EFFECTS OF EXOGENOUS DIGESTIVE ENZYMES ON NILE TILAPIA FED PRACTICAL DIETS

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Abstract
A study was carried out to evaluate the effects of exogenous digestive enzymes on all male Nile tilapia (Oreochromis niloticus) fed practical diets. The tilapia (29.52±0.3g, 40 fish per tank) was fed one of diets supplemented with phytase (75 mg kg^{-1}), protease (300 mg kg^{-1}), xylanase (250 mg kg^{-1}) and control diet. The tilapia was fed at 3 % biomass per day in triplicate tanks per treatment for 8 weeks. The final body weight of tilapia fed xylanase supplemented diet was significantly higher ($P < 0.05$) than that of tilapia fed the remaining three diets (136.42±0.41, 137.09±1.5 and 135.26 g fish^{-1} for control, phytase and protease supplemented diets respectively). Also, the protein efficiency ratio was highest ($P < 0.05$) in tilapia fed xylanases supplemented diet. However, there was no significant ($P > 0.05$) difference observed in specific growth rate, somatic indices, survival and haemato-immunological parameters of tilapia fed the experimental diets. The histological assessment of mid-intestine sections taken from the tilapia also showed no significant difference in their perimeter ratio and number of secretory goblet cells per 100 µm. It could therefore be concluded that tilapia does not benefit markedly from a practical diet supplemented with costly exogenous digestive enzymes.

Keywords: growth, haemato-immunology, light microscopy, intraepithelial leucocytes

Introduction

As the demand for aquafeed increases due to rise in aquaculture production, the aquafeed industry is constantly seeking to optimise product quality in a cost-effective manner. Hence, the importance of functional ingredients (such as yeast products, phytogenics, organic acids, prebiotics, probiotics, exogenous digestive enzymes, etc.) as supplements to optimise the performance of aquafeed at little or no extra cost. The potential of exogenous digestive enzymes as functional ingredients (as reported by Adeola and Cowieson (2011), Kumar *et al.*, (2012), Jiang *et al.*, (2014), Castillo and Gatlin (2015), Adeoye *et al.*,(2016a), Adeoye *et al.*, (2016b), Wallace *et al.*, (2016)) has been demonstrated in different fish species to mitigate the effects of anti-nutritional factors (ANFs) common in cost effective feed ingredients and subsequently enhance the ingredients' nutritional value thereby leading to improved nutrients' utilisation and improved fish growth performance. Unlike other functional feed additives and supplements (such as antibiotic growth promoters) which may have adverse impact on human health and the environment, exogenous digestive enzymes are perceived to be harmless, environment friendly and natural (Liu and Baidoo, 1997) with proven benefits in poultry and swine (Adeola and Cowieson, 2011). However, research and development are useful to further establish the efficacy of functional feed supplements in aquafeed and aquaculture species. Therefore, the objective of the study was to evaluate the efficacy and effects of exogenous digestive enzymes (phytase, protease and xylanase) on Nile tilapia (*O. niloticus*) under practical diet conditions.

Materials and Methods

The trial was conducted in a freshwater flow-through aquaculture system (12 circular concrete tanks of 580 L capacity each supplied with freshwater sourced from a local river system at 3.98L min^{-1} flow rate) at the Division of Animal Production Technology and Fisheries of King Mongkut's Institute of Technology Ladkrabang, Bangkok – Thailand. Four hundred and eighty all male Nile tilapia (*O. niloticus*) of mean weight 29.52±0.15g obtained from Charoen Pokphand farm in Thailand were randomly distributed (40 fish per tank) into the 12 tanks after four weeks of acclimatization. The photoperiod and water temperature (30.83±0.29 °C) were maintained at ambient condition. The pH (6.48±0.34) and dissolved oxygen levels (>5.0 mgL^{-1}) in water system were monitored daily using an HQ40d pH meter and dissolved oxygen multiparameter meter (HACH Company, Loveland, USA). NH_3 (0.34±0.1 mgL^{-1}), NO_2 (0.008±0.005 mgL^{-1}) and NO_3 (1.28±0.12 mgL^{-1}) were also monitored on a weekly basis using a nutrient analyser (SEAL AQ2 Analyser, Hampshire, UK). A commercial diet (35% protein, 5% lipid, containing fishmeal, soybean meal, full-fat soybean meal, yeast, corn meal, broken rice, rice bran, wheat bran, dicalcium phosphate, salt, vitamins, minerals, amino acids, fish oil and preservatives) was obtained from INTEQC Feed Co. Ltd., Thailand and was used as the basal formulation. The diets were prepared and cold extruded as described in our previous study (Adeoye *et al*., 2016b) and exogenous digestive enzymes added separately; phytase at 75mg kg^{-1}, protease at 300mg kg^{-1} and xylanase at 250mg kg^{-1} (Table 1). The diets were analysed for proximate composition (AOAC, 1995). The tilapia was fed the experimental diets for eight weeks at 3% biomass per day in three equal rations. Total fish (biomass) in individual tanks were batch weighed each week after 24 h starvation and feeding rate was adjusted weekly to the fish biomass.

Growth performance, feed utilisation and somatic indices as well as haemato-immunological parameters and histological appraisal of mid-intestine of tilapia fed the experimental diets were carried out as described by Adeoye *et al.*,(2016b).

Statistical analysis was carried out using SPSS for Windows (SPSS Inc., 22.0, Chicago, IL, USA). Unless otherwise stated, all data were reported as mean ± standard deviation. All data were checked for normality and equality of variance using Kolmogorov-Smirnov and Bartlett's test, respectively. Where normality assumptions were met, data were analysed using one-way analysis of variance followed by a post-hoc Duncan test to determine significant differences. Where data violated these conditions, a Kruskal- Wallis test was used on log transformed data. Differences between treatments were then determined using a Mann-Whitney U-test. All percentage data were transformed using arcsine function prior to statistical analysis. In all cases significance was accepted at $P < 0.05$.

Results

Growth and feed performance was assessed by means of final body weight, weight gain, specific growth rate, feed conversion ratio and protein efficiency ratio. Tilapia in all treatments showed excellent growth performance with survival in each treatment exceeding 99%.Tilapia fed the xylanase supplemented diet showed a significantly ($P < 0.05$) higher final body weight, improved feed conversion ratio and better protein efficiency ratio when compared to the control group (Table 2).None of the dietary treatments affected the somatic indices and survival of the tilapia.

The haemato-immunological status of tilapia fed the enzyme supplemented diets was assessed by the measurement of haematocrit, haemoglobin, blood cells count, MCV, MCH, MCHC and serum lysozyme activity. Tilapia in all treatments displayed good haemato-immunological status compatible with profiles for this species (Table 3). No significant ($P > 0.05$) differences were observed in the haemato-immunological status of the fish fed different experimental diets, except white blood cells count.

The mid-intestine of the tilapia was examined under light microscope after eight weeks of feeding experimental diets. Tilapia from all treatments displayed intact epithelial barrier with extensive mucosal folds which extend into the lumen. Each fold consists of simple lamina propria that house abundant intraepithelial leucocytes (IELs) and goblet cells (Plate 1). No differences were observed in mid-intestine perimeter ratio and number of goblet cells in the epithelial of tilapia fed the experimental diets (Table 4). However, tilapia fed the control diet had the highest ($P < 0.05$) IELs abundance when compared to tilapia fed the protease and xylanase supplemented diets.

Table 1. Dietary formulation and proximate composition of experimental diets

	Control	Phytase	Protease	Xylanase
Commercial feed[a]	100	99.9925	99.97	99.975
Phytase[b]	0	0.0075	0	0
Protease[c]	0	0	0.03	0
Xylanase[d]	0	0	0	0.025
Proximate composition (% as fed basis)				
Moisture	8.03±0.04	6.87±0.14	8.06±0.06	6.63±0.09
Protein	34.32±0.28	34.78±0.09	34.43±0.13	34.56±0.08
Lipid	5.49±0.04	5.33±0.10	6.38±0.70	5.22±0.08
Ash	13.13±0.11	13.13±0.17	13.16±0.04	13.4±0.04
Energy (MJ kg^{-1})	17.06	17.56±0.01	17.31±0.04	17.66±0.21
Fibre	3.65±0.06	3.15±0.12	3.15±0.07	3.21±0.05

[a]No. 461, INTEQC Feed Co Ltd., Thailand
[b]RONOZYME® Hiphos (contains 10,000FYT g^{-1}) from DSM Nutritional Products
[c]RONOZYME® ProAct (contains 75,000 PROT g^{-1}) from DSM Nutritional Products
[d]RONOZYME® WX (contains 1000 FXU g^{-1}) from DSM Nutritional Products

Table 2. Growth, feed utilisation and somatic indices of tilapia fed the experimental diets

	Control	Phytase	Protease	Xylanase
IBW (g fish^{-1})	29.57±0.76	29.31±0.55	29.73±0.58	29.33±0.17
FBW (g fish^{-1})	136.42±0.72^{a}	137.09±2.59^{a}	135.26±1.74^{a}	140.43±0.54^{b}
WG (%)	461.62±13.9^{ab}	467.74±3.99^{ab}	455.19±12.56^{a}	478.89±1.54^{b}
SGR (% day^{-1})	3.18±0.06^{ab}	3.21±0.02^{ab}	3.16±0.06^{a}	3.26±0.01^{b}
FI (g fish^{-1})	100.95±0.38	100.53±2.25	99.52±1.46	101.28±0.59
FCR	0.99±0.01^{a}	0.97±0.00^{b}	0.98±0.01^{ab}	0.96±0.01^{b}
PER	2.47±0.04^{a}	2.53±0.01^{ab}	2.48±0.04^{a}	2.57±0.02^{b}
HSI	2.01±0.27	2.30±0.53	2.04±0.35	2.69±0.32
K-factor	1.97±0.08	2.02±0.14	1.98±0.02	2.14±0.17
VSI	20.57±2.55	18.70±1.03	19.61±0.32	20.05±1.77
Survival (%)	100	99.17±1.44	100	100

Means in the same row with different superscripts are significantly different (P < 0.05). IBW, initial mean body weight; FI, daily feed intake; FBW, final mean body weight; WG, weight gain; SGR, specific growth rate; FCR, feed conversion ratio; PER, protein efficient ratio; HSI, hepato-somatic index and VSI, viscero-somatic index.

Table 3: Haemato-immunological parameters of tilapia fed the experimental diets

	Control	Phytase	Protease	Xylanase
Haematocrit, (%PCV)	48.00±2.84	44.56±0.84	45.44±3.15	47.33±3.84
Haemoglobin, (g dL^{-1})	13.03±1.11	13.33±1.03	13.49±1.39	15.16±1.15
RBC (10^{6} µL^{-1})	1.43±0.16	1.69±0.33	1.54±0.26	1.58±0.28
WBC (10^{3} µL^{-1})	20.49±1.55^{a}	22.81±0.87^{b}	23.14±0.97^{b}	22.32±0.72^{ab}
MCV (fL)	349.9±65.86	284.3±68.10	301.9±45.79	312.7±67.70
MCH (pg)	93.97±7.40	84.95±23.77	89.49±15.49	99.59±27.29
MCHC (gdL^{-1})	27.28±3.88	29.89±1.78	29.66±1.05	32.08±2.89
Lymphocytes (%)	92.64±2.05	94.11±0.72	93.21±0.71	93.84±2.56
Monocytes (%)	4.34±1.47	2.69±0.39	3.99±0.53	3.78±2.36
Granulocytes (%)	3.02±0.60	3.20±0.40	2.81±0.18	2.37±0.39
Serum lysozyme (U)	77.81±6.93	94.31±54	94.09±48.66	145.79±46.85

Figures in each row with similar superscript are not significantly different (P > 0.05). RBC, red blood cells; MCV, mean corpuscular volume (haematocrit (%PCV) x 10)/RBC 106 µL-1); MCH, mean corpuscular *haemoglobin (haemoglobin (g dL-1) x 10)/RBC (106 µL-1); MCHC, mean corpuscular haemoglobin concentration (haemoglobin (g dL-1) x 100)/haematocrit (%PCV); U is lysozyme activity unit (activity mL-1 min-1); %, mean percentage of total leucocytes*

Table 4: Intestinal histology of tilapia fed the experimental diets

	Control	**Phytase**	**Protease**	**Xylanase**
Perimeter ratio	4.14±1.55	3.85±0.20	4.00±0.61	4.55±0.74
Goblet cells (per 100µm)	5.42±1.38	4.61±1.26	3.82±1.07	4.01±0.83
IELs (per 100µm)	36.45±0.90[a]	31.45±5.01[ab]	26.96±3.29[b]	26.83±1.71[b]

Values with different superscripts indicate significant differences (P < 0.05). IELs, Intraepithelial leucocytes

Discussion

For cost and sustainability reasons, commercial aquafeeds are increasingly being modified to contain more plant-based materials. However, the presence of ANFs in plant materials could impair full optimisation of nutrients and consequently result in reduced production and growth performance. Supplementation with functional feed additives may enhance the optimisation of nutrients present in the plant materials.

In this study, a commercial aquafeed was supplemented with three exogenous digestive enzymes (phytase, protease and xylanase) separately to assess the most effective enzyme(s) in terms of nutrient utilisation, haemato-immunology and intestinal histology of tilapia. The final body weight of tilapia fed xylanase supplemented diet was the highest compared to tilapia fed other experimental diets. Xylanase, a non-starch polysaccharides (NSPs) degrading enzyme can hydrolyse cell wall components in the plant materials, efficiently reducing NSP content of the plant materials and consequently releasing the bound nutrients. Jiang *et al.*,(2014) also reported improved growth performance in Jian carp (*Cyprinus carpio var. Jian*) fed xylanase (800mg kg^{-1}) supplemented plant-based diet. Improved growth performance was also reported in Japanese sea bass when fed plant-based diet supplemented with xylanase (Ai *et al.,* 2007). It is important to note however that the high-quality diet used in this study could have possibly masked the potential impacts of other exogenous digestive enzymes (phytase and protease) that might have occurred on lower quality diet as reported by Wallace *et al.,* (2016).

The haemato-immunological status of tilapia was good and within the healthy range for this species. No differences were observed in the parameters measured (except white blood cells count). The mode of action of exogenous digestive enzymes is mainly in the digestive process to enhance better digestibility and utilisation of nutrients rather than modulation of immune system. Also, there is no established interaction between the exogenous digestive enzymes and the tilapia haematology and/or immune system.However, the level of abundance of IELs (components of gut associated lymphoid tissue) was significantly lower in tilapia fed protease and xylanase supplemented diets compare to tilapia fed control diet. To the author's knowledge, this is the first time this parameter was measured in tilapia fed exogenous supplemented diets. This requires further study to establish the effect of exogenous digestive enzymes in tilapia immune-stimulation or immune-depression.

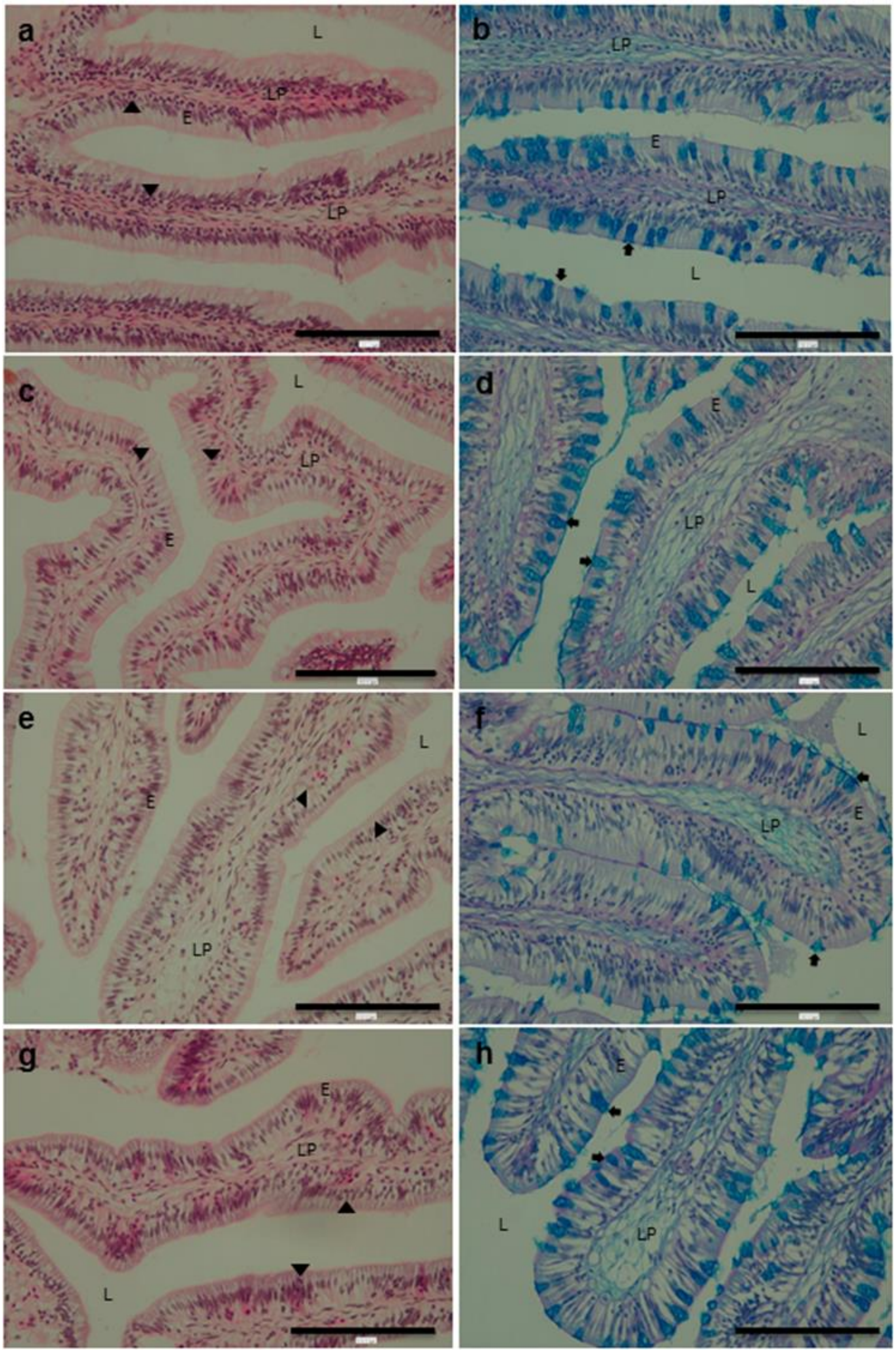

Plate 1: **Light micrograph of the mid-intestine of tilapia fed control (a & b), phytase (c & d), protease (e & f) and xylanase (g & h) diets. Goblet cells (arrows) are in all treatments and abundant IELs (arrowheads) are present in the epithelia. Abbreviations are E enterocytes, LP lamina propria and L lumen. Light microscopy staining: [a, c, e & g] H & E; [b, d, f & h] Alcian Blue-PAS. Scale bars = 100 µm.**

Conclusion

This study provides further information on the efficacy of exogenous digestive enzymes in practical diet and Nile tilapia.It could be inferred that more pronounced effects would more likely be obtained when inferior diets formulated with poor quality ingredients are supplemented with exogenous digestive enzymes. Such plant by-products having higher fibre and NSPs may be more sensitive to the effects of exogenous digestive enzymes supplementation in tilapia feed.

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