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Effects of an Enzyme Complex-Treated Rice Protein Concentrate on Growth Performance and Feed Utilization of Rainbow Trout *Oncorhynchus mykiss* Juveniles

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Abstract: This study investigated the effect of the inclusion of an enzyme complex-treated rice protein concentrate (RPC) in an extruded diet of juvenile rainbow trout (*Oncorhynchus mykiss*). A mixture of RPC, corn gluten and soybean meal was pretreated with an enzyme complex before extrusion processing of the diets. An enzyme complex-pretreated RPC (5, 10 and 15%) was formulated with 20% fish meal. A diet without RPC was used as a control. A total of 240 rainbow trouts with an average body weight of 6.04 g were placed in 12 rectangular glass aquaria and fed one of the experimental diets at apparent satiation for 12 weeks. There were no significant differences in growth, feed intake and survival among the groups. Slightly inferior weight gain and specific growth rate were observed in the RPC15E group compared to those in the other groups. The protein and ash contents of the whole body of the final fish were not significantly different among all groups, but the lipid content was significantly lower than that of the control when the dietary RPC level was >10%. Protein digestibility was negatively affected by increased RPC levels in diet. Significantly lower phosphorus digestibility was observed in fish fed the diet containing 5% enzyme-treated RPC than the control. Although there was no significant difference in lipid digestibility in all groups, significantly lower lipid retention was observed in fish fed a diet formulated with more than 10% RPC. These results suggest that the inclusion of RPC in the diet affects lipid retention and the content of rainbow trout. It also decreased protein digestibility. In conclusion, rainbow trout can be fed an extruded diet formulated with 20% fishmeal and 10% enzyme-treated RPC without negative effects on fish growth.

Keywords: plant protein sources; *Oryza sativa*; fishmeal; animal feed; digestibility; nutrient retention

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1. Introduction

Global fishmeal production is approximately 5 million tons, but increasing demand for aquafeeds, competitiveness and rising prices have limited its availability [1]. To cover the limited availability of fishmeal for aquafeeds, the increasing use of plant protein sources such as pea [2], soybean [3,4], lupine [5], corn gluten [4–6], canola/rapeseed [7] and cotton seed [8] meals has become a global trend [2]. The global crop production is estimated to be over 9 billion tons, and according to the United States Department of Agriculture (USDA), the world rice production in 2021/2022 is estimated to reach 513.02 million metric tons [9], becoming the third most produced crop after maze and wheat [9]. Because rice is produced for human food, its safety is guaranteed.

Although the protein content of rice is only 6.3–7.1%, rice protein concentrate (RPC), a by-product of rice starch production, contains more than 65%. In the general manufacturing process of rice starch, polished rice is dissolved in an alkaline solution, and a starch fraction is collected after centrifugation. Rice protein dissolved in a supernatant can be isolated after neutralization [10].

Although RPC is commonly used in the animal feed industry [11,12], there are limited reports on the use of RPC as a fishmeal alternative in aquafeeds. Soybean meal and corn gluten meal are the most popular plant protein alternatives to fishmeal, and soybean and corn prices are closely related because they are commonly affected by similar factors such as inflation, crude oil price, and production [12]. On the other hand, the association between rice and other crop prices is low [12]. Thus, the replacement of soybean and corn gluten meal with rice by-products could contribute to the price stability of the diet. RPC has been tested as a protein source for the black spot bream (*Pagellus bogaraveo*), blunt snout bream (*Megalobrama amblycephala*), Nile tilapia (*Oreochromis niloticus*), rainbow trout (*O. mykiss*), Siberian sturgeon (*Acipenser baerii*), hybrid sturgeon (*Acipenser naccarii* × *Acipenser baeri*) and Pacific whiteleg shrimp (*Litopenaeus vanamei*) [6,13–18]. Palmegiano et al. (2006) tested the usefulness of RPC in the diet of rainbow trout [13] and concluded that 20% RPC could be included in the diet of rainbow trout without a negative impact on fish growth [13]. However, their diet contains 35% fishmeal. Normally, the fishmeal inclusion level in an aquafeed determines the acceptable upper limit of the inclusion level of alternative protein sources in a diet. Thus, it is not clear whether 20% RPC can be included without a negative impact on the growth of the rainbow trout fed a diet containing less than 35% of fishmeal. In addition, there were no reports on the replacement of soybean and corn gluten meal with RPC in a rainbow trout diet.

Enzyme treatment and fermentation are popular techniques for improving the performance of plant protein-based diets for fish [19]. *Aspelgillus niger* is naturally isolated from rice fermentation and widely used for the production of rice wine and soy sauce [20,21]. Thus, the enzyme complex produced from *A. niger* seems to effectively digest rice protein. However, there have been no reports on the treatment of RPC by the enzyme complex. In addition, one concern regarding an enzyme complex is the lower efficacy of an enzyme complex when it is a supplement in the diet for cold-water species because an enzyme complex may not be effective below 18 °C; therefore, a simple addition of an enzyme complex to a diet for cold-water species seems to be less effective. However, we hypothesized that the pretreatment of plant protein sources with an enzyme complex at an optimal temperature could improve the feed performance of cold-water species. Therefore, we pretreated RPC with the enzyme complex at 45 °C for 18 h before formulating an extruded diet. The objective of this study was to investigate the effect of a diet containing 5–15% of an enzyme complex-pretreated RPC on growth performance, nutrient digestibility and nutrient retention in juvenile rainbow trout.

2. Material and Methods

2.1. Diets

RPC contained approximately 69% crude protein (Table 1). Four different experimental diets (Table 2) with each containing 20% fishmeal and 5%,10% and 15%RPC were formulated. Replacement level of soybean meal and corn gluten meal by RPC was 15, 30 and 45% in the RPC5E, RPC10E and RPC15E, respectively. To observe the effects of enzyme treatment, mixture of RPC, soybean meal and corn gluten meal were moistened with 30% distilled water and treated with commercially available enzyme complex (SSF, Alltech Inc., Lexington, KY, USA) containing amylase, amyloglucosidase, cellulase, protease, pectinase, xylanase and glucoamylase for 18 h under humid condition at 45 °C, and crystalline amino acids were supplied to balance the indispensable amino acids in the diet. These ingredients were processed into isoproteic (48%) and isolipidic (15%) extruded pellets at the Scientific Feed Laboratory (Table 2). Pellets were made using a twin-screw extruder (OEE-8, Amandus Kahl GmbH & Co. KG, Reinbek, Germany), and the ingredients were introduced into the hopper of the extruder at 33 kg/h to obtain a stable cooking temperature of 120 ± 2 °C. The resultant pellet met the indispensable amino acid requirement of rainbow trout (Table 3) [22]. The diet was maintained at 4 °C until further use.

Table 1. The proximate and amino acid composition of RPC.

Moisture	13.6
Crude protein	69.1
Crude lipid	13.8
Crude ash	3.4
Arginine	5.97
Lysine	2.28
Histidine	1.78
Phenylalanine	3.93
Tyrosine	3.84
Leucine	6.00
Isoleucine	2.93
Methionine	1.89
Valine	4.19
Alanine	3.95
Glycine	3.11
Proline	3.44
Glutamic Acid	12.80
Serine	3.60
Threonine	2.58
Asparagic acid	6.35
Tryptophan	0.97
Cystine	1.61
Moisture	13.6
Crude protein	69.1

Table 2. Formula and analyzed proximate composition of the experimental diets [1].

(%)	Control	RPC5E	RPC10E	RPC15E
Fishmeal	20	20	20	20
Soybean meal	20	17	14	11
Corn gluten meal	20	17	14	11
Rice protein conc. [2]	0	5	10	15
Wheat flour	10	10	9	8
Poultry feather meal	9.2	9.2	9.2	9.2
Fish oil	4	4	4	4
Soybean oil	6	6	6	6
Amino acids [3]	1.5	1.5	1.5	1.5
Cellulose	0.4	1.4	3.4	5.4
Vitamin mix [4]	3	3	3	3
Mineral mix [5]	1	1	1	1
Monobasic calcium phosphate	0.8	0.8	0.8	0.8
Choline chloride	0.5	0.5	0.5	0.5
Chromic oxide	0.5	0.5	0.5	0.5
Pre-gelatinized starch	2	2	2	2
Vitamin E	0.1	0.1	0.1	0.1
Moisture (%)	3.3	2.9	3.5	2.1
Protein (%)	47.6	48.4	48.1	48.6
Lipid (%)	15.5	16.0	16.3	15.7
Ash (%)	8.2	7.7	7.7	7.2

[1] Sum of all ingredients was 99%, as 1% of the enzyme complex (SSF, Alltech Inc. US.) was used for pretreatment of a mixture of RPC, soybean meal and corn gluten meal. [2] Rice protein concentrate (made in China, Herbmax Co., Osaka, Japan). [3] Lysine: Methionine = 1:1. [4] Vitamin mix (mg/kg; Kohkin Chemical Co., Ltd., Osaka, Japan): calcium ascorbate 368,902 mg; vitamin B1 3630 mg; vitamin B2 6050 mg; vitamin B6 2420 mg; vitamin B12 6 mg; niacin 24,200 mg; Ca-pantothenate 6050 mg; inositol 121 × 103 mg; biotin 363 mg; folic acid 908 mg; p-aminobenzoic acid 3.025 mg; vitamin K3 3025 mg; vitamin A 2,420,000 IU; vitamin D3 2,420,000 IU. [5] Mineral mix (g/100 g): Sodium chloride 5.0 g; Magnesium sulfate heptahydrate 74.5 g; Ferric citrate n-hydrate 12.5 g; cellulose 3.0 g; trace element mixture 5.0 g [Zinc sulfate heptahydrate 35.3 mg; Manganese sulfate pentahydrate 16.2 mg; Copper sulfate pentahydrate 3.1 g; Aluminum chloride hexahydrate 1.0 g; Cobaltous chloride hexahydrate 0.1 g; Potassium iodate 0.3 g; Cellulose 44.0 g].

Table 3. Essential amino acid composition of experimental diet.

	Control	RPC5E	RPC10E	RPC15E	Requirement *
Threonine	2.94	2.76	2.56	2.50	1.44
Valine	2.36	2.33	2.09	2.08	1.26
Methionine	1.92	1.69	1.55	1.60	0.81
Isoleucine	1.77	1.70	1.51	1.49	0.98
Leucine	6.68	6.09	5.58	5.13	1.75
Phenylalanine	3.39	3.16	2.97	2.89	1.26
Histidine	1.26	1.23	1.16	1.09	0.63
Lysine	3.98	3.70	3.48	3.29	2.10
Tryptophan	0.51	0.40	0.55	0.58	0.21
Arginine	3.97	3.94	3.82	3.90	1.40

* According to Ogino (1980) [22].

2.2. Fish Husbandry and Rearing

Animal experiment was conducted according to Handling Rules for Animal Experiments, Tokyo University of Marine Science and Technology (TUMSAT) (13 March 2020, TUMSAT Regulations No. 8) based on Basic Guidelines for Conducting Animal Experiments at Research Institutes (Ministry of Education, Culture, Sports, Science and Technology, Japan). The trials were performed in the Laboratory of Fish Nutrition, TUMSAT, Tokyo, Japan. Juvenile rainbow trout were purchased from a local hatchery and land transported to the Laboratory of Fish Nutrition, TUMSAT. They were acclimated in the experimental environment for 2 weeks with commercial pellets (Trout feed, 2.5C, Feed One Co., Tokyo, Japan). A total of 240 rainbow trout juveniles were randomly distributed in 60 L rectangular glass aquaria. The average weight of the fish in each experimental tank was 6.04 $\pm$ 0.10 g. They were fed one of the four diets in triplicate at apparent satiation for 12 weeks at 16.3 $\pm$ 1.17 °C. Fish were weighed every 3 weeks in order to monitor growth performance with a precision scale (UX4200H, Shimadzu Co., Kyoto, Japan). Before measuring body weight, the fish were anesthetized with 0.2 mL/L 2-phenoxyethanol (Wako Fujifilm Co., Osaka, Japan). Feces were collected via a Tokyo University of Fisheries (TUF) column system attached to each glass aquarium [23]. Fecal material was collected, lyophilized using a freeze dryer (RLE-206, Kyowa Vacuum Co., Saitama, Japan), and subjected to protein, lipid, chromium oxide and phosphorous analysis. At the end of the 12-week trial, the fish were starved for 24 h, euthanized with an overdose of 2-phenoxyethanol, and weighed as described above. The carcasses were maintained at −30 °C. They were defrosted and minced using a knife and a centrifugal mill (ZM500, Retsch Co., Haan, Germany). For chemical analysis, the processed material was freeze-dried (RLE-2, Kyowa Vacuum Co., Saitama, Japan) overnight and stored at −30 °C.

2.3. Calculation

Calculation of the growth and feed performance parameters was made as follows: Calculation of the growth parameters was made as follows:

$$\begin{aligned}&(\text{Apparent digestibility coefficient (ADC) of nutrient } (\%) = 1\\&-\left[\left(\frac{\text{Chromium oxide in diet } (\%)}{\text{Chromium oxide in feces } (\%)}\right)\times\left(\frac{\text{Nutrient in feces } (\%)}{\text{Nutrient in diet } (\%)}\right)\right]\times 100\end{aligned}$$

$$\text{Daily feed intake (DFI) } (\%/\text{day}) = \left[\frac{\text{Total feed intake}}{\text{Mean body weight}} \times \text{Experimental period (days)}\right] \times 100$$

$$\text{Feed conversion ratio (FCR)} = \frac{\text{Total feed intake (g)}}{\text{Weight gain (g)}}$$

$$\text{Nutrient retention } (\%) = \left(\frac{\text{Nutrient gain (g)}}{\text{Nutrient intake (g)}}\right) \times 100$$

$$\text{Protein efficiency ratio (PER)} = \frac{\text{Weight gain (g)}}{\text{Protein intake (g)}}$$

$$\text{Specific growth rate (SGR, \%/ day)} = \left[\frac{\text{lnfinal body weight} - \text{lninitial body weight}}{\text{Experimental period (days)}}\right] \times 100$$

$$\text{Survival rate (\%)} = 100 \times \frac{\text{Final number of fish}}{\text{Initial number of fish}}$$

$$\text{Weight gain (WG) (g)} = \text{Final body weight} - \text{Initial body weight}$$

2.4. Chemical Analyses

The proximate composition of the test diet, feces and the fish body were analyzed by the standard method. Protein content was determined by the Kjeldahl method and multiplying by the nitrogen coefficient by 6.25 [24]. Crude fat was extracted and weighed according to Folch et al. (1957), using a mixture of chloroform and methanol (2:1) [25]. Diet was digested with 4M methanesulfonic acid for 22 h under vacuum conditions by the method described by Simpson et al. (1976) for total amino acid analysis [26]. The digested solution was passed through a membrane filter (0.45 µm), and then the amino acid composition was analyzed by a fully automatic amino acid analyzer (JLC-500/v; JEOL Co., Tokyo, Japan). Moisture was determined by drying in an oven under normal pressure at 105 °C. The samples were weighed using a balance after an interval of every 1–2 h until obtaining a constant weight. The ash content was determined gravimetrically after incineration at 650 °C for more than 8 h in a muffle furnace (FO200, Yamato Co., Tokyo, Japan). For digestibility, the ADC of nutrients was calculated based on ratio of amount of chromium oxide to nutrients in the diet and feces. Phosphorus was analyzed using a UV-VIS spectrophotometer (UV-2550, Shimadzu) as described by Lowry and Lopez (1946) [27]. Chromic oxide concentration was determined after acid digestion [28] using a UV-VIS spectrophotometer.

2.5. Statistical Analysis

Growth performance and feed utilization data were tested for normality using the Kolmogorov–Smirnov test and subjected to one-way analysis of variance (ANOVA) using the SPSS software for Windows (Version 20; SPSS, Inc., Chicago, IL, USA). If significant differences were observed ($p < 0.05$), Tukey's honestly significant difference (HSD) test was used to determine the differences between means. Spearman's test was used to test the significance of the correlation between dietary RPC levels and growth/feeding parameters. Regression analysis was conducted to determine the relationship between the RPC content in diets and the growth parameters, such as FCR and DFI.

3. Results

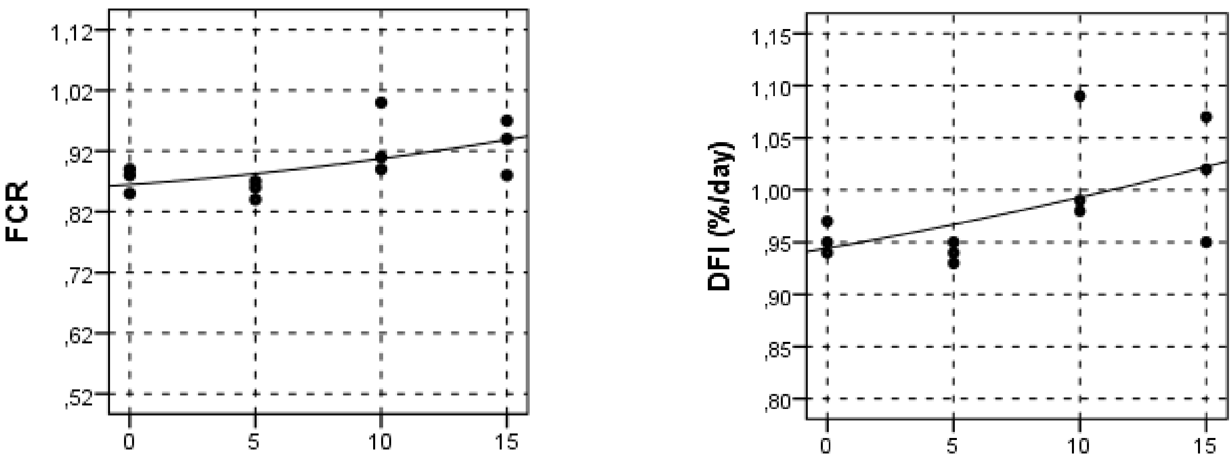
3.1. Growth Performance

There was no significant difference in all parameters analyzed, such as final body weight, weight gain, survival rate, SGR, PER, FCR and daily feed intake (Table 4, $n = 3$, $p > 0.05$, Tukey's test). Although there was no significant difference in all groups, slightly lower final weight, weight gain and SGR were observed in the RPC15E group than in the other groups. Regression analysis suggested that DFI and FCR decreased with increasing dietary RPC levels (Figure 1).

Table 4. Growth performance of rainbow trout juveniles after 12 weeks.

	Control	RPC5E	RPC10E	RPC15E	*p*-Value
Final body weight (g)	66.9 ± 1.69	68.4 ± 1.70	66.5 ± 1.29	64.0 ± 1.44	0.255
Weight gain (g)	60.9 ± 3.12	62.2 ± 1.59	60.5 ± 1.80	57.9 ± 2.21	0.644
Survival rate (%)	100	100	100	100	
Specific growth rate (%/day)	2.9 ± 0.06	2.9 ± 0.02	2.9 ± 0.03	2.8 ± 0.04	0.522
Protein efficiency ratio	2.4 ± 0.12	2.4 ± 0.06	2.2 ± 0.07	2.2 ± 0.08	0.361
Feed conversion ratio	0.9 ± 0.01	0.9 ± 0.01	0.9 ± 0.03	0.9 ± 0.03	0.063
Daily feed intake (%/day)	1.0 ± 0.01	0.9 ± 0.01	1.0 ± 0.03	1.0 ± 0.01	0.064

No significant difference was observed ($n = 3$, $p > 0.05$, Tukey's HSD test).



R^2 Quadratic =0.354

p-value: 0.041

y=0.000133x2+0.003x+0.865

FCR of fish and dietary RPC content

R^2 Quadratic =0.335

p-value: 0.040

y=0.000067x2+0.004x+0.944

DFI of fish and dietary RPC content

Figure 1. Growth performance parameters with dietary RPC levels.

3.2. ADC of Protein, Lipid and Phosphorus

Significant differences were observed in the ADC of protein and phosphorus (Table 5, $p < 0.05$, Tukey's HSD test). Protein digestibility was negatively affected by increased RPC levels in the diet. Phosphorus absorption was significantly lower in the RPC5E group than in the control. However, there were no significant differences in the ADC of phosphorous between the other RPC groups and the control group ($p > 0.05$). There were no significant differences in the ADC of lipids among the groups ($p > 0.05$).

Table 5. ADC of protein, lipid and phosphorus (%).

	Protein	Lipid	Phosphorous
Control	89.8 ± 1.52^{b}	71.5 ± 3.62	68.3 ± 2.31^{b}
RPC5E	$86.5 \pm 1.49^{a,b}$	67.9 ± 3.92	39.1 ± 3.83^{a}
RPC10E	82.1 ± 2.42^{a}	64.1 ± 3.78	$49.9 \pm 2.89^{a,b}$
RPC15E	82.8 ± 1.18^{a}	71.6 ± 2.66	$54.6 \pm 2.67^{a,b}$
p-value	0.016	0.205	0.041

Values with different superscript letters are significantly different ($p < 0.05$, Tukey's HSD test).

3.3. Nutrient Retention

Significantly lower lipid retention was observed in fish fed the RPC10E and RPC15E diets than in the control (Table 6, $p < 0.05$, Tukey's HSD test). No significant difference was observed in protein retention in any of the groups (Table 6, $p > 0.05$, Tukey's HSD test).

Table 6. Nutrient retention in whole body of fish (%).

	Protein	Lipid
Control	31.19 ± 0.59	89.15 ± 1.38^{b}
RPC5E	30.47 ± 0.08	83.34 ± 1.26^{b}
RPC10E	29.99 ± 2.77	69.35 ± 2.42^{a}
RPC15E	30.66 ± 1.05	74.61 ± 1.63^{a}
p-value	0.668	0.016

Values with different superscript letters are significantly different ($p < 0.05$, Tukey's HSD test).

3.4. Fish Body Composition

There were no significant differences in the proximate composition of the final fish body, excluding lipids (Table 7, $n = 3$, $p > 0.05$, Tukey's HSD test). However, protein content tended to increase with increasing RPC in the diet. Similarly, a decreasing trend in lipid content was observed when RPC levels increased (Table 7, $n = 3$, $p < 0.05$, Tukey's HSD test).

Table 7. Proximate composition (% wet weight basis) of rainbow trout fed experimental diets.

	Control	RPC5E	RPC10E	RPC15E	*p*-Value
Moisture	67.8	67.9	69.3	68.8	0.147
Protein	17.0	17.1	16.2	16.3	0.598
Lipid	13.8^{b}	13.4^{b}	11.4^{a}	11.9^{a}	0.029
Ash	2.2	2.4	2.1	2.2	0.232

Values with different superscript letters are significantly different ($n = 3$, $p < 0.05$, Tukey's HSD test).

4. Discussion

Gaylord et al. (2010) and Gaylord and Barrows (2008) examined the gross nutrient and amino acid availability in rainbow trout from 25 feed ingredients in an extruded diet [29,30] and demonstrated low digestibility of crude protein, and low amino acid availability from RPC among the 16 plant ingredients examined [29,30], suggests a relatively low availability of RPC as a protein source for an extruded diet for rainbow trout. There were no significant differences in any of the growth parameters examined in rainbow trout. Although no significant difference was observed in fish-fed diets formulated with 15% RPC, a marked decrease was observed in the final weight, weight gain and SGR in this group. This also suggests that the inclusion of 15% RPC negatively affects fish growth. Considering previous experiments on the rainbow trout testing effects of RPC in a diet, it has been observed that with an increase in the percentage of RPC, the growth performance of fish is negatively affected [13]. A significant decrease in growth performance was observed in Pacific whiteleg shrimp as the dietary RPC level increased in the experimental diets [16]. It can be hypothesized that by increasing the RPC level in the diet of fish, the growth performance may be adversely affected. On the contrary, no negative impact was reported on the growth performance of the tilapia-fed diet, replacing all fishmeal in the diet with RPC [15]. Additionally, a lysine-supplemented RPC-based non-fishmeal in the diet did not show adverse effects on the growth of blunt snout bream [14]. This could be explained by the different feeding habits of the species; tilapia and blunt snout bream are omnihervivorous species and accept a relatively wider range of feedstuffs than carnivorous species such as rainbow trout. In addition, they observed a higher feed intake of fish fed a higher inclusion level of RPC than that of fish fed a fishmeal diet [15]. However, such a higher feed intake was not observed in the rainbow trout in the present study. We thought that there were two possible explanations for the reasons for decreased feed intake; (1) an increasing level of RPC in the diet and (2) decreasing level of soybean and corn gluten meal accompanied by an increasing RPC level in the diet. Unfortunately, it was not clear whether RPC or soybean and corn gluten meal were more palatable for rainbow trout in the present study. Considering the greater negative impact of dietary RPC on DFI than FCR, it was inferred that one of the reasons for the decreased FCR was the decreased diet consumption.

In this experiment, although no significant difference in lipid digestibility was observed in any of the groups, a significant decrease in phosphorus digestibility was observed in the RPC5E group. Soybean, corn and rice contain phytate-phosphorous [31], which is not utilized as a phosphorous source in monogastric animals, including fish, without the use of exogenous phytase; however, in this study, we used an enzyme complex containing phytase. Therefore, phytate phosphorous in our diet can be a useful source of phosphorous. The phosphorus sources in our diet were monobasic calcium phosphate, fishmeal, soybean meal, corn gluten meal and RPC. Because we included the same level of fishmeal and monobasic calcium phosphate commonly in all diets, they could not be the cause of lower phosphorous availability in RPC15E. Different concentrations of soybean meal, corn gluten meal and RPC were formulated in RPC diets and treated with enzyme complexes before extrusion processing. The efficacy of phytase in liberating free phosphorous from different plant ingredients could differ depending on the feed formulation [32]. The presence of higher levels of soybean meal and corn gluten meal in the RPC15E diet could be one of the causes of lower phosphorous absorption in the RPC5E group. The growth performance of the 10E and 15E groups could be suppressed, in part, due to the lower protein digestibility in the diet in our study. Rice contains serine protease inhibitors as well as oryzacystatin, another protease inhibitor [32,33]. These antinutritional factors limit protein digestibility and availability of RPC in a diet with a lower fishmeal percentage. However, the inclusion of a high percentage of fishmeal in a basal diet may mask the negative impact of protease inhibitors in RPC in rainbow trout diets. This idea is supported by the fact that no negative effect of RPC on the growth performance of rainbow trout was observed when it is formulated with 35% fishmeal in an extruded diet [13]. Considering the optimal RPC level in an extruded diet for rainbow trout, 20% RPC can be included when the fishmeal content is 35% [13]. Here, we also estimated that the optimal RPC level in the extruded diet for rainbow trout was approximately 5% when the dietary fishmeal level was 20%. Considering the differences in RPC and fishmeal content in the diet in the previous and present studies, when a fishmeal content is 15% higher than that of the RPC level in the diet, it does not seem to affect the growth of rainbow trout negatively.

Protein in rice is a seed storage protein called glutelin or prolamin and has a role in storing carbon, nitrogen and sulfur until it is used for seed germination [34]. Rice protein in milled rice is composed of 5–8% prolamin, 15% albumin and globulin and approximately 80% glutelin [11]. Seed storage proteins are hydrolyzed and utilized during germination but are protected by protease inhibitors until germination initiation [34]. Therefore, when rice proteins are isolated and concentrated, these protease inhibitors are included together with prolamin and glutelin. Therefore, it is considered that the protease inhibitor contained is a contaminant in the RPC, thus reducing the digestion and absorption rate of the RPC protein.

The major focus of fishmeal replacement studies is monitoring growth performance, probably because it is directly associated with economic aspects. However, relatively few studies have reported its impact on the nutrient content of fish-fed alternative protein sources. It was reported that the inclusion of more than 20% RPC in the trout diet reduces nutrient digestibility and thus leads to lower growth [13]. Here, we observed significantly lower lipid content in fish fed more than 10% RPC in the diet of the rainbow trout. This was also reflected in the low lipid retention of the fish. Lower lipid digestibility was observed in rainbow trout fed a diet containing more than 20% RPC. The reason our result did not match with those of the previous study could be explained by a different inclusion level of RPC; 5–15% in the diet used in the present diet vs. 20–53% RPC in the diet used by Palmegiano et al. [13]. Higher levels of RPC in the diet had a negative impact on lipid digestibility in rainbow trout. Another explanation is that the diet in the present study contained RPC pretreated with enzyme complex. Enzyme pretreatment of RPC may partially reduce the negative impact of the antinutritional factors on lipid digestibility. It was also reported that protein digestion of the fish fed the RPC-based diet decreased linearly in rainbow trout [13]. Our study also observed a negative impact of RPC on protein digestion. As previously discussed, the existence of a protease inhibitor has been suggested in RPC [33,34]. In addition, prolamin, one of the major components of the RPC, is resistant to pepsin digestion [35]. The inclusion of protease inhibitors and the digestion-resistant nature of the protein component of RPC is considered to lead to lower protein digestion in fish fed the RPC-formulated diet in our study.

5. Conclusions

The present results suggest that enzyme complex pretreatment could have a limited impact on the feed performance of an extruded diet containing RPC, and the inclusion of less than 10% RPC is recommended for a 20% fishmeal diet for rainbow trout.

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