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EFFECT OF DIETARY BETAINE SUPPLEMENTATION ON SOME PRODUCTIVE TRAITS OF RAINBOW TROUT (*ONCORHYNCHUS MYKISS W.*) CULTIVATED IN RECIRCULATION SYSTEM

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ABSTRACT

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 rainbow trout (*Oncorhynchus mykiss W.*), cultivated in recirculation system. The fish were fed with extruded pellets "Aqua UNI", with size 2 mm. Betaine in amount of 1% and 3% was added in the feed of the rainbow trout from the experimental groups EG_1 and EG_2 respectively, the control group (CG) received no betaine supplementation of the diet. In regards to the survival of the fish from different groups no discrepancies were observed. The average individual gain of the rainbow trout was as follows: CG-70.23 ± 1.42 g, EG_1-71.93 ± 1.33 g and EG_2-73.91 ± 1.30 g, the differences were significant ($P<0.01$). The best feed conversion ration had the rainbow trout from EG_2 - 1.04 ± 0.01% and it was lower than that of the groups EG_1 and CG by 1.07 ± 0.01% and 1.13 ± 0.02% respectively, the differences were significant ($P<0.01$). The economic conversion ratio of fish production in the groups, receiving 1% and 3% dietary betaine supplementation was identical 3.47, that is lower than these ones of the fish from control group by 4.32%.

Keywords: Betaine, *Oncorhynchus mykiss W*, Growth traits, Feed conversion ratio, Economic 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 (Rumsey, 1991; Virtanen *et al.* 1994), pikeperch (Rahimabadi *et al*. 2012), common carp (Zhelyazkov *et al*., 2015), 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 common 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 rainbow trout (*Oncorhynchus mykiss W*.), cultivated in recirculation system.

Materials and Methods

One hundred sixty-two rainbow trouts were divided in three experimental variant, as each of them had two replicates, consisting twenty-seven fish per group. The average initial live weight of trouts from both replicates of the control (CG) and experimental group EG_1 and EG_2 were 26.07 ± 1.30 g, 27.11 ± 1.01 g and 26.26 ± 1.06 g, respectively. The fish were cultivated in plastic tanks with an effective water volume of 0.3 m^3, which were part of the recirculation system. Rainbow trouts were fed with extruded feed " Aqua UNI", a product of "Aqua garant", with 2 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 rainbow trout from the control group (CG) received feed without added betaine. The content of nutrients in the extruded feed for different variants is presented in Table 1. Fish from all groups were fed 3 times/daily with the diet, based on 3% of biomass. The trial period was 60 days.

Hydrochemical analysis

The hydrochemical parameters in the recirculation system of the rainbow trout (*Oncorhynchus mykiss W*.) were determined, using methods, adapted for fish farming. They are as follows:

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

S NO	Item	Groups		
		CG	EG_1	EG_2
1	Crude protein, %	45	45	45
2	Crude lipids, %	16	16	16
3	Crude fiber, %	2.4	2.4	2.4
4	Crude ash, %	8	8	8
5	Ca, %	1.6	1.6	1.6
6	P, %	1.2	1.2	1.2
7	Betaine, %	-	1	3
8	ME, MJ/kg	18.5	18.5	18.5

*1 kg feed contains: vitamin A–10000 IE; vitamin D_3–1500 IE; vitamin E–200 mg; vitamin C-150 mg; ** 1 kg feed contains: Fe–62 mg; Mn–26 mg; Cu–5 mg; Zn–103 mg; I–2.6 mg; Se–0.3 mg

Water temperature°C-MultiLine P4; Quantity of the dissolved oxygen, mg.l^{-1}–MultiLine P4; pH–MultiLine P4; Electrical conductivity, µS.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. The above water parameters were daily measured.

Growth of the rainbow trout

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

Economic analysis

In order to analyse the economic efficiency of the betaine supplementation in the diet of rainbow trout (*Oncorhynchus mykiss W.*), 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 growing of rainbow trout.

The hydrochemical parameters during the trial are presented in Table 2. Water temperature for the three experimental groups was 14.20 ± 0.60°C, and dissolved oxygen content - 9.87 ± 0.43 mg.l^{-1}. Water pH in the recirculation system in the different tanks was 7.58 ± 0.36. Nitrate content was 0.30 ± 0.04 mg.l^{-1}, and nitrites-0.007 ± 0.001 mg.l^{-1}. Electric conductivity of water during the experiments was 626.00 ± 4.62 µS. cm^{-1}.

Growth of the rainbow trout

The average initial live weight of the rainbow trout from both replicates of the control and experimental group EG_1 and EG_2 were 26.07 ± 1.30 g, 27.11 ± 1.01 g and 26.26 ± 1.06 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 (51.46 ± 2.64 g and 52.13 ± 2.86 g when compared to the control group - 50.20 ± 2.21 g) (Table 3). The same tendency existed at the end of the trial, as the live weight was the highest in the rainbow trout from EG_2-

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

Parameter	n	$\bar{x}$ ± SD	Optimum values (Regulation NO 4/2000)
Temperature °C	60	14.20 ± 0.60	12.0-16.0
Dissolved oxygen, mg.l^{-1}	60	9.87 ± 0.43	>9
pH	60	7.58 ± 0.36	6.0-9.0
Nitrates, mg.l^{-1}	60	0.30 ± 0.04	<2.0
Nitrites, mg.l^{-1}	60	0.007 ± 0.001	<0.01
Electric conductivity, µS.cm^{-1}	60	626.00 ± 4.62	-

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	54	26.07 ± 1.30	27.11 ± 1.01	26.26 ± 1.06	NS
Body weight in the middle of the trial, g	54	50.20 ± 3.21	51.46 ± 2.64	52.13 ± 2.86	NS
Final body weight, g	54	96.30 ± 4.48	99.04 ± 3.54	100.17 ± 3.25	NS
Survival rate, %	54	100	100	100	NS
Average individual weight gain, g	54	70.23 ± 1.42^a	71.93 ± 1.33^b	73.91 ± 1.30^b	**
FCR	54	1.13 ± 0.02^a	1.07 ± 0.01^b	1.04 ± 0.01^b	**

**P ≤ 0.01; NS–non significant.

100.17 ± 3.25 g, followed by this one of trouts from EG_1-99.04 ± 3.54 g. The fish from the control group displayed the lowest live weight-96.30 ± 4.48 g.

The survival rate of the rainbow trout 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 trouts from 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 rainbow trout from the two replicates of CG was 70.23 ± 1.42 g, which is 1.42% and 5.24% lower, than the fish, receiving betaine in amounts 1% and 3%, as the difference was significant (P<0.01) (Table 3).

The analysis of the feed, fed to fish from replicates of the control and the experimental groups was done. The feed conversion ratio of the rainbow trout, receiving 3% betaine was 1.04 ± 0.01 and it was 2.80% lower, than that of the fish, fed 1% betaine, while 7.96% lower, compared to this parameter of individuals from the control group (Table 3). The differences were significant between the experimental groups and this one without betaine supplementation (P<0.01).

Economic analysis

The price of the extruded feed for a rainbow trout was 3200.00 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 rainbow trout in the groups, fed 1% and 3% dietary betaine supplementation was identical 3.47, which was lower, when compared to these ones of the fish from control group by 4.32%.

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. According Regulation NO 4/ 20.10.2000 for the trout farms these parameters must be up to 2 $mg.l^{-1}$ and 0.01 $mg.l^{-1}$ respectively and they were considerably higher, than those, maintained in the water during the experimental period. The optimal values of the traits for all the studied groups are due to the fact that the rainbow trout 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 10% 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 rainbow trout 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 rainbow trout revealed, that it was 70.23 ± 1.42 g in the control group, which was 1.42% and 5.26% lower, than these ones of the fish in the experimental groups, fed 1% and 3% betaine, as the differences were significant (P<0.01) (Table 3).

At the end of the trial period, the feed conversion ratio of the rainbow trout, cultivated in recirculation system and fed 3% betaine was 1.04 ± 0.01. This value is 2.80% lower, than that of the fish, receiving 1% betaine in the diet and 7.96% 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<0.01). 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; Polat and Beklevik, 1999; Przyby *et al*., 1999; Rahimabadi *et al*., 2012).

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

Item	CG	EG_1	EG_2
Price, BGN/t feed (VAT excluded)	3200.00	3245.00	3335.00
Price, BGN/kg feed (VAT excluded)	3.20	3.25	3.34
ECR	3.62	3.47	3.47

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 rainbow trout 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 these ones from the control. The average live weight of the rainbow trout from both replicates of the group, fed 3% betaine was 52.13 ± 2.86 g and it was 1.30% higher, than this one of the fish, receiving 1% betaine and 3.84%, than the values of this parameter of rainbow trouts 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 rainbow trout in the two replicates of EG_2 was 100.17 ± 3.25 g and it was 1.14% higher, that the EG_1 and 4.57%, than CG (P>0.05) (Table 4). 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, Anguila (Mackie and Mitchell, 1982) and common carp (Zhelyazkov *et al*., 2015). 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 groups, fed 1% and 3% betaine had identical economic conversion ratio. In regards of this trait our results were confirmed by those of Virtanen *et al*. (1994) and Przyby *et al*. (1999).

Conclusion

The study showed that the betaine might successfully be used as a feed additive to the diet of rainbow trout. 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.

References

Can, K., Sener, E. (1992). Effect of betaine-added starter feeds on the growth of rainbow trout (*Oncorhynchus mykiss*) fry. *Journal of Aqua Products*, 6(1), 95-104.

Goh, Y., Tamura, T. (1980). Effect of amino acids on the feeding behaviour in red sea bream. *Comparative Biochemistry and Physiology,* 66(2),225-229.

Mackie, A.M., Mitchell, A.I. (1982). Further studies on the chemical control of the feeding behaviour in the Dover sole, *Solea solea*. *Comparative Biochemistry and Physiology*. 73(1), 89-93.

Meyers, S.P. (1987). Aquaculture feeds and chemoattractants. INFOFISH Marketing Digest, 1(87), 35-37.

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 Brufau J. (ed.), Tacon A. (ed.). Feed Manufacturing in the Mediterranean Region: Recent Advances in Research and Technology. Zaragoza: CIHEAM-IAMZ. 217-220.

Przybyl, A., Mazurkiewicz, J., Madziar, M., Hallas, M. (1999). Effect of betafin addition on selected indices of carp fry rearing in ponds. *Archives of Polish Fisheries*, 7(2), 321-328.

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 **NO** 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).

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., Smith, P. (1953). Labile methyl group and its synthesis de novo in relation to growith in chicks. *Journal of Biological Chemistry*, 203, 763-773.

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(1-2), 220.

Zhelyazkov, G., Staykov Y., Georgiev, D. (2015). Effect of dietary betaine supplementation on some productive traits of common carp (*Cyprinus carpio* L.) cultivated in recirculation system. Conference proceedings of 7th International conference "Water and fish", Belgrade-Zemun, Serbia, 518-525.