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THE HISTOLOGY AND MUCIN HISTOCHEMISTRY OF THE FARMED JUVENILE AFRICAN CATFISH DIGESTIVE TRACT (*Clarias gariepinus* B)

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ABSTRACT: The microanatomy of the juvenile African catfish was studied. This study was carried out to provide baseline study on the digestive tract of this important culture seed in the commercial catfish aquaculture in Nigeria, as there is dearth of information on it from available literature to help understand its digestive physiology. The result showed that the oro-pharyngeal wall and tongue were lined by stratified squamous epithelium but the tongue lacked taste buds, thus making it a non-gustatory organ. The lamina propria contained irregular collagen fibres. The oesophageal longitudinal folds was lined by stratified mucous epithelium containing eosinophilic club cells, with the lamina propria core containing regular collagen fibres. The oesophageal tunica muscularis contained skeletal muscles in an inner longitudinal and outer circular arrangement. The stomach was lined by simple columnar epithelium with apical neutral mucin. The gastric epithelium contained intraepithelial lymphocytes. The stomach cardiac and fundic regions contained gastric glands in the lamina propria while none was seen in the pyloric region. The intestinal simple absorptive epithelium contained intraepithelial lymphocytes and goblet cells that increased in number towards the rectum. Mucin histochemistry revealed the presence neutral mucin in the oesophageal mucous cells and this is associated with pre-gastric digestion. Acid mucin present in the intestinal goblet cells are involved with the local defense against pathogenic organisms in the digestive tract. This study provides data for further investigative researches and will help clinicians in diagnosis of its diseases thus helping aquaculture growth.

Keywords: lymphocytes, club cells, histology, mucin. nigeria

INTRODUCTION

Fish is an essential source of protein for mankind in several nations (Hussain et al., 2011; Rubani et al., 2011). However, fish catch in freshwaters and oceans is depleting due to population increase causing overfishing; and also as a consequences of climate change effects like drought and flooding (UNEP, 2004; Limburg, et al., 2011). To make up for this deficit, fish culture in ponds has increased greatly. In Nigeria the most popular culture species is the fishes of the genus Clarias (Bard et al., 1976; Adewumi and Olaleye, 2011). The increasing interest of farmers in catfish culture has produced scarcity of adequate supply of good seed for commercial aquaculture farms (Huisman et al., 1976), some farmers now rely on smaller culture ponds for the supply of juveniles African catfish for their grow out business. This new business model will raise problems of feed management, feed compounding, size of feed, and also issues of diseases associated with intensive rearing of animals. Whereas there are documented researches in the juveniles of other teleosts (Sinha, 1976; Ellis and Reigh, 1991; Zhenzhen et al., 2007), there is dearth of information in available literature on the basic biology including the digestive tract of these juvenile fish from commercial farms in Nigeria, hence paper this paper aimed at providing the baseline on this species digestive tract.

The information obtained from this research fill the knowledge gap, help us inunderstanding its digestive physiology, serve as base line for further investigative research to improve feed conversion ratio, help juvenile catfish nutritionist in formulating compound feed that will help reduce cost of production (Cahu and Zambonio-Infante,2001), and aid clinicians in diagnosis.

MATERIALS AND METHODS

Ten apparently healthy juvenile African catfish of different sex sourced from a commercial aquaculture in Eastern Nigeria were used for the study. They weighed an average of 30.76g and measured a standard body length of 12.03cm. The fish were humanely immobilized. The body cavity was cut open through mid ventral incision and the alimentary tract dissected out. The specimen under study – the digestive tract was excised and sections of oro- pharyngeal wall, tongue, oesophagus, stomach, intestines (proximal, middle, distal and rectum) were immediately fixed in 10% neutral buffered formalin.

The tissues were passed through graded ethanol, cleared in xylene, impregnated and embedded in paraffin wax. Sections 5µm thick were obtained with Leitz microtome model 1512. They were stained with haematoxylin and eosin for light microscopy examination (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;

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Ikpegbu et al., 2011). In addition, the PAS technique was employed in combination with AB for neutral and acid mucin demonstration (Bancroft and Stevens, 1977). Sections were examined with microscope and photomicrographs were taken with – Motican 2001 camera (Motican UK) attached to Olympus microscope.

RESULTS

The oro-pharyngeal wall histology presented stratified squamous epithelium containing PAS positive mucous cells. The lamina propria-submucosa contained dense collage fibres and adipose tissue beneath it (Fig.1). The tongue was lined by stratified squamous epithelium, the lamina propria-submucosa contained collagen fibres while the skeletal muscles of the tunica muscularis were oriented mostly in longitudinal direction (Fig. 2). Hyaline cartilage was present at the base of the tongue.

Grossly, the oesophagus was a short narrow tube connecting the oro-pharyngeal cavity to the stomach. Histologically, the tunica mucosa was modified into longitudinal folds. The folds were lined by stratified mucous epithelium containing eosinophlic club cells and lymphocytes (Fig.3). The mucous cells secreted both neutral and acid mucins but the acid mucins were more concentrated at the base of the longitudinal fold (Fig.4,5). The lamina propria core of the longitudinal fold contained collgen fibres. The tunica muscularis was of inner longitudinal and outer circular skeletal muscles (Fig.6). Cranially, tunica adventitia was seen but the caudal portion was covered by tunica serosa. Blood vessels were seen in the adventitia.

The J-shaped stomach histologically presented three regions from oesophagus to the intestine: cardia (glandular), fundus (glandular) and the most developed portion for gastric digestive activity, pyloric (non-glandular) but has the deepest gastric pit (Fig.7 and 8). The lining epithelium of the entire stomach was simple columnar with a basal nucleus and apical cytoplasm filled with only PAS positive mucin (fig.9). The laminar propria of the cardiac and fundic regions contained gastric glands. The submucosa contained loose connective tissue, lymphocytes and blood vessels. The tunica muscularis contained smooth muscle in a inner circular and outer longitudinal orientation. Myenteric plexus was seen between the two smooth muscle coats.

The intestine was separated from the stomach by the pyloric sphincter containing smooth muscle cells. The intestine was segmented into a short thick linear proximal portion, coiled middle and distal parts and straight rectum opening into the anus. No valve separated the distal intestine from the rectum. The genital and digestive systems had two separate openings hence, no cloaca was observed. The mucosa of the intestine was covered by simple columnar epithelium containing goblet cells and intraepithelial lymphocytes. The mucosal folds of the proximal intestine was modified into branched labyrinthine anastomosing network resembling a honey comb appearance (Fig.10), but the mucosal folds in the other sections of the intestine were simple with the goblet cell number increasing towards the rectum (Fig.11). The goblet cells secreted both neutral and acid mucin (Fig.12) but the acid mucin predominated towards the rectum. The lamina propria core contained collagen fibres and blood vessels. Muscularis mucosae of smooth muscle cells were observed. The submucosa contained loose collagen fibres. The tunica muscularis contained smooth muscles of inner circular and outer longitudinal layers, with myenteric plexus in-between them. Tunica serosa was of simple squamous cells.

The histochemical reaction of the mucosubstances present in the epithelium of the entire digestive tract is presented in table 1.

DISCUSSION

The digestive tract histology under study is very similar to that other teleost (Sis et al., 1979, Grau et al., 1992; Petrinec et al., 2005). The stratified epithelium containing mucous cells in the oro-pharyngeal wall and tongue is for protection (Diaz *et al*., 2008). The absence of taste buds on the tongue makes it a non gustatory organ unlike mammalian tongue that is a gustatory organ.

The modification of the oesophageal mucosal folds into longitudinal fold is for distensiblity to help easy passage of food. (Arellano *et al*., 2001). The large mucin seen is for lubrication and protection of the tract since teleosts are known to lack salivary glands (Kozaric *et al*., 2008; Micale and Mugha, 2011). The neutral mucin is associated with pre-gastric digestion (Domeneghini et al., 1998). The large quantity of the acid mucin at the base of the longitudinal folds may be an adaptation to protect the organ against bacteria accumulation (Neuhaus *et al*., 2007). The eosinophilc club cells are associated with flight response in fish (Singh and kapoor, 1967). The lymphocytes seen are involved in specific defense mechanism (Ezeasor, 1984; Diaz *et al*., 2008). The collagen fibres are for support and strengthening of the longitudinal folds. The tunica muscularis of inner longitudinal and outer circular skeletal muscles may be an adaptation for re-enforcement of contraction force during deglutition.

The gastric mucosa of simple columnar epithelium is for absorption of easily digestible molecules with the aid of neutral mucin (Grau et al., 1992). The mucosubstances are also involved in protecting the gastric mucosa from mechanical destruction by feed materials (Petrinec et al., 2005). The gastric glands in the lamina propria of cardiac and fundic region are oxynticopeptic cells that produce both pepsinogen and hydrochloric acid (Xiong *et al*., 2011). The absence of gastric gland in the pyloric region seen in this study has been reported in other teleost (Chen *et al*., 2006; Cao and Wang 2009). But the absence of gastric gland in the cardia stomach has been reported also (Hernandez et al., 2009). The well developed fundic region must be the major site for gastric digestion. The myenteric plexus is for nervous stimulation (Ezeasor, 1981).

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The smooth muscles in the pyloric sphincter are for involuntary regulation of feed movement into the proximal intestine.

The branched anastomosing mucosal folds seen in the proximal intestine is an adaptation for increased surface area for nutrient absorption, and also reduce the speed of intestinal feed flow. The reduced height of the simple mucosal fold towards the rectum is to allow easy movement feacal materials (Moitra and Ray, 1979; Hernandez et al., 2009). The mucin from increasing goblet cells number towards the rectum is to help lubricate and protect the mucosa from abrasion by fecal materials (Ghosh *et al*., 2011).The absence of a cloaca agrees with the finding of Smith (1978). No valve separated the posterior intestine from the rectum but a valve has been reported in the *Dentex dentex* (Carasson et al., 2006). In conclusion this study for the first time in available literature present the histology of farmed juvenile African catfish digestive tract. Being an important culture species, the findings will help in understanding the digestive physiology of this species juvenile stage- an important step towards sustainable feed formulation for the commercial aquaculture source of stocking seed. It will facilitate further investigative research and help pathologist in disease diagnosis.

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Table 1

Mucin histochemical reaction of epithelial mucosubstances in different portions of the digestive tract of juvenile *Clarias gariepinus*

Procedure	T	BC	E	S	PI	MI	DI	R
PAS	+	+	+	+	+	+	+	+
AB (PH 2.5)	+	±	+	-	++	++	++	+
AB/PAS	AB	AB	PAS	PAS	AB	AB	AB	AB

KEY: AB, Alcian blue; PAS, Periodic Acid Schiff; AB/PAS, combined Alcian blue with PAS procedure after diastase treatment; BC, oro-pharyngeal wall; T, Tongue; E, Esophagus; S, Stomach; Pl, proximal intestine; MI, Middle intestine; DI, distal intestine; R, Rectum; (-), no staining observed; (±) poorly stained (+), low; (++), medium; (+++), high; AB, AB dominance; PAS, PAS dominance; AP, equal coloration .

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FIGURES

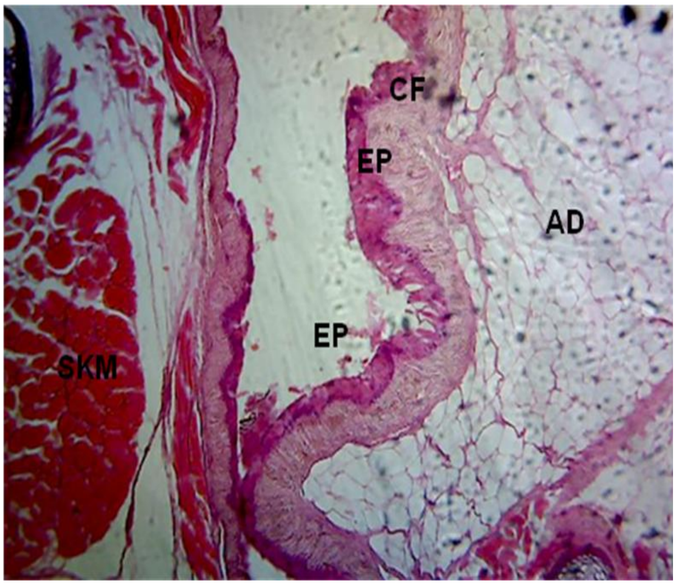

Fig.1 Section of juvenile oro-pharyngeal wall showing stratified epithelium (EP), collagen fibre (CF) and adipose tissue (AD) in the lamina propria-submucosa. Note the skeletal muscle (SKM) contained in the tunica muscularis. H. & E. X100.

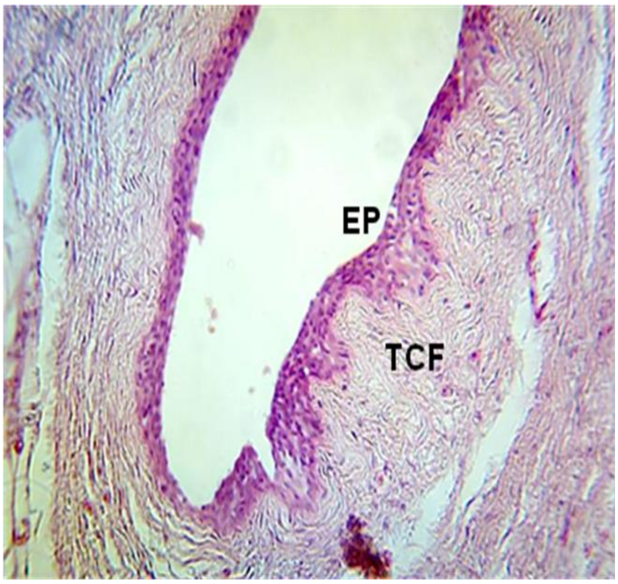

Fig. 2 Section of juvenile tongue showing stratified epithelium (EP), collagen fibres (TCF) in the lamina propria. H. & E. X 400.

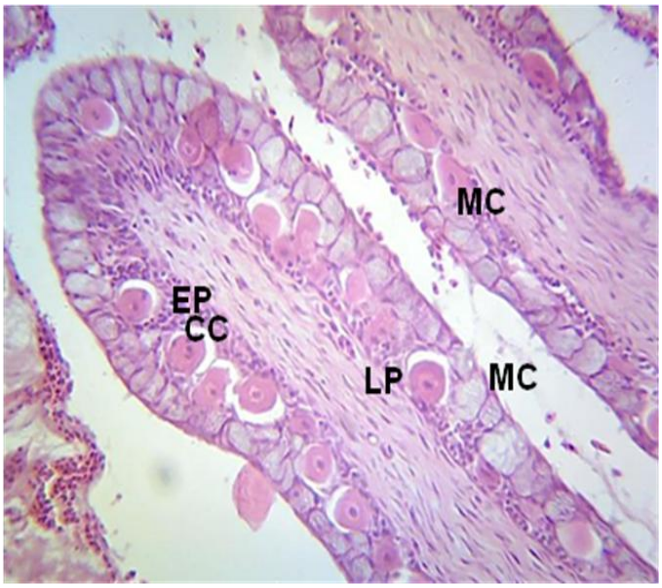

Fig.3 Section of juvenile esophagus longitudinal folds showing stratified mucous epithelium (EP) containing eosionphilic club cells (CC).MC- mucous cells. The lamina propria (LP) contained collagen fibres . H. & E. x400

Fig.4 Section of juvenile oesophagus showing longitudinal fold mucous cells that contained a combination of acid and neutral mucin (C), acid (A), neutral mucins(N).AB/PAS X100

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Fig. 5 Section of juvenile oesophagus showing longitudinal fold mucous cells that contained a combination of acid and neutral mucin (C), acid (A), neutral mucin (N).AB/PAS X400

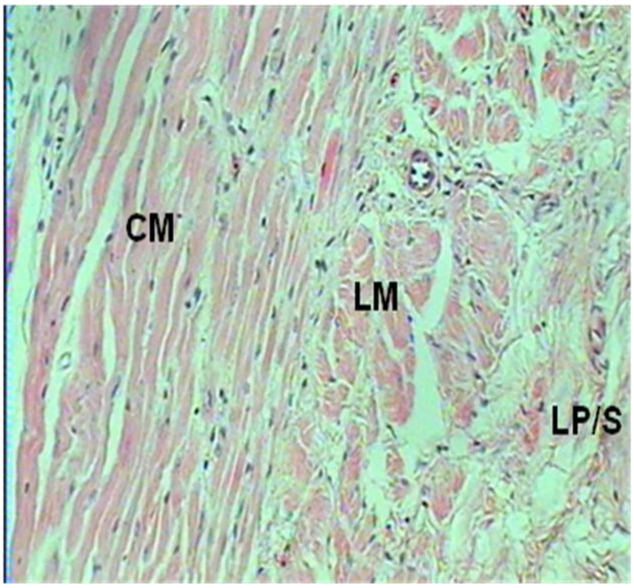

Fig. 6 Transverse section of juvenile esophagus showing loose connective tissue in the lamina propria/ submucosa(LP/S). The tunica muscularis contained inner longitudinal skeletal muscle(LM) and outer circular skeletal muscle(CM). H. & E. x400

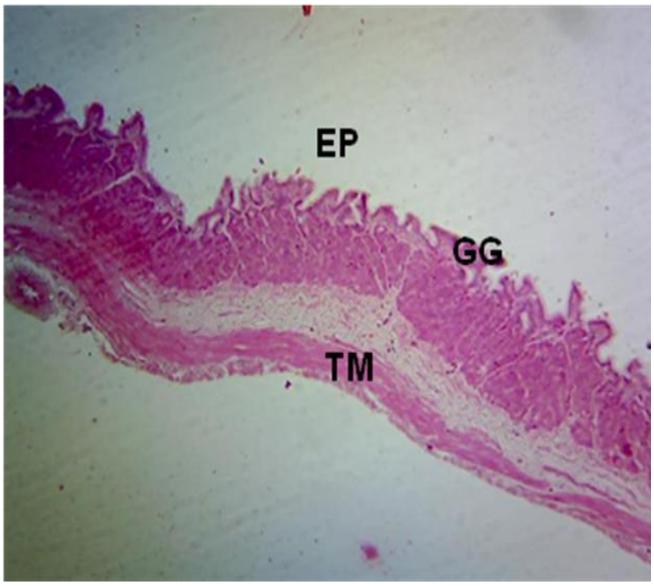

Fig. 7 Section of juvenile fundic stomach showing simple epithelium (EP), gastric gland (GG) in the lamina propria, and tunica muscularis (TM). Note shallow gastric pits. H. & E. x100

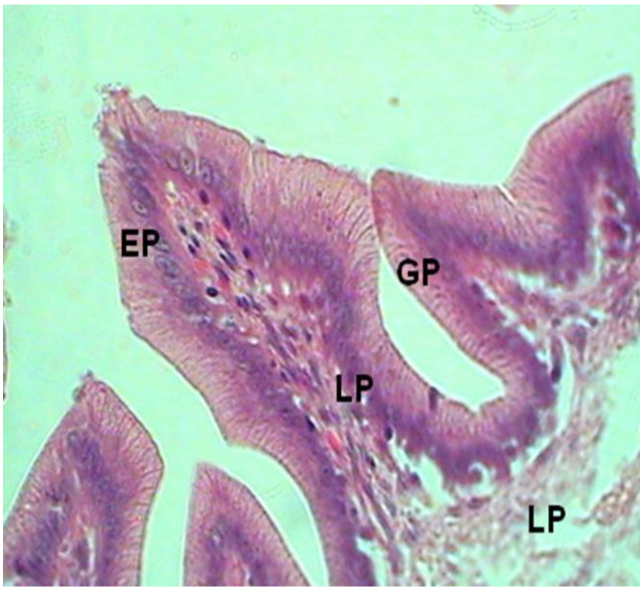

Fig. 8 Section of juvenile pylorus showing simple columnar epithelium (EP) with apical mucin. Observe the absence of gastric glands in the lamina propria (LP). Note the deep gastric pits (GP).H. & E. x1000

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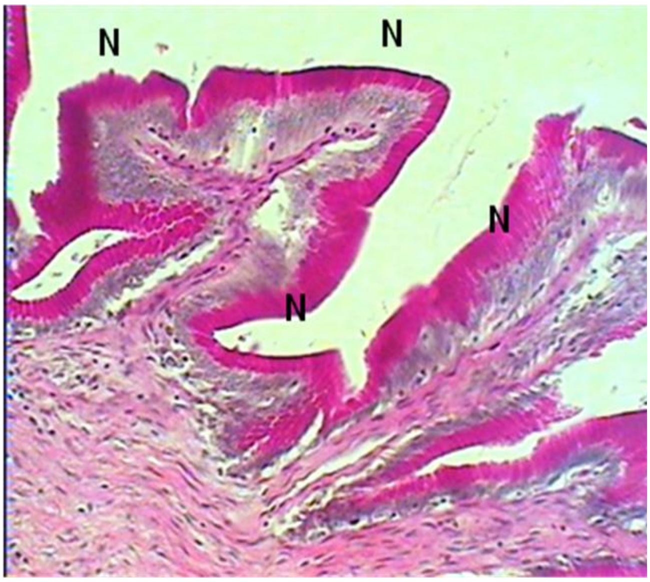

Fig. 9 Section of juvenile pylorus showing epithelia containing only neutral (N) mucin. AB/PAS X 400

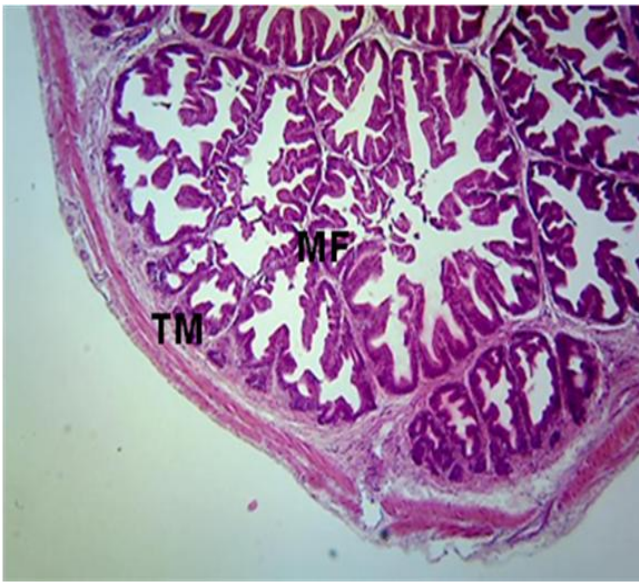

Fig.10 Section of juvenile proximal intestine showing complex branched mucosal folds (MF). TM-tunica muscularis . H. & E. X100

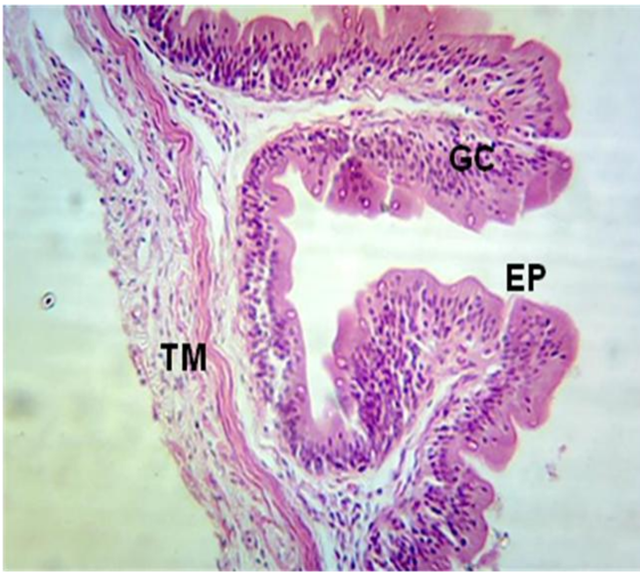

Fig. 11 Section of juvenile middle intestine showing simple columnar epithelium (EP), with interspersed goblet cells (GC).Note tunica muscularis (TM). H. & E. x400

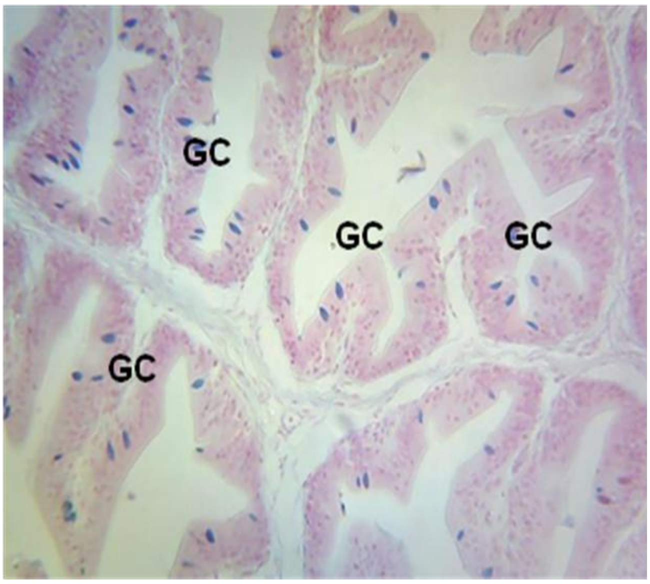

Fig. 12 Section of juvenile proximal intestine showing AB positive goblet cells (GC).AB X 400

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