THE INVESTIGATION OF THE POSSIBILITY OF USING RED CALIFORNIA EARTHWORM (EISENIA FETIDA) MEAL AS AN ALTERNATIVE PROTEIN SOURCE IN RAINBOW TROUT (ONCORHYNCHUS MYKISS) DIET

Article *in* Fresenius environmental bulletin · April 2020

CITATIONS
2

READS
408

4 authors, including:

Huriye Ariman Karabulut

28 PUBLICATIONS **74** CITATIONS

SEE PROFILE

İlker Zeki Kurtoğlu

Recep Tayyip Erdoğan Üniversitesi

48 PUBLICATIONS **296** CITATIONS

SEE PROFILE

Turan YUKSEK

Recep Tayyip Erdogan University Faculty of Engineering and Architecture

67 PUBLICATIONS **488** CITATIONS

SEE PROFILE

© by PSP Volume 29 – No. 04A/2020 pages 2951-2959 Fresenius Environmental Bulletin

THE INVESTIGATION OF THE POSSIBILITY OF USING RED CALIFORNIA EARTHWORM (*EISENIA FETIDA*) MEAL AS AN ALTERNATIVE PROTEIN SOURCE IN RAINBOW TROUT (*ONCORHYNCHUS MYKISS*) DIET

Huriye Ariman Karabulut[1,*], Ilker Zeki Kurtoglu[1], Turan Yuksek[2], Zeynep Bayraktar[1]

[1] Faculty of Fisheries and Aquaculture, Recep Tayyip Erdogan University, Rize, 53020, Turkey
[2]Department of Landscape Architecture, Faculty of Fine Art, Design and Architecture, Recep Tayyip Erdogan University, Rize, Turkey

ABSTRACT

This study was carried out to determine the effect of earthworm meal usage on growth performance and body composition in fry rainbow trout feed. The 360 fish with an average weight of 5.05 ± 03.61 g were used in the study. In the experiment, four different feeding groups were designed with 3 replications. These groups are SU0 (Control), SU1, SU2 and SU3 groups which contain 0, 10, 20 and 30% earthworm meal (SU), respectively. Fish were fed three times a day for 90 days with these experimental feeds which were prepared to be 2.5% of the fish live weight. At the end of the study, SU3 group reached the highest weight gain with 259.40%, followed by SU2, SU1 and SU0 (211.17%, 182.76%, 113.68%, respectively). The difference between the SU3 group and the other groups was statistically significant in terms of live weight gain, specific growth rate, feed conversion ratio and protein efficiency ratio ($P < 0.05$). Hepatosomatic index was statistically significant between all groups ($P < 0.05$). As a result, considering the growth performance measurement criteria, it was determined that the use of 20-30% earthworm meal in the rations of fry rainbow trout could increase fish growth significantly.

KEYWORDS:
Earthworm meal, fishmeal, fish nutrition, growth performance, ration preparation, *Oncorhynchus mykiss*

INTRODUCTION

The consumption of fish and fishery products is of great importance in healthy and balanced nutrition of people [1]. Global consumption of fish and fishery products has increased more than two-fold in the last 50 years and has exceeded 20 kg per capita per year in 2015 [2]. Aquaculture capacity in Turkey reached 314.537 tonnes in 2018 while it was 79 thousand tons in 2000 [3]. Despite this rapid development of aquaculture in Turkey in recent years, it has not reached the desired level due to some factors such as feed cost which constitutes 50-75% of production expenses. In order to ensure the sustainability of aquaculture, it is of utmost importance that diversified, healthy and high quality fish feeds are put into use of the industry. In the last 15-20 years, an intensive study program has been conducted on fish feeds, on the alternative animal and plant proteins, and on the fat sources in order to achieve sustainable production in the aquaculture sector. Studies on the advantages and disadvantages arising from the fact that alternative protein sources (especially of plant protein sources) contain different amounts of amino acids and fatty acids from fish meal continue increasingly [4, 5].

Source and quality of the protein in fish feeds is an important issue. The deficiency of protein quality found in vegetative feeds is tried to be compensated with ingredients of animal origin. In addition to essential amino acids such as methionine, lysine and tryptophan, which are missing in the vegetal feeds, some mineral substances are available much more in the feeds of animal origin. The presence of anti-food effects in vegetal feeds restricts the use this kind of feeds in the aquaculture sector [6].

In recent years, it has become more prominent in the aquaculture sector to reduce production costs, increase the efficiency of production systems and promote environmental sustainability. Considering that fish feeds are among the most expensive inputs in the aquaculture sector, the production of cost-effective, environmentally friendly and nutritious fish feeds is of great importance for the sustainable aquaculture sector [7].

Trout farming has a large share in the aqua cultural sector of Turkey. It is important to include feed ingredients of animal origin as protein requirement in the rations prepared for feeding trout [8]. Vermicompost production (vermiculture) has become an increasingly prevalent sector because of the widespread zero waste policies and the use of earthworms in the conversion of organic waste into rich fertilizer for agricultural activities. Earthworms, which are the final product in vermiculture

production, have significant protein values. It has been reported that the addition of worms to pet diets as meal or fresh material increases productivity in farm animals such as fish, chicken, duck [9, 10, 11]. A similar effect of high nutritional earthworm meal on other farm animals has a high potential for rainbow trout farming. The use of high nutritional worms obtained from vermicomposting production as a complementary ingredient in trout feeds is likely to contribute to the development performance of fingerling rainbow trout. Although there are many studies about rendering products, mollusc meal as a source of animal protein; there are very few studies on the use of worms in place of fish meal as an alternative source of protein [12].

In this study, we intended to determine the effect of using earthworm meal in the feed of fingerling rainbow trout (*Oncorhynchus mykiss*) with different ratios on the growth performance and on composition of the fish flesh.

MATERIALS AND METHODS

Fish and Experimental Conditions. This study was carried out at Iyidere Fisheries Research and Application Center of Recep Tayyip Erdogan University. The study was carried out in spring in natural photoperiod with water taken from the stream with its natural flow. A total of 360 fingerling rainbow trout were used, with an average weight of 5.05 ± 03.61 g, with no previous infection and no exposure to toxic substances. Fish were obtained from a private trout farm operating in the Eastern Black Sea region. In the statistical calculations, it was verified that the groups formed at the beginning of the experiment were statistically similar in terms of average fish weights ($P > 0.05$). They were placed randomly in each tank so as to include 30 fry fishes, with 3 repetitions, and a total of 4 different study groups were formed, one being the control group. Thus, a test medium was created in 12 fiberglass tanks with 100 L volume and the study was carried out in a volume of 80 litres of water for 90 days. Their developments were observed in the groups with 0% earthworm meal (SU0), 10% earthworm meal (SU1), 20% earthworm meal (SU2) and 30% earthworm meal (SU3). In order to achieve the adaptation of the fish fingerlings, they were fed with control feed for 10 days. With this process, the adaptation of the fish to trial conditions, water temperatures and feeds was achieved. To determine periodical fish growth during the experiment, individual fish weights were measured with a digital scale with ± 0.1 g sensitivity, and their sizes were measured using Von Bayer method [13] trough with ± 1 mm sensitivity. In periodic measurements, sedation was applied to the fish with 50 mg/L clove oil. At the end of the experiment, the fish were subjected to deep anaesthesia with 100 mg/L to take the meat sample [14]. The water temperature in the tanks was calculated as 15.85 ± 2.03 °C at the end of the experiment, by measuring the water temperature twice a day, in the morning and evening during the experiment period. Dissolved oxygen values were measured as 7.84 mg/L and pH was 7.9 on average throughout the study.

Experimental Diets. In the preparation of trial feeds; fish meal, earthworm meal, bonkalit, corn gluten, soybean meal, vitamin and mineral mixes and pellet binder were used.

The essential nutrient contents of the ingredients to be used in the production of trial feeds (amounts of nitrogen-free extract, crude protein, crude lipid, crude cellulose, crude ash) were determined (Table 1).

Using these values, 4 different isonitrogenic (50% crude protein) trial feeds were prepared with the following ratios; control 0% (SU0), earthworm meal 10% (SU1), earthworm meal 20% (SU2) and earthworm meal 30% (SU3) (Table 2). Firstly, the dry feed ingredients, which form the rations, were milled and sieved through a sieve with 500 μm mesh. After the sieving process, dry ingredients were weighed separately in ± 1 g precision digital scale and placed into the mixing vessel, and after the mixture was homogenised, they were passed through meat mincer and prepared as 3 mm diameter pellets. The prepared feeds were dried for 24 hours in an oven set at 60 ºC. Dried feeds were put into the bags and labelled after they were cooled to room temperature, and were kept at +4 ºC in the refrigerator until they were served to the fish. Prepared feeds were calculated at a rate of 2.5% as per the tank biomass every 15 days and were given to the fish three times a day in the morning, noon and

TABLE 1
Proximate analyses of feed ingredients (%)

Ingredients *	Moisture	CP	CL	CC	CA	NFE[1]
Fish meal	9.1	71.4	11.5	0.1	7.6	0.3
Earthworm meal	10.0	59.0	9.0	0.26	17.0	4.74
Bonkalit	9.1	14.3	2.45	9.3	1.9	62.95
Corn gluten	9.8	64.0	2.25	2.2	1.5	20.25
Soybean meal	10.9	45.5	2.8	4.46	5.3	31.04

CP: Crude protein CL: Crude lipid CC: Crude cellulose CA: Crude ash NFE: Nitrogen free extracts
[1]Nitrogen free extracts = matter - (crude lipid + crude cellulose + crude ash + crude protein)
*The results of the analysis in the feed laboratory of the Recep Tayyip Erdoğan University, Faculty of Fisheries.

evening. Unconsumed feeds were removed from the tank by siphon, then weighted and recorded.

Essential amino acid (Table 3) and fatty acid analyses (Table 4) of the experimental feeds were carried out in Kazlıçesme R&D Test Laboratory, Istanbul, Turkiye.

Sample Collection and Analysis. At the end of the experiment, samples taken randomly from each tank were weighed with precision digital balance and their weights were retained and their total lengths were measured. To determine the efficiency of the slaughter, carcass weight, fin weight, internal organ weight, and liver weight were measured, and hepatosomatic index and viscerosomatic index values were calculated for the fish which were exposed to deep anaesthesia [14]. In addition, dry matter, ash, lipid, protein and moisture analyses were performed in the laboratory and the data obtained were evaluated. Dry matter and moisture ratio of the fish meats was calculated according to "TS 1743 (110 ± 1 °C)"; crude protein according to the "Kjeldahl Method"; crude lipid according to the "Soxhlet Method"; crude ash according to "TS 1746" (550 ± 1 °C)" [18, 19, 20].

Statistical Analysis. The results obtained are shown as the standard deviation (SD) of the mean. SPSS 22.0 software was used to evaluate the results, ANOVA test was used to reveal the difference in the groups and Duncan Test was used to find out from which groups this difference was arisen. Differences between groups were evaluated using significance level of $P < 0.05$.

TABLE 2
Formulation and proximate analyses of the experimental diets

Ingredients (%)	SU0	SU1	SU2	SU3
Fish meal	40	30	20	10
Earthworm meal	0	10	20	30
Bonkalit	13.9	12.8	9.9	7.4
Corn gluten	18	19.1	22	24.5
Soybean meal	19	19	19	19
Fish oil	8	8	8	8
Vitamin mixture[1]	0.5	0.5	0.5	0.5
Mineral mixture[2]	0.1	0.1	0.1	0.1
Molasses	0.5	0.5	0.5	0.5
Total	100	100	100	100
Proximate composition (%)				
Dry matter	90.2	90.5	90.1	90.3
Crude protein	50.71	50.02	50.22	50.21
Crude lipid	13.86	13.62	13.36	13.11
Crude ash	8.2	8.27	8.83	8.90
NFE[3]	17.43	18.59	17.69	18.08
Gross energy (kcal/g)[4]	490.437	488.864	483.934	483.062
Digestible energy (kcal/g)[5]	413.15	409.86	406.72	405.2

[1]Vitamin mixture**:** Included per kg; Vitamin A 20.000.000 IU, Vitamin D3 2.000.000 IU, Vitamin E 200.000 mg, Vitamin K312.000 mg, Vitamin B1 20.000 mg, Vitamin B2 30.000 mg, Vitamin B6 20.000 mg, Vitamin B12 50 mg, Vitamin C 200.000 mg, Niacin 200.000 mg, Cal.D.Panth. 50.000 mg, Folic acid 6.000 mg, D-Biotin 500 mg, Cholin Chloride 300.000 mg.

[2] Mineral mixture: Included per kg; 60 mg manganese, 80 mg zinc, 60 mg ferro, 5.000 mg copper, 2.000 mg iodine, 1.000 mg cobalt, 200 mg selenium, 50 mg magnesium

[3] Nitrogen free extracts (NFE) = matter - (crude lipid + crude ash + crude protein)

[4] Gross energy (GE), calculated according to 23.7 kJ/g protein, 39.5 kJ/g lipid, 17.2 kJ/g NFE [15].

[5]Digestible energy (DE), calculated on an estimated 5.0 kcal/g protein; 9.0 kcal/g lipid; 2.0 kcal/g carbohydrate [16].

TABLE 3
Amino acid content of experimental diets (% of dietary protein)

Essential amino acids	SU0	SU1	SU2	SU3	Rainbow trout* Requirements*
Leucine	7.00	7.61	7.77	7.92	3.5
Lysine	7.98	7.99	8.10	8.30	4.5
Methionine	3.09	2.81	2.98	3.11	3.5
Phenylalanine	3.58	3.76	4.06	4.45	4.5
Tryptophan	1.07	1.04	1.03	1.50	0.5
Valine	7.64	5.93	5.28	5.64	3.2
Arginine	7.72	6.85	6.76	6.95	5.0
Histidine	2.41	3.46	3.95	4.00	1.8
Isoleucine	4.10	5.09	5.80	5.93	2.0

* [17].

RESULTS

Growth Performance. In this study, growth and feed evaluation results obtained from control groups; (SU0) free of earthworm meal; 10% earthworm meal added (SU1); 20% earthworm meal added (SU2); and 30% earthworm meal added (SU3) has been shown in Table 5. The highest increase in weight was detected in the SU3 group.

TABLE 4
Fatty acid composition (% total fatty acids, mean ± SEM, *n* = 3) of feeds used in the experiments

Fatty acids	SU0	SU1	SU2	SU3
C16:0	0.04	0.13	nd	nd
C18:0	0.01	0.03	nd	0.02
[1]Σ SFA	0.05	0.16	nd	0.02
C16:1n7	0.02	0.03	nd	0.10
C18:1n9	nd	0.03	0.13	nd
[2]Σ MUFA	0.20	0.06	0.13	0.10
C18:2n6	0.16	0.3	0.15	0.10
C20:4n6	0.03	0.04	0.23	0.03
[3] Σ n-6 PUFA	0.19	0.34	0.38	0.13
C18:3n3	0.09	0.16	0.12	0.05
C20:5n3	0.09	0.14	0.14	0.05
C22:6n3	0.30	0.43	0.12	0.14
[4]Σ n-3 PUFA	0.48	0.73	0.38	0.24
[5]Σ PUFA	0.67	1.08	0.76	0.37
[6]Σ n-3 HUFA	0.39	0.57	0.26	0.19
n-3/n-6	0.40	0.47	1.00	1.85

SFA saturated fatty acid, MUFA monounsaturated fatty acid, PUFA polyunsaturated fatty acid, HUFA highly unsaturated fatty acids, nd: non-detectable
See Table 2 for rate of inclusion of the four oils in the experimental diets
[1]ΣSFA includes Palmitic acid (16:0) and Stearic acid (18:0)
[2]Σ MUFA includes Palmitoleic acid (16:1n-7) and Oleic acid (18:1n-9)
[3] Σ n-6 PUFA includes Linoleic acid (18:2n-6) and Arachidonic acid (20:4n-6)
[4]Σ n-3 PUFA includes Linolenic acid (18:3n-3), Eicosapentaenoic acid (20:5n-3) and Docosahexaenoic acid (22:6n-3)
[5]Σ PUFA includes Pn-6 PUFA and Pn-3 PUFA
[6]Σ n-3 HUFA includes 20:5n-3 and 22:6n-3. The only n-6 HUFA detected was 20:4n-6

TABLE 5
Growth performance of rainbow trout fed on different levels of earthworm meal ($\overline{X}$ ± SD).

	SU0	SU1	SU2	SU3
IBW (g/fish)	5.04±0.12 [a]	5.05±0.11 [a]	5.02±0.15 [a]	5.05±0.12 [a]
FBW (g/fish)	10.77±2.04 [a]	14.28±1.66 [b]	15.62±1.98 [c]	18.15±2.10 [d]
WG (g)	5.74±3.21 [a]	9.22±2.45 [b]	10.61±3.07 [c]	13.12±2.66 [d]
[1]WG (%)	113.68±1.32 [a]	182.76±1.09 [b]	211.17±1.14 [c]	259.40±2.20 [d]
[2]SGR (% per) day)	0.84±0.33 [a]	1.15±0.15 [b]	1.26±0.08 [c]	1.42±0.31 [d]
[3]FI (%BW per) day)	12.30±3.10 [a]	13.76±0.78 [b]	13.38±1.62 [c]	13.32±1.06 [cd]
[4]FCR	2.15±0.24 [a]	1.49±0.75 [b]	1.26±0.21 [c]	1.02±0.44 [d]
[5]PER	0.12±0.72 [a]	0.20±0.09 [b]	0.23±0.24 [c]	0.28±0.33 [d]
[6]CF (%)	1.43±0.44 [a]	1.39±2.21 [ab]	1.16±0.09 [c]	1.05±0.21 [d]
[7]SR (%)	60.02±0.11[a]	62.21±0.23 [ab]	73.34±1.15 [c]	74.45±0.07 [cd]
[8]HSI (%)	2.43±0.18[a]	1.80±0.37[b]	1.66±0.28[c]	1.51±0.09[d]
[9]VSI (%)	8.99±1.11[a]	8.97±0.78[a]	8.96±1.16[a]	8.93±2.01[b]

Values are means ± SD (*n* = 3); values with different superscript letters in the same rows are significantly different (P < 0.05).
IBW and FBW are initial body weight and final body weight
[1]Weight gain (WG, %)=100×(final body weight−initial body weight)/initial body weight
[2]Specific growth rate (SGR, %per day)=100×(ln final weight−ln initialweight)/days of the experiment
[3]Feed intake (FI,%BWper day)=100×dry feed intake/(days×(initial body weight+final body weight+death body weight)/2)
[4]Feed conversion ratio (FCR)=dry feed consumed/wet weight gain
[5]Protein efficiency ratio (PER) = wet weight gain/protein intake
[6]Condition factor (CF,%) = (wet weight/total length^3) × 100
[7]Survival rate (SR, %) = 100 × (final fish number)/(initial fish number)
[8]Hepatosomatic index (HSI, %) = liver weight × 100/body weight [21].
[9]Viscerosomatic index (VSI, %) = viscera weight × 100/body weight

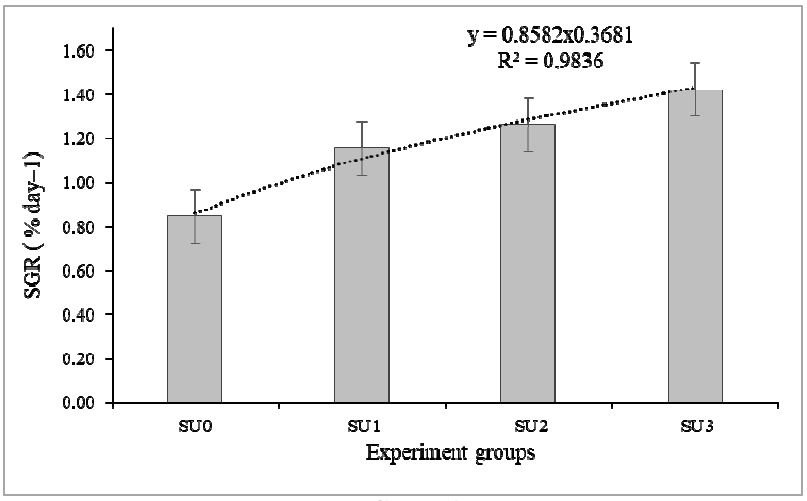

FIGURE 1
Specific growth rate of rainbow trout fed the experimental diets (mean ± SD, n = 3 tanks diet^{-1})

TABLE 6
Body composition of rainbow trout fed with earthworm meal (*n* = 5, $\overline{X}$ ± SD).

	SU0	SU1	SU2	SU3
Dry matter (%)	21.39±0.02 ^{a}	22.18±0.03 ^{a}	22.79±0.01 ^{a}	23.01±0.02 ^{a}
Crude protein (%)	16.98±0.42^{a}	17.16±0.11^{b}	18.04±0.23^{c}	18.22±0.09^{cd}
Crude lipid (%)	3.35±0.17^{a}	2.71±0.22^{b}	2.24±0.11^{c}	2.08±0.31^{cd}
Crude ash (%)	1.09±0.11 ^{a}	1.07± 0.62 ^{a}	1.07±0.31 ^{a}	1.06±1.02 ^{a}

Values in the same column with different superscripts are significantly different (P < 0.05). Data represented as mean ± *SD* of triplicate tanks.

SGR of fingerling rainbow trout was evaluated during the experiment and the lowest SGR was found to be 0.84 ± 0.33% in the control groups. The highest SGR was found in SU3 group with 1.42 ± 0.31%, followed by SU2 with 1.26 ± 0.08% (Figure 1).

According to the results of the experiment; when the condition factor (CF) was examined, there was no significant difference between SU0 and SU1 groups (P > 0.05), but it was found that the difference between SU0 and SU2 and SU3 groups was statistically significant with respect to CF (P < 0.05). The amount of feed consumed by the fish for 90 days according to their body weight percentages feed conversion ratio (FCR) in the SU0, SU1, SU2 and SU3 groups was 2.15 ± 0.24, 1.49 ± 0.75, 1.26 ± 0.21 and 1.02 ± 0.44 respectively, and there was a statistically significant difference between all groups (P < 0.05). The best FCR was found to be 1.02 ± 0.44 for SU3 group fish and the worst FCR was 2.15 ± 0.24 for SU0 group fish. Protein efficiency ratios (PER) for the experimental groups were found as follows. The highest one was found in the SU3 group with 0.28 ± 0.33, the lowest one was in the SU0 group with 0.12 ± 0.72, and a significant difference was found between the two groups (P < 0.05). For the trout fingerlings fed with feeds supplemented with different amounts of earthworm meal, the highest survival rate (SR) was found in SU3 group with 74.45 ± 0.07% ratio, the lowest **SR** with 60.02 ± 0.11**%** in the SU0 group. There was a statistically significant difference between the groups with respect to survival rate (P < 0.05) (Table 5).

At the end of the experiment, hepatosomatic index and viscerosomatic index values were calculated (Table 5). The highest HSI value was obtained from SU0 group with the 2.43 ± 0.18%. When HSI values were examined, it was found that the difference between all groups was significant (P < 0.05). Highest VSI value was obtained in the control group SU0 with 8.99 ± 1.11%. When VSI values were examined, it was found that S3 group was different from all other groups (P < 0.05), but the difference between S0, S1 and S2 groups was found to be insignificant (P > 0.05).

Body Proximate Composition. At the end of the experiment, the crude protein, crude ash, dry matter and crude lipid ratios calculated as a result of the analyses performed in the muscle tissue of 5 fish randomly sampled from each tank are given in Table 6. At the end of the experiment, as the result of the biochemical analysis of fish flesh, the highest value was obtained from SU3 group with 18.22 ± 0.09% for crude protein and the highest value was 3.35 ± 0.17% with control SU0 group for crude lipid. As a result of statistical analysis, it was found that the difference between SU0 control group and SU3 group was significant (P < 0.05).

DISCUSSION

In this study, the effects of adding earthworm meal to fish feed on the growth performance of fingerling rainbow trout, the hepatosomatic index and viscerosomatic index values and on the chemical composition of fish meat were investigated. When the growth performance data in fish were evaluated, it was determined that earthworm meal had a positive effect on development. In this study, when the data related to the growth were evaluated at the end of the 3-month feeding experiment, the highest live weight average was obtained in SU3 group and the lowest one was obtained in control SU0 group. The difference between weight increases of the groups having similar initial weights was found to be statistically significant ($P < 0.05$) at the end of the experiment. When the specific growth rate (SGR) among the growth parameters was evaluated, the best SGR was found in the SU3 group with 1.42 ± 0.31% and the lowest one was found in the SU0 group with 0.84 ± 0.33%. In order to determine the growth performance of fish correctly, specific growth rate is used commonly. Specific growth rate decreases as fish grows. If SGR is above 1%, it can be said that the fish grows well [22**].** Generally, in most of the studies, fingerling ones of the different fish species are used as it is the case with this study. In these studies, where growth performance was the subject, feeds supplemented with earthworm meal have been reported to have a positive effect on parameters such as weight gain rate of fish, protein efficiency ratios (PER), feed conversion ratio and specific growth rate [23, 24, 25]. In a study carried out for rohu fish (*Labeo rohita*) with average 0.7 g weight, they have fed them with 3 different diets (plainly cooked earthworm D1, mixed ground earthworm D2 and pellet feed produced from dried earthworm D3); at the end of the study they have found that best growth was in D3 with SGR 4.21% and in D1 with SGR 3.38%, and the results of the experiment suggested that pelleted feed supplemented with earthworm meal can be used in the breeding of fingerlings of rohu fish [25]. In this study, when the data related to feed conversion ratio were evaluated, the best feed utilisation was found in the SU3 group with 1.02 ± 0.44 and the worst value was found in the control group with 2.15 ± 0.24. The difference between the groups was found statistically significant ($P < 0.05$). [23], in their studies, added 0% (Control), 25%, 50%, 75% and 100% earthworm meal to the feed of catfish, and they reported that the highest average weight gain was observed in those fed with 75% earthworm meal. Highest specific growth rate was observed with the rate 1.5 in the feed prepared with 25% earthworm meal and the lowest one was 1.2 in the control group. The best feed conversion rate was seen in the feed supplemented with 50% earthworm meal with the rate of 1.6, and the worst FCR with the rate of 3.1 was seen in the feed supplemented with 100% earthworm meal. In catfish, feeds containing earthworm meal and fish meal were found appropriate for optimal growth performance and feed utilisation. In a study, 95, 190 and 380 g/kg of earthworm meal was added to the diets prepared for rainbow trout, which had an average weight of 208.6 g, close to the marketing weight, as against to 250, 500 and 100 g / kg of fish meal respectively, and at the end of 53 days of experiment, a significant increase in weight and daily specific growth rate was determined in groups fed by the feed containing 95 g/kg *E. fetida* meal, but in dietary groups containing 380 g/kg *E. fetida* meal, a decrease in growth performance was detected, and in the same study, it has been reported that carcass-fat rate of the group in which 100% earthworm meal was used was decreased by 16.7% in comparison to control group where 100% fish meal was used. As a result of this literature study, for the feeding of the fish in the later commercial breeding period, it has been reported that *E. fetida* meal can be added successfully to the feeds of trout instead of high amounts of fish meal [26]. [27], have prepared 3 different rations (earthworm meal T1, frozen earthworm T2 and live earthworm T3) for fingerling African Snakehead (*Parachanna obscura)* with an average weight of 2.7 ± 0.15 g, and served them to the fish together with the pellets prepared with frozen and live earthworm. At the end of the experiment, they have detected that the best protein efficiency ratio (PER) 1.58 and the best feed conversion ratio (FCR) 1.41 were found in T3 group which was fed with live earthworm, followed by the T1 group which was fed by the ration prepared with earthworm meal. They also achieved a 100% survival rate from these two groups contrary to the T2 group. Earthworms survive with microorganisms in soil and water. However, they are not affected much by these pathogens. Antimicrobial substances in coelom fluid are described as important elements of the defence system [28]. In feeding studies carried out with worms, one of the successes due to antimicrobial substances in worms is to increase the survival rate and improve stress symptoms. In the study, the best survival rate for the groups was found as 74.45 ± 0.07%. in group SU3 containing earthworm meal, and 60.02 ± 0.11% in the control group that does not contain earthworm meal. Statistically significant differences were found between the groups with respect to the survival rate ($P < 0.05$). In a study carried out by [29], they have added different ratios of earthworm meal (**0%**, 15%, 25%, 35% and 50%) into the feed of fingerling *Clarias gariepinus* fish, and they have determined that the feed supplemented with earthworm meal with 25% and 35% ratios provided higher survival rate compared to the control group which did not contain earthworm meal.

In this study, the best condition factor was found to be 1.05 ± 0.21**%** in group SU3 containing earthworm meal, and it was found to be 1.43 ± 0.44% in the control group without earthworm meal. According to the results of the experiment, when the condition factor (CF) was examined, there was no significant difference between SU0 and SU1 groups ($P > 0.05$), but it was found that the difference between SU0 and SU2 and SU3 groups was statistically significant ($P < 0.05$). The lowest hepatosomatic index (HSI) was detected as 1.51 ± 0.09% in the group containing SU3 earthworm meal**.** In statistical analyses, it was found that the difference between SU0 group and SU3 group was significant with respect to HSI values ($P < 0.05$). In a study carried out by [30], five different groups of feeds with an average weight of 4.43 g (E0, E25, E50, E75 and E100) were prepared for hybrid hetero-clarias fingerlings and they fed them for 8 weeks. At the end of the experiment, the best average weight was 6.77 g in those fed with E50 diet, and in the control group without earthworm meal, and fish weight was found to be 6.04 g. In the same study, they obtained the lowest condition factor (KF) value from the control group as 1.369%, and from E50 group with the highest value as 1.996%. Thus, they found that earthworm meal supplement is a good alternative protein source in fish feed. [21], fed fingerling carp fish weighing an average of 8.1 g with 4 different diets containing animal protein (control, EW20, EW70 and EW100) for 8 weeks, and at the end of the study they evaluated the morphological parameters. They obtained highest hepatosomatic index (HSI) in the control group with the value of 2.08%, and the lowest HSI from the EW70 group with 1.83%.

We think that the reasons of why the results obtained in this experiment differ from some literature data [21, 23, 25, 26, 27, 28, 29, 30]. might be different fish species, fish size, amount of feed, form of the feeding, experiment period, different feed ingredients and ratios in the rations, different serving methods of experiment materials, water temperature, stock density, environment conditions and so on.

As a result, it was found that the use of 30% earthworm meal in the feeds of fingerling rainbow trout improves growth performance and that earthworm meal can be used over 30% in fish feeds considering other literature studies.

ACKNOWLEDGEMENTS

Earthworm material requirement of this study has been supplied by LAZUTIM Organik Tarım Ltd., and the study itself has been supported by Department of Scientific Research (BAP), Recep Tayyip Erdogan University with the project number FLY-2017-753. We are thankful to Ozay KOSE for his attentiveness and support.

COMPLIANCE WITH ETHICAL STANDARDS

The animal ethics committee permission for the study was received from Recep Tayyip Erdogan University Local Ethics Committee for Animal Experiments (Decision No:2015/6, Date: 16/01/2015) and the rules of the committee were followed during the investigations.

REFERENCES

[1] Hosomi, R., Yoshida, M., Fukunaga, K. (2012) Seafood consumption and components for health. Global Journal of Health Science. 4(3), 72–86.

[2] Food and Agriculture Organization of the United Nations (FAO). (2018) The State of World Fisheries and Aquaculture, Meeting the Sustainable Development Goals. Retrieved on September 20, 2019 from.

[3] Arıman Karabulut, H., Kose, O., Kurtoglu, I.Z. and Kırım, B. (2019) Use of Alternative Feed Sources in Sturgeon Fish Feed and Importance of Aquaculture. I. International Science and Innovation Congress, Pamukkale, Denizli/ Turkiye, 26-29 August 2019, 345-349.

[4] Akiyama, T. (1995) Nutritive Value of Alternative Protein Sources. In: New Feeds for Fish Culture Utilization of Alternative Protein Sources. Suisangaku Series 102 Koseisha-Koseikaku, Tokyo, 45-42.

[5] De Silva, S.S. and Anderson, T.A. (1995) Fish Nutrition in Aquaculture. Chapman and Hall, London, 319.

[6] Lim, S.J., Kim, S.S., Ko, G.Y., Song, J.W., Oh, D.H., Kim, J.D., Kim, J.U., Lee, K.J. (2011) Fish meal replacement by soybean meal in diets for Tiger puffer, *Takifugu rubripes*. Aquaculture. 313 (1-4), 165-170.

[7] Musyoka, S.N., Liti, D.M., Ogello, E., Waidbacher, H. (2019) Utilization of the earthworm, *Eisenia fetida* (Savigny, 1826) as an alternative protein source in fish feeds processing: A review. Aquaculture Research. 50, 2301–2315.

[8] Aras, N.M., Kocaman, E.M. and Aras, M.S. (2000) General Fisheries and Fundamental Principals of Aquaculture. Ataturk University, Faculty of Agriculture Publications No: 216, Erzurum, Turkiye, 115-118.

[9] Sabine, J.R. (1978) The nutritive value of earthworm meal. In: Hartenstein R. (Eds.), Utilization of Soil Organisms in Sludge Management. National Technical Information Services, Springfield. 285-296.

[10] Lourdumary, A.J.B. and Uma, K. (2012) Nutritional Evaluation of Earthworm Powder (*Lampito mauritii*). Journal of Applied Pharmaceutical Science. 3(03), 82.
[11] Grdišaa, M., Gršićb, K. and Grdišac, M.D. (2013) Earthworms-role in soil fertility to the use in medicine and as a food. Invertebrate Survival Journal. 10 (1), 38-45.
[12] Arıman Karabulut, H, Kurtoglu, I.Z., Yüksek, T. and Osmanoglu, M.I. (2016) Use of Worm meal as Animal Protein Source in Fish Feed. Journal of Anatolian Environmental and Animal Sciences. 1(2), 64-69.
[13] Piper, R.G., McElwain, L.B., Orme, L.F., McCraden, J.P., Fowler, L.G. and Leonard. J.R. (1983) Fish Hatchery Management. US Fish and Wildlife Service, Washington DC, 544.
[14] Coyle, S.D., Durborow, R.M. and Tidwell, J.H. (2004) Anesthetics in Aquaculture. USDA Southern Regional Aquaculture Center (SRCA), Puplication No: 3900.
[15] Stafford, E.A. (1984) The use of earthworms as a food for rainbow trout (*Salmo gairdneri*). Institute of Aquaculture University of Stirling, Scotland.
[16] Cowey, C.B., Pope, J.A., Adron, J.W. and Blair, A. (1972) Studies on the nutrition of marine flatfish. The protein requirement of plaice (*Pleuronectes platessa*). The British Journal of Nutrition. 8(3), 447-456.
[17] Hardy, R.W. (2002) Rainbow trout, *Oncorhynchus mykiss*. In: Webster and Lim CE (Eds.): Nutrient requirements and feeding of finfish for aquaculture. The Haworth Press, Binghamton, New York, USA, 184-202.
[18] Green, B. (1980) *Fish Feed Technology*. FAO (Eds.), Rome: ADCP/REP/80/11, 395.
[19] Lovell, R.T. (1981) Laboratory manual for fish feed analysis and fish nutrition studies. Auburn University, US, 65.
[20] AOAC (Association of Official Analytical Chemists). (2005) Official methods of analysis of the association of official analytical chemists international (18th ed.). Gaithersburg, Maryland, USA, AOAC International.
[21] Ngoc, T.N., Pucher, J., Becker, K. and Focken, U. (2016) Earthworm powder as an alternative protein source in diets for common carp (*Cyprinus carpio* L.). Aquaculture Research. 47, 2917-2927.
[22] Korkut, A.Y., Kop, A., Demirtas, N. Cihaner, A. (2007) Determination methods of growth performance in fish feeding, Ege Journal of Fisheries and Aquatic Sciences. 24, (1-2), 201–205.
[23] Monebi, C.O. and Ugwumba, A.A.A. (2013) Utilization of the earthworm, *Eudrilus eugeniae* in the diet of Heteroclarias fingerlings. International Journal of Fisheries and Aquaculture. 5(2), 19-25.
[24] Chiu, S.T., Wong, S.L., Shiu, Y.L., Chiu, C.H., Guei, W.C. and Liu, C.H. (2016) Using a fermented mixture of soybean meal and earthworm meal to replace fish meal in the diet of white shrimp, *Penaeus vannamei* (Boone). Aquaculture Research. 47(11), 3489-3500.
[25] Mohanta, K.N., Subramanian, S. and Korikanthimath, V.S. (2016) Potential of earthworm (*Eisenia fetida*) as dietary protein source for rohu (*Labeo rohita*) advanced fry. Cogent Food and Agriculture. 2(1), 1138594.
[26] Velasquez, L., Ibañez, I., Herrera, C., Oyarzun, M. (1991) A note on the nutritional evaluation of worm meal (*Eisenia fetida*) in diets for rainbow trout. Animal Science. 53(1), 119-122.
[27] Vodounnou, J.V., Kpogue, D.N.S., Mensah, G.A. and Didier, F.E. (2017) Earthworm amino acids and effect of 3 different forms of earthworm (meal, frozen and living) on growth, feed utilization and survival rate of African Snakehead fingerlings (*Parachanna obscura)* reared in captivity. International Journal of Fisheries and Aquatic Studies. 5(1), 268-273.
[28] Wang, C., Sun, Z., Zheng, D. (2006) Research advance in antibacterial immunity ecology of earthworm. The Journal of Applied Ecology. 17(3), 525-529.
[29] Dedeke, G.A., Owa, S.O., Olurin, K.B., Akinfe, A.O., Awotedu, O.O. (2013) Partial replacement of fish meal by earthworm meal (*Libyodrilus violaceus*) in diets for African catfish, (*Clarias gariepinus*). International Journal of Fisheries and Aquaculture. 5(9), 229-233.
[30] Olele, N.F. (2011) Growth response of Heteroclarias fingerlings fed on earthworm meal in hatchery tanks. Journal of Life Sciences. 3(2), 131-136.

Received: **07.11.2019**
Accepted: **17.01.2020**

CORRESPONDING AUTHOR

Huriye Ariman Karabulut
Department of Aquaculture,
Faculty of Fisheries,
Recep Tayyip Erdogan University,
Rize, 53100, Turkey.

e-mail: huriye.ariman@erdogan.edu.tr