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STUDIES ON THE USE OF BRANCHED CHAIN AMINO ACIDS IN THE DIET FOR SALMONIDS

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ABSTRACT

Rainbow trout *(Salmo gairdnerii)* and Atlantic salmon *(Salmo salar)* were fed low protein diets, with or without supplementation by mixtures of branched chain amino acids. Groups receiving standard high protein diets were included as controls.

In both species of fish the high protein groups gained weight more rapidly than the experimental low protein groups, but the differences were not great. No consistent improvement of growth was observed when branched chain amino acids were given as a supplement to the low protein diets.

No differences were found between groups in the muscle tissues activities of the branched chain amino acid metabolizing enzymes, leucine: α-keto glutarate aminotransferase and branched chain α-keto acid dehydrogenase, in either species. These enzyme activities were lower in salmon muscle than in trout muscle.

INTRODUCTION

Most essential amino acids are deaminated and metabolized in the liver, but this is not so for the branched chain acids, leucine, isoleucine and valine (Odessey and Goldberg, 1979). In the rat, muscle and kidney tissues are rich in branched chain amino acid transaminase, and rat skeletal muscle contains an active branched chain α-keto acid dehydrogenase, an enzyme which is regulated by phosphorylation and dephosphorylation (Odessey, 1980; Paul and Adibi, 1983). We have found high activity of the branched chain amino acid transaminase in dark muscle and kidney from rainbow trout (Teigland and Klungsøyr, 1983), and the dark muscle from rainbow trout is also rich in branched chain α-keto acid dehydrogenase (Ottesen and Klungsøyr, 1984).

Thus, the branched chain amino acids may be regarded as an energy source, committed for use mainly in the dark muscle. This might be one reason why the trout needs high levels of protein in its diet for optimal growth (Christiansen and Klungsøyr, 1987).

Briefly, our working hypothesis was that branched chain amino acids in part may replace protein for muscular energy metabolism. As a consequence, salmonids should grow as well on a reduced protein diet supplemented with branched chain amino acids, as on a high protein diet. The present experiments were performed to test this hypothesis.

Hughes et al. (1984), in experiments with lake trout fingerlings, showed that high levels in the diet of one of the branched chain amino acids, leucine, was actually growth inhibitory if dietary isoleucine and valine were low. We arrived at similar conclusions based on preliminary experiments with rainbow trout.

EXPERIMENTAL

For experiment 1, rainbow trout, *Salmo gairdnerii,* weighing approximately 200 g at the start, were distributed into 5 duplicate groups of 50 fish in tanks of 1500 l sea water. Feed was given by automatic feeders, *ad libitum,* but care was taken not to overfeed, and the tanks were regularly inspected for excess feed. The fish were weighed at the start of the experiment, after 48 days, and after 95 days (the end of the experiment).

The composition of the diets are presented in table 1. Group 1 received a diet containing 42% protein from fish meal, while groups 2 and 3 received diets with 28% fish meal protein. Group 3 received in addition a mixture of branched chain amino acids calculated to correspond to the amounts present in 10.4% protein, which was the calculated difference in protein levels from the control group.

The protein source for groups 4 and 5 was mixed from fish meal and blood meal, as the latter is rich in leucine and valine, and calculated to approxi-

Table 1. Composition of the diets (g/kg).

	Expt. 1					Expt. 2		
Group	1	2	3	4	5	1	2	3
Fish meal	570	400	400	165	165	611	335	335
Blood meal	–	–	–	175	175	–	–	–
Capelin oil	180	220	220	230	230	139	213	213
Cooked wheat	220	350	350	400	400	60	60	60
Corn starch	–	–	–	–	–	155	357	322
Vit. + min. mix	30	30	30	30	30	35	35	35
Amino acid addition:								
Valine	–	–	5.9	–	–	–	–	11
Leucine	–	–	8.5	–	–	–	–	15
Isoleucine	–	–	5.1	–	5.3	–	–	9
Protein (calc., N · 6.25)	425	270	295	–	–	453	264	287

mately 28% protein. Group 5 received in addition sufficient isoelucine to bring the total amounts of branched chain amino acids up to the level corresponding to the group 1 feed.

For experiment 2, Atlantic salmon, *Salmo salar,* weighing approximately 200 g at the start, were distributed into 3 duplicate groups and fed the diets given in table 1, by the same procedures as for the fish in experiment 1. The fish were weighed after 34 and 76 days.

Enzyme assays: The α-ketoglutarate: branched chain amino acid aminotransferase and the branched chain α-keto acid dehydrogenase activities were measured in individual samples of the dark muscle of 6 fish from each dietary group after completion of the feeding experiments. The dehydrogenase assays were carried out in lysed mitochondria as described by Ottesen and Klungsøyr (1984). When preparing the mitochondria, samples of the homogenates were saved for determination of the aminotransferase activities by the method of Teigland and Klungsøyr (1983). Protein in the homogenates and the mitochondrial preparations was determined according to Klungsøyr (1969).

In experiment 1 the concentrations of free amino acids were determined in extracts of dark and white muscle samples from one fish in each tank. Samples of tissues were homogenized in equal volumes of 67 mM phosphate buffer, pH 7.0, and centrifuged. The supernatants were mixed with equal volumes of 6% sulfosalicylic acid and analyzed on an Amino Analyzer Liquimat III (Kontron) using norleucine as an internal standard. The values reported are the group averages (n = 2).

At the start and the completion of the feeding experiments the fish were analyzed for protein, fat, ash and water. Five fish from each tank were killed, ground, and the mass was pooled and mixed. The mixed samples as well

Table 2. Weight gain (g, ± S.D.) in rainbow trout from experiment 1.

Group	Day 0	Day 48	Day 95
1a	198.6 ± 56.8 (50)	280.5 ± 75.6 (47)	406.6 ± 107.7 (41)
b	198.3 ± 61.3 (50)	269.8 ± 88.3 (46)	387.1 ± 129.9 (42)
2a	185.6 ± 63.2 (50)	251.0 ± 85.8 (42)	348.2 ± 125.3 (41)
b	197.7 ± 56.9 (50)	254.8 ± 80.8 (40)	350.8 ± 111.0 (38)
3a	210.2 ± 65.9 (50)	290.2 ± 85.3 (43)	401.3 ± 129.0 (43)
b	217.7 ± 61.2 (50)	296.7 ± 88.7 (47)	403.3 ± 118.5 (45)
4a	214.0 ± 55.4 (50)	255.4 ± 68.6 (47)	311.6 ± 87.4 (43)
b	200.3 ± 58.5 (50)	248.7 ± 71,1 (43)	332.4 ± 106.0 (38)
5a	212.7 ± 56.8 (50)	253.0 ± 74.1 (47)	323.9 ± 95.4 (45)
b	200.9 ± 55.8 (50)	246.2 ± 72.3 (41)	316.8 ± 94.7 (38)

as samples of the feeds were analyzed for protein by the Kjeldahl method with mercuric oxide as a catalyst, and calculated as N · 6.25. Fat was determined by Soxhlet extraction with diethyl ether, followed by removal of the solvent, drying and weighing of the extracted fraction. Water was determined by drying at 103° for 4 hours if the water content was low, and for 16 hours when the samples contained much water. Ash was weighed after ashing at 500° for 4 hours.

Table 3. Weight gain in salmon from experiment 2 (g ± S.D.).

Group	Day 0	Day 34	Day 76
1a	195.5 ± 21.9 (60)	250.7 ± 42.4 (60)	303.4 ± 74.2 (59)
b	196.7 ± 26.4 (60)	250.4 ± 33.0 (59)	316.2 ± 53.6 (59)
2a	191.7 ± 27.9 (60)	227.5 ± 37.0 (60)	287.8 ± 43.3 (58)
b	188.7 ± 24.9 (60)	237.6 ± 29.5 (59)	283.3 ± 54.5 (58)
3a	195.8 ± 26.1 (60)	236.8 ± 27.5 (60)	284.6 ± 48.2 (59)
b	196.4 ± 25.2 (60)	227.7 ± 30.4 (58)	282.9 ± 41.9 (58)

RESULTS

Experiment 1. Growth values are presented in Table 2. Group 4 and 5, receiving blood protein, grew poorly compared to group 1 (control). Group 3, receiving low protein and extra branched chain amino acids grew as well as the control group, while group 2, low protein without amino acids, gained weight more slowly during the first 48 days of the experiment. However, during the last 47 days, group 2 gained weight more rapidly, at about the same rate as group 1 and 3. There were no differences in composition of the fish in groups 1, 2 and 3 at the end of the experiment. No differences in the activities of leucine: α-keto glutarate amino transferase and branched chain α-keto acid dehydrogenase were found in the dark muscle from fish of groups 1, 2 and 3 at the end of the feeding period (Table 4).

The concentrations of the free amino acids in white and dark muscle samples from the 5 groups showed no effects of the diets except for elevated histidine values in the groups given blood meal (Table 5).

Experiment 2. The low protein diet supplemented with branched chain amino acids supported growth in the salmon no better than the unsupplemented low protein diet (Table 3). Both groups had a growth of about 80% of the control group. The difference was small, considering that the low protein diets supplied only 26 per cent of the energy as protein. In the control feed, 45 per cent of the energy came from protein. There were no differences in the protein content of the fish between groups at the end of the experiment, but as expected from the feed composition, group 1 contained slightly less fat than groups 2 and 3 (7,2% against 8,8%).

No significant differences between groups 2 and 3 were observed in the activities of amino transferase and α-keto acid dehydrogenase in the dark muscle (Table 4). However, α-aminotransferase activity in group 1 was significantly higher than in groups 2 and 3, showing the effect of reduced dietary protein in the latter groups, regardless of the added branched amino chain acids in group 3. The enzyme activities were low, compared to the values observed in dark muscle from rainbow trout (Expt 1.). It is not known whether the difference is incidental, or due to a species difference.

Table 4. Activities of branched chain amino acid metabolizing enzymes in dark muscle tissues (nanomoles per min and mg protein, ± S.D., n = 6).

Group	Leucine/α-ketoglutarate aminotransferase	α-keto acid dehydrogenase
	Expt. 1	
1	18.3 ± 2.9	3.7 ± 0.4
2	17.0 ± 2.3	4.4 ± 1.3
3	18.0 ± 3.3	4.1 ± 0.7
	Expt. 2	
1	4.02 ± 0.21	1.00 ± 0.34
2	3.17 ± 0.48	0.83 ± 0.08
3	2.85 ± 0.79	0.98 ± 0.51

Table 5. Free amino acid contents in white and dark muscle from rainbow trout in experiment 1 (mg per g tissue).

Group	Tau	Gly	His	Val	Ile	Leu
1 dark	714.7	15.9	21.5	2.9	2.6	3.2
white	19.4	127,6	62,8	5.0	2.4	2.0
2 dark	686.9	25.4	24.6	0.9	2.8	3.5
white	18.0	179.0	52.0	5.8	2.6	2.8
3 dark	660.4	25.7	23.2	2.0	2.5	2.7
white	28.6	118.4	43.3	4.0	1.9	2.6
4 dark	488.5	34.6	79.3	2.6	1.5	2.3
white	21.1	114.4	131.1	3.5	1.5	1.5
5 dark	609.9	15.8	39.8	3.8	2.3	2.7
white	14.2	115.7	166.1	4.4	2.3	2.1

DISCUSSION

It has been demonstrated that leucine stimulates protein synthesis in muscle tissue in the rat (Hong and Layman, 1984), but the mechanism of this effect is not clear. The branched chain amino acids are believed to share the transport vehicle into muscle, and also the initial enzymes of their metabolism.

Branched chain amino acids are not changed by their passage through the liver, but are metabolized in the kidney and the dark muscle tissue. Dark muscle cells of pelagic fish have metabolic activities similar to those in the heart. Trout are rarely completely at rest, so their dark muscle have a steady supply of oxidizable metabolites and oxygen. It is not known whether the dark muscle depends upon the supply of branched chain amino acids, or if fatty acids and glucose alone can cover the need. This might influence the absolute protein requirement of the animal.

In mammals, branched chain amino acids have a common transaminase (Aki and Ichihara, 1970) and α-keto acid dehydrogenase (Parker and Randle, 1978). Presumably, this is also the case in fish. Chance et al. (1964) found an antagonism between the different branched chain amino acids for growth of chinook salmon. Similary, Robinson et al. (1984) observed growth inhibition by leucine in channel catfish, when the diet was deficient in isoleucine or valine. Hughes et al. (1984) studied the effects in lake trout fingerlings of high levels of each of the three branched chain amino acids and found a competition between the individual branched chain amino acids, probably both at the point of uptake into the cells, and in the intracellular metabolic processes.

The difference in growth rates of rainbow trout in expt. 1. was small between protein levels (82% weight of the control groups in 95 days in the low protein group). During the first period of the experiment the addition of branched chain amino acids seemed to make up for the lower protein content of the food. However, this effect did not persist during the last part of the experiment. The effect must therefore be considered to be spurious.

Blood protein, with or without isoleucine addition, gave much poorer growth than the control feed, especially during the last part of the experimental period. Possibly, this is caused by low acceptability of the feeds containing blood protein.

The supplementation of branched chain amino acids to the low protein feed, gave no increase in growth of salmon in expt. 2. Also, the low protein content resulted in a weight gain rate (50% in 77 days) that was only a little less than that seen with control feed containing 45% protein (58% in the same period). This difference is small and may possibly be explained by a low absorption or poor utilization of absorbed glucose from the low protein feed, as corn starch was used to balance the decreased protein energy.

In conclusion it must be stated that we found no evidence for a positive effect upon the growth of trout or salmon by the addition of branched chain amino acids to a diet which should be suboptimal in protein for growth.

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