

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

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22 **ABSTRACT**

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

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

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

44 **INTRODUCTION**

45

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

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

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

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

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

94 **Materials and methods**

95 **Diet formulation and preparation**

Table 1~2

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

107 In preparing the diet, all dried ingredients were thoroughly mixed by a horizontal
108 mixer (ACM-50 LAT model, Aicoasha, Saitama, Japan). Feed ingredients were
109 ground in order to reduce the particle size to less than 500 μm . Chromic oxide
110 (Cr_2O_3) was used at 5 g kg^{-1} in all the diets as an inert marker for the study of
111 digestibility. The ingredients were mixed in a horizontal mixer, added to deionized
112 water (30%), and pelleted to 3 sizes (\emptyset , 2.3, 3.2 and 4.8 mm) using a laboratory pellet
113 maker machine (OMC-22B model, Omichi, Gunma, Japan). The pellet was dried
114 using a vacuum freeze-drier (RLE-206, Kyowa Vacuum Engineering, Tokyo) and
115 stored at $-30\text{ }^\circ\text{C}$ until use. Crude protein (41.2-44.5%) and crude lipid contents
116 (18.8-21.7%) were similar among all diets (Table 4). The essential amino acid
117 contents in test diet met the requirement of rainbow trout except lysine [6]. The free
118 histidine content of FM diet is higher than the other experimental diets. Free
119 methionine content of PFB and PFBDC diets are higher than the other diets due to
120 the supplementation of the DL-methionine (Table 5).

Table 4-5

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

Table 3

125

126 **Experimental fish and feeding**

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

143 **Sample collection and preparation**

144 *Initial and final carcass chemical composition*

145 At the beginning and the end of the growth experiment, 10 and 5 fish were
146 randomly selected from the experimental stock and from the experimental tank in

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

158

159 *Haematological parameters and fish body and blood indices*

160 In order to investigate the postprandial effect, five fish were sampled at 24 hr
161 after the last meal and anesthetized by 2-phenoxyethanol. Blood samples were
162 collected by the heparinized syringe from the caudal vein, pooled, and analyzed two
163 replicates. A small fraction of heparinized blood was immediately used for blood
164 indices, namely: haematocrit (Hct); haemoglobin level (Hb). The remained blood
165 was centrifuged for the plasma by centrifugation at 3000 g at 4 °C for 15 min using a
166 high-speed refrigerated centrifuge (SRX – 201 model, Tomy, Tokyo, Japan). The
167 plasma was separated and kept at - 35 °C until analysis. It was reported that the

168 activities of glutamic oxaloacetic transaminase (GOT), and glutamic pyruvic
169 transaminase (GPT) was reflected by dietary protein intake and increase together
170 with excretion of nitrogen [22]. GOT and GPT in blood plasma were analyzed using
171 an automatic blood chemistry analyzer (7020 model; Hitachi, Tokyo, Japan). After
172 collecting the blood samples, digestive tracts (except heart) and fillet were dissected
173 out from the fish body, weighed individually to get the body indices. Liver and fillet
174 of fish in each tank were pooled and stored at – 30 °C until analyzed.

175

176 **Chemical analyses**

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

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

186 The samples were hydrolyzed in 4 mol l⁻¹ methanesulfonic (Sigma-Aldrich,
187 Missouri, Mo, USA) for 22 hours at 110 °C prior to total amino acid analysis. The
188 total amino acids compositions were determined using an automatic amino acid

189 analyzer (JLC-500/v, JEOL, Tokyo, Japan). The free amino acid of experimental
190 diets were deproteinized with 2% sulphosalicylic acid (w/v) and centrifuged 3000 g
191 for 15 min (4°C). The supernatants were also analyzed using the same automatic
192 amino acid analyzer.

193

194 **Calculations**

195 Fish growth performances were calculated according to the formula below

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

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

198 Specific growth rate (SGR %·day⁻¹) = [ln (final weight) – ln (initial weight)]
199 × 100/ day

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

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

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

203 The apparent digestibility coefficient (ADC) of nutrients was calculated according to
204 the method described by [19, 23] as:

205 $ADC (\%) = 100 - \{100 \times (\% Cr_2O_3 \text{ in diet} / \% Cr_2O_3 \text{ in feces}) \times (\% \text{ nutrient in}$
206 $\text{feces} / \% \text{ nutrient in diet})\}$

207 $ADC_{\text{test ingredient}} = ADC_{\text{test diet}} + \{(ADC_{\text{test diet}} - ADC_{\text{reference diet}}) \times (0.7 \times D$

208 $\text{reference}/0.3 \times D_{\text{ingredient}}\}$

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

211

212 **Statistical analyses**

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

218

219 **Results**

220 The growth performance and nutrient utilization in rainbow trout are presented
221 in Table 6. Fish fed the diets that replaced 75% and 100% fish meal with the
222 combination of PBM+FEM+BM showed better or similar weight gain and SGR
223 compared with fish fed the control diet. Although final weight and SGR were lower
224 than the other treatment when fish were fed the FM+DC and PFBDC diets, being not
225 significantly different from those of the fish fed the control diet ($p>0.05$).

Table 6

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

229 FCR in FM group was significantly lower than the other groups ($P<0.05$).

230 PER in FM+PFB, FM+PFBDC and PFB groups were significantly lower than FM
231 and FM+DC groups ($P<0.05$).

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

Table 7

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

Table 8

242 Table 9 shows the results of the blood indices and plasma chemistry of rainbow
243 trout fed the different diets. There were no significant differences in the hematocrit
244 and hemoglobin among fish fed the experimental diets ($p>0.05$). Significantly lower
245 level of the GOT was found in the fish fed the FM diet than the FM+DC and PFBDC
246 groups ($p<0.05$). GOT value of the PFBDC group was significantly higher than the
247 FM, FM+PFB, and FM+PFBDC groups ($p<0.05$).

Table 9

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

251 significantly lower in PBM and FEM than DSM and CGM ($p<0.05$, Table 11).

252

Table 10~11

253 **Discussion**

254 In the present experiment, 75% and 100 % fish meal replacement by PBM,
255 FEM and BM resulted in no negative impact on final body weight, weight gain, SGR,
256 survival and FI., blood indices and liver enzyme activities of rainbow trout. Schulz et
257 al. [25] reported that better growth performance of rainbow trout fed mixture of
258 PBM, FEM, and BM at 33.3%: 33.3%: 33.3% than 50% PBM and 50% FEM as
259 dietary protein. Alexis et al. [26] also reported that complete replacement of fish
260 meal with 30% PBM with 20% CGM and 12% carob seed germ meal showed
261 significantly higher growth in rainbow trout than herring meal base diet. Sealey et al.
262 [27] examined effect of total replacement of fish meal with three kinds of poultry
263 products (chicken concentrate, PBM, and chicken and egg concentrate) on growth
264 performance of rainbow trout. They found that growth performance of the fish fed
265 PBM based diet was comparable with fish meal based diet, although FCR of fish fed
266 PBM based diet was significantly inferior to that fed fish meal based diet. These
267 results strongly suggest that PBM is a suitable protein source for rainbow trout diet
268 and complete replacement of fishmeal with PBM in rainbow trout diet without
269 negative impact on final body weight, weight gain, SGR, survival and FI.

270 In the present study, lower WG and SGR were observed in the fish fed the
271 FM+DC and PFBDC diets compared with the FM+PFB and FM+PFBDC. With the

272 same trend of the growth, the FI showed the lower result in the FM+DC and PFBDC.
273 Regarding FI, significantly higher FI was recorded in FM+PFB, FM+PFBDC and
274 PFB groups than the control (FM group). Reduced palatability has been showed
275 responsible to the reduced feed intake of fish fed the diets in which high levels of
276 FM was replaced with plant protein source [28]. Therefore, inclusion of DSM and
277 CGM in FM+DC and PFBDC diets seem to reduce FI in FM+DC and PFBDC
278 groups due to low palatability of DSM and CGM. Adding the feeding stimulants can
279 improve the palatability of fish diet [29]. Supplementation with feed stimulants in
280 FM+DC and PFBDC diets might suggest to improve FI and induce better growth
281 performance of FM+DC and PFBDC groups. Highest FI were found in FM+PFB
282 and FM+PFBDC groups in current study. Palatability of fish feed is reported to be
283 mainly depended on amino acid and nucleotide [6]. It was also reported that
284 L-proline, L-hydroxyproline, L-alanine, L-phenylalanine, and L-leucine could
285 stimulate taste sense of rainbow trout and L-proline was most potent among the
286 amino acids [30-31]. However, no much difference was observed in free amino acid
287 composition among all diets. It was reported that nucleotide content is higher in
288 animal protein sources than plant products
289 [[http://en.engormix.com/MA-pig-industry/nutrition/articles/nucleotides-yeast-extract](http://en.engormix.com/MA-pig-industry/nutrition/articles/nucleotides-yeast-extract-potential-t340/141-p0.htm)
290 [-potential-t340/141-p0.htm](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
291 the diets were not analyzed in this experiment, nucleotide included in FM+PFB and
292 FM+PFBDC diets may enhance FI of rainbow trout. Another explanation of lower

293 growth performance and lower FI of FM+DC and PFBDC groups may be attributed
294 to lower lysine content of these diets. Since lysine is one of the indispensable amino
295 acid, FM+DC and PFBDC diets contained a little bit lower lysine than the
296 requirement of rainbow trout [6]. In addition, it was reported that lower FI in
297 rainbow trout fed amino acid imbalanced diet than fed amino acid balanced diet [32],
298 suggesting lower FI of FM+DC and PFBDC groups could be induced by lower
299 lysine content of the diet.

300 Hemoglobin and hematocrit are related to physiological status of body fluid
301 and immune response, where high hemoglobin can be taken as an indication of good
302 condition [33]. In the present study, replacement of fish meal with rendered animal
303 protein and plant protein source did not influence the hemoglobin and hematocrit.
304 GOT and GPT are amino acid transferase in liver and higher activities of these
305 enzyme were suggested to be related to consumption of protein for energy
306 production [34]. Fish fed the FM and PFBDC diets had the lower and higher GOT
307 level than fish fed the other experimental diets, respectively. Although there was no
308 significant difference, similar trend was found in GPT value of fish in FM and
309 PFBDC groups. These results may indicate protein consumption for energy
310 production in fish fed alternative protein sources based diet such as PFBDC.

311 The HSI in fish fed the FM diet was significantly lower than fish fed the
312 FM+DC and PFBDC diets. It seems that the hepatic fat deposition indeed is higher
313 when fish fed FM+DC and PFBDC diets. The IFR in fish fed the experimental diets

314 tended to be higher than that fed the control diet. Previous studies reported that fish
315 meal replaced by the alternative protein sources including BM, DSM and PBM
316 increased the IFR [16, 35-38]. Our results were in agreement with these previous
317 studies. MR in fish fed the FM+PFBDC diet was also higher level than the other
318 groups.

319 High ADC of protein in PBM was observed in this study (87.8%). This is in
320 agreement with previous studies that reported 87-91% of ADC of protein in PBM [2,
321 17, 39-40]. ADC of protein of test diets was not different among treatments
322 (88.3-94.3%) except the significant different between FM+FPB and FM+DC.
323 Similarly, ADC of EAA in the experimental diets was similar among treatments
324 except methionine. Different growth performance of fish fed these diets could reflect
325 different FI and/or amino acid composition of the diets.

326 Considering ADCs of protein and EAA in test protein sources, values of PBM
327 and FEM were lower than DSM and CGM. ADCs of EAA in FEM were less than
328 90% except arginine (Table 11). Digestibility analysis of the test protein sources
329 revealed that rendered animal protein sources (PBM and FEM) except BM were less
330 digestible compared to plant protein sources such as CGM and DSM. Fish fed the
331 FM and FM+DC diets performed the better PER than fish fed the FM+PFB,
332 FM+PFBDC and PFB diets. Poor PER in these three groups could be due to higher
333 percentage of rendered animal protein sources in the diets. On the contrary, BM was
334 highly digestible in rainbow trout. This is well agreement with these previous studies

335 [2, 9]. These results suggested that inclusion of high level of rendered animal protein
336 sources in rainbow trout diets could result in growth retardation. In this study, we
337 used combination of rendered animal protein sources in order to confer well
338 balanced amino acid in the test diets. As a result, there was no marked difference in
339 digestibility of experimental diets in the growth experiment (Table 10). With respect
340 to high digestibility of plant protein sources such as CGM and DSM, inclusion of
341 high level of these protein sources may lead better growth in rainbow trout. However,
342 fish fed the diets with DSM and CGM showed growth retardation. This could be due
343 to less palatability of these diets. Considering these results altogether, rendered
344 animal protein sources can be used to support high palatability in low or non fish
345 meal diet for rainbow trout. It is suggested that addition of feed stimulants in plant
346 protein based diets could improve feed performance of rainbow trout diets. However,
347 addition of feed stimulants may elevate production cost of the feed. Therefore,
348 inclusion of rendered animal protein sources could be cost effective solution to
349 produce low or non-fish meal diet for rainbow trout with well growth.

350 The results of this study demonstrated that PBM, FEM and BM have good
351 nutritive value for rainbow trout diets. Very high inclusion levels of the combination
352 of the rendered animal protein successfully might be used in the formulated fish diet
353 for rainbow trout.

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524

525 Table 1 Proximate composition of the experimental ingredients used as protein
 526 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
<i>EAA</i> s (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
<i>NEAA</i> s (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

527

528 Table 2 Formulation of the experimental diets for growth of rainbow trout (%)
 529

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

530 ^a Blood meal: porcine blood meal.

531 ^b Vitamin mixture (amount kg⁻¹): thiamin hydro-chloride, 3.025 g; riboflavin, 3.63 g;
 532 pyridoxine hydrochloride, 2.42 g; cyanocobalamin, 0.006 g; ascorbic acid, 368.902
 533 g; niacin, 24.2 g; calcium pantothenate, 6.05 g; inositol, 121 g; biotin, 0.363 g; folic
 534 acid, 0.908 g; p-aminobenzoic acid, 3.025 g; vitamin K3, 6.05 g; vitamin A acetate,
 535 2,420,000 IU; vitamin D3, 2,420,000 IU.

536 ^c Mineral mixture composition (g kg⁻¹): sodium chloride, 50; magnesium sulfate,
 537 745; iron (III) citrate n-hydrate, 125; trace element mix, 50; cellulose, 30. (The trace
 538 element mixture contains (g kg⁻¹): zinc sulfate heptahydrate, 353; manganese sulfate,
 539 162; copper (II) sulfate pentahydrate, 31; aluminium chloride hexahydrate, 10;
 540 cobalt chloride, 3; potassium iodate, 1; cellulose, 440)

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

Ingredients	Amount g 100g ⁻¹ diet as fed	
	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 ₂ O ₃	0.5	0.35
Vitamin E	0.1	0.07
Cellulose	1.4	0.98
Test ingredient		30.0

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

545 ^a Composition of vitamin premix is the same as in Table 2.

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

547

548 Table 4 Proximate and amino acids composition of the experimental diets for
 549 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
<i>EAA</i> s						
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
<i>NEAA</i> s						
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

550

551

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

	FM	FM+PFB	FM+PFBDC	FM+DC	PFB	PFBDC
<i>EAA</i> s						
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
<i>NEAA</i> s						
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

554

555

556 Table 6 Growth parameters and nutrient utilization in rainbow trout fed the
 557 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

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

560

561 1. Specific growth rate (SGR %·day⁻¹) = [ln (final weight) – ln (initial weight)] ×

562 100/ day

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

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

565 Table 7 Proximate composition of the whole body of rainbow trout fed the
 566 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

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

569

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

571

	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

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

573 Values are mean± SEM. Means with the same letter in a same row are not

574 significantly different ($P>0.05$).

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

576

	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

577 Hb (Haemoglobin); Hct (Haematocrit); GOT (Glutamic oxaloacetic transaminase);

578 GPT (Glutamic pyruvic transaminase)

579 Values are mean± SEM. Means with the same letter in a same row are not

580 significantly different ($P>0.05$).

581

582 Table 10 Apparent digestibility coefficient (ADC) of nutrients in the experimental
 583 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
<i>EAA</i> s						
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

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

586 Table 11 Apparent digestibility coefficient (ADC) of nutrients in the test ingredient
 587 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
<i>EAA</i> s					
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

588 Values are mean± SEM. Means with the same letter in a same row are not
 589 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 ± 0.1 g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)を組み合わせることで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。