Research Article

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Age induced changes in the microscopic anatomy of the digestive system of Japanese quails (*Coutrnix japonica*)

Razia Kausar*, Anas Sarwar Qureshi, Malik Zohaib Ali, Muhammad Khalil Ateeq and Muhammad Usman

Department of Anatomy, Faculty of Veterinary Science, University of Agriculture, Faisalabad, **Pakistan**, 38040.
***Corresponding author**

A total of 24 clinically healthy Japanese quails (*Coutrnix japonica*) of each sex and three different age groups i.e. group A (4 weeks of age), group B (> 8 weeks of age) and group C (> 12 weeks of age) were used in the present study. The samples of the organs of digestive system (Esophagus, crop, proventriculus, gizzard, small intestine, pancreas, large intestine, ceaca, rectum, Liver) were collected from each bird. The microscopic studies of different parts of the digestive system revealed highly significant differences ($p<0.01$) among all the three groups. The esophagus was lined by non-keratinized st. squamous epithelium. The epithelial height was significantly higher ($p<0.01$) in group C (208.15 ± 30.16µm) compared to groups A (87.69 ± 23.88µm) and B (171.06 ± 29.63µm). The histological structure of crop was similar to esophagus except mucous glands which were found absent in lamina propria. The glandular stomach was lined by simple columnar to cuboidal epithelium and mucosa was abundant with large compound tubular glands. The gizzard or muscular stomach was lined by a thick layer of keratinoid. The thickness of keratin was significantly ($p<0.01$) higher in group C (201.63 ± 41.42 µm) as compare to group A (134.43 ± 7.89µm) and B (164.43 ± 12.75µm). The epithelium was low to tall columnar that invaginate into the lamina propria. The dudoneum, jejunum and ilium were found similar in histological structure except the height of villi, which were larger in duodenum and then become shorter and thicker caudally. The ceaca exhibit non-significant ($p>0.05$) difference among all three groups.

Key words: Crop, ceaca, gizzard, proventriculus, liver, microscopy.

Today, poultry industry is one of the leading industries in our country. It is the fast growing part of our agriculture and it adds in our economy. The pace of poultry is tremendous. Problems are also abounding this growing industry. Among the birds the Japanese quails are farmed for meat and eggs in America, India and Europe (Panda and Singh, 1990; Baumgartner, 1994). These birds are also important as experimental animals used in our research projects. The Japanese quails get early maturity (Qureshi, 1996) and physiologically they resemble with domestic fowl (Wilson et al. 1961). These are fairly resistant to poultry disease and are considered hardy in nature (Randall and Bolla, 2008). Developmental data is essential to describe adaptive change in a population (Bell et al. 2007). Birds are structurally very different than mammals (Getty, 1975) and this difference is so striking in the arrangements of their digestive systems. Birds have a number of features of the digestive system that distinguish them from mammals. These include the lack of teeth and a soft palate and a feeding strategy that allows for maximum ingestion of food in a short time. As birds need to keep down weight to fly, most cannot afford the luxury of prolonged food storage or digestion. The postnatal gastrointestinal development depends upon many factors including genetic diversification, age, sex, diet, hormones secreted from intestine and from other body organs and various growth factors (Anthony et al. 1991; Szczepanczyk and Wesolowska,

2008).

The present experiment was designed to study the age related changes in Japanese quails. This bird is frequently used as a model for experiment in poultry and the results thus obtained from this study can be extrapolated on other avian species like chicken, pigeon etc.

MATERIALS AND METHODS

A total of 24 clinically healthy Japanese quails (*Coutrnix japonica*) including both sex and 3 different age groups i.e. group A (4 weeks of age), group B (above 8 weeks of age) and group C (above 12 weeks of age) were used in the present project. The samples of the organs of digestive system (esophagus, crop, proventriculus, gizzard, small intestine, pancreas, large intestine, ceaca, rectum, and liver) were collected from each bird immediately after slaughtering the birds.

Microscopic Anatomical Methods:

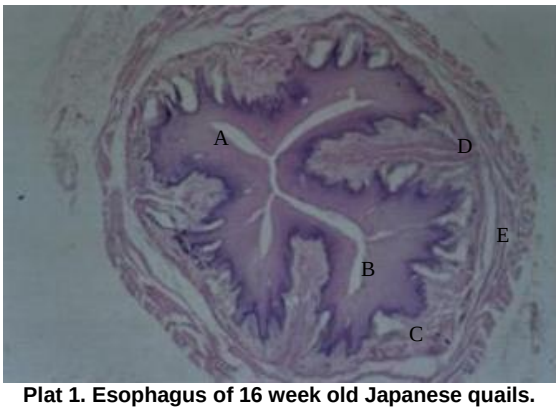
For microscopic studies 1 to 2 cm tissue samples of organs of digestive system were taken. The tissues were marked and fixed in the neutral buffer formaldehyde solution for histological studies. After fixation, sections of 5to 6 µm thickness were taken. The fixed tissues were further processed by the paraffin tissue preparation technique as described by Bancroft and Stevens (1990). These sections were stained with heamatoxyline and eosin for microscopic studies

Statistical Analysis: Descriptive statistics was calculated for each parameter investigated with the help of computer software Microsoft Excel. The means of parameters of all the organs of digestive system were compared with individual 95% CIs. Statistical analysis was performed by using the statistical computer software Minitab (Mtb13). Values are presented as mean ± SD.

RESULTS AND DISCUSSION

The oesophagus of Japanese quails was lined by a thick, non-keratinized stratified squamous epithelium (Plate 1).

The epithelial height was significantly higher ($p<0.01$) in group C with value 208.15 ± 30.16µm and 87.69 ± 23.88, 171.06 ± 29.63µm in groups A & B respectively. Das and Biswal (1967) reported that no keratinization of the stratified squamous epithelium was present in chicken. The mucous glands were more numerous in the goose than in the chicken. Similarly in quails no keratinization was present in the esophageal epithelium. The lamina propria has mucous glands.



Plat 1. Esophagus of 16 week old Japanese quails.

A. Lumen
B. Non Keratinized St. Squamous epithelium
C. Lamina propria
D. Stratum circularis
E. Stratum longitudinal

Muscularis externa was composed of smooth muscles. The means ± SD of esophagus parameters are shown in Table 1. These values were highest in group C and lowest in group A. The histological structure of crop was similar to esophagus except mucous glands which were found absent in lamina propria. The mucous glands in crop only present close to the esophagus (Plate 2).

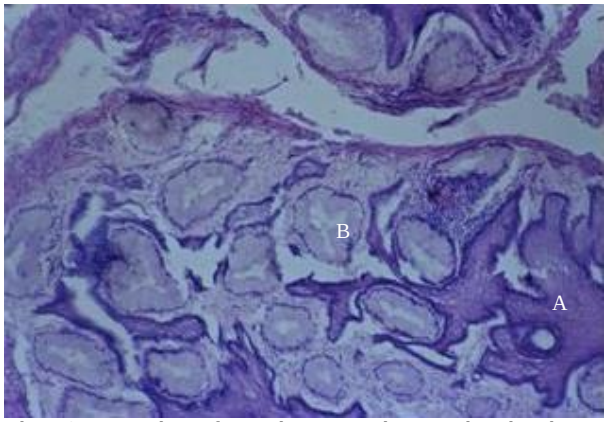


Plate 2. Junction of esophagus and crop showing large number of mucous glands in lamina propria.

A. Stratified squamous non keratinized epithelium
B. Mucous Glands

The thickness of epithelium of crop differs significantly ($p<0.01$) among all the three groups studied. It was highest in group C i.e., 278.30 ± 53.83µm. The means ± SD are shown in table 1.

The stomach of Japanese quails was divided into two parts as in other birds and reported by Getty (1975).

Table: 1. Micrometric parameters (mean ± SD) of digestive system of Japan quails under three different age groups.

Parameters Organs		Epithelium Height (µm)	Lamina propria (µm)	Muscularis mucosa (µm)	Stratum circularis (µm)	Stratum longitudinal(µm)
esophagus	**Group A**	87.69 ± 23.88a	23.33 ± 3.97a	29.33 ± 25.40a	11.21 ± 2.87a	16.96 ± 3.23a
	Group B	171.06 ± 29.63b	55.49 ± 9.87 b	28.46 ± 4.42a	60.38 ± 7.04 b	42.83 ± 2.1b
	Group C	208.15± 30.16c	72.16 ± 18.46 c	60.66 ± 28.75 b	102.06 ± 20.53 c	42. 8 ± 2.107 b
Crop	**Group A**	85.39 ± 20.40a	25.88 ± 6.36a	14.37 ± 3.63a	27.31± 3.12ab	23.57± 2.94a
	Group B	192.05 ± 36.37b	43.70 ± 9.44ab	26.450±4.76bc	32.20 ± 3.48ab	31.33 ± 3.87ab
	Group C	278.30 ± 53.83c	72.16 ± 19.12 c	31.913 ± 4.67bc	107.81±11.69c	58.36 ± 10.85c
Proventriculus	**Group A**	92.29 ± 9.8a	87.71±18.51a	----------	8.00 ±1.53a	10.43 ±1.72a
	Group B	112.00 ± 5.42ab	190.86 ± 33.66b	----------	10.0 ± 2.31 ab	14.43 ± 1.51ab
	Group C	150.71 ± 30.26c	306.38 ±14.85c	----------	52.13 ± 92.14c	62.25 ± 88.03 c
Duodenum	**Group A**	13.22 ± 3.63a	219.94 ± 8.42a	8.91 ± 1.47a	24.43 ± 4.06a	10.063 ±1.71a
	Group B	20.41 ± 1.47b	310.50 ±72.21ab	12.075 ± 1.62b	34.21± 4.15bc	18.68 ±1.47b
	Group C	28.46 ± 3.46c	564.65 ± 112.8c	15.52 ± 2.039c	35.65 ± 1.73bc	18.68 ± 1.47b
Jejunum	**Group A**	10.063 ± 2.99a	166.18 ± 22.02a	8.91 ± 3.11ab	18.11 ± 3.11a	8.62 ±1.62a
	Group B	10.35 ± 1.73a	212.7 ± 14.02b	11.21 ± 2.86ab	25.87 ± 4.21b	12.07 ± 3.42ab
	Group C	23.0 ± 3.477b	252.71 ± 5.56c	18.4 ± 2.45c	43.7 ± 3.47c	13.22 ±2.94ab
Ileum	**Group A**	8.62 ± 1.62a	126.21 ± 11.82a	8.33 ± 1.19ab	14.37 ± 2.94a	7.47±1.62a
	Group B	9.77 ± 2.94ab	213.61 ± 10.46bc	8.62 ±1.62ab	19.26 ± 2.99b	7.76 ±1.19ab
	Group C	17.82 ± 2.67c	224.54 ± 8.24bc	15.52 ± 2.38c	37.37 ± 2.94c	12.36 ± 2.01c
Ceaca	**Group A**	11.21 ± 1.91a	88.26 ± 6.38a	10.63 ± 1.19a	19.26 ± 2.44a	9.5 ± 1.9a
	Group B	18.11 ± 2.59b	118.4 ± 13.07b	12.93 ± 2.73ab	20.7 ±1.73ab	17.0 ± 4.2ab
	Group C	24.72 ± 3.42c	134.55 ± 7.78c	15.23 ± 2.73bc	29.03 ± 3.23c	122.8 ± 325.9b

The glandular stomach was lined by simple columnar to cuboidal epithelium (Plate 3).

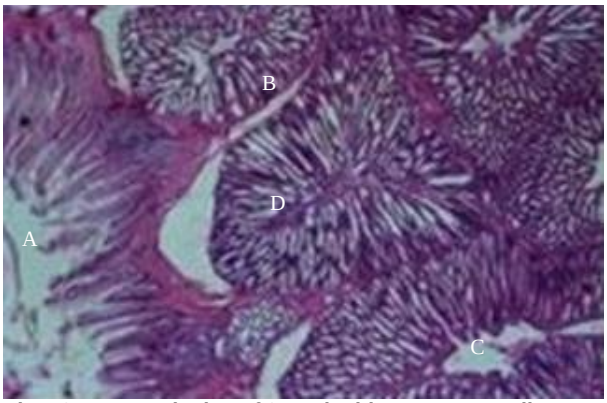


Plate 3: Proventriculus of 8 week old Japanese quails.
A. Lumen
B. Promary duct
C. Secondary Duct
D. Glands

The epithelial height was significantly higher ($p<0.01$) in group C, i.e., 150.71 ± 30.26µm, as compare to 92.29 ± 9.8µm, 150.71 ± 30.26µm in group A and B respectively. The mucosa was arranged into plica. It was abundant with large compound tubular glands. The lamina propria has primary, secondary and tertiary ducts which joins the lumen of the proventriculus (Plate 3).

These ducts were also lined by columnar epithelium. Salender and Toner (1963) studied that the glands of proventriculus had only one type of cells which had features of both parietal and zymogen cells of the mammalian stomach. Similar findings were also recorded in quail's proventriculus. The means ± SD are shown in Table 1.

The gizzard or muscular stomach was lined by a thick layer of keratinoid. The thickness of keratin was significantly ($p<0.01$) higher in group C with value 201.63 ± 41.42 µm as compare to 134.43 ± 7.89µm and 164.43 ± 12.75µm in group A and B. The epithelium was low to tall columnar that invaginate into the lamina propria (Plate 6). The epithelial height was significantly ($p<0.01$) differ among all the three groups studied with values, 220.13 ± 24.54µm, 195.86 ± 5.2µm and 188.57 ± 7.18µm in groups A, B and C respectively (Table 2). Muscularis externa consist of thick layers of smooth muscle cells separated by thick sheets of connective tissue (Plate 4).

The muscular thickness was significantly ($p<0.01$)higher in group B and C and lower in group A, with value 761.3 ± 11.9 µm, 797.3± 55.9µm, 833.5 ± 228.9µm in group A, B and C respectively. Matthias et al. (2003) reported that the thickness of gizzard muscle was 180.53 ± 42.15 mm^2 in Japanese quails at two weeks of age (Table 2). Within each muscle layer, the smooth muscle cells show the typical elongated spindle shape with a peripheral compartment of contractile fibers and the perinuclear cytoplasm.

Table 2: Micrometric parameters of gizzard of Japanese quails

parameters		Keratin (µm)	Epithelium (µm)	Muscular layer (µm)
Gizzard	**Group A**	134.43± 7.89ab	188.57±7.18a	761.3± 11.9a
	Group B	164.43±12.75ab	195.86±5.2ab	797.3± 55.9bc
	Group C	201.63±41.42c	220.13± 24.54 c	833.5± 228.9bc

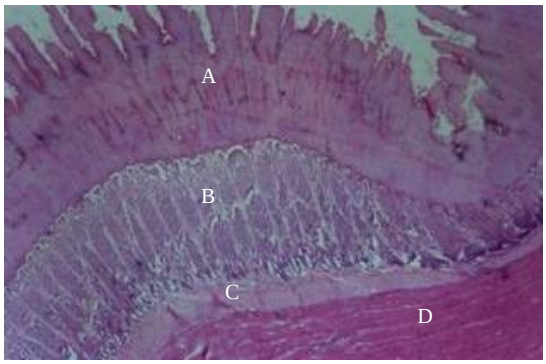
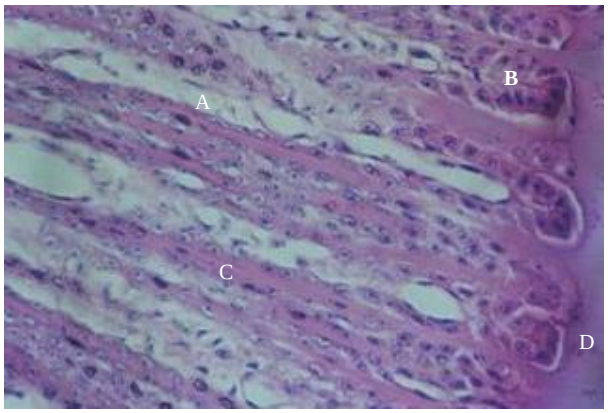


Plate 4. Muscular stomach of 16 week old Japanese quail.
A. Keratinoid
B. Simple columnar epithelium
C. Lamina propria
D. Muscularis externa

The muscular thickness was significantly ($p<0.01$)higher in group B and C and lower in group A, with value 761.3 ± 11.9 µm, 797.3 ± 55.9µm, 833.5 ± 228.9µm in group A, B and C respectively. Matthias et al. (2003) reported that the thickness of gizzard muscle was 180.53 ± 42.15 mm^2 in Japanese quails at two weeks of age (Table 2). Within each muscle layer, the smooth muscle cells show the typical elongated spindle shape with a peripheral compartment of contractile fibers and the perinuclear cytoplasm. The nucleus is sigar shaped, about five times as long as wide. Chief cells were present in the upper and mid regions of the gastric glands (Plate. 5).

The dudoneum, jejunum and ilium were found similar in histological structure except the height of villi, which were larger in duodenum and then become shorter and thicker caudally. These findings were in concordance with the findings of Hilton (1902) and Kaiser (1925). They observed that villi of duck duodenum had a square thin base and a distal triangular part with a pointed apex. The two villi were closely fit to each other, the edge of one villi was thickened and each thick edge touches the thin edge of the other villi. The wall of the jejunum of the duck and goose was similar to the duodenum, but in the jejunum the villi were shorter and oblong shaped .The small intestine was lined by simple columnar epithelium with goblet cells (Plate 6).



Plat 5. Ventriculus of the quails showing the gastric pits and glands.
A. Chief cells
B. Surface epithelium
C. Gastric pits
D. Kerationied

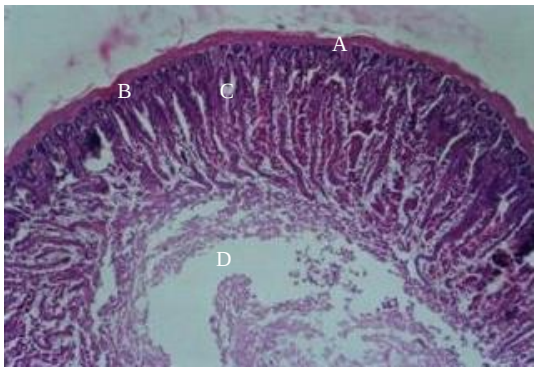


Plate 6. Small intestine of 4 weeks old Japanese quails.
A. Muscularis externa
B. Glands
C. Simple columnar epithelium
D. Lumen

Partha et al. (2002) observed the topographic positions of duodenum in all birds were similar and only the length and diameter varied. Histologically all the five layers of the duodenum were present in fowls, ducks and quails. Villi were present in all layers of the duodenum of the three bird species within a variation in sizes and shapes. The comparisons of three parts of small intestine are shown in table 1. The result shows that the measurements of the three parts of small

intestine significantly differ ($p<0.01$) among all three groups ($p< 0.01$). Aptekmann et al. (2001) observed the effect of dietary calcium on the villus height and reported the villus height was 818.80 ± 62.64µm, 430.13 ± 27.50µm and 366.53 ± 18.23µm in duodenum, jejunum and ileum, respectively. Two ceaca were present at the junction of small and large intestine. It was lined by simple columnar epithelium with goblet cells. The microscopic measurements of ceaca non significantly differ ($p>0.05$) among all the three groups, with highest value in group C i.e., 122.8 ± 325.9µm. The villi were shorter and broader in both the ceaca. Ceacal tonsils were present at the proximal portion. These findings were in line with those of Chen et al. (2002). He studied that the villi of ceaca showed finger like, peak like or tongue like shapes. The height of the villi decreased far from the proximal ceaca. Kappelhoff (1959) observed villi in the proximal part of the ceacum, the mucous membrane of the middle and distal parts forms fold. The wall of each ceacum was thinner than in other parts of the intestinal tract and contains lymphoid tissue which was especially well developed in the proximal part where there was a ceacal tonsil.

The liver is covered by a thin connective tissue capsule. These connective tissue septa radiate in to the interlobular spaces and give support to the vascular system formed by lymph vessels, hepatic artery, portal vein and bile duct. A fine network of reticular fibers surrounds the cells and sinusoids (Plate 7).

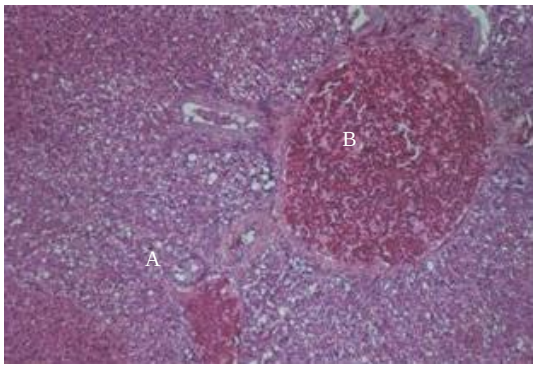


Plate 7. Liver of 16 weeks old Japanese quail
A. Central Vein
B. Lobule

The parenchyma of liver is formed by hepatocytes, these cells have spherical nucleus. Bile canaliculi are the minute microscopic canals present between apposed hepatocytes. Purton (1969) studied that the parenchyma of the liver consists of anastomotic plates of hepatic cells enclosing sinusoids. Unlike the hepatic plates in the mammals, which were one cell thick, those of the chicken were two cells thick.

CONCLUSION

Most organs of digestive system showed significant higher growth in terms of epithelial height, lamina properia, tela submucosae, muscularis mucose in Japanese quails with age more than 12 weeks.

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