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

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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 (Hct); haemoglobin level (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 glutamic oxaloacetic transaminase (GOT), and 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)}

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

$_{reference}/0.3 \times D_{ingredient})\}$

where: $D_{reference}$ = % nutrient of reference diet; $D_{ingredient}$ = % 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. Although final weight and SGR were lower than the other treatment when fish were fed the FM+DC and PFBDC diets, being not significantly different from those of the fish fed the control diet (p>0.05).

Table 6

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

Table 9

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 and hematocrit are related to physiological status of body fluid and immune response, 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 influence the hemoglobin and hematocrit. GOT and GPT 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 GOT level than fish fed the other experimental diets, respectively. Although there was no significant difference, similar trend was found in GPT 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 BM, DSM and PBM increased the IFR [16, 35-38]. 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, 39-40]. 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)

	Diets					
Parameters	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
GOT ($U\ L^{-1}$)	194.5±33.2c	233±35.4bc	243.5±41.7bc	314.5±23.3ab	295.5±14.8abc	372.5±14.8a
GPT ($U\ L^{-1}$)	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).

動物性タンパク質および植物性タンパク源による魚粉の代替がニジマスの成長、生物学的特性およびアミノ酸の消化吸収に及ぼす影響

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ニジマス用実用飼料において魚粉の 75％または 100％を養鶏副産物(PBM)、フェザーミール(FEM)、血粉(BM)、脱脂大豆粕(DSM)およびコーングルテンミール(CGM)により代替した飼料を平均体重 16.7±0.1g のニジマスに 12 週間給餌した。動物性タンパク質(PFB、PBM+FEM+BM の混合物)を組み合わせて魚粉を代替した飼料を給餌した魚の成長は正タンパク質効率および飼料効率以外は対照区と同等であった。FM+PFB 飼料および FM+PFBDC 飼料の摂餌量は他の試験区よりも高かった。以上から PBM、FEM、BM の混合により成長や健康状態に悪影響を及ぼさずに魚粉を代替できることが示唆された。