https://doi.org/10.17221/120/2020-VETMED

Histological and histomorphometric study of the cranial digestive tract of ostriches (*Struthio camelus*) with advancing age

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Citation: Umar Z, Qureshi AS, Shahid R, Deeba F (2021): Histological and histomorphometric study of the cranial digestive tract of ostriches (*Struthio camelus*) with advancing age. Vet Med-Czech 66, 127–139.

Abstract: The present study was conducted to determine the histological and histomorphometric variations in the tongue, oesophagus, proventriculus, and gizzard of ostriches (*Struthio camelus)* with regards to the sex and advancing age. A total of 40 healthy ostriches of both sexes and five age groups; young (up to 1 year, 1 to 2 years and 2 to 3 years) and adult (3 to 4 years and above 4 years) in equal numbers (n = 8) were used in this study. The organs under study were collected immediately after slaughtering the birds. Overall, the colour, shape, weight and various dimensions (length, width, and diameter) of the collected organs were recorded. The mean values of the gross anatomical variables of the studied organs increased (P < 0.05) among all the young groups (i.e., from 1 to 2 years, 2 to 3 years). Similarly, the organs under study in the adult groups (birds aged 3 to 4 years and above 4 years) grew (P < 0.05) as well. However, the differences between the adults were not significant. The histological analysis and histometric measurements were conducted on paraffin embedded tissue sections with Image J® analysis software. The statistical analysis revealed a significant increase in the thickness of the different tunics of the digestive organs in all the groups except those the adult groups. These findings may be of importance for the strategic manipulation of feed and nutrition to enhance the growth rate and also to diagnose pathological processes.

Keywords: biometry; oesophagus; gizzard; histometry; proventriculus; tongue

Globally, ostrich farming is gaining importance on the market with food animal commodities, making it more diversified. However, there is still a gap in our knowledge of precise nutrient requirements of ostriches. Their meat production depends on the development of the entire digestive tract, including the stomach. A detailed study of the gastrointestinal tract (GIT) limited to the early postincubation period, up to day 72, in ostrich chickens showed a rapid growth and development of its individual parts (Iji et al. 2003).

The crop, a storage place for feed, is absent in ostriches when compared to other avian species. The proventriculus of ostriches is large enough to store the food and, hence, compensates for the function of the crop.

The bird's stomach consists of two parts: the glandular (pars glandularis) and muscular (pars muscularis) part. The true stomach of the ostrich is sac-shaped and located in the cranial part of the abdomen in the left hypochondrium (Bezuidenhout 2001). In this part, protein digestion begins through

https://doi.org/10.17221/120/2020-VETMED

the secretion of pepsinogen and hydrochloric acid (Camiruaga et al. 2003). These two parts of the stomach are separated by constriction, called *isthmus gastris* (Rossi et al. 2005). The deep glandular region (regio glandularis) is situated on the greater curvature of the proventriculus. The junction between the oesophagus and the proventriculus has a narrowed cranial end and a wide rounded caudal end.

The thick-walled gizzard is situated to the left of the midline, and it contains grit (small pieces of stone) important for grinding and mixing of food (Speer 2006; Oliveira et al. 2008). The pars muscularis of the gizzard is bilaterally curved with a complex structure. In most birds, the gizzard develops with two-layers of smooth musculature. Moreover, the innermost layer is covered by koilin, a substance formed by protein secreted from the glands combined with entrapped sloughed cells and cellular debris (Shanawany and Dingle 1999). A simpler gizzard is observed in piscivorous and pray birds. The caudodorsal and caudoventral areas of the thick muscles of the ostrich gizzard are particularly well developed, and its thickness is 5.2–6.5 cm, in hens, its thickness is only 1.5–2.1 cm (Sales 2006).

To the best of our knowledge, the biometrical and histometric variations in the tongue, oesophagus, proventriculus, and gizzard of ostriches (*Struthio camelus)* with regards to the advancing age and sex have not been studied yet in detail beyond the early postincubation period.

Therefore, this study was designed to perform the biometry and histometry of the digestive organs in ostriches over a life span longer than four years, concerned with the sex and progressing age, to illustrate the changes also at the tissue and cellular levels.

MATERIAL AND METHODS

Ethical concern

This research was conducted according to the standards of the research ethics committee of the University of Agriculture, Faisalabad-Pakistan (Letter No. 962).

No bird was otherwise harmed and no health hazard was caused to the handlers during the slaughtering process.

Table 1. Grouping of the young and adult birds

Groups	Age	Male	Female	Total
	up to 1 year	4	4	8
Young groups	1–2 years	4	4	8
	2–3 years	4	4	8
Adult groups	3–4 years	4	4	8
	above 4 years	4	4	8
Total		20	20	40

Experimental groups

A total of 40 clinically healthy ostriches of either sex (20 males, 20 females) comprising five age groups of equal size, namely young (up to 1 year, 1 to 2 years and 2 to 3 years) and adult (3 to 4 years and above 4 years) in equal numbers (n = 8) were used in this study (Table 1).

Collection of samples

All the cranial digestive tract organs studied (tongue, oesophagus, proventriculus, gizzard) were collected from the Signature Meat Shop Lahore and Riphah College of Veterinary Sciences, Lahore, Pakistan. Immediately after slaughtering, the organs were washed with normal saline for use in the anatomical and histological studies.

Biometric characteristics

Following the collection, the gross features (i.e., shape, colour), weight, biometric characteristics (length, width, and diameter) of the collected organs were measured with the help of a measuring tape. The length of the tongue (cm) was measured from the apex to the root, and the width (cm) was measured from the basis of the tongue, as shown in Figure 1. The oesophageal length (cm) was taken from the pharyngeal opening to the proventricular opening, and outer diameter was also measured. The width (cm) of the proventriculus and gizzard were recorded dorsoventrally, whereas the length (cm) of proventriculus was determined from the oesophageal distal termination to the proventricular-gizzard (PG) junction. Similarly, the gizzard length (cm) was measured from the PG junction to the duodenal opening (Figure 1). An electronic weighing balance was used to weigh (g) the organs.

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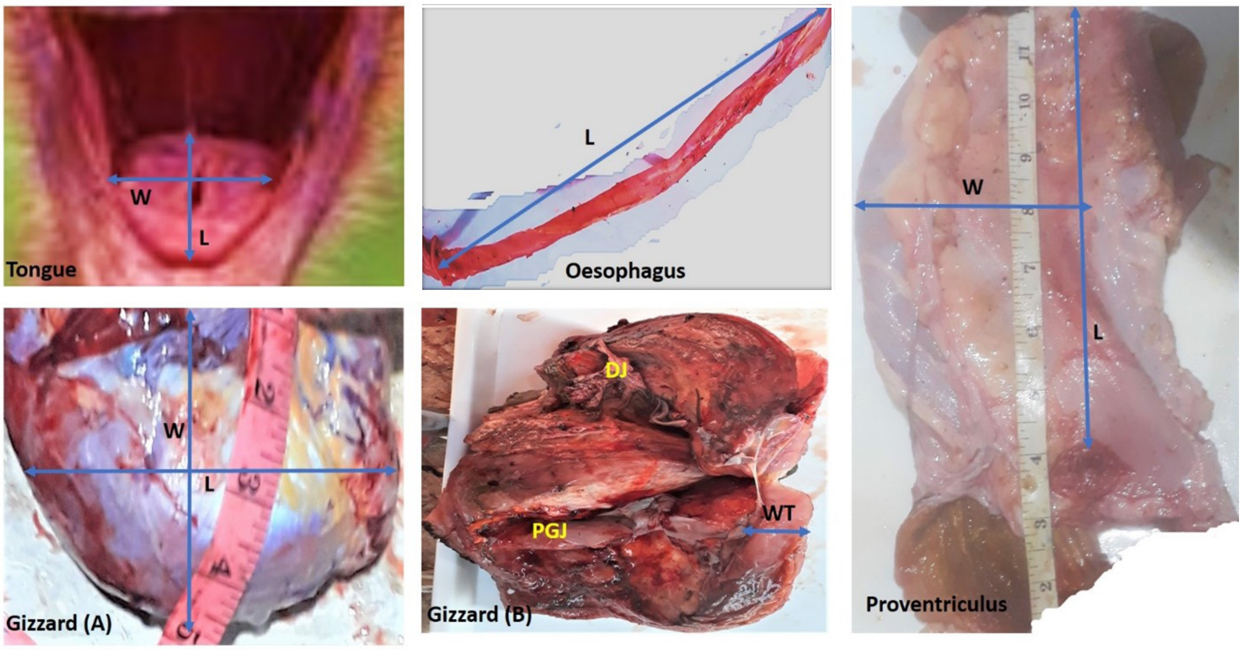

Figure 1. Photomicrograph of the different digestive organs of ostriches
The length of the tongue (L) was measured from the apex to the root, the width (W) was measured from the base of the tongue. The oesophageal length (L) was taken from the oesophageal distal termination to the proventricular opening, and the outer diameter was also measured. The width (W) of the proventriculus and gizzard was measured dorsoventrally while the length (L) of the proventriculus was determined from oesophageal opening to the proventricular-gizzard junction (PGJ). Similarly, the gizzard length (L) was measured from the proventricular-gizzard junction (PGJ) to the duodenal opening (DJ). The fibrous muscular wall thickness (WT) of gizzard was also measured

Histological study

The collected organs (tongue, oesophagus, proventriculus and gizzard/ventriculus of each bird) were cut into small cubes of 1 cm^3 using a sharp knife/blade. These pieces were washed in normal saline. All the organs were fixed in neutral buffered 10% formalin, followed by the formation of paraffin blocks by an embedding technique (Bancroft and Gamble 2008). Thin slices of 5–7 µm thickness were obtained using a rotary microtome. These sections were mounted on glass slides and stained with haematoxylin and eosin. The stained sections were observed under × 100 (10 × 10) magnification.

Histometric analysis

Photomicrographs of each sample of each studied organ were captured using a Nikon optiphot microscope at × 100 magnification These photos were used to determine the thickness of four histological layers, namely the tunica mucosa (epithelium, lamina propria, lamina muscularis), tela submucosa, tunica muscularis, and tunica adventitia/serosa of each organ with the help of the automated image analysis system Image J® v1.80 (Research Service Branch, National Institute of Mental Health, Bethesda, Maryland, USA).

Statistical analysis

A factorial one-way analysis of variance (ANOVA) was used to compare the means of the parameters. Tukey's honest significance test was used to compare the group means at a 5% level of significance (Rushing et al. 2013).

RESULTS

Biometric characteristics

The mean ± SEM values of weight (g), length (cm), width (cm), diameter, and wall thickness (cm) of the selected digestive organs (i.e., the tongue,

Veterinarni Medicina, 66, 2021 (04): 127–139
https://doi.org/10.17221/120/2020-VETMED

Table 2. Mean (± SEM) of the weight, length, width, diameter and the wall thickness of the digestive organs of the ostriches *(Struthio camelus)* of the different age-groups and sexes

Organs	Parameters	Mean ± SEM (n = 40)	Sex groups		Age groups				
			male (n = 20)	female (n = 20)	up to 1 year (n = 8)	1–2 years (n = 8)	2–3 years (n = 8)	3–4 years (n = 8)	above 4 years (n = 8)
Tongue	weight (g)	3.95 ± 0.21	3.94 ± 0.20^a	3.94 ± 0.19^a	2.89 ± 0.02^a	3.38 ± 0.14^b	3.75 ± 0.15^c	4.80 ± 0.06^d	4.93 ± 0.09^d
	length (cm)	5.56 ± 0.33	3.55 ± 0.23^a	3.54 ± 0.22^a	1.77 ± 0.11^a	2.82 ± 0.21^b	3.97 ± 0.12^c	4.58 ± 0.08^d	4.63 ± 0.07^d
	width (cm)	2.33 ± 0.16	2.34 ± 0.17^a	2.32 ± 0.15^a	1.50 ± 0.04^a	1.73 ± 0.05^b	2.54 ± 0.12^c	2.92 ± 0.01^d	2.94 ± 0.01^d
Oesophagus	weight (g)	198.89 ± 11.44	192.66 ± 10.87^a	193.44 ± 10.67^a	118.0 ± 4.16^a	142 ± 8.19	195 ± 2.89^c	218.33 ± 6.01^d	223.33 ± 6.17^d
	length (cm)	118.23 ± 12.15	122.74 ± 8.89^a	121.72 ± 8.85^a	77.57 ± 2.29^a	101.00 ± 3.52	115.11 ± 4.67^c	131.13 ± 7.12^d	132.96 ± 7.55^d
	diameter (cm)	3.13 ± 0.14	3.12 ± 0.13^a	3.11 ± 0.11^a	2.52 ± 0.05^a	2.73 ± 0.06	3.00 ± 0.05^c	3.70 ± 0.06^d	3.72 ± 0.12^d
Proventriculus	weight (g)	687.2 ± 21.9	750 ± 28.12^a	752 ± 29.11^a	586.00 ± 6.66^a	620.51 ± 9.27^b	681.62 ± 5.49^c	777.3 ± 28.3^d	776.45 ± 30.7^d
	length (cm)	26.07 ± 0.51	27.16 ± 0.61^a	27.06 ± 0.61^a	23.46 ± 0.23^a	24.77 ± 0.04^b	25.97 ± 0.35^c	28.01 ± 0.57^d	28.16 ± 0.64^d
	width (cm)	12.89 ± 0.18	11.99 ± 0.15^a	12.71 ± 0.17^a	11.93 ± 0.20^a	12.47 ± 0.02^b	12.97 ± 0.04^c	13.57 ± 0.06^d	13.67 ± 0.07^d
	wall thickness (cm)	3.30 ± 0.11	3.27 ± 0.12^a	3.26 ± 0.12^a	2.762 ± 0.02^a	3.06 ± 0.09^b	3.51 ± 0.01^c	3.66 ± 0.005^d	3.67 ± 0.008^d
Gizzard	weight (g)	1 086.2 ± 39.3	1 250.1 ± 6.81^a	1 251 ± 6.80^a	888.3 ± 1.74^a	984.56 ± 6.84^b	1 050.4 ± 21.10^c	1 253.8 ± 6.92^d	1 254.1 ± 6.71^d
	length (cm)	26.45 ± 0.61	27.1 ± 0.71^a	27.12 ± 0.70^a	23.3 ± 0.06^a	24.6 ± 0.25^b	26.83 ± 0.56^c	28.5 ± 0.31^d	29.03 ± 0.56^d
	width (cm)	16.72 ± 0.31	16.77 ± 0.42^a	16.75 ± 0.39^a	14.80 ± 0.05^a	16.13 ± 0.15^b	16.97 ± 0.07^c	17.73 ± 0.17^d	17.96 ± 0.11^d
	wall thickness (cm)	3.92 ± 0.19	3.99 ± 0.21^a	3.97 ± 0.20^a	2.87 ± 0.01^a	3.34 ± 0.12^b	3.96 ± 0.02^c	4.68 ± 0.06^d	4.75 ± 0.04^d

$^{a–d}$Mean values having a different letter of the alphabet differ significantly from one another ($P \leq 0.05$)

https://doi.org/10.17221/120/2020-VETMED

oesophagus, proventriculus, and gizzard) of either sex and five age progressive groups of ostriches (Table 2). The statistical analysis showed that the age is directly related to the macroscopic values of the organs. The mean values of the gross anatomical variables of the studied organs increased ($P < 0.05$) among all young groups (i.e., up to 1 year, 1 to 2 years, 2 to 3 years). The values for young groups were significantly ($P < 0.05$) lower than those for the adult groups of birds (3 to 4 years and above 4 years). The mean values of gross anatomical parameters of studied organs followed an increasing trend ($P < 0.05$) within young group, however, mean values were significantly ($P < 0.05$) lower than that of adults group (Table 2). The sex did not affect any of the variables within the same age group (Table 2). A rapid increase ($P < 0.05$) in the morphometrical parameters of the cranial digestive organs was observed during the young age period, but a non-significant ($P > 0.05$) increase was recorded in the adults (3 to 4 years and > 4 years).

Histometric characteristics

The histometric analysis of the organs under study, including the tongue, oesophagus, proventriculus, and gizzard of the different groups of ostriches, are presented in Figures 2, 3, 4-A, B, C and 5-A, B.

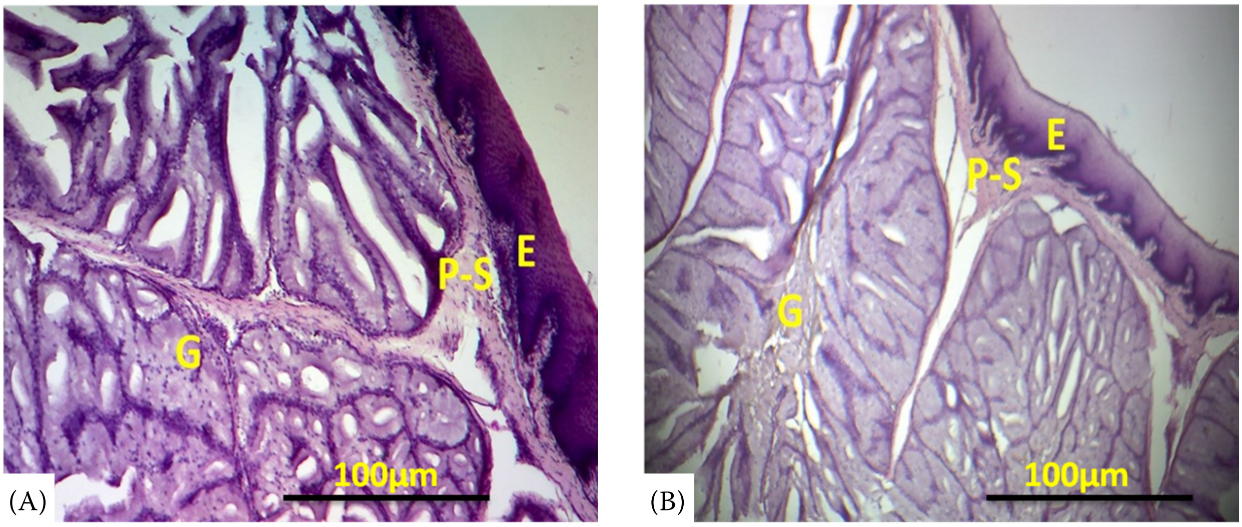

Figure 2. Tongue photomicrograph (A) of an adult ostrich. Tongue photomicrograph (B) of a young ostrich; Haematoxylin and eosin (H&E) × 100

E = epithelium; G = salivary glands; P-S = propria-submucosa

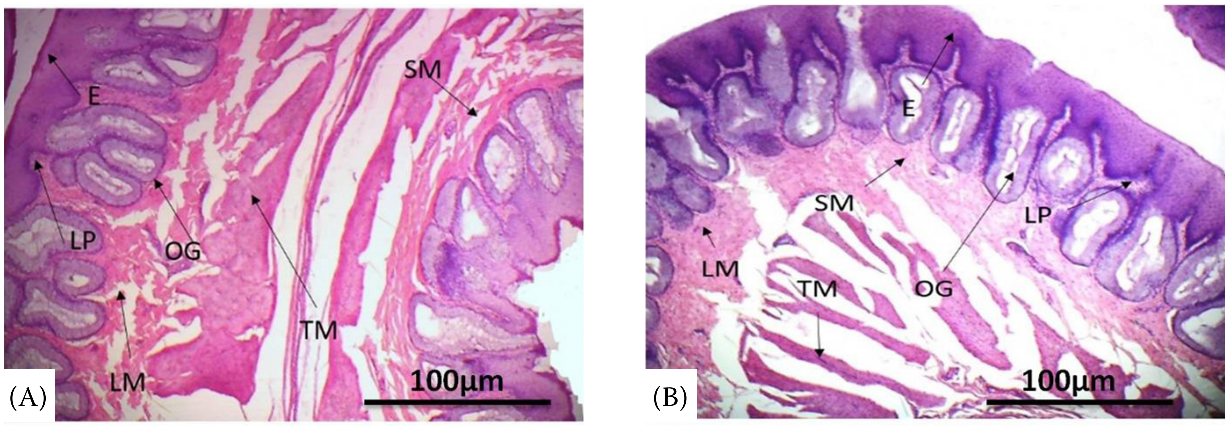

Figure 3. Oesophagus photomicrograph (A) of an adult ostrich. Oesophagus photomicrograph (B) of a young ostrich; Haematoxylin and eosin (H&E) × 100

E = epithelium; LM = lamina muscularis; LP = lamina propria; OG = oesophageal glands; SM = submucosa; TM = tunica muscularis

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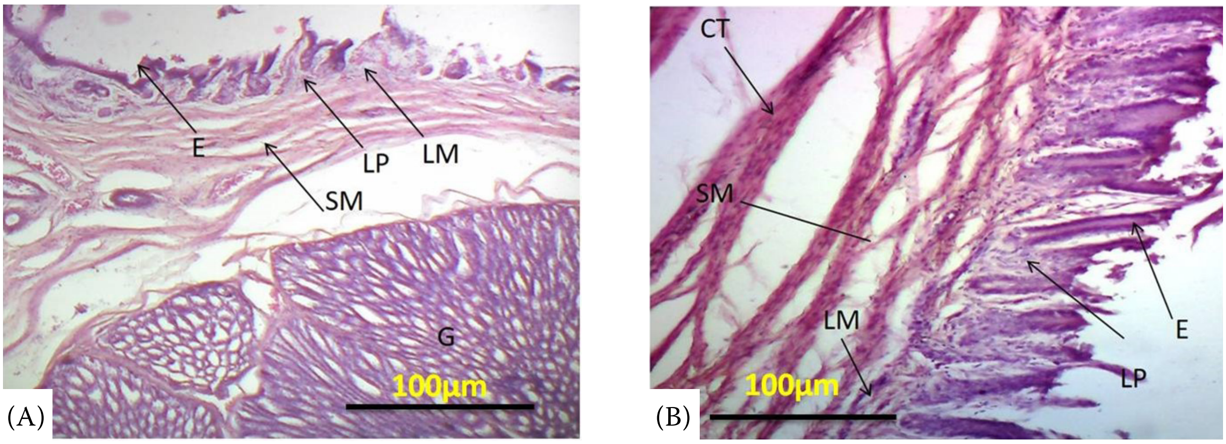

Figure 4-A. Proventriculus (glandular part) photomicrograph (A) of a young ostrich. Proventriculus (glandular part) photomicrograph (B) of an adult ostrich; Haematoxylin and eosin (H&E) × 100

CT = connective tissue; E = epithelium; G = gastric glands; LM = lamina muscularis; LP = lamina propria; SM = sub mucosa; TM = tunica muscularis

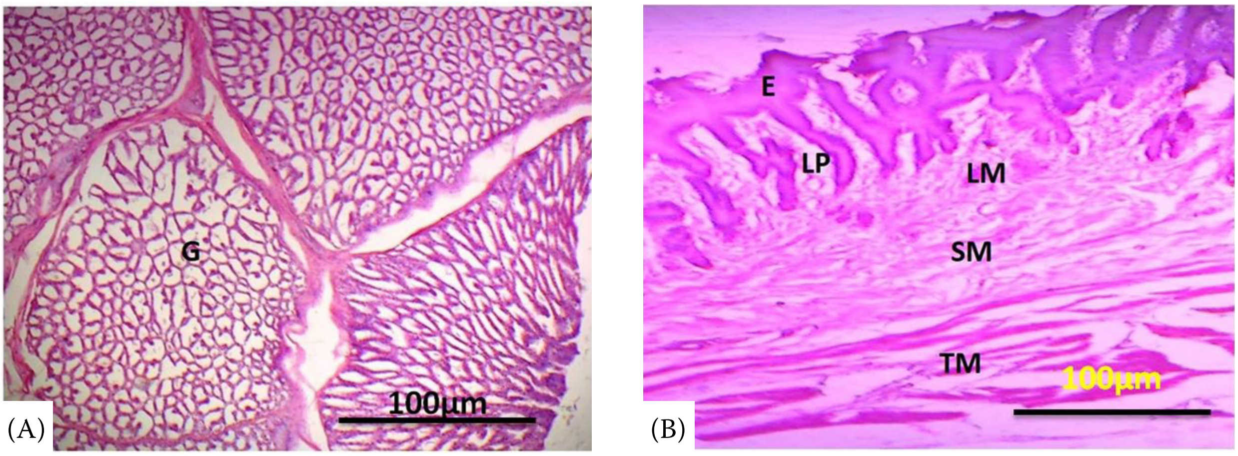

Figure 4-B. Proventriculus (glandular part) photomicrograph (A) of an adult ostrich. Proventriculus (non-glandular part) photomicrograph (B) of an adult ostrich; Haematoxylin and eosin (H&E) × 100

E = epithelium; G = gastric glands; LM = lamina muscularis; LP = lamina propria; SM = sub mucosa; TM = tunica muscularis

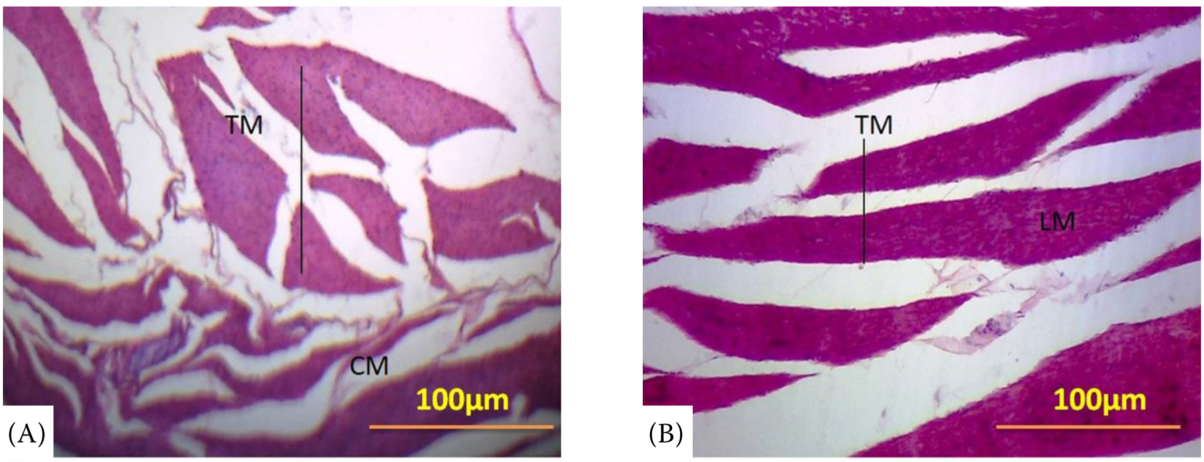

Figure 4-C. Proventriculus photomicrograph (A) of tunica muscularis of a young ostrich. Proventriculus photomicrograph (B) of tunica muscularis of an adult ostrich; Haematoxylin and eosin (H&E) × 100

CM = circular muscularis; LM = longitudinal muscularis; TM = tunica muscularis

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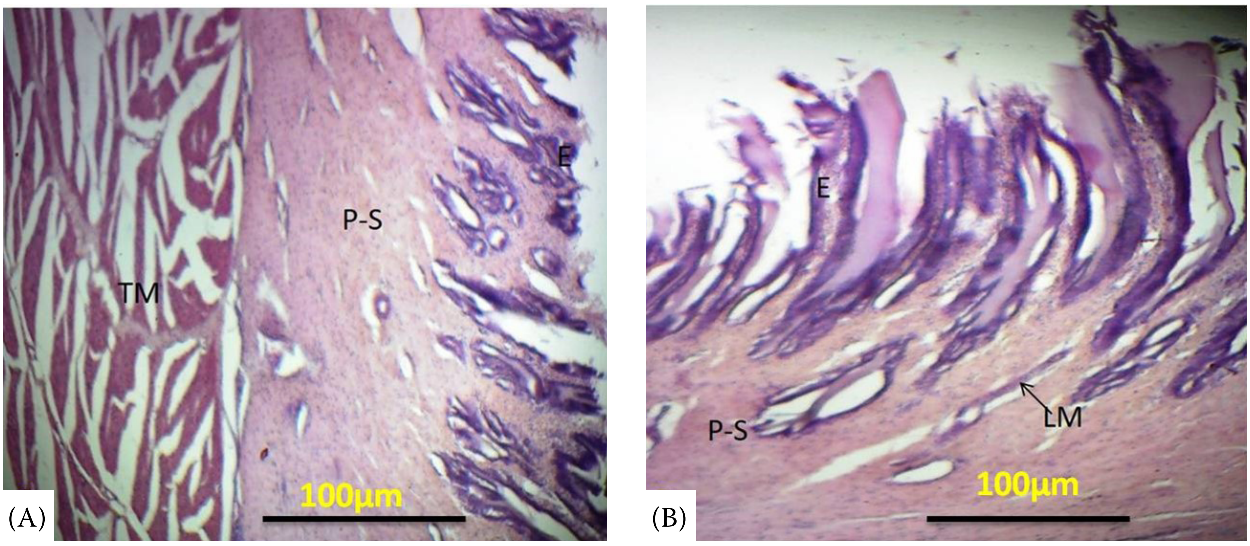

Figure 5-A. Gizzard photomicrograph (A) of a young ostrich. Gizzard photomicrograph (B) of an adult ostrich; Haematoxylin and eosin (H&E) × 100

E = epithelium; LM = lamina muscularis; P-S = propria-submucosa; TM = tunica muscularis

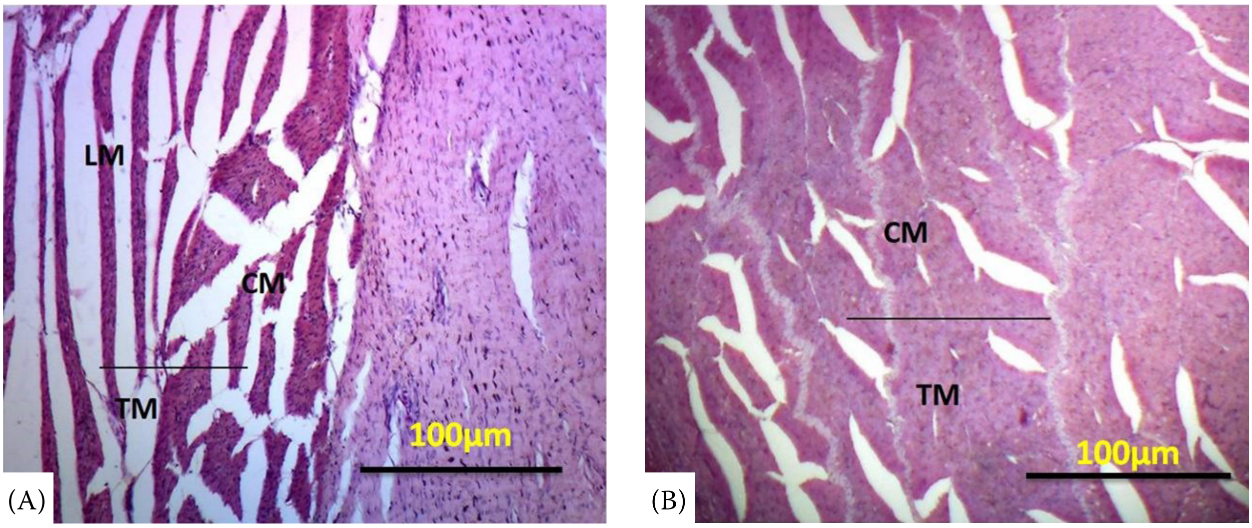

Figure 5-B. Gizzard photomicrograph (A) of a young ostrich. Gizzard photomicrograph (B) of an adult ostrich; Haematoxylin and eosin (H&E) × 100

CM = circular muscles; LM = longitudinal muscles; TM = tunica muscularis

The different histometric parameters, including the thickness of the epithelium, cartilage, lamina muscularis propria-submucosa, inner circular, outer longitudinal, and tunica adventitia/serosa in the young and adult ostriches, are presented in Table 3.

The statistical analysis revealed that the mean values of the thickness of all layers of all groups grew ($P < 0.05$) except between the adult (3 to 4 years and above 4 years) age groups, but these values were non-significantly ($P > 0.05$) different between the sexes (Table 3).

DISCUSSION

The ostrich (*Struthio camelus*), the largest non-flying bird, is a preferred meat source due to its very low cholesterol and fat contents.

Ostrich by-products, feathers and skin, are in great demand in the leather industry because it requires less acreage in comparison to livestock. The meat production of animals is directly related to different factors, like the health status, metabolic processes and digestive tract development. Therefore, the digestive tract of ostrich was sub-

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Table 3. Mean (± SEM) thickness (μm) of various cellular layers of the digestive organs of the ostriches *(Struthio camelus)* at different ages and both sexes

Organs	Parameters		Sex groups		Progressive age groups				
		mean ± SEM (*n* = 40)	male (*n* = 20)	female (*n* = 20)	up to 1 year (*n* = 8)	1–2 years (*n* = 8)	2–3 years (*n* = 8)	3–4 years (*n* = 8)	above 4 years (*n* = 8)
Tongue	epithelium	96.56 ± 9.33	121.55 ± 8.23^{a}	120.54 ± 8.22^{a}	57.77 ± 4.11^{a}	63.82 ± 5.21^{b}	98.97 ± 6.36^{c}	131.58 ± 7.08^{d}	130.63 ± 7.06^{d}
	cartilage	458.89 ± 39.21	449 ± 35.8^{a}	448.9 ± 34.23^{a}	168.4 ± 13.9^{a}	230.2 ± 57.4^{b}	372.2 ± 25.1^{c}	472.1 ± 40.5^{d}	472.6 ± 41.5^{d}
	propria submucosa	498.45 ± 31.16	485.34 ± 29.17^{a}	484.32 ± 29.15^{a}	211.4 ± 23.5^{a}	306.4 ± 25.2^{b}	496.55 ± 7.85^{c}	522.3 ± 24.5^{d}	524.4 ± 25.2^{d}
	tunica muscularis (circular)	373.4 ± 45.4	381.4 ± 37.4^{a}	383.4 ± 38.4^{a}	127.26 ± 7.38^{a}	167.26 ± 10.38^{b}	291.3 ± 30.3^{c}	383.4 ± 35.4^{d}	393.4 ± 36.4^{d}
	tunica muscularis (longitudinal)	333.4 ± 24.6	349.4 ± 29.6^{a}	348.4 ± 29.5^{a}	130.99 ± 9.33^{a}	269.17 ± 11.96^{b}	339.5 ± 14.7^{c}	359.5 ± 19.7^{d}	359.4 ± 19.6^{d}
	tunica adventitia	168.26 ± 11.38	156.36 ± 10.38^{a}	157.26 ± 10.37^{a}	115.68 ± 9.70^{a}	130.02 ± 9.16^{b}	159.4 ± 23.0^{c}	199.3 ± 19.6^{d}	198.3 ± 19.9^{d}
Oesophagus	epithelium	45.92 ± 15.625	47.92 ± 16.62^{a}	48.92 ± 15.25^{a}	9.92 ± 0.625^{a}	24.11 ± 1.63^{b}	36.66 ± 4.89^{c}	49.71 ± 5.92^{d}	49.92 ± 5.63^{d}
	lamina propria	73.36 ± 8.02	77.36 ± 7.02^{a}	76.36 ± 6.02^{a}	11.36 ± 1.02^{a}	47.07 ± 1.20^{b}	70.09 ± 2.20^{c}	79.95 ± 4.09^{d}	78.36 ± 4.02^{d}
	laminae muscularis	40.68 ± 15.92	42.68 ± 8.89^{a}	41.68 ± 15.97^{a}	44.68 ± 5.92^{a}	43.7 ± 5.11^{b}	101.46 ± 8.32^{c}	99.74 ± 8.08^{d}	44.68 ± 5.92^{d}
	submucosa	140.02 ± 24.20	144.02 ± 14.20^{a}	143.02 ± 14.10^{a}	46.02 ± 4.20^{a}	89.58 ± 8.39^{b}	122.6 ± 15.9^{c}	151.2 ± 14.4^{d}	146.02 ± 14.20^{d}
	tunica muscularis (circular)	221.1 ± 19.21	224.1 ± 8.81^{a}	222.1 ± 8.41^{a}	67.1 ± 10.2^{a}	136.00 ± 7.28^{b}	204.95 ± 7.24^{c}	227.84 ± 9.42^{d}	226.1 ± 9.21^{d}
	tunica muscularis (longitudinal)	286.1 ± 31.0	291.1 ± 29.80^{a}	290.1 ± 29.70^{a}	76.1 ± 12.0^{a}	178.9 ± 14.0^{b}	250.1 ± 26.3^{c}	292.8 ± 20.1^{d}	296.1 ± 21.0^{d}
	tunica serosa	110.47 ± 20.47	112.47 ± 14.41^{a}	111.77 ± 13.68^{a}	40.47 ± 2.48^{a}	70.12 ± 7.17^{b}	91.6 ± 10.7^{c}	114.6 ± 13.1^{d}	114.47 ± 12.48^{d}
Proventriculus	epithelium	89.07 ± 23.80	92.27 ± 13.31^{a}	92.11 ± 13.31^{a}	39.07 ± 3.80^{a}	79.95 ± 5.35^{b}	84.49 ± 6.94^{c}	95.8 ± 11.3^{d}	99.07 ± 13.80^{d}
	lamina propria	80.50 ± 39.51	85.50 ± 18.51^{a}	84.50 ± 19.24^{a}	57.50 ± 2.51^{a}	69.22 ± 3.69^{b}	78.47 ± 4.49^{c}	88.11 ± 9.93^{d}	87.50 ± 9.51^{d}
	laminae muscularis	75.93 ± 23.54	78.83 ± 13.64^{a}	77.30 ± 13.54^{a}	48.93 ± 3.54^{a}	63.78 ± 4.30^{b}	71.98 ± 6.69^{c}	82.8 ± 13.1^{d}	83.93 ± 13.54^{d}
	submucosa	500.7 ± 47.2	505.23 ± 25.2^{a}	506.7 ± 25.2^{a}	360.7 ± 19.2^{a}	417.0 ± 20.2^{b}	456.9 ± 21.9^{c}	514.6 ± 26.7^{d}	510.7 ± 27.2^{d}
	tunica muscularis (circular)	512.8 ± 59.6	518.8 ± 28.6^{a}	517.67 ± 27.6^{a}	144.2 ± 24.1^{a}	327.8 ± 29.6^{b}	465.6 ± 13.3^{c}	513.6 ± 26.4^{d}	522.8 ± 29.6^{d}
	tunica muscularis (longitudinal)	517.8 ± 49.1	520.8 ± 29.91^{a}	521.8 ± 30.21^{a}	365.8 ± 21.1^{a}	450.1 ± 28.9^{b}	495.1 ± 33.9^{c}	528.7 ± 38.0^{d}	527.8 ± 39.1^{d}
	tunica serosa	145.21 ± 41.78	150.21 ± 13.78^{a}	151.21 ± 14.64^{a}	75.21 ± 4.58^{a}	105.21 ± 5.78^{b}	142.6 ± 9.27^{c}	157.7 ± 10.7^{d}	158.21 ± 11.78^{d}
Gizzard	epithelium	290.6 ± 49.0	294.6 ± 54.0^{a}	2933.6 ± 57.0^{a}	114.6 ± 11.70^{a}	210.5 ± 14.81^{b}	255.3 ± 27.9^{c}	293.3 ± 46.1^{d}	294.6 ± 47.0^{d}
	laminae muscularis	201.2 ± 45.6	210.2 ± 25.6^{a}	208.2 ± 25.56^{a}	110.2 ± 12.6^{a}	142.0 ± 13.4^{b}	64.0 ± 14.6^{c}	199.9 ± 15.9^{d}	216.2 ± 15.6^{d}
	propria submucosa	214.9 ± 56.2	220.9 ± 16.2^{a}	219.7 ± 16.241^{a}	124.9 ± 10.2^{a}	156.07 ± 17.06^{b}	198.5 ± 22.8^{c}	222.6 ± 25.7^{d}	224.9 ± 26.2^{d}
	tunica muscularis (circular)	979.2 ± 71.6	982.2 ± 51.4^{a}	981.2 ± 51.89^{a}	406.2 ± 25.6^{a}	654.0 ± 35.4^{b}	842 ± 42.80^{c}	1 012.7 ± 52.5^{d}	999.2 ± 51.6^{d}
	tunica muscularis (longitudinal)	877.1 ± 69.6	878.1 ± 36.6^{a}	880.1 ± 39.6^{a}	656.1 ± 29.6^{a}	794.5 ± 33.1^{b}	826.49 ± 47.7^{c}	886.7 ± 59.4^{d}	887.1 ± 59.6^{d}
	tunica serosa	80.83 ± 30.56	93.13 ± 14.68^{a}	92.53 ± 14.56^{a}	58.83 ± 4.86^{a}	63.25 ± 8.81^{b}	79.37 ± 9.06^{c}	90.17 ± 10.89^{d}	98.83 ± 10.86^{d}

$^{a–d}$Mean values having a different letter of the alphabet differ significantly from one another ($P \leq 0.05$)

https://doi.org/10.17221/120/2020-VETMED

jected to morphometric and histological evaluation in order to illustrate the changes at tissue and cellular level.

Tongue

The tongue of the ostriches was quite thick and short. The gross biometric results revealed that the tongue was significantly ($P < 0.05$) longer among all the young groups and also the mean values of the adult group were significantly ($P < 0.05$) higher than the young group's readings and remained non-significant ($P > 0.05$) between the sexes in the same age group (Table 2). These findings were similar to studies by Rossi et al. (2005), who measured the length of the tongue in adult partridges (1.70 ± 0.08 cm in males and 1.65 ± 0.072 cm in females) and Tadjalli et al. (2008), who measured the length of two-year-old ostriches (1.92 ± 0.086 cm). In this study, these findings were determined in immature (> 2 years) as well as in adult. There was no significant difference in tongue length between different sexes all ages group which is in line with the findings of Rossi et al. (2005). The current study indicated that the width of the tongue was significantly ($P < 0.05$) wider in the adult ostriches than in the young ostriches. The tongue width did not differ between males and females. Tadjalli et al. (2008) measured the width of the tongue (1.92 cm) in young ostriches. In this study, the weight of the tongue was determined in the young and adult ostriches. The mean values remained non-significant ($P > 0.05$) between the sexes in a group, but were highly significantly ($P < 0.05$) different between the young and adult groups. A larger length and width of the tongue may be linked with the higher production of saliva which helps the digestion of food. The strong intrinsic muscles control the movement of the tongue (Beason 2003; Olsen et al. 2011). Tactile and temperature receptors are present on the surface of their tongue, which helps to position and identify (sensitive to cold and hot) the food before swallowing it (Beason 2003; Olsen et al. 2011).

The present study revealed that the epithelium of the tongue had squamous shaped cells with stratifications and are not keratinised. The propria-submucosa contained salivary glands, dense irregular connective tissue (DICT), arteries, veins and lymphatics. The tunica muscularis was comprised of skeletal muscles that were divided into two layers, inner circular and outer longitudinal ones, which can be a link to its strong prehension and mixing of the food with the saliva. The tunica adventitia was composed of loose connective tissue. Jackowiak and Ludwig (2008) and Guimaraes et al. (2009) also described similar findings in three- to five-year old and one and a half year old ostriches, respectively.

Oesophagus

The gross biometrical results revealed that the mean oesophagus length differed significantly ($P < 0.05$) among all the young groups and also the mean values of the adult group were significantly ($P < 0.05$) higher than the young group's readings and remained non-significant ($P > 0.05$) between the sexes in the same age group (Table 2). No literature is available on the oesophagi of ostriches to compare these findings to. Swallowing is accomplished by oesophageal peristalsis, and, in most birds, appears to be aided by the extension of the neck. This study conflicted with the study of Bailey et al. (1997), who described the oesophagus as being significantly longer in male bustards when compared to females. The present study indicated that the oesophagus was significantly ($P < 0.05$) longer in the young ostriches than in the adult ostriches (225.60 ± 8.13 g) without any ingesta.

The oesophagus of ostriches consisted of four layers, i.e., mucosa, sub-mucosa, muscularis and adventitia. The oesophagus epithelium was composed of squamous shape cells. Simple straight tubular glands and connective tissue (loose to dense) were present in the lamina propria. The lamina muscularis layer was formed of a well-developed layer of longitudinally arranged smooth muscles. The tela submucosa was a thin layer and consisted of a DICT, the sub-mucosal nerve and vascular plexus. Similar trends were found in other species of birds like the duck, chicken, geese, and pheasant (Srisai et al. 2002; Nagy et al. 2005; Shiina et al. 2005; Qureshi et al. 2017). Guimarraes et al. (2009) also supported analogous findings in ostriches. The muscularis tunic was composed of smooth muscles and divided into two layers according to the orientation, either inner circular and outer longitudinal, the same as reported in chickens (Rossi et al. 2005). Shiina et al. (2005) reported that the

https://doi.org/10.17221/120/2020-VETMED

oesophagus consisted of two regions (cervical and thoracic) in all birds and was composed of smooth muscles. In this study, the thickness of all the layers was measured in the ostriches. All the layers were (P < 0.05) thicker in the adult group as related to the young group, but these values did not change within the sexes of the ostriches. Qureshi et al. (2017) also reported congruent findings in ducks.

Proventriculus and gizzard

Ostriches do not have a crop. The proventriculus and gizzard can fulfil the crop's function (storage) (Bezuidenhout 2001). The shape of the proventriculus was elongated and oval, so that it was not constricted in the middle like other avian species. Its wall thickness was variable, the cardiac region's thickness had a minimum and membranous thickness, contrarily, but the half pyloric region was thin-walled, yet half of it was thick-walled. The proventriculus gland was dumbbell-shaped. The same organ shape was reported in doves and owls by Mol (2010) and in falcons by Abumandour (2013). Similar results were reported in ostriches by Mahdy (2009). Hassan and Moussa (2012) revealed that the proventriculus was cone-shaped in the pigeon. The actual stomach of the ostrich was sac-shaped and located at the cranial part of the abdomen in the left hypochondrium (Bezuidenhout 2001). In this part, the protein digestion begins by the secretion of pepsinogen and hydrochloric acid (Camiruaga et al. 2003), but the main function of the gizzard is mechanical grinding of the hard food particles. The present study indicated that the proventriculus was significantly (P < 0.05) longer from one to the next age group and the mean values of adult group were also (P < 0.05) higher than the young group's readings and remained non-significant (P > 0.05) between the sexes in the same age group (Table 2). Hassan and Moussa (2012) reported the length of the proventriculus in pigeons to be 26 ± 2.16 mm and was 60 ± 5.16 mm in ducks. The length of the proventriculus in the Muscovy duck was 56 ± 6.43 mm, as reported by Madkour (2015). For ostriches, the efficiency of the dry feed digestibility is enhanced due to the presence of the large glandular parts with a thin wall stomach. This is related, in large birds like the ostrich and emu, to the fact that crops are not present in these birds and the stomach plays its part and stored the feed and water for up to twenty hours. In older birds and larger young birds with a slower metabolism, these water reserves may be stored for an even longer time. The present study indicated that the proventriculus length and weight were (P < 0.05) increased among all the young groups and also the mean values of the adult group were significantly (P < 0.05) higher than the young group's readings and remained non-significant (P > 0.05) between the sexes in the same age group (Table 2). Wu et al. (2010) reported the proventriculus length and weight was 60 mm and 35.9 g, respectively, in pheasants.

The shape of the gizzard was like a biconvex lens and was located left to the midline of in the body cavity. The internal membrane of the gizzard, the koilin layer was very rigid, and detachable. Its colour was yellowish-green, which was due to the bile reflux and its feed content. Elnagy and Osman (2010) revealed that the gizzard was an elongated dilation between the oesophagus and the intestines of the digestive tract in rabbits. Wu et al. (2010) reported a dark purple colour of the gizzard in the pheasant, which was due to its feed. These length and weight values in the gizzard and proventriculus are different in different species because it depends upon the feed intake and body size. Szczepanczyk (2005) reported that the shape and length of the gizzard and proventriculus of the long-tailed duck depended upon its feed intake. Nasrin et al. (2012) revealed that the weight of the gizzard was 5.32 g in broiler chickens.

In comparison, the ostrich gizzard shape looked like a hen's gizzard, but the ostrich gizzard weight (1 001–1 150 g) was 12 times heavier than the weight in the hen's gizzard (52.0–81.0 g). The comparative analysis with other running birds revealed that the area of the glandular part of the ostrich was proportionally more extensive than the area of the muscular part and the area of the deep glandular region of the ostrich proventriculus was smaller than that of other running birds as well as the stomach mucosal surface area was only 25% as compared to other running birds (Cooper and Mahroze 2004; Sales 2006). Ostriches have two parts of the stomach; the proventriculus secretes gastric juice and the gizzard acts like a grinder. Variations have occurred in many stomach characteristics in different species of animals during the development of the digestive tract (Fukuda and Yasugi 2005; Mason et al. 2013; Garcia et al. 2014).

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The proventriculus epithelium (in ostrich) was composed of columnar shape cells. The present study results revealed that the epithelium was significantly ($P < 0.05$) higher the adult groups when compared to the young groups, but these values were non-significant ($P > 0.05$) within both ostrich sexes. The advancing age was positively correlated with the thickness of all the layers of the proventriculus and the gizzard. Qureshi et al. (2017) reported comparable findings in ducks. The lamina muscularis was composed of well-developed layers of longitudinally arranged smooth muscles. The data in Table 3 may serve as orientation values e.g. in diagnosis of gastric impaction of ostriches which is a life-threatening condition (Irfan et al. 2020). The lamina muscularis in the gizzard was significantly ($P < 0.05$) thicker in the adult groups when compared to the young age groups. The results of this study were similar to those of Wang et al. (2017). They measured the thickness of the proventriculus glands and muscularis mucosae in ostriches. The tunica muscularis was composed of thick layers of smooth muscles and divided into two layers according to the orientation, outer longitudinal and inner circular. The mean thickness values of circular and longitudinal muscles were significantly ($P < 0.05$) thicker within young group, however, mean values were significantly ($P < 0.05$) lower than that of adults group (Table 3). The layers of the tunica muscularis were found to be positively related to the progressing age. Rahman et al. (2003) measured the muscularis tunic's thickness of gizzards in two-week-old quail. Qureshi et al. (2017) and Starck and Rahman (2003) reported similar results for the tunica muscularis in immature, adult and old age quail groups. The tunica serosa was composed of connective tissues and a layer of mesothelial cells. The outermost layer tunica serosa was also significantly ($P < 0.05$) developed in the adults as compared to the young groups. Wang et al. (2017) reported data similar to these results on tunica serosa in ostriches. All the layers of the proventriculus and gizzard were thicker ($P < 0.05$) in the adult groups when compared with immature groups, but these values were not significantly different between males and females.

The current study was aimed at a comprehensive histomorphometric evaluation of the organs of the cranial digestive system in ostriches of both sexes and various ages along with the basic measures of the cranial GIT segments. Close scrutiny of the data indicates that the development and growth of the cranial digestive organs is rapid during the young age; however, it maintains a plateau with minor increments in the adult age. The sex of the birds had no significant effect on the development and growth of the different cranial digestive organs in ostriches (*Struthio camelus*).

These findings can serve e.g. for the manipulation in ostrich feeding and nutrition, and diagnosis of pathological processes.

Acknowledgement

We are grateful to the Signature meat shop Lahore and Riphah Veterinary College, Lahore in Pakistan for allowing us to carry out the study and their practical support.

We also thank Dr. Muhammad Usman and Dr. Adeel Sarfraz for help in the conduct of laboratory work.

Conflict of interest

The authors declare no conflict of interest.

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Received: May 29, 2020
Accepted: December 16, 2020