PERFORMANCE OF *Clarias gariepinus* FED NATIONAL INSTITUTE FOR FRESHWATER FISHERIES RESEARCH (NIFFR) FEED SUPPLEMENTED WITH NUTRASE XYLANASE

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ABSTRACT
Eight weeks feeding experiment was conducted to evaluate xylanase inclusion into un-extruded and extruded diets of Clarias gariepinus fingerlings in a 2 x 3 factorial design. Triplicated groups of 10 fingerlings with mean weight of 5.3 g ± 0.5 were used at National Institute for Freshwater Fisheries Research, New Bussa, Niger State, Nigeria. There were significant differences (P < 0.05) in final weight, specific growth rate and weight gain of the fish. The fish that fed on the diets produced by cold pelleting supplemented with either yeast or xylanase were not significantly different (P > 0.05) in growth, but were significantly lower than those on diet with enzyme produced by extrusion (P < 0.05). However the groups that fed on diets with xylanase had higher mean weight gain, 20.76 versus 22.39 g fish^{-1} for yeast and xylanase respectively. There was a reduction in cost per kg of feed with xylanase supplementation compared to yeast as an additive. It was observed that xylanase resulted in better performance which could be due to its effect on the B,1- 4 bonds to expose and reduce the polymers of nutrients that might have been bound up in the cell wall for the available endogenous enzyme. It was concluded that xylanase supplementation in diets of C. gariepinus results in significantly (P < 0.05) higher performance. It will be necessary to carry out further studies on this species.

Keywords: *Clarias gariepinus*, xylanase, performance, supplementation

INTRODUCTION
Feed is one of the major variable inputs in fish production and has been identified to contribute 60 – 70% cost of fish production (NRC, 2000). Foreign feed constitutes the largest quantity of feed used by fish farmers in Nigeria even as aquaculture develops in the country to meet the target of the MDG on aquaculture. The availability of good quality locally produced fish feed is just gaining ground in Nigeria despite the several researches on the various nutrient requirements of fish in the past several decades. The aquaculture farmers are with the perception that fish eat and grow better on floating feed and it is recently that, standard indigenous floating fish feed are found in the Nigeria market. For quite a long time the fish farmer had depended on foreign floating fish feed. To reduce over dependence on foreign feed was the objective for the development of the un-extruded NIFFR floating fish feed with optimum performance not significantly different from Coppens® foreign fish feed (Ibiyo *et al*., 2011). The un-extruded floating feed has a performance with FCR of 0.78 and 0.98 - 1.35 for fingerlings and grow out respectively.

There is possibility of enhancing the utilization and reduction in cost of the feed with the use of an exogenous enzyme. Enzymes are catalysts that lowers amount of activation energy and speed up a reaction. The earlier constraint to the use of exogenous enzymes in feeds is that enzymes are generally heat-labile. However, recently, thermostable microbial enzymes have been developed that can withstand the effects of high temperature during feed processing (Officer, 2000). Inclusion of exogenous enzymes as additives in plant-based feeds has greatly improved feed utilization in terrestrial animals (Bedford, 1995; Castanon *et al.*, 1997; Bedford, 2000) due to its effect in overcoming the antinutritional effect of non-starch polysaccharides (NSPs) associated with plant cell wall (Crawford *et al*., 2005).

Commercial enzymes typically contain endoglucanase and endoxylanase which hydrolyze the β-1,4-bonds in cellulose and xylan polymers respectively thereby reducing the size of soluble NSPs and subsequently viscosity within the digestive tract (Crawford *et al*., 2005). In aquaculture, addition of NSP-degrading enzymes to canola meal diets has been shown to improve growth rates of juvenile Tiger prawns (Buchanan *et al.*, 1997). These studies indicated that endoxylanase enzymes could play a significant role in the utilization of plant materials in crustacean diets (Crawford *et al*., 2005) which might also be possible in *Clarias gariepinus.* One of the benefits from increasing efficiency with which

nutrients are obtained from a feed is the reduction in faecal nutrient level (Officer, 2000). Information on enzyme use in *C. gariepinus* is scarce. This study was designed to investigate the performance of *C. gariepinus* fed NIFFR floating feeds supplemented with xylanase with the objectives of further improvement in the aroma, utilization and cost effective fish feed for the production of fast growing *Clarias gariepinus*.

MATERIALS AND METHODS

A 2 x 3 factorial design with three replicates groups of 10 fingerlings per experimental tank (aquarium) was used to study the response of *Clarias gariepinus* to the recommended levels of xylanase enzyme (10g/100Kg diet) included in NIFFR floating feed (Table I). The fish were subjected to the experimental treatments after two weeks acclimatization.

Two forms of NIFFR floating feeds were used as controls (unextruded and extruded). The yeast in the feed was replaced with manufacturer's recommended level (10 g xylanase/100 kg feed) of Xylanase enzyme pelleted and extruded. The third group of treatment was formulated with reduction in the fishmeal level by 15% with increase in plant protein sources and also supplemented with enzyme. The fish were fed 5% body weight divided into two and given twice daily (morning and evening; 8:00 – 9:00 am and 6:00 – 7:00 pm). The morning feeding was carried out after siphoning the waste materials from the aquaria with fresh water added to reduce pollution.

Fortnight sampling was adopted and subsequent feed adjustment with new weight. Water quality was also monitored by the limnology unit of NIFFR. Digestibility trial was carried out at the sixth week into the experiment with the use of chromic-oxide as a marker. Faecal samples were collected for three days from the fish through hand press after weighed quantities of feed were fed and allowed to digest. At the end of eight weeks final sampling was carried out. Four fish were randomly selected from each replicate for blood samples, whole body proximate composition and hepatosomatic index. Chemical analysis on proximate composition of fish, feed and faecal samples were in accordance to AOAC (2000).

Haematological parameters were measured following standard methods (Joshi *et al*., 2002b and 2002c) for packed cell volume (haematocrit method) and haemoglobin (Hb) concentration (cyanmethaemoglobin method).

Feed cost was calculated based on the prevailing price of ingredients at the time of experimentation.

Data obtained were subjected to Multivariate analysis using SPSS version 15 for windows with the principle of Steel and Torie (1980) and where significant differences were identified, means were separated with Duncan Multiple Range Test by setting type -1 error at 5% level of significance (Duncan, 1955). Bar charts of means with standard deviation were plotted.

Table I: Composition of the basal and experimental diets

Ingredients	% Inclusion level in Diets (Treatments)					
	1 Unextruded	2 Extruded	3 Unextruded	4 Extruded	5 Extruded	6 Extruded
Baker's Yeast	2.00	2.00	0.00	0.00	0.00	0.00
Xylanase	0.00	0.00	0.01	0.01	0.01	0.01
Silica	0.00	0.00	1.99	1.99	1.99	1.99
Clupeid Fishmeal	40.00	40.00	40.00	40.00	25.00	25.00
Other feedstuffs and Premix*	58.00	58.00	58.00	58.00	73.00	73.00
Total	100.00	100.00	100.00	100.00	100.00	100.00

***** Provides per kg diet: Vitamin A, 125000 IU, Vitamin D_3 2500 IU, Vitamin E 40mg, Vitamin K 2mg; Vitamin B_1 3mg; Vitamin B_2 5.5mg; Choline chloride 500mg; Niacin 35mg; Vitamin B_6 5mg; Vitamin B_{12} 0.025mg; Folic acid 1mg; Biotin 0.08mg; Maganese 120mg; Iron 100mg; Zinc 80mg; Iodine 1.8 mg; Calcium pantothenate 11.5mg; Copper 8.5mg; Cobalt 0.3 mg; Selenium 0.12 mg; vitamin C 2000mg, Antioxidant 120 mg.

RESULTS

There were significant differences in final weight, specific growth rate and weight gain of the fish ($P < 0.05$; Fig. 1). The fish that fed on the diets produced by cold pelleting supplemented with either yeast or enzyme were not significantly different ($P > 0.05$) but fish that fed on cold pelleted diets were significantly lower than those on diet with enzyme produced by extrusion ($P < 0.05$). However the xylanase group had higher values in those parameters irrespective of production method though not significantly different. The group that fed on diet with enzyme inclusion and produced by extrusion consumed significantly higher quantity of feed ($P < 0.05$) compared to the other groups. The group that fed on the diet with 15% reduction in fish meal by increasing plant protein sources supplemented with enzyme and extruded was not significantly different from the other groups ($P > 0.5$).

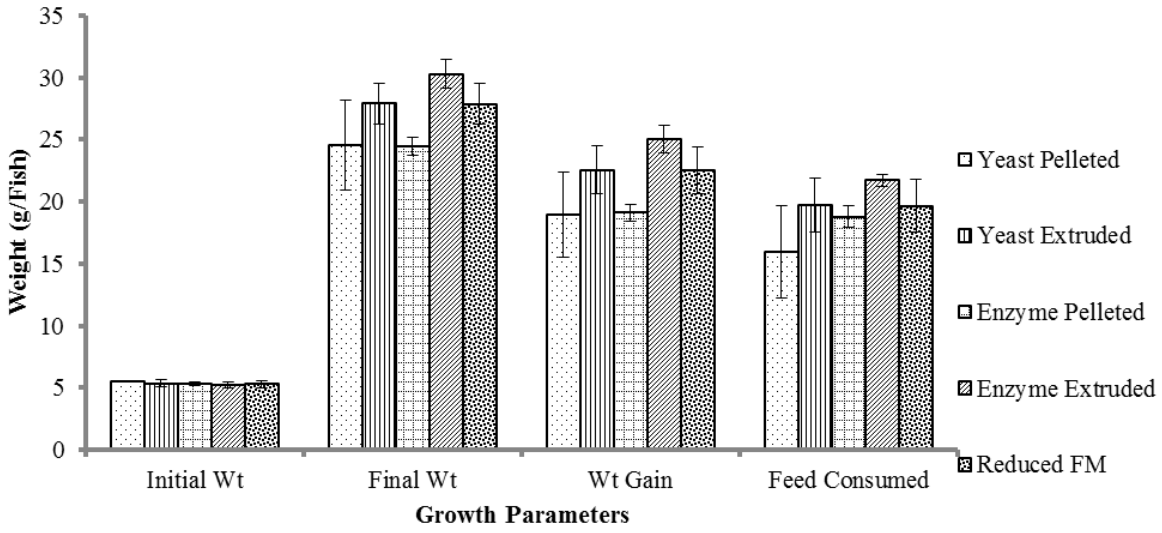

Fig. 1: Growth performance of fish fed diets with bakers yeast compared with enzyme inclusions

The digestibility of the diets, survival of the fish, hepatosomatic index (HI) and the haematological parameters were not significantly different ($P > 0.05$) (Figure 2). The HI ranged from 0.2 - 0.31 while haemoglobin (Hb) ranged from 9.53 - 10.5 mg/dl. The results of water quality parameters assessed ranged as follows; Temperature (28 - 30.2 ^{o}C), pH (6.5 - 7.3 units), dissolved oxygen (5.8 - 7.0 mg/l) and conductivity (300 - 480). The conductivity was noted to be high in the course of experimentation but was always reduced by the addition of fresh water in the aquaria. Most of the parameters were within the range for good performance of freshwater fish (Boyd, 1990).

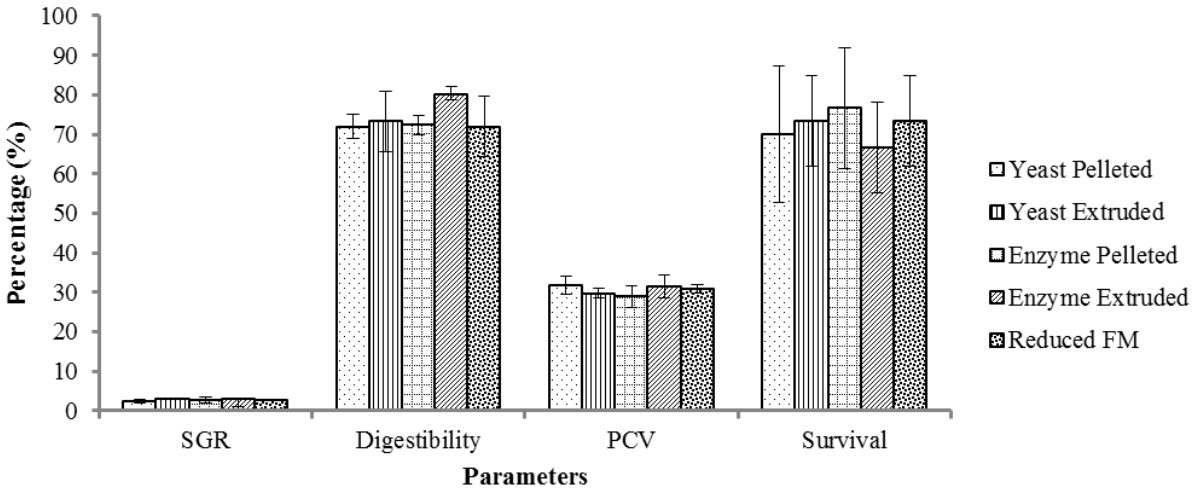

Fig. 2: Specific Growth Rate, Digestibility, PCV and Survival of fish fed diets with yeast or xylanase inclusion

The feed cost analysis indicated that a kg of the diets amounted to ₦261.05, ₦261.05, ₦250.59, ₦250.59 and ₦230.00 for 1, 2, 3, 4, and 6 respectively based on prevailing prices of ingredients at the time of experimentation. Group 5 was excluded from the report due to human factor in the course of experimentation to avoid bias. The use of enzyme led to a 4% decrease in price per kg feed while the interaction of the enzyme with increase in plant protein sources to reduce the quantity of fish meal resulted to 11% decrease in cost per kg feed.

DISCUSSION

The significant differences observed in final weight, specific growth rate and weight gain of the fish might be attributable to extrusion process as the fish that fed on the diets produced by cold pelleting supplemented with either yeast or enzyme were not significantly different but were significantly lower than those on diet with enzyme produced by extrusion (Deguara, 1997). However, the xylanase group had higher values in those parameters even in the groups not significantly different. The group that fed on diet with enzyme inclusion and produced by extrusion consumed significantly higher quantity of feed compared to the other groups. This might be attributed to better gelatinization of the feed that occurred in extrusion process and possible increase in palatability of the diets. Surprisingly the group that fed on the diet with 15% reduction in fish meal by increasing plant protein sources supplemented with enzyme and extruded performed well as those on diet 4. The xylanase must have effectively exposed and reduced the polymers of nutrients that might have been bound up in the cell wall to the available endogenous enzyme with improved nutritive value (Bedford, 2000; Francis *et al.*, 2001; Watanabe 2002). This is likely due to its effect on the β,1-4 bonds. There by enabling the fish access to as much nutrients as those on higher level of fish meals. There was also an indication that another way of improving the efficiency of feed utilisation other than ingredient quality is manufacturing process. While extrusion is more expensive as a technology, the advantages brought about by extrusion with regard to feed efficiency and reduced pollution seems to have given the process an edge over pressing/cold pelleting (Deguara, 1997). This was confirmed from the higher values obtained from the groups that fed on the extruded diets in either of the treatments in this study.

The observed digestibility of the diets, survival of the fish, hepathosomatic index (HI) and the haematology which were not significantly different are indications of equal performance with respect to these parameters. The range of 0.2 - 0.31 and 9.53 - 10.5 mg/dl for HI and haemoglobin (Hb) respectively were similar to the observation of Deguara (1997) when processing methods were studied. Though digestibility and survival showed no significant deference, better values were obtained in the groups that had xylanase supplementation (Buchanan *et al.*, 1997). Most of the water quality parameters were within the range for good performance of freshwater fish (Boyd, 1990). The conductivity which was noted to be high in the course of experimentation was always reduced by the addition of fresh water in the aquaria.

The feed cost analysis which indicated that a kg of the diets amounted to ₦261.05, ₦261.05, ₦250.59, ₦250.59 and ₦230.00 for 1, 2, 3, 4, and 6 respectively provides opportunity to identify the cost effectiveness of the diets. The use of enzyme led to a 4% decrease in price per kg feed while the interaction of the enzyme with increase in plant protein sources to reduce the quantity of fish meal resulted to 11% decrease in cost per kg feed. This reduction in cost of feed production is very important in profit oriented fish farming venture. From the feed consumed and weight gained, extrusion as a processing method seems to be beneficial to the production process (Deguara, 1997). Fish consumes more and had better gain which resulted to more fish output with better profit from the group that fed on the diets extruded using the same feedstuffs. It was also observed that the use of the enzyme had no effect on the fishy aroma of the feed which was encouraging as opposed to baker's yeast. Based on the results obtained from this study it can be recommended that xylanase enzyme inclusion is necessary to reduce NIFFR feed production cost due to yeast additive inclusion, improvement in utilization by the fish and reduce pollution due to waste. There is need to also study other enzymes and their effect on this species.

CONCLUSION

It could be concluded from the improved performance and reduction of cost per kg feed obtained from this study that xylanase enzyme inclusion in *Clarias gariepinus* feeds might be beneficial in its production. It is advisable that the manufacturer's recommended level (100g/tonne i.e 0.1g/Kg of feed) is adhered to for cost benefit actualization. Also, xylanase was useful in situation of high plant protein use in fish feed to reduce high use of fish meal and famers could exploit the additive.

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