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Histology of the small intestine of broiler chicks

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KEYWORDS

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Tunica muscularis
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ABSTRACT

This study was conducted to investigate the histology and scanning electron microscopy of the small intestine of the broiler. Samples were taken from different regions of the small intestine of twenty broiler chicks (Ross308) at 42 days of age. The histological observations in the present study revealed that the mucosal layer of the small intestine of the broiler chicks had neither discernible muscularis mucosae nor tunica submucosa. Thus, the arrangement remained a distinctive feature and the intestinal layers were identified, from inside out, as tunica mucosa, tunica muscularis and tunica serosa. Moreover, the histological and scanning electron microscopic findings in this study showed branched villi which were mainly located in the duodenum and rarely in the jejunum and ileum. The tunica muscularis in all parts of the small intestine presented three smooth muscle layers; inner longitudinal, middle circular, and outer longitudinal layers. The middle circular layer was broad and most often appeared separated by small amount of connective tissue into vast outer part and a tiny inner or accessory segment. It is concluded that the wall of the small intestine of broiler chicks had remarkable histological structure, particularly the mucosa, which suggested to improve nutrient absorption capability

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INTRODUCTION

Many researches carried out on the small intestine of avian, including chicken and ostrich, have pointed out that the histological structure of the small intestine is composed of four layers namely from the lumen outwards, tunica mucosa, tunica submucosa, tunica muscularis and tunica serosa (Calhoun, 1954; Hodges, 1974; Kadhim *et al.*, 2014). However, these studies showed controversial results mainly in the arrangement and the number of layers that constitute the intestinal wall. For example, studies carried out on different chicken breeds have indicated for the absence of tunica submucosa (Gabella, 1985; Kachave *et al.*, 2009; Rana *et al.* 2015).

It is well known that the small intestine is highly adapted to nutritional and environmental challenges in order to have efficient nutrients' digestibility and absorption (Mitchell and Moretó, 2006). This adaptation is recognized, among others, by alterations in mucosal histology, and villus and microvillus surface (Mitchell and Moretó, 2006). As a result, several studies have indicated for close relationship between histological structure and intestinal function (Shamoto and Yamauchi, 2000; Samanya and Yamauchi, 2002; Yamauchi, 2010; Sittiya and Yamauchi, 2014). Thus, the development of the small intestine is essential to health and performance of chicken (Kawalilak *et al.*, 2010).

Artificial genetic selection has successfully enhanced the growth rate and food conversion efficiency in the commercial broiler chickens. In order to support the increased growth rate of the 'demand organs' such as muscle, bone, fat, skin and feathers, appropriate adaptations must occur in the 'supply organs' such as intestine and liver (Lilja, 1983; Lilja *et al.*, 1985;). Nevertheless, many of the physiological mechanisms mediating such adaptations have not been fully understood (Scanes and Pierzchala-Koziec, 2014).

Taken together, in attempt to bridge the gap of knowledge, the present study was conducted to investigate the histology and scanning electron microscopy of the small intestine of broiler chicks.

MATERIALS AND METHODS

The study was conducted on twenty unsexed broiler chicks (Ross308) reared in littered floor, open-sided, house from day-old to day 42 of age. Management practices and feed formulation were applied in accordance with the guidelines given by company breeder. At day 42 of age, birds were slaughtered humanely. All procedures of birds rearing and slaughter method were approved by the Sudan Veterinary Council (Ethical approval No. EA/0035/2019).

Tissue samples of the small intestine were then collected for histology and scanning electron microscopy. The samples were taken from the middle part of the duodenum, jejunum and ileum of each bird. The demarcation between jejunum and ileum was determined by the Vitelline diverticulum. For histology, the samples were washed in phosphate buffer (PH 7.4) and fixed in 10% neutral buffered formalin for 24 hours. Tissues were then processed routinely and embedded in paraffin wax. Sections 3-4µm thick were cut by a rotary microtome (Lieca, Germany) and placed on glass slides coated with 0.1% poly-L-Lysine. Different staining methods were then applied on tissue sections: Haematoxylin and Eosin (H&E), Masson's Trichrome, Verhoeff's and Gomori's reticulin stains.

For scanning electron microscopy, the collected samples (about 5 mm^2 each) were immediately washed with 0.1 M phosphate buffer (PH 7.4) and fixed in 2.5% gluteraldehyde buffered with 0.2 M cacodylate buffer (PH 7.4) for 24 hours. Samples were then washed in 0.2 M Cacodylate buffer (PH 7.4) for three times, 10 minutes each. Afterwards, samples were washed in the same buffer and post-fixed in 2% osmium tetraoxide for 2 hours. Samples were then washed in 0.2 M Cacodylate buffer (PH 7.4), and dehydrated in ethanol (30, 50, 70, 80, 90 and 100%), dried and sputter-coated with gold in a vacuum coater for 3 minutes. Then, samples were microphotographed with Jeol scanning electron microscope (JSM- 6390 LA, Japan).

RESULTS

The wall of the small intestine (duodenum, jejunum, and ileum) of the broiler chicks was formed of tunica mucosa, tunica muscularis and tunica serosa. There was no tunica submucosa and the mucosal layer had no discernible muscularis mucosae (Fig. 1).

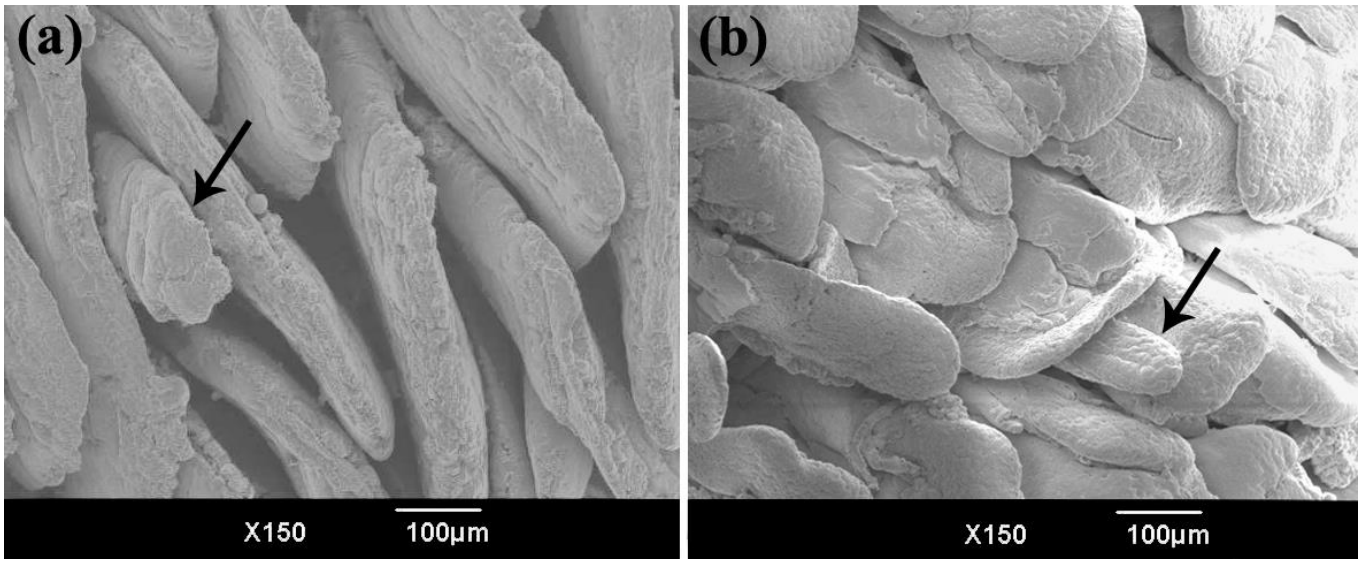

Figure 1: Scanning electron micrographs from the jejunum (a) and ileum (b) of broiler chick at 42 days of age, showing small villi (arrows) among the large ones.

Tunica mucosa

The tunica mucosa was characterized by numerous projections, the villi, which exhibited various sizes and shapes along the different intestinal segments. There were also simple tubular glands (intestinal crypts) situated between the villi. In the three segments of the small intestine, smaller villi were occasionally seen between the large ones (Fig. 1). In the duodenum, some of the villi showed branches, which were either arranged at an acute angle or widely separated forming T-shaped appearance (Fig.2 a); some other villi showed several branches (Fig.2 b). The villi also displayed irregular network by anastomosis which represented by intervillous bridges (Fig. 3). In the jejunum and ileum, branched villi were rarely observed and when seen, they were similar to those of the duodenum.

The mucosa in the different parts of the small intestine was composed of lining epithelium and lamina propria. The luminal surface of the villi as well as intestinal crypts was lined by columnar cells, the enterocytes, with basally located oval nuclei and striated surface which represented by prominent microvillus brush border. The borders were interrupted by the openings of goblet cells that encountered between the enterocytes. The basement membrane of the enterocytes in the villi and crypts regions exhibited a remarkable amount of reticular fibres. The lamina propria, which constituted the core of the villi in the three intestinal segments, was composed of loose connective tissue containing a well-developed network of reticular fibres (Fig.2 a) and scarce amount of collagenous fibres. Elastic fibres were not observed neither within the villus core nor amongst the intestinal crypts.

Tunica muscularis

The tunica muscularis in all regions of the small intestine presented three smooth muscle layers; inner (longitudinal), middle (circular) and outer (longitudinal) layers (Fig. 4 a, b, c). They were separated by a thin layer of intermuscular connective tissue, which was composed mainly of collagenous and reticular fibres, and sparse amount of elastic fibres. The middle circular layer was broad and most often appeared separated by small amount of connective tissue into vast outer part and a tiny inner, or accessory, segment. (Fig. 4 b). The outermost layer of the tunica muscularis was composed of longitudinal smooth muscle bundles enclosed by a network of

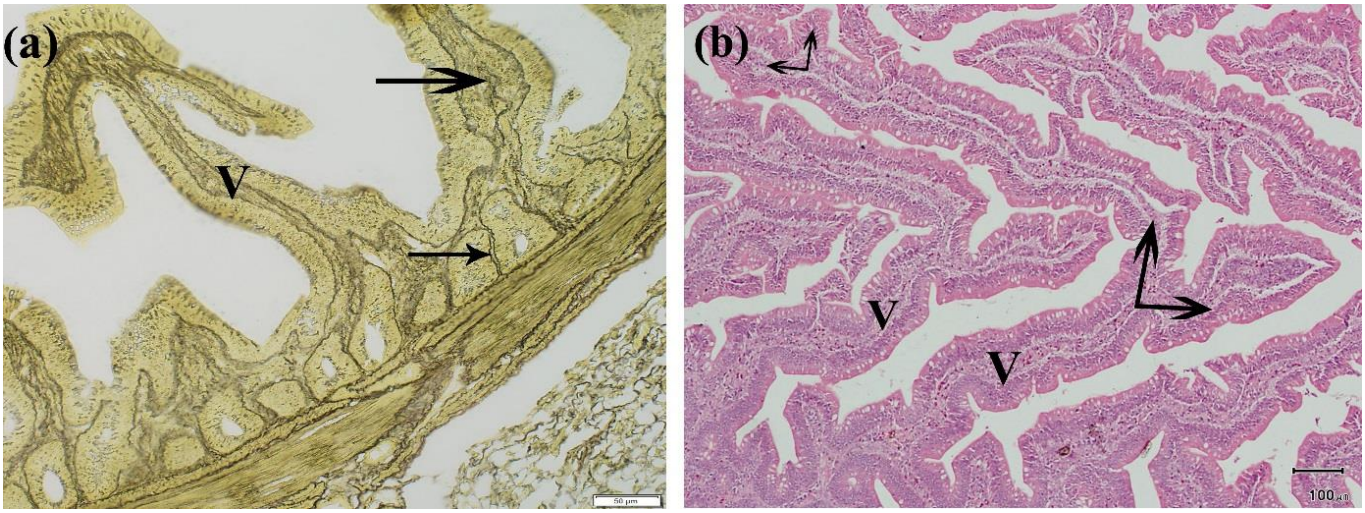

Figure 2: Photomicrographs of the duodenum of broiler chicks showing branched villi. a: T-shaped villus (V) and reticular fibres forming well-developed network within the lamina propria of the villus (large arrow). Large amount of reticular fibres are also present in basement membrane of the enterocytes of the intestinal crypts (small arrow). **Gomori's stain**. b: villus (V) with several branches (arrows). **H&E stain**.

dense intramuscular connective tissue; mainly collagenous fibres that probably arised from the connective tissue of the tunica serosa. In addition, numerous blood vessels, were located between the outer longitudinal and the middle circular muscular layers. These blood vessels course along the intestinal wall and gave off branches that penetrated obliquely the middle and inner layers of the tunica muscularis reaching the lamina propria (Fig. 4 c).

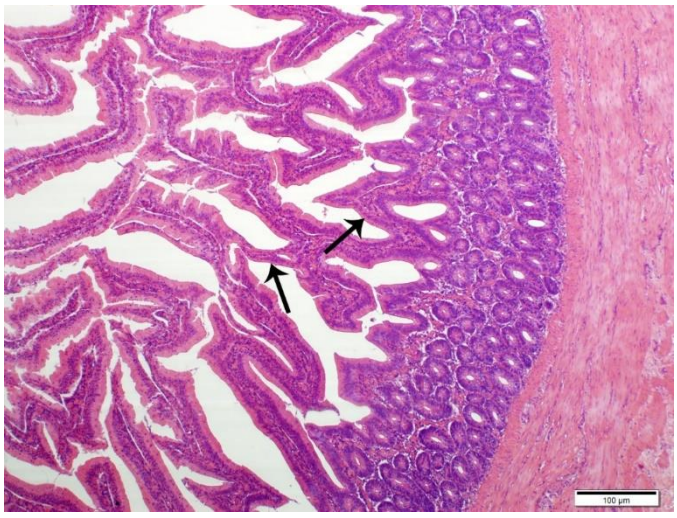

Figure 3: Photomicrograph of the duodenum of broiler chick showing: irregular network of villi formed by anastomosis; intervillous bridges (arrows). **H&E stain.**

Tunica serosa

The outermost serous layer, the tunica serosa, in all parts of the small intestine was composed of a single layer of mesothelial cells resting on a basement membrane, and a well-developed submesothelial connective tissue. Occasionally, the tunica serosa together with the subjacent outer longitudinal muscular layer, were seen undulated. This corrugation was more obvious towards the distal parts of the small intestine (Fig. 4 d). The submesothelial connective tissue was comprised mainly of dense irregular collagenous fibres. There were no evident elastic and reticular fibres seen in tunica serosa.

DISCUSSION

The wall of the small intestine was formed of tunica mucosa, tunica submucosa, tunica muscularis and tunica serosa in domestic mammal (Eurel and Frappier, 2006) and in chicken (Calhoun, 1954; Hodges, 1974; Kadhim *et al.*, 2014). However, histological observations in the present study revealed that the mucosal layer of the small intestine of the broiler chicks had neither discernible muscularis mucosae nor tunica submucosa. Thus, the arrangement remained a distinctive feature and the intestinal layers were identified, from inside outwards, as tunica mucosa, tunica muscularis and tunica serosa. This is in

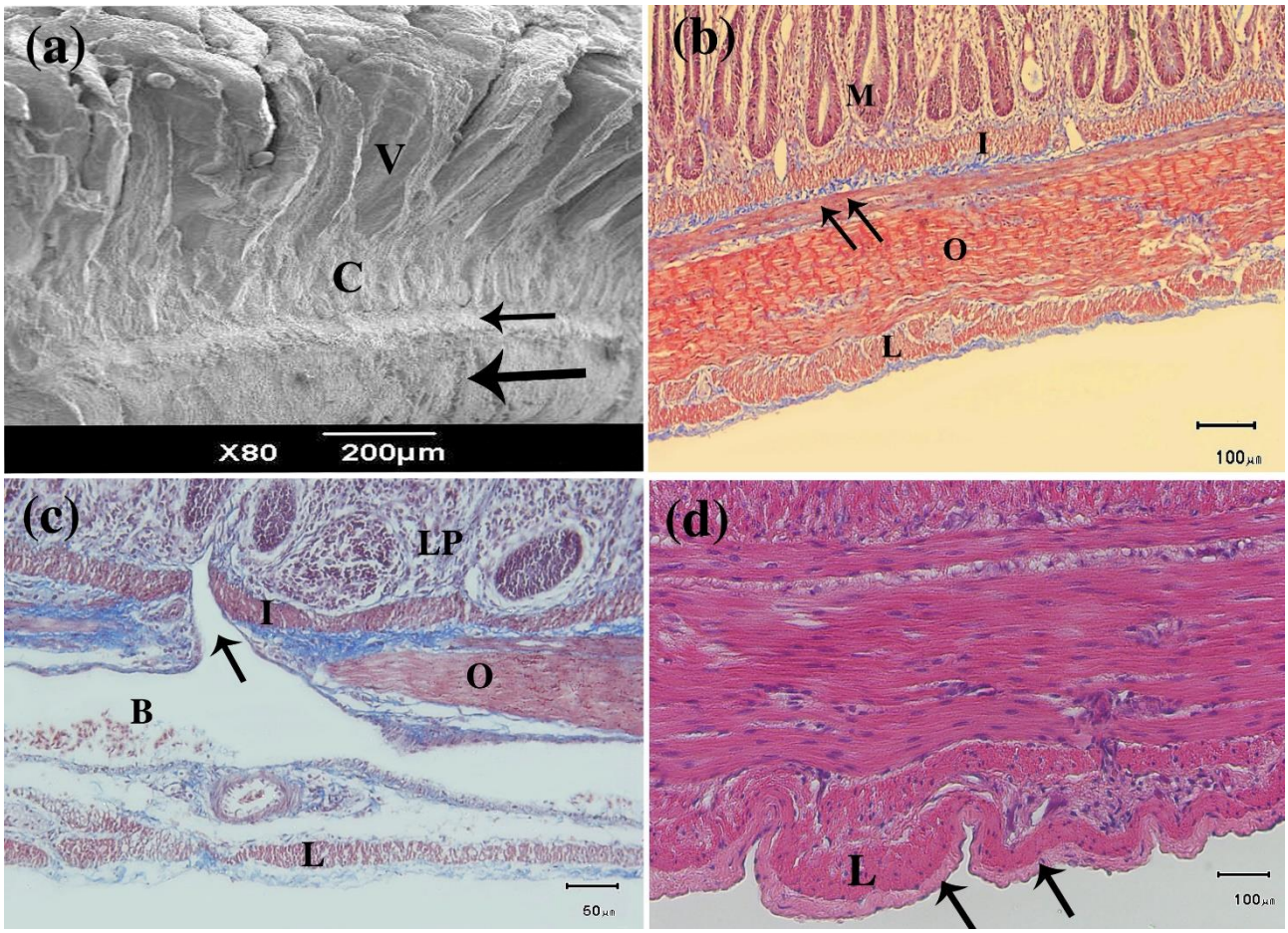

Figure 4: Scanning electron micrograph of a longitudinal section (a), and photomicrographs of cross sections (b, c, d) from the wall of the jejunum of broiler chick at 42 days of age. a: tunica mucosa showing intestinal villi (V) and crypts (C); inner (small arrow) and middle (large arrow) layers of tunica muscularis. b: inner longitudinal (I), middle circular (O) and outer longitudinal (L) layers of the tunica muscularis. The middle circular layer branched into broader outer layer and a narrower inner layer (arrows).**H&E stain**. c: longitudinal blood vessel (B) showing a branch (arrow) that penetrating the middle (O) and inner (I) muscular layers to reach the lamina propria (LP). The outer longitudinal muscular layer (L). **Masson's trichrome stain**. d: undulation of tunica serosa together with the subjacent outer lingitudinal muscular layer (arrows). **H&E stain.**

agreement with findings of Gabella (1985) and Rana *et al.* (2015) in chicken and Bezuidenhout and Van Aswegen (1990) in ostrich.

This study showed that the histological structure of the tunica mucosa of the small intestine of broiler is similar to the earlier findings reported in chickens (Yamauchi, 2002). However, the histological and scanning electron microscopic observations in this study showed branched villi which were mainly located in the duodenum and rarely in the jejunum and ileum. This observation was closely similar to the findings of Bezuidenhout and Van Aswegen (1990) who stated that the small intestine of ostrich contained long villi that branched into secondary and tertiary villi to form a labyrinthine. It is assumed that the branched villi might provide more surface area which may subsequently lead to more efficient absorption of nutrients (Okpe *et al.*, 2016; Elhassan *et al.*, 2021). However, the factors which caused branching of the villi remain to be explained. This study also revealed an anastomosis between the villi in the duodenal region. Similar findings have been reported in craw in which the presence of such anatomical feature suggested to

increase the surface area (Okpe et al, 2016). However, in the current study, the anastomosis was mostly represented by intervillous bridges. Similar finding has been reported by Nakamura and Kumoro (1983) in primitive villi of rat duodenum. Honda (1997) considered these structures as a sign for activated epithelial cells turnover. It is plausible that the role of these epithelial bridges in the present study is not only to increase the absorptive surface area but act as a support for the large duodenal villi of broilers as well. Nonetheless, the mechanism by which the intervillous bridges are formed still needs to be elucidated (Honda, 1997).

In the current findings, the tunica muscularis in all parts of the small intestine of all birds studied, presented three smooth muscle layers; inner longitudinal, middle circular and outer longitudinal layers. This was similar to the findings reported by Gabella (1985) in non-laying *pullets* at age 8-12 weeks. In addition, Gabella (1985) has pointed out for the presence of accessory inner circular muscle layer throughout the different parts of the small intestine. In the present study, however, such muscle layer was found but inconstant in broilers of 42 days of age. This inconsistency in the appearance of the accessory muscle layer may be attributed to the difference in breeds or may be linked with the advance in age.

CONCLUSION

It is concluded that the wall of the small intestine of broiler chicks had remarkable histological structure which suggested improving nutrient absorption.

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