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EFFECT OF ENZYME SUPPLEMENTATION IN NILE TILAPIA DIETS ON GROWTH PERFORMANCE AND HEALTH

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ABSTRACT

This study was carried out to evaluate the effects of dietary supplementation of exogenous enzymes (protease or carbohydrases) at o.1gm/kg on growth performance, whole body composition, serum metabolities and enzymes activity of Nile tilapia fingerlings. One hundred ninety six (196) Nile tilapia fingerlings were allocated to seven dietary treatment groups. Each treatment was duplicated and had an average body weight of 10.14 gm/fish and ranged from 8 to 11 gm. Nile tilapia fingerling groups were fed control diet(G1) contained 32% CP and 3000 kcal DE/kg, group 2 (G2) control diet supplemented with protease enzyme, group 3 (G3) control diet supplemented with NSPases enzymes (β-glucanase, β-mannase, xylanase, pectinase, cellulase and hemicellulase), group 4 (G4) low CP diet (30%), group 5 (G5) low CP diet (30%) supplemented with protease, group 6 (G6) low DE diet (2900 kcal/kg) and group 7 (G7) low DE diet (2900 kcal/kg) supplemented with NSPases for aperiod of 84 days. Growth performance (body weight (BW), body weight gain (BWG),feed consumption (FI), feed conversion ratio (FCR), protein efficiency ratio (PER) and specific growth rate (SGR)), whole body composition (moisture, crude protein, ether extract and ash), serum metabolities (total protein, albumin, globulin, creatinine and urea) and enzymes activity (ALT and AST)were determined. The results indicated that control diet supplemented with NSPases enzymes (G3) significantly ($p<0.05$) improved growth performance parameters of Nile tilapia fingerlings (BW, BWG, FCR). In conclusion, supplementation of carbohydrases enzymes to the control diet (32% CP and 3000 kcal DE/kg) had a significant effect on body weight and body weight gain and decreased feed conversion ratio. Also, Nile tilapia can tolerate diets with low CP (30%) and low DE (2900 kcal/kg diet) supplemented with protease and carbohydrases respectively.

Keywords: protease, carbohydrases, growth performance, Nile tilapia.

INTRODUCTION

It has been reported that the aquafeeds constitute 50-60% of return cost in intensive aquaculture production and protein is the most expensive component of the feed. Fish meal has been considered traditional and one of the main protein sources in aquafeeds and fishmeal content ranging from 30-50% because it is considered a good source of essential amino acids, essential fatty acids, high nutrient digestibility, general lack of anti-nutritional factors (ANFs) and palatable **(Davies and Gouveia, 2008; NRC, 2011; Tacon and Metian, 2013)**. The increasing cost of fish meal is a limiting factor that affect continuous supply of fish meal to support aquaculture growing production. Therefore, fish meal has moved from being a commodity to a specialized ingredient due to the increasing

demand and the unstable supply as the amount of whole fish used to produce fish meal has been decreased **(IFFO, 2013)**.

As a result, researchers and aquaculture industry have focused on the identification and the use of suitable and cost-efficient ingredients as alternative to fish meal **(Adeoye, 2016)**. Grains and oilseed by-products are among the most promising alternative ingredients for diets of cultured fish species in the future. This is due to their certain characteristics including their low cost, increasing abundance, ability to increase production, greater sustainability and lower health risks than other alternatives **(Hardy et al., 2009)**. However, it has been investigated that grains, oilseed by-products and some plant-derived feedstuffs have some disadvantages making them not fully suitable for use in aquafeed as substitutes for fish meal. Plant-derived feedstuffs contain much more indigestible organic matter present in form of insoluble carbohydrates (non-starch polysaccharides (NSP)) and fiber compared to fish meal, resulting in increasing levels of excretion and waste products from fish into the environments **(Adeoye, 2016)**.

It has been demonstrated that exogenous digestive enzymes as feed additives have the ability to deactivate ANFs, optimize nutrient utilization and also lower P and N excretion into the environment. Moreover, exogenous enzymes are harmless, environmentally friend, natural and represent the most powerfull tool of all the methods and techniques used for improving nutritional value of plant materials. Also, they consider a natural way of converting complex feed components into more absorbable nutrients **(Kumar et al., 2012b; Castillo and Gatlin, 2015)**. Proteases are protein-digesting enzymes that have the ability to degrade protein-based ANFs (lectins or trypsin inhibitors) resulting in a fast absorption rate and increased growth rate **(Isaksen et al., 2010)**. Carbohydrases or NSP-degrading enzymes (e.g. cellulase, xylanase, β-glucanase, α-amylase, etc) are enzymes that have the ability to disrupt plant cell wall integrity and reduce molecular weight characteristics of NSPs. So, this results in a rapid digestion by reducing viscosity in the gut and increase digestibility of energy-yielding nutrients **(Castillo and Gatlin, 2015)**.

Therefore the objectives of this study were to determine the effects of enzymes supplementation (protease or carbohydrases) to Nile tilapia fingerlings diets (basal control diet, low crude protein (CP) diet supplemented with protease enzyme and low digestible energy (DE) diet supplemented with carbohydrases (NSPases) on growth performance and general health.

MATERIALS AND METHODS

1. Diet preparation:

In this study, seven dietary treatments were used (Table 1), group 1 (control diet) contained 32% CP and 3000 kcal DE/kg, group 2 control diet supplemented with protease enzyme, group 3 control diet supplemented with NSPases enzymes (β-glucanase, β-mannase, xylanase, pectinase, cellulase and hemicellulase), group 4 low CP diet (30%), group 5 low CP diet supplemented with protease, group 6 low DE diet (2900 kcal/kg) and group 7 low DE diet supplemented with NSPases. The enzymes were added at 0.1 gm/kg. Diets were prepared in the form of water stable pellets of 2-3 mm and stored in plastic bags in refrigerator during the time of use.

2. Experimental design:

One hundred ninty six (196) Nile tilapia fingerlings were set for seven dietary treatments. Each treatment was duplicated and had an average body weight of 10.14 gm/fish. Fish were stocked in 14 glass aquaria (80 cm length, 35 cm width & 40 cm height) 2 aquaria per treatment. Daily feed intake (on air-dry basis) was introduced to fish at 3% of BW/fish/aquarium thereafter, fish were fed twice daily (at 9:00 am & 3:00 pm) to minimize over feeding and waste of feed in the aquarium. During the experiment, photoperiod used was 8 to 12 hrs light/12 hrs dark cycle and the temperature during the experiment was fluctuated between 24 to 28ºC. Each aquarium was cleaned day by day with partial replacement with tap water in which dechlorinating agent was added. The experiment extended for 12 weeks.

2.3.Samples collection and chemical analysis:

Samples of experimental diets were analyzed for moisture, crude protein, ether extract, and ash by standard methods according **AOAC (1995)**.Random fish (6 fish/group) were collected at the end of the experiment. These fish samples were minced, dried at 70 Cº for 72 hrs to be analyzed for whole body chemical composition (moisture, DM, CP, EE and ash) according to **AOAC (1995)**. Also, blood samples were collected from 6 random fish of each group at the end of the experiment from the heart. Blood samples were coagulated, the sera obtained were centrifuged at 3000 rpm for 15 minutes. The collected sera were frozen at -20 Cºin a deep freeze until used for biochemical determination of serum total proteins (**Yatzidis, 1987**), albumin (**Young, 2001**), alanine aminotransferase (ALT), aspartate aminotransferase (AST) activities **(Tietz, 1986),** urea and creatinine **(caraway, 1963)** using prepared analyzing chemical kits, after the instructions of the producer (Vitro and Diamond). The difference between total proteins and albumin was calculated as globulin concentration.

2.4. Growth Parameters Measurements:

The following equations were used to evaluate fish growth performance:

Weight gain (gm) = Mean final weight (gm) - Mean initial

FCR = Feed intake (gm) / Weight gain (gm)

PER % = Weight gain (gm) / protein intake

SGR % = (Ln (W2) – Ln (W1) / t x 100

Feed intake was calculated as the total weight of diet introduced in a period of time (2 weeks) divided by the weight of survival fish in the aquarium.

2.5. Statistical Analysis:

The results were subjected to a one-way ANOVA to test the impact of supplementation of exogenous enzymes (protease or carbohydrases) to Nile tilapia diets on growth performance, whole fish body composition, serum metabolities and enzymes activity. Data were analyzed using statistical SPSS v20 (SPSS Inc., Chicago, IL, USA). Differences between dietary groups means were compared using Duncan's multiple range test. Differences due to dietary treatments were considered significant if P-value for the effect was < 0.05.

RESULTS

At the end of the experiment (84 days period) most of fish were survival and mortality percentage were negligible. The growth performance parameters of the fish groups fed the experimental diets are presented in Table 2. Theproximate chemical composition of whole body of Nile tilapia fingerlings is presented in Table 3. Serum metabolites and enzymes activity of Nile tilapia fingerlings are presented in Table 4.

Table 1. Ingredients percentages and nutrient composition of the experimental diets.

	Group 1	Group 2	Group 3	Group 4	Group 5	Group 6	Group 7
Ingredients (%)							
Corn, yellow	14.48	14.48	14.48	17.06	17.06	14.53	14.53
Wheat bran	32.5	32.5	32.5	34.44	34.44	33.85	33.85
Soybean meal 47	34.6	34.6	34.6	30.0	30.0	36.55	36.55
Fish meal	10.0	10.0	10.0	9.5	9.5	9.1	9.1
Corn gluten meal	3.0	3.0	3.0	3.0	3.0	2.0	2.0
Soybean oil	1.65	1.65	1.65	2.2	2.2	0.19	0.19
Gelatin	2.0	2.0	2.0	2.0	2.0	2.0	2.0
Min.&vit.premix**	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Common salt	0.50	0.50	0.50	0.50	0.50	0.50	0.50
Vit C	0.05	0.05	0.05	0.05	0.05	0.05	0.05
Antioxidant	0.02	0.02	0.02	0.02	0.02	0.02	0.02
Methionine	0.19	0.19	0.19	0.22	0.22	0.21	0.21
Protease	--	0.1 gm/kg	--	--	0.1gm/kg	--	--
NSP enzymes	--	--	0.1gm/kg	--	--	--	0.1gm/kg
Chemical composition							
Crude protein%*	32.13	32.13	32.13	30.15	30.15	32.07	32.07
DE(Kcal/kg)*	3000	3000	3000	3000	3000	2900	2900
Crude fat%	6.15	6.15	6.15	6.22	6.22	6.11	6.11
Crude fiber%	4.84	4.84	4.84	4.93	4.93	5.02	5.02
Ash%	5.88	5.88	5.88	5.65	5.65	5.87	5.87
Calcium%	0.66	0.66	0.66	0.63	0.63	0.62	0.62
Phosphorus%	0.94	0.94	0.94	0.92	0.92	0.93	0.93
Chemical analysis							
Crude protein%	31.60	31.60	31.60	29.75	29.75	31.98	31.98
EE%	9.22	10.78	10.84	11.71	11.71	9.31	9.31
Ash%	7.61	7.31	6.93	6.95	7.54	6.95	8.52
Moisture%	9.17	9.24	8.52	11.15	9.88	8.93	8.87

* DE (kcal/kg) and Crude protein%values are calculated from the feed composition tables, nutrient requirement of fish **(NRC, 1993).**

Trace minerals & vitamins premixes were supplemented to cover the levels of the microminerals & vitamins for tilapia fish as recommended by **(NRC, 1993).Vitamins premix (IU or mg/kg diet); vit. A 5000, Vit. D3 1000, vit. E 20, vit. k3 2, vit. B1 2, vit. B2 5, vit. B6 1.5, vit. B12 0.02, Pantothenic acid 10, Folic acid 1, Biotin 0.15, Niacin 30.Mineral mixture (mg/kg diet); Fe 40, Mn 80, Cu 4, Zn 50, I 0.5, Co 0.2 & Se 0.2.

G1 = Control diet (32% CP, 3000 kcal DE/kg)
G2 = Control + protease
G3 = Control + NSPases
G4 = Low CP (30% CP)
G5 = Low CP + protease
G6 = Low DE (2900 kcal/kg)
G7 = Low DE + NSPases

Table 2. Allover effect of dietary supplementation of exogenous enzymes (protease or carbohydrases) on growth performance parameters (Means ± standard error) of Nile tilapia (O. niloticus) fingerlings, (12 weeks period).

	Experimental diet						
Items	**G1**	**G2**	**G3**	**G4**	**G5**	**G6**	**G7**
Initial BW (gm)	10.14±0.36	10.13±0.38	10.14 ± 0.39	10.15 ± 0.35	10.14±0.36	10.15 ± 0.35	10.13±0.34
Final BW (gm)	39.05^{b}±1.75	40.09^{ab}±1.27	43.40^{a}±1.62	37.12^{b}±1.79	38.76^{b}±1.11	37.28^{b}±1.61	38.67^{b}±1.85
BWG (gm)	28.91^{b}±0.90	29.96^{b}±0.73	33.26^{a}±0.86	26.97^{b}±1.19	28.62^{b}±0.59	27.13^{b}±0.82	28.54^{b}±0.89
Feed consumption (gm)	55.80^{ab}±1.42	58.72^{a}±0.96	59.53^{a}±1.29	54.75^{b}±1.46	58.96^{a}±0.78	55.34^{b}±1.29	58.80^{a}±1.54
FCR	1.93^{b} ± 0.039	1.96^{ab}±0.039	1.79^{c}±0.034	2.03^{a}±0.053	2.06^{a}±0.039	2.04^{a}±0.038	2.06^{a}±0.44
PER	1.62^{b}±1.42	1.60^{bc}±0.96	1.74^{a}±1.29	1.64^{b}±1.46	1.62^{b} ± 0.78	1.53^{c}±1.29	1.52^{c}±1.54
SGR	1.49±0.23	1.52±0.27	1.61±0.24	1.43±0.30	1.49 ± 0.26	1.44±0.27	1.49±0.23

[abc] Means in each row followed by different superscripts are significantly different (P<0.05).

G1 = Control diet (32% CP, 3000 kcal DE/kg)
G2 = Control + protease
G3 = Control + NSPases
G4 = Low CP (30% CP)
G5 = Low CP + protease
G6 = Low DE (2900 kcal/kg)
G7 = Low DE + NSPases

Table 3. Effects of dietary supplementation of exogenous enzymes (protease or carbohydrases) on proximate chemical composition of whole body of Nile tilapia (O. niloticus) fingerlings fed control, low CP and low DE diets for 12 weeks.

	Experimental diets						
Items	**G1**	**G2**	**G3**	**G4**	**G5**	**G6**	**G7**
Moisture%	74.41 ± 1.19	73.64 ± 1.12	73.63 ± 0.54	73.45 ± 0.59	73.81 ± 0.63	73.15 ± 1.22	75.16 ± 0.59
DM%	25.69 ± 1.19	26.36 ± 1.12	26.37 ± 0.54	25.55 ± 0.59	26.19 ± 0.63	25.45 ± 1.22	25.84 ± 0.59
CP%	15.93^{b} ± 1.85	16.58^{a} ± 1.50	16.37^{ab}± 0.57	15.88^{b} ± 0.67	16.14^{ab}± 1.23	15.73^{b} ± 0.56	15.95^{ab}± 1.09
Fat%	6.05^{b} ± 0.60	6.21^{ab} ± 0.53	6.44^{a} ± 1.09	6.06^{b} ± 1.21	6.37^{a} ± 0.58	6.15^{ab} ± 0.91	6.32^{a} ± 0.56
Ash%	3.71 ± 0.57	3.57 ± 1.12	3.58 ± 0.60	3.61 ± 1.21	3.68 ± 1.03	3.57 ± 0.60	3.57 ± 0.51

[abcd] Means in each row followed by different superscripts are significantly different (P<0.05).

G1 = Control diet (32% CP, 3000 kcal DE/kg)
G2 = Control + protease
G3 = Control + NSPases
G4 = Low CP (30% CP)
G5 = Low CP + protease
G6 = Low DE (2900 kcal/kg)
G7 = Low DE + NSPases

Table 4. Effects of dietary supplementation of exogenous enzymes (protease or carbohydrases) on serum metabolites and enzymes activity of Nile tilapia (O. niloticus) fingerlings at the end of the experimental period (12 weeks period).

Experimental diets							
Items	**G1**	**G2**	**G3**	**G4**	**G5**	**G6**	**G7**
Total protein (gm/dl)	3.00^{b} ±0.13	3.52^{b} ± 0.22	3.23^{b} ± 0.27	3.42^{b} ± 0.026	4.17^{a} ± 0.026	3.19^{b} ± 0.21	4.10^{a} ± 0.13
Albumin (gm/dl)	1.52^{b} ±0.10	1.74^{b} ± 0.08	1.67^{b} ± 0.17	2.23^{a} ± 0.33	2.01^{ab}±0.093	1.73^{b} ± 0.122	1.83^{b} ± 0.06
Globulin (gm/dl)	1.48^{b} ±0.23	1.78^{b} ± 0.31	1.56^{b} ± 0.043	1.19^{c} ± 0.35	2.16^{a} ± 0.119	1.46^{b} ± 0.088	2.27^{a} ± 0.13
Creatinine (mg/dl)	0.72 ± 0.11	0.65 ± 0.037	0.72 ± 0.13	0.65 ± 0.078	0.69 ± 0.051	0.66 ± 0.00	0.73 ±0.095
Urea (mg/dl)	1.78 ± 0.65	2.03 ± 0.50	1.86 ± 0.37	2.01 ± 0.48	1.80 ± 0.58	2.12 ± 0.47	2.09 ± 0.58
ALT (U/L)	7.00 ±1.00	6.33 ± 2.20	7.23 ± 0.98	7.5 ± 1.50	8.5 ± 1.50	6.7 ± 1.25	9.00 ± 1.00
AST (U/L)	84.00±5.00	76.00 ±13.00	78.00 ±11.00	77.5 ± 11.5	70.25 ± 1.25	79.00 ±10.00	83.75 ±5.25

^{abc}Means in each row followed by different superscripts are significantly different (P<0.05).

ALT = Alanine aminotransferase
AST = Aspartate aminotransferase
G1 = Control diet (32% CP, 3000 kcal DE/kg)
G2 = Control + protease
G3 = Control + NSPasesG4 = Low CP (30% CP)
G5 = Low CP + protease
G6 = Low DE (2900 kcal/kg)
G7 = Low DE + NSPases

DISCUSSION

Effect of dietary supplementation of exogenous enzymes (protease or NSPases) on growth performance:

The growth data (BW development (gm), absolute BWG, FI, FCR, PER and SGR) of Nile tilapia fingerlings fed control diet (CP 32% & 3000 kcal DE/kg) and diets with low CP level (30%) or low DE level (2900 kcal/kg) supplemented with exogenous enzymes (protease or NSPases) at 0.1 gm/kg diet are presented in Table 2. The means of intial body weight of the experimental groups of Nile tilapia fingerlings were not significantly different. There were no significant differences of the means of BW of Nile tilapia fingerlings fed the experimental diets supplemented with enzymes or not during the first 8 weeks of experiment. However, at the last 4 weeks of experiment, the best overall growth response was significantly ($p<0.05$) obtained in Nile tilapia fingerlings fed control diet supplemented with carbohydrases enzymes (G3). In addition, fish groups fed the low CP or low DE were the lowest (non significant) in growth response compared with the control group. Supplementing the diet with protease or NSPases enzymes improved growth development near to the result obtained by the control group.

Effect of dietary supplementation of NSPases enzyme on performance:

Allover effects of feeding low DE diet and low DE diet supplemented with NPSases of Nile tilapia on growth performance is presented in Table 2. The results showed that there were no significant differences in growth performance (BW, BWG, FCR, SGR) between control group and the groups fed low DE diets (G6, G7).The results of the present study

indicated that the best growth performance (BW, BWG, FCR) were observed in the group of fish fed the control diet supplemented with NSPases enzymes.

With the same concept, **Yildirim and Turan (2010)**found a significant improvment in growth performance and feed utilization in African catfish fed diet supplemented with exogenous enzymes (Farmazyme® containing fungal xylanase, β-gluconase, pentosonase, β-amylase, fungal β-gluconase, hemicellulase, pectinase and cellulase) at 0.5 and 0.75 gm/kg in comparison with control and o.25 gm/kg of carbohydrase supplemented diet. Also, it has been postulated that supplementation of exogenous NSP enzymes reduced the anti-nutritional effect of NSP and thus increased growth for Japanese sea bass, large yellow croaker and tilapia (**Zhang et al. 2009; Li et al. 2009)**.Moreover, **Ai et al. (2007)** observed that growth of Japanese seabass increased when fed diets supplemented with NPS enzymes (β-glucanase, pentosanase, cellulosase and xylanase), suggesting that NSP enzymes were effective to resist the anti-nutritional effect of NSP and increased growth. Also, NSP-degrading enzymes (e.g. cellulase, xylanase, etc.) are capable of disrupting plant cell wall integrity thereby reducing molecular size characteristics of NSPs. Consequently, this enhances rapid digestion by reducing viscosity in the gut (**Zijlstra et al., 2010; Bedford and Cowieson, 2012)**.

On contrary, **Yigit and Olmez (2011)** observed that cellulase supplementation in soybean-based meal and canola-based meal did not affect growth response of tilapia (Oreochromisniloticus). Also, **Kazerani and Shahsavani (2011)** showed no significant effects on growth rate and feed conversion of the carp fed diets supplemented with low level of carbohydrases (glucanase, xylanase, cellulase and hemicellulose), (0.25-1 gm kg^{-1} feed), while higher level of the supplement (2-3 gm kg^{-1} feed) reduced weight gain in a level dependent manner, although the reduction was not significant. However, it has been claimed that low levels of enzymes may increase viscosity of the digesta by increasing the soluble non-starch polysaccharides via solubilizing the insoluble carbohydrate fraction, resulting in reduced digestibility and absorption (**Castanon et al., 1997**). **Mahmoud et al. (2014)** recorded that the overall growth response of Nile tilapia fish obtained in control diet were better than those fed low DE diet supplemented with multi-enzymes and those fed control diet supplemented with multi-enzymes. With this concept, it is worthy to mention that present experimental diets were formulated to contain considerable amount of wheat bran (32.5 to 34.44%) and soybean meal (30.0-36.55%) which contain high amounts of non-starch polysaccharides and anti-nutritional factors.

Effect of dietary supplementation of protease enzyme on performance:

Allover effects of protease supplementation on growth performance parameters are presented in Table 2. There were no significant differences in growth performance of Nile tilapia fish fed control (32% CP) diet supplemented with protease (G2) and the control group (40.09 vs 39.05 gm). In spite of decreasing the dietary CP level from 32 to 30% (G4) body weight development did not significantly decrease, at the end of the experimental period, compared to the control group (37.12 vs 39.05 gm). However, supplementing the low CP diet with protease (G5) did not increase final body weight than that of the control group (38.76 vs 39.05 gm). This finding may indicate that protease supplementation of Nile tilapia diet could overcome an unconditional little decrease in total dietary CP with keeping expected rate of body development.

In agreement with the present results, **Adeoye et al. (2016)** found that there were no significant differences (P>0.05) in growth performance and nutrient utilization between the fish group fed control diet and those fed diet supplemented with protease enzyme. Also,

Dalsgaard et al. (2012) showed that there were no significant differences in growth parameters including FCR on addition of protease to soybean meal diet in rainbow trout. Moreover, **Ayhan et al. (2008)** reported that addition of protease to soybean meal diets in seabream had no effects on growth and protein digestibility. However, **Li et al. (2016)** reported significantly better weight gain and FCR of shrimp fed a low fish meal diet supplemented with a protease compared to those fed the same diet without supplementation. Also, **Li et al. (2015)** detected that supplementing 175 mg/kg exogenous protease in pelleted diet containing 30 gm/kg fish meal significantly increased WG and decreased FCR of tilapia.

Furthermore, **Chowdhury et al. (2017)** studied the effect of protease enzymes supplementation with different levels (125, 150 and 175 mg/kg) on juvenile Chinese mitten crab Eriocheirsinensis diets and those authors found that growth parameters (WG, FCR, SGR, survival and PER) were not different ($p>0.05$) among the treatments except for the feed intake per crab (FIC) fed the diet supplemented with 175 mg/kg protease which was lower than those fed positive control diet (high fish meal). However, **Dias et al. (2012)** found that growth performance of juvenile Nile tilapia were significantly improved ($p<0.05$) when fed 26% CP diet or 28% CP diets supplemented with 200, 400 and 600 mg/kg protease enzyme. Similarly, **Ragaa et al. (2017)** showed that growth performance parameters of O. niloticus were significantly ($p<0.05$) improved when 26% CP and 28% CP diets were supplemented with protease enzyme at 200 and 400 mg/kg in comparing with the non supplemented groups. Those authors reported that protease effect is likely to be more pronounced in a low level of crude protein diet.

Effects of dietary supplementation of exogenous enzymes (protease or carbohydrases) on whole body composition:

The proximate chemical composition (moisture, CP, fat & ash) of the whole body of juvenile Nile tilapia fish as affected by enzymes supplementation or feeding low CP or low DE diets is presented in Table 3. The results showed that there were no significant effects for exogenous enzymes supplementation to control diet or to diet low in CP level or DE on moisture percentages. There were no differences in DM percentages of the whole fish body of the different groups which ranged from 25.45% for the fish group fed the low DE diet (G6) to 26.37% for the fish group fed the control diet supplemented with protease (Table 3). Also, the highest value (6.44%) of the ether extract content of the whole body is reported for the fish group fed the control diet supplemented with protease (G3). Reviewing the whole data presented in Table 3 detected that composition of DM of the whole fish body (CP, EE and ash contents) were nearly similar in all fish groups fed diets supplemented or not supplemented with digestive enzymes. **Adeoye et al. (2016)** showed that supplementation of exogenous enzymes (protease or carbohydrases) did not affect whole body composition of tilapia or any of protein, ether extract or ash contents. However, body moisture content of tilapia fed the protease supplemented diet was higher ($P<0.05$) than those fed the control one. Furthermore, **Khalafalla and EL-Hais (2013)**showed that there was no effect on whole body composition (dry matter, protein, ether extract and ash contents) of Nile tilapia fingerlings fed diets supplemented with Nutrasexylam enzyme (mixture of β-xylanase and α-amylase) in comparison with control groups. Our results are in agreement with previous results reported by **Lin et al. (2007)** who showed that there were no significant differences ($P>0.05$) in whole body moisture, protein, total lipid and ash of tilapia fish fed diets supplemented with exogenous enzymes (neutral protease, β-glucanase and xylanase). Similarly, **Yigit et al. (2016)** found that there were no differences among rainbow trout fed diet supplemented with protease and control group in the body

composition (DM, crude protein, total lipid and ash). Moreover, **Shi et al. (2016)** observedno significant differences in whole body composition of gibel carp fed high fishmeal diet and low fishmeal diets supplemented with protease enzyme at different levels.

However, it has been recorded that the body composition of fish is primarily influenced by diet composition, feeding practices and fish size and can be controlled through nutrition (**Burtle, 1990**). In addition, **Danicke et al. (2003)** concluded that the absolute amounts of protein which were synthesised daily and accreted in muscle increased with xylanase supplementation.

Effects of dietary supplementation of exogenous enzymes (protease or carbohydrases) on serum metabolites and enzymes activity:

Effects of feeding Nile tilapia fingerlings control, low CP and low DE diets supplemented with exogenous enzymes on serum metabolities and enzymes activity are presented in Table 4. The results showed that fish fed the low CP diet supplemented with protease (G5) had elevated serum total protein, albumin and globulin levels than other fish groups. Similarly, the fish group fed the low DE diet supplemented with carbohydrases (G7) had serum total protein, albumin and globulin levels higher than the other fish groups (fed the control diets). However, the results showed that there were no significant differences in serum levels of total protein, albumin, globulin, creatinine and serum urea between the fish groups fed control diet, enzyme supplemented control diets or low CP and low DE diets. Also, reviewing the results showed that serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activity were not significantly different between all fish groups fed the experimental diets supplemented with enzymes or not for 12 weeks period. With the same concept**, Ragaa et al. (2017)** clarified that the protease supplementation had no significant effect on serum concentrations of creatinine, urea, ALT and AST compared with control groups of Nile tilapia fish. Our results were partially similar to that of**Khalafalla and EL-Hais (2013)** whofound insignificant influence ($p \geq 0.05$) due to Nutrasexylam enzyme (mixture of β-xylanase and α-amylase) addition on serum parameters of proteins (total protein, albumin and globulin) and liver activity indices (AST and ALT activity). In addition, **Mahmoud et al. (2014)** showed that there were no significant differences in serum metabolites (total protein, albumin, uric acid, and creatinine) and serum activity of ALT and AST of Nile tilapia fish fed control diet, control plus multi-enzymes and low energy diet (2760 DE kcal/kg) with multi-enzymes. Also, **Shi et al. (2016)** detected no significant differences in serum total protein and albumin of gibel carb fed low fishmeal diets supplemented with different levels of protease enzyme and high fishmeal diet. On the other hand, **Goda et al. (2012)** found that total plasma protein and total plasma globulin levels were significantly ($p \leq 0.05$) highest in all treatments receiving mixture of Saccharomyces cerevisiae and exogenous digestive enzymes (pepsin, papain and α-amylase) supplemented diets. Therefore, digestive enzymes supplementation may be a way to improve nutrients metabolism for anabolic functions to improve growth and feed utilization. **Helmy et al. (1974)** postulated that the increase in serum protein would be resulted when anabolic processes exceeded catabolic ones and reserved protein is being produced in greater quantity to meet the increased metabolic requirements of fish. Thus, an increase in catabolic rate may be a cause of decrease in serum protein level and the cyclic nature of the total serum protein is an indicator of the changes taking place in the serum globulin fraction.

CONCLUSION

From the present study, it could be concluded that supplementation of carbohydrases enzymes to the control diet (32% CP and 3000 kcal DE/kg diet) had a

significant effect on body weight and body weight gain and decreased feed conversion ratio. Also, Nile tilapia can tolerate diets with low CP (30%) and low DE (2900 kcal/kg diet) supplemented with protease and carbohydrases respectively.

REFERENCES

Adeoye, A. (2016). The effects of selected bio-active feed additives on Nile tilapia (Oreochromisniloticus) production and health. Published by University of Plymouth.

Adeoye, A. A., Taramillo-Torres, A., Fox, S. W., Merrifield, D. L. and Davies, S. J. (2016). Supplementation of formulated diets for tilapia (Oreochromisniloticus) with selected exogenous enzymes: Overall performance and effects on intestinal histology and microbiota. Animal Feed Science and Technology, 215:133-143.

Ai, Q. H., Mai, K. S., Zhang, W. B., Xu, W., Tan, B. P., Zhang, C. X. and Li, H. T., (2007). Effects of exogenous enzymes (phytase, non-starch polysaccharide enzyme) in diets on growth, feed utilization, nitrogen and phos- phorus excretion of Japanese seabass, Lateolabrax japonicas. Comp BiochemPhysiolA 147:502-508.

AOAC, (1995). Official Methods of Analysis 16th Ed. Associations of Official Analytical Chemists, Arlington, VA.

Ayhan, V., Diler, I., Arabaci, M. and Sevgili, H. (2008). Enzyme supplementation to soybean based diet in gilthead sea bream (Sparusaurata): effects on growth parameters and nitrogen and phosporus excretion. Kafkas Univ. Vet. Fak. Derg., 14:161-168.

Bedford, M. R. and Cowieson, A. J. (2012). Exogenous enzymes and their effects on intestinal microbiology. Animal Feed Science and Technology 173: 76-85.

Burtle, G. J. (1990). Body composition of farmraisedcatfishcanbecontrolled by attention to nutrition. Foodstuffs. 62:68-70.

Caraway, W.T. (1963). Standard Method of Clinical Chemistry. Academic Press, New York; PP239.

Castanon, J. I. R., Flores, M. P. and Pettersson, D. (1997). Mode of degradation of non-starch polysaccharides by feed enzyme preparations. Anim. Feed. Sci. Tech., 68: 361-365.

Castillo, S. and Gatlin, D. M. (2015). Dietary supplementation of exogenous carbohydrase enzymes in fish nutrition: a review. Aquaculture, 435, 286-292.

Chowdhury , M. A. K., Zhu, J., Cai, C., Ye, Y. and He, J. (2017). Dietary protease modulates nutrient retention efficiencies and hepatopancreatic protease activity in juvenile Chinese mitten crab Eriocheirsinensis. AquacultNutr. 2017; 00:1-7. https://doi.org/10.1111/anu.12627.

Dalsgaard, J., Verlhac, V., Hjermitslev, N. H., Ekmann, K. S., Fischer, M., Klausen, M. and Pedersen, P. B. (2012). Effects of exogenous enzymes on apparent nutrient digestibility in rainbow trout, Oncorhynchusmykiss fed diets with high inclusion of plant-based protein. Animal Feed Science and Technology 171:181-191.

Danicke, S., Simon, O., Jeroch, H. (2003). Effects of dietary fat type and non-starch polysaccharide hydrolysing enzyme addition to rye-based diets on muscle protein turnover in broilers. Br PoultSci 44:245-255.

Davies, S. J. and Gouveia, A. (2008). Enhancing the nutritional value of pea seed meals (Pisumsativum) by thermal treatment or specific isogenic selection

with comparison to soybean meal for African catfish, Clariasgariepinus. Aquaculture 283(1-4):116-122.

Dias, J., Rema, P., Nunes, C., Vogel, K. and Verlhac, V. (2012). Efficacy of dietary protease supplementation on growth performance and nutrient digestibility and utilization of juvenile Nile tilapia fed low fishmeal diets with variable crude protein level. FEEDINFO News Service. http://www.feedinfo.com/console/PageViewer.aspx?page=3105895.

Goda, A., H., Mabrouk, M. A. E. H., Wafa, and T. M., El-Afifi (2012). Effect of using baker's yeast and exogenous digestive enzymes as growth promoters on growth, feed utilization and hematological indices of Nile tilapia, Oreochromisniloticus fingerlings. Journal of Agricultural Science and Technology B 2:15-28.

Hardy, R., Burnell, G. and Allan, G. (2009). Aquaculture feeds and ingredients: an overview. New technologies in aquaculture: improving production efficiency, quality and environmental management, 178, 370.

Helmy, A. M., Badawi, H. K. and El-Bishry, A. (1974). Seasonal variations in the protein composition of blood serum of Anguilla vulgaris and Mugillcephalus, Egyptian Journal of Aquatic Research, 4, 369-375.

IFFO (2013). Present supply status of fish meal and fish oil. Global Aquaculture Advocate.

Isaksen, M., Cowieson, A. and Kragh, K. (2010). Starch-and Protein-degrading Enzymes: Biochemistry, Enzymology and Characteristics Relevant to Animal Feed Use. Enzymes in Farm Animal Nutrition, 85-95.

Kazerani, H. R. and Shahsavani, D. (2011). The effect of supplementation of feed with exogenous enzymes on the growth of common carp (Cyprinuscarpio). Iran. J. Vet. Res., 12, 127-132.

Khalafalla, M. M and El-Hais, A. M. (2013). The influence of Nutrasexylam© enzyme on growth, carcass composition and plasma indices of Nile tilapia fingerlings. Journal of Fisheries and Aquaculture ISSN: 0976-9927 & E-ISSN: 0976-9935, Volume 4, Issue 3.

Kumar, V., Sinha, A., Makkar, H., De Boeck, G. and Becker, K. (2012b). Phytate and phytase in fish nutrition. Journal of animal physiology and animal nutrition, 96, 335-364.

Li, J. S., Li, J. L. and Wu, T. T. (2009). Effects of non-starch polysaccharide enzyme, phytase and citric acid on activities of endogenous digestive enzymes of tilapia (Oreochromisniloticus X Oreochromisaureus). AquacNutr 15:415-420.

Li, X. Q., Chai, X. Q., Liu, D. Y., Chowdhury, M. A. K. and Leng, X. J. (2016). Effects of temperature, pH and feed processing on protease activity and dietary protease on growths of white shrimp, Litopenaeusvannamei, and tilapia, Oreochromisniloticus × O. aureus. Aquaculture Nutrition, 22, 1283-1292.

Li, X. Q., Chai, X. Q., Liu, D. Y., Chowdhury, M. A. K. and Leng, X. J. (2015). Effests of temperature and feed processing on protease activity and dietary protease on growths of white shrimp, Litopenaeusvannamei, and tilapia, Oreochromisniloticus X O.aureus. Aquacult. Nutr. http://dx.doi.org/10.111/anu.12330.

Lin, S., Mai, K. and B., Tan (2007). Effects of exogenous enzyme supplementation in diets on growth and feed utilization in tilapia, Oreochromisniloticus x O. aureus. Aquac. Res., 38:1645-1653.

Mahmoud, M. M. A., Kilany, O. E. and Dessouki, A. A. (2014). Effects of fish meal replacement with soybean meal and use of exogenous enzymes in diets of Nile tilapia (Oreochromisniloticus) on growth, feed utilization, histopathological changes and blood parameters. Life Sci J, 11(2): 6-18.

NRC (1993). Nutrient requirements of fish. National Academy Press, Washington, D. C.

NRC (2011). National Research Council. Nutrient Requirements of Fish and Shrimp, The National Academies Press, Washington D. C.

Ragaa, N. M., Abu Elala, N. M., Kamal, A. M. and Kamel, N. F. (2017). Effect of a serine-protease on performance parameters and protein digestibility of cultured Oreochromisniloticus fed diets with different protein levels. Pak. J. Nutr., 16:148-154.

Shi, Z., Li, X. Q., Chowdhury, M. A. K., Chen, J. N. and Leng, X. J. (2016). Effects of protease supplementation in low fish meal pelleted and extruded diets on growth, nutrient retention and digestibility of gibel carp, Carassiusauratusgibelio. Aquaculture, 460, 37-44.

Tacon, A. G. and Metian, M. (2013). Fish matters: Importance of aquatic foods in human nutrition and global food supply. Reviews in Fisheries Science, 21, 22-38.

Tietz, N.W. (1986). Text Book of Clinical Chemistry. Philadelphia: W.B. Saunders.

Yatzidis, H. L. (1987). J. Clin. Chem. 23/908.

Yigit, N. O. and Olmez, M. (2011). Effects of cellulase addition to canola meal in tilapia, Oreochromisniloticus, diets. Aquaculture Nutrition 17:494-500.

Yigit, N. O., Koca, S. B., Didinen, B. I. and Diler, I. (2016). Effect of protease and phytase supplementation on growth performance and nutrient digestibility of rainbow trout (Oncorhynchusmykiss, Walbaum) fed soybean meal-based diets. Journal of Applied Animal Research, 46:1, 29-32.

Yildirim, Y. B. and Turan, F. (2010). Effects of exogenous enzyme supplementation in diets on growth and feed utilization in African catfish, Clariasgariepinus. J Anim Vet Adv 9(2):327–331.

Young, D. S. (2001). Effects of disease on Clinical Lab. Tests, 4th ed. AACC.

Zhang, L., Ai, Q. H., Mai, K. S., Li, J., Li, H. T, Zhang, C. X. and Zheng, S. X. (2009). Effects of phytase and non-starch polysaccharide enzyme supplementation in diets on growth and digestive enzyme activity for Japanese seabass, LateolabraxJaponicus C. ActaHydrobiol Sin 33:82-88 (In Chinese with English Abstract).

Zijlstra, R., Owusu-Asiedu, A. and Simmins, P. (2010). Future of NSP-degrading enzymes to improve nutrient utilization of co-products and gut health in pigs. Livestock Science, 134, 255-257.

الملخص العربي

تأثير استخدام انزيمات الهضم في علائق البلطي النيلي على معدلات النمو والصحة

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اجريت هذه الدراسة لتقييم اضافة نوعين من الانزيمات (انزيمات هضم البروتين وانزيمات هضم الكربوهيدرات من غير السكريات والنشا) بنسبة ٠,١ جرام لكل كجم علف.

اجريت التجربة علي مجموعة من اسماك البلطي النيلي (١٩٦سمكة) وزنها يتراوح من ٨-١١ جرام قسمت الي سبع مجموعات متساوية في الوزن واستخدمت الاحواض الزجاجية (عدد ١٤ حوض) بواقع حوضين لكل مجموعة (١٤ سمكة بالحوض) واجريت التجربة لمدة ١٢ اسبوع.تم اضافة الانزيمات كلا علي حدة للمجموعة الضابطة التي تحتوي علي ٣٢% بروتين خام و ٣٠٠٠ كيلو كالوروي طاقة هضم لكل كجم علف.واستخدمت عليقة منخفضة البروتين (٣٠%) وعليقة قليلة الطاقة (٢٩٠٠ كيلو كالوري لكل كجم علف) بدون اضافة انزيمات اليهما. كما تم اضافة انزيمات هضم البروتين لعليقة منخفضة في البروتين الخام (٣٠% بروتين) وتم اضافة انزيمات هضم الكربوهيدرات لعليقة قليلة في طاقة الهضم (٢٩٠٠ كيلو كالوري لكل كجم علف).تم قياس: الوزن كل اسبوعين – العلف المستهلك يوميا – نسبة تحويل العلف – كفاءة تحويل البروتين وفي نهاية التجربة اخذت عينات من دم الاسماك (عدد ٦ من كل مجموعة) لفصل مصل الدم وقياس كل من البروتين الكلي – الالبيومين – الجلوبيولين – الكرياتينين – اليوريا – وانزيمات الكبد. كما اخذت عينات من الاسماك من كل مجموعة (عدد ٦) لقياس مكونات الجسم من الرطوبة والدهون والرماد.

ادت التجربة الي النتائج التالية:

اضافة انزيمات هضم الكربوهيدرات للمجموعة الضابطة التي تحتوي علي ٣٢% بروتين خام و ٣٠٠٠ كيلو كالوروي طاقة هضم لكل كجم علف أدت الي تحسن معدلات النمو (زيادة الوزن - أفضل معدل تحويل علف – افضل معدل تحويل بروتين). لم تسجل فروق معنوية واضحة في معدلات النمو بين المجموعة الضابطة والمجموعات الخمس الاخري. لم تسجل فروق معنوية واضحة في معظم قياسات مصل الدم (انزيمات الكبد - الكرياتنين - اليوريا) بين المجموعة الضابطة والمجموعات التجريبية الاخري. زيادة معنوية واضحة في مستوي البروتين الكلي والجلوبين في مصل الدم في المجموعة الخامسة (قليلة البروتين مع اضافة انزيمات هضم البروتين) والمجموعة السابعة (قليلة الطاقة مع اضافة انزيمات هضم الكربوهيدرات) مقارنة بالمجموعات التجريبية الاخري. لا توجد فروق معنوية في نسبة البروتين الخام - الدهون - الرماد في جسم السمك المطحون في المجموعات التجريبية.

وخلصت نتائج التجربة الي:

اضافة انزيمات هضم الكربوهيدرات (من غير السكريات والنشا) الي علائق البلطي ادت الي تحسين معدلات النمو دون تأثير علي مكونات مصل الدم و الكبد ومكونات الجسم. كما ان اضافة الانزيمات ادت الي المحافظة علي معدلات النمو في حالة تغذية علائق منخفضة في البروتين والطاقة.