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Effect of Dietary Inclusion of Green Tea (*Camellia sinensis*) Powder on Growth Performance, Feed Utilization and Body Composition in Rainbow Trout (*Oncorhynchus mykiss*)

Özlem Bilgin[1,*], Huriye Arıman Karabulut[2], Serap Ustaoglu Tırıl[3]

[1]Sinop University, Scientific and Technological Research Application and Research Center (SUBİTAM), TR57000, Sinop, Türkiye.
[2]Recep Tayyip Erdoğan University, Faculty of Fisheries and Aquaculture, TR53000, Rize, Türkiye.
[3]Sinop University, Faculty of Fisheries and Aquaculture, TR57000, Sinop, Türkiye.

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Corresponding Author
E-mail: ozlmbilgin28@gmail.com

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Abstract

This study was conducted to evaluate the effect of dietary inclusion of green tea powder on growth performance, feed utilization and body composition of rainbow trout. A total of 546 fish with an average body weight of 40.4±0.01 g were divided in the seven experimental treatments. The fish were fed with diets containing 0%(control), 0.25%(diet1), 0.5%(diet2), 1%(diet3), 2%(diet4), and 3%(diet5) of dried green tea (GT) powder containin 5.3±0.04% epigallocatechin gallate-3-gallate (EGCG), 1%(diet6) green tea dust (GTD) containing 6.5±0.07% EGCG for 60 days. Fish fed with diet 2 had higher weight gain, specific growth rate, apparent net protein retention, and protein efficiency ratio than other groups, and had a lower feed conversion ratio than other groups, but not significantly changed than control group ($P>0.05$). Crude lipid content of liver and fillet in fish fed experimental diets were significantly lower than of fish fed the control diet ($P<0.05$). These results showed that dietary supplementation of GT powder in trout feed promoted the growth performance and feed utilization, and also administration of 1% level of GTD and different doses of GT powder in the experimental diets showed lipid-lowering effects for rainbow trout.

Introduction

The rainbow trout, *Oncorhynchus mykiss*, is one of the most important commonly fish species for aquaculture in Türkiye and for last decades, the average annual production is 138,636.0±12,915.1 tons (between 410,435,5.0 and 221,046.0 tons), which constitute about 23.5±1.19% of the total marine and inland water aquaculture production of Türkiye (TUIK, 2014-2023). The use of plants and their derived products for improving growth and feed utilization of farmed fish species such as rainbow trout, have attracted a lot of attention globally and have become a subject of fisheries sciences investigations (Bulfon et al., 2015; Hai, 2015; Hernández-Contreras and Hernández, 2020; Mbokane and Moyo, 2022; Yu et al., 2024). Bulfon et al. (2015) reviewed about 105 scientific publications available in literature and reported that more than 60 different plant species have been studied for the improvement of cultured fish growth, feed utilization, health and disease management in aquaculture. The results of these studies showed that plants or their derived products have a broad spectrum of growth promotion, appetite stimulation, antimicrobial, immunostimulant, antiinflammatory, antistress and anticancer properties. Moreover, dietary inclusion in fish diets of these plants or their derived products reported as different multiple functions such as

probiotics, antioxidants (Yu et al., 2024), lipid derivatives (Welker et al., 2017) and aromatic compounds, etc. (Goda, 2008; Bulfon et al., 2015; Hai, 2015). Whole plants, their parts (leaf, root, or seed), or extracted compounds can be incorporated into aquaculture practices, either through water applications or as feed additives, etc. (Bulfon et al., 2015; Hai, 2015; Hernández-Contreras and Hernández, 2020).

Tea, a beverage originating from leaves and buds of a single species of plant, *Camellia sinensis*, is grown in about 30 countries and is the second most consumed beverage in the world after water with a per capita worldwide consumption of approximately 0.12 liter per year (Graham, 1992; Vinson et al., 1998). Tea plants show lanceolated or elliptic leaves 2-3 cm wide that vary in size length depending on the species and variety. In the last decade, the amount of produced fresh tea leaves obtained from tea farmers changed between 1104 and 1328 thousand tons (mean: 1,194.5±25.9 thousand tons) in Türkiye. The quantity of dry tea leaves obtained from 100 kg fresh tea leaves called as the efficiency of tea and this value is between 18.8% and 19.0% (mean: 18.6±0.14%) for Turkish green tea (Kacar, 2010). Main compositions of green tea include tea polyphenols, protein, vitamins, carbohydrates, alkaloids such as caffeine and theophylline, lipids and inorganic elements such as N and P (Kacar, 2010). Fresh green tea are rich in the flavanol group of polyphenols known as catechins which may constitute up to 36% of the dry green tea leaves weight (Tosun and Karadeniz, 2005; Turkmen and Velioğlu, 2007). Pharmacological properties of green tea are primarily due to its compound of catechins (Friedman et al., 2005; Kacar, 2010; Tounekti et al., 2013), which are divided into four primary compounds, epigallocatechin gallate (EGCG), epigallocatechin (EGC), epicatechin gallate (ECG), epicatechin (EC), and four secondary compounds, gallocatechin gallate (GCG), gallocatechin (GC), catechin gallate (CG) and catechin (C). EGCG is also known as a predominant catechin in green tea leaves with a level approximately 48–55% of total polyphenols (Perva-Uzunalić et al., 2006; Kacar, 2010; Mahmood et al., 2010; Tounekti et al., 2013). It was also reported that green tea compenents may be varied depending on variety of tea species, age of the leaves (plucking position), plucking seasons or harvest time, region and altitude where grown, climatic conditions, horticultural practices, etc (Lin et al., 1996; Turkmen and Velioğlu, 2007; Erturk et al., 2010; Tounekti et al., 2013; Welker et al., 2017).

Because of the aforementioned properties, green tea used in several studies, suggest a beneficial impact in human and several species of animals. Furthermore, green tea has recently received much interest and attention because of its multiple functions in the aquaculture as well. Only a few studies with green tea were carried out to fisheries sciences prior to 2010 (Kono et al., 2000; Ishihara et al., 2002; Suzuki et al., 2006; Cho et al., 2007; Cho and Kim, 2009). However, the number of studies have gone up after 2010 so far, and different studies have just been performed to determine the effect of green tea and/or extract compounds on growth, fish antioxidant defense and immune system, protection against pathogens and blood chemistry in different farmed fish species such as rainbow trout, *O. mykiss* (Thawonsuwan et al., 2010; Sheikhzadeh et al., 2011; Nootash et al., 2013; Welker et al., 2017), nile tilapia, *Oreochromis niloticus* (Abdel - Tawwab et al., 2010; Zheng et al., 2017; Van Doan et al., 2019; Qian et al., 2021; Daniel et al., 2024), gilthead sea bream, *Sparus aurata* (Pérez-Jiménez et al., 2013), chum salmon, *O. keta*, and masu salmon, *O. masou* (Suzuki et al., 2006), coho salmon, *O. kisutch* (Yu et al., 2024), grass carp, *Ctenopharyngodon idella* (Rizwan et al., 2021), kelp grouper, *Epinephelus bruneus* (Harikrishnan et al., 2011), olive flounder, *Paralichthys olivaceus* (Cho et al., 2007; Cho and Kim, 2009), black rockfish, *Sebastes schlegeli* (Hwang et al., 2013), yellowtail, *Seriola quinqueradiata*, and ayu, *Plecoglossus altivelis* (Kono et al., 2000), barramundi, *Lates calcarifer* (Ahmadi et al., 2022) and blue gourami, *Trichogaster trichopterus* (Paulpandian et al., 2023).

Different studies in fish species mentioned above showed the positive effects of green tea derivatives on growth, immune system, protection against some pathogens and blood chemistry. The potential benefits of tea make it a candidate as dietary supplement to be used in fish nutrition, namely by helping to modulate lipid deposition in aquaculture fish such as *P. olivaceus* (Cho et al., 2007; Cho and Kim, 2009) that tend to accumulate high lipid concentrations. Adding surplus lipid as an energy sources generally are applied to reduce protein availability in formulated fish diet for improving growth performance in aquaculture. But, the dietary surplus lipid application in fish diet generally leads to a reduction in the edible portion of the fish as a result of increasing body and liver lipid content or also decreasing in storage time as a result of easy oxidation of high levels of lipid after feed utilization, and to prevent the lipid oxidation of feed, therefore, antioxidant is commonly included in fish diets (Cho et al., 2007; Cho and Kim, 2009; Qian et al., 2021).

In addition, the effect of green tea catechins on growth performance were reported as dose depended for different experimental animals such as rats (Kao et al. 2000; Lin and Lin-Shiau, 2006). However, the studies related to the use of tea plant as a dietary raw material in rainbow trout still do not currently provide sufficient data on the components of the tested green tea plant and their usability level as raw materials (note that: the amount of polyphenols and also catechins contents in the tea plant may affected by different factors mantioned in the discussion section).

Moreover, it is imperative to assess the potential of green tea dust, generated as a by-product in tea factories, for its incorporation into aquaculture diets. Evaluating this application not only leverages waste material from the tea industry but also could enhance

the nutritional profile of fish feed, thereby potentially contributing to improved growth performance and health outcomes in aquatic species. Such considerations may facilitate a more sustainable approach to feed formulation within the aquaculture sector, promoting resource efficiency and the utilization of by-products in effective dietary strategies.

This study was performed to determine the effect of the dietary inclusion of different doses (0.25%, 0.50%, 1%, 2% and 3%) of green tea powder containing 11.0±0.09% total catechins (EGCG: 5.3±0.04%) and also 1% level of green tea dust (a factory by-product) containing 14.5±0.16% total catechins (EGCG: 6.5±0.07%), which is manufactured green tea waste by ÇAYKUR (General Directorate of Tea Enterprises) in Türkiye, on growth, body composition, crude lipid content of liver and fillet and feed utilization of rainbow trout, *O*. *mykiss* with an average body weight of 40.4±0.01 g.

Materials and Methods

Experimental Animal, Rearing Conditions

Rainbow trout (*Oncorhynchus mykiss*) used in the experiments were obtained from Recep Tayyip Erdoğan University Iyidere Fisheries Research Center in the centre of Rize, Türkiye. Fish were held in a flow-through fiberglass tank systems for 15 days to acclimate to laboratory conditions prior to experiments. During the acclimation period, fish were fed twice a day on commercial trout pellets with about similar content to the control diet (crude protein: 45% and crude lipid: 18%). A total of 546 rainbow trouts with an average body weight of 40.4±0.01 g were used in the experiment.

Obtaining of Green Tea (GT) and Green Tea Dust (GTD)

The samples of fresh green tea (GT), *Camellia sinensis*, were supplied from a local farmer's garden (at an altitude of about 400 m) and also manufactured green tea dust (GTD) were obtained from Directorate General of Tea Enterprises (ÇAYKUR) Cumhuriyet tea-processing factory in the centre of Rize, Türkiye. The fresh GT were dried into the fan oven during 30 minutes at 90°C temperature for use in fish diets as feed raw materials. Dry tea were homogenized by using a 180 W BOSCH mark coffee grinder homogenizer and sifted into sieve with a 500 µm mesh size. In this way, green tea was ground into powder and made ready to be added to the ration. The obtained GT and GTD used in the design of the experimental diets.

Components Analysis of GT and GTD

GT and GTD phenolic content (%) analysis include gallic acid, caffeine and catechins were performed three replicates by colorimetric method as described to the International Organization for Standardization (ISO) 14502-1 protocol (Anonymous, 2005a). Furthermore, GT and GTD proportion of catechins (%) analysis include epigallocatechin (EGC), catechin (C), epigallocatechin-3-gallate (EGCG), epicatechin (EC) and epicatechin gallate (ECG) was performed three replicates using HPLC (High Pressure Liquid Chromatography) by the ISO 14502-2 protocol (Anonymous, 2005b).

The ultraviolet (UV) absorption spectrums of GT (Figure 1A) and GTD (Figure 1B) phenolic and catechins content are determined three replicates by measuring the absorbance at 278 nm. Content of total polyphenols and catechins analysis in GT and GTD were conducted in the ÇAYKUR Atatürk Tea and Horticultural Research Institute, Rize, Türkiye. Components of GT and GTD used as the diet raw material in the experimental diets showed in Figure 2 and Figure 3, respectively.

Experimental Diets and Chemical Analysis

A control diet was formulated to contain about 44.5% protein and 17.5% lipid based on fish meal and fish oil as main protein and lipid sources. Six other diets were formulated similar to the control but supplemented with 0.25%, 0.50%, 1%, 2%, 3% GT and with 1% GTD. Seven experimental diets (3 mm diameter), including the control diet, were prepared using a mincing machine (ENAZON mark, 32 No meat mincer, 380V industrial electricity three phase, 2.2 Kw 3 Hp, chuck mouth 3mm) and dried into the oven (POL-EKO-APARATURA SP. J. SLW 400 STD) during 12 hours at 60°C temperature until the moisture content drops below 10% level.

At the beginning of the experiment, 30 fish from the stock tank were picked randomly for initial chemical composition analyses. At the end of the study, 3 fish were removed randomly from each tank (9 per treatment) for chemical composition analyses. Moreover, at the end of the feeding trial, liver of 9 rainbow trouts were also weighed to calculate the hepatosomatik index values for each group.

Chemical composition of diets, fish fillet and liver were analyzed at the nutrition laboratory of the Faculty of Fisherie, Recep Tayyip Erdoğan University. Moisture content of experimental diets, fish fillet and liver were determined by oven drying about 3-5 g sample at 105°C until a constant weight was obtained (AOAC, 1995, Method 985.14). Protein content was determined by the AOAC (1980) method 2.507. Crude lipid content was determined using a solvent extractor Velp SER 148/6 (Velp Scientifica, Milano, Italy) with petroleum ether (130°C). Ash was also determined by the AOAC (1980) method 7.009. Note that ash and crude protein contents of liver were not being performed through the lack of sample.

The prepared experimental diets stored in a freezer at -20˚C until use. Ingredient and proximate composition of the experimental diets with different of GT levels and with 1% GTD level showed in Table 1.

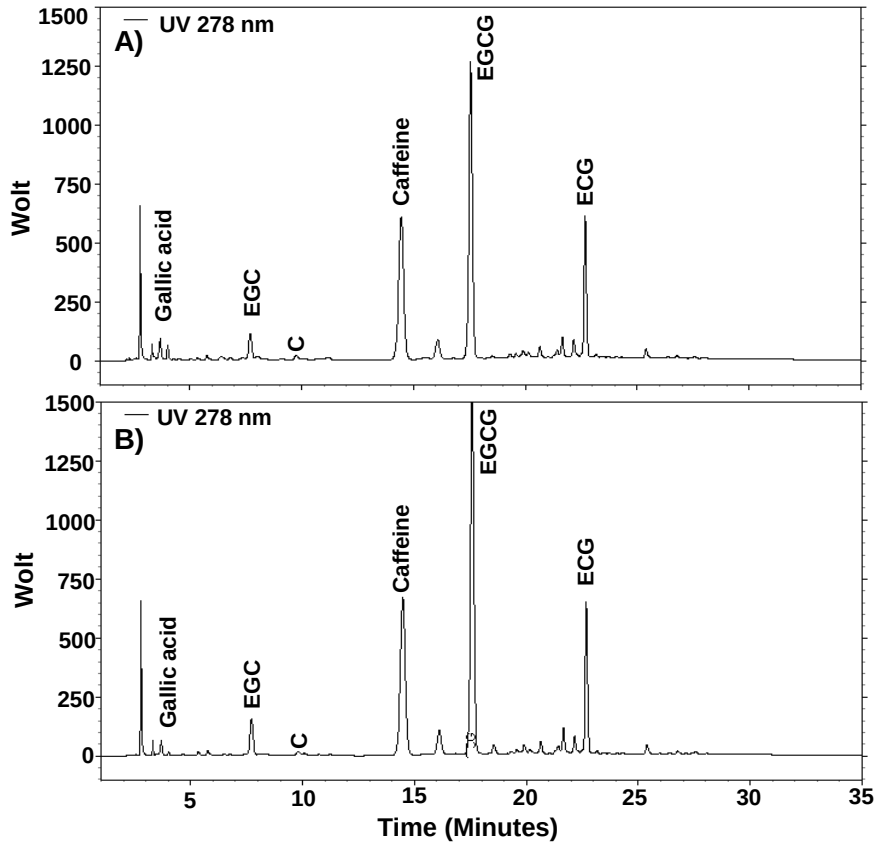

Figure 1. The ultraviolet (UV) absorption spectrum of A: green tea (GT) and B: green tea dust (GTD) phenolic and catechins content measuring the absorbance at 278 nm. EGC: Epigallocatechin, C: catechin, EGCG: epigallocatechin-3-gallate, EC: epicatechin and ECG: epicatechin gallate.

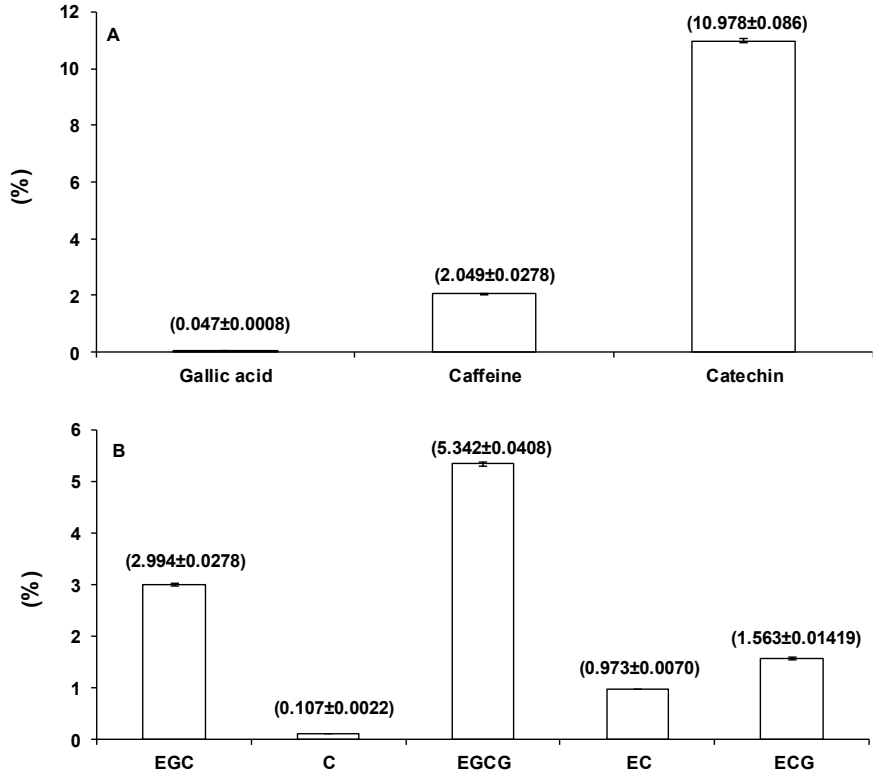

Figure 2. Components of green tea (GT) used as the diet raw material. A: The proportion of GT phenolic content (%) including gallic acid, caffeine and catechins. B: The proportion of GT catechins (%) including epigallocatechin (EGC), catechin (C), epigallocatechin-3-gallate (EGCG), epicatechin (EC) and epicatechin gallate (ECG). Data are mean±standard error (SE).

Experimental Design

After acclimation, twenty-six rainbow trouts were randomly chosen from the stock tank and than were transferred to each one of 21 fiberglass tanks containing 100 L of flow-through well water. A total of 546 rainbow trouts with an average body weight of 40.4±0.01 g were divided in the seven experimental treatments (three replicates each).

Feeding Regime, Feed Utilization and Growth Performance

The fish were fed the equivalent of 2% of their body weight, two times daily (at 0800 and 1600) for 60 days. Fish were weighed every 15 days and daily feeding rate was updated. While interim weighings were made in 15-day periods, fish were sedated with clove oil at a dose of 2-5 mg/L. The fish were fed slowly with small amounts by hand. The appetites of the fish were monitored carefully during the feeding to avoid wasting any food. At the beginning and at the end of the feeding trial, body weight (BW) and total length (TL) were measured for the fish in each tank. Interm live weight measurements of all fish were also obtained every two weeks to monitor growth and to determine the amount of feed to be fed to fish.

Specific growth rate (SGR), hepatosomatic index (HSI), condition factor (CF), feed conversion ratio (FCR), protein efficiency rate (PER), apparent net protein retention (ANPR), and protein intake (PT) were calculated as follows:

$$SGR = [(\ln \text{mean final BW} - \ln \text{mean initial BW})/(\text{time interval, days}]\times 100$$

$$HSI = [(\text{Liver weight/BW})]\times 100$$

$$CF = [(BW)/(TL)^3]\times 100$$

$$FCR = [(\text{Final BW} - \text{Initial BW})/(\text{Feed intake})]\times 100$$

$$PT = [(\text{Individual feed intake} \times \text{Feed crude protein})]/100$$

$$PER = [(\text{Final BW} - \text{Initial BW})/(\text{Protein intake})]$$

$$ANPR = [((\text{Final BW} \times \text{Final protein in fish}) - (\text{Initial BW} \times \text{Initial protein in fish}))/((\text{Total feed intake/Number of fish in treatment}) \times (\text{Protein in feed}))]\times 100$$

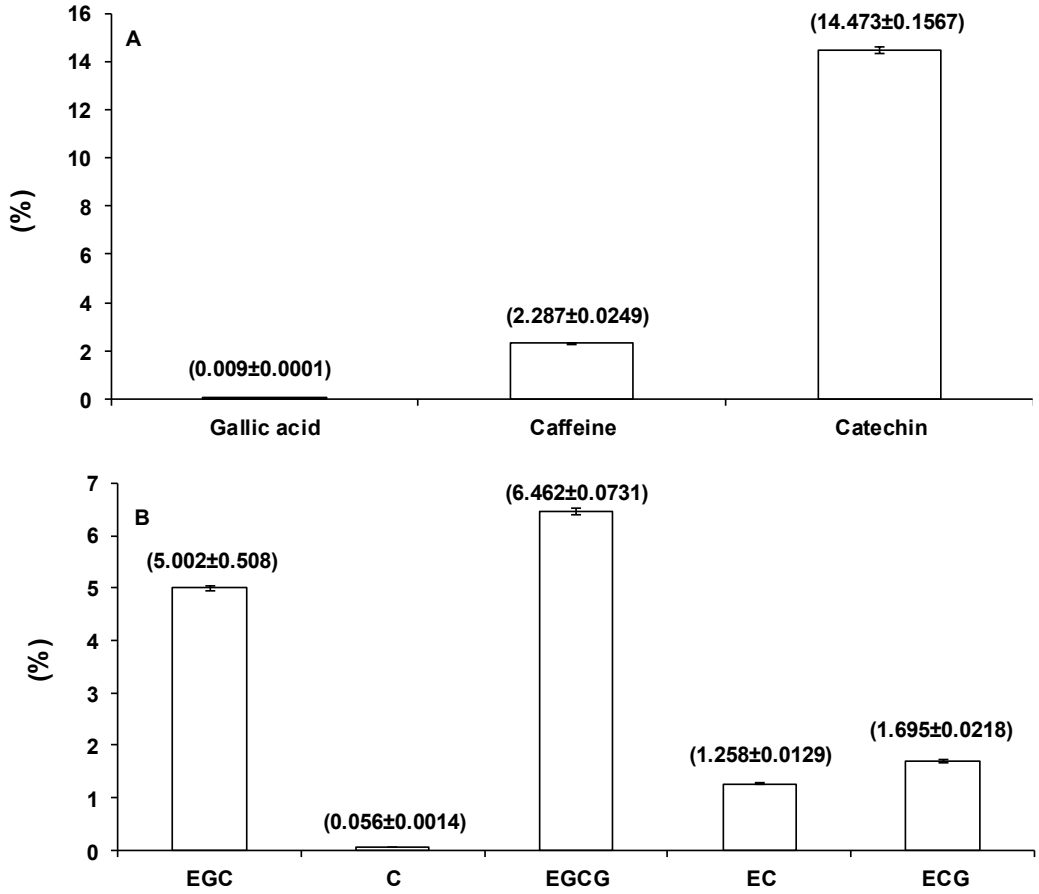

Figure 3. Components of green tea dust (GTD) produced in the tea factory used as the diet raw material. A: The proportion of GTD phenolic content (%) including gallic acid, caffeine and catechins. B: The proportion of GTD catechins (%) including epigallocatechin (EGC), catechin (C), epigallocatechin-3-gallate (EGCG), epicatechin (EC) and epicatechin gallate (ECG). Data are mean±standard error (SE).

Statistical Analysis

Experimental data were presented as mean±standard error (SE) and analyzed using one-way ANOVA followed by Tukey multiple range test to compare the means between the different experimental diet groups in PAST ver 1.75b software package (Hammer et al., 2001). Differences were considered statistically significant when P<0.05.

Results

During the feeding period, the experimental diets were equally accepted by the rainbow trouts. No apparent evidence of disease was observed and subsequently, preventive veterinary measures were not needed. The survival rate of fish feeding experimental diets without and with GT or GTD supplementation was 100%. Ingredients and proximate composition of experimental diets with various levels (0%, 0.25%, 0.50%, 1%, 2% and 3%) of GT and 1% level of GTD showed in Table 1. The chemical compositions of prepared diets were estimated as statistically similar to each other (P>0.05). The proportion of catechins (10.98±0.086%) in GT were significantly lower than the value in GTD (14.47±0.157%) (P<0.05). Moreover, the proportion of catechins (EGC, C, EGCG, EC and ECG) in dried GT used in experimental diets were significantly different than catechin proportion in GTD (P<0.05) (see: Figure 2 and Figure 3).

Growth performance and feed utilization parameters of the rainbow trout fed the experimental diets with various levels of GT and 1% level of GTD during the experimental time (60 days) presented in Table 2. GT and GTD did not exhibit any significant negative improvement in growth and feed utilization at any concentrations except for a group fed with 3% GT supplemented diet. Variance in final weight gain, SGR, HSI, FCR and PER were not effected significantly by the GTD and GT (P>0.05) except for a group fed with 3% GT supplemented diet group (P<0.05).

The mean final weight (104.49±0.443 g), SGR (1.58±0.007%), PT (31.95±0.367 g), PER (2.01±0.010%) and ANPR (36.75±0.615%) values were observed at maximum and FCR (1.12±0.006) value was observed at optimum level for a group fed with 0.5% GT supplemented diet than other groups. But, any significant differences were not observed for these parameters between control and a group fed with 0.5% GT supplemented diet (P>0.05).

The chemical composition of fillet and liver of trout fed experimental diets with different levels of GT and 1% level of GTD showed in Table 3. Crude protein and crude lipid contents in fillet and crude lipid contents in liver were significantly different between the control and all GT/GTD supplemented diet groups (P<0.05). Moreover, dry matter and ash contents in fillet and dry matter content in liver were not affected significantly by the GT/GTD experimental diets (P>0.05). At the end of the feeding trial, fillet crude protein content was increased and fillet crude lipid content was decreased, depending on the increasing of GT level in the experimental diets (Table 3). Moreover, the highest crude protein content (18.53±0.090%) and the lowest crude lipid content (2.68±0.005%) were determined in the fish fillet fed with 3% GT supplemented diet group. The lowest crude lipid content in liver was also detected in the fish fed diets containing 2% (2.06±0.031) and 3% (2.06±0.045) GT.

Table 1. Ingredient and proximate composition of experimental diets with various of green tea (GT) and with green tea dust (GTD) levels. Data for percentage of proximate composition in diets are mean±standard error (SE)

	Groups						
	GT levels						GTD level
Ingredients (%)	Control (0)	0.25%	0.5%	1%	2%	3%	1%
Fish meal	30	30	30	30	30	30	30
Green tea	-	0.25	0.5	1	2	3	-
Green tea dust	-	-	-	-	-	-	1
Bonkalit flour	18	17.75	17.5	17	16	15	17
Corn gluten meal	18	18	18	18	18	18	18
Soybean meal	20	20	20	20	20	20	20
Fish oil	12.5	12.5	12.5	12.5	12.5	12.5	12.5
Vitamin premix*	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Mineral premix**	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Molasses	0.9	0.9	0.9	0.9	0.9	0.9	0.9
Chemical composition of diets							
Dry matter (%)	93.7±0.04	93.8±0.10	90.8±0.11	93.8±0.13	94.0±0.02	95.0±0.08	93.7±0.02
Crude protein (%)	44.5±0.13	44.5±0.05	44.4±0.06	44.6±0.09	44.7±0.07	44.8±0.07	44.7±0.03
Crude lipid (%)	17.6±0.06	17.5±0.02	17.2±0.04	17.6±0.04	17.7±0.05	17.7±0.09	17.5±0.07
Ash (%)	7.4±0.03	7.3±0.05	7.3±0.01	7.4±0.13	7.4±0.03	7.4±0.03	7.7±0.02
Crude fiber (%)	1.94±0.01	2.0±0.01	2.0±0.03	2.05±0.01	2.1±0.03	2.3±0.05	2.3±0.02

*: Vitamin mix (mg/kg mix): vitamin D3, 2500000 IU; vitamin A, 12500000 IU; vitamin K3, 125000 mg; vitamin E, 200000 mg; vitamin C mono, 250000 mg; vitamin B6, 20000 mg, vitamin B2, 25000 mg; vitamin B12, 30 mg; vitamin B1, 20000 mg; niacin, 150000 mg, inositol, 250000 mg; folic acid, 8000 mg; d-bioton, 600 mg; calcium-d-pantothenate, 50000 mg; antioxidant, 150000 mg.

**: Mineral mix (mg/kg mix): Selenium, 300 mg, Mangan, 20000 mg; Cobalt, 3500 mg; Iodine, 2500 mg; Zinc, 10000 mg; Copper, 6000 mg

Table 2. Growth performance and feed utilization of the rainbow trout fed the experimental diets with various levels of green tea (GT) and green tea dust (GTD) for 60 days

Parameters	Groups						
	GT levels						GTD level
	Control (0)	0.25%	0.5%	1%	2%	3%	1%
IMBW (g)	40.47±0.043^{a}	40.37±0.141^{a}	40.43±0.041^{a}	40.38±0.050^{a}	40.49±0.045^{a}	40.41±0.054^{a}	40.47±0.030^{a}
FMBW (g)	99.27±1.545^{ac}	98.84±1.139^{ac}	**104.49±0.443**^{c}	96.02±0.706^{a}	95.33±1.109^{a}	**89.24±1.944**^{b}	95.92±0.863^{a}
SGR (%)[1]	1.50±0.028^{ab}	1.49±0.014^{ab}	1.58±0.007^{b}	1.44±0.014^{a}	1.43±0.021^{a}	1.32±0.036^{c}	1.44±0.015^{a}
HSI (%)[2]	1.63±0.024^{a}	1.55±0.075^{ab}	1.54±0.082^{ab}	1.49±0.052^{ab}	1.36±0.043^{b}	1.36±0.053^{b}	1.40±0.048^{ab}
CF (%)[3]	1.23±0.033^{ab}	1.32±0.028^{a}	1.20±0.027^{b}	1.22±0.024^{ab}	1.17±0.027^{b}	1.21±0.015^{ab}	1.17±0.023^{b}
FCR[4]	1.19±0.022^{ab}	1.17±0.011^{ab}	**1.12±0.006**^{b}	1.22±0.021^{a}	1.24±0.014^{a}	1.36±0.038^{c}	1.24±0.016^{a}
PT (g)[5]	31.06±0.306^{ab}	30.62±0.318^{ab}	**31.95±0.367**^{a}	30.33±0.190^{b}	30.47±0.291^{b}	29.76±0.399^{b}	30.65±0.146^{ab}
PER (%)[6]	1.89±0.035^{ab}	1.91±0.017^{ab}	**2.01±0.010**^{a}	1.83±0.032^{b}	1.80±0.020^{b}	1.64±0.045^{c}	1.81±0.022^{b}
ANPR (%)[7]	31.55±0.535^{d}	35.00±0.336^{ab}	**36.75±0.615**^{b}	33.72±0.881^{ad}	34.38±0.530^{abc}	32.22±0.580^{cd}	34.40±0.304^{abc}
Survival	100	100	100	100	100	100	100

Data are mean±standard error (SE). Values with different superscripts in the same line are significantly different (P<0.05). IMBW: initial mean body weight (g), FMBW: final mean body weight (g). [1]expressed as [(ln mean final BW – ln mean initial BW)/(time interval, days]x100. [2]expressed as [(liver weight/BW)]x100. [3]expressed as [(BW)/(total length)^{3}]x100. [4]expressed as [(final BW – initial BW)/(feed intake)]x100. [5]expressed as [(individual feed intake x feed crude protein)]/100. [6]expressed as [(final BW – initial BW)/(protein intake)]. [7]expressed as [((final BW x final protein in fish) – (initial BW x initial protein in fish^{+}))/((total feed intake/n*) x (protein in feed))]x100. n*: number of fish in treatment. $^{+}$expressed as value of (17.2%)

Table 3. The chemical composition of trout fillet and liver of trout fed experimental diets with different levels of green tea (GT) and green tea dust (GTD) for 60 days

Time	Groups (GT/GTD levels)	Chemical composition in fillet (%)			
		Dry matter	Crude protein	Crude lipid	Ash
IE		22.33±0.422	17.17±0.108	3.62±0.016	1.16±0.011
FE	Control	22.59±0.287^{a}	16.88±0.070^{a}	4.28±0.038^{a}	1.08±0.023^{a}
	0.25% GT	22.63±0.584^{a}	17.86±0.089^{b}	3.30±0.027^{b}	1.17±0.037^{a}
	0.5% GT	22.71±0.508^{a}	17.88±0.138^{b}	3.31±0.004^{b}	1.20±0.037^{a}
	1% GT	22.89±0.730^{a}	17.87±0.191^{b}	3.35±0.018^{b}	1.23±0.025^{a}
	2% GT	22.78±0.725^{a}	18.28±0.070^{bc}	3.28±0.027^{b}	1.22±0.031^{a}
	3% GT	22.36±0.283^{a}	18.53±0.090^{c}	2.68±0.005^{c}	1.15±0.039^{a}
	1% GTD	22.31±0.496^{a}	18.24±0.044^{b}	2.80±0.002^{c}	1.22±0.034^{a}
Time	Groups (GT/GTD levels)	Chemical composition in liver (%)			
		Dry matter	Crude protein	Crude lipid	Ash
IE		22.52±0.281	-	1.78±0.118	-
FE	Control	22.73±0.161^{a}	-	2.64±0.010^{a}	-
	0.25% GT	22.92±0.266^{a}	-	2.21±0.087^{b}	-
	0.5% GT	23.31±0.311^{a}	-	2.16±0.083^{b}	-
	1% GT	23.12±0.141^{a}	-	2.22±0.065^{b}	-
	2% GT	22.34±0.099^{a}	-	2.06±0.031^{b}	-
	3% GT	22.97±0.175^{a}	-	2.06±0.045^{b}	-
	1% GTD	22.59±0.159^{a}	-	2.15±0.065^{b}	-

IE: initial of experiment, FE: final of experiment. Data are mean±standard error (SE). Values with different superscripts in same column are significantly different (P<0.05)

Discussion

The present study results showed that administration of GT especially at 0.5% level in rainbow trout diet promoted the growth performance and fed utilization even though these parameters were not significantly affected by the experimental diets except for a diet supplemented with 3% GT level. Administration of GT in different doses in trout diets was also showed hypolipidemic effects. Comparison results of the effect of GT or GT-derived products used by experimental diets on the growth performance and feed utilization in different cultured fish species showed in Table 4. In addition that, the comparison of proximate composition of whole body, dorsal muscle and liver for different cultured fish species fed test diets concerning various levels of GT or GT-derived products showed in Table 5. Different or similar results to the present study have been previously reported regarding the influence of tea or tea-derived products on growth performance and feed utilization in different cultured fish species (Table 4). For example, dietary inclusion of GT extract was reported by Cho et al. (2007) as the most effective way to enhance growth performance and feed utilization of olive flounder, *Paralichthys olivaceus*, among the various sources of green tea. Namely, WG,

Table 4. Comparison of the effect of tea or tea-derived products used by experimental diets on the growth performance and feed utilization in different cultured fish species

					Growth performance				Feed utilization			
Species	Tea-derived products	Diet levels	Time(days)	InitialWeight	WG	SGR	CF	HSI	FCR	FER	PER	Ref.
Paralichthys olivaceus	GT	5%	49	52.5 g	↓	↓	=	↓	↓	↓	↓	[1]
	Dry GT	5%			↓	↓	=	↓	=	=	=	
	By-product GT	5%			↓	↓	↓	↓	↓	↓	↓	
	GTE	5%			=	=	=	↓	=	=	=	
Paralichthys olivaceus	GTE	1%	56	12.9 g	=	=				=	=	[2]
	By-product GT	1%			=	↓				=	=	
Oreochromis niloticus	GT	0.0125%	84	1.2 – 2 g	=	=			=		=	[3]
		0.025%			↑	↑			=		↑	
		0.05%			↑	↑			↓		↑	
		0.1%			=	=			=		=	
		0.2%			=	=			=		=	
Oncorhynchus mykiss	EGCG	0.002%	48	145±3.8 g	=	=		=	=			[4]
Oncorhynchus mykiss	GT	0.002%	35	23.5±2.6 g	=	=			=			[5]
		0.01%			=	=			=			
		0.05%			=	=			=			
Sebastes schlegeli	GTE	1%	56	8.1±2 g	=	=	↓	=		=	=	[6]
		3%			=	=	↓	↓		=	=	
		5%			↓	↓	↓	↓		↓	=	
Sparus aurata	WT	2.9%	30	35 g	↓	↓		↓		=	=	[7]
Seriola quinqueradiata	GT	3.6%	56	400 g	↓		=			↓		[8]
	GTE	0.7%			↓		=			↓		
Plecoglossus altivelis	GT	1%	36	33.9 g	↓		=			↓		
	GTE	5%			↓					↓		
Oncorhynchus kisutch	GT polyphenol	0.005%	70	180.6±0.20 g	↑	↑	↓	↑	↓			[9]
		0.01%		180.3±0.13 g	↑	↑	↓	↑	↓			
		0.02%		180.5±0.2 g	↑	↑	↑	=	↓			
		0.04%		180.7±0.05 g	↑	↑	↓	↑	↓			
Oncorhynchus mykiss	GT	1%	60	34.4±1.5 g	=			↓	=		=	[10]
		2%			=			↓	=		=	
		4%			↓			↓	↑		↓	
Oncorhynchus mykiss	GT	0.25%	60	40.37±0.141 g	=	=	=	=	=		=	PS
		0.5%		40.43±0.041 g	=	=	=	=	=		=	
		1%		40.38±0.050 g	=	=	=	=	=		=	
		2%		40.49±0.045 g	=	=	=	↓	=		=	
		3%		40.41±0.054 g	↓	↓	=	↓	↑		↓	
	GTD	1%		40.47±0.030 g	=	=	=	=	=		=	

GT: green tea, EGCG: epigallocatechin-3-gallate, GTE: green tea extract, GTD: green tea dust; WT: white tea. WG: weigth gain; SGR: Specific growth rate; CF: condition factor; HSI: hepatosomatic index; FCR: feed conversion ratio; FER: protein efficiency rate; PER: protein efficiency rate. [1]: Cho *et al.* (2007); [2]: Cho and Kim (2009); [3]: Abdel-Tawwab *et al.* (2010); [4]: Thawonsuwan *et al*. (2010); [5]: Nootash *et al.* (2013); [6]: Hwang *et al*. (2013); [7]: Pérez-Jiménez *et al.* (2013); [8]: Kono *et al*. (2000). [9]: Yu *et al*. (2024); [10]: Welker *et al*., (2017); PS: present study; Statistical variations in treated fish groups compared to control groups; [↑]: significant increase, [↓]: significant decrease, [=]: no significant changes.

SGR, FCR, FER and PER values of *P*. *olivaceus* (mean 52.5 g) fed the control diet and experimental diet containing 5% level GT extract were higher than those of juvenile *P*. *olivaceus* fed the diets containing various sources of GT (raw GT 5% and by-product GT 5%) for 7 weeks. However, GT extract and by-product GT at level 1% did not change the WG, FER and PER values of *P*. *olivaceus* (mean 12.9 g) during the 8 weeks (Cho and Kim 2009). In other studies, the effect of different levels of dietary GT ethanol extracts (1%, 3% and 5%) supplementation on juvenile black rockfish, *Sebastes schlegeli*, (mean 8.1±2 g) diet showed that WG, SGR, FER and PER were not negatively effected at lower doses (1% and 3%) (Hwang et al., 2013). But WG, SGR, CF, HSI and FER values for *S. schlegeli* were lower than control group at higher dose level (5%) (Hwang et al., 2013).

Pérez-Jiménez et al. (2013) reported that WG, SGR and HSI negatively changed but FER and PER parameters were not affected when *Sparus aurata* (mean 36 g) fed containing 2.9% white tea for 30 days. The an other cultured fish species, *Oreochromis niloticus*, (initial mean weight: 1.1-2 g) fed different levels (0.0125%, 0.025%, 0.05%, 0.1% and 0.2%) of GT during the 84 days and WG, SGR and PER values were improved at 0.025% and 0.05% levels than others (Abdel-Tawwab et al., 2010). Conversely, Nootash et al. (2013) reported that WG, SGR and FCR values of *O. mykiss* (mean 23.5±2.6 g) fed the control diet and experimental diet containing 0.002%, 0.01% and 0.05% levels GT did not change for 35 days. Similarly heavier *O. mykiss* (mean 145±3.8 g) fed containing 0.002% epigallocatechin-3-gallate were not affected in respect to growth performance and feed

Table 5. Comparison of proximate composition of whole body, dorsal muscle and liver in different cultured fish species fed test diets with various level of tea or tea-derived products

Species		Diet levels	Time (days)	Initial Weight (g)	Proximate composition												Ref.
					Whole body				Dorsal muscle				Liver				
					M	CP	CL	Ash	M	CP	CL	Ash	M	CP	CL	Ash	
Sebastes schlegeli	GTE	1%	56	8.1±2	=	=	=	=	=	=	=	=	=	=	=	=	[1]
		3%			=	=	=	=	=	=	=	=	=	=	↓	=	
		5%			=	=	↓	↑	=	=	=	=	=	=	=	=	
Sparus aurata	WT	2.90%	30	35	=	=	↓	=							↓		[2]
Oreochromis niloticus	GT	0.0125%	84	1.2 – 2	=	↑	=	↓									[3]
		0.0025%			=	↑	=	↓									
		0.0500%			=	↑	=	↓									
		0.1000%			=	↑	↑	↓									
		0.2000%			=	↑	↑	↓									
Paralichthys olivaceus	Raw GT	5%	49	52.5	↑	=	=	=					↑	↑	↓		[4]
	Dry GT	5%			↑	=	=	=					↑	↑	↓		
	By-product GT	5%			↑	=	=	=					↑	↑	↓		
	GT Extract	0.05			=	=	=	=					=	↑	=		
Paralichthys olivaceus	GTE	1%	56	12.9									=	=	=		[5]
	By-product GT	1%											↑	=	↓		
Seriola quinqueradiata	GTE	0.2%	28						=	=	=	=					[6]
		0.02%							=	=	=	=					
Oncorhynchus kisutch	GT polyphenol	0.005%	70	180.6±0.20 g	=	=	=	↓									[7]
		0.01%		180.3±0.13 g	=	↓	↓	↓									
		0.02%		180.5±0.2 g	=	=	=	↓									
		0.04%		180.7±0.05 g	=	=	=	↓									
Oncorhynchus mykiss	GT	1%	60	34.4±1.5 g	=		↓										[8]
		2%			=		↓										
		4%			=		↓										
Oncorhynchus mykiss	GT	0.25%	60	40.37±0.141 g	=	↑	↓	=					=		↓		PS
		0.5%		40.43±0.041 g	=	↑	↓	=					=		↓		
		1%		40.38±0.050 g	=	↑	↓	=					=		↓		
		2%		40.49±0.045 g	=	↑	↓	=					=		↓		
		3%		40.41±0.054 g	=	↑	↓	=					=		↓		
	GTD	1%		40.47±0.030 g	=	↑	↓	=					=		↓		

M: Moisture; CP: Crude protein; CL: Crude lipid; GT: green tea; GTE: green tea extract; GTD: green tea dust; WT: white tea. [1]: Hwang *et al*. (2013); [2]: Pérez-Jiménez *et al*. (2013); [3]: Abdel-Tawwab *et al*. (2010); [4]: Cho *et al*. (2007); [5]: Cho and Kim (2009); [6]: Ishihara *et al*. (2002); [7]: Yu *et al*. (2024); [8]: Welker *et al*., (2017); [PS]: present study.
Statistical variations in treated fish groups compared to control groups; [↑]: significant increase, [↓]: significant decrease, [=]: no significant changes.

utilization (Thawonsuwan et al., 2010). Administration of green tea polyphenols in the cultured yellowtail, *Seriola quinqueradiata*, diets (at 0.02% and 0.2% levels) for 4 weeks did not change the growth performance, feed utilization and fish body components (Ishiara et al., 2002). Growth performance (WG) and feed utilization (FER) parameters were also negatively effected for two cultured fish species, *Seriola quinqueradiata* (mean 400 g) fed diets containing 3.6% GT, 0.7% GT extract for 56 days, and *Plecoglossus altivelis* (mean 36 g) fed diets containing 1% GT and 5% GT extract for 36 day (Kono et al., 2000). Kono et al (2000) used GT and GT extract as a raw materials in the experimental diets concerning 3.6% GT (total catechin 6.3% and epigallocatechin gallate 2.85%) and 0.7% GT extract (total catechin 31% and epigallocatechin gallate 17%) in the fish diets. Welker et al. (2017) reported that green tea had a growth promoting effect on *O. mykiss* (34.4±1.5 g) and that adding a lower dose of 1% to 2% of green tea (total catechin 1.7-3.9% and epigallocatechin gallate 0.4-1.01%) to the diet allowed for the most effective growth and feed utilization. However, the addition of high doses (4%) of green tea to the feed reduces the growth performance of *O. mykiis*. Our conclusions are consistent with the results obtained by Welker et al. (2017) (see: Table 4).

The safety and efficiency of GT and GTD supplementation in rainbow trout diets were also evaluated by lipid profiles of fish fillet and liver by crude lipid analyzis. GT and GTD supplementation in the experimental diets effect on fish hematology and biochemistriy including blood cholesterol levels (hypolipidemic effect) will be presented and discussed in another study. Our results based on proximately analyzis for liver and fish fillet showed that administration of 1% level of GTD and different doses of GT especially at higher doses (e.g. 3% GT) in the experimental diets showed markedly lipid-lowering effect for rainbow trout. Similar results have been previously reported regarding the liver and body fat reduction effect for different farmed fish species (Table 5). For example, effect of reducing the body and crude liver lipid contents was observed for *O. mykiss* fed with a diet concerning 1%, 2% and 4% levels of GT (Welker et al., 2017), for *S. schlegeli* fed with a diet concerning 3% and 5% levels of GTE (Hwang et al., 2013), for *S. aurata* fed with a diet concerning 2.9% level of WTL (Pérez-Jiménez et al., 2013), for *P. olivaceus* fed a diet concerning various sources of GT at 5% level (Cho et al., 2007) and at 1% by-product GT (Cho and Kim, 2009). Lipid-lowering and/or hypolipidemic effect of tea was also observed different animal species such as rats after long-term feeding (27 and 63 weeks) due to contents of tea polyphenols (Kuo et al. 2005) and green tea leaves (Lin et al., 1998).

Among factors that may influence the effectiveness of plant products (considering GT) in fish, their dose and the duration of administration are crucial (Bulfon et al. 2015). In the present study, the proportion of catechins (10.98±0.086%) in dried GT were lower than the GTD (14.47±0.157%) and also the proportion of catechins (EGC, C, EGCG, EC and ECG) in dried GT used in experimental diets were significantly different than GTD ($P<0.05$). The polyphenols are the most significant group of tea components, especially certain catechins (major green tea catechins: EGCG, EGC, ECG, EC and C), which they are the main compounds in green tea (Erturk et al., 2010; Mahmood et al., 2010; Tounekti et al., 2013). The effect of green tea catechins, EGCG, on growth performance were reported as dose depended for mammal such as rats (Kao et al. 2000; Lin and Lin-Shiau, 2006). Namely, the effect of EGCG (26 and 53 mg/kg BW EGCG) were not effective or were less effective in reducing the body weight than 85 mg/kg BW (Kao et al. 2000).

The studies, mentioned above except for Kono et al. (2000) and Welker et al. (2017), relating to the use of GT in aquaculture do not provide any data about the chemical composition of the tested GT or GT-derived products such as extracts, polyphenols, catechins, EGCG. Amount of polyphenols and also catechins contents in the GT affected by variety of tea species, different harvest times or seasons, the age of the leaf (plucking position), geographical origin and climatic factors, growth altitude, horticultural practices etc. (Lin et al., 1996; Turkmen and Velioğlu, 2007; Erturk et al., 2010; Tounekti et al., 2013). Despite the use at the same rate of GT or GT products (e.g. EGCG) in the experimental diets, the content of polyphenols and also catechins of tea may be different due to most of above mentioned factors affecting GT polyphenol content. For that reason, total polyphenols and also catechin contents including major catechins of GT or GT products used in the ingredient of experimental diets should be given to compare of the results of the different studies. Moreover, factors influencing the effectiveness of plants and plant-derived products in cultured such as GT and GT extract were reported as concentration and duration of administration, bioactive compounds contained in plants and plant extracts, solvent used for plant extraction and also synergism or antagonism between plants (Bulfon et al., 2015). As seen the previous studies results mentioned in above and summarized in Table 4 and Table 5, GT and tea-derived products, applied doses and time, fish species, fish life stage and initial body weight can be considered as important parameters to obtained reliable results to suggest in application green tea or tea derived products supplementation in aquaculture.

The present study results primarily revealed that the use of GT as a feed additive for rainbow trout could be recommended to stimulate growth and feed utilization. Improved diet utilization efficiency was seen in rainbow trout (initial body weight 40.4±0.01 g) fed diets containing at least 0.5% GT for 60 days.

Our study did not explore the potential growth effect of dietary green tea at different life stage, which may be a useful application of green tea in aquaculture.

Further research is needed to detect the mode of action of GT or GT extract (especially the most important antioxidant catechin, epigallocatechin gallate-3-gallate) doses at different or all stages of life in farmed fish species, especially rainbow trout. Moreover, research on mode of action, stability of plant materials such as GT in aquatic environment and digestibility in farmed fish species as well as in vitro and in vivo toxicological tests are prerequisites for their safe application (Bulfon et al. 2015).

Conclusion

According to the results of this study, it can be concluded that dietary Inclusion of green tea (*C. sinensis*) and dust, as a factory by-product green tea waste, to rainbow trout feeds promote the growth performance and feed utilization, and also application of 1% dust and green tea (e.g. up to 3%) in diets have lipid-lowering effects for rainbow trout. That is, green tea and dust can be used as a raw material for rainbow trout feeds. The recommended dietary inclusion level of green tea in rainbow trout feed is 0.5%. However, conducting further research that also takes fish size (e.g. >1.25 kg, known as Turkish somon in Türkiye) into account would be beneficial in order to more definitively demonstrate the advantages of adding green tea or its waste, referred to as 'dust', to rainbow trout feed.

Ethical Statement

All process and experimental protocols of this study have been checked and approved by Recep Tayyip Erdogan University Animal Experiments Local Ethics Committee (Decision No: 2014/14 Date: 21.02.2014). 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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Author Contribution

First Author (OB): writing – original draft, conceptualization, data curation, visualization, methodology. Second Author (HAK): supervision, conceptualization, review and editing, validation. Third Author (SUT): supervision, conceptualization, review and editing, validation.

Conflict of Interest

The authors declare that they have no known competing financial or non-financial, professional, or personal conflicts that could have appeared to influence the work reported in this paper.

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