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## A study on the use of tea (*Camellia sinensis*) flower extract in the feed of rainbow trout (*Oncorhynchus mykiss*): Growth, body composition, and hematological blood parameters

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**ABSTRACT:** This study investigated the effects of supplementing rainbow trout (*Oncorhynchus mykiss*) feed with tea flower (*Camellia sinensis*) extract (TFE) on growth performance, body composition and hematological blood parameters. A total of 360 rainbow trout with a mean length of  $12.2 \pm 0.21$  cm (min 11.5 cm; max 13.00 cm) and a mean weight of  $20.3 \pm 0.26$  g (min 18.00 g; max 22.00 g) were used in the experiment. The fish were distributed into 12 fiberglass tanks, each with a volume of 100 L (adjusted to 80 L), equipped with a flow system (water flow rate of 9 L/min) and air support. Thirty fish were placed in each tank. The study involved supplementing a commercial trout feed with TFE at four different concentrations: 0 mL/kg (control), 10 mL/kg (TFE-10), 20 mL/kg (TFE-20), and 40 mL/kg (TFE-40). Each treatment group was replicated three times and randomly assigned to the tanks. The results demonstrated that the TFE-10 group exhibited significantly higher final body weight, weight gain, weight gain rate, specific growth rate, feed conversion ratio, protein efficiency, survival rate, and meat yield compared to the control group ( $P < 0.05$ ). Additionally, TFE supplementation was found to increase the protein content and reduce the fat content in the fish flesh. Hematological analysis revealed that all blood parameters, except for platelet count (PLT), were significantly higher in the TFE-10 group compared to the control group at the end of the study ( $P < 0.05$ ). Based on cubic polynomial regression analysis, the optimal TFE supplementation level for maximizing fish growth was calculated to be  $12.3 \pm 3.81$  mL/kg.

**Keyword:** *Camellia sinensis*; growth performance; hematological parameters; *Oncorhynchus mykiss*; polynomial regression.

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## INTRODUCTION

Reports indicate that more than 820 million people worldwide are affected by chronic hunger, with approximately 2 billion facing food insecurity to some extent (FAO et al., 2022). At the same time, the global population is projected to exceed 9.7 billion by 2050, which will increase the demand for nutritious and sufficient food (United Nations, 2022). Based on these reports, it can be said that there will be serious problems with access to nutritious and sufficient food in the future. The livestock sector, which provides high nutritional value products, plays a critical role in addressing these challenges by contributing to food security and nutrition. Among these sectors, aquaculture stands out as one of the fastest growing sectors with an average annual growth rate of 5.3% over the last decade (FAO, 2022). Aquaculture has become indispensable, accounting for 17% of the global population's animal protein intake and 7% of total protein consumption worldwide (FAO, 2022). However, the aquaculture sector faces numerous challenges, including issues related to cultivation technologies, feed and feed ingredients, fish nutrition, disease management, climate change, and environmental interactions (Shen et al., 2021). A primary problem is the high cost of feed due to the imbalance between the supply and demand of fish meal and fish oil, which are the main ingredients of fish feed. In aquaculture, it is desirable for fish to get the most out of the feed they are fed. For this reason, most of the recent research in aquaculture has focused on feed additives with natural compounds that improve the growth and digestive system of cultured species, increase appetite, and regulate hematological and chemical blood parameters.

It has been reported that many plant extracts or their by-products promote growth, support the immune system and increase appetite due to the biologically active compounds they contain (e.g. flavonoids, phenolic compounds, essential oils, steroids, saponins, tannins, terpenoids) (Jeney et al., 2009; Chakraborty et al., 2011; Reverter et al., 2014; Kose and Ariman Karabulut 2022). Tea (*Camellia sinensis*) is one of the most widespread industrial crops in the world, grown in tropical and subtropical climates and used as a beverage by boiling the leaves and buds in water. It has been reported that the chemical content of tea flowers is similar to that of tea leaves, but the amounts are different (Chen et al., 2018). Tea flowers are rich in bioactive compounds such as proteins, polysaccharides, saponins, amino acids, catechins,

and flavonoids. However, the composition and concentration of these metabolites vary depending on the developmental stage of the flowers (Yang et al., 2007; Chen et al., 2020a). Although total flavonoid content was reported to be higher in leaves (Chen et al., 2020a), flavonoid content was also reported to be higher in tea flowers than in leaves (Yang et al., 2007; Yurteri et al., 2022). Catechin content in flowers has been reported to increase after budding, peaks when the petals begin to crack, and decreases to minimum levels during the flowering stage (Robin Joshi et al., 2011). Additionally, it has been reported that tea flowers possess bioactivities, including antioxidant, anticancer, anti-inflammatory, anti-obesity, and hypoglycemic effects (Hamao et al., 2011; Wang et al., 2012; Chen et al., 2012; Sil et al., 2021).

In recent studies, it has been reported that tea flower extract has various biological activities including gastroprotective, hypoglycemic, antioxidative, hypolipidemic and antiproliferative effects (Chen et al., 2020b). The high levels of polysaccharides and catechins (epigallocatechin 3-O-gallate, epicatechin 3-O-gallate) in tea flowers have been reported to contribute to its antioxidant properties (Yang et al., 2009; Wang et al., 2012).

Although studies have been carried out with different parts of the tea plant (*Camellia sinensis*) in the field of aquaculture (Cho et al. 2007; Chen et al. 2012; Boran et al. 2015; Kakoolaki et al. 2016; Altınterim et al. 2018; Chen et al. 2018; Chen et al. 2020a, 2020b; Khodadadi and Monfared, 2021), no study on the potential use of tea flower in fish feed has been found. Therefore, in this study, the effects of adding tea flower extract to rainbow trout feed on growth performance, body composition and blood parameters were investigated.

## MATERIALS AND METHODS

### Experimental area, plant and fish material, environmental factors

This study was carried out at Recep Tayyip Erdoğan University, Faculty of Fisheries, Iyidere R&D unit (41°01'17"N 40°22'39"E; altitude 2m) between November 2023 and February 2024. A total of 360 rainbow trout (*Oncorhynchus mykiss*) with a mean length of 12.2±0.21 cm (min 11.5 cm, max 13.00 cm) and a mean weight of 20.3±0.26 g (min 18.00 g; max 22.00 g) were used in the study. Throughout the study, water temperature (ISOLAB Laborgerate GmbH thermometer), pH (KN MASTER pH-100), conductivity and total dissolved solids (TDS) (KN

MASTER TE-300 Ec) were measured daily and dissolved oxygen (HACH HQ 40D digital multimeter) was measured weekly and presented as mean  $\pm$  standard error ( $\pm$ SE) (water temperature  $10.40 \pm 0.85^\circ\text{C}$ , pH  $6.6 \pm 1.04$ , conductivity  $132.54 \pm 34.49 \mu\text{S}/\text{cm}$ , TDS  $66.27 \pm 17.24 \text{ ppm}$ , and dissolved oxygen  $11.3 \pm 0.91 \text{ mg}/\text{L}$ ). Tea flowers (*Camellia sinensis*) were collected from tea gardens in Rize province, Central Camidağı ( $40^\circ 58' 39.4''\text{N}$   $40^\circ 33' 45.4''\text{E}$ ; altitude 230 m) and Kanboz villages of Güneysu district ( $40^\circ 59' 03.0''\text{N}$   $40^\circ 36' 42.7''\text{E}$ ; altitude 183 m) in October 2023.

#### Preparation of tea flower extract (TFE)

Tea flowers (*Camellia sinensis*) were extracted according to the method described in our previous study (Kose and Ariman Karabulut, 2022; Köse et al., 2024). Briefly, fresh tea flowers were dried at  $40^\circ\text{C}$  for 3-4 days, ground and sieved through a  $500 \mu\text{m}$  sieve. A mixture of methanol (Sigma-Aldrich, CAS: 67-56-1, Germany) and distilled water (3:7 v/v) was used as the solvent. The mixture of 100 g ground tea flowers was filled up to 1000 ml with solvent, treated in a sonicator (Sonics VCX-130 Vibra-Cell Ultrasonic Liquid Processor) for 20 min (20kHz wavelength, 5-sec active, 5-sec deactivate) and kept at  $60^\circ\text{C}$  for 24 h in an automatic shaking water bath machine (NÜVE, ST 402). It was then filtered through Whatman no 5 filter papers, and the solvent was removed by an evaporator (Hei-VAP Advantage, Hei Dolph, Germany) at  $60^\circ\text{C}$ . The prepared tea flower extract (TFE) was stored at  $-20^\circ\text{C}$  until it had been used.

#### Formation of experimental groups and preparation of experimental feeds

In the experiment, 30 fish were placed in 12 fiber-glass tanks of 100 L each (80 L adjusted), with a flow system (water flow 9 L/min) and air support. Four experimental groups were formed with three replications (control group 0 ml/kg, 10 ml/kg (TFE-10); 20 ml/kg (TFE-20) and 40 ml/kg (TFE-40) tea flower extract/kg feed) and randomly distributed. All groups were fed three times a day (9:00 am, 1:00 pm, and 4:00 pm) for 87 days until the fish were satiated and 12 hours of light and 12 hours of darkness were applied.

In the study, 4 mm commercial trout feed (45% crude protein, 20% fat, 1.7% crude cellulose and 9.5% ash, Gümüş Doga Su Ürünleri A.Ş., Mugla, Türkiye) was ground and pelletized by adding TFE. Briefly, first, stock solutions were prepared by add-

ing up to 350 ml of pure water to 10, 20 and 40 ml of TFE. Ground commercial feeds were then kneaded with these stock solutions and the resulting dough was passed through a meat grinder with 3 mm discs to produce pellets. The prepared pellets were dried at  $45^\circ\text{C}$  in a fan-assisted drying oven (POL-EKO-APARATURA SP. J. SLW 400 STD) until the moisture content was below 10% and stored at  $-18^\circ\text{C}$  until it had been used.

#### Proximate composition of fish flesh

The analyses were carried out at Recep Tayyip Erdoğan University, Faculty of Fisheries, Food and Feed Technology Laboratory. At the end of the experiment, 3 fish from each group were randomly sampled and the muscle from the skinless and awnless dorsal regions of these fish were used for analysis. Analyses of dry matter, crude protein, crude oil (ether extract, EE) and crude ash were performed according to AOAC (2005). In brief, dry matter analysis was performed by drying the samples at  $105^\circ\text{C}$  to constant weight. Crude protein analysis was carried out by the Kjeldahl method ( $\text{N} \times 6.25$ , Method No. 978.04) using a Behr S5 Distillation Unit and TitroLine Easy apparatus. Ether extraction was carried out with Velp SER 148/6 (VelpScentfca. Milan, Italy) using petroleum ether ( $40-60^\circ\text{C}$ ) (method no. 930.09) and crude ash was determined by burning 3 g of the sample in porcelain crucibles at  $550^\circ\text{C}$  for 6 hours (method no. 930.05).

#### Analysis of hematological blood parameters

During the experiment, blood samples were collected from 4 randomly selected fish every 21 days using a 5 ml sterile syringe and a 22G (black) syringe needle. All samples were taken from the caudal vein and collected in K2EDTA (VACUSERA 2 ml K2E  $13 \times 75 \text{ mm}$ , Izmir, Türkiye) blood collection tubes which were then gently inverted 5-6 times. Analyses were performed at Recep Tayyip Erdogan University Fish Diseases Laboratory using a PROKAN PE-6800 VET fully automated hematology analyzer. This device counts and sizes blood cells by electrical impedance method and determines hemoglobin by colorimetric method. Other blood parameters are automatically calculated according to formulas stored in the system.

#### Data collection and calculation of growth parameters

Fish length and weight were measured every 21 days. Fish were sedated with clove oil at a dose of 2-5 mg/L before measurement. Fish lengths were



measured using a von Bayer ruler with an accuracy of  $\pm 1$  mm and weights were measured using a digital scale with an accuracy of  $\pm 0.1$  g. The following formulae were used to calculate growth parameters and some indices.

Weight gain (WG) = [(final body weight - initial body weight)], (Köse et al., 2024)

Weight gain rate (WGR, %) =  $100 \times [\text{final body weight} - \text{initial body weight}] / \text{initial body weight}$ , (Köse et al., 2021)

Specific growth rate (SGR, % day<sup>-1</sup>) =  $100 \times [\ln(\text{final body weight}) - \ln(\text{initial body weight}) / \text{day}]$ , (Sonay and Kavuk, 2023)

Feed Conversion Ratio (FCR) = Dry Feed Intake / Wet Weight Gain, (Cimagil and Sonay, 2025)

Protein efficiency ratio (PER) = Wet weight gain / protein intake (Karabulut et al., 2021)

Condition factor (CF, g/cm<sup>3</sup>) =  $100 \times [(\text{body weight, g}) / (\text{body length, cm})^3]$ , (Sonay et al., 2024)

Survival rate (SR, %) =  $100 \times [\text{final number of fish} / \text{initial number of fish}]$  (Çimagil and Sonay, 2025)

Viscera-Somatic index (VSI, %) =  $100 \times (\text{viscera weight}) / (\text{total body weight})$ , (Kose and Karabulut, 2022)

Hepato-Somatic index (HSI, %) =  $100 \times (\text{liver weight}) / (\text{total body weight})$ , (Kose and Karabulut, 2022)

Meat yield (MY, %) =  $100 \times (\text{fillet weight}) / (\text{whole body weight})$ , (İzciand Ümit., 2023)

### Statistical Analyses

Means and standard errors ( $\pm$ SE) of the data obtained in the study were calculated using Microsoft Office Excel 2016 Pro. The Sigma plot 14.0 package (Systat Software Inc., San Jose, CA, USA) was used for statistical evaluation of the data. The normal distribution of the data was confirmed by the Shapiro-Wilk test ( $P < 0.05$ ). One-way analysis of variance (ANOVA) was applied to all data. Differences between groups were determined by the Tukey test ( $P < 0.05$ ) for growth parameters and the Holm-Sidak test ( $P < 0.001$ ) for hematological blood parameters. To determine the optimal TFE level, Spearman rank correlation ( $P < 0.0001$ ) was first applied to the data, and the optimal extract level (X-value) was determined by polynomial cubic regression ( $f = y_0 + ax + bx^2 + cx^3$ ).

## RESULTS

### Growth performance, nutrient utilization and body indices

The values obtained for final body weight (FBW), weight gain (WG), weight gain rate (WGR), specific growth rate (SGR), feed conversion ratio (FCR), feed intake (FI), protein efficiency ratio (PER), survival rate (SR), condition factor (CF), hepato-somatic index (HSI), viscera-somatic index (VSI), meat yield (MY) and comparisons between the data obtained at the end of the experiment are presented in Table 1. Weight gain rates (WGR) determined at the measurement periods during the study are shown in Figure 1 and specific growth rates are shown in Figure 2. The results showed significant differences in the TSE-10 group compared to the control group ( $P < 0.05$ ). Accordingly, the highest FBW, WG, WGR, SGR, SR and MY values and the lowest FCR, HSI and VSI values were found in the TSE-10 group. While the TFE-10 group differed from all groups in terms of WG ( $P < 0.05$ ), the TFE-20 and TFE-40 groups were similar to each other ( $P > 0.05$ ) but differed from the control group ( $P < 0.05$ ). For FCR, PER, HSI and MY, the TFE groups were similar to each other, whereas the TFE-20 and TFE-40 groups were similar to the control group. In SGRs, the TFE-10 and TFE-20 groups were similar and the TFE-20 and TFE-40 groups were similar to the control. In FI values, all groups were statistically different from each other ( $P < 0.05$ ). For the SR values, the TFE groups were similar to each other, while the values of TFE-10 and TFE-20 were different from the control group ( $P < 0.05$ ). For VSI values, the TFE-10 group was different from all groups ( $P < 0.05$ ), while the other groups were similar.

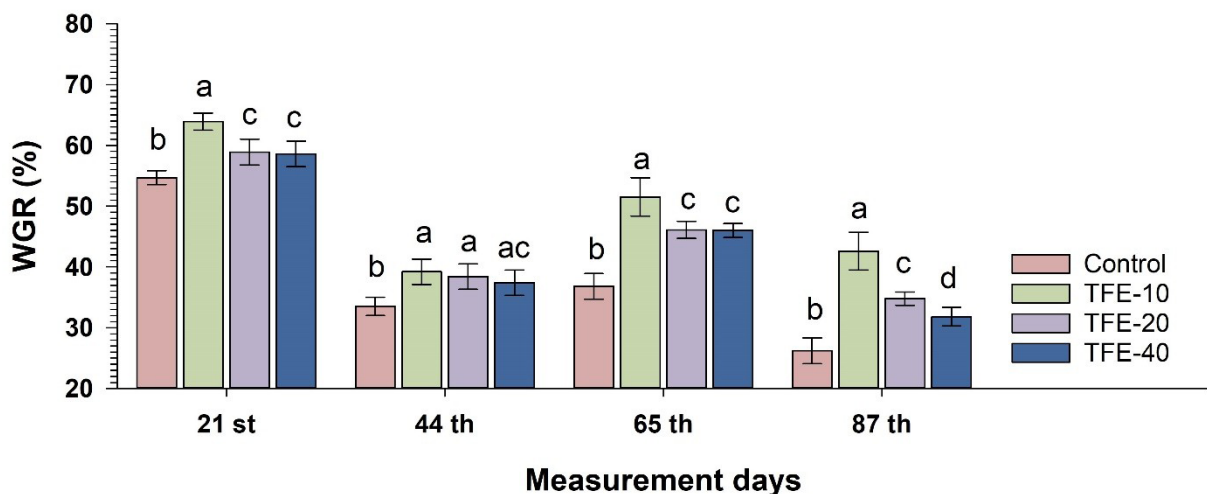
In the measurement periods, the statistical difference of WGR ratios in all TFE groups compared to the control group was significant in all periods ( $P < 0.05$ ). Except for the 44th day, the difference of TFE-10 from TFE-20 and TFE-40 groups was significant in all periods ( $P < 0.05$ ), while TFE-20 and TFE-40 were similar between them on the 65th day ( $P > 0.05$ ) and different on the 87th day ( $P < 0.05$ ) (Figure 1).

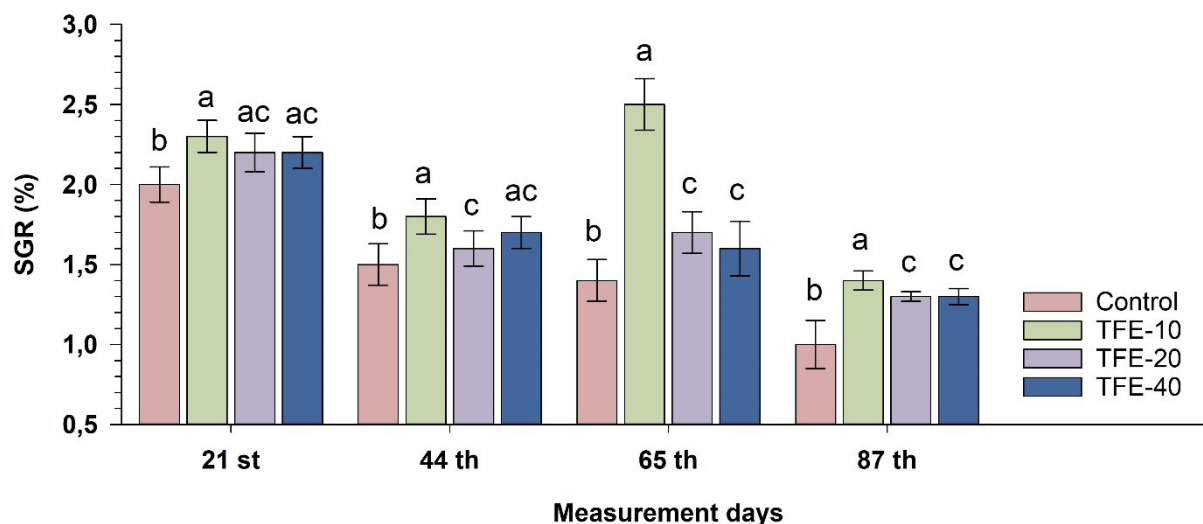
In the measurement periods, all TFE groups were found to be different from the control group in SGR ratios at all periods ( $P > 0.05$ ). While the TFE-10 group was similar to TFE-20 and TFE-40 on the 21st day and TFE-40 on the 44th day ( $P > 0.05$ ), it was found to be different from TFE-20 and TFE-40 on the 65th and 87th day ( $P < 0.05$ ). The TFE-20 and

**Table 1.** Changes in growth parameters, nutrient utilization and body indices of rainbow trout (*Oncorhynchus mykiss*) fed feed supplemented with different levels of tea flower extract (TFE) at the end of the study

Parameters	Control	TFE-10	TFE-20	TFE-40
IBW (g)	20.0±0.25	20.3±0.26	20.3±0.25	20.4±0.28
FBW (g)	73.0±1.29 <sup>b</sup>	84.4±1.45 <sup>a</sup>	77.6±1.18 <sup>c</sup>	76.9±1.58 <sup>c</sup>
IBL (cm)	12.7±0.10	12.7±0.10	12.6±0.11	12.6±0.09
FBL (cm)	18.1±0.21	18.8±0.13	18.5±0.16	18.6±0.17
WG (g)	53.0±0.17 <sup>b</sup>	64.2±0.07 <sup>a</sup>	57.3±0.11 <sup>c</sup>	56.8±0.12 <sup>c</sup>
WGR (%)	265.1±0.33 <sup>b</sup>	316.3±0.41 <sup>a</sup>	283.2±0.29 <sup>c</sup>	277.6±0.56 <sup>bc</sup>
SGR (%)	1.5±0.02 <sup>b</sup>	1.7±0.01 <sup>a</sup>	1.6±0.03 <sup>ab</sup>	1.5±0.02 <sup>b</sup>
FCR	1.3±0.11 <sup>b</sup>	1.0±0.04 <sup>a</sup>	1.1±0.12 <sup>ab</sup>	1.2±0.11 <sup>ab</sup>
FI (g)	69.0±0.45 <sup>a</sup>	64.1±0.21 <sup>b</sup>	63.1±0.27 <sup>c</sup>	68.1±0.36 <sup>d</sup>
PER	1.2±0.07 <sup>b</sup>	1.4±0.10 <sup>a</sup>	1.3±0.08 <sup>ab</sup>	1.3±0.09 <sup>ab</sup>
CF (%)	1.2±0.04	1.2±0.03	1.2±0.04	1.2±0.02
SR (%)	92.9±0.96 <sup>b</sup>	94.6±1.04 <sup>a</sup>	93.8±1.39 <sup>a</sup>	93.6±1.12 <sup>ab</sup>
HSI (%)	1.8±0.04 <sup>b</sup>	1.5±0.03 <sup>a</sup>	1.6±0.08 <sup>ab</sup>	1.7±0.02 <sup>ab</sup>
VSI (%)	13.8±0.18 <sup>b</sup>	12.8±0.16 <sup>a</sup>	13.0±0.24 <sup>b</sup>	13.0±0.14 <sup>b</sup>
MY (%)	60.2±3.16 <sup>b</sup>	70.9±2.03 <sup>a</sup>	67.6±0.56 <sup>ab</sup>	66.8±0.46 <sup>ab</sup>

All data are expressed as mean ±SE (n=3). Data with different letters in the same row are statistically significant (P<0.05, TUKEY). IBW: Initial body weight, FBW: Final body weight, IBL: Initial body length, FBL: Final body length, WG: Weight gain, WGR: Weight gain rate, SGR: Specific growth rate, FCR: Feed conversion ratio, FI: Feed intake, PER: Protein efficiency ratio, CF: Condition factor, SR: Survival rate, VSI: Viscera-Somatic index, HSI: Hepato-Somatic index and MY: Meat yield. Control, TFE-10, TFE-20 and TFE-40 are the experimental groups containing 0-, 10-, 20- and 40-ml tea flower extract (TFE)/kg feed, respectively.

**Figure 1.** Weight gain rate (WGR, %) values of rainbow trout (*Oncorhynchus mykiss*) fed feed supplemented with different levels of tea flower extract (TFE) during the study periods. Bar values are the mean ±SE of three replicates (n=3). Different letters on the bars indicate the difference between the experimental groups (P<0.05, TUKEY).



**Figure 2.** Specific growth rate (SGR, %) values in rainbow trout (*Oncorhynchus mykiss*) fed feed supplemented different levels of tea flower extract (TFE) during the study periods. Bar values are the mean  $\pm$ SE of three replicates (n=3). Different letters on the bars indicate the difference between the experimental groups ( $P < 0.05$ , TUKEY).

TFE-40 groups were similar in all periods ( $P > 0.05$ ) (Figure 2).

#### Determination of optimum TFE level

Since WG, FCR, SGR and FI are key indices for monitoring fish growth parameters and the TFE-10 group was found to have the best values, this group was taken as a reference and used in a cubic polynomial regression to determine the optimum extract value. The values obtained are shown in Figure 3. Accordingly, if the TFE level was reduced from 10.00 ml/kg to 9.68 ml/kg, the  $WG = 64.21g$  ( $y = 0.003x^3 - 0.17x^2 + 2.588x + 53$ ) if it was increased from 10.00 ml/kg to 11.09 ml/kg, the  $FCR = 0.99$  ( $y = 0.000054x^3 + 0.004x^2 - 0.061x + 1.3$ ); if it was increased from 10.00 ml/kg to 10.47 ml/kg, the  $SGR = 1.701\%$  ( $y = 0.000042x^3 - 0.003x^2 + 0.043x + 1.5$ ); and if it was increased from 10 ml/kg to 17.96 ml/kg, the FI would be 63.04g ( $y = -0.00019x^3 + 0.025x^2 - 0.724x + 69$ ). The optimal TFE level was  $12.3 \pm 3.81$  ml/kg when taking the arithmetic mean of the optimal growth points (x-value) corresponding to the optimal WG, FCR, SGR and FI values.

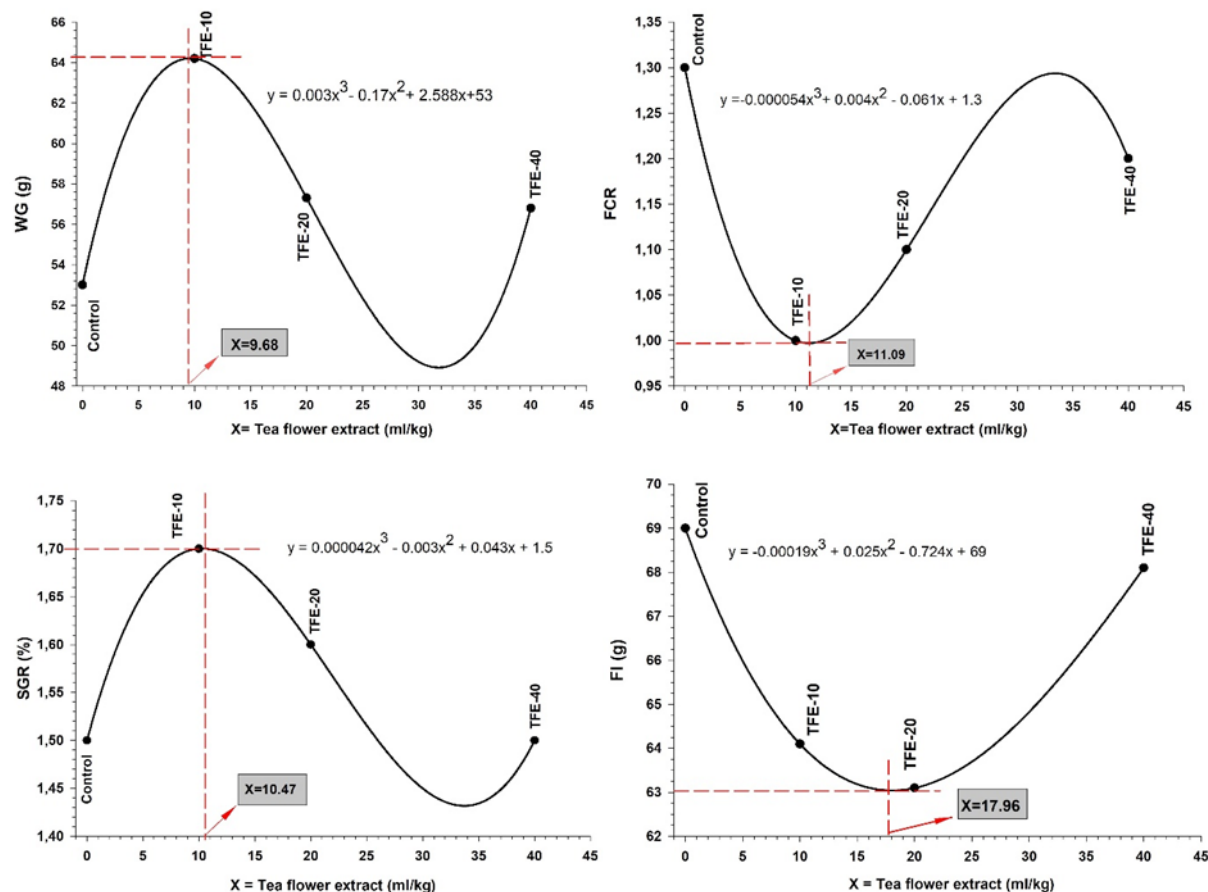
#### Proximate composition of fish flesh

Fish flesh proximate results showed that TFE-10 group protein was similar to TFE-20 ( $P > 0.05$ ) but different from control and TFE-40 ( $P < 0.05$ ). Control, TFE-20 and TFE-40 groups were also similar

to each other. The fat content of fish flesh was found to be different from the control in all TFE groups ( $p < 0.05$ ). Also, the TFE-10 group was statistically different from the TFE-40 group. While the moisture content of the TFE-10 group was different from all other groups ( $P < 0.05$ ), the control, TFE-20 and TFE-40 groups were similar to each other. The ash contents of the TFE groups were similar to each other. However, they differed significantly from the control group ( $P < 0.05$ ) (Table 2).

#### Hematological blood parameters

White blood cell (WBC, leukocyte), red blood cell (RBC, erythrocyte), hemoglobin (HGB), hematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), platelet (PLT) and platelet distribution width (PDW) values and their comparisons at the end of the study are shown in Table 3. The results showed that WBC was different from the control group in all TFE groups ( $P < 0.05$ ). In RBC, the TFE-40 group differed from the TFE-10 and TFE-20 groups ( $P < 0.05$ ) but was similar to the control group. For HGB, a difference was found only between the TFE-10 and control groups ( $p < 0.05$ ). In HCT, the TFE-20 group differed from the control and TFE-10 groups and the control group differed from the TFE-40 group ( $P < 0.05$ ). The TFE-10 group was different from all groups in MCV ( $P < 0.05$ ), while the control group was different from all groups in



**Figure 3.** Significant cubic relationships and polynomial regression analysis ( $P < 0.0001$ , Spearman rank correlation) between feed intake (FI), feed conversion ratio (FCR), weight gain (WG) and specific growth rate (SGR) in rainbow trout (*Oncorhynchus mykiss*) fed different levels of tea flower extract (TFE). The trend line equation ( $f = y_0 + ax + bx^2 + cx^3$ ) shown in each graph represents the 'x' value corresponding to the peak of the equation. The x value refers to the calculated TFE level estimated for optimal growth.

MCH. In addition, the TFE-10 group differed from the TFE-20 and TFE-40 groups in MCH ( $P < 0.05$ ). While the MCHC values of all TFE groups were

similar to each other ( $P > 0.05$ ), they differed from the control group ( $P < 0.05$ ). PLT was found to be different between all groups ( $P < 0.05$ ). In PDW, while the

**Table 2.** The proximate composition of fish flesh (100 g) determined at the end of the study in rainbow trout (*Oncorhynchus mykiss*) fed diets supplemented with different levels of tea flower extract (TFE)

Parameters	Control	TFE-10	TFE-20	TFE-40
Moisture (%)	77.7±0.14 <sup>b</sup>	76.3±0.15 <sup>a</sup>	77.3±0.57 <sup>b</sup>	77.6±0.11 <sup>b</sup>
Protein (%)	18.1±1.04 <sup>b</sup>	19.0±1.08 <sup>a</sup>	18.7±0.94 <sup>ba</sup>	18.5±1.05 <sup>b</sup>
Fat (%)	8.8±1.01 <sup>a</sup>	6.9±1.35 <sup>b</sup>	6.1±1.25 <sup>bc</sup>	5.7±1.16 <sup>c</sup>
Ash (%)	1.2±0.05 <sup>a</sup>	1.3±0.10 <sup>b</sup>	1.3±0.05 <sup>b</sup>	1.3±0.51 <sup>b</sup>

Values are the mean ± SE of three replicates (n=3). Means in the same row with different letters indicate significantly different ( $P < 0.05$ , TUKEY). Control, TFE-10, TFE-20 and TFE-40 are the experimental groups containing 0-, 10-, 20- and 40-ml tea flower extract (TFE)/kg feed, respectively.



**Table 3.** Mean values of hematological blood parameters determined at the end of the study in rainbow trout (*Oncorhynchus mykiss*) fed diets supplemented with different levels of TFE

Parameters	Control	TFE-10	TFE-20	TFE-40
WBC ( $10^3/\mu\text{L}$ )	103.7 $\pm$ 0.49 <sup>b</sup>	109.1 $\pm$ 0.45 <sup>a</sup>	106.0 $\pm$ 0.39 <sup>c</sup>	108.4 $\pm$ 0.41 <sup>ac</sup>
RBC ( $10^6/\mu\text{L}$ )	1.3 $\pm$ 0.05 <sup>ab</sup>	1.5 $\pm$ 0.03 <sup>a</sup>	1.4 $\pm$ 0.05 <sup>a</sup>	1.2 $\pm$ 0.05 <sup>b</sup>
HGB (g/dL)	15.9 $\pm$ 0.26 <sup>b</sup>	18.8 $\pm$ 0.32 <sup>a</sup>	16.8 $\pm$ 0.35 <sup>ab</sup>	17.2 $\pm$ 0.05 <sup>ab</sup>
HCT (%)	22.0 $\pm$ 0.41 <sup>a</sup>	21.5 $\pm$ 0.17 <sup>ac</sup>	20.7 $\pm$ 0.16 <sup>b</sup>	20.2 $\pm$ 0.36 <sup>bc</sup>
MCV (fL)	167.8 $\pm$ 0.34 <sup>b</sup>	174.8 $\pm$ 0.29 <sup>a</sup>	171.2 $\pm$ 0.24 <sup>cb</sup>	169.7 $\pm$ 0.28 <sup>bc</sup>
MCH (pg)	125.5 $\pm$ 0.40 <sup>b</sup>	155.5 $\pm$ 0.52 <sup>d</sup>	158.9 $\pm$ 0.30 <sup>a</sup>	157.2 $\pm$ 0.57 <sup>ad</sup>
MCHC (g/dL)	98.7 $\pm$ 0.19 <sup>b</sup>	107.9 $\pm$ 0.23 <sup>a</sup>	106.6 $\pm$ 0.24 <sup>a</sup>	105.3 $\pm$ 0.54 <sup>ca</sup>
PLT ( $10^3/\mu\text{L}$ )	69.7 $\pm$ 0.43 <sup>a</sup>	52.3 $\pm$ 0.36 <sup>b</sup>	55.7 $\pm$ 0.57 <sup>c</sup>	62.2 $\pm$ 0.53 <sup>d</sup>
PDW (%)	16.4 $\pm$ 0.17 <sup>b</sup>	17.8 $\pm$ 0.17 <sup>a</sup>	17.6 $\pm$ 0.21 <sup>a</sup>	17.3 $\pm$ 0.22 <sup>ab</sup>

All data are presented as mean  $\pm$ SE (n=4). Data with different letters in the same row are statistically significant ( $P<0.001$ , Holm-Sidak test). WBC: White blood cell, RBC: Red blood cell, HGB: Hemoglobin, HCT: Hematocrit, MCV: Mean corpuscular volume, MCH: Mean corpuscular hemoglobin, MCHC: Mean corpuscular hemoglobin concentration, PLT: Platelet, PDW: Platelet distribution width. Control, TFE-10, TFE-20 and TFE-40 are the experimental groups containing 0-, 10-, 20- and 40-ml tea flower extract (TFE)/kg feed, respectively.

TFE groups were similar ( $P>0.05$ ), only the TFE-10 and TFE-20 groups differed from the control group ( $P<0.05$ ).

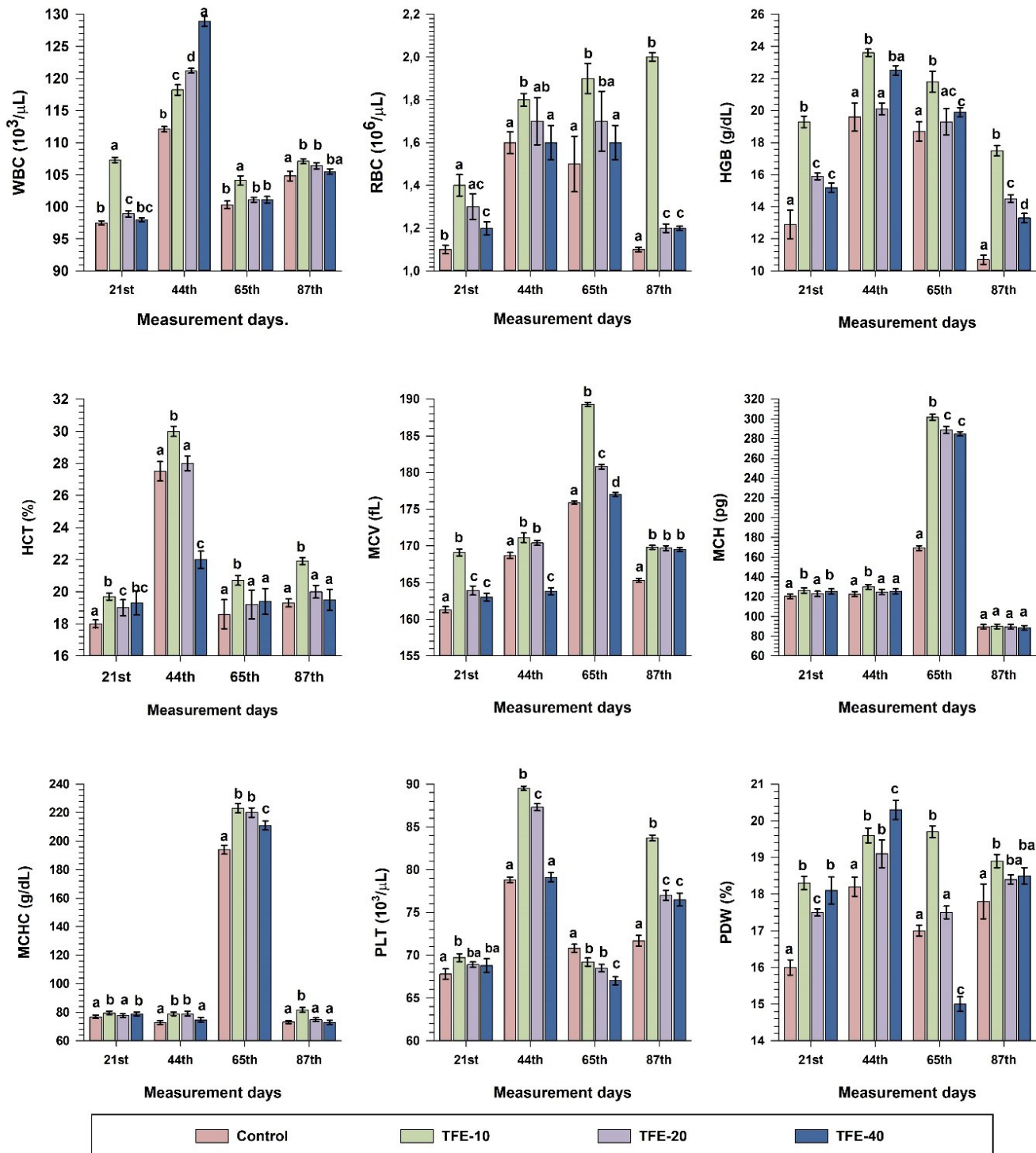
Figure 4 shows the hematological values of the blood samples taken during the measurement days throughout the study. The results showed that the TFE groups had higher values than the control group for all WBC, RBC, HGB, HCT, MCV, MCHC, PLT and PDW values. All hematological values of the TFE-10 group (except MCH on day 87) were found to be significantly different from the control group ( $P<0.05$ ). On day 65, MCV, MCH and MCHC were notably raised in all groups compared to the other measurement days, and the TFE groups were found to be significantly different from the control ( $P<0.05$ ).

## DISCUSSION

Tea is one of the most common agricultural products and its leaves are commonly used. Tea flower has been neglected and even considered industrial waste, except for the obtaining of tea seeds. Studies carried out in the Far East countries over the last two decades have shown that the tea flower contains biological metabolites (catechins, flavonols, caffeine, amino acids, etc.) that are as valuable as the leaves. Studies on the usability of some tea products (green tea, tea seeds, processed tea leaves) in aquatic feeds are available in the literature (Cho et al., 2007; Kakoolaki et al., 2016; Bilgin, 2017; Altınterim et al., 2018; Khodadadi and Monfared, 2021). However,

no studies on tea flower extract (TFE) were found in the literature. In the present study, changes in growth performance, body composition, survival rates and hematological blood parameters of juvenile rainbow trout were investigated by feeding trout diets supplemented with TFE at different levels (10, 20, 40 ml TFE/kg feed) for 87 days, and the optimal amount of TFE was determined by polynomial cubic regression.

In our study, WG, WGR, SGR, PER, SR and MY were significantly higher in the TFE groups and FCR, FI, HSI and VSI were significantly lower compared to the control group. In a study in which green tea was tested as fresh, dried, by-product and extract in *Paralichthys olivaceus*, FBW, WG, SGR, PER, CF and HSI were reported to be lower in the fresh, dried and by-product groups compared to the control group, similar to the control in the extract group, while FCR and SR were reported to be high (Cho et al., 2007). The study shows a partly different trend than our study. On the other hand, the SR and HSI values are in line with our study. The study in which green tea leaves were supplemented to the diet of *Oreochromis niloticus* was consistent with our study (except for FI) (Abdel-Tawwab et al., 2010). Similarly, a previous study on the growth performance of green tea extract in *Pterophyllum scalare* showed the same trend as the present study (Yilmaz, 2017). In another study with green tea oil, WG and WGR trends differed from this study, while FI and FCR values were similar (Altınterim et al., 2018). In



**Figure 4.** Changes in hematological blood parameters of rainbow trout (*Oncorhynchus mykiss*) fed diets supplemented with different levels of TFE (n=4). Bar values are mean  $\pm$  SE. Different letters on the bars indicate that the difference between groups is significant ( $P < 0.001$ , Holm-Sidak test). WBC: White blood cell, RBC: Red blood cell, HGB: Hemoglobin, HCT: Hematocrit, MCV: Mean corpuscular volume, MCH: Mean corpuscular hemoglobin, MCHC: Mean corpuscular hemoglobin concentration, PLT: Platelet, and PDW: Platelet distribution width.

the present study, fish were fed three times a day until satiation and, contrary to expectations, lower feed intake but better growth performance was observed in the TFE-10 and TFE-20 groups. This can be explained by the reports of Chen L et al. (2020) and Chen et al. (2020a) who explained that the bioactive compounds of TFE increased nutrient digestibility. We think that the bioactive compounds in TFE may have had a positive effect on the microbiota of the digestive system, resulting in an increase in beneficial microorganisms and possibly an increase in microbial enzymes. In our study, CF values were found to be similar in all groups, confirming this assumption. In fact, CF has been reported as one of the best criteria for monitoring the development and nutrient utilization of farmed fish (Ontario Ministry of Agriculture, 2023).

In the present study, FI, HSI and VSI levels decreased as a result of feeding trout feed supplemented with TFE. In addition, the fat content of the fish meat decreased while the protein content increased. Similar findings were also reported by Yılmaz (2017). Chen et al (2011) reported that green tea could increase lipid metabolism and prevent cholesterol accumulation in fish. Similarly, a clinical study in mice reported that methanolic extract from flower buds reduced visceral adiposity by increasing serotonin release, suppressing appetite signaling pathways in the hypothalamus and reducing food intake (Hamao et al, 2011). The low HSI, VSI and FI values found in our study can be explained by the findings of Hamao et al. (2011). The high protein ratios and associated high MY levels found in fish muscle in the TFE-fed groups may also be related to protein synthesis and the resulting growth rate and muscle accumulation rate (Abdel-Tawwab et al., 2010; Gao et al., 2012; Hasanpour et al., 2019).

Hematological parameters can provide information on the health and physiological status of fish, the water quality in which they live and their nutritional conditions. Regular measurements of RBC, WBC, HGB and PDW levels have been recommended for the monitoring of stock health in fish farms (Fazio et al., 2013). Leukocytes (WBCs) are used to evaluate the physiological and pathological status of the organism as they protect the organisms against pathogenic microorganisms (Mohammadi et al., 2020). High blood WBC levels have been reported as an indicator of fish health (Yılmaz, 2015). However, they may also indicate the presence of inflammation in the organism (Czech et al, 2009). Erythrocytes (RBCs)

contain HGBs, which carry oxygen to the tissues. Erythrocyte (RBC) count is significantly influenced by environmental factors, particularly temperature and dissolved oxygen levels (Javeed et al., 2022). Additionally, erythrocyte counts may vary between fish species. For instance, Hrubec and Smith (2010) reported that erythrocyte counts can range from  $0.03 \times 10^3$  to  $5.3 \times 10^6$  cells/ $\mu$ l. In healthy rainbow trout, the erythrocyte count was reported to range from 0.28 to  $1.34 \times 10^6$  cells/ $\text{mm}^3$  (Rehulka et al., 2004). HGB levels may decrease due to some problems in the antioxidant system (Abdel-Tawwab et al, 2018; Hoseini et al, 2018; Hajirezaee et al, 2020). Hematocrit (HCT) defines the volumetric percentage of RBCs and increases or decreases in proportion to the number and size of RBCs (Mohammadi et al., 2020). RBC, HGB and HCT values provide information about anemic conditions (Yılmaz, 2015). MCV refers to the state of cell divisions of RBC during erythropoiesis (Zorriehzahra et al., 2010) and is important in the detection of anemia together with MCH and MCHC (Yılmaz, 2015). Platelets (PLT) are responsible for blood clotting. Platelet levels in fish can vary depending on sex, physical and chemical properties of the water, age and feeding period. Depending on the species, platelet (PLT) count varies between 2.000 and 78.000  $\mu$ l (Dias and Oliveira, 2009; Hrubec and Smith, 2010). Platelet distribution width (PDW) is defined as a platelet index and is one of the parameters obtained from automated complete blood count devices. PDW is one of the biomarkers of platelet activation (Larsen et al., 2014).

In the present study, higher WBC, RBC, HGB, HCT, MCV, MCH, MCHC, and PDW levels were observed in the TFE groups (except PLT in TFE-10 and TFE-20) than in the control group, both during the measurement periods and at the end of the experiment. Similar hematological results were reported in studies conducted with both *Camellia sinensis* (Abdel-Tawwab et al., 2010; Hasanpour et al., 2017; Alinterim et al., 2018) and different plant extracts (Goda, 2008; Kose and Ariman Karabulut, 2022). The use of green tea powder as an immunostimulant reduced the levels of hematological parameters in rainbow trout, as reported by Bilgin (2017). In another study conducted with *Mugil cephalus*, the trends in HGB, RBC and WBC values were reported to be similar to our study, while the trends in MCV, MCH and MCHC values were different in the same study (Kakoolaki et al., 2016). In light of these reports, it can be said that the addition of TFE to the diet in our

study has a good effect on hematological parameters without a negative effect on fish health. This view is supported by the fact that SR ratios were found to be higher in the TFE groups in our study. A large number of fish species (approximately 23.500) differ in terms of hematological values (Fazio, 2019). This is because blood parameters are influenced by many factors such as species (Ikechukwu and Obinnaya, 2010), age (Orun and Umit Erdeml, 2002), temperature (Magill and Sayer, 2004), stress (Cnaani et al., 2004), nutritional status (Lim and Klesius, 2003), water quality (Fazio et al., 2012), and stocking density and health status (Vazquez and Guerrero, 2007). These factors may be the reason why the results obtained in this study differ from those reported in other literature.

## CONCLUSION

*Camellia sinensis*, an industrial plant, is an important source of income in the countries where it is grown and its by-product, tea flower, is under-utilized. This study may provide new alternative ideas for the use of tea flowers in aquaculture. The results of the study indicate that the addition of TFE to rainbow trout diets has a positive effect on growth performance, nutrient composition and changes in the blood parameters of the fish. Cubic polynomial regression results showed that the optimal TFE level should be  $12.3 \pm 3.81$  ml TFE/kg in the TFE-10 group, which was the best treatment group. Considering the SR ratios, it was concluded that the changes in

hematological parameters did not have an adverse effect on the fish and therefore TFE can be safely used. Low FCR, HSI and VSI values and high WG, WGR and SGR values in the TFE groups suggest that TFE may affect microbial enzyme activity and lipid metabolism by affecting the gut microbiota, and it is concluded that more comprehensive studies on this topic are needed.

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## CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

## ETHICAL STANDARDS

This research was checked and approved by Recep Tayyip Erdoğan University Animal Experiments Local Ethics Committee (Decision No: 2022/21, Date: 30.06.2022). All procedures subject to the research were carried out within the scope of all guidelines for the scientific use of animals.

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