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Research Article

Histomorphological and histochemical study of the cecum in moorhen, *Gallinula chloropus*, in Iraq

Rafal YOUSUF*, Azhar Saleem KHALAF
Department of Anatomy, Histology and Embryology, College of Veterinary Medicine, Baghdad University, Iraq.
Email: azhar.s@covm.uobaghdad.edu.iq

Abstract: The goal of the current investigation is to understand the cecum's histological and histochemical composition in moorhens (*Gallinulla chloropus*). This study involved 10 birds that were used for the histological examinations of the ceca, which also included ceca determination. Hematoxylin and Eosin, Periodic Acid Schiff, and Alcian blue-(PH2.5) stain were used for staining the prepared histological slides. According to the findings, the cecum is a muscular tube made up of the four major tunics of the digestive tract: the tunica mucosa, submucosa, muscularis externa, and serosa. In the proximal portion of the ceca, the mucosa was created by the villi, and in the middle, it altered into folds or mounds that resembled flat mucosa. Simple columnar cells with a brush border and goblet cells lined the cecal mucosa. This epithelium expanded to line the Lieberkühn crypts in the folds and bases of the villi, which were situated between the villi and that which filled the lamina propria.

Keywords: Large intestine, Rectum, Histological, Histochemical, Moorhen.
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Introduction

The avian has the second-highest number of species among all vertebrates. For their various food sources, all birds have adapted to their various surrounding environment. Birds have distinct feeding patterns that reflect their diverse lifestyles, and their digestive systems also differ according to their feeding behaviors (Dhyaa et al. 2014; Süzer et al. 2018). The avian digestive tract, like that of all other vertebrates, is a double-ended open tube that begins with food entering through the beak or bill and ends with trash exiting through the vent (Kardong 2006; Ghali & Dauod 2014; Taai Suhaib & Nasif 2020). The shape of the avian gut varies from one species to another because the avian digestive system has undergone physiological changes unsuitable for other animals to fit the physical and chemical characteristics of a wide array of food kinds. It was believed that these variations resulted from different species' dietary patterns (Hamdi et al. 2013; Jawad Salman 2016).

The cecum and rectum make up the large intestine in birds. The intersection of the small and large intestines (King & Mclelland 1975; Allouse 1961) is where the ceca are located in the alimentary canal. Birds vary in the number, size, and shape of their cecum; some have two, some only one, and some do not have any at all. The rectum, which makes up the other portion of the large intestine in birds, is typically small and straight and connects the ileum with the cloaca in a vast number of birds (Shafeq 1983; Mohammed et al. 2018). This study aimed to investigate the histological and histochemical characteristics of the moorhen (*Gallinula chloropus*) rectum in Iraq.

Materials and Methods

The current study was performed with a collection of ten moorhens purchased from local suppliers in Baghdad markets. Prior to dissection, birds were euthanized with an intravenous dose of sodium

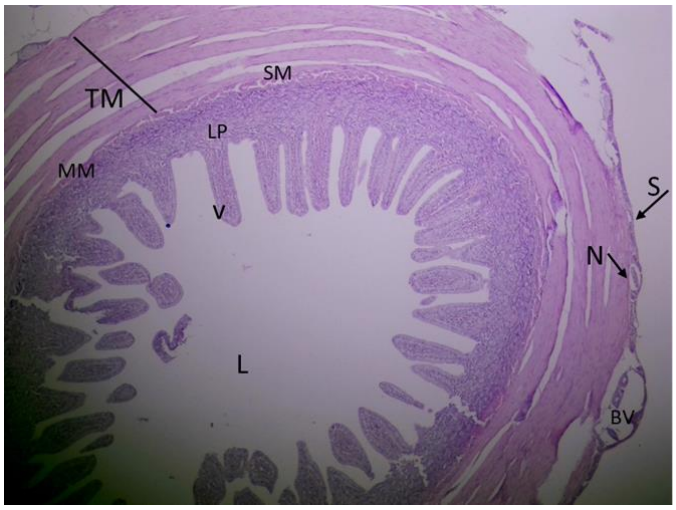

Fig.1. Histological section in the proximal part of cecum wall in moorhen show: Mucosal villi (V), Lamina propria (LP), Mscularis mucosa (MM), Tunica muscularis (TM), Auerbach's plexus (N), Blood vessels (BV), Tunica serosa (S), and Lumen(L) (H&E 4X).

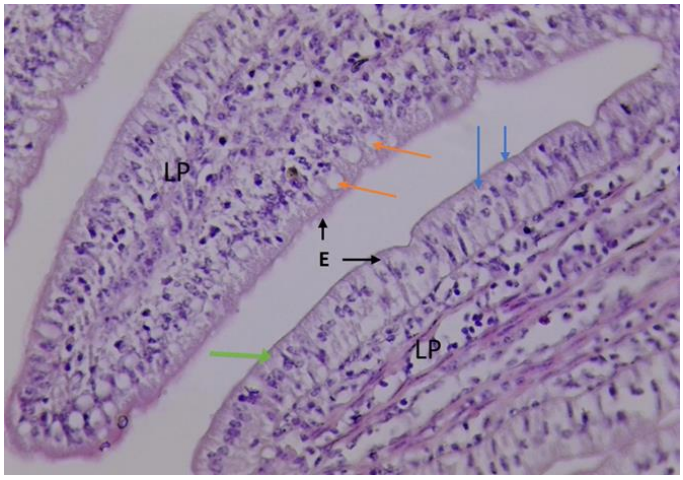

Fig.2. Histological section in the proximal part of the cecum wall in moorhen show: Epithelium (E), Mucosal villi (green arrow), Goblet cells (orange arrows), Columnar cells (blue arrows), and lamina propria (H&E, X400).

pentobarbitone (80 mg/kg), then fixed on a dissecting board. To observe the coelomic viscera, an incision was created in the midline of the abdominal wall. To remove blood or other adherent debris, the cecum was rinsed with a normal saline solution. The contents of the cecum were extracted with mild pressure and then rinsed with normal saline (Khalaf & Merhish 2010; Iman & Abdul Razzaq 2012).

The tissues were preserved in 10% neutral buffered formalin for the histopathological investigation. After fixation, the specimens were dehydrated by passing them through a series of ascending ethanol for 2 h (70, 80, 90, and 100%) and then cleared in xylene for 1 h before being embedded in paraffin wax and sectioned at 6µ thickness and stained with one of the following stains: Mayer's hematoxylin and eosin routine stain for identifying general feature (Luna 1968; Al-Saffar & Al-Samawy 2016; Khaleel et al. 2017).

The fixed parts were sectioned at 5µm and stained with one of the stains listed above to conduct the histochemical investigation. The PAS alone was used for the illustration of the goblet cells and the basement membranes of the epithelial lining the cecum, and for the determination of the acidic mucin Alcian blue (AB) (pH 2.5) (Luna 1968). The prepared slides were subsequently examined and photographed by a microscope, equipped with a digital camera.

Results and Discussion

The ceca in moorhens had a similar histological structure to that of chicken and goose, but it was of the opposite intestinal type as described by Clench & Mathias (1995) in carnivorous and granivorous birds, in which the ceca may function as a secondary lymphatic tissue and as part of the immune system. These variations may result from the different ways that the ceca functions in relation to the feeding habits of these birds (AL-Aredhi 2013). This intestinal type is histologically identical to the small intestine found in herbivores and the majority of granivorous birds, where the ceca's function includes the microbial fermentation of complex carbohydrates not digested in the small intestine used for absorption (Al-Zaydy 2011). Additionally, it demonstrated that the mucosa, submucosa, muscularis externa, and serosa made up the ceca (Figs. 1, 2). In contrast to findings of Al-Zaydy (2011), in the cecum of chickens and geese, the submucosa appeared as a thin layer of connective tissue containing blood and lymph arteries, different findings were found by Hamdi (2013) in *Elanus caeruleus*, in which the wall of the ceca lacks the submucosa.

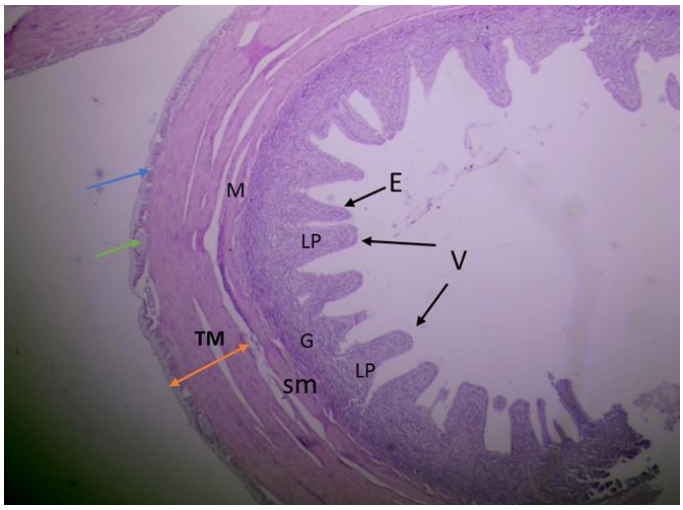

Fig.3. Histological section in the middle part of the cecum in Moorhen show: Epithelium (E), Villi (V), Crypt of lieberkuhn (G), Lamina propria (LP), Muscularis mucosa (M), Tunica sub mucosa (SM), Tunica muscularis (TM), Auerbach's plexus (Green arrow), and serosa (blue arrow) (H&E, X40).

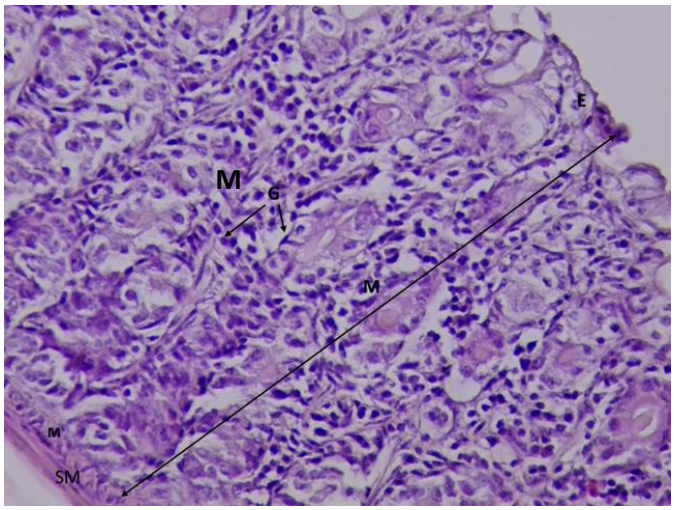

Fig.4. Histological section in the distal part of the cecum shows: Mucosa (M), Epithelium (E), mucosal gland (G), Muscularis mucosa (M), and Submucosa (SM), (H&E, X400).

The mucosa of the cecum was thrown up into a variety of sizes and forms of villi, first appearing as finger- or leaf-shaped villi at the proximal section (Figs. 1, 2), before changing into mounds or folds in the middle (Fig. 3), and finally becoming flattened mucosa in the distal half (Fig. 4). This finding contrasted with those findings of Jawad Salman (2016) in common kestrels and AL-Nassiri (2012), which had lymphoid-type cecums with immunological and defensive functions. While Clench (1999) pointed out that the proximal cecum may have an absorptive role and serve as a habitat for symbiotic bacteria that help with the digestion of fibrous dietary components. Similar findings in geese and chicken (AL-nassiri 2012) may be explained by the fact that in Galliformes and grainivorous birds - herbivorous birds that feed on low levels of protein and nitrogenous materials with high levels of fibrous nutrients - the ceca are regarded as a functional extension of the small intestine's functional surface area (AL-Nassiri 2012).

The deep crypts of Lieberkühn, which were just basic branched tubular glands (Figs. 3, 4), were lined with a simple columnar epithelium with many goblet cells (Fig. 2, 5). While goblet cells have a narrow base and a rounded or inflated apical region,

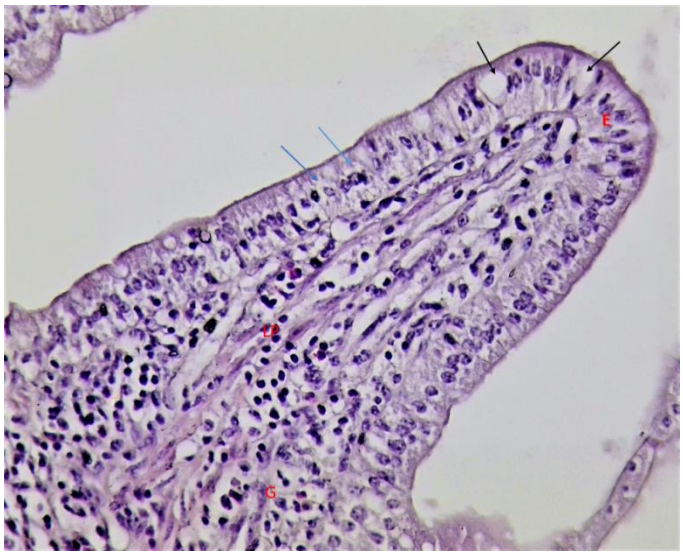

Fig.5. Histological section in the Middle part of the cecum in moorhen show: Epithelium (E), Goblet cells (Black arrows), Columnar cells (blue arrows), and Lamina propria (LP) (H&E, X400).

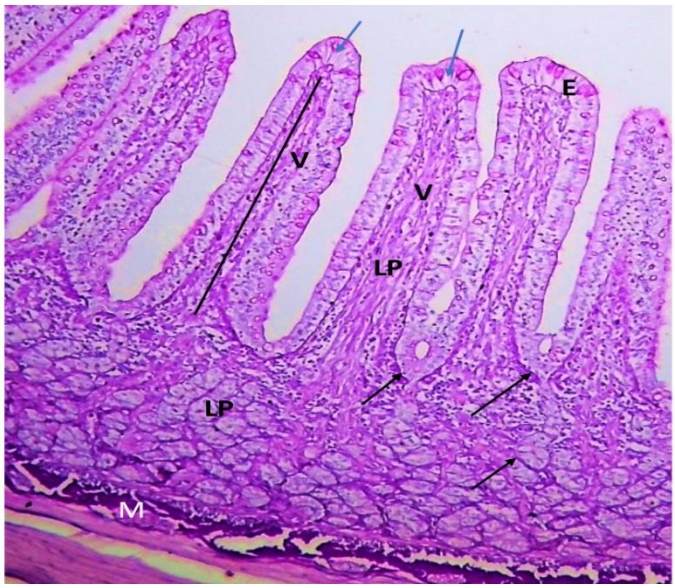

Fig.6. Histological section in the proximal part of the cecum wall in moorhen show: Epithelium (E), Positive reaction mucosal gland (black arrows), Goblet cells (Blue arrows), Lamina propria (LP), and Muscularis mucosa (M) (PAS stain, X40).

columnar cells contain transparent cytoplasm and elongated nuclei (Fig. 5). The goblet cells responded positively to PAS stain, which revealed the presence of mucin in the villi and crypts (Figs. 6, 7, 8), and to Alcian-PH2.5, which highlighted the acidic mucin released by the goblet cells (Figs. 9, 10, 11). Similar findings in domestic pigeons were reported by Al-Saffar & Al-Samawy (2016). This finding demonstrated that the goblet cells release mucus that aids in the lubrication and protection of the mucosa (Aitken 1988). This outcome is in line with Clench et al. (1989) for poultry.

Numerous crypts Lieberkühn were present throughout the lamina propria along the cecum (Figs. 3, 4, 7, 8). As shown in Figures 1, 2, 3, 5, and 6, the lamina propria also expanded into the villi and folds of the cecum. At the proximal region of the cecum, the muscularis mucosa is observed as a thin layer of smooth muscle fibres that gradually becomes very thin and interrupted (Figs. 1, 3, 4). This discovery was made by AL-Aredhi (2013) in Green-winged teal Anas crecca and by AL-Nassiri (2012) in broilers.

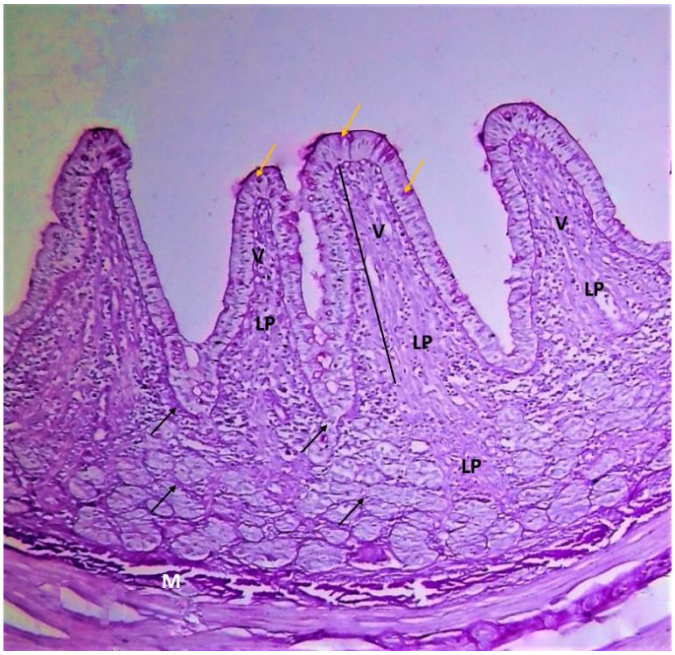

Fig.7. Histological section in the middle part of the cecum wall in moorhen show: Epithelium (E), Positive reaction mucosal gland (black arrows), Goblet cells (yellow arrows), Lamina propria (LP), and Muscularis mucosa (M) (PAS stain, X40).

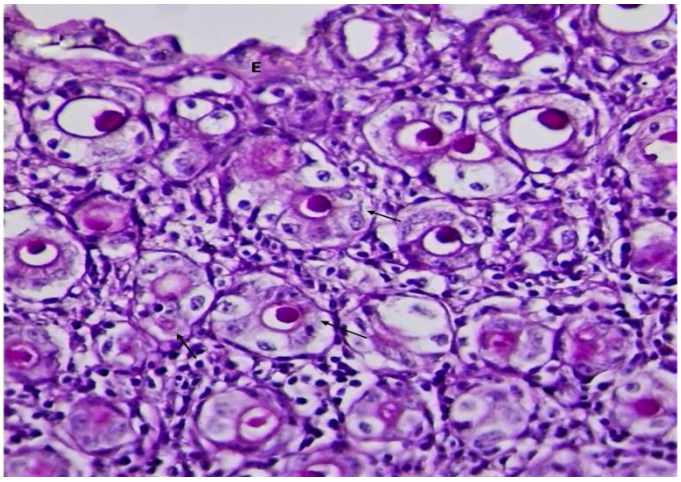

Fig.8. Histological section in the distal part of cecum wall in moorhen show: Epithelium (E), Positive reaction mucosal gland (black arrows) (PAS stain, X400).

The lamina propria is surrounded by a layer of smooth muscle fibres allowing the emptying of the contents of crypts of lieberkuhn by its contractibility.

The muscularis served as a support for the submucosa and mucosa (Fig. 1, 3). The muscularis externa is made up of a thick layer of smooth muscle fibres oriented into an outer longitudinal layer and an inner circular layer that form a sphincter at the proximal part of the cecum, then gradually thin out along the middle and distal parts of the cecum (Figs. 3, 4). This discovery was made by AL-Aredhi (2013) in Green-winged teal Anas crecca and by AL-nassiri (2012) in broilers.

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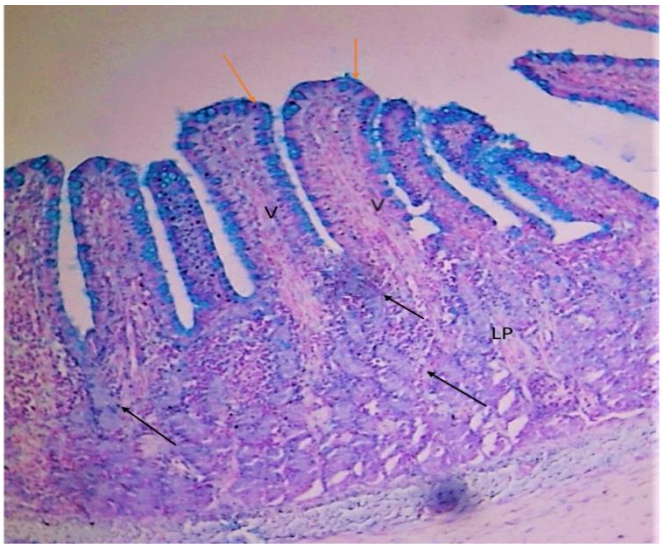

Fig.9. Histological section in the proximal part of the cecum wall in moorhen show: Villi (V), Positive reaction mucosal gland (black arrows) and Epithelia Goblet cells (orange arrows) (Alcian blue stain, X10).

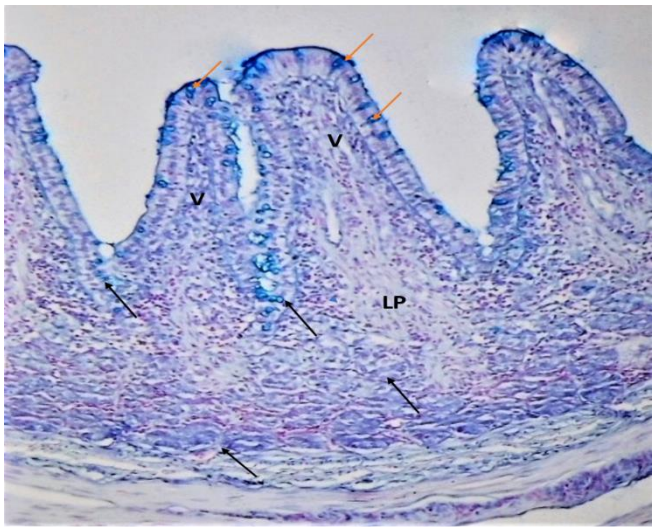

Fig.10. Histological section in the middle part of the cecum wall in moorhen show: Villi (V), positive reaction mucosal gland (black arrows) and Epithelia Goblet cells (orange arrows) (Alcian blue stain, X10).

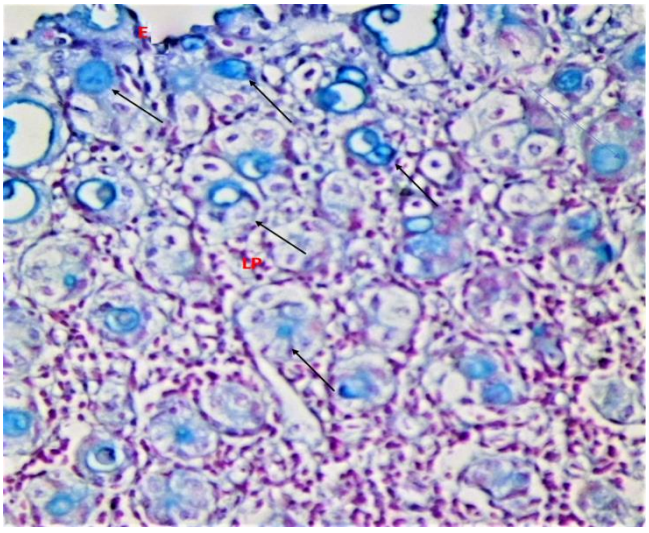

Fig.11. Histological section in the distal part of the cecum wall in moorhen shows Epithelia (E), Positive reaction mucosal gland (black arrows) and Epithelia Goblet cells (Alcian blue stain, X400).

along the middle and distal parts of the cecum (Figs. 3, 4).

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