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THE INFLUENCE OF NUTRASEXYLAM© ENZYME ON GROWTH, CARCASS COMPOSITION AND PLASMA INDICES OF *Nile tilapia* FINGERLINGS

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Abstract- This study was carried out to investigate effect of Nutrasexylam© enzyme on growth performance, whole body composition and hematological indices of *Nile tilapia*, *Oreochromis niloticus* fingerlings. A total of 120 *Nile tilapia* fingerlings with an average body weight of 15.29 ±1.10 g were fed diets supplemented with at 0, 0.05, 0.10 and 0.15 g/kg diet of Nutrasexylam© enzyme. Experimental diets were formulated as dry pellets to contain about 30.11% crude protein and gross energy about 4.43 kcal/ g. Results show that, Nutrasexylam© enzyme supplementation had a significant effect ($p \leq 0.05$) on all growth parameters of experimental fish groups. However, the whole body contents were no affected significantly ($p \leq 0.05$) by supplementation. The same trend of insignificant effect ($p \leq 0.05$) of enzyme was detected with hematological indices of experimental fish. So it can be concluded that, the supplementation of Nutrasexylam© especially, at 0.10 g/kg diet, detected the preferable results for growth performance without harmful effects on hematological characteristics and liver function of experimental fish.

Keywords- Nutrasexylam©, *Oreochromis niloticus*, feed additive, growth parameters

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Introduction

Feed is the major operational cost for most aquaculture enterprises. Formulation of well-balanced diets and their adequate feeding are the most important for successful aquaculture. The nutritive value of formulated feeds depends on the digestibility of the individual components. Thus, the first task in evaluating the potential of any foodstuff for inclusion in a diet is the measurement of its digestibility. The usual strategy in formulating diets is to reduce costs by maximizing the inclusion of carbohydrate while simultaneously sparing expensive protein. At present, artificial diets are available but they have been designed primarily for penaeid species and can contain up to 50% of relatively expensive animal based ingredients such as fishmeal. Cost-effective artificial diets specifically formulated for mud crabs will be required to ensure the development of a sustainable culture industry in the future. For many aquaculture species, fish meal replacement has become a priority issue due to increasing price of fish meal and unsustainable pressures on wild fisheries to satisfy demand for this product [1-3]. Nowadays, exogenous enzymes are extensively used all over the world as additives in fish diets to improve the nutritional value of fish feeds, especially with the raise of using plant proteins in aqua feeds and reduce water pollution [4]. A wide variety of exogenous enzyme cocktails able to degrade non-starch polysaccharides (NSPs), which consider indigestible compounds to fish and other monogastric animals. Some enzymes present in these cocktails may also neutralize some of the anti-nutritional factors present in soybean meal. This may help mitigate the negative effects of high levels of soybean meal on growth and feed utilization by fish.

Tilapia are mainly lacustrine fish that can be reared in both freshwater and seawater; they produce high yields and are thus an important human protein source. Tilapia aquaculture is important, particularly for the lesser-developed countries in the tropics [5]. *Nile tilapia*, *Oreochromis niloticus* are considered as the most common and popular fish in Egypt. Egypt is a country where, arguably, the farming of tilapia has its roots [6]. Tilapia occupies the 10th order concerning the world production from aquaculture [7] and consists 78% of the Egyptian tilapia production from fish culture [8]. So, the present study aims to determine the effect of Nutrasexylam© enzyme on the growth, whole body composition and plasma indices of *Nile tilapia*, *Oreochromis niloticus* fingerlings.

Materials and Methods

This study was performed at Wet Fish Laboratory, Department of Animal Production, Faculty of Agriculture, Kafr El-Sheikh University, Egypt.

Experimental Diets and Design

The experimental diets formulation and chemical composition are provided in [Table-1]. The control diet was formulated from locally

available ingredients including herring fish meal, soybean meal, yellow corn, rice bran, wheat bran, sunflower oil and vitamins and were supplemented with graded enzyme at levels 0, 0.05, 0.10 and 0.15 g/kg diet. Nutrasexylam© enzyme was obtained from Nutrexnv, Achterstenhoek 5, 2275 Lille, Belgium. Nutrasexylam© enzyme contained a mixture of β- xylanase (6300u/g) and α-amylase (40000u/g). Diets were formulated as dry pellets to contain about 30.11 % crude protein and gross energy about 4.43 kcal/ g and offered to fish in equal proportions, six days a week at a rate of 3% of fish biomass daily for a period of 12 weeks [9]. Quantity of the supplementary feed was re-adjusted biweekly according to the increase in fish body weight.

Fresh tap water was stored in fiberglass tanks for 24h under aeration for dechlorination. One third of all aquaria water was replaced daily. Air stones were used for aerating the aquaria water. Feces and feed residues were removed daily by siphoning. Fish from each replicate were weighted at the start of the experiment, counted and weighted every two weeks throughout the experimental period (12 weeks).

Table 1- Composition and proximate analysis of the experimental diet

Items	Control diet (On DM basis, %)
Feed ingredients	
Herring fish meal, 72% CP	10
Soybean meal, 44% CP	42
Yellow corn	24
Rice bran	10
Wheat bran	10
Sunflower oil	3
Vitamins[1]	0.5
Minerals premix[2]	0.5
Total	100
Chemical composition %	
Dry matter	88.98
Crude protein	30.11
Ether extract	8.19
Crude fiber	7.5
Total ash	7.71
Nitrogen free extract[3]	46.49
Calculated energy value	
GE (kcal/kg)[4]	4437
DE (kcal/kg)[5]	3328
P/E,mg/kcal[6]	90.47

[1]Mineral premix consisted of (mg kg−1 premix): 2600 mg Mn, 600 mg Cu, 6000 mg Fe, 4600 mg Zn, 50 mg Se, 100 mg Iu, 50 mg Co, 100,000 mg choline chloride, up to 1 kg carrier (Local market).
[2]Vitamin premix consisted of (mg kg−1 premix): 1,200, 000 IU Vitamin A, 400,000 IU Vitamin D3, 3000 IU Vitamin E, 1200 mg K3, 5400 mg C, 200 mg 2, 200 mg B1, 3360 mg B2, 7200 mg B3, 9000 mg B5, 2400 mg B6, 600 mg B9, 4 mg B12(Local market).
[3]Nitrogen free extract concluded by difference
[4]GE (Gross energy) was calculated according to [13] by using factors of 5.65, 9.45 and 4.22 K cal per gram of protein, lipid and carbohydrate, respectively.
[5]DE (Digestible energy) was calculated by applying the coefficient of 0.75 to convert gross energy to digestible energy according to [36].
[6]P/E (protein energy ratio) = crude protein x 1000 / digestible energy/100g
***Diets: D1 (control): without supplements*
***Values of diets content were within the range suggested for tilapia by [9] and [13].*

Experimental Fish

Nile tilapia, *Oreochromis niloticus* fingerlings (n=120) used in this experiment were brought from a fresh water commercial farm in Motobas, Kafr El-Sheikh Governorate, Egypt. Prior to the start of the experiment, fish were placed in a fiberglass tank and randomly distributed into 12 glass aquaria to be adapted to the experimental condition until starting the experiment. Fish were fed on the basal diet for two weeks, during this period healthy fish at the same weight were replaced instead of died ones. Fish were measured and weighted individually at the beginning of the experiment and divided into twelve groups with a stocking density of ten fish per aquarium with mean initial body weight of 15.29 ±1.10 g. All the experimental treatments were conducted under an artificial photo period equal to natural light/darkness period (12h light: 12h darkness). Fish feces and feed residue were removed daily by siphoning.

Water Quality

Water temperature and dissolved oxygen were measured every other day using an YSI Model 58 oxygen meter. Water samples were taken each two days for ammonia and pH analysis. Analytical methods were done according to the American Public Health Association [10].Total alkalinity and chloride was monitored twice weekly using the titration method; pH was monitored twice weekly using an electronic pH meter (pH pen; Fisher Scientific, Cincinnati, OH). During the 12 weeks feeding trial, the water-quality parameters averaged (± SD): water temperature, 27.7 ± 0.8 ºC: dissolved oxygen, 6.20 ± 0.5 mg l-1: water ammonia 0.05 ± 0.03 mg l-1: and pH, 7.2 ± 0.3. It noticed that, the estimated water quality parameters were within the normal ranges for fish growth [11].

Chemical Analysis

Proximate chemical analyses were made for diet ingredients and a sample of fish at the beginning and end of the experiment according to standard methods [12] for dry matter, crude protein, ether extract, crude fiber and ash. Gross Energy (GE) contents of the experimental diets and fish samples were calculated by using factors of 5.65, 9.45 and 4.22 kcal/g of protein, lipid and carbohydrates, respectively [13].

Blood Parameters Determination

At the end of the experiment fish in each aquaria were weighed and three blood samples were taken randomly from the caudal vein for blood analysis and differential leukocyte count, Anti coagulated blood samples were prepared immediately for counting red and white blood cells etc.

Red blood cells count (RBCs×10^6|mm^3) and white blood cells count (WBCs×10^3|mm^3): were measured on an A bright- line Haemocytometer model (Neubauer improved, Precicolor HBG, Germany) by using a commercial kits (Ranox company, Germany) according to the method described by Stoskopf [14]. Hemoglobin concentration (Hb gm/dl): was determined according to the method of Zinkl [15]. Packed cell volume (PCV %): was estimated by the microhaematocrite method as described by Dacie & Lewis [16].

Total proteins (TP) was measured according to the method of Henry [17] using reagent kits obtained from Diamond Diagnostic Company (Egypt). ALT (U|L) and AST (U|L): Alanine Aminotransferase (ALT) and Aspartate Aminotransferase (AST) activities were assayed according to the method of Reitman & Frankel [18] using reagent kits purchased from Randox Company (UK).

Statistical Analysis

The obtained numerical data were statistically analyzed using SPSS [19] for one-way analysis of variance. When F-test was significant, least significant difference was calculated according to Duncan [20].

Results

Growth Performance

Growth performance parameters of *Nile tilapia*, fingerlings fed on the experimental diets treated with different Nutrasexzylam® enzyme levels are presented in [Table-2].

Table 2- Growth performance parameters of Nile tilapia (O. niloticu s) fed on the experimental diets

Items	Diets No (On DM basis, %)				SE*
	D_1, Control	D_2	D_3	D_4	
Initial weight, g/fish	15.32	15.28	15.34	15.22	1.1
Final weight, g/fish	45.13b	46.79b	50.16a	49.84a	1.11
Average total gain[1], g/fish	29.81b	31.51b	34.82a	34.62a	1.88
Average daily gain[2], g/fish/day	0.35b	0.38ab	0.41a	0.41a	0.08
Specific growth rate[3] (SGR % /day)	1.29b	1.33b	1.41a	1.41a	0.22
Survival rate[4], %	93	97	100	100	1.98
Feed intake (FI), DM g/fish	57.86a	53.94b	51.61b	53.10b	2.04
Feed conversion ratio[5] (FCR)	1.73a	1.52b	1.32c	1.36c	1.02
Protein efficiency ratio[6] (PER)	1.92c	2.18b	2.52a	2.43a	0.25
Protein productive value[7] (PPV, %)	31.23c	34.74b	39.42a	40.20a	1.48
Energy retention[8] (ER, %)	17.50a	8.00c	13.40b	10.30bc	0.7

**Means in the same rows having different superscript letters were significantly different at 0.05 levels*

1. ATG (g/fish) =Average final weight (g) – Average initial weight (g).
2. ADG (g/fish/day) = [ATG (g)/experimental period (d)].
3. SGR (%/day) = 100(Ln final weight–Ln initial weight)/experimental period (d).
4. SR =100 [Total No of fish at the end of the experimental/Total No of fish at the start of the experiment].
5. FCR = DM Feed Intake (g)/Live weight gain (g).
6. PER = Live weight gain (g)/ Protein intake (g).
7. PPV (%) =100 [Final fish body protein (g)–Initial fish body protein (g)]/crude protein intake (g).
8. ER (%) = (%Energy in fish carcass (kcal) at the end - Energy in fish carcass (kcal) at the start) ×100 /Energy intake (kcal).

Data of the present study explained that addition of enzyme to the experimental *Nile tilapia* diets had a significant effect ($p \leq 0.05$) on final weight, ATG, ADG, SGR, FCR and ER estimations. The acceptable tendency of growth parameters were detected with D_3 and D_4. Also, the highest SR of the experimental fish was observed with 0.10 and 0.15% enzyme supplementation (100%). While, the lowest FI was obtained with D_3 followed by D_4, D_2 and D_1 (51.61, 53.10, 53.94 and 57.86, respectively). On the other hand, for protein parameters, it was reported that tilapia groups fed diets containing Nutrasexylam© enzyme at 0.10 or 0.15 gkg-1 had higher PER ratio (2.52 and 2.43) and PPV % (39.42 and 40.20%) than the 0 (control, 1.92 and 31.23%) and 5 g kg-1 (2.18 and 34.37%) groups. Enzyme addition decreased energy retention percentage significantly ($p \leq 0.05$), especially with 0.05 gkg-1 diet (D2).

Body Composition

The influence of Nutrasexzylam® on whole-body composition of *Nile tilapia*, *O. niloticus* fingerlings is shown in [Table-3].

Table 3- Effect of Nutrasexylam© on Nile tilapia body composition (%, on DM basis)

Items	Initial fish	Diets				SE*
		D_1,Control	D_2	D_3	D_4	
Dry matter (%)	23.01	26.51	26.11	26.32	26.28	0.22
Crude protein (%)	53.48	56.22	56.48	56.78	56.71	1.24
Ether extract (%)	17.92	20.21	19.62	19.88	19.76	0.52
Ash (%)	12.61	15.42	15.32	15.5	15.49	0.27
Energy (Kcal/100g)	539	543	540.7	541.8	541.1	4.51

**Means in the same columns having different superscript letters were significantly different at 0.05 levels.*

In general Nutrasexylam© enzyme had no significant ($p \geq 0.05$) influence on whole body composition of experimental fish as dry matter, protein, ether extract, ash (%) contents and gross energy (kcal 100g-1/ body weight). The control diet (D1) had the highest DM followed by D3, D4 and D2 (26.51, 26.32, 26.28 and 26.11%, respectively). However, D3 diet recorded higher CP and ash contents (56.78 and 15.50%, respectively). A slight decrease was observed for fish body EE content as 2.92, 1.63 and 2.23% by enzyme addition at 0.05, 0.10 and 0.15%, respectively compared with control fish. The same trend of fish body EE was recoded with D1 which gave the highest value of fish body energy (543 Kcal/100g).

Hematological and Biochemical Parameters

Hematological and biochemical parameters of *Nile tilapia* fed on the experimental diets containing different levels of Nutrasexzylam® are showing in [Table-4].

Table 4- Hematological and biochemical parameters of Nile tilapia fed on the experimental diets containing different levels of Nutrasezylam®

Items	Treatments				SE*
	Control, D1	D2	D3	D4	
Blood picture					
T. W. BC[1]s (10^3/ mm^3)	21.8	22.14	22.33	22.1	2.18
T. R. BC[2]s (10^6 / mm^3)	3.55	3.65	3.59	3.65	0.09
Hb[3] (g / dl)	7.4	7.54	7.39	7.48	0.4
PCV[4] (%)	21.22	22.4	21.9	22.16	2.42
Biochemical parameters Protein					
Total Protein (g/dl)	3.2	3.24	3.42	3.33	0.24
Albumin (g /dl)	1.35	1.41	1.33	1.34	0.09
Globulin (g/dl)	1.9	1.83	2.09	1.99	0.1
Liver function					
AST[5] (U /L)	60.21	58.45	59.55	60.54	2.45
ALT[6] (U /L)	20.87	20.67	21.08	20.84	0.18

**Means in the same columns having different superscript letters were significantly different at 0.05 levels.*
***TWBC[1] = Total white blood cell, TRBC[2]= Total Red blood cells, Hb[3] = Hemoglobin concentrations; PCV[4] = Mean corpuscular volume, ALT[5] = Alanine Aminotransferase and AST[6] =Aspartate Aminotransferase.*

The present study reflected that, Nutrasexzylam® enzyme had no significant influence ($p \geq 0.05$) on blood serum parameters including TWBCs, TRBCs, Hb, and PCV. The higher count of TWBCs was detected with D3 (22.33 103/mm3), while the higher count of TRBCs was found with D2 and D4 (3.65 103/mm3).The same trend of insignificant influence ($p \geq 0.05$) of enzyme addition was obtained with parameters of serum protein (Total protein, albumin and globulin). In general, enzyme addition at 0.10 gkg-1 had the best value of blood total protein and globulin (3.42 and 2.09 g/dl). Also, liver activity indices as AST and ALT concentration was not affected significantly ($p \geq 0.05$) by enzyme addition.

Discussion

In this study the tested fish readily accepted the experimental diets. The results showed that, Nutrasexylam© enzyme addition improved ($p \leq 0.05$) growth performance of tilapia fish significantly. The acceptable tendency of growth parameters and SR were detected with additional Nutrasexylam© enzyme at 0.10 and 0.15 gkg-1levels. Khalafalla, et al [21] reported that, the addition of cocktail of Amylease, Xylanase, Protease, Cellulose, Lipase, Phytase, B-Glucanase and Alphagalactosidase enzymes (amecozyme®) in the diets at levels of 0.5 and 1.0% improves and enhance the growth performance of *Nile tilapia*, *Oreochromis niloticus*, fingerlings. Also,

for feed intake and growth rate, it was reported that Carp fed diets containing multi-enzyme premix at 5 or 10 gkg-1 had 12.3 and 27.5% faster growth rate than the control [22]. It can explained that the improved performances in monogastics fed enzyme supplemented plant protein/leaf meals is also due to breakdown of compounds, which would have increased digesta viscosity and resultant low digestibility. So, Increase in digesta viscosity slow the rate of digestion and absorption [23]. Also, Arnesen, et al [24] suggested that a large fraction of the potential carbohydrate energy is not available to salmonids because most of the soybean polysaccharides cannot be absorbed. This carbohydrate fraction is unavailable because salmonids only have the enzyme necessary to digest starch and starch makes up less than 1% of soybean meal. This carbohydrate fraction is unavailable because salmonids only have the enzyme necessary to digest starch and starch makes up less than 1% of soybean meal. In agreement with the pervious results, one such product, designed specifically for use in high-wheat feeds for poultry, contains endo-xylanase, which breaks down pentose sugars. A similar product breaks down glucans found in wheat, barley, triticale and rye, releasing glucose [25].

Data of the present experiment explained that, no significant ($p \geq 0.05$) differences were detected among different fish groups for whole body composition as DM, protein, ether extract, ash contents (%) and gross energy (kcal 100g-1/ body weight) by Nutrasexylam© addition. Similar trend was detected by Ng & Chong [26] who indicated that exogenous enzyme Superzyme® enzyme (cocktail of xylanase, amylase, cellulase, protease and β-glucanase enzymes) supplementation in the diets did not have any effect on the whole body composition of tilapia. They explained that, no significant effect ($p \geq 0.05$) of soybean meal inclusion, Superzyme® enzyme or interaction between SBM and enzyme supplementation on crude protein, lipid, ash and phosphorus of whole body carcass of fish fed the experimental diets. Superzyme® enzyme supplementation had a significant ($p \geq 0.05$) effect on gross energy content of whole body carcass but no effect of SBM inclusion or interaction between SBM and enzyme supplementation on energy content of whole body carcass was observed [27].

Lin, et al [28] Showed that the pervious result is in agreement with the study on tilapia fed by exogenous enzyme [commercial enzyme complex (neutral protease, b-glucanase and xylanase)] show no significant difference ($p \leq 0.05$) in whole body moisture, protein, lipid and ash. On the other hand, Osman & Nour [29] suggested that, the chemical constituents of amecozyme® (Amylease, xylanase, protease, cellulose, lipase, phytase, B-glucanase and alphagalactosidase) had slight positive influence on fish body composition. Also, significant differences ($p \leq 0.05$) were obtained in DM and CP contents of tilapia at the end of the experimental period while, the differences between different levels of amecozyme® enzymes were low [21]. However, EE, ash and GE had insignificant ($p \geq 0.05$) differences. These results in this study may be detected because the experimental period (12 weeks) is not enough to appear the effect of enzyme supplementation on whole body composition of tilapia fish.

The same trend of insignificant effect ($p \leq 0.05$) of Nutrasexylam© enzyme was observed with hematological characteristics as TWBCs, TRBCs, Hb, and PCV. The biochemical composition of fish serum protein including total protein, albumin and globulin values also were not influenced significantly ($p \leq 0.05$). On the other hand, Goda, et al [30] found that red blood cells counts, hematocrit and hemoglobin 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. The same trend was observed for total plasma protein and total plasma globulin levels. Also, Dobšíková, et al [31] obtained similar values of WBC and differential cell counts for normal healthy common carp. Helmy, et al [32] reported that the increase in serum protein would result when anabolic processes exceeded catabolic ones, and reserved protein is being produced in greater quantity to meet increased metabolic requirements of fish. Thus, an increase in catabolic rate may due to decreases 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. In this experiment globulin concentration was a higher with R3 ration than the other tested rations. Globulin level in blood serum may be a preferable indicator for antibody bodies and so the level of specific immunoglobulin [33]. However, Tizard [34] reported that most techniques employed to investigate the immune state of animals are those that depend on detection and measurement of antibody in blood serum and other body fluids. Thus, the significant reduction in these parameters is an indication of severe anemia [35]. Moreover, the measurement of AST and ALT in plasma is of considerable diagnostic value in fish, as it relates to general nutritional status as well as the integrity of the vascular system and liver function. The present results referred that, concentrations of AST and ALT in plasma were in a normal range, so enzyme supplementation had no adverse effect on fish health.

Conclusion

So it can be summarized that, under the same condition of this study. The supplementation of Nutrasexylam© especially, at 0.10 g/kg diet, detected the preferable results for growth performance without harmful effects on hematological characteristics and liver function of experimental fish.

Conflicts of Interest : None declared.

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