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Effect of fish meal replacement by blood meal in fingerling rainbow trout (*Oncorhynchus mykiss*) on growth and body/fillet quality traits

Roghayeh Bahrevar, Hamid Faghani-Langroudi

Department of Fisheries, Tonekabon Branch, Islamic Azad University, Tonekabon, Iran. Corresponding author: H. Faghani-Langroudi, hamid_faghani1@yahoo.com

Abstract. A feeding trial was conducted to evaluate the potential of replacing fish meal by processed blood meal, in practical diets for fingerling rainbow trout *Oncorhynchus mykiss*. Fish meal was replaced by 0%, 10%, 20% and 30% of blood meal. The diet with 0% blood meal was used as control. Fingerling rainbow trout were reared in 12 ponds. Each dietary treatment was tested in groups of 30 fish per pond arranged in a completely randomized design. Fish were fed by the diets for 8 weeks. Percentage weight gain, specific growth rate, survival rate, feed conversion ratio and body composition of fish were estimated. There were significant differences ($p < 0.05$) in growth performance among fish fed by diets (0–30% fish meal replacement) with those fed diet control as feed. No significant difference was observed in survival rate among fish fed by the experimental diets. These results showed that blood meal is not a suitable protein source as fish meal replacement for fingerlings rainbow trout. Growth, nutrient utilization and body composition were either not improved or were significantly influenced by gradually replacing fish meal by blood meal.
Key Words: FCR, SGR, diet, protein source, carcass analyze.

Introduction. In recent years, many researchers have tried different kind of animal meal (bone meal, blood meal, hemoglobin meal, poultry meal, and meat meal) to substitute fish meal in diets and the percentage of substitution has varied according to species, fish size and feeding habits (Zhou et al 2004; Barnes et al 2012; Dedeke et al 2013). Initially, the alternative raw materials were selected because they were less expensive and more available than fish meal and fish oil, but it is currently also necessary to consider raw materials with an adequate balance in amino acid profile, good digestibility, high level protein content, and a suitable palatability be considered good protein sources in fish diets (Lunger et al 2007; Antolović et al 2012; Bayraktar & Bayır 2012). Fish meal is a major protein source in aqua feeds, especially for carnivorous fish species because it is an excellent source of essential nutrients such as indispensable amino acids, essential fatty acids, vitamins, minerals, attractants and unknown growth factors (Zhou et al 2004; Sheen et al 2014). However, increasing demand, unstable supply and high price of the fish meal with the expansion of aquaculture made it necessary to search for alternative protein sources (Lunger et al 2007; FAO 2010).

Blood meal is one of the alternative protein sources for fish meal in diets for many fish species, due to its high protein content, reasonable price and steady supply (El-Sayed 1999). Blood meal, a lysine-rich ingredient (6–8% lysine), is produced using a wide variety of processing techniques (Bureau et al 1999).

Some researchers have tried a mixture of vegetable and animal meals, e.g., Martínez-Llorens et al (2008), who replaced 10% of fish meal by blood or hemoglobin meal, with a constant quantity of poultry meal and soybean meal, and obtained better results with a 5% blood meal inclusion. In most studies, commercial fish weight is not reached, so the effect of protein source dietary inclusion on sensorial changes in fish muscle cannot be evaluated. Some authors reported this effect on fillet quality traits (De Francesco et al 2007; Martínez-Llorens et al 2009). Estimates of lysine availability in blood meals appear to be quite highly variable across species and/or studies, even for the same type of drying technique. The objective of the present study is evaluating effects of fish-meal replacement by blood-meal on growth performance and carcass analyze, in diets for fingerling rainbow trout (*Oncorhynchus mykiss*).

Material and Method. A feeding trial was conducted at the reproduction and breeding center of rainbow trout located in Tonekabon, north of Iran, using fingerling rainbow trout, *O. mykiss*. The study was done from February to April 2014. In total 360 fingerlings were acclimatized to the rearing conditions for a 1-week period prior to the feeding trial.

For the eight week feeding trial, homogenous groups of 30 rainbow trout with an average initial weight of 10±1g (Mean ± SD) were randomly distributed among twelve ponds. Each pond was supplied with a water flow of 1.5-2 L S^{-1}. During the experiment period, the quality and quantity of water including oxygen, pH and temperature, were measured and controlled. Biometry was performed at the end of each week. Formulation of diets according to then nutritional requirements of fish was determined by the UFFDA software (Table 1). Amount of energy and protein in diets were considered identical.

Table 1

Composition of the experimental diets on a dry matter basis in 100 g dry diet

Ingredients	*Diets (replacement percentage)*			
	1 (0%)	*2 (10%)*	*3 (20%)*	*4 (30%)*
Fish meal	50.00	45.00	40.00	35.00
Blood meal	0.00	5.00	10.00	15.00
Meat meal	13.86	16.60	20.21	23.20
Wheat meal	2.59	2.29	2.20	2.72
Maize meal	27.09	24.65	20.13	16.62
Maize oil	4.00	4.00	5.00	5.00
Pellet binder	1.00	1.00	1.00	1.00
Mineral mix	0.50	0.50	0.50	0.50
Vitamin mix	0.50	0.50	0.50	0.50
Collin chloride	0.36	0.36	0.36	0.36
Antioxidant	0.10	0.10	0.10	0.10

At the end of the feeding trials, 8 specimens of each treatment were used for carcass analyze. Analyses of crude protein, moisture and ash in the diets were performed by standard methods (AOAC 2000). Dietary lipid was determined by the method of Kinsella et al (1977). Growth, biometric and economic indexes considered were as follows (Xue et al 2006): body weight increase (BWI) = 100 (W1 – W0) $(W0)^{-1}$; growth rate (GR) = (W1–W0) $(t)^{-1}$; feed conversion ratio (FCR) = [feed offered (g)] $[\text{weight gain (g)}]^{-1}$; specific growth rate (SGR) = 100 (lnW1–lnW0) $(t)^{-1}$; condition factor (CF) = 100 (W1) $(FL^3)^{-1}$; survival rate (SR) = 100 (N1) $(N0)^{-1}$; final body weight (FBW) = W1–W0. Where W0 and W1 were wet body weight of fish at the start and end of the experiment, respectively; FL and t were fork length and duration of experiment (8 weeks), respectively; N0 and N1 were number of fish at the start and end of the experiment, respectively.

Statistical analyses of data were carried out with SPSS 16.0 software package (SPSS; Chicago, IL). Normality was tested using Kolmogorov-Smirnoff test. Homogeneity was checked using the absolute residuals according to Levene's test. Effect of treatment was carried out using one-way ANOVA followed by a post hoc Duncan's multiple range test. In all statistical tests used, $p < 0.05$ was considered statistically different.

Results. The fish readily accepted all diets. At the end of 8 weeks of the feeding trial, negative effects on growth performance were observed when 10% of fish meal protein was replaced by blood meal protein. Also FCR was increased during the feeding trial. Significant differences were found in weight gain, FCR, SGR, GR, CF and FBW when the replacement level for fish meal protein was increased from 0 to 10% (Table 2). The percentages of BWI were significantly decreased from 19.32±4.06 to 14.13±2.30 after 8 weeks, when the replacement level for fish meal protein was increased from 0 to 30% (Table 2). Among the treatments, numerically the higher growth performance was found in fish fed fish meal free diet (blood meal 0%). Similarly, SGR, CF, and PR were also higher in blood meal 0% group and these parameters were significantly different between the treatments. No difference was also found in SR among different treatments. Survival ranged from 100% to 99.72% in fish fed the test diets and there were no differences among treatments at the end of the feeding trial.

Table 2

Growth performance of fingerlings rainbow trout fed by different levels of fish and blood meals in experimental diets for 8 experimental weeks

Growth factor	*Diet group (replacement percentage)*			
	1 (0%)	*2 (10%)*	*3 (20%)*	*4 (30%)*
BWI	19.32±4.06[c]	17.64±3.58[b,c]	16.02±3.14[a,b]	14.13±2.30[a]
SGR	1.09±0.21[c]	1.00±0.18[a,b]	0.92±0.17[b]	0.82±0.12[a]
FCR	1.06±0.05[a]	1.18±0.06[b]	1.31±0.06[c]	1.52±0.07[d]
SR	100.00±0.00[a]	100.00±0.00[a]	99.86±0.68[a]	99.72±0.96[a]
GR	0.55±0.14[d]	0.47±0.11[c]	0.41±0.08[b]	0.33±0.07[a]
CF	1.65±0.04[d]	1.43±0.05[c]	1.32±0.05[b]	1.04±0.14[a]
FBW	40.94±1.32[d]	36.59±1.27[c]	32.75±1.40[b]	27.81±0.71[a]

Different letters indicate significant differences ($p \leq 0.05$).

The whole body proximate composition of fish at the start and end of the feeding trial are shown in Table 3. All the fish showed a change in the analyzed parameters compared to those of the initial values. There was significant difference ($p < 0.05$) in the final whole body proximate composition among the groups of fish fed the different experimental diets (Table 3).

Table 3

Proximate composition of fingerlings rainbow trout fed by different levels of fish and blood meals in experimental diets for 8 experimental weeks

Carcass analyze	*Diet group (replacement percentage)*			
	1 (0%)	*2 (10%)*	*3 (20%)*	*4 (30%)*
Ash	2.20±0.50[a]	2.77±0.22[b]	1.74±0.12[a]	1.69±0.02[a]
Moisture	72.99±1.00[a]	74.44±1.29[a,b]	75.08±1.01[a,b]	75.51±1.00[b]
Lipid	5.69±0.01[c]	5.63±0.01[b]	5.62±0.01[a,b]	5.61±0.01[a]
Protein	17.24±0.01[a]	17.55±0.00[b]	17.78±0.00[c]	17.77±0.00[c]

Different letters indicate significant differences ($p \leq 0.05$).

Discussion. Fishery products, either in the form of low-value trash fish or rendered as fish meal, are presently the major sources of protein in the grow-out culture of most fish species and constitutes up to 70% by weight of their diet (Tacon 1995). As the demand for fish meal and marine fishery products for aquaculture increases while their availability decreases, the cost is expected to rise (Tacon 1995; Fasakin et al 2005). Reduction of the fish meal dependency is becoming more important for the sustainability and profitability of commercial fish farming. Individual rendered animal protein meals, such as blood meal often have deficiencies or excesses in essential amino acids that may affect the overall productivity of cultured fish (Fasakin et al 2005). Moreover, these diets are not always representative of what is commonly used in the industry and the use of rendered animal protein ingredients needs to be evaluated in more practical diets. This study has demonstrated that replacement of fish meal protein by processed animal by-product meals for example blood meal allowed growth rates reduce than those exhibited by the control groups.

Obtained results indicated the fingerling rainbow trout readily accepted the diets at all levels of fish meal replacement by blood meals as shown by the high feed conversion ratios of 1.06–1.52. This conforms to another study where diets with inclusion levels of meat meal ranging from 30 to 70% as substitute for fish meal have been readily accepted by rainbow trout (Watanabe et al 1993). Possible reasons for the reduced growth of fingerling rainbow trout at total replacement by blood meal may be due to deficiencies in essential nutrients such as essential amino acids. The dietary essential amino acid requirements of rainbow trout have not been defined. In the absence of amino acid requirement data, the amino acid profile of the whole body tissue of the animal has been used as an index of the essential amino acid requirements (Wilson & Poe 1985). Fish meals, in general, have been reported to have good essential amino acid profiles for fish (Cuzon & Guillaume 1991). On the other hand, the animal by-product meals were lower in some essential amino acids (methionine, lysine, and isoleucine). Deficiencies in essential amino acids may explain the decline in growth performance of fingerlings rainbow trout particularly at full replacement levels of fish meal by blood meal.

Other possible explanation for the low performance at increasing levels of fish meal substitution by blood meal may be the resulting effect on diet digestibility. Several authors have mentioned that poor growth and feed utilization of fish fed feeds containing alternatives of fish meal e.g., spray-dried blood meal, may be due to low protein digestibility and essential amino acid deficiency (mainly to deficient processing of rendered meals) (Bureau et al 1999; De Francesco et al 2007; Martínez-Llorens et al 2008, 2009). Millamena (2002) has shown that apparent digestibility values of blood meal were generally lower than for fish meals for *Epinephelus coioides*.

From the economic standpoint, replacement of fish meal by cheaper animal by-product meals in a practical diet for rainbow trout can alleviate the problem of low fish meal availability and high cost. Fish survival was not affected by experimental diets and similar results were obtained by Millamena (2002).

The results of this study clearly demonstrated that growth performances of fish in the dietary treatments in the present experimental condition were not satisfactory. The growth rate was comparable to that observed in other studies (Sevgili & Erturk 2004; Azevedo et al 2004; Kikuchi et al 2007).

In this study, the whole body proximate composition of fish at the start and end of the feeding trial showed a change in the analyzed parameters compared to those of the initial values. There was significant difference in the final whole body proximate composition among the groups of fish fed the different experimental diets. The increasing in moisture content from 72.99% to 75.51% and the decreasing in ash content from 2.20% to 1.69% with increasing levels of blood meal were reflected in the proximate analysis of the diets. This increase of moisture level after 8 weeks revealed decreasing levels of lipid.

Conclusions. Based on overall performances of fish, blood meal is not a suitable protein source as fish meal replacement for fingerling rainbow trout. Growth, nutrient utilization and body composition were either not improved or were significantly influenced by gradually replacing fish meal with blood meal. Further studies to determine the long-term effect on the performance of fish fed the fish meal replacement diet under on-farm conditions is suggested.

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Authors:
Roghayeh Bahrevar, Department of Fisheries, Tonekabon Branch, Islamic Azad University, P. O. Box: Tonekabon 46815-559, Tonekabon, Iran, e-mail: r.bahrevar@yahoo.com
Hamid Faghani-Langroudi, Department of Fisheries, Tonekabon Branch, Islamic Azad University, P. O. Box: Tonekabon 46815-559, Tonekabon, Iran, e-mail: hamid_faghani1@yahoo.com

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