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TOWARD MORE ENVIRONMENTALLY FRIENDLY AQUACULTURE: EFFECT OF DIETARY EXOGENOUS ENZYMES (NATUZYME®) ON NITROGEN AND PHOSPHORUS RETENTION AND EXCRETION IN NILE TILAPIA (*Oreochromis niloticus*)

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Abstract

The present study investigated the effect of commercial enzymes mixture, Natuzyme® (NZ) in all-plant based diets on the retention and excretion of both nitrogen (N) and phosphorus (P) in Nile tilapia (*Oreochromis niloticus*). Five iso-nitrogenous (28 % crude protein) and iso-energetic (425 kcal / 100 g) diets were formulated. Fishmeal-based diet (FM-diet) served as a positive control and four soy bean meal-based diets (SBM-diet) were supplemented with 0, 0.5, 1 and 1.5 g NZ/kg diet, respectively. The diets were offered twice daily to apparent satiation in triplicate fish groups (2.52g/fish) for 12 weeks. The N and P supply were significantly higher ($p \leq 0.05$) with FM-diet, while the lowest nutrients supply was recorded with SBM+NZ_1 diet. The apparent retention rate % (ARR%) for N and P showed a positive relation with the level of NZ. The highest ARR% for N and P were obtained with SBM+NZ_4 diet, while the lowest ARR% values were recorded with SBM+NZ_1 diet. The highest fish N content was measured with FM-diet, whereas the highest P content was achieved with SBM+NZ_4 diet. The excreted N% and P g/aquarium showed an opposite trend of ARR%. The NZ had a significant reducing effect on the inorganic fraction of both excreted N and P, where it reduced the dissolved in-organic nitrogen (DIN) as a percent of excreted N and orthophosphate as a percent of excreted P. This study provided fundamental insight to enhance N and P retention efficiency of Nile tilapia and to reduce their excretion through using "environmentally-friendly" additive NZ in plant based diets which in turn limit or at least postpone the eutrophication problem.

Keywords: Nile tilapia, *O. niloticus*; all-plant diets; exogenous enzymes; nitrogen and phosphorus retention and excretion; sustain environment.

INTRODUCTION

To meet the future fish demands, aquaculture must continue to grow up and intensified. However, aquaculture growth is being challenged by the environmental pollution problem, mainly with N and P, as a result of fish feeds addition (Cho and Bureau, 1997). That constrains aquaculture sustainability and push for more responsible production within the framework of legislative and regulatory controls.

Both nitrogen (N) and phosphorus (P), in aquaculture wastewater, originate mainly from fish feeds supplementation (Cho and Bureau, 1997) due to their high content of fishmeal (FM), which is rich in nitrogen (4.8 -8 % N, Trushenski *et al.*, 2006) and P (2 - 4% P, Sugiura and Hardy, 2000). Several studies mentioned that the high N and P content of fish meal-based diets are not well utilized by many fish and crustacean species. Schuenhoff *et al.* (2003) reported that more than 80% of the feed N content and more than75% of the feed-P content are exerted in the water in both dissolved and particulate forms. Abou *et al.* (2010) in concrete tanks found that 80% of total N and 90% of total P supplied were wasted by Nile tilapia. Investigating Salmonid cage culture, Phillips *et al.* (1985) found 79% loss of N and 85% loss of P supplied with feed. Abou *et al.* (2012) reported values ranging from 69.5% to 80.7% and from 89.6% to 91.2%, of N and P loss, respectively. Indeed, the fish intensification leads to the release of higher amounts of N and P, which are known to enrich and promote eutrophication in aquatic ecosystems (GESAMP IMO 1996). As a result, FM-based diets supplementation are considered as a primary cause of aquatic ecosystems pollution coincided with aquaculture that became a critical constrain for sustainability and future expansion of aquaculture industry (Sugiura *et al.*, 2000).

Therefore, and due to the decreasing fishmeal production and increasing demand and price of produced FM, the use of plant based feed in aquaculture is inevitable in the near future (Cho and Bureau, 2001 and Ai *et al.*, 2007). However, plant ingredients have their own restrictions because of the presence of anti-nutritional factors including phytate protease-inhibitors and indigestible

carbohydrates such as non-starch polysaccharides (NSP) (Francis *et al.,* 2001 and Azarm and Lee, 2014).The presence of phytate in the aqua feeds is reported also to have a negative effect on growth performance, nutrient, protein and energy utilization, and mineral uptake as reported by Kumar *et al.* (2012). To escape from anti-nutrient behavior of phytic acid and improve the nutritive value of plant-based diet in aquaculture exogenous phytase enzymes should be supplied to fish feeds (Gabaudan *et al.*, 2006; Baruah *et al.,* 2007 and Hlophe-Ginindza *et al.,* 2016).

It is clear that supplemental phytase can enhance the digestibility and bio-availability of N, P and other minerals and markedly decrease N and P load to aquatic environment (Kung *et al.,* 2000; Cao *et al.,* 2007 and Kumar *et al.*, 2012). However, the impact of phytase on protein and amino acids bioavailability and utilization in fish is somewhat contentious (Teskeredzic *et al.*, 1995; Storebakken *et al.*, 2000; Castillo and Gatlin, 2015; Lemos and Tacon, 2015 and Adeoye *et al.,* 2016). However, Clifford (1989) ; Graham and Inborr (1993) reported that using purified enzymes does not bring about as good an improvement in fish performance as using a number of different enzymes together ('cocktails').

Accordingly, the present study was conducted to define the impact of the commercial enzymes mixture (Natuzyme®) on the N and P retention and excretion in water by Nile tilapia (*O. niloticus*) fed on all-plant based diets.

MATERIALS AND METHODS

Experimental design and set-up:

The present study was conducted to investigate the N and P utilization, nutrient retention and excretion as well as the inorganic fraction of excreted nutrients by Nile tilapia fry, *O. niloticus* fed on all-plant based diets supported with gradual levels of a commercial enzymes mixture (Natuzyme®) in Aquaria. The experimental system that was applied to achieve this goal was described in details in Amer (2017). Fifteen glass aquaria were randomly assigned to 5

triplicate treatments. Each treatment attributed to one of the experimental diets. Diets are prepared to be iso-nitrogenous (28 % crude protein) and iso-energetic (425 Kcal/100g diet) and the dietary P content (% DM) ranged from 0.66 and 0.78%. The experimental diets were formulated to be fish meal-based diet, (FM-diet), or soybean meal-based diet, (SBM-diets). The SBM diets were supplied with 4 gradual levels of a commercial enzymes mixture (Natuzyme®, produced by Bioproton Pty Ltd., Sunnybank, Queensland, Australia) represented as NZ_1, NZ_2, NZ_3 and NZ_4 (to contain zero, 0.5, 1.0 and 1.5 g/Kg diet respectively). Formulation and proximate composition of experimental diets are given in Table (1). Nile tilapia fry (2.52± 0.08 g) from a same cohort were stocked at a density of 20 fry per 100-L aquarium provided with continuous aeration. Fry were hand fed twice daily (9AM and 1 PM) at apparent satiation. Daily feedings were divided into two parts, 6 days a week for 12 weeks.

About half of the water volume in each aquarium was changed daily through the experiment to maintain acceptable water quality in the aquaria. Only in three occasions, water and faces were kept within the aquaria without changing, each for three days and water samples were taken and faces collected to determine the fish nutrients excretion rates.

Chemical analysis:

Samples of the experimental diets were taken in a powder form, and preserved in well-sealed plastic bags in a refrigerator for the chemical analysis. Fish samples at the initiation and the termination of the experiment were also taken and preserved frozen for the chemical analysis. These chemical analysis included dry matter (drying samples in an oven at 85˚C (AOAC, 1990) and nitrogen content was determined applying micro-Kjeldahl method using a micro-Kjeldahl apparatus (Labconco Corporation, Kansas, Missouri,USA). Phosphorus content in fish and diets was determined by persulphate digestion (Gross and Boyd, 1998) with boric acid and sodium hydroxide. After wet digestion, P was analyzed by molybdate-vandate and spectrophotometric (420nm) method (AOAC 1990).

Table 1. Formulation and proximate composition (%, on dry matter basis) of the experimental diets.

Diets	FM-diet	SBM-diet			
		NZ_1	NZ_2	NZ_3	NZ_4
Ingredients (g/100g diets):					
Fish Meal (FM)	10.2	0	0	0	0
Soy Bean meal (SBM)	35	55	55	55	55
Yellow corn	26.0	20.0	20.0	20.0	20.0
Wheat bran	19.5	14.0	14.0	14.0	14.0
Corn oil	3.3	5	5	5	5
Starch	3	3	2.95	2.9	2.85
Mineral mix premix[1]	2	2	2	2	2
Vitamins premix[2]	1	1	1	1	1
Natuzyme® (NZ)[3]	0	0	0.05	0.1	0.15
Proximate composition:					
Dry Matter (DM)	89.73	89.11	89.25	89.25	89.12
Total nitrogen (% DM)	4.51	4.51	4.51	4.51	4.51
Crude Lipid (% DM)	7.72	7.35	7.38	7.36	7.32
Fiber (% DM)	6.8	7.43	7.51	7.50	7.54
Ash (% DM)	9.91	10.0	10.21	10.10	10.12
Phosphorus (% DM)	0.78	0.66	0.66	0.66	0.66
NFE%[*]	47.38	47.03	46.70	46.85	46.81
GE (kcal/ 100 g diet)[**]	425.28	424.73	424.82	424.68	424.85

1, 2: The constituents of mineral and vitamins premixs is reported in Amer (2017).

3: Commercial enzyme mixture (NZ) includes: Cellulase, Xylanase, β-glucanase, protease, α-amylase, phytase and pectinase at the activity (unit/g at 30°C and pH 7.2) of 5000, 10000,1000,6000,1800,500 and 140, respectively. Activity (unit/g): the amount of the enzyme that catalysis the conversion of 1µM of the substrate per minute under specified conditions (temperature and pH).

*NFE is nitrogen free extract =100- (% crude protein+% crude lipid+% crude fiber+% crude ash).

** GE is gross energy (was calculated according to NRC1993)

Routinely, water samples were collected periodically throughout the experimental period from each aquarium for water quality measurements. Water temperature and dissolved oxygen were measured with an YSI model 58 oxygen meter (Yellow Spring Instrument Co., Yellow Spring, Ohio, USA). The pH was measured using a pH–meter (Digital Mini-pH Meter, model 55, Fisher Scientific, USA). Total alkalinity and total hardness were determined weekly according to Boyd and Tucker (1992).

At the beginning and end of the specified three occasions, water samples were collected and total ammonia, nitrite, nitrate and orthophosphate were determined according to the analytical methods of AOAC (1990) in each aquarium.

Calculations:

Apparent retention rate (%) of N or P, nutrient supplied, and the amount of N and P retained and that is discharged into water as wastes (in all forms as faecal + urinary + gill) by fish were evaluated according to Cho *et al.* (1994) as follow:

Apparent retention rate (ARR, %) of N and P = $100 \times (TN_f - TN_i) / (N_{supplied}, g)$

Nutrient supplied (NS, g) = $N_{feed} \times TFI$

Nutrient retained (NR, g) = $NS \times ARR / 100$

Total nutrient waste (TNW, g) = NS - NR

Where:

TN_f and TN_i are the final and initial nutrient (N or P) body content in g,

$N_{supplied}$ is the amount of nutrient (N or P) (g) in the total introduced feed,

N_{feed} is the amount of nutrient (N or P) (g) in introduced feed,

TFI is the total feed intake.

Statistical Analysis:

Data were submitted to one-way ANOVA and were expressed as mean ± SD of the replicates. Differences were considered significant if P was less than 0.05. All statistical analyses were performed using SAS, (SAS Inc. 1988). Significant differences ($P \leq 0.05$) among means were tested by the method of Duncan's new multiple ranges test (Duncan 1955).

RESULTS

Fish survival, growth performance and feed utilization data are presented and discussed in a previous article (Amer, 2017).

During the study span, water temperature ranged from 28.00 to 28.8°C, while pH ranged from 7.4 to 7.8. Dissolved oxygen level (DO) was higher than

5.85 mg DO/l. Total alkalinity and total hardness values ranged from 125 to 165 mg/l and 165-180 mg/l as $CaCO_3$, respectively. No significant differences were detected among the treatments in water quality parameters.

Table (2) showed the amount of supplied feed, N and P supply (g/fish), N and P retention rate (ARR %), retained N and P (NR g/fish), as well as N and P excretion (TNW g/fish). Significant differences ($P \leq 0.05$) were noticed in feed supply among treatments, with the order of FM-diet > $SBM+NZ_3$>$SBM+NZ_4$> $SBM+NZ_2$>$SBM+NZ_1$. Subsequently, the same trend with significant differences was noticed in both supplied N and P among treatments with the same order.

With respect to N and P apparent retention rate % (ARR %) and retained N and P (NR as g/fish), there were significant differences ($P \leq 0.05$) among treatments with a positive relationship with the level of NZ. The highest value of ARR% for N was recorded at the $SBM+NZ_4$ followed by that at FM-diet treatment; both did not differ significantly. However, the other treatments showed lower values significantly ($P \leq 0.05$), with lowest value at $SBM+NZ_1$treatment. It is also worthy to mention that the N ARR% at $SBM+NZ_3$ did significantly differ from that of FM-diet.

With respect to the P ARR %, significant ($P \leq 0.05$) highest values were noticed at the $SBM+NZ_4$ treatment, followed by that at $SBM+NZ_3$. FM-diet was lower significantly ($P \leq 0.05$) than the former. It was noticeably, that the value of P ARR% at FM-diet and $SBM+NZ_2$ did not significantly differ. The lowest value was recorded at the $SBM+NZ_1$.

Table 2. Overall nitrogen and phosphorus budget from Nile tilapia fed different experimental diets for 12 weeks.

Diets	FM-diet	SBM-diet+NZ_1	SBM-diet+NZ_2	SBM-diet+NZ_3	SBM-diet+NZ_4
Feed supplied (g/fish)	33.69 ±0.09 [a]	26.83 ±0.25 [d]	30.44 ±0.14 [c]	32.23 ±0.03 [b]	32.21 ±0.05 [b]
N supplied (g/fish)	1.52 ±0.004 [a]	1.21 ±0.011 [d]	1.38 ±0.006 [c]	1.46 ±0.001 [b]	1.46 ±0.002 [b]
P supplied (g/fish)	0.263 ±0.001[a]	0.177 ±0.002[d]	0.201 ±0.001[c]	0.213 ±0.000[b]	0.213 ±0.000[b]
N apparent retent-ion rate % (ARR%)	35.98 ±0.724[ab]	31.58 ±0.546[c]	32.67 ±1.324[c]	34.64 ±0.951[b]	37.17 ±1.124[a]
P apparent retent-ion rate % (ARR%)	33.258±0 .908[c]	26.967 ±0.769[d]	32.985 ±2.461[c]	37.144 ±1.701[b]	44.048 ±0.993[a]
N retained (g/fish) NR	0.547 ±0.011 [a]	0.383 ±0.010 [d]	0.450 ±0.017[c]	0.505 ±0.014[b]	0.541 ±0.017[a]
P retained (g/fish) NR	0.087 ±0.002 [b]	0.048 ±0.002 [e]	0.066 ±0.005 [d]	0.079 ±0.004 c	0.094 ±0.002 a
Total N waste (g/fish) TNW	0.975 ±0.011 [a]	0.830 ±0.001 [d]	0.928 ±0.022 [bc]	0.952 ±0.013 [ab]	0.915 ±0.016 [c]
Total P waste (g/fish) TNW	0.175 ±0.003 [a]	0.129 ±0.002 [b]	0.135 ±0.005 [b]	0.134 ±0.004 [b]	0.119 ±0.002 [c]

In each line, different superscripts letters mean significantly differed values ($P \leq 0.05$). Data are means (Mean±SD) of three replicates.

The NR (g/fish) for both nutrients (N and P) showed significant ($P \leq 0.05$) differences among the treatments. For N, the highest value of NR was recorded at FM-diet followed by SBM+NZ_4, which were not significantly different. For P, the significant ($P \leq 0.05$) highest NR value was noticed at SBM+NZ_4. The lowest values of NR for both N and P were obtained at SBM+NZ_1.

In regard to the nutrients (N and P) excretion (TNW as g/fish) there were also significant differences ($P \leq 0.05$) with highest values at FM-diet, while lowest values recorded at SBM+NZ_1 and SBM+NZ_4 for N and P excretion, respectively. The addition of NZ reduced the excreted P % from 73 % at SBM-diet to 56% SBM+NZ_4.

During the specified occasions, feed supply showed significant differences ($P \leq 0.05$) among the treatments, with the highest supply at the FM-diet and the lowest at the SBM+NZ_2 (Table 3). Similarly, both N and P supply showed significant differences ($P \leq 0.05$) among the treatments, in a same manner of the feed supply.

Table 3. Nitrogen and phosphorus excretion in water (as measured in the three occasions) by Nile tilapia fed different experimental diets for 12 weeks.

Diets	FM-diet	SBM-diet+NZ_1	SBM-diet+NZ_2	SBM-diet+NZ_3	SBM-diet+NZ_4
Total feed intake (g/aquarium)	27.389 ±2.25^{a}	20.079 ±1.66^{c}	24.252 ±1.16^{b}	25.096 ±2.13^{b}	25.536 ±2.251^{b}
Total nitrogen supplied (g/aquarium)	1.238 ± 0.102^{a}	0.907 ±0.075^{c}	1.096 ± 0.052^{b}	1.134 ±0.096^{b}	1.154 ± 0.102 ^{b}
Retained N (g/aquarium)	0.445 ±0.035^{a}	0.286 ±0.025^{d}	0.358 ±0.025^{c}	0.393 ±0.035^{b}	0.429 ±0.038^{a}
excreted nitrogen (g/aquarium)	0.792 ±0.067^{a}	0.621 ±0.051^{c}	0.738 ±0.032^{ab}	0.741 ±0.063^{a}	0.725 ±0.066^{b}
% of nitrogen excretion	64.017 ±0.627^{d}	68.425 ±0.473^{a}	67.332 ±1.147^{b}	65.36 ±0.824^{c}	62.829 ±0.973^{e}
% NH_4 of excreted N	11.117 ±7.718^{a}	9.444 ±6.812^{a}	7.126 ±3.122^{a}	7.076 ±2.937^{a}	7.955 ±4.764^{a}
% NO_2 of excreted N	1.443 ±0.706^{b}	2.273 ±1.336^{a}	1.183 ±0.678^{b}	0.501 ±0.148^{c}	0.432 ±0.23^{c}
% NO_3 of excreted N	17.923 ±4.3^{ab}	18.692 ±1.364^{a}	17.373 ±6.033^{ab}	14.232 ±5.19^{b}	3.596 ±2.607^{c}
% DIN of excreted N	30.484 ±8.233^{a}	30.408 ±8.507^{a}	25.682 ±8.374^{ab}	21.809 ±4.895^{b}	11.629 ±5.814^{c}
Total supplied P (g/aquarium)	0.214 ±0.018^{a}	0.143 ±0.005^{c}	0.174 ±0.009^{b}	0.18 ±0.001^{b}	0.182 ±0.004^{b}
Retained P (g/aquarium)	0.071 ±0.009^{b}	0.04 ±0.001^{d}	0.057 ±0.007^{c}	0.067 ±0.005^{b}	0.081 ±0.003^{a}
excreted Phosphorus (g/aquarium)	0.143 ±0.01^{a}	0.097 ±0.007^{c}	0.108 ±0.004^{b}	0.106 ±0.011^{b}	0.093 ±0.01^{c}
OP (mg/aquarium)	12.33 ±2.724^{a}	10.893 ±3.402^{a}	6.286 ±1.632^{b}	5.747 ±0.952^{b}	6.046 ±1.475^{b}
OP% of excreted P	8.82 ±2.592^{b}	11.078 ±2.633^{a}	5.837 ±1.385^{c}	5.532 ±0.921^{c}	6.076 ±2.076^{c}

In each line, different superscripts letters mean significantly differed values ($P \leq 0.05$).
Data are means (Mean±SD) of three replicates.

With respect to the retained N (NR g/aquarium), significant differences ($P \leq 0.05$) were noticed among the treatments in a similar behavior as overall retained N, with the highest value at FM-diet (0.445±0.035) followed by the SBM+NZ_4 (0.429±0.038). The lowest value was obtained at the SBM+NZ_1 (0.286±0.025). Likewise the overall N excretion, the excreted N during the three occasions (TNW g N/aquarium) significantly ($P \leq 0.05$) differed among the treatments with highest value at FM-diet (0.792±0.067 g N/aquarium) followed by that at SBM+NZ_3 (0.741±0.063 g N/aquarium), while the lowest value was recorded at SBM+NZ_1 (0.621±0.051 g N/aquarium). The excreted N% showed significant differences ($P \leq 0.05$) among the treatments with a negative relation with the level of NZ. The lowest value of N excretion % was recorded at the SBM+NZ_4 (62.83±0.973%). Total Ammonia as a percent of excreted N did not show significant differences ($P > 0.05$) among treatments; however, the highest value was noticed at FM-diet and lowest value at SBM+NZ_3. Interestingly, both nitrite and nitrate as a percent of excreted N showed significant ($P \leq 0.05$) differences among the treatments as it showed negative relationship with the levels of NZ. The dissolved inorganic nitrogen (DIN), similarly, showed a significant negative response with the level of NZ, with highest percent at FM-diet (30.5%), and lowest percent at SBM+NZ_4 (11.6%). The addition of NZ reduced the DIN by more than 61% comparing with both controls.

In regard to retained P (NR), it showed a significant positive response with the level of NZ as well as significant differences ($P \leq 0.05$) among the treatments with highest value at the SBM+NZ_4 and lowest one at SBM+NZ_1. The excreted P also showed significant ($P \leq 0.05$) differences among the treatments with highest value at the FM-diet and lowest one at SBM+NZ_4, with a negative behavior with NZ addition. The orthophosphate (as a percent of excreted P) revealed significant ($P \leq 0.05$) differences with highest value at SBM+NZ_1, while lowest value was observed at SBM+NZ_3. The addition of NZ resulted in a reduction in OP% by more than 45% comparing with SBM+NZ_1 negative control diet.

Significant ($P \leq 0.05$) differences among the treatments were observed with respect to N and P body contents (g/fish) in favor of the FM-diet and SBM+NZ_4, respectively (Table 4). The lowest body N and P contents were measured at SBM+NZ_1. The body N content reflexes the differences in ARR % of nitrogen among the treatments; however, highest content was noticed at the FM-diet (0.616 g N/fish), followed by that at SBM+NZ_4 (0.609 g N/fish). The lowest N content was noticed at the negative control diet SBM+NZ_1 (0.451 g N/fish). The body P content also was a mirror for ARR%, where the significantly ($P \leq 0.05$) highest content was determined at SBM+NZ_4 (0.154 g P/Fish), followed by that at FM treatment (0.148 g P/fish), whereas, lowest body P content was recorded in the fish at SBM+NZ_1 (0.109 g P/fish).

Table 4. Nitrogen and Phosphorus content (% fresh matter basis) in Nile tilapia fed on different experimental diets for 12 weeks.

Fish body composition	FM-diet	SBM-diet +NZ_1	SBM-diet +NZ_2	SBM-diet +NZ_3	SBM-diet +NZ_4	Initial
Nitrogen (g/fish)	0.616 ±0.012^{a}	0.451 ±0.010^{d}	0.518 ±0.017^{c}	0.572 ±0.014^{b}	0.609 ±0.017^{a}	0.068 ±0.001
Phosphorus (g/fish)	0.148 ±0.002^{b}	0.109 ±0.002^{e}	0.127 ±0.005^{d}	0.139 ±0.004^{c}	0.154 ±0.002^{a}	0.061 ±0.001

Initial average fish weight: 2.53 g/fish & Final average fish weight for T_1, T_2, T_3, T_4, T_5 was 24.06, 17.56, 21.10 and 23.06 g/fish, respectively.

In each row, different superscripts letters mean significantly differed values ($P \leq 0.05$). Data are means (Mean ±SD) of three replicates.

DISCUSSION

Tilapia are capable of utilizing nutrients from plant feedstuffs, such as soybean meal (Maina *et al*., 2002 and Siti-Norita *et al.*, 2015); however, plant-protein sources contain anti-nutritional factors (ANFs) that can reduce nutrients utilization efficiency of this fish, which is reflexed in nutrients excretion into water (Baruah *et al*., 2004; Azarm and Lee, 2014). Therefore, the apparent nutrients utilization, nutrients retention and excretion by Nile tilapia fed on all-plant diets supplemented with exogenous multi-enzymes, Natuzyme® (NZ) were investigated in the present study.

In the present study the feed consumption was significantly proportional with the level of dietary NZ. However the feed consumption at the highest NZ addition was significantly lower than that at FM-diet. Similarly, Vielma *et al.*, (1998) stated that increasing the palatability and conversion rate of a diet may be due to enhanced release of nutrients of plant-based diets by breaking down the bonds between phytate-protein and phytate-minerals.

In a previous paper Amer (2017) studied the responses of growth performance of Nile tilapia fry to plant-based diets supported with gradual levels of NZ and found that the addition of NZ to soybean meal based diets improve feed intake, feed utilization (FCR) and growth performance(PER, PPV% and ER%).

Our results showed that supplementation SBM diets with NZ significantly enhanced the ARR% of both P and N, by 63.3% and 17.7% respectively, compared with the negative control (SBM without NZ). Furthermore, at highest NZ addition (1.5g/Kg diet) the ARR% of P and N were superior over that of the positive control (FM-diet), although the fish consumed less feed. They were 44% vs 33% for P and 37 % vs 36% for N, respectively.

The lower ARR% of P at the negative control can be attributed to the presence of phytate-P in soybean meal, where soybean meal contain about 0.67% total phosphate, out of which 50-80% is in phytate-P form (Ravindran *et al.*, 1995; Tyagi and Verma, 1998 and Godoy *et al.*, 2005). Phosphorus in this form is not bioavailable to monogastric and gastric animals because they lack the intestinal digestive enzyme, phytase, required to separate P from the phytate molecule (Jackson *et al.*, 1996).

The higher P ARR% in treatments (NZ_2-NZ_4) can be attributed to breaking phytate-P complex and increasing the bioavailability of P as a result of including NZ in SBM-diets. On consistency, literatures documented the enhancement of P and other nutrients bio-availability as a result of adding phytase enzyme to plant-based diets of Nile tilapia (Furuya *et al.*, 2001; Phromkunthong and Gabaudan 2006; Lin *et al.*, 2007; Cao *et al.*,

2008; Mahmoud *et al.*, 2014 and Castillo and Gatlin, 2015). Selle *et al.* (2007) reported that phytase can generate 282 g inorganic P from each kg of dietary phytate. The ARR% of P in the present study was enhanced by 63.3% comparing with that of the negative control treatment, which is corresponded with the finding of Riche and Brown (1996) who found that, the P bioavailability to rainbow trout from various plant foodstuffs significantly increased to 46.2% - 75.6% on supplementation with phytase.

On the other hand, lower P ARR % at FM-diet comparing with NZ_3 and NZ_4 can be attributed to the lower P bio-availability in FM-diet, although FM is rich in P; it contains 2-4% P (Sugiura and Hardy, 2000). Similarly, Sugiura and Hardy (2000) and Hernández *et al.* (2004), reported that P provided by FM-based diets generally surpasses the minimal requirements needed for optimal fish growth however in fact are less utilized by some cultivated species.

With respect to the nitrogen, the low ARR% and retention rate of N in the negative control can be explained as a result of phytate presence in soybean meal, which binds with cation sites on protein and amino acids and renders it less available and less digestible. Kumar *et al*. (2012) reported that phytate can integrate with cation groups on protein, amino acids, starch and lipids in feed stuff reducing their digestibility in fish. Also, it has been observed that high dietary phytate levels lead to depressed growth, and protein utilization, which may be a result of complexing with cations (Spinelli *et al.*, 1983; Richardson *et al.*, 1985 and Francis *et al.*, 2001) so that protein bioavailability reduced.

The higher N ARR% and N retention in NZ_{2-4} treatments may be attributed to the improvement in protein digestion and absorption and decreasing phytate-protein complex as a result of adding NZ which includes phytase as well as protease enzymes (Kumar *et al.*, 2012). Also, it may be attributed to enhanced bio-availability of P. Nile tilapia showed higher protein content and lower lipid content when feed on diets supplemented with phosphorus (Hung, 1989; Wee and Shu, 1989 and Sarkar *et al.*, 2004). The presence of NZ in the plant-based diets reduce the viscosity of non-starch

polysaccharides (NSP), thus enhance the hydrolysis of the diets and nutrients liberation, and increasing endogenous enzymes activities (Hlophe-Ginindza *et al.*, 2016). Dephytinization of dietary phytate by exogenous phytase accounts for increased protein utilization in common carp (Schafer *et al.*, 1995), European seabass (Oliva-Teles *et al.*, 1998), Nile tilapia (Heindl, 2002), and in Atlantic salmon in seawater (Storebakken *et al.*, 2000) by degrading the pre-formed phytate–protein complexes. However, Teskeredzic *et al.* (1995) reported a decrease in rapeseed protein quality by dephytinization of soy protein in rainbow trout.

Overall excretion of P and N percentages as well as during the three occasions showed significant differences among treatments with a negative relationship with the level of NZ in the diet. Also, the excretion P and N amount at the highest level of NZ were significantly lower than those at FM diets. Baruah *et al.* (2004) showed that as a consequence of low digestibility of phytate by fish, most of the phytate-P ends up being excreted into the water and may cause algal bloom pollution. Also, high N excretion in SBM negative control can be explained as a result of containing soybean meal phytate, which binds with protein in a digestion-resisting complex, and enzymes inhibitors (Norton, 1991; Liener, 1994; Ravindran *et al.*, 1995 and Francis *et al.*, 2001).

Supplementation of phytases to plant-based diets in in various studies reduce P load to the environment by 30-50% in the red sea bream, Nile tilapia, salmon, rainbow trout, carp and channel catfish (Omogbenigun *et al.*, 2003; Biswas *et al.*, 2007and Dechavez and Serrano, 2012). Similarly, in the present study, addition of NZ results suggest potential environmental benefits to the extent of 23% reduction in P excretion and to the extent of 8.2% reduction in N excretion, comparing with negative control diet. Interestingly the addition of NZ caused a reduction by 61.8% of DIN of excreted N and 45% of orthophosphate, comparing with SBM negative control. Moreover, supplementation with NZ resulted in lower excretion percent of both total excreted and inorganic fraction of N and P compared with FM-diet, which mean less pollution to the aquatic ecosystem.

In the present study, supplementation SBM diets with NZ had significant influence on the P and N body content comparing with the negative control. Moreover, the highest NZ addition (1.5g/kg diet) yielded significant higher P content and comparable N content with fish fed on FM diet. Similarly, Vielma *et al.*, (2004) and Hlophe-Ginindza *et al.* (2016) found that protease supplementation can improve protein digestibility by degrading complex proteins in the diet into usable amino acids and peptides. On contrary, Khalafalla and El-Hais (2013) didn't find any significant differences in whole body composition of Nile tilapia fed diets supplemented with exogenous multi-enzyme preparations. Hassaan *et al.* (2013) indicates that phytase supplementation positively affected chemical composition of body and vertebra when combined with 0.6 Ca/P ratio. Also, Mahmoud *et al.*(2014) documented that exogenous enzymes can improve nutrient digestibility of plant-based fish diets and result in better growth performance feed efficiency and higher crude protein content. This inconsistency may be attributed to differences in feed ingredients, nutritional quality of plant ingredients, water quality, fish species, size and culture conditions.

CONCLUSIONS

The replacement of fishmeal with extensively available plant or grain is getting increased attention for the development of low-cost fish feed. To maximize the nutritional value of plant-based diets and improve nutrients (N&P) utilization, exogenous enzymes mixture incorporation is an efficient means as it could not be negligible in preventing eutrophication in the aquatic environment.

The results of the present study are of utmost benefits in economic and environmental management of semi-intensive and intensive tilapia cultures, where feed costs and ecosystems pollution are the major factors limiting fish production. The present study findings show the great potential for using exogenous multi-enzymes (Natuzyme®) in plant protein based diets, as it (at a rate 1.5 g /Kg of Nile tilapia diets) gives the possibility of total replacement of

fishmeal by soybean meal, enhance the bioavailability and ARR% of P and N, reduce the amount of N and P excretion (TNW %) as well as it can effectively reduce inorganic P and N fractions of the excreted nutrients. So, it can limit or at least postpone the eutrophication problem.

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نحو استزراع أكثر صداقة للبيئة: تأثير إضافة الإنزيمات الخارجية (ناتوزيم) على احتجاز وإخراج النيتروجين والفوسفور فى أسماك البلطى النيلى

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الملخص العربى

هذه الدراسة قامت ببحث تأثير إضافة الإنزيمات الخارجية (ناتوزيم) إلى العلائق النباتية على كفاءة الاحتجاز و الإخراج لكل من النيتروجين و الفوسفور لأسماك البلطى النيلى. تم إعداد خمس علائق متساوية فى محتوى البروتين (٢٨٪) والطاقة (٤٢٥ كيلو كالورى/ ١٠٠ جم غذاء). العليقة الأولى احتوت على مسحوق السمك (كنترول ١) ، الأربع علائق الأخرى احتوت على مسحوق فول الصويا وتم إضافة مخلوط الإنزيمات إليها بمعدل ٠ ، ٠.٥ ، ١ ، ١.٥ جم/ كجم عليقة على التوالى. غُذيت الأسماك مرتين يومياً إلى حد الإشباع فى ثلاث مكررات من مجموعات الأسماك (متوسط وزن السمكة ٢.٥٢ جم) لمدة ١٢ أسبوع. أظهرت المدخلات من النيتروجين و الفوسفور أعلى قيم ذات دلالة إحصائية مع العليقة المحتوية على مسحوق السمك بينما تم تسجيل أقل قيم لهما مع العليقة النباتية بدون إضافة إنزيمات. معدل الاحتجاز الظاهرى لكل من النيتروجين و الفوسفور أظهر علاقة إيجابية مع مع مستوى إضافة الإنزيمات فى العلائق، و تم الحصول على أعلى قيمة له مع العليقة النباتية المضاف إليها ناتوزيم بمعدل ١.٥ جم/كجم بينما تم تسجيل أقل قيمة له مع العليقة النباتية بدون إضافة الناتوزيم. تم تسجيل أعلى محتوى من النيتروجين مع العليقة المحتوية على مسحوق السمك بينما سُجل أعلى محتوى من الفوسفور مع العليقة النباتية مع إضافة ناتوزيم بمعدل ١.٥ جم/كجم. أظهرت قيم الأخراج من النيتروجين و الفوسفور اتجاه معاكس لمعدل الاحتجاز الظاهرى. تشير النتائج إلى أن ناتوزيم له تأثير فعال فى تقليل نسبة النيتروجين و الفوسفور الغيرعضوى فى المخرج من النيتروجين و الفوسفور. هذه الدراسة تشير إلى إمكانية تحسين كفاءة احتجاز أسماك البلطى النيلى للنيتروجين والفوسفور فى العلائق وتقليل المخرج منهما عن طريق استخدام الإنزيمات الخارجية ناتوزيم بمعدل ١.٥ جم/كجم عليقة فى العلائق النباتية مما يؤدى بالتالى إلى الحد أو على الأقل تأجيل مشكلة التلوث بالنيتروجين و الفوسفور.