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Effects of Dietary Protein Source on Growth Performance, Feed Utilization and Digestive Enzyme Activity in Rainbow Trout (Oncorhynchus Mykiss)

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Abstract

In this paper the effect of replacing fish meal with different protein sources was tested on growth performance, feed utilization and digestive enzyme activity in rainbow trout. Six experimental diets containing different protein sources were tested: 1) 100% fish meal (FM), 2) 60% fish meal + 40% plant protein (60FM/40PP), 3) 30% fish meal + 70% plant protein (30FM/70PP), 4) 100% plant protein (PP), 5) 50% poultry by-product meal protein + 50% plant protein (50PP/50PM) and 6) 100% poultry by-product meal (PM). Rainbow trout with a mean initial weight of 15±2 g were fed experimental diets for 60 days. Results showed that 40% fish meal replacement with plant protein did not negatively affect the growth indices, feed utilization and muscle proximate composition. However, 70% and 100% replacement of fish meal with different protein sources resulted in significantly decreased growth, feed utilization and total fillet protein, but significantly increased total fillet lipid. The alkaline protease activity in 60FM/40PP, 50PM/50PP and PM groups were not significantly different with control, but significantly lower in 30FM/70PP and PP groups. The fish fed 100% plant protein (PP) resulted in decreased lipase activity compared to other feeding treatments, but no significant differences in lipase activity among other groups.

Key words: Protein source, Growth, Digestive enzyme activity, Rainbow trout

INTRODUCTION

Feed cost is the major expense in fish culture. One of the challenges is to develop less wasteful and more economic diets. Fish meal has long been the major protein source in feeds for trout, salmon, and marine fish. In order to reduce feed costs and improve sustainability of culture of these fishes, fish meal is increasingly being replaced by more economical protein sources. The production of successful fish feed formula which rely less on fish meal, requires accurate information on the nutritive value of more economical protein sources. The increasing demand of ingredients for aquaculture feeds all over the world has driven an important research effort towards the nutritive evaluation of other protein sources.

Many plant protein sources can be used to partially or almost totally replace dietary fish meal [21-37], provided that the essential amino acid requirements of the fish species are met, the palatability of the diet is improved and the levels of anti-nutritional factors (ANFs) are reduced [13]. Another alternative ingredient to fish meal is poultry by-product meal (PBM). PBM is made of ground and clean parts of the carcass of slaughtered poultry.

Previous works have shown good potential of the combination of PBM, FM and blood meal (BM) [10], PBM and FM [43] and PBM, meat and bone meal (MBM), BM and FM [46] in diet of various fish species. Fowler (1991) reported PBM could replace about 50% of fish meal in the diets for chinook salmon and rainbow trout. Higss et al. (1979) found that defatted PBM and PBM mixed with hydrolysed feather meal could replace up to 33% and 75 % of fish meal, respectively, in coho salmon diets. About 50 % of fish meal was successfully replaced with PBM in chinook salmon and rainbow trout [43]. Moreover, PBM has been tested at varying success so far in sea bream [29], European eel [4], channel catfish [39], common carp [17] and sunshine bass [47].

One of the main limitations to the use of plant protein sources is the presence of antinutritional factors that may reduce the activity of fish digestive enzymes [40]. Provided that fish proteases are highly sensitive to such inhibitors, the assessment of the nutritional value of vegetable foodstuffs (particularly through the determination of the apparent digestibility coefficient of proteins) should consider the interactions between the antinutritional factors and fish digestive enzymes.

Generally, distribution of enzymes and their activity in the digestive tract of fish vary with their feeding habits and the morphology of their intestine [45]. Therefore changing the diet may induce changes in the enzymatic activity. An understanding of the functioning of the digestive enzymes helps to explain nutrient digestibility [22]. In short, studies on digestive secretions in fish can elucidate certain aspects of its nutritive physiology and help resolve nutritional problems, such as the matching of an artificial diet to the nutritive capabilities of fish. The knowledge of how different feed ingredients may affect enzyme activity is important, and this would provide information on if and how the choice of ingredients in feed formulations could allow a better efficiency of digestive enzymes [8].

Since there are few researches regarding the effects of fish meal replacement with PMB and combination of different protein sources on digestive enzyme activity in rainbow trout, the present study was performed to study the probable effects of replacement of fish meal with different protein sources on growth performance, feed utilization and digestive enzyme activity in pyloric caeca of rainbow trout.

MATERIALS AND METHODS

Fish husbandry and diet preparation

Fish were purchased from a local trout farm and acclimated for 2 weeks during which they were fed commercial diet. Forty fish with average weight of 15±0.2 g were stocked in 18 polyethylene tanks (300 L) supplied with freshwater at a flow rate of 7.5 L min^{-1}. Light/dark cycle was 12 L:12 D. The pH (7.3-7.7), temperature (14-15˚C) and dissolved oxygen level (6.8-7.5 mgL^{-1}) of each tank were monitored daily

Five experimental diets with similar protein, lipid and energy content were formulated to contain different protein sources to replace fish meal (Table 1). The control diet contained only the Kilka meal (*Clupeonella* sp.) as the primary sources of protein (FM). The experimental diets contained: (1) 60% fish meal + 40% plant protein (60FM/40PP), (2) 30% fish meal + 70% plant protein (25FM/75PP), (3) 100% plant protein (PP), (4) 50% poultry by-product meal protein + 50% plant protein (50PP/50PM), (5) 100% poultry by-product meal protein (PM). The experimental plant protein sources included wheat gluten, corn gluten and soybean meal.

Briefly, all dry ingredients were thoroughly mixed in a mixer. Oil was added and thoroughly mixed for 5 min and then moistened by adding cold distilled water until stiff dough yielded. The wet dough was grinded and converted to strands (3 mm in diameter) using a meat grinder. The strands were dried at 50°C for 8 h using an oven, manually crumbled into appropriate size and sieved. Pellets were stored at 4°C during the experiment. Fish were fed three times per day at 3% body weight for 8 weeks.

Table 1. Ingredient and proximate composition of experimental diets

Ingredients (g kg^{-1} diet)	*Dietary treatment* [1]					
	FM	*60FM/40PP*	*30FM/70PP*	*PP*	*50PP/50PM*	*PM*
Kilka fish meal	*582.5*	*350*	*182.5*	-	-	-
Wheat gluten	-	*155*	*260*	*420*	*160*	-
Corn gluten	-	*55*	*110*	*100*	*100*	-
Soybean meal	-	*150*	*150*	*150*	*150*	-
Poultry by-product	-	-	-	-	*320*	*500*
Blood meal	*40*	*40*	*40*	*40*	*60*	*200*
Kilka fish oil	*128.9*	*140.6*	*161.3*	*185.7*	*128*	*110*
Wheat meal	*145*	-	-	-	-	*50*
Wheat starch	*52.5*	*49.4*	*8*	-	*26*	*60*
Filler	-	-	*28.2*	*37.3*	-	*24*
Zeolite	*5*	*5*	*5*	*5*	*5*	*5*
Vitamin premix[1]	*15*	*15*	*15*	*15*	*15*	*15*
Mineral premix[2]	*10*	*10*	*10*	*10*	*10*	*10*
L-methionine	*12*	*12*	*12*	*12*	*12*	*12*
L-lysine	*0*	*8*	*8*	*15*	*4*	*4*
Di-calcium phosphate	*5*	*5*	*5*	*5*	*5*	*5*
Calcium carbonate	*5*	*5*	*5*	*5*	*5*	*5*
Proximate composition (% dry matter)						
Moisture	*8.1*	*7.6*	*8.2*	*8.1*	*7.7*	*7.9*
Crude protein	*45.3*	*44.5*	*45.1*	*45.5*	*45*	*45.4*
Crude lipid	*19.9*	*19.8*	*20.1*	*19.8*	*20.1*	*20.1*
Crude starch	*14.9*	*15*	*14.9*	*15.4*	*15.2*	*15.1*
Gross energy (kcal/g)[3]	*5.04*	*5.03*	*5.05*	*5.04*	*5.04*	*5.04*
Digestible energy (kcal/g)[4]	*4.3*	*4.3*	*4.3*	*4.3*	*4.3*	*4.3*

[1]Vitamin mixture: (mg or IU/kg of diet) Vitamin A (as acetate) 1600000 IU; vitamin D3, 400000 IU; choline chloride.12000; niacin, 4000; riboflavin, 8000; pyridoxine, 4000; folic acid, 2000; vitamin B12, 8000; biotin, 1; inositol, 20000; vitamin C, 60000; vitamin H2, 2.4; vitamin B2, 8000; vitamin K3, 2000; vitamin E,40000.

[2]Mineral mixture (g/kg): zinc, 12.5 g; iron, 26 g; manganese, 15.8 g; copper, 4.2 g; cobalt, 0.48 g; selenium, 2 g; iodine, 1 g.

[3]Calculated on the basis of 5.64, 9.43, and 4.11 (kcal/g diet) for protein, fat, and carbohydrate, respectively (NRC 1993).

[4]Calculated using apparent coefficients of digestibility of 0.9, 0.85, and 0.8 for crude protein, crude fat, and carbohydrates (NFE), respectively.

Growth parameters and feed utilization indices

On the first and the last day of the experiment fish were weighed (W ± 0.01 g) and total lengths were measured (TL ± 0.1 cm). The following parameters were calculated:

Specific growth rate (SGR, $\%d^{-1}$) $=100\times[(\ln W_f-\ln W_i)\times T^{-1}]$; Daily growth rate (DGR, g d^{-1}) $= (W_f-W_i)\times T^{-1}$; Condition factor (CF) $= 100\times(W\times TL^{-3})$; days reared; Feed conversion ratio (FCR) $= TFI\times(FB-IB)^{-1}$; Protein efficiency ratio (PER) $= (FB-IB)\times TFP^{-1}$; Feed efficiency ratio (FER) = wet gain×(dry feed intake)$^{-1}$; Protein efficiency ratio (PER) = weight gain (g)/protein intake (g); Protein production value (PPV) = fish protein gain (g)/protein intake (g); Lipid efficiency ratio (LER) = weight gain (g)/lipid intake(g); Lipid production value (LPV) = fish lipid gain (g)/lipid intake (g) [26]. Hepatosomatic index (HSI, %) $= 100\times(LW\times W^{-1})$; Viscerosomatic index (VSI, %) $= 100\times(VW\times W^{-1})$.

Where: W_f and W_i are the final and initial body weights (g), T—time of rearing (days), FB and IB are the final and initial absolute weights (g), TFI—total feed intake (g), FBP—final body protein content (%), IBP—initial body protein content (%), TFP—total protein intake (g), FBL—final body lipid content (%), IBL—initial body lipid content (%), TFL—total lipid intake (g), LW—liver weight (g), VW—viscera weight (g).

Collection of samples

Before starting the experiment, five randomly selected fish samples were anaesthetised with clove oil (concentration: 0.1 ml L^{-1}), killed by a sharp blow to the head and stored at −22°C prior to chemical analysis. At the start of the experiment and at the end of the growth period, 12 fish/tank were collected to measure the total length and weight. At the end of experiment, four fish per tank were tested for carcass analyses (moisture, ash, protein and fat content) and nutrient retention calculation. Nine fish per tank were collected to weigh the liver and digestive tract for measurement of hepatosomatic index (HSI) and viscerosomatic index (VSI) and fillets were stored at -40°C for muscle fatty acid determination.

Proximate composition of diets and fish

Feeds and muscle moisture were determined by drying in oven (Iran khodsaz Co, Iran) at 105°C for 25 h to a constant weight; ash was determined by incineration in a muffle furnace (Iran Khodsaz Co, Iran) at 600°C for 6 h; crude protein was determined by the Kjeldahl method (N × 6.25) using an automatic Kjeldahl system (Behrotest WD 40, Germany); Crude lipid content determination was conducted by ether extraction and carbohydrate was calculated by the following formula [1]:

Carbohydrate$(gkg^{-1})=1000-(protein+lipid+moisture+ash)$.

Gross energy content of the diets and feces were calculated on the basis of 5.64, 9.43, and 4.11 (kcal/g diet) of protein, fat, and carbohydrate, respectively [31].

Enzyme assay

Sampling and Enzymatic analysis

Three fish were collected from each tank at the end of 60 days of feeding trial. After 24 h of starvation, the fish were killed and their digestive tracts (pyloric caeca) were removed, frozen in liquid nitrogen and stored at -80°C until analysis.

Analytical methods

Preparation of extracts and determination of soluble protein

Digestive tract (pyloric caeca) were weighed and homogenized in an equal volume of ice-cold 50mM Tris-HCl buffer, pH 7.5 (w/v), using a Polytron PT 1300 D homogenizer (Kinematica AG, Littau-Lucerne, Switzerland) with a 7 mm generator at a setting of 20000 rpm for 3 × 30 s. The homogenate was centrifuged at 10000 g for 20min at 4°C and the supernatant was collected in small aliquots (300-500 mL) and stored at -80°C until analysis [7]. All procedures were carried out at 4°C. Total soluble proteins were determined in the supernatant by the Bradford method using bovine serum albumin as the standard [5].

Alkaline protease

The specific alkaline proteolytic activity of the samples were assayed in quadruplet using 2% azocasein as a substrate according to a modification of García-Carreño & Haard (1993). Briefly, samples (20 µL) of enzyme preparation were mixed with 0.5mL of the substrate in 50mMTris-HCl, pH 7.5, at 25 °C. The reaction was stopped 10 min later by the addition of 0.5mL of 20% trichloroacetic acid (TCA). The reaction mixture was centrifuged for 5 min at 6500 g. The supernatant was separated from the undigested substrate and the absorbance was recorded at 360 nm for the released dye. The activity unit was the change in absorbance (360 nm) $min^{-1}mg^{-1}$of protein.

Lipase

Lipase activity was determined by hydrolysis of n-nitrophenyl myristate. Each assay (0.5 ml) contained 0.53 mM n-nitrophenyl myristate, 0.25 mM 2-methoxyethanol, 5 mM sodium cholate and 0.25 M Tris–HCl (pH 9.0). Incubation was carried out for 15 min at 30°C, and the reaction was terminated by adding 0.7 ml of acetone/n-heptane (5:2, v/v). The reaction mixture was vigorously mixed and centrifuged at 6080 g for 2 min. The absorbance was measured in the resulting lower aqueous layer at 405 nm. The extinction coefficient of n-nitrophenol was 16,500 $M^{-1}cm^{-1}$ L^{-1}. One unit of enzyme activity was defined as 1 µmol of n-nitrophenol released per min [20].

Amylase

Amylase activity was determined by the 3,5-dinitrosalicylic acid (DNS) method [48]. Starch substrate (1% w/v) was diluted in a buffer at pH 6.9, 0.02 M Na2HPO4 and 0.006 M NaCl. The substrate (250 µl) was incubated with crude extract (50 µl) and buffer solution (250 µl) for 3–4 min at 25 °C. Then 0.5 ml of 1% dinitrosalicylic acid (DNS) solution was added and boiled for 5 min. After boiling, 5 ml of distilled water was added to the mixture and the absorbance of the cooled solution was recorded at 540 nm. Blanks were similarly prepared, but without the crude enzyme extracts. Maltose (0.3–5 μMml^{-1}) was used for the preparation of the standard curve. The α-amylase specific activity was defined by the µmol of maltose produced min^{-1} mg^{-1} protein at the specified condition.

Statistical analysis

The results were analysed using analysis of variance, ANOVA, for which the homogeneity of variances and the normal distribution were tested according to the Levene and Shapiro_Wilk tests, and comparison among the means was made using Duncan's multiple range test (DMRT) [42]. All statistical analyses were conducted using SPSS (version16) and tested at $P < 0.05$.

RESULTS

Growth indices in different treatments are shown in table 2. No significant differences were detected in growth of fish fed control diet (FM) and those fed on diet containing 60% fish meal protein + 40% plant protein (60FM/40 PP). However fish fed 70% plant protein (30FM/70PP), 100% plant protein (PP), 50% plant protein + 50% poultry meal protein (50PM/50PP) and 100% poultry meal protein (PM) resulted in decreased WG, SGR, and DGR and increased FCR. No significant differences were observed in hepatosomatic index (HIS) and viscera somatic index (VSI) among feeding treatments. Condition factor (CF) was significantly lower in fish fed diet with plant protein sources (PP) compared to other feeding treatments ($P < 0.05$).

Chemical composition of muscle and feed efficiency in different treatments are shown in Table 3. Muscle protein content in fish fed 60FM/40PP, 30FM/70PP, 50PM/50PP and PM showed no significant differences compared to that in control fish (FM). However, muscle protein content in PP group was significantly lower ($P < 0.05$). Muscle lipid content in 60FM/40PP group had no significant differences with control; whereas, lipid content in fish fed 100% fish meal replacement diets (PP and PM) were significantly higher compared to control group ($P < 0.05$). Muscle ash content in groups fed 60FM/40PP and 50PM/50PP did not show any significant differences compared to the control group (FM). Nevertheless, this parameter was significantly lower in 30FM/70PP and PP groups and significantly higher in PM group compared to other feeding treatments ($P < 0.05$).

No significant differences were observed in protein production value (PPV), protein efficiency ratio (PER), and lipid efficiency ratio (LER) in 50FM/50PP group compared to control fish. Nevertheless, these parameters were significantly lower in fish fed other protein sources. Lipid production value (LPV) did not show any significant differences among different treatments.

Table 2. Growth indices of rainbow trout fed experimental diets for 60 days (n=9 fish/tank).

Performance parameters	*Dietary treatment* [1]					
	FM	*60FM/40PP*	*30FM/70PP*	*PP*	*50PP/50PM*	*PM*
Initial body weight (g)	15.6 ± 0.2^{a}	15.5 ± 0.3^{a}	15.1 ± 0.1^{a}	15.5 ± 0.1^{a}	15.2 ± 0.1^{a}	15.1 ± 0.1^{a}
Final body weight (g)	71.1 ± 1.8^{a}	69 ± 1.5^{a}	56.9 ± 0.1^{c}	47.9 ± 3.2^{e}	64.4 ± 0.2^{b}	52.1 ± 0.7^{d}
Weight Gain (g/fish)	55.4 ± 2.0^{a}	53.5 ± 1.2^{a}	41.7 ± 1.0^{c}	32.4 ± 3.2^{e}	49.13 ± 0.8^{b}	36.87 ± 0.7^{d}
Feed intake (g/fish)	54 ± 2.6^{a}	56 ± 1.5^{a}	49 ± 0.4^{b}	46 ± 2.1^{bc}	55 ± 0.9^{a}	44 ± 2.8^{c}
FCR[2]	0.97 ± 0.07^{d}	1.04 ± 0.03^{cd}	1.17 ± 0.03^{b}	1.33 ± 0.04^{a}	1.33 ± 0.04^{bc}	1.33 ± 0.04^{b}
DGR[3]	2.82 ± 0.07^{a}	2.77 ± 0.05^{ab}	2.36 ± 0.04^{c}	1.96 ± 0.13^{e}	2.20 ± 0.04^{b}	2.36 ± 0.05^{d}
SGR[4]	1.13 ± 0.02^{a}	1.11 ± 0.01^{a}	0.99 ± 0.01^{b}	0.84 ± 0.05^{d}	1.09 ± 0.04^{a}	0.93 ± 0.02^{c}
HIS[5]	1.49 ± 0.04^{a}	1.42 ± 0.03^{a}	1.49 ± 0.11^{a}	1.46 ± 0.08^{a}	1.48 ± 0.02^{a}	1.43 ± 0.07^{a}
VSI[6]	14 ± 0.3^{a}	14.2 ± 0.5^{a}	14.5 ± 0.6^{a}	14.4 ± 0.2^{a}	14.3 ± 0.1^{a}	14.8 ± 0.9^{a}
CF[7]	1.14 ± 0.01^{ab}	1.14 ± 0.05^{ab}	1.18 ± 0.04^{a}	1.06 ± 0.05^{c}	1.12 ± 0.02^{b}	1.17 ± 0.02^{ab}

Values are means ±S.D. Values with the same superscripts within the same row are not significantly different ($P < 0.05$). [1]See Table 1 for diet abbreviations.
[2]FCR, food conversion ratio; [3]DGR, Daily growth rate; [4]SGR, specific growth rate; [5]HIS, Hepatosomatic index; [6]VSI, viscerosomatic index; [7]CF, Condition factor.

Table 3. Proximate composition of muscle (%) and nutrient retention in rainbow trout fed experimental diets for 60 days (n=4 fish/tank).

	Dietary treatment [1]					
	FM	*60FM/40PP*	*30FM/70PP*	*PP*	*50PP/50PM*	*PM*
Moisture (%)	77.4 ± 0.4^{a}	70.3 ± 2.9^{c}	71.5 ± 0.1^{bc}	69 ± 2.7^{c}	76.0 ± 2.6^{a}	69 ± 0.5^{ab}
Crude protein (%DM)	70.7 ± 0.1^{ab}	69 ± 0.9^{c}	69 ± 1^{bc}	65.1 ± 2^{d}	69.5 ± 1^{b}	72.0 ± 1^{a}
Crude lipid (%DM)	13.7 ± 0.8^{c}	13.9 ± 0.4^{c}	15.7 ± 0.7^{b}	18.1 ± 0.7^{a}	16.0 ± 0.4^{b}	15.9 ± 0.4^{b}
Ash (%DM)	7.4 ± 0.3^{bc}	6.9 ± 0.1^{cd}	6.4 ± 0.1^{d}	4.7 ± 0.2^{e}	8.2 ± 0.9^{ab}	8.6 ± 0.2^{a}
PPV[2]	0.38 ± 0.02^{a}	0.35 ± 0.02^{ab}	0.31 ± 0.01^{b}	0.22 ± 0.03^{c}	0.32 ± 0.01^{b}	0.31 ± 0.02^{b}
PER[3]	2.25 ± 0.12^{a}	2.12 ± 0.06^{ab}	1.89 ± 0.03^{c}	1.55 ± 0.18^{d}	1.95 ± 0.06^{bc}	1.83 ± 0.08^{c}
LPV[4]	0.17 ± 0.02^{a}	0.15 ± 0.01^{a}	0.16 ± 0.01^{a}	0.16 ± 0.02^{a}	0.17 ± 0.01^{a}	0.16 ± 0.01^{a}
LER[5]	$5.15\pm0.42a$	4.7 ± 0.15^{ab}	4.3 ± 0.09^{c}	3.5 ± 0.42^{d}	4.5 ± 0.15^{bc}	4.2 ± 0.19^{c}

Values are means ± S.D. Values not sharing the same superscript letters within the same row are significantly different ($P < 0.05$). [1]See Table 1 for diet abbreviations.
[2]PPV, protein production value; [3]PER, protein efficiency ratio; [4]LPV, lipid production value; [5]LER, lipid efficiency ratio.

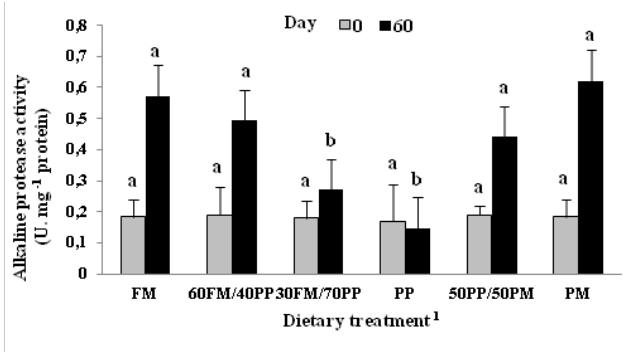

Figure 1. The Alkaline protease activity in pyloric caeca of rainbow trout fed experimental diets for 60 days. Values are expressed as mean ± SEM (n=3 fish.tank^{-1}). Values in the same column with the same superscript are not significantly (P > 0.05) different using Duncan's multiple comparison. [1]See Table 1 for diet abbreviations.

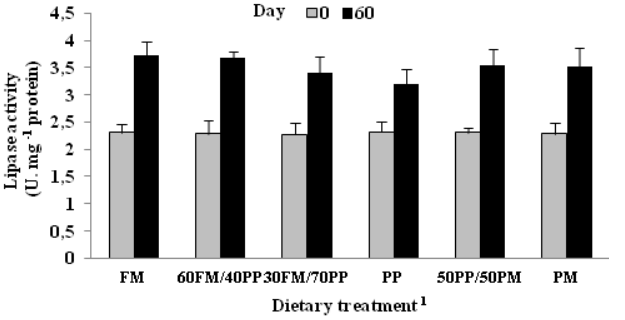

Figure 2. The lipase activity in pyloric caeca of rainbow trout fed experimental diets for 60 days. Values are expressed as mean ± SEM (n=3 fish/tank). No significant differences were observed among different dietary treatments (P > 0.05). [1]See Table 1 for diet abbreviations.

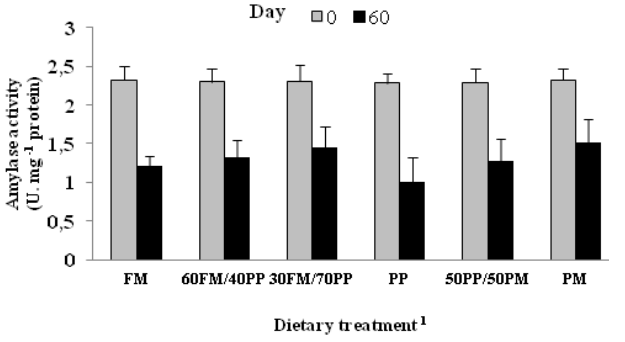

Figure 3. The amylase activity in pyloric caeca of rainbow trout fed experimental diets for 60 days. Values are expressed as mean ± SEM (n=3 fish/tank). No significant differences were observed among different dietary treatments (P > 0.05). [1]See Table 1 for diet abbreviations.

The alkaline protease, lipase and amylase activity on day 0 of the experiment were 0.18 ± 0.05, 2.30 ± 0.18 and 2.32 ± 0.16 U respectively. The alkaline protease activity on day 60 in 60FM/40PP (0.49 ± 0.05 U), 50PM/50PP (0.52 ± 0.09 U) and PM (0.49 ± 0.12 U) groups had no significant differences with control (0.57 ± 0.06 U); however this parameter was significantly lower in 30FM/70PP (0.27 ± 0.08 U) and PP (0.15 ± 0.04 U) groups compared with other feeding treatment (P < 0.05) (Figure 1). No significant differences on day 60 were detected in lipase activity of FM (3.73 ± 0.02 U), 60FM/40PP (3.67 ± 0.12 U), 30FM/70PP (3.40 ± 0.03 U), 50PM/50PP (3.43 ± 0.03 U) and PM (3.66 ± 0.03 U) groups. But fish fed with 100% plant protein PP (3.18 ± 0.02 U) resulted in decreased lipase activity compared to other feeding treatments (Figure 2). The amylase activity did not show any significant differences among different treatments (P > 0.05) (Figure 3).

DISCUSSION

Results showed that substituting 40% fish meal with gluten-based protein (60 FM/40PP) does not adversely affect the fish growth. This is in accordance with replacing 30 and 35% fish meal with wheat gluten in Atlantic salmon and Atlantic halibut respectively [18-44] and 50% fish meal with corn gluten in Atlantic salmon [27]. However, growth indices decreased significantly by substitution of 70 (30FM/70PP) and 100% (PP) of fish meal with plant protein sources [9-12-32-40], combination of plant protein and PBM [20] and PBM as sole source of protein in the diet of rainbow trout [10-14-43]. It is proposed that inclusion of higher levels of plant ingredients and PBM in salmonids diets has adverse effect on fish performance.

Recent studies on concentrated plant protein inclusion in rainbow trout diet showed that it can potentially replace whole dietary fish meal with either no reduction or just a slight reduction in growth [21], which is in contrast with our findings. There are several explanations for undesirable effects of higher levels of plant derived ingredients in salmonids diet such as higher carbohydrate content which is not generally well digested by salmonids [41]. However, it is not conceivable that carbohydrate could have noticeable effects on fish growth in this study, since all diets had balanced carbohydrate content. We incorporated wheat and corn gluten because of their higher content of protein, lower amounts of fiber and starch and relatively void of any ANFs [38]. Moreover, wheat and corn gluten have proved higher digestibility coefficients (99 and 95-96%) in salmonids [34-49].

Essential amino acids are necessary for optimal growth rate and better fish performance [16]. Another problem arisen from higher plant derived ingredients in aquafeed is their lower protein and essential amino acids contents compared to fish meal. However, gluten based protein contains high protein levels but it is deficient in some essential amino acids such as lysine and methionine [35]. The animal by-product meals such as PMB also contain less amounts of methionine, lysine, and isoleucine [28]. However, it was observed that supplementation of 0.4% lysine and 1.2% methionine alone does not support fish growth indices which is in agreement with findings of Francesco et al. (2004) and Steffens (1994) in rainbow trout, and with results of fowler (1982) on Chinook salmon fry. However, in another study a maximum level of 20% fishmeal replacement by PBM was found to be a practical diet for juvenile fall Chinook salmon [10].

Effects of different levels of dietary fish meal replacement with plant protein on digestive enzyme activities of rainbow trout were studied by Santigosa et al. (2008), where they found that total protease activity of fish fed diet containing fish meal as protein source reached its highest value 3 hours after

feeding, while in fish fed diet containing 50 and 70% plant protein postprandial protease activity increased slowly. Group with total fish meal replacement did not ever reach the highest protease activity. They reported decreased digestive enzyme activities can to some extent interpret the lower fish growth rates observed in groups fed diet with 75 and 100% fish meal replacement with plant protein.

In the present study, the protease activity significantly decreased with increase in dietary plant protein levels. The decreased proteases activity can be due to the presence of ANFs in some plant proteins. In addition the protease activity may be influenced by the nutrient quality and quantity [23].

The protein source did not have significant effect on amylase activity in this study. However, the lipase activity significantly decreased in diet with 100% plant protein. Similar observation was reported by López-López et al. (2005). In this regard, modifications in digestive activity may occur during individual fish life in response to food availability even if morphological and histological characteristics of intestine are genetically determined along evolution according to natural diet [6]. The knowledge of how different feed ingredients may affect enzyme activity is important, and this would provide information on if and how the choice of ingredients in feed formulations could allow a better efficiency of digestive enzymes [8].

Moreover, the results of present study revealed that 100% replacement of fish meal with PBM or combination of plant protein sources and PBM did not show any significant effect on enzymatic activity in pyloric caeca of rainbow trout. Therefore we may conclude that lower growth rate in most of the treatments could be due to quality and composition of essential amino acids in protein sources.

Fish fed different protein sources did not show any significant differences in hepatosomatic index which is in compliance with some existing literature on rainbow trout feed with plant protein [9-31] and PBM [2]. Similarly there were no significant differences in VSI amongst fish of different dietary groups, which may be attributed to the shorter experimental period in this study. In contrast to our findings, 24 week and 96 days experiments on inclusion of plant protein in rainbow diet [12, 32 respectively] resulted in significantly higher VSI.

Increasing plant protein inclusion upto 70% did not significantly affect fillet protein content. While fish fed 100% fish meal replacement diet with plant protein, PBM and combination of plant and PBM protein showed significantly decreased fillet protein content, PPV, PER and LER and significantly increased fillet lipid content. These findings were in accordance with existing literature on salmonids feed with high plant protein [18-32-33] and PMB [2-43].

A lower crude protein concentration and a trend towards an increase in the crude lipid concentration, as more wheat gluten was added to the diet may however, indicate a problem in amino acid metabolism. Our results support the findings of Pfeffer et al. (1992) who reported a decrease in the crude protein concentration and an increase in the crude lipid content in rainbow trout fed a diet containing wheat gluten as sole dietary protein source compared to those fed a combination of fish meal and wheat gluten and PBM protein [36]. However, Fowler (1991) reported that the increased body lipid and lowered body protein levels of the fish indicate that the quality of protein in PBM was not as good as that in fish meal.

CONCLUSION

Based on the results obtained from present study it was concluded that supplementation of 40% fish meal with combination of plant proteins (wheat and corn gluten and soybean meal) improves growth, enzymatic activity and muscle composition as high as control diet. However, inability of rainbow trout to use higher levels of plant protein in their diet could be due to scarcity of some essential amino acids and low alkaline protease enzyme activity. PBM together with plant protein sources (50%:50%) did not decrease digestive enzyme activity and had promising growth results. Therefore it may be used in rainbow trout feed after properly balancing its essential amino acids composition.

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