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Effect of Dietary Betaine on Growth Performance and Body Composition of *Oreochromis aureus* Reared in Fresh and Sea WaterA Comparative Study

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Abstract: This study was performed to examine the effects of betaine on growth performance and body composition of *Oreochromis aureus* reared in fresh and sea water comparatively. Fish was fed with diets containing graded levels of betaine (0, 1, and 2%) during 43 days in fresh water (Experiment I). Some fish cultivated for 43 days in fresh water conditions were transferred to the seawater tanks. Remaining fish were left in same fresh water tanks. In both conditions, fish were fed to same diets during 60 days (Experiment II). Regarding findings of Experiment I, betaine supplementation did not improve the growth of *Oreochromis aureus*. Final weight of fish fed the control diet (0% betaine) showed better performance compared to fish fed 1 and 2 % betaine ($p<0.05$). On the other hand, live weight gain, feed conversion ratio, and survival rates of all groups were found similar at the end of 43 days ($p>0.05$). In experiment II, final weights of control groups in both fresh and sea water conditions were relatively higher than that of the treatment groups for 60 day-feeding period. Fish reared with same treatment groups in both fresh and seawater were observed similar in terms of their final mean weights. Live weight gain, feed conversion and survival rates of fish fed control and 1 and 2 % betaine groups showed no difference in both fresh and seawater conditions ($p>0.05$).

Key words: Betaine, *Oreochromis aureus*, sea water, growth

INTRODUCTION

The use of dietary feeding attractants within compound aquafeeds has received considerable attention for their promoting food intake, providing additional nutrients for protein and energy metabolism and good water quality due to low feed wastage. Betaine (glycine betaine, trimethylglycine) is a highly water soluble and therefore, diffusible compound which has the ability to stimulate the olfactory bulb of fish. It is also considered a positive flavor component for several species of fish[1-5] and a flavor additive in diets or rearing water of crustaceans[6-8]. Betaine is found in high quantities within marine invertebrates[9] micro-organisms and some plants. Furthermore, betaine constitutes an important part of the natural diet of marine carnivorous fish and crustacean species. However, under culture conditions, farmed fish usually have little or no chance of obtaining sufficient quantities of betaine from aquafeeds composed of conventional feed ingredients unless the diet is specifically supplemented with exogenous betaine[10].

Betaine has an important role on osmoregulation and methyl donation, as well. This function is vital when transporting fish such as salmon from a freshwater to a seawater environment, since osmotic stress may result in a reduction in betaine synthesis within the fish liver mitochondria while the uptake of betaine to mitochondria increases[11]. Previous research also reported that feeding with betaine and amino acid supplemented diets promoted seawater adaptation and growth performance in fish[12-16].

Considering the reported functions of betaine, the present study aimed to determine the effects on growth and body composition of *Oreochromis aureus,* a commercially important culture fish, in sea and fresh water environment, comparatively.

MATERIALS AND METHODS

This study was conducted through two separate experiments. Experiment I, was performed in circular tanks (in diameter of 4 m and divided into 4 equal parts with net), in triplicates with randomized block experimental design, at the Fresh Water Fish Culture and Research Station of Fisheries Faculty of Cukurova University, Turkey. 150 fish (mean initial body weight were 1.10±0.41g) were stocked into each side of the tank

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(except one side of each tank was left empty). Fresh water was obtained from an irrigation channel of the Seyhan Dam Lake. In fresh water tanks, water temperature was maintained between 23.5°C and 29.8°C. In this stage, fish were fed ad libitum three times a day (in the morning, noon and late afternoon) with diets containing 0, 1 and 2% betaine during 43 days. Experimental diets were prepared by adding betaine (from Merck) into the commercial carp feed (28-30 % crude protein, Pinar, Izmir, Turkey) at the rates of 0% (control), 1 and 2%. Betaine was dissolved in distilled water because of its high solubility in the water and then pulverized over the feed. Betaine added feeds were dried by using a ventilator in a shadow place until all water was evaporated and stored. Fish was weighed (using a 0.01 g sensitive scales) at the end of 43 days.

Experiment II was carried out both at Fresh Water Fish Culture and Research Station and Yumurtalik Marine Research Station, Fisheries Faculty of Cukurova University, Turkey. Fish (90 of 150) fed for 43 days in fresh water conditions were transferred to seawater tanks. Remaining fish (60 fish of 150) were fed at the same fresh water tanks. Fish in both fresh and sea water tanks were fed in same diets and feeding protocol during 60 days. Ninety fish transported to sea water station were stocked into each tanks with same dimensions and design and acclimated to sea water within seven days. To acclimate the fish, water's salinity was increased gradually during seven days, at the rate of 5 ppt in each day by adding the seawater[17]. Sea water was pumped directly from the sea. Water was pumped into the tanks at 10 l min^{-1}. Water temperature, dissolved oxygen and salinity were measured twice a day. Dissolved oxygen, temperature, dissolved oxygen and salinity varied 7.00-7.72 mg l, 24.1-30.2 °C, 7.34-7.56 mg l, 34.98-35.30 ppt, respectively.

20% of fish from of each tank were weighed (using a 0.01 g sensitive scales) every 20 days. At the end of 60-day experiment, all fish fed at fresh and sea water tanks were harvested and 5 fish from each tank were pooled and stored frozen (-20° C) for proximate analysis. Chemical analyses were performed for both fillet and whole body samples. The proximate compositions of the fish were analyzed according to AOAC[18] procedures as follows: moisture was determined by oven-drying at 105°C for 24 h, crude protein (N×6.25) by the Kjeldahl method and crude ash by combustion in a muffle furnace at 550°C for 16 h. Total lipid concentration was determined by extract with the chloroform-methanol method described by Bligh and Dyer[19].

Final fish weight, live weight gain, survival, feed conversion ratio, carcass composition values were all subjected to one-way analysis of variance to determine if significant differences occurred among dietary treatments. Data were statistically analyzed with one-way ANOVA and Duncan's multiple range tests. Effects with a probability of $p<0.05$ were considered significant. Statistical analyses were performed using SPSS for Windows (Standard Version 9.0 SPSS Inc. Chicago, Illinois). Data were expressed as mean values±SD.

RESULTS

In experiment I, betaine supplementation did not improve the growth. Final weight of fish fed control diet showed better performance compared to fish fed 1% and 2% betaine ($p<0.05$), but live weight gain, feed conversion ratio, and survival rates of all groups were found similar at the end of 43 days ($p>0.05$) Table 1.

In experiment II, final weights of control groups in both fresh and sea water conditions were generally higher than that of the treatment groups for 60-day feeding period Table 2. Final mean weights of fish reared with same treatment groups in both fresh and seawater were found similar. Live weight gain, feed conversion, and survival rates of fish fed control and 1 and 2% betaine groups showed no difference in both fresh and seawater conditions ($p>0.05$) Table 2.

Fillet dry matter and protein contents of fish reared in both fresh and seawater conditions did not differ significantly. But in seawater condition 2% betaine group had the higher protein and dry matter content than that of control and 1% groups ($p<0.05$). The lowest lipid were detected in fresh water control group and this also was significantly different from the seawater control group.Whole body protein content in seawater groups were found lower than fresh water groups generally and 2% betaine groups differed from each other in two environments ($p<0.05$). Both fresh and seawater betaine groups showed generally higher lipid contents compared

Table 1: Mean growth performance of *Oreochromis aureus* fed with diets containing different levels of betaine in fresh water phase during the first 43 days

	Inclusion level (%)		
Parameters	0	1.0	2.0
Initial weight (g)	1.10±0.41	1.10±0.41	1.10±0.41
Final weight (g)	8.88±2.74^{a}	7.92±2.67^{b}	7.43±2.57^{b}
Live weight gain (g)	7.76±0.77	6.82±1.61	6.33±1.26
Feed conversion ratio	0.99±0.04	1.00±0.21	0.97±0.10
Survival (%)	92.67±3.05	94.43±3.16	95.97±3.55

Mean values in rows with different superscript are significantly different ($p<0.05$)

Table 2: Mean growth performance of *Oreochromis aureus* fed with diets containing different levels of betaine in fresh water and seawater phase during the 60 days

	Fresh water			Seawater		
	Inclusion level(%)					
Parameter	0	1	2	0	1	2
Initial weight (g)	8.88±2.74^{a}	7.92±2.67^{b}	7.43±2.57^{b}	8.88±2.74^{a}	7.92±2.67^{b}	7.43±2.57^{b}
Final weight (g)	34.62±12.73^{ab}	30.65±10.71^{b}	31.76±10.06^{ab}	36.34±10.35^{a}	33.75±9.35^{ab}	30.47±9.19^{b}
Live weight gain (g)	25.75±3.25	22.72±4.40	24.33±2.60	27.47±1.32	25.78±1.79	23.04±4.58
Feed conversion ratio	0.99±0.04	1.00±0.21	0.97±0.10	1.17±0.22	1.20±0.16	0.95±0.11
Survival (%)	83.67±14.50	89.43±7.86	90.00±10.00	85.57±8.65	90.73±8.64	87.80±8.40

Mean values (Mean ±SD) in rows with different superscript are significantly different ($p<0.05$)

Table 3: Fillet and whole body composition of *Oreochromis aureus* fed with diets containing different levels of betaine in fresh water and seawater phase during the 60 days

		Fresh water			Seawater		
	Inclusion level (%)	0	1	2	0	1	2
Fillet	Dry matter	23.25±0.13^{ab}	23.23±0.11^{ab}	23.30±0.14^{ab}	22.65±0.23^{a}	22.92±0.31^{a}	23.62±0.23^{b}
	Protein	20.46±0.13^{ab}	20.29±0.12^{ab}	20.42±0.19^{ab}	19.69±0.14^{a}	20.09±0.25^{a}	20.72±0.28^{b}
	Lipid	0.98±0.12^{a}	1.02±0.04^{ab}	1.02±0.02^{ab}	1.14±0.05^{b}	1.12±0.09^{b}	1.15±0.02^{b}
	Ash	1.33±0.12	1.30±0.07	1.36±0.05	1.38±0.01	1.34±0.03	1.39±0.03
Whole body	Dry matter	27.42±0.40^{a}	28.39±0.26^{b}	28.08±0.23^{ab}	27.68±0.30^{ab}	28.41±0.16^{b}	28.27±0.09^{ab}
	Protein	17.06±0.07^{ab}	17.58±0.15^{b}	17.42±0.17^{b}	16.60±0.31^{a}	16.97±0.10^{ab}	16.77±0.09^{a}
	Lipid	4.85±0.58^{ab}	5.36±0.11^{b}	5.22±0.17^{b}	4.56±0.31^{a}	5.21±0.16^{b}	5.35±0.20^{b}
	Ash	3.51±0.15^{a}	3.72±0.13^{a}	3.65±0.16^{a}	4.51±0.14^{b}	4.36±0.07^{b}	4.32±0.11^{b}

Mean values (Mean ±SD) in rows with different superscript are significantly different ($p<0.05$)

to control groups and seawater betaine groups significantly differed from the control. In addition, all groups in seawater had higher crude ash content than those in fresh water ($p<0.05$) Table 3.

DISCUSSION

Betaine was used as a feed attractant and growth promoter for fish and crustaceans and it was reported that it enhanced the growth performance in aquaculture. It has been documented that diets containing 1.5 and 2% betaine increased the growth performance compared to control group for rainbow trout significantly[13,16]. Also, 2% inclusion level of betaine improved the growth performance of African catfish larvae[20]. This growth trend was also similar for fresh water prawn, *Macrobrachium rosenbergii*, that were reared in water added betaine-HCL[8]. Clarke *et al.*, [14] reported faster growth rate for Chinook salmon fed betaine/amino acid additive (1% inclusion), but plasma sodium concentration was measured significantly lower than control group at the end of the seawater stage. For this reason, they indicated that the improved growth performance was due to a reduction in the number of salmon growing poorly. Castro *et al.*,[15] also indicated positive effect of betaine/amino acid mixture (FinnStim) on specific growth rate, feed conversion ratio and survival of Coho salmon after seawater adaptation period. In addition, *Panaeus monodon* fed 1% FinnStim had a significantly higher weight gain and feed conversion ratio compared to control group[7]. However, in the present experiment, betaine supplementation decreased the final weights in both fresh and seawater conditions for *Oreochromis aureus*, while live weight gains for all groups did not differ significantly. This result paralleled Polat *et al.*,[21] as they stated that 1.5% betaine had no effect on growth and body composition of *Oreochromis aureus* reared in fresh water conditions.

The role of betaine on whole body and fillet compositions of *Oreochromis aureus* cultured in fresh and seawater conditions has not been investigated before. In this study, chemical components did not differ generally, an increase was detected in fillet protein content in 2% betaine group for seawater condition, and whole body lipid contents for fresh and seawater conditions were increased. In general, betaine supplementation is expected to decrease body lipid content, as a methyl donor[10] unexpectadely, results from the present study did not support this function.

CONCLUSION

This study represented the first experiment where the level of betaine in rearing *O. aureus* was controlled both in freshwater and sea water conditions as previous studies have been conducted in only one of the water settings. Contrary to the results of previous research on different fish and crustaceans[1-8] this study

did not reveal any positive effects of betaine as a feed attractant and osmotic regulator on *O. aureus* growth in either freshwater or sea water environments. On the other hand, present results encouraged *O. aureus* culture in seawater conditions due to similar growth performance with fresh water conditions.

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