Studies on availability of rendered animal protein sources to rainbow trout Oncorhynchus mykiss

STUDIES ON AVAILABILITY OF RENDERED ANIMAL PROTEIN SOURCES TO RAINBOW TROUT *Oncorhynchus mykiss*

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DECLARATION

I do hereby declare that this thesis has been achieved by myself and is the result of my own investigations. It has neither been accepted, nor is being submitted, for any other degree or qualification. All sources of information have been duly acknowledged.

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［課程博士］

Doctoral Course

博士学位論文内容要旨

Abstract

専　攻 Major	応用生命科学専攻	氏　名 Name	Feng Lu
論文題目 Title	Studies on availability of rendered animal protein sources to rainbow trout *Oncorhynchus mykiss*		

Fish meal has long been the major protein source in feeds for trout, salmon, and marine fish. However, the cost of fishmeal and the negative impact on the environment of fish farming site could limit the expected growth of aquaculture. Therefore, fish meal is increasingly being replaced by more economical protein sources. Alternative proteins, including plant and animal protein, have been studied by many fish nutritionists. However, plant protein inclusion has normally been limited due to deficiencies in essential amino acid, anti-nutrients factors and poor palatability. Rendered animal protein ingredients, poultry by-products meal (PBM), feather meal (FEM), blood meal (BM) and pork and poultry by-product meal (PPM), are potential fish meal substitutes for formulating fish diet.

Compared to fish meal, methionine and lysine and methionine, lysine and histidine is limited in poultry by-product meal and feather meal, respectively. Blood meal is rich in lysine, and can be used to balance dietary lysine content when poultry by-product meal and feather meal are used alone or in combination as fish meal

substitutes.

In the first experiment, a 12-week feeding trial was carried out to investigate partial and full replacement of fish meal with PBM, FEM, BM, defatted soybean meal (DSM) and corn gluten meal (CGM) in practical type diets for rainbow trout. Duplicate treatments of rainbow trout (mean initial weight; 16.7±0.1g) were fed six isonitrogenous (43.7% crude protein) diets. Fish meal based diet (56% anchovy meal) was designated as control. In the other five diets, fish meal was replaced by rendered animal protein and/or plant protein sources at the levels of 75% and 100%.

Fish fed the diets replacing fish meal with the combination of rendered animal protein at levels of 75% and 100% showed comparable growth performance with fish fed the control diet except the protein efficiency ratio and feed conversion ratio. Higher apparent crude protein digestibility coefficients were observed in the FM+DC treatment when compared with fish fed the FM+PFB diet.

In the second experiment, the apparent digestibility coefficients of amino acid in PBM, FEM, BM, DSM and CGM were determined for juvenile rainbow trout. A reference diet (RF) and test diets (consisting of 70% RF and 30% of the test ingredients) were used. Fish were randomly selected and reared in each of two tanks at the density of 15 fish per tank for 2 weeks. Each diet was hand-fed to apparent satiation twice a day to fish at 14.1±1.0 ºC. It was found that FEM showed the lower crude protein digestibility than the plant protein sources. Also the PBM and FEM showed the

lower amino acid availability (methionine and lysine) than the DSM and CGM, but might increase the feed intake for rainbow trout.

In the third experiment, A 12-week feeding trial by using rainbow trout (mean initial weight; 23.6±0.1g) was carried out to assess the effect of replacing fish meal with rendered animal protein sources (PBM, FEM, and BM) in the practical diets. Fish meal diet was designated as control. In the other four diets, fish meal was replaced completely by the combination of PBM+FEM+BM at the different levels of 60/20/20, 70/20/10, 80/10/10, 90/10/0. Synthetic lysine and methionine were supplemented to satisfy the essential amino acid in all experimental diets.

Fish fed the control diet exhibited high weight gain and specific growth rate than the other treatment. Fish fed the diet with the combination of PBM+FEM+BM at 60/20/20 showed the significantly higher feed intake than fish fed the combination of PBM+FEM+BM at 90/10/0. Also better feed conversion ratio was shown in the control than the fish fed the PBM+FEM+BM at 70/20/10 and 80/10/10. No significant differences were found in hepatosomatic index among the diet treatments.

In the fourth experiment, a 10-week feeding trial by rainbow trout (mean initial weight; 20.6±0.1g) was conducted to determine the effect of replacing poultry by-product meal with four levels of PPM on the fish growth performance, amino acid availability of experimental diets. Five experimental diets contain the same level crude protein (44.3%). The study found that increased the PPM inclusion levels of fish diet

resulted in reduction of the growth performance. The lysine digestibility of the experimental diets decreased when increased the PPM inclusion levels. It seems that using PPM to replace the PBM did not exhibit the better growth for trout.

In conclusion, very high inclusion levels of the combination of the rendered animal protein successfully might be used in the formulated fish diet for rainbow trout. The combination of PBM, FEM and BM at 60/20/20 could be suitable to replace the fish meal in practical diets for rainbow trout.

CHAPTER 1
GENERAL INTRODUCION

Aquaculture is an extremely diverse industry, it is estimated that more than 200 species of fish, crustaceans, and molluscs are cultured around the world. World aquaculture has risen in the last six decades. From a production of less than one million tons in the early 1950s, production in 2009 was reported to have elevated to 55.1 million tons (FAO, 2010).

Growth of aquaculture throughout the world requires increased production of aqua-feeds (Tacon & Metian 2008). Fish meal is the major protein contributor to the aqua-feeds due to its high protein content, excellent amino acid composition, high nutrient digestibility and high availability for farmed aquatic animals (Gatlin et al. 2007). Alternate protein sources can lower the cost of aqua-feeds, reduce the amount of wild fish used as protein, and reduce the nutrient levels in effluent waste.

Many plant proteins have been currently used to manufacture fish diets, including meals from soybean, corn and pea. Plant protein products are successfully used as the protein source in fish diets (e.g. rainbow trout). However, the study of growth in rainbow trout fed diets which in plant products have resulted in variable results (Davies & Morris 1997; Mambrini, Roem, Carvedi, Lalles & Kaushik 1999). The use of plant proteins in fish feed is limited by deficiencies in several amino acids, such as methionine (Lim, Webster & Lee 2008). The potential of using different protein sources, including poultry by-product meal, meat and bone meal, blood meal and feather meal have been used successfully in feeds for various fish species, such as

Chinook salmon (Fowler, 1991), rainbow trout (Bureau et al., 2000), red drum (Kureshy et al., 1997), Australian silver perch (Allan et al., 2000) and Siberian sturgeon (Zhu et al., 2011).

The objectives of the current study were to examine the growth performance of rainbow trout fed rendered animal protein and plant protein diets, to investigate apparent digestibility coefficients of nutrients and amino acid availability in the ingredients and diets, to examine the different ratio among the rendered animal protein.

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CHAPTER 2

LITERATURE REVIEW

Fish meal

Fish meal is a high-protein-content feed ingredient and is also an excellent source of essential amino acids. It has been used as the main protein source in aquafeed for many reasons including a good source of fatty acid, vitamins, minerals (Sauvant et al. 2002; Lim et al. 2008). At the present, dietary Fish meal inclusion within aquafeed ranges between 20 to 60% of the diet (Watanabe, 2002). Over the past 25 years, the percentage of annual global production of fish meal being used in aquafeed has increased from approximately 15%-65% (Hardy, 2010). However, the freight costs and the Peruvian government decision to slow down the fish catch to replenish anchovy supplies resulted in the higher price of fish meal (Goettl, 2003). These reasons are leading to find the alternative protein sources to replace the fish meal as the protein sources in aquafeed.

Plant protein sources

Plant protein sources have been extensively used in carnivorous fish species as replacements of fish meal. However, plant protein sources have several problems as protein source for fish feed such as amino acid imbalance, less palatability and inclusion of anti-nutritional factors. These weak points of plant protein sources can be overcome by addition of crystalline amino acid, extrusion processing and enzyme supplementation (NRC, 2011).

Rendered animal protein sources

Rendered animal protein sources have been used in aquaculture feeds for

several decades. The use of rendered animal protein sources was limited in the 1970s and 1980s because a small number of studies indicated that some of these ingredients had poor digestibility for fish or were of highly variable quality (Cho and Slinger, 1979; Cho et al., 1982; NRC, 1993). Today, ingredients produced appear to be of much higher quality than those produced 20 to 30 years ago. Many studies on the nutritive value of rendered animal proteins for aquaculture species have been contributed in the scientific literature over the past 30 years.

Poultry by-product meal

Poultry by-product meal (PBM) is produced from waste generated from poultry processing plants, exclusive of feathers and the contents of gizzards and intestines (Hardy and Barrows, 2002). Comparison of the results of Dong et al. (1993), Sugiura et al. (1998), and Bureau et al. (1999) suggests improvements in the digestibility of protein in regular poultry by-product meal for rainbow trout.

High digestibility of crude protein for PBM appears to be observable in the marine fish. Lupatsch et al. (1997) observed the digestibility of crude protein of about 80 percent for PBM fed to gilthead seabream (*Sparus aurata*). In red drum, *Sciaenops ocelatus*, Kureshy et al. (2000) were able to successfully raise juvenile fish on a diet with 66.7% of fish meal replaced by PBM. For the other species, Gibel carp, *Carassius* auratus gibelio, grew well on diets with 50% of the fish meal replaced by PBM protein (Yang, 2004).

Feather meal

Feather meal (FEM) is pressure-cooked, clean undecomposed feathers from slaughtered poultry. Not less than 75 percent of its crude protein content must be digestible by the pepsin digestibility method. Modern processing methods that cook the feathers under pressure with live steam hydrolyze the protein and break the keratinaceous bonds that account for the unique structure of feather fibers.

Lee et al. (2002) examined that the digestibility of crude protein of FEM was about 79 percent for rockfish (*Sebastes schlegeli*). And Bureau et al. (2000) suggested that about 15% feather meal could be incorporated in the diet of rainbow trout without effect on growth and feed efficiency of the trout.

Blood meal

Blood meal (BM) is a dry product made from clean, fresh animal blood, exclusive of all extraneous materials. Blood meal contains high protein and is considered to be an excellent source of lysine. Cho et al. (1982) observed that a flame-dried blood meal had crude protein digestibility of only about 12 %, whereas the protein in spray-dried blood meal was almost completely digestible. The study with rainbow trout suggested that the bioavailability of lysine in spray-dried blood meal was slightly higher than that of L-lysine HCL (El-Haroun and Bureau, 2004).

Rainbow tro*ut (Oncorhynchus mykiss)*

Rainbow trout is a highly commercial sport and market fish (Woynarovich et al. 2011). Rainbow trout require highly oxygenated water and optimal temperature of this species ranges from 13-18°C. In the natural environment rainbow trout feed on

various invertebrates including plankton, larger crustaceans, fish, insects, snails, and leeches. Nutritional requirements of rainbow trout have been well studied (NRC, 1993). Rainbow trout feeds generally are formulated to contain between 42% and 48% crude protein and 16–24% lipid, depending upon life-history stage.

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Chapter 3

Replacement of fish meal with rendered animal protein and plant protein sources on growth response, biological indices and amino acid availability of rainbow trout *Oncorhynchus mykiss*

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ABSTRACT

Duplicate groups of rainbow trout (mean initial weight; 16.7±0.1g) were fed six isonitrogenous (43.7% crude protein) diets for 12 weeks. Fish meal based diet was designated as control. In the other five diets, 75% and 100% of fish meal was replaced by combination of poultry by-product meal (PBM), hydrolyzed feather meal (FEM), spray-dried blood meal (BM), defatted soybean meal (DSM) and corn gluten meal (CGM).

Fish fed the diets replacing 75% and 100% fish meal with the combination of rendered animal protein showed comparable growth performance with fish fed the control diet except the protein efficiency ratio and feed conversion ratio. Feed intake of the fish fed combination of fish meal and rendered animal protein based diets with or without plant protein was significantly higher than that on the fish meal based diet. Apparent crude protein digestibility coefficients were significantly higher in fish fed the 75% fish meal replaced by the combination of plant protein sources than that on the combination of rendered animal protein sources ($P < 0.05$). These results suggested that the combination of PBM, FEM and BM was able to replace most of the fish meal in practical feed for rainbow trout.

Keywords: Rainbow trout; Rendered animal protein; Plant protein; Amino acid digestibility

INTRODUCTION

In aquaculture, fish meal is used as a major protein source in the diet in the world. However, the cost of fish meal (FM) and the negative impact on the environment of fish farming site might limit the expected growth of aquaculture. Using low cost, reasonable plant or animal protein sources to replace fish meal can reduce feed cost of aquaculture production. Many studies focused on assessing the potential to reduce fish meal level in formulated fish diets [1-3].

Defatted soybean meal (DSM) and corn gluten meal (CGM) have been extensively studied as fish meal replacer because of their stable production and cost effectiveness [4-5]. However, plant protein sources have several problems as protein source for fish feed such as amino acid imbalance, less palatability and inclusion of anti-nutritional factors [6]. These weak points of plant protein sources can be overcome by addition of crystalline amino acid, extrusion processing and enzyme supplementation [6]. However, these additional treatments could elevate cost of the plant protein sources. In addition, price of corn products have been elevated for this decade because of its use for bioethanol production [7]. Compared to plant protein sources, use of animal protein sources such as poultry by-product meal (PBM), meat and bone meal (MBM), hydrolyzed feather meal (FEM), and blood meal (BM) has not been studied in detail. Several report suggested that animal protein source can be used as fish meal replacement. The potential of using different protein sources, including PBM, BM, FEM, DSM and CGM as dietary protein sources have been

investigated in rainbow trout [2], Atlantic salmon *Salmo Salar* [8], Japanses flounder *Paralichthys olivaceus* [9], European seabass *Dicentrarchus labrax* [10], sunshine bass *Morone chrysops*×*M.saxatilis* [11] and malabar grouper *Epinephelus malabricus* [12]. Yamamoto et al. (2003) [13] suggested that MBM can be used as partial replacement of fish meal when combined with SBM and CGM. Bureau et al. (2000) [2] also suggested that usefulness of MBM as fish meal replacer. However, MBM contains relatively higher ash content and this may reduce digestibility of this ingredients and decrease P availability [14]. In addition, after spreading bovine spongeform encephalopacy (BSE) in the worldwide around 1990's, public concerns on use of MBM in animal feed has been provoked, and eventually ruled out from fish feed ingredient in Japan from October, 2001 [http://www.maff.go.jp/j/syouan/douei/bse/b_nikukopp/pdf/h131001.pdf]. Poultry by-product is one of the other animal protein sources which can be used for fish meal replacer. Considering successful replacement of fish meal, one of the main problems of alternative protein source is amino acid imbalance. Because of similarity of amino acid composition of PBM and FM [2, 15-17], PBM was used as main animal protein source to replace fish meal in this study. It was thought that PBM can be major fish meal replacer in rainbow trout. However, it is unclear that complete fish meal replacement can be achieved by PBM as main protein source.

Avoidance of essential amino acid (EAA) deficiency is one of the most critical issue for the successful utilization of most inexpensive alternative proteins in fish feed.

A blend of several protein sources could be a promising way to replace a higher level of dietary fish meal, and this strategy has been successfully implemented in different species [12, 18]. The objectives of the current study were (i) to examine the growth performance of rainbow trout fed rendered animal protein and plant protein diets, (ii) to investigate apparent digestibility coefficients of nutrients and amino acid availability in diets, and (iii) to determine the amino acid availability and crude protein digestibility of rendered animal protein sources for rainbow trout.

Materials and methods

Diet formulation and preparation

Table 1~2

PBM, FEM, BM, DSM and CGM were employed for replacement of FM. PBM and FEM were purchased by Nangoku Kosan (Miyazaki, Japan). The proximate analysis, amino acid profiles of fish meal, and rendered animal protein and plant protein ingredients are shown in Table 1. Similar amino acid composition was observed in FM and PBM except low histidine in PBM (Table 1). The composition of the experimental diets is shown in Table 2. Anchovy meal based diet (FM) was arranged as control. In order to increase lysine and histidine, BM was formulated in all diets except FM (control) and FM+DC diets (Table 2). 75 and 100% anchovy meal was replaced by combination of PBM, FEM, and BM or further combination with SBM and CGM (Table 2). In 75% FM replacement group, FM+DC were designated to examine effect of diet without animal protein sources such as PBM, FEM, and BM.

In preparing the diet, all dried ingredients were thoroughly mixed by a horizontal mixer (ACM-50 LAT model, Aicohsha, Saitama, Japan). Feed ingredients were ground in order to reduce the particle size to less than 500 μm. Chromic oxide (Cr_2O_3) was used at 5 g kg $^{-1}$ in all the diets as an inert marker for the study of digestibility. The ingredients were mixed in a horizontal mixer, added to deionized water (30%), and pelleted to 3 sizes (ø, 2.3, 3.2 and 4.8 mm) using a laboratory pellet maker machine (OMC-22B model, Omichi, Gunma, Japan). The pellet was dried using a vacuum freeze-drier (RLE-206, Kyowa Vacuum Engineering, Tokyo) and stored at -30 ºC until use. Crude protein (41.2-44.5%) and crude lipid contents (18.8-21.7%) were similar among all diets (Table 4). The essential amino acid contents in test diet met the requirement of rainbow trout except lysine [6]. The free histidine content of FM diet is higher than the other experimental diets. Free methionine content of PFB and PFBDC diets are higher than the other diets due to the supplemention of the DL-methionine (Table 5).

Table 4-5

After the feeding trial, the apparent digestibility of the ingredients was investigated. The control diet was used as a reference diet (Table 3). The test diet was then formulated by 700 g kg $^{-1}$ of control diet (as reference diet) and 300 g kg $^{-1}$ test ingredient, following the method described by Cho *et al* [19].

Table 3

Experimental fish and feeding

The rainbow trout juveniles were obtained from Oizumi Station, Field Science Center, Tokyo University of Marine Science and Technology (TUMSAT), Yamanashi and reared under laboratory conditions at Laboratory of Fish Nutrition, TUMSAT, Japan. Prior to experiment, all fish were acclimatized to the experimental condition by feeding a commercial diet (Nippai, Kanagawa, Japan) for two weeks. Fish with an average body weight of about 16.7±0.1 g were randomly sampled from stock and distributed into 60 L glass rectangular tank at the density of 25 fish per tank. Duplicate groups were assigned to each experimental diet. Feed intake (FI) was monitored daily. Tanks were supplied with dechlorinated tap water at 0.6 l/min, and the water temperature was controlled at 14.1±1.0 °C with a thermostat (RHUP250A2, Hitachi, Tokyo, Japan) in a semi-recirculating system during the experimental period. The feeding experiment was conducted for 14 weeks including the digestibility experiment (2 weeks) in the water recirculating system with a constant water supply at a rate 0.5 l/min and aeration provided by sand aerator. The fish were hand-fed twice (10:00 and 17:00) a day, 6 days a week to an apparent satiation. Determination of growth changes and feed performance calculations were examined every 3 weeks.

Sample collection and preparation

Initial and final carcass chemical composition

At the beginning and the end of the growth experiment, 10 and 5 fish were

randomly selected from the experimental stock and from the experimental tank in each treatment, respectively for whole body analysis. Sampled fish were euthanized with an overdose of 2-phenoxyethanol (Wako Pure Chemical Industries, Osaka, Japan). Fish body samples were ground using a centrifugal mill (Retsch, Haan, Germany) fitted with 0.5-mm screen, and homogenized. Homogenized samples were then dried by a freeze-drying machine and stored at – 30 ºC until analyzed. Apparent digestibility coefficients values of nutrients and amino acids were determined by using the Tokyo University of Fisheries (TUF) column system as the method decribed in Sarker *et al*. [20]; Satoh *et al*. [21]. After feeding, uneaten feed and residue were siphoned out and the TUF faeces collector was immediately installed. Faeces were collected from each tank within each dietary treatment on the next day in 12 days, and pooled in order to collect enough material for analyses.

Haematological parameters and fish body and blood indices

In order to investigate the postprandial effect, five fish were sampled at 24 hr after the last meal and anesthetized by 2-phenoxyethanol. Blood samples were collected by the heparinized syringe from the caudal vein, pooled, and analyzed two replicates. A small fraction of heparinized blood was immediately used for blood indices, namely: haematocrit value (Hct); haemoglobin concentration (Hb). The remained blood was centrifuged for the plasma by centrifugation at 3000 *g* at 4 °C for 15 min using a high-speed refrigerated centrifuge (SRX – 201 model, Tomy, Tokyo,

Japan). The plasma was separated and kept at - 35 °C until analysis. It was reported that the activities of aspartate amino transferase (AST, former glutamic oxaloacetic transaminase: GOT), and alanine amino transferase (ALT, former glutamic pyruvic transaminase : GPT) was reflected by dietary protein intake and increase together with excretion of nitrogen [22]. GOT and GPT in blood plasma were analyzed using an automatic blood chemistry analyzer (7020 model; Hitachi, Tokyo, Japan). After collecting the blood samples, digestive tracts (except heart) and fillet were dissected out from the fish body, weighed individually to get the body indices. Liver and fillet of fish in each tank were pooled and stored at – 30 °C until analyzed.

Chemical analyses

Pooled faecal samples were dried by a vacuum freeze-drying machine (REL 206 model, Kyowa, Tokyo, Japan) for 22 h and kept at - 30°C until analyzed. Samples were digested using nitric- perchloric acid, and chromic oxide was measured at 350 nm by using a spectrophotometer (UV-2550, Shimadzu, Kyoto, Japan) [23].

Analyses of moisture and ash were conducted by using the method described by [24], the crude protein content in samples was determined by semi-micro Kjeldahl method (N* 6.25) using Kjeldahl analyzer (FOSS Kjeltec^{TM} 2400, FOSS, Tokyo, Japan), and crude lipid content was analyzed using a gravimetric method after lipid extraction using chloroform-methanol (2:1, v/v), respectively.

The samples were hydrolyzed in 4 mol L^{-1} methanesulfonic (Sigma-Aldrich, Missouri, Mo, USA) for 22 hours at 110 ºC prior to total amino acid analysis. The total amino acids compositions were determined using an automatic amino acid analyzer (JLC-500/v, JEOL, Tokyo, Japan). The free amino acid of experimental diets were deproteinized with 2% sulphosalicylic acid (w/v) and centrifuged 3000 *g* for 15 min (4°C). The supernatants were also analyzed using the same automatic amino acid analyzer.

Calculations

Fish growth performances were calculated according to the formula below

Feed conversion ratio (FCR) = feed intake (g)/ body weight gain (g)

Protein efficiency ratio (PER) = live weight gain (g)/ protein intake (g)

Specific growth rate (SGR %.day^{-1}) = [ln (final weight) – ln (initial weight)] × 100/ day

Hepatosomatic index (HSI, %) = liver mass (g) ×100/ fish mass (g)

Intraperitonial fat ratio (IFR) = peritoneal fat mass (g) × 100/ fish mass (g)

Muscle ratio (MR) = muscle weight (g) × 100/ fish mass (g)

The apparent digestibility coefficient (ADC) of nutrients was calculated according to

the method described by [19, 23] as:

ADC (%) =100 – {100 × (% Cr_2O_3 in diet/% Cr_2O_3 in feces) × (% nutrient in feces /% nutrient in diet)}

$ADC_{\text{test ingredient}} = ADC_{\text{test diet}} + \{(ADC_{\text{test diet}} - ADC_{\text{reference diet}}) \times (0.7 \times D_{\text{reference}}/0.3 \times D_{\text{ingredient}})\}$

where: $D_{\text{reference}}$ = % nutrient of reference diet; $D_{\text{ingredient}}$ = % nutrient of test ingredient

Statistical analyses

The differences among treatments with respect to each parameter were determined by one-way analysis of variance. When significant differences were detected, Tukey's multiple-range test was used to detect the difference between means among the treatments using SPSS (version 17.0). *P*-values less than 5% were considered significant.

Results

The growth performance and nutrient utilization in rainbow trout are presented in Table 6. Fish fed the diets that replaced 75% and 100% fish meal with the combination of PBM+FEM+BM showed better or similar weight gain and SGR compared with fish fed the control diet. But final weight and SGR were lower than the other treatment when fish were fed the FM+DC and PFBDC diets, being not

Table 6

significantly different from those of the fish fed the control diet (p>0.05).

Feed intake of the FM+PFB and the FM+PFBDC groups were significantly higher than the other groups (p<0.05). Feed intake of the PFB group was significantly higher than the PFBDC group (p<0.05).

FCR in FM group was significantly lower than the other groups (P<0.05). PER in FM+PFB, FM+PFBDC and PFB groups were significantly lower than FM and FM+DC groups (P<0.05).

Body composition of rainbow trout fed experimental diets is shown in Table 7. The crude protein content of the fish whole body was affected by the experimental diets. Crude protein content of fish fed the FM diet was significantly higher than fish fed the other experimental diets except the FM+PFB group (p<0.05). Fish fed the FM diet showed the lowest level crude lipid when compared with fish fed the other experimental diets (p<0.05). **Table 7**

Table 8 shows the results of body indices of rainbow trout fed the experimental diets. Low hepatosomatic index was observed in the fish fed the FM diet when compared with fish fed the FM+DC and PFBDC diets (p<0.05). Highest muscle ratio was found in the trout fed the FM+PFBDC diet. **Table 8**

Table 9 shows the results of the blood indices and plasma chemistry of rainbow trout fed the different diets. There were no significant differences in the hematocrit and hemoglobin among fish fed the experimental diets (p>0.05). Significantly lower level of the GOT was found in the fish fed the FM diet than the FM+DC and PFBDC

Table 9

groups (p<0.05). GOT value of the PFBDC group was significantly higher than the FM, FM+PFB, and FM+PFBDC groups (p<0.05).

The ADCs of crude protein were significantly higher when 75% fish meal was replaced by the combination of DSM and CGM than fish fed the FM+PFB diet (p<0.05, Table 10). The ADCs values of all the essential amino acid examined were significantly lower in PBM and FEM than DSM and CGM (p<0.05, Table 11).

Table 10~11

Discussion

In the present experiment, 75% and 100 % fish meal replacement by PBM, FEM and BM resulted in no negative impact on final body weight, weight gain, SGR, survival and FI., blood indices and liver enzyme activities of rainbow trout. Schulz et al. [25] reported that better growth performance of rainbow trout fed mixture of PBM, FEM, and BM at 33.3%: 33.3%: 33.3% than 50% PBM and 50% FEM as dietary protein. Alexis et al. [26] also reported that complete replacement of fish meal with 30% PBM with 20% CGM and 12% carob seed germ meal showed significantly higher growth in rainbow trout than herring meal base diet. Sealey et al. [27] examined effect of total replacement of fish meal with three kinds of poultry products (chicken concentrate, PBM, and chicken and egg concentrate) on growth performance of rainbow trout. They found that growth performance of the fish fed PBM based diet was comparable with fish meal based diet, although FCR of fish fed PBM based diet was significantly inferior to that fed fish meal based diet. These

results strongly suggest that PBM is a suitable protein source for rainbow trout diet and complete replacement of fishmeal with PBM in rainbow trout diet without negative impact on final body weight, weight gain, SGR, survival and FI.

In the present study, lower WG and SGR were observed in the fish fed the FM+DC and PFBDC diets compared with the FM+PFB and FM+PFBDC. With the same trend of the growth, the FI showed the lower result in the FM+DC and PFBDC. Regarding FI, significantly higher FI was recorded in FM＋PFB, FM+PFDC and PFB groups than the control (FM group). Reduced palatability has been showed responsible to the reduced feed intake of fish fed the diets in which high levels of FM was replaced with plant protein source [28]. Therefore, inclusion of DSM and CGM in FM+DC and PFBDC diets seem to reduce FI in FM+DC and PFBDC groups due to low palatability of DSM and CGM. Adding the feeding stimulants can improve the palatability of fish diet [29]. Supplementation with feed stimulants in FM+DC and PFBDC diets might suggest to improve FI and induce better growth performance of FM+DC and PFBDC groups. Highest FI were found in FM+PFB and FM+PFBDC groups in current study. Palatability of fish feed is reported to be mainly depended on amino acid and nucleotide [6]. It was also reported that L-proline, L-hydroxyproline, L-alanine, L-phenylalanine, and L-leucine could stimulate taste sense of rainbow trout and L-proline was most potent among the amino acids [30-31]. However, no much difference was observed in free amino acid composition among all diets. It was reported that nucleotide content is higher in animal protein sources than plant

products

[http://en.engormix.com/MA-pig-industry/nutrition/articles/nucleotides-yeast-extract-potential-t340/141-p0.htm accessed 24 Aug 2014]. Although nucleotide content in the diets were not analyzed in this experiment, nucleotide included in FM+PFB and FM+PFBDC diets may enhance FI of rainbow trout. Another explanation of lower growth performance and lower FI of FM+DC and PFBDC groups may be attributed to lower lysine content of these diets. Since lysine is one of the indispensable amino acid, FM+DC and PFBDC diets contained a little bit lower lysine than the requirement of rainbow trout [6]. In addition, it was reported that lower FI in rainbow trout fed amino acid imbalanced diet than fed amino acid balanced diet [32], suggesting lower FI of FM+DC and PFBDC groups could be induced by lower lysine content of the diet.

Hemoglobin value and hematocrit concentration are related to health condidition of fsih, where high hemoglobin can be taken as an indication of good condition [33]. In the present study, replacement of fish meal with rendered animal protein and plant protein source did not cause anemia and hematocrit value. AST and ALT are amino acid transferase in liver and higher activities of these enzyme were suggested to be related to consumption of protein for energy production [34]. Fish fed the FM and PFBDC diets had the lower and higher AST level than fish fed the other experimental diets, respectively. Although there was no significant difference, similar trend was found in ALT value of fish in FM and PFBDC groups. These

results may indicate protein consumption for energy production in fish fed alternative protein sources based diet such as PFBDC.

The HSI in fish fed the FM diet was significantly lower than fish fed the FM+DC and PFBDC diets. It seems that the hepatic fat deposition indeed is higher when fish fed FM+DC and PFBDC diets. The IFR in fish fed the experimental diets tended to be higher than that fed the control diet. Previous studies reported that fish meal replaced by the alternative protein sources including DSM and PBM increased the IFR [16, 35-37]. Our results were in agreement with these previous studies. MR in fish fed the FM+PFBDC diet was also higher level than the other groups.

High ADC of protein in PBM was observed in this study (87.8%). This is in agreement with previous studies that reported 87-91% of ADC of protein in PBM [2, 17, 38-39]. ADC of protein of test diets was not different among treatments (88.3-94.3%) except the significant different between FM+FPB and FM+DC. Similarly, ADC of EAA in the experimental diets was similar among treatments except methionine. Different growth performance of fish fed these diets could reflect different FI and/or amino acid composition of the diets.

Considering ADCs of protein and EAA in test protein sources, values of PBM and FEM were lower than DSM and CGM. ADCs of EAA in FEM were less than 90% except arginine (Table 11). Digestibility analysis of the test protein sources revealed that rendered animal protein sources (PBM and FEM) except BM were less digestible compared to plant protein sources such as CGM and DSM. Fish fed the

FM and FM+DC diets performed the better PER than fish fed the FM+PFB, FM+PFBDC and PFB diets. Poor PER in these three groups could be due to higher percentage of rendered animal protein sources in the diets. On the contrary, BM was highly digestible in rainbow trout. This is well agreement with these previous studies [2, 9]. These results suggested that inclusion of high level of rendered animal protein sources in rainbow trout diets could result in growth retardation. In this study, we used combination of rendered animal protein sources in order to confer well balanced amino acid in the test diets. As a result, there was no marked difference in digestibility of experimental diets in the growth experiment (Table 10). With respect to high digestibility of plant protein sources such as CGM and DSM, inclusion of high level of these protein sources may lead better growth in rainbow trout. However, fish fed the diets with DSM and CGM showed growth retardation. This could be due to less palatability of these diets. Considering these results altogether, rendered animal protein sources can be used to support high palatability in low or non fish meal diet for rainbow trout. It is suggested that addition of feed stimulants in plant protein based diets could improve feed performance of rainbow trout diets. However, addition of feed stimulants may elevate production cost of the feed. Therefore, inclusion of rendered animal protein sources could be cost effective solution to produce low or non-fish meal diet for rainbow trout with well growth.

The results of this study demonstrated that PBM, FEM and BM have good nutritive value for rainbow trout diets. Very high inclusion levels of the combination

of the rendered animal protein successfully might be used in the formulated fish diet for rainbow trout.

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Table 1 Proximate composition of the experimental ingredients used as protein sources (%)

Ingredients	Anchovy meal	Poultry by-product meal	Feather meal	Blood meal	Defatted soybean meal	Corn gluten meal
Dry matter	89.4	98.5	93.1	91.5	86.5	91.0
Crude protein (dry basis)	72.5	59.0	92.2	93.8	52.2	64.3
Crude lipid (dry basis)	9.1	17.9	5.2	1.1	2.0	4.0
Ash (dry basis)	16.5	20.6	1.4	5.3	7.0	1.3
EAAs (dry basis)						
Arginine	3.5	4.3	6.5	3.1	3.7	2.3
Histidine	2.0	1.1	0.8	4.4	1.3	1.3
Isoleucine	1.6	1.6	5.0	0.5	2.4	2.5
Leucine	4.2	4.0	8.5	8.8	4.0	11.7
Lysine	4.7	3.2	2.5	6.0	3.2	1.2
Methionine	1.8	1.2	0.8	0.7	0.7	1.8
Phenylalanine	2.3	2.3	6.3	4.9	2.8	4.2
Threonine	2.6	2.2	5.7	2.8	1.9	2.5
Valine	1.9	1.9	6.7	3.8	2.4	3.3
NEAAs (dry basis)						
Alanine	4.3	4.9	4.4	6.2	2.1	5.3
Aspartic acid	6.0	5.2	5.5	9.3	5.2	4.0
Glutamic acid	8.5	7.8	9.4	7.7	7.9	12.4
Glycine	4.2	6.8	5.9	3.7	2.3	1.7
Serine	2.8	2.7	8.7	4.2	2.4	3.5
Tyrosine	2.0	1.8	2.5	2.0	1.4	1.2
Proline	3.3	5.5	8.3	3.2	2.3	4.2

Table 2 Formulation of the experimental diets for growth of rainbow trout (%)

Ingredient	FM	FM+PFB	FM+PFB DC	FM+DC	PFB	PFBDC
Peruvian anchovy meal	56.0	14.0	14.0	14.0	-	-
Poultry by-product meal	-	28.0	14.0	-	34.0	21.0
Feather meal	-	7.0	5.0	-	8.0	5.0
Blood meal [a]	-	9.0	8.0	-	14.0	9.0
Defatted soybean meal	-	-	10.0	25.0	-	12.0
Corn gluten meal	-	-	8.0	24.0	-	10.0
Wheat flour	20.0	14.0	10.0	10.0	15.0	15.0
Pre-gelatinized starch	4.5	4.5	4.5	4.5	4.5	4.5
Fish oil	5.0	6.0	6.0	6.0	6.0	6.0
Soy bean oil	8.0	8.0	9.0	10.0	9.0	10.0
Vitamin mixture [b]	3.0	3.0	3.0	3.0	3.0	3.0
Monocalcium phosphate	-	0.8	0.8	0.9	1.7	1.6
DL-Methionine	-	-	-	-	0.5	0.5
Mineral mixture [c]	1.0	1.0	1.0	1.0	1.0	1.0
Choline chloride	0.5	0.5	0.5	0.5	0.5	0.5
Vitamin E	0.1	0.1	0.1	0.1	0.1	0.1
Cellulose	1.4	3.6	5.6	0.5	2.2	0.3
Chromic oxide	0.5	0.5	0.5	0.5	0.5	0.5
Total	100.0	100.0	100.0	100.0	100.0	100.0

[a] Blood meal: porcine blood meal.

[b] Vitamin mixture (amount kg^{-1}): thiamin hydro-chloride, 3.025 g; riboflavin, 3.63 g; pyridoxine hydrochloride, 2.42 g; cyanocobalamin, 0.006 g; ascorbic acid, 368.902 g; niacin, 24.2 g; calcium pantothenate, 6.05 g; inositol, 121 g; biotin, 0.363 g; folic acid, 0.908 g; p-aminobenzoic acid, 3.025 g; vitamin K3, 6.05 g; vitamin A acetate, 2,420,000 IU; vitamin D3, 2,420,000 IU.

[c] Mineral mixture composition (g kg^{-1}): sodium chloride, 50; magnesium sulfate, 745; iron (III) citrate n-hydrate, 125; trace element mix, 50; cellulose, 30. (The trace element mixture contains (g kg^{-1}): zinc sulfate heptahydrate, 353; manganese sulfate, 162; copper (II) sulfate pentahydrate, 31; aluminium chloride hexahydrate, 10; cobalt chloride, 3; potassium iodate, 1; cellulose, 440)

Table 3 Reference and test diet formulation for the determination of digestibility coefficients of ingredients in juvenile rainbow trout

	Amount g $100g^{-1}$ diet as fed	
Ingredients	Reference diet	Test diet
Peruvian anchovy meal	56.0	39.2
Wheat flour	20.0	14.0
Pre-gelatinized starch	4.5	3.15
Fish oil	5.0	3.5
Soybean oil	8.0	5.6
Vitamin premix [a]	3.0	2.1
P- free mineral premix [b]	1.0	0.7
Choline chloride	0.5	0.35
Cr_2O_3	0.5	0.35
Vitamin E	0.1	0.07
Cellulose	1.4	0.98
Test ingredient		30.0

Test ingredient: Poultry by-product meal; Feather meal; Blood meal; Defatted soybean meal; Corn gluten meal

[a] Comosition of vitamin premix is the same as in Table 2.

[b] Composition of P- free mineral premix is the same as in Table 2.

Table 4 Proximate and amino acids composition of the experimental diets for growth (%)

Component	FM	FM+PFB	FM+PFBDC	FM+DC	PFB	PFBDC
Dry matter	95.8	93.9	92.1	97.6	90.2	95.1
Crude protein	43.9	43.1	43.3	41.2	44.5	43.1
Crude lipid	18.8	20.9	21.2	21.4	21.7	21.4
Ash	10.6	9.7	7.9	5.5	9.3	7.5
EAAs						
Arginine	2.0	1.9	2.2	1.9	2.2	2.0
Histidine	1.1	0.9	1.0	0.8	1.0	0.9
Isoleucine	1.2	1.1	1.2	1.1	1.1	1.1
Leucine	2.4	2.5	3.5	3.0	3.1	3.4
Lysine	2.5	2.4	2.4	1.9	2.4	1.9
Methionine	0.9	0.7	0.7	0.7	1.0	1.0
Phenylalanine	1.4	1.5	1.9	1.5	1.8	1.8
Threonine	1.5	1.3	1.6	1.2	1.5	1.4
Valine	1.2	1.3	1.6	1.2	1.5	1.4
NEAAs						
Alanine	2.4	2.2	2.7	2.0	2.7	2.1
Aspartic acid	3.3	3.0	3.6	2.8	3.5	3.1
Glutamic acid	5.9	5.3	6.1	6.0	5.6	6.1
Glycine	2.5	2.8	2.7	2.0	3.0	2.4
Serine	1.7	2.0	2.3	1.7	2.5	2.0
Tyrosine	1.0	1.0	1.3	1.2	1.0	1.3
Proline	1.8	2.7	2.6	1.7	2.6	2.2

Table 5 Free amino acids composition of the experimental diets for growth (g/100g d.b.)

	FM	FM+PFB	FM+PFBDC	FM+DC	PFB	PFBDC
EAAs						
Arginine	0.05	0.02	0.04	0.07	0.01	0.04
Histidine	0.44	0.10	0.10	0.10	0.01	0.01
Isoleucine	0.03	0.02	0.02	0.01	0.02	0.01
Leucine	0.04	0.04	0.03	0.02	0.03	0.02
Lysine	0.06	0.04	0.03	0.02	0.03	0.02
Methionine	0.01	0.01	0.01	0.01	0.46	0.48
Phenylalanine	0.02	0.02	0.02	0.01	0.02	0.01
Threonine	0.02	0.02	0.01	0.01	0.03	0.04
Valine	0.03	0.02	0.02	0.02	0.02	0.02
NEAAs						
Alanine	0.06	0.06	0.06	0.04	0.05	0.04
Aspartic acid	0.02	0.02	0.02	0.02	0.02	0.02
Glutamic acid	0.05	0.03	0.03	0.03	0.03	0.03
Glycine	0.02	0.02	0.02	0.01	0.02	0.02
Serine	0.02	0.01	0.01	0.01	0.03	0.01
Tyrosine	0.02	0.01	0.01	0.01	0.01	0.01
Proline	0.02	0.03	0.03	0.01	0.02	0.01

Table 6 Growth parameters and nutrient utilization in rainbow trout fed the experimental diets for 12 weeks (mean± standard deviation)

Parameters	Diets					
	FM	FM+PFB	FM+PFBDC	FM+DC	PFB	PFBDC
Initial weight (g)	16.7±0.0	16.8±0.0	16.7±0.0	16.6±0.1	16.7±0.2	16.7±0.1
Final weight (g)	135.0±2.0ab	148.5±3.3a	143.9±4.5a	123.1±6.0b	134.9±2.8ab	120.3±6.2b
Weight gain (g)	118.3±2.1ab	131.7±3.3a	127.2±4.5a	106.5±6.0b	118.2±2.9ab	103.5±6.3b
SGR (%/day)[*1]	2.49±0.02ab	2.60±0.03a	2.56±0.04a	2.38±0.05b	2.49±0.04ab	2.35±0.07b
Feed intake (g/fish)	98.6±0.0bc	121.1±0.7a	119.2±1.8a	97.1±4.9bc	107.3±2.1b	96.9±2.7c
FCR[*2]	0.83±0.01b	0.92±0.02a	0.94±0.02a	0.91±0.01a	0.91±0.00a	0.94±0.03a
PER[*3]	2.67±0.05a	2.42±0.05b	2.46±0.05b	2.66±0.02a	2.39±0.01b	2.49±0.08ab
Survival (%)	100.0	100.0	100.0	100.0	100.0	98.0

Values are mean± SEM. Means with the same letter in a same row are not significantly different (P>0.05).

1. Specific growth rate (SGR %.day^{-1}) = [ln (final weight) – ln (initial weight)] × 100/ day
2. Feed conversion ratio (FCR) = feed intake (g)/ body weight gain (g)
3. Protein efficiency ratio (PER) = live weight gain (g)/ protein intake (g)

Table 7 Proximate composition of the whole body of rainbow trout fed the experimental diets for 12 weeks (% on a wet weight basis)

Component	FM	FM+PFB	FM+PFBDC	FM+DC	PFB	PFBDC
Crude protein	16.8±0.2a	16.3±0.3ab	15.4±0.5bc	14.6±0.1c	15.2±0.1c	14.8±0.1c
Crude lipid	10.5±0.4c	11.9±0.2b	11.6±0.3b	13.7±0.2a	12.0±0.1b	13.2±0.3a
Ash	1.9±0.1a	2.5±0.1b	1.7±0.1a	2.0±0.2a	1.8±0.2a	1.9±0.1a
Moisture	71.6±0.3a	68.8±0.1c	70.5±0.1b	69.2±0.6c	69.8±0.1bc	68.9±0.2c

Values are mean± SEM. Means with the same letter in a same row are not significantly different (P>0.05).

Table 8 Body indices of rainbow trout fed experimental diets for 12 weeks (%)

	FM	FM+PFB	FM+PFBDC	FM+DC	PFB	PFBDC
HSI*	1.1±0.0c	1.3±0.0abc	1.2±0.1bc	1.3±0.1ab	1.2±0.1bc	1.5±0.0a
IFR*	5.4±0.4b	6.3±0.3ab	7.3±0.5a	7.1±0.2ab	6.5±0.6ab	6.6±0.6ab
MR*	53.0±1.0ab	48.5±1.6abc	54.6±2.7a	45.0±1.6c	46.9±1.6bc	46.6±0.8bc

HSI, hepatosomatic index; IFR, intraperitoneal fat ratio; MR, muscle ratio

Values are mean± SEM. Means with the same letter in a same row are not significantly different (P>0.05).

Table 9 Blood indices and plasma chemistry of rainbow trout fed experimental diets

	FM	FM+PFB	FM+PFBDC	FM+DC	PFB	PFBDC
Hb (%)	14.2±0.6	13.6±0.4	14.5±0.8	15.4±0.7	13.7±0.9	13.0±0.0
Hct(%)	51.8±4.6	45.5±0.7	53.5±1.4	49.5±4.2	49.5±4.9	49.0±7.8
AST (U L^{-1})	194.5±33.2c	233±35.4bc	243.5±41.7bc	314.5±23.3ab	295.5±14.8abc	372.5±14.8a
ALT (U L^{-1})	5.5±0.7	11.0±1.4	7.5±3.5	12.5±4.9	11.0±2.8	16.0±1.4

Hb (Haemoglobin); Hct (Haematocrit); GOT (Glutamic oxaloacetic transaminase); GPT (Glutamic pyruvic transaminase)

Values are mean± SEM. Means with the same letter in a same row are not significantly different (P>0.05).

Table 10 Apparent digestibility coefficient (ADC) of nutrients in the experimental diets for growth (%)

Component	FM	FM+PFB	FM+PFBDC	FM+DC	PFB	PFBDC
Dry matter	79.4±2.2	79.1±1.0	80.3±0.4	82.5±1.0	83.4±0.1	81.1±1.1
Crude protein	91.8±0.5ab	88.6±0.5b	91.5±0.7ab	94.3±0.7a	91.2±0.5ab	91.5±0.5ab
EAAs						
Arginine	95.5±0.0	89.6±0.8	92.5±1.7	94.6±2.1	90.3±2.3	92.2±1.3
Histidine	96.9±0.1	92.7±1.8	94.0±2.1	92.8±3.4	93.8±2.1	94.0±1.4
Isoleucine	93.7±2.1	87.2±4.3	90.9±0.3	90.6±5.3	88.6±1.8	90.4±0.2
Leucine	96.2±0.2	91.2±2.0	92.9±1.9	93.3±3.4	91.1±2.7	92.6±2.0
Lysine	97.1±0.1	89.7±0.9	93.1±0.5	91.2±3.8	90.0±3.1	91.2±0.7
Methionine	97.3±0.3ab	91.7±0.6c	94.2±1.8bc	100.0±0.0a	94.7±1.4bc	96.6±1.4ab
Phenylalanine	95.3±0.2	91.1±1.2	92.0±2.2	92.4±3.8	91.4±3.0	92.9±1.4
Threonine	95.5±0.3	93.0±0.8	93.4±0.8	91.7±4.7	89.4±2.6	92.2±0.1
Valine	94.6±0.5	90.2±3.1	92.6±0.2	90.1±4.9	90.8±2.8	92.5±0.3

Values are mean± SEM. Means with the same letter in a same row are not significantly different (P>0.05).

Table 11 Apparent digestibility coefficient (ADC) of nutrients in the test ingredient for rainbow trout (%)

Component	Poultry by-product meal	Feather meal	Blood meal	Defatted soybean meal	Corn gluten meal
Crude protein	87.8±0.4b	84.1±0.9c	89.0±0.3b	90.2±0.1ab	92.3±1.1a
EAAs					
Arginine	84.9±0.2d	90.9±0.1c	96.5±0.4a	95.6±0.3ab	94.6±0.3b
Histidine	88.4±0.2d	89.2±0.4cd	90.7±0.6c	98.0±0.6a	93.9±0.5b
Isoleucine	81.6±0.6d	88.6±0.6c	89.9±1.0bc	97.0±0.6a	92.4±1.2b
Leucine	83.7±0.4d	85.7±0.7d	91.0±1.1c	94.8±0.3b	97.6±0.6a
Lysine	86.4±1.6b	86.2±1.1b	90.8±0.8ab	93.5±1.3a	92.2±1.4a
Methionine	82.8±0.2d	82.9±0.1d	92.4±0.3b	95.3±0.7a	89.7±0.7c
Phenylalanine	83.0±0.6d	87.7±0.7c	91.8±0.3b	96.0±0.2a	95.6±0.8a
Threonine	83.3±0.4b	87.1±2.1b	91.5±0.8a	94.2±0.5a	94.0±0.8a
Valine	81.6±0.4c	86.5±0.8b	82.5±0.8c	96.0±0.7a	94.5±0.9a

Values are mean± SEM. Means with the same letter in a same row are not significantly different (P>0.05).

Chapter 4

Effect of replacement of fish meal by poultry by-product meal, feather meal and blood meal on growth response of rainbow trout (*Oncorhynchus mykiss*)

Abstract

A 12-week feeding trial by using rainbow trout (mean initial weight; 23.6±0.1g) was carried out to assess the effect of replacing fish meal with rendered animal protein sources (PBM, FEM, and BM) in the practical diets. Fish meal diet was designated as control. In the other four diets, fish meal was replaced completely by the combination of PBM+FEM+BM at the different levels of 60/20/20, 70/20/10, 80/10/10, 90/10/0. Synthetic lysine and methionine were supplemented to satisfy the essential amino acid in all experimental diets.

Fish fed the control diet exhibited high weight gain and specific growth rate than the other treatment. Fish fed the diet with the combination of PBM+FEM+BM at 60/20/20 showed the significantly higher feed intake than fish fed the combination of PBM+FEM+BM at 90/10/0. Also better feed conversion ratio was shown in the control than the fish fed the PBM+FEM+BM at 70/20/10 and 80/10/10. No significant differences were found in hepatosomatic index among the diet treatments. These results were suggested that the combination of PBM, FEM and BM at 60/20/20 could be suitable to replace the fish meal in practical diets for rainbow trout.

Introduction

Fish meal is conventional source of protein in fish feed and it has been highly evaluated as its balanced amino acids, palatability and growth factors (Tacon, 1993). Because of increasing cost of high quality fish meal required for aqua-feeds and declining stocks of fish from capture fishery and competition of feed ingredients with livestock industry (Tacon, 1993), replacement of fishmeal with less expensive protein sources would be beneficial in reducing feed costs.

A draw back to use of plant protein sources in fish diets is the presence of a variety of anti-nutritional factors (Tacon, 1993), the need to investigate the use of animal protein sources to feed the fish has become very important. Rendered animal protein ingredients, such as poultry by-product meal (PBM), blood meal (BM) and feather meal (FEM) have been used successfully in feeds for various fish species.

The aim of this study is to investigate the effect of replacing fish meal with the different combination ratios of the rendered animal protein sources on the growth performance, biological indices and plasma free amino acid of rainbow trout.

Materials and methods

Ingredients and diets

All diets were produced at the Laboratory of Fish Nutrition, Tokyo University of Marine Science and Technology (Shinagawa campus), Tokyo, Japan. PBM, FEM and BM were employed for replacement of FM. PBM and FEM were purchased by Nangoku Kosan (Miyazaki, Japan). The composition of the experimental diets is shown in Table 1. Anchovy meal based diet (FM) was arranged as control. In the other four diets, fish meal was replaced completely by the combination of PBM+FEM+BM at the different levels of 60/20/20, 70/20/10, 80/10/10, 90/10/0. In preparing the diet, all dried ingredients were thoroughly mixed by a horizontal mixer (ACM-50 LAT model, Aicohsha, Saitama, Japan). Feed ingredients were ground in order to reduce the particle size to less than 500 μm. The ingredients were mixed in a horizontal mixer, added to deionized water (30%), and pelleted to 2 sizes (ø 3.2 and 4.8 mm) using a laboratory pellet maker machine (OMC-22B model, Omichi, Gunma, Japan). The pellet was dried using a vacuum freeze-drier (RLE 206-II, Kyowa Vacuum Engineering, Tokyo) and stored at -30 ºC until use. Crude protein (43.1-44.9%) and crude lipid contents (18.9-19.3%) were similar among all diets (Table 2).

Design and fish management

The rainbow trout juveniles were obtained from Oizumi Station, Field Science Center, Tokyo University of Marine Science and Technology (TUMSAT), Yamanashi and reared under laboratory conditions at Laboratory of Fish Nutrition, TUMSAT, Japan. Prior to experiment, all fish were acclimatized to the experimental condition by feeding a commercial diet (Nippon Formulation Feed Co. Ltd., Kanagawa, Japan) for two weeks. Fish with an average body weight of about 23.6±0.2 g were randomly sampled from stock and distributed into 60 L glass rectangular tank at the density of 15 fish per tank. Duplicate groups were assigned to each experimental diet. Feed intake (FI) was monitored daily. Tanks were supplied with dechlorinated tap water at 0.6 l/min, and the water temperature was controlled at 14.5±1.0 °C with a thermostat (RHUP250A2, Hitachi, Tokyo, Japan) in a semi-recirculating system during the experimental period. The feeding experiment was conducted for 12 weeks in the water recirculating system with a constant water supply at a rate 0.5 l/min and aeration provided by aerator. The fish were hand-fed twice (10:00 and 16:30) a day, 6 days a week to an apparent satiation. Determination of growth changes and feed performance calculations were examined every 3 weeks.

Sample preparation and chemical analysis

Initial and final carcass chemical composition

At the beginning and the end of the growth experiment, 10 and 5 fish were randomly selected from the experimental stock and from the experimental tank in each treatment, respectively for whole body analysis. Sampled fish were euthanized

with an overdose of 2-phenoxyethanol (Wako Pure Chemical Industries, Osaka, Japan). Fish body samples were ground using a centrifugal mill (Retsch, Haan, Germany) fitted with 0.5-mm screen, and homogenized. Homogenized samples were then dried by a freeze-drying machine and stored at – 30 ºC until analyzed.

Haematological parameters and fish body and blood indices

In order to investigate the postprandial effect, five fish were sampled at 36 hr after the last meal and anesthetized by 2-phenoxyethanol. Blood samples were collected by the heparinized syringe from the caudal vein, pooled, and analyzed two replicates. The blood was centrifuged for the plasma by centrifugation at 3000 *g* at 4 °C for 15 min using a high-speed refrigerated centrifuge (SRX – 201 model, Tomy, Tokyo, Japan). The plasma was separated and kept at - 35 °C until analysis. After collecting the blood samples, digestive tracts (except heart) and fillet were dissected out from the fish body, weighed individually to get the body indices.

Analyses of moisture and ash were conducted by using the method described by Boonyoung *et al*. (2012) the crude protein content in samples was determined by semi-micro Kjeldahl method (N* 6.25) using Kjeldahl analyzer (FOSS Kjeltec^{TM} 2400, FOSS, Tokyo, Japan), and crude lipid content was analyzed using a gravimetric method after lipid extraction using chloroform-methanol (2:1, v/v), respectively.

The samples were hydrolyzed in 4 mol L^{-1} methanesulfonic (Sigma-Aldrich, Missouri, Mo, USA) for 22 hours at 110 ºC prior to total amino acid analysis. The

total amino acids compositions were determined using an automatic amino acid analyzer (JLC-500/v, JEOL, Tokyo, Japan).

Calculations

Fish growth performances were calculated according to the formula below

Feed conversion ratio (FCR) = feed intake (g)/ body weight gain (g)

Specific growth rate (SGR %.day^{-1}) = [ln (final weight) – ln (initial weight)] × 100/ day

Hepatosomatic index (HSI, %) = liver mass (g) ×100/ fish mass (g)

Intraperitonial fat ratio (IFR) = peritoneal fat mass (g) × 100/ fish mass (g)

Muscle ratio (MR) = muscle weight (g) × 100/ fish mass (g)

Statistical analysis

All statistical analyses were carried using SPSS (version 17.0) and *P*-levels < 0.05 were considered to be significant. Effects of dietary treatment were tested for significance by one-way analysis of variance (ANOVA). Differences between treatment means were compared using Tukey's multiple-range test.

Results

The results of the feeding experiment are shown in Table 3. Fish fed the control diet exhibited higher weight gain and specific growth rate than the other treatment. Fish fed the diet with the combination of PBM+FEM+BM at 60/20/20 showed the significantly higher feed intake than fish fed the combination of PBM+FEM+BM at 90/10/0. Also better feed conversion ratio was shown in the control than the fish fed the PBM+FEM+BM at 70/20/10 and 80/10/10. No significant differences were found in hepatosomatic index among the diet treatments.

Proximate composition of the whole body of rainbow trout fed the experimental diets is shown in Table 4. The protein content of the fish fed the PFB4 diet showed significant lower than those of the others. On the other hand, there was no significant difference in crude lipid, ash and moisture content.

Body indices of rainbow trout fed experimental diets are shown in Table 5. As elevation of dietary poultry by-product meal, MR was decreased. IFR was decreased by the replacement of fish meal by poultry by-product meal significantly. However, there are no significant differences in HSI.

Plasma free essential amino acid concentration (nmol g^{-1}) in rainbow trout fed experimental diets are presented in Table 6. Free His, Lys, Met concentration in plasma were significantly decreased as elevation of dietary inclusion of poultry by-product meal. However, there were no differences in the other plasm free amino

acids.

Discussion

Alexis et al. (1985) reported that complete replacement of fish meal with 30% PBM, 20% CGM and 12% carob seed germ meal showed significantly higher growth response in rainbow trout than herring meal base diet. Sealey et al. (2011) conducted the effect of total replacement of fish meal with three kinds of poultry products on growth performance of rainbow trout. They found that growth performance of the fish fed PBM based diet was comparable with fish meal diet. However, the growth of current feeding experiment decreased significantly as elevation of dietary poultry by-product meal inclusion. However, FCR was not influenced by the dietary levels of poultry by-product meal inclusion. On the other hand, feed intake was decreased by dietary inclusion of poultry by-product meal. Thus, it was suggested that the growth was greatly influenced by feed intake in current study.

Histidine is considered one of the feed stimulating amino acid to several fish species. Histidine concentration of plasma free amino acid in the fish fed the experimental diet was significantly decreased by the elevated inclusion of dietary poultry by-product meal. The dietary histidine content also decreased as elevation of inclusion of poultry by-product meal. Therefore, the decent of histidine concentration in plasma was influenced by two factors, dietary histidine content and feed intake.

The ash content in feed ingredient, such as fish meal was reported to be a factor of decreasing the digestibility of nutrient and growth (Satoh et al., 1984). In current study, the ash content was markedly higher in poultry by-product meal than that of fish meal or other ingredients. It was suspected that such higher ash content might influence the growth of fish fed PFB4 diet.

In current study, FCR was significantly lower in the fish fed all the non-fish meal feed than that of fish meal feed. Moreover, protein content in fish deceased slightly as elevation of poultry by-product meal. Those phenomena might be suggested that the availability of protein contained in poultry by-product meal used in this study might be lower than that of fish meal. Namely, replacement with poultry by-product meal alone is not recommended, therefore the combination with the other ingredients such as feather meal and blood meal is recommended.

The results of the current study were suggested that the combination of PBM, FEM and BM at 60/20/20 could be suitable to replace the fish meal in practical diets for rainbow trout.

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Table 1 Formulation of the experimental diets for growth of rainbow trout (%)

Ingredient	FM	PFB1	PFB2	PFB3	PFB4
Peruvian anchovy meal	46.0	-	-	-	-
Poultry by-product meal	-	27.0	31.5	36.0	40.5
Feather meal	-	9.0	9.0	4.5	4.5
Blood meal	-	9.0	4.5	4.5	-
Corn gluten meal	8.0	7.0	9.0	10.0	12.0
Wheat flour	16.0	14.0	14.0	14.0	14.0
Pre-gelatinized starch	5.0	5.0	5.0	5.0	5.0
Fish oil	5.0	6.0	6.0	6.0	6.0
Soy bean oil	8.0	9.0	8.0	8.0	7.0
Vitamin mixture [a]	3.0	3.0	3.0	3.0	3.0
Monocalcium phosphate	-	1.3	1.3	1.2	1.1
DL-Methionine	-	0.5	0.5	-	-
Lys-HCL	-	-	0.5	0.5	0.5
Mineral mixture [b]	1.0	1.0	1.0	1.0	1.0
Choline chloride	0.5	0.5	0.5	0.5	0.5
Vitamin E	0.1	0.1	0.1	0.1	0.1
Cellulose	7.4	7.6	6.1	5.7	4.8
Total	100.0	100.0	100.0	100.0	100.0

[a] Vitamin mixture (amount kg^{-1} diet): thiamin hydro-chloride, 3.025 g; riboflavin, 3.63 g; pyridoxine hydrochloride, 2.42 g; cyanocobalamin, 0.006 g; ascorbic acid, 368.902 g; niacin, 24.2 g; calcium pantothenate, 6.05 g; inositol, 121 g; biotin, 0.363 g; folic acid, 0.908 g; p-aminobenzoic acid, 3.025 g; vitamin K3, 6.05 g; vitamin A acetate, 2,420,000 IU; vitamin D3, 2,420,000 IU.

[b] Mineral mixture composition (g kg^{-1}): sodium chloride, 50; magnesium sulfate, 745; iron (III) citrate n-hydrate, 125; trace element mix, 50; cellulose, 30. (The trace element mixture contains (g kg^{-1}): zinc sulfate heptahydrate, 353; manganese sulfate, 162; copper (II) sulfate pentahydrate, 31; aluminium chloride hexahydrate, 10; cobalt chloride, 3; potassium iodate, 1; cellulose, 440)

1 Table 2 Proximate and amino acids composition of the experimental diets for growth
2 (%)

Component	FM	PFB1	PFB2	PFB3	PFB4
Dry matter	97.5	97.1	97.2	96.0	98.4
Protein	43.1	43.9	44.9	44.6	44.9
Lipid	19.2	19.3	18.9	19.2	18.9
Ash	9.7	7.1	7.7	7.7	9.5
EAAs					
Arginine	2.0	2.0	2.1	1.6	2.1
Histidine	1.2	0.8	0.6	0.6	0.6
Isoleucine	1.0	1.3	1.1	1.1	1.2
Leucine	2.3	4.0	3.3	3.2	3.4
Lysine	2.4	2.4	2.4	2.4	2.4
Methionine	0.8	0.8	0.9	0.7	0.7
Phenylalanine	1.5	2.3	1.9	1.8	1.9
Threonine	1.6	1.7	1.5	1.4	1.4
Valine	1.3	2.0	1.5	1.4	1.4

3

4

Table 3 Growth parameters and nutrient utilization in rainbow trout fed the experimental diets for 12 weeks (mean± standard deviation)

Parameters	Diets				
	FM	PFB1	PFB2	PFB3	PFB4
Initial weight (g)	23.7±0.1	23.6±0.1	23.6±0.0	23.5±0.2	23.4±0.2
Final weight (g)	176.2±1.1a	163.7±2.1b	159.5±0.9bc	158.2±0.1c	152.4±0.6d
Weight gain (g)	152.4±0.9a	140.1±2.3b	135.8±1.0bc	134.6±0.5c	129.0±0.8d
SGR (%/day)[*1]	2.39±0.01a	2.30±0.03b	2.27±0.01bc	2.27±0.02bc	2.23±0.01c
Feed intake (g/fish)	157.6±1.6a	151.6±1.9ab	147.8±0.7b	148.6±2.6b	140.0±1.6c
FCR[*2]	1.03±0.01b	1.08±0.01ab	1.09±0.01a	1.10±0.02a	1.09±0.01ab
Survival (%)	100.0	100.0	100.0	100.0	100.0

Values are mean± SEM. Means with the same letter in a same row are not significantly different (P>0.05).

4. Specific growth rate (SGR %.day^{-1}) = [ln (final weight) – ln (initial weight)]* 100/ day
5. Feed conversion ratio (FCR) = feed intake (g)/ body weight gain (g)

Table 4 Proximate composition of the whole body of rainbow trout fed the experimental diets for 12 weeks (% on a wet weight basis)

Component	FM	PFB1	PFB2	PFB3	PFB4
Crude protein	16.0±0.2a	15.9±0.1a	15.8±0.3ab	15.6±0.1ab	15.2±0.2b
Crude lipid	12.2±0.2a	12.3±0.2a	11.9±0.3a	12.0±0.2a	12.0±0.3a
Ash	2.0±0.1a	2.0±0.2a	1.9±0.1a	2.1±0.2a	2.2±0.2a
Moisture	70.2±0.3a	69.8±0.1a	70.3±0.2a	69.6±0.4a	69.8±0.3a

Values are mean± SEM. Means with the same letter in a same row are not significantly different (P>0.05).

18 Table 5 Body indices of rainbow trout fed experimental diets for 12 weeks (%)
19

	FM	PFB1	PFB2	PFB3	PFB4
HSI*	1.32±0.19a	1.41±0.03a	1.32±0.03a	1.29±0.03a	1.34±0.06a
IFR*	5.0±0.4b	6.0±0.5a	5.8±0.4a	6.1±0.3a	5.7±0.6a
MR*	53.8±1.0a	52.0±1.2ab	51.6±2.0ab	48.3±1.5b	48.9±1.7b

20 HSI, hepatosomatic index; IFR, intraperitoneal fat ratio; MR, muscle ratio

21 Values are mean± SEM. Means with the same letter in a same row are not
22 significantly different (P>0.05).

23 Table 6 Plasma free essential amino acid concentration (nmol g^{-1}) in rainbow trout
24 fed experimental diets

Component	FM	PFB1	PFB2	PFB3	PFB4
36h post-absorptive period					
Arginine	100.4±25.2a	111.4±8.7a	116.5±4.2a	95.4±28.1a	100.5±14.1a
Histidine	157.1±17.8a	162.0±19.4a	114.8±7.3ab	102.9±4.2b	97.2±1.7b
Isoleucine	100.1±13.4a	108.0±4.9a	120.4±13.9a	90.0±14.1a	101.3±26.5a
Leucine	200.5±42.4a	190.7±14.4a	210.0±7.8a	180.2±28.3a	190.3±27.9a
Lysine	248.6±13.6a	253.1±14.0a	243.0±23.2ab	186.1±8.1bc	162.5±14.4c
Methionine	92.7±6.4abc	120.5±7.8a	117.5±17.7ab	75.0±7.9c	79.3±4.4bc
Phenylalanine	140.3±20.9a	163.8±12.4a	150.5±14.2a	130.5±7.0a	124.7±20.1a
Threonine	115.5±7.0a	117.3±3.2a	125.5±35.3a	95.8±8.1a	100.2±28.0a
Valine	230.0±28.3a	250.2±14.4a	260.9±13.6a	251.6±8.4a	260.2±28a

25 Values are mean± SEM. Means with the same letter in a same row are not
26 significantly different (P>0.05).

Chapter 5

Nutritional assessment of pork and poultry by-product meal in rainbow trout (*Oncorhynchus mykiss*) diet

Abstract

A 10-week feeding trial was conducted with fingerlings of rainbow trout (mean initial weight; 20.6±0.1g) in 10 indoor rectangular 60-L tanks (20 fish per tank) with duplicate per each diet. Low fish meal diet was arranged as control diet (FM14%), and five isonitrogenous (44 % crude protein) and isolipidic (20 % total lipid) diets were formulated, in which protein from poultry by-product meal was substituted at levels of 0%, 33%, 50% and 100% by pork and poultry by-product meal. DL-Methionine was supplemented to satisfy the essential amino acid in all experimental diets.

The study found increased the pork and poultry by-product meal inclusion levels of fish diet resulted in reduction of growth performance. Furthermore, the amino acid digestibility of the experimental diets decreased when increased the pork and poultry by-product meal inclusion levels. These results suggested that using the pork and poultry by-product meal to replace the PBM did not lead to the better growth for rainbow trout.

Introduction

Fishmeal is the major protein sources in aquafeed (Suárez et al., 2009). The limited supply together with increased demand of fishmeal elevated the cost of fishmeal (Drew et al., 2007), and the price of aqua feeds has been consequently expensive (Azaza et al., 2009). Thus it is necessary to replace the fish meal by using alternative protein sources, such as rendered animal protein.

Rendered animal protein sources are initially waste products from livestock and poultry production. They have been used for decades in salmonid feeds, however inclusion has been limited due to the poor digestibility and variations in quality (Ayadi et al., 2012).

There is no report on the evaluation of pork and poultry by-product meal (PPM) as animal protein sources in a diet for rainbow trout. Therefore, the study is to determine the effect of replacing poultry by-product meal with four levels of pork and poultry by-product meal on the fish nutritional responses, and amino acid availability of the experimental diets.

Materials and methods

Experimental diets

PBM, FEM and PPM were purchased by Nangoku Kosan (Miyazaki, Japan). The proximate analysis, amino acid profiles of fish meal, and rendered animal protein are shown in Table 1. The formulation of the experimental diets is shown in Table 2. Anchovy meal based diet (FM) was arranged as control. In order to investigate the improvement of protein and amino acid utilizations, the experimental diets were formulated to equivalent protein and lipid specifications.

In preparing the diet, all dried ingredients were thoroughly mixed by a horizontal mixer (ACM-50 LAT model, Aicohsha, Saitama, Japan). Feed ingredients were ground in order to reduce the particle size to less than 500 μm. Chromic oxide (Cr_2O_3) was used at 5 g kg^{-1} in all the diets as an inert marker for the study of digestibility. The ingredients were mixed in a horizontal mixer, added to deionized water (30%), and pelleted to 3 sizes (ø, 2.3, 3.2 and 4.8 mm) using a laboratory pellet machine (OMC-22B model, Omichi, Gunma, Japan). The pellet was dried using a vacuum freeze-drier (RLE 206-II, Kyowa Vacuum Engineering, Tokyo) and stored at -30 ºC until use. Crude protein (43.5-44.8%) and crude lipid contents (19.8-20.3%) were similar among all diets (Table 3).

Experimental conditions

Rainbow trout were obtained from the Oizumi station, Tokyo University of

Marine Science and Technology, Yamanashi, Japan and acclimatized for 2 weeks before starting. 20 fish with 20.6 ± 0.1 g $fish^{-1}$ were randomly allocated to either of 10 tanks with duplicate groups per treatment. The feeding experiment was conducted for 12 weeks including the digestibility experiment (2 weeks) in the water recirculating system with a constant water supply at a rate 0.5 l/min and aeration provided by aerator. Fish were hand fed until visual satiation two times daily and 6 days per week and the temperature of system was controlled at 14.5 ± 1.0 °C during experimental period.

Sample preparation and chemical analysis

At the beginning and the end of the growth experiment, 10 and 5 fish were randomly selected from the experimental stock and from the experimental tank in each treatment, respectively for whole body analysis. Sampled fish were euthanized with an overdose of 2-phenoxyethanol (Wako Pure Chemical Industries, Osaka, Japan). Fish body samples were ground using a centrifugal mill (Retsch, Haan, Germany) fitted with 0.5-mm screen, and homogenized. Homogenized samples were then dried by a freeze-drying machine and stored at – 30 ºC until analyzed. Another 5 fish were randomly selected from each treatment to collect the body indices, digestive tracts (except heart) and fillet were dissected out from the fish body, weighed individually to get the body indices. Liver and fillet of fish in each tank were pooled and stored at – 30 °C until analyzed.

During weeks 11 – 12, the digestibility of experimental diets was determined using Tokyo University of Fisheries (TUF) column system as described by Satoh et al. (1992). After feeding, uneaten feed and residue were siphoned out of the tank and a TUF column was immediately installed. Feces were collected from each tank within each dietary treatment on the next day in 14 consecutive days, and pooled in order to collect enough material for analyses.

Pooled faecal samples were dried by a vacuum freeze-dryer (REL 206-II model, Kyowa, Tokyo, Japan) for 22 h and kept at - 30°C until analyzed. Samples were digested using nitric- perchloric acid, and chromic oxide was measured at 350 nm by using a spectrophotometer (UV-2550, Shimadzu, Kyoto, Japan) (Satoh et al 1987).

Analyses of moisture and ash were conducted by using the method described by AOAC (1993), the crude protein content in samples was determined by semi-micro Kjeldahl method (N* 6.25) using Kjeldahl analyzer (FOSS Kjeltec^{TM} 2400, FOSS, Tokyo, Japan), and crude lipid content was analyzed using a gravimetric method after lipid extraction using chloroform-methanol (2:1, v/v), respectively.

The samples were hydrolyzed in 4 mol L^{-1} methanesulfonic (Sigma-Aldrich, Missouri, Mo, USA) for 22 hours at 110 ºC prior to total amino acid analysis. The total amino acids compositions were determined using an automatic amino acid analyzer (JLC-500/v, JEOL, Tokyo, Japan).

Data calculation and statistical analysis

Fish growth performances were calculated according to the formula below

Feed conversion ratio (FCR) = feed intake (g)/ body weight gain (g)

Specific growth rate (SGR %.day^{-1}) = [ln (final weight) – ln (initial weight)] × 100/ day

Hepatosomatic index (HSI, %) = liver mass (g) ×100/ fish mass (g)

Intraperitonial fat ratio (IFR) = peritoneal fat mass (g) × 100/ fish mass (g)

Muscle ratio (MR) = muscle weight (g) × 100/ fish mass (g)

Condition factor = fish mass (g) × 100/ (body length, cm)^3

The apparent digestibility coefficient (ADC) of nutrients was calculated according to the method described by Cho et al. (1985) & Satoh et al. (1987) as:

ADC (%) =100 – {100 × (% Cr_2O_3 in diet/% Cr_2O_3 in feces) × (% nutrient in feces /% nutrient in diet)}

The differences among treatments with respect to each parameter were determined by one-way analysis of variance (one-way ANOVA). When significant differences were detected, Tukey's multiple-range test was used to detect the difference between means among the treatments using SPSS (version 17.0 IBM Corporation, NY, USA), and a probability level of less than 0.05 was considered to be significant.

Results

The result of the feeding experiment is shown in Table 4. Fish fed the control diet exhibited higher weight gain and specific growth rate than the other treatment. As elevation of dietary inclusion of park and poultry by-product meal, the weight gain, SGR of fish decreased significantly. The feed intake also showed same trend. On the other hand, FCR showed slight increase as elevation of dietary inclusion of park and poultry by-product meal.

The proximate composition of the whole body of rainbow trout fed the experimental diets is shown in Table 5. As fish meal was replaced by pork and poultry by-product meal in the experimental diets, the crude protein content decreased slightly. On the other hand, there were no significant difference in crude lipid, ash and moisture content.

Body indices of rainbow trout fed experimental diets were shown in Table 6. HIS and IFR increased slightly as elevation of dietary inclusion of pork and poultry by-product meal, On the other hand, MR and CF significantly decreased by the replacement of poultry by-product meal with pork and poultry by-product meal.

Apparent digestibility coefficient (ADC) of nutrients in the experimental diets for growth is shown in Table 7. The digestibility of crude protein, histidine, lysine, and threonine decreased significantly as the replacement rate of poultry by-product meal with pork and poultry by-product meal increased. However, there was no difference in the other amino acid digestibility.

Discussion

The growth and feed intake of current feeding experiment decreased significantly and drastically as elevation of dietary pork and poultry by-product meal inclusion. And Feed conversion ratio was slightly increased as the elevation of dietary pork and poultry by-product meal levels. Thus, it was suggested that the growth was greatly influenced by feed intake in current study.

The protein content was significantly decreased as elevation of dietary inclusion of pork and poultry by-product meal. And the lipid content in fish fed PPM4 diet which contained the highest pork and poultry by-product meal in this study was lowest amongst the dietary treatment. Muscle ratio was also decreased as elevation of dietary pork and poultry by-product meal inclusion. Those phenomena might suggest that the decent of protein intake induced muscle synthesis.

In current study, all the test diets replaced with rendered animal meal were not supplemented with taurine. It is suggested that the dietary taurine of them might be not contained at all. Boonyoung (2012) reported that supplementation with taurine to soy protein concentrated based diet might enhance the palatability to rainbow trout. Thus, taurine supplementation might increase the palatability and increase feed intake of the diet with pork and poultry by-product meal.

ADC of protein of test diets was different among treatments (87.7-92.2%). However, ADC of EAA in the experimental diets was similar among treatments

except histidine, lysine and threonine. Bureau et al. (2000), Cheng and Hardy (2002, 2004) reported high ADC of protein in different rendered animal protein for rainbow trout, therefore, the ADC of protein and EAA in PPM should be examined in the future study.

These results of the current study suggested that using the pork and poultry by- product meal to replace the PBM did not lead to the better growth for rainbow trout.

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1 Table 1 Proximate composition of the experimental ingredients used as protein
2 sources (%)

Ingredients	Anchovy meal	Poultry by-product meal	Feather meal	Blood meal	Pork and poultry by-product meal
Dry matter	89.4	98.5	93.1	91.5	95.8
Crude protein (dry basis)	72.5	59.0	92.2	93.8	57.6
Crude lipid (dry basis)	9.1	17.9	5.2	1.1	14.3
Ash (dry basis)	16.5	20.6	1.4	5.3	19.6
EAAs (dry basis)					
Arginine	3.5	4.3	6.5	3.1	4.0
Histidine	2.0	1.1	0.8	4.4	1.3
Isoleucine	1.6	1.6	5.0	0.5	1.3
Leucine	4.2	4.0	8.5	8.8	4.1
Lysine	4.7	3.2	2.5	6.0	3.0
Methionine	1.8	1.2	0.8	0.7	0.9
Phenylalanine	2.3	2.3	6.3	4.9	2.6
Threonine	2.6	2.2	5.7	2.8	2.1
Valine	1.9	1.9	6.7	3.8	2.0

3

Table 2 Formulation of the experimental diets for growth of rainbow trout (%)

Ingredient	FM	PFB1	PFB2	PFB3	PFB4
Peruvian anchovy meal	14.0	-	-	-	-
Poultry by-product meal	28.0	42.0	28.0	21.0	-
Feather meal	7.0	7.0	7.0	7.0	7.0
Blood meal	9.0	9.0	9.0	9.0	9.0
Pork and poultry by-product meal	-	-	14.0	21.0	42.0
Wheat flour	14.0	15.0	16.0	16.0	17.0
Pre-gelatinized starch	4.5	4.5	4.5	4.5	4.5
Fish oil	6.0	6.0	6.0	6.0	6.0
Soy bean oil	8.0	8.0	8.0	8.0	7.0
Vitamin mixture [a]	3.0	3.0	3.0	3.0	3.0
Monocalcium phosphate	0.8	1.1	1.3	1.3	1.5
DL-Methionine	-	0.5	0.5	0.5	0.5
Mineral mixture [b]	1.0	1.0	1.0	1.0	1.0
Choline chloride	0.5	0.5	0.5	0.5	0.5
Vitamin E	0.1	0.1	0.1	0.1	0.1
Cellulose	3.6	1.8	0.6	0.6	0.4
Chromic oxide	0.5	0.5	0.5	0.5	0.5
Total	100.0	100.0	100.0	100.0	100.0

[a] Vitamin mixture (amount kg^{-1} diet): thiamin hydro-chloride, 3.025 g; riboflavin, 3.63 g; pyridoxine hydrochloride, 2.42 g; cyanocobalamin, 0.006 g; ascorbic acid, 368.902 g; niacin, 24.2 g; calcium pantothenate, 6.05 g; inositol, 121 g; biotin, 0.363 g; folic acid, 0.908 g; p-aminobenzoic acid, 3.025 g; vitamin K3, 6.05 g; vitamin A acetate, 2,420,000 IU; vitamin D3, 2,420,000 IU.

[b] Mineral mixture composition (g kg^{-1}): sodium chloride, 50; magnesium sulfate, 745; iron (III) citrate n-hydrate, 125; trace element mix, 50; cellulose, 30. (The trace element mixture contains (g kg^{-1}): zinc sulfate heptahydrate, 353; manganese sulfate, 162; copper (II) sulfate pentahydrate, 31; aluminium chloride hexahydrate, 10; cobalt chloride, 3; potassium iodate, 1; cellulose, 440)

17 Table 3 Proximate and amino acids composition of the experimental diets for growth
18 (%)

Component	FM	PPM1	PPM2	PPM3	PPM4
Dry matter	96.2	95.2	97.2	94.0	93.8
Protein	44.8	44.2	43.5	44.4	44.4
Lipid	19.7	19.7	20.1	20.3	19.8
Ash	9.7	11.0	11.4	10.9	10.8
EAAs					
Arginine	1.9	1.6	1.8	1.7	1.7
Histidine	0.9	1.0	0.9	1.1	1.1
Isoleucine	1.1	1.1	1.2	1.2	1.1
Leucine	2.5	2.6	2.6	2.7	2.8
Lysine	2.4	2.2	2.1	2.0	2.0
Methionine	0.7	1.1	1.0	1.1	1.0
Phenylalanine	1.5	1.6	1.6	1.7	1.7
Threonine	1.3	1.1	1.1	1.2	1.1
Valine	1.2	1.2	1.3	1.3	1.3

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Table 4 Growth parameters and nutrient utilization in rainbow trout fed the experimental diets for 10 weeks (mean± standard deviation)

Parameters	Diets				
	FM	PPM1	PPM2	PPM3	PPM4
Initial weight (g)	20.5±0.2	20.6±0.1	20.6±0.1	20.5±0.1	20.6±0.0
Final weight (g)	97.5±0.9a	85.6±0.7b	82.7±0.3b	75.7±0.8c	67.7±1.0d
Weight gain (g)	76.9±1.1a	65.0±0.8b	62.1±0.3b	55.1±0.9c	47.1±1.0d
SGR (%/day)[*1]	2.23±0.03a	2.04±0.02b	1.98±0.00b	1.86±0.02c	1.70±0.02d
Feed intake (g/fish)	82.1±0.3a	74.1±2.5b	72.1±1.1bc	66.6±1.9c	58.2±0.6d
FCR[*2]	1.07±0.02b	1.14±0.05ab	1.16±0.02ab	1.21±0.02a	1.24±0.04a
Survival (%)	100.0	100.0	100.0	100.0	100.0

Values are mean± SEM. Means with the same letter in a same row are not significantly different (P>0.05).

6. Specific growth rate (SGR %.day^{-1}) = [ln (final weight) – ln (initial weight)]* 100/ day
7. Feed conversion ratio (FCR) = feed intake (g)/ body weight gain (g)

29 Table 5 Proximate composition of the whole body of rainbow trout fed the
30 experimental diets for 10 weeks (% on a wet weight basis)

Component	FM	PPM1	PPM2	PPM3	PPM4
Crude protein	16.5±0.3a	15.9±0.4a	15.6±0.2ab	15.4±0.4ab	15.0±0.1b
Crude lipid	12.1±0.2a	12.0±0.1a	12.4±0.2a	12.0±0.2a	11.6±0.2b
Crude ash	2.5±0.1b	2.8±0.1a	2.9±0.2a	2.7±0.2ab	2.7±0.1ab
Moisture	69.8±0.1a	70.2±0.3a	69.7±0.4a	69.8±0.3a	70.1±0.2a

31 Values are mean± SEM. Means with the same letter in a same row are not
32 significantly different (P>0.05).
33

Table 6 Body indices of rainbow trout fed experimental diets for 10 weeks (%)

	FM	PPM1	PPM2	PPM3	PPM4
HSI*	1.2±0.1b	1.3±0.1b	1.3±0.1b	1.5±0.1a	1.6±0.0a
IFR*	6.0±0.4b	6.1±0.3b	6.2±0.5ab	6.6±0.2a	6.7±0.1a
MR*	49.0±0.9a	50.0±1.8a	49.3±2.0a	46.0±1.7b	43.9±0.6c
CF*	1.4±0.0a	1.3±0.0ab	1.3±0.1bc	1.2±0.0bc	1.2±0.0c

HSI, hepatosomatic index; IFR, intraperitoneal fat ratio; MR, muscle ratio; CF, condition factor.

Values are mean± SEM. Means with the same letter in a same row are not significantly different (P>0.05).

Table 7 Apparent digestibility coefficient (ADC) of nutrients in the experimental diets for growth (%)

Component	FM	PPM1	PPM2	PPM3	PPM4
Crude protein	90.5±0.2ab	92.2±0.7a	90.3±0.7ab	88.2±0.5c	87.7±0.4c
EAAs					
Arginine	90.6±0.6a	91.5±1.7a	90.1±2.1a	90.3±2.0a	89.4±1.8a
Histidine	92.8±1.2a	91.2±1.9a	89.8±3.0ab	88.7±2.6ab	87.4±1.9b
Isoleucine	88.2±1.3a	89.9±1.5a	90.6±2.4a	87.6±1.9a	88.2±0.7a
Leucine	91.6±2.0a	91.0±1.9a	91.7±3.2a	89.5±2.8a	88.4±2.4a
Lysine	91.0±0.9a	89.8±0.5ab	87.8±0.8b	86.1±1.0c	85.8±1.1c
Methionine	91.7±0.5a	91.2±1.6a	92.0±0.8a	91.7±1.4a	90.5±1.0a
Phenylalanine	91.9±1.2a	90.1±2.0a	92.5±2.8a	90.4±3.1a	89.7±1.9a
Threonine	93.4±0.9a	93.0±0.8a	93.7±3.4ab	91.4±2.9ab	90.1±0.4b
Valine	91.3±2.1a	92.8±1.2a	91.1±1.9a	90.8±2.0a	90.9±1.4a

Values are mean± SEM. Means with the same letter in a same row are not significantly different (P>0.05).

Chapter 6

General Conclusion

The results from the current study found a lot of scientific information in rainbow trout. It can be summarized as the following;

- The results of this study demonstrated that PBM, FEM and BM have good nutritive value for rainbow trout diets. Very high inclusion levels of the combination of the rendered animal protein successfully could be used in the formulated fish diet for rainbow trout.
- The combination ratio of PBM, FEM and BM at 60/20/20 could be suitable to replace the fish meal in practical diets for rainbow trout.
- For aquatic protein sources, the nutritional value of each rendered product is unique and will depend upon the quality or freshness and composition of the product processed (i.e. PPM).