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Anatomy and Histology of Muscular Stomach (Gizzard) in Iraqi Wild Bird Laughing Dove (Streptopelia Senegalensis L.)

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Summary

A lot of research that related to an Iraqi wild bird was interested in the physiological and ecological side, but the histological sides of wild birds did not receive much attention in the country, particularly with regards to the histological differences of the digestive tract specially stomach in the different wild species, therefore, the work amid To provide a morphological description of the stomach (muscular portion) Iraqi wild grainivorous birds, Laughing dove, *Streptopelia senegalensis*, also to link microscopic findings to the morphology or anatomy and formulate postulations for function .

Ten healthy birds with (75.6-97.00) gm in weight without interest to sex (male and female) were obtained by capturing from different regions of Wasit province. Birds were divided randomly into two groups for anatomical and histological studies, five birds for each group. All birds were anesthetized and opened at abdomen area carefully, then the stomach was separated from its attachments. Gross descriptions of muscular stomach and calculation of it's weight, and the ratio of the stomach weight to body weight were recorded.

Anatomical study referred that the stomach of the studied bird consisted of two distinguishable chambers; a glandular region, the proventriculus and muscular region, ventriculus or gizzard. Appeared as a disc form structure, or approximately spherical in shape and it was easy to distinguish proventriculus and gizzard by presence a constriction between them. It was located on the left of the midline plane after the proventriculus and covered partially by the left lobe of liver. The internal lining of the gizzard was covered by a cuticle layer greenish in color and the gizzard also contains grit and gravel particulates.

Microscopically examination of the gizzard revealed that the wall of muscular stomach of studied bird was lining by simple columnar epithelium and its wall consisted of four tunics differ in their thickness, according to histological measurments, tunica muscularis was thickness than other tunica, where the rate of thickness (1314.5 ± 268, 6). Tunica mucosa of gizzard was invaginated into the lamina propria forming gizzard pits or gastric pits. It contains glands lining with short columnar cells which represent chief cells.

Tunica muscularis is muscle fibers, arranged into two layers, an outer longitudinal, and an inner circular. Tunica serosa is loose connective tissue carried the blood vessels, lymphatic vessels, nerves and adipose tissue. The connective tissue is surrounded by mesothelial cells.

Key words: Laughing Dove, Streptopelia senegalensis L., Gizzard, Histological,

الخلاصة

كثير من الابحاث المتعلقة بالطيور البرية في العراق ركزت على الجانب الوظيفي والبيئي بدون ان تبدي اهتمام للجانب النسجي باستثناء القليل منها في القطر، وخصوصا فيما يتعلق بالاختلافات النسجية للقناة الهضمية ومنها المعدة العضلية (القانصة) لذا هدفت الدراسة الى تسليط الضوء على الوصف التشريحي للمعدة العضلية في طائر فاختة النخيل او ما يسمى بالحمام او اليمام الضاحك وربطه بالجانب المجهري وما يتعلق بوظيفته.

تضمنت الدراسة عشرة طيور ذات أوزان تتراوح ما بين (75.6-97.00) غم جمعت بغض النظر عن للجنس (ذكور وإناث) تم الحصول عليها بصيدها من مناطق مختلفة من محافظة واسط. تم تقسيم الطيور عشوائيا إلى مجموعتين للدراستين التشريحية والنسيجية تضمنت خمس طيور لكل مجموعة. خدرت جميع الطيور وفتحت في منطقة البطن بعناية بعدها فصلت المعدة عن ملحقاتها وسجل تسجيل الأوصاف العيانية للمعدة العضلية وحساب الوزن الكلي، ونسبة وزن المعدة إلى وزن الجسم.

أشارت الدراسة التشريحية إلى أن المعدة في طائر الفاختة تتكون من غرفتين او ردهتين متمايزة، منطقة غدية ومنطقة عضلية (قانصة) تظهر بشكل قرصي او دائرية تقريبا ومن السهل التمييز بين المنطقين بواسطة تخصر يفصل بينهما. تقع القانصة خلف الخط الوسطي بعد المعدة الغدية وتكون مغطاة جزئيا بواسطة الفص الايسر للكبد، اظهرت نتائج الفحص العياني وجود طبقة خضراء من الكيوتكل تبطن التجويف البطني للقانصة وتكون حاوية ايضا على حصى ودقائقيات من الطين.

مجهريا أشارت الفحوصات الى ان جدار المعدة العضلية للطائر يبطن بطلائية عمودية بسيطة حيث تكونت بطانة الجدار من الطبقات الاربعة الاساسية التي تتكون منها المعدة النموذجية مختلفة في السمك، حسب القياسات النسجية ظهرت الطبقة العضلية الخارجية اسمك الطبقات الاربعة بلغ معدل سمكها (1314.5 ± 268,6) تكون الطبقة الاولى المخاطية من انبعاجات داخل الصفيحة الاصيلة مكونة نقر او ما يسمى بالحفر المعدية، تظهر حاوية على غدد مبطنة بخلايا عمودية قصيرة تمثل الخلايا الرئيسة.

اما الطبقة العضلية فتكونت من مجموعة من الالياف العضلية مرتبة بشكل طبقتين خارجية اليافها مرتبة بصورة طولية وداخلية ترتبت اليافها بصورة دائرية. فيما يخص الطبقة المصلية فظهرت مكونة من نسيج ضام رخو يضم أوعية دموية ولمفية بالإضافة الى الاعصاب ونسيج دهني، يحاط نسيجها الضام بخلايا ميزوثيلية.

Introduction

Organ functional capacities may limit sustained metabolic rate because they act as the metabolic machinery that supports maintenance metabolism, production, reproduction, and activity (1). Birds consume more food in proportion to their size than most animal due to the high metabolic rates (2). The extent to which an animal can utilize food energy depends largely on the anatomical and physiological properties of the digestive tract (3).

Digestive system and gut morphology show considerable variation among animals, depending on phylogenetic, diet quality, the size of the animal and differing environmental pressures. The avian digestive system has a larger number of organs, which have greater interorgan cooperation, than do mammals (4). Modifications to the general avian digestive tract may occur as an adaptation to diet (5).

The most active part of the digestive system of birds is a stomach which is formed of two distant parts; the glandular portion, proventriculus and the muscular portion ventriculus or gizzard which is located caudal to the proventriculus (6). Histologically the wall of the stomach (proventriculus and ventriculus) is constituted by the following layers: tunica mucosa,

submucosa, muscular and tunica serosa (7).

Proventriculus presents an elliptical shape, which is a relatively thick-walled structure located at the distal end of the esophagus and containing both simple mucous-secreting glands and compound submucosal tubuloalveolar glands. The gizzard is a thick muscular bulb, the mucosa of which contains simple glandular cells which secrete a tough, horny layer (8).

The glandular stomach (proventriculus) varies in size between species, being relatively small in graminivores, but often quite large and distensible in carnivores that ingest large food items and in ostriches which use it for water storage, In most species, however, food passes rapidly through the glandular stomach and is held in the muscular stomach where in the actions of the gastric secretions (pepsin, HCL, and mucus) occur (9).

The muscular stomach is complex in most species and consists of two pairs of opposing muscles (10). Ventriculus lined on its mucosal surface by a cuticle layer, the koilin, a keratine like protein (11) which serves as a grinding surface, enabling the ventriculus to mechanically digest food (12).

Gizzard functions to grind up the food with the aid of gastroliths, as the birds typically do not have teeth specialized for chewing and grinding. Whereas the gizzard may compensate for the lack of teeth in birds, which still functions as a region of storage and digestion of proteins (13).

The Laughing dove, *S. senegalensis*, belongs to family Columbidae (14). This family contain fruit or grain eating birds of forest, woodland or grassland. This species has a distribution that extends across Africa (15). It's also found in India, Afghanistan and found locally in Palestine, Syria, Lebanon (16), Emirates (17), Egypt (18). In Iraq laughing dove was a very rare species until 1960 then become common, through years 1970, 1980, 1990 and 2005, Lahony *et al*., (2008) record presence of this dove in Sulaimaniyah, Kirkuk, Baaqoba and Baghdad. This dove was coming from Iran toward north east of Iraq and from this point it was spread all over the country (19).

Materials and Methods

The Experimental Animals

Ten healthy birds (*S. senegalensis*) were captured from different regions of Wasit province and kept in individual cages, for a short period 3-5 days (20). They were fed with grains similar to those existent in the area of capture. Five birds were chosen from (male and female) in weights (75.6-97.0) gm. Then all birds were anesthetized by chloroform inhalation, and left for (2-5) minutes to ensure fully drugged to prepare for anatomical and histological studies.

Anatomical Study

Five birds were used for anatomical clarifications include: body weight, stomach weight and position of the stomach. Both body and stomach weight were recorded using sensitive balance. The body weight was recorded before the birds were sacrificed. Each bird anesthetized by chloroform inhalation. Then the bird was killed and fasted in anatomy plate by fixing pins in its four limits to obtain a good position for anatomical work. The stomach weighted after it's removing from its location.

Histological Study

After birds anesthetized they were sacrificed. From each bird, the stomach

was removed, parts of ventriculus were catted fixed in two different fixatives, formalin and Bouin's fluid for 18-24 hours. Then dehydrated in an increased concentration of ethyl alcohol until absolute alcohol, to clearing, infiltration and embedding in paraffin wax, then catted using rotary microtome, sectioned at (5-6 µm) thickness. Cross sections were made and other Longitudinal. Then sections adhesive on slides by using Mayer's glycerol albumin. Sections were stained with Mayer's hematoxylin and alcoholic eosin for general examination, periodic acid Schiff (PAS) for identification of acidic mucous and Masson's Trichrome Stain for detection of connective tissue.

Microscopic Examination and Histological Measurements

Tissue sections of ventriculus were examined using different magnifications power of Microscope, different histological parameters were used to study the structures of stomach wall in the studied bird. Using the ocular micrometer, parameters reading were taken include thickness of the four gizzard layers, diameter of submucosal glands.

Statistical Analysis

The data of present work were analyzed by SPSS 12.0, Statistical analysis was obtained standard error.

Results

Anatomical Findings

The anatomical results showed that the stomach of laughing dove was appeared as a muscular organ distinct into two parts, glandular elongated, spindle-shaped organ with thick walls represents the proventriculus or true stomach, and it lies in the left ventral part of the body cavity between the esophagus and the gizzard and covered by the left lob of liver. (Figs. 1 and 2). There is no distinct macroscopic boundary between the lower esophagus and the beginning of the proventriculus, while the posterior extension of the proventriculus is a very short portion represents the isthmus, which is the intermediate zone between the proventriculus and the gizzard. (Fig. 3). A muscular portion of the stomach, the ventriculus or gizzard that appeared as a disc form structure, or approximately spherical in shape (Fig. 2), it was easy to distinguish proventriculus and gizzard by presence a constriction between them (Fig. 3). It was located on the left of the midline plane after the proventriculus and covered partially by the left lobe of liver. The internal lining of the gizzard was covered by a cuticle layer greenish in color and the gizzard also contains grit and gravel particulates (Fig. 4).

Weight of the Stomach organ

The study revealed that the mean of body weight of bird was about (89.55 ± 1.15 gm), while the mean of organ weight was about (3.32 ± 0.12) gm. and the ratio of the organ weight to the body weight was about (3.7 %) (Table 1).

The Histological Study

The microscopic examination of stomach in laughing dove bird showed that presence of junction between proventriculus and gizzard, at this area, the proventricular glands terminated abruptly. The histological examination showed that the histological structure of gizzard wall in laughing dove consists of four tunics: mucosa, submucosa, muscularis and serosa (Fig. 4).

Tunica Mucosa

Tunica mucosa was invaginated into the lamina propria forming

different size of gizzard pits or gastric pits (Figs. 6 and 7). The epithelium was simple columnar and continuous within the simple straight tubular mucosal glands that found in the lamina propria (Fig. 8). The lamina propria appeared as loose connective tissue while the muscularis mucosa appeared as a band of smooth muscle fibers extends between the mucosal glands (Figs. 7 and 8). The histological measurements showed that the total thickness of this layer without koilin was about (244.6 µm), (Table 2).

Tunica submucosa

This layer consisted of loose connective tissue, containing blood vessels, lymphatic vessels and nerves (Figs. 5, 6 and 7). The thickness of this tunica was about (97.8 µm), (Table 2).

Tunica Muscularis Externa

This tunica was smooth muscle fibers, arranged into two layers, an outer longitudinal layer, and an inner circular layer (Figs. 7, 9, 10 and 11). The total thickness of this layer was about (1280.6 µm), (Table 2).

Tunica serosa

This tunica was loose connective tissue carried the blood vessels, lymphatic vessels and nerves. The connective tissue was surrounded by mesothelial cells (Figs. 10 and 11). The thickness of this layer was about (178.8 µm), (Table 2).

Discussion

The Anatomical Study

The anatomical results of the present work revealed that the stomach in laughing dove bird was a muscular organ distinct into two parts: glandular part, proventriculus and muscular part, ventriculus. These two parts were connected by narrow portion represents isthmus as mentioned by Turk, (1982) in *Gullus gullus* bird and *Gullus domesticus*, Bailey *et al*., (1997) in different type of captive bustards, Duke et al., (1997) in *Falco sparverius* which is American carnivorous bird also Rossi *et al*., (2005) in *Rhynchotus rufescens* which is omnivorous bird.

Muscular portion of the stomach, the ventriculus or gizzard in laughing dove appeared as a disc form structure, or approximately spherical in shape, with two a pair of powerful muscles, This finding was accepted with Rossi *et al*., (2005) on *Rhynchotus rufescens*; Klasing, (1998); Sturkie, (1986).

Results showed that the dove have a well developing muscular stomach, gizzard that enclosed by a pair of thick and thin smooth muscles and the internal lining of the gizzard was covered by a greenish cuticle layer and contains grit and gravel particulates. This findings were confirmed by the previous studies done by Sturkie, (1986): Klasing, (1998) and Rossi *et al*.,(2005) on *Rhynchotus rufescens*.

The presence of powerful muscles in laughing dove is necessary because this bird swallowed the seeds without breaking it, so the developing gizzard and its hardening lining with the aid of grit particles working to break up and grinding of grains as well as mixing food with pepsin enzyme secreted from the proventriculus, while Hrabar and Perrin, (2002) referred that in Agapornis Fischer and *Padda oryzivora* which are granivorous Parrots that peeling and breaking grains with their peaks before ingested. These parrots possess a small and less developed gizzard as compared with the birds that don’t peeling grains.

The present work showed that it was easy to distinguish the proventriculus from the gizzard in laughing dove bird through the clear distinction between the two parts of stomach while in white breasted kingfisher the distinction between the two parts was difficult to determine. This finding was agreed with Pesek, (1999) and Konishi, (2001) who noticed that in meat eaters' birds, the distinction between the proventriculus and ventriculus is difficult to determine, while it is easy to determine in seed eaters.

The present findings showed that the gizzard in bird was located as unilateral, to the left side of intestinal peritoneal cavity, behind the proventriculus, and found close to the median plane, partially between the two lobes of liver, this finding is in agreement with Dyce et al., (2002) in avian.

Also our findings certain by what King and Mclelland, (1975) in carnivores that the gizzard which extremely variable in its muscularity dependent on the type of diet, The muscles tend to be well developed in graminivores and herbivores e.g. domestic fowl, while in carnivore's birds such as a hawk the gizzard tends to be a thin walled bag. The development in muscularity in the wall of gizzard could be concerned to the function of gizzard in these birds. This finding pointed by King and Mclelland, (1975) who mentioned that the main function of the muscular stomach in graminivores and herbivorous species is to triturate the food in preparation for gastric proteolysis. This is achieved by powerful asymmetrical contractions of the muscles. In other species the muscular compartment plays a much less important role in the physical digestion of food and in carnivorous at least it functions mainly as a storage organ where the gastric juice can act.

Histological Study

The analysis of histological sections, under light microscopy, showed that the wall of the ventriculus of bird was consisted of four layers: tunica mucosa, submucosa, tunica muscularis and tunica serosa, the same finding was observed by many previous studies done by Hodges (1974) on fowl; Rossi et al., (2005) on *Rhynchotus rufescens* and Illanes *et al*., (2006) on *Struthio camelus*. In addition to the four layers, an internal secretory lining layer called koilin, found above the mucosa, this finding was accepted with Selven *et al*., (2008) on Guinea fowl.

The surface lining of the tunica mucosa in studied bird was simple columnar epithelium. This mucosal epithelium form a finger like projections refer to plicae that is Parallel with each other and restrict between them gastric pits that extended more of the half of tunica mucosa as mentioned by Rocha and Lima, (1998). The pits extend along the glandular layer. This finding was in agreement with the previous results of Akaster, (1986) and Hodges, (1974) who mentioned that the lamina propria is rather obscured by the glandular tubules which penetrate down through it's thickness and terminate at the level of the sub mucosa.

The present histological examination revealed that the luminal surface was lined with secretory product of the mucosal glands, which solidifies at the surface to form a hard cuticle of koilin, which is made from both carbohydrates and proteins secreted from the inner epithelial cells of the gizzard. This finding was mentioned by King and Mclelland ,(1984) who said that the gizzard is lined with a keratin- like layer of koilin, known as the cutica gastrica. This layer shows great variation, and is most elaborate in graminivorous and herbivorous. This finding was in

agreement with Konishi, (2001), who mentioned that in the bird species with thin sac- like gizzards, the cuticle is present but softer in composition. On the other hand the present study was in disagreement with Al-A'araji, (2007) and Alsheshani, (2006) who mentioned that the koilin layer did not found covering the epithelium of gizzard in kestrel and Accipiter nisus. This thick layer of the gizzard is concerned with grinding the food, but also it may be protect the underlying tissues from the chemical and mechanical abrasions.

This work was agreed with Calhoun (1954) and Banks (1993) who mentioned that the lamina propria composed of loose connective tissue, and contained blood vessels lymphatic vessels, nerve, and glands.

Catroxo *et al*., (1997) and Eglitis and Knouff (1962) mentioned that there was no muscularis mucosa in the gizzard of red-capped cardinal and chicken. Which disagreement with present findings. On the other hand, Rocha and Lima, (1998) referred that there is a development muscularis mucosa in the gizzard of *Speotyto cunicularia*, Cooper and Mahroze, (2004) who mentioned the same in the gizzard of ostrich.

Tunica muscularis externa presented some variation regarding the number and the arrangement of its layers. The present findings showed that studied bird, this tunic consisted of two muscular layers: inner circular layer and outer longitudinal layer of muscular fibers. This result was agreed with DeSperoni and Chikilian, (1996) in three species of Tinamous bird, Hodges, (1974) in chickens and Jain, (1976) on frugivorous, carnivorous and omnivorous species of birds, While it is in disagreement with Espinola and Galliussi (1990); Ahmed *et al*., (2011) and Kadhim (2011) who mentioned that in addition to these layers, oblique muscle fibers formed the most internal layer of the tunica muscularis in the ventriculus of red jungle fowl.

There are great variations as far as the development of the muscle tunic among the kinds of birds. Akester, (1986) stated that in graminivores birds, the gizzard presents much developed muscles than carnivores and frugivores. Singanallur et al., (1976) stated that in Pelecanus phillippensis (piscivorous bird) the well-developed and thick musculature of gizzard associated with the function of triturating and crushing of the body of the fish.

Tunica serosa was consisted of loose connective tissue lined by mesothelium, containing blood vessels, nerve elements of the serous plexus and adipose cells in both studied birds as mentioned by Catroxo *et al*., (1997) and Rocha; De Lima (1998).

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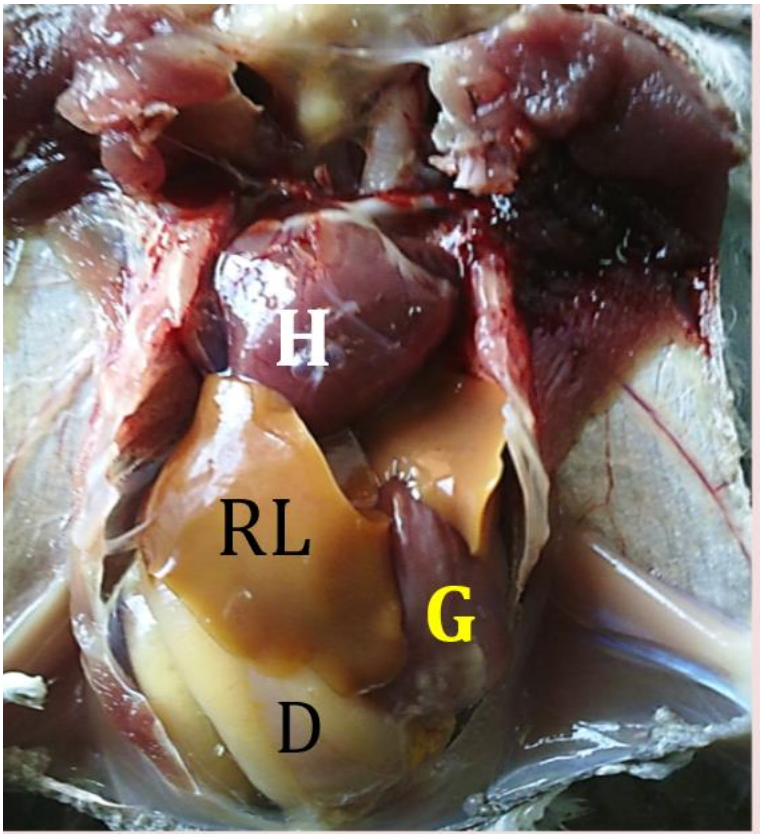
Table (1): Shows the body weight (gm.), the gizzard weight (gm.), and the ratio of gizzard weight / body weight in laughing dove.

Bird	Body Weight (Mean ± SE)	Stomach weight (Mean± SE)	Ratio of Stomach Weight/ Body Weight
Laughing dove	89.55 ± 1.15	3.32 ± 0.12	3.70 %

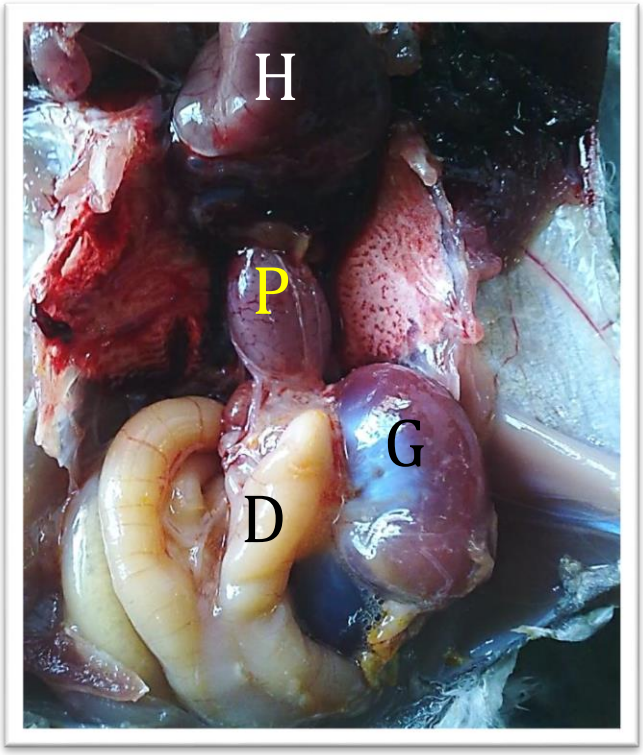
Table (2): Shows the thickness of the four tunics of the gizzard wall (micrometers) in laughing dove.

Bird	mucosa (Mean ± SE)	Submucosa (mean ± SE)	Muscularis externa (mean ± SE)	Serosa (mean ± SE)
Laughing dove	244.6 ± 38.0	97.8 ± 21.2	1314.5 ±268.6	178.8 ± 24.2

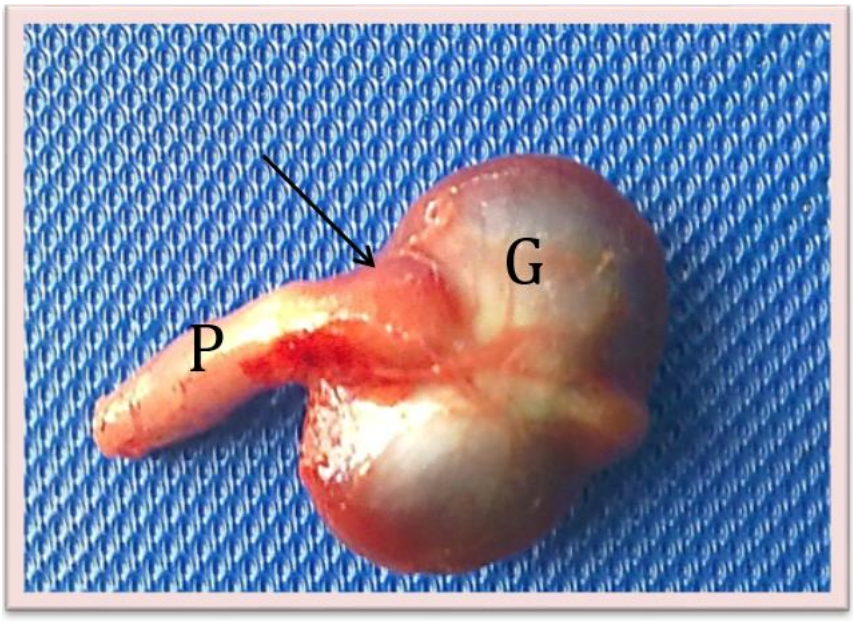
SE: Standard error , n= 10



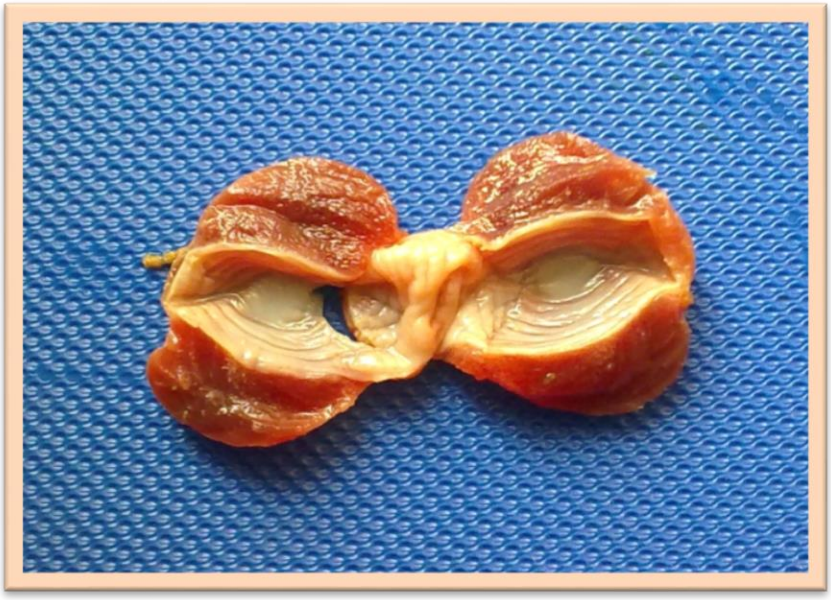
(Fig. 1): Ventral view of the thorax and abdomen of the laughing dove bird shows the heart (H), Gizzard (G), Duodenum (D),Left lobe of liver (LL), Right lobe of liver (RL).



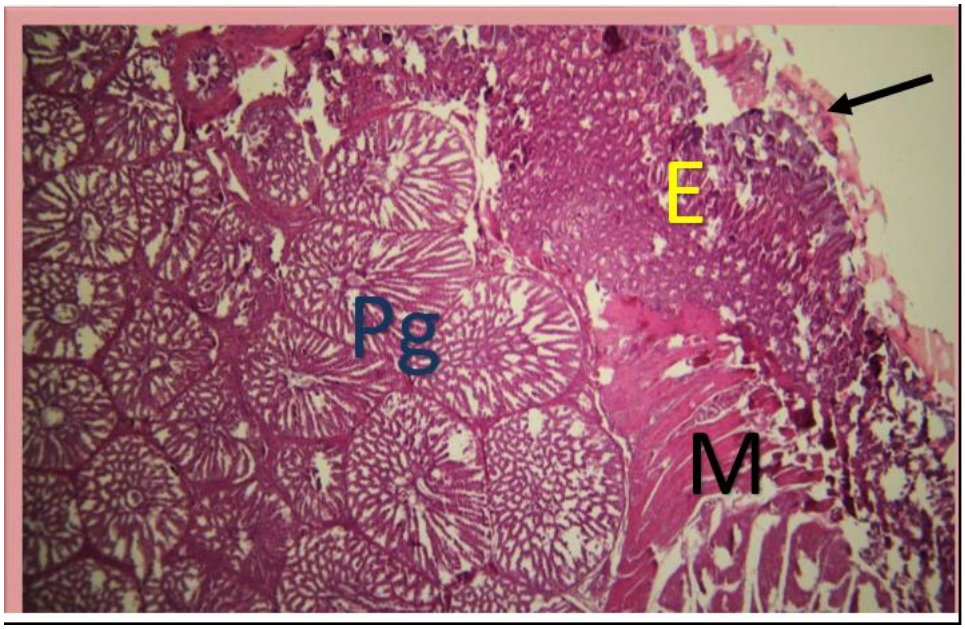
(Fig. 2): Shows the shape and position of stomach in laughing dove, Heart (H), Proventriculus (P), Gizzard (G), Duodenum (D)



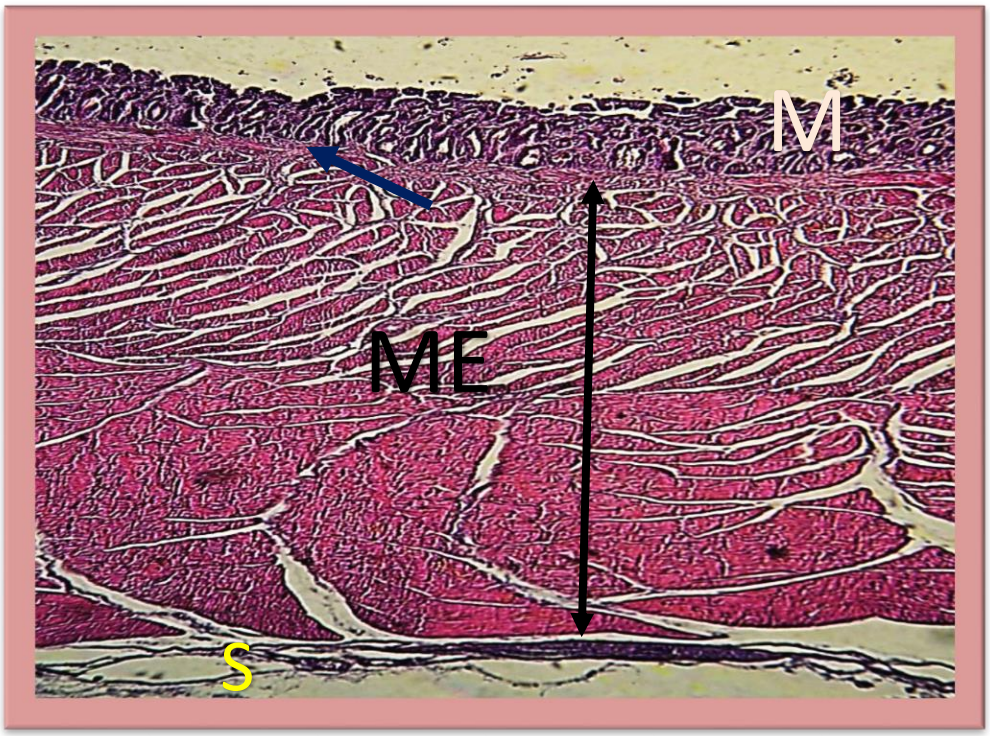
(Fig. 3): Portions of stomach in laughing dove show, Proventriculus (P) and Gizzard (G), isthmus (Arrow).



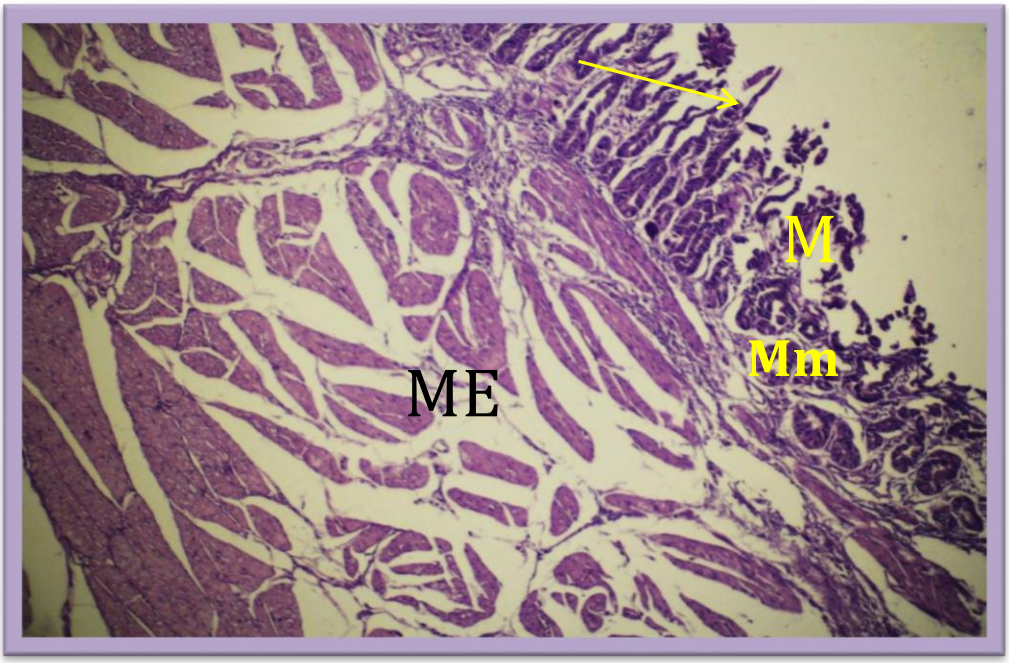
(Fig. 4): The internal surface of gizzard with removed koilin in laughing dove.



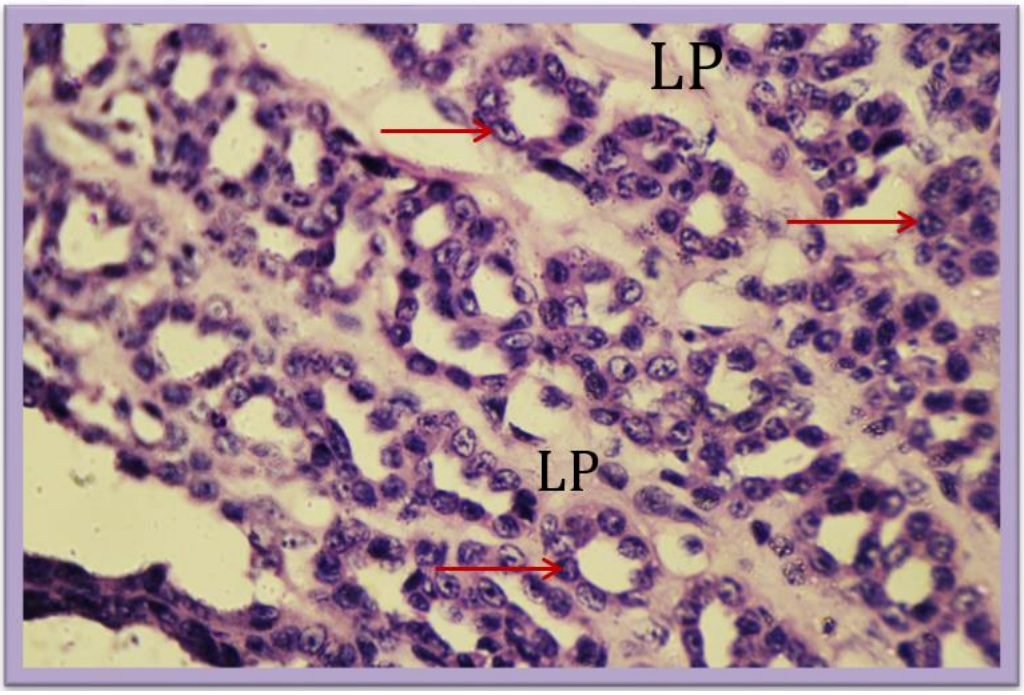
(Fig. 5): Photomicrography of the proventriculus-gizzard junction in laughing dove shows Proventriculus glands (Pg), Muscular layer (M), Epithelial lining (E), Keratinized layer (Arrow). (H&E stain, 40X)



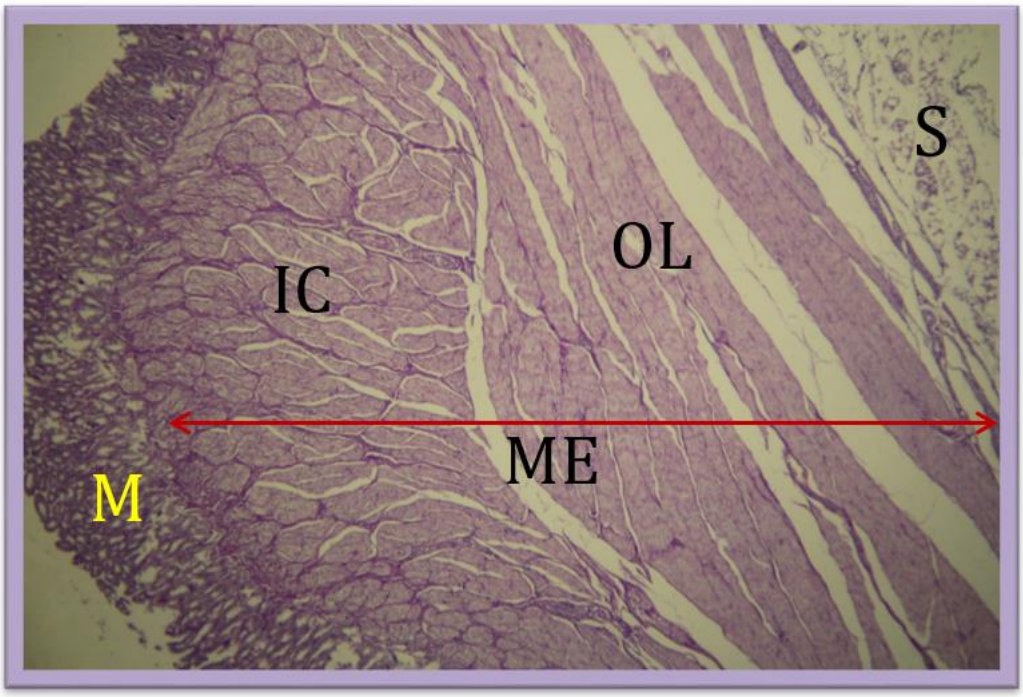
(Fig. 6): Photomicrography of the Gizzard wall in laughing dove shows the mucosa layer (M), muscularis externa (ME), Serosa (S) (H&E stain, 40X).



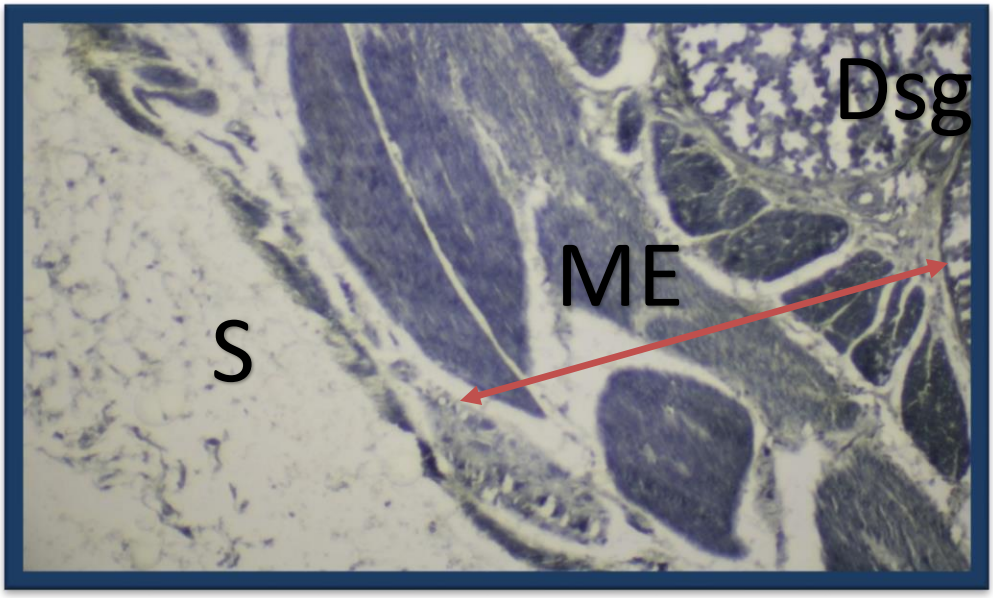
(Fig.7): Photomicrography of the Gizzard wall shows the mucosa layer (M), muscularis mucosa (Sm), muscularis externa (ME), gastric pits (arrow) (PAS stain, 200X).



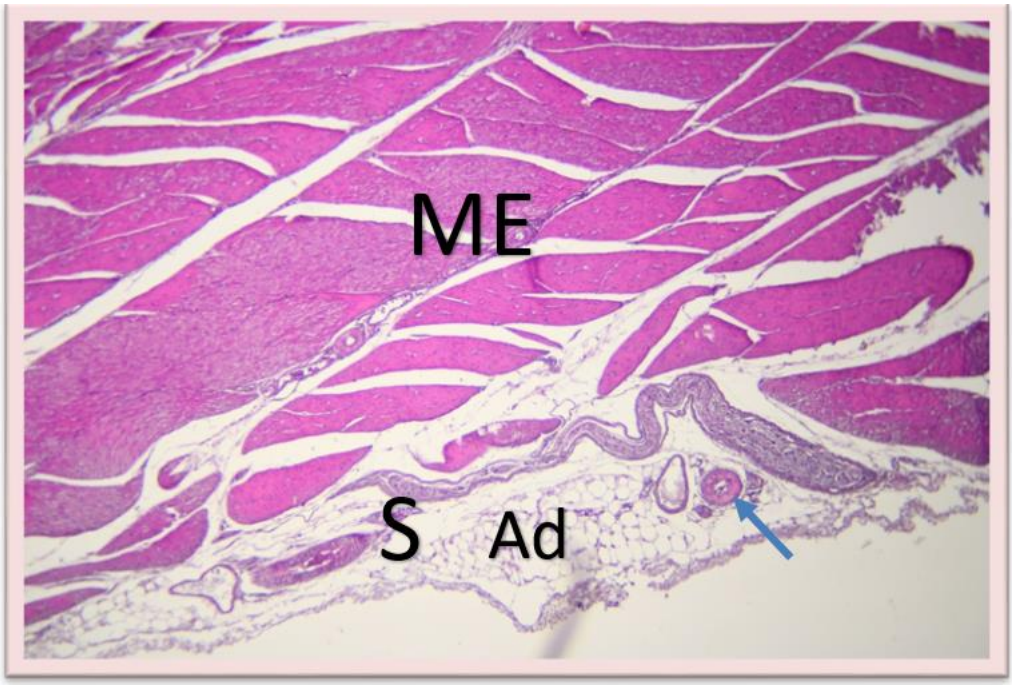
(Fig. 8): Photomicrography of the mucosa in laughing dove gizzard shows, Lamina propria (LP), Simple gastric gland (Arrow), (H&E stain, 1000X)



(Fig. 9) : Longitudinal section of the gizzard wall in laughing dove shows mucosa layer (M), muscularis externa (EX), inner circular layer (IC), outer longitudinal layer (OL), serosa (S), (PAS stain, 100X)



(Fig. 10): Photomicrography of gizzard wall shows deep submucosal gland (Dsg), muscularis externa (ME), serosa (S). (Masson's Trichrome Stain, 200X)



(Fig. 11): Photomicrography of the gizzard wall shows muscularis externa (ME), tunica serosa (S), Adipose cells (AD), Artery (Arrow). (H&E stain, 100 X)