



## RESEARCH ARTICLE

### Effects of Dietary Fish Meal Replacement by Red Lentil Meal on Growth and Amino Acid Composition of Rainbow Trout (*Oncorhynchus mykiss*)

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#### ABSTRACT

The purpose of this study was to determine the effects of replacing fish meal with red lentil meal (RLM) as an alternative plant protein source in diets for juvenile rainbow trout (10.14±0.04 g mean initial weight) on growth performance and amino acid composition of fish. Four iso-nitrogenous and iso-lipidic experimental diets were prepared to include 15% (RLM15), 20% (RLM20) and 25% (RLM25) of fish meal. At the end of the 60 day feeding trial, the highest mean individual weight gain (30.55±0.08 g) of fish was found in control group but not significantly different from RLM15. Crude protein level of whole body/fillet gradually decreased with increase in RLM percentages in the diets. Generally, essential amino acid (EAA) profiles of whole body/fillets reflected the dietary EAA profile. EAA profile of fish fed RLM15 diet was close to control group (P>0.05). However, lysine levels of fish decreased with increasing dietary RLM levels. RLM20 fed fish had the highest body contents of phenylalanine (P<0.05). Naturally, EAA levels of fillets were higher than whole body's EAA levels. Histidine levels of fillets were highest in control group and the lowest in RLM20 group. In contrast, isoleucine levels of fillets were highest in RLM20 group whereas the control group had the least level (P<0.05). Leucine and valine values of fish fed the control diet were lower than the other experimental groups. Threonine level was highest in fish fed the RLM25 diet (P<0.05). Results of the present study showed that 15% of dietary fish meal can be replaced by RLM in diets of juvenile rainbow trout without any adverse effects on growth performance and body amino acid composition.

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#### Introduction

Aquaculture is one of the most rapidly developing sectors in the world (Şener & Yıldız 2003; FAO 2016). According to estimates, the capacity of global aquaculture to cope with an enhancement demand for fish meal has reached the limited supplies (Sargent & Tacon 1999; Naylor et al. 2000; FAO 2016). As a risk reduction strategy, the identification, development

and use of alternatives to fish meal in aqua feeds remain a high priority (Hardy 2010). Fish meal is an excellent but costly protein source for fish feed formulation and is generally count in to 40-50% in commercial feeds for carnivorous fish species (Tacon and Metian 2008; Hardy 2010; Larsen et al. 2012).

Given the global needs of fish meal for aquaculture, there is an increasing demand for more insight into the potential of

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alternative protein sources in fish feeds (Kaushik et al. 2005). Among the plant protein sources, soybean meal (SBM) is considered the most cost-effective alternative for high-quality fish meal in feeds of many aquaculture animals, because of its high content of available protein with a relatively well-balanced amino acid profile, high digestibility, reasonable price, steady supply and low phosphorus content (Tan et al. 2005; Biswas et al. 2007). Although, soy protein concentrates and isolates are expensive, and the use of less soybean meals is limited in fish and other aquatic animals by anti-nutritional factors, higher crude fibre and unavailable carbohydrate concentrations (Brown 2008; Brown et al. 2008). Alternative plant protein sources are needed to reduce the current dependence on fish meal and soybean meal as the primary protein sources for aquatic animal diets (Reigh 2008). For this reason, numerous studies have been undertaken to examine the effects of replacing fish meal by other sources of proteins such as plant proteins or animal by-products in diets of rainbow trout (Dabrowski et al. 1989; Watanabe et al. 1993; Gomes et al. 1995; Kaushik et al. 1995; Xie & Jokumsen 1997; Ustaoglu-Tiril et al. 2009; Bilguven & Baris 2011; Øverland et al. 2013; Ouraji et al. 2013; Hauptman et al. 2014; Bahrevar & Faghani-Langroudi 2015; Dogan & Bircan 2015; Lee et al. 2015; Tenamura et al. 2016; Craft et al. 2016; Gerile & Pirhonen, 2017).

The use of grain products in aquaculture feeds is now common in the diet formulations of many aquatic animals (Gatlin et al. 2007). Among those grain raw materials frequently being used are red lentil meal. Red lentil meal is an important and inexpensive source of carbohydrate and protein

for the human diet (Frias et al., 1996). However, there are no reports on the nutritional value of red lentil meal when fed to rainbow trout except for Ustaoglu-Tiril's (2009) research. Therefore, the objective of the present study was to determine the effects of dietary replacement of fish meal with red lentil meal as an alternative plant protein source on growth performance and whole body/fillets amino acid composition in rainbow trout.

## Materials and Methods

### Experimental Diets

Four iso-nitrogenous and iso-lipidic experimental diets were formulated to contain graded levels of red lentil meal (RLM) to replace fish meal. The control diet contained only fish meal as the main protein source. The other experimental diets RLM15, RLM20 and RLM25, contained 150, 200 and 250 g kg<sup>-1</sup> of red lentil meal respectively. Wheat gluten and corn gluten in the diet were used to create a protein balance. The amino acid profile and proximate composition of protein sources in diets are presented in Table 1. Formulation and proximate compositions of experimental diets are shown in the Table 2. The amino acid composition of diets and essential amino acids requirement for rainbow trout are given in Table 3. Experimental diets (2 - 3 mm diameters) were produced at the Sapanca Inland Waters Research Center (Adapazari, Turkey) of Istanbul University as steam pressured pellets using a laboratory feed mill (KAHL-L, 173). Diets were kept in plastic storage bags at -20 °C until used.

**Table 1.** Amino acid and proximate compositions (dry weight basis) of fish meal, wheat gluten, corn gluten and red lentil meal

	Fish Meal	Wheat Gluten	Corn Gluten	Red Lentil Meal
<b>Essential Amino Acids (EAA, g/100g)</b>				
Arginine	3.74±0.04 <sup>a</sup>	2.47±0.03 <sup>b</sup>	1.85±0.23 <sup>c</sup>	1.73±0.18 <sup>c</sup>
Histidine	2.07±0.07 <sup>a</sup>	1.43±0.05 <sup>b</sup>	1.35±0.10 <sup>b</sup>	0.57±0.01 <sup>c</sup>
Isoleucine	2.40±0.00 <sup>a</sup>	1.99±0.00 <sup>b</sup>	1.86±0.11 <sup>b</sup>	0.73±0.08 <sup>c</sup>
Leucine	4.78±0.08 <sup>b</sup>	4.90±0.00 <sup>b</sup>	10.04±0.77 <sup>a</sup>	1.73±0.03 <sup>c</sup>
Lysine	5.08±0.46 <sup>a</sup>	1.35±0.18 <sup>b</sup>	1.11±0.03 <sup>b</sup>	1.61±0.10 <sup>b</sup>
Methionine	1.61±0.49 <sup>a</sup>	0.96±0.20 <sup>a</sup>	1.26±0.47 <sup>a</sup>	0.21±0.04 <sup>a</sup>
Phenylalanine	2.76±0.00 <sup>b</sup>	3.91±0.01 <sup>a</sup>	3.89±0.07 <sup>a</sup>	1.16±0.06 <sup>c</sup>
Threonine	3.07±0.03 <sup>a</sup>	2.04±0.03 <sup>b</sup>	2.52±0.27 <sup>ab</sup>	0.90±0.03 <sup>c</sup>
Tryptophan	0.02±0.00	0.02±0.00	0.02±0.00	0.02±0.00
Valine	2.79±0.15 <sup>a</sup>	2.33±0.12 <sup>b</sup>	2.14±0.09 <sup>b</sup>	0.89±0.03 <sup>c</sup>
Total EAA	28.33±0.19 <sup>a</sup>	21.44±0.16 <sup>c</sup>	26.05±0.20 <sup>b</sup>	9.58±0.29 <sup>d</sup>
<b>Non- Essential Amino Acids (NEAA, g/100g)</b>				
Alanine	4.13±0.27 <sup>b</sup>	2.08±0.09 <sup>c</sup>	5.81±0.49 <sup>a</sup>	1.06±0.04 <sup>c</sup>
Asparagine	0.02±0.00	0.02±0.00	0.02±0.00	0.02±0.00
Aspartic acid	5.71±0.01 <sup>a</sup>	2.19±0.18 <sup>c</sup>	3.55±0.27 <sup>b</sup>	2.62±0.22 <sup>c</sup>
Citrulline	0.02±0.00	0.02±0.00	0.02±0.00	0.81±0.73
Cystine	0.44±0.08 <sup>a</sup>	0.28±0.06 <sup>ab</sup>	0.33±0.00 <sup>ab</sup>	0.13±0.10 <sup>b</sup>
Glycine	3.47±0.24 <sup>a</sup>	2.45±0.03 <sup>b</sup>	1.89±0.01 <sup>c</sup>	1.01±0.02 <sup>d</sup>
Glutamic acid	9.12±0.24 <sup>bc</sup>	28.52±3.67 <sup>a</sup>	14.78±1.29 <sup>b</sup>	4.39±0.22 <sup>c</sup>
Hydroxyproline	0.26±0.00	0.03±0.00	0.03±0.00	0.03±0.00
Ornithine	0.03±0.00	0.00±0.00	0.03±0.00	0.03±0.00
Proline	3.24±0.32 <sup>c</sup>	11.48±0.70 <sup>a</sup>	7.42±0.25 <sup>b</sup>	1.21±0.02 <sup>d</sup>
Sarcosine	0.02±0.00	0.02±0.00	0.02±0.00	0.02±0.00
Serine	2.50±0.04 <sup>c</sup>	3.45±0.07 <sup>a</sup>	3.17±0.00 <sup>b</sup>	1.21±0.03 <sup>d</sup>
Tyrosine	2.39±0.05 <sup>a</sup>	2.13±0.12 <sup>a</sup>	2.91±0.31 <sup>a</sup>	0.37±0.27 <sup>b</sup>
Total NEAA	31.37±0.44 <sup>c</sup>	52.70±4.00 <sup>a</sup>	39.99±1.12 <sup>b</sup>	12.94±0.29 <sup>d</sup>
<b>Proximate composition</b>				
Dry matter	88.06±0.00 <sup>b</sup>	92.70±0.00 <sup>a</sup>	93.61±0.00 <sup>a</sup>	92.23±0.01 <sup>a</sup>
Crude Protein	68.16±0.01 <sup>c</sup>	81.9±0.00 <sup>a</sup>	70.03±0.01 <sup>b</sup>	27.24±0.00 <sup>d</sup>
Crude Lipid	11.25±0.00 <sup>a</sup>	1.53±0.00 <sup>c</sup>	0.67±0.00 <sup>d</sup>	1.95±0.00 <sup>b</sup>
Ash	11.32±0.00 <sup>a</sup>	0.52±0.00 <sup>d</sup>	1.69±0.00 <sup>c</sup>	2.15±0.00 <sup>b</sup>

Data are reported as mean ± SD of three replicates (n = 3). Means with different superscript letter in a row are significantly different (P<0.05).

**Table 2.** Ingredients and proximate composition of the four experimental diets

	Diets			
	Control	RLM15	RLM20	RLM25
<b>Ingredients (g kg<sup>-1</sup> dry weight)</b>				
Fish meal	600	300	150	0
Soybean meal	125	20	0	0
Corn gluten	80	150	140	90
Wheat gluten	0	155	270	410
Lentil Meal	0	150	200	250
Gelatin	50	50	50	50
Fish oil (Anchovy oil)	85	115	130	140
Mineral premix <sup>a</sup>	30	30	30	30
Vitamin premix <sup>a</sup>	30	30	30	30
<b>Analyzed proximate composition(g kg<sup>-1</sup>)</b>				
Dry matter	90.65±0.17	92.08±0.08	92.62±0.22	93.82±0.09
Crude protein	49.31±0.53	49.36±0.52	47.24±0.24	46.55±0.27
Lipid	15.48±0.58	15.11±0.48	15.00±0.42	14.75±0.52
Ash	7.92±0.16	4.70±0.06	2.91±0.05	1.32±0.07
Crude cellulose	1.76±0.01	2.03±0.11	2.51±0.31	2.64±0.30
NFE <sup>b</sup>	16.88±0.42	21.56±1.22	25.79±1.54	29.45±2.27
Metabolizable energy (kJ g <sup>-1</sup> )	14.36±0.29	14.56±0.14	14.46±0.02	14.51±0.07

<sup>a</sup>Premix of vitamins and minerals according to NRC (1993) recommendations for fish.

<sup>b</sup>NFE: nitrogen-free extract calculated by difference.

**Table 3.** Amino acid composition in the four experimental diets (g/100 g protein)

	Diets			
	Control	RLM15	RLM20	RLM25
<b>Essential Amino Acids (EAA, g/100g)</b>				
Arginine	3.00±0.12 <sup>a</sup>	2.69±0.01 <sup>b</sup>	2.42±0.06 <sup>bc</sup>	2.26±0.05 <sup>c</sup>
Histidine	1.46±0.06 <sup>a</sup>	1.31±0.09 <sup>ab</sup>	1.18±0.13 <sup>ab</sup>	1.07±0.03 <sup>b</sup>
Isoleucine	1.84±0.03 <sup>a</sup>	1.71±0.04 <sup>ab</sup>	1.59±0.12 <sup>ab</sup>	1.44±0.07 <sup>b</sup>
Leucine	4.24±0.02	4.52±0.03	4.27±0.11	3.85±0.33
Lysine	3.36±0.02 <sup>a</sup>	2.27±0.11 <sup>b</sup>	1.86±0.04 <sup>c</sup>	1.39±0.06 <sup>d</sup>
Methionine	1.41±0.01 <sup>a</sup>	1.16±0.06 <sup>b</sup>	0.97±0.07 <sup>b</sup>	0.67±0.01 <sup>c</sup>
Phenylalanine	2.31±0.07	2.55±0.01	2.58±0.14	2.52±0.12
Threonine	2.42±0.26 <sup>a</sup>	1.96±0.05 <sup>ab</sup>	1.77±0.09 <sup>b</sup>	1.53±0.03 <sup>b</sup>
Tryptophan	0.34±0.04 <sup>a</sup>	0.02±0.00 <sup>b</sup>	0.02±0.00 <sup>b</sup>	0.02±0.00 <sup>b</sup>
Valine	2.16±0.01 <sup>a</sup>	2.02±0.02 <sup>ab</sup>	1.87±0.11 <sup>b</sup>	1.62±0.01 <sup>c</sup>
Total EAA	22.58±0.51 <sup>a</sup>	20.23±0.20 <sup>ab</sup>	18.55±0.88 <sup>bc</sup>	16.41±0.60 <sup>c</sup>
Alanine	3.57±0.27 <sup>a</sup>	3.22±0.00 <sup>ab</sup>	2.75±0.03 <sup>b</sup>	2.13±0.07 <sup>c</sup>
Asparagine	0.02±0.00	0.02±0.00	0.02±0.00	0.02±0.00
Aspartic acid	4.77±0.42 <sup>a</sup>	3.75±0.03 <sup>b</sup>	3.11±0.12 <sup>bc</sup>	2.45±0.18 <sup>c</sup>
Citrulline	0.02±0.00	0.02±0.00	0.21±0.00	0.02±0.00
Cystine	0.25±0.18	0.28±0.25	0.32±0.30	0.36±0.38
Glycine	3.09±0.29 <sup>a</sup>	2.97±0.18 <sup>a</sup>	2.57±0.24 <sup>a</sup>	2.46±0.15 <sup>a</sup>
Glutamic acid	7.22±0.23 <sup>c</sup>	10.22±0.73 <sup>b</sup>	11.89±0.23 <sup>b</sup>	14.37±0.79 <sup>a</sup>
Hydroxyproline	0.26±0.00	0.26±0.00	0.26±0.00	0.26±0.00
Ornithine	0.03±0.00	0.03±0.00	0.03±0.00	0.03±0.00
Proline	3.60±0.50 <sup>c</sup>	4.52±0.20 <sup>c</sup>	6.25±0.35 <sup>b</sup>	8.35±0.49 <sup>a</sup>
Sarcosine	0.02±0.00	0.02±0.00	0.02±0.00	0.02±0.00
Serine	2.41±0.11 <sup>a</sup>	2.59±0.06 <sup>a</sup>	2.65±0.01 <sup>a</sup>	2.60±0.10 <sup>a</sup>
Tyrosine	1.77±0.05	1.84±0.06	1.64±0.15	1.53±0.08
Total NEAA	27.05±2.06 <sup>b</sup>	29.75±1.44 <sup>ab</sup>	31.75±0.36 <sup>ab</sup>	34.38±2.25 <sup>a</sup>

Data are reported as mean ± SD of three replicates (n = 3). Means with different superscript letter in a row are significantly different (P<0.05).

### Experimental Conditions and Measurements

Juvenile rainbow trout (*Oncorhynchus mykiss*), with a mean initial body weight of 10.14±0.04 g, were obtained and stocked randomly (50 fish tank<sup>-1</sup>) into 8 cylindro-conical tanks of 1000 L capacity in the Sapanca Inland Waters Research Center (Adapazari, Turkey). The tanks were supplied with freshwater

having an average temperature of 12.3±0.2 °C. Dissolved oxygen was maintained around 9.9±0.1 mg L<sup>-1</sup>. 12 h light: 12 h dark photoperiod regimen was utilized throughout the study. Before starting the experiment, fish were acclimatized to the experimental feeding regimen using a commercial diet for 2 weeks (trout commercial pellet 2 mm in diameter). During the

study, fish were fed to apparent satiation by hand twice per day at 09:00 and 17:00 h. Bulk fish live weight increments were measured every 2 weeks and feed intake was recorded daily throughout the study. At the end of the study, fish were taken individually weight and length for determining growth performance parameters. In addition, 15 fish per tank (30 fish per diet) were collected for chemical analyses. Fish samples were kept at -80 °C until proximate composition and amino acid profile analysis. Growth performance measured are listed below and the calculations were according to Ricker (1979);

$Weight\ gain\ (\%) = [(final\ weight - initial\ weight) / initial\ weight] \times 100;$

$Specific\ growth\ rate\ (SGR) = [(\ln\ final\ weight - \ln\ initial\ weight) / days] \times 100;$

$Condition\ factor\ (CF) = 100 \times [(body\ weight\ (g) / length^3\ (cm));$

$Feed\ efficiency\ ratio\ (FER) = wet\ weight\ gain\ (g) / feed\ intake\ (g);$

$Protein\ efficiency\ ratio\ (PER) = wet\ weight\ gain\ (g) / protein\ intake\ (g);$

$Hepatosomatic\ index\ (HSI) = (liver\ weight / body\ weight) \times 100;$

$Viscerosomatic\ index\ (VSI) = 100 \times (viscera\ weight / body\ weight).$

### Chemical Analyses

Feed ingredients, experimental diets, and fish samples were analyzed for proximate composition (protein, lipid, ash and dry matter) according to standard AOAC (1998) procedures. Dry matter was obtained by weight loss after drying samples in an oven at 105°C until constant weight. Crude protein was determined as total nitrogen (N) by using a semi-automatic Kjeldahl (Gerhardt Vapodest, 45s) technique (N×6,25). Crude lipid was extracted according to Soxhlet (Velp Scientifica Ser, 148) method with petroleum ether. Ash content was obtained from the weight loss after incineration of dried samples at 550 °C for about 12 h in a Muffle Furnace. All samples were analyzed as triplicates.

Amino acid levels of feed ingredients, experimental diets and fish were hydrolyzed with 6 mL of 6 N HCl at 110 °C for 22 h in an evacuated sealed tube to determine amino acids composition. The hydrolysate was dried under nitrogen gas to remove HCl, re-dissolved in 0.1 N HCl loading buffer, and filtered through a 0.22 µm polyethersulfone ultrafiltration membrane. The filtrate was loaded on a high-performance liquid chromatography system (LC1200, Bilim Laboratory A.S., Istanbul) equipped with an Agilent ZORBAX Eclipse Plus C18 column (150 × 5 µm). Signals of 16 amino acids were detected

after derivatization with ophthaldialdehyde. Asparagine, glutamine, proline, and tryptophan were not within the determination range. The HPLC conditions followed the protocol for the Agilent ZORBAX Eclipse Plus C18 column.

### Statistical Analyses

Statistical analyses of data were subjected to one-way ANOVA, and a subsequent comparison of means by Tukey's multiple range test. All of the above mentioned statistical analyses were performed using SPSS (Version 10 for Windows). Differences were considered statistically significant at  $P < 0.05$ .

### Results

#### Amino Acid Composition of Dietary Ingredients and Experimental Diets

EAA levels of red lentil meal were significantly lower than EAA levels of fish meal, wheat gluten and corn gluten (Table 1). EAA levels of fish meal were higher than other protein sources ( $P < 0.05$ ).

The amino acid composition of experimental diets is presented in Table 2. Amino acid composition changes among the experimental diets reflected the replacement of fish meal with RLM. EAA levels were gradually decreased with increasing dietary levels of fish meal replacement except for leucine and phenylalanine. In contrast, NEAA levels of diets increased with increasing RLM inclusion ( $P < 0.05$ ).

#### Growth Performance

At the end of the experiment, final weight of the control group was higher than the other experimental fish groups ( $P < 0.05$ ). However, the final weight of RLM15 fish group were close to control group ( $P > 0.05$ ). Fish weights were similar in the RML20 and RLM25 groups ( $P > 0.05$ ) and these were lower than the other experimental groups ( $P < 0.05$ ). Fish fed the RML25 diet had the lowest SGR and the highest PER levels ( $P < 0.05$ ). All experimental groups had similar FCR, CF and HSI values ( $P > 0.05$ ). VSI value of fish fed with control diets was lower than other experimental. However, there were no significant differences in VSI among the RLM fed fishes (Table 4;  $P < 0.05$ ).

**Table 4.** Growth performance values of rainbow trout fed four experimental diets

	Dietary Treatments			
	Control	RLM15	RLM20	RLM25
Initial weight (g fish <sup>-1</sup> ) <sup>1</sup>	10.15±0.06	10.14±0.00	10.15±0.08	10.12±0.03
Final weight (g fish <sup>-1</sup> ) <sup>1</sup>	40.70±0.15 <sup>a</sup>	40.00±0.12 <sup>b</sup>	39.40±0.17 <sup>c</sup>	39±0.04 <sup>c</sup>
Weight gain (%) <sup>1</sup>	300.98±0.08 <sup>a</sup>	294.48±0.11 <sup>b</sup>	288.18±0.25 <sup>c</sup>	285.37±0.08 <sup>c</sup>
SGR <sup>1</sup>	2.35±0.01 <sup>a</sup>	2.31±0.01 <sup>ab</sup>	2.29±0.02 <sup>ab</sup>	2.28±0.01 <sup>b</sup>
FCR <sup>1</sup>	0.95±0.04	0.93±0.03	0.93±0.04	0.91±0.01
PER <sup>1</sup>	1.82±0.00	1.88±0.01	1.88±0.02	2.04±0.00
CF <sup>1</sup>	1.12±0.03 <sup>a</sup>	1.12±0.02 <sup>a</sup>	1.12±0.03 <sup>a</sup>	1.07±0.04 <sup>b</sup>
HSI <sup>2</sup>	1.79±0.29 <sup>c</sup>	1.92±0.21 <sup>b</sup>	2.02±0.32 <sup>a</sup>	2.01±0.26 <sup>a</sup>
VSI <sup>2</sup>	18.17±1.13 <sup>b</sup>	20.59±1.96 <sup>a</sup>	20.24±2.65 <sup>a</sup>	20.25±1.41 <sup>a</sup>

Data are reported as mean ± SD of three replicates (n = 3). Means with different superscript letter in a row are significantly different ( $P < 0.05$ ).

<sup>1</sup> n = 60 × 2

<sup>2</sup> n = 20

### Proximate Composition of Whole Body/Fillets

Proximate composition of whole body and fillet were significantly affected by dietary treatments (Table 5). Fish fed the RLM25 had the highest fillet dry matter levels ( $P<0.05$ ). Crude protein was highest in fish fed the control diet. In particular, crude protein level of whole body and fillet gradually decreased with the increase red lentil meal percentages in the diets ( $P<0.05$ ). In contrast, the crude lipid and dry matter levels of fish fillet increased with the increase red lentil meal in diets ( $P<0.05$ ). However, whole body crude lipid levels were similar to fish fed the control and RLM25 diets and these groups had higher level of crude lipid than the other experimental groups ( $P<0.05$ ). The crude lipid levels of the fish livers decreased with increasing red lentil meal percentages in the diets ( $P<0.05$ ).

### Amino Acid Composition of Whole Body/Fillets

Whole body amino acid compositions were shown Table 6. There were no significant differences EAA levels among the initial, control and the other experimental groups ( $P>0.05$ ), except for lysine and phenylalanine. Lysine levels of fish fed with increased RLM in the diets were gradually decreased.

Phenylalanine level was the lowest in control group and the highest in RLM20 group ( $P<0.05$ ).

The amino acid composition of fillets is given in Table 7. Generally, EAA levels of fillets were higher than that of whole body. Fillet histidine concentration were highest in control group and whereas the RLM20 group had the lowest content. In contrast, isoleucine levels of fillet were highest in RLM20 group while the control group recorded the lowest fillet content ( $P<0.05$ ). Leucine and valine contents of fish fed the control diet were lower than the other experimental groups. Threonine level of fish fed the RLM25 diet was the highest. There were no significant differences in total EAA levels between control and other experimental groups for both whole body and fillets. In addition EAA levels of whole bodies were lower than fillets EAA levels.

Essential amino acid requirement of rainbow trout is given in Table 8. The EEA requirement of rainbow trout levels of arginine, histine, methionine, tryptophan and valine were found higher than the four experimental diets. However, isoleucine, leucine, lysine, phenylalanine and threonine levels in the experimental diets were higher than the requirement values.

**Table 5.** Whole body/fillets proximate composition and crude lipid in liver of rainbow trout fed four experimental diets

	Dietary Treatments			
	Control	RLM15	RLM20	RLM25
<b>Whole Body</b>				
Dry matter	26,41±0,18 <sup>b</sup>	26,92±0,69 <sup>b</sup>	26,25±0,27 <sup>b</sup>	28,43±0,39 <sup>a</sup>
Crude Protein	16,75±0,70 <sup>a</sup>	15,84±0,41 <sup>ab</sup>	15,51±0,15 <sup>b</sup>	14,18±0,14 <sup>c</sup>
Crude Lipid	13,88±0,74 <sup>a</sup>	12,35±0,19 <sup>b</sup>	12,27±0,48 <sup>b</sup>	14,24±0,62 <sup>a</sup>
Ash	1,10±0,17 <sup>b</sup>	1,65±0,27 <sup>a</sup>	1,08±0,12 <sup>b</sup>	1,09±0,20 <sup>b</sup>
<b>Crude lipid of liver</b>	3,70±0,44 <sup>a</sup>	2,65±0,35 <sup>ab</sup>	2,63±0,00 <sup>ab</sup>	1,82±0,02 <sup>b</sup>
<b>Fillet</b>				
Dry matter	20,74±0,14 <sup>c</sup>	22,24±0,47 <sup>b</sup>	22,55±0,15 <sup>b</sup>	25,96±0,73 <sup>a</sup>
Crude Protein	19,58±0,32 <sup>a</sup>	18,77±0,14 <sup>b</sup>	18,03±0,23 <sup>c</sup>	16,44±0,34 <sup>d</sup>
Crude Lipid	3,56±0,03 <sup>d</sup>	4,37±0,39 <sup>c</sup>	5,56±0,13 <sup>b</sup>	7,97±0,09 <sup>a</sup>
Ash	0,86±0,07	0,92±0,07	0,81±0,07	0,79±0,04

Data are reported as mean ± SD of three replicates (n = 3). Means with different superscript letter in a row are significantly different ( $P<0.05$ ).

**Table 6.** Amino acid composition (dry weight basis) in whole body of rainbow trout fed four experimental diets

Amino Acids	Fish Groups				
	Initial	Control	RLM15	RLM20	RLM25
<b>Essential Amino Acids (EAA, g/100g)</b>					
Arginine	0.85±0.02	0.75±0.08	0.78±0.02	0.78±0.04	0.84±0.03
Histidine	0.39±0.03	0.36±0.06	0.33±0.03	0.38±0.01	0.37±0.04
Isoleucine	0.47±0.03	0.39±0.03	0.37±0.01	0.48±0.05	0.45±0.01
Leucine	1.03±0.04	0.88±0.04	0.93±0.07	0.90±0.08	0.89±0.02
Lysine	1.05±0.03	0.89±0.04	0.93±0.08	0.72±0.10	0.71±0.01
Methionine	0.42±0.01	0.21±0.14	0.21±0.14	0.34±0.03	0.37±0.01
Phenylalanine	0.58±0.03	0.49±0.03	0.51±0.03	0.66±0.07	0.64±0.00
Threonine	0.65±0.00	0.58±0.06	0.59±0.03	0.59±0.03	0.60±0.03
Tryptophan	0.19±0.06 <sup>b</sup>	0.11±0.02 <sup>b</sup>	0.10±0.01 <sup>b</sup>	0.22±0.00 <sup>a</sup>	0.17±0.01 <sup>b</sup>
Valine	0.57±0.03	0.52±0.05	0.52±0.03	0.54±0.06	0.51±0.01
Total EAA	6.21±0.28	5.20±0.27	5.29±0.16	5.64±0.40	5.57±0.09
<b>Non-Essential Amino Acids (NEAA, g/100g)</b>					
Alanine	0.87±0.01	0,74±0,06	0,79±0,00	0.75±0.05	0.80±0.01
Asparagine	0.02±0.00	0.02±0.00	0.02±0.00	0.02±0.00	0.02±0.00
Aspartic acid	1.15±0.04	0.95±0.05	0.99±0.06	1.00±0.05	1.08±0.01
Citrulline	0.26±0.34	0.02±0.00	0.02±0.00	0,04±0,03	0.02±0.00

Amino Acids	Fish Groups				
	Initial	Control	RLM15	RLM20	RLM25
Cystine	0.06±0.00	0.08±0.02	0.05±0.03	0.06±0.06	0.05±0.05
Glycine	0.93±0.04	0.74±0.06	0.85±0.09	0.66±0.03	0.75±0.01
Glutamic acid	1.43±0.05 <sup>b</sup>	1.21±0.06 <sup>c</sup>	1.28±0.06 <sup>c</sup>	1.75±0.11 <sup>a</sup>	1.78±0.03 <sup>a</sup>
Hydroxyproline	0.26±0.00	0.26±0.00	0.26±0.00	0.26±0.00	0.26±0.00
Ornithin	0.03±0.00	0.03±0.00	0.03±0.00	0.03±0.00	0.03±0.00
Proline	1.28±0.25 <sup>a</sup>	0.72±0.17 <sup>b</sup>	0.87±0.01 <sup>b</sup>	0.76±0.18 <sup>b</sup>	0.86±0.11 <sup>b</sup>
Sarcosine	0.02±0.00	0.02±0.00	0.02±0.00	0.02±0.00	0.02±0.00
Serine	0.70±0.01	0.61±0.03	0.66±0.03	0.60±0.04	0.63±0.02
Tyrosine	0.48±0.06	0.42±0.05	0.40±0.03	0.44±0.06	0.43±0.03
Total NEAA	7.50±0.03	5.85±0.50	6.26±0.05	6.41±0.44	6.75±0.23

Data are reported as mean ± SD of three replicates (n = 3). Means with different superscript letter in a row are significantly different (P<0.05).

**Table 7.** Amino acid composition (dry weight basis) in fillets of rainbow trout fed four experimental diets

Amino Acids	Fish Groups			
	Control	RLM15	RLM20	RLM25
<b>Essential Amino Acids (EAA, g/100g)</b>				
Arginine	1.02±0.03	1.10±0.08	1.07±0.02	1.19±0.06
Histidine	0.53±0.01 <sup>a</sup>	0.55±0.00 <sup>a</sup>	0.37±0.01 <sup>b</sup>	0.59±0.04 <sup>a</sup>
Isoleucine	0.59±0.01 <sup>b</sup>	0.66±0.04 <sup>ab</sup>	0.79±0.01 <sup>a</sup>	0.73±0.08 <sup>ab</sup>
Leucine	1.25±0.04 <sup>b</sup>	1.37±0.08 <sup>ab</sup>	1.50±0.03 <sup>a</sup>	1.48±0.04 <sup>a</sup>
Lysine	1.14±0.04	1.21±0.11	1.34±0.06	1.35±0.18
Methionine	0.36±0.32	0.61±0.01	0.57±0.09	0.36±0.39
Phenylalanine	0.84±0.02	0.94±0.06	0.94±0.01	0.98±0.07
Threonine	0.81±0.01 <sup>b</sup>	0.88±0.03 <sup>ab</sup>	0.80±0.02 <sup>b</sup>	0.95±0.03 <sup>a</sup>
Tryptophan	0.20±0.06	0.23±0.02	0.06±0.03	0.12±0.11
Valine	0.65±0.01 <sup>b</sup>	0.74±0.03 <sup>ab</sup>	0.83±0.01 <sup>a</sup>	0.81±0.06 <sup>a</sup>
Total EAA	7.41±0.56	8.32±0.43	8.30±0.07	8.58±0.35
<b>Non-Essential Amino Acids (NEAA, g/100g)</b>				
Alanine	1.02±0.03 <sup>b</sup>	1.15±0.07 <sup>ab</sup>	1.04±0.03 <sup>b</sup>	1.22±0.01 <sup>a</sup>
Asparagine	0.02±0.00	0.02±0.00	0.02±0.00	0.02±0.01
Aspartic acid	1.59±0.03	1.75±0.13	1.57±0.06	1.76±0.18
Citrulline	0.02±0.00	0.02±0.00	0.11±0.11	0.38±0.46
Cystine	0.07±0.03	0.11±0.05	0.12±0.07	0.05±0.01
Glycine	0.71±0.02	0.80±0.11	0.80±0.01	0.85±0.07
Glutamic acid	2.48±0.13	2.71±0.21	2.41±0.07	2.78±0.11
Hydroxyproline	0.26±0.00	0.26±0.00	0.26±0.00	0.26±0.00
Ornithin	0.03±0.00	0.03±0.00	0.03±0.00	0.03±0.00
Proline	0.72±0.06	0.87±0.03	0.69±0.04	0.98±0.32
Sarcosine	0.02±0.00	0.02±0.00	0.02±0.00	0.02±0.00
Serine	0.75±0.03	0.83±0.07	0.70±0.01	0.88±0.08
Tyrosine	0.58±0.01 <sup>b</sup>	0.65±0.01 <sup>ab</sup>	0.62±0.01 <sup>b</sup>	0.68±0.00 <sup>a</sup>
Total NEAA	8.28±0.34 <sup>b</sup>	9.24±0.62 <sup>ab</sup>	8.41±0.27 <sup>ab</sup>	9.93±0.15 <sup>a</sup>

Data are reported as mean ± SD of three replicates (n = 3). Means with different superscript letter in a row are significantly different (P<0.05).

**Table 8.** Amino acid requirement of rainbow trout (NRC, 2011)

Amino acid requirement of rainbow trout (g/100 g protein)	
Arginine	3.3
Histidine	1.6
Isoleucine	0.9
Leucine	1.6
Lysine	2.0
Methionine	2.2
Phenylalanine	2.1
Threonine	0.9
Tryptophan	0.5
Valine	3.1

## Discussion

The effects of dietary fish meal replacement by alternative plant protein sources on the growth performance, feed utilization rate and body composition were investigated in the present study. There are many studies that have evaluated the use of plant protein sources in diets for rainbow trout (Dabrowski et al. 1989; Watanabe et al. 1993; Gomes et al. 1995; Kaushik et al. 1995; Xie & Jokumsen 1997; Ustaoglu-Tiril et al. 2009; Bilguven & Baris 2011; Øverland et al. 2013; Ouraji et al. 2013; Hauptman et al. 2014; Bahrevar & Faghani-Langroudi 2015; Dogan & Bircan 2015; Lee et al. 2015; Tenamura et al. 2016; Craft et al. 2016; Gerile & Pirhonen, 2017). However, information on the dietary replacement of

fish meal by RLM is non-existent, except the study by Ustaoglu-Tiril et al. (2009).

It has been reported in several studies that the use soybean meal in dietary fish meal replacement does not negatively affect growth performance in cultured fish species (Refstie et al. 1997; Davies et al. 1997; Carter&Hauler 2000; Opstvedt et al. 2003; Zhou et al. 2011). However, some studies have reported a decrease in the growth performance of fish fed diets when fish meal was replaced by alternative protein sources other than soy meal (Xie & Jokumsen 1997; Luo et al. 2006; Palmegiano et al. 2006; Romarheim et al. 2006; Øverland et al. 2009; Slawski et al. 2011; Bullerwell et al., 2016; Anderson et al., 2018). Similarly, an increasing inclusion of RLM in diets in the present study led to a decrease in weight gain of rainbow trout. At the end of the experiment, the control group had the highest weight gain (30.55±0,08 g) while the RLM25 group had the least weight gain (28,89±0,08 g). These results indicate that fish use less lentil meal than fish meal. Congruently, Kasiga and Brown (2019) were found weight gain decreased with increased fish meal replacement by carinata (*Brassica carinata*) meal.

At the end of the present trial, feed utilization ranged from 0.91-0.95 (P>0.05). These results show that fish can use all of the experimental feeds effectively. Similar results were reported by Kasiga and Brown (2019), Glencross et al. (2011) and Cheng and Hardy (2002) in juvenile rainbow trout when dietary fish meal was replaced with pods and cotton seeds respectively. However, Ustaoglu-Tiril et al. (2009) found feed utilization rates of 1.61 for rainbow trout by feeding at 30% of red lentil meal in the test diet. Feed utilization has been reported to decrease with increasing inclusion of plant protein sources diets of rainbow trout (Xie and Jokumsen 1997; Adelizi et al. 1998; Cheng et al. 2003; Lou et al. 2006; Ustaoglu-Tiril et al. 2009).

Crude protein levels in fish fillet decreased with increasing dietary fish meal replacement. (P<0.05). The liver lipid of fish showed a gradual decrease with increasing substitution of fish meal (P<0.05). Similarly, lipid content in fish fillet increased with increasing dietary RLM inclusion (P<0.05). Previous studies have showed that protein content of fish fillet is reduced whereas fillet lipid is increased when carnivorous fish species, including rainbow trout, are fed alternative diets with plant sources providing dietary protein (Palmegiano et al. 2006; Deng et al. 2006; Shafaeipour et al. 2008; Güroy et al. 2011).

The examination of the amino acid composition of test diets showed a reduction in EAA with increasing inclusion of plant protein sources (P<0.05). It appears that the reduction in dietary EAA in experimental diets was due to the inclusion of RLM as all the other dietary ingredients from plant sources had higher EAA contents than RLM. In comparison to the EAA dietary requirements of rainbow trout, replacement of fish meal with plant protein sources led to reduced dietary levels of arginine, histidine, methionine, tryptophan and valine. RLM20 and RLM25 were found to be deficient in lysine levels in feed groups. However, the levels of phenylalanine, isoleucine, leucine and threonine in the control and in all three

experimental diets were above the levels required for fish. Naturally, EAA levels in fillets were higher than whole body's. Because whole body was included all fish parts such as skin, bones, gills and skull but the fillet was only pure fish meat. Non-essential amino acids are important for rainbow trout's nutrition as in all fish. Barely, it is well known that fish can synthesized NEAA in direction of their needs. For this reason, there was not much discussion on NEAA.

The present study showed that the RML15 can be used in juvenile rainbow trout feed. In addition to we thought that RML20 and RML25 groups may be used with synthetic amino acids. Because EAA composition in experimental feeds as well as amounts of EAA that seem to be inadequate, especially due to the increase in red lentil meal. In the future, research on synthetic amino acids is expected to continue.

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