Journal of Animal and Veterinary Advances 9 (2): 327-331, 2010
ISSN: 1680-5593

Effects of Exogenous Enzyme Supplementation in Diets on Growth and Feed Utilization in African Catfish, *Clarias gariepinus*

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Abstract: The effects of various supplemental exogenous enzyme on the growth performance in African catfish *Clarias gariepinus* (initial mean weight 46.32±0.29 g) were examined for 12 weeks feeding trial. A multi enzyme complex (containing fungal xylanase, β-glucanase, pentosonase, β-amilase, fungal β-glucanase, hemicellulase, pectinase, cellulase, cellubiase), was included at the level of 0.0 (control group), 0.25, 0.5 and 0.75 g enzyme complex kg^{-1} diet in four test diets. Each of the four experimental diets was randomly assigned to triplicate groups of fish and accidentally stocked into 100 L aquaria at a density of 10 fish per aquarium. After 90 days of rearing, the survival rate was ranged from 86.67-93.33% and there was no statistical difference between experimental and control groups. Growth rate significantly increased in fish fed with enzyme complex supplemented diets in comparison with the control groups ($p<0.001$). Specific growth rate was ranged from 1.09±0.03 (control group) to 1.23±0.01 (0.75 g kg^{-1} enzyme complex supplemented group). The best specific growth rate was observed at the group receiving 0.75 g kg^{-1} enzyme complex group. Also, food conversion ratio, protein efficiency ratio and apparent net protein utilization were significantly higher in all enzyme complex groups than that with control ($p<0.01$). The highest value of protein content (21.75%) was observed at 0.75 g kg^{-1} enzyme complex group. The results suggested that enzyme supplementation can significantly improve growth performance and feed utilization in African catfish.

Key words: African catfish, *Clarias gariepinus*, exogenous enzyme complex, growth performance, control group

INTRODUCTION

Commercial fish feeds usually contain high fish meal as the major protein source, ranging from 30-50% (Hardy, 1995). But now-a-days, fish meal is generally avoided in the feed due to its scarcity and high cost. This is of particular concern to tropical countries, which are dependent on fish meal imports such as the African continent and Southeast Asia with fish farming operations based on carp, tilapia and catfish species, which contribute high value protein for human consumption in these regions (Goda *et al*., 2007; Davies and Gouveia, 2008). Hence, aquaculture nutrition have been trying to improve the nutritional value of fish feed by enzyme supplementation, to find suitable alternatives to fish meal, since last decades. Exogenous enzymes are now extensively used throughout the world as additives in animal diets. The digestibility of all nutrients, however, including protein, carbohydrates and minerals, seems to be affected (Forster *et al*., 1999; Yavuz, 2001; Felix and Selvaraj, 2004). Also, supplementation with enzymes can help to eliminate the effects of antinutritional factors and improve the utilization of dietary energy and amino acids, resulting in improved performance of fish (Farhangi and Carter, 2007; Lin *et al*., 2007; Soltan, 2009).

The effects of enzyme supplementation on growth and survival of several cultured fish species have been demonstrated by several researchers; channel catfish (Jackson *et al*., 1996), *Pangasius pangasius* (Debnath *et al*., 2005), *Clarias batrachus* x *Clarias gariepinus* (Giri *et al*., 2003), tilapia (Drew *et al*., 2005) and salmon (Refstie *et al*., 1999) but, there are few published reports on the effect of adding enzyme to African catfish diets on feed utilization, growth performance or the secretion of endogenous enzymes (Ng *et al*., 1998; Van Weerd *et al*., 1999; Ng and Chen, 2002).

African catfish (*Clarias gariepinus*) is a major species for aquaculture in Africa and has also been introduced in Europe and Southern Asia such as Israel, Syria and South of Turkey. It is cultured for its omnivorous feeding habit, high growth rate and its resistance to handling and stress (Bureau and De la Noue, 1995; De Graaf and Janssen, 1996; Fagbenro and Davies, 2001; Turan and Akyurt, 2005).

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The objective of this study was to investigate the effect of a commercially prepared exogenous multi enzyme complex on growth, feed utilization, survival and carcass characteristics in African catfish, *Clarias gariepinus*.

MATERIALS AND METHODS

Experimental fish: Full-sibling fish (initial mean weight 46.32±0.29 g), produced in Mustafa Kemal University Fisheries Research Unit were randomly stocked into 100 L aquaria at a density of 10 fish per aquarium. The aquaria were equipped with aeration and supplied with continuously flowing water (2 L min^{-1}) and controlled temperature (25±1°C). The photoperiod was maintained on a 12 h light: 12 h dark schedule.

Experimental diets: African catfish were fed with trout diets (Aquamaks, Turkey: 48% protein, 18% lipid). Proximate composition of the experimental diets is determined by analysis (AOAC, 1990). Multi enzyme complex (Farmazyme® 2010, Farmavet, Turkey), containing fungal xylanase, β-glucanase, pentosonase, β-amilase, fungal β-glucanase, hemicellulase, pectinase, cellulase, cellubiase was used as a feed supplement.

The 0.0, 0.25, 0.5 and 0.75 g enzyme complex kg^{-1} diet was incorporated into the trout diet to formulate four experimental diets, respectively. In the preparation of experimental diet, multi enzyme complex were mixed with a pulverized trout diet in which water (450 mL kg^{-1}) were added and extruded through a food grinder with a 2 mm diameter die plate. Three different dosages of red clover (0.25, 0.5 and 0.75 g enzyme complex kg^{-1} diet) were used in the experiment. The control diet was also mixed with 450 mL water. These pelleted diets were dried in the open air for 24 h. The dry pellets were placed in covered plastic containers and stored in a refrigerator at 5°C (Lin *et al*., 2007).

Experimental procedure: Each of the four experimental diets was randomly assigned to triplicate groups of fish and all the groups were fed with their respective diet to ca. 3% body weight day twice daily for 90 days. Also, water parameters were recorded at every week. Besides, ammonia and pH were found out as 0.08±0.005 mg L^{-1} and 7.5±0.17, respectively. The level of dissolved oxygen was supplied 7.35±0.23 mg L^{-1} throughout the study.

Calculations and statistical analysis: During experiment, the mortality was recorded daily and fish in each aquarium were counted and weighed individually at bimonthly intervals after anesthetized for 2.5 min in water that contained 0.4 g L^{-1} Tricaine Methane Sulphonate (TMS) and 0.8 g L^{-1} sodium bicarbonate as a buffer. Growth were monitored to determine the growth in each treatment groups during the experiment. Each fish was individually weighed and measured (total length) to the nearest 0.01 g and 0.01 cm, respectively. Specific Growth Rate (SGR), percent of weight gain, Food Conversion Ratio (FCR), Protein Efficiency Ratio (PER), Apparent Net Protein Utilization (ANPU) and survival rate were calculated according to Castell and Tiews (1980) in this experiment.

At the start of experiment, 15 fish randomly were treated with an overdose of phenoxyethanol (1.5 mg L^{-1}) solution and stored at 20°C for the determination of body proximate composition. At the end of the feeding trial, 5 fish from each dose group (n = 20 fish/dose) were analyzed for final whole body proximate composition. Proximate composition of body was analyzed following AOAC (1990) methods. In the experiment, all data were assessed by one-way analysis of variance using SPSS 13.0 for Windows software ($p<0.05$). Significant differences among means were determined by the subsequent use of Duncan's multiple range ad-hoc test (Dytham, 1999).

RESULTS AND DISCUSSION

At the start of experiment, there was no statistical difference between experimental and control groups in initial weights meaning the conditions of the groups were the quite similar. Growth response of African catfish, *Clarias gariepinus* fed diets with graded levels of enzyme complex for 12 weeks are shown in Table 1.

After 90 days of rearing the survival rate was ranged from 86.67-93.33% and there was no statistical difference between experimental and control groups. Growth rate significantly increased in fish fed with enzyme complex-supplemented diets in comparison with the control groups ($p<0.001$). Among the enzyme complex-supplemented groups, the catfish fed diets with 0.5 and 0.75 g kg^{-1} enzyme complex exhibited significantly higher growth rate than those supplemented 0.25 g kg^{-1} enzyme complex (Table 1). These results indicated that the increase in the enzyme complex to some extent in diets enhance weight gains in African catfish, *Clarias gariepinus*.

Specific Growth Rate (SGR) was ranged from 1.09±0.03 (control group) to 1.23±0.01 (0.75 g kg^{-1} enzyme complex supplemented group). Analysis of variance of SGR between groups showed that 0.75 g kg^{-1} enzyme complex-supplemented group were significantly different from the control and 0.25 g kg^{-1} enzyme complex supplemented group ($p<0.01$). The best specific growth rate was observed at the group receiving 0.75 g kg^{-1} enzyme complex group (Table 1).

Table 1: The growth response of African catfish-fed diets with graded levels of multi enzyme for 12 weeks*

Parameters	Multi enzyme supplementation level (g kg^{-1}) 0	0.25	0.5	0.75
Weight gain (g)	77.28±1.29^a	80.01±1.76^b	90.37±0.74^c	90.58±0.61^c
SGR	1.09±0.03^a	1.15±0.02^b	1.20±0.01^{bc}	1.23±0.01^c
FCR	2.59±0.04^c	2.35±0.05^b	2.21±0.02^a	2.13±0.01^a
PER	0.81±0.01^a	0.88±0.02^b	0.94±0.01^c	0.97±0.01^c
ANPU (%)	23.17±0.11^a	26.53±0.71^b	29.90±0.97^c	30.53±0.76^c
Survival (%)	90.00±5.74^a	93.33±3.34^a	86.67±3.34^a	93.33±3.34^a

*Values (mean±SE of triplicate) with different superscripts in each line indicate significant differences (p<0.001) (p<0.01 for SGR), WG (Weight Gain) (g) = Final weight-Initial weight, SGRW (Specific Growth Rate Weight) (%) = [(lnW^2-lnW^1)\(T^2-T^1)] ×100, where W^1 and W^2 are mean body weight at times, when the first and second samples were taken (T^1 and T^2), FCR (Food Conversion Ratio) = Dry feed intake (g)/wet weight gain (g), PER (Protein Efficiency Ratio) = Live body weight gained (g)/protein intake (g), ANPU (Apparent Net Protein Utilization)(%) = [protein retained/unit of protein intake] ×100

Table 2: The carcass characteristics of whole-body African catfish-fed diets with graded levels of multi enzyme for 12 weeks*

Chemical composition (%)	Initial	Multi enzyme supplementation level (g kg^{-1}) 0	0.25	0.5	0.75
Moisture	75.70±0.55	73.71±0.35^a	74.80±0.42^a	74.60±0.40^a	73.67±0.34^a
Crude protein	17.05±0.21	18.27±0.23^a	19.84±0.32^a	21.74±0.74^b	21.75±0.57^b
Crude lipid	2.20±0.08	4.26±0.35^a	5.01±0.43^a	5.27±0.45^a	4.77±0.35^a
Ash	1.10±0.03	1.07±0.06^a	1.18±0.10^a	1.05±0.04^a	1.07±0.05^a

*Values (mean±SE of triplicate) with different superscripts in each line indicate significant differences (p<0.01), body composition data presented on a wet basis

Also, Food Conversion Ratio (FCR), Protein Efficiency Ratio (PER) and Apparent Net Protein Utilization (ANPU) were significantly higher in 0.25, 0.5 and 0.75 g kg^{-1} enzyme complex groups than that with control (p<0.01, Table 1). The best food conversion ratio and other growth parameters were observed at the group receiving 0.75 g kg^{-1} enzyme complex.

The effects of supplemental enzyme complex on carcass characteristics of the whole-body African catfish, *Clarias gariepinus* are given in Table 2.

Protein contents of the 0.5 and 0.75 g kg^{-1} enzyme complex-supplemented groups were significantly higher than protein content of the control and other enzyme complex group (p<0.01). The highest value of protein content (21.75%) was observed at 0.75 g kg^{-1} enzyme complex group (Table 2). No significant differences were shown in whole body moisture, lipid and ash among the dietary treatments (p>0.05).

In the present study, fish fed the control diet exhibited lower growth and FCRs than diets supplemented enzymes, indicating that enzyme is beneficial for the growth of African catfish. Growth response and feed utilization were improved with enzyme supplementation. This is the first report to the knowledge regarding the use of Farmazyme® in catfish culture. Research in to the use of Farmazyme® had generally concentrated on the direct application of this exogenous enzyme into plant-based poultry and pig feeds. Depending on the feed ingredients that were used, significant improvements in growth, feed utilization efficiency and nutrient availability have been reported (Kitchen, 1997; Schang *et al*., 1997). Boonyaratpalin *et al*. (2000) reported that nile tilapia, *Oreochromis niloticus* L. fed diets with palm kernel meal (pretreated with a feed enzyme, Ronozyme VP9) showed significantly higher weight gain and ANPU than fish fed a similar level of untreated palm kernel meal in their diets.

The results of the current study showed that optimum growth performance and feed efficiency were obtained at 0.75 g kg^{-1} enzyme complex-supplemented diet. This result is in agreement with some previous studies on channel catfish (Jackson *et al*., 1996), *Pangasius pangasius* (Debnath *et al*., 2005), *Clarias batrachus* x *Clarias gariepinus* (Giri *et al*., 2003), *Oreochromis niloticus* x *O. aureus* (Lin *et al*., 2007) and rainbow trout (Farhangi and Carter, 2007). A significant body weight improvement was also reported in turkeys and poultry by Leeson *et al*. (1996) and Ghazi *et al*. (2003) in diet supplemented with exogenous enzymes (xylanase).

Dietary enzymes can be used to supplement the fish and broiler's own enzyme production including amylases to improve starch digestibility, proteases to improve protein digestibility and lipases to improve lipid digestibility (Lin *et al*., 2007; Zhou *et al*., 2009). A similar trend has been demonstrated by this study, which is supported by some other studies (Ng *et al*., 1998; Van Weerd *et al*., 1999; Ng and Chen, 2002). These results indicated that the exogenous enzyme supplementation can promote the secretion of endogenous enzymes.

Recent reserach with broilers and fish suggest that not only are the types of enzymes critical but also their concentration to the size and direction (positive or negative) of an animal response to supplementation (Bedford and Inborr, 1993; Lin *et al*., 2007). This suggests that enzyme concentration plays an important role in diets.

CONCLUSION

The addition of multi enzyme complex (Farmazyme) resulted in significant improvements in body weight gain and feed efficiency in catfish. Other possible effects of the enzyme supplement, such as an action on intestinal microbial flora or an improvement in growth by the release of a growth-enhancing factor, cannot be disregarded. Further investigations can be carried out to focus on the substrates themselves in combination with management practices, which may also affect the results. Much more research is necessary before exogenous enzymes should be made available for commercial fish culture.

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