International Journal of Chemistry and Chemical Processes E-ISSN 2545-5265 P-ISSN 2695-1916,
Vol 10. No. 5 2024 www.iiardjournals.org Online Version

Histochemistry of the Gastrointestinal Tract Segments of Age-Related African Catfish (*Clarias gariepinus)* Exposed to Graded Concentrations of Urea Fertilizer

A.G. Ruma;[1]J.W. Wade;[2]and B.S. Audu;[2.]
[1]Department of Agricultural Science Education,
Federal University of Education, Pankshin, Plateau state Nigeria.
[2]Department of Zoology, Faculty of Natural Sciences, University of Jos, Nigeria.
Correspondence Author: A. G. Ruma, ruma.aaron@gmail.com
10.56201/ijccp.v10.no5.2024.pg101.122

Abstract

The study examines changes in the histochemistry of the gastrointestinal tract segments of age-related African catfish (Clarias gariepinus) exposed to sub-lethal concentrations of Urea fertilizer. The study was carried out in the Postgraduate Research Laboratory, Department of Zoology, University of Jos, Nigeria. One hundred each of apparently healthy C. gariepinus fingerlings, juveniles and adults of mixed sex were purchased from a reputable fish farm in Jos, Plateau State, North Central Nigeria and transported in an aerated plastic Jeri cans and acclimated for two weeks. During the acclimation period, fish were fed with commercial pelleted feed as reference diet. Three different types of Urea fertilizer: : Indorama- N46%; Dangote - N46% and Notore - N46% were procured from Plateau Agricultural Development Program (PADP) while two types of foreign fish feeds: : Copen and Skretting were procured from reliable marketers. Solubility and stability test for Urea fertilizers and fish feeds were determined. The selected Urea fertilizer was weighed into different sub lethal concentrations as in Ajima, Audu, Mane, Okeke& Varghese. (2017); 0.75g/L, 1.50g/L and 3.00g/L. One kilogram each of the test feed (skretting) according to age of fish was poured into 0.75g/L, 1.50g/L and 3.00g/L concentrations of Urea fertilizer to enable the feed absorb the Urea fertilizer solution for 30 minutes then sieved out and allowed to dry under laboratory condition. Each of the dried feed was labelled: 0.75g/L, 1.5g/l and 3.00g/l accordingly. The experiments were conducted in twenty four (24) plastic bathes according to C. gariepinus age groups. A total of three test concentrations: 0.75g/L, 1.5g/l and 3.00g/l of fish feed each for C. gariepinus fingerlings, Juveniles and adults and control each duplicate replicate were labeled A^1, A^2, B^1, B^2, C^1, C^2, F^1, F^2 for the three age groups. The 24 containers were grouped into eight according to age of fish labeled and randomly distributed on a platform in the laboratory. Ten (10) apparently healthy each of acclimated C. gariepinus age groups were exposed to each of the 24 plastic containers accordingly and each filled with dechlorinated water. The test fish were fed for 62 days within which behavioral changes were observed. Water quality parameters were determined bi-weekly throughout the exposure period using the methods of APHA. (2005). The gastrointestinal tract segment of the C, gariepinus age groups were excised and processed routinely for variations inhistochemistry using periodic acid Schiff (PAS). Findings

revealed that significant differences ($p<0.05$) was recorded in the histochemical changes of the GIT segments of C. gariepinus age groups exposed to sub-lethal concentrations of Urea fertilizer and control. In conclusion, the results showed that sub-lethal concentrations of Urea fertilizer (0.75, 1.50 and 3.00g/L) has effect on the histochemistry of the GIT segmentsof age-related African catfish.

Key words: Histo-chemistry, Gastrointestinal tract, Urea Fertilizer, Sub-lethal concentration

INTRODUCTION

The gastro intestinal segments of vertebrates have been considered to employ different cellular mechanisms in response to diet quantity and quality (Gisbert *et al.,* 2008). Characteristics of the digestive apparatus in fishes are closely dependent on the nature of their foods, the characteristics of their habitats, their nutritional state and the developmental stages of the individual (Trindade & Queiroz, 2012). Fishes as in other verterbrates, the alimentary tract consists of the oesophagus, the stomach, and the intestine (Ikpegbu, *et al*., 2013). Each segment of the alimentary tract as earlier mentioned differs according to age and feed intake. Several authors such as Sinlapachai, Genten, Terwinghe&Danguy (2008); Wannee&Njwat (2015) reported that the gatro-intestinal tract of vertebrates from the cranial end to the caudal end is formed by four distinctive layers: Mucosa, sub mucosa, musclaris and serosa or adventitia. Each of the four layers plays vital role in the histo-architecture of the GIT segments.

Urea fertilizer when mixed with water was found to diminish fish production, cause motility and cause pernicious physiological changes in fish (Soma & Susanta, 2014). When fishes are exposed to chemical pollutant such as Urea, there is either increase or decrease in hematological level and these changes depend on fish species, age and other characteristics of the fish. Similarly, Chanda (2020) reported that Urea fertilizer as a result of agricultural run-off posed a serious effect that induced various histopathological and cytogenitical changes in fish. Finding by Omoregie et al. (2009) revealed that higher concentration of chemical fertilizers in water may cause mortality to fishes as well as impairment of the physiological responses of fish. Several studies on histochemistry of the GIT of different fish species have been documented by Ikpegbu, Nlebedum&Ibe (2014); Marcella, et al., (2015); Manisha, et al., (2015) among others. However, no research work has been documented onhistochemistry of age-related changes in the GIT segments of African catfish exposed to graded concentrations of Urea fertilizer. In line with the above, this study seeks to investigate changes in thehistochemistry of age-related African catfish GIT segments exposed to sub-lethal concentrations of Urea fertilizer.

MATERIALS AND METHODS

The study on effect of sub-lethal concentrations of Urea fertilizer on histochemistry of the gastro-intestinal tract(GIT) segments of African catfish was investigated in Post graduate Laboratory, Applied Hydrobiology and fisheries, Department of Zoology, University of Jos, North Central, Nigeria. One hundred each of apparently healthy *C. gariepinus* fingerlings, juveniles and adults

mixed sex with mean weight 4.22±0.15; 22.67±1.10 and 643.96±48.75g respectively, mean total length 8.77±0.25; 12.80±0.21 and 38.93±0.39cm respectively and mean standard length 7.60±0.21; 10.63±0.24 and 24.93±0.45cm respectively were purchased from Global fish expert ltd, Kangang, Jos South Local Government Area (LGA), Plateau State, North Central Nigeria. The fish were transported in an aerated plastic container to post graduate Hydrobiology and fisheries laboratory, Department of Zoology, University of Jos, Nigeria. The fish were acclimated for two weeks in plastic containers separately according to age groups. During the acclimation period, fish were fed with commercial pelleted feed (skretting; crude protein- 42%, crude fat- 10%, crude fibre- 3.2%, ash- 7%).

Source and types of Urea: three different types of Urea (Indorama Urea - N46%; Dangote Urea - N46% and Notore Urea - N46%) fertilizer from three different companies were procured from Plateau Agricultural Development Program (PADP).

Source and types of fish feeds; two types of foreign fish feeds (Copen & Skretting)

were procured from reliable marketers.

Solubility test: Five grams of the different types of Urea; A B C were dissolved in five litres of water and stirred with a glass rod. Each of the three Urea types procured above dissolved within two minutes.

Stability test for fish feeds: Five grams of feed procured; A and B were dropped in five litres of water and observed for thirty minutes to determine which one is more stable in water for use in feeding the experimental fish throughout the research period.

Preparation of stock solution: Urea fertilizer (Notore- N46%) was weighed into different sub lethal concentrations (0.75g/L, 1.50g/L and 3.00g/L) as in Ajima et al. (2017). One kilogram each of the test feed (skretting) according to age of fish was poured into 0.75g/L, 1.50g/L and 3.00g/L concentrations of Urea and enable the feed to absorb the Urea solution for 30 minutes, stirred with a glass rod to obtain a homogenous mixture then sieved out and allowed to dry under laboratory condition.. Each of the dried feed was labelled0.75g/L, 1.5g/l and 3.00g/l accordingly.

Experimental Design: The experiments were conducted in twenty-four (24) plastic bathes according to age. A total of three test concentrations; 0.75g/L, 1.5g/l and 3.00g/l of fish feed each for *C. gariepinus* fingerlings, Juveniles and adults and two control each duplicate replicate were labelled A^1, A^2, B^1, B^2, C^1, C^2, F^1, F^2 each for the three age groups. The 24 containers were grouped into eight according to age of fish labelled and randomly distributed on a platform in the laboratory.

Experimental Procedures: Ten (10) apparently healthy acclimated fish were exposed to each of the aforementioned test concentrations in the 24 plastic containers; *C. gariepinus* fingerlings, Juveniles and adults. Each of the container was filled with dechlorinated water. The test fish were fed for 62 days within which behavioral changes were observed. The test fish were not fed 24 hours a day prior to commencement of the experiment.

Determination of water quality parameters: Water quality parameters were determined bi-weekly throughout the exposure period using the methods of APHA. (2005). The water quality parameters determined were; temperature, free carbon dioxide, dissolved oxygen, hydrogen ions (pH), alkalinity, nitrite, ammonia and nitrate.

Histochemistry of theGIT of *C. gariepinus* age groups

The periodic acid Schiff (PAS) technique was carried out using procedure of Blachall&Daisley (1973). Waxed sections of various segments of GIT segments from the different catfish groups were dewaxed in xylene for 5 minutes and rinsed consecutively in 100%, 96% and 70% alcohol for 1minute each. This was followed by placing rinsed tissues in distilled water and subsequently treating with undiluted periodic acid for 10 minutes. The treated tissues were washed in eight changes of distilled water, exposed to Schiff's solution for 1hour and washed in running tap water for 10 minutes. The nuclei were distinctly stained with Lilly Mayer's heamatoxylin for 1 min and further differentiation was avoided. Bluing of the tissues was done under tap water for 10 minutes. This was followed by tissue dehydration in 96% and 100% alcohol, cleared in xylene and mounted in Entellan. When viewed under microscope, parts of the tissue that were positive for glycogen stained magenta while the nuclei stained bluish. Furthermore, the respective photomicrographs of the PAS-stained slides were quantified using Image J software (NIH, Bethesda, MD, USA).

STATISTICAL ANALYSIS

Two-way analysis of variance (ANOVA) was used to evaluate significant differences across groups and the values of $P<0.05$ was considered significant. A Turkey post hoc test was further used to evaluate the significant differences between and within groups. Statistical package for social sciences (SPSS) version 17.

RESULT

Generally, the trend in oesophageal PAS intensity across the different age group of *C. gariepinus* exposed to grades of Urea fertilizer seemed to increase from *C. gariepinus* fingerling to adulthood (Plates 1-3). Though, an exception to this was the significant decrease ($p<0.05$) displayed by *C. gariepinus* adult exposed to the highest concentration(3.00g/L) of Urea fertilizer.

The PAS positive regions within the cardiac region of the stomach of different age-groups of *C. gariepinus* exposed to various concentrations of Urea fertilizer were demonstrated in the mucosal epithelial surface and in the submucosal region (Plates4-6).The cardiac stomach PAS intensity trend within each age category of *C. gariepinus* exposed to varied concentrations of Urea fertilizer showed a consistently significant decrease ($p<0.05$) values with the increasing grades of Urea concentration when compared to their respective controls. Similarly, the cardiac stomach PAS intensity variation across all the age groups of *C. gariepinus* showed a progressive significant decrease ($p<0.05$) value from *C. gariepinus* fingerling to adult. Although, the PAS intensity values in *C. gariepinus* juvenile and adult cardiac segments exposed to 1.5 and 3.0 g/L of Urea fertilizer were not significantly different ($p>0.05$).

The glycogen rich regions (PAS positive) of the fundic segment of the stomach of the various age groups of *C.gariepinus* exposed to grades of Urea fertilizer concentrations were observed in the mucosal epithelial surface and in the submucosal region (Plates7-9).

The fundic stomach PAS intensity within each age group of *C. gariepinus* exposed to different concentrations of Urea fertilizer showed similar pattern of a consistently significant decrease ($p<0.05$) values observed for cardiac segment with the increasing grades of Urea fertilizer concentrations relative to their controls. Also, the fundic stomach PAS intensity variation across all the age groups of *C. gariepinus* seemed to decrease from fingerlings to juvenile and subsequently increased in the *C. gariepinus* adult. Although, the PAS intensity values in *C. gariepinus* juvenile and adult fundic segments exposed to 0.75 g/L of Urea fertilizer were not significantly different ($p>0.05$).

The PAS positive region of the pyloric segment of the stomach of different age category of *C. gariepinus* exposed to grades of Urea fertilizer concentrations was confined to the mucosal epithelial surface (Plates10-12). The observed PAS intensity pattern in the pyloric segment is similar to the cardiac segment intensity. There was a consistently significant reduction ($p<0.05$) in the PAS intensity values with the increasing concentrated grades of Urea fertilizer in each of the age group of the exposed catfish when compared to their respective controls. Similarly, the trend of pyloric segment PAS intensity across all the age groups in this study appeared to decrease from *C. gariepinus* fingerlings to adult.

The glycogen-rich (PAS positive) areas of the proximal intestinal segment in the different age category of *C. gariepinus* exposed to grades of Urea fertilizer concentrations were restricted to the mucosal epithelial surface and in the submucosal region (Plates13-15).The proximal intestinal PAS intensity in each of the age group of *C. gariepinus* exposed to concentrated grades of Urea fertilizer was observed to decrease significantly ($p<0.05$) with the rising Urea fertilizer concentrations compared to their respective controls. Also, the trend of proximal intestinal PAS intensity across all the age groups was noticed to increase significantly ($p<0.05$) from the *C. gariepinus* fingerlings to juvenile and then decreased in the *C. gariepinus* adult fish).

The PAS positive regions of the middle intestinal segment in the different age category of *C. gariepinus* exposed to grades of Urea fertilizer concentrations were observed in the mucosal epithelial surface, in the submucosal and muscular regions (Plates16-18).

The middle intestinal PAS intensity in each of the age-group of *C. gariepinus* displayed a consistent significant decrease ($p<0.05$) values with the increasing grades of Urea fertilizer concentrations when compared to their corresponding controls (Table 3). In addition, the trend of middle intestinal PAS intensity across all the age groups was observed to decrease significantly ($p<0.05$) from the *C. gariepinus* fingerlings to juvenile and then increased in the *C. gariepinus* adult.

The glycogen-rich (PAS positive) areas of the distal intestinal segment in the different age category of *C. gariepinus* exposed to grades of Urea fertilizer concentrations were observed in the mucosal epithelial surface, the submucosal and muscular regions (Plates19-21).

The distal intestinal PAS intensity showed a progressive significant reduction ($p<0.05$) with the increasing grades of Urea fertilizer in each of the age group of the exposed catfish when compared to their respective controls. In addition, the trend of the distal intestinal PAS intensity across all the age groups was observed to increase significantly ($p<0.05$) from the *C. gariepinus* fingerlings to juvenile and then decreased in the *C. gariepinus* adult. There was no significant difference ($p>0.05$) in the PAS intensity of *C. gariepinus* juvenile and adult exposed to the higher concentrations (1.5 and 3.0 mg/L) of urea fertilizers.

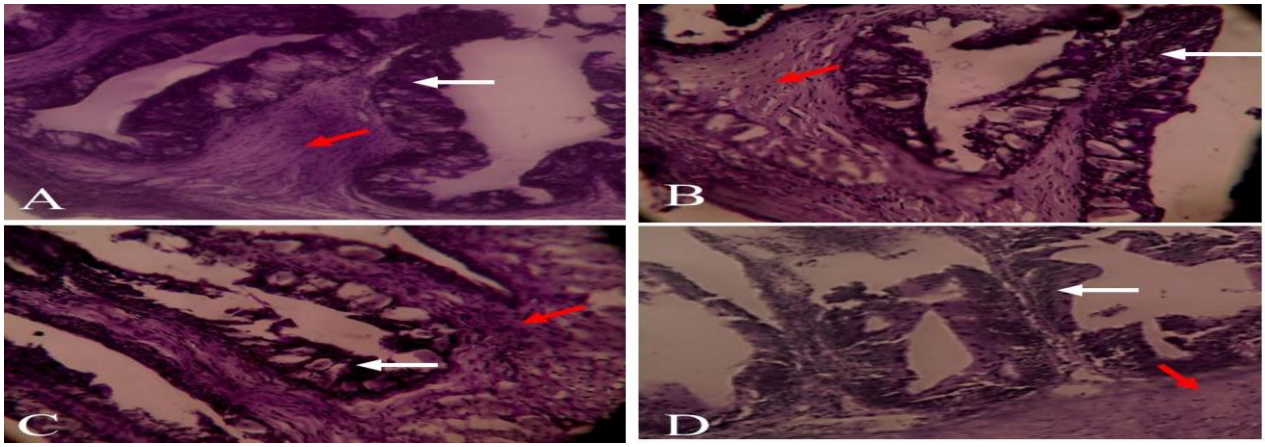

Plate1. The PAS Staining of the Oesophagus of *Clarias gariepinus* Fingerlings Exposed to Different Concentrated Grades of Urea Fertilizer. A. Control B. 0.75 g/L C. 1.5 g/L D. 3.0 g/L. Note the demonstration of PAS positive areas withinoesophageal mucosal epithelial surface (white arrow) and in the submucosal region (red arrow). Magnification: X400.

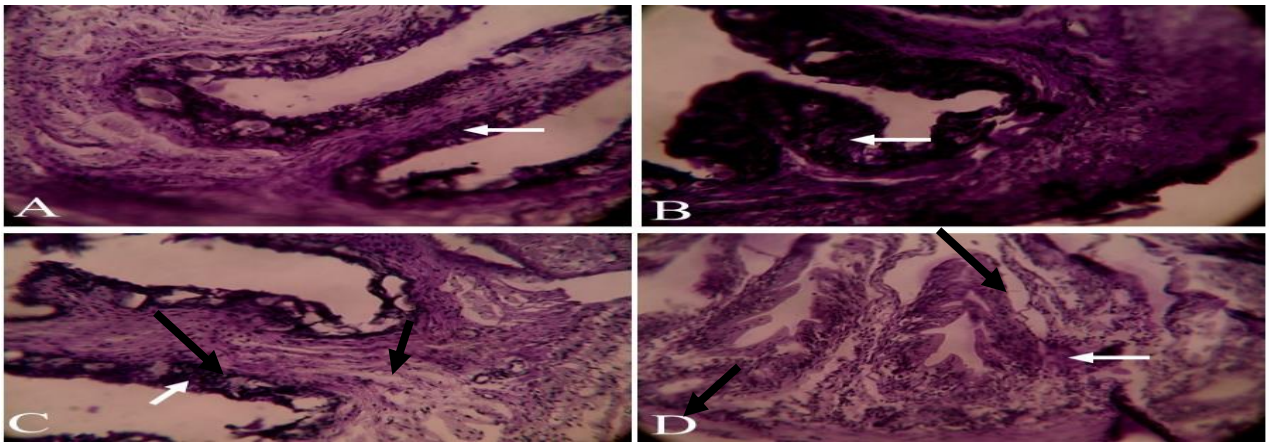

Plate 2. The PAS Staining of the Oesophagus of *Clarias gariepinus* Juvenile Exposed to Different Concentrated Grades of Urea Fertilizer. A. Control B. 0.75 g/L C. 1.5 g/L D. 3.0 g/L. Note the demonstration of PAS positive areas withinoesophageal mucosal epithelial surface (white arrow) and in the submucosal region (black arrow). Magnification: X400.

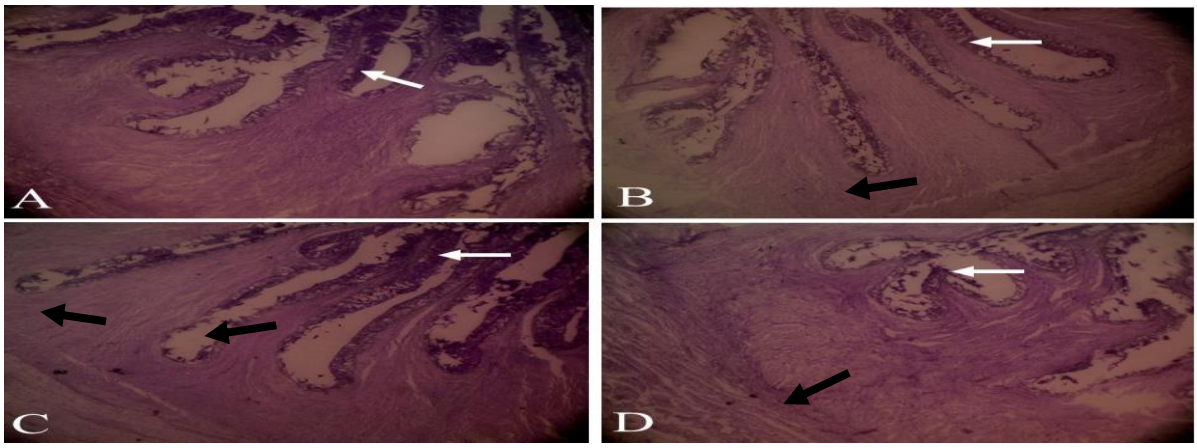

Plate 3. The PAS Staining of the Oesophagus of *Clarias gariepinus* Adult Exposed to Different Concentrated Grades of Urea Fertilizer. A. Control B. 0.75 g/L C. 1.5 g/L D. 3.0 g/L. Note the demonstration of PAS positive areas withinoesophageal mucosal epithelial surface (white arrow) and in the submucosal region (black arrow). Magnification: X100.

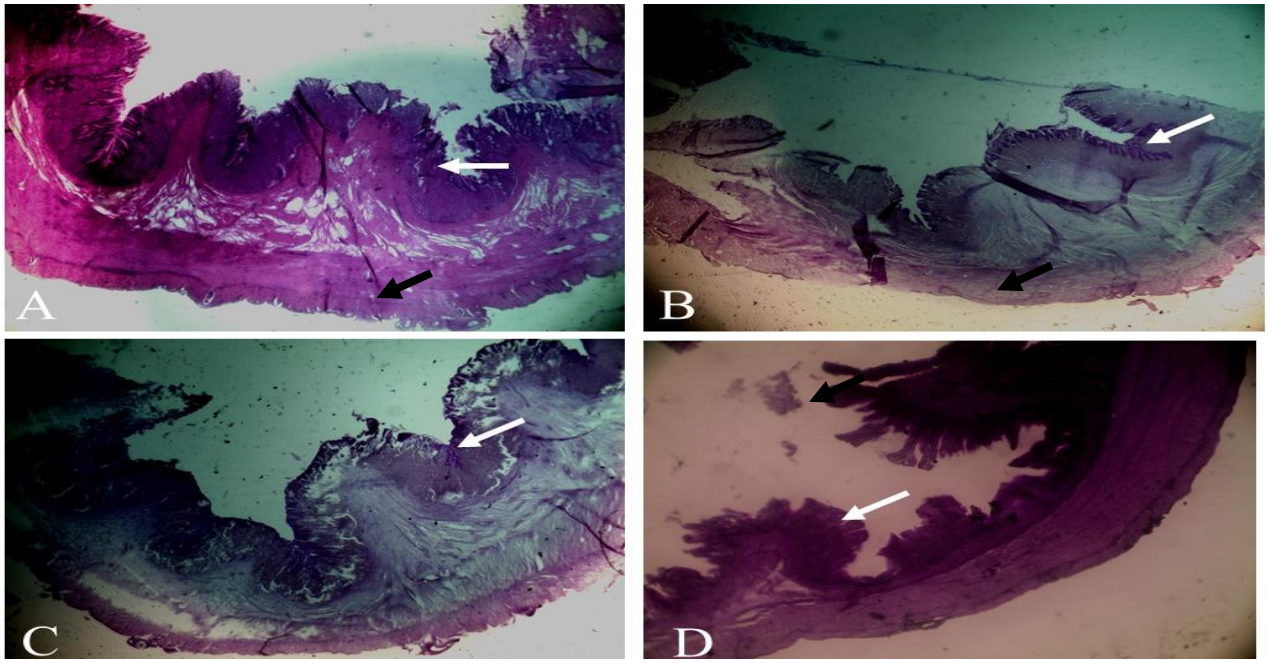

Plate4. The PAS Staining of the Cardiac Segment of the Stomach of *Clarias gariepinus* Fingerlings Exposed to Different Concentrated Grades of Urea Fertilizer. A. Control B. 0.75 g/L C. 1.5 g/L D.

3.0 g/L. Note the demonstration of PAS positive areas within mucosal epithelial surface (white arrow) and in the submucosal region (black arrow). Magnification: X100.

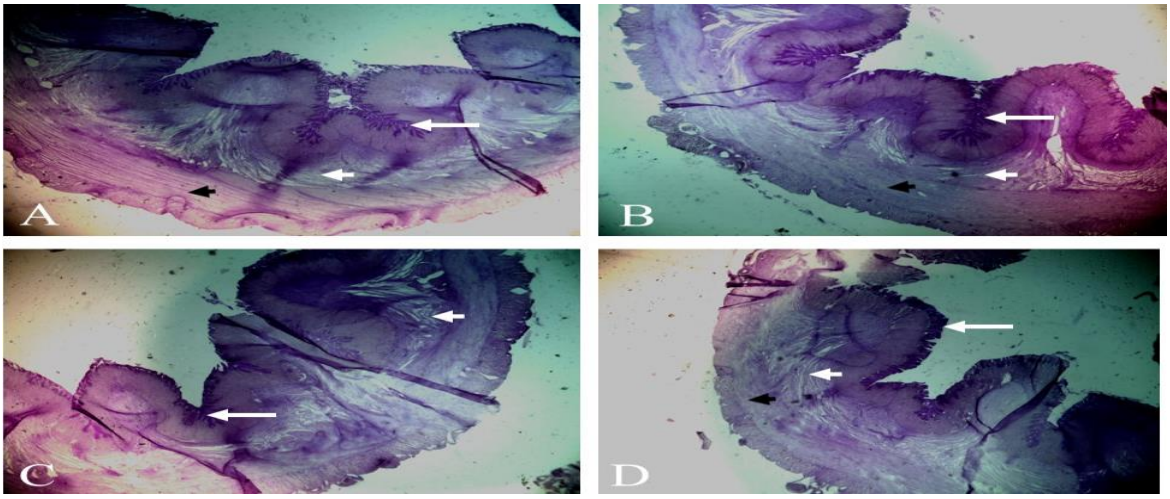

Plate5. The PAS Staining of the Cardiac Segment of the Stomach of *Clarias gariepinus* Juvenile Exposed to Different Concentrated Grades of Urea Fertilizer. A. Control B. 0.75 g/L C. 1.5 g/L D. 3.0 g/L. Note the demonstration of PAS positive areas within mucosal epithelial surface (white arrow) and in the submucosal region (white short arrow). Magnification: X100.

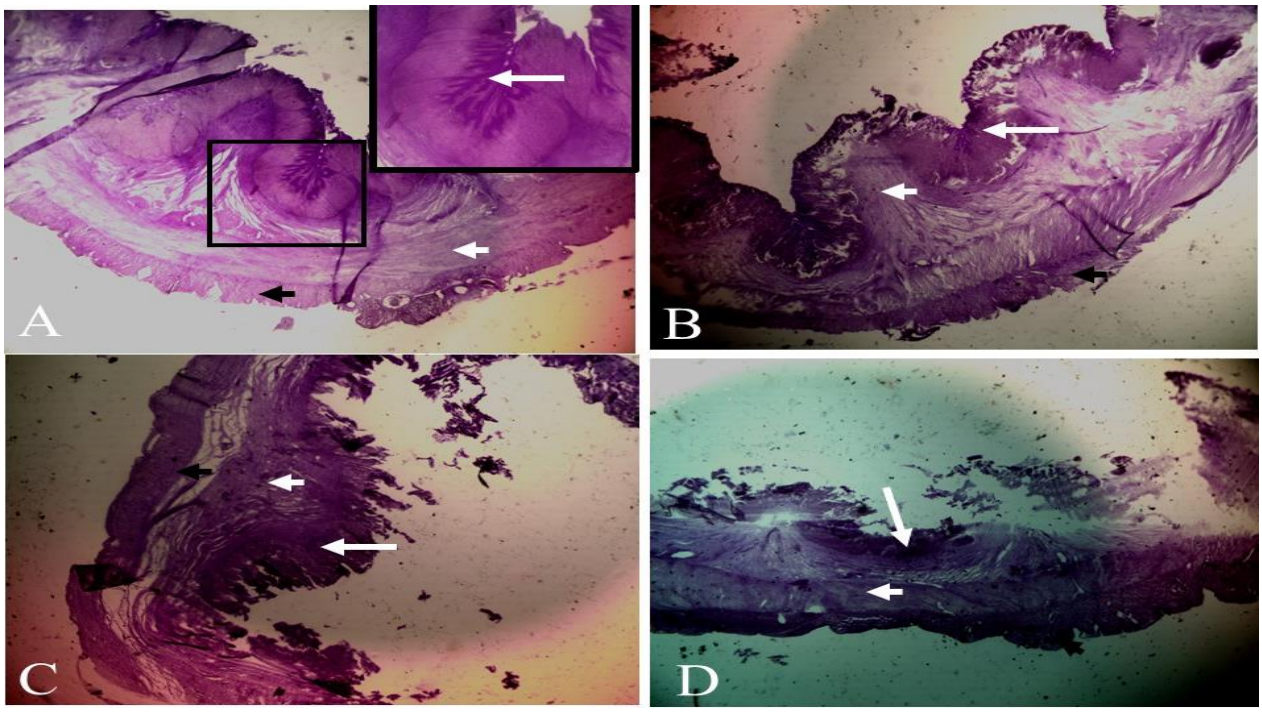

Plate6. The PAS Staining of the Cardiac Segment of the Stomach of *Clarias gariepinus* Adult Exposed to Different Concentrated Grades of Urea Fertilizer. A. Control B. 0.75 g/L C. 1.5 g/L D. 3.0 g/L. Note the demonstration of PAS positive areas within mucosal epithelial surface (white arrow) and in the submucosal region (white short arrow). Magnification: Main: X100; Inset: X400.

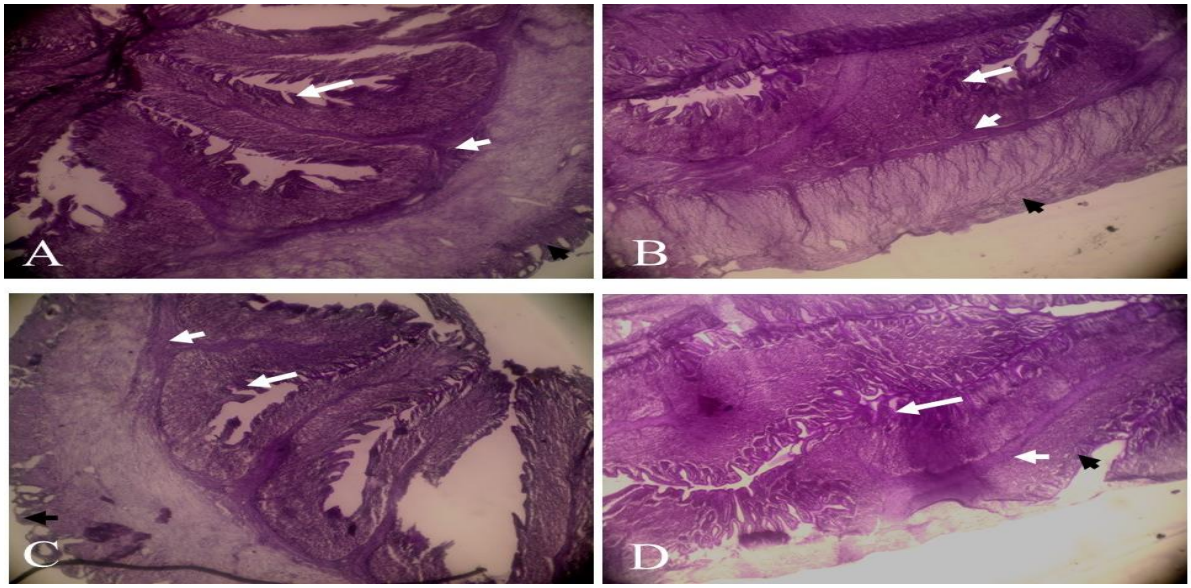

Plate7. The PAS Staining of the Fundic Segment of the Stomach of the *Clarias gariepinus* Fingerlings Exposed to Different Concentrated Grades of Urea Fertilizer. A. Control B. 0.75 g/L C. 1.5 g/L D. 3.0 g/L. Note the demonstration of PAS positive areas within mucosal epithelial surface (white arrow) and in the submucosal region (white short arrow). Magnification: X100.

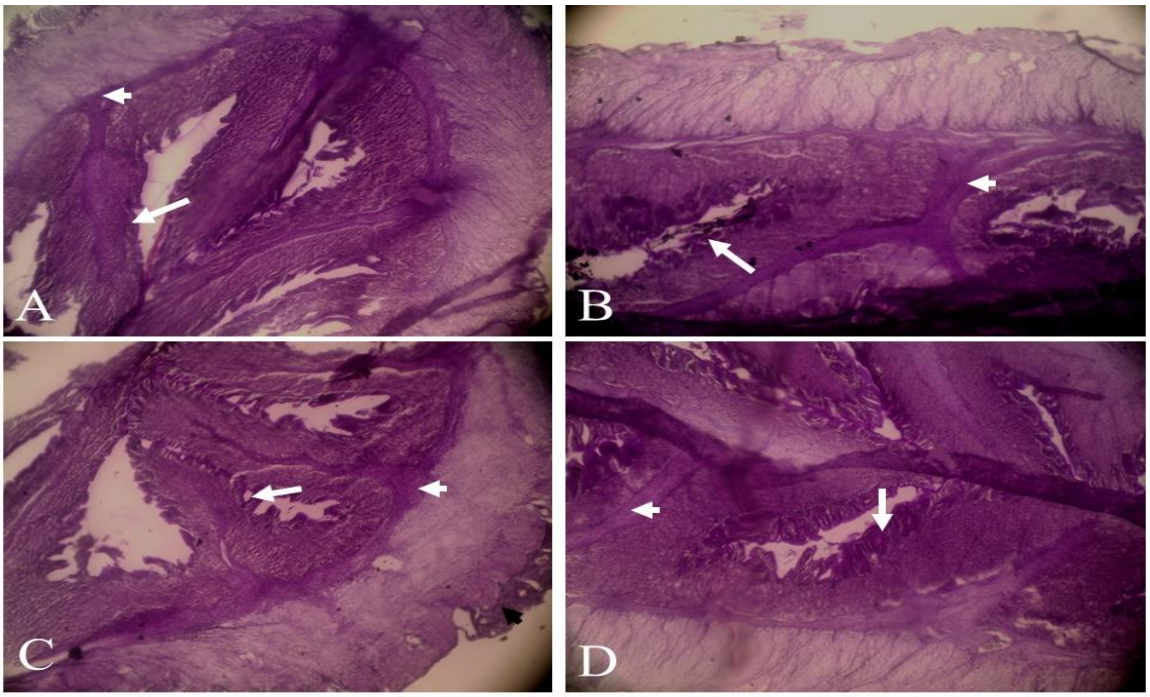

Plate8 The PAS Staining of the Fundic Segment of the Stomach of *Clarias gariepinus* Juvenile Exposed to Different Concentrated Grades of Urea Fertilizer. A. Control B. 0.75 g/L C. 1.5 g/L D. 3.0 g/L. Note the demonstration of PAS positive areas within mucosal epithelial surface (white arrow) and in the submucosal region (white short arrow). Magnification: X100.

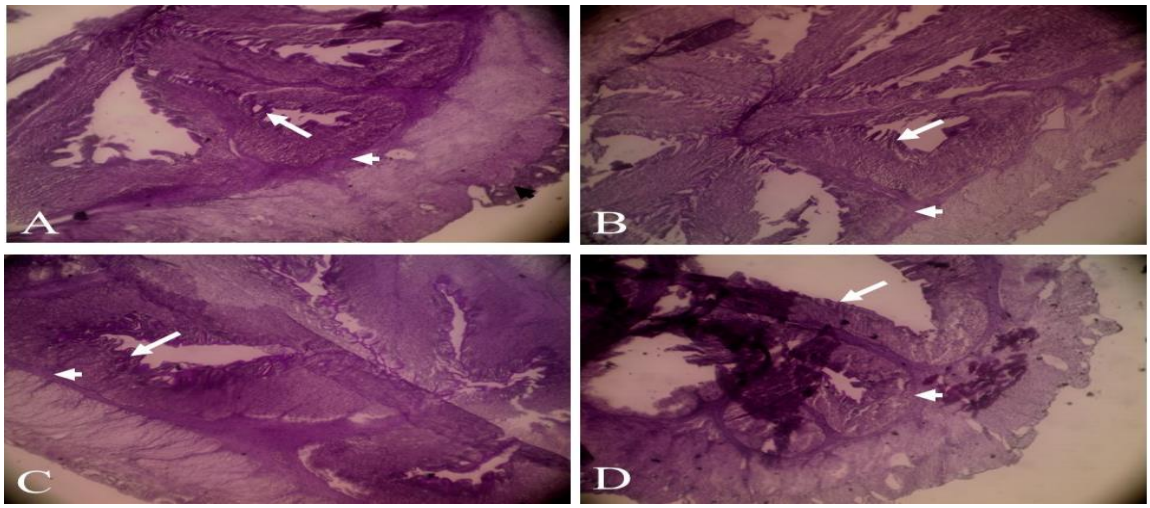

Plate9 The PAS Staining of the Fundic Segment of the Stomach of *Clarias gariepinus* Adult Exposed to Different Concentrated Grades of Urea Fertilizer. A. Control B. 0.75 g/L C. 1.5 g/L D. 3.0 g/L. Note the demonstration of PAS positive areas within mucosal epithelial surface (white arrow) and in the submucosal region (white short arrow). Magnification: X100.

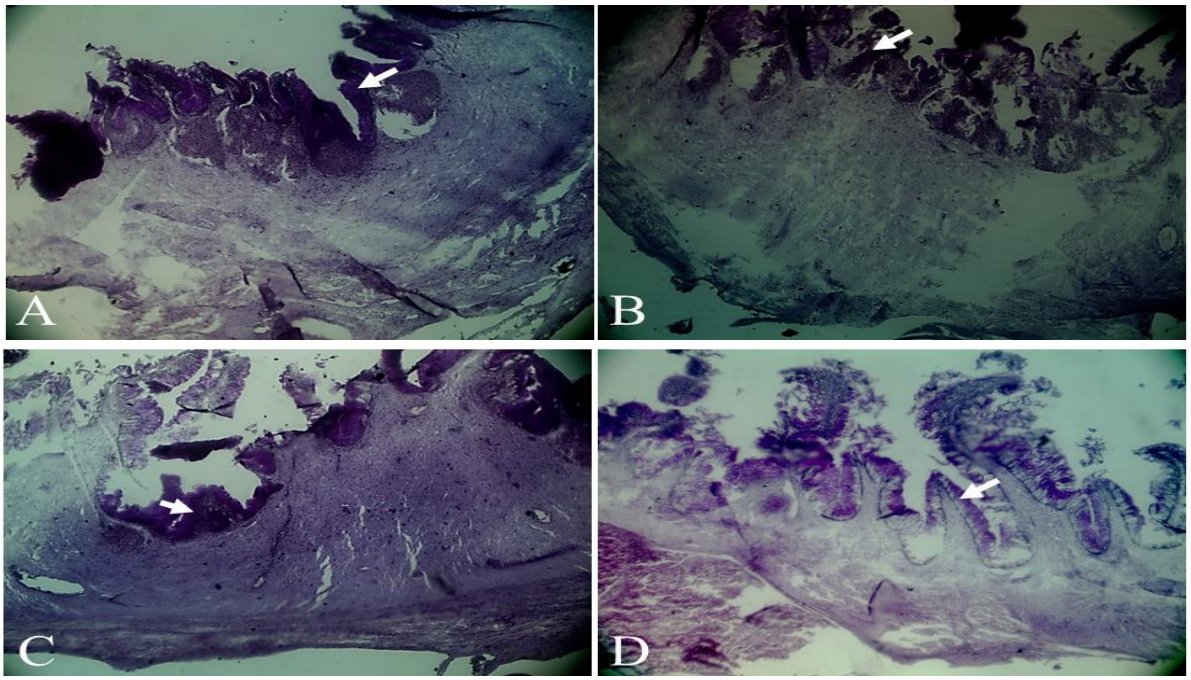

Plate10 The PAS Staining of the Pyloric Segment of Stomach of *Clarias gariepinus* Fingerlings Exposed to Different Concentrated Grades of Urea Fertilizer. A. Control B. 0.75 g/L C. 1.5 g/L D. 3.0 g/L. Note the demonstration of PAS positive areas within mucosal epithelial surface (white arrow). Magnification: X100.

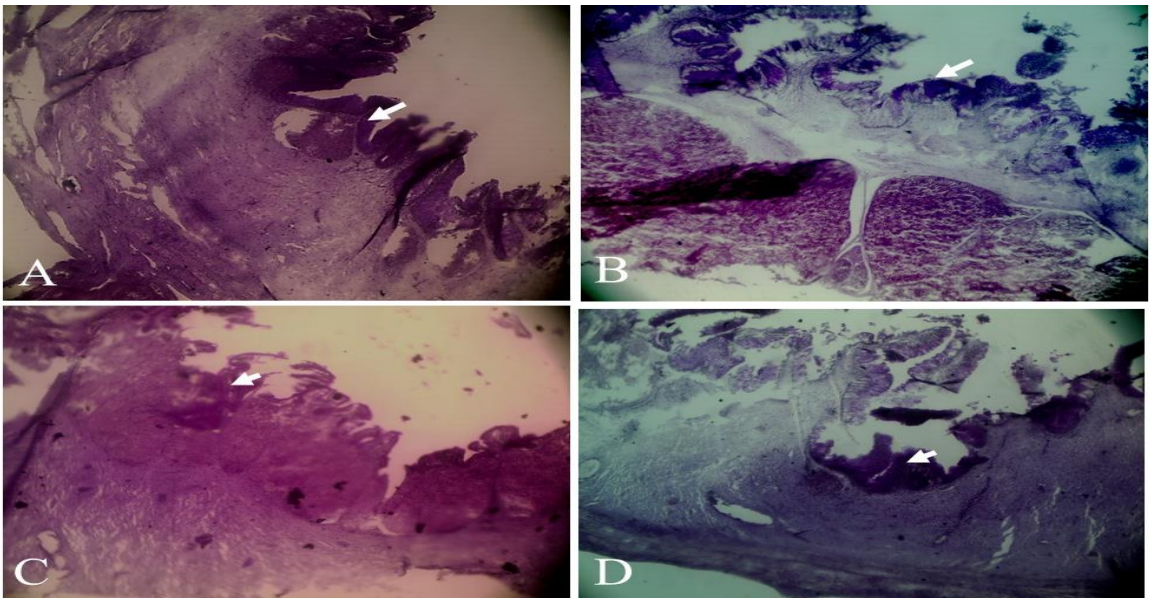

Plate11 The PAS Staining of the Pyloric Segment of Stomach of the *Clarias gariepinus* Juvenile Exposed to Different Concentrated Grades of Urea Fertilizer. A. Control B. 0.75 g/L C. 1.5 g/L D.

3.0 g/L. Note the demonstration of PAS positive areas within mucosal epithelial surface (white arrow). Magnification: X100.

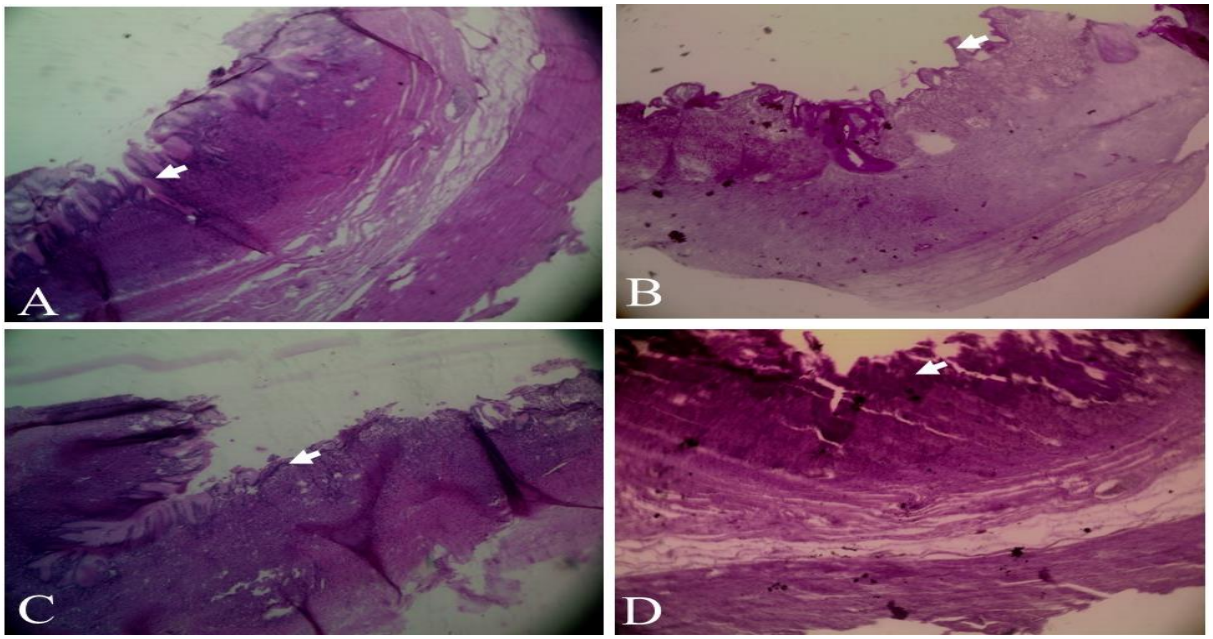

Plate12 The PAS Staining of the Pyloric Segment of the Stomach of *Clarias gariepinus* Adult Exposed to Different Concentrated Grades of Urea Fertilizer. A. Control B. 0.75 g/L C. 1.5 g/L D. 3.0 g/L. Note the demonstration of PAS positive areas within mucosal epithelial surface (white arrow). Magnification: X100.

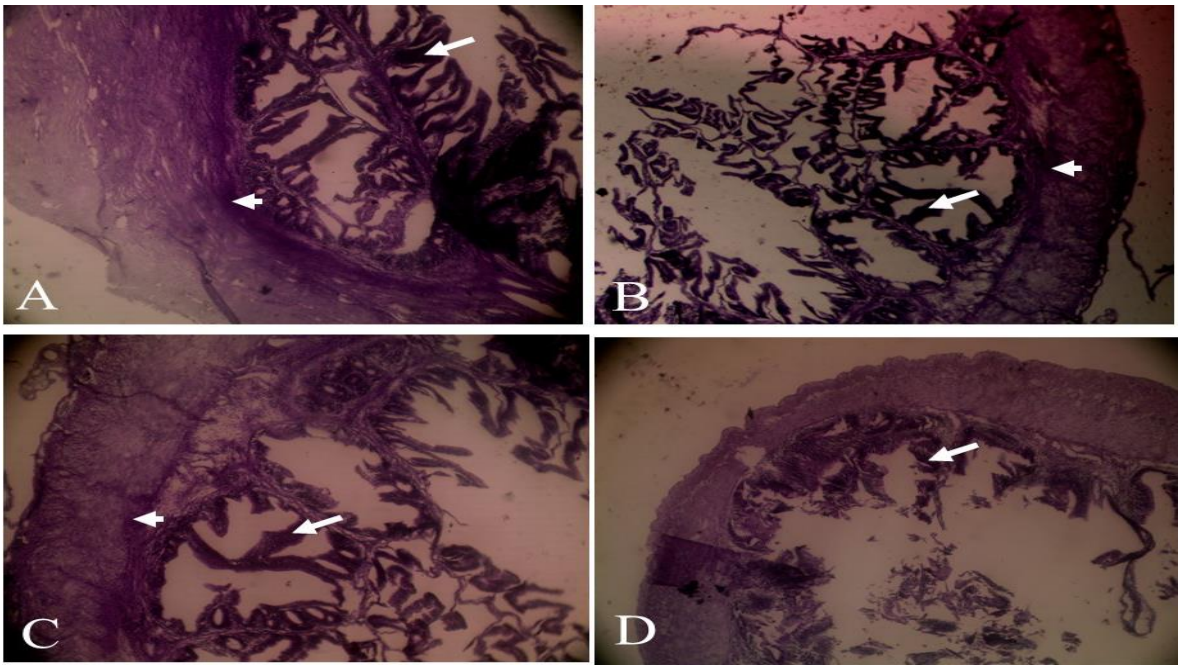

Plate13 The PAS Staining of the Proximal Intestinal Segment of *Clarias gariepinus* Fingerlings Exposed to Different Concentrated Grades of Urea Fertilizer. A. Control B. 0.75 g/L C. 1.5 g/L D. 3.0 g/L. Note the demonstration of PAS positive areas within mucosal epithelial surface (white arrow) and in the submucosal region (short arrow). Magnification: X100.

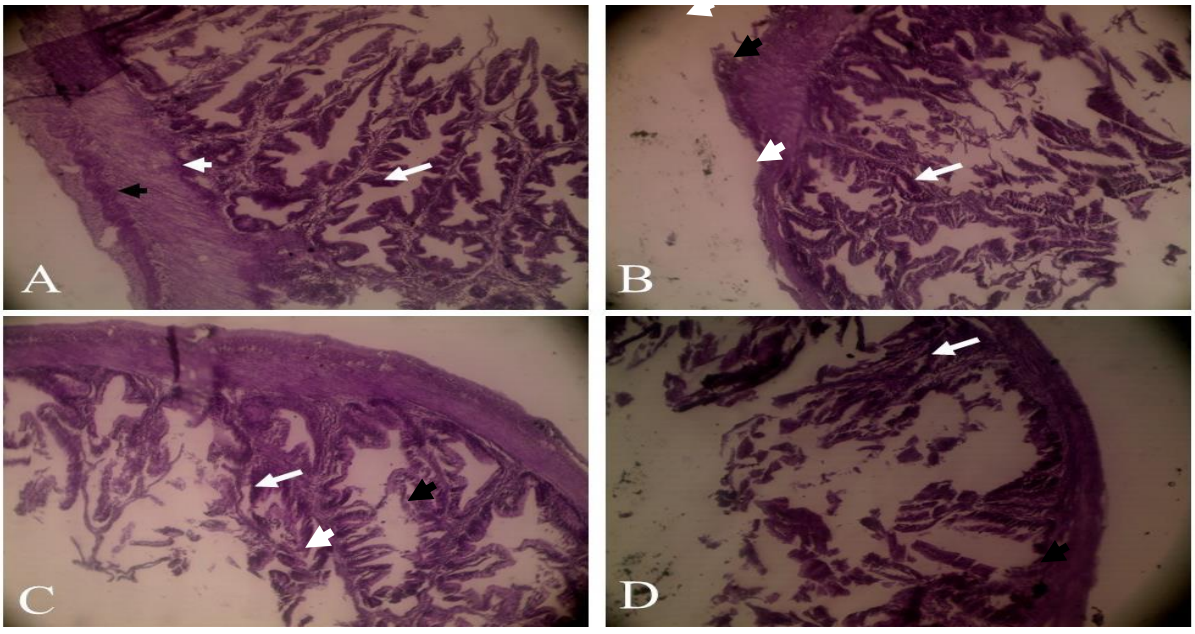

Plate14 The PAS Staining of the Proximal Intestinal Segment of *Clarias gariepinus* Juvenile Exposed to Different Concentrated Grades of Urea Fertilizer. A. Control B. 0.75 g/L C. 1.5 g/L D. 3.0 g/L. Note the demonstration of PAS positive areas within the mucosal epithelial surface (long white arrow), in the submucosal and muscular regions (short white arrow) as well as in the serosal layer (short black arrow). Magnification: X100.

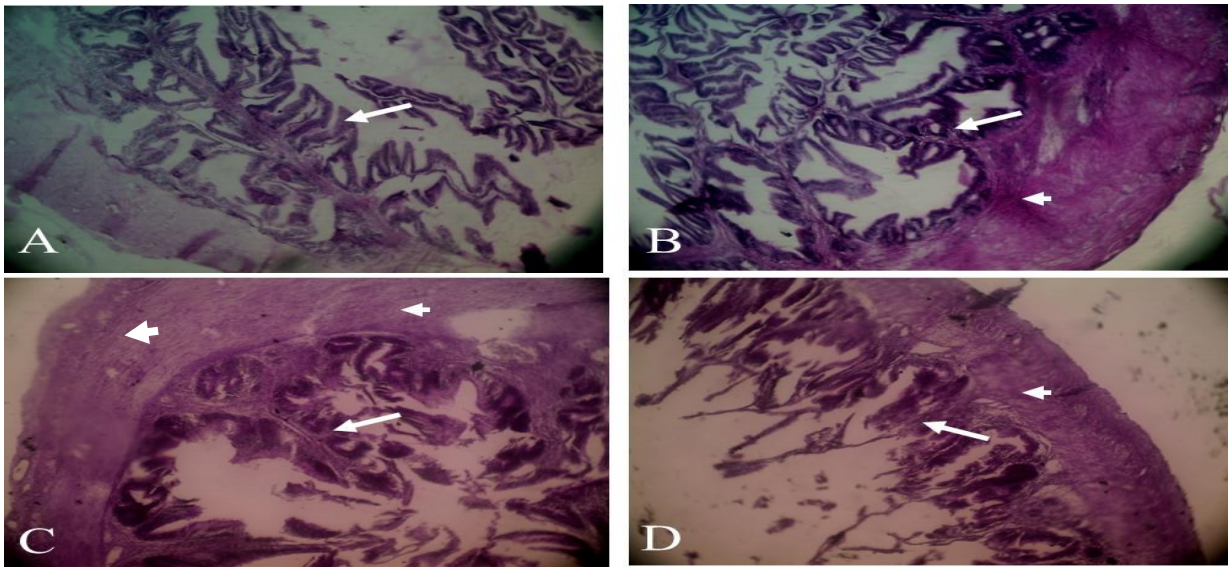

Plate15 The PAS Staining of the Proximal Intestinal Segment of *Clarias gariepinus* Adult Exposed to Different Concentrated Grades of Urea Fertilizer. A. Control B. 0.75 g/L C. 1.5 g/L D. 3.0 g/L. Note the demonstration of PAS positive areas within the mucosal epithelial surface (long white arrow) and in the submucosal and muscular regions (short white arrow). Magnification: X100.

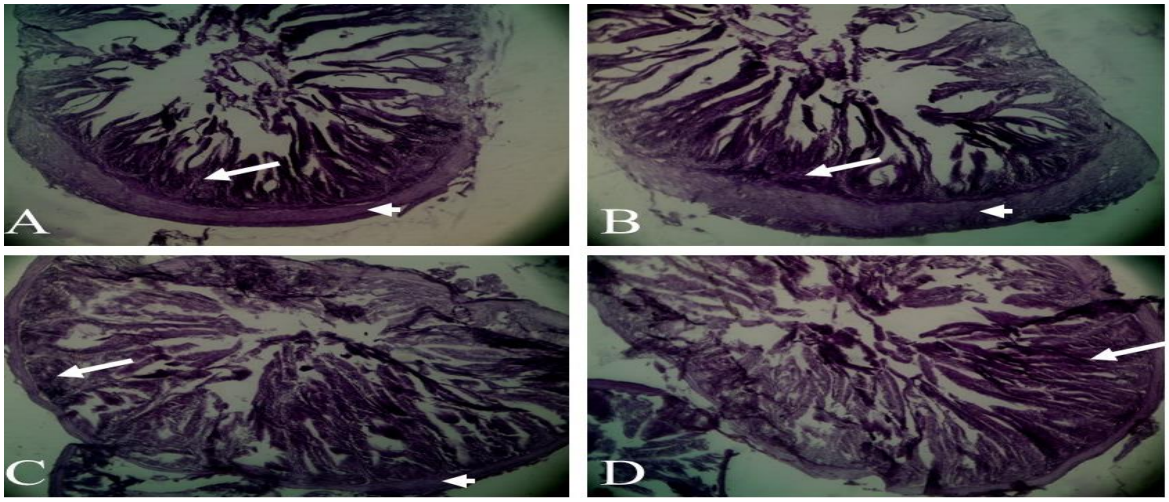

Plate16 The PAS Staining of the Middle Intestinal Segment of *Clarias gariepinus* Fingerlings Exposed to Different Concentrated Grades of Urea Fertilizer. A. Control B. 0.75 g/L C. 1.5 g/L D.

3.0 g/L. Note the demonstration of PAS positive areas within the mucosal epithelial surface (long white arrow) and in the submucosal and muscular regions (short white arrow). Magnification: X100.

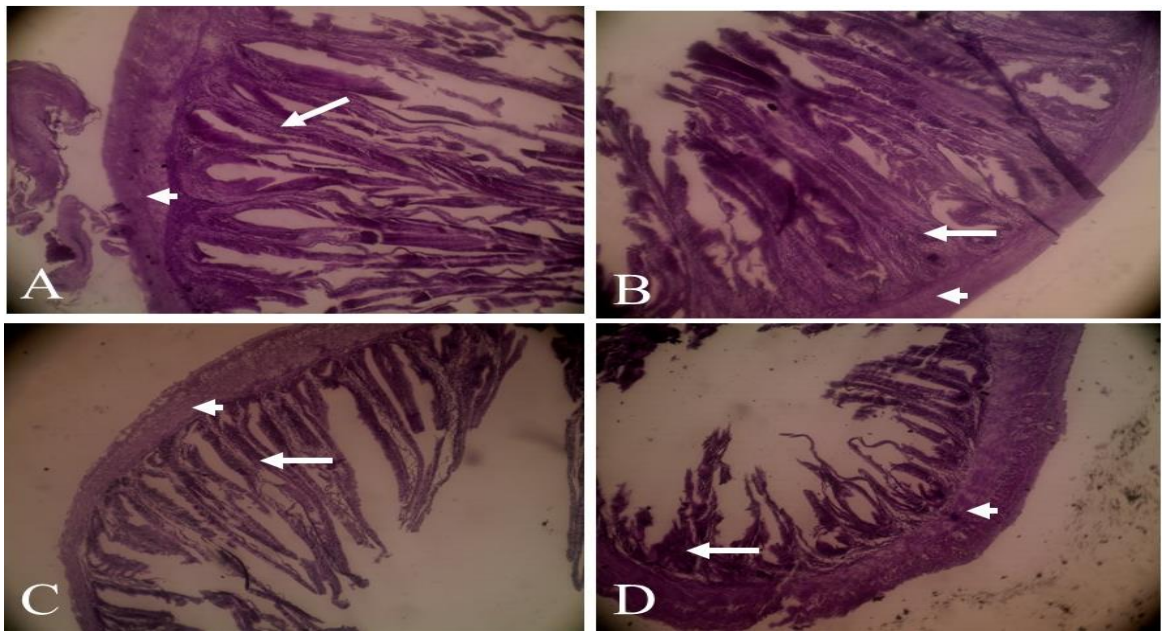

Plate17 The PAS Staining of the Middle Intestinal Segment of *Clarias gariepinus* Juvenile Exposed to Different Concentrated Grades of Urea Fertilizer. A. Control B. 0.75 g/L C. 1.5 g/L D. 3.0 g/L. Note the demonstration of PAS positive areas within the mucosal epithelial surface (long white arrow) and in the submucosal and muscular regions (short white arrow). Magnification: X100.

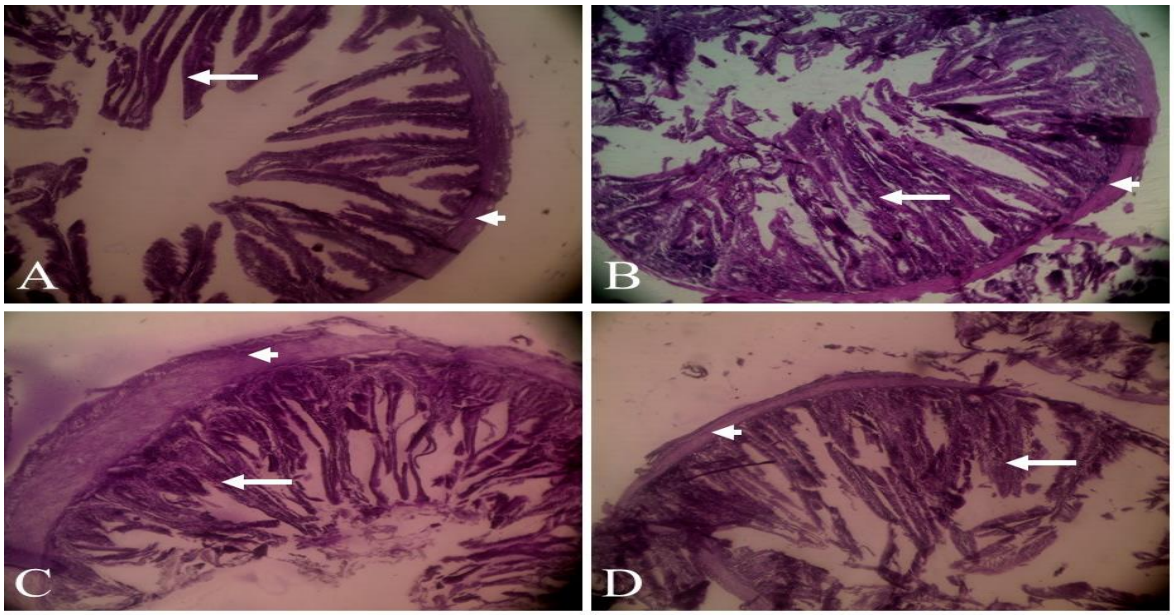

Plate18 The PAS Staining of the Middle Intestinal Segment of the *Clarias gariepinus* Adult Exposed to Different Concentrated Grades of Urea Fertilizer. A. Control B. 0.75 g/L C. 1.5 g/L D. 3.0 g/L. Note the demonstration of PAS positive areas within the mucosal epithelial surface (long white arrow) and in the submucosal and muscular regions (short white arrow). Magnification: X100.

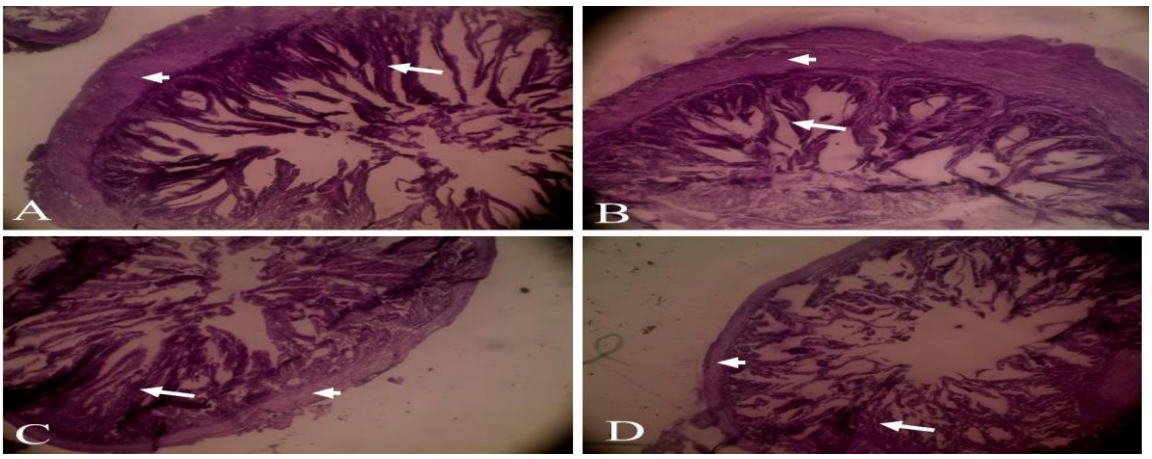

Plate19 The PAS Staining of the Distal Intestinal Segment of *Clarias gariepinus* Fingerlings Exposed to Different Concentrated Grades of Urea Fertilizer. A. Control B. 0.75 g/L C. 1.5 g/L D. 3.0 g/L. Note the demonstration of PAS positive areas within the mucosal epithelial surface (long white arrow) and in the submucosal and muscular regions (short white arrow). Magnification: X100.

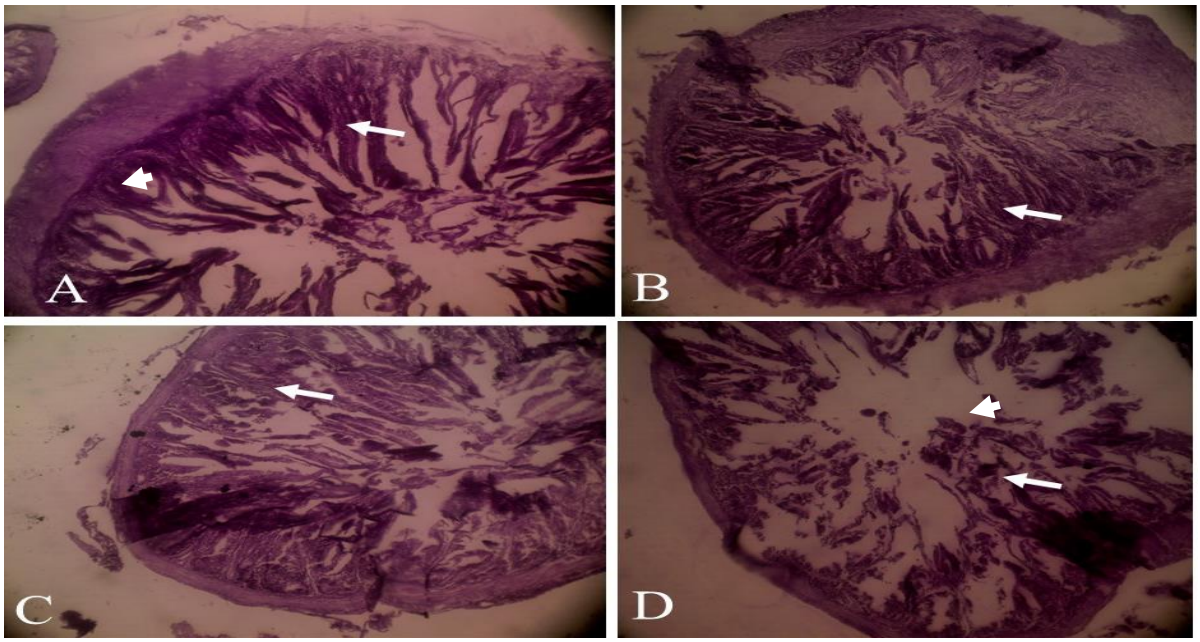

Plate20 The PAS Staining of the Distal Intestinal Segment of *Clarias gariepinus* Juvenile Exposed to Different Concentrated Grades of Urea Fertilizer. A. Control B. 0.75 g/L C. 1.5 g/L D. 3.0 g/L. Note the demonstration of PAS positive areas within the mucosal epithelial surface (long white arrow) and in the submucosal and muscular regions (short white arrow). Magnification: X100.

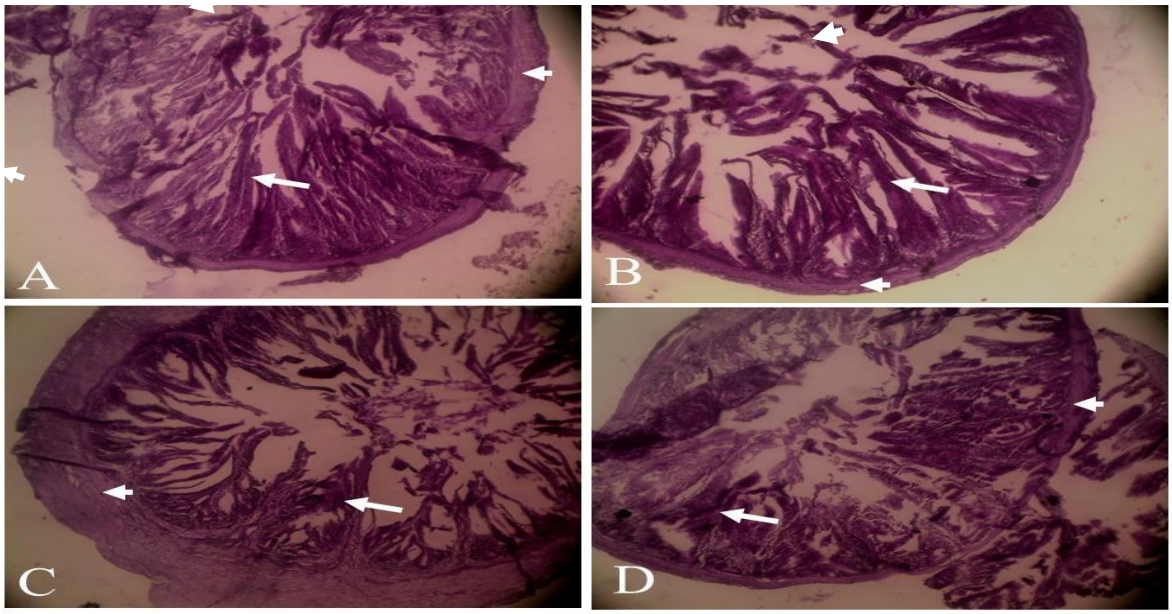

Plate21 The PAS Staining of the Distal Intestinal Segment of *Clarias gariepinus* Adult Exposed to Different Concentrated Grades of Urea Fertilizer. A. Control B. 0.75 g/L C. 1.5 g/L D. 3.0 g/L.

Note the demonstration of PAS positive areas within the mucosal epithelial surface (long white arrow) and in the submucosal and muscular regions (short white arrow). Magnification: X100.

Discussion

Histochemistry study on the oesophagus of *C. gariepinus* in the present study of all the age groups exposed to graded concentrations of Urea fertilizer and normal treatment shows that they were located only to the oesophageal mucosal epithelial surface and in the sub mucosa. The study shows that PAS intensity trend of the oesophageal mucosal epithelial surface in all the age groups, recorded a decrease value with increasing concentration of Urea fertilizer. The present of PAS+ cells in tissues often indicates the present of mucins and has been reported by Wolczuk et al. (2015). The finding was in line with that of Akuthe and Bhomela (2020) who recorded that the oesophagus of carnivorous and omnivorous fish contain numerous mucus cells. The present of acid mucin in the oesophageal mucosal epithelial surface and in the sub mucosa of the fish under study may confer high viscosity to the mucus, which would be good for rapid and consistent lubrication of food particles during swallowing. The present of mucin will equally help in trapping of foreign particles and protecting the epithelium against bacteria and other viral infections as reported by Diaz et al. (2008)..

Histochemistry studies on the cardiac stomach of all the *C. gariepinus* age groups exposed to graded concentrations of Urea fertilizer shows that the PAS+ cells were observed in the mucosal epithelial surface and in the submucosa region. The study shows that PAS+ intensity trend of the cardiac stomach in all the age groups and in all the treated concentrations decreased with increasing grades of Urea concentration than their respective control.The PAS+ intensity of the epithelial cells in the stomach of *C. gariepinus* age groups exposed to graded concentrations of Urea fertilizer under study suggest the predominance of neutral mucins. Similar finding was reported by Akuthe and Bhomela (2020). Histochemistry study in the current work revealed that the PAS+ intensity values of the fish under study exposed to 1.5 and 3.0g/L concentration of Urea fertilizer were similar but lower than the normal and those exposed to 0.75 g/L concentrations of Urea fertilizer. Neutral mucin serves to protect mucosal surface against microorganisms and high acidity of the stomach content. This agrees with findings by Ikpegbu, et al. (2013).Histochemistry studies on the fundic stomach of the entire *C. gariepinus* category exposed to graded concentrations were restricted to PAS+ in the mucosal epithelial surface and the submucosa. PAS+ intensity values of fundic stomach observed in *C. gariepinus* juveniles and adults exposed to 0.75g/L Urea concentrations were significantly the same.

The intensity of PAS+ value recorded in the fundic stomach in the current study was higher in the *C. gariepinus* juveniles and adult exposed to graded concentrations. The above finding was similar to that of Akuthe and Bhomela (2020). High value of PAS+ intensity in the fundic stomach may help to aid the passage of food as well as adjusting the pH of food.

Histochemistry study in the pyloric stomach of all the *C. gariepinus* age groups exposed to Urea concentrations of fertilizer were observed to confirned to the mucosal epithelial surface. The finding is in tandem with that of Ikpegbu, el al. (2014) who reported same in *C. gariepinus* fingerlings.Study on the histochemistry of all the age groups of *C gariepinus*age groups shows that significant reduction in the PAS+ intensity values was recorded with increasing concentrations of

Urea fertilizer compared to their respective controls. The significant reduction in the intensity of PAS+ value level in the pyloric stomach may be an indicative of a food retentive function more than a digestive function as equally reported by Raji and Norouzi. (2010). The present of the mucus cells in the pyloric region of the stomach in the fish age groups may help in the free passage of rough food substances into the intestinal tract segment.

Study on the histochemistry of the proximal intestine of all the *C. gariepinus* age groups exposed to graded concentrations of Urea fertilizer and control shows strong concentration of PAS+ acid mucins and were confined to the mucosal epithelial surface and submucosa region. This finding was in line with early works by Ikpegbu, et al. (2013); AkutheandBhomela (2020). The present of strong acid mucin in the anterior intestine may imply the need for increase viscosity which is associated with lubrication of the intestinal mucosa and undigested foods towards the middle and posterior intestine. The above accersion agreed with that of Chirde and Gadhikar (2014). The PAS+intensity of proximal intestine of all *C. gariepinus* age groups exposed to different concentrations of Urea fertilizer decreased with increasing Urea fertilizer concentrations compared to their respective control.Study on the histochemistry of the middle intestine of all the *C. gariepinus* age groups exposed to graded concentrations of Urea fertilizer shows concentration of PAS+ acid mucins confined in the mucosal epithelial surface, submucosa and muscular regions.This agrees with early works by Raji and Norouzi (2010). PAS intensity values of the middle intestine of all the *C. gariepinus* age groups in all the concentrations of Urea fertilizer consistently decreases with increasing concentrations of Urea fertilizer compared to their respective controls.PAS intensity of the entire *C.gariepinus* age groups decreased from the fingerlings to juveniles and increased in the *C.gariepinus* adult.Histochemistry studies in the distal intestine of all the three *C. gariepinus* age groups were restricted in the mucosal epithelial surface, submucosa and muscularis region. Similar finding was reported by Okuthe and Bhomela (2020). Histochemistry study revealed that the PAS intensity of the distal intestine registered a progressive decrease with increasing exposure to concentrations of Urea fertilizer compared to their respective controls. The PAS intensity of the distal intestine across all the three age groups of *C. gariepinus* exposed to different concentrations of Urea fertilizer were observed to increase from fingerlings to juveniles while they decreased in the adult *C. gariepinus*. The presence of PAS acid mucin in the GIT segments may confer high viscosity to mucus which would be essential for rapid and consistent lubrication of food particles during swallowing and egestion.

Conclusion: The results showed that sub-lethal concentrations of Urea fertilizer (0.75, 1.50 & 3.00g/L) have effect on the histochemistry of the GIT segments of age-related African catfish.The PAS intensity of the GIT segments decrease with increasing exposure to concentrations of Urea fertilizer compared to their respective controls.

REFERENCES

Ajima, M. N. O., Audu, B. S., Mane, A. M., Okeke, L. & Varghese, T. (2017). Alteration in activities of some selected metabolic enzymes and hematological profile of African catfish, *Clarias gariepinus* chronically treated with Urea fertilizer. *Research in Environmental and Life Science*,*10*(2), 129-134.

American Public Health Association [APHA] (2005): Standard methods for the examination of water and waste water. (21st edition), Washington, D.C.,pp 2042.

Arellano, J. M., Storch, V., &Sarasquete, C. (2015).A histological and histochemical study of the oesophagus and oesogaster of the Senegal sole, *Soleasenegalensis*. *European Journal of Histochemistry,* 45(3), 279-294.

Blachall, P.C &Daisley, K.W. (1973).Practical histochemistry.*Journal of Histochemistry and cytochemistry*, 21, 1-12.

Bocina, I., Santic, Z., Restovic, I., & Topic, S. (2017). Histology of the digestive system of the garfish *Belonebelone* (*Teleostei: Belonidae)*. *The European Zoological Journal*,*84*(1), 89-95.

Chanda, K. (2020). Geno toxic effects of synthetic fertilizer urea in *Channapunctatus*.*International Journal of Scientific Development and Research*, *5*(1), 237-242.

Germano, R. M., Stabille, S. R., Mari, R. B., Pereira, J. N. B., Faglioni, J. R. S., & Miranda, N. M. H. (2014). Mophorlogical characteristics of the *Pterodorasgranulosus*(Valenciennes, 1821) digestive tube (Osteichthyes, Dovadidae). *Acta Zoological*, *95*(2), 166-175.

Gisbert, E., Ortiz-Delgado, J. B., &Sarasquete, C. (2008). Nutritional cellular biomakers in early life stages of fish. *Histology and Histopathology*,*23*(12), 1525-1539.

Gupta, D.,& Kumari, A. (2021). Effect of urea [$CO(NH_2)_2$] on histopathology of kidney, liver, intestine and gonads in *Anabas testidineous* (Bloch). *International Journal of Current Microbiology and Applied Sciences*, 10(6), 106-115.

Ikpegbu, E., Nlebedum, U. C., Nnadozie, O., &Agbakwuru, I. O. (2012c). Histological structures of the accessory glands of the digestive system in adult farmed African catfish (*Clarias gariepinus B.*).*I0SR Journal of Agriculture and Veterinary Science*, 1(6), 41-46.

Ikpegbu, E., Ezeasor, D.N., Nlebedum, U.C., &Nnadozie, O. (2013).Morphological and histological observations on the Oesogaster of the domestic African catfish (*Clarias gariepinus Burchell*, 1822).*International Journal of Vetrinary Medicine*. 16(2), 88-95.

Ikpegbu, E., Nlebedum, U.C., &Ibe, C.S. (2014).The histology and Mucin Histochemistry of the farmed juvenile African catfish digestive Tract (*Clarias gariepinus). StudiaUniversitatis "VasileGoldis" SeriaStiinteleVietii,* 23(1), 125-131.

Lokka, G., Austbo, L., Falk, K., Bierkas, I. S., & Koppang, E.O. (2013).Intestinal morphology of the wild Atlantic salmon (*Salmosalor*).*Journal of Morphology,* 274(8), 859-876.

Manisha, R. D., Sudhir, G. C., &Gadhikar, Y. A. (2015).Histological and histochemical study on the stomach and intestine of catfish (*heteropneustesfossilis)* (BLOCH 1979).*Global Journal of Biology, Agriculture and Health* Science. *4*(1), 16 – 23.

Marcella, L. D. S., Fabio, P. A., Tiayo, C. P., & Jose, E. D. S. (2015). Morphology, histological and histochemical of the digestive tract of *Trachelyopterusstriatulus*(*Siluriformes: Auchenipteridae*).*Zoologia,* 32 (4), 296-305.

Nazlic, M., Paladin, A., &Bocina, I. (2014).Histology of the digestive system of the black scorpion fish *ScorpeanaporcusL.ActaAdriatica,* 55(1), 65-74.

Omoregie, E., Ajima, M. N. O., Keke, R.I. &Wieski, K. (2009). Effect of single superphosphate fertilizer on survival and respiratory dynamics of Nile tilapia, *Oreochromisniloticus* (Actinopferygii: Perciformes: cichlidae). *ACTA Ichilyologyet Piscatorial,* 39(2), 103-110.

Pollyanna, M. F. F., Debora, W. C., Analucia, S., Sirlene, S. R. S., Jerusa, M. O., Alex, J. S. C.,&Jeneri, A. S. Z. (2015). Intestinal and liver morphometry of the yellow Tail Tetra (*Astyanaxaltiparanae)* fed with oregano oil. *Annals of the BrazilianAcademy of Science,* 88(2), 911-922.

Raji, A. R., & Norouzi, E. (2010), Histological and histochemical study on the alimentary canal in walking catfish (*Clarias batrachus)* and piranha (*Serresalmus nattereri). Iranian Journal of Veterinary Research*, 3(32), 255-261.

Sinlapachai, S., Jes, K., Wannee, J., &Njwat, K. (2015).Structural classifications in the digestive tract of short mackerel, *Rastrelligerbrachysoma*(Bleeker, 1851) from upper Gulf of Thailand.*Songklanakarin Journal of Science Technology,* 37(5), 561-568.

Soma, M.,& Susanta, N. (2014).Toxic impact of urea on the hematological parameters of air breathing fish, *Heteropneustesfossilis* (Bloch).*American-Eurasion Journal of Agriculture and Environmental Sciences*, 14(4), 336-342.

Thayappan, K., Maghil, D., Annadurai, R. A. R.,& Narayanasamy, S. (2014). Histological study of the intestine and liver tissues in fish *Oreochromismossambicus* exposed to Cypermethrin. *Journal of Modern Biotechnology,* 3(4), 48-54.

Trindade, M. E. J., & Queiroz, H. L. (2012).Feeding ecology and morphometry of the digestive tract of *Astronotusocellatus* (Circhlidae) in Varzea environment of the middle solim region, central Amazon, Brazil.*Taxonomy, Morphology and Ecology,* 8 (1), 45-57.

Tulika, A. K., &Gadhikar, Y. A. (2017).Histological and ultrastructural study of intestine of Asiatic knife fish, *Notopterusnotopterus. International Journal of Fisheries and Aquatic Studies*, 5(1), 18-22.

Wilson, J. M., & Castro, L. F. C. (2010).Morphology diversity of the gastrointestinal tract in fish. In M. Grosell, A.P Farrell, C.J Brauner (Eds).The multifunctional gut of fish. *Academic Press as an Imprint of Elsevier*, 30, 1-55.

Xiong, D., Zhang, L., Yu, H., Xie, C., Kong, Y., Zeng, Y., Huo, B., & Liu, Z. (2011).A study of morphological and histology of the alimentary tract of *Glyptosternummaculatum (Sisoridae, siluriformes*).ACTA*Zoologia*, 92(2), 161-169.