PRELIMINARY ASSESSMENT OF DIGESTIVE ENZYMES ON NILE TILAPIA PRODUCTION AND EFFECT ON ITS LIVER MORPHOLOGY

ADEOYE A.A.
Department of Aquaculture and Fisheries Management, Federal University of Agriculture, Abeokuta, Nigeria
Corresponding Author: adeoyeaa@funaab.edu.ng; +2349035473524

ABSTRACT
A feeding trial was done to assess the dietary effects of digestive enzymes on Nile tilapia (Oreochromis niloticus) production and their effects on its liver morphology. Each of the tilapia treatments (38.74±0.51 g fish-1) were dispensed each experimental diet; one as a control and others were supplemented with phytase, protease, and carbohydrase at 0.03 %, 0.02 %, and 0.03 %, respectively. After six weeks, the fish fed the phytase supplemented diet had higher final body weight and SGR (P < 0.05) than fish fed the control and protease supplemented diets. In terms of feed conversion and protein efficiency ratios, the fish fed phytase and carbohydrase diets performed better than the control group (P < 0.05). The fish liver tissue revealed that cellular structure had a regular arrangement with clear boundaries without any severe degradation in any of the dietary treatments. The hepatic density was higher (P < 0.05) in the fish fed protease and carbohydrase supplemented diets than those fed the phytase diet, though not different from the control group. It could be concluded therefore that dietary supplementation of phytase and carbohydrase at the current dosage can improve the performance and nutrient utilisation of Nile tilapia (O. niloticus) without deleterious effect on liver morphology.
Keywords: Phytase, Protease, Carbohydrase, Hepatic function

INTRODUCTION

The inclusion of exogenous digestive enzymes (such as phytase, non-starch polysaccharides degrading enzymes, and protease) in aquafeeds offers the potential to overcome negative effects of antinutritional factors and improve the digestion of dietary components of plant ingredients leading to better nutrients utilisation and subsequently improved performance (Encarnação, 2016). The successful use of phytase as a digestive enzyme to disintegrate phytate for improved minerals and nutrients digestibility has been reported (Cao *et al*., 2008, 2007; Kiarie *et al*., 2013; Kiarie and Nyachoti, 2010). Similarly, enzymes such as cellulase, β-glucanase, α-amylase and xylanase which are known for their capacity to degrade non-starch polysaccharides have been recognised for their potential to promote quick digestion in animals by reducing viscosity in the gastrointestinal tract (Bedford and Cowieson, 2012; Zijlstra *et al*., 2010). The inclusion of protease in the diet also has the potential to increase the utilisation of plant proteins by improving the digestibility and availability of the proteins and amino acids in the diets.

Although, the enzymes have been used in the manufacture of aquaculture feeds to enhance the digestion and utilisation of nutrients, till now there has not been any study showing the effect of exogenous digestive enzymes on the liver, one of the largest glands associated with the digestive system and gastrointestinal tract of fish. To this end, this study was carried out as a preliminary study to evaluate the dietary effect of phytase, protease and carbohydrase on tilapia (*Oreochromis niloticus*) growth performance and their effects on its liver morphology.

MATERIALS AND METHODS

The feeding trial was carried out in a freshwater recirculating aquaculture system (RAS) of Aquatic Animal Nutrition and Health Research Group, University of Plymouth, Plymouth, United Kingdom. The RAS contains 12 rectangular tanks (72 L capacity each) and were supplied with fresh water from the municipal authority. Three hundred and sixty (360) genetically male tilapia (*O. niloticus*) of average weight 38.74 ± 0.51 g (sourced from North Moore Tilapia, Goxhill, UK) were randomly allocated (30 fish per tank) into the 12 tanks after acclimatisation. The fish were held at 26.3 ± 0.8 ºC. Water quality parameters were monitored at pH 6.2 ± 0.7 and dissolved oxygen >6.0 mg L−1, ammonium, nitrite, and nitrate levels were monitored weekly and water changes (~444.6 L, an equivalence of ~20 % system volume) were undertaken weekly to minimise accumulation of these compounds.

Four experimental diets were formulated to be iso-nitrogenous and iso-lipidic (Table 1). Three of the experimental diets were supplemented with phytase, protease, and carbohydrase at 0.03 %, 0.02 %, and 0.03 %, respectively and a diet with no enzyme supplementation served as the control diet. The ingredients used for the experimental diets were well mixed, moistened, and then pelleted to produce 2mm pellet-sized diets. The diets were dried at 45 ºC in an oven for 24 hrs. The proximate composition of the experimental diets were analysed (Table 1) using AOAC protocol (AOAC, 1996). The experimental diets were stored in airtight containers and stored in a cool dry place until use. The tilapia were assigned to respective dietary treatment (n = 3 tanks per treatment) and the fish were fed the respective diet at ~3.5 % biomass per daily in equal proportions at

0900, 1300, and 1700 hr for 42 days. Daily feeding was adjusted weekly by batch weighing the fish per tank after a 24-hr feed deprivation period.

The growth performance, feed utilisation, and somatic parameters were evaluated as previously described by Adeoye *et al.* (2016). On termination of the feeding tria, two tilapia per tank (n = 6) were obtained for liver histological assessment. The liver tissues were fixed in 10 % neutral buffered formalin, processed in graded ethanol and xylene. The processed tissues were embedded in paraffin wax, sectioned (5 mm thick), and stained with haematoxylin and eosin. The liver tissues were imaged using a light microscope (BX53, OlyBPus Life Science, Tokyo, Japan) and the total number of hepatic cell nuclei was counted in five non - overlapping fields-of-view (following a random selection) under a 40x magnification (Wang *et al*., 2019).

All data are presented as mean values with their corresponding standard deviation. Normality and equality of variance of the data were confirmed. Data were analysed using one-way ANOVA and differences among the means were determined using a post-hoc Duncan multiple range test. Differences were considered significant when $P < 0.05$.

RESULTS

The performance, feed utilisation and somatic parameters of Nile tilapia (*O. niloticus*) fed the phytase, protease and carbohydrase supplemented diets were evaluated using mean final weight (FBW), SGR), FCR, PER, survival, condition factor, hepatosomatic and viscerosomatic indices (Table 2). With regards to FBW, the phytase group performed better ($P < 0.05$) than the control and protease group. The fish subjected to the phytase dietary treatment also displayed improved feed utilisation (FCR and PER) when compared to the fish subjected to the control and protease dietary treatments. However, the tilapia subjected to the phytase and carbohydrase dietary treatments had the same growth performance; they had similar FBW and SGR ($P > 0.05$). The survival of fish recorded in all the dietary treatments were above 90 %. The somatic indices (condition factor, hepatosomatic and viscerosomatic indices) of the Nile tilapia were not significantly affected by the dietary supplementation of the exogenous digestive enzymes (i.e. phytase, protease and carbohydrase, $P > 0.05$).

All the fish sampled for liver histological analysis showed that the liver cellular structure had regular arrangements with clear boundaries (Figure 1). The dietary treatments (protease and carbohydrase) did not have a significant effect on the total number of liver nuclei (Table 3, $P > 0.05$) of the Nile tilapia. However, the total number of liver nuclei was found to be lower ($P < 0.05$) in tilapia that were subjected to the phytase dietary treatment, compared to the tilapia subjected to the protease and carbohydrase dietary treatments but not statistically different from those fed the control diet with no enzymes supplementation.

DISCUSSION

The application of digestive enzymes to improve the production and yield of aquaculture species by releasing potentially bound nutrients in plant ingredients have been established. However, to the understanding of the author, no study has been conducted on the possible impact of dietary supplementation of digestive enzymes on the hepatic function of Nile tilapia. To this end, this study was carried out to know the dietary effects of digestive enzymes on the production performance and liver morphology of Nile tilapia (*O. niloticus*). Supplementation of diet with enzymes (such as carbohydrase and phytase) could reduce the deleterious effects of anti-nutrients, increase the bioavailability of previously locked-up nutrients and subsequently increase the nutritional quality of diet. in a similar vein, the dietary supplementation of protease has the potential to increase protein digestibility by reducing the complexity of such proteins. In this study, the improved mean final weight and specific growth rate of Nile tilapia subjected to phytase dietary treatment confirm an increase in the diet nutritional quality and nutrients' bioavailability. Further confirmation was the improvement observed in the feed conversion and protein efficiency ratios observed in the tilapia groups subjected to phytase and carbohydrase dietary treatments, compared to those fed the control diet. The dietary effect of phytase could be explained by its liberation effects on previously bound phytate and associated nutrients, and subsequent utilisation of the available nutrients. In another study, improved growth performance, feed conversion, and protein efficiency ratios were reported in the Nile tilapia fed diet supplemented with phytase (Cao *et al*., 2008). This finding also agrees with Portz and Liebert (2004), Liebert and Portz (2005), and Nwanna and Schwarz (2007), who all observed better nutrients' digestibility and improved performance in the Nile tilapia subjected to phytase dietary treatment. On the contrary, Cao *et al*. (2007) reported that there was no significant effect of phytase supplementation on fish nutrient utilisation and growth performance. The insignificant effect of the phytase supplementation was explained by the possible enhancement of other anti-nutritional factors (once phytate is removed), which consequently prevents further degradation of amino acids or reduction of water-soluble components (Cao *et al*., 2007). In another vein, the non-effect of phytase supplementation could also be due to the dosage of the enzyme and type of substrate provided for the enzyme(s). The Nile tilapia subjected to phytase and carbohydrase dietary treatments in this study had a similar mean final weight and specific

growth rate. This similarity in performance irrespective of the dietary treatment could be a result of the molecular size reduction effect of carbohydrase on non-starch polysaccharides and subsequent improvement in the digestion and absorption of the nutrients (Castillo and Gatlin, 2015). On the other hand, Yigit and Olmez (2011) thought that dietary supplementation of carbohydrase did not confer a beneficial effect on the growth of tilapia. In the present, it was observed that dietary supplementation of protease did not have a significant effect on fish nutrient utilisation and performance. However, Dias *et al.* (2012) reported that the inclusion of protease in a low protein diet improved the performance of tilapia. It could be deduced from this contrasting observation that the dietary effect protease is very likely to be readily noticed in a diet with low levels of protein and fish meal; the protein in the diet used in the present study is relatively higher than what was reported by Dias *et al.* (2012).

The liver is one of the largest organs associated with the gastrointestinal tract in fish and its roles include nutrients assimilation, bile production, detoxification, maintenance of body metabolic homeostasis such as the processing of carbohydrates, proteins, lipids, and vitamins (Genten *et al*., 2009). The hepatic function in this study was assessed by histological examination of the tilapia liver tissue and the total number of liver nuclei. The histological assessment of the liver tissue revealed progressive lipid vacuolisation but did not show severe degradation in any of the dietary treatments. The total number of liver nuclei representing hepatocyte density appears to be significantly lower in the Nile tilapia fed phytase supplemented diet when compared to those fed diets supplemented with protease and carbohydrase even though not different from the control group. From this study, there is a possible inverse relationship between the hepatocyte density and nutrient utilisation of tilapia; the tilapia fed phytase diet (with lower hepatocyte density) had higher nutrient utilisation (FCR and PER) than those (with higher hepatocyte density) fed diets supplemented with protease and carbohydrase. On the other hand, there seems to be a direct relationship between the total number of liver nuclei and the tilapia hepatosomatic index and condition factor; the hepatosomatic index and condition factor of tilapia (higher hepatocyte density) fed diets supplemented with protease and carbohydrase are nominally higher than those (lower hepatocyte density) fed diet supplemented with phytase, though not statistically significant. It might be interesting for a future study to expand the scope of hepatic function study to establish the mode of action between liver functionality and exogenous digestive enzymes.

From this study, it could be concluded that dietary supplementation of phytase and carbohydrase can enhance the growth performance and nutrient utilisation of Nile tilapia (*O. niloticus*) without deleterious effect on liver morphology.

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TABLES

Table 1. Formulation and composition of the experimental diets

Ingredients (%)	**Control**	**Phytase**	**Protease**	**Carbohydrase**
Soybean protein meal[a]	35.30	35.30	35.30	35.30
Narrow-leafed lupin meal[b]	25.00	25.00	25.00	25.00
Corn starch[c]	20.99	20.97	20.98	20.97
Herring meal LT94[d]	10.00	10.00	10.00	10.00
Corn oil	2.17	2.17	2.17	2.17
Fish oil	2.00	2.00	2.00	2.00
Lysamine pea protein concentrate[e]	2.00	2.00	2.00	2.00
Vitamin & mineral premix[f]	2.00	2.00	2.00	2.00
CMC-binder[e]	0.50	0.50	0.50	0.50
Phytase[g]	0.00	0.03	0.00	0.00
Protease[h]	0.00	0.00	0.02	0.00
Carbohydrase[i]	0.00	0.00	0.00	0.030
BHT[f] (mg)	0.008	0.008	0.008	0.008
Ethoxyquin[f] (mg)	0.0008	0.0008	0.0008	0.0008
Alpha tocopherols[f]	0.02	0.02	0.02	0.02
Total	100.00	100.00	100.00	100.00
Composition (%)				
Moisture	7.04	7.43	6.49	5.98
*Crude protein	40.63	40.86	40.65	41.01
*Lipid	7.77	7.49	8.24	7.85
*Ash	6.35	6.48	6.50	6.46
Energy, MJ kg^{-1}	19.18	19.18	19.10	19.34
*NFE[j]	19.03	18.57	19.03	19.36

[a]Hamlet HP100, Hamlet Protein, Denmark.
[b]Soya UK
[c]Sigma- Aldrich Ltd., UK.
[d]Herring meal LT94 – United Fish Products Ltd., Aberdeen, UK.
[e]Roquette Frêres, France.
[f] Premier Nutrition Products vitamin/mineral premix contains: 121 g kg^{-1} calcium, Vit A 1.0 µg kg^{-1}, Vit D3 0.1 µg kg^{-1}, Vit E (as alpha tocopherol acetate) 7.0 g kg^{-1}, Copper (as cupric sulphate) 250 mg kg^{-1}, Magnesium 15.6 g kg^{-1}, Phosphorus 5.2 g kg^{-1}
[g] RONOZYME® Hiphos (contains 10,000FYT g^{-1}) from DSM Nutritional Products
[h] RONOZYME® ProAct (contains 75,000 PROT g^{-1}) from DSM Nutritional Products
[i] ROXAZYME® G2 (contains 2700U g^{-1} xylanase, 700U g^{-1} β-glucanase and 800U g^{-1} cellulose) from DSM Nutritional Products
[j]Nitrogen - free extracts (NFE) = dry matter – (crude protein + crude lipid + ash)
*composition on dry weight basis

Table 2. Growth, feed utilisation and somatic indices of Nile tilapia (*O. niloticus*) fed the experimental diets

	Control	Phytase	Protease	Carbohydrase
IBW (g $fish^{-1}$)	38.64±0.84	38.89±0.34	38.56±0.60	38.87±0.52
FBW (g $fish^{-1}$)	82.63±1.68[a]	94.87±3.28[b]	85.58±0.17[a]	89.36±5.72[ab]
SGR (% day^{-1})	2.11±0.1[a]	2.48±0.08[b]	2.21±0.05[a]	2.31±0.18[ab]
FCR	1.68±0.09[a]	1.36±0.05[c]	1.55±0.03[ab]	1.50±0.1[b]
PER	0.80±0.06[a]	1.08±0.06[c]	0.88±0.03[ab]	0.94±0.11[b]
HIS	1.65±0.09	1.50±0.04	1.68±0.28	1.73±0.19
VSI	11.47±0.28	10.07±0.96	10.54±0.75	10.24±0.98
K	1.97±0.09	1.93±0.03	2.02±0.18	1.94±0.17
Survival (%)	90±8.82[a]	100[b]	100[b]	97.78±1.92[ab]

Means in the same row with different superscripts are significantly different ($P < 0.05$). IBW, initial body weight; FBW, mean final weight; SGR, specific growth rate; FCR, feed conversion ratio; PER, protein efficient ratio; HSI, hepatosomatic index; VSI, viscera-somatic index; and K, condition factor

Table 3. Liver histology of Nile tilapia (*O. niloticus*) fed the experimental diets

	Control	Phytase	Protease	Carbohydrase
Liver nuclei (No/FV)	368±73[ab]	312±77[a]	433±93[b]	395±73[b]

Liver nuclei (No/FV) is the total number of liver nuclei (per field of view under 40x objective magnification)

FIGURE

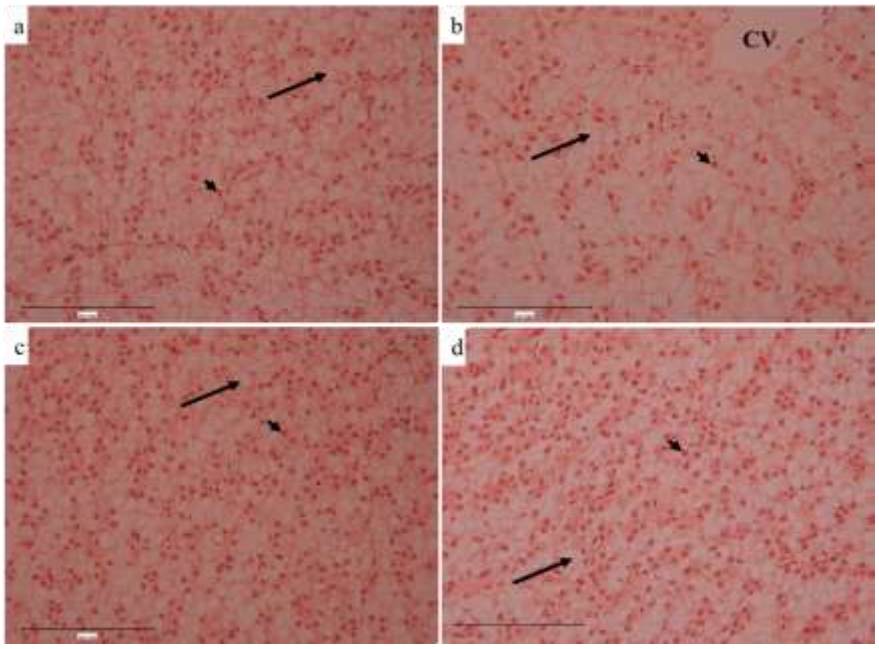

Figure 1. The liver structure of Nile tilapia (*O. niloticus*) fed control (a), phytase (b), protease (c) and carbohydrase (d) diets. The arrows indicate a hepatic cell and their nuclei (short arrow) showing progressive lipid vacuolisation (long arrow). CV is central venous. Stained with Haematoxylin and Eosin (Scale bar = 100 µm).