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Effects of Enzyme Suplementation in Diets on Growth and Feed Utilization in Angel Fish, *Pterophyllum scalare*

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Abstract: A 12-weeks feeding trial was conducted to investigate the effects of commercial cellulase enzyme products on the nutritive value of Canola Meal (CM) determined in angel fish fries. Nine isocaloric and isoprotein experimental diets (44% protein and 3500 kcal kg^{-1}) were prepared by adding cellulase enzyme (0.50 and 1.00 g kg^{-1}) at two different levels to feed including 7.20 and 35.99% canola meal instead of fish meal at basal diet. All diets were fed *ad libitum*. Weight gain, feed conversion ratio, body composition and nutrient digestibility were measured. High canola+enzyme diet gave significantly lower growth rates (1.51±0.02 g) ($p<0.05$). The low canola diet also resulted in higher weight gain but adding of cellulase enzymes in different ratios to diets showed no effect in growth parameter and nutrient digestibility.

Key words: Angel fish, *Pterophyllum scalare*, cellulase enzyme, growth, nutrient digestibility, canola meal

INTRODUCTION

The angel fish (*P. scalare*) is one of the most popular aquarium species, as this species commands a higher price compared with most freshwater food species and other ornamental fish. In spite of the economic importance of angel fish in ornamental fish culture, there has been neither research nor development of cost-effective feed for the intensive culture of this species. Presently, farmers rely on live food such as artemia, tubifex, daphnia and mosquito larvae and freshly prepared feeds. Production of live foods and conservation possibilities are quite limited in comparison with the formulated dry feeds. All ornamental fish feeds are 10-60 times higher in price than aquaculture feeds for food species. Second the price of the feed targeted for a single ornamental species vary dramatically compared to the price of food fish feeds, each of which is targeted for a specific species (Tamaru and Ako, 2000). For this reason, formulation of convenient feed rations for ornamental fish carry importance for aquarium sector (Sales and Janssens, 2003).

Fish meal is still a preferred protein source for fish feeds because of its high protein quality (NRC, 1993). However, due to high cost and limited availability in many contries (Lin *et al*., 2007). The high cost is mainly due to the high dietary protein requirement of carnivore fishes. Depending on the species of angel fish the dietary protein requirement varies between 40-50% (Degani, 1993). Suitable alternative feed ingredients such as grains and oilseed by products are the most promising source of protein and energy for aquafeed in the future (Hardy, 2000). Canola seeds are primarily grown for oil production for human consumption. Currently, considerable amounts of canola meal (17.7 million tons) are available for use in animal production (Kocher *et al*., 2000). However, the use of such plant-derived ingredients in aqua-feed is limited because of the presence of a wide variety of antinutritional substance (De Silva and Anderson, 1995). The digestibility in these plant sources is generally lower compared with fish meal in diets of fish (Lin *et al*., 2007). Exogenous enzymes are often used to increase the nutritive value of feed ingredients of plant origin in animal feeds (Buhcanan *et al*., 1997).

There are studies on enzyme supplemantation in diets for fish (Lin *et al*., 2007; Kolkovski *et al*., 1993; Buhcanan *et al*., 1997; Yan *et al*., 2002; Cavero, 2004; Debnath *et al*., 2005; Jakson *et al*., 1996; Drew *et al*., 2005; Zhong and Zhou, 2005; Papatryphon and Soares, 2001) but there is no published study till date on enzyme supplemantation in diets for ornamental fishes. Therefore, the present study was conducted to determine the effects of a commercially prepared exogenous enzyme on growth, nutrient digestibility and body composition in angel fish fry fed on canola meal based diets.

MATERIALS AND METHODS

Diet preparation and analysis: Using fish meal, canola meal, soybean meal, corn germ meal and blood meal as protein sources, corn starch, wheat meal as carbohydrate

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Table 1: Formulation of experimental diets and proximate analysis

	Groups								
	Control			CM (7.20%)			CM (35.99%)		
Ingredients	0[1]	0.5	1.0	0	0.5	1.0	0	0.5	1.0
Fish meal (68.53% protein)	45.52	45.52	45.52	41.88	41.88	41.88	27.31	27.31	27.31
Canola meal (34.67% protein)	-	-	-	7.20	7.20	7.20	35.99	35.99	35.99
Soybean meal (46.44% protein)	2.50	2.50	2.50	2.50	2.50	2.50	2.50	2.50	2.50
Corustarch	19.38	19.38	19.38	15.32	15.32	15.32	-	-	-
Coru gluten	12.00	12.00	12.00	12.00	12.00	12.00	12.00	12.00	12.00
Blood meal	6.60	6.60	6.60	6.60	6.60	6.60	6.60	6.60	6.60
Vegetable oil	10.00	10.00	10.00	10.50	10.50	10.50	11.60	11.60	11.60
Wheat meal	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50
Vitamin[2]	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00
Mineral[3]	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Cr_2O_3	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50
Cellulase Enzyme (g kg^{-1})	0.0	0.50	1.0	0.0	0.50	1.0	0.0	0.50	1.0
Proximate analysis									
Dry matter	93.39	93.67	93.46	93.83	93.61	93.73	93.56	93.56	93.64
Crude protein	44.53	44.27	44.43	44.36	44.85	44.17	44.62	44.36	44.49
Crude fat	12.20	13.03	12.53	13.53	13.35	13.36	14.26	14.40	24.28
Crude fiber	0.41	0.39	0.40	1.41	1.41	1.34	4.79	4.42	4.51
Crude ash	12.13	12.51	12.38	10.49	10.33	10.61	9.80	9.60	9.22
Digestible energy (kcal kg^{-1})[4]	3500.00	3500.00	3500.00	3500.00	3500.00	3500.00	3500.00	3500.00	3500.00

Control: Group containing no canola (fish meal based basal diet) control diet, CM (7.20%): group contain 7.20% canola meal, CM (35.99%): group contain 35.99% canola meal, [1]Cellulase enzyme level (g kg^{-1}), [2]Vitamin premix contained the following per kilogram; 4,000,000 IU vitamin A, 400,000 IU vitamin D3, 40,000 mg vitamin E, 2,400 mg vitamin K3, 4,000 mg vitamin B1, 6,000 mg vitamin B2, 40,000 Niacin mg, 10,000 mg Cal-D-Pantothenate:, 4,000 mg vitamin B6, 10 mg vitamin B12, 100 mg D-Biotin, 1200 mg Folic asit, 40,000 mg vitamin C (Stay C), 60,000 mg inositol. [3]Mineral premix contained the following per kilogram; 60,000 mg manganese, 80,000 mg zinco, 5,000 mg copper, 200 mg cobalt, 1000 mg iodine, 150 mg selenium,80,000 mg magnesium, 60,000 mg iron. [4]Digestible energy value was calculated from published values for the diet ingredients (NRC, 1993)

sources and vegetable oil as lipid source a basal diet was formulated to contain protein level of 44% (Table 1). The commercial enzyme used is a proprietary fungi fermentation product.

The enzyme (cellulase FG II) was obtained from Enzyme Development Corporation, USA and added to the low canola and high canola diets as an inclusion at two different levels of (0.5-1.00 g kg^{-1}) dry matter, as recommended by the company. Chromium oxide was added to each diet at a concentration of 0.5% as an inert marker for digestibility determinations. All ingredients were mixed thoroughly in a mixer for 30 min after mixing the diets were formed into spaghetties of 1.0 mm diameter by a laboratory pelet machine. Feed was then dried (20°C) for 16 h in a convection oven. After drying, the diets were broken up into appropriate (1 mm) pellet sizes. All diets were frozen (-20°C) until prior to use (Webster *et al*., 1997). At the end of the feeding trial, 5 fish per aquarium were sacrificed by a lethal dose of anesthesia (500 mg L^{-1} MS-222), homogenized in a blender, stored at -20°C for subsequent protein, lipid, ash and moisture analysis. Samples (diets, fish and feces) were analyzed for dry matter, crude protein, crude fibre and ash using standard methods (AOAC, 1995). These samples were analysed for dry matter at 65°C for 24 h in a vacuum oven. Crude protein was determined by measuring Nitrogen (N×6.25) using the Kjeldahl method and fiber by drying and ashing after the extraction with 0.5 M H_2SO_4 and 0.5 M NaOH. Ash content was determined after incineration at 550°C for 12 h in a muffle furnace. Crude lipid was determined using a chloroform-methanol extraction procedure (Folch *et al*., 1957). Faecal samples were collected twice daily 4 h after feeding for 84 days. Droppings from the same tank were pooled together in a bowl, pocketed in cellophane bags and stored in a freezer. Uneaten diet was siphoned out using a 2 cm pipe 20 min after feeding. Fish whole body and feces were determined using the ammonium-molybdate method described content of Cr_2O_3 in diet and feces were determined spectrophotometrically according to Furukawa and Tsukahara (1966). Two Apparent Digestibility Coefficients (ADC) were calculated according to Cho *et al*. (1982):

$$ADC = 0\text{-}100 \text{ (Marker in diet (\%)/Marker in feces (\%))} \times \text{(Nutrient in feces (\%)/Nutrient in diet (\%))}$$

Experimental procedure: Angel fish fry were obtained from Ortaca Vocational School University of Mugla. This experiment was carried out in 27 (80×40×40 cm) glass aquariums and was performed in triplicate. A static water system with continuous aeration and daily water changes (20% of volume) to maintain water quality was used. Twenty five angel fish fries (mean weight 0.91±0.01) were stocked into each aquarium. The total feeding period was 12 weeks. Water quality parameters, the dissolved oxygen level, temperatures, pH above 6.80±0.05 mg L^{-1}, 27±1°C,

7.80±0.10 were recorded throughout the experiment, respectively, as well as the levels of nitrite and nitrate were recorded NO_2 (0.015±0.004 mg L^{-1}) and NO_3 (7.03±0.30 mg L^{-1}).

Calculations and statistical analysis: Growth and feed utilization performances were determined based on these parameters:

$$\text{Survival (\%)} = \left(\frac{\text{Final number of fish}}{\text{initial number of fish}}\right)\times 100$$

Weight gain (g) = Mean final weight-mean initial weight

Specific Growth Rate

$$\text{(SGR \% per day)} = \left(\frac{(\ln W_t - \ln W_{t-1})}{T}\right)\times 100$$

where:

W_t = The mean final weight
W_{t-1} = Mean initial weight
T = Total experimental feeding days

$$\text{Protein Efficiency Ratio (PER)} = \frac{\text{Weight gain of fish (g)}}{\text{Total protein given (g)}}$$

$$\text{Feed Conversion Ratio (FCR)} = \frac{\text{Total feed fed (g)}}{\text{Total wet weight gain (g)}}$$

The data were analyzed by two-way ANOVA, using cellulose and canola meal concentrations as the two factors (SPSS, version 14.0). Where, two-way ANOVA showed a significant interaction between the two factors was used to identify significantly different means using Duncan multiple range test comparison. Differences were considered significant at $p<0.05$.

RESULTS AND DISCUSSION

Growth parameters: Percentage weight gain, specific grow rate, Feed Conversion Ratio (FCR) and Protein Efficiency Ratio (PER) of *P. scalare* fingerlins fed various test diets containing different levels of canola and cellulase are shown in Table 2. Highest weight gain and SGR were found in the CM-0.5 group and these values were significantly higher ($p<0.05$) than those of CM (7.20) and CM (35.99) groups. A significant decrease in weight gain was recorded with the increase in canola concentration. The adding of cellulase enzymes in different rations to diets showed no effect on growth parameters.

In general, enzyme supplemented diets exhibited a significant increase in weight gain ($p<0.05$) Buhcanan *et al.* (1997) in *prawn* and Debnath *et al.* (2005) in *Pangasius pangasius*, but contradicted by the results of Yan *et al.* (2002) with channel catfish. Although, the lowest FCR (2.96±0.07) was recorded in the CM-0.5 group, it was not significantly different ($p>0.05$) from that of other control groups and CM (7.20%)-0 group. Maximum FCR was recorded in CM (35.99%)-0.5, CM (35.99%)-1.0 groups. Enzyme supplemented diets exhibited a significant decrease in FCR ($p<0.05$) compared with the control group supporting the result of Cavero (2004), in Pirarucu but in contrast to Jakson *et al.* (1996).

Table 2: Growth parameters of angel fish fed with cellulase supplemented diets

	Parameters					
Groups	Initial weight (g $fish^{-1}$)	Final weight (g $fish^{-1}$)	WG (g $fish^{-1}$)	FCR	SGR (% day^{-1})	PER
CM -0	0.99±0.01^{a}	3.28±0.12^{a}	2.28±0.15^{a}	3.06±0.27^{a*}	1.41±0.08^{a*}	0.68±0.04^{a}
CM -0.5	0.96±0.02^{a}	3.46±0.09^{a}	2.50±0.08^{a}	2.96±0.07^{a}	1.53±0.03^{a}	0.69±0.03^{a}
CM -1.0	0.98±0.01^{a}	3.45±0.29^{a}	2.47±0.28^{a}	3.05±0.43^{a}	1.49±0.10^{a}	0.72±0.10^{a}
CM (7.20)-0	0.97±0.01^{a}	3.38±0.13^{a}	2.41±0.14^{a}	3.31±0.22^{a}	1.49±0.06^{a}	0.59±0.06^{ab}
CM (7.20)-0.5	0.98±0.01^{a}	2.62±0.26^{b}	1.64±0.27^{b}	5.10±0.64^{b}	1.16±0.13^{b}	0.42±0.06^{c}
CM (7.20)-1.0	0.99±0.01^{a}	2.46±0.03^{b}	1.48±0.03^{b}	4.54±0.26^{b}	1.09±0.01^{b}	0.45±0.05^{bc}
CM (35.99)-0	0.98±0.00^{a}	1.81±0.04^{c}	0.82±0.03^{c}	5.27±0.08^{b}	0.72±0.02^{c}	0.36±0.02^{cd}
CM (35.99)-0.5	0.97±0.01^{a}	1.68±0.07^{c}	0.71±0.07^{c}	6.74±0.70^{c}	0.65±0.05^{c}	0.29±0.04^{cd}
CM (35.99)-1.0	0.96±0.01^{a}	1.51±0.02^{c}	0.55±0.03^{c}	7.68±0.22^{c}	0.54±0.03^{c}	0.22±0.003^{d}
Main effects						
Canola meal (%)						
0	0.98±0.02^{a}	3.40±0.10^{a}	2.41±0.10^{a}	3.02±0.15^{a}	1.47±0.42^{a}	0.70±0.03^{a}
7.20	0.98±0.01^{a}	2.82±0.17^{b}	1.84±0.17^{b}	4.32±0.34^{b}	1.25±0.73^{b}	0.49±0.04^{b}
35.99	0.97±0.01^{a}	1.67±0.05^{c}	0.69±0.04^{c}	6.56±0.41^{c}	0.64±0.33^{c}	0.29±0.03^{c}
Enzyme (g kg^{-1})						
0	0.99±0.00^{a}	2.82±0.26^{a}	1.84±0.26^{a}	3.88±0.36^{a}	1.21±0.12^{a}	0.54±0.05^{a}
0.5	0.97±0.01^{a}	2.59±0.27^{ab}	1.62±0.27^{ab}	4.93±0.61^{b}	1.11±0.13^{ab}	0.47±0.06^{a}
1.0	0.98±0.01^{a}	2.47±0.29^{b}	1.50±0.29^{b}	5.09±0.70^{b}	1.04±0.14^{b}	0.46±0.08^{a}
$(\text{Canola}\times\text{Enzyme})_{int}$	ns	*	*	*	*	ns

$^{a-c*}$Values in the column having the same superscript are not significantly different ($p>0.05$), WG (g) = (final body weight, g-initial body weight, g), FCR = (total feed intake, g)/(final body weight, g - initial body weight, g), SGR (% day^{-1}) = [(ln final body weight - ln initial body weight)/days] ×100, PER = (fish weight gain, g)/protein fed, g), ns: not significant, *$p<0.05$

Protein efficiency ratios of CM -1.0 were higher than those of other groups but were not significantly different in all control groups ($p>0.05$). PER in high canola and enzyme-supplemented groups were significantly ($p<0.05$) lower than in the control groups. PER found in enzyme supplemented groups were significantly higher ($p<0.05$) than in the control group by Buhcanan *et al.* (1997). Poor protein efficiency was reported by Yan *et al.* (2002) when channel catfish was fed enzyme-added diets.

Digestibility: Nutrient digestibility is presented in Table 3. High canola meal levels in the diets were associated with reduced apparent dry matter, protein, ash and cellulose digestibility but there was no significant difference in lipid digestibility among groups. The nutrient digestibility was not improved significantly by the supplementation of enzyme in treatment groups compared with the control group. In rainbow trout, the use of a commercial protease improved the nutrient digestibility of coextruded canola (rapeseed meal) and pea (1:1) (Drew *et al*., 2005). In tilapia *O. niloticus* multienzyme PS had significant positive effects on the growth performance, protein digestibility (Zhong and Zhou, 2005). Kolkovski *et al.* (1993) also indicated that the porcine pancreatic supplementation may influence digestion, assimilation and growth in seabream *Sparus aurata*. In contrast, Papatryphon and Soares (2001) could not support any improvement in

Table 3: Mean percent apparent dry matter, crude protein, crude oil, crude ash nutrient digestibility of angel fish fed with cellulase supplemented diets

	Parameters (%)				
Groups	Dry matter	Crude protein	Crude lipid	Crude ash	Crude cellulose
CM -0	85.73±0.26^{a}	92.92±0.26^{a}	88.09±2.57^{a}	62.23±1.33^{a}	36.68±0.39^{a}
CM -1	86.02±0.66^{a}	93.64±0.70^{a}	87.91±3.00^{a}	58.79±0.44^{ab}	37.39±3.27^{a}
CM -5	86.92±0.19^{a}	93.80±0.05^{a}	88.52±1.83^{a}	56.71±1.14^{abc}	37.65±0.07^{a}
CM (7.20)-0	83.20±1.53^{a}	92.78±0.82^{a}	85.49±0.81^{a}	51.73±7.55^{abcd}	37.30±3.00^{a}
CM (7.20)-0.5	83.62±0.75^{a}	93.61±0.20^{a}	86.97±0.92^{a}	49.63±0.26^{bcd}	36.66±0.70^{a}
CM (7.20)-1.0	82.30±0.45^{a}	92.79±0.40^{a}	85.50±0.80^{a}	45.94±2.71^{cde}	36.55±0.92^{a}
CM (35.99)-0	74.37±2.78^{b}	89.77±0.25^{b}	88.05±1.95^{a}	43.76±4.28^{de}	19.80±0.13^{b}
CM (35.99)-0.5	72.29±2.09^{b}	88.94±0.16^{b}	87.02±2.11^{a}	35.03±0.79^{ef}	18.88±1,85^{b}
CM (35.99)-1.0	71.03±1.30^{b}	88.02±1.13^{b}	85.24±0.45^{a}	28.96±3.61^{f}	17.22±0.02^{b}
Main effects					
Canola meal (%)					
0	86.22±0.09^{a}	93.45±0.26^{a}	88.17±1.13^{a}	59.24±1.12^{a}	37.24±0.87^{a}
7.20	83.04±0. 52^{b}	93.06±0.30^{a}	85.98±0.49^{a}	49.10±2.33^{b}	36.84±0.97^{a}
35.99	72.56±1.14^{c}	88.91±0.44^{b}	86.77±0.91^{a}	35.91±3.08^{c}	18.63±0.67^{b}
Enzyme (g kg^{-1})					
0	81.10±2.33^{a}	91.82±0.69^{a}	87.21±1.02^{a}	52.57±4.07^{a}	31.26±3.71^{a}
0.5	80.64±2.74^{a}	92.06±1.01^{a}	87.30±0.99^{a}	47.81±4.38^{ab}	30.97±3.95^{a}
1.0	80.08±3.01^{a}	91.53±1.17^{a}	86.42±0.85^{a}	43.87±5.25^{b}	30.47±4.20^{a}
(Canola×Enzyme)$_{int}$	ns	ns	ns	ns	ns

$^{a-c}$Values in the column having the same superscript are not significantly different ($p>0.05$), ns: not significant

Table 4: Dry matter, crude protein, crude oil, crude ash and crude cellulose in body composition of angel fish fed with cellulase supplemented diets

	Parameters (%)				
Groups	Dry matter	Crude protein	Crude lipid	Crude ash	Moisture
CM -0	23.65±1.34^{ab}	21.02±0.96^{a}	5.27±2.00^{a}	2.29±0.19^{b}	76.35±1.34^{ab}
CM -1	28.11±5.66^{ab}	19.69±0.17^{ab}	5.72±0.16^{a}	3.04±0.20^{ab}	71.90±5.66^{ab}
CM -5	23.03±0.95^{ab}	18.52±0.02^{b}	5.22±1.28^{a}	3.30±0.36^{a}	76.97±0.95^{ab}
CM (7.20)-0	32.94±6.96^{a}	19.36±0.36^{ab}	5.12±1.42^{a}	2.90±0.08^{ab}	67.07±6.96^{b}
CM (7.20)-0.5	21.26±0.05^{ab}	18.14±0.92^{b}	5.32±1.38^{a}	3.09±0.09^{ab}	78.74±0.05^{ab}
CM (7.20)-1.0	21.46±0.51^{ab}	18.79±0.92^{ab}	5.91±0.61^{a}	3.13±0.11^{ab}	78.55±0.52^{ab}
CM (35.99)-0	27.11±2.77^{ab}	18.64±1.18^{ab}	5.00±0.04^{a}	3.46±0.22^{a}	72.90±2.55^{ab}
CM (35.99)-0.5	20.74±0.02^{b}	18.01±0.33^{b}	5.72±0.60^{a}	3.20±0.60^{ab}	79.27±0.02^{a}
CM (35.99)-1.0	24.08±2.81^{ab}	18.19±0.43^{b}	5.57±1.67^{a}	3.48±0.00^{a}	75.93±2.81^{ab}
Main effects					
Canola meal (%)					
0	24.93±1.83^{a}	19.74±0.52^{a}	5.40±0.62^{a}	2.88±0.22^{a}	75.07±1.83^{a}
7.20	25.22±3.03^{a}	18.76±0.41^{ab}	5.45±0.55^{a}	3.04±0.06^{a}	74.78±3.03^{a}
35.99	23.97±1.55^{a}	18.28±0.36^{b}	5.43±0.48^{a}	3.38±0.17^{a}	76.03±1.55^{a}
Enzyme (g kg^{-1})					
0	27.90±2.61^{a}	19.67±0.60^{a}	5.13±0.63^{a}	2.88±0.23^{a}	72.10±2.61^{a}
0.5	23.37±2.09^{a}	18.61±0.43^{a}	5.59±0.40^{a}	3.11±0.17^{a}	76.63±2.09^{a}
1.0	22.85±0.91^{a}	18.50±0.28^{a}	5.57±0.58^{a}	3.30±0.12^{a}	77.15±0.91^{a}
(Canola×Enzyme)$_{int}$	ns	ns	ns	ns	ns

^{a}Values in the column having the same superscript are not significantly different ($p>0.05$), ns: not significant

apparent dry matter and protein digestibility in striped bass reported by Storebakken *et al*. (1998). Analysis of fish body composition (Table 4) at equal weight indicated no significant differences in lipid concentrations, However dry matter, protein content displayed significant differences.

Survival was not affected by supplementation of cellulase enzyme and canola meal to angel fish diets. Survival rate in trial groups were 77-93% in all treatments.

CONCLUSION

The results of this study indicate that supplement of exogenous enzymes in order to improve the nutritional value of angel fish feeds with high levels of canola meal was not effective. Additional research is needed to improve the nutritional value of feeds.

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