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Effects of Green Tea Extracts on freshwater angelfish, *Pterophyllum scalare* Growth Performance.

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ABSTRACT

The study was conducted to investigate the effects of dietary green tea extract (GTE) supplementation in diet on growth performance, feed utilization and biometric indexes in freshwater angelfish, *Pterophyllum scalare*. The fish (mean body weight, 2.61±0.01 g) were fed fish meal diets that included 0% (control), 2.5 and 5% GTE for 8 weeks. The results showed, the addition of green tea extract did not have a positive effect on growth performance and other variables. Addition of green tea extract did not change the amount of viscerosomatic index and hepatosomatic index.

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Introduction

Ornamental fish farming is an important primary industry (Lim & Wong, 1997). The freshwater angelfish (*Pterophyllum scalare*) is one of the South American cichlids that originate from the Guyana, the Orinoco and the Amazon River basins (Ortega-Salas, 2009).

Like other aquarium fish market, producers of the freshwater angelfish also prefer live feed such as artemia, tubifex, and daphnia in grower feed. When both production

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techniques and storage opportunities are considered, the production of live feed is much harder and more difficult compared to dry fodders. For this reason, it is highly important to search the ingredients of feeds that can provide nutritional requirements of aquarium fishes and enable the optimum growth (Sales & Janssens, 2003). Feeds containing herbal matters improve growth performance are also natural, and easy and cheap to supply (Harikrishnan et al., 2011). Plants' role in improving immune system can be related to the components they contain. Catechin compound contained by green tea, showed significant antioxidant features. Additionally, it has been reported that they can provide improvement in feed conversion ratio and provide high protein synthesis by improving digestibility of plant extracts and availability of nutrition (Reverter et

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al., 2014).

It has been stated that green tea decreases specific growth rate in *Paralichthys olivaceus and Sebastes schlegeli* fishes (Cho et al., 2007; Hwang et al., 2013). The aim of this study is to determine the dosage of green tea extract, which is necessary to support the growth of angelfish, and to search its effects on the growth performance and survival rate.

Material and Methods

The experiment was set up in Sera Aquarium in Aydın city, Turkey. Feed analyzes were made in the Tarbiyomer unit of Adnan Menderes University. In this experiment, 9 aquariums (65×30×40 cm) and 180 P. scalare (mean body weight 2.61±0.01 g) were used. Each trial group was carried out with triplicate. Green tea extracts (Talya herbal product) were added to a commercial feed (Gemma chichlid granul green, Turkey). Degani (1993) stated that the feed that will provide optimum growth for angelfish should include 44% crude protein and 10% raw oil. The herbs extracts were added to the feed at 2.5% (Group GTE1) - 5% (Group GTE2). The addition of extract to the feed was carried out with alcohol via spraying method. With the aim of protecting the activities of the components in plant extracts, feeds were prepared on a weekly basis and stored in glass flacons at +4°C. Additionally, a control group was fed a diet without herbal extract supplementation (Sonmez et al., 2015). Fishes were fed two times a day with the feeds containing green tea extract for period of eight weeks. Water temperature, dissolved oxygen and pH were measured 26°C, 7.02 mg·L⁻¹, 7.01, respectively.

Proximate analyses of commercial feed were performed using standard methods (Latimer, 2016). Moisture was detected after drying at 105° C until a constant weight was achieved. Crude protein was analyzed by the Kjeldahl method, and crude ash by incineration at 525° C in a muffle furnace for 12 h. Crude fat was analyzed by methanol/chloroform extraction (Folch et al., 1957) (Table 1).

Table 1. Commercial fish granule feed ingredients.

Parameters	Values (%)			
Proximate analyses				
Crude Protein	56			
Crude Lipid	10			
Crude Cellulose	0.5			
Crude Ash	10			
Macro elements				
Total Phosphor	1.5			

Note: Ingredients: Fish meal processed under low temperature, crustacean fish meal, fish oil, cereal seeds gluten, cornstarch, yeast, garlic, soy lecithin, algae, herbal products, vitamins and minerals.

Growth Performance

Growth performance and feed utilization were calculated according to the formulae given below:

$$SGR = \frac{ln final weight - ln initial weight}{days} \times 100$$
$$FCR = \frac{feed intake}{weight gain}$$

Biometric indices were calculated from the following formulae:

HSI= wet weight of liver wet body weight - wet weight of liver

VSI= wet weight of viscera and associated fat wet body weight - wet weight of viscera and associated fat

In these formulae; WG indicates weight gain, SGR indicates specific growth rate, FCR indicates feed conversion ratio, HSI indicates hepatosomatic index, VSI indicates viscerosomatic index, BSI indicates Bilesomatic index.

Statistical Analysis

Each value was expressed as mean \pm standard error of mean (SEM). Statistical significance was determined by oneway analysis of variance (ANOVA) followed by a DUNCAN multi comparison test with SPSS 21.0 package software. Statistical significance was established at *P*<0.05.

Results

There were not any significant differences of weight increase, feed conversation ratio and specific growth rate between the experimental group and the control group (Table 2, P>0.05).

Table 2. Growth performance of *P. scalare* fed the test diets with various levels of GTE for 8 weeks (n=6).

Parameters	Control	GTE1	GTE2
Initial fish weight (g)	2.60±0.024	2.61±0.002	2.63±0.005
Final fish weight (g)	3.17±0.020	3.19±0.038	3.21±0.023
Weight gain (%)	11,39±0.712	11.63±0.800	11.55±0.356
FCR	1.02±0.008	1.01±0.016	1.01±0.002
SGR (%/d)	0.11±0.007	0.12±0.007	0.11±0.003

There were not any significant differences for crude protein, crude lipid, crude ash and crude moisture between

the experimental group and the control group (Table 3, P>0.05).

At the end of 8 weeks, VSI and HSI values were found lower in the group fed with green tea extract compared to the control group but the values were not statistically significant (Table 4, P>0.05).

Table 3. Whole-body proximate composition (%) of *P.* scalare fed diets with different levels of green tea extract for 8 weeks. (n=6).

Composition (%)	Control	GTE1	GTE2
Crude Protein	17.68±0.82	17.98±0.60	17.99±0.69
Crude Lipid	5.32±0.76	4.94±0.15	5.05±0.27
Crude Ash	2.47±0.31	2.58±0.26	2.33±0.38
Moisture	74.40±0.17	74.95±0.79	74.18±0.95

Table 4. Viscerosomatic index (VSI) and Hepatosomatic index (HSI) of *P. scalare* fed different diets containing green tea extract for 8 weeks.

Index	Control	GTE1	GTE2
VSI	8.19±0.56	7.62±0.62	7.75±1.14
HSI	2.20±0.22	2.05±0.19	2.03±0.06

Discussion

In recent years, in order to increase the growth of fishes and to protect fishes from illnesses, the use of natural products as alternatives to synthetic chemicals and antibiotics is researched and studied (Csép et al., 2010). With this aim, the usability of aromatic plants, herbs, spices, and plant extracts -known to be beneficial for human health- in aquaculture is emphasised. Plant extracts are begun to be widely used in animals, as well as in humans (Gabor et al., 2010).

In our study, it was seen that the addition of green tea extract to *P. scalare* feeds did not positively affect growth parameters. Similarly, Cho et al. (2007) stated that there was no positive effect of green tea extract on growth parameters in juvenile olive flounder, *Paralichthys olivaceus*. Zhou et al. (2016) also reported no change in growth parameters of *Ctenopharyngodon idellus* that was fed with a diet containing 5% green tea.

Biometric indices may increase or decrease due to factors such as unhealthy conditions (Hadidi et al., 2008), feeding levels, or feeding ration (Company et al., 1999). However, in the present study, no mortality or disease in fish from any of the treatments were observed.

It can be said that with the decrease in HSI amount, liver can continue to function in a healthier way. In this study, it has been observed that VSI and HSI values of the fishes fed with green tea extract decreased compared to the control group; however, there was not any statistically significant difference. It was reported in a study conducted among *Paralichthys olivaceus* that a type of green tea (*Camellia sinensis*) decreased HSI amounts (Cho et al., 2007). In the same study, it was reported *Ctenopharyngodon idellus*, a type of green tea and green tea waste (*Camellia sinensis*), decreased HSI amounts (Zhou et al., 2016).

In a study carried out among *Sebastes schlegeli* fishes, it has been reported that green tea extract decreased HSI and VSI amounts (Hwang et al., 2013). In the same study, the decrease in HSI rate in parallel with VSI amount may result from green tea's effect of decreasing fat in liver. Also, it can be said that with the decrease in HSI amount, liver can continue to function in a healthier way.

Conclusion

In accordance with the results acquired from the study, it has been found that green tea extract does not have any negative effects on the metabolism of angelfish. In future studies, the effects of green tea extract on different sizes of angelfish can be investigated. The use of this plant in feeds of other aquarium fishes can be searched. Green tea is a widely found herbal resource in Turkey. Therefore, it is easy and cheap to find. In addition to this, considering the positive results acquired from angelfish, as well as from many animals and human, green tea extract can be used by feed companies.

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