Effect of Different Diets on Productivity and Biochemical Values of Rainbow Trout Eggs (*Oncorhynchus mykiss* Walbaum, 1972)

Birol Baki and Dilara Kaya Öztürk*

ABSTRACT

The nutritional status of broodstock is an important determinant of egg quality and fry viability in fish species. This study investigated the effects of different broodstock diets on egg productivity and quality in rainbow trout (*Oncorhynchus mykiss*). Rainbow trout broodstocks (average initial weight 731.25±0.39 g) were fed one of three diets for 75 days: two commercial rainbow trout diets with different protein and fat contents (C and F1 groups), and a semi-wet diet composed of a mixture of trout fry feed and fresh horse mackerel (F2 group). At the end of the study, the C group showed the highest specific growth rate and feeding day growth coefficient values. There were no significant differences in absolute and relative egg production between the dietary groups ($p>0.05$). However, diet did impact egg nutritional composition and color. The C group eggs had higher levels of omeg-3 and omeg-9 fatty acids, while omeg-6 fatty acids were highest in the F1 group eggs. Total amino acid content, including aromatic, branched-chain and essential amino acids, was highest in the F2 group eggs. The C group eggs also displayed greater chroma, yellowness, and redness compared to the F1 and F2 eggs ($p<0.05$). In summary, while egg output was similar between rainbow trout broodstock dietary groups, the amino acid profiles and lipid profiles of the eggs differed by diet. Specifically, the Σomega-3 PUFA, DHA and Σomega-3/Σomega-6 ratios in feeds impacted measures of egg quality like color and nutritional value. These findings highlight the importance of broodstock nutrition in determining the composition and quality of rainbow trout eggs.

Keywords: Amino acid, Color parameter, Egg productivity, Fatty acid, *Oncorhynchus mykiss*

INTRODUCTION

The quality of eggs produced by farmed fish remains a critical issue impeding the development of the aquaculture sector (Bobe, 2015). Even in fish that have invested much in culture and incubation systems, such as the Salmonids, egg mortality rates of up to 50% are still common (Bromage *et al*., 1992; Brooks *et al*., 1997; Bobe and Labbe 2010). In aquaculture, egg quality is determined by the survival rate or the number of fertilized eggs that reach the eyed stage of development and successfully hatch (Brooks *et al*., 1997; Bobe and Labbe, 2010). The quality of eggs varies greatly depending on external factors such as feeding regime, feed quality, age, ambient circumstances, and year's season (Čeřovský *et al*., 2009; Jacyno *et al*., 2009; Stolc *et al*., 2009; Wolf and Smital, 2009; Bezdíček *et al*., 2010; Strapak *et al*., 2010; Ingthamjitr *et al*., 2017). Past research has revealed that a variety of parameters, including egg size, shape, and biochemical composition, can be utilized to predict egg quality (Brooks *et al*., 1997; Bobe and Labbe, 2010). Bobe and Labbe (2010) revealed that a variety of variables can influence egg quality at various stages of development. In fact, it has been reported that the feeding of the broodstock, exposure to stress, and the maturation period of the eggs

Department of Aquaculture, Faculty of Fisheries, Sinop University, Sinop, Turkey
*Corresponding author. E-mail address: dilara.kaya55@gmail.com
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directly affect the egg quality indirectly (Watanabe 1985; Bromage *et al*., 1992; Campbell *et al*., 1992). Reports have shown that certain dietary elements might affect egg quality, including lipids, fatty acids, protein, and trace minerals (Brooks *et al*., 1997; Izquierdo *et al*., 2001).

The composition of broodstock diets significantly influences the reproductive and egg quality of fish (Brooks *et al*., 1997). Therefore, broodstock diets should be carefully formulated to meet all the nutritional requirements of the species being raised (Migaud *et al*., 2013). Neglecting this aspect can have adverse effects on both the condition of the broodstock and egg quality. Washburn *et al*. (1990) discovered that rainbow trout fed a diet high in carbohydrate (with low protein) produced eggs with higher survival up to the eyed stage, hatchability and relative fecundity than the group fed high protein, low carbohydrate diet.

Typically, rainbow trout broodstock are fed extruded feeds with special rations. However, some farmers opt for alternative practices due to the high cost of these specialized feeds, the prioritization of fry/fingerling/fattening feeds by manufacturers, and the distance of hatcheries from urban centers. Instead of purchasing broodstock feed, farmers sometimes feed the fish with farm by-products such as internal organs, or blend inexpensive fish with their existing feed to nourish the broodstock. Consequently, these feeding practice can impact both the quality and productivity of eggs. Therefore, this study aims to evaluate the egg quality and productivity of rainbow trout broodstock fed diets with varying protein-lipid ratios, including homemade semi-wet feed.

MATERIALS AND METHODS

Experimental fish and dietary experimental conditions

The experiment was carried out in the Research and Application Center, Fisheries Faculty, Sinop University, Turkey, using 3-year-old rainbow trout (*Oncorhynchus mykiss*, Walbaum, 1972) broodstock during gonanal maturation. The feeding experiment was conducted under natural light conditions (10L:14D) from December to February, 75 days before spawning. The fish were reared in 4 m^3 tanks supplied with natural spring water at atemperature of 12.2±0.5 °C. Three groups of broodstock were hand-fed twice daily to apparent satiation at 9:00 a.m. and 4:00 p.m. The rainbow trout broodstock, with an average initial weight of 731.25±0.39 g were used. The fish were divided into 3 groups and 3 repetitions, each consisting of 9 fish. During broodstocks selection, preference was given to individuals without abnormalities, free from disease, displaying active movement, and in good health. Before stripping, biometric measurements (weight-length) of the broodstock were taken. Fish and eggs were weighed using a precise electronic scale with a sensitivity of 0.001 g. Prior to stripping, the fish were towel-dried to prevent slipping. Stripping was performed by gently massaging the abdomen from the chest to the tail whilw holding the tail and head of the broodstock. The developed eggs were collected in a plastic container.

Experimental broodstock diets

In the study, rainbow trout broodstocks were fed three different feeds. The control group (C) received feed with a crude protein/crude lipid ratio of 41.80/28.0%. The F1 group received feed with a crude protein/crude lipid ratio of 44.50/21.40%, both produced by a commercial company (Biomar-Sagun) in 4.5 mm pellets. The third group (F2) was fed a semi-wet feed consisting of a blend of trout fry feed (0.8 mm) (50%) with a crude protein/crude lipid ratio of 45/20% and ground horse mackerel (*Trachurus trachurus*) meat (50%) with a crude protein/crude lipid ratio of 17.21/5.06%. Biochemical, amino acid, and fatty acid compositions of the feeds used for feeding rainbow trout broodstocks are listed in Table 1.

Egg productivity analysis

After the stripping process, eggs from each broodstock were randomly gathered into three plastic containers, and their count, weight, and diameter were measured. Absolute egg productivity (AEP, pieces·$fish^{-1}$) was calculated by first measuring the overall weight of the eggs and then counting 10-g

Table 1. The biochemical, amino acid and fatty acid compositions of the three feed types used for feeding rainbow trout broodstocks in the present study.

Composition	C	F1	F2
Biochemical composition (%)			
Crude protein	41.80±0.25	44.50±0.48	35.58±0.42
Crude lipid	28.0±1.05	21.40±0.40	9.44±0.32
Crude ash	5.9±0.30	7.30±0.12	11.48±0.51
Dry matter	92.25±0.18	89.84±0.69	53.25±01.13
Fatty acid composition (%)			
C14:0	3.44±0.07	3.92±0.10	5.70±0.10
C15:0	0.39±0.01	0.74±0.06	0.96±0.02
C16:0	10.17±0.36	10.56±0.16	16.12±0.16
C18:0	3.95±0.38	6.81±0.63	6.75±0.04
C20:0	1.18±0.01	1.55±0.01	0.88±0.02
C16:1	0.35±0.01	0.67±0.05	0.96±0.01
C18:1n-9c	23.85±1.11	19.64±0.30	14.90±0.10
C20:1n-9c	5.09±0.12	5.55±0.09	4.34±0.05
C22:1n-9	4.57±0.07	5.08±0.10	4.38±0.02
C24:1	1.29±0.06	1.52±0.06	2.07±0.08
C18:2n-6c	15.13±0.34	12.66±0.36	6.64±0.07
C18:3n-3	8.35±0.13	7.20±0.21	2.75±0.05
C20:4n:6	0.83±0.02	1.05±0.01	1.46±0.02
C20:5n-3	5.17±0.20	5.16±0.09	8.56±0.19
C22:6n-3	5.93±0.02	6.99±0.18	14.18±0.11
ΣSFA	21.48±0.52	26.22±0.20	32.45±0.25
ΣMUFA	38.71±0.98	36.50±0.71	32.24±0.21
ΣPUFA	39.76±0.69	36.40±0.78	34.93±0.11
Σ n-3	20.12±0.35	20.76±0.33	25.94±0.03
Σ n-6	16.89±0.33	14.55±0.26	8.23±0.11
Σ n-9	36.29±0.85	33.28±0.69	27.98±0.15
n-3/n-6	1.19±0.01	1.43±0.01	3.15±0.04
n-6/n-3	0.84±0.01	0.70±0.01	0.32±0.01
EPA/DHA	0.87±0.03	0.74±0.01	0.60±0.02
EPA+DHA	11.10±0.22	12.15±0.26	22.74±0.11
PUFA/SFA	1.85±0.05	1.39±0.02	1.08±0.01
AI	0.32±0.01	0.37±0.01	0.59±0.01
TI	0.20±0.01	0.24±0.01	0.28±0.01
HI	3.78±0.06	3.17±0.08	2.10±0.03

Table 1. Continued

Composition	C	F1	F2
Amino acid composition (g·100 g^{-1})			
Ala	2.13±0.01	2.66±0.03	1.52±0.01
Arg	2.35±0.01	1.93±0.01	1.43±0.01
Asp	4.78±0.01	2.51±0.02	3.18±0.01
Glu	7.29±0.01	6.76±0.05	4.29±0.01
Gly	1.94±0.01	3.83±0.01	1.53±0.01
His	1.06±0.01	0.69±0.02	0.72±0.01
Ile	0.87±0.01	1.65±0.01	0.60±0.01
Leu	3.09±0.03	3.10±0.01	1.92±0.02
Lys	3.54±0.01	4.91±0.01	2.27±0.01
Met	0.89±0.01	0.75±0.01	0.83±0.01
Phe	1.81±0.02	1.93±0.01	1.05±0.01
Pro	2.55±0.01	2.35±0.02	1.25±0.01
Ser	2.32±0.01	1.88±0.01	1.57±0.01
The	1.55±0.01	1.74±0.01	1.05±0.01
Tyr	1.13±0.01	1.34±0.02	0.65±0.01
Val	1.50±0.01	2.40±0.01	0.89±0.01
ΣAA	38.76±0.01	40.40±0.17	24.73±0.02
ΣEAA	16.63±0.01	19.09±0.04	10.76±0.03
ΣSEAA	3.40±0.01	2.62±0.01	2.15±0.01
ΣNEAA	22.13±0.01	21.31±0.13	13.98±0.01
ΣBcAA	5.45±0.03	7.15±0.02	3.41±0.02
ΣSAA	0.89±0.01	0.75±0.01	0.83±0.01
ΣArAA	2.94±0.02	3.27±0.03	1.70±0.01
ΣBAA	6.94±0.02	7.53±0.01	4.42±0.01
ΣAAA	12.07±0.02	9.27±0.07	7.47±0.01
EAA/NEAA	0.75±0.01	0.46±0.01	0.77±0.01
EAAI	1.22±0.01	1.28±0.01	1.03±0.01

Note: Value means mean±standard deviation (SD); C = commercial feed (crude protein/crude lipid ratio [CP/CL] = 41.80/28.0%); F1 = commercial feed (CP/CL = 44.50/21.40%); F2 = semi-wet feed (50% trout fry feed [CP/CL = 45/20%] + 50% ground horse mackerel [CP/CL = 17.21/5.06%])

Essential amino acids (EAA) = Histidine + Lysine + Phenylalanine + Methionine + Threonine + Leucin E+ Isoleucine + Valine + Arginine; Semi-Essential amino acids (SEAA) = Histidine + Arginine; Non-Essential amino acids (NEAA) = Alanine + Aspartic acid + Glutamic acid + Tyrosine + Glycine + Serine + Proline; Branched-chain amino acid (BcAA) = Leucine + Isoleucine + Valine; Sulphur-containing amino acids (SAA) = Cystine + Methionine; Aromatic amino acids (ArAA) = Phenylalanine + Tyrosine; Basic (alkaline) amino acids (BAA) = Lysine + Arginine + Histidine; Acidic amino acids (AAA) = Aspartic acid + Glutamic acid; Saturated fatty acid (SFA) = C12:0 + C13:0 + C14:0 + C15:0 + C16:0 + C17:0 + C18:0 + C20:0 + C21:0 + C22:0 + C23:0 + C24:0; Monounsaturated fatty acid (MUFA) = C14:1 + C15:1 + C16:1 + C17:1 + C18:1n-9c + C18:1n-9t + C20:1n-9c + C22:1n-9 + C24:1; Polyunsaturated fatty acid (PUFA) = C18:2n-6t + C18:2n-6c + C18:3n-3 + C18:3n-6 + C20:2 + C22:2 + C20:3n-6 + C20:5n-3 + C20:4n-6 + C22:6n-3; ΣOmega-3 (n-3) = C18:3n-3 + C20:3n-3 + C20:5n-3 + C22:5n-3 + C22:6n-3; ΣOmega-6 (n-6) = C18:2n-6t + C18:2n-6c + C18:3n-6 + C20:4n-6 + C20:3n-6; ΣOmega-9 (n-9) = C18:1n-9c + C18:1n-9t + C20:1n-9c + C22:1n-9; Index atherogenicity (AI) = [(C12:0 + (4×C14:0) + C16:0)] / (MUFA + Omega-3 + Omega-6); Index thrombogenicity (IT) = (C14:0 + C16:0 + C18:0) / [(0.5×MUFA) + (0.5×Omega-6) + (3×Omega-3) + (Omega-3 / Omega-6)]; Hypocholesterolaemic/hypercholesterolaemic ratio (HH) = (C18:1n-9 + C18:2n-6 + C18:3n-3 + C20:4n-6 + C20:5n-3 + C22:6n-3) / (C14:0 + C16:0); Essential amino acids index (EAAI) =√aa1/AA1 x aa2/AA2 x…. Aan/AAn (aa1 = EAA of diets, AA1 = EAA of fish, n = number of EAA)

eggs, using a weighting technique. Relative egg productivity (REP, pieces·kg^{-1}) was determined by dividing the total number of eggs by the weight of the broodstock (Bromage *et al*., 1992). The average egg diameter (mm) was computed by randomly taking 100 samples from each broodstock. For the egg productivity analysis, three replications were conducted. Prior to analysis, egg samples were kept in a deep freezer (WiseCryo/WUF-D500-80 °C), and samples were delivered to the lab using a cold chain.

Biochemical, amino acids and fatty acids composition analysis

According to established AOAC (1995) methods, the Aquaculture Laboratory at the Faculty of Fisheries carried out the analysis of the dry matter (DM), crude protein (CP) and crude ash (CA) in the diet. Soxhlet methods were used to calculate the crude lipids (CF). The amino acids in eggs and meals were examined using the Jasem LCMS/MS amino acid assay kit. Derivatization of the samples' lipids in a gas chromatography apparatus (Thermo Scientific Trace 1310) for fatty acid analysis resulted in the creation of methyl esters. The samples were examined using a GC/MS system from Thermo Scientific, ISQ LT model. The composition of amino acids and fatty acids was measured at the labs of Sinop University Scientific and Technological Research Center. According to Ulbricht and Southgate (1991), Santos-Silva *et al*. (2002), and Li *et al*. (2009) were calculated total amino acid and fatty acids quality.

Growth performance

The data for growth performance, viscerosomatic index, hepatosomatic index, carcass yield, and condition factor were calculated using the following formulas. (Skalli and Robin, 2004; Hoşsu *et al*., 2005; Cui *et al*., 2006; Turchini *et al*., 2011).

Specific Growth Rate (SGR), % = [(Ln Final weight (g)-Ln Initial weight (g))/Day]×100

Daily Growth Coefficient (DGC) = (Final weight (g)-Initial weight (g))/The number of trial days

Feeding Day Growth Coefficient (FDGC) = (Final weight, (g)-Initial weight (g))/The number of feedig days

Feed Conversion Rate (FCR) = Total consumed amount of feed (g)/Total weight gain (g)

Protein Efficiency Rate (PER), % = (Live weight gains (g)/Protein intake (g))×100

Carcass Yield (CY), % = (Edible fillet weight (g)/Total body weigh (g))×100

Viscerosomatic index (VSI), % = (Visceral weight (g)/Total body weight (g))×100

Hepatosomatic index (HSI), % = (Liver weight (g)/Total body weight (g))×100

Gonadosomatic index (GSI), % = (Weight of the ovary/Weight of the fish)×100

Condition Factor (CF) = (Weight/Leight^3) ×100

Color analysis

The color values of the egg were measured according to Commission Internationale de l´Eclairage (CIE) (1976) using a Minolta Chroma Meter (CR400, Konica Minolta, Marunouchi, Tokyo, Japan) and the white plate as a standard (Standard values for white plate L* = 91.97; a* = 1.4; b* = 2.0, Standard C222326). According to Nickell and Bromage (1998), L* stands for brightness (lightness-darkness), a* for redness-greenness, and b* for yellowness-blueness values. The Chroma (C*) refers to the color's intensity or level of saturation, whereas the Hue describes what is typically thought of as the genuine color. Using the a* and b* values, the angle of Hue and chroma (C*) was computed (Kestin and Warriss, 2001).

$$C^*=\sqrt{(a^2+b^2)}$$

$$\text{Hue} = \arctan(b^*/a^*)$$

Statistical analysis

One-way analysis of variance (ANOVA) was used to evaluate the effects of feed types on each parameters. Since the samples were equal, the subsequent mean comparisons were made using the Tukey test. Regression analysis was used to determine the association between the biochemical values of the egg and feed. The significance level was taken as $p<0.05$. The IBM SPSS 21 statistical package program (IBM Corp., Armonk, NY) was used for the statistical analysis.

RESULTS

Growth performance

The growth performances of the broodstocks at the end of the 75-day study are given in Table 2. At the end of the study, the difference between the weights of the fish was not significant ($p>0.05$). Specific growth rate (SGR), daily growth coefficient (DGC), and feeding day growth coefficient (FDGC) values were prominent in the control group (C).

The feed conversion ratio (FCR) was the best (lowest) in the control group (C) and was statistically significant ($p<0.05$). The highest protein efficiency rate (PER) was in the F2 group, but the statistical difference between the groups was not significant ($p>0.05$). Among the biometric indices, the carcass yield (CY) was highest in the control group ($p<0.05$), while the gonadosomatic index (GSI) value was in the F2 group ($p>0.05$).

Egg productivity

Data on the egg productivity (absolute egg productivity, relative egg productivity, egg diameter and weight) of the broodstock are given in Table 3. In the study, no anomalies were detected in the morphology of the eggs obtained from the broodstocks. There were no significant differences among the fish fed with different feeds regarding absolute and relative egg productivity values ($p>0.05$). Egg weights and diameters were the highest in the F2 group ($p<0.05$) followed by F1 and the control ($p<0.05$).

Fatty acid and amino acids composition of eggs

The fatty acids, and amino acid compositions of the eggs from the broodstock fed with different feeds are given in Table 4 and 5, respectively. Among the saturated fatty acids (SFAs), C16:0, the most abundant in eggs, exhibited the highest levels in the control group (C) ($p<0.05$). The C18:0

Table 2. The growth performances and biometric indices of the rainbow trout broodstocks fed three different diets.

	C	F1	F2
Initial weight (g)	730.32±27.84^{a}	731.88±40.07^{a}	731.56±35.23^{a}
Final weight (g)	1153.78±311.30^{a}	1100.80±248.92^{a}	1061.04±241.71^{a}
SGR (%)	0.61±0.05^{b}	0.54±0.04^{ab}	0.50±0.04^{a}
DGC	5.65±0.49^{b}	4.92±0.38^{ab}	3.39±0.34^{a}
FDGC	11.45±1.00^{b}	9.97±0.77^{ab}	8.91±0.70^{a}
FCR	1.13±0.10^{a}	1.45±0.22^{ab}	1.62±0.12^{b}
PER	0.97±0.09^{a}	0.96±0.15^{a}	1.03±0.08^{a}
CY (%)	41.07±4.24^{b}	40.16±4.03^{b}	37.55±1.97^{a}
VSI (%)	16.67±4.66^{a}	16.11±6.45^{a}	17.35±8.31^{a}
HSI (%)	1.09±0.18^{a}	1.12±0.19^{a}	1.41±0.20^{b}
GSI (%)	9.36±6.26^{a}	8.24±7.88^{a}	10.32±5.41^{a}
CF	1.56±0.17^{a}	1.57±0.19^{a}	1.57±0.21^{a}

Note: Mean±standard deviation (SD) within a row superscripted with different lowercase letters are significantly ($p<0.05$) different; Detail of feed as shown in Table 1

Table 3. Egg productivity of rainbow trout broodstock fed three different diets.

	C	F1	F2
Absolute egg productivity (eggs per fish)	2263±883[a]	2840±615[a]	2634±356[a]
Relative egg productivity (eggs per kg)	1962±1113[a]	2580±693[a]	2482±764[a]
Egg diameter (mm)	4.35±0.44[a]	4.42±0.10[a]	4.65±0.40[b]
Egg weight (mg)	63.17±0.02[a]	65.22±0.01[b]	77.61±0.03[c]

Note: Mean±standard deviation (SD) within a row superscripted with different lowercase letters are significantly ($p<0.05$) different; Detail of feed as shown in Table 1

Table 4. Fatty acid composition of eggs (%) obtained from the rainbow trout broodstock fed three different diets.

Fatty acids	C	F1	F2
C14:0	1.37±0.01[a]	1.78±0.01[b]	1.62±0.01[c]
C15:0	0.32±0.01[a]	0.43±0.01[c]	0.39±0.01[b]
C16:0	11.25±0.07[b]	10.41±0.28[a]	10.46±0.07[a]
C18:0	7.34±0.02[c]	6.45±0.06[a]	6.62±0.03[b]
C20:0	0.23±0.01[a]	0.32±0.01[c]	0.27±0.01[b]
C16:1	0.30±0.01[a]	0.48±0.03[b]	0.42±0.01[b]
C18:1n-9c	19.31±0.15[b]	18.66±0.26[a]	18.08±0.22[a]
C20:1n-9c	3.07±0.01[a]	3.65±0.05[c]	3.38±0.01[b]
C22:1n-9	1.55±0.01[a]	1.54±0.01[a]	1.50±0.02[a]
C24:1	0.17±0.01[a]	0.21±0.02[b]	0.17±0.01[a]
C18:2n-6c	11.84±0.02[a]	14.08±0.25[c]	12.79±0.05[b]
C18:3n-3	4.53±0.02[b]	4.88±0.08[c]	4.21±0.02[a]
C20:4n:6	3.15±0.01[a]	3.30±0.05[b]	4.17±0.02[c]
C20:5n-3	5.96±0.04[c]	4.80±0.12[a]	5.36±0.05[b]
C22:6n-3	17.59±0.02[c]	15.51±0.38[a]	16.41±0.10[b]
ΣSFA	21.11±0.04[b]	20.13±0.19[a]	20.20±0.05[a]
ΣMUFA	28.09±0.12[b]	28.27±0.33[b]	27.40±0.17[a]
ΣPUFA	50.63±0.10[a]	51.39±0.32[b]	52.25±0.19[c]
Σn-3	30.97±0.07[c]	28.11±0.41[a]	29.60±0.16[b]
Σn-6	15.98±0.05[a]	18.27±0.29[b]	18.12±0.02[b]
Σn-9	27.26±0.12[b]	26.96±0.32[b]	26.34±0.20[a]
n3/n6	1.94±0.01[c]	1.54±0.04[a]	1.63±0.01[b]
n6/n3	0.52±0.01[a]	0.65±0.02[b]	0.61±0.01[b]
EPA/DHA	0.34±0.01[a]	0.31±0.01[a]	0.33±0.01[a]
EPA+DHA	23.55±0.03[c]	20.31±0.42[a]	21.77±0.14[b]
PUFA/SFA	2.40±0.01[a]	2.55±0.03[b]	2.59±0.01[b]
AI	0.22±0.01[a]	0.24±0.01[a]	0.23±0.01[a]
TI	0.17±0.01[a]	0.17±0.01[a]	0.17±0.01[a]
HI	4.61±0.03[a]	4.66±0.10[ab]	4.74±0.03[b]

Note: Mean±standard deviation (SD) within a row superscripted with different lowercase letters are significantly ($p<0.05$) different; Detail of feed as shown in Table 1

Table 5. Amino acid composition of eggs (g·100 g^{-1}) obtained from the rainbow trout broodstock fed three different feeds.

Amino acids	C	F1	F2
Alanine	1.82±0.01^{b}	1.34±0.01^{a}	1.91±0.01^{c}
Arginine	1.24±0.01^{b}	0.94±0.01^{a}	1.24±0.01^{b}
Aspartic acid	2.86±0.01^{b}	2.22±0.01^{a}	3.04±0.01^{c}
Glutamic acid	2.41±0.01^{c}	1.84±0.01^{a}	2.32±0.01^{b}
Glisine	0.29±0.01^{a}	-	0.37±0.01^{b}
Histidine	0.54±0.01^{b}	0.40±0.01^{a}	0.59±0.01^{b}
Isoleucine	0.64±0.01^{b}	0.50±0.01^{a}	0.70±0.01^{c}
Leucine	1.88±0.02^{b}	1.60±0.01^{a}	1.93±0.01^{b}
Lysine	2.04±0.01^{b}	1.49±0.01^{a}	2.26±0.01^{c}
Metionine	0.66±0.01^{b}	0.56±0.01^{a}	0.64±0.01^{b}
Phenylalanine	1.04±0.01^{b}	0.88±0.06^{a}	1.12±0.04^{b}
Proline	1.25±0.01^{b}	1.06±0.01^{a}	1.37±0.01^{c}
Serine	1.43±0.01^{b}	1.16±0.01^{a}	1.47±0.01^{b}
Threonine	1.02±0.01^{b}	0.76±0.01^{a}	0.96±0.01^{b}
Tyrosine	0.81±0.01^{a}	0.78±0.01^{a}	0.86±0.01^{b}
Valine	1.00±0.01^{b}	0.85±0.01^{a}	1.13±0.01^{c}
ΣEAA	10.05±0.01^{b}	7.97±0.06^{a}	10.57±0.04^{c}
ΣSEAA	1.78±0.01^{b}	1.34±0.01^{a}	1.83±0.01^{c}
ΣNEAA	10.86±0.01^{b}	8.40±0.01^{a}	11.33±0.01^{c}
ΣAA	20.91±0.02^{b}	16.36±0.04^{a}	21.90±0.04^{c}
ΣBCAA	3.52±0.02^{b}	2.95±0.01^{a}	3.76±0.01^{c}
ΣSAA	0.66±0.01^{b}	0.56±0.01^{a}	0.64±0.01^{b}
ΣArAA	1.84±0.01^{b}	1.66±0.05^{a}	1.98±0.03^{c}
ΣBAA	3.82±0.01^{b}	2.83±0.01^{a}	4.09±0.01^{c}
ΣAAA	5.27±0.01^{b}	4.06±0.01^{a}	5.36±0.01^{c}
EAA/NEAA	0.93±0.01^{a}	0.95±0.01^{a}	0.93±0.01^{a}
EAAI	1.00±0.01^{b}	0.90±0.01^{a}	1.02±0.01^{b}

Note: Mean±standard deviation (SD) within a row superscripted with different lowercase letters are significantly ($p<0.05$) different; Detail of feed as shown in Table 1

value were ranked as C>F2>F1, with statistically significant differences between the groups ($p<0.05$). While the total SFA (ΣSFA) values of the F1 and F2 groups did not differ significantly ($p>0.05$), the C group exhibited a statistically significant difference compared to the other two groups ($p<0.05$). The highest levels of C18:1n-9 were observed in the C group ($p<0.05$). Additionally, the highest values of C18:2n-6 and C18:3n-3 were found in the F1 group, with statistical significant differences ($p<0.05$). Moreover, the C20:5n-3 and C22:6n-3 values of eggs belonging to group C were higher than those in the other groups ($p<0.05$). The total monounsaturated fatty acid (ΣMUFA) content of the eggs was highest in the F1 group ($p<0.05$). Regarding the total polyunsaturated fatty acids (ΣPUFA) content, the order was F2>F1>C, with statistically significant differences ($p<0.05$). Furthermore, the total omega-3 and omega-9 values of the eggs were higher in the C group (C>F2>F1 [$p<0.05$] for omega-3; C , F1>F2 for omega-9), while the omega-6 values were higher in the F1 group (F1, F2>C [$p<0.05$]).

The relationships between fatty acid level in feed and in the eggs were determined for C20: 5n-3 (EPA), C22:6n-3 (DHA), C20:4n:6 (ARA), C18:1n-9c (Oleic acid), C18:2n-6c (Linoleic acid) and C18:3n-3 (α- Linolenic acid) (Figure 1). The C18:1n-9c value of eggs of the control ($r^2 = 0.98$, $p = 0.043$) and F2 groups ($r^2 = 0.98$, $p = 0.047$) was highly affected by the C18:1n-9c value of the feed. The relationship between the linoleic acid value in the feed and especially the linoleic acid value of F2 group eggs was positive and very strong ($r^2 = 0.99$, $p = 0.035$). While the relationship between the α- Linolenic acid value of the C group eggs and the α- Linolenic acid value of the feed was positive and very strong ($r^2 = 0.96$, $p = 0.017$), no relationship was between the α- Linolenic acid value of the F2 group eggs and the α- Linolenic acid value of the feed ($r^2 = 0.01$, $p = 0.901$).

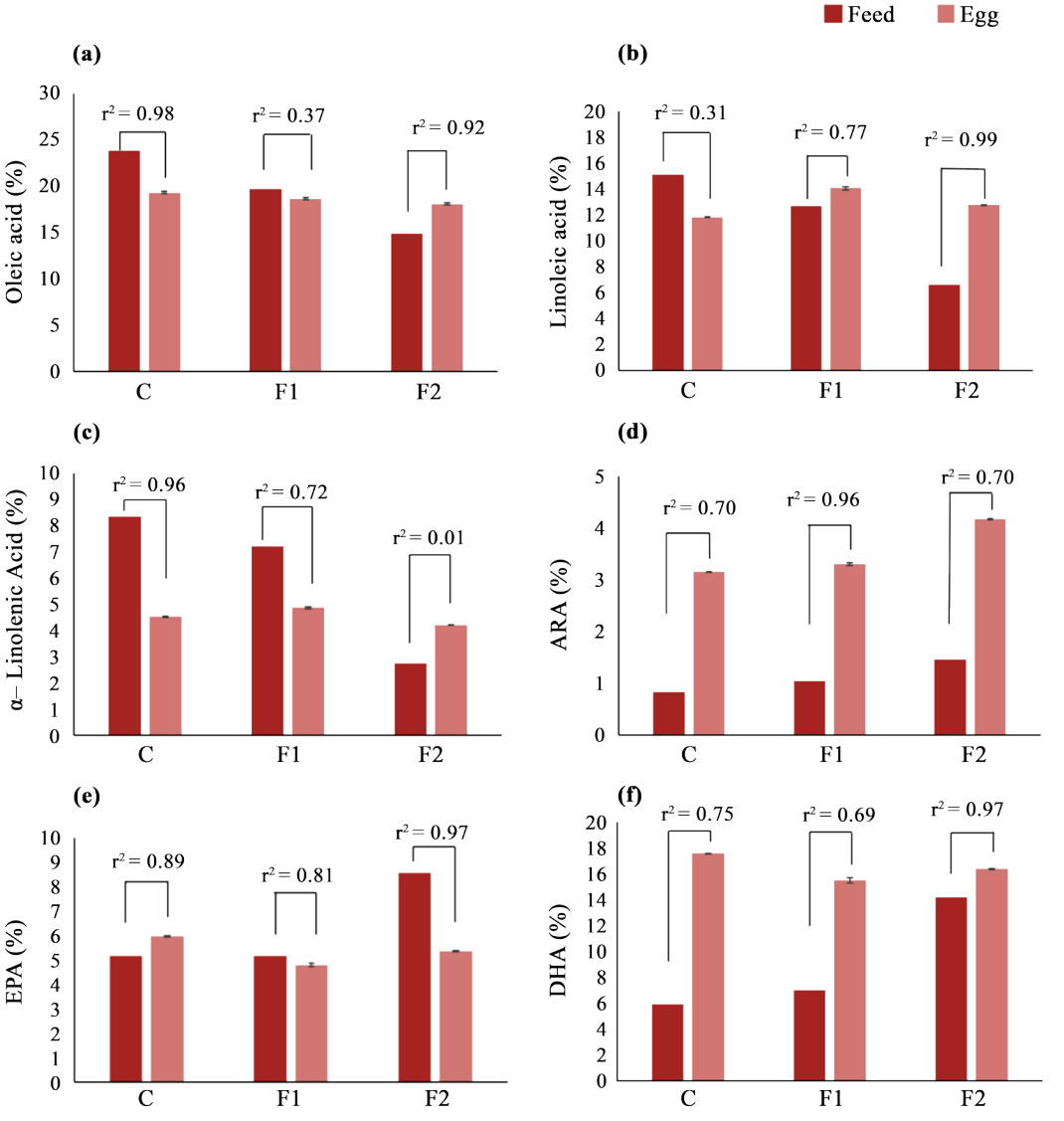

Figure 1. The bar graphs showing mean of fatty acids content (%) in feed and the eggs of the rainbow trout broodstock fed three different diets, and r^2 values showing relationship between each fatty acid in feed and eggs: (a) Oleic acid; (b) Linoleic acid; (c) α– Linolenic Acid; (d) ARA; (e) EPA; (f) DHA; Detail of feed as shown in Table 1.

Aspartic acid was the most determined non-essential amino acid in eggs and the difference between groups was significant ($p<0.05$). Lysine and leucine were the most abundant essential amino acids in all egg groups.

All amino acid values (essential, semi-essential, non-essential, branched-chain, aromatic, basic and acidic amino acids), except for those containing total sulfur, were highest in F2 group, and mostly with statistical supports ($p<0.05$). While the EAA/NEAA ratio was highest in the F1 group ($p>0.05$), the EAAI value was higher in the F2 group ($p<0.05$).

Color analysis of eggs of rainbow trout

Table 6 shows the L*, a*, b*, c*, and Hue values of eggs used in the research. The order of L* values of eggs fed with different feeds was F2>C, F1, and the statistical difference between the groups was significant ($p<0.05$) only between the F2 group and the others. The a*, b* and C* values of the C group were found to be higher and statistically significant than the F1 and F2 groups ($p<0.05$). Hue values were found to be similar in all three groups ($p>0.05$).

Table 6. L*, a*, b*, C* and Hue values of eggs obtained from the rainbow trout broodstock fed three different feeds.

	C	F1	F2
L*	33.64±1.19^{a}	32.75±1.02^{a}	37.57±1.21^{b}
a*	18.60±1.28^{b}	10.73±1.09^{a}	11.30±1.10^{a}
b*	15.60±1.59^{b}	9.38±1.08^{a}	9.23±0.98^{a}
C*	24.49±1.89^{b}	14.33±1.45^{a}	14.47±1.35^{a}
Hue	0.66±0.03^{a}	0.66±0.06^{a}	0.67±0.05^{a}

Note: Mean±standard deviation (SD) within a row superscripted with different lowercase letters are significantly ($p<0.05$) different; Detail of feed as shown in Table 1

DISCUSSION

The effects of rainbow trout broodstocks fed with different feeds on egg production and quality were evaluated. Several factors influence egg production and size in fish, including broodstock weight, age, genetic structure, and nutrition (diet quality and quantity) (Bromage *et al*., 1992; Kurtoğlu *et a*l., 1998). In general, egg yield and size increase with the size and age of the brood fish. This characteristic is particularly evident in Salmonids. The feeding rainbow trout broodstocks with different feeds had no effect on the absolute and relative productivity of eggs, it had an impact on the weight and height of the eggs. It was concluded that F2 group eggs fed with semi-wet feed with lower protein/fat content had higher length and weight. In this study, the absolute egg yields of 3-year-old broodstocks with average live weights varying between approximately 1,061.04±80.57 and 1,153.78±98.44 g were determined as 2,263±361 and 2,840±356 eggs·fish^{-1}. These values were similar to research on rainbow trout absolute egg production (Sharma *et al*., 1989; Estay *et al*., 1994; Kurtoğlu *et al*., 1998).

Fish embryos and larvae utilize the fatty acids found in the eggs as a source of energy and as a structural component to develop. During larval and embryonic development, some fatty acids are added to the structural lipids in the larval tissues, while other fatty acids are catabolized (Anderson *et al*., 2011). PUFA and SFA, which are catabolized in the early stages of development, have reportedly been shown to be utilized for energy generation in the later stages (de Mello *et al*., 2022). For fish to function properly metabolically, certain fatty acids must be present in the diet much like protein and amino acids. For instance, it is claimed that omega-3 fatty acids, particularly DHA (22:6n-3), are crucial for the growth and reproduction of rainbow trout (Lazzarotto *et al*., 2015). Additionally, sufficient fatty acid levels in the food are crucial for both the gonad development of broodstocks and the embryonic development of the eggs produced from these broodstocks (March, 1993).

When compared to wild fish, farmed fish have lower amounts of arachidonic acid (C20:4n-6, ARA), and resulted in worse egg quality (Salze *et al*., 2005). Additionally, ARA levels have been associated with improved egg quality traits and successful hatching (Pickova *et al*., 1997). In Sawanboonchun's (2009) research looked at the impact of ARA addition in broodstock diets for various lengths of time and found no improvement in egg quality. In this study, ARA values determined in eggs were high (Table 4) and the relationship between arachidonic acid values determined in feeds and eggs was positive and strong (Figure 1). The fatty acid composition of rainbow trout eggs was reported previously (Ballestrazzi *et al*., 2003; Chavez-Mendoza *et al*., 2014; Kaya Öztürk *et al*., 2019; Baki *et al*., 2021). The predominance of n-3 in rainbow trout eggs, namely DHA and EPA, revealed in this study is consistent with earlier reports. Even though they were fed with a low-fat diet, the eggs of the F2 group were determined to contain high amounts of EPA, DHA, and n-3 fatty acids. Sargent *et al*. (2003) reported that the fatty acid content of eggs is typically more preserved and less altered by diets than that of other fish tissues, indicating the relevance of gamete-specific composition. The fatty acid composition of eggs, however, has been found in several investigations to be comparable to broodstock diets from both marine and freshwater species (Furuita *et al*., 2000; Li *et al*., 2005; Henrotte *et al*., 2010; Zakeri *et al*., 2011). In this study, the DHA values of the eggs were higher than the DHA values of the broodstock diets (Table 1 and Table 4). In different studies, the DHA values of the eggs were higher than the DHA values in the broodstock diets and it was found to be compatible with our study (Mazorra *et al*., 2003; Lund *et al*., 2008). Moreover, the EPA value of the eggs was high only in the C group, where the EPA of the broodstock diets was high. In other groups, the EPA value of the eggs was lower than the EPA value of the diet feeds (Table 1 and Table 4). Similarly, the EPA value of eggs was greater in gilthead sea bream (*Sparus aurata*) than in their broodstock diets (Fernández-Palacios *et al*., 1995). These findings imply that species-based differences in the rates of DHA and EPA deposition in the eggs may exist.

Proteins and the amino acids that make them up affect fish reproduction. Amino acids are principally important as metabolic energy sources for larvae; they are necessary for proper tissue and organ development and play critical roles in fertilization and embryonic development. (Fyhn and Serigstad, 1987; Fyhn, 1989; de-Silva and Anderson, 1995; Sivaloganathan *et al*., 1998; Rønnestad *et al*., 1998; 1999; Parra *et al*., 1999; Ohkubo and Matsubara, 2002). At the end of the study, the crude protein and amino acid values of the diets had an effect on the egg amino acid values. In general, in this study, the amino acid values of rainbow trout eggs (F2) fed with low protein diets were higher than most of the other two groups, and some amino acid values remained constant.

In this study, L* (brightness) values in the eggs of broodstocks fed with different diets were higher in the F2 group, and a* (redness) and b* (yellowness) values in the C group. It is thought that the differences in the color parameters of the eggs are due to the diet ingredients. In different studies, color analysis of fish eggs was determined as a quality criterion (Baki *et al*., 2019) and carotenoids in fish eggs were reported to affect embryonic development and the survival rate of larvae (George *et al*., 2001; Palace and Werner, 2006; Sawanboonchun *et al*., 2008).

CONCLUSION

As a consequence in this study, there were differences in the amino acid levels of rainbow trout eggs obtained from broodstock fed with various diets. Data indicate that n-3 PUFA, primarily DHA, and diet DHA/EPA and n-3/n-6 ratios impact egg quality in rainbow trout. This work is expected to give a baseline to guide both the composition of broodstock diets and future research into egg quality for this species in order to maximize production and provide healthy eggs.

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