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COMPARISON OF THE COMPOSITION AND COLOR CHARACTERISTICS OF HAPLOID AND DIPLOID RAINBOW TROUT (*Oncorhynchus mykiss*) EGGS PRODUCED UNDER DIFFERENT REARING CONDITIONS

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Abstract

This study investigated the biochemical composition and color characteristics of haploid (n) and diploid (2n) eggs from rainbow trout (*Oncorhynchus mykiss*) broodstock reared at two farms in Seydikemer, Muğla. Four-year-old females and three-year-old males were used, and eggs were analyzed for proximate composition, fatty acid profile, and colorimetric parameters. While no significant differences were observed in dry matter, crude protein, crude fat, or ash content ($p>0.05$), diploid eggs exhibited higher total saturated and monounsaturated fatty acids, greater omega-3 levels, and higher EPA+DHA content. Haploid eggs showed higher omega-6 levels, DHA/EPA ratio, and hypocholesterolemic/hypercholesterolemic (HH) ratio ($p<0.05$). Color analysis revealed that diploid eggs had higher L* (lightness) and b* (yellowness), whereas haploid eggs were redder (higher a*, $p<0.05$). These results indicate that ploidy affects egg fatty acid composition and color, which may influence embryonic development and selective breeding programs.

Keywords: Egg quality, Fatty acid, Lipid composition, Rainbow trout, Trout egg

Introduction

Aquaculture has become a rapidly growing global sector, playing a strategic role in meeting the food demands of the world's increasing population (FAO, 2020). Key drivers of this growth include the depletion of natural fish stocks, the need for sustainable food production, and the high productivity offered by aquaculture. In this context, rainbow trout (*Oncorhynchus mykiss*) stands out as one of the most widely farmed cold-water fish species worldwide due to its rapid growth performance, adaptability to environmental conditions, economic return, and ease of production (Escamilla-Rosales et al., 2024). Furthermore, this species possesses a rich nutritional profile with high-quality protein, omega-3 fatty acids, and various vitamins and minerals, making it highly valued for its nutritional value and significantly contributing to the continuously increasing consumer demand (Xu et al., 2022; Mahato et al., 2023). Its widespread acceptance in the consumer market is further bolstered by its soft texture, desirable white-to-pink flesh color, and mild flavor, enhancing its appeal as a high-quality animal protein source (Janampa-Sarmiento et al., 2020).

In trout farming, production success largely depends on egg quality, as the chemical composition of the eggs directly determines embryonic development, fry survival rates, and overall productivity (Izquierdo et al., 2001; Brooks et al., 1997). In this context, the amino acid and fatty acid profiles of trout eggs are critically important and are generally at levels that support embryonic development. In particular, omega-3 polyunsaturated fatty acids such as EPA and DHA are essential components for fry growth, immune function, and physiological development (Tocher, 2010; Baki et al., 2021). The levels of these fatty acids in eggs largely depend on the broodstock diet. Differences in lipid sources used in broodstock diets can directly affect the fatty acid composition of the eggs; some vegetable oil-enriched feeds have been reported to increase EPA, ARA, and DHA levels, positively impacting fry development and survival rates (Mazorra et al., 2003; Yıldız et al., 2020).

Grčević et al. (2019) emphasized that egg color should be considered as one of the important quality criteria in fish breeding studies. Since fish cannot synthesize carotenoids, these pigments must be obtained entirely from external sources via feed (Bjerkeng, 2008). In addition to giving the egg yolk a bright and vibrant color, carotenoids contribute to healthy embryonic development by protecting the embryo against oxidative damage thanks to their strong antioxidant properties. Therefore, sufficient carotenoid availability plays a critical role in improving embryonic development and increasing the survival rate of juvenile fish. Furthermore, the attractive color appearance of the egg significantly contributes to the marketability of the product by enhancing the perception of quality in both aquaculture facilities and consumer markets (Nakano & Wiegertjes, 2020; Shastak & Pelletier, 2023).

Due to the influence of feed composition, environmental conditions, and genetic factors, significant differences arise in the chemical and biochemical properties of eggs obtained from different production centers (Bobe, 2015). Therefore, comparative analyses of rainbow trout eggs raised under various conditions are crucial for ensuring quality control and conducting improvement efforts in the industry (Baki et al., 2019; Baki et al., 2021).

The aim of this study was to comparatively investigate the basic chemical composition, fatty acid profile and color parameters of haploid and diploid rainbow trout (*Oncorhynchus mykiss*) broodstock eggs obtained from different trout fish farms.

Material and Method

Broodstock Spawning and Fertilization Process

This study was conducted in two separate rainbow trout (*Oncorhynchus mykiss*) hatcheries located in the Seydikemer district of Muğla province. Four female and two male broodstock were used in the study. Dry spawning and fertilization were performed. The weight and length of the fish, which were stunned with phenoxyethanol, were measured. Haploid (n) eggs obtained from two four-year-old female broodstock were weighed, measured, labeled, and transported to the laboratory in styrofoam boxes covered with ice trays. Sperm from a three-year-old male broodstock, checked for motility under a microscope, was added to the eggs and mixed. For the analysis of rainbow trout sperm, a Nikon Eclipse microscope equipped with a 20x objective and phase contrast was used. Sperm motility was examined using D532 buffer (1 mM $CaCl_2$, 20 mM Tris, 30 mM glycine, 125 mM NaCl, pH 9.0). During the examination, 2 µL of milt was mixed with 398 µL of activation solution at 6°C, and 0.7 µL of this mixture was placed on a glass slide, covered with a coverslip, and motility was observed from activation until cessation (Billard, 1977, Dietrich et al., 2008). Fertilization was achieved by contacting the mixture with water, and the eggs were allowed to rest for 30 minutes. The same procedures were followed for diploid (2n) eggs. All samples were stored at -80°C, and care was taken to ensure cleanliness and labeling of the materials. Similar procedures were performed at the other facility (Emre & Kürüm, 2007).

Fecundity (Egg Production)

Fecundity was estimated by dividing the number of eggs counted in the subsample by the total weight of the gonad (Le Cren, 1951; Avşar, 2005; Serezli, 2017).

F = Fecundity (number),

n = Number of eggs in the subsample (number),

Wg = Gonad weight (g),

g = Weight of the subsample (g).

The equation F = n x Wg/g was used.

Determination of Chemical Composition of Egg Samples

Approximately 10-15 grams of egg sample were used for the analyses. Moisture analysis was performed by drying at 105°C. Crude protein content was determined by the Kjeldahl method using an EFLAB device equipped with an infrared combustion system. Following this, distillation (EFLAB) and titration with 0.1 mol HCl were performed. Ash analysis was performed by combustion at 600°C, and crude fat was determined using a Soxhlet apparatus (VELP SCIENTIFICA SER 148 model). After extraction with ether, the fat-ether mixture was distilled in the apparatus to separate the solvent from the fat (AOAC, 1998).

Lipid Extraction and Fatty Acid Compositions

Total lipids were extracted according to the method of Bligh & Dyer (1959). Fatty acid composition was determined after lipid extraction and methylation. Fatty acid methyl esters (FAMEs) were prepared by mixing 0.25 g of extracted lipid with 4 mL of heptane and 0.4 mL of 2 N KOH in methanol. The mixture was vortexed for 2 minutes and centrifuged at 5000 rpm for 5 minutes, and the clear supernatant was transferred into GC vials. Fatty acid compositions of the samples were analyzed using a GC/MS (Thermo Scientific ISQ LT) instrument equipped

with an automated sampler. The capillary column used was a Trace Gold TG-WaxMS (60 m) column with an inner diameter of 0.25 µm and a film thickness of 0.25 µm. The column temperature was initially held at 100°C for 3 minutes, then increased to 240°C at a rate of 4°C/min after an initial hold time of 6 minutes. Helium was used as the carrier gas at a flow rate of 1 mL/min, with a split ratio of 1:20. The injection block temperature was set to 240°C, and the MS transfer line and ion source temperatures were set to 250°C and 240°C, respectively. The mass spectrometer was operated in electron impact ionization mode (70 eV). For identification and comparison, a standard FAME mixture (Supelco, 37 components, Bellefonte, PA, USA) was used (Çorapcı et al., 2021; Kocatepe et al., 2025).

Total fatty acids were calculated using the following formulas:

ΣSFA=C6:0 + C8:0 + C10:0 + C11:0 + C12:0 + C13:0 + C14:0 + C15:0 + C16:0 + C17:0 + C18:0 + C20:0 + C21:0 + C22:0 + C23:0 + C24:0
ΣMUFA=C14:1 + C15:1 + C16:1 + C17:1 + C18:1n-9c + C18:1n-9t + C20:1n-9c + C22:1n-9 + C24:1
ΣPUFA=C18:2n-6t + C18:2n-6c + C18:3n-3 + C18:3n-6 + C20:2 + C22:2 + C20:3n-3 + C20:3n-6 + C20:5n-3 + C20:4n-6 + C22:6n-3
ΣOmega-3 (ω3)=C18:3n-3 + C20:3n-3 + C20:5n-3 + C22:6n-3
ΣOmega-6 (ω6)=C18:2n-6t + C18:2n-6c + C18:3n-6 + C20:4n-6 + C20:3n-6
ΣOmega-9 (ω9)=C18:1n-9c + C18:1n-9t + C20:1n-9c + C22:1n-9
Atherogenicity (AI) Index: [(C12:0 + (4 × C14:0) + C16:0)] / (MUFA + Omega-3 + Omega-6)
Thrombogenicity (IT) Index: (C14:0 + C16:0 + C18:0) / [(0.5 × MUFA) + (0.5 × Omega-6) + (3 × Omega-3) + (Omega-3 / Omega-6)]
Hypocholesterolemic/Hypercholesterolemic Ratio (H/H) = (C18:1n-9 + C18:2n-6 + C18:3n-3 + C20:4n-6 + C20:5n-3 + C22:6n-3) / (C14:0 + C16:0)

Color Analysis

Egg color was measured using a color spectrophotometer (ColorFlex EZ, HunterLab, USA). The L∗, a∗, and b∗ parameters are as follows: L* represents brightness (lightness-darkness), a* represents redness-greenness, and b* represents yellowness-blueness. Three replicate readings were taken for each sample.

Statistical Analysis

Data obtained in the experiment were analyzed using the IBM SPSS 21 statistical program. The fatty acid levels of broodstock feeds and the fatty acid composition of haploid and diploid eggs were evaluated using a t-test. The independent sample t-test was applied to the groups in the study. First, the Levene test was used to check whether the variances were equal. If the variances were equal, the significance value in the "Equal variance assumed" line was used. If the variances were not equal, the t-test result was determined by examining the significance value in the "Unequal variance assumed" line. If the sig (2-tailed) value was less than 0.05, a significant difference was concluded between the two groups. If this value was greater than 0.05, there was no significant difference between the groups (Yazıcıoğlu & Erdoğan, 2014).

Nutrient content, fatty acid composition, and color analyses of haploid and diploid eggs were analyzed using the Tukey test. Data were analyzed using one-way analysis of variance (ANOVA) using the SPSS 21 statistical program and subjected to Tukey's multiple comparison test. Differences between groups were evaluated as $p<0.05$ (Logan, 2010).

Results

Water quality parameters were measured during the winter months at two different trout farms in Seydikemer. Temperature, pH, and dissolved oxygen levels were measured as 12.25±0.53°C, 7.75±0.18, and 8.15±0.25 mg/L in facility 1. Temperature, pH, and dissolved oxygen levels were measured as 11.50±0.35°C, 7.40±0.15, and 8.50±0.30 mg/L in facility 2.

Table 1. Metric measurements of the trial fish.

		Weight (g)	Length (cm)	Spawned Eggs (g)	Post-spawned weight (g)	Gonad Weight (g)
Station 1	1. fish	3.010	64	408	2.602	602
	2. fish	3.470	64	572	2.898	694
Station 2	1. fish	2.912	55	527	2.385	582.4
	2. fish	2.900	57	411	2.489	580

According to the metric measurements, the trial fish at Station 1 exhibited body weights of 3.010-3.470 g, a length of 64 cm, and gonad weights ranging from 602 to 694 g. At Station 2, the fish showed body weights of 2.900-2.912 g, lengths of 55-57 cm, and gonad weights between 580 and 582.4 g (Table 1).

Table 2. Haploid and diploid trout egg composition and estimated fertility at two stations

Egg Composition	Sample (count)	Sample (gram)	Diameter (mm)	Fecundity (count)
Station 1				
Haploid	63	5.668±0.32	4.5±0.15	6.691±0.27
Diploid	78	7.634±0.45	5.5±0.20	7.713±0.35
Total				14.404
Station 2				
Haploid	58	4.490±0.28	5±0.18	7.523±0.31
Diploid	57	6.060±0.38	5.5±0.22	7.492±0.33
Total				15.015

In the comparison between haploid and diploid eggs, the sample weights of diploid eggs were measured as 7.634±0.45 g and 6.060±0.38 g, while those of haploid eggs were 5.668±0.32 g and 4.490±0.28 g, respectively.

Diameter measurements showed that diploid eggs had values of 5.5±0.20 mm and 5.5±0.22 mm, whereas haploid eggs measured 4.5±0.15 mm and 5±0.18 mm. Regarding fecundity, diploid eggs exhibited values of 7.713±0.35 and 7.492±0.33, compared to haploid eggs, which were 6.691±0.27 and 7.523±0.31. These results indicate that diploid eggs surpass haploid eggs in terms of weight, diameter, and fecundity (Table 2).

Table 3. Chemical composition of broodstock feeds

Chemical Composition of Feeds	Station 1	Station 2
Crude Protein (%)	50	45.3
Crude Fat (%)	14	19.4
Crude Fiber (%)	2.5	0.8
Crude Ash (%)	8.5	8.9
Gross Energy (GE; kJ/g)	21.58	22.71

The chemical composition of the feeds given to broodstock fish varied among the stations. The feed used at the first station contained 50% crude protein, 14% crude fat, 2.5% crude fiber, and 8.5% crude ash, yielding a gross energy value of 21.58 kJ/g. The feed used at the second station contained 45.3% crude protein, 19.4% crude fat, 0.8% crude fiber, 8.9% crude ash, and a gross energy value of 22.71 kJ/g (Table 3).

Table 4. Analysis of chemical composition of diploid and haploid rainbow trout eggs from various location

Chemical Composition of Eggs	Haploid (1st station)	Haploid (2st station)	Diploid (1st station)	Diploid (2st station)
Dry matter (%)	29.75±1.30	28.76±1.64	30.3±2.35	29.72±3.56
Crude Protein (%)	22.09±1.63	21.02±1.37	22.37±2.48	20.98±0.67
Crude Fat (%)	3.69±0.56	3.43±0.32	3.65±0.08	4.26±1.48
Crude Ash (%)	3.96±0.62	4.30±0.24	4.29±0.35	4.47±1.41

Significant differences ($p<0.05$) exist between values in the same rows that are indicated by different letters. The values (n=3) are presented as mean ± SEM.

Analysis of the chemical composition of diploid and haploid rainbow trout eggs revealed no statistically significant differences in dry matter, crude protein, crude fat or crude ash contents ($p>0.05$). Dry matter ranged from 28.76±1.64% to 29.75±1.30% in haploid eggs and from 29.72±3.56% to 30.3±2.35% in diploid eggs. Crude protein was similar between groups; haploids ranged from 21.02±1.37% to 22.09±1.63% and diploids ranged from 20.98±0.67% to 22.37±2.48%. Crude fat content ranged from 3.43±0.32% to 3.69±0.56% in haploids and from 3.65±0.08% to 4.26±1.48% in diploids. Raw ash content also did not show any significant difference, varying between 3.96±0.62% and 4.30±0.24% in haploids and 4.29±0.35% and 4.47±1.41% in diploids (Table 4).

Fatty acid analysis of trout broodstock feeds from the first and second stations revealed significant differences in several saturated, monounsaturated, and polyunsaturated fatty acids. Specifically, C14:0, C16:0, C17:0, C18:0, C20:0, C22:0, C23:0, ΣSFA, C17:1, C18:1n9t, C20:1, ΣMUFA, C18:2n6t, C18:2n6c, C18:3n3, C20:2, C20:3n-3, C20:5n-3, C20:3n-6, C20:4n-6, C22:6n-3, C22:2, C22:1n9, ΣPUFA, ΣOmega-3, ΣOmega-6, ΣOmega-9, ω3/ω6, ω6/ω3, EPA/DHA, and DHA/EPA showed significant differences ($p<0.05$) between the two

stations. In contrast, C15:0, C15:1, C16:1, C21:0, C24:0, C24:1, C14:1, and C18:3n6 did not differ significantly (p>0.05) (Table 5).

Table 5. Comparison of fatty acid levels of different broodstock feed groups (Independent Sample T-Test)

Fatty Acids	Groups	N	X	ss	t-test		
					t	sd	p
C14:0 (%)	1st Stn.	3	2.87	0.05	-10.161	4	0.001
	2nd Stn.	3	4.60	0.29	-10.161	2.127	0.008
C15:0 (%)	1st Stn.	3	0.48	0.02	1.006	4	.371
	2nd Stn.	3	0.45	0.04	1.006	2.748	.395
C16:0 (%)	1st Stn.	3	15.19	0.14	11.345	4	.000
	2nd Stn.	3	12.25	0.42	11.345	2.476	.003
C17:0 (%)	1st Stn.	3	0.78	0.01	17.482	4	.000
	2nd Stn.	3	0.50	0.02	17.482	2.616	.001
C18:0 (%)	1st Stn.	3	2.87	0.05	-10.161	4	.001
	2nd Stn.	3	4.60	0.29	-10.161	2.127	.008
C20:0 (%)	1st Stn.	3	1.40	0.02	19.400	4	.000
	2nd Stn.	3	1.07	0.02	19.400	3.994	.000
C21:0 (%)	1st Stn.	3	0.03	0.02	-2.457	4	.070
	2nd Stn.	3	0.07	0.01	-2.457	3.200	.086
C22:0 (%)	1st Stn.	3	1.31	0.05	17.717	4	.000
	2nd Stn.	3	0.54	0.05	17.717	3.980	.000
C23:0 (%)	1st Stn.	3	0.14	0.02	-3.280	4	.031
	2nd Stn.	3	0.21	0.02	-3.280	3.816	.033
C24:0 (%)	1st Stn.	3	0.58	0.01	.898	4	.420
	2nd Stn.	3	0.57	0.03	.898	2.580	.445
ΣSFA (%)	1st Stn.	3	32.26	0.22	8.995	4	.001
	2nd Stn.	3	27.07	0.97	8.995	2.219	.009
C14:1 (%)	1st Stn.	3	0.21	0.00	-.354	4	.742
	2nd Stn.	3	0.22	0.03	-.354	2.129	.756
C15:1 (%)	1st Stn.	3	0.09	0.00	1.768	4	.152
	2nd Stn.	3	0.07	0.01	1.768	2.560	.191
C16:1 (%)	1st Stn.	3	0.42	0.00	2.457	4	.070
	2nd Stn.	3	0.38	0.03	2.457	2.148	.125
C17:1 (%)	1st Stn.	3	0.45	0.01	-9.865	4	.001
	2nd Stn.	3	0.73	0.04	-9.865	2.528	.004
C18:1n9t (%)	1st Stn.	3	1.61	0.04	-9.099	4	.001
	2nd Stn.	3	1.94	0.04	-9.099	3.906	.001
C20:1 (%)	1st Stn.	3	2.68	0.03	110.363	4	.000
	2nd Stn.	3	0.35	0.00	110.363	2.102	.000
C24:1 (%)	1st Stn.	3	1.16	0.29	1.012	4	.369
	2nd Stn.	3	0.98	0.03	1.012	2.057	.416
ΣMUFA (%)	1st Stn.	3	34.34	0.35	6.126	4	.004
	2nd Stn.	3	30.01	1.17	6.126	2.367	.017
	1st Stn.	3	0.22	0.00	-23.702	4	.000

Fatty Acids	Groups	N	X	ss	t-test		
					t	sd	p
C18:2n6t (%)	2nd Stn.	3	0.40	0.01	-23.702	2.941	.000
C18:2n6c (%)	1st Stn.	3	18.50	0.06	30.207	4	.000
	2nd Stn.	3	14.41	0.22	30.207	2.287	.001
C18:3n3 (%)	1st Stn.	3	4.05	0.05	-39.588	4	.000
	2nd Stn.	3	6.78	0.10	-39.588	3.152	.000
C18:3n6 (%)	1st Stn.	3	0.17	0.01	-2.055	4	.109
	2nd Stn.	3	0.21	0.03	-2.055	2.322	.158
C20:2 (%)	1st Stn.	3	1.65	0.01	-20.348	4	.000
	2nd Stn.	3	1.95	0.02	-20.348	2.941	.000
C20:3n-3 (%)	1st Stn.	3	1.98	0.02	-98.508	4	.000
	2nd Stn.	3	4.88	0.04	-98.508	3.298	.000
C20:5n-3 (%)	1st Stn.	3	1.90	0.02	-82.575	4	.000
	2nd Stn.	3	5.76	0.07	-82.575	2.569	.000
C20:3n-6 (%)	1st Stn.	3	0.49	0.00	7.155	4	.002
	2nd Stn.	3	0.44	0.01	7.155	2.941	.006
C20:4n-6 (%)	1st Stn.	3	0.80	0.01	-23.888	4	.000
	2nd Stn.	3	1.24	0.03	-23.888	2.424	.001
C22:6n-3 (%)	1st Stn.	3	3.39	0.16	-33.310	4	.000
	2nd Stn.	3	6.68	0.05	-33.310	2.456	.000
C22:2 (%)	1st Stn.	3	0.20	0.00	5.892	4	.004
	2nd Stn.	3	0.11	0.02	5.892	2.00	.028
C22:1n9 (%)	1st Stn.	3	0.11	0.00	6.364	4	.003
	2nd Stn.	3	0.08	0.00	6.364	4.00	.003
ΣPUFA (%)	1st Stn.	3	33.38	0.18	-52.247	4	.000
	2nd Stn.	3	42.90	0.25	-52.247	3.709	.000
ΣOmega-3 (%)	1st Stn.	3	11.33	0.15	-98.888	4	.000
	2nd Stn.	3	24.12	0.16	-98.888	3.974	.000
ΣOmega-6 (%)	1st Stn.	3	20.19	0.05	25.970	4	.000
	2nd Stn.	3	16.71	0.22	25.970	2.254	.001
ΣOmega-9 (%)	1st Stn.	3	31.99	0.15	5.917	4	.004
	2nd Stn.	3	27.61	1.27	5.917	2.056	.026
ω3/ω6	1st Stn.	3	0.561	0.00	-67.569	4	.000
	2nd Stn.	3	1.44	0.02	-67.569	2.421	.000
ω6/ω3	1st Stn.	3	4.56	0.05	81.798	4	.000
	2nd Stn.	3	2.12	0.00	81.798	2.046	.000
EPA/DHA	1st Stn.	3	0.56	0.03	-15.605	4	.000
	2nd Stn.	3	0.86	0.00	-15.605	2.100	.003
DHA/EPA	1st Stn.	3	1.78	0.10	10.537	4	.000
	2nd Stn.	3	1.15	0.00	10.537	2.019	.009

N: Number of samples in each group, $\bar{X}$: Mean. ss: Standard deviation, sd: Degrees of freedom, p: Significance level

Table 6. Fatty acid composition comparison between diploid and haploid rainbow trout eggs from various locations

Fatty acids	Haploid (1st station)	Haploid (2st station)	Diploid (1st station)	Diploid (2st station)
C14:0 (%)	1.25±0.02^{c}	1.85±0.06^{a}	1.42±0.01^{b}	1.18±0.08^{c}
C15:0 (%)	0.28±0.02^{ab}	0.26±0.00^{b}	0.30±0.00^{a}	0.16±0.01^{c}
C16:0 (%)	13.44±0.38^{ab}	10.92±0.03^{c}	13.88±0.07^{a}	12.85±0.23^{b}
C17:0 (%)	0.64±0.01^{a}	0.41±0.01^{c}	0.57±0.01^{b}	0.30±0.00^{d}
C18:0 (%)	10.11±0.20^{b}	8.14±0.04^{c}	10.60±0.10^{a}	8.31±0.00^{c}
C20:0 (%)	0.27±0.01^{b}	0.15±0.00^{c}	0.29±0.00^{a}	0.13±0.01^{d}
C21:0 (%)	0.07±0.13^{ab}	0.02±0.04^{b}	0.25±0.00^{a}	0.02±0.01^{b}
C22:0 (%)	0.26±0.03^{a}	0.02±0.00^{b}	0.34±0.07^{a}	0.13±0.06^{b}
C23:0 (%)	0.20±0.05^{b}	0.21±0.01^{ab}	0.29±0.02^{a}	0.01±0.00^{c}
C24:0 (%)	1.04±0.03^{a}	0.76±0.03^{b}	1.04±0.11^{a}	0.43±0.06^{c}
ΣSFA (%)	27.61±0.56^{b}	22.80±0.12^{c}	29.04±0.10^{a}	23.24±0.18^{c}
C14:1 (%)	0.03±0.02^{a}	0.04±0.00^{a}	0.04±0.00^{a}	0.02±0.00^{a}
C15:1 (%)	0.02±0.01^{a}	0.02±0.00^{ab}	0.02±0.00^{ab}	0.01±0.00^{b}
C16:1 (%)	0.30±0.02^{a}	0.24±0.00^{b}	0.31±0.00^{a}	0.16±0.02^{c}
C17:1 (%)	0.38±0.04^{a}	0.25±0.02^{b}	0.41±0.04^{a}	0.12±0.00^{c}
C18:1n9c (%)	19.64±0.04^{c}	20.04±0.16^{b}	21.50±0.03^{a}	15.93±0.11^{d}
C18:1n9t (%)	2.03±0.13^{a}	1.70±1.19^{a}	2.00±0.03^{a}	2.33±0.04^{a}
C20:1 (%)	2.24±0.08^{b}	0.18±0.00^{d}	2.47±0.04^{a}	0.44±0.02^{c}
C24:1 (%)	0.14±0.00^{b}	0.07±0.01^{c}	0.13±0.02^{b}	1.07±0.01^{a}
ΣMUFA (%)	24.98±0.07^{b}	22.85±1.08^{c}	27.09±0.06^{a}	20.17±0.16^{d}
C18:2n6t (%)	0.49±0.01^{b}	0.32±0.01^{c}	0.56±0.01^{a}	0.18±0.00^{d}
C18:2n6c (%)	0.03±0.00^{b}	13.31±0.08^{a}	0.03±0.00^{b}	13.17±0.06^{a}
C18:3n3 (%)	3.11±0.02^{c}	3.27±0.01^{b}	3.48±0.01^{a}	2.32±0.09^{d}
C18:3n6 (%)	1.54±0.02^{b}	0.58±0.00^{c}	1.72±0.08^{a}	0.41±0.03^{d}
C20:2 (%)	4.46±0.02^{c}	4.81±0.02^{ab}	4.85±0.01^{a}	4.72±0.06^{b}
C20:3n-3 (%)	1.56±0.04^{b}	1.30±0.00^{c}	1.81±0.00^{a}	0.01±0.00^{d}
C20:5n-3 (%)	6.30±0.06^{b}	5.58±0.13^{d}	6.79±0.03^{a}	5.81±0.05^{c}
C20:3n-6 (%)	6.37±0.03^{a}	3.47±0.04^{c}	*	4.28±0.04^{b}
C20:4n-6 (%)	7.94±0.11^{b}	5.89±0.01^{c}	8.35±0.03^{a}	5.45±0.03^{d}
C22:6n-3 (%)	15.47±0.31^{c}	15.28±0.11^{c}	16.01±0.11^{b}	19.81±0.14^{a}
C22:2 (%)	0.17±0.00^{b}	0.04±0.00^{c}	0.21±0.02^{a}	0.02±0.00^{c}
C22:1n9 (%)	0.06±0.08^{b}	0.12±0.00^{ab}	0.20±0.01^{a}	0.07±0.02^{b}
ΣPUFA (%)	45.28±0.47^{c}	53.87±0.08^{b}	43.85±0.12^{d}	56.21±0.11^{a}
ΣOmega-3 (%)	26.45±0.40^{b}	25.43±0.13^{c}	28.10±0.11^{a}	27.95±0.09^{a}
ΣOmega-6 (%)	16.40±0.07^{b}	23.58±0.12^{a}	10.68±0.10^{c}	23.51±0.02^{a}
ΣOmega-9 (%)	23.98±0.21^{b}	22.05±1.10^{c}	26.17±0.03^{a}	18.78±0.15^{d}
ω3/ω6	1.61±0.01^{b}	1.07±0.01^{d}	2.63±0.03^{a}	1.19±0.00^{c}
ω6/ω3	0.62±0.00^{c}	0.92±0.00^{a}	0.38±0.00^{d}	0.84±0.00^{b}
EPA/DHA	0.40±0.00^{a}	0.36±0.01^{b}	0.42±0.00^{a}	0.29±0.00^{c}
DHA/EPA	2.45±0.03^{c}	2.74±0.07^{c}	2.35±0.01^{b}	3.41±0.05^{a}
AI	0.31±0.00^{b}	0.35±0.01^{a}	0.35±0.00^{a}	0.33±0.00^{ab}
TI	0.24±0.00^{a}	0.20±0.00^{b}	0.24±0.00^{a}	0.20±0.00^{b}
HH	3.74±0.13^{c}	5.12±0.12^{a}	3.60±0.02^{c}	4.46±0.05^{b}
EPA+DHA	21.78±0.36^{c}	20.86±0.15^{d}	22.80±0.11^{b}	25.62±0.11^{a}

*Statistical analysis does not include this. Significant differences ($p<0.05$) exist between values in the same rows that are indicated by different letters. The values (n=3) are presented as mean ± SEM.

Total saturated fatty acid (SFA) and monounsaturated fatty acid (MUFA) levels were found to be higher in diploid eggs compared to haploid eggs ($p<0.05$). The amount of omega-3 (Σω-3) fatty acids was higher in diploid eggs, while omega-6 (Σω-6) levels were significantly higher in haploid eggs. This resulted in a higher omega-3/omega-6 (ω3/ω6) ratio in diploids and a higher ω6/ω3 ratio in haploids, and these differences were statistically significant ($p<0.05$). The EPA/DHA ratio was generally higher in diploid eggs, which was also reflected in the DHA/EPA ratio; the DHA/EPA ratio was higher in haploids ($p<0.05$). The Hypocholesterolemic/Hypercholesterolemic (HH) ratio was higher in haploid eggs ($p<0.05$). Additionally, total EPA+DHA levels were found to be higher in diploid eggs ($p<0.05$) (Table 6).

Table 7. L*. a*. b* values in feeds (Independent Sample T-Test).

Parameters	Groups	N	X	ss	t-test		
					t	sd	p
L*	1st Stn.	3	4.56	0.00	-58.308	4	.000
	2nd Stn.	3	5.61	0.03	-58.308	2.143	.000
a*	1st Stn.	3	2.08	0.01	27.569	4	.000
	2nd Stn.	3	1.06	0.06	27.569	2.238	.001
b*	1st Stn.	3	2.44	0.06	-2.625	4	.058
	2nd Stn.	3	2.55	0.04	-2.625	3.496	.067

N: Number of samples in each group, $\bar{X}$: Mean. ss: Standard deviation, sd: Degrees of freedom, p: Significance level

L* and a* values were significantly different between feed groups ($p<0.05$), but no significant difference was observed in terms of b* values ($p>0.05$) (Table 7).

Table 8. L*. a*. b* values in eggs.

Parameters	Haploid (1st station)	Haploid (2st station)	Diploid (1st station)	Diploid (2st station)
L*	30.19 ± 0.04^{c}	30.30 ± 0.03^{b}	30.90 ± 0.05^{a}	30.98 ± 0.02^{a}
a*	39.49 ± 0.05^{a}	38.14 ± 3.50^{ab}	34.24 ± 0.03^{bc}	33.56 ± 0.08^{c}
b*	52.05 ± 0.07^{b}	52.23 ± 0.06^{b}	53.27 ± 0.12^{a}	51.15 ± 0.16^{c}

Significant differences ($p<0.05$) exist between values in the same rows that are indicated by different letters. The values (n=3) are presented as mean ± SEM.

There is a significant difference between haploid and diploid eggs in the L* value; diploid eggs are higher ($p<0.05$). There is also a significant difference between haploid and diploid eggs in the b* value; diploid eggs are higher ($p<0.05$). There is a significant difference between haploid and diploid eggs in the a* value; haploid eggs are higher ($p<0.05$) (Table 8).

Discussion

The chemical composition of the egg is frequently examined to assess egg quality, as it must meet the nutritional needs for embryonic and larval development (Parrish et al., 1994; Bell et al., 1997; Furuita, 2002). This study compared the chemical composition of haploid and diploid rainbow trout eggs obtained from different sources. No significant differences were found between haploid and diploid eggs in terms of dry matter, crude protein, crude fat, or crude ash. The results indicate that haploid and diploid eggs are similar in chemical quality for aquaculture, and both species are suitable in terms of nutritional value.

Diploid eggs generally have a higher total saturated fatty acid (ΣSFA) content than haploid eggs. SFA components such as C16:0 (palmitic acid) and C18:0 (stearic acid) are higher in diploid eggs. The high levels of SFAs (especially C16:0 and C18:0) in diploid eggs may provide an adaptive advantage in meeting the energy needs of the embryo during development (Loften et al., 2014). This suggests that diploid eggs may have a higher energy density than haploid eggs. Total monounsaturated fatty acid (ΣMUFA) levels are significantly higher in diploid eggs than in haploid eggs. Important MUFA components, such as C18:1n9c (oleic acid, cis-9-octadecenoic acid) and C20:1 (gondoic acid, cis-11-eicosenoic acid), were particularly abundant in diploids. MUFAs are crucial not only as energy sources but also for cell membrane fluidity and structural integrity (Torres et al., 2021). Therefore, the high MUFA levels in diploid eggs may be related to membrane stability and metabolic requirements. This enhanced membrane integrity, supported by higher C18:1n9c and C20:1 abundance, may contribute to improved oocyte quality and developmental potential (Chen et al., 2020). Haploid eggs are generally higher in total PUFA (ΣPUFA) content than diploid eggs. This difference in PUFA content between ploidy levels indicates a potential factor that may affect reproductive success, as n-3 long-chain PUFAs (n-3 LC-PUFA) are known to be directly related to egg viability and larval survival rates (Ferosekhan et al., 2020). Diploid fish eggs generally have higher levels of ω-3. In particular, DHA (C22:6n-3) levels are higher in diploids, which may indicate a greater capacity to incorporate or retain long-chain polyunsaturated fatty acids during oogenesis, which may lead to better embryo development, membrane fluidity, and oxidative stability compared to lower ω-3 genotypes (e.g., triploids). Similarly, diploid Atlantic salmon fillets were found to exhibit higher absolute amounts of EPA and DHA than triploids (Murray et al., 2018). On the other hand, haploid eggs are richer in omega-6 fatty acids, which play a role in immune responses and cellular signaling pathways. They contribute to the regulation of inflammatory processes and immune responses, particularly through arachidonic acid (C20:4n-6)-derived eicosanoids (prostaglandins, thromboxanes, and leukotrienes). Therefore, alterations in the omega-6/omega-3 balance may affect the development of embryonic immunity and early metabolic regulation (Murray et al., 2018; Innes & Calder, 2018; Rainuzzo, 2020). The ω-3/ω-6 ratio is significantly higher in diploid eggs, indicating a more balanced and healthy fatty acid profile. A high omega-3/omega-6 ratio is generally associated with anti-inflammatory effects because it reduces the production of pro-inflammatory eicosanoids such as prostaglandins and leukotrienes, while promoting the synthesis of anti-inflammatory lipid mediators such as resolvin and protectin, derived from EPA and DHA (Zivkovic et al., 2011; Calder, 2017). Although EPA (C20:5n-3) and DHA (C22:6n-3) are present at high levels in both groups, the total EPA + DHA content is higher in diploid eggs. The higher DHA/EPA ratio in haploid eggs indicates relatively more DHA in this group, while the higher EPA/DHA ratio in diploids suggests a more balanced distribution of omega-3 compounds. This balanced profile may support properties such as membrane flexibility, cellular signaling, and resistance to lipid peroxidation (Luo et al., 2015). Atherogenic Index (AI) and Thrombotic Index (TI) values were generally found to be close to each other in haploid and diploid fish eggs, which provides a similar assessment of cardiovascular health for both groups (Attia et al., 2015).

Comparison of L*, a*, and b* values between haploid and diploid trout eggs demonstrates that ploidy has a significant effect on egg color. Diploid eggs exhibited higher L* values, indicating lighter coloration, while haploid eggs showed higher a* values, reflecting a more pronounced reddish tint. B* values, indicating yellowness, were generally higher in diploid eggs, likely due to greater accumulation of yellow pigment. These differences in color measurement parameters suggest underlying metabolic and genetic variations in pigment synthesis and accumulation pathways between haploid and diploid individuals (Lukanov et al., 2015; Gomes et al., 2019; Salazar-González et al., 2020; Meng et al., 2022). Such variations in L*, a*, and b* values may reflect different carotenoid profiles and accumulation rates influenced by ploidy, affecting both the visual appeal and nutritional quality of the eggs. Furthermore, these colorimetric differences have potential implications for marketability, as consumer preference for seafood is often heavily influenced by visual appearance. Similar observations have been made in triploid rainbow trout, where altered fat metabolism and increased growth resulting from the reallocation of energy from gonadal development also affect fillet quality characteristics such as color and texture (Everson et al., 2021). Specifically, triploid salmon have been observed to be significantly paler and less yellowish compared to diploids, although redness may not be consistently affected (Lerfall et al., 2017).

Despite the comprehensive analysis of the chemical composition, fatty acid profiles, and color characteristics of haploid and diploid rainbow trout eggs, this study has several limitations. The environmental conditions and feed composition of the broodstock were not fully standardized, which may have influenced egg quality parameters. Although analyses were performed in triplicate (n = 3), the relatively small sample size may limit the generalizability of the findings. The study primarily focused on descriptive biochemical and colorimetric measurements, and the underlying molecular mechanisms, such as gene expression or protein activity related to lipid metabolism and pigment synthesis, were not investigated. Additionally, seasonal variations and maternal effects, which may affect egg quality, were not controlled. Future studies integrating transcriptomic and proteomic approaches with larger sample sizes under controlled conditions would provide a deeper understanding of the physiological and genetic factors influencing egg quality across different ploidy levels.

Conclusion

This study has shown that egg ploidy in rainbow trout (*Oncorhynchus mykiss*) has significant effects on the chemical composition and physical properties of eggs. While haploid and diploid eggs show similarities in basic nutritional components, they differ in fatty acid profiles and color characteristics. Diploid eggs have higher levels of saturated and monounsaturated fatty acids, omega-3, and EPA+DHA, while haploid eggs exhibit a higher omega-6 ratio, DHA/EPA, and HH values. Color analysis revealed that diploid eggs are lighter and yellowish, while haploid eggs have more reddish tones. These findings suggest that ploidy may have a direct impact on egg quality, embryonic development, and fry health. Consequently, the biochemical and physical differences between haploid and diploid eggs provide important information for optimizing broodstock selection and production strategies.

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Ethical approval

This study was approved by the local ethics committee for animal experiments of Aydın Adnan Menderes University 24.08.2023 dated and numbered 64583101/2023/138.

Informed consent

Not available

Data availability statement

The authors declare that data are available from authors upon reasonable request.

Conflicts of interest

The authors declare no conflict of interest.

Contribution of authors

Necmettin Küçük : Formal analysis, Writing original draft

Ebru Yılmaz: Project administration, Conceptualization, Data curation, Investigation, Methodology, Writing original draft

References

AOAC (1998). Official Methods of Analysis of AOAC International. Gaithersburg, MD: AOAC International.Attia, Y.A., Al-Harthi, M.A., Korish, M.A., & Shiboob, M.M. (2015). Fatty acid and cholesterol profiles and hypocholesterolemic, atherogenic, and thrombogenic indices of table eggs in the retail market. *Lipids in Health and Disease*, 14(1), 136. https://doi.org/10.1186/s12944-015-0133-z

Avşar. D. (2005). Balıkçılık Biyolojisi ve Popülasyon Dinamiği. Akademisyen Kitabevi. https://doi.org/10.37609/akya.561

Baki, B., Kaya Öztürk, D., & Tomgişi, S. (2019). The use of egg color of rainbow trout (*Oncorhynchus mykiss*) as a quality criterion. *Journal of Engineering Research and Application*, 9(3), 43-46.

Baki, B., Ozturk, D.K., & Tomgisi, S. (2021). Comparative analysis of egg biochemical composition and egg productivity rainbow trout (*Oncorhynchus mykiss* Walbaum, 1792) in different stations in Turkey. *Aquaculture Studies*, 21(3), 117-127. http://doi.org/10.4194/2618-6381-v21_3_04

Bell, J.G., Farndale, B.M., Bruce, M.P., Navas, J.M., & Carillo, M. (1997). Effects of broodstock dietary lipid on fatty acid compositions of eggs from sea bass (*Dicentrarchus labrax*). *Aquaculture*, 149(1-2), 107-119. https://doi.org/10.1016/S0044-8486(96)01436-6

Billard, R. (1977). A new technique of artificial insemination for salmonids using a sperm diluent. *Fisheries*, 1, 24-25. https://doi.org/10.1577/1548-8446-2-1

Bjerkeng, B. (2008). Carotenoids in Aquaculture: Fish and Crustaceans. In: Britton, G., Liaaen-Jensen, S., Pfander, H. (eds) Carotenoids. Carotenoids, vol 4. Birkhäuser Basel. https://doi.org/10.1007/978-3-7643-7499-0_12

Bligh, E.G., & Dyer, W.J. (1959). A rapid method of total lipid extraction and purification, *Canadian Journal of Biochemistry and Physiology*. 37(8). 911-917. https://doi.org/10.1139/o59-099

Bobe, J. (2015). Egg quality in fish: Present and future challenges. *Animal Frontiers*, *5*(1), 66-72.

Brooks, S., Tyler, C.R. & Sumpter, J.P. (1997) Egg quality in fish: what makes a good egg?. *Reviews in Fish Biology and Fisheries* 7, 387-416 https://doi.org/10.1023/A:1018400130692

Calder, P.C. (2017). Omega-3 fatty acids and inflammatory processes: from molecules to man. *Biochemical Society Transactions*, 45(5), 1105-1115. https://doi.org/10.1042/BST20160474

Chen, X., Han, F., Zhu, K., Punt, A.E., & Lin, D. (2020). The breeding strategy of female jumbo squid *Dosidicus gigas*: energy acquisition and allocation. *Scientific Reports*, 10(1), 9639. https://doi.org/10.1038/s41598-020-66703-5

Çorapci. B., Köstekli. B., Eyüboğlu. A., & Kocatepe. D. (2021). The effect of different application methods of sumac (*Rhus coriaria*) and tarragon (*Artemisia dracunculus*) on nutritional composition, fatty acids and TBARS values of marinated sea bream (*Sparus aurata* L., 1758). *Journal of Food Processing and Preservation*, 45(2), e15102. https://doi.org/10.1111/jfpp.15102

Dietrich, G.J., Wojtczak, M., Słowińska, M., Dobosz, S., Kuźmiński, H., & Ciereszko, A. (2008). Effects of ovarian fluid on motility characteristics of rainbow trout (*Oncorhynchus mykiss* Walbaum) spermatozoa. *Journal of Applied Ichthyology*, 24(4), 503-507. https://doi.org/10.1111/j.1439-0426.2006.01130.x

Emre, Y., & Kürüm, V. (2007). Havuz ve Kafeslerde Alabalık Yetiştiriciliği Teknikleri. (2. Baskı). Minpa Matbaacılık, Ankara, Türkiye.

Escamilla-Rosales. M.F., Pérez-Escalante. E., Jara-Gutiérrez. C.E., Santana-Sepúlveda. P.A., Álvarez-Álvarez. C.A., Castañeda-Ovando. A., & González-Olivares. L.G. (2024). Rainbow trout (*Oncorhynchus mykiss*) proteins as a source of antioxidant peptides with promising anticancer activity. *Future Foods*. 10. 100509. https://doi.org/10.1016/j.fufo.2024.100509

Everson, J.L., Weber, G.M., Manor, M.L., Tou, J.C., & Kenney, P.B. (2021). Polyploidy affects fillet yield, composition, and fatty acid profile in two-year old, female rainbow trout, *Oncorhynchus mykiss*. *Aquaculture*, 531, 735873. https://doi.org/10.1016/j.aquaculture.2020.735873

FAO. (2020). The State of World Fisheries and Aquaculture 2020. Sustainability in Action. Food and Agriculture Organization of the United Nations. Rome.

Ferosekhan, S., Xu, H., Turkmen, S., Gómez, A., Afonso, J.M., Fontanillas, R., ... & Izquierdo, M. (2020). Reproductive performance of gilthead seabream (*Sparus aurata*) broodstock showing different expression of fatty acyl desaturase 2 and fed two dietary fatty acid profiles. *Scientific reports*, 10(1), 15547. https://doi.org/10.1038/s41598-020-72166-5

Furuita, H., Tanaka, H., Yamamoto, T., Suzuki, N., & Takeuchi, T. (2002). Effects of high levels of n-3 HUFA in broodstock diet on egg quality and egg fatty acid composition of Japanese flounder, *Paralichthys olivaceus*. *Aquaculture*, 210(1-4), 323-333. https://doi.org/10.1016/S0044-8486(01)00855-9

Gomes, A.D., Vieira, V.A.R.O., Tabata, Y.A., Takahashi, N.S., Moreira, R.G., & Ribeiro, C. D.S. (2019). Ploidy influences on metabolic substrate deposition of rainbow trout. *Acta*

Scientiarum. Animal Sciences, 42, e44580. https://doi.org/10.4025/actascianimsci.v42i1.44580

Grčević, M., Kralik, Z., Kralik, G., & Galović, O. (2019). Effects of dietary marigold extract on lutein content, yolk color and fatty acid profile of omega-3 eggs. *Journal of the Science of Food and Agriculture*, 99(5), 2292-2299. https://doi.org/10.1002/jsfa.9425

Innes, J.K., & Calder, P.C. (2018). Omega-6 fatty acids and inflammation. *Prostaglandins, Leukotrienes and Essential Fatty Acids*, 132, 41-48. https://doi.org/10.1016/j.plefa.2018.03.004

Izquierdo, M.S., Fernandez-Palacios, H., & Tacon, A.G.J. (2001). Effect of broodstock nutrition on reproductive performance of fish. *Aquaculture*, 197(1-4), 25-42. https://doi.org/10.1016/S0044-8486(01)00581-6

Janampa-Sarmiento, P.C., Takata, R., Freitas, T.M.D., Freire, L.D.S., Pereira, M.M.D.B., Lugert, V., ... & Pereira, M.M. (2020). Modeling the weight gain of freshwater-reared rainbow trout (*Oncorhynchus mykiss*) during the grow-out phase. *Revista Brasileira de Zootecnia*, 49, e20190028. https://doi.org/10.37496/rbz4920190028

Kocatepe, D., Şengör, G.F.Ü., Turan, H., Çorapcı, B., Altan, C.O., & Köstekli, B. (2025). Innovative versus traditional cooking methods: Impacts on the nutritional profile of large rainbow trout (*Oncorhynchus mykiss*) cultured in the Black Sea, Türkiye. *Journal of Food Composition and Analysis*, *148*, 108273. https://doi.org/10.1016/j.jfca.2025.108273

Le Cren, E.D. (1951). The length-weight relationships and seasonal cycle in gonad weight and condition in Perch (*Perca fluviatilis*). *The Journal of Animal Ecology*. 201-219. https://doi.org/10.2307/1540

Lerfall, J., Hasli, P. R., Skare, E. F., Olsen, R. E., Rotabakk, B. T., Roth, B., ... & Egelandsdal, B. (2017). A comparative study of diploid versus triploid Atlantic salmon (*Salmo salar* L.). The effects of rearing temperatures (5, 10 and 15° C) on raw material characteristics and storage quality. *Food Chemistry*, *225*, 37-44. https://doi.org/10.1016/j.foodchem.2017.01.012

Loften, J.R., Linn, J.G., Drackley, J.K., Jenkins, T.C., Soderholm, C.G., & Kertz, A.F. (2014). Invited review: Palmitic and stearic acid metabolism in lactating dairy cows. *Journal of dairy science*, 97(8), 4661-4674. https://doi.org/10.3168/jds.2014-7919

Logan M. (2010). Biostatistical design and analysis using R: a practical guide. Wiley-Blackwell. London. 546 p.

Lukanov, H., Genchev, A., & Pavlov, A. (2015). Colour traits of chicken eggs with different eggshell pigmentation. *Trakia Journal of Sciences*, 2, 149-158. https://doi.org/10.15547/tjs.2015.02.007

Luo, L., Ai, L., Li, T., Xue, M., Wang, J., Li, W., & Liang, X. (2015). The impact of dietary DHA/EPA ratio on spawning performance, egg and offspring quality in Siberian sturgeon (*Acipenser baeri*). *Aquaculture*, 437, 140-145. https://doi.org/10.1016/j.aquaculture.2014.11.036

Mahato, I.S., Timalsina, P., Gurung, G.B., Paudel, K., Shrestha, A., Bhusal, C., & Kunwar, P. (2023). Effects of dietary supplementation of a multi-strain commercial probiotic on growth performance, feed conversion ratio, and survival rate in rainbow trout (*Oncorhynchus mykiss*). *International Journal of Fisheries and Aquatic Studies*, 11(6), 96-101. https://doi.org/10.22271/fish.2023.v11.i6b.2881

Mazorra, C., Bruce, M., Bell, J. G., Davie, A., Alorend, E., Jordan, N., ... & Bromage, N. (2003). Dietary lipid enhancement of broodstock reproductive performance and egg and larval quality in Atlantic halibut (*Hippoglossus hippoglossus*). *Aquaculture*, 227(1-4), 21-33. https://doi.org/10.1016/S0044-8486(03)00493-9

Meng, Y., Liu, X., Guan, L., Bao, S., Zhuo, L., Tian, H., ... & Ma, R. (2022). Does dietary lipid level affect the quality of triploid rainbow trout and how should it be assessed?. *Foods*, 12(1), 15. https://doi.org/10.3390/foods12010015

Murray, D.S., Kainz, M.J., Hebberecht, L., Sales, K.R., Hindar, K., & Gage, M.J. (2018). Comparisons of reproductive function and fatty acid fillet quality between triploid and diploid farm Atlantic salmon (*Salmo salar*). *Royal Society Open Science*, 5(8), 180493. https://doi.org/10.1098/rsos.180493

Nakano, T., & Wiegertjes, G. (2020). Properties of carotenoids in fish fitness: a review. *Marine drugs*, 18(11), 568. https://doi.org/10.3390/md18110568

Parrish, C.C., Castell, J.D., Brown, J.A., Boston, L., Strickland, J.S., & Somerton, D.C. (1994). Fatty acid composition of Atlantic halibut eggs in relation to fertilization. Bulletin of the Aquaculture Association of Canada, 2 (36-38).

Rainuzzo, J.R. (2020). Fatty acid and lipid composition of fish egg and larvae. In *Fish Farming Technology* (pp. 43-48). CRC Press.

Salazar-González, C.Y., Rodríguez-Pulido, F.J., Stinco, C.M., Terrab, A., Díaz-Moreno, C., Fuenmayor, C., & Heredia, F.J. (2020). Carotenoid profile determination of bee pollen by advanced digital image analysis. *Computers and electronics in agriculture*, 175, 105601. https://doi.org/10.1016/j.compag.2020.105601

Serezli, E.E. (2017). Sapanca derelerindeki balık çeşitliliği ile baskın bir türün bazı büyüme ve üreme özelliklerinin belirlenmesi (Yayın No: 480585), [Yüksek Lisans tezi., İstanbul Üniversitesi]. YÖK Ulusal Tez Merkezi.

Shastak, Y., & Pelletier, W. (2023). Captivating colors, crucial roles: Astaxanthin's antioxidant impact on fish oxidative stress and reproductive performance. *Animals*, 13(21), 3357. https://doi.org/10.3390/ani13213357

Tocher. D.R. (2010). Fatty acid requirements in ontogeny of marine and freshwater fish. *Aquaculture Research.* 41(5). 717-732. https://doi.org/10.1111/j.1365-2109.2008.02150.x

Torres, M., Parets, S., Fernández-Díaz, J., Beteta-Göbel, R., Rodríguez-Lorca, R., Román, R., ... & Escribá, P.V. (2021). Lipids in pathophysiology and development of the membrane lipid therapy: new bioactive lipids. *Membranes*, 11(12), 919. https://doi.org/10.3390/membranes11120919

Xu, H., Shi, C., Ye, Y., Mu, C., & Wang, C. (2022). Photoperiod-independent diurnal feeding improved the growth and feed utilization of juvenile rainbow trout (*Oncorhynchus mykiss*) by inducing food anticipatory activity. *Frontiers in Marine Science*, 9, 1029483. https://doi.org/10.3389/fmars.2022.1029483

Yazıcıoğlu. Y., & Erdoğan. S. (2014). SPSS Uygulamalı Bilimsel Araştırma Yöntemleri. (4. Baskı). Detay Yayıncılık. Ankara. Türkiye.

Yıldız, M., Ofori-Mensah, S., Arslan, M., Ekici, A., Yamaner, G., Baltacı, M.A., ... & Korkmaz, F. (2020). Effects of different dietary oils on egg quality and reproductive performance in rainbow trout *Oncorhynchus mykiss*. *Animal Reproduction Science*, 221, 106545. https://doi.org/10.1016/j.anireprosci.2020.106545

Zivkovic, A.M., Telis, N., German, J.B., & Hammock, B.D. (2011). Dietary omega-3 fatty acids aid in the modulation of inflammation and metabolic health. *California agriculture*, 65(3), 106. https://doi.org/10.3733/ca.v065n03p106