Morphological, histochemical and histological study of the ileum in the Iraqi Black Partridge (*Francolinus francolinus*)

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**Abstract.** Objective: This project aimed to study the morphological description and histological structure of ileum in Iraqi black partridge (*Francolinus francolinus*). Methods: To conduct this investigation, 20 healthy Iraqi black partridge were collected from local suppliers. Birds were euthanized, dissected and then specimens were processed for histological and histochemical staining techniques. Results: A morphological study showed that the ileum appears as a short narrow tubular structure. It extends from the wide end of the jejunum and ends in the Ileo-caecal junction region. Histologically, the wall ileum consists of four tunica mucosa, submucosa, muscularis and serosa. The mucosa was lined by simple columnar epithelium with goblet cells. The muscularis mucosa is arranged in two thick layers of smooth muscle fibers, outer longitudinal and inner circular bundles. The tunica submucosa was a thin layer of fibrous connective tissue. Tunica serosa was loose connective tissue covered by mesothelium. The goblet cells gave a positive reaction with PAS stain. Conclusion: Goblet cells neutral mucopolysaccharide secretions; in fact, the latter stain is an indicator mucin substance which are very important in digestion and absorption and subsequent body growth of the bird.

**Keywords.** Histomorphological study, Ileum, Iraqi Black Partridge.

1. **Introduction**

The food mechanism is an important determinant of the success of the adaptation of birds in their environment, also the nature of nutrition and the way it is obtained is considered as an effective factor on the morphological and histological composition of the digestive tracts parts [1, 2]. The digestive system in birds is necessary to convert the food intake by the bird into simple materials used by the body to carry out its daily activities. Food is then transformed into crude materials that synthesis, nutrition and restore the cells of the body through the destruction of food molecules, absorption and transmission through the bloodstream and the body disposes of its remains. The small intestine is the first site concerned with the breakdown of enzymes, in addition to absorption of carbohydrates, fatty acids, and amino acids [3]. The small intestine in birds is characterized by its length and frequent wrap, the small intestine begins from the back end of the stomach and ends with the ileo-caecal junction and divides into three parts: duodenum (part one), jejunum (middle part) and ileum (last part)
[4, 5], Iraqi black partridge (*Francolinus francolinus*) returns to Phasianidae family of Galliformes order, and all of this family’s birds are wild and are called the same name to the male and female as well as they rely on the grains as food (Graniivorous) [6]. The aim of study is designated to determine the Morphological description and histological structure of ileum in Iraqi black partridge (*Francolinus francolinus*).

2. Materials and Methods

2.1. Birds Collection

Twenty of Iraqi black partridge (*Francolinus francolinus*) collected from local suppliers in Baghdad Province.

2.2. Morphological Study

Gross morphology, topographical relationship in situ and other anatomical observations were studied. The ileum was identified and photographed in situ using a Digital Sony camera.

2.3. Histological and Histochemical Preparation

For histological study, the specimens were fixed in neutral buffered formalin of 10% concentration for 48 hours. After well fixation, the specimens were dehydrated by passing them through a series of ascending ethanol concentrations (70, 80, 90 and 100 %) and then the specimens were cleared by xylene. After that, they were embedded in paraffin wax, then the blocks were sectioned at 3-5 µm thickness and stained with the following stains: Hematoxylin and eosin routine stain for general features identification. The histological examination was done by using light microscope (Kruss) and photographed using (14.1) mega pixels power digital Sony camera. In the histochemical study, sections were stained with Periodic Acid Schiff (PAS) and was used for the illustration of the goblet cells and the basement membranes of the epithelial lining of the ileum [7].

3. Results and Discussion

3.1. Morphological Study

The results of the gross examination showed that the ileum appears in the form of a short tubular composition of a thin diameter, the jejunum is associated with ileum by a fold of mesenteric membrane to form together a group of coils or irregularly shaped open twists. The ileum begins at the wide end of the jejunum and ends with the ileo-caecal junction. The caeca appear conjoined along the sides of the ileum, as the ileum appears less diameter at the bottom near the area of contact with the caecum (Figure 1). Because of the absence of vitlline diverticulum or what is known as Meckel's diverticulum, there is no separation border between the jejunum and the ileum but there is a clear difference in diameter (Figure 2). The results of the current study are consistent with those of [8] in *Coturinx coturnix* and are contrary to the results of [9] in *Anas platyrhynchos*, which stated that the jejunum and the ileum were arranged in large parallel rings taking the letter U overlapping with each other.
Figure 1. The position of jejunum and ileum is illustrated within the body of Iraqi black partridge, jejunum (J), ileum (I), ileo-caecal junction (ICJ), mesenteric membranes (MeM), the rectum (R), the cloaca (CI).

Figure 2. Difference between jejunum (J), ileum (I), caecum (C), duodenum (D), pancreas (Pa), mesenteric membranes (MeM) in the Iraqi black partridge.

3.2. Light microscopic observation

The ileum wall consists of four main tunicae represented by the tunica mucosa, the tunica submucosa, tunica muscularis, and tunica serosa (Figure 3), which is consistent with the results of [10] study. However, it contradicts the results of [11] when the researcher studies the Japanese Quail (*Coturnix Coturnix Japonica*) study which showed that the ileum consists of three tunicae represented by mucosa, muscularis and serosa. This variation in compatibility seems to have something to do with the structural composition based on functional need as well as the specificity of the bird in this study.
3.2.1. Tunica Mucosa

Tunica mucosa consists of three secondary layers represented by the lining epithelium, lamina propria and muscularis mucosa (Figure 4) this result in agreement with [12] when the researcher studies the guinea fowl, while differ from those of Corvus albus study [13], which showed the absence of muscularis mucosa layer in the tunica mucosa.

3.2.1.1. Lining epithelium

The tunica mucosa consists of villi coated by simple columnar epithelium tissue, in which the main columnar cells have a pale cytoplasm, and the nucleus is spherical clearly pigmented, located in the last third of the cell, and based on a membrane basement membrane. The apical surface of the cell is characterized by the presence of a striated border and spread between the columnar cells. Many of the goblet cells possess a swollen apical surface (Figure 5) and this result was identical to the results of [12]. Epithelial cells absorb sugars, amino acids, fatty acids, water and other useful substances [14].

The results of histochemical study showed a positive reaction with periodic acid Schiff (PAS) stain (Figure 5) and used this reagent to confirm the presence of glycoprotein and polymucosaccharides in the goblet cells, basal membrane and striated border. Therefore, the mucin substances stained by purple color and this result came with similar of [15] study. The villi in ileum is characterized by different lengths and some of them take a finger shape (Figure 6) while a few of them show a leaf shape containing some meanders (Figure 7). This result was in full compliance with the results of Tyto alba and Columba palumbus [16] and Anas platyrhynchos [17]. The presence of meanders in leaf shape villi may be due to the fact that nutrient absorption is more efficient when the villi are arranged in this way than if they were parallel; as well as the passage of nutrition will take less time than if the surface was plane. Moreover, the connection between the nutrients and the surface of the cells would be better [18]. The results of the current study differ from those of the Japanese quail Coturnix coturnix japonica study [11], where the villi appeared in the spatula shape. The goblet cells produce a mixture of glycoproteins called mucinogen that acts to lubrication of the intestinal tract as well as protecting them from infection with pathogens, viruses and stomach acidity [19].

3.2.1.2. Lamina propria

The lamina propria is composed of loose connective tissue in which blood vessels, lacteal lymphatic vessels, smooth muscle fibers and nuclei of connective tissue cells are spread as well as positioned intestinal glands (Figure 4). This finding is consistent with the results of Ara ararauna [20], while contrary to [11] study in the Japanese quail Coturnix coturnix japonica, as it was shown that the lamina propria is composed of irregular dense connective tissue contain on reticular fiber, smooth muscle fiber and many blood vessels. This variation in tissue type may be due to the tissue structure associated with the functional specificity performed by the small intestine. The connective tissue that is the component of the lamina propria in partridge at the current study is composed of most connective tissue cells whose main function is support as well as the collagenous and elastic fibers that gain strength and flexibility. Intestinal glands, or Crypts of Lieberkuhn, open in the base of the villi which are simple tubular gland and their wall consists of low columnar cells with pale cytoplasm and spherical nuclei located near the base of the cell. The presence of goblet cells with mucin secretion and basal cells (Stem cells) these cells are based on a basement membrane and surround these cells with a small cavity (Figure 8) and this result corresponds to [12, 21]. These glandular units produce a large number of digestive enzymes whose role lies in facilitating the digestion and absorption of food such as peptidase enzymes and disaccharides that digest dietary bonds [22]. Enteroendocrine cells that appear within the cells lining the intestinal glands are few in number, and are characterized by their pyramidal shape and nucleus spherical shape and occupy a base position of the cell. These cells are characterized by the presence of secretion granules near the bottom of the nucleus present mainly in...
the crypts and occasionally on the villi, these Enteroendocrine cells are of different types. (Figure 8). The histological examination also showed the presence of a limited number from Paneth cells in the bases of the intestinal glands. Paneth cells are characterized as pyramidal cells with cytoplasm containing secretory granules receptive to acidic stains (Figure 9). The importance of enteroendocrine cells is that they are responsible for the secretion of hormones that control the movement of the intestine are organized. Paneth cells are responsible for the production of lysozyme enzymes, which play an important role in the elimination of pathological bacteria [22].

3.2.1.3. Muscularis mucosa

This layer consists of two layers of smooth muscle fibers, longitudinal and circular. The longitudinal muscle fibers lie outwards in the form of bundles thicker than circular muscle fibers. Circular muscle fibers located inward the layers of muscle are separated with auerbach’s plexus of nerves (Figure 10). The results of the current study are similar to the results of Anas platyrhynchos [17] and are contrary to the results of Otus scolbrocie study because of this layer is missing [23]. The importance of this secondary layer lies in the mixing process and the movement of nutrients through contraction within the gut, particularly in the small intestine [24].

3.2.2. Tunica Submucosa

It consists of areolar connective tissue which contains blood capillaries, lymphatic and nerves in the ileum (Figure 10) and this result is consistent with the results of the Guinea fowl study [12].

3.2.3. Tunica Muscularis

This tunica consists of two smooth muscle fiber layers, where the inner smooth muscle fiber sits in a circular order and thicker. While the second layer appears with a longitudinal order and a lower thickness located outward, as interspersed between these two secondary layers are thin tapes of fibrous connective interspersed with blood and lymphatic vessels and nerves (Auerbach’s plexus) as well as collagenous and elastic fibers (Figure 10 and 11). This result is consistent with the result of Tyto alba [16] and a contrast with Japanese quail Coturnix coturnix japonica study, as the tunica muscularis consists of three secondary layers interior and external longitudinal arrangement, while the middle is circular in order [11]. Circular muscles contribute to the expansion of the small intestine diameter while the longitudinal muscles work in the mechanism of pushing food towards the large intestine, and therefore we find that both layers come together to complement the other to complete digestion.

3.2.4. Tunica Serosa

This layer is represented by loose connective tissue. The areolar connective tissue is covered by simple squamous mesothelium interspersed with blood vessels, nerves and adipose tissue and bordered from the outside by a row of mesothelium cells (Figure 11). This result was consistent with the results of [18], in the brown falcon Falco berigora.
Figure 3. Cross section in the ileum wall for the Iraqi black partridge showing the four tunicae: mucosa (M), submucosa (Sm), muscularis (Mu), serosa (S), lumen (L) (H&E x4).

Figure 4. Transverse section in the ileum wall for the Iraqi black partridge showing the three secondary layers of mucosa tunica: lining epithelium (LE), lamina propria (LP), muscularis mucosa (MM) (H&E x 40).

Figure 5. Cross section in the ileum wall for the Iraqi black partridge showing the components of the lining epithelium and the medullary of the villi (CV), columnar cell (CC), goblet cell (GC), a striated border (SB), base membrane (BM) (PAS x100).
Figure 6. Cross section in the wall of the ileum for the Iraqi black partridge showing the finger shape of the villi (V) (H&E x10).

Figure 7. Cross section in the wall of the ileum for the Iraqi black partridge showing the leaf shape of the villi (V) (PAS x40).

Figure 8. Transverse section in the ileum wall for the Iraqi black partridge showing the cells that formed the intestinal glands and the enteroendocrine cell (EC), columnar cell (CC), goblet cell (GC), base cell (BC), basement membrane (BM) (H&E x100).
Figure 9. Cross section in the intestinal gland bases of the ileum wall for the Iraqi black partridge showing Panith cell (PC) (H&E x100).

Figure 10. Transverse section in the ileum wall for the Iraqi black partridge showing the muscularis mucosa layer (MM) with its longitudinal muscular layer (Lml) and circular muscle layer (Cml), as illustrated by tunica submucosa (Sm) and tunica muscularis (Mu) by its longitudinal muscular layer (LML) and circular muscular layer (CML), blood vessel (BV), Auerbach's plexus (AP) (H&E x40).

Figure 11. Cross section in the ileum wall for the Iraqi black partridge showing tunica mucosa (Mu), circular muscular layer (CML), longitudinal muscular layer (LML), tunica serosa (S), blood vessels (Bv) (H&E x10).
4. References

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