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Effect of feeding frequency on growth, fecundity, and meat quality of Abant Trout (*Salmo abanticus* Tortonese, 1954) broodstock

Mehmet Zeki ALKAN[1,*], **Nadir BAŞÇINAR**[2]
[1]Department of Food Technology, Maçka Vocational High School, Karadeniz Technical University, Trabzon, Turkiye
[2]Department of Fisheries Technology Engineering, Faculty of Marine Sciences, Karadeniz Technical University, Çamburnu, Trabzon, Turkiye

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Abstract: This study was carried out to investigate the growth data, egg production and changes in the biochemical structure of meat of Abant trout depending on feeding frequency (once a day, twice a day, three times a day, four times a day) in order to contribute to the economy through the private sector. All fish in the groups were planned to be of similar weight at the beginning of the study. Individual fish weights were measured at the beginning of the study, and the average was found to be 224.87 ± 0.69 g. At the end of the study, the weights of the fish groups that were fed differently were 536.77 ± 37.46 g, 693.52 ± 67.53 g, 770.47 ± 37.29 g, and 810.63 ± 42.09 g, respectively. The absolute numbers of eggs taken per brood from the once meal group to the four-time meal group were found as 707 ± 38, 999 ± 77, 1036 ± 110, and 1053 ± 60 eggs/broodstock, respectively. In terms of fatty acids, which are an important contributor to meat quality, the second, third, and fourth meal groups were found to be advantageous compared to the first meal group. According to the data obtained at the end of this study, which lasted 392 days, it was seen that the groups which received meals twice, three times, and four times could be cultured by the private sector because of the good results. Especially the three-time meal group yielded the best results in terms of growth, egg production, biochemical data, and meat quality. It has been concluded that breeding and species-specific feed production studies will contribute to the cultivation studies.

Key words: Abant trout, *Salmo abanticus*, feeding frequency, growth, body composition, fatty acids

1. Introduction

Fish has been accepted as one of the most important sources of healthy and balanced nutrition all over the world. In line with population growth, the demand for fish increases, whereas there is a decrease in fishing resources due to overfishing and pollution. In this case, options such as more efficient use of available resources, the use of innovative technologies or the search for alternative aquaculture sources can help meet increased global demand. Especially in terms of using innovative technologies, triploidization applications have gained importance in trout production [1]. Cultivation of alternative species can make a different contribution to breeding with definite species. It can support sustainable fish farming. It can contribute to the presentation of different flavours to consumers. Therefore, it can help the economy of countries and protect natural fish stocks. In Türkiye, the second most widelycommonly produced species is rainbow trout (*Oncorhynchus mykiss*) [2] which is mainly farmed in the seas, dams, and inland waters. Also, a small amount of Black Sea trout (*Salmo trutta labrax* Pallas, 1811) is grown in similar farming areas. However, the Abant trout (*Salmo abanticus* Tortonese, 1954), an endemic fish species living in the Bolu Yedigöller region, is not farmed commercially but only farmed for fishing purposes. However, bringing this species to commercial farming will add a different aspect to trout farming with definite species. It will offer a different flavour to consumers in the national and international arena and will help protect and continue the extinction of this endemic species. The aquaculture studies conducted on Abant trout are extremely limited [3,4,5,6]. These studies were conducted mostly for scientific purposes and have remained far from meeting the expectations of the private sector. Determining the biological and economic advantages through the application of feeding frequency of Abant trout will also contribute to the selection of the most suitable aquaculture technique. In addition, we believe that the results obtained from this study, especially related to growth and feed evaluation, will be effective in the decision-making process of enterprises that will be engaged in commercial cultivation.

2. Materials and methods

2.1 Aquaculture system, fish, and diets used

The study was carried out at Prof. Dr. Ibrahim OKUMUŞ Research and Application Unit of the Sürmene Faculty of Marine Sciences, KTU. The first measurements were taken on December 2019. It was completed on January 2021 with a total duration

* Correspondence: mzalkan@ktu.edu.tr

of 392 days. The fish used for this research were obtained at the Prof. Dr Ibrahim OKUMUŞ Research and Application unit. In the selection of breeding fish, the following criteria have been considered: appropriate body form, no signs of disease, and structural characteristics of the species. In the research, 480 candidates breeding Abant trout were examined. The mean length of the fish was measured as 27.55 ± 1.9 cm (24.5–31.5 cm), and the weight was measured as 224.87 ± 47.5 g (168.4–349.7 g) (Table 1).

The tanks used for stocking have a volume of approximately 400 L. They are square in shape with rounded edges and are made of fibreglass. The temperature values of the water used during the research varied between 5 °C and 21 °C. Oxygen values have always been higher than 7 mg/L. The pH value was between 7.2 and 8.4. Approximately 0.3–0.5 L/s of water was supplied to each tank, depending on the size of the fish and the water temperature.

To meet the nutrient requirement of the trout used in the study, 6 mm commercial trout feed was used (Table 2).

The length and weight of the fish were measured every 8 weeks from the beginning of the study. The fish were stunned while weighing and measuring. 50 ppm benzocaine solution was used as an anaesthetic agent. In the incubation of fertilized eggs, aluminium incubation cabinets with top water inlet, vertical flow, and two compartments were used. There were 10 hatching trays in each compartment of these cabinets. Each of the aluminium hatching trays was 40 cm in diameter and had a surface area of approximately 1250 cm^2. While 37% formaldehyde was used for egg disinfection, wooden forceps were used in the selection of dead eggs.

The formulas given below were used to evaluate the data.

Specific Growth Rate by length; $SBO_L = [(\ln L_f - \ln L_i) / Day] \times 100$ [7]

Specific Growth Rate by weight; $SBO_W = [(\ln W_f - \ln W_i) / Day] \times 100$ [7]

Condition Factor (CF) = $(W / L^3) \times 100$ [7]

Feed Conversion Rate; FCR = Total feed intake (kg) / total weight gain (kg), (including dead fish) [7]

On December 2019, the study started by measuring the length and weight of the experimental fish. Forty fish were stocked in each tank (12 tanks in total). The tanks were divided into four homogenous groups: one-meal, two-meal, three-meal, and four-meal. As a result, each group was formed with three times simultaneously. The one-meal group was fed at 08:00, the two-meal group at 08:00 and 17:00, the three-meal group at 08:00, 12:30, and 17:00, and the four-meal group at 08:00, 11:00, 13:30, and 17:00. Feeding was done manually in all groups until satiation. Separate feed containers were used for each tank. Until the end of the study, the amount of feed put in and consumed in each feed container was weighed and recorded. The water temperature was measured with a digital thermometer (±0.1 °C) twice a day, in the morning and in the evening, and recorded. In case of death in the tanks until the end of the fieldwork, the height and weight of the dead fish or alive fish were measured and recorded. From the beginning of the study, all the fish were stunned using 50 ppm benzocaine solution, and their height and weight were measured every 8 weeks. After the data were collected, they were put into clean water and sobered up. Eggs were collected from the stocked experimental fish as of January 01, 2021. The abdominal massage method was used for egg and sperm retrieval. To avoid an unsuccessful fertility process, sperm from at least 2 male fish were used for the eggs taken from each female fish. In order to determine their hatching performance, the eggs of 20 randomly selected female fish from each group were placed in a separate compartment in the hatching cabinets. After the fertilization of the eggs, the hatching period was observed. Eggs that died within the first 36 h were separated; they were taken with the help of forceps and counted and removed from the environment after recording. In order to prevent fungal infection that may occur due to dead eggs, eggs were disinfected by applying 37% formaldehyde prepared at a concentration of

Table 1. The initial average weights, average lengths, and standard deviations of the fish in the study.

Parameters	One meal a day	Two meals a day	Three meals a day	Four meals a day
Average length (cm)	27.84 ± 0.46	27.59 ± 0.81	27.73 ± 1.31	27.03 ± 0.52
Average weight (g)	224.56 ± 1.24	225.01 ± 1.38	224.15 ±1.37	225.77 ± 0.62

Table 2. Biochemical content of trout feed (6 mm) (company statement).

Content	Ratio	Content	Ratio
Water	10%	Vitamin D3	2500 IU
Dry matter	90%	Vitamin E	250 mg/kg
Crude protein	45%	Vitamin C	200 mg/kg
Crude oil	20%	Calcium	2.00%
Crude ash	11%	Total phosphorus	1.50%
Crude cellulose	1.5%	Sodium	0.45%
Vitamin A	12,000 IU		

1–2 mg/L with a plastic injector every 2 days.

The following procedures and formulas were used to evaluate egg production.

Total fecundity (TF) = Number of eggs / female

Relative fecundity (RF) = Number of eggs / kg female

2.2. Biochemical analysis

2.2.1. Crude ash, crude protein, and crude oil determination

Ash determination was performed according to the method described in [8,9]. For this purpose, the crucibles containing the sample were burned for 12 h at 550 °C in the ash furnace, reduced to ash, cooled for 30 min in the desiccator, and then weighed. The following formulas were used to evaluate the results [10].

The Amount of Ash (%) = [(S2 - S0) / (S1 - S0)] × 100

S0: Croze weight(g),

S1: Croze weight + sample weight before the burning process (g),

S2: Croze weight + sample weights after the burning process (g).

Crude protein analysis was performed using the Kjeldahl method [8]. The evaluation of the results was calculated according to the following formula [8].

Crude protein (%) = 0.1 N H2SO4 (ml) × 0.0014 × 6.25 × 100 / sample (g)

Crude oil analysis was performed using the soxhlet method [8,9]. The following formula was used to evaluate the data [8,9].

Crude Oil (%) = (S2 - S1) × 100 / S0

S0: Sample weight (g),

S1: Extraction balloon + boiling stones weights (g),

S2: Extraction balloon + boiling stones + oil weights are (g).

2.2.2. Fatty acid composition

Fatty acid analysis (FAME Analysis) was performed according to Tufan [11]. According to this method, after weighing 10 mg of oil in a tube, 2 mL of n-heptane and 2M methanolic potassium hydroxide (KOH) were added. After shaking the tube in the vortex for 2 min, it was centrifuged at 4000 rpm for 10 min, and the n-heptane portion on it was taken and injected into the Gas Chromatography device (GC). The fatty acid composition was calculated by means of chromatograms obtained with fatty acid standards [11].

3. Results

During the study, the temperature of the water for the tanks where the fish were stocked was 13.90 ± 4.39 (4.0–21.3) °C (Figure). The pH value of the water was 7.5–8, while the amount of dissolved oxygen content was above 8 mg/L during the study period.

The length and weight measurements of all fish in the tanks were recorded every 56 days from the beginning of the field study. At the beginning of the study, the aim was to have a similar length for all the groups, so they were measured, and groups were formed based on these measurements. The measurements of the one-meal/day group to four-meal/day groups were 27.84 ± 0.46 cm, 27.59 ± 0.81 cm, 27.73 ± 1.31 cm, and 27.03 ± 0.52 cm, respectively. After the second measurement date, changes in the lengths were observed. At the end of the study, there was a length difference between the experimental fish from the four-meal/day group and those from the one-meal/day group.

At the end of the study, the average length of the fish in the groups increased from one meal/day group to four meals/day group. This trend in length increase was also observed in weight measurement. With a similar approach, measurements of 4 groups were made at the beginning of the study and the groups were tried to be formed with similar weight values. In the weight measurements made at the end of the study, the body weight of the fish increased from one meal/day group to four meals/day group (Table 3).

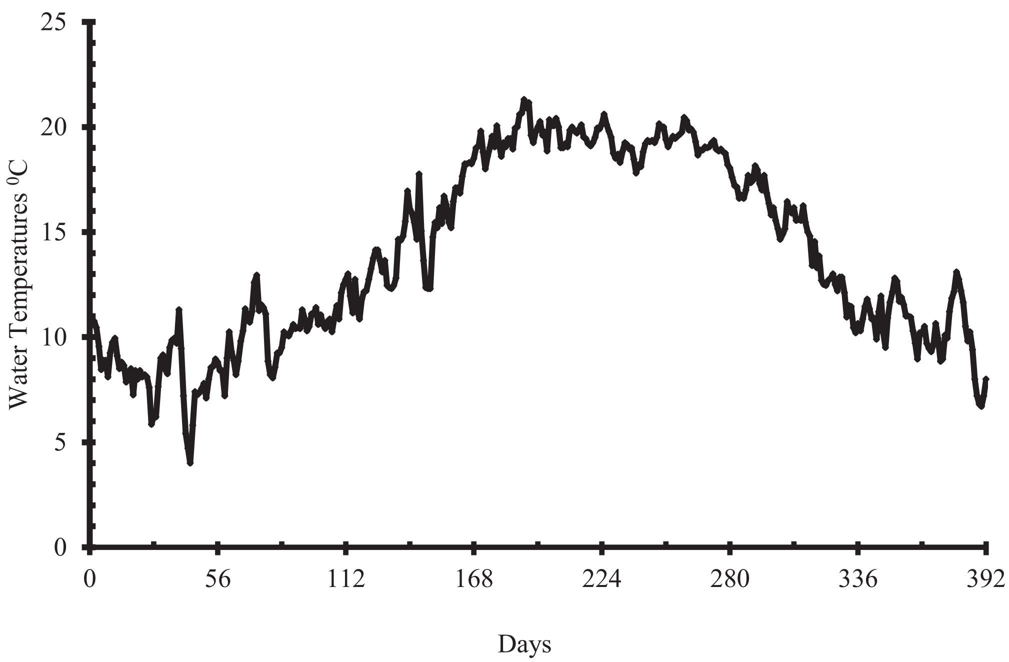

Figure. Water temperatures were measured during the research.

Table 3. Survival rates with growth and feed conversion parameters measured and standard deviations at the beginning and end of the study.

Parameters*	İ/F/A	One-meal	Two-meal	Three-meal	Four-meal	ANOVA	F
Average Length	Li	27.84 ± 0.46	27.59 ± 0.81	27.73 ± 1.31	27.03 ± 0.52	>0.05	0.55
	Lf	36.04 ± 0.23^{a}	40.25 ± 0.63^{b}	42.89 ± 1.17^{bc}	43.61 ± 1.41^{c}	<0.01	25.23
Average weight	Wi	224.56 ± 1.24	225.01 ± 1.38	224.15 ± 1.37	225.77 ± 0.62	>0.05	1.01
	Wf	536.77 ± 37.46^{a}	693.52 ± 67.53^{b}	770.47 ±37.29^{bc}	810.63 ± 42.09^{c}	<0.01	14.05
SGR_L	Final	0.466 ± 0.028^{a}	0.675 ± 0.042^{ab}	0.779 ± 0.110^{bc}	0.854 ± 0.092^{c}	<0.05	10.22
SGR_W	Final	1.554 ± 0.111^{a}	2.005 ± 0.166^{b}	2.203 ± 0.086^{b}	2.281 ± 0.091^{b}	<0.01	17.02
Condition factor	Initial	1.148 ± 0.016	1.140 ± 0.058	1.142 ± 0.036	1.062 ± 0.143	>0.05	0.71
	Final	1.291 ± 0.034	1.371 ± 0.087	1.392 ± 0.045	1.417 ± 0.058	>0.05	1.74
FCR	Average	1.543±0.018^{a}	1.806±0.151^{ab}	2.139±0.208^{b}	2.712±0.129^{bc}	<0.01	28.31
Survival rate	Final	92.50 ± 10.61	83.33 ± 7.64	86.67 ± 9.46	82.50 ± 10.00	>0.05	0.55

*Li; initial length. Lf; final length. Wi; initial weight. Wf; final weight. SGR_L; length-specific growth rate.
SGR_W; weigth-specific growth rate. FCR; feed conversion rate. İ; initial. F; final. A; average.
^{abc} There is a statistical difference between data with different superscripts in the same row (Lf, Wf, SGR_L, SGR_W, and FCR).

When the condition factor, which is an indicator of good nutrition in trout, was calculated, it was seen that there was no statistically significant difference between the groups at the end of the study. However, there were statistical differences in the condition factor between the groups in the 2nd, 3rd, and 4th measurement periods of the growth period ($p < 0.05$). Throughout the study, average feed conversion rates were calculated as 1.543 ± 0.018 for the one-meal groups, 1.806 ± 0.151 for two-meal group, 2.139 ± 0.208 for three-meal group, and 2.712 ± 0.129 for four-meal group, respectively. At the end of the growth period, statistical differences between the groups were revealed ($p < 0.05$).

Survival rates were calculated as 92.50 ± 10.61% in the one-meal group, 83.33 ± 7.64% in the two-meal group, 86.67 ± 9.46% in the three-meal group, and 82.50 ± 10.00% in the four-meal group, respectively. At the end of the growth period, similarities emerged between the groups regarding survival rates.

3.1. Fecundity and hatching efficiency

The average daily temperature values of the incubation water were 10.57 ± 2.03 °C. Milking of mother fish (egg intake) started on February 01, 2021. Milking control was carried out every week until March 16, 2021 when milking and fertilization process was completed. The total number of milked broodstocks is 202, including 50 in the one-meal group, 45 in the two-meal group, 51 in the three-meal group, and 56 in the four-meal group.

The number of eggs given per broodstock (total fecundity) was calculated as 707 ± 38 number/broodstock in the one-meal group; 999 ± 77 number/broodstock in the two-meal group; 1036 ± 110 number/broodstock in the three-meal group, and 1053 ± 60 number/broodstock in the four-meal group. One-meal group results differed from those of the other groups ($p < 0.01$), and the other groups were found to be similar to each other. The egg size (diameter and weight) and the relative egg yields were statistically similar. The mean eggs were observed at 294.1 ± 2.8 day-degrees (30–33 days) and opened at 463.1 ± 6.0 day-degrees (48–51 days). The larvae reached the free swimming stage at a degree of 702.3 ± 1.4 (80–82) days. The egg opening rates were calculated as 73.71 ± 15.81% in one-meal group, 60.96 ± 18.01% in two-meals group, 65.67 ± 21.72% in three-meals group and 71.60 ± 17.46% in four-meals group and the averages were similar (Table 3

3.2. Determination of biochemical composition

At the beginning of the study, crude protein, crude fat, and crude ash values of randomly selected fish from the population where all fish were stocked were calculated. When the values obtained at the end of the study were examined, it was seen that the values calculated in terms of crude protein and crude fat showed a statistically significant difference between the groups ($p < 0.05$ and $p < 0.01$). On the other hand, there was no statistically significant difference between the groups in terms of crude ash values ($p > 0.05$).

All of the fish used in the study were first put into a pond. Then five fish were randomly selected, and saturated fatty acids (∑SFA), monounsaturated fatty acids (∑MUFA), and polyunsaturated fatty acids (∑PUFA) values of selected fish were measured. In conclusion, at the beginning of the study, the mean ∑SFA value was 634.5 ± 28.9 mg / 100 g, the mean ∑MUFA value was 1113.5 ± 46.7 mg / 100 g, and the mean ∑PUFA value was 1143.1 ± 59.2 mg / 100g. The calculations yielded the following values: saturated fatty acids palmitic acid (C16:0) 326.6 ± 17.9 mg / 100 g; stearic acid (C18:0) 121.9 ± 2.7 mg / 100 g; monounsaturated fatty acids oleic acid (C18:1n - 9) 942.1 ± 34.9 mg/100 g; polyunsaturated fatty acids, linoleic acid (C18:2n-6) 580.7 ± 16.8 mg / 100 g; gamma-linolenic acid (C18:3n-6) 5.7 ± 0.2 mg/100g; high unsaturated fatty acids arachidonic acid (C20:4n-6) 32.4 ± 15.0 mg / 100 g; eicosapentaenoic acid (EPA; C20:5n-3) 68.7 ± 6.3 mg / 100 g; docosahexaenoic acid (DHA; C22:6n-3) 448.2 ± 59.1 mg /100 g; total n-3 fatty acids (∑n3) (omega-3) 524.3 ± 63.2 mg / 100 g; total n-6 fatty acids (∑n6) (omega-6) 618.8 ± 27.4 mg / 100 g.

Table 4. Average length of female fish, total weight, weight after egg intake, total egg weight, egg weight and egg diameter, egg yield data, standard deviations, limits of change, and statistical analysis.

Parameters	One-meal (n = 50)	Two-meal (n = 45)	Three-meal (n = 51)	Four-meal (n = 56)	ANOVA
Length (cm)	33.52 ± 0.94[a]	37.00 ± 1.34[b]	37.70 ± 1.42[b]	38.12 ± 0.48[b]	<0.01
Weight (g)	486.54 ± 41.70[a]	694.93 ± 81.13[b]	763.28 ± 96.25[b]	756.19 ± 24.20[b]	<0.01
Weight after egg intake (g)	424.61 ± 37.64[a]	605.39 ± 70.10[b]	675.67 ± 84.49[b]	662.39 ± 20.89[b]	<0.01
Total egg weight (g)	65.10 ± 1.97[a]	95.91 ± 10.25[b]	96.98 ± 11.50[b]	98.57 ± 7.00[b]	<0.01
Egg weight (mg)	92.8 ± 2.44	96.6 ± 2.57	95.0 ± 1.40	94.2 ± 1.24	>0.05
Egg diameter (mm)	5.40 ± 0.017	5.45 ± 0.025	5.47 ± 0.046	5.44 ± 0.023	>0.05
Total fecundity	707 ± 38[a]	999 ± 77[b]	1036 ± 110[b]	1053 ± 60[b]	<0.01
Relative fecundity	1518 ± 67	1473 ± 76	1392 ± 63	1397 ± 60	>0.05

[ab] There is a statistical difference between data with different superscripts in the same row (length, weight, weight after egg intake, total egg weight, and total fecundity).

Table 5. The crude protein, crude oil, and crude ash ratios (%) of the fish feed used in the study and the fish fillets taken at the beginning and end of the study.

Content (%)	Used food	İnitial	Final				
			One-meal	Two-meal	Three-meal	Four-meal	Anova
Crude protein	44.32 ± 1.99	18.14 ± 0.09	15.15 ± 1.07[a]	15.79 ± 0.89[ab]	16.80 ± 0.77[bc]	17.02 ± 0.86[c]	<0.01
Crude oil	22.30 ± 0.09	4.12 ± 0.03	4.61 ± 0.82[a]	5.60 ± 1.14[b]	6.18 ± 1.13[b]	6.21 ± 1.56[b]	<0.05
Crude ash	5.63 ± 0.05	0.89 ± 0.06	1.21 ± 0.02	1.22 ± 0.02	1.23 ± 0.02	1.21 ± 0.04	>0.05

[abc] There is a statistical difference between data with different superscripts in the same row (crude protein and crude ash).

At the end of the study, fatty acids were calculated in all groups. Total saturated fatty acids were calculated as 976.1 ± 14.0 mg in the one-meal group, 1182.8 ± 30.4 mg in the two-meal group, 1352.7 ± 23.8 mg in the three-meal group, and 1328.7 ± 33.2 mg in the four-meal group (per 100 g of fish meat). Total monounsaturated fatty acids were calculated as 1900.8 ± 44.8 mg in the one-meal group, 2380.9 ± 56.1 mg in the two-meal group, 2606.2 ± 54.3 mg in the three-meal group, and 2633.6 ± 75.8 mg in the four-meal group (for 100 g of fish meat). Polyunsaturated fatty acids were calculated as 1476.5 ± 25.5 mg in the one-meal group, 1701.5 ± 32.0 mg in the two-meal group, 1865.1 ± 37.0 mg in the three-meal group, and 1877.6 ± 51.1 mg in the four-meal group (per 100 g of fish meat).

Palmitic acid, one of the saturated fatty acids, (C16:0) was found in the meal groups (for 100 g sample) as follows; 498.6 ± 71.6 mg in the one-meal group, 597.7 ± 155.5 mg in the two-meal group, 698.7 ± 116.2 mg in the three-meal group, and 684.8 ± 177.8 mg in the four-meal group. Stearic acid (C18:0) in meal groups (for 100 g sample) was found as follows: 152.0 ± 23.8 mg in the one-meal group, 166.7 ± 33.4 mg in the two-meal group, 201.0 ± 43.5 mg in the three-meal group, and 182.7 ± 48.9 mg in the four-meal group. Oleic acid (C18:1n-9), one of the monounsaturated fatty acids, was found in the meal groups (for 100 g sample) as follows: 1589.7 ± 37.1 mg in the one-meal group, 1961.0 ± 44.4 mg in the two-meal group, 2149.9 ± 46.7 mg in the three-meal group, and 2161.3 ± 62.1 mg in the four-meal group. Linoleic acid (C18:2n-6), one of the polyunsaturated fatty acids, was found in the meal groups (for 100 g sample) as follows: 787.7 ± 16.5 mg in one-meal group, 920.6 ± 21.5 mg in the two-meal group, 1001.9 ± 25.7 mg in the three-meal group, and 996.5 ± 27.4 mg in the four-meal group. Gamma linolenic acid (C18:3n-6) was found in meal groups (for 100 g sample) as follows: 9.9 ± 1.5 mg in th e one-meal group, 13.0 ± 3.8 mg in the two-meal group, 13.7 ± 4.6 mg in the three-meal group, and 14.3 ± 4.3 mg in the four-meal group. Arachidonic acid (C20:4n6), one of the highly unsaturated fatty acids, was found in the meal groups (for 100 g sample) as follows: 24.7 ± 4.9 mg in the one-meal group, 28.0 ± 3.2 mg in the two-meal group, 29.4 ± 7.4 mg in the three-meal group, and 31.4 ± 10.6 mg in the four-meal group. Eicosapentaenoic acid (EPA; C20:5n-3) was found in meal groups (for 100 g sample) as follows: 101.9 ± 13.9 mg in the one-meal group, 117.1 ± 23.3 mg in the two-meal group, 129.5 ± 9.1 mg in the three-meal group, and 137.4 ± 38.9 mg in the four-meal group.

Docosahexaenoic acid (DHA; C22:6n-3) was found in meal groups (for 100 g sample) as follows: 536.9 ± 96.1 mg in the one-meal group, 601.2 ± 92.0 mg in the two-meal group, 668.1 ± 99.7 mg in the three-meal group, and 675.6 ± 105.3 mg in the four-meal group. Total n-3 fatty acids (∑n3) were found in meal groups (for 100 g sample) as follows: 654.2 ± 11.0 mg in the one-meal group, 739.8 ± 11.1 mg in the two-meal group, 820.2 ± 11.4 mg in the three-meal group, and 835.4 ± 24.2 mg in the four-meal group. Total n-6 fatty acids (∑n6) meal groups (for 100 g sample) were found as follows: 822.3 ± 16.2 mg in the one-meal group, 961.7 ± 22.2 mg in the two-meal group, 1044.9 ± 26.4 mg in the three-meal group, and 1042.2 ± 28.1 mg in the four-meal group. The differences between the results found were statistically significant ($p < 0.05$), excluding EPA (Table 6). Percentage values of fatty acid methyl ester (FAME %) in oil are given in Table 6.

4. Discussion

4.1. Growth performance

It was reported that the growth of brown trout occurs at 4–19 °C and the best growth occurs at a water temperature of 13 °C [12]. During the period of the study, the water temperature rose up to 21.3 °C in summer. The growth performance of the fish and the feed evaluation rate were negatively affected by this situation.

Due to the fact that Abant trout is an endemic species and there are few growth studies conducted on it, it was not possible to compare its growth parameters. The fish sizes and research periods used in cultural studies [5,13,14] are quite different from this study. Aras [13] reported that the average length and weight of Abant trout (31.7 cm and 270.7 g) reached 33.3 cm in length and 361.8 g in weight as a result of a six-month study. It was reported that the total individual weight increase was 96.8 g during the study period, the larvae did not adapt to artificial feeding, and as a result, Abant trout will not be economical. Similarly, Uysal [14] stated that the growth performance of Abant trout is not profitable from the point of view of aquaculture, since it is about 5 g at the end of the first year after hatching from the egg. Unlike other researchers, Kocabaş and Başçınar [15] reported that Abant trout larvae reached a weight from 0.40 g to 19.95 g in 228 days and stated that culture characteristics should be investigated in detail.

The specific growth rates of Abant trout by weight have been found to be different by different researchers. While SGR_W was found between 0.07 and 0.24 by Aras [13], it was found as 1.24 ± 0.19 in larvae and juveniles by Uysal et al. [4]. The same parameter was found to be 0.94 ± 0.038 by Kocabaş [5]. Altınok et al. [16] reported that they found this parameter as 0.609 ± 0.07. The lowest SGR_W obtained in this study was determined in the one-meal group (1.554 ± 0.111) and it was found to be higher than the values reported by other researchers.

Kocabaş [5] and Altınok et al. [16] reported that the FCR ratio ranged between 0.94 and 2.54 in Abant trout. In our study, the highest FCR value was calculated in fish fed four meals a day. This situation shows that the FCR value increases with the increase in feeding, as Erbaş [17] reported in his study. Kurtoğlu [18], in his study on Black Sea trout, reported that the FCR value varied between 0.6 and 3.5 in fish in the freshwater group and between 1.3 and 4.4 in fish in the seawater group. Başçınar et al. [19], in their study with Black Sea trout, reported that the lowest FCR value was obtained in fish fed once a day (1.45), and the highest FCR value was obtained in fish fed three times a day (1.77). Among the reasons for the different FCR values found in different studies, the size of the fish used in the study, the content of the feed used, and the quality criteria of the water used may be effective.

In our study, the condition factor value increased with increasing fish weight. Aras [13] reported that these values ranged from 0.84 to 0.98. Kocabaş [5] reported that the condition factor value in Abant trout fry ranged between 0.86 and 1.24. In this study, the lowest value was calculated in the one-meal group (1.267–1.315), which is higher than the values found by Aras [13] and Kocabaş [5] but similar to the value reported by Altınok et al. (1.39) [16].

Ruohonen et al. [20] reported that rainbow trout in the range of 400–700 g should be fed at least 3 meals a day for growth as a result of a 1-, 2-, and 4-meal nutrition study. When this study is evaluated in terms of growth rates by weight, it is seen that fish fed 2, 3, and 4 times a day exhibit higher values than fish fed 1 time.

Petursdottir [21], investigated the effects of feeding frequency on the height and weight distribution of *Salvelinus alpinus* (L.). Petursdottir [21], divided trial fish with an average weight of approximately 145 g into 4 groups fed 24 h, fed twice a day (morning and evening), fed once a day (morning), and fed every 2 days. The study lasted 130 days. Petursdottir [21] reported at the end of the study that there was no significant difference between the final weights and specific growth rates of the fish. At the end of our study, which lasted 392 days, it was seen that there were statistically significant differences between the average final weights and specific growth rates of the fish. The reason for this situation is the short time between feeding frequencies in the study and the growth differences occurring.

When the obtained data are evaluated in terms of CF, SBO_W, and FCR, it is seen that the number of meals has an effect on these values. For trout, which is an important criterion for nutrition and development in fish, values between 1.14 and 1.53 are considered good [22]. The calculated conditioning factor is similar to that of other studies. Expressed as the logarithm of the difference in growth by weight in a given time period, SGR_W by weight increases depending on the excess of the number of meals in certain time periods and has a similar trend with other studies. The feed conversion rate, which shows the conversion of the feed taken to meat, also increases with the increase in the number of meals in each study. Tunçelli and Pirhonen [23] conducted a similar study with rainbow trout in a closed and fully controlled system under laboratory conditions. Looking at the results obtained from the study, it is seen that the growth performance data where water and stocking conditions are effective are in the same trend but better.

Investigating how the number of meals has an effect on the growth of rainbow trout fry, Sönmez et al. [24] evaluated the results of feeding 1, 2, and 3 meals a day to fish with a live weight of 15.78 ± 0.76 g. As a result, similar to the results obtained in this study, the final weights and SBO_W increased in parallel with the increase in the number of daily meals, but the FCR decreased. The feed evaluation rate increased in parallel with the increase in the number of meals in this study. Başçınar et al. [19] reported that feed consumption increased with the frequency of feeding. Erbaş [17] stated that the FCR increases in direct proportion with the increase in feeding. The FCR may vary depending on different criteria such as fish size, environmental living conditions, feeding amount and frequency, and feed content. In general, the feed evaluation results we found in this study are at a level that can

Table 6. Fatty acid composition in the feed and fish meat used in the study (FAME%).

Fatty acid*	Feed	Initial	One-meal	Two-meal	Three-meal	Four-meal	ANOVA
C14:0	1.71 ± 0.03	0.96 ± 0.19	1.40 ± 0.27	1.66 ± 0.20	1.61 ± 0.27	1.69 ± 0.17	p > 0.05
C15:0	0.11 ± 0.01	0.15 ± 0.01	0.21 ± 0.04	0.20 ± 0.05	0.20 ± 0.04	0.21 ± 0.03	p > 0.05
C16:0	9.76 ± 0.04	10.45 ± 0.59	10.89 ± 0.88	11.37 ± 0.55	10.60 ± 0.51	11.07 ± 0.36	p > 0.05
C16:1	2.39 ± 0.10	1.95 ± 0.27	2.73 ± 0.35	3.37 ± 0.46	3.21 ± 0.42	3.57 ± 0.26	p > 0.05
C17:0	0.13 ± 0.03	0.16 ± 0.01	0.21 ± 0.05	0.21 ± 0.03	0.21 ± 0.04	0.24 ± 0.02	p > 0.05
C18:0	3.59 ± 0.04	3.90 ± 0.11	3.31 ± 0.17	3.25 ± 0.17	2.99 ± 0.09	2.95 ± 0.15	p > 0.05
C18:1n9	38.53 ± 0.36	30.15 ± 1.03	34.28 ± 1.71	34.71 ± 0.79	34.98 ± 1.15	34.65 ± 1.19	p > 0.05
C18:2n6	19.19 ± 0.09	18.58 ± 0.59	17.05 ± 0.87	16.11 ± 0.97	16.41 ± 0.48	16.03 ± 0.90	p > 0.05
C18:3n6	0.11 ± 0.00	0.18 ± 0.01	0.22 ± 0.03	0.22 ± 0.03	0.23 ± 0.02	0.23 ± 0.01	p > 0.05
C20:0	0.51 ± 0.02	0.28 ± 0.04	0.35 ± 0.06	0.35 ± 0.06	0.28 ± 0.15	0.26 ± 0.13	p > 0.05
C20:1	4.19 ± 0.03	3.10 ± 0.14	3.58 ± 0.46	3.73 ± 0.24	3.87 ± 0.17	3.67 ± 0.14	p > 0.05
C21:0	6.16 ± 0.05	2.37 ± 0.12	2.78 ± 0.09	2.74 ± 0.14	2.89 ± 0.05	2.86 ± 0.11	p > 0.05
C22:0	1.63 ± 0.04	2.03 ± 0.16	2.15 ± 0.07	2.20 ± 0.21	2.21 ± 0.08	2.22 ± 0.08	p > 0.05
C20:3n3	0.47 ± 0.01	0.24 ± 0.11	0.33 ± 0.02	0.36 ± 0.04	0.39 ± 0.01	0.36 ± 0.02	p > 0.05
C20:4n6	0.21 ± 0.01	1.03 ± 0.47	0.54 ± 0.02	0.47 ± 0.04	0.51 ± 0.07	0.50 ± 0.05	p > 0.05
C20:5n3	2.46 ± 0.01	2.20 ± 0.22	2.23 ± 0.24	2.14 ± 0.29	2.10 ± 0.08	2.23 ± 0.30	p > 0.05
C22:6n3	3.06 ± 0.02	14.35 ± 2.01	11.72 ± 1.56	10.90 ± 0.77	10.92 ± 1.47	10.88 ± 1.06	p > 0.05
C24:1	0.44 ± 0.01	0.44 ± 0.03	0.42 ± 0.02	0.32 ± 0.03	0.36 ± 0.05	0.35 ± 0.04	p > 0.05
Σ SFA	23.60 ± 0.13	20.30 ± 0.90	21.29 ± 1.20	21.97 ± 0.62	20.99 ± 0.96	21.50 ± 0.59	p > 0.05
Σ MUFA	45.55 ± 0.24	35.63 ± 1.35	41.00 ± 1.93	42.12 ± 0.85	42.42 ± 1.32	42.24 ± 1.04	p > 0.05
Σ PUFA	25.51 ± 0.06	36.59 ± 2.17	32.09 ± 1.35	30.19 ± 0.97	30.55 ± 1.46	30.23 ± 0.85	p > 0.05
Σ n3	6.00 ± 0.03	16.79 ± 2.16	14.28 ± 1.69	13.40 ± 0.99	13.41 ± 1.53	13.47 ± 1.30	p > 0.05
Σ n6	19.51 ± 0.09	19.80 ± 0.84	17.81 ± 0.85	16.80 ± 1.02	17.15 ± 0.44	16.76 ± 0.88	p > 0.05
Σ n3 / Σn6	0.31 ± 0.01	0.85 ± 0.12	0.81 ± 0.12	0.80 ± 0.09	0.78 ± 0.10	0.81 ± 0.11	p > 0.05
Σ EPA+DHA	5.52 ± 0.03	16.55 ± 2.23	13.95 ± 1.71	13.03 ± 1.03	13.02 ± 1.53	13.11 ± 1.32	p > 0.05
Other	5.35 ± 0.19	5.63 ± 0.22	5.63 ± 0.23	5.73 ± 0.42	6.04 ± 0.63	6.04 ± 0.25	p > 0.05

* Σ SFA; total saturated fatty acids. Σ MUFA; total monounsaturated fatty acids. Σ PUFA; total polyunsaturated fatty acids. Σ EPA; eicosapentaenoic acid. Σ DHA docosahexaenoic acid.

be considered high for fish weighing 200–250 g, which is called portioned, while it can be said that it is within acceptable limits for breeding fish in which we conducted the study. The fact that some of the feed received by breeding fish is devoted to the production of sperm and eggs is a fact that explains this situation.

4.2. Egg yield and egg data (diameter and size)

Even if the type of fish is the same, there are some factors that affect egg yield and size. These are reported as intraspecies individual differences, broodstock size, and environmental and cultural conditions [25]. Therefore, different data have been reported by various researchers. The absolute egg yield calculated in this study is (707 ± 38 to 1053 ± 60). The results were higher than the values found by Kocabaş [5] (623 ± 515), close to the values found by Kalaycı [26] (1118 ± 314), and lower than the values found by Kavuk [6] (2749 ± 956). The relative egg yield (1392 ± 63 to 1518 ± 67) was close to the values found by Kocabaş [5] (1871 ± 742), higher than the values found by Kalaycı [26] (1083 ± 518), and lower than the values found by Kavuk [6] (3303 ± 1218). The egg diameter (5.40 ± 0.017 to 5.47 ± 0.046) was determined to be higher than the values found by Kocabaş [5] (4.91 ± 0.036), lower than the values found by Kalaycı [26] (5.55 ± 0.32) and higher than the values found by Kavuk [6] (4.76 ± 0.75).

Different researchers have calculated the egg sizes (diameters) of *Salmo trutta* differently in their studies. The egg diameters of *Salmo trutta* were determined by McFadden et al. [27] as 3.05–4.67 mm, by Gunnes and Gjedrem [28] as 5.2 mm, by Landergren [29] as 5.33 ± 0.17 mm, by Estay et al. [30] (3, 4, 5 years of age) as 4.64 ± 0.11, 4.77 ± 0.27, and 5.24 ± 0.12 mm. Tatar [31] reported that the egg diameters of the *Salmo trutta* in the Munzur Stream located in the city of Tunceli increased with the size of the fish, and the egg diameters were calculated between 4.83 and –5.20 mm. Tabak et al. [32] explained that the egg diameter of Black Sea trout caught from nature is 5.48 ± 1.101 mm, but the egg diameter of Black Sea trout grown is 5.8 ± 0.03 mm. Kurtoğlu [18] reported in his study that the egg diameters of *Salmo trutta* are similar in the river ecotype and the Black Sea ecotype. In the study they conducted in the Ceyhan River, Alp and Kara [33] stated that the egg diameter for Anatolian trout (in broodstocks over the second age) varies between 2.33 and –5.93 mm and has an average diameter of 4.18 mm. Table 7 has been edited to indicate what the data obtained in this study correspond to in order to make an assessment in terms of commercial trout farming. The results of this study were adapted according to the sample of producing 1,000,000 Abant trout eggs.

When calculations are made according to one-meal / day group, since the relative egg yield of this group is 1518 pieces / kg, 658.76 kg of female broodstock fish are needed to obtain 1,000,000 eggs. The proportional growth rate of the first group of fish by weight was found to be 138.94%. Therefore, with the environmental conditions in which the work is carried out (water quality, temperature, feed, stocking, and maintenance conditions, etc.), it is possible to reach a broodstock fish mass of 275.70 kg with a weight proportional growth of 138.94% at the end of 392 days to around 658.76 kg. As a result, 1,000,000 eggs can be taken from 658.76 kg of female fish with a purchase amount of 1518 eggs per kg. Taking into account the study period (392 days), an approximate weight difference of 383.06 kg can occur between the first and last live weights of broodstock fish. According to these data, approximately 591.06 kg of feed can be consumed with a feed evaluation rate of 1.54 caught in the one-meal group. If a similar adaptation is made within other groups, it is possible to reach the values given for all meal groups given in Table 7.

If the table is interpreted based on the values given, it is seen that in the one-meal group, a starting weight of 275.70 kg and a 591.06 kg meal are needed to obtain 1,000,000 eggs, while in the four-meal groups, a starting weight of 199.35 kg and a 1400.67 kg meal are needed to reach the same number of eggs. In other words, for the starting weights, a meal group needs 38% more live weight than a four-meal group, while this time the four-meal group needs 134% more food than a meal group to reach the final weights.

4.3. Incubation efficiency of eggs

The average incubation water temperature used in this study was 10.57 ± 2.03 °C and varied between 7.3–14.9 °C. Teufel et al. [34] reported that water temperatures of 7–12 °C are required during the hatching period of *Salmo trutta* fish. Ojanguren and Brana [35] reported that the optimum water temperature for optimum embryonic development in brown trout was 8–10 °C and the mortality rate increased above 14 °C. Due to the fact that the

Table 7. The amount of female breeding fish required and the average amount of feed consumed according to the data derived from the study to obtain 1,000,000 eggs (egg yield, proportional growth by weight, feed conversion rate).

Parameters*	One-meal	Two-meal	Three-meal	Four-meal
Relative fecundity (eggs/kg)	1518	1473	1392	1397
Required broodstock weight	658.76 kg	678.89 kg	718.39 kg	715.82 kg
Proportional growth by weight	138.94%	208.16%	243.74%	259.08%
Initial weights	275.70 kg	220.30 kg	208.99 kg	199.35 kg
FCR	1.54	1.81	2.14	2.71
Weight gain	383.06 kg	458.58 kg	509.40 kg	516.74 kg
Average feed needs	591.06 kg	828.20 kg	1089.60 kg	1400.67 kg

,* FCR; feed conversion rate.

brood water values used in the last period of this study exceeded the values given in the literature, an increase in egg mortality was observed.

The survival rate of salmonid eggs during the incubation period is between 0% and 100% [36]. A big fry comes out of a big egg. This increases the survival rate of large fry compared to small fry in the natural environment [27]. Jonsson and Jonsson [37] report that the size of the egg is related to the survival rate, and the survival rate of the offspring that will hatch from a large egg is higher. In contrast to these researchers, Springate and Bromage [38] report that egg size does not matter at the rate of fertilization, observation, hatching, and life up to swimming.

In this study, the eye pigmentation of Abant trout eggs was determined as 294 day-degree. This value is lower than the 330 day-degree reported by Kocabaş [5], but higher than the 248 day-degree reported by Kalaycı [26] and the 229–278 day-degree reported by Kavuk [6]. The hatching time of the Abant trout larvae was determined as 463 days-degrees. This value is lower than the 500 day-degree reported by Kocabaş [5]. It is higher than the 429 day-degree reported by Kalaycı [26] and the 393–458 day-degree reported by Kavuk [6]. Although water temperature is very important for the growth of *Salmo trutta*, water temperature has little effect on the survival of their eggs. One of the reasons for the differences in the survival rate in this study with the studies conducted by different researchers may be the temperature of the water used in the incubation phase and the incubation unit [39].

4.4. Biochemical composition

Türker and Yıldırım [40] investigated the crude protein, crude fat, and ash values of rainbow trout fed 2, 3, 4, and 6 times a day in the study of feeding frequency in seawater. At the end of the study, they found the highest crude protein, crude oil, and ash values in fish fed 6 times a day and 18.4%, 8.6%, and 2.2%, respectively. In our study, the highest crude protein, crude fat, and ash values were found in the four meals/day groups and were 17.2%, 6.21%, and 1.21%, respectively. When these two studies were compared in terms of crude protein and crude fat, it was determined that the increase from fewer meals to more meals was similar. However, from the point of view of ash ratios, the highest rate was found in the group that was fed three meals/day with a value of 1.23% in our study.

Palmitic acid (16:0) is the main saturated fatty acid (SFA) in fish, and C16:0 was reported to be the most abundant SFA in different types of trout, natural or cultured [41-45]. It was reported that the SFA ratio of Abant trout oil is high compared to many marine fish and low compared to some fish [46]. In contrast, it is similar to Black Sea trout and spring trout [47].

Oleic acid (18:1 n9) is a monounsaturated fatty (MUFA) acid analysed for trout and other fish species [42,44,48,49]. The lowest MUFA value was calculated in the group that was fed 1 meal/day in this study ($p < 0.05$). The ΣMUFA value is applied to brook trout and Black Sea trout [47], also rainbow trout [50] and it was found to be high compared to many marine fish [46].

The addition of n3 PUFA to food increases its nutritional value and can protect against diseases [51]. DHA and EPA have been shown to have preventive effects on human coronary artery disease. For this reason, fish has been proposed as a key ingredient for a healthy diet in humans [52]. In this study, EPA values were found to be similar in all Abant trout groups, while DHA levels were determined to be slightly lower in the group that was fed 1 meal/day. The minimum recommended PUFA/SFA ratio value is 0.45 [53], PUFA/SFA ratios are well above the recommended level in all groups. This level is higher than that of many marine species [46,51,54,55]. It was stated that the ratio of unsaturated fatty acids n3/n6 is 1:1 to 1:5, which is necessary for a healthy human food [56]. The n3/n6 ratios calculated in all four groups carried out in the study are within the recommended values. The n6/n3 ratio is important in fish oils and the ideal n6/n3 ratio is recommended to be maximum 4.0 [53] and it is suggested that higher values have a negative effect on health and may promote heart diseases [51]. In this study, this ratio is lower than the ideal ratio, so Abant trout oil has an important nutritional value in terms of the n6/n3 ratio.

5. Conclusion

When the data obtained from the whole study are evaluated, it is seen that the growth data of Abant trout are acceptable; it has a significant egg yield and has a meat quality that can be considered equivalent to the like. Developments in aquaculture techniques, increasing information technologies, and developments in feed technology may have an impact on achieving values higher than the values previously achieved in the study conducted by Uysal [14] about Abant trout. According to these results, it was found that Abant trout has the culturability, fertility, good growth rate, moderate FCR, and good meat quality that commercial fish breeders are looking for. As a result, it was concluded that Abant trout, which has the growth/cost/meat quality data requested by commercial fish breeders, can be grown commercially under culture conditions.

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Data availability statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Conflict of interest

The authors declare there are no competing interests.

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