MORPHO-HISTOCHEMISTRY OF THE DISTAL INTESTINES AND RECTUM OF AFRICAN CATFISH (CLARIAS GARIEPINUS)

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Abstract: The morphology of the farmed African catfish distal intestines and rectum was investigated. The samples were dissected out and the tissue slices were passed through graded ethanol, cleared in xylene, embedded in paraffin wax, sectioned and stained for light microscopy. Grossly, the intestine was subdivided into proximal, middle, distal and rectal regions based on palpable thickness and diameter of the regions. No intestino-rectal valve was observed; hence the rectum was not well defined. The histology revealed the presence of tunica mucosa of simple columnar cells, lamina propria of collagen fibres and muscularis mucosae of smooth muscle fibres; submucosa of loose connective tissues and blood vessels; muscularis of smooth muscles in an inner circular and outer longitudinal arrangement; and a serosa in all regions except the rectum and anus that presented tunica adventitia. The mucosal folds were finger-like to orange leaf shaped ingrowths into the lumen on a transverse section. They decreased in complexity and height but increased in width towards the rectum. The lining epithelium of the intestinal tract was comprised of the absorptive simple columnar cells containing goblet cells and intraepithelial lymphocytes. Some rectal simple columnar epithelium contained supranuclear vacuoles while others contained non-vacuolated cytoplasm. The goblet cells contained neutral and acid mucins, and increased in number progressively towards the rectum. The anal tunica muscularis contained mostly skeletal muscles.

Key words: histology, histochemistry, intestine, farmed African catfish.

Introduction

The intestine has attracted a lot of research interest, especially the use of its length in correlating feeding habits. The herbivores have the longest while the carnivores have the shortest length of the intestine. The omnivores have values in between these two (Kramer and Bryant, 1995).

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The histology of the intestine in many teleosts has also been studied (Ezeasor and Stokoe, 1981; Park and Kim, 2001; Cinar and Senol, 2006; Diaz et al., 2008). The structure and function of the stratum compactum seen in the submucosa have been reported in teleost intestine. The cells of the gut associated lymphoid tissues which include eosinophilic granular cells, intraepithelial lymphocytes and wandering leukocytes have been documented as part of intestinal local defense mechanism (Ezeasor and Stokoe, 1981; Dorin et al., 1993; Powell et al., 1993; Delashoud et al., 2010). The nature and form of teleost mucosal folds have also been reported (Reifel and Travill, 1979), even their role in nutrient absorption (Ezeasor and Stokoe, 1981).

Despite the abundance of information on the teleost intestine, no report, from available literature, was found on the basic histology of farmed African catfish intestine from Nigerian commercial aquaculture, hence the need for the investigation of its intestinal morphology. The information obtained will help researchers to understand its digestive physiology. It will also help aquaculture feed industry to maximize feed conversion ratio techniques and clinicians to diagnose fish diseases.

**Material and Methods**

Twenty adult African catfish *Clarias gariepinus* of both sexes from a commercial aquaculture in Eastern Nigeria were used for the study. The fish aged 8 months, measured an average weight of 803±28.31 g and measured a standard body length of 44.91±0.79 cm. The fish were humanely immobilized by deep chloroform sedation before decapitation. The body cavity was cut open through mid ventral incision and the alimentary tract dissected out. The specimen under study – the intestine, was excised from the pyloric sphincter (Figure 1) and sections of proximal, middle, distal intestines and the rectum were immediately fixed in 10% neutral buffered formalin for thirty hours.

The tissues were passed through graded ethanol series, cleared in xylene, impregnated and embedded in paraffin wax. Sections of 5- to 6-µm thickness were obtained with Leitz 1512 microtome (Wetzlar, Germany). They were stained with haematoxylin and eosin for light microscopy (Bancroft and Stevens, 1977). Mucins were demonstrated using alcian blue (AB) at pH 2.5 (Steedman, 1950; Lev and Spicer, 1964) and periodic acid Schiff (PAS) procedure with and without prior digestion with diastase (Lillie and Greco, 1947; Ikpegbu et al., 2011). In addition, the PAS technique was employed in combination with AB for neutral and acid mucin (Bancroft and Stevens, 1977). Photomicrographs were taken with Moticam 2001 camera (Moticam, UK) attached to Olympus CX22 microscope (Tokyo, Japan).
Results and Discussion

Grossly, the pylorus was separated from the proximal intestine by a constriction (Figure 1). The proximal intestine coursed cranially and then turned right coursing caudally to about two-third of the length of the right border of stomach and left border of the gall bladder. The intestine at this point referred to as middle segment was seen coiled just like the distal intestinal segment. The distal intestine made contact with the cranial one-third of the length of the testis in males and cranial pole of the ovary in females and continued as the straight rectum in between the gonads, to the anus. No intestino-rectal valve was observed, but the mucosal folds quickly changed from honey-comb appearance in the proximal intestine to longitudinal folds in the rectum (Figure 2). The proximal intestine on palpation was firmer than the rest of the intestinal segments.

Figure 1. Dissected adult digestive tract showing oesophagus (OE), stomach (S), pyloric sphincter (PS), proximal intestine (PI), middle intestine (MI), distal intestine (DI), rectum (rec), anus (AN). Note the black lines demarcate the different intestinal regions. Scale bar = 5 cm.

Figure 2. Dissected section of adult distal intestine (DI), rectum (REC) and anus (AN). Scale bar = 5 cm.
The complex mucosal fold of the proximal intestine is already described in Ikpégbo et al. (2014).

Middle intestine: The luminal tunica mucosa was modified into mucosal folds. These mucosal folds appear finger-like to orange leaf shaped ingrowths into the lumen on a transverse section (Figure 3). The mucosal folds contained covering epithelium and a lamina propria core. The epithelium was of the simple columnar type and contained goblet cells. A brush border was present at the apical region of the simple columnar epithelium (Figure 4). The lamina propria core contained collagen fibres and blood vessels. The muscularis mucosae were of smooth muscle fibres. The tunica muscularis was composed of inner circular and outer longitudinal layers of smooth muscle cells (Figure 3). The tunica serosa was made up of simple squamous cells.

Distal intestine: The luminal mucosal fold of this segment was broader and shorter than that observed in the middle intestine (Figure 5). The mucosal fold contained simple columnar epithelium with brush border, goblet cells and intraepithelial lymphocytes (Figure 6). The distal intestine contained more goblet cells than the middle intestine. The lamina propria core of the mucosal fold contained collagen fibres, blood vessels and leukocytes (Figure 6). Muscularis
mucosae of smooth muscle cells were observed. The submucosa contained collagen fibres, smooth muscle, leukocytes and blood vessels (Figure 6). The tunica muscularis contained smooth muscles of inner circularly and outer longitudinally oriented fibres.

Figure 5. Section of adult distal intestine showing more goblet cells (white arrow) interspersed simple columnar epithelium (EP). Note the muscularis mucosae (black arrow). Also note migratory leukocytes (ML), blood vessel (BV) in submucosa. H. & E. Scale bar = 40 µm.

Figure 6. Section of adult distal intestine showing brush border (BB) on the simple columnar epithelium (SCE). Note intraepithelial lymphocytes (white arrow) and goblet cell (arrow) in the epithelium. Observe blood vessel (BV) in submucosa. H. & E. Scale bar = 100 µm.
Rectum: The lumen of the rectum on transverse section was compartmentalized due to the in-growth of the whorl-like shaped mucosal folds (Figure 7). The epithelium of simple columnar cells also contained goblet cells and leukocytes. The simple columnar cells of the epithelium were of two cell types. Some were vacuolated while others were non-vacuolated. The vacuolated columnar cells contained ovoid to fusiform vacuoles in the supranuclear region of the cytoplasm (Figures 8, 10). The non-vacuolated columnar cells contained pale eosinophilic apical cytoplasm. The lamina propria core of the mucosal fold was significantly larger because of the presence of dense regular collagen fibres. Blood vessels and leukocytes were also present in the lamina propria. The muscularis mucosae were of smooth muscle cells. The submucosa contained smooth muscle cells, loose collagen fibres, leukocytes and blood vessels. The smooth muscle fibres of the tunica muscularis were arranged in inner circular and outer longitudinal layers. The tunica adventitia contained loose connective tissue fibres and blood vessels.

Anus: The anal histology was very similar with that of the rectum. The exception was thick layers of smooth muscle bundles surrounding the anal region. Adipose tissue and skeletal muscle fibers could also be seen.

Figure 7. Section of adult rectum showing whorl-like mucosal fold. Note simple epithelium (EP) with lamina propria (LP) core. Observe lamina propria/submucosa (LP/SUB), inner circular (CM) and outer longitudinal (LM) smooth muscles. H. & E. Scale bar = 15 µm.
Figure 8. Section of adult rectum showing goblet cells (GC) and vacuolated columnar cells (black arrow) in the epithelium (EP). Note muscle fibres and blood vessel (BV) in the submucosa (SB). H. & E. Scale bar = 40 µm.

Figure 9. Section of adult distal intestine showing abundant PAS positive goblet cells (white arrow). PAS. Scale bar = 40 µm.
Histochemistry revealed that the goblet cells in the intestine were PAS positive (Figures 9, 10), and AB positive (Figures 11, 12, 13). Combined AB-PAS procedures revealed that the goblet cells contained both neutral and acid mucins, but the acid mucin dominated (Figure 14).

Figure 10. Section of adult rectum showing PAS positive goblet cell (white arrow). Note the vacuolated simple columnar epithelium (black arrow) and non-vacuolated simple columnar cells (NC). PAS. Scale bar = 40 µm.

Figure 11. Section of adult middle intestine showing AB positive goblet cells (white arrow). AB. Scale bar = 40 µm.
Figure 12. Section of adult distal intestine showing abundant AB positive goblet cells (white arrow). AB. Scale bar = 40 µm.

Figure 13. Section of adult rectum showing abundant AB positive goblet cells (white arrow). AB. Scale bar = 100 µm.
The intestinal mucosal folds that caudally were of decreasing height, increasing width with denser collagen fibre core from proximal to distal intestine have been reported in literature (Jaroszewska, et al., 2008; Delashoud et al., 2010). This large core may help in withstanding the increased weight as fecal material is formed. The absence of regional differences apart from decreasing mucosal fold height, broadening width and increasing goblet cell number from the proximal to distal intestine has also been reported (Banan-Khojasteh et al., 2009; Xiong et al., 2011).

The increased number of goblet cells seen, especially towards the rectum, is associated with lubrication of the tract against mechanical harm by fecal materials and protection against infectious agents – especially bacteria. This finding has also been reported in the literature (Falk-Petersen and Hansen, 2001; Cinar and Senol, 2006; Banan-Khojasteh et al., 2009; Trevino et al., 2011).

The brush border seen at the apical border of intestinal simple columnar epithelium serves to increase surface area for digestion and absorption (Ezeasor and Stokoe, 1981; Sarieyyupoglu et al., 2000; Hamlin et al., 2000; Jaroszewska et al., 2008; Kozaric et al., 2006, 2008; Banan-Khojastech et al., 2009).

The vacuolated simple columnar epithelium seen in the rectum may be involved in increasing protein digestion intracellularly, thus helping to maximize protein digestion, that was not extracellularly hydrolysed in the stomach and
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intestine. This feature of protein pinocytosis in the vacuolated rectal epithelium is usually seen in teleosts that require high protein content in feed like the rainbow trout (Ezeasor and Stokoe, 1981). However, some authors claim that distal intestine has an osmoregulatory function and does not add to protein digestion (Rombout et al., 2011). The intraepithelial lymphocytes are associated with local defense mechanism (Park et al., 2003; Banan-Khojasteh et al., 2009).

Muscular arrangement of inner circular and outer longitudinal smooth muscles is involved in involuntary contraction that aids food movement caudally. Tunica muscularis has been reported also in Hypophthalmichthys nobilis (Albrecht et al., 2001; Delashoud et al., 2010). The myenteric plexus observed in this study serves as a source of nerve stimulation (Park et al., 2003; Delashoud et al., 2010; El-Bakary and El-Gammal, 2010; Xiong et al; 2011).

In teleosts that lack a well defined rectum as seen in this study, there is neither a change in the diameter of the intestine nor a valve like structure at the beginning of the rectum. The intestino-rectal valve when present prevents the reflux of the posterior intestinal content (Jaroszewska et al., 2008). Such a well defined rectum according to Ezeasor and Stokoe (1981), presented numerous annular mucosal folds which appeared like a stack of caudally-directed funnels under the electron microscope. The presence of skeletal muscles in the anus will help in voluntary control of the rate and time of fecal material passage to the environment. This is important for complete absorption of all nutrients especially the proteins in the rectal region.

Intestinal neutral mucosubstances participate in enzymatic food digestion and absorption (Ribeiro et al., 1999; Kozaric et al., 2008). The higher concentration of the goblet cells at the base of the mucosal fold has been reported (El-Bakary and El-Gammal, 2010). This accumulation may be due to the need to lubricate and protect the basal region as more food and bacteria are likely to stay longer here during digestive contraction. The predominating acid mucin is associated with biofilm formation to protect against parasitic or other pathogens (Neuhaus et al., 2007).

**Conclusion**

The main result presented in this study is absence of intestino-rectal valve in farmed African catfish, a feature that will allow easy passage of food materials to the rectum where intracellular digestion and absorption of protein is done, as was seen in its histology. This assertion is supported by the presence of vacuolated columnar cells in the rectum – a characteristic feature of teleosts that require high protein content in feeds for their upkeep and growth. This information will help catfish nutritionist to increase protein content of African catfish feed to achieve maximum growth.
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Rezime

Istraživana je morfologija distalnog dela creva i rektuma afričkog soma. Uzorci su izolovani disekcijom i delovi tkiva su dehidrirani kroz rastuće koncentracije etanola, prosvetljeni u ksilenu, ukalupljeni u parafinski vosak, isečeni na mikrotomu i obojeni za svetlosnu mikroskopiju. Makroskopski, crevo je podeljeno na proksimalni, srednji, distalni i rektalni region na osnovu palpatorne debljine i prečnika regiona. Crevno-rektalni zalistak nije uočen, pa stoga rektum nije dobro definisan. Histologijom je otkriveno prisustvo: sluzokože od jednostavnih visokoprizmatičnih celija; krzna sa kolagenim vlakanima i muscularis mucosae od glatko mišićnih celija; podsluzokože od rastresitog vezivnog tkiva i krvnih sudova; mišićnog omotača od glatkih mišića sa unutrašnjim kružnim i spoljašnjim longitudinalnim rasporedom; i seroznog sloja u svim delovima osim u rektalnom i analnom delu koji imaju vezivni omotač - tunica adventitia. Mukozni nabori su bili prstoliki ili oblika pomorandžinog lista koji štire u lumen na poprečnom preseku. Složenost i visina nabora su se smanjivali, dok se širina povećavala prema rektumu. Epitel crevnog trakta se sastojao od apsorpcionih jednostavnih visokoprizmatičnih celija, peharastih celija i intaepitelnih limfocita. Neki delovi epitelnog lista sluzokože rektuma sadržali su supranuklearne vakuole dok su drugi imali citoplazmu bez vakuola. Peharaste celije su sadržale neutralne i kisele mucine i njihov broj se povećavao progresivno prema rektumu. Mišićni omotač analnog dela je sadržao uglavnom skeletne mišiće.

Ključne reči: histologija, histohemija, crevo, gajeni afrički som.

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