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Effect of Acorus calamus L. extract on growth performance and blood parameters of common carp (*Cyprinus carpio* L.) cultivated in a recirculation system

Ефект на Acorus calamus L. екстракт върху растежа и кръвните параметри на обикновен шаран (*Cyprinus carpio* L.), отглеждан в рециркулационна система

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ABSTRACT

The aim of this study was to determine the effect of the sweet flag (*A. calamus* L.) extract addition of growth performance, haematological (white blood cells, red blood cells, hemoglobin, hematocrit) and biochemical (glucose, urea, creatinine, total protein, albumin, ASAT, ALAT, Ca, P, Mg, triglycerides, cholesterol) blood parameters of common carp (*Cyprinus carpio* L.) cultivated in recirculation system. Carps were randomly selected and distributed into four concrete tanks for the growth trial (8 fish/tank). Fish were divided into experimental (E) and control groups (C), each of them with two replicates and mean initial weight 908.5±171.2 g and 913.8±147.4 g, respectively. They were fed with pelleted carp feed with 25% crude protein, produced by the "Top mix" company, having a granule size of 6 mm. To the fish feed on the experimental group (E) was added 1% aqueous extract of *A. calamus* root, as well as oiling the pellets with 5 mL of sunflower oil for every 100 g of feed. Carps from control group (C) were fed with feed only greased with the same amount of sunflower oil. The daily ration that the studied fish received was 1.8% of their live weight. The duration of the trial period was 45 days. At the end of the experiment with 5.2% was measured a higher average live weight in the fish receiving the sweet flag supplement compare to the value of this parameter of carps from control group, and the differences were statistically significant ($P<0.05$). The survival rate was 100% in control and experimental variants. At the end of the experiment, the average individual weight gain of the experimental fish fed with the supplement was higher with 53.2% compared to this one of carps from control. The feed conversion ratio in experimental carps, supplemented with 1% sweet flag extract was with 16.1% lower than that of control fish, although the differences were not relevant ($P>0.05$).Better blood biochemical and haematological parameters were measured in carp fed with sweet flag supplement.

Keywords: common carp, growth, haematological and biochemical blood parameters, sweet flag

АБСТРАКТ

Целта на това проучване е да се определи ефектът от добавянето на екстракт от блатен аир (*A. calamus* L) върху растежа, хематологични (бели кръвни клетки, червени кръвни клетки, хемоглобин, хематокрит) и биохимични (глюкоза, урея, креатинин, общ протеин, албумин, ASAT, ALAT, Ca, P, Mg, триглицериди, холестерол) кръвни параметри на обикновен шаран (*Cyprinus carpio* L.), отглеждан в рециркулационна система. Рибите се разделят на експериментални (E) и контролни групи (C), всяка от тях с две повторения и средно тегло 908.5 ± 171.2 g и 913.8 ± 147.4 g, съответно. Те са хранени с гранулиран фураж за шарани с 25% суров протеин, произведен от фирма "Top mix", с размер на гранулите 6 mm. Към храната за рибите от експерименталната група (E) се прибавя 1% воден екстракт от корен на *A. calamus*, както и омасляване на пелетите с 5 мл слънчогледово масло на всеки 100 г фураж. Шараните от контролната група (C) са хранени с фураж, само омаслен със същото количество слънчогледово масло. Дневната дажба на изследваната риба е 1.8% от тяхното живо тегло. Продължителността на експерименталния период е 45 дни. В края на експеримента с 5.2% се измерва по-високо средно живо тегло на рибата, получаваща добавката с блатен аир, в сравнение със стойността на този параметър за шараните от контролната група, като разликите са статистически значими ($P<0,05$). Преживяемостта е 100% в контролните и експерименталните варианти. В края на експеримента средният индивидуален прираст на теглото на експерименталната риба, хранена с добавката, беше по-висок с 53.2% в сравнение с този при шараните от контрола. Хранителният коефициентът при експерименталните шарани, с добавка 1% екстракт от блатен аир, е с 16.1% по-нисък от този на контролните риби, въпреки че разликите не са статистически доказани ($P>0,05$). По-високи биохимични и хематологични параметри на кръвта са измерени при шараните, хранени с добавка на блатен аир.

Ключови думи: блатен аир, шаран, растеж, хематологични и биохимични кръвни параметри

INTRODUCTION

The introduction of new biotechniques in aquaculture stimulated the demand for effective feeds that meetthe nutritional needs of individual species (Sargent and Tacon, 1999). The application of phytotherapy in the field of aquaculture is a relatively newarea of research which is still developing. The herbs may be used in many ways such as fresh, dried, powdered,extracted (in various solvents like water, alcohol, acetone, ether, etc),or essential oils (Raman, 2017). The advantage of herb therapy is that it does not stress the fish. It is interesting to trace the influence of extracts of herbs that lead to increased appetite. One of these herbs is *Acorus calamus* (sweet flag) a known herbal drug commonly used in traditional medicine. It was found that *A. calamus* has a wide range of pharmacological activities, such as anti-diabetic (Prisilla et al., 2012), central nervous system depressant (VengadeshPrabu et al., 2009), anti-inflammatory (Kim et al., 2009), antioxidant (Subathraa and Poonguzhali, 2012), antispasmodic (Gilani et al., 2006), antibacterial (Manikandan et al., 2010), antifungal (Begum et al., 2007), and cardiovascular (Shah and Gilani, 2012) and insecticidal agent (Nalamwar et al., 2009). A large number of studies predominate on the effects of herbs on the treatment of various infectious and parasitic diseases in fish (Mazurkiewicz, 2009; Raman, 2017). There are no studies aimed at determining the effects of phyto-additions on the growth indicators and the use of feed in different fish species. Sweet flag is an herb used for the appetite and as an aid to the digestion. The nutritional properties present in *A. calamus* can cause an increase in growth and health of fish when they consume this plant, through a variety of mechanisms including increasing digestibility, binding of various nutrients or upgrade the intestinal wall, and thereby raising digestive efficiency. On the other hand, the most common aquaculture products are freshwater, omnivorous fish, most of which come from the Cyprinidae family. The aim of this study was to determine the effect of the sweet flag (*A. calamus*) extract addition of growth performance, haematological and biochemical blood parameters of common carp (*C. carpio*) cultivated in recirculation system.

MATERIAL AND METHODS

Fish and feeding

Carps were randomly selected and distributed into four concrete tanks for the growth trial (8 fish/tank). Fish were divided into experimental (E) and control groups (C),each of them with two replicates and mean initial weight 908.5±171.2 g and 913.8±147.4 g, respectively. The concrete tanks have an effective water volume of 0.8 m^3, which are part of a recirculation system.The fish were fed with pelleted feed for carp with 25% crude protein produced by the "Top mix" company, having a granule size of 6 mm. To the fish feed on the experimental group (E) was added 1% aqueous extract of *A. calamus* root, as well as oiling the granules with 5 mL of sunflower oil for every 100 g of feed. Carps from control group (C) were fed with feed only greased with the same amount of sunflower oil. The nutrient content in the feed of the two groups is: 25% crude protein, 3.1% crude lipids, 6% crude fiber, 8% crude ash, 1.04% P, 11.1 MJ/kg ME, 4,800 IU/kg Vitamin A, 900 IU/kg Vitamin D. The daily ration that the studied fish received was 1.8% of their live weight and they were fed three times per day. The duration of the trial period was 45 days.

Growth performance

The average individual weight (g) of the fishwas calculated at the start and end of experiment in order to study the sweet flag influence on the weight gain and feed conversion ratio in the common carp (*C. carpio*), 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.

Water quality

The oxygen content (mg/L), pH, temperature andelectrical conductivity (µS/cm) were measured dailywith a portable meter (HQ30D) accordingly with LDO combined probe for measurement of oxygen and temperature, pH (liquid) and conductivity electrodes.

Haematological and biochemical blood parameters

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 haematological and biochemical blood parameters were investigated: glucose (mmol/L), urea (mmol/L), creatinine (µmol/L), total protein (g/L), albumin (g/L), ASAT (U/L), ALAT (U/L), Ca (mmol/L), P (mmol/L), Mg (mmol/L), triglycerides (mmol/L), cholesterol (mmol/L), white blood cells (x10^9 L), red blood cells (x10^{12} L), hemoglobin (g/L) and hematocrit (%).

Statistical analysis

The data received from the trial were statistically analysed with ANOVA single factor (MS Office, 2010).

RESULTS AND DISCUSSION

Water quality

The water quality during the trial period are presented in Table 1. They are in the optimal limits for common carp cultivating in the recirculation system. Water temperature was 24±1.48 °C, and dissolved oxygen content was 6.52±0.22 mg/L during the experiment. Water pH in the recirculation system in the different tanks was 7.88±0.21. Electric conductivity of water during the experiments was 678±15 µS/cm. Every day the tanks were cleaned and fresh water is added every day to an amount 10% of the total volume of the recirculation system. Principal role for optimum water chemical parameters had biofilter and mechanical filter.

Growth of the common carp

At the beginning of the trial the average individual weight of common carp was 908.5±171.2 g for control group and 913.8±147.4 g for experimental, as the differences were not statistically significant (P>0.05) (Table 2). The survival rate was 100% in control and experimental variants.

Table 1. Water quality in the recirculation system during the experiment with common carp

Parameter	n	$\bar{\chi}$±SD	Optimum values (Regulation № 4/2000)
Temperature (°C)	45	24±1.48	22-26
Dissolved oxygen (mg/l)	45	6.52±0.22	>5
pH	45	7.88±0.21	6.5-8.5
Electric conductivity (µS/cm)	45	678±15	-

At the end of the trial period with 5.2% was measured a higher average live weight in the fish receiving the sweet flag supplement compare to control group, and the differences were statistically significant (P<0.05).

The average individual weight gain of carps from the two experimental replications (supplemented with1% sweet flag aqua extract) was134.1±1.76 g vs 87.5±51.02 g in non-supplemented fish but the differences were not statistically significant (P>0.05) (Table 2). At the end of the experiment, the average individual weight gain of the experimental fish fed with the supplement was higher with 53.2%, compared to values of these parameters of carps from control.

At the end of the trial, the analysis of consumed feed amount showed that feed conversion ratio in the group supplemented with1% sweet flag aqua extract was 3.02±0.04 and in control carps: 3.6±0.06 (P>0.05; Table 2).

The feed conversion ratio in experimental carps, supplemented with 1% sweet flag extract was with 16.1% lower than that of control fish (Table 2), although the differences were not relevant (P>0.05).

Haematological and biochemical blood parameters

A. calamus is an herb used for the appetite and as an aid to the digestion. Furthermore, it has antidiabetic, anticellular, immunosuppressive, antidiarrheal, insulin-sensitizing activity (Shetty and Shruthi, 2015) and for this reason blood samples were investigated from the studied fish. The values of blood test parameters are presented in Table 3.

According to Coz-Rakovac et al. (2005) the blood glucose level may vary by season and water temperature, also glucose concentration decreases are depending on age and size of fish. Si et al. (2010) obtained four fractions from the radix of *A. calamus* and were used for insulin

Table 2. Growth performance of common carp

Parameter	n	$\bar{\chi}_C$ ±SD	$\bar{\chi}_E$ ±SD
Initial body weight (g)	16	908.5±171.2	913.8±147.4
Final body weight (g)	16	996.09±153.4*	1047.9±38.3*
Survival rate (%)	16	100	100
SGR % per day	16	0.2±0.11	0.3±0.003
Average individual weight gain (g)	16	87.5±51.02	134.1±1.76
FCR	16	3.6±0.06	3.02±0.04

* P<0.05, SGR – specific growth rate, FCR – feed conversion ratio.

releasing or alpha-glucosidase inhibitory action. In this study, the glucose level was 57.8% lower in carp fed with sweet flag supplement (Table 3). The urea level was 23.4% lower for carp fed with sweet flag supplement compare to control variant. The creatinine level was 20% higher in the control carp compare to versus fed with the sweet supplement. The level of the total protein was higher by 23.3% in the carp from the control compared to those fed with the supplement. The increased concentration of plasma protein may be due to structural liver alternations that reduce aminotransferase activity, with a concurrent decrease in deamination capacity (Kavadias et al., 2004). According to Hrubec et al. (2001) the striped bass protein level increased with age. The amount of albumin in the fish blood in control (17.85±13.2 mg/L) and experiment groups (17.35±1.8 mg/L) were very close. Slightly elevated ALAT values 38.8±12.7 mg/L in blood experimental group are probably due to the proven antihepatotoxic activity of the ethanolic extract of the plant is due to the increase in the level of the serum level of hepatic enzymes and total bilirubin levels (Palani et al., 2009). According to Hill (1982) with increasing fish size the cholesterol concentrations expansion. The measured cholesterol and triglyceridesare lower respectively with 37% and 18% in the sweet flag supplement experimental group compared to control. The change in blood electrolytes may bring disturbances in the normal vital physiological functions of the fish and its growth rate (Prasad et al., 2011). Calcium is a very important mineral element and presents in larger amounts compared to the other electrolytes. It

Table 3. Blood parameters of fishes

Blood parameters	n	C	E
Glu (mmol/L)	6	9.54±7.5	4.03±19.1
Urea (mmol/L)	6	1.58±0.23	1.21±0.38
Crea (µmol/L)	6	6±5.6	4.8±21.1
TP (g/L)	6	37.3±58.6	28.6±39.1
Alb (g/L)	6	17.85±13.2	17.35±1.8
ASAT (U/L)	6	7.5±7.2	7.8±11.2
ALAT (U/L)	6	19±37.2	38.8±12.7
Ca (mmol/L)	6	2.04±0.2*	2.6±0.1*
P (mmol/L)	6	5.34±3.4	4.68±0.8
Mg (mmol/L)	6	1.65±0.04	1.67±0.05
TG (mmol/L)	6	0.95±0.3	0.78±0.1
CHOL (mmol/L)	6	2.2±0.12	1.39±1.5
WBC x10^9 L	6	164.4±12.92	180.5±78.2
RBC x10^{12} L	6	0.7±0.3	0.71±0.2
HGB (g/L)	6	92.3±13.3	97.2±28.7
HCT (%)	6	11.73±6.1	12.58±21.4

* $P<0.05$

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 (Kulkarni, 2015). In this study the blood calcium is higher with 22% in the fish from experimental group compare to control and the differences was statistically proven (P<0.05). Magnesium and phosphorus levels are similar in both studied groups. Haematological and biochemical parameters have been used as valuable tools for fish health monitoring. Unfortunately, the normal ranges of the key biochemical parameters are still undefinedfor different species in different aquaculture conditions (Satheeshkumar et al., 2011). In this study higher RBC (red blood cells) and WBC (white blood cells) count were reported in experimental group. The hemoglobin and hematocrit blood content are higher respectively with 5.04% and 6.16% in the sweet flag supplement experimental group compared to control.

CONCLUSIONS

Better growth performers, better blood biochemical and haematological parameters were measured in carp fed with sweet flag supplement. This is probably due to phytochemical and biological activities in *A. calamus* extract. The literature review did not establish studies with a sweet flag extract as a feed additive for fish. This allows other types of fish to be tested with other *A. calamus* concentrations in the future.

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