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Effects of the Feeds Containing Different Amounts of Hazelnut Meal, Soybean Meal, Fish Meal and Phytase on the Growth of Rainbow Trout (*Oncorhynchus mykiss*) Juveniles

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Research Article

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Abstract

This study aimed to examine the effects of six trial diets (containing different amounts of fish meal, hazelnut, and soybean meal protein, also without the enzyme phytase “0 FTU” and with “1000 FTU”) on the rainbow trout (*Oncorhynchus mykiss*) juveniles. The study was conducted as three replicates. Live weight gain, feed consumption, feed conversion ratio (FCR), specific growth ratio (SGR) and the protein efficiency ratio (PER) of the trial groups were calculated as 52.8-64.3g, 64.1-72.1g/fish, 1.12-1.22, 1.42-1.6% and 1.79-2.0, respectively ($P<0.05$). The dietary factors did not produce a significant effect on the organ indexes, body chemical composition, digestibility ratios of diets in the fish ($P>0.05$). But phytase enzyme affected significantly the dietary phosphorus ratio and the digestible energy ratio ($P<0.05$). Dry matter, crude ash, crude lipid, crude protein and phosphorus components in the fish bodies were found to be 30.7-31.8%, 2.2-2.3%, 11.8-12.8%, 14.5-15.2% and 0.56-0.62%, respectively. In the trial groups fish, values of the total nitrogen and phosphorus consumption, gain and retention were calculated as 1139-1173 mg/kg versus 248-279 mg/kg, 315-350 mg/kg versus 73-82 mg/kg, and 27.2-30.6% versus 26-33%, based on the average daily weight. Generally, the interaction between factors was found to be statistically insignificant ($P>0.05$).

Keywords: Diet proteins, enzyme, retention, growth, trout.

Farklı Oranlarda Fındık, Soya Küspesi, Balık Unu ile Birlikte Fitaz İçeren Rasyonların Gökkuşağı Alabalığı (*Oncorhynchus mykiss*) Yavrularının Gelişimi Üzerine Etkileri.

Özet

Bu çalışmada, balık unu protein (BUP), fındık küspesi proteini(FKP) ve soya küspesi protein (SKP) ile birlikte fitaz enzimini 0 (-) ve 1000 FTU (+) farklı oranlarda içeren altı adet deneme rasyonunun gökkuşağı alabalığı (*Oncorhynchus mykiss*) yavrularının (27,82±0,04g) büyüme parametrelerine, vücut kimyasal kompozisyonuna, organ indekslerine, yemin sindirilebilirliğine etkilerinin belirlenmesi amaçlanmıştır. Çalışma 3 tekerrürlü olarak yürütülmüştür. Canlı ağırlık artışı 52,8-64,3g, yem tüketimi 64,1-72,1g/balık, yem dönüşüm oranı 1,12-1,22, spesifik büyüme oranı % 1,42-1,6 ve protein etkinlik oranı 1,79-2,0 arasında değişmiştir($P<0.05$).Denemede rasyon protein kaynağı ve fitaz enzim içeriği gökkuşağı alabalığı yavrularının organ indeksleri, vücut kimyasal kompozisyonları ve rasyon sindirim oranları üzerine etkilerinin önemsiz, ancak rasyon fosfor ve enerjinin sindirilme oranı üzerine rasyon fitaz enzim seviyesi etkisinin önemli olduğu saptanmıştır ($P<0,05$). Deneme sonu balık vücutlarının kuru madde (KM), ham kül (HK), ham yağ (HY), ham protein (HP) ve fosfor bileşenleri sırasıyla %30,7-31,8, %2,2-2,3, %11,8-12,8,%14,5-15,2 ve %0,56-0,62 olarak bulunmuştur. Deneme grubu balıkların toplam azot, tüketimi, kazancı ve tutumu günlük ortalama ağırlık üzerinden 1139-1173 mg/kg, 315-350 mg/kg, %27,2-30,6 ve toplam fosfor tüketimi, kazancı ve tutumu 248-279 mg/kg, 73-82 mg/kg ve %26-33 olarak hesaplanmıştır. Tüm hesaplamalarda (“rasyon protein kaynağı*Fitaz”) faktörleri arasında etkileşimin önemsiz olduğu bulunmuştur ($P>0,05$).

Anahtar kelimeler: Rasyon proteinleri, enzim, vucütta tutulma, büyüme, alabalık.

INTRODUCTION

Aquaculture is the fastest-growing sector among the agricultural and food fields (Granada et al., 2016). The growth of the fish farming sector has also led to an inevitable increase in the demand for the fish meal (Orisasona et al., 2017), and using higher amounts of plant-based raw materials in fish feeds (Ruohonen et al., 2007; Granada et al., 2016).

It is commonly reported that soybean protein and other vegetable protein may be used in the rainbow trout farming instead of a part of fish meal in the diet in order to reduce aquafeeds environmental pollution without compromising on live weight gain,feed conversion ratios, specific growth rate (Vielma et al., 2000; Morris et al., 2005; Dalsgaard et al., 2009; Pratoomyot et al., 2010; Harlıoğlu, 2011; Burr et al., 2012; Diler et al., 2012; Zhang et al., 2012; Orisasona et al., 2017). Atalayoğlu and Çakmak (2010) reported that hazelnut (10%) meal could be substituted for some parts of the fish meal without having a problem. Doğan and Bircan (2010) reported that the rate of hazelnut meal (increasing this ratio to 30%) in the feed of rainbow trout did not affect the feed conversion ratio, the protein efficiency ratio, the condition factor. Karabulut et al. (2017) reported that when the hazelnut meal was greater than 35%, the protein efficiency ratio decreased and the feed conversion ratio significantly reduced in angelfish (*Pterophyllum scalare*) juveniles. Important findings have been obtained from the use of hazelnut meal at certain ratios instead of fish meal (Emre et al., 2008a; Emre et al., 2008b; Sevgili et al., 2009a; Sevgili et al., 2009b). Harlıoğlu (2011) found that the feeds have significant effects on the phosphor and nutrients digestibility rates of rainbow trout ($p<0.05$).

The use of the enzyme phytase, for example, provided an increase in the digestion rate of minerals, protein-amino acid, and starch in the feeds and an improvement in feed conversion ratio, reducing the pollution from the metabolic waste and faeces (Cheng et al., 2004; Cao et al., 2007; Dalsgaard and Pedersen, 2011). The optimum level of phytase supplementation in rainbow trout diets was approximately 500 mUkg diet (Cheng et al., 2004). The addition of fungal phytase diet could not increase nutrient digestibility in trout Orisasona et al. (2017). Phytase activity is defined as FTU, FTT, PU and U (Selle and Ravindran, 2007). The addition of fungal enzyme of phytase, as well as the increase in phosphorus level in feed, did not significantly affect the growth and feed conversion ratios (Dalsgaard et al., 2009). The use of microbial enzyme in biotechnological applications is promising to allow for higher availability (Chung, 2001; Maenz, 2001). Besides, use of 2000 FTU phytase enzyme in the diet significantly reduced the phosphorus waste output (Biswas et al., 2007).

Doğan and Bircan (2010) reported that the rate of hazelnut meal in the feed of rainbow trout did not affect the hepatosomatic index, and viserosomatic index. In all experimental groups, protein (90.93% - 9.176%) and fat (96.78% -98.33%) digestibility rates were quite high, but no statistical difference was found between them. In another report, use of different ratios of canola meal instead of fish meal in *Lateolabrax japonicus* diets and the increase in this ratio negatively affected feed conversion ratio, specific growth rate slowed down digestive enzyme activity and significantly reduced the digestibility of dietary dry matter, protein, lipid and phosphorus (Cheng et al., 2010).

Özgür Yigit et al. (2018) reported that feeds containing protease and phytase did not produce a significant effect on the growth, feed conversion ratio, protein and lipid digestibility in the rainbow trout groups. Vielma et al. (1998) found that on the phosphorus utilization in rainbow trout had been reduced by feed containing phytase and cholecalciferol.

Hernandez et al. (2004) observed that the phosphorus retention efficiency of rainbow trout juveniles was higher than that of the group fed with the feed containing high level of fish meal, suggesting that nitrogen retention rates were similar for all experimental groups. In adult fish, phosphorus and nitrogen retention rates in the body were found to be lower than the juveniles.

In this study, it is aimed to investigate the effects of diets containing different amounts of soybean and hazelnut meal, fish meal, and phytase on the development, body chemical structure and the feed digestibility in rainbow trout (*O. mykiss*) juveniles. It is hypothesized that an increase in feed digestibility rate should improve the growth and feed conversion rate in the fishes, and more environmentally sustainable aquaculture practices are needed to be performed by sticking to the natural and economical principles.

MATERIAL and METHODS

Experiment location, duration, and fish species

In the study, 450 rainbow trout (*O. mykiss*) juveniles (27.82 ± 0.04 g as mean live weight) obtained from a private company were used. Trials were conducted at Kepez Trout Unit of the Mediterranean Fisheries Research, Production and Training Institute. The fish were used in the trial after being kept in quarantine for 15 days in order to ensure that they were in good health for the trials. Trial duration was considered as 75 days. In order to determine the feed digestibility after the trials, the fish were fed *ad libitum*. Fecal samples were collected twice a week for 3 weeks.

Experimental diets and design

The raw materials used in the trial diets were purchased from Kağsan Karadeniz Food and Agriculture Industry Inc. Trial diets used to feed rainbow trout juveniles contain different amounts of fish meal protein (FMP), hazelnut meal protein (HMP) and soybean meal protein (SMP), without the enzyme phytase “0 FTU” (-) and with “1000 FTU” (+). Six equally prepared trial diets (D) with 42% of isonitrogenous crude protein values (CPV) were formulated as follows (Table 1), (D_{1-}, D_{1+} = 30% FMP+ 35% HMP+ 35% SMP; D_{2-}, D_{2+} = 40% FMP+ 30% HMP+30% SMP; D_{3-}, D_{3+} = 50% FMP+ 25% HMP+25% SMP).

The study was carried out in three replicates in 18 trial tanks (400 L). 25 juvenile fish were randomly allocated to each of the six groups (Table 1).The fish were fed with the feed corresponding to approximately 2% of their daily live weight in the morning and evening. Each tank was supplied with 12L water per minute. In the trials, water with a temperature of 17.5 ± 0.52ºC, dissolved oxygen of 9.2 ± 0.55 mg / L, and pH of 8.0 ± 0.08 was used. In addition, 0.5% of chromic oxide was added as an indicator in order to determine nutrient digestibility in the trial diets. Prepared trial diets were stored at + 4°C until use.

Table1. Distribution of raw material and nutrient contents according to crude protein structure of trial diets (%).

Feed raw materials		**Distributions of raw materials according to raw material contents of trial diets**					
		D_1	**D_1+**	**D_2**	**D_2+**	**D_3**	**D_3+**
Phytase (FTU)		0(-)	1000(+)	0(-)	1000(+)	0(-)	1000(+)
Fish meal		18		24		30	
Soybean meal		28.8		24.8		20.4	
Hazelnut meal		33		27.8		23	
Corn gluten		2.4		1.7		1	
Wheat meal		1.64		2.99		5.49	
Corn meal		0		3.85		7.05	
Vitamin mixture		0.5		0.5		0.5	
Mineral mixture		0.11		0.11		0.11	
Fish oil		14.4		13.1		11.3	
Carboxymethyle cellulose		0.5		0.5		0.5	
Choline chloride		0.15		0.15		0.15	
Chromic acid		0.5		0.5		0.5	
Unit feed		100		100		100	
Proximate analysis: Diets by dry matter (%)	Moisture	6.39	6.61	6.32	6.22	5.93	6.55
	DM	93.1	93.39	93.68	93.78	94.07	93.45
	CA	8.53	8.71	8.74	8.81	8.93	9.13
	L	17.64	21.09	14.32	18.79	15.04	18.72
	CP	42.64	42.92	42.54	42.44	42.02	42.86
	Total	68.80	72.72	65.60	70.04	65.99	70.71

Dry matter ‘DM’, Crude Ash ‘CA’, Crude Lipid ‘CL’, Crude protein ‘CP’, D_1 [%30 FMP+ (%35 SMP + %35 HMP)], D_2 [%40 FMP + (%30 SMP + %30 HMP)], D_3 [%50 FM + (%25 SMP + %25 HM)]. Phytase 0 FTU ‘-‘, 1000 FTU ‘ + ’,

Analysis and calculations of growth parameters

This study investigated the effects of diets containing equal ratios of hazelnut meal and soybean meal and different ratios of fish meal and phytase on the growth parameters of rainbow trout juveniles and the digestibility of nitrogen and phosphorus in feeds. In periodical intervals (15 days), the fishes were anesthetized with 0.3 mL / L phenoxyethanol.

Then, their total length (mm) was measured with the aid of a caliper, and their live and internal organs weights (g) were measured with an electronic scale with 0.01 precision. Feed requirements were determined by calculating the feed conversion rates periodically. For the digestion analysis, the anesthetized fishes were dried with blotting paper. Then, the feces samples were collected by rubbing the abdominal area and pressing on it. This process was repeated every three days. All collected feces samples were combined in a way to represent the average, and then stored in the deep freeze until the analysis.

The analyses of dry matter (DM), crude protein (CP) and crude lipid (CL) (James, 1999), crude ash (CA) (AOAC, 2000), fecal chromium oxide (Furukawa and Tsukahara, 1966) and phosphor amount (James, 1999), and the calculations of total energy (Henken et al., 1986), dry matter digestion (Cheng et al., 2004), nutrient digestion coefficient, retained nutrient rate (Vielma et al., 2002), nutrient gain, nutrient uptake and mean weight gain were performed in trial diets. Live weight gain (LWG), feed conversion ratio (FCR), condition factor (CF), specific growth rate (SGR) and protein efficiency ratio (Akpınar et al., 2012) and viscerosomatic index (VSI), hepatosomatic index (HSI), and carcass yield (CY) were calculated (Metailler, 1987).

Statistical Analysis

After the arcsin transformation, Shapiro-Wilk W test for data normality and Bartlett test for data homogeneity were performed. Then, all percentages were calculated. Differences between the averages of the trial group were analyzed with One Way Variance Analysis (ANOVA) followed by Tukey multiple comparisons. Two-way ANOVA was used to test whether the addition of phytase to the feed has an effect on the parameters included in the study. Tukey multiple comparisons were performed to determine whether there is any difference among groups, $P<0.05$ was considered significant. Data were analyzed using the JMP 8.0 Statistical Package Program (SAS Institute and Inc., 2008) Analysis results are presented as the mean standard error (mean ± SE).

RESULTS

Growth and feed conversion ratio

Trial diets (D_1, D_2 and D_3) contain fish meal protein (30%, 40% and 50% respectively) as the protein of animal origin and semi soybean meal protein+semi hazelnut meal protein, (70%, 60% and 50%, respectively) as plant-based protein, without the phytase enzyme (0FTU) and with the phytase enzyme (1000FTU) (Table 1).

The effects of trial diets, contents of which are given above, on the growth parameters, body chemical composition, organ indexes, and nutrient digestibility in rainbow trout juveniles are summarized in Table 2-6. The fishes having an initial weight of 27.85-27.88 g gained 80.63-92.20 g at the end of the experiment. The most live weight gain (LWG) was in the group fed with D_{3}-, while the least live weight gain was in the group fed with D_{1+}. Furthermore, phytase enzyme added to the feeds (D_1, D_2 and D_3) did not shown any significant difference ($P>0.05$).

Table 2. Effects of trial feeds on the growth parameters and feed conversion ratio in rainbow trout juveniles (#).

Diets	IW(g/fish)	FW (g/fish)	LWG (g/fish)	F Con. g/fish)	FCR	SGR (%/day)	PER
D_1-	27.8±0.02	87.84±0.61[abc]	59.96±0.62[abc]	67.86±0.56[abc]	1.13±0.02[abc]	1.53±0.01[ab]	1.94±0.04[ab]
D_{1+}	27.8±0.04	80.63±3.16[c]	52.80±3.19[c]	64.12±1.67[c]	1.22±0.05 [c]	1.42±0.05[b]	1.79±0.07[b]
D_2-	27.8±0.04	82.80±1.84[bc]	54.97±1.88[bc]	66.56±1.16[bc]	1.21±0.02 [bc]	1.45±0.03[ab]	1.82±0.03[ab]
D_{2+}	27.8±0.05	83.86±2.37[abc]	56.06±2.34[abc]	67.87±1.62[abc]	1.21±0.02 [abc]	1.47±0.04[ab]	1.82±0.04[ab]
D_3-	27.8±0.05	92.20±0.33[a]	64.35±0.38[a]	72.17±0.19[a]	1.12±0.01 [a]	1.60±0.01[a]	2.00±0.02[a]
D_{3+}	27.8±0.03	90.20±1.10[ab]	62.35±1.12[ab]	70.29±0.44[ab]	1.13±0.02 [ab]	1.57±0.02[a]	1.93±0.03[ab]
Two-way Anova							
DPS		0.002	0.002	0.001	0.016	0.004	0.048
F		0.099	0.104	0.139	0.159	0.125	0.008↓
DPS * F		0.121	0.125	0.111	0.205	0.139	0.165

(#)Different letters in the same column indicate significant differences between group averages (P<0.05).
¤ Diet 'D', Dietary protein source 'DPS', Phytase 'F', 0 FTU '-', 1000 FTU '+', Initial weight 'IW', Final weight 'FW'. Live weight gain 'LWG', Feed consumption 'F Con', Feed conversion ration 'FCR', Specific growth ratio 'SGR', Protein efficiency ratio (PER).

'DPS*F' interaction was found to be non-significant on the feed consumption of trial groups (P>0.05). However, differences in the average feed consumption per fish at the end of the experiment were found significantly (P <0.05) depending on the trial diet with a protein of the plant-based or animal origin and on their rates (P<0.05).The most feed consumption was observed in the D_3- group where the growth was the most, while the least feed consumption was in the D_{1+} having the least growth (Table 2).

The interaction between the 'DPS*F' factors on the specific growth rate of the trial groups was found to be non-significant (P>0.05).At the end of the experiment, the greatest SGR was in the D_3- group (1.60), whereas the least SGR was in the D_{1+} group (1.42) (P<0.05).

In terms of FCR, D_3- group had the highest value with 1.12 while D_{1+} group exhibited the lowest value with 1.22.In FCR, the difference between (D_2>D_3) = D_1 group was found significantly. At the end of the experiment, differences between FCR values in the groups were significantly (P <0.05), depending on the source and rates of plant- or animal-based proteins used in the trial diets (P<0.05).Increased amount of fish meal protein in diets positively affected FCR (P<0.05). However, the presence or absence of the phytase enzyme in the experimental diets presented no difference between the groups (P> 0.05).

The interaction between the 'DPS*P' factors on the protein efficiency ratio (PER) in the experimental groups was found non-significant (P> 0.05). At the end of the experiment. D_3- group had the highest PER value (2.00), while D_{1+} displayed the lowest PER value (1.79).Diet protein source and phytase of the trial diets affected PER values of experimental groups (P<0.05). Adding phytase to the feeds reduced significantly PER.

Organ indexes

Values of viscerosomatic index (VSI), carcass yield (CY), condition factor (CF), and hepatosomatic index (HSI) of the experimental groups are given in Table 3 At the end of the experiment, interaction between 'DPS*F' factors on the VSI, CY, CF, and HSI and the effects of dietary protein source and phytase were found to be similar for all groups (P>0.05).

Table3. Effects of trial diets on the organ indexes in rainbow trout juveniles.

Diets	Viscerosomatic index (%)	Hepatosomatic index (%)	Condition factor	Carcass Yield (%)
D_1-	13.28±0.27	1.26±0.02	1.40±0.02	85.10±0.28
D_{1+}	13.02±0.28	1.17±0.08	1.41±0.04	85.38±0.36
D_2-	13.39±0.63	1.34±0.16	1.40±0.06	85.07±0.70
D_{2+}	13.70±0.58	1.40±0.12	1.45±0.04	84.64±0.65
D_3-	14.09±0.13	1.27±0.01	1.43±0.01	83.98±0.13
D_{3+}	12.98±0.24	1.22±0.07	1.45±0.06	85.24±0.30

The lowest VSI (12.98%) was observed in D_{3+} group having a supplement of the lowest phytase, while the highest VSI (14.09%) was found in D_3- group without phytase. The lowest HSI was in D_{1+} with 1.17%, as the highest HSI was recorded in D_2- with 1.40%. In terms of condition factors, D_1- had the lowest value (1.40), whereas D_{3+} had the highest value (1.45). When the Table 3 is examined in terms of carcass yield, the lowest value was found in the D_3-group with 83.98%, as the highest value was recorded in D_{1+} with 85.38%.

Body composition

The initial and final body compositions of the groups are given in the Table 4. At the end of the experiment, interaction between 'DPS*F' factors on the dry matter, crude ash, crude lipid, crude protein and phosphor in rainbow trout juveniles and the effects of dietary protein source and phytase were similar for all groups (P>0.05). Selected factors also had no significant effects on the body composition, although there was a proportional difference between the initial and final body compositions.

Table4. Effects of trial diets on the body composition in rainbow trout juveniles (%).

Diets	Dry matter	Ash	Lipid	Protein	Phosphor
Before experiment	27.18	2.45	9.87	13.63	0.62
D_1-	31.15±0.50	2.28±0.01	12.47±0.15	14.84±0.18	0.59±0.03
D_{1+}	31.66±0.48	2.37±0.10	12.45±0.52	14.67±0.70	0.59±0.01
D_2-	31.84±0.48	2.30±0.05	12.87±0.47	14.59±0.35	0.62±0.00
D_{2+}	30.84±0.12	2.35±0.03	12.11±0.35	15.21±0.09	0.60±0.00
D_3-	31.22±0.37	2.22±0.15	12.30±0.11	14.82±0.24	0.59±0.03
D_{3+}	30.73±0.09	2.21±0.05	11.81±0.13	14.71±0.06	0.56±0.02

Digestibility rates of trial diets

Analysis results of digestion in the diets are given Table 5. It was found that the digestibility coefficients of dry matters in trial diets ranged from 50.83% to 62.34% and that the interaction between 'DPS*F' factors and the effects of dietary protein source and phytase were similar among the groups (P>0.05).

Digestion coefficients for dry matters were higher in those containing the phytase than in those without it among D_1, D_2 and D_3. It is most likely that the digestibility coefficients of dry matter were positively affected by the presence of phytase enzyme.

It was observed that the digestibility coefficients of dietary lipid varied from 93.23% to 96.33 % and that interaction between 'DPS*F' factors on digestibility coefficients of dietary lipid and the effects of dietary protein source were non-significant among the groups (P>0.05). However, phytase enzyme had an important effect on the digestibility rate of dietary lipid, suggesting that there was a statistically significant difference between the trial groups ($D_3 = D_1 > D_2$) (P<0.05).

Table5. Digestibility rates of diets in trial group fish ($^{\#}$).

Diets	DM	Lipid	Protein	Phosphor	Energy
D_1-	60.90±3.87	95.22±0.93 ^{a}	83.07±1.43	64.43±2.85^{ab}	73.80±2.55 ^{ac}
D_{1+}	62.34±3.57	94.79±0.53 ^{a}	84.37±1.12	74.38±1.19^{a}	74.99±2.35 ^{ac}
D_2-	53.08±5.64	93.23±0.33 ^{b}	79.70±2.12	55.80±6.73^{b}	66.87±3.78^{b}
D_{2+}	57.85±0.61	93.76±0.21 ^{b}	81.47±0.16	74.11±0.38^{a}	76.45±0.28 ^{a}
D_3-	50.83±5.96	94.58±0.79 ^{a}	78.11±2.61	55.37±4.00^{b}	70.21±3.18^{bc}
D_{3+}	60.73±2.98	96.33±0.16^{a}	82.40±1.19	64.99±2.74^{ab}	73.33±1.85^{ac}
Two-way ANOVA					
DPS	0.289	0.212	0.106	0.074	0.507
F	0.141	0.012	0.091	0.001↑	0.048↑
DPS*F	0.606	0.203	0.629	0.422	0.272

($^{\#}$) Different letters in the same column indicate significant differences between group averages (P<0.05).

Digestibility coefficients of dietary protein in trial fishes ranged from 78.11% to 84.37% and interaction between 'DPS*F' factors and the effects of dietary protein source and phytase were similar among the groups (P>0.05).

Digestibility rate of dietary phosphorus varied from 55.37% to 74.38 %, and interaction between 'DPS*F' factors on digestibility coefficients of dietary phosphor and the effect of dietary protein source was non-significant among the groups (P>0.05). On the contrary, the phytase enzyme present in the feed had a positive effect on the digestibility rate of phosphorus in the experimental groups (P<0.05).

The digestible energy ratio varied between 66.87% and 76.45%. The interaction between 'DPS*F' factors on the digestible energy ration and the effect of dietary protein source were found to be statistically non-significant (P >0.05). However, phytase enzyme had a significant effect on the digestible energy ratio (P<0.05), suggesting that it positively affected the groups being fed with D_{1+}, D_{2+} and D_{3+} diets containing the phytase.

Nitrogen and phosphor utilization

Table 6 indicates the values of nitrogen and phosphorus consumption, gain and retention in the diets used to feed the trial group fishes. The interaction between 'DPS*F' factors on consumption, gain and retention of dietary nitrogen, and the effect of dietary protein source and phytase were statistically non-significant among the groups (P>0.05).

The highest nitrogen consumption, gain and retention values were 1173.4 mg / kg average weight (AW) / day, 350.8 mg / kg AW / day and 30.63%, respectively, while the lowest nitrogen consumption, gain and retention values were 1139.8 mg / kg AW / day, 315.9 mg / kg AW / day, and 27.29%, respectively.

The highest phosphorus consumption, gain and retention values were 279.6±1.4 mg / kg average weight (AW) / day, 83.03 mg / kg AW / day, and 33.07%, respectively. While the lowest phosphorus consumption, gain and retention values were 248.8 mg / kg AW / day, 73.69 mg / kg AW / day, and 26.53%, respectively. While the interaction between 'DPS*F' factors on the dietary phosphorus consumption was statistically non-significant (P>0.05), the effects of feed protein source and phytase on dietary phosphorus consumption were found to be statistically significant (P<0.05).

The results of two-way ANOVA analysis showed statistically significant differences between the groups in terms of 'dietary protein source' (P<0.05).In addition, the interaction between 'DPS*F' factors and the effect of "dietary protein source" were statistically non-significant (P>0.05). The interaction between "DPS *F" factors on the phosphorus retention and the effect of 'dietary protein source' were found to be statistically non-significant (P>0.05). However, the results of two-way ANOVA analysis indicated that adding the phytase to the diets significantly affected the phosphorus retention, leading to a reduction in D_1, D_2, and D_3 groups (P<0.05).

Table6. Values of nitrogen and phosphorus consumption, gain and retention in the diets used to feed the trial group fishes (#).

Diets	N Con. (mg/kg OA/day)	P Con. (mg/kg OA/day)	N gain (mg/kg OA/day)	P gain (mg/kg OA/day)	N retention (%)	P retention (%)
D_{1-}	1139.8±15.0	248.9±3.3^b	340.4±6.3	80.15±6.38	29.88±0.83	32.27±2.99^a
D_{1+}	1159.5±11.0	277.7±2.6^a	315.9±3.2	73.69±2.13	27.29±2.79	26.53±0.70^b
D_{2-}	1165.5±2.5	252.9±0.6^b	319.4±5.8	83.03±1.58	27.41±0.54	32.82±0.59^a
D_{2+}	1173.4±5.8	279.6±1.4^a	342.5±9.4	79.81±1.75	29.19±0.76	28.55±0.67^b
D_{3-}	1145.6±5.3	248.8±1.2^b	350.8±8.2	82.33±5.61	30.63±0.83	33.07±2.11^a
D_{3+}	1165.3±8.2	272.4±1.9^a	342.2±4.9	74.40±4.14	29.37±0.57	27.33±1.61^b
			Two-way ANOVA			
DPS	0.116	0.049	0.393	0.546	0.409	0.752
F	0.051	<0.001↑	0.776	0.103	0.532	0.003↓
DSP*F	0.756	0.461	0.259	0.841	0.271	0.884

(#) Different letters in the same column indicate significant differences between group averages (P<0.05). Nitrogen'N', Phosphorus 'P', Consumption 'Con.', Average weight/day 'AW/d'.

DISCUSSION

Although the interaction between 'dietary protein source (DPS) and phytase (F)' on the final live weight, LWG, SGR and PER in the trial groups was statistically non-significant (P>0.05), these parameters were affected by variations in the amount and rates of fish meal protein, hazelnut meal protein and soybean meal protein found in the diets.

In addition, the increased amount of fish meal in the diet had a positive effect on the final live weight and live weight gain, SGR and PER parameters improved depending on the increased amount. Improvement in FCR also seems to support these increases (Table 2, P<0.05). These results are consistent with those of Cheng et al. (2004); Cao et al.(2007); Cheng et al. (2010); Pratoomyot et al. (2010); Doğan and Bircan (2010); Diler et al. (2012); Zhang et al. (2012); Karabulut et al. (2017); Orısasona et al. (2017) and Özgür Yigit et al. (2018). However, our findings are inconsistent with the results of Vielma et al. (2000); Ruohonen et al. (2007); Cheng et al. (2010); Pratoomyot et al. (2010); Burr et al. (2012); Zhang et al. (2012).

Presence or absence of phytase in diets containing the same rate of plant-protein did not create any marked difference in these characteristics of the groups (P> 0.05). These results are in line with those of Dalsgaard et al. (2009) and Özgür Yigit et al. (2018). However, our findings are inconsistent with the results of Vielma et al. (2000); Ruohonen et al. (2007); Burr et al. (2012). One of the possible reasons why phytase has no effect may be that water temperature is not optimal for the enzyme activity.

Moreover, it is understood from these results that feed structures inappropriate for the nutrient requirements of carnivorous fishes due to various concerns in terms of fish production and feed costs have a negative impact on the fish growth and feed digestibility. These results are consistent with those of Biswas et al. (2007); Cao et al. (2007); Cheng et al.(2010); Pratoomyot et al. (2010); Burr et al. (2012).

This study showed that the most feed consumption was in the group D_{3-} (72.17±0.19g) where the growth was the highest and our findings are inconsistent with the results of Cheng et al. (2010); Pratoomyot et al. (2010). Our study also revealed that the groups fed with D_{3-} and D_{3+} diets containing more amount of fish meal protein had higher final live weight, live weight gain, FCR, SGR, and PER values than the other groups (Table 2).

It can be inferred from the current data that the distribution of raw material contents and ratios of the D_{3-} and D_{3+} is relatively more important than the other diets in terms of biological fulfillment of fish nutrient requirements and fish welfare. Aquaculture should be performed appropriately for the fish biology. Therefore, its main objective should be to reduce the feed costs, improve the product quality, increase the biomass, and reduce the waste output released into a receiving environment.

It was found that the effects of factor levels on the final VSI, CY, CF, and HSI were similar for all groups, and there was no interaction between them (P >0.05). These results are in line with those of Doğan and Bircan (2010). We think that the absence of difference between body indexes of fishes in the experimental groups maybe due to the fact that the trial diets were prepared suitably for the natural nutrient requirements of the fish, and feeding ratio and frequency were few.

It has been observed that the lowest ratio of HSI was in the D_{1+} with 1.17%, while the highest HSI value was in the D_{2+} with 1.40%. As the amount of plant-based raw materials in carnivorous diets increases, the value of HSI in living organism also increases.

While CF was the lowest in the D_{1-} group with 1.40, it was the highest in the D_{3+} group with 1.45. The fact that CF remains at this level also suggests that the feeding ratio is relatively low.

The carcass yield was found to be the lowest in the D_{3}-group with 83.98% and the highest in the D_{1+} group with 85.38%. It is believed that one reason for not being able to detect a difference between the final VSI, CY, CF and HSI values may be the trial duration. Values of final crude protein, crude lipid, and dry matter in the groups are increased slightly when compared to the baseline values (Table 4), suggesting that the final values are affected more positively by the present feeding regimes than the pre-trial feeding profiles. These results are consistent with those of Morris et al. (2005). Levels of dietary protein source and phytase did not produce a significant effect on the body phosphorus levels of fishes in the experimental group. Even it has been found that these values are relatively lower in the experimental groups. It is thought that this is similar to the level of pre-trial mineralization in the fish body, and joint evaluation of the calcium and phosphorus relationship would be useful.

The effects of dietary protein source on the digestibility of these nutrients present in the trial diets were found to be similar (P>0.05). These results are consistent with those reported by Cheng et al. (2004); Doğan and Bircan (2010); Özgür Yigit et al. (2018), despite being inconsistent with the results of Harlıoğlu (2011). Digestibility ratio of dietary protein in D_1, D_2 and D_3 fed with the diets containing phytase enzyme was found to be relatively higher than that of other groups.

The phytase enzyme present in the diets had a significant positive effect on the digestible energy, lipid and phosphorus digestibility (P<0.05). Our findings are inconsistent with the results of Cao et al. (2007). These values were shown to be higher in all the groups fed with D_{1+}, D_{2+} and D_{3+} diets containing phytase enzyme. It is clear from the results shown in Table 5 that the aquaculture studies would contribute significantly to the reduction of the environmental impacts of organic phosphorus burden since phytase enzyme added to the diets improves the digestibility ration of dietary phosphorus (P <0.05). These results are also supported by those reported by Cheng et al. (2004); Biswas et al. (2007); Cao et al. (2007); and Dalsgaard and Pedersen (2011). We believe that the digestibility ratios of dietary dry matter used in terms of the relationship between fish production amount, unit cost per growth and environment should be further improved.

It was determined that the effects of factor levels on the dietary nitrogen consumption, gain, and retention in fishes of the experimental group were statistically non-significant (P> 0.05). These results are different from those of Chung (2001). The effects of DPS and F factor levels on consumption of dietary phosphorus were found to be statistically significant (P <0.05). These results are in line with those of Vielma et al. (1998); Maenz (2001); Chung (2001); Cheng et al. (2004); Hernandez et al. (2004) Biswas et al. 2007; Cao et al. 2007; Harlıoğlu (2011), despite being inconsistent with those of Dalsgaard et al. (2009). Regardless of which proportion plant-derived proteins are used in the diets, phytase-added diets provided a higher amount of phosphorus consumption than the phytase-free ones (P<0.05). These results are also supported by those reported by Biswas et al., 2007; Cao et al., 2007.

The two-way ANOVA analysis revealed statistically significant differences between the groups in terms of "dietary protein source" factor levels (P<0.05). The increase in the amount of fish meal protein in the trial diets also positively affected the quantity of phosphorus consumed by the fishes in the experimental group. These results vary depending on whether the diets contain dietary protein source and phytase enzyme (P<0.05). Effect of factor levels on dietary phosphorus gain was found to be statistically non-significant in experimental group fishes (P>0.05). A significant reduction was shown in the dietary phosphorus gain and retention in the groups fed with $D_{1+,}$ D_{2+} and D_{3+} containing the phytase enzyme. Biswas et al. (2007); Cao et al. (2007). This suggests that the parameter 'phosphor gain' would be more meaningful because the fishes are able to benefit from the dietary phosphorus more efficiently. These results are consistent with those of Biswas et al. (2007); Cao et al. (2007), despite being inconsistent with those of Vielma et al. (2000); Ruohonen et al. (2007); Zhang et al. (2012).

In conclusion, the decreased amount of plant-based hazelnut meal and soybean meal protein added to the six trial diets used for feeding rainbow trout juveniles had a positive effect on the growth parameters (P<0.05), but the level of phytase enzyme did not produce any effect on these parameters. D_3- and D_{3+} diets may be recommended for the fish biology and growth. It should also be noted that all diets produce similar effects on organ indexes and body chemical components of fish but they will be in the order of D_1>D_2>D_3 in terms of feed cost. Vegetable source protein ingredients contain anti-nutritional factors that reduce the uptake of nutrients and minerals, especially in carnivorous fish, by increasing the waste output in this way. The effect of dietary protein source on the digestibility ratios of dietary nutrients is insignificant, but the diets containing phytase achieve a significant effect in some groups. Depending on whether or not the diets contain the phytase enzyme, it produces an effect on dietary nitrogen and phosphorus consumption, gain and retention.

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