

Full replacement of fishmeal by poultry by-product meal in rainbow trout, *Oncorhynchus mykiss* (Walbaum, 1972) diet

Keramat Amirkolaie A.^{1*}; Shahsavari M.²; Hedayatyfard M.²

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Abstract

Along the plant ingredients, rendered animal protein sources such as blood meal, meat and bone meal and poultry by-product meal have potential to be replaced by fishmeal in rainbow trout feed. Therefore, the main objective of the present study is to determine the effect of full replacement of fishmeal by poultry by-products meal (PBM) on fish performance, nutrient digestibility and also liver characteristics in rainbow trout. Four experimental diets were formulated to contain graded levels of PBM at 0 (control diet), 33 (PBM33), 66 (PBM66) or 100% (PBM100), respectively. The four treatments were randomly assigned to each of 12 tanks, having three replicates for each treatment. Rainbow trout juveniles with an average initial weight of 50 ± 0.42 g were reared for two months. The fish gained lower weight and specific growth rate at PBM 66% and 100%. FCR was recorded larger for rainbow trout feeding on PBM 66% and 100% in comparison to PBM 33% and control diets ($p < 0.05$). The whole exchange of fishmeal by PBM (PBM100%) reduced dry matter, fat and protein digestibility ($p < 0.05$). An increase in PBM content of diet also resulted in larger fat content of the fish liver ($p < 0.05$). Body fat content reduced and moisture content increased by increasing PBM level ($p < 0.05$). In conclusion, PBM can be included in rainbow trout feed as an alternative for fishmeal up to 33%. A larger fat content of liver at PBM 100% may indicate a negative impact of PBM on rainbow trout health at full replacement level.

Keywords: Digestibility, Fat content, Fishmeal, Rendered animal protein

1-Department of Fisheries, Faculty of Animal Science and Fisheries, Sari Agricultural and Natural Resources University, Km 9 Darya Boulevard, P.O.Box: 578, Sari, Iran.

2-Department of Fisheries, Islamic Azad University, Ghaemshar Branch, Gjaemshar, Iran

*Corresponding author's email: amirkola@yahoo.com

Introduction

Aquaculture production has expanded around four-fold by weight (from 15 to 64 million tonnes) between 1992 and 2011 (FAO, 2012). This growth rate needs a similar sustainable supply of feed input to meet the demand (Tacon and Metian, 2008). Along with the increasing demand, the prices of fish feed are also increasing. Today, feed is the principal operating cost (El-Sayed, 1999; EL-Haroun *et al.*, 2009) in fish production and it can amount to 50% or more of the variable cost of most fish culture operations (Jory and Darry, 2000). The high price of aqua-feed is often caused by shortage and rising the price of fishmeal (Olsen and Hasan, 2012), which has traditionally been the main protein source of aqua-feed.

Nutritional research in the past decades has paid major attention to alternative protein sources for fishmeal. Most experiments focused on replacement of fishmeal with plant ingredients by considering the growth factors or sometimes by measuring digestibility (Quartararo *et al.*, 1998; Thiessen *et al.*, 2004; Gatlin *et al.*, 2007). Along the plant ingredients, rendered animal protein sources such as blood meal (BM), meat and bone meal (MBM) and PBM have potential to be replaced by fish meal (Bureau *et al.* 1999; Rawles *et al.*, 2006). These feed ingredients have been supplemented in the diets for a number of fish species such as Chinook salmon, *Oncorhynchus tshawytscha*, (Fowler, 1990 and 1991), rainbow trout, *O. mykiss*, (Steffens, 1994; Bureau *et al.*, 2000), Australian snapper, *Pagrus auratus*,

(Quartararo *et al.*, 1998), Nile tilapia, *Oreochromis niloticus*, (El-Sayed, 1998; Fasakin *et al.*, 2005), and Cuneate drum, *Nibea miichthioides*, (Wang *et al.*, 2006).

Among the rendered meals, PBM has a potential to be included in the feed of carnivorous fish species such as rainbow trout because of its relatively high protein content and lower price compared to fishmeal (Shapawi *et al.*, 2007). Moreover, rainbow trout is able to digest well PBM nutrients (Bureau *et al.*, 1999). Although many studies have been done on replacement of fishmeal with PMB in rainbow trout in last two decades (Steffens, 1994; Bureau *et al.*, 1999; EL-Haroun *et al.*, 2009), the replacement of PBM did not exceed 50%. Nowadays, improved quality of the products allows us to incorporate a bigger fraction of PMB in fish feed.

Feeding on a new feed ingredient may have an impact on organ health mainly liver. Liver can act as an indicator organ to show physiological and nutritional status of fish (Storch and Juario, 1983; Segner and Juario, 1986). A number of authors have described liver alterations caused by different nutritional factors (Godino *et al.*, 1990; Tucker *et al.*, 1997) or even pathological conditions in livers as result of dietary lipid imbalances (Bautista and De la Cruz, 1988; Watanabe *et al.*, 1989). While some literature is available on replacement of fish meal by PMB, little attention is paid on the nutrient availability from PBM and liver health status.

Therefore, the main objective of the present study is to determine the effect of

full replacement of fishmeal by PBM on fish performance, nutrient digestibility and also liver characteristics in rainbow trout.

Materials and methods

Experimental diets

Four experimental diets were formulated to contain graded levels of PBM. Nutrient composition of the diets was based on the data available for feeding rainbow trout

(Hardy, 2002). Diet one was a control with fishmeal as the main protein source. Diets two to four were formulated by replacing fishmeal with a locally sourced feed-grade PBM (Poultry By-product Provider Company, Ghaemshar, Iran) with 51% protein and 14% fat contents at 33% (PBM33), 66% (PBM66) or 100% (PBM100), respectively.

Table 1: Ingredient composition and nutrient content of the experimental diets in percentage wet weight. Each value is the mean of three sub-samples.

Diet	Control	PBM33%	PBM66%	PBM100%
Ingredients:				
Fishmeal	45	30	15	0
Poultry meal	0	15	30	45
Soybean meal	20	20	20	20
Wheat gluten	5	9	10	13
Wheat flour	6	4	4	2
Maize flour	5	3.5	2	1
Fish oil	5.6	5.6	6	6
Maize oil	8.4	8.4	9	9
Molasses	1.5	1	0.5	0.5
² Mineral	1.5	1.5	1.5	1.5
¹ Vitamin	1.5	1.5	1.5	1.5
Cr ₂ O ₃	0.5	0.5	0.5	0.5
Nutrient composition of the experimental diets in percentage				
Dry matter	93.6	93.6	94.5	94.9
Crude protein	44.5	43.9	43.3	42.7
Crude fat	19.9	21.2	22.2	22.5
Crude ash	10.0	10.8	11.7	12.4

¹Vitamin premix consisted of (g or IU/kg premix): 1200000IU Vitamin A, 400000IU D3, 3000IU E, 1200 mg K3, 5400mg c, 200 mg H2, 200 mg B1, 3360 mg B2, 7200 mg B3, 9000 mg B5, 2400 mg B6, 600 mg B9, 4 mg B12, 500 mg Antioxidant, up to 1 kg carrier (Mazandaran Animal & Aquatic Feed, Sari, Iran).

²Mineral premix consisted of (g/kg premix): 2600 mg Mn, 600 mg Cu, 6000 mg Fe, 4600 mg Zn, 50 mg Se, 100 mg IU, 50 mg Co, 100000 mg choline chloride, up to 1 kg carrier (Mazandaran Animal & Aquatic Feed, Sari, Iran).

This led to the production of four experimental diets containing almost similar levels of protein and fat. Wheat gluten was exchanged with wheat flour to maintain similar protein levels for the four experimental diets. The formulation and chemical composition of the four experimental diets are presented in Table 1. All ingredients were finely ground, mixed, and pelletized. To allow for digestibility studies, chromic oxide (Cr_2O_3) was used as the inert marker. A pellet size of 2 mm was used for the diets. Pellets were air-dried for 8 h at 45°C and stored at -20 °C until use.

Experimental system and fish

Rainbow trout juveniles with an average initial weight of 50 ± 0.42 g were reared for two months. The experiment started after two weeks of acclimation to the system. Afterward, rainbow trout were randomly distributed in groups of 18 fish into 12 circular tanks, with a volume of around 500L, a height of 120 cm and a diameter of 80 cm. Water quality was checked twice a week, two hours after feeding. The measured parameters were: temperature, pH, oxygen content, and NH_4^+ . The water temperature and pH ranged between 13-16°C and 7.3-7.9, respectively, during the experiment. Oxygen concentration was always above 7.1 mg/l and ammonium was below 0.15 mg/l during the study. A continuous flow-water (10-12 l/min) was directed to the experimental and control tanks throughout the experiment. The

photoperiod regime was 12h dark and 12h light.

Experimental procedure

The treatments were randomly assigned to each of the 12 tanks, having three replicates for each treatment. During the experiment (two months), fish were fed manually at 2% body weight, two times per day (starting at 08.00 and 17.00hrs).

On the last day of the experiment, all fish were weighed individually. Afterward, three fish were randomly selected from each tank and sacrificed using overdosed clove essence solution (400 mg/l) for analysis of body part composition. The whole viscera were removed and livers were separated from the viscera. In addition, five fish were randomly collected from each tank to analyze final body composition. Fish weight was measured during the study for adjustment of feeding level. All fish were starved the night before weighing.

Chemical analysis

Feed, feces and fish samples were analyzed for dry matter by drying samples for 24h at 103°C until constant weight (ISO, 1983). Ash content was determined by incineration in a muffle furnace for 4h at 550°C (ISO, 1978). Crude protein ($\text{N} \times 6.25$) was measured by the Kjeldahl method after acid digestion, according to ISO (1979). Lipid was extracted by petroleum ether extraction in a Soxhlet apparatus. Chromic oxide was measured Spectrophotometrically by the use of the

method described by Furakaw and Tsukahara (1966).

Digestibility measurements

After weight measurement, all fish were returned into the same tank for feces collection from each tank separately. Feces were collected by pipetting from tank bottom. Daily feces samples were pooled for each tank until desirable amount of feces collected. Apparent digestibility coefficients of nutrients in the diets were determined using the indicator method with Cr_2O_3 as a marker (5g/kg). Apparent digestibility (%) is expressed as a fractional net absorption of nutrients from the diet and was calculated according to:

$$\text{ADC} = (1 - [\text{Mar}_{\text{diet}} / \text{Mar}_{\text{feces}} \times \text{Nutr}_{\text{feces}} / \text{Nutr}_{\text{diet}}]) \times 100$$

where ADC = apparent digestibility coefficient; Mar_{diet} = dietary chromic oxide concentration; $\text{Mar}_{\text{feces}}$ = fecal chromic oxide concentration; $\text{Nutr}_{\text{diet}}$ = Nutrients of the diet; and $\text{Nutr}_{\text{feces}}$ = Nutrients of the feces.

The apparent dry matter digestibility (DMD) in the test ingredient (PBM) was calculated according to:

$$\text{DMD in PBM (\%)} = [(\text{ADC}_{\text{TD}} - (\% \text{RD}) \times (\text{ADC}_{\text{RD}}))] / \% \text{ of test diet}$$

Where ADC_{TD} is the apparent digestibility coefficient of the test diet, TD is the test diet, ADC_{RD} is the apparent digestibility coefficient of the reference diet, RD is the reference diet and TI is the test ingredient (PBM).

The TI ADC of protein, ash, and fat (%) was calculated by the use of formula applied by Sugiura *et al.* (1998):

$$\text{ADC (\%)} = [\text{TD}_{\text{nutrient}} \times \text{ADC of TD}_{\text{nutrient}}] - [\% \text{RD} \times \text{ADC of RD}_{\text{nutrient}} \times \text{RD}_{\text{nutrient}}] / (\% \text{ TI} \times \text{TI}_{\text{nutrient}})$$

where $\text{TD}_{\text{nutrient}}$ is the nutrient concentration in the test diet, $\text{RD}_{\text{nutrient}}$ is the nutrient concentration in the reference diet and $\text{TI}_{\text{nutrient}}$ is the nutrient concentration in the test ingredient (PBM).

Fish performance and statistical analysis

Weight gain was determined by the difference between total initial and final body weights. Feed conversion ratio (FCR) was calculated per tank from feed intake data and weight gain. Specific growth rate (SGR) was calculated from the natural logarithm of the mean final weight minus the natural logarithm of the mean initial weight and divided by the total number of experimental days expressed as a percentage per day. Protein efficiency ratio (PER) was calculated per tank by dividing total weight gain to total protein consumed during the experiment. Hepatosomatic and visceral somatic indices were calculated according to the following formulas.

$$\text{Hepatosomatic index (\%)} = 100 \times (\text{liver weight/body weight})$$

$$\text{Visceral somatic index (\%)} = 100 \times (\text{visceral weight/body weight})$$

Data are presented as means of each treatment with standard deviation. All data were verified for normality after transformation (ArcSin). One-way ANOVA was used to determine the effects of PBM levels on fish performance and digestibility using GLM procedure of SAS,

Institute Inc. (2002). Tukey's test was used to compare differences between the means at 0.05% probability. For all statistical analyses, each tank was considered as the experimental unit.

Results

Replacement of fishmeal by the PBM influenced growth related parameters during two-month experiment (Table 2; $p < 0.05$). Rainbow trout gained lower

weight with increasing PBM content. FCR was recorded larger for rainbow trout feeding on PBM66% and PBM100% in comparison to PBM33% and control diets ($p < 0.05$). Similar to other growth parameters, SGR reduced when rainbow trout fed on PBM 66% and PBM 100% diets ($p < 0.05$).

Table 2 Growth performance in rainbow trout feeding on different levels of PBM products over : a 60-day experimental period. All values are means of three replicates (tanks)/treatment \pm standard deviation.

Growth Parameters	Diet			
	Control	PBM33%	PBM66%	PBM100%
Initial weight (g)	51.3 \pm 1.3	48.5 \pm 2.1	52.4 \pm 2.3	50.0 \pm 1.5
Final weight (g)	122.7 \pm 2.65 ^c	122.5 \pm 2.63 ^c	108.26 \pm 2.08 ^b	82.0 \pm 2.26 ^a
Biomass gain (g)	72.5 \pm 2.78 ^c	72.5 \pm 2.63 ^c	56.6 \pm 3.13 ^b	32.0 \pm 2.26 ^a
SGR (%/day)	0.64 \pm 0.01 ^c	0.64 \pm 0.01 ^c	0.54 \pm 0.02 ^b	0.35 \pm 0.01 ^a
FCR	1.32 \pm 0.01 ^a	1.26 \pm 0.03 ^a	1.57 \pm 0.50 ^b	2.42 \pm 0.18 ^c
PER	1.73 \pm 0.07 ^c	1.79 \pm 0.04 ^c	1.46 \pm 0.04 ^b	0.97 \pm 0.07 ^a

Different superscript letters show significant differences

Relative liver weight was not affected by the diets, but relative visceral weight was larger at PBM 33% than that of PBM 100%. An increase in PBM

content of diet resulted in larger fat content of the fish liver (Table 3).

Table 3: Organ characteristics in rainbow trout feeding on different levels of PBM over a 60-day experimental period. All values are means of three replicates (tanks)/treatment \pm standard deviation.

Organ characteristics	Diet			
	Control	PBM33	PBM66	PBM100
HSI (%)	1.21 \pm 0.07	1.1 \pm 0.11	1.15 \pm 0.4	1.24 \pm 0.14
VSI (%)	11.69 \pm 2.9 ^{ab}	9.15 \pm 0.9 ^a	11.85 \pm 0.9 ^{ab}	12.53 \pm 0.8 ^b
Liver fat (%)	10.83 \pm 1.04 ^a	14.53 \pm 0.50 ^b	18.0 \pm 1.0 ^c	20.0 \pm 1.73 ^c

Different superscript letters show significant differences.

Nutrients digestibility were affected by inclusion of PBM (Table 4). The whole exchange of fishmeal by PBM (PBM 100%) reduced fat and protein digestibility ($p < 0.05$). Dry matter digestibility also followed a similar trend. Protein digestibility of PBM was not significantly

different at different inclusion levels while dry matter and fat digestibility was affected by PBM inclusion levels ($p < 0.05$; Table 5). Fat and dry matter digestibility of PBM declined substantially at full replacement of fishmeal by PBM ($p < 0.05$).

Table 4: Apparent digestibility coefficients in rainbow trout feeding on different levels of PBM over a 60-day experimental period. All values are means of three replicates (tanks)/treatment \pm standard deviation.

Parameters	Diets			
	Control	PBM33	PBM66	PBM100
Dry matter (%)	90.1 \pm 0.24 ^b	87.3 \pm 0.06 ^b	87.2 \pm 0.08 ^b	74.6 \pm 0.31 ^a
Protein (%)	98.8 \pm 0.04 ^b	97.4 \pm 0.01 ^b	97.2 \pm 0.01 ^b	95.1 \pm 0.05 ^a
Fat (%)	98.2 \pm 0.02 ^c	98.0 \pm 0.01 ^{bc}	98.2 \pm 0.01 ^b	96.2 \pm 0.08 ^a

Different superscript letters show significant differences.

Table 5: Apparent digestibility coefficients of PBM in rainbow trout over a 60-day experimental period. All values are means of three replicates (tanks)/treatment \pm standard deviation.

Parameters	PBM		
	PBM33	PBM66	PBM100
Dry matter (%)	74.5 \pm 4.21 ^b	81.9 \pm 2.60 ^b	54.9 \pm 7.08 ^a
Protein (%)	68.5 \pm 3.74	70.6 \pm 1.79	66.6 \pm 2.50
Fat (%)	71.8 \pm 0.50 ^c	68.8 \pm 0.18 ^b	61.9 \pm 0.17 ^a

Different superscript letters show significant differences.

Table 6: Body composition in rainbow trout feeding on different levels of PBM over a 60-day experimental period. All values are means of three replicates (tanks)/treatment ± standard deviation.

Parameters	Diets				
	Initial	Control	PBM33	PBM66	PBM100
Protein	16.68	17.90±0.10	17.80±0.11	17.75±0.22	17.70±0.17
Fat	7.3	7.98±0.11 ^c	7.63±0.09 ^{bc}	7.45±0.080 ^b	7.0±0.28 ^a
Moisture	75.94	75.64±2.73 ^a	77.58±2.93 ^{ab}	77.87±0.65 ^a	80.87±0.22 ^b
Ash	3.22	2.62±0.06	2.26±0.06	2.04±0.18	2.78±1.54

Different superscript letters show significant differences

The body composition results demonstrated that the inclusion of PBM affected fat and moisture contents of carcass in rainbow trout (Table 6; $p < 0.05$). Body fat content reduced and moisture content increased by increasing PBM levels ($p < 0.05$). However, addition of PBM did not change protein and ash contents of rainbow trout.

Discussion

Inclusion of 33% PBM had no negative impacts on growth related parameters during a 60-day experiment, but further exchange of fishmeal by PBM sharply reduced these parameters. This finding is similar to most previous works on the potential of PBM as an alternative protein source for fish diet (Nengas *et al.*, 1999; Millamena, 2002; Rossi and Davis 2012). Deficiencies in essential amino acids such as lysine and methionine may be a possible reason for reduced growth of juvenile rainbow trout at high replacement with PBM (Millamena, 2002; EL-Haroun *et al.*, 2009). Similarly, Tiews *et al.* (1976) showed that full replacement of fishmeal by PBM in rainbow trout was possible using lysine, D,L-methionine and tryptophan supplementation.

Most of previous works suggested a larger replacement of fishmeal by PBM (50% in European eel, *Anguilla anguilla*;

Gallagher and Degani, 1988; and Chinook salmon, *Oncorhynchus tshawytscha*;; Fowler, 1991; 75% in gilthead seabream, *Sparus aurata*, : Nengas *et al.*, 1999; 66.5% in gibel carp, *Carassius gibelio*;; Yang *et al.*, 2006) without a significant reduction in growth performance. However, the current results suggested a lower replacement rate (33%). It appears that the replacement rate should be adjusted according to the quality of PBM, especially protein content and/or quality; i.e. the more qualified protein of PBM the higher replacement rate. Protein content of PBM tested in the current study was 51% whereas previous experiments included PBM with larger content of protein (69 and 65%: Bureau *et al.*, 1999, 69%: Shapawi *et al.*, 2007, 63%: Rossi and Davis, 2012). Organ characteristic measurement revealed that although HSI were not affected by dietary inclusion of PBM, fat content of liver was increased with increasing PBM. This condition may suggest that rainbow trout cannot handle well poultry fat and that organ health would be threatened by inclusion of high levels of PBM in fish feed. There are evidences showing that diet composition affects lipid deposition of liver. Aksnes and Mundheim (1997) observed a high content of lipid in the hepatocytes of halibut fed fishmeal produced from spoiled raw material

compared with fresh raw fish. Lipid deposition in the liver of cod (*Gadus morhua* L.) fed natural prey was much lower in comparison to fish-based diet (36.4 versus 66.8%: dos Santos *et al.*, 1993). Liver can act as an indicator organ to show physiological and nutritional status of fish (Storch and Juario, 1983; Segner and Juario, 1986). High-fat content of livers as a result of PBM diets may affect liver histology (Sargent *et al.*, 1989) characterized by displaced nuclei and large lipid droplets in the cytoplasm (Caballero *et al.*, 1999). PBM inclusion had also a negative impact on dry matter and nutrients digestibility. This finding is similar to that of Shapawi *et al.* (2007) who observed a depression in digestibility of dry matter and crude protein in humpback grouper, in which fishmeal was replaced with PBM at 75 and 100%. A reduction in digestibility was probably the major contributing factor to the low growth performance of rainbow trout fed PBM66 and 100%. Dry matter digestibility values (74 – 90%) for PBM-based diets observed in the present study were slightly better than the values reported in PBM-based diets for hybrid striped bass, *Morone saxatilis*, (Rawles *et al.*, 2006) and in gibel carp, *Carassius gibelio*, (Yang *et al.*, 2006) indicating that rainbow trout is capable to utilize efficiently PBM diets.

Lower estimates of protein digestibility of PBM diets in previous experiments (74 and 85%: Hajen *et al.* 1993; 64 and 78%: Dong *et al.*, 1993; 81 and 82%: Pfeiffer *et al.*, 1995) are probably related to the type of feces collection method. In the current study, feces were collected by pipetting from tank bottom. This method is always associated to disintegration/separation of

feces and leaching of nutrients from the feces (Amirkolaie *et al.*, 2005) leading to larger estimates of digestibility. These different results, therefore, suggest that it is necessary to report the method used to collect fecal material when comparing estimates of apparent digestibility among studies. In conclusion, PBM resulted from poultry by-products have a potential as feed ingredients to replace fishmeal in rainbow trout diet up to 33%. Low fish performance may be caused by shortage of essential amino acids such as lysine and methionine. Digestibility and growth performance reduced sharply with increasing the inclusion level (66% and beyond). Different recommended ratios of PBM for certain species result from differences in the quality of PBM, thereby any replacement really depends on nutrient composition of PBM. A larger fat content of rainbow trout's liver at PBM 100% may suggest that the full replacement of fishmeal by PBM should be conducted with further precaution.

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