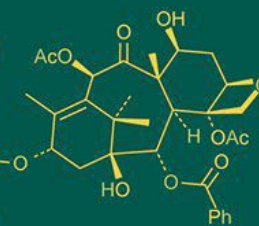
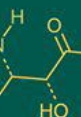
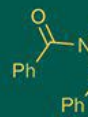
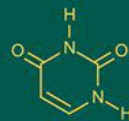
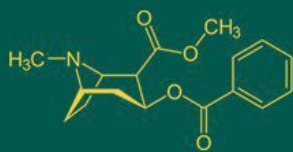


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Microscopic and micrometric analysis of jejunal development in Japanese quail

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Abstract

The present study was conducted to record age-wise postnatal developmental changes in the jejunum of 48 Japanese quails. Various dimensions, including the thickness of different layers of the jejunal wall, villus height and width, the height of columnar cells at the villus and crypt, the diameter of intestinal glands, and the lumen diameter of the jejunum, were measured at 7, 14, 21, and 42 days post-hatch. Histologically, the thicknesses of the tunica mucosa, tunica muscularis, and tunica serosa, as well as villus height and width, the height of columnar cells at the villus and crypt, the diameter of intestinal glands, and the lumen diameter, increased significantly with age. The surface epithelium was simple columnar and comprised chief cells, goblet cells, intraepithelial lymphocytes, and globular leukocytes. Ultra structurally, the principal absorptive cells were tall, columnar-shaped, with a striated border on the luminal surface made up of numerous microvilli and a large, basally located prominent nucleus. Goblet cells were scattered over the surface of the villi. The majority of goblet cells appeared as empty cells containing some secretory material inside.

Keywords: Japanese quail, histology, jejunum, post-hatch development

Introduction

Japanese Quails (*Coturnix coturnix japonica*) is catching attention in the poultry industry due to their low fat and soft delicious meat. They are farmed for meat and eggs in America, India and Europe (Panda and Singh, 1990 ^[18]; Baumgartner, 1994) ^[3]. They are fast-growing birds as compared to other poultry birds. Since, the quail is the most important type of bird after chicken from an economical aspect (Sharma *et al.*, 2000) ^[22]. The jejunum, which continues to be the small intestine's longest segment and its greatest surface, is important for fat digestion and assimilation in its final phases. Lema *et al.* (2020) ^[12]. The main intestinal region for lipid absorption in the Gastrointestinal system is the jejunum (Lema *et al.*, 2020) ^[12]. There has been only a little research on the typical variability of the avian jejunum, and there is no information on the histogenesis of the quail jejunum throughout post-hatch development. In order to learn more about microscopical and micrometrical observations of the quail jejunum, the present investigation was conducted.

Materials and methods

The present study was conducted on 48 Japanese quail birds (*Coturnix coturnix japonica*) irrespective of sex were procured from authenticated source was reared on poultry farm of College of Veterinary and Animal Science, Parbhani under standard managemental quail rearing practices. The jejunum was collected from 12 (twelve) birds each at end of 7th, 14th, 21st and 42nd day of age. The collected organs were washed with normal saline and Tissue samples of 3-5 mm size were taken from jejunum and fixed in 10 % neutral buffer formalin, 10% formal saline, and Bouins fluid. Thereafter, these tissue samples were processed routinely and 5 μ thick paraffin sections were obtained. These were stained with Harris hematoxylin and Eosin stain for general Histology and Van - geison's stain for collagen fibers (Singh and Sulochanna, 1996) ^[23], Masson's trichrome for collagen fibers and Verhoeff's stain for elastic fibers (Luna, 1968) ^[14], Silver impregnation staining technique for reticular fibers (Mukherjee, 1988) ^[16].

Under the Olympus microscope, photomicrography was used to document the measurements, such as villous height, villous width, the height of columnar cells at villus and crypt,

the diameter of intestinal glands, diameter of lumen, and thickness of tunica mucosa, tunica muscularis, and tunica serosa layer. The widths of the villi were measured in the center, and their heights were determined from tip to base. The measured data were statistically evaluated using the proposed techniques of Panse and Sukhatme (1967) [19].

Results and Discussion

Histologically, the jejunum of Japanese quail exhibited a typical three-layered structure comprising tunica mucosa, tunica muscularis, and tunica serosa, from the innermost to outermost layers. Notably, the tunica submucosa was absent in the jejunum of Japanese quail (Fig. 1). These findings align with previous studies by Ahmad *et al.* (2012) [1] and Rana *et al.* (2015) [21], who reported the presence of three layers in the jejunum of Japanese quail and Uttara fowl, respectively. Conversely, Zaher *et al.* (2012) [33] documented the presence of four layers—serosa, muscosa, submucosa, and mucosa—in the jejunum of common quail, indicating some variation among avian species.

The tunica mucosa consisted of lamina epithelialis mucosa and lamina propria, with the lamina muscularis mucosa being absent across all age groups examined (Fig. 2). The mucosal lining was characterized by elongated villi, primarily tongue-shaped, with a few spatula-shaped villi, consistent with Zaher *et al.* (2012) [33]. In European starling and precocial Japanese quail, Stark (1996) observed that the mucous membrane was not yet separated from the inner circular muscle coat by a distinct muscularis mucosa and submucosa. The height of the villi increased significantly with age, from an average of $336.42 \pm 33.70 \mu\text{m}$ at 7 days to $520.52 \pm 26.22 \mu\text{m}$ at 42 days (Table 1). Similar age-related increases in villus height have been reported by Liman *et al.* (2002) [13] in the large intestine of geese and Wang and Peng (2008) [30] in African ostrich chicks, whereas Stark (1996) noted a decrease in villus height with age in European starlings and Japanese quail. Longer villi are generally associated with an increased absorptive surface area, facilitating enhanced nutrient absorption, as supported by de Verdal *et al.* (2010) [5]. The width of the villi also increased from $2.36 \pm 0.17 \mu\text{m}$ at 7 days to $4.15 \pm 0.29 \mu\text{m}$ at 42 days (Table 1). This trend aligns with findings by Nasrin *et al.* (2012) [17] in broiler chickens. The height of columnar epithelial cells lining the villi was $23.61 \pm 1.50 \mu\text{m}$ at 7 days and increased to $31.37 \pm 1.60 \mu\text{m}$ at 42 days, with the cells being taller in the villi compared to crypt cells (Table 1). Ahmad *et al.* (2012) [1] reported similar observations, noting maximum columnar cell height at the sixth week of age. The diameter of intestinal glands increased from $36.76 \pm 1.61 \mu\text{m}$ at 7 days to $47.76 \pm 2.82 \mu\text{m}$ at 42 days, indicating enhanced cell proliferation with age (Table 1). Xia *et al.* (2004) [31] also reported that larger crypt areas reflect increased cellular activity.

The jejunal wall demonstrated the thickest tunica mucosa, followed by tunica muscularis and tunica serosa across all age groups (Table 1). All parameters increased significantly with age, corroborating findings by Rana *et al.* (2015) [21] and Kausar *et al.* (2016) [10], who reported progressive thickening of the mucosa and muscular layers during development.

The epithelial lining comprised simple columnar cells with prominent goblet cells, enterochromaffin cells, and chief cells (Fig. 3). Goblet cells, scattered among the epithelial cells, produced mucous that covered the villar surface, often

appearing as empty cells with some secretory material inside. These cells varied in shape, being rounded or almond-shaped, with smaller nuclei compared to principal cells. They lacked microvilli and were observed in two forms: swollen and thinner, darkly stained, considered regenerating goblet cells, consistent with Hodges (1974) [7]. Principal absorptive cells were tall, columnar, with a striated border of microvilli, and possessed large, basally located nuclei with prominent nucleoli. Their cytoplasm contained numerous mitochondria, endoplasmic reticulum, ribosomes, lysosomes, and other organelles (Fig. 10). Tight junctions between neighboring cells and desmosomes below these junctions were clearly visible, in agreement with Turk (1982) [26].

Microvilli projecting from the luminal surface contained fibrous structures extending along their length, with a terminal web (terminal bar) beneath. The nuclei of principal cells were large, basally located, with prominent nucleoli. These ultrastructural features align with observations by Turk (1982) [26]. The core of the villus housed connective tissue with a dense reticular fiber network and numerous blood vessels, lymphatic vessels, macrophages, plasma cells, erythrocytes, eosinophils, and lacteals (Fig. 7). The basement membrane, comprised of a thin, electron-dense basal lamina and a reticular lamina, separated the epithelium from underlying connective tissue, consistent with Verma *et al.* (1999) [29].

Connective tissue fibers included collagen and elastic fibers; collagen fibers were larger and less numerous in the muscularis layer (Fig. 5), while elastic fibers were less evident in early age groups but occasionally visible at 42 days (Fig. 6). Verma *et al.* (1999) [29] reported increased elastic fiber density with age, whereas Vaish (2005) [27] observed an absence of elastic fibers in Kadaknath fowl. The villus core also contained lymphatic tissue with lymphocytes, plasma cells, macrophages, and blood vessels. The crypts of Lieberkühn were short, simple tubular structures, extending from the muscularis mucosa to the bases of the villi, and contained various cell types, including goblet cells, enterochromaffin cells, and chief cells (Fig. 8). These crypts opened into the intestinal lumen and exhibited cell proliferation activity, as described by Al-Samawy *et al.* (2017) [2] and Hodges (1974) [7].

The epithelial lining of the intestinal glands was predominantly composed of tall columnar enterocytes with microvilli, along with goblet and enterochromaffin cells (Fig. 10). Goblet cells, scattered throughout, secreted mucus, while enterochromaffin cells produced serotonin-like substances. The cells exhibited characteristic ultrastructural features, such as apical microvilli and tight junctions, consistent with Turk (1982) [26] and Zaher *et al.* (2012) [33]. The absence of a distinct submucosal layer and lamina muscularis mucosa was consistent with prior reports by Ahmad *et al.* (2012) [1] and Rana *et al.* (2015) [21], although Zaher *et al.* (2012) [33] reported a thin submucosa in Japanese quail.

The tunica muscularis was composed of three layers: a thick middle circular layer and two thin outer longitudinal layers, in agreement with Ahmad *et al.* (2012) [1]. However, Kadhim *et al.* (2010) [8], Zaher *et al.* (2012) [33], Rana *et al.* (2015) [21], and Al-Samawy *et al.* (2017) [2] reported only two layers, i.e., an outer longitudinal and an inner circular layer. The myenteric plexus and blood vessels were observed within the muscular layers, with nuclei of smooth muscle cells elongated in the circular layer and rounded in

the longitudinal layers (Fig. 9), consistent with Hodges (1974) [7]. The tunica serosa appeared as a thin connective tissue layer with adipose tissue, blood vessels, and mesothelial cells, supporting previous observations by Starck (1996) [25], Kalita (2009) [9], and Zaher *et al.* (2012) [33].

Ultrastructurally, the epithelial cells of the jejunum in Japanese quail across all age groups comprised mainly absorptive (principal) cells and goblet cells. Goblet cells, scattered among epithelial cells, secreted mucous and varied

in shape from rounded to almond-shaped, with small nuclei, and lacked microvilli on their apical surface. Their appearance ranged from swollen to thinner, darker cells, indicating regeneration (Fig. 10). Principal cells were tall, columnar with a striated border of microvilli, and contained numerous mitochondria, endoplasmic reticulum, and other organelles. Tight junctions sealed neighboring cells, with desmosomes located below (Fig. 10). Microvilli contained fibrous structures and terminal web, with nuclei large and basally located.

Table 1: Villus height and width, height of columnar cells at villus and crypt, diameter of intestinal glands, lumen diameter, and wall thickness of the jejunum across different age groups of Japanese quail (values in μm).

Age groups	Jejunum										
	Height of villi (μm)	Width of villi (μm)	Height of columnar cells at villus (μm)	Height of columnar cells at crypt (μm)	Diameter of intestinal gland (μm)	Diameter of lumen (μm)	Tunica mucosa (μm)	Innermost longitudinal layer of tunica muscularis (μm)	Middle circular layer of tunica muscularis (μm)	Outer longitudinal layer of tunica muscularis (μm)	Tunica Serosa (μm)
7 th day	336.42 ^c ± 33.70	70.13 ^c ± 4.95	23.61 ^c ± 1.50	10.20 ^c ± 0.78	36.76 ^b ± 1.61	2009.52 ^c ± 63.24	373.68 ^b ± 36.96	4.83 ^b ± 0.40	16.10 ^c ± 1.43	9.53 ^c ± 0.85	4.83 ^c ± 0.49
14 th day	375.87 ^b ^c ± 24.25	88.76 ^{bc} ± 5.15	25.95 ^{bc} ± 0.96	13.69 ^b ± 0.90	42.61 ^{ab} ± 1.75	2145.62 ^b ± 60.06	419.70 ^b ± 20.82	6.31 ^b ± 0.24	24.42 ^b ± 1.40	12.88 ^b ± 0.59	6.31 ^{bc} ± 0.70
21 st day	453.68 ^b ± 26.02	106.30 ^a ^b ± 10.30	28.18 ^{ab} ± 1.44	14.25 ^b ± 0.83	44.89 ^a ± 1.86	2302.55 ^{ab} ± 113.43	515.04 ^a ± 23.84	9.26 ^a ± 0.63	29.52 ^b ± 1.98	15.83 ^a ± 0.74	7.65 ^b ± 0.77
42 nd day	520.52 ^a ± 26.22	122.73 ^a ± 11.60	31.37 ^a ± 1.60	16.78 ^a ± 0.83	47.76 ^a ± 2.82	2495.58 ^a ± 72.27	590.65 ^a ± 28.38	11.00 ^a ± 0.99	36.23 ^a ± 2.25	17.44 ^a ± 1.22	9.53 ^a ± 0.54

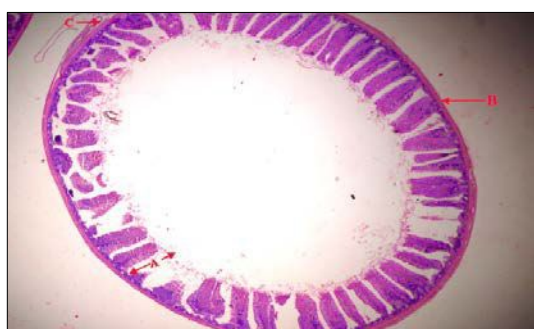


Fig 1: Photomicrograph of jejunum at 21st day of age

- a. Tunica mucosa
 - b. Tunica muscularis
 - c. Tunica serosa
- (Haematoxylin and Eosin, X 40)

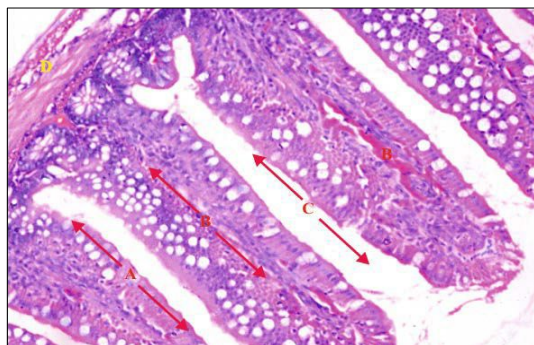


Fig 2: Photomicrograph of jejunum at 14th day of age

- a. Lamina Epithelialis Mucosa
 - b. Lamina Propria
 - c. Villus
 - d. Tunica muscularis
- (Haematoxylin and Eosin, X 400)

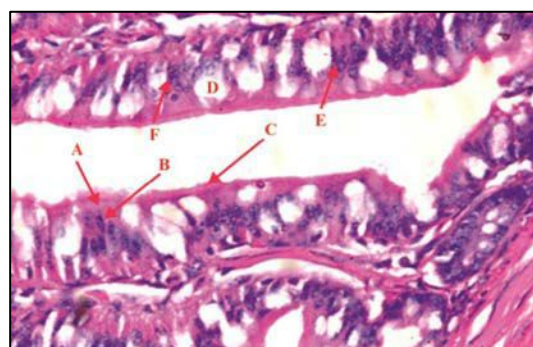


Fig 3: Photomicrograph of jejunum at 14th day of age

- a. Chief cells
 - b. Nucleus of chief cells
 - c. Striated border
 - d. Goblet cells
 - e. Enterochromaffin cell
 - f. Globular leucocyte
- (Haematoxylin and Eosin, X 1000)



Fig 4: Photomicrograph of jejunum at 42nd day of age

- a. Reticular fiber
 - b. Inner reticular lamina
 - c. Outer basal lamina
 - d. Reticular fibers in tunica muscularis.
- (Silver impregnation stain X400)

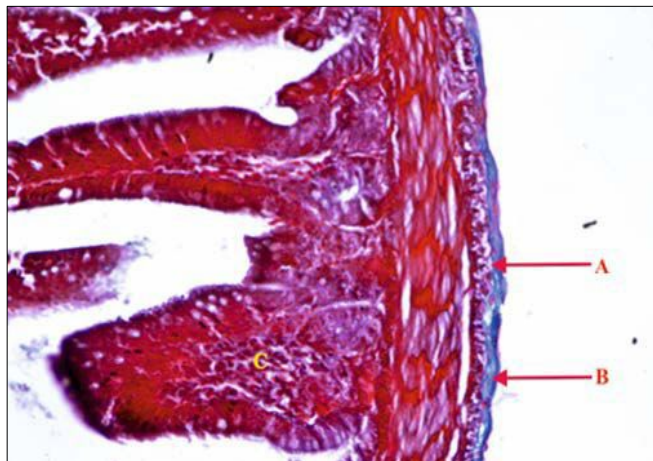


Fig 5: Photomicrograph of jejunum at 42nd day of age.

- a. Collagen fiber
 - b. Tunica serosa
 - c. Lymphatic tissue
- (Masson's Trichrome, X 100)

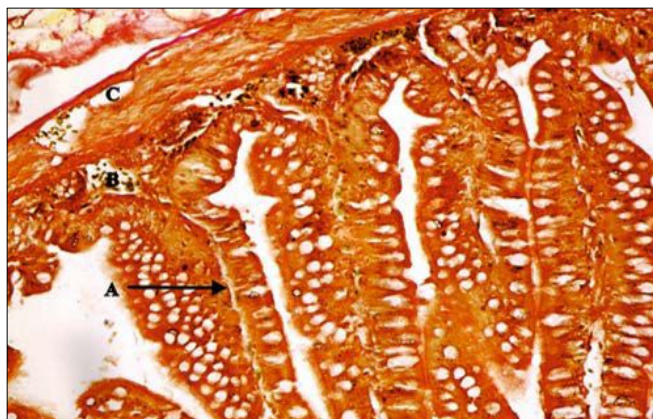


Fig 6: Photomicrograph of jejunum at 7th day of age

- a. Elastic fiber
 - b. Nucleated RBC
 - c. Blood vessels in inner circular layer of tunica muscularis
- (Verhoeff's stain, x 100)

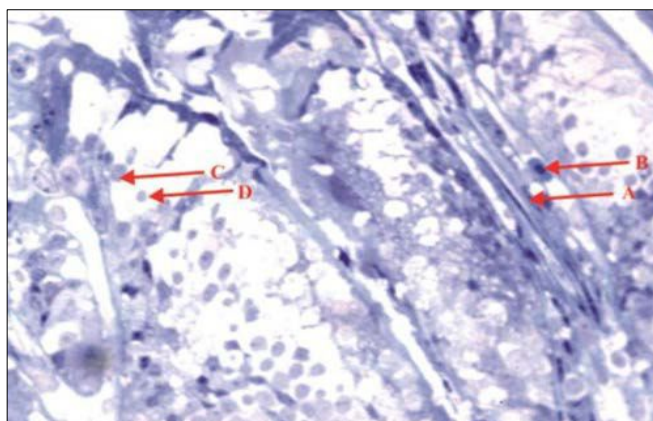


Fig 7: Photomicrograph of jejunum at 14th day of age

- a. Smooth muscle fibres

- b. Erythrocytes
 - c. Large lymphocyte
 - d. Small lymphocyte
- (Toluidine blue, X1000)

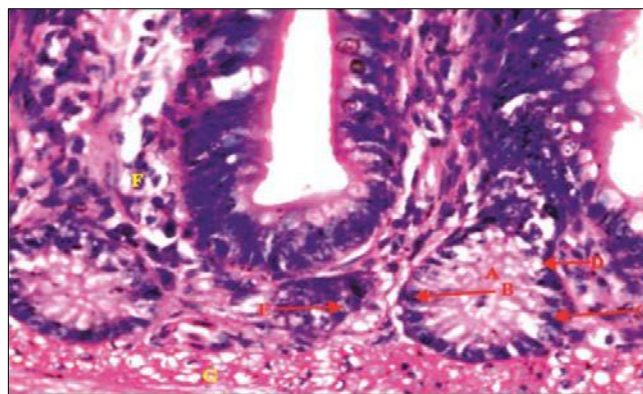


Fig 8: Photomicrograph of jejunum at 42nd day of age

- a. Intestinal gland
 - b. Chief cell
 - c. Enterochromaffin cell
 - d. Goblet cell
 - e. Vacuolated cell
 - f. Interglandular lymphatic tissue
 - g. Inner longitudinal muscle layer
- (Haematoxylin and Eosin, X 100)

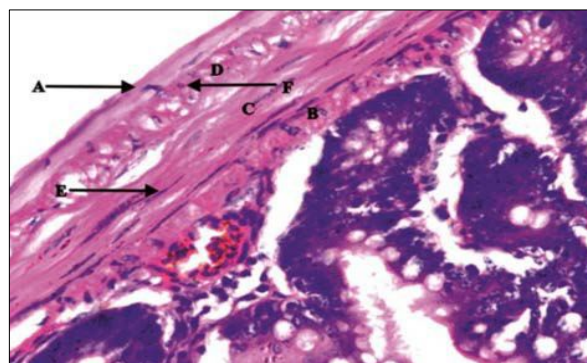


Fig 9: Photomicrograph of jejunum at 42nd day of age

- a. Tunica serosa
 - b. Inner longitudinal layer
 - c. Middle circular layer
 - d. Outer longitudinal layer
 - e. Elongated nucleus
 - f. Rounded nucleus
- (Haematoxylin and Eosin, X 100)

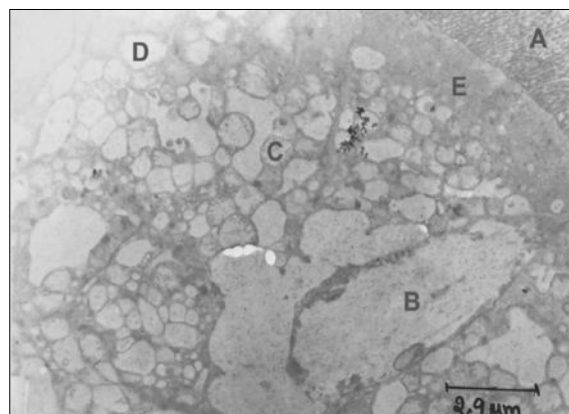


Fig 10: Transmission electron photomicrograph of jejunum at 42nd day of age

- a. Microvilli
- b. Goblet cell
- c. Vesicular mitochondria
- d. Membrane bounded vesicle
- e. Terminal web

(Transmission electron microscope, X 10060)

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