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Effects of rare earth elements and exogenous multi-enzyme supplementation to plant protein enriched diet on growth performance, digestibility and economic efficiency of Nile tilapia, *Oreochromis Niloticus*

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Abstract

This study aimed to improve the nutritional value and utilization of plant protein enriched diet for Nile tilapia, *Oreochromios Niloticus*. The effects of Rare Earth Element (REEs) and two supplemental exogenous multi-enzymes on growth performance, body composition, nutrients digestibility and economic efficiency of Nile tilapia fingerlings were examined over 12-week period. Nile tilapia fingerlings (n=400) averaging 22.61± 0.07g divided into four groups. The 1st group received control diet with no additives the 2nd received the control diet supplemented with 0.05% REEs, 3rd and 4th groups received the control diet supplemented with Roxazyme G2® at 100mg/kg and Avizyme 1500 at 1 gm/kg, each with 0.5 gm/kg phytase, respectively. All growth parameters and condition factor of Nile tilapia were improved by supplementing REEs, Roxazyme and Avizyme when compared with control group. The highest value of Tilapia protein content was observed by addition of these additives. On other hand, the nutrients digestibility were enhanced after addition of Avizyme, Roxazyme and REEs. Additionally, better digestibility for CF was recorded with Avizyme and Roxazyme groups followed by REEs group. Moreover, these additives had positive returns when compared with control group. So, it could be recommend that adding of REEs and exogenous multi-enzymes improve fish growth and profitability by improving the nutrients digestibility of such feed ingredients consequently increasing fish gain.

Keywords: Growth performance, Digestibility, Enzyme supplement, Nile tilapia, Rare earth element.

Introduction

Aquaculture has a great prospective in food production industry and utilization of plant based proteins in aqua feed has an economic impact. However, a number of problems aroused from their inclusion which include the presence of anti-nutritional factors (ANFs), non-starch polysaccharides (NSP) and protease inhibitors which may impair nutrient digestibility and utilization and fish performance[6]. On other hand, Oil seed meals as sun flower meal have been found to have Considerable economic potential[12]. Fish don't have endogenous enzymes for hydrolysis of NSP as xylanase and glucanase which present in highly fibrous feed stuff.

Supplementation of exogenous enzymes can improve nutrient digestibility and eliminate the effects of ANFs in plant-based feedstuffs resulted in better performance of fish [10]. Additionally, REEs are the 15 lanthanide elements which are in group III A of the periodic table. The role of REEs in enhancing performance at low concentrations have been described for approximately all animals and fish[18].

The use of carbohydrases has not been as nearly as common in aquatic species and the same for REEs. Therefore, our study was designed to evaluate the effects of REEs and commercially prepared exogenous multi- enzyme plus phytase on growth performance, nutrient utilization, digestibility and economic efficiency of Nile tilapia fed on plant protein enriched diet.

Materials and methods

Experimental Fish and Culture:

All experimental protocols were approved by the animal care and use committee at Faculty of Veterinary Medicine, Zagazig University. A total of 400 Nile tilapia fingerlings averaging 21.61±0.07 g (mean ± SD) were divided into equal four groups (each group divided into 5 replicate, 20 fish each). Each fish group was stocked in its corresponding cage in clean concrete pond. The mean water temperature, dissolved oxygen found to be 26±1 °C, 5.5±.05mg/l, pH, ammonium (NH4), nitrite and nitrate were measured and found to be 7.1 ±0.03, 0.2 mg/l, 0.025 mg/ l and 6 mg/ l, respectively.

Feeding management and Digestibility trial:

The 1st group received control diet with no additives as described in table 1. The 2nd received the control diet supplemented with 0.05% REEs, 3rd and 4th groups received the control diet supplemented with Roxazyme G2® at 100mg/kg and Avizyme 1500 at 1 gm/kg, each with 0.5 gm/kg phytase, respectively. Roxazyme G® obtained from DSM Nutritional Products (UK) Ltd and consists of cellulose beta-glucanase and xylanase. Avizyme 1500 purchased from the nutritional additive company in Egypt (multivita Co.) and contained xylanase, protease, and amylase. REEs supplied from ThyssenKrupp Metallurgical Products GmbH, Germany. Fish groups were fed their respective diets at a level of 3% of body weight. Fish were weighed every 3 week through the feeding trial. After the end of feeding trail,

Table 1. Dietary formulation and proximate composition of the control diet for Nile tilapia.

Ingredients	%	Calculated Composition	(%)
Fish meal	20	DM	89.60
Soy bean meal	26	CP	32.30
Sunflower rmeal	12.4	EE	9.22
Yellow corn	21	CF	6.02
Rice bran	15	Ash	8.38
Fish oil	3.2	NFE	42.38
L-Lysin HCL	0.1	lysine	2.05
D L-Methonine	0.2	methonine	0.82
Throenine	0.1	throenine	0.64
Calcium dibasic phosphate	0.5	DE (Kcal/ kg)[b]	2901.48
Vitamin & mineral premix[a]	1.5		
Total	**100**		

[a]Vitamin and mineral premix (per kg of diet): vitamin A, 4500 IU; vitamin D3, 3200 IU; vitamin E, 4200 mg; vitamin B1, 10 mg; vitamin B2, 15 mg; vitamin B6, 40 mg; vitamin B12, 0.08 mg; vitamin K3, 15 mg; ascorbic acid, 750 mg; nicotinic acid, 200 mg; Ca-pantothenate, 110 mg; folic acid, 4 mg; biotin, 3 mg; inositol, 500 mg; p-amino benzoic acid, 200 mg; Ca, 2.1 g; Fe, 240 mg; Mn, 35 mg; Zn, 55 mg; I, 4 mg; Cu, 13 mg; Se, 0.3 mg; Co, 1 mg.

[b]Digestible energy calculation based on values of protein 3.5 kcal/gm, fat 8.1 kcal/gm, NFE, 2.5 kcal/gm. as reported previously[14]

apparent digestibility was determined by addition of chromic oxide at a rate of 0.5%. Fish fed on diets containing chromic oxide for one week as an adaptation period, then for two weeks as a collection period.

Chemical analysis:

Proximate chemical analyses of feed ingredients and fish were done according to AOAC[2]. Digestibility measurements, were made as stated by Petry and Rapp[13].

Calculation of growth indices and digestibility:

Final body weight, body gain, SGR, FCR, PER and condition factor were calculated as described previously[3]. Apparent digestibility coefficient (DC) was calculated according to NRC[11].

Economic efficiency calculation: Economic efficiency was calculated according to the following equation: Y= [(A-B)/B X 100] where, A is the selling cost of obtained gain and B is the feeding cost of obtained gain [5].

Statistical analysis:

The experimental data were tested by one-way analysis of variance (ANOVA), using spss 18 and Excel 2013 in Microsoft. Differences among treatment were determined by Duncan's multiple range test at a ($P< 0.05$) level of significance.

Results

Growth performance and feed utilization efficiency:

All over growth performance are shown in Table 2. The results cleared that addition of REEs and Roxazyme, Avizyme plus phytase to Nile tilapia diet had achieved an improvement on all over growth performance and feed utilization. In spite of higher growth rate, the cumulative feed intake was decreased after addition of such feed additives when compared with control group.

Table 2. Effects of REEs and Exogenous multi-enzymes Supplementation on all over growth performance of Nile tilapia (means ±SE).

Parameters	Control	REEs	Roxazyme +Phytase	Avizyme +Phytase
Initial BW (g)	22.62 ± 0.17	22.51 ± 0.19	22.68 ± 0.13	22.63 ± 0.10
Final BW (g)	43.84 ^{d} ± 0.32	47.41 ^{c}± 0.64	53.57 ^{b} ± 0.38	56.15 ^{a} ± 0.68
Body gain (g)	21.22^{d} ± 0.17	24.90 ^{c} ± 0.45	30.89^{b} ± 0.46	33.52 ^{a} ± 0.62
SGR (%)	0.79^{d} ± 0.00	0.89 ^{c} ± 0.01	1.02 ^{b} ± 0.01	1. 08 ^{a} ± 0.01
Feed intake (g)	33.08^{a} ± 0.17	31.43^{bc} ± 0.43	31.85 ^{b} ± 0.25	30.80 ^{c}± 0.11
FCR	2.07 ^{a} ± 0.00	1.90 ^{b} ± 0.01	1.73 ^{c} ± 0.01	1.68 ^{d} ± 0.01
Condition factor	2.06 ^{c} ± 0.11	2.20^{bc} ± 0.05	2.39 ^{ab} ± 0.03	2.45 ^{a} ± 0.03
PER	1.38 ^{d} ± 0.00	1.50 ^{c} ± 0.01	1.64^{b} ± 0.01	1.70 ^{a} ± 0.01

Within-row different superscript letters denote significant difference ($P<0.05$)

Nutritive value of fish meat:

According to the body analysis composition data Table 3. at the first and at the end of the Experiment. The DM and CP content were significantly ($P< 0.05$) increased in Fish supplemented with Avizyme, Roxazyme and REE when compared with control group.

Table 3. Effects of REE and Exogenous multi-enzymes Supplementation on body composition of Nile tilapia (means ±SE).

Nutrient (%)	Initial	Control	REE	Roxazyme + Phytase	Avizyme + Phytase
Dry matter	19.22± 0.14	21.00^{c} ±0.29	23.89^{b} ±0.11	25.57^{a} ± 0.73	24.88^{ab}± 0.05
Protein	48.38± 0.30	56.76^{b} ±0.58	60.46^{a} ±0.30	61.59^{a} ± 0.63	61.82^{a}± 0.55
Fat	15.54± 0.28	16.37 ± 0.22	16.43 ±0.12	16.18 ± 0.11	16.33 ± 0.14
Ash	14.33± 0.16	16.17^{c} ±0.11	16.80^{bc} ± 0.15	17.27^{b} ± 0.39	18.83^{a}± 0.42

Within-row different superscript letters denote significant difference ($P<0.05$)

Digestibility studies:

Diets supplemented with REEs and Roxazyme, Avizyme plus phytase had positive effects on nutrients digestibility Table 4. The maximum digestibility for DM, CP and starch increased significantly ($P <0.05$) in the group supplemented with Avizyme followed by groups supplemented by Roxazyme and REEs, in comparison with the control group. While the digestibility of CF and fat was increased ($P <0.05$) with Avizyme and Roxazyme groups followed by REEs group, in comparison with the control group.

Economic efficiency evaluation:

Data on economic performance of Nile Tilapia in different groups are presented in Table 5. Addition of Avizyme, Roxazyme and REEs had a great impact on economic efficiency when compared with control group

Table 4:Effect of Effects of REEs and Exogenous multi-enzymes Supplementation on Economic efficiency.

Parameters	Control	REEs	Roxazyme + Phytase	Avizyme + Phytase
Diet cost (L.E^{c}/kg diet)	4.32	4.60	4.47	4.91
Feeding cost of obtained gain (L.E)	0.14	0.15	0.14	0.15
Fish selling cost of obtained gain (L.E /kg live weight)	14	14	14	14

[c] L.E Egyptian Pound.

Discussion

The present study showed that dietary inclusion REEs and multi-exogenous enzymes with phytase markedly improved growth performance, nutrients digestibility as well as economic efficiency of Nile tilapia fingerlings fed on diet rich in plant protein source. These results in accordance with, Hlophe-Ginindza *et al.*[9] who indicated that addition of cellulase, xylanase and phytase up to 0.5 g / kg to a kikuyu-based diet of *Oreochromis mossambicus* had had higher growth performance, protein digestibility, highest digestive enzyme activities and higher profits due to increased growth. Similarly, Zamini *et al.*[20] reported that inclusion of protease, β-glucanase, α-amylase, xylanase, cellulase, pectinase, lipase by 0.5 g/kg of each caused significant improvement on growth performance and feed utilization of Caspian salmon. Within the same trend, Nile tilapia, *Oreochromis niloticus* fed on Corn gluten and soybean meal Supplemented by pepsin, papain and α-amylase significantly enhanced Growth performance, feed utilization[7]. Moreover, Zhou *et al*.[21] described that exogenous cellulase promoted growth and digestive enzyme activities of grass carp as cellulose have broken down the fibrous components and released bound nutrients for digestion by the fish. Yildirim and Turan[19] described that addition of commercial multi-enzyme complexes increased the African catfish protein content. These all above positive effects of multi- exogenous enzymes in different fish species may be attributed to augmentation of fish's own enzyme production by supplementation of theses enzymes. In this aspect, addition of exogenous proteases in plant based diets increases protein digestion by improving protein hydrolysis[14] or by destroying anti-nutrients such as lectins and trypsin inhibitors[7]. Furthermore, Sinha *et al*.[16] suggested that there are three mechanisms by which exogenous NSP-degrading enzymes enhance nutrient digestion and utilization from plant proteins in fish 1. Disruption of cell wall integrity 2. Reduction of digesta viscosity and 3. Stimulation of bacterial population. Many results indicated that positive effects exogenous carbohydrases inclusion depending on the type and ratio of used feed ingredients and levels of these enzymes. Reduced growth performance and decreased enzyme activities have recently been reported in carp fed diets with exogenous enzymes above the optimal level[10]. All the same, enzyme supplementation reduced feed cost per kg of weight gain by reduction in feed intake and improving feed conversion efficiency as reported by Ani and Omeje[1] on the other hand, inclusion REEs increased weight gain by improving utilization of nutrient and increasing the secretion of digestive enzymes as described by Xu *et al*.[17]. Addition of feed additives resulted in more economically tilapia production[4]

In conclusion, Addition of REEs and multi-enzyme complexes result in improved growth and nutrient utilization of Nile tilapia fed on plant protein enriched diet by increasing nutrient digestibility and utilization efficiency. Furthermore, this response may mediated to some extent by the level and quality of plant protein source used in these diets. As a final point, the economics of REEs and exogenous enzymes was more encouraging where supplemented groups produced more profit than that of control group.

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