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Effects of Betaine Supplementation in Plant Protein Based Diets on Feed Intake and Growth Performance in Rainbow Trout (*Oncorhynchus mykiss*)

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Abstract

The effects of betaine supplementation on feed intake and growth performance in rainbow trout fed a plant-protein based diet were evaluated. Triplicate groups of rainbow trout (130 g) were fed the plant-protein basal diet, the basal diet with 1.5% betaine supplementation, or a fishmeal-based diet (control) for 80 days. Feed intake was significantly better ($p<0.05$) in fish fed the betaine-supplemented diet (161.2 g/fish) than in fish fed the basal diet (131.6 g/fish) and not significantly different from fish fed the control (152.8 g/fish). Weight gain was significantly higher ($p<0.05$) in fish fed the betaine-supplemented diet (117.0%) than in fish fed the basal diet (95.9%). Results indicate that, in diets based on plant proteins that often have low palatability, betaine supplementation may improve feed acceptability and growth performance in rainbow trout.

Introduction

Feed is the single largest variable expense in intensively managed aquaculture systems. Fishmeal is a major ingredient in fish feeds because of its high protein quality and palatability. However, the increasing demand, high cost, and uncertain availability of fishmeal may necessitate its partial or total replacement with low-cost plant protein sources. Currently, commercial diets for carnivorous fish, such as rainbow trout, contain high levels of animal protein, most often fishmeal, but, due to their poor palatability, low levels of plant feedstuffs.

Few studies have attempted complete replacement of fishmeal although it has been recommended when a palatability enhancer is used (Higgs et al., 1995; Kissil et al., 2000). Low-molecular-weight metabolites, including free amino acids, nucleotides and nucleosides, quaternary ammonium bases, and organic

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acids, act as attractants or feeding stimulants (Toften and Jobling, 1997). Attractants are often prepared from extracts from the tissues of common prey (Toften and Jobling, 1997; Toften et al., 2003). The attractants proline, lysine, histidine, glycine, taurine, and betaine are used to promote feed intake and enhanced growth rates have been reported in sea bass, *Dicentrarchus labrax* (Gomes et al.,1997; Brotons Martinez et al., 2004), striped bass, *Morone saxatilis* (Papatryphon and Soares, 2000a, 2001), and gibel carp, *Carassius auratus gibelio* (Xue and Cui, 2001).

The amino acid betaine is found in microorganisms, plants, and animals and is a significant component of many foods, including wheat, shellfish, spinach, and sugar beets. Betaine is a zwitterionic quaternary ammonium compound also known as trimethylglycine, glycine betaine, lysine, and oxyneurine. It is a methyl derivative of the amino acid glycine with a formula of $(CH_3)_3N^+CH_2COO^-$ and a molecular weight of 117.2. It is characterized as a methylamine because of its three chemically reactive methyl groups. Betaine was first discovered in the juice of sugar beets (*Beta vulgaris*) in the nineteenth century and has subsequently been found in other organisms (Craig, 2004).

Betaine has a detectable flavor when added to diets for some fish and crustacean species. Betaine is a positive flavor component in feeds for *Sparus auratus* (Kolkovski et al., 1997), *M. saxatilis* (Papatryphon and Soares, 2000a), *Oreochromis niloticus* (Kasper et al., 2002), and *C. auratus gibelio* (Xue and Cui, 2001). The aim of the present study was to investigate the effect of betaine supplementation in a plant-based diet on feed intake and growth performance of rainbow trout (*Oncorhynchus mykiss*).

Materials and Methods

Fish and maintenance. Experimental rainbow trout were obtained from a commercial farm, Kuzey Su Urunleri Inc., in Bafra-Samsun, and acclimated in the Ondokuz Mayis University Fisheries Faculty in Sinop (Turkey) for two weeks before the start of the experiment. During acclimation, the fish were fed a commercial diet twice per day to satiation. Fish were stocked in centrally drained 300-l circular fiberglass tanks (water depth 80 cm) in a flow-through water system in an indoor facility during acclimation and during the experiment.

After acclimation, the fish (avg 130 g) were subjected to a fast of 24 h, weighed to the nearest 0.1 g, and randomly stocked in nine experimental tanks at 22 fish per tank with no statistical differences in weight between treatments (p>0.05). Water inflow was adjusted to 4 l/min and supplemental aeration was provided via airstone diffusers. Water quality parameters were monitored daily and averaged 11.3±1.3°C, 6.8±0.1 mg/l dissolved oxygen, pH 7.5. The light:dark cycle was 12 h:12 h. At the start of the experiment, 15 fish were homogenized and analyzed for muscle composition. At the end of the experiment, five fish from each tank were analyzed for muscle composition.

Experimental diets. Three diets were prepared from ingredients obtained from a local fish feed manufacturer (SIBAL Inc.; Table 1). Diet I, the basal diet, contained approximately 57.0% plant feedstuffs and 29.8% fishmeal. Diet II consisted of the basal diet, supplemented with 1.5% betaine. Diet III, the control diet, contained 44.6% fishmeal and 42.3% plant feedstuffs. Chromic oxide was incorporated into the test diets as a marker to assess apparent digestibility of the diets. Ingredients were thoroughly mixed, homogenized, moistened by the addition of 35% boiling water, and pelleted (3.0 mm) in a mincing machine. The pellets were dried at 70°C for 18 h, cut into pieces approximately 5 mm in length, and stored in plastic bags in a refrigerator.

Feeding and fecal collection. Fish were fed experimental diets by hand to apparent satiation twice a day (09:00 and 15:00), six days a week. All possible care was taken during feeding so that no uneaten feed settled on the tank bottoms. Feed for each tank was weighed daily to a constant amount (100 g) and feed consumption in each tank was determined by subtracting unconsumed feed from the ration. Tanks were thoroughly cleaned after each feeding. Starting on day 7 of the experiment, fecal matter was collected daily

Table 1. Composition of the experimental diets.

	Basal diet	*Betaine diet*	*Control diet*
Ingredient (g/kg)			
Fishmeal	298.0	290.0	446.5
Fish oil	122.0	122.0	121.0
Full fat soybean meal	210.5	208.0	183.0
Extracted soybean meal	42.0	41.5	40.0
Wheat meal	60.0	60.0	130.0
Maize gluten	130.0	127.0	10.0
Sunflower meal	128.0	127.0	60.0
Vitamin premix[1]	2.0	2.0	2.0
Mineral premix[1]	1.5	1.5	1.5
Vitamin C	0.6	0.6	0.6
Vitamin E	0.4	0.4	0.4
Cr_2O_3	5.0	5.0	5.0
Betaine[2]	-	15.0	-
Proximate composition (%)			
Dry matter	94.2	94.2	93.5
Crude ash (% dry matter)	6.8	6.6	7.2
Crude lipid (% dry matter)	20.0	19.7	20.1
Crude protein (% dry matter)	40.8	41.8	41.7
NFE+fiber[3]	32.4	31.9	31.0
Gross energy (kJ/g)	23.3	23.3	23.2
Cr_2O_3	0.36	0.37	0.36
Amino acids (% of diet)			
Alanine	2.84	2.95	2.67
Histidine	0.79	0.79	0.80
Isoleucine	2.13	2.16	2.25
Leucine	4.75	4.74	3.76
Lysine	2.28	2.23	1.91
Methionine	0.92	0.94	0.78
Phenylalanine	2.59	2.50	2.22
Threonine	1.86	1.91	2.11
Tyrosine	1.76	1.69	1.54
Valine	2.33	2.44	2.62

1 Per kg feed: 12500 IU vitamin A; 2500 IU vitamin D3; 10 mg vitamin K3; 10 mg vitamin B1; 20 mg vitamin B2; 15 mg vitamin B6; 0.03 mg vitamin B12; 250 mg vitamin C; 200 mg niacin; 1 mg biotin; 10 mg folic acid; 60 mg pantothenic acid; 1000 mg Ca; 130 mg ethoxyquin; 600 mg magnesium; 450 mg potassium; 90 mg zinc; 12 mg manganese; 5 mg Cu.

2 Betafin®S1-DANISCO, a crystalline product containing 96% betaine and 1% calcium stearate to improve flowability

3 Nitrogen-free extract = 100 - (%crude ash + %crude lipid + %crude protein)

between 11:00 and 12:00 and between 16:00 and 17:00 by slow siphoning with an 8-mm plastic tube. Fecal samples were immediately frozen and stored at -20°C pending analysis. The experiment lasted 80 days.

Chemical analyses. Chemical composition of dried samples of fish, diets, and feces were analyzed by standard methods (AOAC, 1995). Crude protein was analyzed according to the Kjeldahl method (N x 6.25), crude lipid by petroleum ether extraction in a Soxhlet apparatus, and ash by incineration at 550°C in a muffle furnace. Chromic oxide in the diet and feces was determined spectrophotometrically according to Bolin et al. (1952). Apparent digestibility coefficients for dry matter, nutrients, and energy were calculated as ADC (%) = 100 - [100(% Cr_2O_3 in diet/% Cr_2O_3 in feces) x (% nutrient in feces/% nutrient in diet)] as per Degani et al. (1997) and Degani (2006); ADC of dry matter (%) = 100 - [100(% Cr_2O_3 in diet/% Cr_2O_3 in feces) as per De Silva and Anderson (1995). Amino acids were analyzed by a hydrolysis method using a Phenomenex EZ Faast GC-FID at the TUBITAK Marmara Research Center in Gebze. All chemical analyses were carried out in duplicate.

Calculations. Growth performance (%) was determined by the formula 100[(final wt - initial wt)/initial wt], specific growth rate as SGR (%) = 100[(ln final wt - ln initial wt)/time in days], feed conversion ratio as FCR = (feed intake/wet wt gain), and protein efficiency ratio as PER = (wt gain/protein intake).

Statistical analyses. Statistical analyses were carried out using SPSS 11.0 for Windows (2001 SPSS Inc.). One-way ANOVA was used to test for significant differences among treatment groups. Differences among treatments were compared using Duncan's multiple range test and considered significant at $p<0.05$.

Results

No mortality occurred during the study. The weight gain of fish fed the betaine-supplemented diet was significantly higher than that of fish fed the basal diet but similar to that of fish fed the control (Table 2). Similar results were observed in specific growth rate. Fish fed the supplemented diet consumed 23% more feed than fish fed the basal diet while fish fed the control diet consumed 16% more. The feed conversion ratio was similar for all treatments.

Table 2. Growth performance and feed efficiency in rainbow trout fed a plant-based diet (basal diet), the basal diet supplemented with betaine, or a fishmeal-based diet (control).

	Basal diet	*Betaine diet*	*Control diet*
Initial body wt (g)	132.6±1.14	131.5±0.59	132.6±0.05
Final body wt (g)	259.9±8.48[a]	285.4±8.05[b]	290.2±4.74[b]
Wt gain (%)	96.0±3.52[a]	117.0±9.94[b]	118.8±6.07[b]
Feed intake (g/fish)	131.6±7.39[a]	161.2±3.47[b]	152.8±3.81[b]
SGR (%)[1]	0.84±0.04[a]	0.97±0.03[b]	0.98±0.02[b]
FCR[2]	1.03±0.02	1.05±0.03	0.97±0.01
PER[3]	2.36±0.06	2.28±0.09	2.47±0.01

Values are means±SEM from triplicate groups of fish. Values in a row with different letters significantly differ ($p<0.05$).

[1] Specific growth rate = 100[(ln final body wt - ln initial body wt)/80 days]

[2] Feed conversion ratio = total diet fed/total wt gain

[3] Protein efficiency ratio = wt gain/protein intake

Apparent digestibility of crude protein and crude lipid were high for all groups and there were no significant differences in ADC of apparent dry matter, crude protein, crude lipid, NFE+crude fiber, or energy (Table 3).

Dry matter and gross energy contents in the dorsal muscle were not affected by the level of plant protein in the diet and were about the same in all experimental groups (Table 4). There appeared to be a decrease in carcass protein and ash as the proportion of plant protein increased. In contrast, the lipid level increased as the proportion of plant protein increased.

Discussion

The addition of betaine to a plant-protein based diet tended to enhance feed intake and growth performance in rainbow trout. The daily intake of fish fed the betaine diet was about 23% higher than the intake of fish fed the basal diet while fish fed the control diet consumed only 16% more feed, indicating that betaine acted as a feeding stimulant in rainbow trout, in agreement with findings in other fish species. Dietary choline:betaine supplementation resulted in a significant increase in feed consumption and weight gain in tilapia (Kasper et al., 2002) while betaine and amino acids had additive effects in striped bass (Papatryphon and Soares, 2000a). Likewise, a betaine supplemented diet resulted in enhanced feed preference in juvenile gibel carp (Xue and Cui, 2001).

When supplemented with the feeding stimulant Finnstim, a betaine-containing palatability enhancer, 66% of the fishmeal in rainbow trout feeds was replaced by rapeseed

Table 3. Apparent digestibility coefficients (%) of the experimental diets.

	Basal diet	*Betaine diet*	*Control diet*
Dry matter	80.3±1.23	82.4±0.27	83.0±0.38
Crude protein	91.6±0.60	92.2±0.16	92.9±0.18
Crude lipid	96.9±0.25	97.3±0.05	97.5±0.15
NFE+crude fiber	68.9±0.65	67.6±1.17	69.5±0.72
Gross energy	86.6±0.85	88.5±0.44	88.6±0.22

Values are means±SEM from triplicate groups of fish and did not differ between treatments.

Table 4. Chemical composition (%) of the dorsal muscle of rainbow trout fed experimental diets

	Initial	*Basal diet*	*Betaine diet*	*Control diet*
Dry matter	25.4	25.0±0.04	25.9±0.78	26.2±0.06
Crude protein*	57.6	57.2±0.88^a	59.8±1.37^{ab}	62.6±1.37^b
Crude lipid*	20.4	24.8±0.25^a	25.0±0.92^a	21.0±0.33^b
Ash*	6.2	4.9±1.15^a	4.6±0.07^a	5.8±0.11^b
Gross energy (kJ/g)	24.4	25.6±0.04	25.7±0.39	24.9±0.04

Values are means±SEM from triplicate groups of fish. Values in a row with different letters significantly differ (p<0.05).
* % of dry matter

protein concentrates without significant reduction in feed intake and growth although, when 100% of the fishmeal was replaced, fish growth was reduced (Teskeredzic et al., 1995). When fishmeal was completely replaced by dephytinized rapeseed protein concentrate in combination with Finnstim and minerals (constituting 59% of the dietary protein), no major adverse effects on growth of rainbow trout were observed (Prendergast et al., 1994). Growth, feed conversion ratio, protein retention efficiency, and energy retention efficiency were improved in rainbow trout fed plant protein diets containing the feeding stimulant taurine (Gaylord et al., 2006).

In our study, fish fed the basal diet grew significantly less than fish fed the betaine-supplemented or control diet while there was no significant difference between the betaine-supplemented and control groups. The lower growth obtained in fish fed the basal diet could be attributed to the lower feed intake, possibly due to lower palatability. A similar reduced weight gain was reported in Asian sea bass fed plant-based diets, a finding attributed to lower palatability and feed intake (Boonyaratpalin et al., 1998). The use of stimulants in fish feeds improves palatability, resulting in improved intake and growth rate (Toften and Jobling, 1997). Stimulant supplementation also enhanced growth when a betaine/amino acid mixture was used in combination with commercial-type diets fed to salmonids (Clarke et al., 1994; Virtanen et al., 1994; Castro et al., 1998). Our results support these findings, suggesting that 1.5% betaine supplementation is sufficient to encourage adequate feed intake and growth in rainbow trout.

Stimulant supplementation can affect not only feed intake and subsequently weight gain, but also FCR, indicating an overall increase in diet efficiency (Papatryphon and Soares, 2000b). However, in our study, there was no difference in FCR between treatments, indicating that all the diets had high nutritive and digestive values. All our diets were well digested and there were no statistical differences between treatments although the basal diet contained approximately 57.0% and the control diet only 42.3% plant feedstuffs. Replacement of up to 66% of fishmeal by plant protein in rainbow trout diets is possible without negative effects on digestibility (Gomes et al., 1995).

The apparent digestibility coefficients in the present study were quite high, indicating the adequate quality and efficiency of the ingredients in the test diets. Digestibility values in fish normally range 75-95% for protein and 85-95% for lipid (NRC, 1993). Our values are similar to those of diets containing fishmeal as well as plant protein (Kaushik et al., 1995; Refstie et al., 1997; Aksnes and Opstvedt, 1998).

In conclusion, feed stimulants or attractants can be added to plant-based fish diets to enhance the acceptance and utilization of these economical and nutritional diets. Our study suggests that betaine is an effective feeding stimulant that can enhance the palatability and intake of plant-protein based diets for rainbow trout. The results cannot be generalized for all plant-protein-rich diets but similar feeds can be supplemented with betaine to improve the palatability and feed intake in rainbow trout. Should ecological considerations mandate the substitution of fishmeal by plant protein in fish diets in the near future, the use of feed attractants in fish feeds may become necessary.

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