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Original Contribution

GROWTH PERFORMANCE, BIOCHEMICAL BLOOD PARAMETERS AND MEAT QUALITY OF RAINBOW TROUT (*ONCORHYNCHUS MYKISS* W.) FED WITH LICORICE (*GLYCYRRHIZA GLABRA* L.) SUPPLEMENTED DIET

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ABSTRACT

Licorice (*Glycyrrhiza glabra)* is medicinal plant characterized with sweet flavor which has been used in Bulgarian traditional medicine to improve the condition of the digestive tract and could positively affect digestibility and assimilation of feed nutrients in human and animal organisms. The studies connected with the effect of licorice in fish are limited. The aim of current study was to find the effect of feed supplemented with licorice *Glycyrrhiza glabra* on growth performance, blood parameters and meet quality in rainbow trout (*Oncorhynchus mykiss* W.), raised in the recirculation system. The fish were fed with two feeds: control feed (CF) - without the addition of supplement and experimental feed (EF), with supplementation of 300 $mg.kg^{-1}$ licorice in pellets. The stocking density of rainbow trout in recirculation system was 50 $pcs.m^{-3}$. The initial average weight of fish in CF variant was 13.30 ±3.07 g and in EF variant was 13.40±3.55 g without differences being statistically significant ($p\geq0.05$). The continuation of the experiment was 60 days. The average final weight, meat quality, and blood biochemical parameters were measured at the end of the experiment. The SGR and FCR were also calculated. Fish from the experimental group fed with a supplement of licorice had with 8.54% higher average final weight compared to the parameter's value in trouts from the control variant ($p<0.05$). SGR in rainbow trout's from experimental variants was higher with 6.9% compared to the value of SGR in fish from CF variant. The blood biochemical parameters in experimental trouts were not significantly affected by supplementation of licorice extract. The average values of glucose, ASAT, and ALAT in control variant were higher respectively with 3.96%, 57.2% and 10.3% compared to values in these parameters measured in the blood of fish from the experimental variant, but differences were not statistically proven ($p\geq0.05$). The meat quality parameters were affected from the addition of licorice in feed for trouts and the moisture, fat, and ash quantities were higher respectively with 2.49%, 36.4% and 12.5% compared with the average values of these parameters found for fish from CF group ($p<0.05$). The extract from licorice added to feed for the feeding of rainbow trout increases the growth of fish and affect the meat quality parameters ($p<0.05$), but did not affect significantly the blood parameters ($p\geq0.05$).

Key words: licorice supplementation, rainbow trout, blood biochemical parameters, growth, meat quality.

INTRODUCTION

The aquaculture is probably one of the fast growing food-production sectors. According to Staykov, 2001 (1) the intensive aquaculture has different advantages i.e. increasing the final productivity, decreasing the area necessary for the cultivation of hydrobionts, decreasing the necessary time for raising of different species. The main disadvantages of high stocking density in intensive aquaculture farms are: increased stress (2), higher susceptibility to diseases (3), reduced growth and feed intake in cultivated hydrobionts (4). One possible solution in the past to answering of mentioned challenges was the treatment of water species with antibiotics (5-8) and hormones (9, 10). The development of sustainable and ecologically friendly

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aquaculture is excluding the application of these groups of substances. The application of antibiotics in aqua production could result in the transfer of drug resistance from fish bacterial diseases to human bacterial diseases and contributing to most significant healthcare problems further (11). On the other hand, the usage of hormones in animal production is highly contradictory and could lead to different diseases in humans and affect the age of puberty in children (12).

One possible alternative to antibiotics and hormones present the usage in aquaculture of different plant's extracts. The evaluation of phytobiotics in aquaculture is a relatively new area of research showing very promising results (13). The possible effects of their application on fish are spread on: stimulation of immune system (14, 15, 16, 17, 18, 19), increase of growth intensity (20, 21) and by their antioxidant effect decrease the stress in hydrobionts (22).

Licorice (*Glycyrrhiza glabra)* is medicinal plant characterized with sweet flavor which has been used in Bulgarian traditional medicine to improve the condition of the digestive tract and could positively affect digestibility and assimilation of feed nutrients in human and animal organisms. The studies connected with the effect of this herb in fish diets are relatively limited (23-25).

The aim of current study was to find the effect of feed supplemented with licorice *Glycyrrhiza glabra* on growth performance, blood parameters, and meet quality in rainbow trout (*Oncorhynchus mykiss* W.) raised in the recirculation system.

MATERIALS AND METHODS

The experimental fish

The rainbow trout in good health condition were chosen and transported from trout farm Bukovetz (Tvyrdica) to experimental aquaculture base (Faculty of Agriculture, Trakia University, Stara Zagora). The fish were split into the following experimental groups:

Experimental feed (EF)-the fish were fed with feed supplemented with licorice's (*Glycyrrhiza glabra)* extract;

Control feed (CF)- the fish were fed with feed without the addition of supplement.

The experiment was carried out in two replications.

The average initial weights of trouts from both repetitions of experimental groups were:

EF – 13.40±3.55 g;

CF - 13.30 ±3.07 g.

The fish was cultivated in experimental tanks at 50 specimens.m^{-3} stocking density, 40 trouts/tank. The trial continued 60 days.

Recirculation aquaculture system

The recirculation aquaculture system (RAS) consisted of 11 cultivation tanks were used in the current trial (**Figure 1**). The cleaning block of the system was consisted of two mechanical filters (settling tank), moving bed biofilter, heating and pump sections (**Figure 2**). The useful volume of tanks for fish cultivation was 0.8 m^3.

Figure 1. View of recirculation aquaculture system used in the current trial

The flow rate in RAS was maintained at 25 l.min^{-1}. Every day the bottoms of fish tanks as well as the bottoms of filter's units were cleaned by siphoning. The water loses connected with cleaning process and evaporation were 20% of total volume of RAS. This quantity of water was refilled daily with clean tap water.

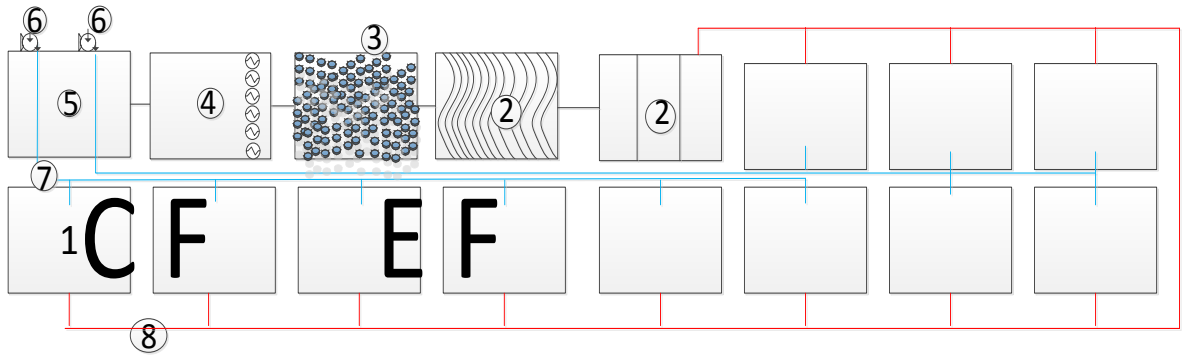

Figure 2. Scheme of RAS used in the trial:1)fish tanks; 2)mechanical filters; 3)moving bed biofilter; 4) tank with heating elements; 5)pump tank; 6)pumps; 7)inlet water; 8)outlet water.

Experimental feed

The experimental fish were fed with a commercial extruded feed produced by the "Aqua-Garant" with the size of pellets 2 mm. The content of commercial trout's feed used in a current trial could be seen in **Table 1**. Licorice extract was added to the granules at a concentration of 300 mg.kg^{-1} fish feed. The sunflower oil at the quantity of 1ml was also added to every 10 g of experimental feed (EF) and the components were very well mixed. The same amount of sunflower oil was added to the control feed (CF) and the pellets and oil were also mixed. Afterward, both tested feeds were left for 12 hours at an air temperature of 20°C and were used for the feeding of experimental fish. The daily diet was maintained at 2.8% of the fish biomass. The feed was supplied manually four times per day.

Table 1 .*Nutritional content of commercial feed used in the trial /according to fish feed producer "Aqua-Garant"/*

Ingredients	**Unit**	**Content**
Crude protein	%	45
Fibers	%	1.5
Fat	%	16
Phosphorus	%	1.05
Vit.A	Thousands IU.kg^{-1}	10
Vit.D_3	IU.kg^{-1}	1500
Vit. E	Mg	200
Digestive energy	MJ.kg^{-1}	18.5

Investigated parameters

Water quality

Daily the water temperature (°C), pH, dissolved oxygen (mg.l^{-1}) and electrical conductivity (µS.cm^{-1}) were measured with portable meter HQ30D (Hach Lange), connected with an appropriate for each parameter probe.

Growth parameters in experimental fish

The mortality cases in experimental tanks were recorded daily during the trial and survival (%) was calculated using the following formulae:

Survival (%) = (final number of fish /initial number of fish) x100 (1)

The experimental fish were weighed at the technical balance with accuracy 0,01g at the start of the trial. The average final weight (g) was measured at the end of the experiment and specific growth rate (SGR) (%.day^{-1}) and FCR were calculated with the following equations:

SGR (%.day^{-1})=[Ln final weight (g)-Ln initial weight(g)]/number of days x 100 (2)

FCR=Fed feed (g)/Weight gain of fish (g) (3)

Blood biochemical parameters in experimental fish

The blood was taken from the hearts of the examined fish (6 specimens per variant) with disposable sterile plastic syringes (3 ml) with a needle**.** Heparin sodium (1%) was used as an anticoagulant. The blood was centrifuged at 3000 rpm for separating the plasma. Afterward the biochemical parameters (glucose (GLU) (mmol.l^{-1}), urea (UREA) (mmol.l^{-1}), total protein (TP) (g.l^{-1}), albumin (ALB) (g.l^{-1}), alanine aminotransferase (ALAT) (U.l^{-1}), aspartate aminotransferase (ASAT) (U.l^{-1}), alkaline phosphatase (ALP), the content of calcium (Ca) (mmol.l^{-1}), phosphorus (P) (mmol.l^{-1}), magnesium (Mg) (mmol.l^{-1}), 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).

Meat quality in experimental fish

The fish fillets from the back side of 6 fish specimens per variant were obtained and homogenized at the end of the trial. The received muscles homogenates were used for analysis of moisture, crude protein, fat and ash in a Central research laboratory (Faculty of Agriculture, Trakia University) according to the following methods:

- Moisture (%) and dry matter (%)-Bulgarian State Standard (BSS)11374-86;
- Crude protein content, % (BSS-ISO 5983, the Kjeldahl method on Kjeltec 8400, FOSS, Sweden);
- Fat content, % (BSS-ISO 6492, Soxhlet extraction method, using Soxtec 2050, FOSS, Sweden);
- Crude ash content, % (BSS 11374-86);

Statistical analysis of data

The data were analyzed statistically with two methods: ANOVA single factor STATISTICA 6.0 software (StatSoft Inc., 2002).

RESULTS AND DISCUSSION

Water quality

The average values of water temperature in experimental tanks were in optimal range according to Regulation No.4/20.10.2000 (26). The difference in this parameter between experimental and control variant was not statistically significant (P≥0.05).The electrical conductivity and oxygen quantities were higher in the water of EF respectively with 0.11% and 2.18% compared with the values of these parameters in tanks of CF, but the differences were not statistically proven (P≥0.05) (**Figure 3**). The pH was lower with 0.37% in EF group, compared with the average value of this parameter measured for CF variant, but the difference was not significant (P≥0.05) (**Figure 3**).

The water quality parameters were in the line with permissible values for this fish species according to Regulation No. 4 of 20.10.2000 (26). The studies retracing the effect of plant's extract supplemented in feed for fish on water quality parameters are limited. The study made with thyme (*Thymus vulgaris*), rosemary (*Rosmarinus officinalis*) and fenugreek (*Trigonella foenum graecum*)(27) and *Y. shidigera* (28) pointed out that level of ammonia decreased and no effect was found on nitrite and dissolved oxygen levels.

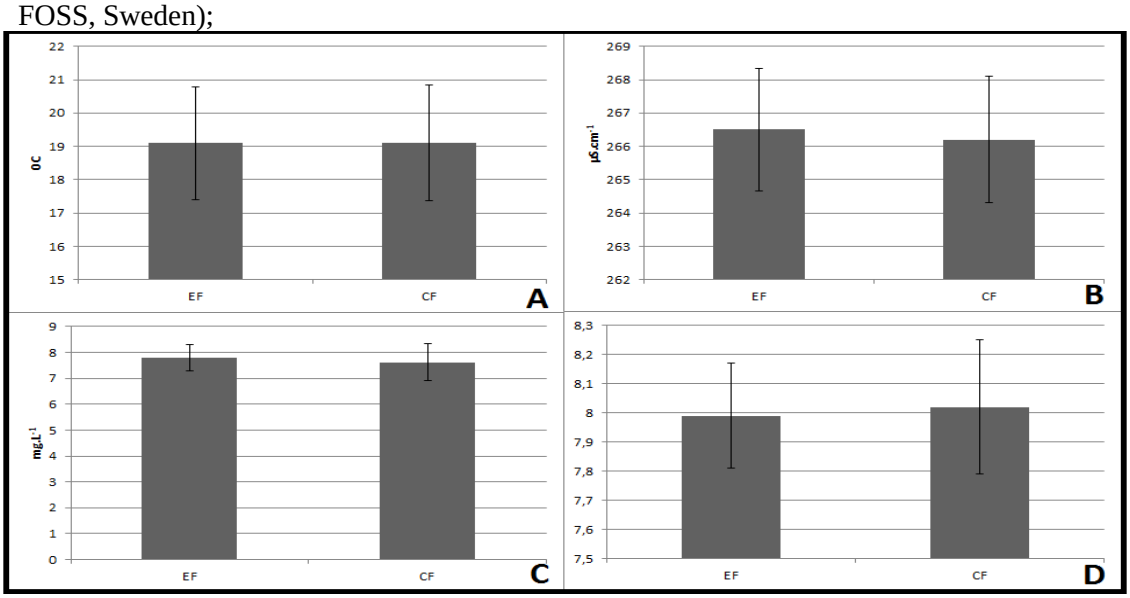

Figure 3. Hydrochemical parameters during the trial-A) Temperature; B) Conductivity; C) Dissolved oxygen; D)pH

Growth parameters in experimental fish

The survival of fish was 100% in both tested variants. The average values of initial weight in rainbow trout from both groups, experimental and control were very close and the difference between them was not significant (P≥0.05) (**Table 2**). The final weight, specific growth rate (SGR) and feed conversion ratio were higher in trouts from the group fed with feed supplemented with licorice (**Table 2**).

The fish from the experimental group, fed with a supplement of licorice had with 8.54% higher average final weight compared to the parameter's value in trouts from the control variant ($p<0.05$). The average value of SGR in rainbow trout from the experimental variant was higher with 6.9% compared to the value of SGR in fish from CF group ($p<0.05$) (**Table 2**). The average value of FCR found for trouts from experimental variant was higher with 15.3% compared with this calculated for fish from control group (**Table 2**).

The received from us results for growth parameters are in confirmation made from Kumar et al., 2007 (29) who found better growth per day in % body weight, average weight gain and FCR in Indian major carp, *Cirrhinus mrigala*, fingerlings when their feed was supplemented with extract of licorice. According to Chakraborty et al., 2013 (30) the better growth performance in fish from licorice addition in their feed was the result of isoflavones content in this herbal could lead to potent antioxidant activity, inhibition of superoxide anion production in xanthine/xanthine oxidase system and protection of mitochondrial function against oxidative stresses. Growth promoting effect was found in yellow perch when in its feed *Astragalus membranaceus* (AM) and *Glycyrrhiza glabra* (licorice) was added (25).

Table 2. *Growth parameters in rainbow trout (O.mykiss) during the trial*

Growth parameters	**n**	**$\bar{x}$ ± SD**	
		CF	**EF**
Initial weight (g)	80	13.30 ±3,07	13.40±3,55
Final weight (g)	80	40.90±1,13	44.80±1,22*
SGR (%body wt gain.day^{-1})	2	1.86	2.05*
FCR	2	1.43	1.69*

*-show significant statistical differences ($p<0.05$)

Table 3. *Blood parameters in rainbow trout (O.mykiss) during the trial*

Blood parameters	**Unit**	**n**	**$\bar{x}$ ± SD**	
			CF	**EF**
GLU	mmol.l^{-1}	6	5,05±0,62	4,88±0,93
UREA	mmol.l^{-1}	6	0,64±0,31	0,85±0,38
TP	g.l^{-1}	6	38,40±8,35	36,75±3,26
ALB	g.l^{-1}	6	17,06±1,65	18,8±3,81
ASAT	U.l^{-1}	6	83,60±70,18	70,28±70,40
ALAT	U.l^{-1}	6	23,20±19,60	20,80±11,90
ALP	U.l^{-1}	6	416,00±206,30	372,16±168,20
Ca	mmol.l^{-1}	6	2,22±0,48	1,84±0,37
P	mmol.l^{-1}	6	4,93±2,02	4,04±1,35
Mg	mmol.l^{-1}	6	0,76±0,12	0,75±0,02
TG	mmol.l^{-1}	6	1,77±0.16	1,70±0,07
CHO	mmol.l^{-1}	6	5,83±2,25	6,50±1,13

*-show significant statistical differences ($p<0.05$)

Blood biochemical parameters in experimental fish

The blood biochemical parameters in experimental fish were not significantly affected by supplementation of licorice extract (P≥0.05) (**Table 3**). The average values of glucose, TP, ASAT, ALAT, ALP, and TG in trouts from the control variant were higher respectively with 3.96%, 4.29%, 57.2%, 10.3%, 10.53% and 3.95% compared to values in these parameters measured in the blood of fish from experimental group, but differences were not statistically proven (P≥0.05). Received from us result for total protein content in rainbow trout fed with supplemented with licorice feed is controversial to data received from to Bhavan et al., 2013 (31) who stated that the quantity of TP was higher in fish from groups of prawn (*Macrobrachium rosenbregii*) fed with the addition of licorice compared to result received for the control group. The hepatoprotective effect (the liver enzymes and the quantity of glucose decreased) was found for the trouts fed with supplemented feed in the current study. Elabd et al. (2016) (25) found the improvement of aspartate aminotransferase (AST) alanine transaminase (ALT) activities, glucose and cortisol concentrations when the diet for the feeding of yellow perch was supplemented with *Astragalus membranaceus* and *Glycyrrhiza glabra*.

Meat quality in experimental fish

The meat quality parameters in experimental fish were significantly affected by the supplementation of feed with licorice. The content of dry matter, fat, and ash in trouts from EF was lower respectively with 7.39%, 36.4% and 12.5% compared with the average values of these parameters found for fish from CF (P<0.05) (**Table 4**). The studies connected with the influence of licorice on meat quality in fish are missing by our understanding. The researches with other animals showed that licorice as animal feed additives decreased lipid oxidation and improve quality of meat in lamb (32).

Table 4. *Meat quality parameters in fish during the trial*

Meat quality parameters	**n**	**$\bar{x}$ ± SD**	
		CF	**EF**
Moisture	6	74,20±0,63	76,10±0,39*
Dry matter	6	25,70±0,63*	23,80±0,52
Crude protein	6	18,60±0,29	18,88±0,14
Fat	6	5,35±0,21*	3,40±0,51
Ash	6	1,75±0,15*	1,53±0,09

*-show significant statistical differences (p<0.05)

CONCLUSION

The extract from licorice (*Gl. glabra*) added to feed for the feeding of rainbow trout (*O.mykiss*) increases the growth of fish and significantly affect the meat quality parameters but did not affect the blood parameters of experimental fish.

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