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Growth efficiency, biochemical blood parameters and meat quality of rainbow trout (*Oncorhynchus mykiss* W.), fed with supplement of sweet flag extract (*Acorus calamus* L.)

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Abstract

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Different plants and their parts can be used as a supplement in food for human and animal. The purpose of this study is to establish the growth rate, meat quality and biochemical blood parameters (glucose, urea, creatinine, total protein, albumin, ASAT, ALAT, Ca, P, Mg, triglycerides, cholesterol) of rainbow trout (*Oncorhynchus mykiss*), fed with sweet flag extract (*Acorus calamus*). The fish from the control group C (without supplement) and the experimental A.c (supplemented with 1% *Acorus calamus* extract), each with two repetitions, were placed in a recirculating system at the Aquaculture Base of the Faculty of Agriculture of the Trakia University. Thirty specimens of rainbow trout with average fish weight of 42.55±7.48g (C) and 45.01±8.94g (A.c) in good health were placed in each tank and cultivated for 60 days. At the end of the experiment, average final weight, specific growth rates, feed conversion factor, meat quality and blood parameters were established. At the end of the experiment, the average live weight of fish fed with sweet flag extract was 6.53% higher than the value of the same indicator of the trout in the control group ($P < 0.05$). The calcium, phosphorus and magnesium levels in the blood of rainbow trout from the experimental group were 71.2%, 37.36% and 37.5% higher respectively than the levels of the same minerals in the control group ($P < 0.05$). Fish fed with sweet flag extract supplement have better growth rate and lower feed conversion ratio.

Keywords: biochemical blood parameters; growth; meat quality; rainbow trout; sweet flag

Introduction

Different plants and their parts can be used as a supplement in food for human and animal (Ganchev et al., 2019). Increasingly experiments have been made in recent years with the addition of various plant extracts to fish feed in order to improve the absorption coefficient, survival rates and meat quality (Gabor et al., 2011, Georgieva et al., 2018). The benefits of plant extracts are that they contain natural and not synthetic active substances, which make them safe for fish and for nature; easily extracted from plants by known methods; no adverse side effects. Since there is not much research in aquaculture, it is interesting to trace the impact of plant extracts as feed additives on different fish species, their growth, and health condition. Experiments with herbal extracts can also be used as substitutes for antibiotics in fish farming (Ali et al., 2018, Georgieva et al., 2019). Therefore it is necessary to conduct research in aquaculture with various herbs and monitored its effect on various parameters in cultivated hydrobionts. One such plant used since ancient times and with proven healing properties is the sweet flag.

Acorus calamus has a range of pharmacological activities, such as central nervous system depressant (Vengadesh Prabu et al., 2009), cardiovascular (Shah & Gilani, 2012), anti-diabetic (Prisilla et al., 2012), antioxidant (Subathraa & Poonguzhali, 2012), anti-inflammatory (Kim et al., 2009), antifungal (Begum et al., 2007), antibacterial (Manikandan et al., 2010), insecticidal agent (Nalamwar et al., 2009). Glycosides, alkaloids, tannins, vitamin C, starch, mucus, resin, and essential oils are found in the leaves and the rhizome of sweet flag. This herb has the ability to enhance the secretion of gastric juice. This property could have a positive impact on the growth and health of fish if they consume this plant by improving the intestinal wall and increasing digestibility.

The purpose of this study is to establish the growth rate, meat quality and biochemical blood parameters (glucose, urea, creatinine, total protein, albumin, ASAT, ALAT, Ca, P, Mg, triglycerides, cholesterol) of rainbow trout (*Oncorhynchus mykiss*), fed with sweet flag extract (*Acorus calamus*).

Material and Methods

Experimental fish and nutrition

Thirty specimens of rainbow trout with an average weight of 42.55 ± 7.48 g (control C) and 45.01±8.94 g (experimental A.c) in good health were placed in tanks and cultivated for 60 days. Concrete tanks have an effective water volume of 0.8 m^3, which is part of a recirculation system. The fish from the control group C (without supplement) and the experimental A.c (supplemented with 1% *Acorus calamus* extract), each with two repetitions, were placed in a recirculating system at the Aquaculture Base of the Faculty of Agriculture of the Trakia University. The fish are fed with 6 mm extruded pellets "Aqua UNI" produced by "Aqua guarant". To the fish feed of the trout from the experimental group 1% aqueous extract of sweet flag was added as well as 5 ml of sunflower oil for every 100 g of pellets. Rainbow trout from the control group are fed with granules coated only with the same amount of sunflower oil. The nutrient content in the feed of the two groups consists of: 45% crude protein, 16% crude lipids, 2% crude fibre, 1% P, 18.5 MJ/kg ME, 10000 IU/kg Vitamin A, 1500 IU/kg Vitamin D3, 200 mg/kg Vitamin E. The daily ration of the fish studied is 1.8% of their weight and are fed three times a day. Tanks are cleaned daily and excreta are released. The light intensity is about 12:12 hours light:dark cycle for a day.

Fish growth parameters

The average individual fish weight (g) is measured at the beginning, middle and end of the experiment to establish the effect of white yarrow extract on weight gain and conversion rate of the rainbow trout grown in the recirculation system. At the end of the experiment, weight gain (g), survival rate (%) and feed conversion ratio were determined.

Biometric calculations were performed according to the following formulas:

Specific Growth Rate (SGR) (Zhou et al., 2006):

$$SGR = \frac{(LnWf - LnWi)}{n} x100 \quad (1)$$

where, SGR - specific growth rate,%; Wi - initial weight, g; Wf - final weight, g; n - number of days

Feed conversion factor (FCR)

$$FCR = \frac{Feed\ given}{Fish\ weight\ gain} \quad (2)$$

where, FCR - feed conversion factor; feed given, g; fish weight gain, g.

Hydrochemical parameters

The oxygen content (mg.l^{-1}), pH, water temperature (° C) and electrical conductivity (µS.cm^{-1}) are measured daily with a portable measuring instrument (HQ30D). Other parameters for water quality, ammonia (mg.l^{-1}) and phosphates (mg.l^{-1}) were observed weekly at Ecolab Faculty of Agriculture, Trakia University, Stara Zagora.

Chemical analysis of meat samples

Fish fillets on the back of 6 fish samples were taken for variant and homogenized at the end of the experiment. The crude protein content (%) is calculated by converting the nitrogen content quantified by the Kjeldahl method using an automated Kjeldahl system (Kjeltec 8400, FOSS, Sweden). The lipid content (%) is determined by the Soxhlet method using an automated system (Soxtec 2050, FOSS, Sweden). The ash content (%) was investigated by burning in a muffle furnace (MLW, Germany) at 550°C for 8 hours. Samples were brought to room temperature and weighed.

Biochemical blood analyses

At the end of the trial, blood is taken directly with sterile disposable syringes (3 ml) with a needle from the heart of the fish being examined. Sodium heparin (1%) is used as anticoagulant. Blood samples were immediately transmitted and analysed in a haematological laboratory (NCPTC - Trakia University) and reported in a Mindray BC -120 haematology analyser. The following biochemical blood glucose parameters were examined: glucose (mmol.l^{-1}), urea (mmol.l^{-1}), creatinine (µmol.l^{-1}), total protein (g.l^{-1}), albumin (g.l^{-1}), ASAT (U.l^{-1}), ALAT (U.l^{-1}), Ca (mmol.l^{-1}), P (mmol.l^{-1}), Mg (mmol.l^{-1}), *triglycerides* (mmol.l^{-1}) and *cholesterol* (mmol.l^{-1}).

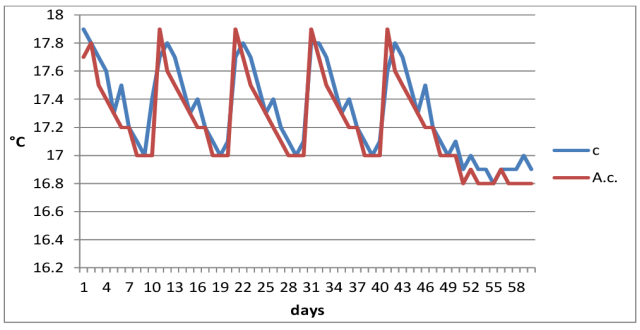
Statistical analysis

Data obtained from the study were statistically analysed with an ANOVA single factor (MS Office, 2010).

Results and Discussion

For the optimal development of cultivated species one of the most important indicators is a water temperature. According to Zaykov & Staykov (2013) the optimal temperature values for rainbow trout is 12.0-16.0°C. During the experiments its values are 16.8-17.9°C in the control and experimental tanks, which are within the optimal values for trout breeding (Fig. 1).

Fig.1. Water temperature in control (C) and experimental (A.c) tanks



The dissolved oxygen during the trial varies between 7.35 mg.l^{-1} and 8.36 mg.l^{-1} (Fig. 2). The values of these parameters during the period were higher with 4.06% in the experimental tanks compared to those of the control tanks. The better effect on dissolved oxygen levels may be due to influence of plant extract supplement in feed for fish.

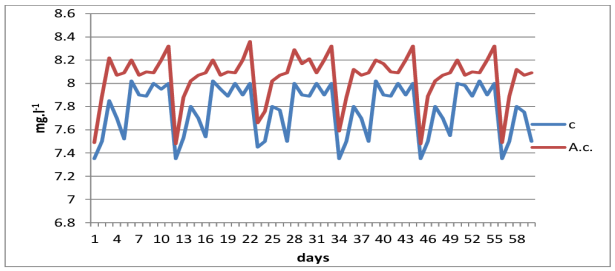


Fig.2. Dissolved oxygen in control (C) experimental (A.c) tanks

The pH values of the water in the recirculation system vary between 8.04 and 7.3, which are slightly alkaline (Fig. 3). It is very important that pH levels are not below 6.5 and above 8.5, because young fish are extremely sensitive to this indicator. The better pH value was observed in the experimental variant which probably due to the action of the plant extract. The water quality parameters were in the line with permissible values for this fish species according to Regulation No. 4 (2000).

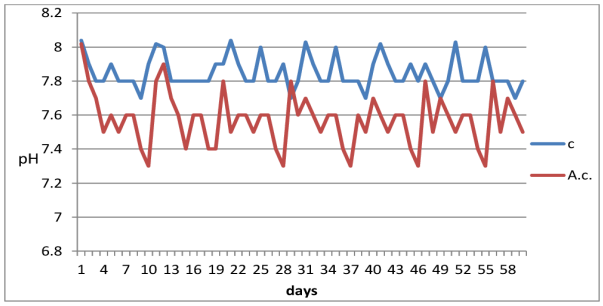


Fig.3. pH in control (C) experimental (A.c) tanks

The electrical conductivity values of water ranged from 262 µS.cm^{-1} to 269 µS.cm^{-1} (Fig. 4). The values of this parameter in the experimental variant were 0.73% higher than those of the control. Analysis of the hydro chemical data shows that during the experiment their values were optimal for the cultivated species.

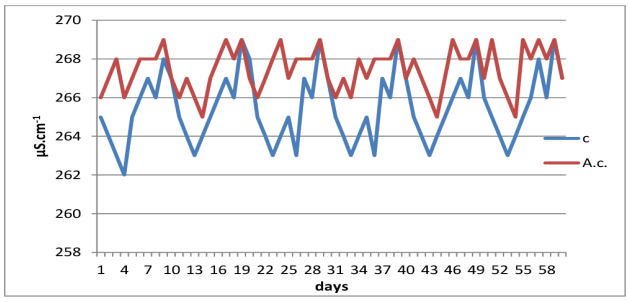


Fig.4. Electric conductivity in control (C) and experimental (A.c) tanks

Three times a day the tanks are cleaned by adding fresh water at a rate of 10% of the total volume of the recirculation system. To maintain optimal water chemical parameters during the experiment mechanical and biological filters are of great importance.

The concentration of ammonia over a period of 60 days was averaged 0.47 mg.l^{-1} in the water of the experimental tanks, and 0.55 mg.l^{-1} in the control tanks, but the differences was not statistically proven (P> 0.05). Phosphorus averaged 0.8 mg.l^{-1}and 0.73 mg.l^{-1}for control and experimental tanks, respectively, but the differences were not statistically proven (P> 0.05) for both groups during the experiment (Table 1).

Table 1. Concentration of ammonia and phosphorus in control (C) and experimental (A.c) tanks

Group	n	NH_4^+, mg/l	P-PO_4^-, mg/l
C	8	0.55±0.12	0.80±0.54
A.c	8	0.47±0.65	0.73±0.23

P>0.05 - ns

The mean initial live weight of rainbow trout from control and experimental variants is 42.55 ± 7.48 g and 45.01 ± 8.94 g, respectively, and the differences are not statistically significant (P> 0.05) (Table 2). The mean live weight of fish fed with sweet flag extract is 122.46 ± 17.92 g at the end of the experiment, which is 6.53% higher compared to the value of the same indicator of the trout in the control group (P <0.05). Survival during the experiment is 100% in fish in the experimental and control groups. The mean individual weight gain of rainbow trout from the group fed with sweet flag extract is 77.45 ± 1.01 g, which is 7.15% higher compared to the control group and the differences are statistically significant (P <0.05). The feed consumption analysis at the end of the study, show that Feed conversion ration of the trouts from the experimental group is 1.35. It is with 7.53% lower than that of the fish from the control group (1.46). The rainbow trout growth parameters are higher in the group fed with sweet flag extract.

As valuable tools for monitoring fish health are used biochemical blood parameters. For different species in different aquaculture conditions have not yet been defined the normal ranges of the main biochemical parameters (Satheeshkumar et al., 2011).

Table 2. Rainbow trout growth parameters in control (C) and experimental (A.c) tanks

Parameter	n	C	A.c
		$\bar{x}$ ± SD	$\bar{x}$ ± SD
Initial body weight, g	30	42.55±7.48	45.01±8.94
Final body weight, g	30	114.46±17.03*	122.46±17.92*
Survival rate, %		100	100
SGR % per day		1.64±0.003	1.66±0.02
Average individual weight gain, g	30	71.91±0.54*	77.45±1.01*
FCR		1.46	1.35

*P<0.05

In the present study, the glucose level is 38.04% higher in the fish from the experimental group, and the differences are statistically proven (P<0.05) (Table 3). The urea level is 40.30% higher in the fish from the experimental group compared to those of the control variant, but without statistically significant differences (P> 0.05). The creatinine level is 23.4% higher in the control group compared to feeding with the sweet flag extract (P> 0.05). The total protein level is 11.15% lower in the rainbow trout fed with supplement compared to the control, although this difference is not statistically significant (P> 0.05). According to Hrubec et al. (2001) the protein level increases with age. Albumin prevents fluid from leaking blood vessels and nourishes tissues and transports hormones, vitamins and substances like calcium in the body (Satheeshkumar et al., 2011). The amount of albumin in the experimental fish blood is 5.85% higher than the control group. ASAT values in the experimental group are 7.09% higher than in the control group (P> 0.05). ALAT values in the experimental group are 24.49% higher than the control group, and the result is not statistically significant (P> 0.05). ALP indicator in blood of the trout in the control group is 74.2% lower than in the experimental group, without statistically significant difference (P>0.05). Cholesterol and triglyceride levels may be affected by pollutants (Adham et al., 2002; Yang & Chen, 2003). Triglyceride concentrations in the serum of rainbow trout fed with supplement are 12.5% higher than in the control fish (P> 0.05). The cholesterol measured is 5.73% lower in the control variant compared to the experimental group but without statistically significant differences (P> 0.05). If there are changes in blood electrolytes this may lead to disturbances in normal vital physiological functions of fish and its growth rate (Prasad et al., 2011). A very important mineral element is calcium and it is present in larger quantities than other electrolytes. This element is contained in bones in combination with phosphorus in the form of calcium phosphate. Ionized calcium is very important for normal muscle excitement and blood clotting (Kulkarni, 2015). In this study, the calcium levels in the blood of rainbow trout from the experimental group are 71.2% higher than the levels of the same mineral in the control group and the differences are statistically proven (P<0.05). Phosphorus levels in the experimental group are 37.36% higher than in the control group with statistically significant differences (P<0.05). Magnesium is 37.5% higher in the fish in the experimental group compared to the control group and the differences are statistically proven (P<0.05).

Table 3. Biochemical blood parameters of rainbow trout in control (C) and experimental (A.c)

Blood parameters/ Groups	n	C	A.c
GLU	6	4.69 ± 0.60*	7.57 ± 1.31*
UREA	6	1.17 ± 0.95	1.96 ± 1.35
CREA	6	10.0 ± 1.20	7.66 ± 0.9
TP	6	45.47 ± 4.21	40.4 ± 19.26
ALB	6	22.5 ± 2.83	23.9 ± 12.39
ASAT	6	153.33 ± 147.41	165.0 ±149.08
ALAT	6	12.33 ± 1.70	16.33 ± 14.80
ALP	6	41.67 ± 16.26	162.00 ± 87.2
CA	6	0.72 ± 1.17*	2.50 ± 1.26*
P	6	5.23 ± 3.52*	8.35 ± 1.18*
Mg	6	0.70 ± 0.49*	1.12 ± 0.18*
TG	6	0.98 ± 0.84	1.12 ± 0.22
CHOL	6	4.27 ± 0.72	4.53 ± 2.38

*P<0.05

Adding sweet flag extract to rainbow trout diet lead to result in 1.12% higher moisture content in rainbow trout fillets compared to that of the control (P<0.01) (Table 4). The protein content of fish in the control is 3.41% higher than the experimental group (P<0.01). The sweet flag extract added to the feed lead to increase in the lipid content of the rainbow trout fillet by 1.26% compared to the control group but it was not statistically proven (P>0.05). The dry matter content in the meat of the experimental group of fish is lower than that of the control group by 3.43% (P<0.01). The ash content of the trout fillets in the control is higher by 14.7% compared to the fish in the experimental group and the difference was statistically significant (P<0.001) (Table 4). There are no essential differences between the two groups in terms of meat quality indicators.

Table 4. Chemical composition of meat from rainbow trout (*O. mykiss*) in control (C) and experimental groups (A.c) in tanks (%)

Group	n	Moisture	Dry matter	Crude protein	Fat	Ash
C	6	74.65±0.51**	25.36±0.51**	19.89±0.16**	3.89±0.48	1.56±0.03***
A.c	6	75.50±0.14 **	24.49±0.14**	19.21±0.23**	3.94±0.20	1.33±0.02***

**P<0.01, ***P<0.001

Conclusion

The effect of sweet flag extract has been studied for the first time as a supplement to the rainbow trout diet. Fish fed with this extract have better growth indicators and lower feed conversion ratio.

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