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Possibilities of Using Poultry By-product Meal Instead of Fish Meal as An Alternative Protein Source in Rainbow Trout (*Oncorhynchus mykiss,* W.) Feeds: Growth Performance and Unit Production Cost

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Abstract : The way to grow cheaper fish is to use protein sources that can be an alternative to fish meal that is considered on the most expensive feed ingredient in fish feeds. For this purpose, poultry by-product meal (PBM) which is locally sourced and cheaper than fish meal, was used to replace 25, 50, 75 and 100 % of fish meal protein in rainbow trout (*Oncorhynchus mykiss*) poultry by-product meal was not included in the control feed. Feeds in this investigation were prepared as similar protein (43% CP) and digestible energy (13.28 MJ. DE kg^{-1}) levels. A total of 300 rainbow trout with 50.6±1.35 g of average initial weight were used in the investigation. All the diets including the control were fed the rainbow trout in triplicate for 70 days. At the end of the trial, the difference between feeds I, II, II, IV and feed V was significant in terms of average live weight gain (ALWG) and feed consumption throughout the investigation ($P<0.05$). No difference was observed in terms of feed conversion ratio (FCR) and protein conversion ratio (PER). However, considering the cost of producing 1 kg of fish, it is obvious that although the cheapest fish is obtained from the group fed with IV and V feeds ($P<0.05$), it will take weeks for the fish in these groups to reach the average live weight of groups I, II and III. As a result, it was concluded that poultry by-product meal could be used instead of 75% of fish meal protein in trout feeds in terms of a normal fish development process.

Keywords: Rainbow trout, fish meal, poultry by-product meal, growth performance, fattening cost

Gökkuşağı Alabalığı (*Oncorhynchus mykiss,* W.) Yemlerinde Alternatif Protein Kaynağı Olarak Balık Unu Yerine Tavuk Unu Kullanma Olanakları: Büyüme Performansı ve Birim Balık Maliyeti

Öz: Daha ucuz balık yetiştirmenin temel yolu, balık yemlerinde önemli bir yeri olan balık unu yerine alternatif olabilecek protein kaynaklarının kullanılmasıdır. Bu amaçla araştırmada gökkuşağı alabalığı (*Oncorhynchus mykiss*) yemlerinde hem yerli üretimimiz hem de balık ununa oranla daha ucuz olan tavuk unu, balık ununun bir kısmı ya da tamamı (balık unu proteininin % 25, 50, 75 ve 100'ü) yerine kullanılmıştır. Kontrol yeminde ise tavuk unu yer almamıştır. Deneme yemleri benzeri protein (%43 HP) ve enerji (13.28 MJ $SE.kg^{-1}$) içeriğine sahip olarak hazırlanmıştır. Ortalama 50,6±1.35 g olan 300 adet balığın kullanıldığı deneme 10 hafta sürmüştür. Deneme sonunda, I, II, II, IV nolu yemler ile V nolu yem arasında besi boyunca ortalama canlı ağırlık artışı (OCAA) ve yem tüketimi açısından bulunan farklılık önemli olmuştur ($P<0.05$). Yemden yararlanma oranı (YYO) ve proteinden yararlanma oranı (PYO) söz konusu olduğunda ise farklılık gözlenmemiştir. Ancak 1 kg balık üretme maliyetine bakıldığında en ucuz balığın IV ve V nolu yemler ile beslenen gruplardan elde edilmesine ($P<0.05$) karşın bu gruplardaki balıkların I, II ve III nolu grupların ortalama canlı ağırlığına ulaşmasının haftalar alacağı aşikardır. Sonuç olarak normal bir balık gelişimi süreci bakımından tavuk ununun alabalık yemlerinde balık unu proteininin % 75'i yerine kadar kullanılabileceği sonucuna varılmıştır.

Anahtar Kelimeler: Balık unu, Gökkuşağı alabalığı, tavuk unu, büyüme performansı, besi maliyeti

1.Introduction

Adequate and balanced nutrition is a serious concern, especially in countries where the population incresing rate is high and the social income balance is not good, and this unfortunately will be a predominant issue the future. In this respect, it is an important issue to make the animal protein sources for human consumption economically is a trend topic producing globally. Fish consumption, on the other hand, is taken into account in terms of both healthy nutrition and the potential to be obtained cheaply. Protein is the most essential nutrient in fish feeds. Fish meal is widely used in aquafeeds specifically for farmed carnivorous fish species. However, despite the increasing demand for fish meal, the shrinkage observed in production every year and the price fluctuation accordingly, due to the different quality caused by the fish it is produced and the production methods, alternative protein sources have been sought.

Poultry by-product (PBM) meal is a feed ingredient that is similar to fish meal in terms of protein digestibility (94% and 98; Hardy, 2000). It can be said

that the use of PBM in fish feeds will increase gradually, since it is domestically produced and close to fish meal in terms of nutrients. Methionine level is higher in PBM than fish meal. In terms of other essential amino acids, the values contained in PBM are very close and balanced to that of fish meal. The high crude fat (CF) content of PBM is also remarkable in terms of salmon and trout feeds, which have high energy content. Although PBM has a good place as a protein source in fish feeds, its quality varies depending on the essential amino acid profile it contains (Davies et al., 1991). In general, PBM contains 55-65% CP, 14-30% CF, 12-21% ash, and the average nutrient content is presented in Table 1 with fish meal (FM), meat meal (MM), meat-bone meal (MBM), blood (BM) meal and hydrolyzed feather meal (HFM) comparatively.

Along with the prohibitions that started in the European Union (EU) countries, within the scope of the harmonization framework with EU laws, important restrictions have been introduced on the inclusion of animal protein sources in the feed of especially terrestrial farm animals. This is due to the prevention of the spread of certain diseases in recent times. There is no such problem for PBM, in which high temperatures are used in its production. (Özaslan, 2004). However, the extruded pellet making process also requires high temperature application.

Since fish meal, which is widely used in fish feeds, is unfortunately not produced enough in Turkey, a large part of the need is met through imports. However, chicken production, chicken meat consumption per capita and, accordingly, PBM production are increasing year by year in our country. In this context, the use of animal protein in feeds used in chicken production is prohibited within the framework of the European Union (EU). If this practice, which is called a feed ban in Turkey within the scope of harmonization with the European Union, comes into effect, its impact on poultry meat integration will also have significant and serious economic dimensions.

Table 1. Average Nutrient Composition of Different Animal Protein Sources, % (Bilgüven, 2002).
Çizelge 1. *Farklı Hayvansal Protein Kaynaklarının Ortalama Besin Madde Bileşimi, % (Bilgüven, 2002).*

Ingredient	PBM	FM	MM	MBM	BM	HFM
Moisture	7.0	8.0	6.0	7.0	9.0	7.0
Digestible Energy (DE), kcal/kg	3916	3567	3311	3233	3599	3882
Crude Protein	58.7	60.0	51.4	50.4	85.6	84.9
Crude Fat	13.1	10.0	9.1	9.7	1.3	2.9
Calcium	3.51	6.25	8.85	10.3	0.48	0.25
Phosphorus	1.81	0.59	4.44	5.1	0.24	0.65
Sulfur	0.52	0.12	0.47	0.25	0.34	1.47
Arginine	3.77	4.0	3.6	3.49	3.57	7.05
Histidine	1.01	1.4	0.96	0.96	5.14	0.99
Isoleucine	2.38	2.6	1.75	1.64	0.9	4.06
Leucine	4.0	4.8	3.19	3.06	10.91	6.94
Lysine	2.89	1.5	3.23	2.9	7.4	2.32
Methionine	1.06	2.4	0.7	0.65	0.87	0.55
Phenylalanine	1.84	2.5	1.81	1.7	5.85	3.05
Threonine	1.94	0.7	1.64	1.65	3.62	3.97
Tryptophan	0.46	0.3	0.34	0.3	1.04	0.52
Valine	2.89	4.5	2.52	2.45	7.48	6.48

In a study conducted by Yiğit et al. (2006) with Black Sea turbot (*Psetta maeotica*) fry (30 g), It was concluded that instead of fish meal, poultry by-product meal can be used at a rate of 25% instead of fish meal protein without any negative effect on growth and feed intake.

Yanık and Aras (1999) reported that 25-50% of poultry by-product meal can be substituted for fish meal in trout feeds.

Yones and Metwall (2015) fed juvenile Nile tilapias (*Oreochromis niloticus*) with feeds used 50, 75 and 100% PBM instead of fish meal and containing %30 CP. PBM was not included in the control feed. No difference was found in terms of final live weight, body weight gain, feed conversion ratio (FCR) and protein efficiency ratio (PER) and specific growth rate, so researchers have reported that up to 100% PBM can be used in the feed of juvenile Nile tilapia.

Chaklader et al. (2020) in their study conducted with an average of 3.58±0.01g Asian sea bass (*Lates calcarifer*) were fed in recirculated sea water tanks for 6 weeks with two different feeds containing 48% CP and 13% CF, in which PBM was not included or 100% contained. At the end of the trial, the final live weight and specific growth rate (SGR) and feed conversion

ratio (FCR) were significantly lower in the group fed with PBM substituted for whole fish meal ($P<0.05$).

Fish meal is widely and intensively used in the feeds of carnivorous fish and is the most important factor in the cost of fish feeds due to its importation and international exchange rate fluctuations. Poultry by-product meal, on the other hand, is a domestic product whose production is gradually increasing due to the increase in chicken consumption. Therefore, in this trial, it was investigated how much of the PBM can be used as a substitute for fish meal and how it affects the 1 kg feed and fish production cost.

2.Materials and Methods

The trial was carried out in a private trout farm in Mersin Elvanlı region. Trial chambers formed with 1 cm mesh stretching on a plastic pipe skeleton of 1x1x1m dimensions were placed In the pool allocated for the trial. 25 fish with an average weight of 50.6±1.35 g were stocked in each compartment and each of the experimental groups was arranged in 3 parallels.

The feeds to be used in the research were made in the feed application unit of Mersin University Faculty of Fisheries. Poutry by-product meal protein was used to replace 0, 25, 50, 75 and 100% of dietary fish meal protein in the experimental feeds, respectively and all the feeds were prepared as isocaloric (13.28 MJ DE kg^{-1}) and isonitrogenic (43% CP) and were pelleted in 2 mm diameter and 6 mm length. The feed ingredients to be used in the feed production process were first ground to medium fineness (max. 0.2 mm). Trial feeds were pressed through discs. After feed production, trial feed samples were analyzed for nutrient content The structure and nutrient analysis of the feeds used in the study are presented in Tables 2 and 3.

Fish were fed twice a day at saturation (*Ad libitum*) level and weighed every two weeks during the experiment. For this purpose, the fish were starved the day before. After these processes, the feeds were also weighed, and the amount of feed consumed in the said period was calculated. Dead fish were recorded regularly, and these individuals were taken into account when calculating feed conversion. Feeding was done slowly and carefully, and it was assumed that all the feeds given were consumed. The trial was completed in 70 days. In the weekly measurements of the water brought to the trial area through open channels, the water temperature was found to be between 12.4-14.8 °C throughout the experiment and the pH was measured as 7,8.

Table 2. Content of Trial Feeds, %
Çizelge 2. *Deneme Yemlerinin İçeriği, %*

Ingredient	Trial Feeds				
	I	II	III	IV	V
Fish Meal	36.0	27.00	18.00	9.00	0
Poultry By-product Meal	0.00	9.00	18.00	27.00	36.00
Meat-Bone Meal	5.00	5.00	5.00	5.00	5.00
Soybean Meal	20.00	20.00	20.00	20.00	20.00
Corn Gluten	11.00	11.00	10.00	10.00	10.00
Wheat Middlings	23.75	24.35	26.05	26.50	27.05
Red Pepper Powder	1.00	1.00	1.00	1.00	1.00
Fish Oil	2.00	1.50	1.00	0.50	0.00
Vitamin Mix.[a]	0.25	0.25	0.25	0.25	0.25
Mineral Mix[b]	0.25	0.25	0.25	0.25	0.25
DL-Methionine	0.10	0.10	0.10	0.15	0.20
L-Lysine	0.15	0.15	0.15	0.15	0.15
Pellet Binder	0.5	0.40	0.20	0.20	0.20
TOTAL	100.0	100.0	100.0	100.0	100.0

[a]Vitamin Mixture (1 kg): Vitamin A: 8.000.000 IU, Vitamin D_3:800.000 IU, Vitamin E: 80.000 mg, Vitamin K_3:4.800 mg, Vitamin B_1:8.000 mg, Vitamin B_2:12.000 mg, Vitamin B_6:8.000 mg, Vitamin B_{12}:20 mg, Vitamin C: 80.000 mg, Niacine: 80.000 mg, Pantothenic acid: 20.000 mg, Folic acid: 2.400 mg, Biotin: 200 mg, İnositol: 120.000 mg.
[b]Mineral Mixture (1 kg): Ca: 672.000 mg, Mg: 16.000, Mn: 24.000 mg, Zn: 32.000 mg, Fe: 24.000 mg, Cu: 2.000 mg, Co: 800 mg, I: 400 mg, Se: 80 mg.

AOAC (1995) standard analysis methods were applied to determine the nutrient composition of the feed and feed ingredients used in the research. Chloroform: methanol extraction (2:1, v:v) was used for crude oil analysis (Bligh & Dyer, 1959).

At the end of the experiment, live weight gain, feed conversion ratio (FCR), protein efficiency ratio (PER) and feed and fish production cost were calculated. PER, which expresses the ratio between the protein consumed and the weight gain of the fish, was calculated according to the following formula (Howe et al., 1965):

$$\text{PER} = \frac{\text{Live Weight Gain (g)}}{\text{Consumed Protein with the diet (g)}} \quad (1)$$

The determined PER value takes into account the crude protein in the feed. In this case, errors that may occur as a result of these changes in fish feeds, which may have different moisture content, can also be corrected.

Protein efficiency rate (PER) reveals how the protein consumed with feed in a certain period is reflected in the live weight gain of fish. The higher this figure, the higher the protein's evaluation. In other words, as the weight gain and feed conversion ratio increase, the PER value also increases. (Steffens, 1989)

Table 3. Nutrient Composition of Trial Feeds, %
Çizelge 3. *Deneme Yemlerinin Bileşimi, %*

Trial Feeds	Moisture	Ash	Crude Protein	Crude Fat	Crude Cellulose	Nitr.Free Extract	Digestible Energy (MJ kg^{-1})
I	8.57	9.95	43.43	8.08	2.61	27.36	13.22
II	9.56	9.34	42.88	8.13	2.88	27.21	13.26
III	8.15	8.73	43.49	7.80	2.84	28.99	13.28
IV	8.08	8.11	43.38	7.67	2.95	29.81	13.30
V	9.01	7.50	43.51	7.45	3.08	29.45	13.32

The wholesale prices in 2022 of the feed ingredients used in trial feeds was determined, then the feed price was calculated by multiplying the feed price with the feed conversion ratio. While expressing the unit fish cost, only feed ingredient prices were taken into account and no other inputs were calculated. The research was carried out in accordance with the "Random Blocks Trial Design" and the SPSS (V.26) program (Anonymous, 2021) was used in the statistical evaluation of the findings collected during the study (Turan, 1995). The groups were compared at the 1% and 5% significance level. (Steel & Torrie, 1981). There is no need for an ethics committee permission report, since no attempt was made on the fish during the experiment.

All fish handling procedures complied with Turkish Ethical guidelines for animal care (No. 28141) set by the Ministry of Food, Agriculture and Livestock, and this study was carried out with the approval of the Mersin University Animal Experiments Ethics Committee (HADYEK) dated 08.02.2021 and number of decisions 06.

3.Results and Discussions

At the end of the experiment, average live weight gain (ALWG), feed conversion ratio (FCR), protein efficiency ratio (PER) values were determined as growth parameters. The results obtained from this study was summarized in Table 4 and Figure 1..

The best average live weight gain of 77.9±6.10 g was obtained from the group fed with feed no. I. This was followed by Groups II, III, IV and V respectively, and The difference between the first 4 groups (I, II, III, IV) and the last group (V) was found to be significant (P<0,05). Group V fish consuming the fishmeal-free feed showed the lowest average body weight gain.

In the experiment conducted by Sevgili and Ertürk (1992) to investigate the most appropriate rate of addition to rainbow trout feeds, the researchers stated that there was no difference in live weight gain between the trout fed with feeds containing up to 20% of PBM and the control group without PBM and also reported that up to 80% of the feed protein can be obtained from this source and attention should be paid to the amino acid levels in PBM.

Gümüş and Aydın (2013) conducted a study with carp (*Cyprinus carpio*) fry with a initial weight of 0.39 g PBM was included in the feeds used at the rate of 25, 50, 75 and 100 %, and it was not used in the control feed. Fish were fed diets containing 34% CP, 9% CF and 15 MJ DE kg^{-1} for 13 weeks. Methionine and lysine have also been added to the feeds in order to provide a better essential amino acid profile. In parallel with the increase in PBM in trial feeds, a decrease in protein utilization and specific growth rate (SGR) was recorded. (P<0,05). No significant difference was observed in body composition.

Wang et al. (2006) used 30 and 50% of PBM instead of fish meal in *Nibea miichthioides* feeds grown in floating net cages and did not find any significance between the groups in terms of final live weight, specific growth rate, feed conversion rate and feed consumption in their research lasted for 8 weeks.

In another study in which 14.1% and 70.2% PBM and hydrolyzed feather meal were substituted for fish meal, Gouveia (1992) reported that body weight gain, feed conversion, specific growth (SGR) and protein efficiency (PER) ratio increased compared to the control group and concluded that chicken meal as a protein source can be included in rainbow trout feeds at a level of 80%.

Findings from the trial showed that PBM protein can be used as a substitute for up to 75% of fish meal protein and is in agreement with the findings obtained from various studies on the subject (Gouveia, 1992; Sevgili & Ertürk 2004; Wang et al., 2006, Gümüş & Aydın 2013). Moreover, Yones and Metwall (2015) stated in their study that PBM can replace 100% of fish meal.

In terms of protein efficiency ratio, the difference between Groups I, II and Groups III, IV, V was significant. (P<0.05). The difference between III, IV and V groups for PER of the was similar. In general, PER value rised up due to the increase in live weight gain and decreased in parallel with the increase in PBM protein in trial feeds. This result is in line with the findings of Gümüş and Aydın (2013), who determined that the special growth and protein efficiency rates decreased significantly due to the increase in the ratio of PBM in the feed. This can be explained by the fact that the biological value of protein in PBM is lower than that of fish meal, despite the addition of synthetic methionine and lysine to feeds containing PBM. Similarly, although I. Group consumed the most feed with 100.80±4.70 g during the fattening period, the best feed conversion ratio was found in Group I and the worst feed conversion

ratio in Group V. In other words, although the fish in Group V consumed proportionally more than the fish in the other groups, the body weight gain was not similar.

The lowest cost feed was the feed of Group V with 9.15 TL due to the decrease in PBM, the cost of feed increased and reached 14.55 TL in feed without PBM. In this respect, the lowest cost of 1 kg of fish production (considering only feed input) was in Group V. However, the average body weights of the fish in this group were followed approximately 30 days behind the average body weights of I, II and III. In this case, a choice who the producer has to make between the gain to be made from the difference in feed cost and the interest income that the gain from the fish sales will provide during the compensation period (ie. the time required for the live weight of the fish consuming the cheapest feed to reach the live weight of the fish consuming the most expensive feed), will be correct.

As a result of the exchange rate fluctuations in the price of fish meal in our country, the decrease in the amount of fish to be processed into fishmeal in the world, and the increase in the demand for fishmeal as a result of the expansion of the livestock sector in which fishmeal is used in parallel with the population increase, fish feed is the most expensive feed all over the world. Therefore, studies investigating the possibilities of using feed ingredients, which can be an alternative to fish meal, in feeds maintain their popularity. In parallel with this situation, the fact that chicken meat is cheaper than red meat in our country has enabled the poultry industry to develop rapidly and to be among the few countries in the world in this regard. Therefore, the production of poultry slaughterhouse residue flour has increased from year to year and the use of animal protein sources in farm animal feeds is prohibited or restricted, It also reveals the necessity of using this product more frequently and intensities in fish feeds.

Table 4. Results of The Experiment.
Çizelge 4. *Deneme Sonuçları.*

Items	Trial Groups				
	I	II	III	IV	V
Trial Period, day	70	70	70	70	70
Total Fish Number	25	25	25	25	25
Survival Rate, %	84	88	80	92	76
Initial Weight, g	49.40±1.21	50.60±1.40	51.40±1.85	50.10±1.33	51.60±0.69
Final Weight, g	127.30±6.93^{a}	119.11±2.05^{a}	122.60±5.52^{a}	118.90±7.31^{a}	96.22±8.12^{b}
Average Live Weight Gain, g	77.90±6.10^{a}	68.51±3.23^{a}	71.19±6.03^{a}	68.85±7.81^{a}	44.62±8.75^{b}
Average Feed Consumption, g	100.80±4.70^{a*}	91.50±3.43^{a}	100.98±6.67^{a}	95.86±8.90^{a}	67.09±6.62^{b}
Feed Conversion Rate (FCR)	1.29±0.06	1.34±0.04	1.42±0.03	1.40±0.09	1.48±0.12
Protein Efficiency Rate (PER)	1.78±0.07^{a}	1.75±0.05^{a}	1.62±0.04$^{bc.}$	1.65±0.03^{bc}	1.52±0.15^{c}
Feed Cost for 1 kg, ₺	14.55	13.17	11.84	10.47	9.15
Fish Cost for 1 kg, ₺	18.84±0.84^{a}	17.57±0.54^{a}	16.83±0.39^{ab}	14.61±0.94^{bc}	13.87±1.60^{c}

* The difference between averages with different letters is significant (P<0.05)

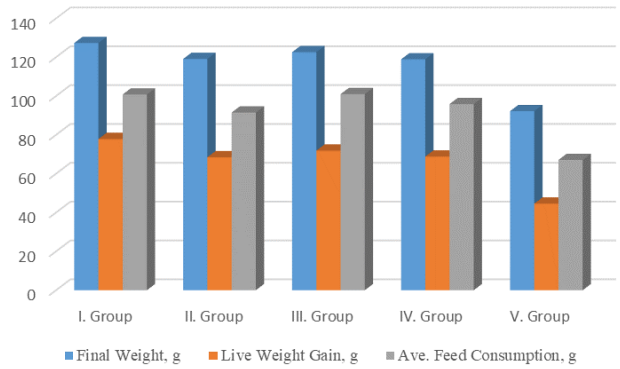

Figure 1. Final Live Weight, Total Live Weight Gain and Feed Consumption At The End of The Trial, g
Şekil 1. Deneme Sonunda Canlı Ağırlık, Toplam Canlı Ağırlık Artışı ve Yem Tüketimi, g

Although the biological value and digestibility of the protein of PBM is lower than that of fish meal, it is a domestic feed ingredient that can be easily used instead of fish meal if its deficiencies are eliminated. Feeds containing PBM can be produced cheaper than the feeds in which fish meal is used at standard levels, and this type of feed has the potential to be easily used in qualitative restricted feeding techniques used in the planning of regular fish sales of the enterprises. In other words, fish producers who can make their own feed, will be able to make year-round sales planning, that is, weight gain control, by obtaining lower weight fish groups at a lower cost, at the rate that they increase PBM in feeds. As it is known, quantitative restricted feeding technique can increase cannibalism due to the fact that trout are carnivorous fish.

Fish deaths occurred after the weighing periods and no relationship was found with the feed groups.

4.Conclusion

It is thought that this study, which aims to use PBM as a substitute for fishmeal protein, will contribute to reducing the demand for fishmeal, which is mostly met by imports. This study, which deals with poultry by-product meal, which is one of the important and domestic feed ingredient of our country, will bring up new research topics to investigate the possibilities of using PBM not only in trout feeds but also in other fish feeds. It is hoped that this trial will provide new recommendations for reducing, at least partially, the dependence on fishmeal.

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