

Effect of dietary phytoextracts supplementation on biochemical blood parameters of common carp (*Cyprinus carpio* L.) cultivated in a recirculation system

Kremena Georgieva*, Georgi Zhelyazkov and Dian Georgiev

Trakia University, Faculty of Agriculture, Department of Biology and Aquaculture, Students campus, 6000 Stara Zagora, Bulgaria

* Corresponding author: georgieva.kremena1983@gmail.com

Abstract

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The present research aimed to examine the effect of dietary phytoextracts supplementation on the biochemical blood parameters (glucose, urea, creatinine, total protein, albumin, ASAT, ALAT) of common carp (*Cyprinus carpio* L.), cultivated in a recirculation system. The fish were divided into 6 groups: one control (C) and five experimental groups in whose food was added phytoextracts of curcumin (EC), paprika (EP), thyme (ET), oregano (EO) and garlic (EG). The addition of phytoextracts had significant influence on total protein content of fish from the experimental groups (EC - $P \leq 0.01$), EP - $P \leq 0.05$, ET - $P \leq 0.05$ and EG - $P \leq 0.05$) compared to control group (C).

Keywords: curcumin; paprika; thyme; oregano; garlic

Introduction

Fish production in Bulgaria is based on two main species - rainbow trout (*Oncorhynchus mykiss* W.) in the cold water and common carp (*Cyprinus carpio* L.) in the warm water direction. These species differ in nutritional requirements, growth indicators, metabolism and nutritional qualities of meat. In the recent years there is a tendency in Bulgaria for the widespread use of medicinal plants in fish feed with the aim to enhance the immunity, stimulate growth, appetite, and increase anti-stress resistance (Stoyanova et al., 2018; Stratev et al., 2018; Georgieva et al., 2018, 2019; Sirakov et al., 2019; Velichkova et al., 2019; Koshinski, 2019, 2020).

Studies on the phytoextracts used in fish farming reveal the following picture:

Curcumin (*Curcuma longa* L.). Xia et al. (2015) established that the inclusion of 60 mg curcumin/kg to the feed of *Megalobrama amblycephala* reduced the ASAT and ALAT levels and increased the albumin content. According to Sahu et al. (2008) the higher amount of total protein and albumin in blood serum of *Labeo rohita* was determined, as a consequence of the addition of curcumin to the feed. The activity of ASAT and ALAT was not significantly affected by the curcumin supplement.

Paprika (*Capsicum annum* L.). In the literature, information is predominantly on the influence of different sources of carotenoids and astaxatin on growth performance

in different species of animals (Lee et al., 2010; Maniat et al., 2014). There is no information on the effect of paprika extract on the hematological and biochemical blood parameters of blood in different fish species.

Thyme (*Thymus vulgaris* L.). The inclusion of 1.0% thyme as a plant feed additive according to Zaki et al. (2012) has a beneficial effect on the biochemical blood parameters of blood of Nile tilapia. This effect is expressed in a lower level of total plasma protein and a lower content of aspartate aminotransferase. The amount of thyme administered results in a significant increase in the blood glucose of the experimental fish. Higher total protein and albumin was found by Emerish & El-Deen (2016) feeding *Clarias gariepinus* with 1.0% thyme and 1.0% fenugreek. On the other hand, Yilmaz et al. (2012) found that urea and creatinine content in blood serum in of *Dicentrarchus labrax* was not affected by the addition of thyme, rosemary and fenugreek. A similar effect on total serum protein, albumin and glucose levels as a result of the inclusion of thyme and vit. E to fodder in *A. stellatus* has also been reported by Dorojan et al. (2015). Garlic (*Allium sativum* L.). Talpur & Ikhwanuddin (2012) reported a higher amount of total protein and albumin in the blood of garlic-fed *Lates calcarifer* and a significant decrease in glucose values. According to Irkin et al. (2014) the addition of garlic in the amount of 2.0 g, 4.0 g and 6.0 g/100 g of feed in the food of the same type of fish led to significantly higher blood albumin content.

An increase in glucose levels was observed in fish fed basic feed and those receiving 2.0 g garlic/100 g feed. According to the authors, the intake of no more than 2.0 g of garlic/100 g of feed has a positive effect on basic blood parameters. Thanikachalam et al. (2010) established that the high amount of protein and albumin was found in the blood of *Clarias gariepinus* stocking material as a result of the addition of 0.5%, 1.0% and 1.5% garlic/kg feed. Similar results for the total protein content of the same species of garlic-eating fish have been reported by Nwabueze (2012). Metwally (2009) found a significant decrease in the levels of aspartate-aminotransferase and alanine-aminotransferase enzymes in the blood serum of *Oreochromis niloticus* when fed with garlic.

The present research aimed to examine the effect of dietary phytoextracts supplementation on the biochemical blood parameters (glucose, urea, creatinine, total protein, albumin, ASAT, ALAT) of common carp (*Cyprinus carpio* L.), 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 №20/2012).

Experimental design

The common carp (*Cyprinus carpio* L.) used in this study were raised in concrete tanks with efficient water volume 0.8 m³, part of a recirculation system. Fish were divided into six groups of 10 fish each: one control (C) and five experimental groups whose feed was supplemented with curcumin (EC), paprika (EP), thyme (ET), oregano (EO) and garlic (EG). Each of the trial variants was with two replications. The carps were fed with extruded pellets “Aqua garant” produced by Garant-Tiernahrung Gesellschaft m.b.H. - Austria, with pellet size 6 mm, three times per day, with 3% of fish’ live weight. The feed of the fish from experimental groups was supplemented with 1% dry extract of the different spices, mentioned above. It was dissolved in 1 ml distilled water and sprayed onto 100 g feed one hour before feeding. The individuals from the control group (C) did not receive any phytoextracts. Phytoextracts of paprika, thyme, oregano and garlic were produced by “Rigana” LTD - Sofia, Bulgaria. The phytoextracts (paprika, thyme, oregano, garlic and curcumin) used in the experiment were supplied by P.I.C.Co LTD - Sofia, Bulgaria. The nutritional content of extruded feed for the different groups was presented in Table 1. The duration of the experiment was 60 days.

Table 1. Nutritional content in the feed for common carp (*Cyprinus carpio* L.), cultivated in a recirculation system

No	Parameters	Experimental groups					
		C	EC	EP	ET	EO	EG
1	Crude protein, %	30.00	30.00	30.00	30.00	30.00	30.00
2	Crude fat, %	8.00	8.00	8.00	8.00	8.00	8.00
3	Crude fibre, %	5.00	5.00	5.00	5.00	5.00	5.00
4	Crude ash, %	8.50	8.50	8.50	8.50	8.50	8.50
5	Calcium, %	1.40	1.40	1.40	1.40	1.40	1.40
6	Phosphorus, %	1.10	1.10	1.10	1.10	1.10	1.10
7	Sodium, %	0.20	0.20	0.20	0.20	0.20	0.20
8	Curcumin, %	-	1.00	-	-	-	-
9	Paprika, %	-	-	1.00	-	-	-
10	Thyme, %	-	-	-	1.00	-	-
11	Oregano, %	-	-	-	-	1.00	-
12	Garlic, %	-	-	-	-	-	1.00
13	Metabolisable energy, MJ/kg	16.60	16.60	16.60	16.60	16.60	16.60

*1 kg compound feed contains: vitamin A – 10000 IU; vitamin D₃ – 1750 IU; vitamin E – 175 mg; vitamin C – 100 mg

** 1 kg compound feed contains: Fe – 80 mg; Mn – 35 mg; Cu – 10 mg; Zn – 107 mg; J – 2.3 mg; Se – 0.4 mg.

C-control group and experimental groups: EC-curcumin, EP-paprika, ET-thyme, EO-oregano and EG-garlic

Table 2. Hydrochemical parameters in the recirculation system during the experiment

Parameters	n	$\bar{x} \pm SD$	Optimal limits (Regulation No 4/2000)
Temperature, °C	60	23.40±1.42	22.00-26.00
Dissolved oxygen, mg/l	60	5.89±0.23	not under 5
pH	60	7.30±0.06	6.50-8.50
Nitrates, mg/l	60	0.57±0.03	to 2.00
Nitrites, mg/l	60	0.029±0.004	to 0.05
Electrical conductivity, $\mu S \cdot cm^{-1}$	60	671.00±2.15	-

The hydrochemical parameters in the recirculation system were maintained within the optimal limits for carp farming throughout the experiment (according to Regulation № 4/2000 for the quality of water for fish farming and for the breeding of shellfish organisms) Table 2.

Growth performance

The effect of added phytoextracts to feed on weight gain of rainbow trouts was determined by control catches in the middle (day 30) and end (day 60) of the experimental period. Individual live weight (g) was measured as the fish were weighed individually – with electronic scale (g). At the end of the trial, weight gain (g), survival rate (%) and feed conversion ratio of the different feeds were calculated and described by Georgieva (2018) and Georgieva et al. (2019).

Biochemical blood parameters

Blood was taken from six fish of each test group (not from all 10 experimental fish) from the caudal vein – at the 60th day of the experimental period with disposable sterile plastic syringes (3 ml) with a needle. An anticoagulant EDTA (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. The following haematological and biochemical blood parameters were investigated: glucose (mmol/l), urea (mmol/l), creatinine (μ mol/l), total protein (g/l), albumin (g/l), ASAT (U/l), ALAT (U/l). The experiment was conducted in accordance with the “Guide to the Care and Use of Experimental Animal Care” (1993, RD - 2020).

Statistical evaluation

The results were statistically evaluated using STATISTICA 6.0 software, t-test, independent by variables (StatSoft Inc., 2002).

Results

Blood parameters of carp cultivated in a recirculating system are presented in Table 3. The addition of curcumin, paprika, thyme, oregano and garlic extracts did not have a significant ($P > 0.05$) effect on carp blood glucose levels. The values reported for this parameter are in the range of 6.41 ± 0.43 mmol / l - 7.00 ± 0.49 mmol / l, with higher content reported in the control group.

Table 3. Biochemical blood parameters of common carp (*Cyprinus carpio* L.), cultivated in a recirculation system

Biochemical blood parameters	n	C	EC	EP	ET	EO	EG
		$\bar{x} \pm SD$	$\bar{x} \pm SD$	$\bar{x} \pm SD$	$\bar{x} \pm SD$	$\bar{x} \pm SD$	$\bar{x} \pm SD$
(Glu) mmol/l	6	6.51±0.59	7.00±0.49	6.54±1.23	6.96±0.30	6.42±0.65	6.41±0.43
Urea mmol/l	6	2.44±0.91	2.59±0.59	2.01±0.50	2.48±0.69	2.17±0.41	2.23±0.89
Creatinin μ mol/l	6	65.95±37.64	36.55±12.37	35.97±9.86	45.00±33.48	33.98±5.89	64.75±33.60
Total protein g/l	6	1.86±0.67 ^{abcd}	2.93±0.53 ^d	2.73±0.44 ^a	2.77±0.69 ^b	2.27±0.30	2.50±0.20 ^e
Albumin g/l	6	3.88±0.33	3.58±0.41	3.75±0.33	3.91±0.21	4.03±0.39	3.85±0.39
ASAT U/l	6	116.50±18.81	94.67±46.03	140.17±44.65	115.67±25.48	115.17±29.39	128.17±28.47
ALAT U/l	6	30.00±13.18	33.00±7.77	31.17±5.46	33.83±8.86	35.17±8.93	37.83±11.72

Different letters in the same row indicated significant difference ($p < 0.05$)

C-control group and experimental groups: EC-curcumin, EP-paprika, ET-thyme, EO-oregano and EG-garlic

The amount of urea in the blood of fish from EC group was 2.59 ± 0.59 mmol/l. It is higher than that of individuals of the C and the experimental groups, by 6.15% (C) and 28.85% (EP), 4.44% (ET), 19.35% (EO), 16.14% (EG), respectively, with no statistical differences between them ($P > 0.05$).

The inclusion of phytoextracts in the feed of fish from the experimental groups did not significantly influence ($P > 0.05$) the creatinine levels and ranged from 33.98 ± 5.89 μ mol/l to 65.95 ± 37.64 μ mol/l. There was a

tendency for lower creatinine levels in the experimental groups compared to the control group (Table 3).

The plasma protein content of the fish from C group was 1.86 ± 0.67 g / l and it was lower compared to the ones from EC, EP, ET and EG groups by 57.52%, 46.77%, 48.92%, 22.04% and 34.40%, respectively.

Significantly higher total protein content was established in fish from EC ($P \leq 0.01$), EP ($P \leq 0.05$), ET ($P \leq 0.05$) and EG ($P \leq 0.05$) groups compared to C group (Fig. 1).

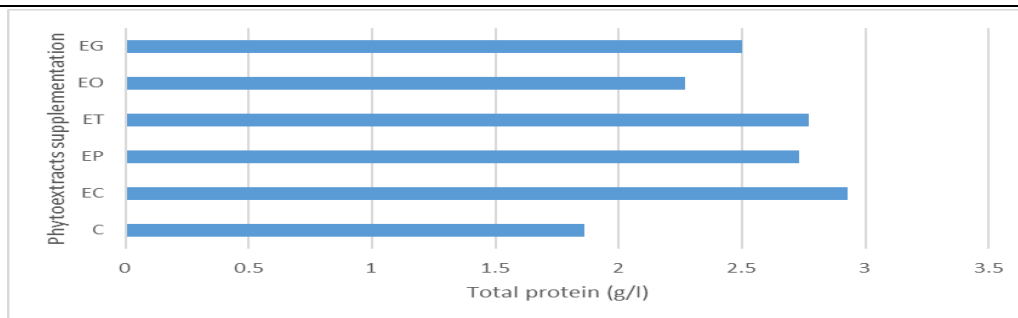


Fig. 1. Total protein content (g/l) in blood of common carp (*Cyprinus carpio* L.), fed by diets with phytoextracts: EC -curcumin, EP-paprika, ET-thyme, EO-oregano, EG-garlic and C-control group without phytoextract supplementation

The inclusion of phytoextracts did not influence significantly the albumin content of fish from experimental groups. The addition of phytoextracts to the feed of fish from experimental groups did not change the albumin content ($P > 0.05$). It was in the range from 3.58 ± 0.41 g / l to 4.03 ± 0.39 g / l, with a higher amount reported in the ET group.

The phytoextracts used did not have a significant ($P > 0.05$) effect on the ASAT values. The level of this parameter of fish from EP group was 140.17 ± 44.65 U / l and it was higher than those from the C and EC, ET, EO, EG groups respectively, by 20.31% and 48.06%, 21.18%, 21.70%, 9.40%.

In regard to the content of alanine aminotransferase, no significant effect of the phytoextracts supplementation was found ($P > 0.05$). It was in the range from 30.00 ± 13.18 U/l to 37.83 ± 11.72 U/l, with trends of higher values in the fish of the experimental groups compared to the control.

Discussion

The addition of phytoextracts to the diet of common carp, cultivated in a recirculation system had no significant effect on glucose levels. According to Dorojan et al. (2015), the inclusion of thyme had no significant influence on the blood glucose level. On the other hand, some authors reported a significantly lower glucose level when feeding different fish species with garlic extract (Metwally, 2009; Talpur & Ikhwanuddin, 2012).

Urea levels were not significantly affected ($P > 0.05$) by the inclusion of phytoextracts in the feed of the experimental groups. Yilmaz et al. (2012) established that the thyme intake had not significant effect on the urea level of *D. labrax*. The addition of curcumin, paprika, thyme, oregano and garlic extracts to the diet of fish from the experimental groups had no significant influence on the creatinine level. According to Yilmaz et al. (2012) the inclusion of thyme extract to the feed of *D. labrax* did not change the values of this parameter.

The addition of curcumin, paprika, thyme and garlic led to significantly higher amount of plasma protein, compared to fish from C group ($P \leq 0.01$, $P \leq 0.05$, $P \leq 0.05$, $P \leq 0.05$). The higher value of that parameter for the group which received curcumin, is probably due to its antioxidant properties. Paprika extract also stimulated the immune system of fish and has antioxidant properties. The main components of thyme and oregano - thymol and carvacol have antioxidant properties and stimulated the immune system, which led to higher plasma protein. The probable cause of the higher protein in garlic-fed fish is the presence of S-allylcysteine or some of its other

ingredients that play a role in stimulating the immune system. According to Dorojan et al. (2015) the total serum protein is the most important indicator about the nutritional status of the body and the health of the fish, influenced by fish species, age, sex, water temperature, quantity and quality of feed. Our results are confirmed by those of Emerish & El-Deen (2016) according to which thyme supplement, increased the protein level of African catfish. Similar effect was found by Sahu et al. (2008) to *L. rohita*, received different curcumin levels. A number of authors have also reported significantly higher levels of plasma protein as a result of the addition of garlic to the diet of *O. niloticus*, *C. gariepinus* and *L. calcarifer* (Metwally, 2009; Thanikachalam et al., 2010; Nwabueze, 2012; Talpur & Ikhwanuddin, 2012).

Regarding albumin content of carps, the addition of phytoextracts had no significant effect ($P > 0.05$). According to some authors, the inclusion of garlic, oregano and thyme in the feed of various fish species led to higher albumin levels (Thanikachalam et al., 2010; Irkin et al., 2014; Haghhigh & Rohani, 2015; Pourmoghim et al., 2015; Emerish & El-Deen, 2016). Our results were similar to those data. Higher albumin was established also by Sahu et al. (2008) to *L. rohita*, as a result of curcumin supplement. The highest albumin was reported in EO group compared to those from EC, EP, ET and EG groups, with no significant differences among groups ($P > 0.05$).

The analysis of experimental data from the present research showed that the addition of curcumin, paprika, thyme, oregano and garlic extracts to the diet had no significant influence ($P > 0.05$) on aspartate aminotransferase. According to Xia et al. (2015) the inclusion different curcumin levels to the feed of *M. amblycephala* decreased the amount of ASAT. The results from the recent study correspond to them. Zaki et al. (2012) also reported for significant lower amount of aspartate aminotransferase to *O. niloticus*, having fed 1.0% thyme extract. According to Metwally (2009) the addition of garlic to the feed of *O. niloticus* led to lower level of this enzyme. Our results were not in agreement with those data. This probably due to the different species of fish and the peculiarities of their metabolism.

In the present study we observed tendency to increase the values of ALAT of fish from the experimental groups. Our results did not correspond with those reported by Metwally (2009), who established significant decrease of alanine aminotransferase to *O. niloticus*, fed with different garlic levels. According to Zaki et al. (2012) and Xia et al. (2015), the inclusion of thyme and curcumin to the feed of *O. niloticus*

and *M. amblycephala* also led to significantly lower values of ALAT. As it was mentioned above for ASAT, the reasons for these differences most likely due to the different fish species and their metabolism.

Conclusion

It was found that the phytoextracts (1% dry extract) in diets of the common carp: (i) influenced significantly (curcumin, paprika, thyme and garlic) on the total protein content, exception is the phytoextract of oregano; (ii) the total protein content of the fish from experimental groups was higher than those from control group, with statistically significant differences between groups (EC - $P \leq 0.01$, EP - $P \leq 0.05$, ET - $P \leq 0.05$, EG - $P \leq 0.05$); (iii) no significant affected ($P > 0.05$) on aspartate aminotransferase, while alanine aminotransferase tends to increase its values; (iv) no changed the glucose, albumin, creatinine and urea levels and differences between the fish from experimental groups and control group were statistically insignificant ($P > 0.05$).

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