AGRICULTURAL SCIENCE AND TECHNOLOGY, VOL. 13, No 3, pp 266-271, 2021
Published by Faculty of Agriculture, Trakia University, Bulgaria
ISSN 1313-8820 (print)
ISSN 1314-412X (online)
http://www.agriscitech.eu
DOI: 10.15547/ast.2021.03.042

Effect of black pepper (*Piper nigrum*) extract on productive traits, economic efficiency and blood biochemical parameters of rainbow trout (*Oncorhynchus mykiss* W.)

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(Manuscript received 5 June 2021; accepted for publication 10 August 2021)

Abstract. *The aim of the present study was to evaluate the effect of the dietary black pepper extract supplement on the survival rate, weight gain, feed conversion ratio (FCR), economic efficiency (ECR) and blood biochemical parameters of rainbow trout, cultivated in a recirculation system. One hundred and twenty rainbow trout were distributed in two experimental variants, with two replicates of 30 fish per each. The average initial live weight of fish from the control group (C) and experimental group (P.n) were 41.55±7.96 g and 41.23±8.38 g, respectively (p>0.05). Trouts were fed extruded feed with granule size of 2 mm. Through lubrication of feed granules with 5 ml sunflower oil per 100 g feed, rainbow trouts from group P.n were supplemented with 1% powdered black pepper extract, whereas control rainbow trouts (C) received feed lubricated with the same amount of sunflower oil. Fish were fed 3 times/daily with the diet, based on 3% of biomass. The trial period was 60 days. Survival rate in trouts from both experimental groups was 100%. At the end of the trial, average live weight of fish from group P.n was by 5.84% higher compared to that of control fish the average weight of which was 117.25±19.15 g, yet differences were not statistically significant (p>0.05). The average individual weight gain of control rainbow trouts was 75.70±11.53 g, which was inferior to that of P.n fish by 8.65% (p>0.05). Better FCR was demonstrated in trouts from the group supplemented with 1% black pepper extract - 0.98±0.02, which was by 10.20% less compared to control fish (p<0.01). The addition of 1% black pepper extract to the feed of rainbow trouts tended to result a trend in better growth performance parameters in this species. ECR of group P.n was by 3.98% better than that of the control fish, which showed an ESR value of 1.51. Blood biochemical parameters of fish from both groups were not consistently different*.

Keywords: black pepper extract**,** feed additive, feed conversion ratio, *Oncorhynchus mykiss*, survival rate, weight gain

Introduction

Dietary supplements in feed are used for improvement of main properties such as taste, aroma, digestibility and slower degradation of granules in water. All this is expected to result in higher weight gain, lower feed conversion and better economic efficiency of aquaculture. Despite numerous studies carried out so far with various phytoextracts, e.g. curcumin, paprika, oregano, garlic (Georgieva et al., 2018, 2019), nutmeg (Zhelyazkov et al., 2018), *Acorus calamus* (Velichkova et al., 2019), *Achillea millefolium* (Koshinski, 2019), *Taraxacum officinale* (Sirakov et al., 2019; Koshinski, 2020) and others, mainly in carps and rainbow trouts, their effects on growth and health of different fish species are still underinvestigated and what is more, there are plenty other potentially useful additives. Phytoextracts are relatively cheap; being natural products, they are safe for both fish and men as ultimate consumers (Gabor et al., 2010, 2011). Thus, scientists believe that they will have an essential role in feed industry in the future as well (Georgieva and Zhelyazkov, 2018; Stoyanova et al., 2018a,b; Zhelyazkov et al., 2018; Georgieva et al., 2018, 2019, 2000; Sirakov et al., 2019; Velichkova et al., 2019; Koshinski, 2019, 2000).

Black pepper (*Piper nigrum*) is a plant from the family Piperaceae, grown for its fruits known as pepper, which are dried and used as spice (Harrison, 2016). It originates from modern South India (Sen, 2004; Hadjeski, 2016), and is cultured commonly there and in other tropical regions. Pepper has strong antioxidant properties due to tocopherols and polyphenols content. It is reported to have gastric, carminative, antioxidant, antibacterial, antimicrobial, immunomodulating, larvicide, antibiotic, anti-inflammatory, anticancer, antipyretic properties (Charles, 2012). Black pepper is top one of spices sold and one of the commonest spices used in global culinary.

The immunomodulating and anticancer properties of piperine were studied *in vitro* (Majdalawieh and Carr, 2010). Its role in the pharmacokinetics of curcumin in animals and men was also investigated (Shoba et al., 1988). The effect of black pepper as dietary supplement was determined in broiler chickens with respect to growth performance, carcass traits, some blood parameters and humoral immune response (Abou-Elkhair et al., 2014). The research on black pepper use in aquaculture are relatively few, use of black pepper seeds as growth enhancer for juvenile of Florida hybrid red tilapia (Shalaby et al., 2014) and *Labeo rohita* (Matiullah et al., 2016), study of the effect of black pepper on Nile tilapia fish weight and the gene expression of growth hormone receptor (GHR) and insulin as growth factor (IGF) (Dowidar et al., 2017), and dietary amino acid utilization and growth performance in common carp

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(Wojno et al., 2021). The metabolism of different fish species is specific, so biologically active substances in plant extracts could have either positive or negative effects, so their influence should be studied in each individual species.

The aim of the present study was to evaluate the effect of the dietary black pepper (*Piper nigrum*) extract supplement on the survival rate, weight gain, feed conversion ratio (FCR), economic efficiency (ECR) and blood biochemical parameters of rainbow trout, cultivated in a recirculation system.

Material and methods

Ethical approval

All applicable international, national, and/or institutional guidelines for the care and use of animals were followed by the authors (Directive 2010/63/EU; Regulation No 20/2012).

Experimental design

One hundred and twenty rainbow trouts were allotted to two experimental variants – control (C) and experimental (*Piper nigrum* - P.n), each of them in two replications with 30 fish. The average initial body weight of fish was 41.55±7.76 g (C) and 41.23±8.38 g (P.n). Fish were housed in concrete tanks with efficient volume of 0.8 m^3, within a recirculation system supplied with mechanical filter and moving bed biofilter in the Aquaculture Base of the Faculty of Agriculture at Trakia University. The flow rate in RAS was maintained at 133 $l.min^{-1}$. The water in the fish tanks was additionally aerated by means of a compressor with a flow rate of 150 $l.min^{-1}$. To compensate for water losses, fresh water was added on a daily basis at the amount of 10% of the recirculation system volume. Trouts were fed extruded feed Aqua garant UNI with granule size of 2 mm, made by Garant-Tiernahrung Gesellschaft m.b.H. - Austria. Through lubrication of feed granules with 5 ml sunflower oil per 100 g feed, rainbow trouts from group P.n were supplemented with 1% powdered black pepper extract (manufactured by P.I.C.Co LTD), whereas control rainbow trouts (C) received feed lubricated with the same amount of sunflower oil. Fish from both groups received feed equal to 3% of their live weight manually, 3 times per day. The trial duration was 60 days, January-March 2018. The nutritional content of feed offered to both experimental groups is presented in Table 1.

Table 1. Nutrient content in the extruded feed for a rainbow trout (*Oncorhynchus mykiss* W.)

No	Item	Groups	
		C	P.n
1	Crude protein, %	45.00	45.00
2	Crude lipids, %	16.00	16.00
3	Crude fiber, %	2.40	2.40
4	Crude ash, %	8.00	8.00
5	Ca, %	1.60	1.60
6	P, %	1.20	1.20
7	Black pepper extract, %	-	1.00
8	ME, MJ/kg	18.50	18.50

*C- control group, P.n- experimental group; 1 kg feed contains: vitamin A- 10000 IE, vitamin D_3- 1500 IE, vitamin E- 200 mg, vitamin C- 150 mg, Fe- 62 mg, Mn- 26 mg, Cu- 5 mg, Zn- 103 mg, I- 2.6 mg; Se- 0.3 mg.

Hydrochemical analysis

Water chemical parameters of the recirculation system during the experiment with rainbow trout (*Oncorhynchus mykiss* W.) were determined as follows:

- Water temperature - °C, dissolved oxygen - $mg.l^{-1}$, pH and electric conductivity - $\mu S.cm^{-1}$ with MultiLineP4 equipment (Xylem Analytics Germany Sales GmbH & Co. KG, WTW);
- Ammonia, $mg.l^{-1}$ - BSS ISO 7150-1:2002;
- Nitrates, $mg.l^{-1}$ - BSS ISO 7890-3:1988;
- Nitrites, $mg.l^{-1}$ - BSS ISO 26777:1997;
- Orthophosphates, $mg.l^{-1}$ - BSS EN ISO 6878:2005.

Throughout the trial, water temperature, dissolved oxygen, pH and electric conductivity were monitored on a daily basis, whereas ammonia, nitrates, nitrites and orthophosphate contents – on a weekly basis.

Determination of growth performance

The effect of black pepper extract added to extruded feed on fish weight gain and feed conversion ratio of rainbow trouts (*Oncorhynchus mykiss* W.) in the recirculation system was evaluated by weighing fish at the beginning, middle and end of the experimental period. Average body weight (g) was determined individually. By the end of the experiment, weight gain (g), survival rate (%), and feed conversion ratio (FCR) were determined.

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

Survival (%) = (FNF/INF).100,

Where: FNF- Final number of fish; INF- Initial number of fish.

The experimental fish were individually weighed on a technical balance with accuracy 0.01g at the beginning, middle and end of the trial. On this basis, the average individual weight gain (WG, g) was determined by the following equation:

WG (g) = AFW - AIW,

Where: AFW- Average final weight; AIW- Average initial weight.

At the end of trial feed conversion ratio (FCR) was also calculated by the following equation:

FCR = Feed fed (g) / Weight gain of fish (g)

Economic analysis

The analysis of the economic efficiency of dietary black pepper extract supplementation of rainbow trouts in a recirculation system was performed with data for feed conversion ratio, survival rate and weight gain. These traits were compared between the experimental groups and extruded feed costs were calculated. The cost of 1 kg weight gain was determined. The economic conversion ratio (ECR) was calculated according to Piedecausa et al. (2007):

ECR = Cost of diet x Feed conversion ratio (FCR)

Determination of blood biochemical parameters

Blood samples of 6 fishes from experimental (P.n) and control (C) group were obtained through caudal vessels puncture using EDTA containers (3 ml). Glucose (GLU), urea, creatinine (CREA), total protein (TP), albumin (ALB), ASAT, ALAT, alkaline phosphatase (ALP), calcium (Ca), phosphorus (P), magnesium (Mg), triglyceride (TG), and cholesterol (CHOL) concentrations were determined by means of BS-120 Chemistry Analyzer (Mindray, China).

Statistical analysis

Statistical analysis was performed using STATISTICA 6.0 software (StatSoft Inc., 2002). Mean, standard deviation and t-test were performed. The statistical significance was determined at $p<0.05$.

Results

Hydrochemical analysis

Average water temperature during the 60-day experimental period was 14.0ºC, within the range from 12.5ºC to 15.5ºC. Dissolved oxygen was maintained higher than 9 $mg.l^{-1}$, and water pH in control (C) and experimental (P.n) groups was 7.64±0.56 and 7.62±0.52, respectively. Water electric conductivity was 485 $\mu S.cm^{-1}$. Detected values of ammonia, nitrates, nitrites and orthophosphates in the recirculation system water, measured on a weekly basis, were far below maximum allowances stipulated by Regulation No 4/2000 (Table 2).

Table 2. Water parameters in the recirculation system during the experiment with rainbow trout (*Oncorhynchus mykiss* W.)

Parameter	n	C	P.n	Significance	Optimum values*
		$\bar{x}$±SD	$\bar{x}$±SD		
Temperature, ºC	60	14.00±1.50	14.00±1.50	NS	12.0-16.0
Dissolved oxygen, $mg.l^{-1}$	60	9.68±0.28	9.63±0.24	NS	> 9
pH	60	7.64±0.56	7.62±0.52	NS	6.0-9.0
Electric conductivity, $\mu S.cm^{-1}$	60	485±35.24	485±38.76	NS	-
Ammonia, $mg.l^{-1}$	8	0.65±0.23	0.66±0.20	NS	< 1.0
Nitrates, $mg.l^{-1}$	8	0.35±0.04	0.33±0.02	NS	< 2.0
Nitrites, $mg.l^{-1}$	8	0.007±0.001	0.008±0.001	NS	< 0.01
Orthophosphates, $mg.l^{-1}$	8	0.253±0.136	0.248±0.129	NS	< 0.40

*Regulation No 4/2000, C- control group, P.n- experimental group, NS- Non-significant.

Determination of growth performance

At the beginning of the trial, average live weight of rainbow trouts from the two replications of groups C and P.n was 41.55±7.76 g and 41.23±8.38 g, respectively, with insignificant differences ($p>0.05$) (Table 3). In the middle of the experimental period, fish from group P.n attained live weight of 71.63±10.68 g, which was by 6.60% higher than the weight of controls ($p>0.05$). The same tendency was preserved by the end of the period, when the weight of fish supplemented with 1% black pepper extract exceeded that of control fish (117.25±19.15 g) by 5.84%, but differences were irrelevant ($p>0.05$). Survival rate in trouts from both groups was 100%. The mean individual weight gain of fish from group P.n was 82.87±11.33 g, e.g. by 8.65% higher than that of fish from group C, with insignificant differences ($p>0.05$). Significantly better feed conversion ratio (FCR) was calculated in rainbow trouts supplemented with 1% black pepper extract (0.98±0.02) - by 10.20% lower than FCR of controls ($p<0.01$) (Table 3).

Table 3. Fish production parameters

Parameter	n	C	P.n	Significance
		$\bar{x}$±SD	$\bar{x}$±SD	
Initial body weight, g	60	41.55±7.76	41.23±8.38	NS
Body weight in the middle of the trial, g	60	66.90±14.77	71.63±10.68	NS
Final body weight, g	60	117.25±19.15	124.10±19.35	NS
Survival rate, %		100	100	
Average individual weight gain, g	60	75.70±11.53	82.87±11.33	NS
Feed conversion ratio, FCR	60	1.08±0.03	0.98±0.02	**

*C- control group, P.n- experimental group, Significant difference: **$p<0.01$, NS- Non-significant.

Economic analysis

The price of extruded feed for rainbow trouts was 1400.00 €/t (VAT excluded). The addition of 1% black pepper extract increased its cost by 78 €/t VAT excluded (Table 4). Better economic conversion ratio (ESR) of 1.45 was found out in the group supplemented with 1% black pepper extract vs ESR of 1.51 in non-supplemented trouts.

Table 4. Economic efficiency of the cinnamon extract supplementation in the feed

Item	C	P.n
Price, €/t feed (VAT excluded)	1400.00	1478.00
Price, €/kg feed (VAT excluded)	1.400	1.478
ECR	1.51	1.45*

*C- control group, P.n- experimental group; ECR= Economic conversion ratio; The lowest value shows the best ECR.

Determination of blood biochemical parameters

The monitoring of health status of rainbow trouts from both studied groups via blood biochemical analysis showed that there were no statistically significant differences with respect to the analysed 13 biochemical indices between control and experimental fish (p>0.05) (Table 5).

Table 5. Blood biochemical parameters of rainbow trouts (*Oncorhynchus mykiss* W.)

Parameter	n	C	P.n	Significance
		$\bar{x}$ ±SD	$\bar{x}$ ±SD	
GLU, mmol/l	6	5.12±0.46	5.14±0.44	NS
UREA, mmol/l	6	0.82±0.08	0.80±0.06	NS
CREA, µmol/l	6	15.58±1.15	15.52±1.09	NS
TP, g/l	6	41.85±1.64	40.92±1.18	NS
ALB, g/l	6	18.86±1.57	18.68±1.46	NS
ASAT, U/l	6	248.19±23.40	250.96±24.54	NS
ALAT, U/l	6	34.73±3.24	35.34±2.82	NS
ALP, U/l	6	555.89±80.67	552.97±80.22	NS
Ca, mmol/l	6	2.30±0.14	2.34±0.12	NS
P, mmol/l	6	5.68±0.49	5.59±0.45	NS
Mg, mmol/l	6	0.69±0.08	0.68±0.07	NS
TG , mmol/l	6	1.88±0.03	1.89±0.02	NS
CHOL, mmol/l	6	6.17±0.54	6.20±0.46	NS

*C- control group, P.n- experimental group, NS- Non-significant; Glu- glucose, Crea- creatinine, TP- total protein, Alb- albumin, ASAT- aspartate aminotransferase, ALAT- alanine transaminase, ALP- alkaline phosphatase, TG- triglycerides, CHOL- cholesterol.

Discussion

During the entire duration of the trial, water chemical parameters (temperature, dissolved oxygen, pH and electric conductivity) in the recirculation system were maintained within the optimum ranges for rainbow trout farming. The same was true for water ammonia, nitrites, nitrates, orthophosphates concentrations that were far below the maximum allowances of Regulation No 4/2000 (Table 2). No statistically significant inter-group differences were found with regard to all studied water parameters (p>0.05) due to the filtration device of the recirculation system and daily addition of 10% fresh water.

The control test on day 30 showed a trend towards higher live weight in rainbow trouts supplemented with 1% black pepper extract – by 6.60% higher than controls. The same tendency was preserved on the 60th day when supplemented fish were by 5.84% heavier on average than controls; nevertheless the observed differences were not consistent (p>0.05) (Table 3). These data supported numerous previous reports about higher live weight in fish supplemented with different phytoextracts with the feed, e.g. black pepper and turmeric in Nile tilapia (Dowidar et al., 2017), black pepper in *Labeo rohita* (Matiullah et al., 2016), black pepper in juvenile of Florida Hybrid Red Tilapia, *Oreochromis niloticus* x *Oreochromis mosambicus* (Shalaby et al., 2014), cinnamon (Dedi et al., 2016; Stoyanova et al., 2018a), savory (Stoyanova et al., 2018b) in carps, thyme, oregano and garlic (Georgieva and Zhelyazkov, 2018), *Achillea millefolium* and *Taraxacum officinale* (Koshinski, 2019, 2020) in rainbow trouts.

The addition of 1% black pepper extract to extruded feed of rainbow trouts farmed in recirculation system had no effect on survival rates, which were 100% in both groups (Table 3). This finding is in line with the reported 100% survival rate of rainbow trouts after dietary supplementation with *Achillea millefolium* and *Taraxacum officinale* extracts (Koshinski, 2019, 2020), but exceeded considerably the rates observed by Georgieva and Zhelyazkov (2018) after addition of curcumin, thyme, oregano and garlic extracts to the feed of the same species (88-93%).

The mean individual weight gain of trouts from group P.n was by 8.65% higher although statistically insignificant than that of group C (p>0.05) (Table 3). These data were comparable with those of Koshinski (2019, 2020) obtained after adding *Achillea millefolium* and *Taraxacum officinale* extracts to trout feed, as well as with the results in rainbow trouts supplemented with thyme, oregano and garlic (Georgieva and Zhelyazkov, 2018). Similar results were demonstrated after addition of cinnamon to the feed of growing and fattened carps (Dedi et al., 2016; Stoyanova et al., 2018a).

At the end of the trial, expenditure of extruded feed demonstrated improved FCR by 10.20% in fish supplemented with 1% black pepper extract compared to controls (p<0.01) (Table 3). These data confirm the reports from experiments with adding black pepper seeds in juvenile of Florida Hybrid Red Tilapia (Shalaby et al., 2014) and *Labeo rohita* (Matiullah et al., 2016), and adding extracts of *Achillea millefolium*, *Taraxacum officinale* (Koshinski, 2019, 2020) and thyme, oregano and garlic in rainbow trouts (Georgieva and Zhelyazkov, 2018). Similar FCR resulted from the addition of cinnamon to the feed of growing and fattened carps (Dedi et al., 2016; Stoyanova et al., 2018a). Better growth performance traits in the group supplemented with 1% black pepper extract could be attributed, in our view, to improved organoleptic quality of feed and its better utilization, as well as to the increased content of vitamins, minerals and phenolic compounds in the black pepper extract.

Economic conversion ratio in the supplemented group was by 3.98% better than that in non-supplemented trouts (ECR 1.51). These findings reflected worse feed conversion and lower growth rates in control fish, in line with values reported by Stoyanova et al. (2018a) in fattened carps after dietary supplementation with cinnamon.

Blood biochemical profiles of fish are influenced by the species, age, sexual maturity, temperature, photoperiod, feeding, water quality and blood sampling technique (Coşkun et al., 2016; Fazio et al., 2016). The addition of 1% black pepper extract had no effect on the studied 13 blood biochemical parameters in rainbow trouts (p>0.05) (Table 5). Available literature does not present any data about the effects of dietary black pepper extracts on blood biochemical indices of fish. Koshinski (2019, 2020) studied blood biochemical parameters in rainbow trouts supplemented with extracts from *Achillea millefolium* and *Taraxacum officinale* and concluded that no

clear effects were found out, in line with our findings. On the contrary, Georgieva and Zhelyazkov (2018) demonstrated elevated blood concentrations of urea after supplementation of rainbow trouts with curcumin, paprika and oregano; higher blood albumin in trouts supplemented with thyme, higher creatinine in fish fed thyme and garlic, elevated ALAT activities after feeding curcumin, oregano and garlic along with lower blood creatinine after addition of paprika, garlic and lower ASAT in trouts that received curcumin, oregano and garlic with feed. Georgieva et al. (2020) reported considerably elevated blood total protein in carps supplemented with curcumin, thyme and garlic, whereas Velichkova et al. (2019) found out higher blood calcium concentrations in carps supplemented with *Acorus calamus*. Sirakov et al. (2019) observed higher blood TG and CHOL in carps whose feed was supplemented with *Taraxacum officinale*. All these data evidence that various phytoextracts had various effects on fish blood biochemistry, therefore research in this field should be extended.

Conclusion

The addition of 1% black pepper extract to the feed of rainbow trouts resulted a trend in better growth performance traits and improved economic conversion when this species was farmed in a recirculation system. The studied blood biochemical parameters were not influenced by dietary black pepper extract. Experiments of black pepper extract as a feed additive to rainbow trout pellets should continue with other concentrations, and its effect in other fish species should be studied.

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