MORPHOLOGY OF THE DIGESTIVE TRACT OF THE FARMED NILE TILAPIA FISH *Orechromis niloticus* (Linnaeus, 1758) FROM EASTERN NIGERIA

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ABSTRACT
The digestive tract morphology of the Nile Tilapia (Orechromis niloticus) was investigated to help understand its digestive biology, provide data for further studies including clinical cases. The lips, tongue, and oesophagus were lined by stratified squamous epithelium containing, mucous cells and lymphocytes. This protective type epithelial covering can be associated with the majorly herbivorous feeding habit. The oesophageal stratified squamous epithelium contained abundant Periodic Acid Schiff (PAS) positive mucous cells. The U-shaped stomach was bound cranially by oesophageal sphincter and pyloric sphincter caudally. The stomach mucosal simple columnar epithelial lining contained PAS positive apical mucin. The laminar propris containing gastric glands were only seen in the cardiac and fundic regions, the pyloric laminar propria contained well developed PAS positive mucous glands with myoepithlial cells in the enveloping connective tissue. These mucous glands with further staining showed a predominance of acid mucin. The intestinal mucosa was lined by simple columnar cells containing abundant PAS positive goblet cells and lymphocytes. The distal intestine contained aggregated lymphoid cells in localised areas of laminar propria. From this study, the digestive tract of the farmed Nile Tilapia from Eastern Nigeria has shown morphological features adaptive for its herbivorous feeding habit in a cultured environment.

Keywords: pyloric sphincter, mucous gland, GALT, stratum compactum.

INTRODUCTION

Anatomical investigations on the digestive tract of freshwater teleosts including Tilapias are considered useful approaches for exploring the mechanisms of obtaining nutrients from feed through the processes of ingestion, digestion, and absorption of food (Xiong *et al*., 2011; Germano *et al*., 2013; Løkka *et al*., 2013; Santos *et al*., 2015., Ikpegbu *et al*., 2018; Salinas *et al*., 2020). The teleost gastrointestinal tract shows characteristic morpho-functional variety, owing to its high biodiversity especially as regards feeding habits (Buddington *et al*., 1997; Diaz *et al*. 2003; Alabssawy *et al*., 2019). Anatomical and histological studies help us relate digestive organs to the feeding habits, and the food processing mechanisms (Rodrigues *et al*., 2009; Ikpegbu *et al*., 2013; Awaad *et al*., 2014).

Nile Tilapia (*Oreochromis niloticus*) is an important fish species in the tropical and sub-tropical waters, due to its great commercial significance in many African states including Egypt, Uganda, and Nigeria (Norman-López and Bjørndal, 2009; FAO, 2016; Mengistu, 2019). Its relative successful production in both wild and farmed conditions has been attributed to its tolerance to poor water quality, a large variety of foods, hardy flesh, and nice typical taste (Fryer and Iles, 1972).

With the increasing demand for Tilapia fish in our meals in Nigeria despite reports of water bodies continue to manifest signs of fish depletion due to overfishing, climate change, and pollution, the need for increased aquaculture production cannot be over-emphasised (Sissenwine *et al*., 2014; Worm, 2016; FAO, 2018). The knowledge obtained from this study will provide data for investigations including improved feed formulation, the baseline for the diagnosis of cultured Tilapia fish diseases, and contribute to ecomorphology having previously explored this species' oropharyngeal cavity morphology from the wild (Ikpegbu *et al*., 2018).

MATERIALS AND METHODS

Twenty-three (23) Nile tilapia adult fishes sourced from Michael Okpara University of Agriculture Umudike Fish Farm, Umuahia, Abia State of Nigeria, were used for the study (Plate1). The fishes were weighed; the total and standard body lengths were measured with a centimetre ruler. The fish were immobilized in MS 222 and the body cavity cut open to expose the viscera and the alimentary tract. The digestive tracts were studied *in situ*, photographed, and dissected out and the absolute and relative intestinal lengths were measured. The mathematical formula for the area of the semi-circle was used to determine the gape size, and the area of the triangle was used to determine the size of the oropharynx. Slices of lips, tongue, pharyngeal pads, oesophagus, stomach, intestine, rectum, and anus were excised and fixed in 10% neutral buffered formalin. The fixed samples under study were sliced, but the lips and pharyngeal pads were decalcified manually with 14% Ethylenediaminetetraacetic acid (EDTA), the tissues were suspended in a Coplin jar with the help of a thread in approximately 100 ml of the EDTA for decalcification. The decalcifying solutions were changed and the pH and temperature of the solutions were maintained daily, for the three days of decalcification. Thereafter, all the tissues were subjected to a routine histological procedure of dehydration in graded concentrations of ethanol, clearing in xylene, and embedding in paraffin wax.

Sections 5µm thick were obtained with Leitz microtome model 1512 and were stained with haematoxylin and eosin for light microscopy examination (Bancroft and Stevens, 1990). The carbohydrate histochemistry was carried out using Periodic Acid Schiff (PAS), Alcian blue (AB); and a combination of AB and PAS procedures (AB/PAS). Collagen and muscle tissue were further demonstrated with Mason Trichrome staining (MT). Stained sections were examined with a Nikon light microscope and photographed with a NOVEL DIGITAL microscope. Mean values of the data (± standard error of mean (SEM) were obtained and a descriptive analysis was computed using Statistical Package for Social Sciences software (SPSS) version 2013.

RESULTS

GROSS ANATOMY:

The oropharyngeal cavity was bounded rostrally by the upper and lower lips; laterally by its wall; dorsally by the hard palate and upper jaw covered by shiny mucus membrane; ventrally by lower jaw and gill apparatus, and caudally by the septum transversum and oesophagus. The mucus membrane coated oropharyngeal cavity contained a triangular-shaped tongue on its ventral floor. The base of the tongue was caudally attached to the gill stem by a cartilaginous structure, while the sharply pointed rostral apex of the tongue was free lying. Two bony mound-like pharyngeal pads were seen attached to the caudo-dorsal aspect of the hard palate about 1cm to the additus oesophagus. The oesophagus entered into the cardiac stomach cranio-dorsally lying dorso-medial to the cranial aspect of the liver (Plate 2).

The stomach and oesophagus formed the dorsal border of the liver. On the left lateral side of the abdominal cavity, the U-shaped stomach was seen as well as the beginning of the proximal intestine exited the pyloric region. The proximal intestine quickly crossed off from the left lateral, then coursed caudally where it made a U-turn cranially and then right lateral part of the abdominal cavity with series turns on itself forming a loop. The proximal intestinal loop lied at the caudal aspect of the liver and continued cranially beyond the cone formed by the intestinal loop making contact with the gall bladder ventro-laterally and the lateral aspect of the fundic stomach and liver dorsally. The proximal intestine turns on itself (second loop of the proximal intestine) on the base of the intestinal coil and then goes laterally again and continued to the middle intestine to make few turns on the cone and formed the distal intestine towards the apex of the cone. The distal intestine moved dorsally to the base of the cone and came out to the medial aspect of the cone as the straight rectum which coursed caudo-ventrally at an acute angle with the mid-ventral floor of the abdominal cavity (Plate 2). The rectum opened to the exterior through the anus located at the mid-ventral aspect of the fish, cranial to the anal fin.

Plate 1. Adult farmed Nile Tilapia was used for the study.

Plate 2. Dissected tilapia showing the gastro-intestinal tract *in situ*. Note the fairly large stomach and convoluted intestine forming a cone-like structure

Morphometry:

Morphometric data were obtained on some parameters as shown in Table 1.

Morphometric parameters	(± Standard Error)
Total Bogy Weight (g)	195.54 ± 9.47
Standard Body Length (cm)	20.31 ± 0.85
Total Body Length (cm)	22.73 ± 1.13
Intestinal Length (cm)	163.16 ± 16.42
Relative intestinal length	8.21 ± 0.99
Intestinal Weight (g)	5.93 ± 1.16
Gape Size (cm^2)	13.84 ± 1.64
Oropharynx Size (cm^2)	21.93 ± 1.07

Histology

LIPS: The upper and lower lips are comprised of stratified squamous epithelium. The presence of a supportive stratum compactum comprised of dense regular connective tissue was observed. These two structures presented an undulating pattern of epidermal pegs and dermal papillae (Plate 3). Melanophores were seen beneath the stratum compactum (Plate4). Canine-like teeth were seen embedded in the upper lip hypodermis.

PHARYNGEAL PAD: The epithelium is stratified squamous epithelium containing taste buds and mucous cells. The teeth were seen erupted from depressions in the epithelium, beneath the teeth were bony core (Plate 5).

OESOPHAGUS: The oesophageal mucosa consisted of stratified squamous epithelium containing PAS and AB positive mucous cells. The lamina propria/submucosa contained dense connective tissue, blood vessels, and pockets of skeletal muscle bundles. The tunica muscularis were of skeletal muscles in a longitudinal orientation that formed the oesophageal sphincter separating it from the cardiac stomach (Figs. 6, 7, and 8).

STOMACH: The stomach lined by simple columnar epithelium was divided into three parts; an initial region close to the entry point of the oesophagus – the cardia; a middle portion forming the bulk of the organ- the fundus; and a terminal portion - the pylorus separated from the proximal intestine by the pyloric sphincter. The cardiac and fundic regions contained gastric glands in the laminar propria (Figs. 9 and 10); while the pyloric region which lacked gastric glands contained numerous PAS and AB positive mucous glands in the laminar propria (Figs. 11, 12, and 13). These ovoid to alveolar shaped mucous glands were coated with collagen fibres and myoepithelial cells. The stomach tunica muscularis contained smooth muscle cells having inner circular and outer longitudinal fibres arrangement coated by tunica serosa.

INTESTINE: The intestine lined by simple columnar epithelium was divided into proximal, middle, distal, and rectal regions. The proximal intestine presented mucosal folds that form the labyrinths lined by villi in the low power. The primary, secondary and tertiary folds were branched (Plate 14). All the folds were lined by simple columnar epithelium containing PAS and AB positive goblet cells (Plate 15); and intraepithelial lymphocytes, the lamina propria contained loose collagen fibres, lymphocytes, and blood vessels. Similar histology was seen in the submucosa. The tunica muscularis contained smooth muscle fibres arranged in inner circular and outer longitudinal layers. The middle intestine mucosal folds had a mound-like modification that extended slightly into the lumen on the transverse section. The simple mucosal folds' epithelium showed similar histology with the proximal intestines except that it presented more epithelial goblet cells (Plate 16). The distal intestine mucosal folds were broader and shorter than that of the middle intestine with the accumulation of lymphoid cells in the laminar propria (Plate 17). The histology was generally similar to the other intestinal regions.

RECTUM: The rectal mucosal folds were broader and taller than that of the distal intestine with similar histology but lacked lymphoid cell accumulation. The submucosa contained a huge investment of loose connective tissue fibres (Plate 18).

HISTOCHEMISTRY

The reaction of the mucin to histochemical procedures of PAS, AB/PAS, and demonstrated the presence of polysaccharides and neutral mucin by their red to magenta colouration. The cells containing acid mucin produced a blue to blue-green colouration, while cells with mixed neutral mucin presented purple colouration while two mucins are present in equal quantities but tended towards the colour of the predominant mucin as the quantity varies. This variation produced bluish colour when acid mucin dominated but red magneta coloration when neutral mucin dominated.

Table 2. Mucin histochemical reaction of epithelial mucosubstances in different portions of the digestive tract of *Oreochromis niloctius*

PROCEDURE	E	S	PI	MI	DI	R
PAS	++	+	+	+++	+	+
AB/PAS pH 2.5	++	+	++	+++	+	-
	AP	A	A	AP	A	N

KEY: PAS, Periodic Acid Schiff; AB, Alcian blue; AB/PAS, combined Alcian blue with PAS procedure after diastase treatment; E,esophagus; S, Stomach; PI, proximal intestine; MI ,middle intestine; DI, distal intestine; R, rectum; (-) no staining observed; (±) poorly stained; (+) low; (++) medium; (+++) high; N,PAS dominace; A, AB dominance; AP, equal presence of Acid and Neutral mucins.

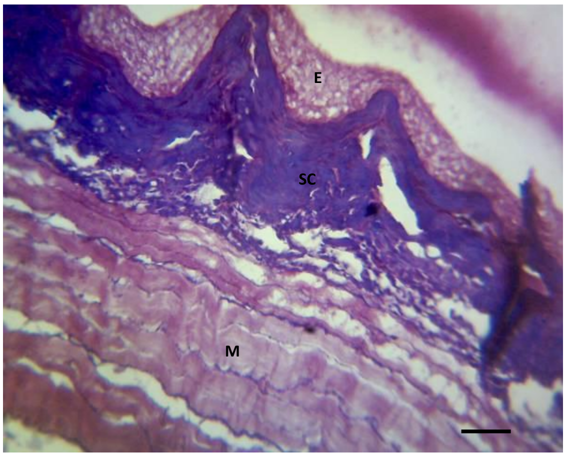

Plate 3. Section of lower lip showing presence of thick dense collagen fibres (SC) supporting the epithelial (E) layer above. Note the muscle fibres beneath the collagen fibres called stratum compactum (SC). MASSON TRICHROME (Scale bar = 40µm).

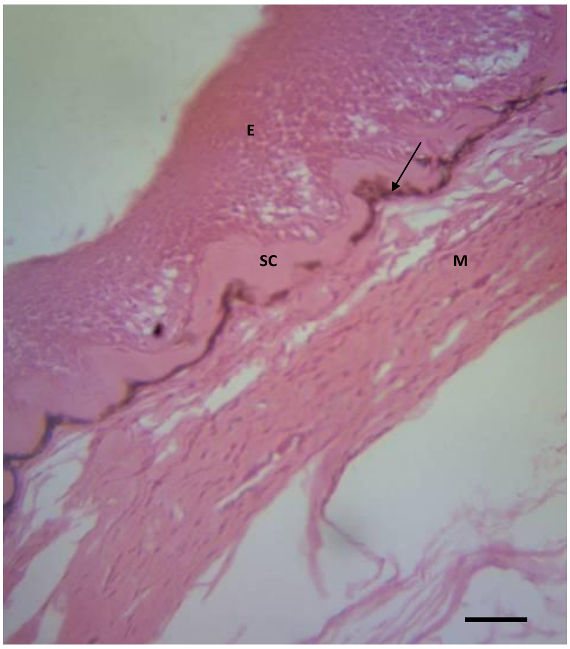

Plate 4. Section of the upper lip showing stratified epithelium (E) supported by dense regular connective tissue stratum compactum (SC). Note the melanophores (Black arrow), and muscle coat (M) underneath. H & E. (Scale bar = 10µm).

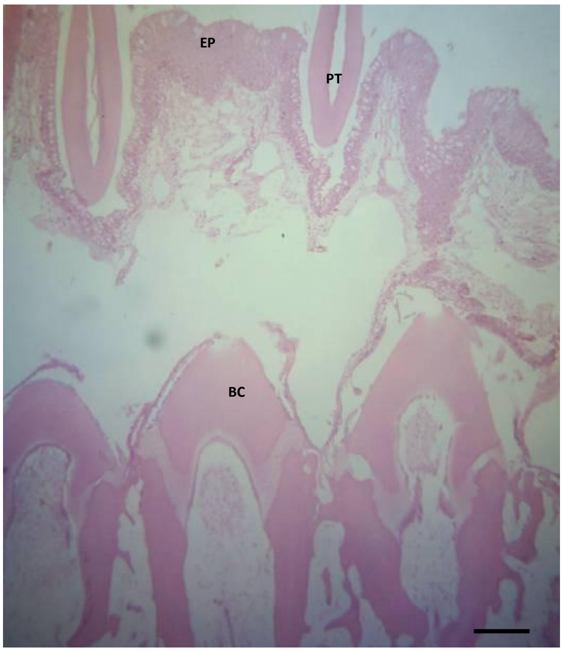

Plate 5. Section of pharyngeal pad showing stratified squamous epithelium (EP) containing taste buds, mucous cells, and erupting teeth (PT). Note beneath the epithelium is the bony core (BC). H & E. (Scale bar = 10µm).

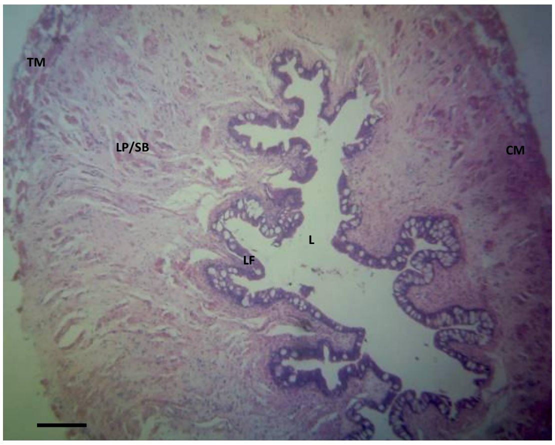

Plate 6. Section of the oesophagus showing longitudinal fold (LF), extending into the lumen. Note the epithelium containing mucous cells. Observe the collagen and muscle fibres in the lamina propria/submucosa; and the tunica muscularis of longitudinal orientation. H & E. (Scale bar = 10µm).

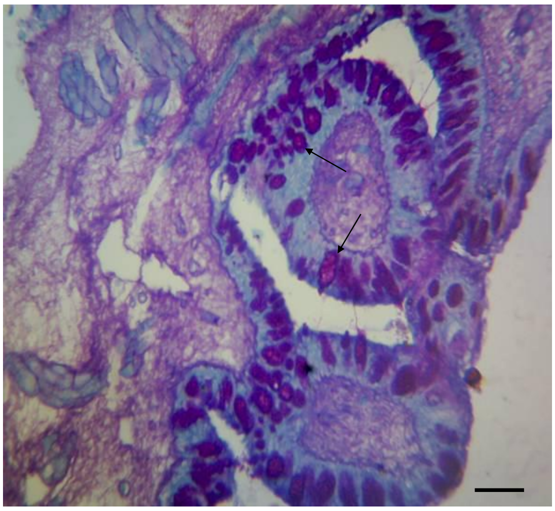

Plate 7. Transverse section of adult oesophagus showing PAS positive mucous cells (black arrow), on the longitudinal fold. PAS (Scale bar = 40µm).

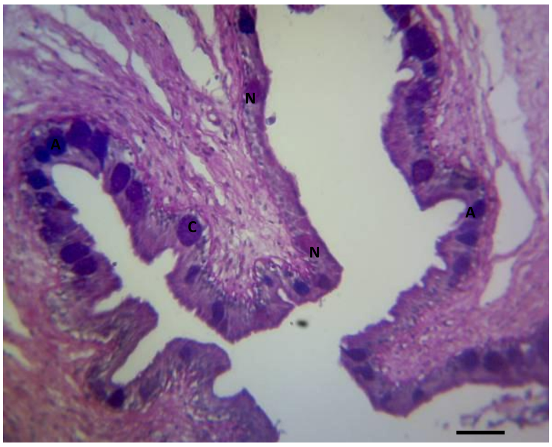

Plate 8. Section of oesophagus showing longitudinal fold epithelium that contained neutral mucin (N) or acid mucin (A) or combination of both (C) AB-PAS (Scale bar = 40µm).

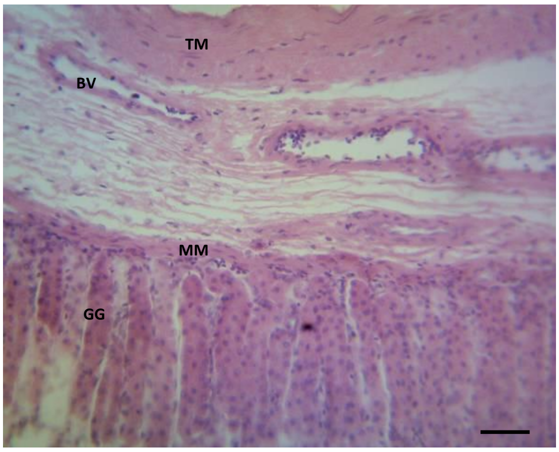

Plate 9. Longitudinal section of adult cardia showing tubular gastric gland (GG) in the laminar propria; muscularis mucosa (MM); blood vessels (BV) in the submucosa. Note the tunica muscularis (TM). H & E. (Scale bar = 40µm).

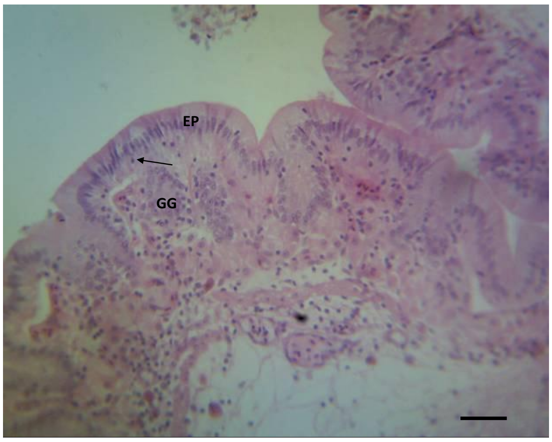

Plate 10. Transverse section of adult fundic stomach showing simple columnar epithelium (EP), intraepithelial lymphocytes (black arrow), and gastric gland (GG). H &E (Scale bar = 40µm).

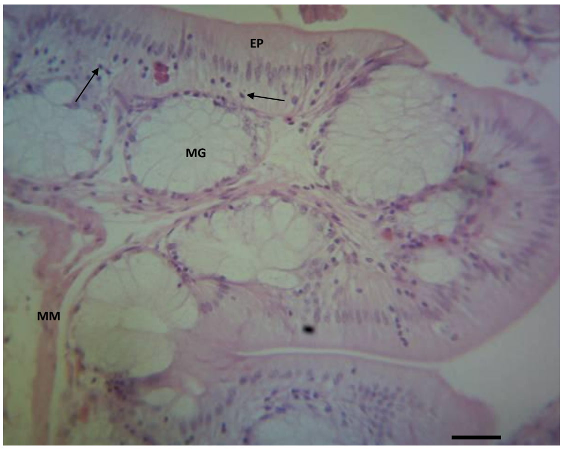

Plate 11. Transverse section of the pyloric stomach showing Simple columnar epithelium (EP) containing intraepithelial lymphocytes (black arrow); mucous gland (MG) in the lamianar propria. Note muscularis mucosa beneath the mucous gland. H & E (Scale bar = 10µm).

Plate 12. Transverse section of adult pylorus stomach showing PAS positive apical mucin (black arrow), and mucous glands (MG). PAS (Scale bar = 40µm).

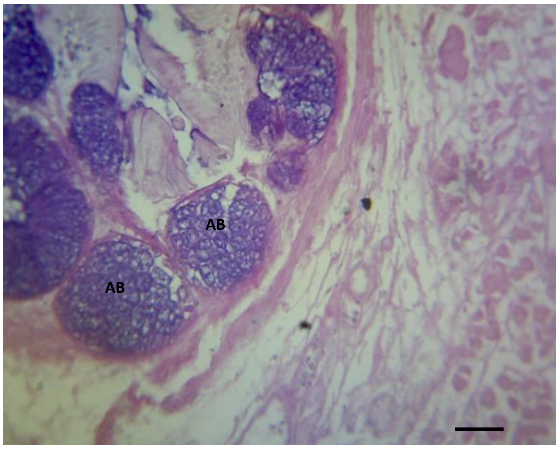

Plate 13. Section pyloric stomach showing the predominance of acid mucin in the mucous glands (AB). AB–PAS (Scale bar = 40µm).

Plate14. Transverse section of adult proximal intestine showing branched mucosal folds (MF). AB-PAS. (Scale bar = 10µm).

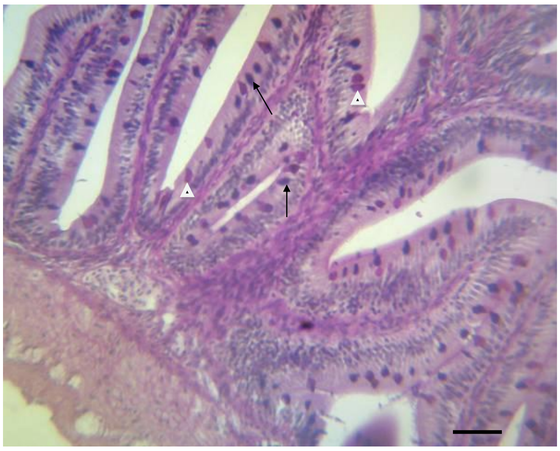

Plate 15. Section of proximal intestine showing the presence of goblet cells that contained neutral (black arrowhead) and acid mucin (black arrow). AB-PAS (Scale bar = 40µm).

Plate 16. Transverse section of the middle intestine showing PAS positive goblet cells (black arrow). PAS (Scale bar = 40µm).

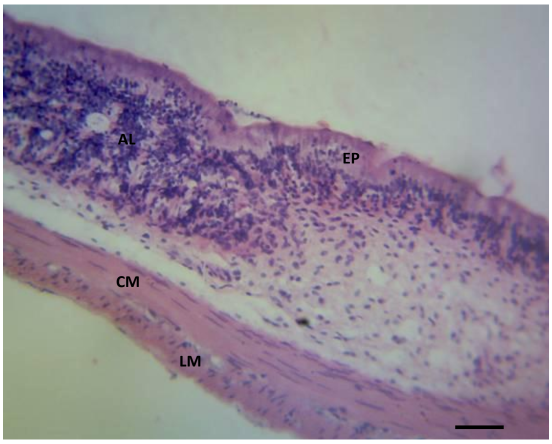

Plate 17. Transverse section of the distal intestine showing simple columnar epithelium (EP); aggregated lymphocytes in the laminar propria (AL). Note the tunica muscularis containing inner circular (CM), and outer longitudinal (LM), smooth muscles. H & E. (Scale bar = 40µm).

Plate18. Transverse section of the rectum showing mucosal folds extended into the lumen. Note the simple epithelium (EP), and lamina propria/ submucosa (LP/SB). Observe the myenteric plexus (black arrow) between the inner circular muscle

(CM) and outer longitudinal (LM) smooth muscle fibres. H & E. (Scale bar = 10µm).

DISCUSSION

This study has revealed that the general plan of the Tilapia fish digestive tract conformed to most teleost of having four tunics –mucosa, submucosa, muscularis, and serosa/adventitia (Wilson and Castro 2011; Awaad, 2014). The oro-pharyngeal organs lined by stratified squamous epithelium associated underlying tissue protection has been reported in the lips of many teleosts including Pebbly Fish, *Alestes baremoze*, and *Trachelyopterus striatulus* (Santos *et al*., 2015; Kasozi *et al*., 2017). The presence of few canine-like teeth suggests that this fish may be tearing feed materials including grasses; since the result of relative intestinal length (8.21 ± 0.99) from our study is very suggestive that it is a herbivorous fish (Canan *et al*., 2012), but more studies on stomach content from both farmed and wild Tilapia fish in the region should be conducted to ascertain the validity of this claim. Whilst the lips, tongue, and wall may be involved with feed prehension, shearing, and trituration; the pharyngeal pad histology of taste bud co-localization with teeth makes it the organ of food selection and rejection (Linser *et al*., 1998). This feature of the pharyngeal pad has also been reported in the Tilapia species from the wild in Afikpo River in eastern Nigeria (Ikpegbu *et al*., 2018).

The oesophageal protective epithelium of stratified squamous cells containing mucous cells seen in many freshwater teleosts may be associated with smooth feed passage into the stomach, unlike marine fish containing simple columnar epithelium lacking mucous cells and related to osmoregulation (Yamamoto and Hirano1978; Suíçmez and Emel, 2005; Ceylan and Kaptaner, 2019). The abundant oesophageal mucous cells have been reported in the Nile Tilapia from the Beni Suef government of Egypt (Awaad *et al*., 2014). The PAS positive mucous cells will aid in lubrication and pre-gastric digestion of carbohydrates as the teleost is known to lack salivary glands (Hafez *et al*., 2013; Fagundes *et al*., 2016). The acid mucins may be protecting the tract from bacterial pathogens (Pérez-Sánchez *et al*., 2013). The predominant longitudinally oriented fibres in tunica muscularis will help in accelerated deglutition (Chaves and Vazzoler, 1984); just as the oro-esophageal sphincter will prevent reflux of food from the stomach into the oesophagus, a dysfunction that may expose the esophageal mucosa to gastric enzymes possible digestion (Orlando, 2010). The presence of esophageal sphincter has been reported in the stomachless puffer fish *Sphoeroides testudineus* and associated with facilitating feed movement from the oesophagus to the intestine (Fagundes *et al*., 2016).

The stomach regional compartments of cardia, fundus, and pylorus with different histological features as seen in this study have been reported previously in Nile tilapia (Caceci *et al*., 1997). Whilst some Tilapia biology researchers have reported only the cardiac and pyloric regions (Abdulhadi, 2005), others suggest the absence of regions in some tilapia stomach. The presence of tubular gastric glands in the cardia (Abdulhadi, 2005) and fundus has been reported (Caceci *et al*., 1997), while the absence of gastric glands in the pylorus has been reported in some teleosts and where it was associated with the reduction of the quantity of gastric juice entering the intestine which has an alkaline pH (Ribeiro *et al*., 1999; Ghosh and Chakrabarti, 2015; Moawad *et al*., 2017). The well-developed PAS positive mucous glands in the pylorus can be regarded as a landmark distinguishing features of the region in this species. The presence of myoepithelial cells surrounding the gland may suggest a need for strong elaboration of the mucous content to help in carbohydrates digestion as well modulate gastric content pH of chyme as it exits the stomach (Solovyev *et al*., 2015; Zhang *et al*., 2016; Moawad *et al*., 2017). The pyloric sphincter may help prevent the abrupt movement of poorly digested food into the intestine (Gomes *et al*., 2014). This pyloric sphincter has been reported in the catfish *Lophiosilurus alexandri* and the Atlantic Halibut *Hippoglossus hippoglossus* associated withholding food shortly in the storage stomach or transiting food directly into the intestine (Gomes *et al*., 2014; Mello *et al*., 2019).

Grossly, the fish under study presented intestinal loops which ended with a rectum emptying through the anus had been reported also in the pufferfish *Sphoeroides testudineus* (Fagundes *et al*., 2016*)*. Histologically, the proximal intestinal mucosal folds were modified into branches believed to be increasing the surface area for increased nutrient absorption since a pyloric caecum with a similar function is lacking from this study (Salinas *et al*., 2020). The presence of branched mucosal folds in the proximal intestine has been reported in African catfish (Ikpegbu *et al*., 2013). The goblet cells will help lubricate the intestine as mostly rough feed materials pass through, as well as help fight bacteria well through the presence of acid mucin in the entire intestinal tract. The intraepithelial lymphocytes are part of local gut-associated lymphoid tissue (GALT) and are involved in immune protection (Miura *et al*. 2012, Salinas, 2015; Salamat, 2018). In the distal intestine, the aggregated lymphoid tissues in the lamina propria can be regarded as integral components of GALT in this species. This aggregated lymphoid tissue in the distal intestines of this species can be speculated to be analogous to other vertebrate lymphoid tissues such as oesophageal tonsils, meckels diverticulum, cecal tonsil, Peyers patches which are all components of the GALT (Sminia and Jeurissen, 1990; Peralta *et al*., 2017).

In conclusion, this study has revealed that the farmed tilapia fish digestive tract has regular features of freshwater teleost and this baseline can help in further investigative researches, especially for the feed formulation industry.

It can also be of benefit to immunologists who can use the digestive tract as a model for an immune response since the fish is highly prolific and adapt well in the tropics.

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