



## RESEARCH ARTICLE

### Macroscopic, Microscopic and Histomorphometric Analysis of Intestine, Liver and Pancreas of Ostrich (*Struthio camelus*) with Advancement of Age and Sex

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#### ABSTRACT

The primary goal of this study was to document the gross morphological and histological structure and histomorphometrical measurements of the intestines and digestive glands of ostrich (*Struthio camelus*) in relation to advancing age and sex. A total of 40 clinically healthy ostriches of either sex [(20 males, 20 females comprising of two age groups, viz, immature (1 month to 2.4 years) and adult (2.5 to 10 years)] of equal size were selected. Immediately after collection, morphological studies were carried out on all organs. Histological and histometrical studies were conducted on paraffin-embedded tissue sections with Image J<sup>®</sup> analysis software. Macroscopic parameters of the intestines, liver and pancreas invariably showed a rapid increase ( $P < 0.05$ ) during immature age but maintained a plateau with negligible increment in an adult age group. Contrary to other avian species, the length of the small intestine in adult ostriches was 706 cm and the large intestine was 1218 cm which means the length of the large intestine is 1.73 times greater than the small intestine. The length of the colon is also larger than the comparable avian species which increases the digestion of fiber. Thicknesses of all layers of intestines and various parameters of digestive glands had a significant ( $P < 0.05$ ) increase in adults compared to immature ostriches. The growth and maturation of digestive organs in ostriches were not related to sex in each age group. These findings can be extremely beneficial for strategic manipulation of feed and nutrition to enhance the growth rate and diagnose pathological processes.

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#### INTRODUCTION

Globally ostrich farming is in continuous growth, making its place in the market. Even with the importance of ostrich, there is a gap in our knowledge of their precise nutrient requirements and diagnosis of pathological processes (Umar *et al.*, 2021). Birds richly depend upon their digestive system to stay healthy and well-nourished. The gastrointestinal tract is involved in intestinal digestion, resorption, secretion and barrier function (Alshamy *et al.*, 2018). The anatomically gastrointestinal tract is fully developed but not fully mature at the time of hatching. The digestive tract bears adequate morphological flexibility for the capacity of variation in nutritional needs (Marc, 2014). The digestive passage of a bird has such anatomy that its milieu is favorable for the

chemical and physical decrease in the size and complex molecular nature of food, which promotes digestion and absorption of the greatly varying quantities of the end-products.

Many metabolic processes are performed by the liver including bile secretion, metabolism of lipid, carbohydrate and protein. The liver can adapt easily to feed and environment alteration. Being at the center of many digestive, metabolic and productive activities, understanding this organ and the factors affecting liver functionality is essential (Zaefarian *et al.*, 2019).

Avian pancreas, accessory digestive gland, showed some differences of structure and function like division of lobes, distribution of islets in lobes, special features in the structure of the ducts and form and frequency of endocrine cell in islets (Saadatfar *et al.*, 2011).

This study aimed to investigate specific morphological and histological characteristics of the intestine and associated digestive glands including liver and pancreas, in consideration of sex and advancing age under local conditions.

## MATERIALS AND METHODS

**Birds and sample management:** Samples of the intestine (duodenum, jejunum, ileum, cecum, colorectum) and associated digestive glands (liver and pancreas) were collected from Signature Meat Shop Lahore (Slaughterhouse) and Riphah Veterinary College Lahore. Before slaughtering, ostriches were kept in farms under optimal managemental conditions. Grass and wheat seeds were fed daily and water was available ad libitum. A total of 40 clinically healthy ostriches (*Struthio camelus*) of either sex (20 males, 20 females) was divided into two age groups of equal size viz., immature (1 month to 2.4 years) and adult (2.5 to 10 years). Soon after slaughtering, macroscopic parameters were measured, and after that, organs dipped in 0.5% saline.

**Macroscopic examination:** Immediately after the slaughter of each bird, organs under investigation were collected and carefully examined for their macroscopic features including color, shape and consistency. The electrical weighing balance was used for the determination of the weight of organs. Length (cm), width (cm) and diameter (cm) were taken by a measuring tape and Vernier caliper. The duodenal length was measured from the start of the gizzard to the ascending and descending part of the duodenum around the pancreas (Fig. 2). Jejunum length was measured from the terminal part of the duodenum to the start of coiling with mesentery. Similarly, ileum length was measured from coiling around the mesentery to the opening into the caecae. Cecael length was measured from apex to root of caecae (Fig. 2). Similarly, Colorectum was measured from the end of the root of the caecae up to the cloacal opening (Fig. 2). The width of the liver was taken as the combined width of the left and right lobe and the length of the liver was measured along the long axis of the

liver (Fig. 3). The pancreas width was measured between the loops of the duodenum and length was measured from the start to the end of the loop (Fig. 3).

**Microscopic and histometric analysis:** All collected samples were fixed in the neutral buffered formalin. Paraffin blocks were prepared by routine embedding technique (Suvama *et al.*, 2018). Tissue blocks were cut at 5-6  $\mu$ m thickness and stained with Hematoxylin and Eosin (H&E). Photomicrographs of each sample were taken with the help of a microscope at 100X. Image J<sup>®</sup>1.80v software was used for histometric measurements.

**Statistical analysis:** Factorial one-way analysis of variance (ANOVA) and Tukey's honest significant test at a 5% level of significance were used to compare the means of parameters (Rushing *et al.*, 2013).

## RESULTS

### Macroscopic examination

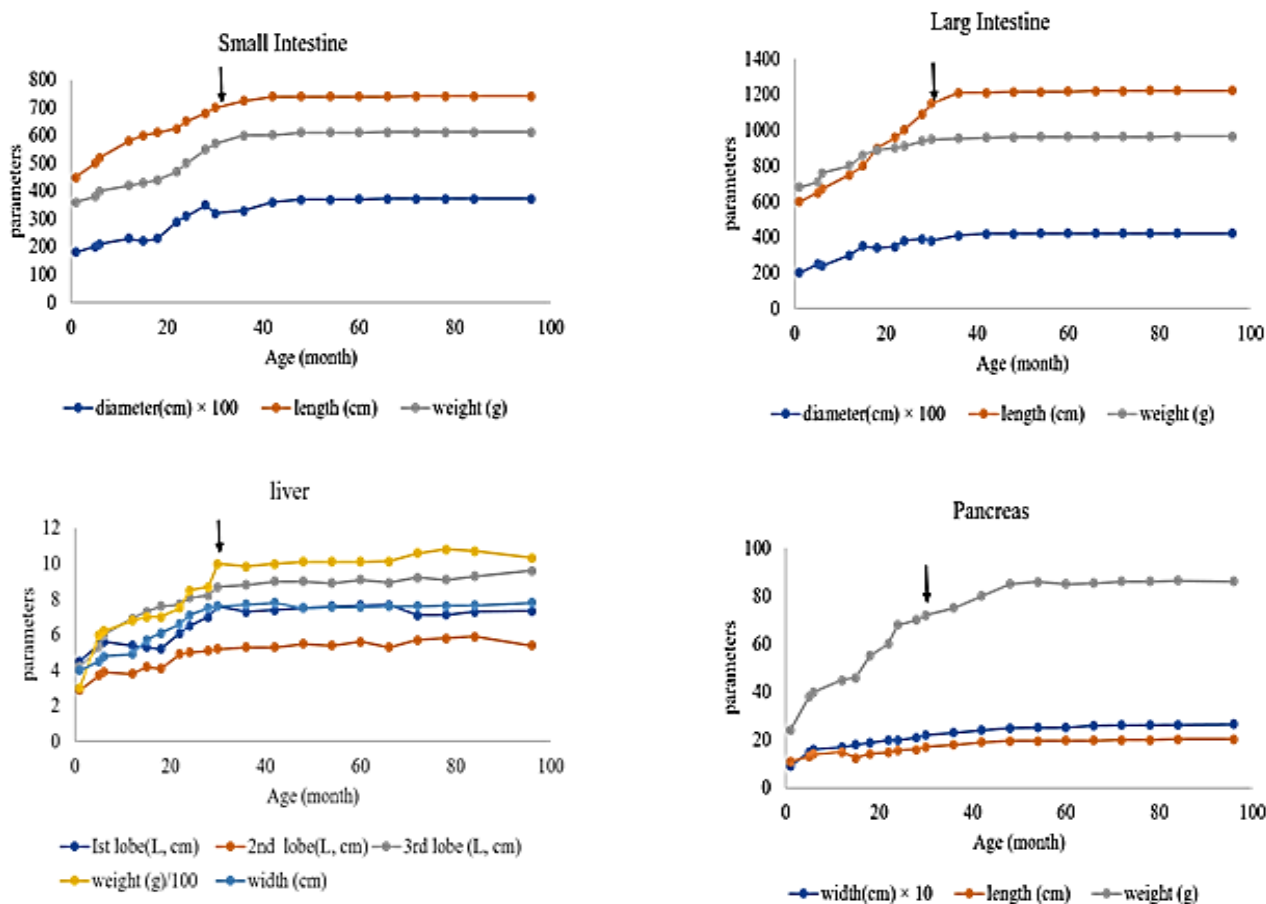
**Intestine:** It was a hollow tube-like structure. The color of the small intestine was pink but toward the ileorectal junction, it gradually changed into darker red-green (Fig. 2). It accounts for 50% of the total digestive tract. The color of the large intestine was dark green internally and lighted pink externally. The colon and rectum were the largest part of the large intestine. The caecae and colon were sacculated, thin-walled and large lumen (Fig. 2). The rectum was thickly walled with the narrow lumen, free of sacculation and containing firm fecal matter in the form of pallets.

**Digestive glands:** The color of the liver was reddish-brown. The liver of the ostrich has three lobes: right, left and intermediate (Fig. 3). The left lobe of the liver was divided into a small caudodorsal lobe and a large caudoventral lobe. The right and intermediate lobes were undivided. There was no gall bladder. The pancreas was located in the mesentery between the loops of the duodenum. The pancreatic duct opened into the ascending limb of the duodenal loop (Fig. 3).

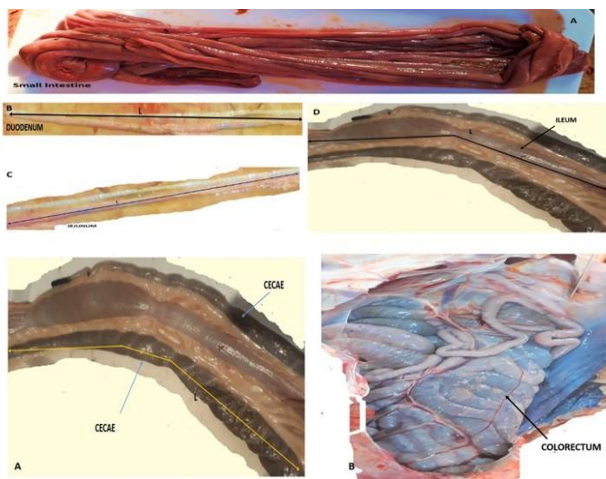
**Table 1:** Means ( $\pm$ SEM) of the macroscopic parameter of the intestine of ostriches (*Struthio camelus*) of two age-groups and sex.

Organs	Parameters	Immature (1 month to 2.4 years)		Adult (2.5 years to 10 years)	
		Male	Female	Male	Female
Duodenum	Diameter (cm)	2.20 $\pm$ 0.07 <sup>a</sup>	2.18 $\pm$ 0.07 <sup>a</sup>	2.98 $\pm$ 0.06 <sup>b</sup>	3.12 $\pm$ 0.14 <sup>b</sup>
	Length (cm)	132.08 $\pm$ 4.64 <sup>a</sup>	134.20 $\pm$ 3.31 <sup>a</sup>	187.80 $\pm$ 2.15 <sup>b</sup>	189.60 $\pm$ 3.22 <sup>b</sup>
	Weight (g)	88.60 $\pm$ 2.60 <sup>a</sup>	90.40 $\pm$ 2.73 <sup>a</sup>	121.20 $\pm$ 3.61 <sup>b</sup>	121.20 $\pm$ 3.61 <sup>b</sup>
Jejunum	Diameter (cm)	2.20 $\pm$ 0.07 <sup>a</sup>	2.36 $\pm$ 0.09 <sup>a</sup>	2.98 $\pm$ 0.06 <sup>b</sup>	3.140 $\pm$ 0.13 <sup>b</sup>
	Length (cm)	243.84 $\pm$ 1.70 <sup>a</sup>	247.76 $\pm$ 2.19 <sup>a</sup>	277.20 $\pm$ 0.86 <sup>b</sup>	282.60 $\pm$ 1.3 <sup>b</sup>
	Weight (g)	177.00 $\pm$ 5.39 <sup>a</sup>	183.00 $\pm$ 5.15 <sup>a</sup>	242.40 $\pm$ 3.50 <sup>b</sup>	237.60 $\pm$ 5.64 <sup>b</sup>
Ileum	Diameter (cm)	2.20 $\pm$ 0.07 <sup>a</sup>	2.44 $\pm$ 0.16 <sup>a</sup>	3.04 $\pm$ 0.051 <sup>b</sup>	3.14 $\pm$ 0.05 <sup>b</sup>
	Length (cm)	249.98 $\pm$ 0.51 <sup>a</sup>	251.67 $\pm$ 2.05 <sup>a</sup>	277.37 $\pm$ 1.07 <sup>b</sup>	277.80 $\pm$ 1.43 <sup>b</sup>
	Weight (g)	181.80 $\pm$ 3.29 <sup>a</sup>	190.40 $\pm$ 2.25 <sup>a</sup>	241.80 $\pm$ 2.89 <sup>b</sup>	237.60 $\pm$ 5.64 <sup>b</sup>
Caecae	Diameter (cm)	2.96 $\pm$ 0.23 <sup>a</sup>	3.04 $\pm$ 0.19 <sup>a</sup>	4.00 $\pm$ 0.07 <sup>b</sup>	4.00 $\pm$ 0.07 <sup>b</sup>
	Length (cm)	145.20 $\pm$ 2.52 <sup>a</sup>	148.28 $\pm$ 1.83 <sup>a</sup>	190.80 $\pm$ 1.69 <sup>b</sup>	192.60 $\pm$ 0.93 <sup>b</sup>
	Weight (g)	80.20 $\pm$ 3.35 <sup>a</sup>	82.80 $\pm$ 3.61 <sup>a</sup>	118.80 $\pm$ 2.42 <sup>b</sup>	118.60 $\pm$ 2.32 <sup>b</sup>
Colon	Diameter (cm)	2.82 $\pm$ 0.15 <sup>a</sup>	3.00 $\pm$ 0.13 <sup>a</sup>	4.06 $\pm$ 0.05 <sup>b</sup>	4.12 $\pm$ 0.06 <sup>b</sup>
	Length (cm)	452.60 $\pm$ 3.31 <sup>a</sup>	457.20 $\pm$ 3.89 <sup>a</sup>	508.40 $\pm$ 2.16 <sup>b</sup>	501.60 $\pm$ 2.58 <sup>b</sup>
	Weight (g)	362.00 $\pm$ 8.46 <sup>a</sup>	363.20 $\pm$ 7.76 <sup>a</sup>	420.00 $\pm$ 7.07 <sup>b</sup>	419.00 $\pm$ 6.02 <sup>b</sup>
Rectum	Diameter (cm)	2.82 $\pm$ 0.15 <sup>a</sup>	2.98 $\pm$ 0.06 <sup>a</sup>	4.06 $\pm$ 0.05 <sup>b</sup>	3.96 $\pm$ 0.05 <sup>b</sup>
	Length (cm)	462.97 $\pm$ 4.05 <sup>a</sup>	468.60 $\pm$ 3.98 <sup>a</sup>	520.00 $\pm$ 1.61 <sup>b</sup>	521.60 $\pm$ 2.25 <sup>b</sup>
	Weight (g)	360.00 $\pm$ 7.07 <sup>a</sup>	370.2 $\pm$ 10.6 <sup>a</sup>	420.20 $\pm$ 8.89 <sup>b</sup>	422.20 $\pm$ 5.13 <sup>b</sup>

ab: Different alphabet superscripts in the mean values showed significant ( $P < 0.05$ ) results from one another ( $P < 0.05$ ).



**Fig. 1:** Mean ( $\pm$ SEM) of length, weight, diameters and width of the intestine and digestive glands of ostriches (*Struthio camelus*) of two different age-groups. 1-30 months: immature; 30-100 months: adult age group (arrow show adult age group in graphs).



**Fig. 2:** The small and large intestine of ostriches. length (L).

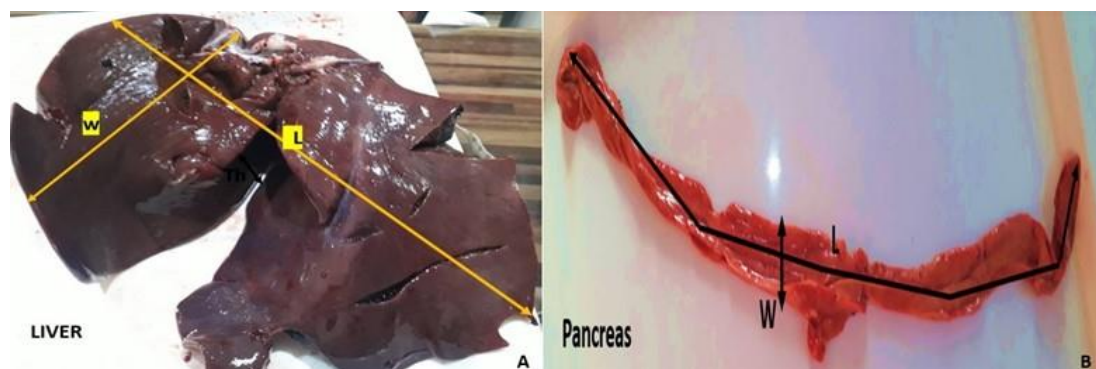
### Histological examination

**Intestine:** It consisted of four layers, i.e., inward to outward, mucosa, submucosa, muscularis tunics and tunica serosa. The small intestine was consisting of villi and the large intestine was consist of plicae circularis. Simple straight tubular gland and connective tissue were distributed in lamina propria. Tunica submucosa was composed of dense irregular connective tissue (DICT), submucosal plexus and blood vessels. Tunica muscularis comprised of smooth muscles that were divided into circular and longitudinal orientation. Between the two layers of tunica muscularis was consist of myenteric

plexus. Tunica serosa was composed of mesothelial cells and connective tissues (Fig. 4, 5).

**Digestive glands:** The liver and pancreas were covered by a connective tissue capsule, which extended into lobes and divided the liver into lobules. The liver was composed of an unlimited number of lobules. Lobules consisted of cords of hepatocyte, central vein, sinusoids and biliary duct system. The hepatocytes were attached to each other in a hexagonal arrangement forming hepatic plates. Ostrich had no gall bladder (Fig. 6). Microscopically, the pancreas had two parts: the exocrine and endocrine portions. The exocrine part was composed of acini and the endocrine part consist of islets of Langerhans (Fig. 6).

**Morphometrical analysis:** Mean  $\pm$  SEM values of morphometric parameters including length (cm), width (cm), diameter (cm) and weight (g) of the small- and large intestine and digestive glands of either sex in immature and adult age groups of ostriches were presented in Table 1 and Table 2. Statistical analysis revealed that age is directly related to the morphometric values of the organs under study. The mean values of each parameter of adult age group were found significantly ( $P < 0.05$ ) greater than the immature group of birds. Sex did not alter any parameter within the same age group (Table 1, 2). A rapid increase ( $P < 0.05$ ) in the morphometrical parameters of the intestine and associated digestive organs were observed in immature birds which, however, maintained a plateau after attaining maturity at the 2.5 years with minor increments (Fig. 1).



**Fig. 3:** The digestive glands of ostriches. length (L); width (W); Thickness (Th).

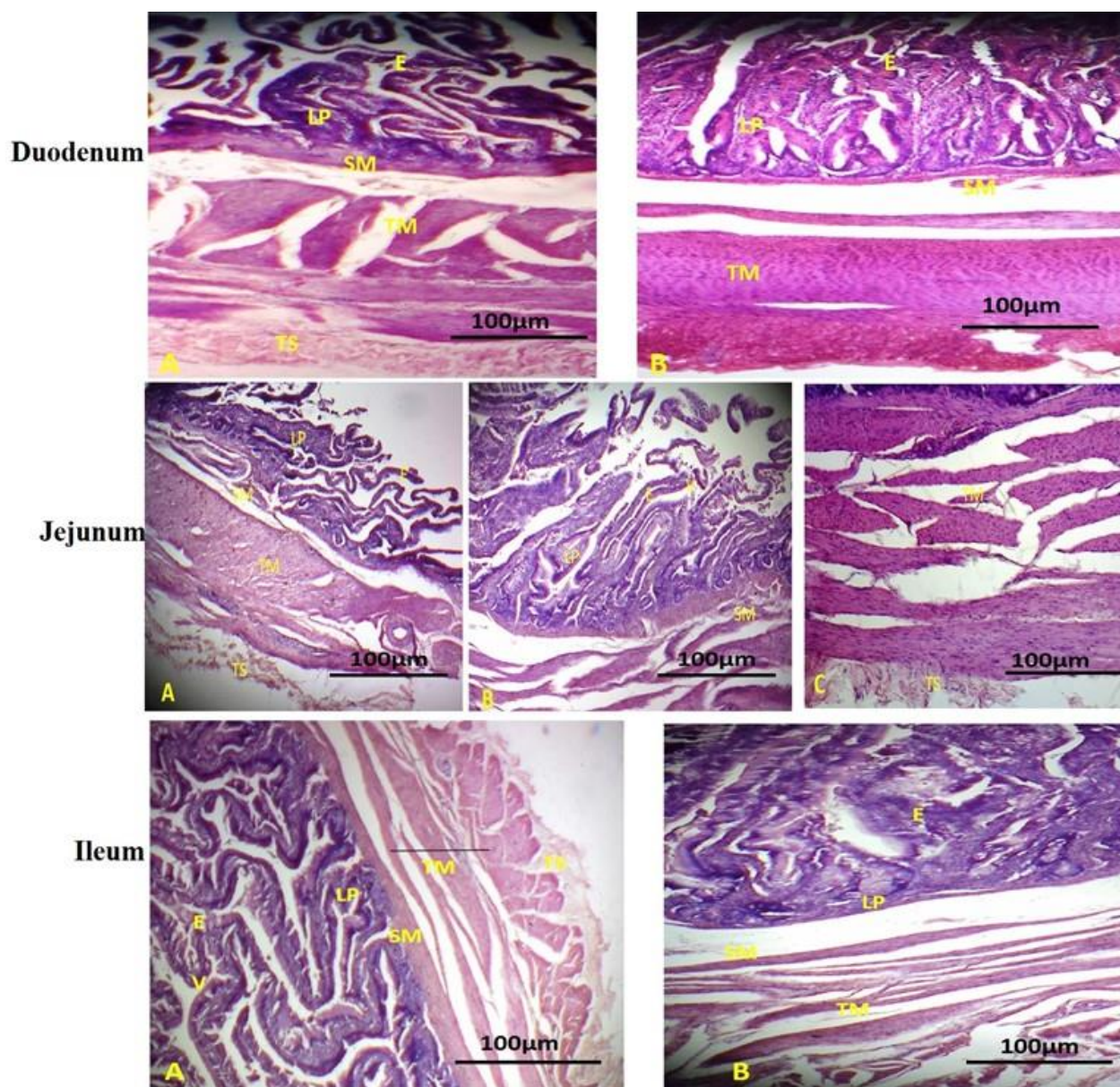
**Table 2:** Means±SEM of the macroscopic parameter of the liver and pancreas of ostriches (*Struthio camelus*) of two age-groups and sex.

Organs	Parameters	Immature (1 month to 2.4 years)		Adult (2.5 years to 10 years)		
		Male	Female	Male	Female	
Liver	Length (cm)	1 <sup>st</sup> Lobe (L)	15.40±0.07 <sup>a</sup>	15.20±0.07 <sup>a</sup>	24.36±0.10 <sup>b</sup>	24.30±0.10 <sup>b</sup>
		2 <sup>nd</sup> Lobe (I)	7.90±0.07 <sup>a</sup>	6.94±0.09 <sup>a</sup>	10.12±0.31 <sup>b</sup>	10.30±0.07 <sup>b</sup>
		3 <sup>rd</sup> Lobe (R)	17.14±0.09 <sup>a</sup>	16.76±0.07 <sup>a</sup>	25.46±0.19 <sup>b</sup>	25.56±0.17 <sup>b</sup>
		4 <sup>th</sup> Lobe (CD)	12.0±0.32 <sup>a</sup>	11.5±0.32 <sup>a</sup>	15±0.04 <sup>b</sup>	14.60±0.07 <sup>b</sup>
		5 <sup>th</sup> Lobe (CV)	8±0.07 <sup>a</sup>	7.8±0.07 <sup>a</sup>	11±0.04 <sup>b</sup>	10.5.60±0.07 <sup>b</sup>
	Weight (gm)	854.00±9.80 <sup>a</sup>	854.00±9.80 <sup>a</sup>	976.20±4.94 <sup>b</sup>	960.00±7.07 <sup>b</sup>	
	Width (cm)	10.68±0.32 <sup>a</sup>	10.68±0.32 <sup>a</sup>	13.62±0.06 <sup>b</sup>	13.60±0.0707 <sup>b</sup>	
	Thickness (cm)	4.90±0.17 <sup>a</sup>	4.90±0.17 <sup>a</sup>	7.16±0.10 <sup>b</sup>	7.31±0.11 <sup>b</sup>	
	Length (cm)	13.10±0.68 <sup>a</sup>	13.10±0.71 <sup>a</sup>	17.92±0.65 <sup>b</sup>	17.90±0.64 <sup>b</sup>	
Pancreas	Weight (gm)	38.60±3.94 <sup>a</sup>	36.40±4.80 <sup>a</sup>	76.40±2.73 <sup>b</sup>	78.40±2.66 <sup>b</sup>	
	Width (cm)	3.52±0.14 <sup>a</sup>	3.60±0.07 <sup>a</sup>	5.20±0.07 <sup>b</sup>	5.30±0.07 <sup>b</sup>	

ab: Different alphabet superscripts in the mean values showed significant (P<0.05) results from one another (P<0.05).

**Table 3:** Mean (±SEM) thickness (µm) in various layers of digestive organs of the ostriches (*Struthio camelus*) at two age-groups and sexes.

Organs	Layers (µm)	Immature (1 month to 2.4 years)		Adult (2.5 years to 10 years)	
		Male	Female	Male	Female
Duodenum	Villi length	121.3±11.9 <sup>a</sup>	182.0±27.1 <sup>a</sup>	309.0±27.4 <sup>b</sup>	297.2±51.2 <sup>b</sup>
	Epithelium	10.079±0.84 <sup>a</sup>	11.90±2.21 <sup>a</sup>	21.75±4.56 <sup>b</sup>	18.27±2.98 <sup>b</sup>
	Lamina Propria	18.05±2.87 <sup>a</sup>	19.27±2.18 <sup>a</sup>	23.8±2.58 <sup>b</sup>	22.0±1.52 <sup>b</sup>
	Laminae Muscularis	10.29±1.16 <sup>a</sup>	14.17±1.98 <sup>a</sup>	25.99±1.51 <sup>b</sup>	28.41±4.68 <sup>b</sup>
	Submucosa	41.68±9.22 <sup>a</sup>	29.7±10.0 <sup>a</sup>	82.94±8.54 <sup>b</sup>	86.37±2.61 <sup>b</sup>
	Tunica Muscularis (C)	75.90±4.06 <sup>a</sup>	81.02±6.57 <sup>a</sup>	119.5±22.0 <sup>b</sup>	145.4±11.2 <sup>b</sup>
	Tunica Muscularis (L)	68.871±0.88 <sup>a</sup>	56.70±5.71 <sup>a</sup>	128.85±1.55 <sup>b</sup>	118.84±6.53 <sup>b</sup>
	Tunica Serosa	29.57±2.61 <sup>a</sup>	32.79±2.79 <sup>a</sup>	45.75±5.49 <sup>b</sup>	64.93±6.44 <sup>b</sup>
	Villi length	188.9±14.0 <sup>a</sup>	186.9±17.6 <sup>a</sup>	272.5±14.9 <sup>b</sup>	268.6±12.6 <sup>b</sup>
	Epithelium	14.45±3.51 <sup>a</sup>	13.52±2.78 <sup>a</sup>	21.48±1.91 <sup>b</sup>	23.06±2.20 <sup>b</sup>
Jejunum	Lamina Propria	17.05±2.37 <sup>a</sup>	19.27±2.15 <sup>a</sup>	22.8±2.48 <sup>b</sup>	21.0±1.42 <sup>b</sup>
	Laminae Muscularis	9.029±0.98 <sup>a</sup>	11.21±0.87 <sup>a</sup>	41.18±1.12 <sup>b</sup>	40.10±2.29 <sup>b</sup>
	Submucosa	46.7±10.1 <sup>a</sup>	50.4±10.1 <sup>b</sup>	80.2±24.2 <sup>b</sup>	82.7±24.3 <sup>b</sup>
	Tunica Muscularis (C)	98.16±8.70 <sup>a</sup>	88.65±9.08 <sup>a</sup>	147.39±9.29 <sup>b</sup>	145.54±6.30 <sup>b</sup>
	Tunica Muscularis(L)	69.49±4.82 <sup>a</sup>	73.84±4.14 <sup>a</sup>	142.0±11.1 <sup>b</sup>	158.1±11.2 <sup>b</sup>
	Tunica Serosa	18.66±1.45 <sup>a</sup>	19.82±3.65 <sup>a</sup>	50.82±4.19 <sup>b</sup>	57.14±2.90 <sup>b</sup>
	Villi length	186.9±17.6 <sup>a</sup>	187.5±17.6 <sup>a</sup>	275.5±15.7 <sup>b</sup>	275.5±15.6 <sup>b</sup>
	Epithelium	13.52±2.78 <sup>a</sup>	14.11±2.70 <sup>a</sup>	28.14±1.51 <sup>b</sup>	27.74±1.46 <sup>b</sup>
	Lamina Propria	19.27±2.15 <sup>a</sup>	19.67±2.36 <sup>a</sup>	54.0±14.2 <sup>b</sup>	51.6±14.2 <sup>b</sup>
	Laminae Muscularis	11.216±0.87 <sup>a</sup>	11.63±0.84 <sup>a</sup>	40.10±2.29 <sup>b</sup>	40.70±2.18 <sup>b</sup>
Ileum	Submucosa	45.5±10.2 <sup>a</sup>	48.67±9.69 <sup>a</sup>	82.7±24.3 <sup>b</sup>	83.7±24.3 <sup>b</sup>
	Tunica Muscularis (C)	88.65±9.08 <sup>a</sup>	89.45±8.94 <sup>a</sup>	145.54±6.30 <sup>b</sup>	145.94±6.53 <sup>b</sup>
	Tunica Muscularis (L)	73.84±4.14 <sup>a</sup>	74.46±4.31 <sup>a</sup>	147.8±10.0 <sup>b</sup>	148.0±10.1 <sup>b</sup>
	Tunica Serosa	19.74±3.68 <sup>a</sup>	20.34±3.65 <sup>a</sup>	57.14±2.90 <sup>b</sup>	57.14±2.90 <sup>b</sup>
	Epithelium	9.80±1.59 <sup>a</sup>	11.431±0.70 <sup>a</sup>	57.8±11.1 <sup>b</sup>	43.9±11.0 <sup>b</sup>
	Lamina Propria	47.4±11.4 <sup>a</sup>	44.1±11.7 <sup>a</sup>	70.09±4.69 <sup>b</sup>	72.57±8.79 <sup>b</sup>
	Laminae Muscularis	16.05±1.78 <sup>a</sup>	14.209±0.63 <sup>a</sup>	27.34±2.42 <sup>b</sup>	27.86±3.22 <sup>b</sup>
	Submucosa	46.20±2.60 <sup>a</sup>	47.34±3.95 <sup>a</sup>	73.7±11.4 <sup>b</sup>	68.2±10.3 <sup>b</sup>
	Tunica Muscularis(C)	78.57±3.14 <sup>a</sup>	82.42±7.85 <sup>a</sup>	199.4±17.9 <sup>b</sup>	186.3±10.5 <sup>b</sup>
	Tunica Muscularis (L)	115.17±1.84 <sup>a</sup>	96.30±8.65 <sup>a</sup>	264.5±22.8 <sup>b</sup>	240.6±20.7 <sup>b</sup>
Cecae	Tunica Serosa	33.71±2.31 <sup>a</sup>	30.398±0.972 <sup>a</sup>	121.85±3.72 <sup>b</sup>	106.2±10.3 <sup>b</sup>
	Epithelium	13.10±4.89 <sup>a</sup>	10.226±0.978 <sup>a</sup>	34.17±3.18 <sup>b</sup>	34.95±3.50 <sup>b</sup>
	Lamina Propria	30.56±1.01 <sup>a</sup>	44.09±6.10 <sup>a</sup>	99.75±6.25 <sup>b</sup>	71.05±8.36 <sup>b</sup>
	Laminae Muscularis	7.54±0.58 <sup>a</sup>	9.22±0.66 <sup>a</sup>	15.22±2.16 <sup>b</sup>	25.24±1.33 <sup>b</sup>
	Submucosa	63.9±13.3 <sup>a</sup>	63.9±13.3 <sup>a</sup>	113.7±20.1 <sup>b</sup>	117.1±24.4 <sup>b</sup>
	Tunica Muscularis (C)	43.75±2.99 <sup>a</sup>	47.35±2.56 <sup>a</sup>	161.0±15.1 <sup>b</sup>	161.0±11.5 <sup>b</sup>
	Tunica Muscularis (L)	32.01±3.02 <sup>a</sup>	33.35±2.61 <sup>a</sup>	111.8±12.0 <sup>b</sup>	123.06±2.89 <sup>b</sup>
	Tunica Serosa	20.94±1.43 <sup>a</sup>	22.60±1.25 <sup>a</sup>	71.86±5.85 <sup>b</sup>	70.76±6.43 <sup>b</sup>
	Hepatocyte	7.62±1.24 <sup>a</sup>	8.96±0.74 <sup>a</sup>	19.93±3.08 <sup>b</sup>	20.51±4.84 <sup>b</sup>
	Central Vein	19.48±1.96 <sup>a</sup>	22.45±2.86 <sup>a</sup>	60.92±3.56 <sup>b</sup>	59.70±3.47 <sup>b</sup>
Pancreas	Islets of Langerhans	60.05±1.45 <sup>a</sup>	59.67±1.79 <sup>a</sup>	67.91±2.91 <sup>b</sup>	68.94±9.39 <sup>b</sup>
	Pancreatic acini	10.02±2.02 <sup>a</sup>	10.68±1.59 <sup>a</sup>	18.25±1.10 <sup>b</sup>	22.74±3.66 <sup>b</sup>



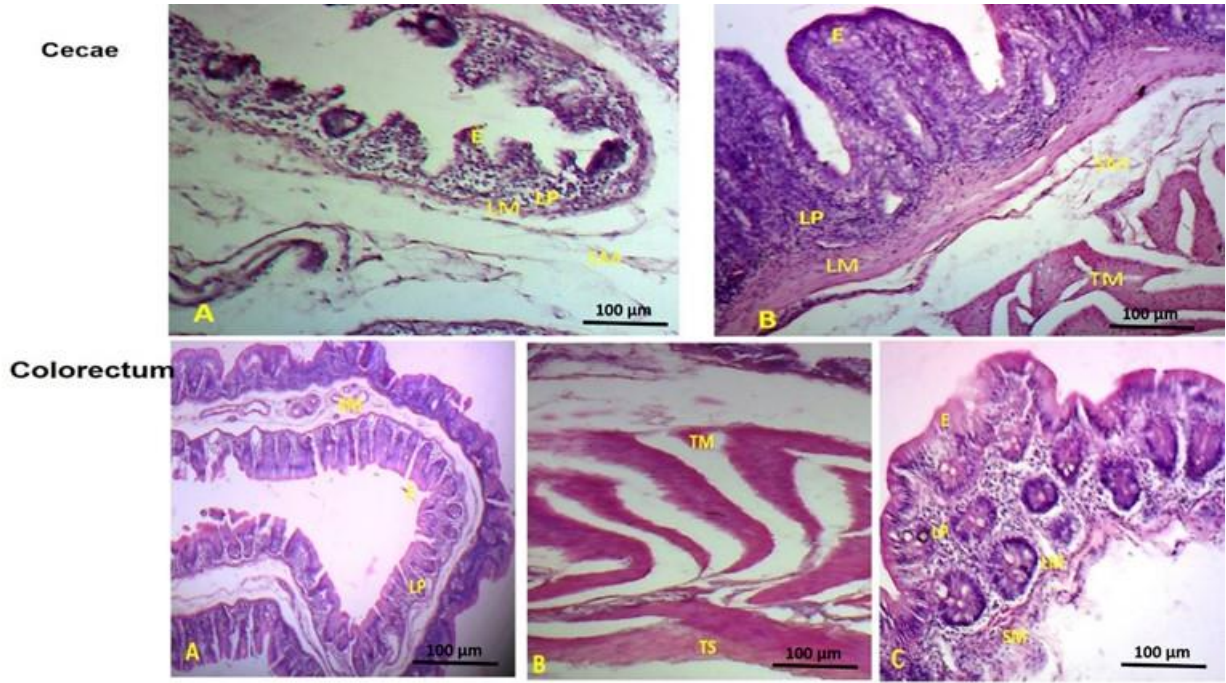
**Fig. 4:** Small intestine of immature ostriches(A); Small intestine of adult ostriches (B, C); V: villi, E: epithelium, LP: lamina propria, Sm: submucosa, TM: tunica muscularis, TS: tunica serosa. (H&E) 100X.

**Histometrical analysis:** Comparison of mean  $\pm$  SEM values of different histometrical parameters of the intestine, liver and pancreas between immature and adult ostriches were depicted in Table 3. According to the statistical analysis, mean values of all measured parameters showed a significant ( $P < 0.05$ ) rise in adult ostriches than the immature ones, however these parameters remained unaltered between both sexes within each age-group (Table 3).

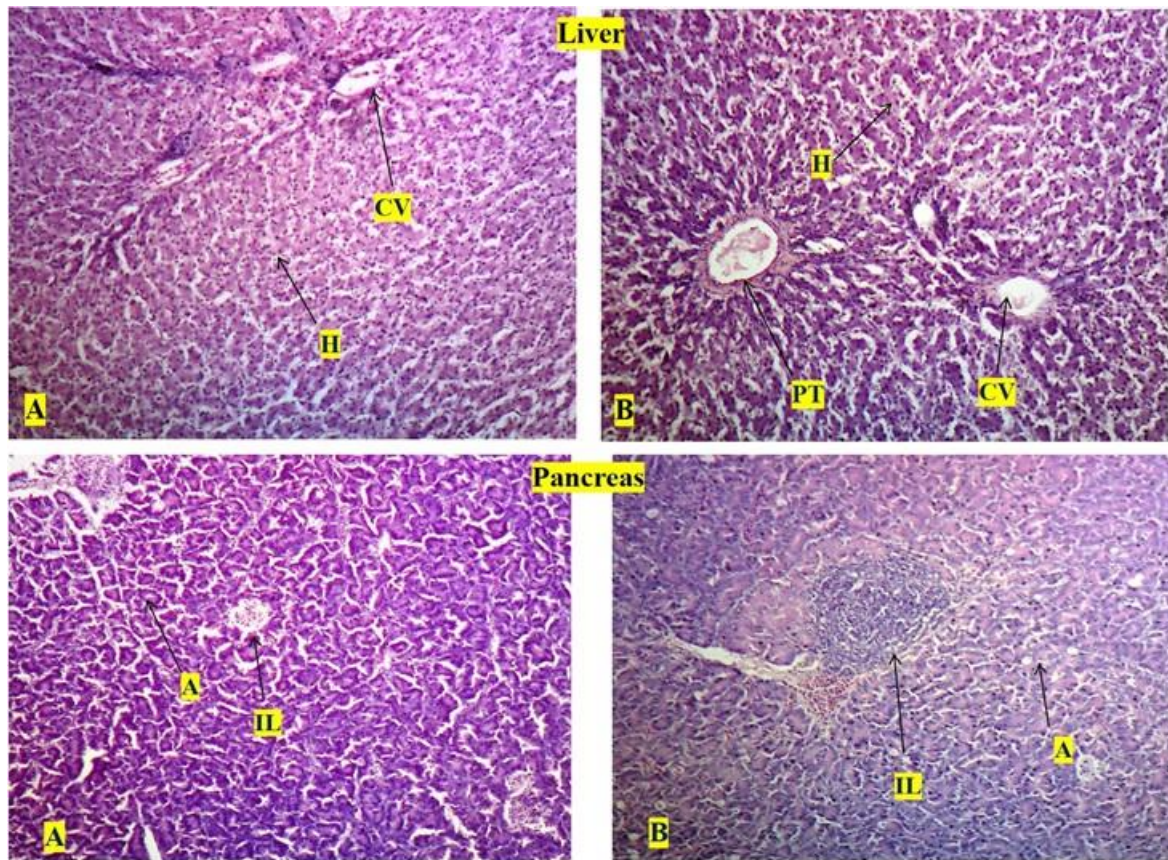
## DISCUSSION

**Intestine:** In this study, the weight and length of the small and large intestines were determined in immature (1 month to 2.4 years) and adult (2.5 to 10 years) ostriches. Iji *et al.* (2003) and Wang and Peng (2008) determined length and weight in three to seventy-two days old and one to ninety days old respectively). Weight and length of the small and large intestines were directly proportional to the age of

ostriches (Wang and Peng, 2008), ducks (Szczepanczyk, 2008) and broilers (Wang *et al.*, 2008). The present study revealed that the length of the small intestine in adult ostriches was 706 cm and the large intestine was 1218 cm. The length of the small intestine in cocks, broiler and duck was 155 cm, 179 cm, 163 cm, and large intestine was 67 cm, 62 cm, 39 cm, respectively (Yang *et al.*, 2013; Mabelebele *et al.*, 2017). These researchers reported the length of the small intestine double as compared to the large intestine of cocks, broiler and duck respectively. In contrast, the current study concluded that the large intestine length is almost 1.72 times longer as compared to the small intestine of ostriches. Although this finding of an ostrich is conflicted with other bird's literature, these findings are in line with Camiruaga *et al.* (2001) who reported that the large intestine of ostrich accounts for 50% and the small intestine accounts for only 35.5% of the digestive tract. In this study, the diameter of the intestines was determined. No literature was available about the diameter of the intestines.



**Fig. 5:** Cecae (A) and Colorectum (A, B) of immature ostriches. Cecae (B) and Colorectum (C) of adult ostriches; E: epithelium, LP: lamina propria, LM: lamina muscularis, SM: submucosa, TM: tunica muscularis. (H&E) 100X.



**Fig. 6:** Liver and Pancreas (A) of immature ostriches. Liver and Pancreas (B) of adult ostriches; IL: islets of Langerhans, A: acini, H: hepatocytes, CV: Central vein, PT: portal triad. (H&E) 100X.

Feed elements in the small intestine are converted into molecules like free fatty acid monosaccharides, amino acids and peptides. These constituents absorbed in the small intestine and through blood reached into the tissues (Kiela and Ghishan, 2016). Intestinal mucosa helps to facilitate the maximal absorption of the feed constituents.

The epithelial cells and villi have apical aspect covered with microvilli having brush border, which increase the surface area (about 600 folds) for the absorption of nutrients (Kiela and Ghishan, 2016). Histologically, the surface epithelium was a simple columnar and covered with villi throughout the length of the small intestine.

Similar findings were reported in fowl, ducks and quails by Das *et al.* (2003) and in ostrich by Predoi *et al.* (2008). These findings agreed with previous studies (Wang *et al.*, 2008; Wang and Peng, 2008). The large intestine of ostriches was comprised of plicae circularis. The surface epithelium of the large intestine was simple columnar. In caecae, lymphatic tissues were absent in the lamina propria of immature ostriches and present in adult ostriches. The present study also supports the findings of Firdous and Lucy (2012). The present study recorded that the thickness of all layers of intestines was expressively ( $P < 0.05$ ) higher in the adult group as related to the immature group (Table 3). In this study, the quantitative thickness of all layers of small and large intestines was measured. Qureshi *et al.* (2017) reported quantitative measurement of the thickness of all layers of small- and large intestines in ducks, these results are in agreement with present findings.

**Digestive glands:** In ostrich, gall bladder was absent, Stornelli *et al.* (2006) and El-Zoghby (2005) also found similar results and they described bile was produced from the liver. The liver had three lobes: right, left and intermediate in ostrich. The left lobe of the liver was divided into a small caudodorsal lobe and a large caudoventral lobe. The right and intermediate lobe were intact. Similar findings were reported in adult ostrich by Moslem (2015). Duck and other relevant birds had two lobes (Stornelli *et al.*, 2006). This study described the hepatic morphometry of ostrich including measurement of each hepatic lobe (Table 2). To date, there is no literature available that explored the macroscopic measurement of the liver in relation to the progressive age. In adult ostrich liver length, weight and width showed significant ( $P < 0.05$ ) difference as compared to immature ostriches (Table 2). A similar trend of weight was documented in adult ostrich in a previous study by Moslem (2015). The current study revealed, the liver weight of mature ostrich was 968g which was 10 times higher as compared to duck (70g) and broiler (49g) reported by Cécile Bonnefont *et al.* (2019) and Kokoszyński *et al.* (2017) respectively. In young bird's liver weight increased rapidly as compared to adult age.

The liver was enclosed by a connective tissue capsule. The liver was composed of an unlimited number of hepatic lobules, difficult to differentiate from one another. Lobules were consisting of cords of hepatocytes, portal triad, central vein, sinusoids and biliary duct system. A similar trend was described in ostriches by Moslem (2015), in *Sturnus vulgaris* by Saddama and Genan (2016) and in other birds (El-Zoghby, 2005). Portal triad consisted of artery, vein and bile duct-like in birds and mammals. Its distribution in the lobules was very scanty as compared to other birds. In this study, these findings were determined in immature and adult as well as in male and female ostriches, Moslem (2015) however described similar findings in adult ostriches aged less than 3 years. There was no literature available about the diameter of hepatocytes and the central vein of the liver in ostriches. The diameter of the hepatocyte and central vein showed a significant ( $P < 0.05$ ) rise in the adult group as compared to the immature group.

The pancreas was located in the mesentery between the loops of the duodenum like other birds. It started from a gizzard and ended into the duodenal loops. The pancreatic duct opened into the ascending limb of the duodenal loop,

near the duodenojejunal junction. In contrast, the hepatic duct opened into the descending limb of the duodenal loop. The pancreas was somewhat narrow, long and pinkish-white in color (Fig. 3). Similar findings were reported in ostrich (Stornelli *et al.*, 2006), goose (Gulmez *et al.*, 2003), duck and mynah (Saadatfar and Asadian, 2009). There was limited literature available regarding quantitative measurements of weight, length and width of the pancreas in ostriches. The present study revealed significantly large pancreatic macroscopic parameters in adult animals when compared with their immature counterparts.

The histological investigation of the pancreas in ostriches consisted of a lobed organ and composed of an unlimited number of lobules. The pancreas was enclosed with a thick capsule of connective tissue. Mobini *et al.* (2009) described similar results in turkey and goose. Present study findings conflict with the work of Al-Agele and Mohammed (2013), which reported a thin connective tissue capsule in birds. Histologically, the exocrine part was composed of acini and the endocrine part consists of islets of Langerhans. The results agreed with the findings of Mobini *et al.* (2009) in turkey and Das *et al.* (2003) in duck. In the present study, the diameter of islets of Langerhans and the diameter of acini were determined in ostriches of both sexes with advancing age. No report available to compare these findings.

**Conclusions:** The current study provided thorough information on the sex and age-related quantitative histomorphometric characteristics in the intestine and digestive glands of ostriches (*Struthio camelus*). It is conceivable from the data that the development and growth of the intestine, liver and pancreas is rapid in the immature age which maintains a plateau with negligible increment till old age, however, sex showed no significant effect in any age group. Further studies should be aimed at the ultrastructure of the intestines and liver. A more profound examination of the liver is very important. A better understanding of gut development and anatomy provides new knowledge to improve feed efficiency and diagnose pathological processes in ostrich.

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**Authors contribution:** This manuscript is from PhD thesis of ZU. ASQ conceived the idea and designed the project. ZU executed the research plan and applied statistics. All authors were involved in data interpretation, write up and final approval of the manuscript. All authors declare no conflict of interest.

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