were recognized. The muscularis mucosa, which was interspersed between the lobules of the proventricular glands, was more distinct than the younger ages (Fig. 4D). Up to 15 days, the muscularis mucosa was increased in thickness at the esophago-proventricular junction toward the esophagus while muscular layer had nearly the same thickness toward both the esophagus and proventriculus (Fig. 4A, C, D).

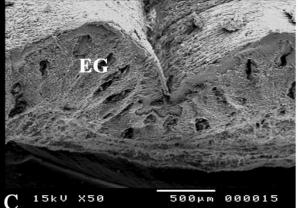


**Fig. 1.** Scanning electron micrographs of the esophago-proventricular junction of 1- (A & B), 15- (C & D) and 60-day-old (E & F) Muscovy ducks. Note; proventriculus (barbed arrow; A, C & E), longitudinal folds (arrow; A-F) crossed by transverse ridges.

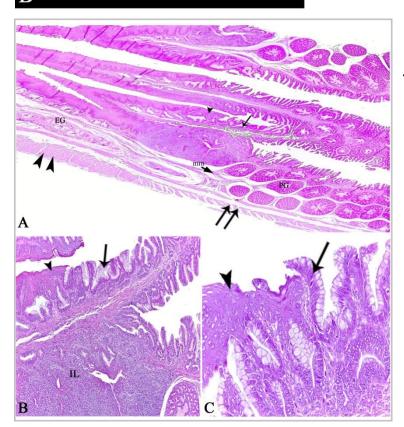
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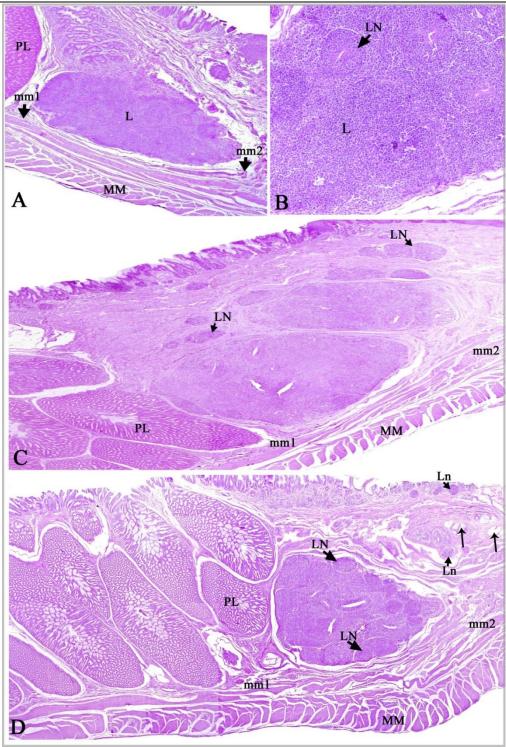
**Fig. 2. Scanning electron micrographs of the esophago-proventricular junction** of 15- (A) and 60-day-old (B, C) Muscovy ducks. Note; demarcation (arrow; A, B) between proventriculus (barbed arrow, A) and esophagus, openings of the esophageal glands (arrowheads; B) and numerous esophageal glands at cross section of the junction (EG; C).



100µm

000013

Fig. 3. Photomicrographs of the esophago-proventricular **junction** of a 1-day-old Muscovy duck stained with H&E (A-C). Note; stratified epithelium squamous of esophagus (arrowhead), simple columnar epithelium of the proventriculus (arrow), proventricular gland (PG), esophageal gland (EG), muscularis mucosa (mm), muscular layer of proventriculus (double arrows) muscular layer and of esophagus (double arrowheads), intraepithelial lymphoid infiltrations (IL). Magnifications; (A) X40, (B) X100 and (C) X1000.



**Fig. 4.** Photomicrographs of the esophago-proventricular junction of 15- (A & B), 30- (C) and 60-day-old (D) Muscovy ducks stained with H&E (A-D). Note; proventricular lobules (PL), diffused lymphoid infiltrations (L), muscularis mucosa of proventriculus (mm1), muscularis mucosa of esophagus (mm2) and muscular layer (MM), indistinct lymph nodule (esophageal tonsil) (LN), esophageal glands (arrows) with lymph nodules (Ln) above and underneath these gland. Magnifications; (A, C & D) X40 and (B) X100.

# *II- Proventricular-gizzard junction:*

Under the scanning electron microscope, the proventricular-gizzard junction was characterized by the presence of few short folds indicating the line of demarcation between the two structures. The cuticle was covering the mucosa of the junction and increased in thickness toward the gizzard. At one day, the proventricular glands were observed underneath the gizzard tubular glands; these glands were well developed at 60 days. In all ages under present study, the muscular layer of the proventricular-gizzard junction increased in thickness toward the gizzard (Fig. 5A-C).

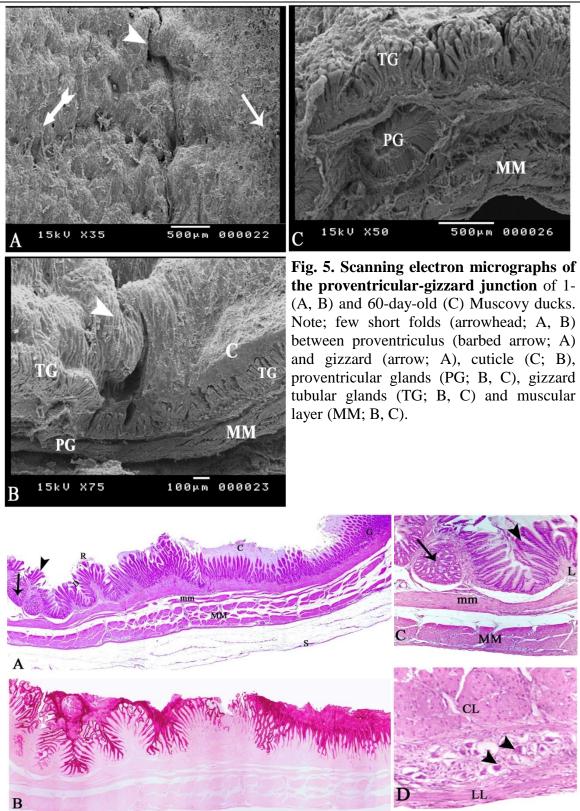
The light microscopy of the proventricular-gizzard junction of one day old one day old duck revealed that the, the wall of the junction between gizzard and proventriculus consisted of: mucosa, musculosa, and serosa, the submucosa was not observed (Fig. 6A). At this junction, the mucosa was folded to form mucosal ridges and deep depressions. The mucosa contained tubular glands of the gizzard and covered by the remaining of cuticle which gradually increased toward the gizzard (Fig. 6A). At the junction between the proventriculus and gizzard, cells of the lamina epithelialis were strong positive for PAS (fig. 6B). Underneath the gizzard tubular glands, the proventricular glands could be observed in the lamina propria which was rich in lymphatic infiltrations (Fig. 6A, C). The muscularis mucosae consisted of longitudinally arranged smooth muscle fibers and was thickened towards the gizzard and merged with the muscular layer of the gizzard (Fig. 6A). The muscular layer at the junction between the proventriculus and gizzard consisted of two layers of smooth muscle fibers; thick inner circular layer which increased in thickness the gizzard, and thin toward outer longitudinal layer which disappeared toward

the gizzard. Blood vessels and aggregations of ganglionic cells were observed between the two layers (Fig. 6C, D). The serosa was mesothelium which was rested on the submesothelial connective tissue layer (Fig. 6A). At 7 and 15 days, there were no distinct changes from the previous age at the junction between proventriculus and gizzard except slight increase in thickness of the muscularis mucosa and muscular layer. At 30 and 60 days, at this junction, the mucosal ridges became more prominent and higher while depressions became much deeper when compared with the previous ages. Moreover, the mucosa was covered by a thicker layer of the cuticle (Fig. 7A, B). At this junction, extensive cellular desquamations of the surface epithelium at this junction were seen towards the proventriculus were seen (Fig. 7D, E). More diffused lymphatic infiltrations were observed in the lamina propria which their penetration and distribution extended to surround the mucosal ridges and depressions. Additionally, abundant lymphatic infiltrations were observed in the gizzard and proventriculus toward the junction (Fig. 7B, C). The muscularis mucosa and tunica muscularis were markedly developed and increased in thickness when compared with those at one day old (Fig.7A).

#### III- Ventriculo-duodenal junction:

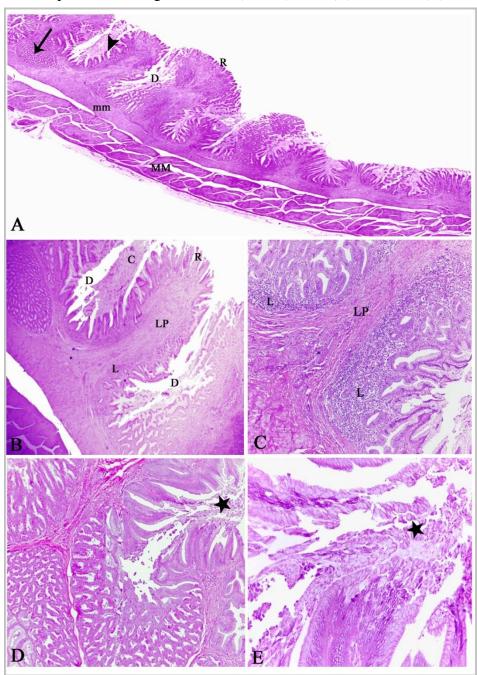
The scanning electron microscopy revealed that at one day the cuticlar thickness at the ventriculo-duodenal junction decreased gradually toward the duodenum. Moreover, the intestinal villi could be recognized. By advancing of the age at 60 days, these villi became longer and, the intestinal glands were observed at their bases (Fig. 8A-B).

Under light microscopy of the gizzardduodenal junction of one day old bird, it was observed that the mucosa of the gizzard was folded, and forming mucosal ridges and depressions near the junction (Fig. 9A).



**Fig. 6. Photomicrographs of the proventricular-gizzard junction** of a 1-day-old Muscovy duck stained with H&E (A, C & D) and PAS (B). Note; mucosal ridges (R), depressions (D) covered by cuticle (C), gizzard (G), gizzard tubular glands (arrowhead), proventricular glands

(arrow), muscularis mucosa (mm), muscular layer (MM) and serosa (S), lymphatic infiltration (L), thick inner circular (CL), thin outer longitudinal (LL) smooth muscle layers and aggregations of ganglionic cells (arrowheads) between 2 layers. In (B) strong PAS reaction for cells of the lamina epithelialis. Magnifications; (A & B) X40, (C) X100 and (D) X400.

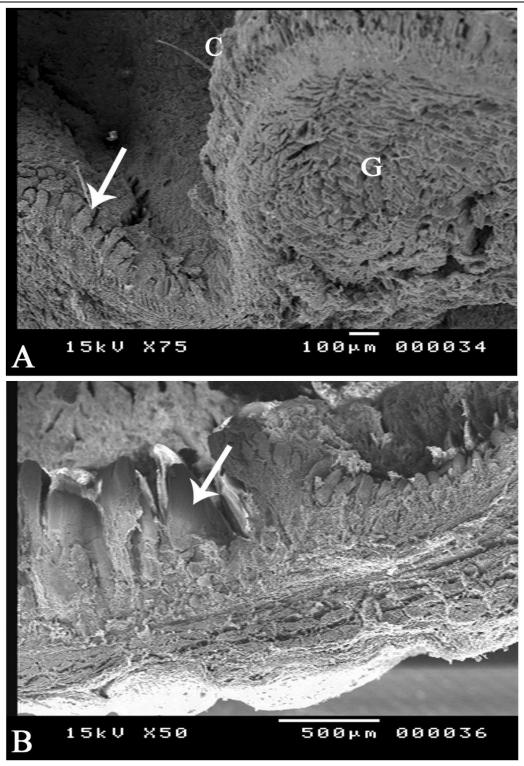


**Fig. 7. Photomicrographs of the proventricular-gizzard junction** of a 60-day-old Muscovy duck stained with H&E (A-E). Note; mucosal ridges (R), depressions (D), gizzard tubular glands (arrowhead), proventricular gland (arrow), muscularis mucosa (mm) and muscular layer (MM), cuticle (C), lamina propria (LP) and lymphatic infiltrations (L), extensive cellular desquamations of the surface epithelium toward the proventriculus (star). Magnifications; (A&B) X40, (C&D) X100 and (E) X400.

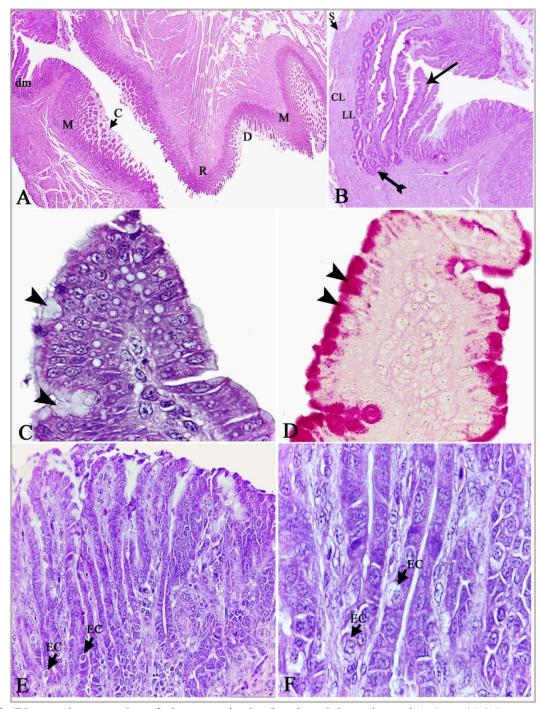
The horny layer covering of the gizzard mucosa was gradually decreased in thickness and only remnants of keratin covering the gastric glands of the gizzard could be observed, which ended abruptly at the beginning of the duodenum (Fig. 9A, B). In the transitional zone, the mucosal layer had been increased in height and the gastric pits also increased in depth as the gizzard glands transformed into long fingerlike projections (intestinal villi). The mucosal glands became shorter and the lining epithelium was columnar cells with rounded basal nuclei and lightly acidophilic cytoplasm (Fig. 9B).

The intestinal villi located at the ventriculo-duodenal junction were lined with intestinal cells however; the tips were covered by mucous secreting cells resembling the surface epithelial covering of the gizzard (Fig. 9C). The mucous secreting cells showed intense reactions for positive substances PAS (Fig. 9D). Intestinal mucous glands were observed at the lamina propria of the intestinal mucosa which were opened at the base of the intestinal villi (Fig. 9B). Numerous endocrine cells were also observed within mucosa of the junction at the terminal part of the gizzard glands. They were oval or elliptical in shape and had a large rounded or oval, eccentrically located nuclei with lightly stained cytoplasm (Fig. 9E, F). Numerous endocrine cells were also observed within mucosa of the junction at the terminal part of the gizzard glands. They were oval or elliptical in shape and had a large rounded or oval, eccentrically nuclei with lightly located stained cytoplasm ((Fig.9E, F).The lamina propria was highly cellular and small lymphatic infiltrations. At the junction between gizzard and duodenum, muscularis mucosa and submucosa were indistinct in all examined age groups.

At this junction, the muscular layer was consisted of circularly arranged smooth muscle fibers towards gizzard. This muscular layer showed a great decrease in thickness toward the duodenum and had been differentiated into two layers of smooth muscles fibers viz. inner thin longitudinal layer and outer thick circular layer. Blood vessels and aggregations of ganglionic cells were observed between two muscular layers of the duodenum (Fig.9B). At the gizzard-duodenal junction serosa mesothelium over a was lined with connective layer with tissue many aggregations of ganglionic cells of the nerve plexus and blood vessels (Fig.9B). At 7-15days, the lamina propria at the junction showed abundant lymphatic infiltrations and more lymphatic aggregations than the previous age. Moreover, the distribution of the lymphatic infiltrations extended to cover the intestinal mucosa and intestinal Branched intestinal villi. villi were demonstrated throughout the intestinal mucosa (Fig. 10A, B). The mucous secreting cells which covered the intestinal mucosa were more observed than those at the previous age and showed mucous substance (Fig. 10C). In further developmental stages at 30-60 days, more diffused lymphatic infiltrations and lymph nodules were observed in the lamina propria. In addition to penetration and distribution of the intraepithelial lymphatic infiltrations throughout the epithelium were increased (Fig. 10D). The gastric pits had been increased in depth gradually towards the ventriculo-duodenal junction (Fig. 10E). At these ages, the muscular layer at the junction between the gizzard and duodenum became well developed than the previous ages.

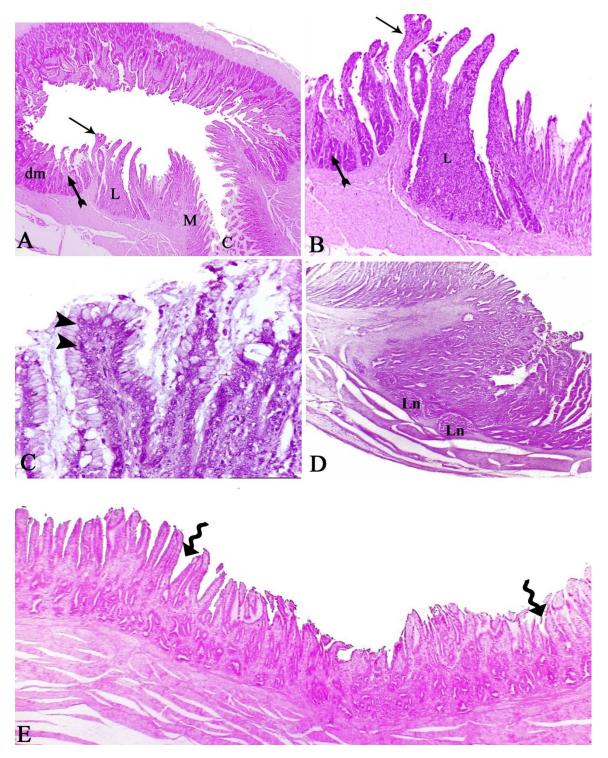


**Fig. 8. Scanning electron micrographs of the ventriculo-duodenal junction** of 1- (A) and 60-day old (B) Muscovy ducks. Note, cuticle (C; A), gizzard (G; A), intestinal villi (arrow; A, B).



**Fig. 9. Photomicrographs of the ventriculo-duodenal junction** of 1-day old Muscovy duck stained with H&E (A, B, C, E &F) and PAS (D). Note; gizzard mucosa (M), mucosal ridges (R) and depressions (D), the cuticle (C) decreased gradually in thickness toward the duodenal mucosa (dm), intestinal villi (arrow), intestinal glands (barbed arrow), thin inner longitudinal (LL) and thick outer circular (CL) of smooth muscle layer and serosa (S), higher magnification of the tip of the intestinal villi covered by mucous secreting cells (arrowheads), numerous endocrine cells (EC) with large rounded or oval eccentrically located nuclei. In (D) intensive

PAS reactions for mucous secreting cells covering intestinal villi (arrowheads). Magnifications; (A) X40, (B) X100, (C, D & F) X1000 and (E) X400.



**Fig. 10. Photomicrographs of the ventriculo-duodenal junction:** of 7- (A-C) and 60-day-old (D-E) Muscovy ducks stained with H&E (A-E). Note; the cuticle (C), the gizzard mucosa (M), duodenal mucosa (dm), lymphatic infiltrations (L) distributed to intestinal villi (arrow),

intestinal glands (barbed arrow), mucous secreting cells which covered the intestinal villi (arrowheads), lymph nodules (Ln) were observed at the lamina propria, gastric pits (barbed arrows). Magnifications;(A) X40, (B, D & E) X100 and (C) X400.

## Discussion

scanning electron microscopic The examination esophago-proventricular of junction in one day old bird revealed that the separated longitudinal folds were bv longitudinal grooves and they were crossed by thin transverse ridges which disappeared gradually toward the proventriculus. With the advancement of the age, these folds became well developed; some of them were branched toward the proventriculus at 60 days age. Hassan and Moussa (2012) mentioned that there was a strong sphincter between esophagus and proventriculus in duck. On the other hand, O'Malley (2005) did not observe any distinct esophageal sphincter and the longitudinal folds were lost at proventriculus in ostrich. Abumandour (2013) reported that the interior of proventriculus was dark brown in color in falcon and it had well-developed muscular folds, while the esophagus was whitish and folded. The present observations showed that stratified squamous epithelium of esophagus abruptly transformed to simple type epithelium of the proventriculus at the esophago-proventricular junction, which was in agreement with Malewitz and Calhoun (1958) in turkey; Klem et al. (1982) in house sparrow; Olsen et al. (2002) in penguins; and Kadhim et al. (2011) in red jungle fowl. Klem et al. (1982) added that the esophagoproventricular junction had a short zone of overlap in which the esophageal glands overlie the lobes of the proventricular glands. The lymphoid infiltrations were observed in lamina propria esophagothe at the proventricular junction of one day old bird and with the advancement of the age, abundant diffused lymphoid infiltrations and lymph nodules (tonsil-like aggregated) called esophageal tonsil were observed. These results were supported by reports of McLelland (1975) in duck and goose;

Malewitz and Calhoun (1958) in turkey. However, Nickel et al. (1977) stated that the esophageal tonsil was prominent in duck, but less well developed in fowl. In addition to the latter authors, Klem et al. (1982) mentioned that the lymphatic tissue occurs in distinct foci surrounded by fibrous connective tissue at esophago-proventricular junction (lymphy noduli esophageales) in house sparrow; Kadhim et al. (2011) stated that the lamina propria at this junction showed many mucous glands with lymphatic infiltration in red jungle fowl. Sagsoz and Liman (2009) quoted that the lymphoid tissues were seen at the distal end of the folds of esophagus, at the esophago-proventricular junction in 5day-old quail.

The proventricular-gizzard iunction (Isthmus gastris) was characterized by presence of few short folds indicating the line of demarcation between the two structures. In falcon, the differentiation between the proventriculus and gizzard by internal structure of them; the proventriculus was characterized by the extensive folds while the gizzard by presence of low gastric folds (Abumandour, 2013). This result was in contrast with the reports made by McLelland (1990) in chicken who stated that the internal surface of the proventricular-gizzard junction was relatively smooth. Similarly, Hassan and Moussa (2012) in duck, Ibrahim (1992) in heron and jackdaw, Bezuidenhout (1986) in ostrich, and Hamdi et al. (2013) in black winged kite also mentioned that, there was no isthmus gastris between the two parts of the stomach.

The junction between gizzard and duodenum showed different morphology from the gizzard and the duodenum. It was distinguished from the gizzard by villous character of the mucosa and decreased length of the gizzard glands and thickness of the keratinized layer which were stopped abruptly at the beginning of the duodenum. It could be distinguished from the duodenum by epithelial cells covering of the mucosa were mucous secreting and absence of muscularis mucosa. These findings somewhat simulate the statement of Ahmed et al. (2011) in quail and Aitken (1958) in chicken.

The histological investigation showed that at one day, the lamina propria at junction between gizzard and duodenum was highly cellular and showed lightly lymphatic infiltrations at one day. At 7-15days age, lymphatic infiltrations abundant and lymphatic aggregations were observed. The junction demonstrated more diffused lymphoid infiltrations and lymph nodules at 30-60days. These findings were in agreement with the description of Ahmed et al. (2011) in quail at 1, 15 and 45days old and of Malewitz and Calhoun (1958) in turkey.

As shown in the present work, numerous endocrine cells were observed within mucosa at the terminal part of the gizzard glands at the junction. In relation with this point, there were several types of endocrine cells at ventriculo-duodenal junction between avian species; five types in duck (Okamoto and Fujii, 1980) and in quail (Yamada et al., 1978); three types in chicken (Larsson et al., 1974) and seven types (Andrew, 1976 a, b) in chicks. Okamoto et al. (1976) mentioned in duck that, the endocrine cells distributed in the whole digestive tract, and described that the frequency of appearance of endocrine cells was the highest in the pyloric region (ventriculo-duodenal junction). On the other hand, no endocrine cells were seen at the junction between the gizzard and the duodenum in quail (Ahmed et al., 2011).

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