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Original Contribution

GROWTH PERFORMANCE, BIOCHEMICAL BLOOD PARAMETERS AND MEAT QUALITY OF RAINBOW TROUT (*ONCORHYNCHUS MYKISS* W.) FED WITH *CNICUS BENEDICTUS* L. EXTRACT

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ABSTRACT

Aquaculture development is influenced by various environmental factors and nutrition with herbal additives can affect the growth in aquaculture and to improve indicators such as digestibility, nutrition effectiveness and food taste. The purpose of this study was to trace growth performance, meat quality and biochemical blood parameters (glucose, urea, creatinine, total protein, albumin, ASAT, ALAT, Ca, P, Mg, triglycerides, cholesterol*)* of rainbow trout (*Oncorhynchus mykiss* W.) fed with additive blessed thistle (*Cnicus benedictus* L.) extract. To achieve the objective a control group (no added) and an experimental (with added 1363 $mg.kg^{-1}$ of blessed thistle extract) option, each with a two repetition, were set in a recirculating system in the Aquaculture Base of the Faculty of Agriculture at the Trakia University. Forty specimens from the fish species rainbow trout with an average weight of fish 13.32+3.07 g (control) and 13.33+2.58 g (experimental) in good health condition were placed in each tank and cultivated for 60 days. At the end of the experiment were calculated average final weight, specific growth rates, feed conversion ratio, meat quality and blood parameters. Trout from the experimental group, fed with supplement had with 8.52% higher average final weight compared to the parameter value of fish from control ($P<0.001$). The blood biochemical parameters ASAT and ALAT in control variant were higher with 27.4% and 44% respectively, compare to values of this parameter of fish from the experimental ($P>0.05$). Experimental fish fed with additive blessed thistle have a higher electrolytes level of magnesium (Mg) with 5.26% compared to control group ($P>0.05$). The protein content in the fish of the control group and experimental were of close value, but not statistically proven. The blessed thistle supplementation in the diet led to lower the lipid content in the fillets of the rainbow trout with 19.7% compare to values of this parameter of fish from control group and was statistically proven ($P<0.05$). This result shows that fish fed with the supplement are more useful and dietetic in their quality as human food. Rainbow trout fed with blessed thistle supplement have better growth performance and blood parameters.

Key words: blessed thistle, biochemical blood parameters, growth, meat quality, rainbow trout

INTRODUCTION

Various plant supplements are used to improve the quality of fish feeds during the last years. They are used to enhance the growth performance of different species, to reduce feed conversion ratio and to increase survival rate. Plant extracts are promising because they are natural products that are safe for both fish and the environment (1). Another important advantage is that they are not expensive. Nowadays, different parts of plants and their extracts are used as spices and for the treatment of various diseases (2, 3). There is still insufficient research on the influence of plant supplements on different fish species, their growth and health. Some of the herbal additives can also be used as an alternative to antibiotics in aquaculture. The plant species are known to have pharmacological properties such as antimicrobial, antiinflammatory, antitumour, antiproliferative effects. The advantage of herb therapy is that it does not stress the fish. Studies that have evaluated the effect of phytoadditives on growth performance and feed conversion parameters

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in different farmed fish species are however few. *Cnicus benedictus* L. was used a cure as for anorexia, as its main constituent - amaroidcninin stimulate the secretion of saliva and gastric juices (4, 5). Blessed thistle leaves, stems and flowers are traditionally used as a "bitter" tonic to enhance appetite and digestion. No data are available on the use of blessed thistle extracts in the cultivation of hydrobionts on a literary review. The purpose of this study was to trace growth performance, meat quality and biochemical blood parameters (glucose, urea, creatinine, total protein, albumin, ASAT, ALAT, Ca, P, Mg, triglycerides, cholesterol) of rainbow trout (*Oncorhynchus mykiss* W.) fed with additive blessed thistle (*Cnicus benedictus* L.) extract.

MATERIAL AND METHODS

Experimental fish and feeding

Forty specimens from rainbow trout with an average weight of 13.32+3.07 g (control) and 13.33+2.58 g (experimental) in good health condition were placed in each tank and cultivated for 60 days. The concrete tanks have an effective water volume of 0.8 m^3, which are part of a recirculation system. Fish from the control group, C (no added) and the experimental, C.b. (with added of blessed thistle extract) option, each with a two repetitions, were set in a recirculating system in the Aquaculture Base of the Faculty of Agriculture at the Trakia University. The fish were fed with 6 mm extruded pellets "Aqua UNI", produced by "Aqua garant". To the fish feed of trouts from the experimental group was added 1363 $mg.kg^{-1}$ extract of blessed thistle, as well as oiling the granules with 5 ml of sunflower oil for every 100 g pellets. Rainbow trout from control group were fed with granules only greased with the same amount of sunflower oil. The nutrient content in the feed of the two groups is: 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 that the studied fish received was 1.8% of their live weight and they were fed three times per day. The tanks were daily cleaned and excreta were siphoned. Light was about 12:12 h light: dark cycle throughout the day.

Fish growth performance

The average individual weight (g) of the fish was calculated at the start, middle and end of experiment in order to study the extract of blessed thistle influence on the weight gain and feed conversion ratio in the rainbow trout, cultivated in recirculation system. At the end of the trial the weight gain (g), survival rate (%) and the feed conversion ratio in fish were determined.

The biometrical calculations were carried out according to the following formulas:
Specific growth rate (SGR) (6):

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

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

Feed conversion ratio (FCR)

$$\boldsymbol{FCR = \frac{Feed\ given}{Fish\ weight\ gain}}$$

where, FCR – feed conversion ratio; feed given, g; fish weight gain, g.

Hydrochemical parameters

The oxygen content ($mg.l^{-1}$), pH, water temperature (°C) and electrical conductivity ($\mu S.cm^{-1}$) were measured daily with a portable meter (HQ30D), accordingly with LDO, pH (liquid) and conductivity electrodes. Other water quality parameters, ammonium ($mg.l^{-1}$) and phosphates ($mg.l^{-1}$) were monitored on a weekly in Ecolab Agriculture Faculty.

Chemical analyses of meat samples

The musculature samples of rainbow trout were determined on atomic absorption spectrometer (AAS) "A Analyst 800" – Perkin Elmer. Crude protein content (%) was calculated by converting the nitrogen content, quantified by Kjeldahl's method, using an automatic Kjeldahl system (Kjeltec 8400, FOSS, Sweden). Lipid content (%) was determined by the method of Soxhlet, using an automatic system (Soxtec 2050, FOSS, Sweden). Ash content (%) was investigated by incineration in a muffle furnace (MLW, Germany) at 550°C for 8 h. Crucibles were brought about the room temperature and weighed.

Biochemical blood analyses

Blood was taken from the examined fish directly from the heart with disposable sterile plastic syringes (3 ml) with a needle. As an anticoagulant Heparine sodium (1%) was used. The blood samples were instantly transmitted and analyzed in a hematological laboratory (NCPTC - Trakia University) and reported in Mindray BC – 120 hematology analyzer. Follow biochemical blood parameters were investigated: glucose ($mmol.l^{-1}$), urea ($mmol.l^{-1}$), creatinine ($\mu mol.l^{-1}$), 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:
The data received from the trial were statistically analysed with ANOVA single factor (MS Office, 2010).

RESULTS AND DISCUSSION

One of the most important indicators for the optimal development of the cultivated species is a water temperature. It was 16.8–17.9°C in control and experimental tanks which were within the optimum values for trout farming (**Figure 1**).

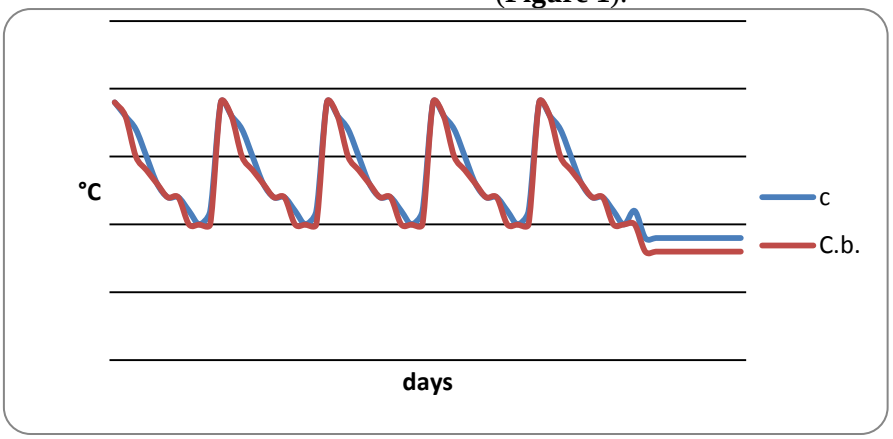

Figure 1. Water temperature in control (C) and experimental (C. b.) tanks

The dissolved oxygen during the experiment ranged between 7.35 mg.l^{-1} and 8.32 mg.l^{-1} (**Figure 2**). The values of this parameters during the trial period were higher with 3.85% in the experimental tanks comparison to these of the controlled.

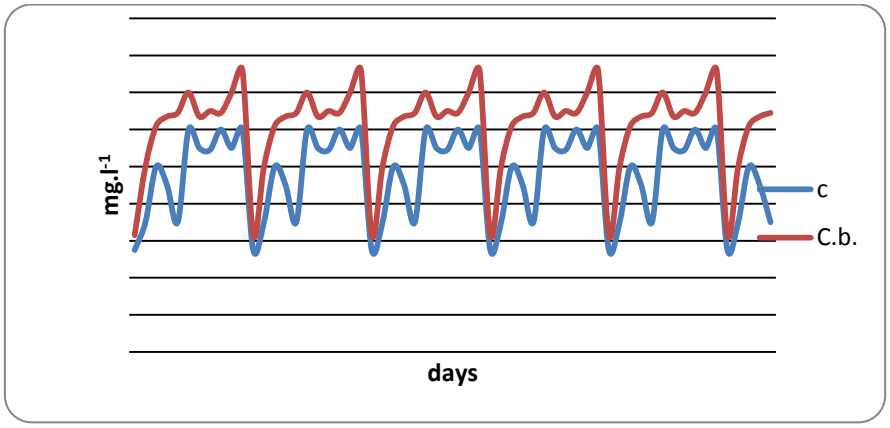

Figure 2. Dissolved oxygen in control (C) and experimental (C. b.) tanks

Young fish are extremely sensitive to pH levels and it is very important not to be under 6,5 and over 8,5. Water pH values in the recirculation system varied between 8.02 and 7.3 which were slightly alkaline (**Figure 3**).

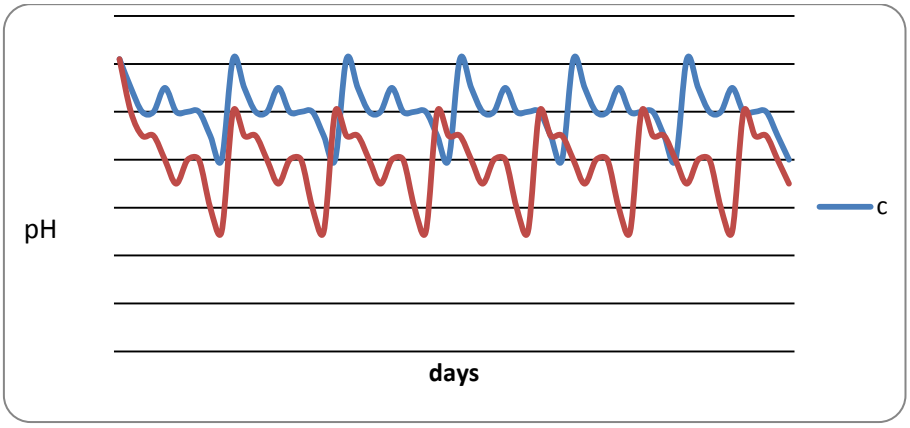

Figure 3. pH in control (C) and experimental (C. b.) tanks

Electric conductivity of water varied from 263 µS.cm^{-1} to 269 µS.cm^{-1} (**Figure 4**). The values of this parameter in experimental variant were with 0.8% higher compared to these one of the control. The analysis of hydrochemical data showed that during the experiment they were optimum for the farmed species.

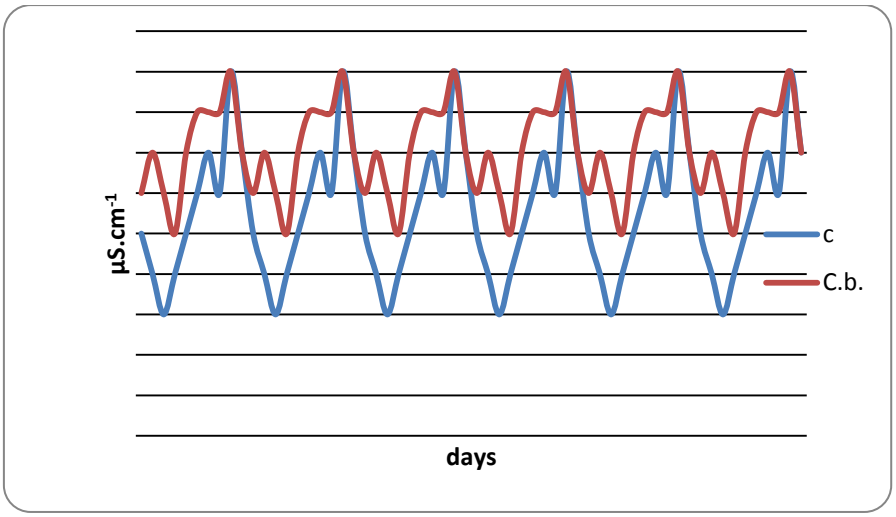

Figure 4. Electric conductivity in control (C) and experimental (C. b.) tanks.

Tanks were cleaned three times per day, with addition of fresh water in amount of 10% from the total recirculation system volume. To maintain the optimum water chemical parameters during the experiment, the mechanical filter and the biofilter in particular was of major significance. This led to good results with respect to survival, weight gain and feed conversion ration in experimental fish.

Amonium concentration was from 0.29 mg.l^{-1} to 1.63 mg.l^{-1} in the water of the two variants for 60 days period. Phosphorus ranged between 0.56 – 1.53 mg.l^{-1} for the both group during the trial (**Table 1**).

Table 1. *Amonium and phosphorus concentration in control (C) and experimental (C. b.) tanks*

Parameters/Group	n	NH4+ mg.l^{-1}	P-PO4- mg.l^{-1}
C	8	0.55±0.12	0.8±0.54
C. b.	8	0.63±0.46	0.91±0.3

Growth performance and feed utilization efficiency

Table 2. *Growth performance of rainbow trout in control (C) and experimental (C. b.) tanks*

Parameter	**n**	**C**	**C. b.**
		$\bar{x}$ ± SD	$\bar{x}$ ± SD
Initial body weight, g	40	13.32±3.07	13.33±2.58
Final body weight, g	40	40.97±0.4*	45.80±0.3*
Survival rate, %		100	100
SGR % per day		1.86±0.16	2.05±0.002
Average individual weight gain, g	40	27.65±1.02*	32.47±1.06*
FCR		1.83	1.54

*P<0.05

The average initial live weight of rainbow trout from control and experimental variants was respectively 13.32±3.07 g and 13.33±2.58 g and the differences were not statistically significant (P>0.05) (**Table 2**).

By the end of the experiment was received average live weight of fish, fed with blessed thistle extract supplemented - 45.8±0.3 g which was with 10.6% higher compared to the value of the same parameter of control trouts (P<0.05) (**Table 2**).

Survival rate during the experiment showed 100% in fish in experimental and control group (**Table 2**).

The average individual weight gain of rainbow trout from the group fed with blessed thistle extract supplemented was 32.47±31.06 g which was with 14.8% higher compared to this one of control fish and the differences were statistically significant (P<0.05) (**Table 2**).

At the end of the trial, the analysis of consumed feed amount showed that feed conversion ratio of trouts in the experimental group was 1.54 and it was 18.83% lower than this one in control fish (**Table 2**). The growth parameters of rainbow trout were higher in the group fed with blessed thistle extract supplemented.

Biochemical blood parameters

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

Blood parameters/Groups	n	C x±SD	C. b. x±SD
Glu mmol.l^{-1}	6	5.06±0.67	5.20±0.79
Urea mmol.l^{-1}	6	0.64±0.31	0.73±0.06
Crea μmol.l^{-1}	6	6.80±4.21	11.50±9.19
TP g.l^{-1}	6	38.42±8.36	39.80±4.03
Alb g.l^{-1}	6	17.10±1.91	19.30±3.82
ASAT U.l^{-1}	6	71.67±107.04	52.00±76.11
ALAT U.l^{-1}	6	23.20±43.89	13.00±18.22
ALP U.l^{-1}	6	416.00±206.35	311.00±148.85
Ca mmol.l^{-1}	6	2.23±0.49	2.18±0.89
P mmol.l^{-1}	6	4.93±2.02	4.59±1.39
Mg mmol.l^{-1}	6	0.76±0.13	0.80±0.23
TG mmol.l^{-1}	6	1.77±0.16	1.69±0.03
CHOL mmol.l^{-1}	6	5.83±2.25	7.10±2.55

The blood glucose level may vary by season and water temperature, also its concentration decreases is depending on age and size of fish (7). In the present study, the value glucose level was 2.7% lower in rainbow trout from control group, but the differences was not statistically proven (P>0.05) (**Table 3**). The urea level was 14.06% lower for control fish compare to these ones from the experimental variant, but with not statistically significant differences(P>0.05). The creatinine level was 69.1% higher in the versus fed with the blessed thistle supplement compare to control group (P>0.05). The level of the total protein was higher by 3.6% in the trouts fed with the supplement compared to control, although this difference did not reach statistical significance (P>0.05). The increased concentration of plasma protein may be due to structural liver alternations that reduce aminotransferase activity, with a concurrent decrease in deamination capacity (8). According to Hrubec et al. (9) the striped bass protein level increased with age. Albumin transports hormones, vitamins, and substances like calcium throughout the body. Also keeps fluid from leaking out of blood vessels and nourishes tissues. The amount of albumin in the experimental fish blood is higher with 12.86% compare to control group. ASAT, ALAT and ALP values are higher with 27.4%, 44% and 25.2% respectively in blood of trouts from control group compare to experimental variant. These results show that the blessed thistle extract has a positive effect on liver function. Cholesterol and triglyceride levels of fish may be affected by pollution agents (10, 11). The triglyceride concentrations in serum of rainbow trout fed with supplement were lower with 4.52% than in control fish. The measured cholesterol are lower with 21.7% in control variant compare to the blessed thistle supplement experimental group, but with not statistically significant differences (P>0.05). The change in blood electrolytes may bring disturbances in the normal vital physiological functions of the fish and its growth rate (12). Calcium is a very important mineral element and presents in larger amounts compared to the other electrolytes. It is contained in the bones in combines with phosphorus under the form of calcium phosphate. The ionized calcium is very important to the normal excitability of muscles and in blood coagulation (13). In this

study the blood calcium and phosphorus levels of rainbow trout are similar in both studied groups. Magnesium is higher with 5.26% in the fish from experimental group compare to control and the differences was statistically proven (P<0.05). Biochemical parameters have been used as valuable tools for fish health monitoring and the normal ranges of the key biochemical parameters are still undefined for water species in different aquaculture conditions (14).

Chemical analyses of meat samples

Table 4. *Chemical composition of the fillets of the rainbow trout (O. mykiss) in control (C) and experimental groups (C. b.) (%)*

Indicator / Group	Moisture $\bar{x} \pm$ SD	Dry matter $\bar{x} \pm$ SD	Grude protein $\bar{x} \pm$ SD	Fat $\bar{x} \pm$ SD	Ash $\bar{x} \pm$ SD
C	74.24±0.63	25.76±0.63	18.66±0.30	5.36±0.22	1.75±0.16
C. b.	75.63±0.42 **	24.38±0.42 **	18.59±0.22	4.30±0.53 **	1.50±0.07 **

**P<0.01

The *C. benedictus* supplementation in the diet led to 1.84% higher moisture content in the fillets of the rainbow trout , compared to that of the control (P <0.01) (**Table 4**). The protein content in the fish of the control group and experimental were of close value, but not statistically proven (P>0.05). The blessed thistle supplementation in the diet led to lower the lipid content in the fillets of the rainbow trout with 19.7% compare to control fish and was statistically proven (P <0.01). This result shows that fish fed with the supplement are more useful and dietetic in their quality as human food. The dry matter content in the meat of the experimental fish group was 24.38 ± 0.42% and it was significantly lower than control group with 5.4% (P <0.01). The ash content of the fillets of trouts in the control were higher with 14.2% compare to this one of individuals from the experimental group (P <0.01) (**Table 4**).

CONCLUSION

For the first time the effect of a *Cnicus benedictus* extract as a supplement to the feed of rainbow trout was tested. Fish fed with blessed thistle supplement have better growth performance, feed conversion ratio and blood biochemical parameters. Trouts fed with the blessed thistle extract have higher meat quality and will be more useful as a human food.

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