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Influence of the food supplement yellow gentian (*Gentiana lutea)* on certain growth indicators, blood parameters and quality of meat in the aquaponic cultivation of rainbow trout (*Oncorhynchus mykiss* W.)

St. N. Stoyanova

Trakia University, Faculty of Agriculture, Department of Biology and Aquaculture, 6000 Stara Zagora, Bulgaria
Corresponding author: st_stoyanova@mail.bg

Abstract

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The use of herbs as supplements in fish feed has shown a stimulating effect on fish growth, utilization of feed, and an increase in digestibility and in fish immunity. The aim of this study is to determine the influence of the supplement yellow gentian (*Gentiana lutea*) added in fish feed on the growth indicators, blood parameters, meat quality and forage utilization of rainbow trout (*Oncorhynchus mykiss* W.), cultivated in an aquaponic system. The experimental period lasted 60 days. At the end of the experiment, the following were calculated: average final weight, specific growth rates, feed utilization rate, meat quality and biochemical blood parameters. The trout fed on feed supplemented with yellow gentian, showed a higher final weight of 4.46%, compared to control group fish fed without it, but the differences between the groups were unreliable ($P > 0.05$). The quality parameters of the meat in the experimental group were not significantly affected by the addition of yellow gentian to the feed and were similar to the results obtained in the control group fish. The addition of yellow gentian to the diet of the rainbow trout affects the biochemical parameters of blood (creatinine, ASAT), the quality of meat (raw protein). Better values in the measurement of trout in the group fed with yellow gentian were reported with an average individual fish weight and a feed conversion factor of 34.91% and 23.03% respectively, compared to control fish.

Keywords: aquaponic system; biochemical parameters of blood; rainbow trout; yellow tint; growth; meat quality
Abbreviations: AAS: atomic absorption spectrometer; ALB: albumin; ALAT: alanine aminotransferase; ASAT: aspartate aminotransferase; C: control group; Ca: calcium; CHOL: cholesterol; CREA: creatinine; D: experimental group; FCR: food conversion ratio; GLU: glucose; Mg: magnesium; P phosphorus; SGR: specific growth rate; TP: total protein; TG: triglyceride; UREA: urea; WG: weight gain

Introduction

The use of recirculation and aquaponic systems in aquaculture ensures compliance with technological parameters and protects fish from the harmful effects of a polluted environment (Valkova et al., 2016; Tzanova et al., 2017). It also ensures the uptake of the beneficial nutritional supplements by the fish introduced through the feed into the system (Staykov & Sirakov, 2018). On the other hand, the use of antibiotics in aquaculture leads to the development of resistant bacteria and to the release of harmful substances into the environment (Baquero et al., 2008). Due to the prohibition of antibiotics use in animal feed by the European Union in 2006, various types of plant supplements have been added to the fodder in recent years (Rochfort et al., 2008; Abdel-Tawwab, 2012; Ogundari, 2012; Doan et al., 2017).

Immunostimulants are increasingly used in the feed industry (Galeoti, 1998; Best, 2002; Li & Gatlin, 2004; Barry & Costa–Pierce, 2010). As a result of the introduction of various types of herbs – garlic, onions, oregano, cumin, basil, anise, dill, licorice, fenugreek, thyme, paprika, cinnamon, etc. – to fish feed, positive results have been established in the growth, feeding coefficient and better uptake of feed (Citarasu et al., 2003; Sivaram et al., 2004; El-Dakar et al., 2007; Sahu et al., 2007; Metwally, 2009; Aly & Mohamed, 2010; Citarasu, 2010; Gabor et al., 2011; Harikrishnan et al., 2011; Saleh et al., 2015; Yilmaz et al., 2012; Yilmaz et al., 2013; Xia et al., 2015). Studies in aquaculture conducted in this area confirm that the use of natural food supplements for humans added to fish food boost the resistance to a number of diseases (Fernandez – Navarro et al., 2006; Ahmad et al., 2011; Setiawati et al., 2016; Staykov & Sirakov, 2018; Koshinski et al., 2018). Extracts of dietary supplements in animal feed reduce total cholesterol levels, lowering LDL cholesterol, increasing HDL cholesterol levels and lowering triglyceride levels (Azima, 2004).

Yellow gentian (*Gentiana lutea*) of the *Gentianaceae* family is a medicinal plant, used to improve the general condition of the digestive tract and the physiological state in human and animal organisms in pharmacology (Sirakov & Sirakov, 2018; Sirakov et al., 2019; Cvetković et al., 2020). The supplement is intended for use in feeding all animal species. According to studies, a maximum concentration of 50 mg of yellow gentian extract/kg is recommended, except in horses, where 200 mg/kg (Bampidis et al., 2021) of whole fodder is recommended. The aim of this study is to establish the effect of feed supplemented by yellow gentian (*Gentiana lutea*) on growth intensity, blood parameters and quality of rainbow trout meat (*Oncorhynchus mykiss* W.), cultivated in the aquaponic system. Information related to the effect of the addition of yellow gentian to the fish feed at this stage is insufficient.

Materials and Methods

Fish and feed

The experimental fish (*Oncorhynchus mykiss* W.) were transported from Bukovets Trout Farm, Tvarditsa to the Experimental Aquaculture Training Base of Trakia University, Stara Zagora. For this study, ten fish were selected in good health and placed in each tank of the aquaponic system divided into two experimental groups – D and C. In experimental group D, the fish were fed with feed supplemented with yellow gentian (*Gentiana lutea*) at a concentration of 6 g/kg feed, while the fish in control group C were fed feed without the plant additive. Sunflower oil in the amount of 5 ml was added to every 100 g of experimental food and the components were mixed very well. The same amount of sunflower oil was added to the control feed and also mixed. Both tested feed types were then left for 12 h at an air temperature of 20°C and used for experimental fish feeding. The experiment was conducted in two repetitions for each variant. The average initial weight of trout in experimental groups D was 107 ± 3.39 g and C, 114.45 ± 2.72 g. The fish were fed with extruded pellets with 45% raw protein produced by "Aqua garant", with a pellet size of 6 mm. The nutritional content of the pelleted trout feed (*Oncorhynchus mykiss* W.) was as follows:

CF (control feed) – Crude protein – 45%; Crude lipids – 16%; Crude fiber – 2.00%; P – 1.00; Vitamin A – 10000 IU/kg; Vitamin D_3 – 1500 IU/kg^{-1}; Vitamin E – 200 mg

DF (experimental, *Gentiana lutea*) – Crude protein – 45%; Crude lipids – 16%; Crude fiber – 2.00%; P – 1.00%; *Gentiana lutea* – 6 g/kg; Vitamin A – 10 000 IU/kg; Vitamin D_3 – 1500 IU/kg, Vitamin E – 200 mg

The daily ration amounted to 1.8% of the total biomass of trout. Food was delivered manually three times a day. The duration of the experiment was 60 days.

Aquaponic system

The aquaponic recirculating system consists of a hydrobiont cultivation system containing 10 tanks, a mechanical (sedimentation tank), a biological filter (a biofilter with a removable layer) and a collection vessel connected to an initial waste pump 14.9 (Wilo®). The volume of each fish tank is 300 L, measuring 1/0.5/0.25 m. The mechanical filter (sedimentation tank) is filled with plastic slats that reduce water speed and help to sink waste particles. The biofilter with a removable layer has 50 kg of filling (plastic rings). The volume of the mechanical and biological filter and the collection vessel is 1 m^3 (each). The flow rate in the recirculating system is maintained at 50 l/min. Fish cultivation vessels and the biological filter are aerated with two aerators. During the test period, the hydrochemical performance values in experimental fish were within optimal limits for the cultivated species, the limit values for the cultivation of rainbow trout during the experiment were maintained by automated mode in the aquaponic system. Water temperature in the tanks was optimized using two heaters with a power of 2000 W, immersed in the collection vessel connected to a temperature probe controller.

Fish growth performance

These mortality cases were recorded daily during the trial and survival was calculated using the following formula:

$$\text{Survival (\%)} = (\text{final of fish}/\text{initial number of fish}) \times 100$$

At the start and the end of the trial the experimental fish were weighed at the technical balance with accuracy of 0.01 g. The average individual weight gain (WG) (g), specific growth rate (SGR) (% of body weight gain/day) and FCR were determined with the following equations:

WG (g) = Average final weight – Average initial weight;

SGR (% body wt gain/day) = [Ln final weight (g) – Ln initial weight (g)] / number or days × 100;

FCR = Fed feed (g) / Weight gain of fish (g).

Biochemical blood parameters

The blood was taken directly from the hearts of the examined fish (6 specimens from experimental variant with disposable sterile plastic syringes (3 mL) with a needle. Heparin sodium (1%) was used as an anticoagulant. Half of the blood was centrifuged at 3.000 rpm to separate the plasma in order to determine the biochemical parameters, while the remaining blood was used for hematological analysis. Glucose (GLU) (mmol/L), urea (UREA) (mmol/L), creatinine (CREA) (µmol/L), total protein (TP) (g/L), albumin (ALB) (g/L), alanine aminotransferase (ALAT)(U/L), aspartate aminotransferase (ASAT) (U/L^{-1}), the content of calcium (Ca) (mmol/L), phosphorus (P) (mmol/L), magnesium (Mg) (mmol/L), as well as triglyceride (TG) ($mmol/L^{-1}$) and cholesterol (CHO) ($mmol/L^{-1}$) in blood plasma were examined by the colorimetric method with blood analyzer (Mindray SC – 120).

Chemical analysis of meat samples

Muscle samples were taken from six experimental fish from each variant. The samples were homogenized and prepared for analysis at the end of the study. The muscle samples of rainbow trout were determined on the atomic absorption spectrometer (AAS) "A Analyst 800"-PerkinElmer in the Research Laboratory of TRU, Stara Zagora by the following methods:

- Moisture (%) and dry matter (%) – Bulgarian State Standard (BSS- 11374-86);
- The crude protein content (%) was calculated by converting the nitrogen content quantified by the Kjeldahl method using the Kjeldahl automatic system (Kjeltec 8400, FOSS, Sweden) (BSS-ISO 5983);
- Fat content, % (determined by the Soxhlet method using Soxtec 2050 automatic system, FOSS, Sweden) (BSS-ISO 6492);
- The ash content (%) was examined by burning in a muffle furnace (MLW, Germany) at 550°C for 8 h. The crucibles were brought to room temperature and weighed (BSS 11374-86).

Statistical analysis

The data received from the trial were statistically analyzed with ANOVA single factor STATISTICA 6.0.

Results and Discussion

Growth performance and feed utilization efficiency

The survival of trout during the experiment is presented in Table 1. The values of this indicator in the subjects from the control group and the experimental group fed with the addition of 6 g/kg of yellow gentian were 100%. The average initial live weight of rainbow trout from the control and experimental variants was 114.45 ± 12.18 g and 107.15 ± 3.39 g, respectively, where the differences were not statistically significant ($P > 0.05$) (Table 1). At the end of the experiment, the fish fed with fodder supplemented with yellow gentian showed a higher final weight of 162.65±0.92, 4.46%, compared to the values of this parameter for the control group trout: 155.7±17.82, but the differences were not statistically significant ($P > 0.05$).

Table 1. Growth performance of rainbow trout in control and experimental tanks

Parameter	n	$\bar{x} \pm SD$	
		CF	DF
Initial body weight, g	20	114.45±12.18	107±3.39
Final body weight, g	20	155.7±17.82	162.65±0.92
Survival rate, %		100	100
SGR, % per day		0.50±0.27	0.70±0.02
Average individual weight gain, g	20	41.25±0.12	55.65±0,15
FCR		2.42	1.79

($P > 0.05$) – no significant statistical differences

The mean SGR for rainbow trout from the experimental variant was higher than the SGR value in control fish. FCR calculated for trout of the experimental variant was 1.79, lower by 26.03% compared to fish in the control group 2.42 (Table 1).

The growth rates of the fish were higher in the group fed with the supplement (*Gentiana lutea*), compared to the trout in the control group.

Blood parameters

The reported results of the tested biochemical parameters of blood in experimental fish were not significantly affected by the addition of yellow gentian (Table 2).

Table 2. Biochemical blood parameters of rainbow trout in control and experimental groups

Blood parameters	Until	n	CF	DF
			$\bar{x}$ ±SD	$\bar{x}$ ±SD
GLU	mmol/L	6	4.01 ±0.76	3.31±0.61
UREA	mmol/L	6	1.18 ±0.83	1.26±0.39
CREA	µmol/L	6	48 ±14.84*	24.00±16.20*
TP	g/L	6	44.45 ±8.18	42.33±19.42
ALB	g/L	6	27.98 ±6.18	23.72±12.16
ASAT	U/L	6	102.5 ±111.22	42.33±65.77
ALAT	U/L	6	13.83 ±11.10	51.83±62.87
ALP	U/L	6	137.40 ±109.48	127.8±98.95
Ca	mmol/L	6	2.74 ±0.74	2.02±0.41
P	mmol/L	6	3.66 ±1.78	4.22±1.49
Mg	mmol/L	6	1.16 ±0.08	1.08±0.22
TG	mmol/L	6	1.51 ±0.40	1.62±0.43
CHOL	mmol/L	6	5.58 ±1.70	5.50±1.54

*P < 0.05 – significant statistical differences; P > 0.05 – no significant statistical differences

The obtained differences between the two groups were not significant (P > 0.05), except for creatinine level. The urea level was 6.78% higher in the experimental individuals, compared to the control variant, yet without a significant statistical difference (P > 0.05). The mean CREA value for trout in the control variant was 50% higher compared to the mean values of this blood parameter measured in the fish of the experimental variant (P < 0.05). This shows the possibility of the yellow gentian supplement to modulate the activity of creatinephosphokinase in the muscles on the one hand and to increase the filtration capacity of the kidneys on the other.

Lower mean values of the total protein, ALB, ALAT, ALP in the control group fish were reported, compared to those in the test group trout, but the differences were not statistically significant (P > 0.05). The ASAT result showed a higher mean in the control group fish, compared to the experimental group fish, but the difference was not significant (P > 0.05). The hepatoprotective properties of the yellow gentian supplement and the regulation of liver marker levels (especially with regard to ASAT) are reported. In addition, optimization of the protein synthesizing ability of the liver was also observed, which is a positive indicator for increasing the productivity of the cultivated trout.

The mean phosphorus result showed that its measured level in the experimental group was higher by 15.3% compared to the fish in the control group, but the difference had no statistical significance (P > 0.05). It is likely, that the yellow gentian supplement enhances energy metabolism and optimizes the synthesis and utilization of adenosine triphosphate.

The magnesium content was 6.8% lower in experimental fish, but without significant statistical differences (P > 0.05). The quantity of triglycerides was 7.3% higher in the blood of yellow gentian-fed fish, compared to the mean recorded in control fish, with no significant difference (P > 0.05). The measured mean value of cholesterol content in the blood of the experimental group fish was lower than the result obtained when taking into account the same parameter in the blood of the control group fish (P > 0.05). An optimization of lipid metabolism was observed in the body of fish fed with a yellow gentian supplement. The reduced level of cholesterol also reflects on the dietary and preventive qualities of trout grown in an aquaponic system. On the other hand, the reduced blood sugar levels in the treated trout also indicate the optimization of their energy metabolism.

Meat quality

The results of the chemical composition of rainbow trout musculature are presented in Table 3.

The reported result for the moisture content of the fish studied was within 77.07± 0.27(C) and 76.43±0.71(D), which was 0.8% in favour of the control group of fish, but the differences were not reliable (P > 0.05). The dry matter content was obtained with a 3.7% higher dry matter content in the fillets of experimental fish fed with added yellow gentian compared to the control fish, but the difference was statistically not significant (P > 0.05). The protein concentration was 5.59% higher in the muscle of rainbow trout of the experimental group compared to that of the control group (P < 0.001). The higher protein level in the muscles of the trout fed with yellow gentian supplement determines the better nutritional value of their meat. The result obtained for the fat content of the experimental trout musculature scored higher in C-variant fish, compared to the same indicator in the trout

Table 3. Chemical composition of the fillets of the rainbow trout (*O. mykiss*) in control (C) and experimental (D) groups (%)

Indicator / Group	n	Moisture $\bar{x}$ ± SD	Dry matter $\bar{x}$ ± SD	Crude protein $\bar{x}$ ± SD	Fat $\bar{x}$ ± SD	Ash $\bar{x}$ ± SD
C	6	77.26±0.26	22.73±0.27	18.61±0.24***	2.51±0.22	1.60±0.02
D	6	76.43±0.71	23.57±0.71	19.65±0.33***	2.28±0.38	1.63±0.06

***P < 0.001 – significant statistical differences group D of the experiment conducted, but the differences were not reliable (P > 0.05).

Conclusion

- Better values in the measurement of trout in the group fed with yellow gentian were reported with an average individual fish weight and a feed conversion factor of 34.91% and 23.03 % respectively, compared to control fish. Better blood parameters were measured in rainbow trout fed with the addition of yellow gentian. Higher crude protein and less fat were reported in trout fed with yellow gentian supplementation by 5.59% and 9.16% respectively, compared to control fish.
- The added yellow gentian to the fodder of rainbow trout cultivated in an aquaponic recirculating system favors the growth indicators of the fish, improves the utilization of feed, positively affects the quality of meat and biochemical indicators of fish blood.

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