Requirements for sulfur amino acids and utilization of D-methionine by rainbow trout *(Oncorhynchus mykiss)*[1]

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ABSTRACT

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The methionine (Met) requirement and replacement values of cystine (Cys) and D-Met for L-Met were estimated at 15°C for fingerling rainbow trout (*Oncorhynchus mykiss*) by feeding diets containing various levels of L-Met, D-Met and L-Cys. Four tanks, each containing 28 (Experiment 1) or 30 (Experiment 2) trout (11–15 g initial weight), were assigned to each diet. Weight gain and nitrogen retention during a 6-week period increased with increasing levels of L-Met up to 0.5% of diet, when the diets contained excess L-Cys (0.5%). Feed efficiency increased as L-Met increased, but no significant ($P>0.05$) differences were detected at levels above 0.4%. With diets containing 0.5% L-Met, the Cys requirement was found to be 0.3% of the diet. Increasing D-Met level above 0.27% in a diet containing 0.23% L-Met did not significantly ($P<0.05$) influence weight gain, feed efficiency or nitrogen retention. Analysis of the results showed that 1. the Met requirement of fingerling rainbow trout is 0.52 (1.49)% of diet (dietary protein), when a diet contains excess Cys; 2. the Cys replacement value (on an equal molar sulfur basis) of L-Cys for L-Met is approximately 42%; 3. the requirement of trout for total sulfur amino acids is about 0.8 (2.3)% of diet (dietary protein); and 4. rainbow trout utilize D-Met as efficiently as L-Met for weight gain but less efficiently for protein accretion.

INTRODUCTION

Methionine requirements of various fish species, in the absence of Cys, have been estimated and are summarized in NRC(1981, 1983) publications : 1.6 % of diet(4.0% of dietary protein) for chinook salmon*(Oncorhynchus tshawytscha)*, 0.56(2.3)% for channel catfish(*Ictalurus punctatus)*, 1.2(3.1) % for carp(*Cyprinus carpio)* and 2.1(4.5)% for Japanese eel(*Anguilla japonica*).

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Walton et al.(1982) reported that the Met requirement of 25-g rainbow trout was between 0.5(1.0) and 1.0(2.1)% of dry diet(protein) in the absence of Cys and that 0.5% was adequate when dietary Cys was 2%. A range of Met requirement values of rainbow trout was reported by Rumsey et al.(1983): 0.55-0.75(1.57-2.14) % of diet(protein) in the presence of adequate Cys. Ogino(1980) has also estimated the amino acid requirements of rainbow trout and carp on the basis of daily increase of protein(amino acids) in the body of fish fed diets containing about 35% protein, and daily excretion of nitrogen by fish fed a protein-free diet. His calculated values for Met and Cys requirements of rainbow trout and carp were 0.7(1.8) and 0.35 (0.9), and 0.62(1.6) and 0.31(0.8)% of diet (protein), respectively.

The variations in the estimated Met requirement values within and among species suggest that more work is needed to pinpoint Met requirements of fishes. Further, studies on the Cys replacement values for L-Met(Harding et al., 1977) and on the utilization of DL-Met(Robinson et al., 1978) have been reported for channel catfish but no other fish species.

Therefore, the following experiments were conducted to determine the Met requirement and the replacement values of L-Cys and D-Met for L-Met in fingerling rainbow trout(*Oncorhynchus mykiss*).

EXPERIMENTAL PROCEDURES

In a preliminary experiment, 14-g rainbow trout fed diets containing 1.18(0.9), 0.58(0.9), 0.23(0.9) or 0.58(0.2)% L-Met(L-Cys) gained 21, 22, 11 and 21 g/fish in 6 weeks, respectively. These results suggested that the Met requirement of fingerling rainbow trout was between 0.23 and 0.58% and the requirement for total sulfur amino acids was no more than 0.78%.

Based on these preliminary findings, Experiment 1 was done to determine the absolute L-Met requirement, excluding any fraction that might be attributable to a need for Cys. Diets used for Experiment 1 contained 0.23(0.5), 0.3 (0.5), 0.4(0.5), 0.5(0.5), 0.6(0.5), 0.8(0.5), 0.4 (0.4), 0.4(0.3) or 0.3(0.3)% L-Met(L-Cys). Of these levels, 0.23% Met and 0.028% Cys were supplied by casein and gelatin.

In Experiment 2, which was done to determine the L-Cys and D-Met replacement values for L-Met, diets contained varying levels of L-Cys from 0.03 to 0.5% along with 0.5% L-Met (diet 1 through 5); 0.03% L-Cys and 0.8% L-Met(diet 6); or 0.23% L-Met plus 0.5% L-Cys and 0.27 or 0.47% D-Met(diet 7, 8 and 9, respectively). Ingredients of the basal diet and the amino acid composition of experimental diets are shown in Tables 1 and 2, respectively.

Four replicate groups, each consisting of 28 (Experiment 1) or 30(Experiment 2) rainbow trout, were assigned to each diet. Osceola strain rainbow trout were provided by the U. S. Fish and Wildlife Service-National Fish Hatchery, Osceola, WI and mean initial body weight±s.e. m. was 15.4±0.02 g for 36 tanks of 28 fish each used for Experiment 1 and 11.2±0.1 g for

TABLE 1

Composition of the basal diet

Ingredient	%	Ingredient	%
IDAA[1]	10.73	Herring oil[5]	10.00
DAA[1]	16.26	DL-α-tocopherol acetate[3]	0.0455
Casein[2,3]	8.91	Carboxy methyl cellulose[4]	1.00
Gelatin (89% CP)[3]	2.00	Vitamin mixture[6]	2.00
Dextrin[3]	27.16	Mineral mixture[7]	5.06
Dextrose[3]	5.00	$CaHPO_4$	2.94
α-cellulose[4]	8.20	Choline chloride[3]	0.70
		Crude protein[8]	35.00

[1] Amounts of IDAA (indispensable amino acids) and DAA (dispensable amino acids) varied as the methionine or cystine level varied (see Table 2).

[2] Vitamin-free, 92% crude protein.

[3] United States Biochemical Corp, Cleveland, OH, USA.

[4] Sigma, St. Louis, MO, USA.

[5] Glencoe Mills, Glencoe, MN, USA.

[6] As mg/kg of diet: vitamin A (500 IU/mg), 50; vitamin D_3 (1000 IU/mg), 4; menadione, 16; D-Ca-pantothenate, 288; d-biotin, 1.6; folic acid, 19.2; vitamin B_{12} (0.1%), 160; thiamin HCl, 64; riboflavin, 144; niacin, 512; pyridoxin HCl, 48; L-ascorbic acid, 1200; myo-inositol, 2500; p-amino benzoic acid, 400; celite, 14 139.

[7] As mg/kg of diet: NaCl, 5842; KI, 1.91; $CaCO_3$, 4010; K_2HPO_4, 15 466; K_2SO_4, 12 984; Na_2HPO_4, 2320; MgO, 4774; $FeSO_4 \cdot 7H_2O$, 1066; $MnCO_3$, 797; $2CuCO_3 \cdot Cu(OH)_2$, 64.9; $ZnCO_3$, 154.7; NaF, 3.82; $CoCl_2 \cdot 6H_2O$, 95.4; citric acid, 3021.

[8] Percent of dry diet.

36tanks of 30 fish each used for Experiment 2. Fish were fed experimental diets three times/day to satiation in tanks containing 100 ℓ of water at 15±0.5℃.

Other procedures including experimental conditions, diet preparations, carcass analysis and statistical analysis are described in Kim et al. (1987).

RESULTS AND DISCUSSION

Weight gain, feed efficiency and nitrogen retention of fish are presented in Table 3. With diets containing 0.5% L-Cys, elevating L-Met from 0.23 to 0.5% increased weight gain, feed efficiency and nitrogen retention, but further elevations had no effect. By applying a broken-line model(Robbins et al., 1977), the Met requirement was estimated at approximately 0.52 % of diet.

A similar value was reported by Walton et al. (1982) who found that 0.5% L-Met was adequate for 25-g rainbow trout, when the dietary Cys level was 2%. Rumsey et al.(1983) estimated the Met requirement of rainbow trout at 0.55-0.75%(1.75-2.14)% of the diet(protein) in the presence of adequate dietary Cys. They also indicated a requirement of 0.3% Cys with a diet marginally deficient in Met. However, the experimental data of Rumsey et al.(1983) clearly show that the Met requirement of 8.8 g rainbow trout is less than 0.55(1.57%) of diet (prorein) in the presence of 0.3% Cys. Rumsey et al(1983) found cataracts in trout fed diets deficient in sulfur amino acids, and most of the fish fed diet 1 containing 0.23% L-Met and 0.5 % L-Cys in the present study had cataracts in either both or one eye. None in the other groups showed an opaque lens in their eyes.

TABLE 2

Amino acid composition (% of dry diet)

Amino acid	From casein + gelatin[1]	From crystalline amino acids	Total[2]
IDAA			
Arginine	0.474	1.803	2.277
Histidine	0.248	0.671	0.919
Isoleucine	0.491	1.435	1.926
Leucine	0.768	2.427	3.195
Lysine	0.715	1.691[3]	2.406
Methionine	0.234	0.948[4]	1.182
Cystine	0.028	0.163[4]	0.191
Phenylalanine	0.427	1.586	2.013
Tyrosine	0.491	1.114	1.605
Threonine	0.412	1.410	1.822
Tryptophan	0.098	0.429	0.527
Valine	0.603	1.746	2.349
DAA			
Alanine	0.397	1.689	2.086
Aspartic acid	0.675	3.088	3.763
Glycine	0.715	0.581	1.296
Glutamic acid	1.956	2.958	4.914
Proline	1.224	0.134	1.358
Serine	0.489	2.283	2.772

[1] Calculated from values provided by the United States, Biochemical Corp., Cleveland, OH, USA.
[2] The amino acid profile simulated that of 35% whole-chicken-egg protein (Robinson et al., 1981).
[3] Added as lysine HCl (2.113% for 1.691%).
[4] Levels of crystalline methionine or cystine were varied at the cost of DAA, and D-methionine was substituted for L-methionine in Experiment 2.

The requirement values of rainbow trout for Met(0.7% of diet) and Cys(0.35%) reported by Ogino.(1980) are likely overestimated because of a low feed-intake level(3.0% of body weight) used to calculate the amino acid requirements. The actual average daily gain of trout fed diets containing about 35% protein supplied from various sources, from egg yolk to casein, was 3.44g/100 g body weight. Given a best feed/gain of 1.20, the average daily feed intake would be 4.13 g. Therefore, his reported Met requirement(0.72%) should be recalculated to be 0.52%(0.72×3.0/4.13) and total sulfur amino acid requirement 0.76%(1.05×3.0/4.13). These values are similar to those found in the present study.

No differences in weight gain, feed efficiency or nitrogen retention were noted between fish fed the 0.3% L-Met diets containing 0.5%(diet 2) or 0.3%(diet 9) Cys, and between trout fed the 0.4% Met diets containing 0.5%(diet 3) or 0.3%(diet 8) Cys(Table 3). This result indicates that the Cys replacement value for L-Met is less than 43%(100×0.3/0.7) by weight or 47 % on an equal molar sulfur basis.

No apparent relationship was observed be-

TABLE 3

Effects of dietary levels of methionine and cystine on weight gain, feed efficiency and nitrogen retention[1]—Experiment 1

Diet	Level (%) of		Weight gain (g/fish)	Feed efficiency (g/g)	Nitrogen retention[2] (%)	Mortality (%)
	Met[3]	Cys[3]				
1	0.23	0.50	$16.5 \pm 0.9^{a,4}$	0.62 ± 0.03^{c}	19.2 ± 1.5^{a}	0
2	0.30	0.50	20.9 ± 0.9^{b}	0.70 ± 0.01^{bc}	21.5 ± 0.8^{a}	1.8
3	0.40	0.50	26.4 ± 0.2^{c}	0.72 ± 0.01^{abc}	29.4 ± 0.7^{bc}	0.9
4	0.50	0.50	30.0 ± 0.5^{de}	0.79 ± 0.01^{ab}	31.3 ± 0.9^{bcd}	0
5	0.60	0.50	30.9 ± 0.9^{e}	0.84 ± 0.01^{a}	35.9 ± 1.2^{d}	0
6	0.80	0.50	32.0 ± 1.2^{e}	0.81 ± 0.01^{ab}	34.2 ± 0.3^{cd}	0.9
7	0.40	0.40	27.2 ± 0.4^{d}	0.80 ± 0.01^{ab}	29.0 ± 1.0^{b}	0.9
8	0.40	0.30	25.2 ± 0.7^{c}	0.77 ± 0.01^{ab}	27.0 ± 0.6^{b}	1.8
9	0.30	0.30	19.2 ± 0.7^{b}	0.67 ± 0.03^{c}	21.1 ± 1.4^{a}	0.9

[1] Means ± s.e.m. of four tanks of 28 fish each over a 6-week period. Mean initial body weight ± s.e.m. was 15.4 ± 0.02 g for 36 tanks.

[2] (Nitrogen gained in carcass/nitrogen intake) × 100.

[3] 0.23% methionine and 0.03% cystine were supplied by casein and gelatin.

[4] Values sharing the same superscripts in the same column are not significantly different ($P>0.05$), when analyzed by the Newman–Keuls test.

TABLE 4

Effects of dietary levels of methionine and cystine on carcass composition[1]—Experiment 1

Diet	Level (%) of		Carcass composition (%)[2]			
	Met[3]	Cys[3]	Dry matter	Protein[4]	Fat[4]	Ash[4]
1	0.23	0.50	$26.9 \pm 0.1^{bc,5}$	51.2 ± 0.1^{a}	36.6 ± 0.4^{f}	9.1 ± 0.1^{a}
2	0.30	0.50	25.9 ± 0.1^{a}	52.2 ± 0.9^{a}	33.0 ± 0.2^{de}	9.5 ± 0.1^{b}
3	0.40	0.50	26.8 ± 0.2^{bc}	58.5 ± 1.0^{bc}	31.4 ± 0.6^{bcd}	9.6 ± 0.1^{b}
4	0.50	0.50	26.1 ± 0.2^{ab}	59.7 ± 0.6^{c}	29.6 ± 0.4^{ab}	9.3 ± 0.1^{ab}
5	0.60	0.50	26.4 ± 0.2^{abc}	61.3 ± 1.0^{c}	28.1 ± 0.4^{a}	9.2 ± 0.1^{ab}
6	0.80	0.50	26.5 ± 0.2^{abc}	60.4 ± 1.1^{c}	28.2 ± 0.8^{a}	9.0 ± 0.1^{a}
7	0.40	0.40	27.2 ± 0.1^{c}	54.6 ± 1.4^{ab}	32.0 ± 0.6^{cd}	9.4 ± 0.1^{ab}
8	0.40	0.30	26.5 ± 0.1^{abc}	54.2 ± 1.2^{ab}	30.7 ± 0.2^{bc}	9.6 ± 0.1^{b}
9	0.30	0.30	26.6 ± 0.2^{abc}	51.8 ± 1.4^{a}	34.3 ± 0.3^{e}	9.6 ± 0.1^{b}

[1] Means ± s.e.m. of four tanks of ten fish each.

[2] Initial carcass composition was 23.6% dry matter, 66.5% protein, 19.8% fat and 10.1% ash.

[3] See footnote 3 to Table 3.

[4] On the dry-matter basis.

[5] Values sharing the same superscripts in the same column are not significantly different ($P>0.05$), when analyzed by the Newman–Keuls test.

TABLE 5

Effects of dietary levels of L- and D-methionine, and cystine on weight gain, feed efficiency and nitrogen retention[1]—Experiment 2

Diet	Level (%) of			Weight gain (g/fish)	Feed efficiency (g/g)	Nitrogen retention[3] (%)	Mortality (%)
	L-Met[2]	D-Met	Cys[2]				
1	0.50	–	0.03	19.0 ± $0.2^{a,4}$	0.79 ± 0.01^{b}	22.3 ± 1.3^{a}	0
2	0.50	–	0.20	21.4 ± 0.6^{bc}	0.85 ± 0.02^{ab}	28.2 ± 0.8^{bc}	0
3	0.50	–	0.30	23.1 ± 0.5^{bcd}	0.86 ± 0.01^{a}	30.6 ± 0.9^{c}	1.7
4	0.50	–	0.40	22.6 ± 0.2^{bcd}	0.88 ± 0.01^{a}	30.4 ± 1.1^{c}	0
5	0.50	–	0.50	23.4 ± 0.3^{cd}	0.88 ± 0.01^{a}	31.5 ± 1.3^{c}	0.8
6	0.80	–	0.03	24.1 ± 0.6^{d}	0.88 ± 0.01^{a}	32.8 ± 0.8^{c}	0
7	0.23	0.27	0.50	21.1 ± 0.7^{b}	0.83 ± 0.01^{ab}	25.7 ± 0.8^{b}	0
8	0.23	0.37	0.50	22.1 ± 0.7^{bcd}	0.83 ± 0.01^{ab}	29.1 ± 1.2^{bc}	0
9	0.23	0.47	0.50	22.9 ± 0.3^{bcd}	0.85 ± 0.01^{ab}	28.8 ± 1.1^{bc}	0

[1] Means ± s.e.m. of four tanks of 30 fish each over a 6-week period.

[2] See footnote 3 to Table 3.

[3] (Nitrogen gained in carcass/nitrogen intake) × 100.

[4] Values sharing the same superscripts in the same column are not significantly different ($P>0.05$), when analyzed by the Newman–Keuls test.

TABLE 6

Effects of dietary levels of L- and D-methionine, and cystine on carcass composition[1]—Experiment 2

Diet	Level (%) of			Carcass composition (%)[2]			
	L-Met[3]	D-Met	Cys[3]	Dry matter	Protein[4]	Fat[4]	Ash[4]
1	0.50	-	0.03	25.1 ± 0.8	53.9 ± $0.7^{a,4}$	32.5 ± 1.0^{de}	10.5 ± 0.2^{b}
2	0.50	-	0.20	26.2 ± 0.2	55.5 ± 0.6^{ab}	31.2 ± 0.8^{bcde}	10.0 ± 0.8^{ab}
3	0.50	-	0.30	25.9 ± 0.2	57.9 ± 1.2^{b}	29.2 ± 0.2^{abc}	10.0 ± 0.2^{ab}
4	0.50	-	0.40	26.0 ± 0.1	56.6 ± 1.1^{ab}	30.9 ± 1.1^{bcd}	9.8 ± 0.1^{a}
5	0.50	-	0.50	25.9 ± 0.2	58.1 ± 0.9^{b}	28.8 ± 0.5^{ab}	9.9 ± 0.2^{ab}
6	0.80	-	0.03	25.6 ± 0.1	59.2 ± 0.5^{b}	27.2 ± 0.4^{a}	9.9 ± 0.1^{a}
7	0.23	0.27	0.50	26.0 ± 0.1	53.6 ± 1.0^{a}	34.3 ± 0.5^{e}	9.7 ± 0.1^{a}
8	0.23	0.37	0.50	26.0 ± 0.2	56.9 ± 0.5^{ab}	31.8 ± 0.2^{cde}	9.7 ± 0.1^{a}
9	0.23	0.47	0.50	26.1 ± 0.2	55.5 ± 0.6^{ab}	31.4 ± 0.7^{bcde}	9.5 ± 0.2^{a}

[1] Means ± s.e.m. of four tanks of ten fish each.

[2] Initial carcass composition was 23.7% dry matter, 67.7% protein, 18.1% fat and 13.5% ash.

[3] See footnote 3 to Table 3.

[4] On the dry-matter basis.

[5] Values sharing the same superscripts in the same column are not significantly different ($P>0.05$), when analyzed by the Newman–Keuls test.

tween dietary levels of Met or Cys, and the content of dry matter or ash in the carcass(Table 4). However, the protein content in the carcass increased, with a concomitant decrease in fat content, as Met levels in the diet increased.

In Experiment 2, increasing the L-Cys level from 0.03 to 0.2% in a diet containing 0.5% L-Met significantly($P<0.05$) improved weight gain and nitrogen retention(Table 5). Additional increases of L-Cys level did not further enhance($P>0.05$) weight gain or nitrogen retention. In the presence of 0.5% L-Met, the Cys requirement of trout appeared to be 0.3% of the diet according to the broken-line model (Robbins et al., 1977). Therefore, the Cys replacement value for L-Met in rainbow trout diets is 38%(100×0.3/0.8) by weight or 42% on an equal molar sulfur basis.

In contrast to our findings, Harding et al. (1977) reported 60% on an equal molar sulfur basis as the replacement value for channel catfish. Whether the difference between the two results is due to differences between species or between experimental methods is not clear at this point. The replacement value will differ within a species depending on the dietary Met level. Our Cys replacement value was obtained with a diet containing Met at a minimum requirement level(0.5%).

Substitution of D-Met for over half of the dietary Met(0.23% L-form and 0.27%D-form) had no significant($P>0.05$) effect on weight gain over the diet containing 0.5% L-Met and 0.3% L-Cys, although trout fed diet 5 contaning 0.5% L-Met and 0.5% L-Cys or diet 6 containing 0.8% L-Met tended to show better growth(Table 5). The result suggested that rainbow trout can use D-Met almost as efficiently as its L-isomer up to the level tested(54%). Similarly, Robinson et al.(1978) reported that channel catfish used DL-Met as well as L-Met.

Effects of dietary levels of L-Met, D-Met and L-Cys on the contents of dry matter, protein, fat and ash in the carcass are shown in Table 6. The dietary level of Met and Cys did not influence the dry matter content in the carcass. However, significant($P<0.05$) differences were found in the content of protein, fat or ash among the treatments. The 0.03% Cys diet(diet 1) decreased the protein content and increased the ash content in the carcass compared to diet 4 containing 0.5% Cys. Interestingly, fish fed diet 6 containing 0.8% L-Met had relatively low fat and high protein contents in the carcass, while fish fed diet 1(deficient in total sulfur amino acids) or diet 7(this diet was considered adequate in total sulfur amino acids but over 50% of the L-Met had been replaced with D-Met) showed an opposite trend.

The level of Met(0.23% L-+0.27% D-Met) in diet 7 apperared to be borderline in meeting the requirement. Comparison of the protein content(53%) in the carcass of trout fed diet 7 with that of fish fed diet 5, in which total sulfur amino acid level was the same as that in diet 7 but no D-Met was present, indicated that D-Met is more prone to oxidation and thus less likely to be used to promote protein accretion than its L-isomer. This differential use of L-and D-Met may partly explain why the results of studies on D-Met utilization in other animal species have been variable and often contradictory(Stegink, 1977; Kim and Bayley, 1983).

Overall results of our studies suggest that the total sulfur amino acid requirement of rainbow trout(about 0.8% of diet) is not much different from that recommended for broiler chicks(0.93%) and young pigs(0.68%) by NRC(1984, 1988), respectively. This similarity supports our contention that the individual amino acid requirements of rainbow trout are not much different from those of rapidly growing farm animals and that the higher protein requirement of fish as compared to farm animals is due to use of protein by fish to meet their energy needs (Kim et al., 1991).

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