

Effects of X-xylanase Incorporated in the Diet on the Growth Performance of Red tilapia (*Oreochromis sp.*)

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Abstract:

The aim of this study was to evaluate the feasibility of using X-xylanase in the diet to improve tilapia growth. Four experimental diets containing X-xylanase (Hostazym[®], Huvepharma, Antwerp, Belgium) at levels of 0 (control), 1050, 1500, and 1950 endopentosanase units (EPU) were used in this study. The growth performance trial was carried out for 56 days. At the end of the study, there were no significant differences between the groups fed by control and the experimental diets in terms of survival rate, condition factor and weight gain. However, the highest weight gain was recorded in fish fed a diet containing 1500 EPU of X-xylanase. Fish fed diets containing 1500 and 1950 EPU X-xylanase for 56 days had significant better feed efficiency compared to those of fish fed the commercial diet and other experimental diets. It was therefore concluded that the addition of X-xylanase to the tilapia diet was able to improve the feed utilization and consequently enhance fish growth.

Keywords: Tilapia; X-xylanase; Growth performance; Feed efficiency

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Introduction

Tilapia (*Oreochromis* sp.) farming is a widespread industry in the world because of its high growth performance and market value, and it has become one of the most important aquaculture species in the world. The production of tilapia is increasing annually, and 5,576,800 metric tons were produced worldwide in 2015. As tilapia aquaculture has dramatically grown over the past decade, feed quality and utilization have become major issues standing in the way of the sustainable development of tilapia aquaculture.

Formulated diets are the primary food source used in tilapia aquaculture. Plant-derived proteins, such as soybean meal, rice gluten meal, rapeseed meal, rice gluten meal, and wheat middling's, are widely used as ingredients in tilapia feed. However, the low nutrient utilization of plant-derived proteins by fish might result in lower growth performances and pollution of pond environment (Leenhouwer *et al.*, 2007). The limited nutrient availability in aquafeed is mainly caused by non-starch polysaccharide (NSP) components (Sinha *et al.*, 2011) and/or other anti-nutritional factors in plant-derived proteins (Cheng *et al.*, 2013). Therefore, issues of anti-nutritional factors in plant-derived proteins must be dealt with to improve nutrient utilization and animal growth to bring down consequent costs of aquafeed.

Microbial enzymes are widely used in feed to minimize the problem of anti-nutritional factors, thereby improving the nutritive value (Forster *et al.*, 1999), nutrient utilization (Cheng and Hardy, 2003; Cheng *et al.*, 2013), animal growth (Sajjadi and Carter, 2004; Cheng *et al.*, 2013; Saputra *et al.*, 2016), and so on. Xylanases are a class of enzymes that degrade linear polysaccharides in plant cell walls into xylose, thus breaking down hemicellulose. They are found in numerous bacteria, yeasts, and fungi and play a role in those organisms' ability to thrive on plant sources by degrading plant matter into usable nutrition. Because of xylanase's capability of enzymatic digestion, it is considered a potential candidate for improving the efficiency of feed utilization and degrading NSPs of plant ingredients in animal feed. Xylanase has been widely used in the poultry (Yang *et al.*, 2008; Pirgozliev *et al.*, 2015) and livestock (Prandini *et al.*, 2014; Passos *et al.*, 2015) industries to enhance energy and nutrient availability of plant ingredients, but little is known about its application in aquaculture. Xylanase-producing yeasts were found in some freshwater fish species, such as Indian major carp (*Cirrhinus mrigala*) and Nile tilapia (*Oreochromis niloticus*), and xylanases released from yeast strains are thus available in the gut of fish and might promote NSP digestibility and utilization by fish (Gatesoupe, 2007; Banerjee and Ghosh, 2014). Purified xylanase was used in a plant protein-enriched diet of Jian carp (*Cyprinus carpio* var. *Jian*), which resulted in improvements in growth, in intestinal enzyme activities, and the microflora (Jiang *et al.*, 2014); similar results were also recorded in Japanese seabass (*Lateolabrax japonicas*) (Ai *et al.*, 2007).

It is well known that xylanases have great potential, as they are widely applied in the feed of terrestrial animals (Prandini *et*

al., 2014; Passos *et al.*, 2015). However, little is known about their application in aquaculture. Therefore, the efficiency of an X-xylanase product in improving feed utilization was evaluated by tracking the growth performance of red tilapia (*Oreochromis* sp.) in this study.

Materials and Methods

Experimental fish

Red tilapia for this experiment were bought from a private farm in Pingtung, Taiwan and then transported to the farm at the Department of Aquaculture, National Pingtung University of Science and Technology. Before the experiment, fish were reared in an outdoor 10-ton cement tank with continuous water flow, and fed by a control diet (**Table 1**) twice daily at around 10% of their body weight. The average weight of fish used was 0.43 ± 0.01 g at the beginning of the experiment.

Preparation of the experimental diets

Table 1 shows ingredients of the experimental diets. Four experimental diets were formulated to contain graded levels of X-xylanase (0 (control), 1050, 1500, and 1950 endopentosanase units (EPU)) using Hostazym® X-xylanase (X 6000, Huvepharma, Antwerp, Belgium). X-xylanase was added to the experimental diets with a corresponding decrease in the amount of inactive-xylanase. Ingredients were ground in a Hammer mill until they passed through a 60-mesh screen. The experimental diets were prepared by mixing the dry ingredients with soybean oil and then adding water until a stiff dough resulted. Each diet was then passed through a mincer with a die, and the resulting spaghetti-like strings were dried to a moisture level of 10% in a drying cabinet at 50°C with an air blower. After drying, the pellets were stored in plastic bins at -4°C until being used. Proximate analysis of the experimental and commercial diet (moisture, crude protein, crude lipid and ash) was performed according to AOAC procedures (1984).

Growth performance trial

A 56-day growth performance trial was conducted in 2-ton cement tanks with continuous water flow and aeration to maintain suitable water quality and dissolved oxygen (DO) of ≥ 5 mg/L. Tilapia juveniles were randomly assigned to four groups, and tests were carried out in triplicate. Each replicate consisted of 50 fish. In addition, a group of fish fed a commercial diet was used as control. Fish were fed twice daily at a ratio of 10% of their body weight at 08:00 and 15:00. Any uneaten feed was collected 1 h after feeding, and then immediately dried in an oven at 80°C. The amount of all diets consumed was calculated by subtracting the uneaten feed and was recorded on a daily basis. The weight of fish was measured at the start of the experiment and at 2-week intervals until the end of the experiment. Water temperature, dissolved oxygen (DO), and pH were respectively determined daily using a thermometer, DO meter, pH meter. Ammonia-N (Solorzano, 1969) and nitrite-N (Bendschneider and Robinson (1952) were measured every 2

Table 1: Ingredients of experimental diets used in the study.

Ingredient	Experimental diets (g/kg)				
	Commercial diet	Control	1050 EPU	1500 EPU	1950 EPU
Soybean meal		350	350	350	350
Fish meal		50	50	50	50
Corn starch		50	50	50	50
Wheat middlings		129.675	129.675	129.675	129.675
Rice bran		250	250	250	250
α -starch		100	100	100	100
Soybean oil		10	10	10	10
Xylanase (X 6000)		0	0.175	0.25	0.325
Inactive-xylanase (X 6000)		0.325	0.15	0.075	0
Mineral premix		30	30	30	30
Vitamin premix		30	30	30	30
Chemical analysis (%)					
Moisture	6.33	2.54	2.34	2.87	2.5
Crude protein	23.92	24.31	24.56	24.48	24.31
Crude lipids	8.03	8.68	9.12	8.24	9.01
Ash	7.8	9.2	9.1	9.05	8.85

Note: EPU-Endopentosanase units.

weeks during the growing-out phase. At the end of the experiment fish were individually weighted. Condition factor, survival rate, weight gain and feed efficiency were determined using following equations:

$$\text{Condition factor} = \text{body weight}/(\text{body length})^3 \times 100\%$$

$$\text{Survival rate (\%)} = (\text{final number of tilapia}/\text{initial number of tilapia}) \times 100\%$$

$$\text{Percentage weight gain (\%)} = [(\text{final weight} - \text{initial weight})/\text{initial weight}] \times 100\%$$

and Feed efficiency = weight gain/total feed intake.

The cost per kg fish production (NT\$) was calculated as following formula: Cost per kg fish production = (Feed cost per kg \times total feed intake (kg))/ weight gain (kg).

Whole-body composition analysis

After the experiment, six fish from each treatment were selected for a whole-body composition analysis. All fish samples were analyzed in duplicate for proximate composition following the standard methods (AOAC, 1984). Moisture was determined by a moisture analyzer (MX-50, A & D Japan). Crude protein was measured by the Kjeldahl procedure using the Kjeltac System (Tecator, Sweden) after acid digestion. Crude lipids were analyzed by the method of Folch *et al.*, 1957. Ash was determined after burning at 550°C in a muffle furnace.

Statistical Analysis

Experimental data were statistically analyzed by a one-way analysis of variance (ANOVA) and Duncan's multiple-range test in SAS software (Cary, NC, USA) to determine variations and the significance of differences between treatments. All percentage data were square-root arcsine-transformed before the statistical

analysis. The level of significance was accepted at $p < 0.05$.

Results and Discussion

Proximate analysis of the experimental diets

Proximate analysis of the experimental diets showed no significant differences in the moisture (2.34%-2.50%), ash (8.85%-9.2%), crude protein (24.31%-24.56%), or crude lipid (8.24%-9.12%) levels among all experimental diets. Compared to the proximate analysis of the commercial diet (crude protein 23.92%, moisture 6.33%, crude lipids 8.03% and ash 7.8%), the experimental diets seemed to have similar nutrient values, which should not have influenced the growth performance of tilapia compared to fish fed the commercial diet.

Growth performance

During the experiment, the water quality did not significantly differ among the treatments. The water temperature ranged 25-26.3°C, pH 7.3-8.3, DO >5 mg/L, ammonia-N 0.002-0.22 mg/L, and nitrite-N 0.013-0.23 mg/L.

Figure 1 shows the growth performance of tilapia fed the different experimental diets for 56 days. Growth performance results indicated that no significant difference was seen in the growth of tilapia fed the experimental diets and commercial diet, which may have been caused by individual growth differences among fish. However, fish fed the diet containing X-xylanase at a level of 1500 EPU seemed to have the highest weight compared to those of fish fed the other experimental diets and the commercial diet, but the growth rate of tilapia was reduced when an excessive dose of X-xylanase in the diet was offered to fish for 56 days in this study (**Figure 1**). Fish fed xylanase-supplemented diets having better growth performances was also found in juvenile Jian

carp (Jiang *et al.*, 2014) and Japanese seabass (Ai *et al.*, 2007). In addition, animals with poor weight gain after consuming a diet with an excessive amount of supplemented xylanase were also recorded in Jian carp (Jiang *et al.*, 2014) and broilers (Mendes *et al.*, 2013), which might have been due to the overproduction of xylooligosaccharides and xylose (Schutte, 1999; Xu *et al.*, 2009).

Xylanase applied in animal feed to improve feed utilization was reviewed (Goswami and Pathak, 2013), and previous studies showed that xylanase was able to improve the growth performance and feed utilization in poultry and pigs (Goswami and Pathak, 2013; Woyengo *et al.*, 2013). In aquaculture, a case of using multiple exogenous enzymes (including xylanase) in the diet of African catfish (*Clarias gariepinus*) resulted in enhanced fish growth performance and feed utilization (Yildirim and Turan, 2010). In Nile tilapia, fish fed a diet containing the xylanase-expressing probiotic, *Bacillus amyloliquefaciens* R8, which expresses the xylanase gene from rumen fungi, also had a significantly better growth performance and health status compared to fish fed a control diet or wild *B. amyloliquefaciens* (Saputra *et al.*, 2016). Similarity, X-xylanase incorporated in the diet at a level of 1500 EPU was able to improve the feed efficiency, a good indicator for evaluating feed quality (Table 2). The improved feed efficiency of fish fed the X-xylanase-supplemented diet at a level of 150 EPU indicated that the product really would be beneficial to tilapia aquaculture by improving feed utilization. At the end of the

experiment, mean weights of fish in the commercial diet, control, 1050 EPU, 1500 EPU, and 1950 EPU groups were 9.36 ± 0.7 , 10.27 ± 0.7 , 9.96 ± 1.15 , 11.07 ± 0.48 , and 9.01 ± 0.57 g, respectively (Table 2).

This study found no significant difference in the body composition of red tilapia, including moisture, protein, lipid, and ash contents among fish fed the commercial, control, and experimental diets. However, fish fed X-xylanase-supplemented diets seemed to have higher protein contents compared to the control and commercial diet groups (Table 3), and the increased tendency of protein content in the fish body was similar to the increase in feed efficiency which was highest at the optimal level (1500 EPU) of xylanase in the diet, and then decreased in the group supplemented with an excessive amount (1950 EPU) of X-xylanase in the diet. Net nutrient retention is considered an accurate and important indicator for evaluating the feed efficiency in fish (Belal, 2005). Results of increased body protein contents and feed efficiency in red tilapia fed X-xylanase-supplemented diets agree with results of Jiang *et al.* (2014). Furthermore, data on the body composition of tilapia in this study were similar to those of tilapia in a previous study (Gao *et al.*, 2015).

Cost analysis

The cost of the experimental diet was estimated to be NT \$31.38/kg. (The average exchange rate is US \$1≈New Taiwan (NT) \$30) (Table 4). No difference in the cost was calculated among the experimental diets because the inactive enzyme was added with a corresponding decrease in the amount of X-xylanase (the cost of X-xylanase was NT \$600 /kg). The cost of feed for 1 kg of tilapia production in the control, and 1050, 1500, and 1950 EPU groups were NT \$44.46 ± 0.30, NT \$43.68 ± 2.07, NT \$38.78 ± 0.46, and NT \$41.62 ± 1.06, respectively. Therefore, producing 1 kg of tilapia using the X-xylanase-supplemented diets at the levels of 1050, 1500, and 1950 EPU could save NT \$0.78, NT \$5.68, and NT \$2.84, respectively, compared to the control. It was thus concluded that using X-xylanase in the diet for tilapia aquaculture would be profitable, and the recommended level is 1500 EPU.

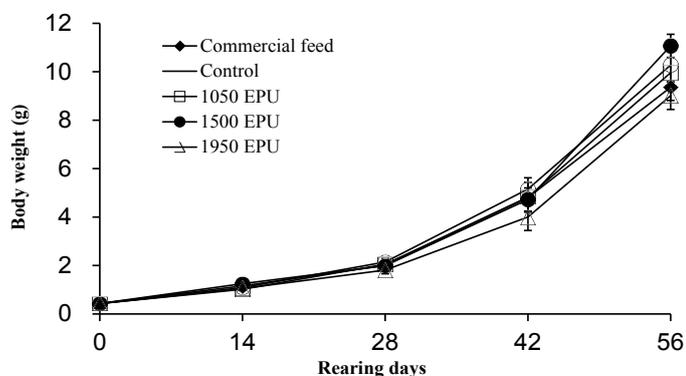


Figure 1: Growth of tilapia during the 56 days of the feeding experiment. EPU-Endopentosanase units.

Table 2: Initial weight, final weight, condition factor, survival, feed efficiency, and percentage weight gain of red tilapia fed by commercial, control and experimental diets. (Data ± standard error; different letters among treatments indicate a significant difference, $p < 0.05$).

	Experimental diets				
	Commercial feed	Control	1050 EPU	1500 EPU	1950 EPU
Initial weight (g)	0.43 ± 0.01	0.43 ± 0.01	0.43 ± 0.01	0.43 ± 0.01	0.43 ± 0.01
Final weight (g)	9.36	10.27 ± 0.7	9.96 ± 1.15	11.07 ± 0.48	9.01 ± 0.57
Condition factor	2.08	2.27 ± 0.03	2.25 ± 0.08	1.90 ± 0.30	2.12 ± 0.06
Survival (%)	98	100	96.67 ± 2.40	97.33 ± 1.76	98.67 ± 0.67
Feed efficiency	0.68 ^c	0.71 ± 0.01 ^{bc}	0.72 ± 0.03 ^{bc}	0.81 ± 0.01 ^a	0.76 ± 0.02 ^b
Percentage weight gain (%)	2076.08	2289.77 ± 162.46	2215.95 ± 266.47	2474.31 ± 110.35	1996.20 ± 133.19

Table 3: Effects of different diets on the body composition of red tilapia.

Experimental diets	Whole body composition (%)			
	Moisture	Protein	Lipids	Ash
Commercial feed	71.12 ± 2.22	17.28 ± 0.83	8.24 ± 0.83	4.72 ± 0.81
Control	70.28 ± 1.38	17.25 ± 0.63	8.32 ± 0.71	4.93 ± 0.25
1050 EPU	70.88 ± 2.27	18.02 ± 1.40	7.92 ± 0.683	4.88 ± 0.66
1500 EPU	71.25 ± 2.11	18.62 ± 0.86	7.81 ± 0.61	5.21 ± 0.91
1950 EPU	70.68 ± 2.47	17.92 ± 0.76	8.76 ± 0.66	5.08 ± 0.35

Note: EPU- Endopentosanase units.

Table 4: Cost analysis of the diets used in this study.

Diets	Cost	Total feed intake	Weight gain	Cost of 1 kg of fish production
	(NT\$)	(kg/fish)	(kg/replicate)	(NT\$)
Control	31.38	0.698 ± 0.061	0.492 ± 0.043	44.46 ± 0.30
1050 EPU	31.38	0.658 ± 0.075	0.476 ± 0.070	43.68 ± 2.07
1500 EPU	31.38	0.658 ± 0.039	0.531 ± 0.029	38.78 ± 0.46
1950 EPU	31.38	0.571 ± 0.059	0.429 ± 0.035	41.62 ± 1.06

Note: EPU-Endopentosanase units. The average exchange rate is US\$1≈New Taiwan (NT)\$30.

Conclusion

The present study clearly shows that X-xylanase is a great exogenous enzyme to improve feed utilization and consequently improve the fish growth performance when added to the diet at a level of 1500 EPU. It is therefore suggested that X-xylanase be used in the diet to improve the feed efficiency and tilapia growth performance at a level of 1500 EPU.

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