EFFECT OF DIETARY BETAINE SUPPLEMENTATION ON SOME PRODUCTIVE TRAITS OF COMMON CARP (*CYPRINUS CARPIO* L.) CULTIVATED IN RECIRCULATION SYSTEM

GEORGI ZHELYAZKOV*, YORDAN STAYKOV, DANIEL GEORGIEV
Department of Biology and Aquaculture, Faculty of Agriculture, Trakia University, Students campus, 6014 Stara Zagora, Bulgaria
**Corresponding author: georgi_ij@abv.bg*

EFEKTI BETAINA KAO DODATKA U ISHRANI NA NEKE PROIZVODNE OSOBINE ŠARANA (*CYPRINUS CARPIO* L.) GAJENOG U RECIRKULACIONOM SISTEMU

Apstrakt

Cilj ovog istraživanja bio je da se ustanove efekti betaina kao dadatka u ishrani na stopu preživljavanja, prirast i konverziju hrane kod šarana (*Cyprinus carpio* L.), gajenog u recirkulacionom sistemu. Četrdeset i osam jedinki šarana čija je prosečna početna težina bila 1238.13±39.19 - 1241.25±29.73 g podeljeni su u tri grupe. Eksperiment je rađen u duplikatu. Ribe su gajene u betonskim tankovima zapremine 0.8 m^3, koji su pripadali recirkulacionom sistemu. Riba je hranjena ekstrudiranom hranom "Aqua VITAL", koju proizvodi "Aqua garant". Veličina peleta bila je 6 mm. 1% betaina dodat je hrani šarana koji je pripadao eksperimentalnoj grupi EG1, dok je hrana za drugu eksperimentalnu grupu (EG2) sadržala 3% betaina. Šaran iz kontrolne grupe (CG) nije dobijao betain u ishrani. Hrana kojom je riba hranjena predstavljala je 2% težine ribe. Ogled je trajao 60 dana, a kontrolni ulov obavljen je 30-og dana da bi se ispitao uticaj betaina kao dodatka u ishrani na prirast i odnos konverzije hrane kod šarana (*Cyprinus carpio* L.), koji se gaji u recirkulacionom sistemu. Težina ribe (g) na kontrolnom ulovu ustanovljena je tako što je svaka jedinka pojedinačno merena. Finalna težina šarana iz eksperimentalnih i kontrolnih grupa iznosila je: EG_1 – 1727.50±40.52 g, EG_2 – 1749.39±33.45 g and CG – 1646.88±26.51 g. Vrednosti ovog parametra kod ribe iz druge eksperimentalne grupe EG2 bile su više nego kod jedinki iz EG1 (1.27%) i CG (6.22%), međutim razlike nisu bile značajne ($P>0.05$). Prosečni individualni prirast kod šarana iz dve replikacione kontrolne grupe bio je 406.25±20.61 g, što je 20.46% i 25.08% niže nego kod riba koje su dobijale 1% i 3% betaina, dok su razlike bile značajne ($P<0.001$). Najbolji koeficijent konverzije hrane bio je kod šarana iz EG2 - 1.49. Ova osobina je imala niže vrednosti nego kod riba iz EG1 (3.36%) i CG (24.16%)

(P<0.001). Kada je reč o stopi preživljavanja riba iz svih grupa, diskrepance nisu uočene. Ekonomska efikasnost grupe koje je dobijala 1% betaina bila je 1.66, što je niže nego kod EG2 (10.34%) i CG (15.66%).

Ključne reči: šaran, Cyprinus carpio L., betain, osobine prirasta, koeficijent konverzije hrane.
Key words: common carp, Cyprinus carpio L., betaine, growth traits, feed conversion ratio.

INTRODUCTION

The use of various supplements in fish feeds has been increasing in the recent years. In addition to improve the quality of the feed, these compounds are intended to enhance the growth of the cultivated species, while at the same time decreasing the feed conversion ratio and augmenting the survival rate. The digestibility of the feeds is also expected to improve, due to the presence of these additives. A lot of experiments have been carried out investigating different supplements and betaine has proved to show a great potential (Polat and Beklevik, 1999).

Betaine (glycinebetaine, trimethylglycine) is highly water soluble and hence diffusing compound, capable of stimulating the olfactory organs of fish. It is contained in high amounts in the sea invertebrates, microorganisms and some plants (Meyers, 1987).

The effect of betaine supplementation has been studies mainly in species like rainbow trout (*Onchorynchus mykiss*) by Rumsey (1991) and Virtanen et al. (1994), however their results were contradictory. Rumsey (1991) reported that exogenous betaine supplementation did not augment the weight of the rainbow trout, while on the other hand Virtanen et al. (1994), found that the weight of the same species was increased by 12 % and the mortality was decreased by 60 % after dietary betaine supplementation in amount of 1 %. Betaine in feed led to enhanced feed consumption and growth in rainbow trout larvae (Can and Sener, 1992; Polat and Beklevik, 1999). Rahimabadi et al. (2012) concluded that betaine might improve the quality of the feed, used for zander larvae (*Sander lucioperca*), as they become easily adapted, when shifting the nutrition from live food to extruded pellets.

Shankar et al. (2008) reported, that betaine supplementation in amount of 0.25 % to the feed of Rohu (*Labeo rohita*) improved considerably the growth, when compared to fish that did not receive the dietary supplement.

Przybyl et al. (1999) concluded that the addition of 0.20 % betaine in the diet of carp larvae enhanced the productive traits.

The aim of the study was to determine the effect of the dietary betaine supplementation on the survival rate, weight gain and feed conversion ratio of common carp (*Cyprinus carpio* L.), cultivated in recirculation system.

MATERIAL AND METHODS

Forty eight common carps with an initial live weight of 1238.13±39.19 - 1241.25±29.73 g were divided in three groups (control, CG and experimental groups: EG_1, EG_2), consisting of eight fish, as each of them had two replicates. The fish were cultivated in concrete tanks

with a volume of 0.8 m^3, which were part of the recirculation system. Carps were fed with extruded feed "Aqua VITAL", a product of "Aqua garant", with 6 mm size of the pellets. Betaine in amount of 1 % was sprayed in the feed of the fish from the experimental group EG_1, while these ones from the second experimental group (EG_2) received 3 % betaine, added to the diet. The carp from the control group (CG) received feed without added betaine. The content of nutrients in the extruded feed for the groups is presented in Table 1. Fish from all groups were fed 3 times/daily, based on 2 % of biomass. The trial period was 60 days.

Hydrochemical Analysis

The hydrochemical parameters in the recirculation system of the carp (*Cyprinus carpio* L.) were determined, using methods, adapted for fish farming (Bessonov and Privezentsev, 1987; Todorov, 1992). They are as follows:

- Water temperature, °C;
- Quantity of the dissolved oxygen, $mg.l^{-1}$ – MultiLine P4;
- pH – MultiLine P4;
- Electrical conductivity, $\mu S.cm^{-1}$ – MultiLine P4 and BDS EN 27888;
- Quantity of nitrates, $mg.l^{-1}$ – BDS 17.1.4.12:1979;
- Quantity of nitrites, $mg.l^{-1}$ – BDS ISO 26777:1997.

Intensity of fish growth

In order to study the betaine influence on the weight gain and feed conversion ratio in the carp (*Cyprinus carpio* L.), cultivated in recirculation system, control catch was carried out at 30th day. The average live weight (g) at the control catch was determined as the fish were weighed individually. At the end of the trial the final body weight (g), the weight gain (g), survival rate (%) and the feed conversion ratio in fish were determined.

Table 1. Nutrient content in the extruded feed for a common carp (*Cyprinus carpio* L.)

№	Item	Groups		
		CG	EG_1	EG_2
1	Crude protein, %	34.00	34.00	34.00
2	Lipids, %	10.00	10.00	10.00
3	Fiber, %	4.00	4.00	4.00
4	Moisture, %	8.74	8.74	8.74
5	Lysine, %	1.43	1.43	1.43
6	Methionine+cysteine, %	0.80	0.80	0.80
7	Ca, %	1.23	1.23	1.23
8	P, %	1.60	1.60	1.60
9	Chlorides, %	0.78	0.78	0.78
10	Betaine, %	-	1	3
11	ME, MJ/kg	17.00	17.00	17.00
12	ME, kcal/kg	4063	4063	4063

** 1 kg feed contains: vitamin A – 10000 IE; vitamin D_3 – 1500 IE; vitamin E – 200 mg; vitamin K – 3 mg; thiamin – 10 mg; riboflavin– 15 mg; pyridoxine – 8 mg; vitamin B_{12} – 0.02 mg; nicotinic acid– 40 mg; folic acid – 3 mg; biotin – 0.3 mg.*

*** 1 kg feed contains: Fe – 145 mg; Mn – 67 mg; Cu – 16 mg; Zn – 68 mg; I – 1.5 mg; Co – 0.5 mg; Se – 0.6 mg*

Economic analysis

In order to analyse the economic efficiency of the betaine supplementation in the diet of carp (*Cyprinus carpio* L.), cultivated in recirculation system, data for feed conversion ratio, weight gain and survival rate were used. Comparisons of these traits were made between the fish of the different experimental groups and the costs for the extruded feed were determined. The price cost for 1 kg weight gain of the fish, cultivated in recirculation systems was determined. The economic conversion ratio (ECR) was calculated, using the following equation (Piedecausa et al., 2007):

ECR = Cost of Diet x Feed Conversion Ratio (FCR)

Statistical evaluation of the data was done by STATISTICA 6.0 software (StatSoft Inc., 2002).

RESULTS

Hydrochemical analysis

During the trial period the hydrochemical parameters of the recirculation system were maintained in the optimal limits for carp. The temperature, dissolved oxygen, pH, nitrates, nitrites and the electric conductivity of the water were daily measured.

The water temperature during the trial is presented in Table 2. For the different groups it was 23.50ºC - 24.50ºC, which is optimal for this species. The amount of the dissolved oxygen was optimal as well. In the different experimental groups the values of this parameter varied between 6.30 $mg.l^{-1}$ and 6.96 $mg.l^{-1}$ /Table. 2/. The pH of the water in the recirculation system for the different experiments was within the range of 7.58-7.73, that was optimal for the common carp (Table 2). The quantity of nitrates during the trial period varied between 0.51 $mg.l^{-1}$ and 0.63 $mg.l^{-1}$, which is below the maximal value, specified in the Regulation №4/20.10.2000. (Table 2). The content of nitrites in the water of the different experimental groups is shown in Table 2 as well. It was within the range of 0.021 $mg.l^{-1}$ - 0.030 $mg.l^{-1}$, which is also below the maximal value, cited in the above regulation. The electric conductivity of the water during the trial period is presented in Table 2 and varied within 635.00 $\mu S.cm^{-1}$ and 714.00 $\mu S.cm^{-1}$, which is optimal for the cultivated species.

Table 2. Water parameters in the recirculation system during the experiment with common carp

Parameter	n	Min.	Max.	Optimum values (Zaykov and Staykov, 2013)
Temperature, ºC	60	23.50	24.50	22.0-26.0
Dissolved oxygen, $mg.l^{-1}$	60	6.30	6.96	> 5
pH	60	7.58	7.73	6.5-8.5
Nitrates, $mg.l^{-1}$	60	0.51	0.63	< 2.0
Nitrites, $mg.l^{-1}$	60	0.021	0.030	< 0.05
Electric conductivity, $\mu S.cm^{-1}$	60	635.00	714.00	-

Growth of the common carp

The average initial live weight of the carp from both replicates of the control and experimental groups were 1240.63±27.31 g, 1238.13±39.19 g and 1241.25±39.19 g, respectively as there were no significant differences between values of different variants (P>0.05) /Table 3/.

In the middle of the experimental period a trend towards higher live weight in the fish, receiving 1 % and 3 % betaine was observed (1456.88±38.64 g and 1473.13±31.86 g when compared to the control group - 1420.60±34.21) (Table 3). The same tendency existed at the end of the trial, as the live weight was the highest in the carp from EG_2 - 1749.39±33.45 g, followed by EG_1- 1727.50±40.52 g. The fish from the control group displayed the lowest live weight.

The survival rate of the carp during the trial is presented in Table 3. The values of this trait in the fish, fed betaine supplemented diet were 100 %. Such was the survival rate in the control group.

At the end of the experiment the weight gain was higher in the fish from the betaine supplemented groups. The average individual weight gain of the carp from the two replicates of CG was 406.25±20.61 g, which is 20.46 % and 25.08 % lower, than the fish, receiving betaine in amounts 1 % and 3 %, as the difference was significant (P<0.001) (Table 3).

During the experimental period the carp were fed three times per day. The analysis of the feed at the replicates of the control and the experimental groups was done. The feed conversion ratio of the carp, receiving 3 % betaine was 1.49 and it was 3.36 % lower, than that of the fish, fed 1 % betaine, while 24.16 % lower in the individuals from the control group (Table 3). The differences were significant (P<0.001) between the experimental groups and this one without betaine supplementation.

Table 3. Fish production parameters

Parameter	n	CG	EG_1	EG_2	Significance
		$\bar{x}$ ± SD	$\bar{x}$ ± SD	$\bar{x}$ ± SD	
Initial body weight, g	16	1240.63±27.31	1238.13±39.19	1241.25±29.73	NS
Body weight in the middle of the trial, g	16	1420.60±34.21	1456.88±38.64	1473.13±31.86	NS
Final body weight, g	16	1646.88±26.51	1727.50±40.52	1749.39±33.45	NS
Survival rate, %	16	100	100	100	NS
Average individual weight gain, g	16	406.25±20.61[a]	489.37±34.15[b]	508.14±48.61[b]	***
FCR	16	1.85±0.09[a]	1.54±0.11[b]	1.49±0.15[b]	***

*** P≤0.001; ** P≤0.01; * P≤0.05; NS – non significant.

Economic analysis

The price of the extruded feed for a common carp was 1038.80 BGN/t (VAT excluded). Liquid betaine was sprayed in the pellets of the fish from two experimental groups, which made the feed more expensive. The increase of the feed price in the group, fed 1 % betaine was 45 BGN/t, while in the group receiving 3 % betaine, it was 135 BGN/t.

The calculated economic conversion ratio for the carp in the group, fed 1 % dietary betaine supplementation was 1.66, which was lower, when compared to these ones of the fish from groups CG and EG_2, respectively by 10.34 % and 15.66 %.

Table 4. Economic efficiency of the betaine supplementation in the feed

Item	**CG**	**EG_1**	**EG_2**
Price, BGN/t feed (VAT excluded)	1038.80	1083.80	1173.80
Price, BGN/kg feed (VAT excluded)	1.04	1.08	1.17
ECR	1.92	1.66	1.74

DISCUSSION

The analysis of the data, concerning the hydrochemical traits (temperature, oxygen, dissolved in water, pH and electric conductivity) during the trial period showed that they were within the optimal range for the particular species. The same could be said for the maximal concentrations of the nitrates and nitrites in the water. For the carp farms these parameters must be up to 2 mg/l and 0.05 mg/l respectively and they were considerably higher, than those, maintained in the water during the experimental period (Regulation № 4/ 20.10.2000; Zaykov and Staykov, 2013). The optimal values of the traits for all the studied groups are due to the fact that the carp were cultivated in optimized technical and technological conditions of the recirculation system. The tanks were cleaned three times per day and fresh water in amount of the total volume of the recirculation system was daily added. For the maintenance of the optimal hydrochemical traits in the system during the experimental period the mechanical filter and particularly biofilter were of critical importance.

The supplementation of betaine to the carp diet in amounts of 1 % and 3 % did not affect the survival rate of the fish. The data obtained at the end of the experiment, as stated above, showed that the values of this variable were 100 % in the individuals of the replicates in the betaine fed groups. The same was observed for the fish from the control group /Table 3/. This was due to the maintenance of the optimal hydrochemical parameters that are required for the cultivation of the species at optimized technological conditions – stocking density, daily diet, feeding frequency.

The analysis of the data for the weight gain of the carp revealed, that it was 406.25±20.61 g in the control group, which was 20.46 % and 25.08 % lower, than these ones of the fish in the experimental groups, fed 1 % and 3 % betaine, as the differences were significant ($P<0.001$) /Table 3/.

At the end of the trial period, the feed conversion ratio of the carp, cultivated in recirculation system and fed 3 % betaine was 1.49. This value is 3.36 % lower, than that of the fish, receiving 1 % betaine in the diet and 24.16 % lower in the individuals from the control group /Table 3/. The differences of this parameter were significant between the experimental groups and the control one ($P<0.001$). The results obtained in this study are due to the

improved metabolism of the nutrients in the fish receiving betaine, since the latter is a donor of methyl groups. This is confirmed by many other studies, reporting that the increase of the methyl groups after betaine supplementation in the diet, enhance the live weight of the fish, achieved with the same quantity of feed (Virtanen et al., 1994; Przyby et al., 1999; Polat and Beklevik, 1999; Rahimabadi et al., 2012).

The better conversion of the extruded feed with betaine, added in amount of 1 % and 3 %, affects positively the growth of the fish from the experimental groups, cultivated in recirculation system. At the beginning of the trial the carp were made equal in weight (P>0.05). In the middle of the experimental period the live weight of the fish from the supplemented groups tended to be higher, when compared to this one of fish from the control. The average live weight of the carp from both replicates of the group, fed 3 % betaine was 1473.13 g and it was 1.12 % higher, than this one of the fish, receiving 1 % betaine and 3.70 %, than the values of this parameter of carps from the control group (P>0.05) /Table 3/. This trend was kept until the end of the experimental period. The average live weight of the carp in the two replicates of EG_2 was 1749.39 g and it was 1.27 % higher, that the EG_1 and 6.22 %, than CG (P>0.05) /Table. 3/. These results confirm the findings of other studies, concerning experiments with different betaine concentrations in different fish species. After an experiment with dietary betaine supplementation in amount of 1.5 % in the feed of rainbow trout, Polat and Beklevik (1999) reported significant influence on the feed, consumed and the growth of the fish. Similar results were reported for betaine, added in the feed of rainbow trout (Can and Sener 1992), red seabream (Goh and Tamura, 1980), *Solea solea* and *Anguilla anguilla* (Mackie and Mitchell, 1982). According to these authors, this was a result of the betaine, which is a donor of methyl groups, used for synthesis of methionine, carnitine, phosphatidylcholine and creatine. These substances are important for the metabolism, although betaine might be synthesized by choline in the mitochondria. Usually the synthesis is not sufficient for the needs of the fast growing hydrobionts (Stekol et al., 1953).

Although the group, receiving betaine in amount 3 % displayed the best feed conversion ratio, the data of the economic analysis showed that the group, fed 1 % betaine had the best economic conversion ratio. In regards of this trait our results were confirmed by those of Virtanen et al. (1994) and Przybyl et al. (1999).

The dietary betaine supplementation in amount of 1 % and 3 % to the extruded feed for carp, cultivated in recirculation system affected positively the following traits:

- Increased the weight gain of the fish from the EG_2 and EG_1 groups, when compared to this one from the control CG, respectively by 25.08 % and 20.46 %.
- Decreased the feed conversion ratio in the carp from EG_2 and EG_1, respectively by 24.16 % and 20.13 %, in comparison to the values of this trait, determined in the fish from the control group;
- Had no effect on the survival rate of the fish in all experimental groups;
- Decreased the economic conversion ratio in the carp from EG_1 and EG_2, respectively by 15.86 % and 10.34 %, when compared to the values of this trait in the fish from the control group.

CONCLUSION

The study showed that the betaine might successfully be used as a feed additive to the diet of common carp. Its supplementation to the extruded pellets influenced positively the

growth, did not have negative effect on the survival rate of the fish, enhanced the weight gain, reduced the feed conversion ratio, as well as the economic conversion ratio. The best economic conversion ratio had fish from the group, fed 1 % betaine.

REFERENCES

Bessonov, N. & Privezentsev, Y. (1987): Fish-farming hydrochemistry M. Agropromizdat. 158 pp. (Ru).

Can, K. & Sener, E. (1992): Effect of betaine-added starter feeds on the growth of rainbow trout (*Oncorhynchus mykiss*) fry. Su-Urum. Degr. J. Aquat. Prod. 6(1): 95-104.

Goh, Y. & Tamura, T. (1980): Olfactory and gustatory responses to amino acids in two marine teleosts-red sea bream and mullet C. omp. Biochem. Physiol. 66: 217-224.

Mackie, A.M. & Mitchell, A.I. (1982): Further studies on the chemical control of the feeding behaviour in the Dover sole, *Solea solea*. Comp. Biochem. Physiol., 73(1): 89-93.

Meyers, S.P. (1987): Aquaculture feeds and chemo attractants. Aquaculture Infofish Marketing Digest, 1/87.

Piedecausa, M.A., Mazón, M.J., García García, B. & Hernández, M.D. (2007): Effects of total replacement of fish oil by vegetable oils in the diets of sharpsnout seabream (*Diplodus puntazzo*). Aquaculture 263: 211–219.

Polat, A. & Beklevik, G. (1999): The importance of betaine and some attractive substances as fish feed additives. In: Feed Manufacturing in the Mediterranean Region: Recent Advances in Research and Technology (J. Brufau and A. Tacon, eds). CIHEAMIAMZ, pp. 217-220.

Przybyl, A., Mazurkiewicz, J., Madziar, M. & Hallas, M. (1999): Effect of betafin addition on selected indices of carp fry rearing in ponds. Vol. 7, Fasc. 2

Rahimabadi, Z. E., Akbari, M., Arshadi, A. & Effatpanah, E. (2012): Effect of different levels of dietary Betaine on growth performance, food efficiency and survival rate of pike perch (Sander lucioperca) fingerlings. Iranian Journal of Fisheries Sciences 11(4): 902-910.

Regulation № 4/20.10.2000: Concerning the quality of the waters for fish farming and for rearing of shell organisms. Ministry of Environment and Waters, Ministry of Agriculture and forests and Ministry of Health (OG, issue 88/ 27.10.2000). (Bg).

Rumsey, G.L. (1991): Choline-betaine requirements of rainbow trout (*Oncorhynchus mykiss*). Aquaculture 91(1-2): 107-116.

Shankar, R., Murthy, H.S., Pavadi, P. & Thanuja, K. (2008): Effect of Betaine as a Feed Attractant on Growth, Survival, and Feed Utilization in Fingerlings of the Indian Major Carp, *Labeo rohita*. The Israeli Journal of Aquaculture – Bamidgeh 60(2): 95-99.

Stekol, J.A., Hsu, P.T., Weiss, S. and Smith, P. (1953): Labile methyl group and its synthesis de novo in relation to growith in chicks. J. Bol. Chem., 203: 763-773.

Todorov, M, & Ivancheva, E. (1992): Manual for works in fish-farming. 86 pp. (Bg).

Virtanen, E. Hole, R., Resink, J.W., Slinning K.E. & Junnila, M. (1994): Betaine/ amino acid additive enhances the seawater performance of rainbow trout (*Oncorhynchus mykiss*) fed standard fish-meal-based diets. Aquaculture 124: 220.

Zaykov, A. and Staykov, Y. (2013): Technologies in the freshwater aquaculture. Academic publisher Trakia University. ISBN 978-945-338-058-9. (Bg).