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HISTOLOGICAL AND HISTOMETRIC ALTERATIONS IN THE DIGESTIVE TRACT AND ACCESSORY GLANDS OF DUCK (*Anasplatyrhynchos)* WITH SEX AND PROGRESSIVE AGE

A. S. Qureshi[1*], T. Faisal[1]and M. K. Saleemi[2] and M. Z. Ali[1]

[1]Department of Anatomy, [2]Department of Pathology, University of Agriculture, Faisalabad
Corresponding author's e-mail: anas-sarwar@uaf.edu.pk

ABSTRACT

The current study was conducted to find morphological and histometrical variations in the digestive system and associated glands of duck (*Anasplatyrhynchos*) as regard to the sex and progressive age. A total of 30 apparently clinically healthy ducks of both sexes and three progressive age groups viz., immature (less than one year), adult (upto one year) and old (above one year) in equal number were used in this study. Digestive organs including tongue, esophagus, proventriculus, gizzard, small intestine, large intestine and associated glands like liver, pancreas and gall bladder were collected immediately after slaughtering the birds for histological studies. The sections of 5-7µm were cut and stained by haematoxylin and eosin stain. Histometrical analysis was done using automated computer software Image J®. The results of this study provide valuable information on the sex and age-related histological and histometrical variations in the digestive organs of ducks (*Anasplatyrhynchos*). Maturation in the organs of the digestive system is rapid in early days of life, that is from immature to adult age while it remains approximately fixed up to the old age.

Keywords: Histomorphometry, digestive organs, ducks, sex, age.

INTRODUCTION

Ducks are reared all over the country for hobby as well asformeat and egg purpose. With increasing demand for duck meat products and by- products, the duck meat industry is expanding rapidly at a growth rate of 10-15% annually; a similar trend is emerging in Pakistan (Qureshi *et al.*, 2016). There are more than 40 breeds of domestic ducks. The white Pekin duck (*Anasplatyrhynchos*) is the most common variety raised for eggs, meat and feather. Ducks are grown to about 7 weeks of age andrise on average 95 g/day with a feed conversion rateof under 2.15 to 1. In other words, 2.15 kg of feed is required to produce 1kg of duck meat (Stein, 2012). Size of the duck egg is 10-15 gram larger than chicken egg. Broiler /green ducks are faster growing than chicken, with better growth rate and feed efficiency (Rajput *et al.,* 2014). Currentlyduck farming increases globally due to economic reasons (King *et al*., 2000). They are also reared at fish farms to have natural control of mollusks and crustaceans, which are the intermediate hosts of many fish parasites, because duck feeds on animal food origin (Szczepanczyk, 2005).

At the time of hatching, digestive system of the birds is fully developed anatomically but not fully mature (Ravindran, 2003). The digestive activities are altered during the development (Wang *et al.,* 2003). Especially rapid changes occur in the growth of villus of the duodenum, jejunum and the ileum (Sklan, 2000). The ability of intestinal tissue to digest and absorb the nutrients also increases more rapidly than the wholebody mass and this continues for a maximum of up to 6-10 days of the age (Galis, 2009).

The gastrointestinal tract (GIT) of birds is physiologically different from other animals. The GIT of a bird is a double-ended open tube beginning at the beak and finishing at the vent (Zaher*et al*., 2012). The bird can take advantage of a wide variety of food due to presence of a glandular proventriculus and a strong gizzard. Also, the digestive system of bird helps to provide conditions for flight; its overall less length in comparison to that of mammals, light-weight beak, lack of teeth, high metabolic rate etc. (Harndi*et al*., 2013).

The published material regarding the GIT system of duck is only very few (Hassan and Moussa, 2012). The present study has been designed to determine the morphohistometry of digestive system of duck with regard to age and sex in order to characterize the differences at the cell and tissue level.

MATERIALS AND METHODS

Sampling: A total of 30 apparently clinically healthy ducks of both sexes were used in this study. For immature group, eggs of ducks were incubated atthe same time,poults(young ducks) were reared for one month and then used for study. The birds were divided into three age-groups having 10 birds each of both sexes as described in the table. Blood and faecal examination was done to confirm the health status.

Histological studies: Collected samples of 1 cm^3 from each organ under study were fixed in neutral buffered

formaldehyde solution. The fixed samples were processed by the paraffin tissue technique, 6 µm thick sections were cut and stained by hematoxylin and eosin (Bancroft and Gamble, 2008).

Histometric analysis: After slide preparation, the thickness of four layers: tunica mucosa (epithelium, lamina propria, lamina muscularis), tunica submucosa, tunica muscularis and tunica adventitia/serosa of all the digestive organs was measured in µm with the help of automated image analysis system Image J® version 1.43n. The thickness of layers was measured from photomicrographs of the digestive organs taken by Nikon Optiphot 2 microscope at 400X. Moreover, diameter of central vein and size of hepatocytes in liver and acinar and pancreatic islet cells in pancreas was determined.

Statistical Analysis: Descriptive statistics was calculated for each parameter under study with the help of computer software Microsoft Excel®. The means of parameters were compared by one-way analysis of variance (ANOVA). Group means were compared with the help of Duncan's multiple range test. The level of significance was $P<0.05$.

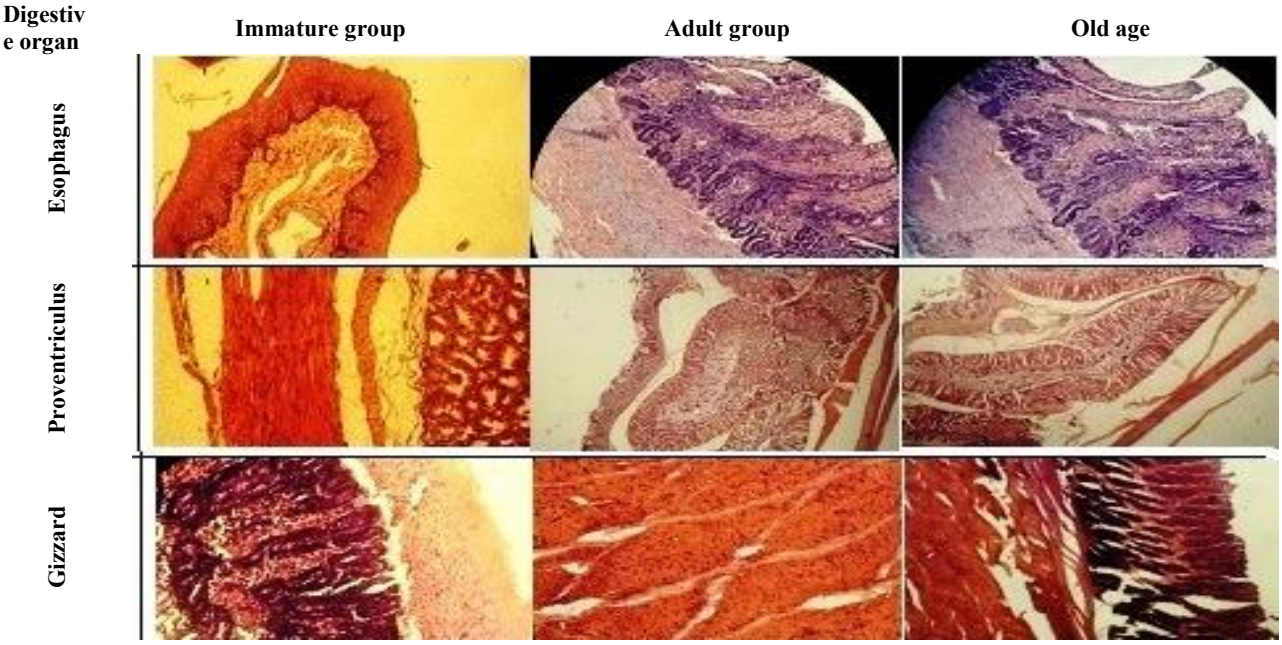
RESULTS

Histological features of study organs including tongue, esophagus, proventriculus, gizzard, small intestine, large intestine and associated glands like liver, pancreas and gall bladder of a total of 30 apparently clinically healthy ducks of both sexes and three progressive age groups viz., immature (less than one year), adult (upto one year) and old (above one year) in equal number are presented in Fig. 1.

Quantitatively, measurements of thickness of various layers ofthe digestive organs are presented in Table 2.These measurements were made with the help of a semi-automated image analysis system using image J®software. Statistical analysis revealed that sex had nosignificant influence on overall mean values of various layers of digestive organs,hence, results in male and female are not presented here. However, overall means of each layer in total birds (n=30) are being included in Table 2.

In contrast, major increase was recorded in early age groupwhich continued until maturity (up to one year). These results were invariably statistically significant (Table 3). Close scrutiny of results indicated that majority of values showed a gradual decline in old age birds but none of these values found statistically significantly low.

Among accessory digestive glands studied, central vein in liver of all the three age groups showed significant differences ($P<0.05$) as depicted in Table 2. Mean values of size of hepatocytes presented non-significant differences among three age groups under study (Table 2). In pancreas, islets and acini showed non-significant differences in their size between two sexes(Table 3) and three age groups (Table 2). Various layers of gall bladder were non-significantly different in adult and old age groups,however,both of these groups were found significantly different ($P<0.01$) from immature age group.



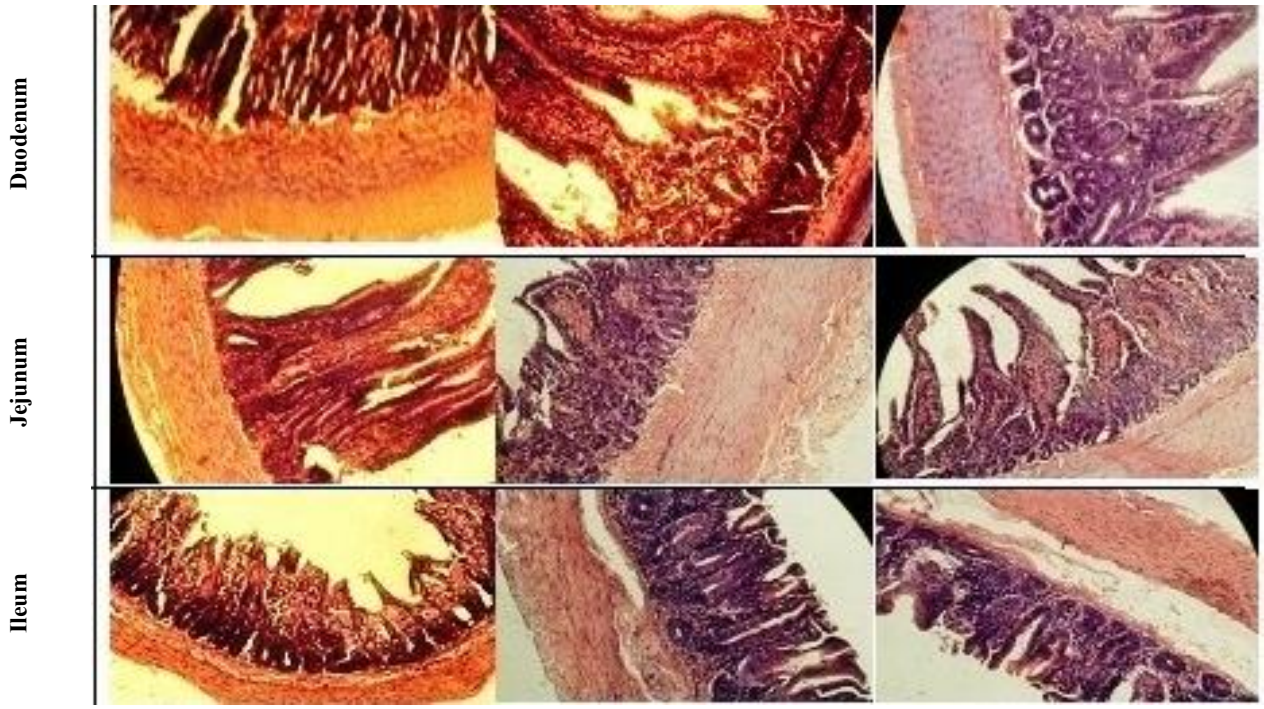


Fig. 1 Photomicrograph of different digestive organs of immature, adult and old age groups of ducks. H & E; 400X.

Table 1.Distribution of 30 study birds according to sex and age.

Age group	Age	Average age	Male	Female	Total no. of birds
Immature	Less than one month	30 days	5	5	10
Adult	Up to one year	366 days	5	5	10
Old	Above 3 years	1099 days	5	5	10
Sex and age group-wise total number of birds			15	15	30

Table 2.Mean ± SEM values of thickness of various layers of gastrointestinal tract.

Organ	Layers (µm)	Overall mean (n = 30)	Immature (< 1month)	Adult (up to 1 year)	Old (> 3 years)
Tongue	Epithelium (Str. squamous)	193.16±24.04	96.13±12.04^{A}	281.1±17.06^{B}	272.22±26.03^{B}
	Propriasubmucosa	417.97±29.66	150.87±0.002^{A}	722.16±0.003^{B}	518.7±0.05^{B}
	Tunica muscularis (Circular)	541.27±76.97	232.67±24.78^{A}	717.8±33.03^{B}	728.34±34.05^{B}
	(Longitudinal)	836.93±120.31	308.45±54.00^{A}	1121.9±49.04^{B}	1150.9±0.05^{B}
Esophagus	Epithelium (Str. Squamous (K)	258.8±6.48	252.12±5.002^{A}	286.16±4.14^{B}	238.13±3.84^{B}
	Lamina propria	156.58±8.61	118.93±7.03^{A}	178.92±12.03^{B}	171.92±10.04^{B}
	Lamina muscularis	44.78±5.3	22.91±6.03^{A}	62.72±6.13^{B}	48.73±4.44^{B}
	Propria submucosa	50.54±4.5	31.16±3.90^{A}	56.72±4.13^{B}	63.73±0.84^{B}
	Tunica muscularis (Circular)	128.93±6.9	98.92±8.04^{A}	139.96±10.05^{B}	147.93±9.04^{B}
	(Longitudinal)	103.98±11.74	52.67±10.04^{A}	124.66±9.88^{B}	134.62±8.04^{B}
	Tunica serosa	308.47±4.52	296.15±3.99^{A}	301.13±1.24^{B}	301.13±1.77^{B}
Proventriculus	Epithelium (Simple columnar)	349.93±21.86	263.2±20.04^{A}	429.27±29.23^{B}	357.26±17.41^{B}
	Lamina propria	470.65±150.05	170.46±30.13^{A}	216.47±24.34^{B}	225.0±27.03^{B}
	Lamina muscularis	38.01±4.54	18.46±3.03^{A}	51.47±3.94^{B}	44.07±7.64^{B}
	Propria Submucosa	1944.58±190.82	1164.1±124.01^{A}	2068.2±93.004^{B}	2068.2±133.04^{B}
	Tunica muscularis (Circular)	54.42±3.72	39.86±3.03^{A}	68.21±5.004^{B}	55.21±4.05^{B}
	(Longitudinal)	70.64±3.18	61.72±3.003^{A}	84.43±4.004^{B}	65.79±3.06^{B}
	Tunica serosa	101.97±2.96	97.47±3.09^{A}	114.8±2.75^{B}	93.67±3.03^{B}

	Epithelium (Simple columnar)	446.32±15.43	411.43±16.04^{A}	513.77±20.10^{B}	413.77±16.04^{B}
	Lamina propria	771.26±14.00	712.53±12.04^{A}	816.63±32.004^{B}	784.63±9.14^{B}
Gizzard	Lamina muscularis	188.88±12.84	154.13±0.004^{A}	244.83±17.94^{B}	167.7±11.66^{B}
	Propriasubmucosa	210.71±9.07	200.83±8.004^{A}	249.14±9.004^{B}	182.16±7.04^{B}
	Tunica muscularis (Circular)	1673.81±83.07	1431.4±88.04^{A}	2031.6±53.04^{B}	1558.4±60.04^{B}
	(Longitudinal)	779.2±32.05	668.67±33.06^{A}	910.24±36.04^{B}	758.79±29.05^{B}
	Tunica serosa	592.54±30.49	490.23±27.04^{A}	718.76±28.04^{B}	568.63±20.04^{B}
	Epithelium(Simple columnar)	25.82±1.17	20.87±1.04^{A}	29.53±1.02^{B}	27.06±1.04^{B}
	Lamina propria	136.78±5.83	112.94±4.004^{A}	156.93±5.04^{B}	140.46±4.06^{B}
	Lamina muscularis	19.7±3.05	9.22±0.04^{A}	32.23±0.004^{B}	17.66±0.004^{B}
Duodenum	Propriasubmucosa	34.49±5.54	24.57±2.001^{A}	56.66±5.01^{B}	54.12±5. 71^{B}
	Tunica muscularis (Circular)	55.56±3.55	42.94±3.82^{A}	69.83±5.72^{B}	53.93±4.02^{B}
	(Longitudinal)	45.41±3.65	31.05±3.00^{A}	53.06±5.65^{B}	52.13±5.04^{B}
	Tunica Serosa	64.55±2.95	51.64±2.46^{A}	72.31±2.55^{B}	69.73±2.36^{B}
	Epithelium (Simple columnar)	59.27±3.30	45.54±2.74^{A}	70.30±3.16^{B}	61.53±2.84^{B}
	Lamina propria	261.26±10.86	217.73±9.04^{A}	300.03±8.04^{B}	266.07±8.04^{B}
	Lamina muscularis	35.42±4.75	14.53±1.94^{A}	45.93±4.74^{B}	45.82±0.45^{B}
Jejunum	Propriasubmucosa	48.99±3.89	36.83±3.04^{A}	65.53±3.004^{B}	44.62±3.16^{B}
	Tunica muscularis (Circular)	55.18±3.27	41.52±2.99^{A}	65.97±3.94^{B}	58.06±2.66^{B}
	(Longitudinal)	52.89±3.57	37.73±3.54B	64.03±3.52^{B}	56.93±2.84^{B}
	Tunica Serosa	76.88±3.89	63.66±2.98.004^{A}	92.93±3.09^{B}	74.05±2.86^{B}
	Epithelium (Simple columnar)	64.38±4.38	48.43±2.15^{A}	81.75±4.01^{B}	62.97±3.94^{B}
	Lamina Propria	250.54±9.5	215.54±8.64^{A}	287.82±8.16^{B}	248.27±6.84^{B}
Ileum	Lamina muscularis	34.67±3.54	19.19±2.66^{A}	40.80±4.17^{B}	44.03±3.94^{B}
	Propria Submucosa	57.79±2.31	47.69±2.37^{A}	64.05±3.04^{B}	61.64±2.96^{B}
	Tunica Muscularis(Circular)	56.08±2.48	45.14±1.96^{A}	61.50±2.07^{B}	61.50±2.46^{B}
	(Longitudinal)	39.16±1.9	30.83±0.24^{A}	43.89±0.26^{B}	42.77±0.54^{B}
	Tunica Serosa	94.84±6.09	73.97±5.76^{A}	119.75±6.18^{B}	90.83±4.91^{B}
	Epithelium (Simple columnar)	61.92±3.52	25.32±3.49^{A}	33.80±2.89^{B}	27.22.2.91^{B}
	Lamina propria	260.72±9.32	260.59±8.06^{A}	296.29±9.73^{B}	225.30±7.17^{B}
	Lamina muscularis	33.25±2.33	23.04±2.31^{A}	38.32±2.47^{B}	38.43±2.15^{B}
Large Intestine	Propriasubmucosa	55.26±3.51	43.96±0.005^{A}	70.04±0.008^{B}	51.78±0.001^{B}
	Tunica muscularis (Circular)	112.09±7.24	87.96±5.05^{A}	142.15±4.94^{B}	106.17±8.06^{B}
	(Longitudinal)	46.14±2.97	34.14±2.95^{A}	56.69±2.26^{B}	47.6±2.53^{B}
	Tunica Serosa	101.62±9.24	79.81±11.04^{A}	142.25±8.76^{B}	82.83±8.17^{B}
	Accessory Digestive Glands				
Liver	Central vein	73.36±3.29	58.92±2.46^{A}	81.64±2.90^{B}	79.51±301^{B}
	Hepatocytes	5.34±0.028	5.22±1.04^{A}	5.41±1.02^{B}	5.38±1.00^{B}
Pancreas	Pancreatic Islets	65.32±1.7	58.01±1.04^{A}	70.48±1.04^{B}	67.49±1.24^{B}
	Acinus	20.2±0.26	19.29±0.34^{A}	21.03±0.34^{B}	20.04±0.54^{B}
	Epithelium (Simple columnar)	0.44±0.03	0.32±0.024^{A}	0.53±0.03^{B}	0.51±0.04^{B}
	Lamina Propria	0.56±0.03	0.40±0.05^{A}	0.66±0.08^{B}	0.62±0.05^{B}
	Lamina muscularis	0.64±0.03	0.51±0.05^{A}	0.73±0.04^{B}	0.67±0.04^{B}
Gall Bladder	Propriasubmucosa	1.4±0.19	0.97±0.15^{A}	1.63±0.14^{B}	1.58±0.45^{B}
	Tunica muscularis (Circular)	2.52±0.15	1.86±0.13^{A}	2.89±0.16^{B}	2.83±0.14^{B}
	(Longitudinal)	1.15±0.03	1.02±0.24^{A}	1.23±0.15^{B}	1.19±0.41^{B}
	Tunica serosa	3.36±0.08	2.98±0.07^{A}	3.56±0.09^{B}	3.52±0.40^{B}

Different superscripts in each row indicate difference at 1% level.

Table 3.Overall mean (± SEM) values of various part of associated digestive organs of different sex groups in 30 ducks.

Sex	N	Liver		Pancreas	
		Central vein(µm)	Hepatocytes(µm)	Pancreatic Islets (µm)	Acinus(µm)
M	15	73.37±3.29ns	4.85±0.023ns	61.93±1.71ns	20.22±0.26ns
F	15	69.35±4.27	5.33±0.058	65.31±1.38	23.21±0.26

ns= non-significant.

DISCUSSION

Esophagus is comprised of all typical layers while epithelium is composed of thick cutaneous stratified squamous cells (Klem*et al.,* 1982, 1984). There are many irregularly shaped mucous glands in the lamina propria which are deeper in case of duck as compared to other avian species. In duck, the esophageal lumen was lined by a layer of stratified squamous epithelial cells as described in other avian species (Srisai*et al.,* 2002; Nagy *et al.,* 2005). This was also supported by the finding of Guimara*et al.* (2009) in ostrich.

Tunica muscularis of esophagus of duck was made up of thick inner circular and narrow outer longitudinal smooth muscle fibers layers. In ducks, epithelium type and the glands of esophagus are not associated with type of the feed (Fedr, 1972). Shiina *et al.* (2004) discovered that the cervical and thoracic regionof esophagus of all the birds were composed of smooth muscles while the esophageal glands were present in the lamina propria of the mucosa.

In the proventriculus side of lumen, epithelium was simple columnar (Chikilian and Noemi, 1996). There were multilobulated tubular glands in the proventriculus. Tunica muscularis was made up of two layers; inner circular and outer longitudinal one of smooth muscles. Tunica serosa consisted of connective tissue, nerves, blood vessels and mesothelium (Rocha and Lima, 1998).

Gizzard was lined internally by yellowish layer of cuticle secreted by gizzard glands. Lamina propria contained many tubular glands which were the protruding lamellae of the glandular cells making elongated crypts. The simple glandular tubules and the simple tubular glands open into the shallow crypts. The tunica muscularis was made up of two layers of smooth muscles; inner circular and outer longitudinal layer. Starck*et al*. (2003) described in quails that muscles of gizzard consisted of smooth muscles which were separated by layers of connective tissue giving an onion structure shape to gizzard muscles. Mathias and Abdul Rahman (2003) described that thickness of gizzard muscles were approximately 180.52 mm in two weeks old Japanese quails.

Histologically, the small and large intestine were approximately similar to other avian species and mammalian species. In caeca, the only histological difference was the absence of lymphatic tissue in the lamina propria of day old birds than in the adult birds. Mucosa was made up of simple columnar epithelium with goblet cells and the number of goblet cells decreased towards the apex of the villi (Firdous and Lucy, 2012).

Applegate *et al.* (2005) described that in the intestine the villus length and maturation increase rapidly during the first week of age in Pekin duckling. Watkins *et al.* (2004) showed that the domesticated ducks found to have greater absorption surface area and elevated digestive enzyme activity as compared to the mallards. Wang and Peng (2008) proved that during the infection of digestive system, the mast cells played an important role as a first line of defense.

The results of this study provide valuable information on the sex and age-related histometrical variations in the digestive organs of ducks (*Anasplatyrhynchos*). Maturation in the organs of the digestive system is rapid in early days of life that is from immature to adult age while it remains approximately fixed up to the old age. This study also shows that there is no significant effect of sex on growth and the maturation of different organs and associated glands of the digestive system in ducks.

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