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EFFECT OF EXOGENOUS ENZYMES SUPPLEMENTATION WITH DIFFERENT LEVELS OF FIBER IN DIETS ON GROWTH PERFORMANCE, FEED UTILIZATION AND HEALTH OF AFRICAN CATFISH *Clarias gariepinus*

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SUMMARY

The effect of various exogenous enzymes on growth performance in African catfish *Clarias gariepinus* (initial mean weight 80 g) were examined in feeding trial for 55 days. A multi enzyme complex (xylanase, amylase and protease) were used with two levels of fiber in the diet (5 and 9%), Eight isonitrogenous and isoenergetic experimental diets were formulated to contain 5, 9 % fiber without multi enzymes (T_1 and T_2) and 5, 9% fiber with 2, 4, 6 % of the diets fiber multi enzymes. Each of the eight dietary treatments was fed to triplicate groups of *Clarias gariepinus* raised in tanks (1×1.5×1 m) and each tank was stocked with 20 fish. *Clarias gariepinus* fed the diets supplemented with enzymes had significantly higher growth and feed intake compared to control groups ($P < 0.05$). Protein efficiency ratio, feed conversion ratio and protein productive value were significantly higher in all enzyme complex groups ($P < 0.05$). Exogenous enzymes significantly affected the whole fish body components ($P < 0.05$), the highest quantity of fat contents was observed in treatments contained exogenous enzyme and high content of fiber (9%) in contrast to T_1. The blood parameters were also affected, as liver enzymes were decreased while total protein and white blood cells were increased in treatments contained exogenous enzymes and low fiber. In conclusion, the results suggested that enzyme supplementation can significantly improve growth performance, feed utilization and health in African catfish *Clarias gariepinus.* The group fed 4% multi enzymes complex with low fibers content (5%) showed the best results.

Keywords: *African catfish, exogenous enzymes, dietary fiber, growth performance, feed utilization and blood parameters.*

INTRODUCTION

In Egypt, as a source of meat, both marine and fresh water fish are of great importance. Of the 32 valid species of the genus Clarias, Clarias gariepinus is the most important species for aquaculture recognized by Teugels (1984). It is probably the most widely distributed fish in Africa (Adham, 2002). It is a carnivorous feeder and a general scavenger with a distinct propensity to graz on detritus and benthic species (Bok and Jongbloed, 1984). The *Clarias gariepinus* catfish of Africa are freshwater fish. As they grow rapidly, they are of great importance; they reach a large size, and edible fish have few spines in their flesh. They can withstand a wide range of environmental conditions, including extreme and low oxygen temperatures. The ability of carnivorous fish to use fibrous structures as an energy source is limited. In their diets, carnivorous fish need high amounts of protein at a time when digesting plant feed stuff that increases the cost of nutrition is an issue. This problem can be solved by complementary enzymes. Feed is one of the major variable costs associated with feed culture systems; reducing feed costs will generate substantial savings (Sookying and Davis, 2013). Due to increased demand, supply restrictions, and increasing social and environmental issues about wild fish extraction practices, fish meal costs have increased over time (Tacon and Metian, 2008). Various plant based products containing high protein content are possible substitute sources of fish meal to grow safe and environmentally friendly aquaculture (NRC, 2011).

Protein sources dependent on plants, such as soybean meal, canola meal, corn gluten meal, are globally available and have a relatively low cost compared to fish meal. Most plant based feedstuffs, such as soybean meal, rapeseed meal, maize gluten meal or wheat gluten, have a broad range of antinutritional factors, such as phytin, non-starch polysaccharides (NSP) and protease inhibitors, which can hinder the use of nutrients, fish efficiency, and digestive tract enzyme activities (NRC 2011; Castillo and Gatlin

2015). However, its use in fish diets has been restricted to a comparatively high amount of fiber, which would slow digestion and nutrient absorption because fish are unable to excrete cellulase and are unable to effectively use complex fibrous carbohydrates as an energy source (Lindsay and Haris, 1980). It has been demonstrated that exogenous enzymes increase the nutritional value of feed and reduce environmental emissions in terrestrial animals (Gdala *et al.,* 1997). Recently, dietary supplementation with exogenous enzymes has also been implemented to improve feed consumption and increased fish water contamination (Dawood *et al.,* 2019). There are different forms of enzymes, including phytase, xylanase, cellulase, lipase, protease, amylase, and many more, which can increase the availability of nutrients, increase the rate of fish growth during digestion, and help fish live in the early stages of life. In reality, it makes the goods more cost effective. In addition, the application of carbohydrases in aquaculture feeding is still relatively new compared to the poultry and swine industry; it is likely that carbohydrases can increase the use of nitrogen and amino acids by increasing access to protein for digestive proteases (Tahir *et al.*, 2008).

The purpose of the present study was to evaluate the effects of various supplemental exogenous enzymes (xylanase, amylase and protease) with two levels of diet fiber content on the growth performance and health in African catfish *Clarias gariepinus*.

MATERIALS AND METHODS

Experimental diets:

Experiments were conducted in this study using a combination of xylanase, amylase and protease as exogenous enzymes in experimental diets. eight experimental diets, including (T_1,T2) control groups, fed on the basal diet only (5% and 9% fiber without exogenous enzymes, respectively); (T_3) contain 5% fiber and 2% exogenous enzymes; (T_4) contain 5% fiber and 4% exogenous enzymes; (T_5) contain 5% fiber and 6% exogenous enzymes; (T_6) contain 9% fiber and 2% exogenous enzymes; (T_7) contain 9% fiber and 4% exogenous enzymes; (T_8) contain 9% fiber and 6% exogenous enzymes where the aim was to raise the sources of plant feeds as well as high fiber. All experimental diets were formulated to have equal protein levels (32% protein) and isocaloric (4.65 Kcal/g). Mixture of enzymes was purchased from Multi-vita Co., Giza, Egypt.

Ingredients were ground into fine powder, all the ingredients were thoroughly mixed with fish oil, Mix enzymes and other additives and warm water was added to produce stiff dough and dried for about 48 hr. in laboratory. After drying, the diets were broken up and sieved into proper pellet size (1.5 × 3.0 mm and were stored until used. Each diet was assigned to triplicate groups of fish in tank.

Experimental procedures:

Experimental fish were obtained from a commercial farm in Fayoum governorate (Beside Qarun Lake). Prior to the start of the experiment, Young cat fish (*Clarias gariepinus*) were reared in tanks (1×1.5×1m), and fed a commercial diet for 2 weeks to acclimate to the experimental conditions. Then fish were fasted for 24 hr. and weighed. Fish of similar sizes (80g) were randomly distributed into 24 tanks and each tank was stocked with 20 fish, fish were hand fed at a rate of 3% of total biomass (9:30 and 1:30 h) daily. A sample of 10 fish at the initiation of the feeding experiment and 5 fish per tank at termination were collected and stored frozen (−20 °C) for determination of proximate carcass composition. Proximate composition analysis of feed ingredients, experimental diets (Table 1) and fish body were performed according to AOAC (1995).

Analysis of water quality parameters:

Dissolved oxygen (DO), temperature, and pH were measured daily (Table 2) by an oxygen meter for dissolved oxygen, The temperature is measured by an ordinary thermometer, pH was determined using a pH meter (Digital Mini pH Meter, Model 55, and Water samples were taken to measure total ammonia-nitrogen (TAN) every week. During the experiment period DO, temperature, pH, TAN, were maintained within acceptable ranges (Boyd and Tucker 1998).

Table (1): Formulation (g/kg) and chemical composition of experimental diets (% on DM).

Ingredient	Fiber level							
	5% of the diet				9% of the diet			
	Exogenous enzymes (% of dietary fiber)				Exogenous enzymes (% of dietary fiber)			
	0	2	4	6	0	2	4	6
Yellow corn	326	325	324	323	102	100.2	98.4	96.6
Wheat bran	200	200	200	200	133	133	133	133
Rice bran	0	0	0	0	215	215	215	215
Cotton seed meal	177	177	177	177	350	350	350	350
Fish meal	252	252	252	252	155	155	155	155
Dried garlic	5	5	5	5	5	5	5	5
Salt	1	1	1	1	1	1	1	1
Vit & min premix[1]	4	4	4	4	4	4	4	4
Dicalcium phosphate	5	5	5	5	5	5	5	5
Fish oil	10	10	10	10	10	10	10	10
Molas(sugar beet)	10	10	10	10	10	10	10	10
Enzymes mixture	0	1	2	3	0	1.8	3.6	5.4
Starch	10	10	10	10	10	10	10	10
Total	1000	1000	1000	1000	1000	1000	1000	100
Proximate analysis (%):								
Dry matter (DM)	88.12	88.03	87.94	87.85	88.21	88.05	87.89	87.73
Crude protein (CP)	31.98	31.99	32	32.02	32.01	32	31.98	32.1
Ether extract (EE)	4.74	4.74	4.73	4.73	5.53	5.53	5.52	5.51
Crude fiber (CF)	5.21	5.28	5.28	5.27	9.02	9.02	9.01	9.01
Ash	5.47	5.47	5.46	5.45	2.81	2.8	2.79	2.78
NFE[2]	52.53	52.53	52.53	52.52	50.62	50.64	50.62	50.59
Methionine	0.756	0.755	0.755	0.754	0.634	0.635	0.632	0.634
Lysine	1.941	1.941	1.941	1.943	1.743	1.743	1.742	1.741
Gross energy (Kj/g)[3]	11.25	11.23	11.20	11.21	11.18	11.25	11.27	11.28

1: Each kg contains: 400000 IU vit. D3, 2000000 IU vit. A, 300 mg vit K3, 4000 mg vit. E, 200 mg vit B1 , 800 mg vit B2, 2.0 mg B12, 4000 mg nicotinic acid, 2000 mg pantothenic acid, 300 mg vit. B6, 200 mg folic acid, 10mg biotin , 100 mg choline
chloride, 1600 mg Cu, 156 mg I, 12800 mg Mn, 9000 mg Zn, 32 mg Se and 53 mg cobalt
2: Nitrogen free extract (NFE %) = 100 – Crude protein – Crude lipid – Crude fiber – Crude ash.
3: Energy values were 5.64, 9.44 & 4.11 k cal/g for protein, lipid and carbohydrates respectively, ***NRC (2011)****.*

Table (2): Water quality parameters during 55 days of rearing period.

Treatment	Parameter			
	Dissolved oxygen(mg/l)	Temperature (Cº)	pH	Total ammonia (mg/l)
T_1	6.78	25.53	7.25	0.033
T_2	6.88	25.38	7.25	0.035
T_3	6.90	26.2	7.34	0.034
T_4	6.91	2570	7.3	0.033
T5	6.87	25.75	7.22	0.034
T6	6.83	25.81	7.31	0.041
T7	6.84	25.61	7.30	0.040
T_8	6.79	25.80	7.30	0.039

Blood sampling:

Five fishes from each treatment were randomly selected for the collection of blood at the end of the experiment and collected from the caudal vein without anticoagulant by special syringe in small plastic vials then transferred to Wassermann tubes. Blood was allowed to centrifuge at 3500 rpm for 20 minute to obtain serum **(Mehrim *et al*., 2014)**. The serum samples were pipetted into Eppendorf tube, labeled and stored in deep freeze at -20º C till assayed. Blood biochemical parameters were aspartate aminotransferase (AST), alanine aminotransferase (ALT), total protein (Tp), albumen (Alb), globulin (Glob) and white blood cells (WBS).

Growth and feed utilization parameters:

Parameters of growth performance and feed utilization were calculated as follows:

Weight gain (g) = final weight (g) - initial weight (g)

$$\text{Specific growth rate (SGR)} = \frac{\text{lin(final weight)} - \text{ln(initial weight)}}{\text{Rearing period (Days)}} \times 100$$

$$\text{Survival (\%)} = \frac{\textit{final count}}{\textit{initial count}} \times 100$$

$$\text{Feed conversion ratio} = \frac{\textit{feed intake (g)}}{\textit{weight gain (g)}}$$

Daily gain (DG), g = TG (g)/ Experimental period (day). Protein efficiency ratio (PER) = Weight gain, g / Protein intake, g. Protein productive value (PPV) % = 100 (Retained protein, g / Protein intake, g).

Statistical analysis:

All collected data were analyzed according to PROC GLM (**SAS, 2011**) by the following model:

$$Y_{ijk} = \mu + F_i + E_j + (FE)_{ij} + e_{ijk}$$

Where: Y_{ijk}: is the observation for a trait µ: is the overall mean, F: the effect of i^{th} dietary fiber, E: the effect of j^{th} exogenous enzymes level, $(FE)_{ij}$: the effect of i^{th} dietary fiber with the j^{th} exogenous enzymes level and eijk: is the random error term. Duncan's multiple range tests was used for the multiple comparisons (Duncan, 1955).

RESULTS AND DISCUSSIONS

Growth performance:

Data on growth performance of African catfish (*Clarias gariepinus)* fed the experimental diets are presented in Table (3).

Growth performance parameters(TG, ADG and SGR) were affected negatively (P<0.05) by increasing fiber in the diet, there were no significant differences in survival rate as a result of fiber levels (5 or 9% of the diet), and all growth parameters were improved significantly with increasing exogenous enzymes mixture (xylanase, amylase and protease) levels compared with basal diets (T_1, T_2), WG, TG, ADG and SGR were increased significantly with increasing dietary enzymes level compared with zero enzymes, the best was a 6% mixture of enzymes. Also, there were no significant differences between zero enzymes and 2% mixture enzymes for survival rate.

With regard to the interaction between levels of enzyme and levels of fiber in the diet the results are as follows; the best growth parameters such as WG, TG, ADG and SGR were recorded with T_4 followed by T_5 and T_8 and the lowest was T_1, T_2, while no significant differences were obtained between T_4, T_5, T_2, T_6 and T_8 for survival rate.

Feed utilization:

Results of Table (4) clearly showed that 9% fiber in the diet decreased (P<0.05) feed consumption compared with 5% fiber and in the same time the better (P<0.05) FCR was obtained with the level 5% fiber. Moreover 5% fiber improved (P<0.05) the protein utilization parameters (PER and PPV). Adding enzymes mixture increased (P<0.05) feed consumption and improved the FCR, PER and PPV. The best FCR, PER and PPV were obtained by the 5% enzymes level while the worest ones were recorded for the zero % enzymes level.

The interaction between fiber levels and enzymes showed that the highest feed consumption was obtained for T_7 (9% fiber, 4% enzymes) whereas the lowest one was recorded for T_2 (9% fiber, zero% enzymes).

The best FCR was found with T_4 (5% fiber, 4% enzymes) while the worst was recorded for T_1 (5% fiber, zero % enzymes).

Table (3): Effect of treatments on growth responses of *Clarias gariepinus*.

Treatment	Initial weight, g	Final weight, g	TG, g	ADG, g	S. Rate	SGR
Fiber (F)	**	**	**	**	N.S	**
F_5	80.42	159.25[a]	78.79[a]	1.43[a]	97.5[a]	0.54[a]
F_9	79.78	154.21[b]	74.43[b]	1.35[b]	97.9[a]	0.52[b]
Enzymes (E)	**	**	**	**	**	**
Zero (E_0)	81.14	147.20[d]	66.08[d]	1.21[d]	97.5[b]	0.47[d]
2 (E_1)	79.68	156.50[c]	76.84[c]	1.39[c]	97.5[b]	0.53[c]
4 (E_2)	80.03	160.90[b]	80.77[b]	1.46[b]	95.8[c]	0.55[b]
6 (E_3)	79.55	162.30[a]	82.75[a]	1.50[a]	100.0[a]	0.56[a]
F×E	**	**	**	**	**	**
T_1	82.50	147.20[f]	64.75[g]	1.17[f]	95.0[b]	0.46[g]
T_3	79.69	160.30[c]	80.71[c]	1.46[b]	95.0[b]	0.55[c]
T_4	80.07	165.80[a]	85.55[a]	1.55[a]	100.0[a]	0.57[a]
T_5	79.43	163.70[b]	84.17[b]	1.54[a]	100.0[a]	0.57[a]
T_2	79.78	147.20[f]	67.40[f]	1.24[e]	100.0[a]	0.48[f]
T_6	79.67	152.60[e]	72.98[e]	1.32[d]	100.0[a]	0.51[e]
T_7	80.00	156.00[d]	76.00[d]	1.36[c]	91.7[c]	0.52[d]
T_8	79.67	161.00[c]	81.33[c]	1.47[b]	100.0[a]	0.56[c]

T_1=(5% fiber of the diet, zero exogenous enzymes), T_3=(5% fiber of the diet, 2% of fiber exogenous enzymes), T_4 =(5% fiber of the diet, 4% of fiber exogenous enzymes), T_5 =(5% fiber of the diet, 6% of fiber exogenous enzymes), T_2=(9% fiber of the diet, zero exogenous enzymes), T_6=(9% fiber of the diet, 2% of fiber exogenous enzymes), T_7=(9% fiber of the diet, 4% of fiber exogenous enzymes) and T_8=(9% fiber of the diet, 6% of fiber exogenous enzymes).

Average in the same row having different superscripts differ significantly $P \leq 0.05$.

Table (4): Effect of treatments on feed utilization efficiency of *Clarias gariepinus*.

Treatment	Feed intake	FCR	PER	PPV
Fiber (F)	**	**	**	**
F_5	155.19[a]	1.98[b]	1.59[a]	34.27[a]
F_9	150.89[b]	2.04[a]	1.54[b]	32.72[b]
Enzymes (E)	**	**	**	**
Zero (E_0)	145.20[d]	2.19[a]	1.42[d]	31.85[d]
2 (E_1)	156.40[b]	2.03[b]	1.54[c]	33.15[b]
4 (E_2)	159.90[a]	2.01[c]	1.57[b]	32.49[c]
6 (E_3)	150.60[c]	1.81[d]	1.72[a]	36.49[a]
F×E	**	**	**	**
T_1	149.00[e]	2.29[a]	1.36[g]	28.55[f]
T_3	167.80[b]	2.07[d]	1.52[d]	33.67[d]
T_4	150.50[d]	1.75[g]	1.77[a]	36.17[b]
T_5	153.40[c]	1.81[f]	1.72[b]	38.70[a]
T_2	141.30[h]	2.09[c]	1.49[e]	35.16[c]
T_6	145.10[g]	2.00[e]	1.56[c]	32.63[e]
T_7	169.30[a]	2.26[b]	1.38[f]	28.82[f]
T_8	147.80[f]	1.81[f]	1.72b	34.28d

Average in the same row having different superscripts differ significantly $P \leq 0.05$

It is widely accepted that fibers are scarcely digested and ingested by fish, and fiber quantities are extremely reduced even if digestion occurs. However, a certain amount of fiber in the feed should be retained (Sun *et al*., 2019), with the gradual rise of plant parts of fish feed formulations, higher quantities of fiber will naturally be added to the feed. Cellulose is the main component of plant fiber (Sinha *et al.*, 2011). On the other hand, cellulose plays a vital role in improving stickiness of the feed, stimulating peristalsis of the digestive tract and promoting the movement of feed, digestion and absorption, and the required content of crude fiber differs greatly between species.

The challenge here is how to raise the plant ingredients in diets especially carnivorous fish without any effect on growth performance. In this study, increasing the fiber level from 5 to 9% led to reduce final weight in the rate of 3.16% (from 159.25 to 154.21) and specific growth rate 3.7(from 0.54 to 0.52), feed conversion ratio was the best with 5% fiber compared with 9%.

According to Anderson *et al*. (1984) high levels of fibers may reduce the use rate of other nutrients. Dioundick and Stom (1990) showed that the best FCR and PER were obtained with 2.5–5 % supplemental fiber, but the growth has been inhibited by 10 % in the cellulose community, Adamidou *et al*. (2011) indicated that up to 5 % fiber content does not affect the sea bream's growth efficiency or nutrient digestibility. The results of this study regarding fiber content are very similar to previous reports. On the other side currently, crude fiber content was assessed predominantly in carnivorous fish feed, and no research evaluated the fiber demand in omnivorous feed.

With regard to enzyme effect, in the present study, fish fed the control diet showed lower growth output than diets supplemented with exogenous enzymes, noting that dietary exogenous enzymes were beneficial for the growth of African catfish *Clarias gariepinus.* Adding a mixture of enzymes led to a higher improvement in FCR especially 6% exogenous enzyme (1.81).

Enzyme supplementation enhanced growth response and feed utilization, indicating that exogenous enzymes have growth stimulating effects and are also successful in eradicating anti-nutritional aspects and enhancing feed use. Several authors have demonstrated the impact of enzyme supplementation on the growth and survival of several species of cultivated fish such as Drew *et al*. (2005); Debnath *et al*. (2005); Lin *et al*. (2007); Yildrim and Turan, (2010a) and Yigit and Olmez (2011). In salmon *Salmo salar* juveniles, positive effects on the growth and protein utilization were recorded when dietary pancreatic enzymes were used (Carter *et al*., 1994). Usage of a commercial protease in rainbow trout increased the nutrient digestibility of coextruded canola (rapeseed meal) and pea (1:1) (Drew *et al*., 2005).

Exogenous carbohydrase supplementation in plant based fish diets increases the digestibility of nutrients and reduces nutrient excretion (Castillo and Gatlin, 2015). Carbohydrases include all enzymes that catalyse a decrease in the molecular weight of polymeric carbohydrates, but in addition, two dominant proteins, xylanase and glucanase account for more than 80% of the global carbohydrase market; other commercially available carbohydrases include alpha amylase, β-mannanase, alpha galactosidase and pectinase (Adeola and Cowieson, 2011). Improved weight gain, SGR, FCR and PER growth efficiency as well as improved body composition along with nutrient utilization were observed in rohu (*Labeo rohita* (Hamilton, 1822)) fingerlings by dietary incorporation of thermostable α-amylase (Ghosh *et al*., 2001). Furthermore, carbohydrases can also enhance the use of nitrogen and amino acids by increasing protein access for digestive proteases (Tahir *et al*., 2008).

Yildirim and Turan (2010a, b) indicated that supplementation with multi-enzyme complexes (fungal xylanase, β-glucanase, pentosonase, alpha-amylase, hemicellulose, pectinase, cellulase, and cellubiase) could significantly boost growth efficiency and feed use in juvenile tilapia (*Oreochromis aureus* Steindachner, 1864) and African catfish (*Clarias gariepinus* Burchell, 1822). Currently, Ghomi *et al.* (2012), Zamini *et al*. (2014) found a positive impact on the growth availability and feed efficiency of African catfish (*Clarias gariepinus*), big sturgeon (*Huso huso*) and Caspian salmon (*Salmo trutta*), respectively, by various commercial multi enzyme complexes (phytase, xylanase, glucanase, amylase, cellulase and pectinase). However, no specified plant based feedstuffs have been used in any of these studies. Moreover, Ai *et al.* (2007) found that Japanese sea bass (*Lateolabrax japonicus*) growth was increased with the addition of carbohydrase enzymes (phytase, glucanase, pentosanase, cellulase and xylanase) to diets where more than 40% of the protein was extracted from plant feedstuffs (soybean meal, rapeseed meal, peanut meal and wheat meal), suggesting that in reducing the anti-nutritional effect of NSP and improving fish efficiency, carbohydrase enzymes were successful. These results regarding exogenous enzymes are also in agreement with some previous studies on *Pangasius pangasius* (Debnath *et al*., 2005), *Carias batrachus* × *Carias gariepinus* (Giri *et al*., 2003), *Oreochromis niloticus* × *O. aureus (*Lin *et al.*, 2007*)* and rainbow trout (Farhangi and Carter, 2007).

Body chemical analysis:

Just as the growth of ***Clarias gariepinus*** is affected by the level of dietary fiber, the body chemical composition also affected as a natural reflection of the effect of nutrition on the carcass composition. No significant differences (P>0.05) were observed with regard to dry matter or total ash, while body protein was negatively affected by increasing dietary fiber, where T_2 was the least body protein contrary to T_1

and these results agree with the work of Lin and Shiau (1995), who stated that body composition should reflect the diets.

Data of Table (5) clearly indicated that exogenous enzymes concentration significantly affected the whole fish body components ($P < 0.05$).

Including dry matter, crude protein and total lipids, the carcass chemical analysis of the whole fish body was dramatically improved due to dietary enzyme supplementation. These results indicated that supplementation of dietary enzymes improved protein synthesis and deposition in the fish 's body when supplemented at optimum levels, increasing body protein content may be due to the higher degree of digestive enzyme production and its beneficial effect on digestion and absorption in the fish gut (Ye *et al.*, 2011; Abdel-Tawwab and Monier, 2018). Dry matter was recorded as 30.05% (highest value) and 27.31% for treatment T_2 and T_1, the highest quantity of fat contents was observed in treatments, which contains exogenous enzyme and high content of fiber (9%), while the lowest in body fat was T_1. The best in body crude protein was treatments T_4 and T_5 followed by treatments T_6, T_7 and the least of them were T_1, regarding ash content adding enzymes compared to the control groups (T_1,T_2) decreased significantly contents of whole body ash which were 23.87 in T_1 (highest) then followed by other treatments.

Table (5): Effect of treatments on body chemical composition of *Clarias gariepinus.*

Treatment	Dry matter	Crude protein	Total lipid	Total ash
Fiber (F)	**	**	**	**
F_5	28.41[a]	66.24[a]	11.65[b]	21.78[a]
F_9	28.31[a]	65.12[b]	12.25[a]	21.85[a]
Enzymes (E)	**	**	**	**
Zero (E_0)	28.68[a]	63.76[c]	11.57[c]	23.48[a]
2 (E_1)	28.24[a]	65.77[b]	12.08[ab]	21.70[b]
4 (E_2)	27.89[a]	66.64[a]	11.83[bc]	21.48[b]
6 (E_3)	28.62[a]	66.54[a]	12.34[a]	20.62[c]
F×E	**	**	**	**
T_1	27.31[b]	64.16[d]	11.26[d]	23.87[a]
T_3	29.09[ab]	65.56[bc]	11.78[bcd]	22.18[c]
T_4	28.25[ab]	67.40[a]	11.60[cd]	20.92[d]
T_5	28.98[ab]	67.82[a]	11.97[bcd]	20.17[e]
T_2	30.05[a]	63.35[a]	11.89[bcd]	23.09[b]
T_6	27.40[b]	65.98[b]	12.37[ab]	21.22[d]
T_7	27.53[b]	65.89[b]	12.05[abc]	22.03[c]
T_8	28.26[ab]	65.25[c]	12.70[a]	21.07[d]

Yildirim and Turan (2010b) reported that body fat was increased in African catfish (*Clarias gariepinus*) when exogenous enzymes are used to feed but this increase was not significant. Ghomi *et al*. (2012) also concluded that sturgeon body fat, *Huso huso*; fingerlings enhanced the highest fat content (34.53 %) when fingerlings were fed exogenous enzyme supplemented diets (500 mg / kg diet). Improving the carcass chemical composition of *Clarias gariepinus* is often due to improved growth and utilization of feed as many studies indicated in Common Carp (*Cyprinus carpio* Linnaeus, 1758) Fingerlings (Patil *et al*., 2019); tilapia (Maas *et al.*, 2018); Striped Catfish *Pangasianodon hypophthalmus* (Khalil *et al*., 2018).

Hematological and biochemical blood parameters:

The blood measurements were affected by the level of fiber in the diets, it was improved by decreasing fiber level, whether in the diets without enzymes (T_1 and T_2) or that contain exogenous enzymes. Data in Table (6) indicate that the adding of exogenous enzymes led to significant differences among treatments, where results indicate an improvement in the treatments, which containing exogenous enzymes, especially those containing lower levels of fibers compared to control groups (T_1, T_2). Plasma total protein (TP), albumen (Alb), globulin (Glob) and white blood cells (WBCs) were significantly (($p \leq 0.05$) increased with increasing the level of exogenous enzymes. With regard to interaction between fiber levels and adding enzymes, the results showed that the highest level of serum total protein was observed in T_4 while the lowest level was recorded in T_2 but no significant differences ($P > 0.05$) were found in T_3, T_5 and T_6, $T_{7.}$

In this study, the results indicated that T_2 recorded the highest level of AST followed by T_6 and T_1, the lowest level of AST was observed in T_5. In the other hand, treatment T_2 reflected the highest level of ALT followed by T_6 and T_8, while the lowest level of ALT was observed in T_5. In this study, high level of addition (exogenous enzymes) led to signicantly ($p\leq0.05$) decrease in blood index (ALT, AST) at the time it led to an increase in blood index (TP, WBC),

Table (6): Effect of treatments on hematological and biochemical blood parameters of *Clarias gariepinus.*

Treatment	AST	ALT	WBC	TP	Alb(g/dl)	Glob(g/dl)
Fiber (F)	**	**	**	**	**	**
F_5	38.91^b	46.28^b	81.42^a	50.91^a	18.99^a	31.76^a
F_9	44.36^a	69.94^a	66.10^b	46.87^b	16.12^b	30.67^b
Enzymes (E)	**	**	**	**	**	**
Zero (E_0)	47.08^a	61.30^a	66.62^d	47.07^d	16.49^d	30.64^c
2 (E_1)	45.57^b	59.60^b	69.85^c	48.67^c	16.98^c	31.25^b
4 (E_2)	39.16^c	57.80^c	78.07^b	50.16^a	19.35^a	30.86^c
6 (E_3)	34.74^d	53.73^d	80.52^a	49.67^b	17.39^b	32.11^a
F×E	**	**	**	**	**	**
T_1	43.15^c	49.45^d	72.06^d	49.08c	17.94^{bc}	31.14^c
T_3	42.83^d	47.50^e	76.65^c	50.57^b	18.03^b	32.05^b
T_4	37.17^f	46.06^f	87.02^b	53.17^a	22.21^a	31.05^{cd}
T_5	32.50^h	42.10^g	89.97^a	50.85^b	17.77^c	32.81^a
T_2	51.02^a	73.14^a	61.18^h	45.06^f	15.05^g	30.15^f
T_6	48.30^b	68.10^b	63.05^g	46.78^e	15.94^f	30.45^{ef}
T_7	41.15^e	73.14^a	69.11^f	47.15^e	16.50^e	30.67^{de}
T_8	36.98^g	65.37^c	71.07^e	48.50^d	17.00^d	31.41^c

Average in the same row having different superscripts differ significantly $P\leq0.05$

Hematological characteristics are the vital physiological health characteristic index; its contents clearly reflected the ordinary metabolism and physiological activity (Yarahmadi *et al.*, 2014; Zahangir *et al.*, 2015; Shahjahan *et al.*, 2018). Nutritional status and adaptability are also expressed in biochemical parameters if fish exhibit physiological or pathological changes caused by external factors (Shi *et al.*, 2012). Stress or in reaction to dietary supplements can also result in changes in blood physiology (Yue *et al.*, 2013; Lin *et al.*, 2015). In fish physiology and pathology, various investigators have identified normal ranges for different blood parameters in fish (Zhou *et al.*, 2009), the present findings partly corroborated the report of Bello *et al.* (2014), where who reported significant changes in hematology of *Clarias gariepinus.*

Liver enzymes are liver generated compounds that can be tested using a blood test alanine aminotransferase (ALT) and aspartate aminotransferase (AST) are two of the enzymes central to test the health of liver function. In general, an increase in these liver enzymes implies organ damage or toxicity (Ghadi, 2000). ALT acts more precisely and is a better indicator of liver damage. Accordingly, it could then be concluded that the current significant decrease in the level of ALT in fish fed graded levels of exogenous enzymes supplemented diets confirmed that the exogenous enzymes had hepato protective function, which is similar to the results obtained by Akpan and Ekpo (2015). Albumin level was also significantly increased in T_4, T_3 and T_5 groups compared with T_2 where the protease enzyme worked in a good internal environment for the fish, especially with a low level of fiber, in addition, the endogenous activity of African catfish cellulase in T_2 is far from adequate to fully digest the ingested fiber, so exogenous cellulase could be a good feed additive. Zhou *et al*. (2009) mentioned the importance of adding enzymes to fish diets, which dietary enzymes can be used to support the fish's own enzyme production including proteases to improve protein hydrolysis and digestibility and amylases to improve starch quality and digestibility, this was found in the treatments including exogenous enzymes and this helpful effects was increased as the dietary fibers decreased.

Such positive findings in hematological and biochemical blood parameters have contributed to an improvement in immune responses and stable status of fish whose diets included higher levels of exogenous enzymes and lower levels of fiber. These positive findings were related to improving the digestive system and reducing stress on fish. In the digestive tract, exogenous enzymes use sufficient substrates for probiotic action (Bedford and Cowieson, 2012). Furthermore, enzymes react positively with the gut microbiota through improved digestibility and thus augment efficient nutrient absorption (Adeoye *et al.,* 2016). In addition, an increased hematological index of pepsin supplementation in Nile tilapia and common carp has been identified (Singh *et al.,* 2011; Goda *et al.,* 2012). Exogenous protease supplementation hydrolyses protein and releases shorter chain peptides in feed, thus increasing protein digestibility and total blood protein, resulting in better feed consumption, utilization and eventually good immunity (Farhangi and Carter, 2007; Singh *et al.,* 2011; Choi *et al.,* 2016).

CONCLUSION

Carnivorous fish like African catfish fish find difficulty in the digestion of plant feeds, especially those that contain high insoluble and indigestible fiber content such as hemicellulose and cellulose (anti-nutritive factors). These effects can be reduced by using exogenous enzymes, applying enzymes in feeds to reinforce feed utilization is an idea that has been explored well in terrestrial animal feeding and to some aquatic fish. Exogenous enzymes increase the feed utilization, digestibility, enhance growth performance, optimize whole body composition and promote immune performance and pathogen resistance, the results of the present study concluded that adding mixed enzymes in *Clarias gariepinus* diets can improve growth performance, feed utilization and health, in where the best treatment were T_4 and T_5 and the least of them was T_1 and $T_{2.}$

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تأثير إضافة إنزيمات خارجية بعلائق القرموط الأفريقي تحت مستويات مختلفه من الألياف علي آداء النمو وكفاءة الإستفادة من الغذاء وبعض المقاييس الصحيه

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تم إجراء هذه التجربة لدراسة تأثير إضافة مخلوط من الإنزيمات الخارجية(اميليز، زيلانيز، بروتيز) على أداء النمو والإستفادة من الغذاء وكذا بعض المقاييس الصحيه في سمك القرموط الأفريقي *Clarias gariepinus* بمتوسط وزن إبتدائي 80جم وكانت مدة التجربة 55 يوم وتمت بالمزرعة البحثية لكلية الزراعة جامعة الفيوم بدمو حيث تم إستخدام مخلوط الإنزيمات مع مستويين من الألياف في النظام الغذائي (5٪ و 9٪)، استخدمت 8 معاملات تجريبية متوازنة من حيث الطاقة والبروتين وبواقع ثلاث مكررات لكل معاملة تجريبية وجاء توزيع المعاملات الغذائية علي النحو التالي: T_1 و T_2 عليقتي المقارنة(5، 9٪ ألياف علي الترتيب بدون إضافة إنزيمات خارجية)، يمثل مستوي الألياف 5٪ معاملات T3 و T4 و T5 مع مخلوط إنزيمات 2، 4، 6 % من الياف العليقة علي التوالي بينما يمثل مستوي الألياف 9% معاملات T6 و T7 و T8 مع مخلوط إنزيمات 2، 4، 6 % من الياف العليقة علي التوالي. تم تسكين كل مكررات المعاملات بتنكات حديدية مربعة وبمقاييس(1×1.5×1م) وبمستوي تخزين 20 سمكة/تانك. أظهرت النتائج فروقا معنوية بين المعاملات حيث أظهرت الأسماك التي إحتوت علائقها علي إنزيمات خارجية معدل نمو أفضل وتناول علف أعلى بكثير من الأسماك التي تغذت على علائق بدون إنزيمات خارجية وذلك عند مستوي معنوية(P<0.05). كذلك جاءت مقاييس الإستفادة من البروتين ومعاملات التحويل الغذائي أفضل وبشكل ملحوظ في جميع المعاملات المتضمنة إنزيمات خارجية مقارنة مع مجموعة المقارنه (P<0.05) كما أثر مخلوط الإنزيمات الخارجية على مكونات جسم السمكة بالكامل (P<0.05)، لوحظت أعلى كمية من محتوى الدهون في المعاملات التي تحتوي على إنزيمات إضافية ومحتوى عالي من الألياف(9٪)، في حين جاءت أقل نسبة في دهون الجسم مع المعاملات ذات المستوي الأقل من الياف الغذاء وبدون إنزيمات خارجية، كذلك أيضا تأثرت صفات الدم حيث تحسنت وظائف الكبد وزاد البروتين الكلي مع إستجابة مناعية جيدة للأسماك التي إحتوي علائقها علي نسب متزايده من خليط الإنزيمات والأقل في الألياف. خلصت هذه الدراسة إلي أن إضافة مخاليط الإنزيمات الخارجية يمكن أن يحسن من أداء النمو بشكل كبير، الإستفاده من الغذاء وبعض المقاييس الصحيه في أسماك القرموط الأفرقي خاصة عند مستوى 4٪ انزيمات مع مستوي الياف 5٪.