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RESEARCH ARTICLE

Histometerical and Morphological Studies of Digestive Tract and Associated Glands in Domestic Pigeon (*Columba livia*) with Regard to Age

Razia Kausar[1]*, Sajid Raza[1], Mumtaz Hussain[1] and Sami Ullah Khan Bahadur[2]

[1]Department of Anatomy, [2]Faculty of Veterinary Science, University of Agriculture, Faisalabad
*Corresponding author: razia.kausar@uaf.edu.pk

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ABSTRACT

Wide description of avian species is available however, limited is known about pigeon anatomy. This study has been carried out in 30 male and female pigeons (*Columba livia*) in equal proportion. The birds were divided into 3 groups, Group A: immature, Group B: adult and Group C: old, each having equal males and females. Birds were slaughtered, digestive tract organs and associated glands were collected and fixed in 10% neutral buffered formalin. For histometerical studies, tissues were processed by the paraffin embedding technique and were stained with Hematoxylin and Eosin. Microscopic studies of the different parts of digestive tract showed the significant differences ($P<0.05$) among all the three groups. Stratified squamous keratinized epithelium of tongue had mean thickness values 84.53±6.53, 269.44± 8.62 and 200.23±13.19µm in group A, group B and group C, respectively. The lumen of the esophagus was consisted of longitudinal folds along the length. Height of these folds increased with the age advancement and esophageal growth. The mean thickness values of esophageal epithelium were 226.37±5.45, 360.87±31.10 and 312.67±6.69µm in group A, B and C respectively, which were significantly different ($P\leq 0.01$). Mean epithelial thickness values of duodenum, jejunum and ileum were 19.09±1.88µm, 35.28±4.02µm and 37.94±5.04µm, respectively. The results showed that the mucosal layers and other layers were the thickest in group B, moderate in group C and lowest in group A. This study of histological parameters of pigeon's digestive system is an addition in the basic knowledge of avian anatomy.

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INTRODUCTION

Birds are an amazing source of entertainment and are considered the most successful animals among the terrestrial vertebrates. Among the birds, pigeon is considered as game bird which is found in all the continents of the world except Antarctica (Gibbs *et al.,* 2001; Goodwin, 1983). Pigeons are game birds which are commonly used in flight competition (Khan, 2004).

The digestive organs develop rapidly in early days of life up to the adult age and then it remains almost the same in old age as in adult age. (Qureshi *et al.,* 2017). Anatomical structures of the birds are very different from the mammals and this difference is very important in the arrangements of their digestive systems. Digestive system of the birds has a number of features that distinguish them from mammals and other vertebrates. These features include absence of teeth, soft palate and having a special feeding strategy that allows them to ingest maximum food in a short time. To keep the body weight down, for flight, most of the birds cannot afford the luxury of prolonged food storage or digestion. Development rate of digestive tract in birds varies from species to species. Development of digestive tract is related with growth rate of the birds so faster development of digestive tract is characteristic feature of high growing birds (Wasilewski *et al.,* 2015). The development of digestive organs starts just after hatching and the intestine develop rapidly as compare to the other organs of digestive system. (Dong *et al.,* 2012)

Digestive tract of the vertebrates varies anatomically as well as physiologically among the different species. Even among the bird species, variation in length and weight of small intestine does exist (Mabelebele *et al.,* 2017). Similarly, anatomical topography of the crop rigorously depends on species (Kierończyk *et al.,* 2016). Light weight and length of intestines is an adaptation by birds for flight

(Al-taee, 2017). The GI tract of the birds provides a very suitable environment which is involved in the reduction of food size and its complex structure with the help of its physical activity and chemical activity. As a result of this digestive activity large complex molecules of food are converted into smaller and simpler molecules which are absorbed into blood (Klasing, 1999). Anatomy and physiology of digestive tract allow to accommodate the variable need of an individual. The morphological plasticity of GI tract makes it to accommodate the nutritional changes in the feed (Klasing, 1998).

Generally, wide description of avian species can be found but a very limited study on pigeon has been carried out so far. That's why, normal anatomical and histological parameters of pigeon (*Columba livia*) are also not known. Based on this scenario, present study has been designed to document the microscopic anatomical features and variations of GI tract and associated glands with the variation of age in pigeon.

MATERIALS AND METHODS

Collection of samples: A total of 30 male and female sailkoti pigeons (*Columba livia*) were purchased from the local market. The birds were divided into 3 groups, Group A: immature (2 months), Group B: adult (One year) and Group C: old aged (≤5 years), each group having equal proportion of male and female birds. The Age of the pigeons were determined according to the method described by Silovsky *et al.,* 1968. The birds were slaughtered according to the rules and regulations of bioethics committee of University of Agriculture, Faisalabad. After slaughtering, the samples of different organs of digestive system and its associated glands from each individual were collected and fixed in 10% neutral buffered formalin. Digestive organs included upper tract (tongue, esophagus and crop), stomach parts (proventriculus and gizzard), small and large intestines, caeca; and associated glands included liver and pancreas. For microscopic studies, tissues of suitable dimensions were further processed by paraffin embedding technique and were stained with hematoxylin and eosin (Bancroft and Gamble, 2008). For the morphometric analysis of these selected digestive organs and associated glands, automated computer adopted image analysis system lucia®, was used.

Statistical analysis: The collected data were analyzed through a suitable statistical analysis. For calculation of statistical parameters like means, standard error and ranges, Microsoft Excel® was used. A comparison between means of parameters and one-way analysis of variance (ANOVA) was made using XL-STAT 2012. Means of different groups were compared by two factor factorials in completely randomized design (CRD) (Steel and Torrie, 1997) using Tukey's HSD test ($\alpha = 0.05$).

RESULTS

Total body weight: Average body weight of all three age groups Group A, Group B and Group C ranges from 32. 00-337.00g. Over all means (±SEM) of total body weight of three age groups was 218.17±23.18. There was a significant difference in the mean body weight of pigeon of three age groups. Maximum weight was observed in the Group C (five years) (317.20±4.38) and minimum was observed in Group A (one month) (43.70±3.18). Total body weight means (± SEM) of all three age groups showed significant difference ($P \le 0.01$) as shown in Table 2.

Histological and Histomorphometrical studies: Tongue of pigeon was covered entirely by a stratified squamous keratinized epithelium (Plate 1). The thickness of the epithelium in group B birds were significantly more ($P \le 0.01$) than in group A and C (Table 1). Tunica muscularis of the tongue was comprised of inner circular and outer longitudinal layers of smooth muscles. Type of

Table 1: Histometric parameters (mean ± SEM) of layers of different organs of digestive system of pigeon in three different age groups.

Organs		Tunica Mucosa			Tela submucosa	Tunica Muscularis		Tunica adventitia
		Epithelium	Lamina propria	Muscularis mucosa		Circular	Longitudinal	
Esophagus	Group A	226.37±5.45^{c}	132.26±6.19^{c}	24.61±3.79^{c}	29.05±2.31^{c}	100.95±3.02^{c}	40.89±14.06^{c}	224.07±33.34^{c}
	Group B	360.87±31.10^{a}	192.20±11.55^{a}	64.71±5.89^{a}	54.71±4.34^{b}	141.07±9.88^{a}	112.62±18.94^{b}	256.72±17.95^{b}
	Group C	312.67±6.69^{b}	185.63±4.88^{b}	50.47±3.88^{b}	61.27±5.20^{a}	133.33±6.58^{b}	122.47±14.45^{a}	229.10±24.99^{a}
Crop	Group A	211.17±11.78^{b}	38.37±4.16^{b}	22.24±3.62^{b}	35.73±9.40^{b}	303.84±9.99^{b}	209.33±10.90^{c}	212.99±12.11^{c}
	Group B	332.73±12.45^{a}	79.81±4.16^{a}	37.57±4.82^{a}	57.85±5.36^{a}	339.57±21.29^{a}	320.44±9.69^{a}	249.37±17.63^{a}
	Group C	337.90±6.44^{a}	74.68±6.74^{b}	36.44±4.41^{a}	45.49±3.35^{b}	338.07±13.86^{a}	261.8±15.99^{b}	242.08±13.39^{b}
Proventriculus	Group A	229.73±11.52^{c}	74.13±5.26^{c}	20.80±1.33^{c}	908.00±20.98^{c}	29.67±2.40^{c}	47.13±2.67^{b}	75.13±4.38^{b}
	Group B	395.31±253.56^{a}	220.07±18.89^{a}	53.04±8.74^{a}	2345.53±109.26^{a}	57.99±2.88^{a}	71.71±4.35^{a}	92.45±10.90^{a}
	Group C	318.57±18.67^{b}	116.12±9.43^{b}	45.63±3.99^{b}	1812.15±71.75^{b}	44.99±6.58^{b}	53.07±2.26^{b}	71.31±5.99^{b}
Gizzard	Group A	367.47±31.76^{b}	644.57±32.68^{c}	126.14±3.95^{c}	207.77±13.99^{c}	1199.87±39.13^{c}	666.67±83.15^{c}	360.53±36.61^{c}
	Group B	469.80±11.11^{a}	748.68±43.44^{a}	216.87±6.82^{a}	256.08±18.24^{a}	1800.07±99.06^{a}	908.23±125.60^{a}	589.04±50.64^{a}
	Group C	369.77±12.97^{b}	716.73±40.31^{b}	139.75±9.23^{b}	189.09±10.82^{b}	1326.78±99.98^{b}	756.77±37.54^{b}	438.93±46.20^{b}
Small intestine (Duodenum)	Group A	14.27±2.90^{b}	108.33±8.09^{c}	11.33±1.34^{c}	20.85±2.05^{c}	30.85±3.49^{c}	21.69±1.76^{b}	31.90±2.03^{b}
	Group B	22.93±2.49^{a}	152.53±7.29^{a}	34.37±5.69^{a}	52.97±5.72^{a}	57.70±5.29^{a}	43.67±3.36^{a}	52.57±5.11^{a}
	Group C	20.08±2.90^{a}	136.48±10.31^{b}	19.79±2.08^{b}	37.20±3.03^{b}	41.91±4.63^{b}	37.73±2.90^{ab}	49.73±4.31^{ab}
Small intestine (Jejunum)	Group A	21.53±2.98^{c}	125.78±9.50^{c}	10.90±0.78^{b}	20.83±1.47^{c}	25.51±1.61^{b}	21.15±2.61^{b}	41.53±1.81^{c}
	Group B	46.30±4.22^{a}	208.10±10.09^{a}	2.29±10.03^{a}	49.52±3.85^{a}	49.95±3.96^{a}	47.41±3.57^{a}	70.78±4.92^{a}
	Group C	38.00±2.97^{b}	174.11±12.31^{b}	42.18±7.04^{a}	28.60±3.72^{b}	42.07±5.58^{ab}	40.34±7.49^{ab}	51.89±5.50^{b}
Small intestine (Ileum)	Group A	22.37±1.47^{c}	132.82±4.19^{c}	10.17±0.90^{b}	27.13±2.30^{b}	28.33±2.05^{b}	21.13±3.70^{b}	38.70±4.24^{c}
	Group B	55.11±4.02^{a}	205.10±4.35^{a}	31.79±6.71^{a}	46.32±4.10^{a}	44.69±5.00^{a}	34.19±5.65^{a}	85.45±4.52^{a}
	Group C	36.35±4.07^{b}	165.55±5.82^{b}	34.01±4.38^{a}	43.63±5.34^{a}	44.79±4.29^{a}	33.07±5.66^{a}	56.55±5.08^{b}
Large intestine	Group A	13.77±2.06^{c}	178.13±17.20^{c}	9.97±0.81^{b}	22.50±2.68^{c}	67.21±4.92^{c}	18.26±1.79^{c}	42.17±2.14^{b}
	Group B	22.27±1.70^{a}	213.83±5.67^{b}	25.27±1.35^{a}	48.55±4.13^{a}	121.40±6.12^{a}	40.82±3.49^{a}	104.60±14.13^{a}
	Group C	16.43±1.79^{b}	142.83±24.61^{a}	25.11±4.35^{a}	30.52±3.51^{b}	85.42±6.91^{b}	31.73±4.51^{b}	45.21±8.63^{b}

LP = Lamina propria; MM = Muscularis mucosae; Different subscripts in the same column indicate statistically different group values at 1 or 5 percent levels.

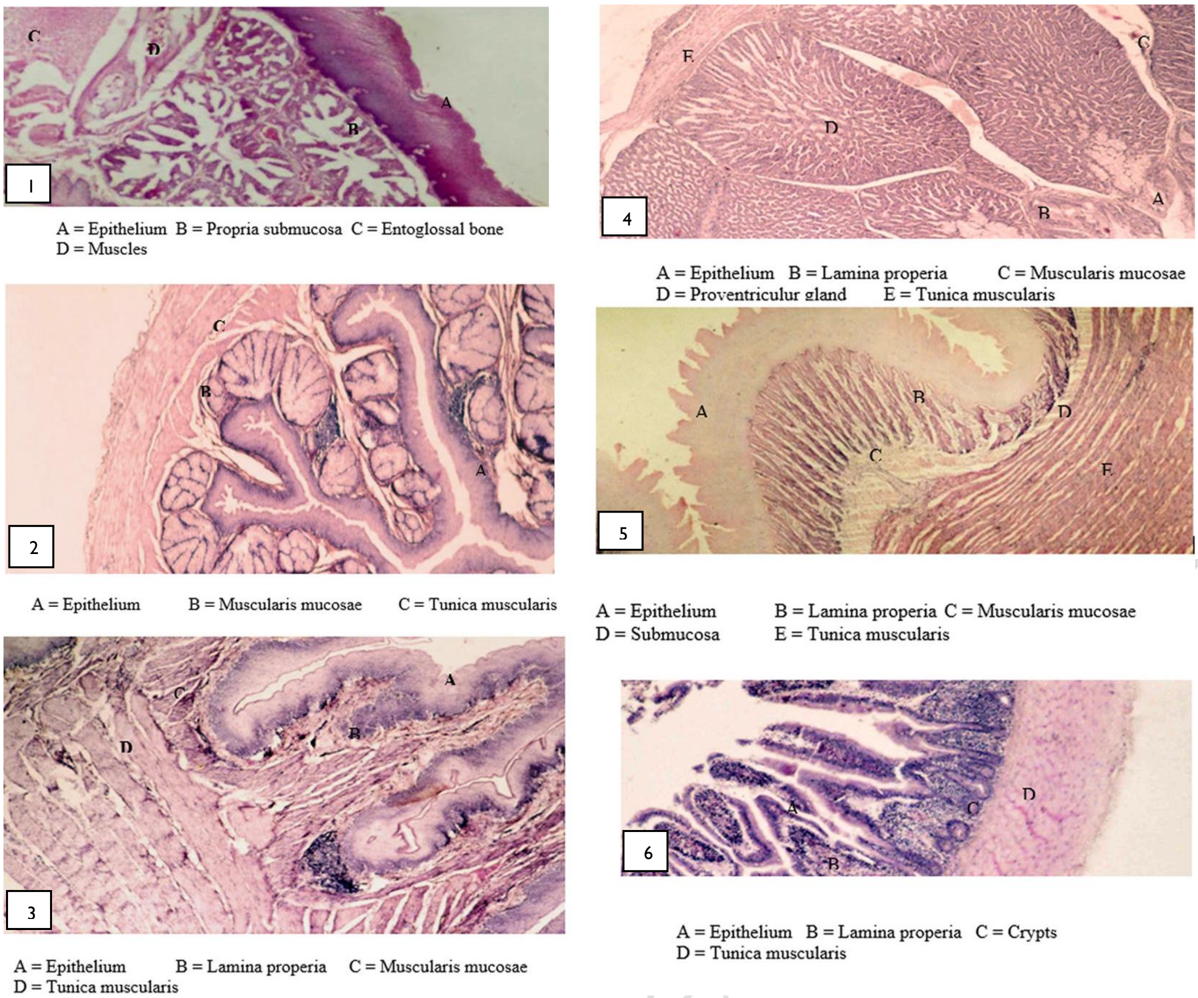

Plate: 1: Microscopic view of tongue of pigeons *(Columba livia)*, Stain: H&E; X40; **2:** Microscopic view of esophagus of pigeons *(Columba livia)* Stain: H&E; X40; **3:** Microscopic view of crop of pigeons *(Columba livia)* Stain: H&E; X40; **4:** Microscopic view of proventriculus of pigeons *(Columba livia)* Stain: H&E; X40; **5:** Microscopic view of gizzard of pigeons *(Columba livia)* Stain: H&E; X40; **6:** Microscopic view of duodenum of pigeons *(Columba livia)* Stain: H&E; X40.

epithelium found in the mucosa of esophagus was stratified squamous. Numerous irregularly shaped mucous glands in tunica propria were present in anterior esophagus which reduced in number in the crop (Plate 2). Tunica muscularis was divided into inner thick circular and outer thin longitudinal muscle fibers. Histometerical parameters revealed variation in the thickness of esophageal layers with the difference of age among three groups of pigeon. Height of epithelium, lamina propria, lamina muscularis and serosa were highest in group B, moderate in group C and lowest in the group A of birds (Table 1). Tunica mucosa of crop had stratified squamous epithelial as innermost layer. Lamina propria, with higher number of mucous glands in esophagus, was lacking in crop. Only longitudinal muscles were present in lamina muscularis while tunica muscularis was composed of two layers: inner circular and outer longitudinal (Plate 3). The most external layer, tunica adventitia consisted of connective tissue supplied with blood and nerves. A significant difference in the thickness of crop histological layers was seen with age variation among three groups of pigeon. Statistical analysis revealed the thickest epithelium in the group C, moderate in group B and thinner in the group A. The mean values (±SEM) also revealed variation in other layers among three groups of birds (Table 1). Epithelium of the proventriculus was columnar having no longitudinal folds unlike the esophagus. Proventriculus contained multilobulated compound tubular glands in tunica propria. The tunica muscularis was composed of inner thin circular and outer longitudinal muscles. Outermost layer was serosa comprising of connective tissue supplied with blood vessels and nerves, also lined by mesothelium (Plate 4). Histometerical parameters revealed that in context of mean values, group B had thickest, group C had moderate and group A had thinnest epithelial layer. These values were significantly different ($P \leq 0.01$) from each other as shown in the Table 1. The simple columnar epithelium, covered by thick layer of cuticle, was present in gizzard. Glands of the gizzard were present in the lamina propria for the secretion of cuticle. Only inner circular layer of smooth muscle in tunica muscularis had been seen in this study (Plate 5). Analysis of variance showed a significant difference ($P \leq 0.05$) in principle layers of gizzard with the variation of age groups of birds as shown in the Table 1. By and large, Intestinal histology of pigeon was similar to other avian and mammalian species (Plate 6). Over all mean values of epithelial thickness of duodenum, jejunum and ileum were 19.09±1.88µm, 35.28±4.02µm and 37.94±5.04 µm respectively. These results indicated an association between age of pigeon and development of the

Table 2: Overall means (± SEM) with ranges and group means of body weight in the pigeons *(Columba livia)* of three different age-groups

Groups	Body Weight
Overall Means	218.17±23.18
Range	32.00-337.00
Group A	43.70±3.18^{c}
Group B	293.60±7.87^{b}
Group C	317.20±4.38^{a}

Different subscripts in the same column indicate statistically different group values at 1 or 5 percent levels.

Table 3: Overall means (±SEM) with ranges and group means of length (cm), width (cm) and weight (gm) of Liver and pancreas of pigeons *(Columba livia)* of three different age-groups under study

Organs	Groups	Length (cm)	Width (cm)	Weight (gm)
Liver	Group A	2.47±0.02^{b}	2.58±0.03^{b}	3.35±0.03^{b}
	Group B	3.47±0.03^{a}	3.60±0.04^{a}	5.76±0.20^{a}
	Group C	3.49±0.01^{a}	3.54±0.03^{a}	5.55±0.04^{a}
Pancreas	Group A	2.79±0.04^{b}	0.45±0.01^{b}	0.29±0.01^{b}
	Group B	4.90±0.02^{a}	0.71±0.01^{a}	0.89±0.01^{a}
	Group C	4.88±0.02^{a}	0.72±0.01^{a}	0.99±0.01^{a}

Different subscripts in the same column indicate statistically different group values at 1 or 5 percent levels.

Table 4: Overall means (± SEM) various parts of liver and pancreas in the pigeons *(Columba livia)* of three different age-groups under study

Groups	Liver		Pancreas	
	Central Vein(µm)	Hepatocytes (µm)	Acinar (µm)	Islet of langerhans (µm)
Group A	58.90±3.31^{b}	4.19±0.26^{b}	17.20±1.32^{b}	39.98±2.18^{b}
Group B	81.68±3.03^{a}	5.03±0.23^{a}	19.90±1.20^{a}	52.47±3.29^{a}
Group C	79.56±1.90^{a}	4.59±0.16^{b}	19.07±1.52^{a}	49.73±2.96^{a}

Different subscripts in the same column indicate statistically different group values at 1 or 5 percent levels.

digestive system and showed fast growth from age of immaturity to maturity while a slower increase has been seen from maturity to old age as shown in the Table. Overall means (±SEM) of histological layers of all parts of digestive tract of pigeon *(Columba livia)*; sample size 30, has been measured as shown in Table 1. Liver had only two lobes with right lobe bigger than left one. Liver was lying in the mid-coelomic cavity of the pigeons with the absence of gall bladder. With respect to length, width and weight of liver, there was significant variation among three different age groups of pigeons. Mean values (± SEM) of these parameters of liver were significantly higher ($P\leq0.01$) in group C as compare to group A. Mean values of length in cm, width in cm, and weight in gm, of pancreas in total 30 birds were 2.61 to 4.98cm, 0.39 to 0.77cm and 0.25 to 1.03g respectively Similar to liver, mean values (± SEM) of weight as well as length and width were significantly higher ($P\leq0.01$) in Group C: old birds than those of group A: immature bird. (Table 3).

The age groups under study showed a significant difference in the size of central vein and size of hepatocytes of liver. The mean values of central vein and size of hepatocytes of liver ranged between 47.52-91.92 and 3.66-5.88. These values were highest in the Group B, moderate in Group C and lowest in the Group A. These findings were also statistically ($P\leq0.01$) secured. The mean values of size of acini of pancreas and size of islet of langerhans were highest in the Group B, moderate in Group C and lowest in the Group A. These findings were also statistically ($P\leq0.05$) secured (Table 4).

DISCUSSION

The body weight increases with the advancement in age and this change was rapid from immature to mature stage and then there is little difference between adult and old age. this study was also supported by Qureshi *et al.*, (2017). The esophagus connects oropharynx with proventriculus (Kadhim and Mohamad, 2015), had thick cutaneous stratified squamous type of epithelium having folds during contracted phase. Chicken has also stratified squamous epithelium but without stratification. Histological layers in esophagus were present in a similar fashion, as present in any other tubular organ (Kum, 2002; Long and Orlando, 1999). The lamina propria had number of amorphous shape mucous glands in anterior esophagus but reduced in crop region. The position of glands varies with species difference i.e. just beneath the epithelium of chicken and very deep in goose and ducks. The histological structure of the crop is similar to the esophagus. The mucous glands are reduced in size and the lymphatic tissues are dispersed in the connective tissue (Juliana *et al*, 2006). The sequence of muscle layers (inner circular and outer longitudinal) found in tunica muscularis of the pigeon's esophagus was similar as described in chicken (Kum, 2002). Unlike other bird species, pigeon and parrot also have a structure, similar to esophageal sphincter, for portioning of feed (Kierończyk *et al*. 2016). Type of epithelial in proventriculus as simple columnar in pigeon is prismatic type in other avian species (Chikilian and Speroni 1996, Said and Mussa, 2012). Tunica propria of pigeon's proventriculus contained multilobulated compound tubular glands. Tunica muscularis of proventriculus was composed of two smooth muscle layers i.e. inner circular and outer longitudinal while there are three layers of smooth muscles in other avian species. Serosal connective tissues of proventriculus were supplied with nerves and blood vessels, similar findings by (Rocha and Lima, 1998). In pigeon, pH of proventriculus and ventriculus is mentioned as 4.8 and 2.0, respectively (Langlois, 2003). Thick cuticle layer secreted by glands of gizzard which covered the simple columnar epithelium in the internal surface of the gizzard. Tubular glands were present in lamina propria of gizzard and these have been named simple tubular glands opening into shallow crypts by simple glandular tubules; crypts terminating in branched tubular glands by protruding lamellae of glandular cells forming elongated crypts (Yasser *et al*., 2011). In this study, only inner circular smooth muscle layer found in tunica muscularis of gizzard has also been described by (Starck and Rahmaan, 2003) in quails and he has termed gizzard's muscle as "onion structure" because smooth muscle layers were separated by thin connective tissue layers. Histology of intestinal layers in pigeon was similar to those of other avian and mammalian species. Mean values of epithelial thickness in small intestine parts of pigeon; duodenum, jejunum and ileum were found 19.09±1.88µm, 35.28±4.02µm and 37.94±5.04µm respectively. (Ahmed, 2010) determined histometerical values of intestinal epithelium in Japanese quails which were similar to these findings. The microscopic structure of small and large intestine was almost alike to other avian species and mammalian species. But the caeca differ histologically, the lymphatic tissue in the lamina propria of day-old birds found absent than in the adult birds. The simple columnar epithelium was found in the mucosa with goblet cells and the apex villi has less number of goblet cells. (Firdous and Lucy, 2012). This study has described variation among intestinal histology in pigeons based on the age. Schaefer *et al*., (2006) have also reported intestinal histological variation in turkey breeder hens of different ages: increase in villi length with increase in age had been noticed.

Liver had only two lobes with right lobe bigger than left one. Liver was lying in the mid-coelomic cavity of the pigeons with the absence of gall bladder. These findings have been supported by (Stornelli *et al*., 2006) where absence of gall bladder is also seen in parrots and ostriches. Two separate bile ducts, from each lobe of liver, were opening in duodenum's ascending loop.

It can be concluded that the digestive system organs were similar to other avian species histologically and showed significantly higher growth in terms of epithelial height, lamina propria, tela submucosae, muscularis mucosa with advancement in age. To our knowledge this is first report on detailed histometerical, histological features and age-related changes in GI tract and associated glands of domestic pigeon (*Columba livia*).

Authors contribution: All authors are contributed equally in conducting this research and writing of manuscript.

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