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Re-evaluation of Dietary Methionine Requirement by Plasma Methionine and Ammonia Concentrations in Surgically Modified Rainbow Trout, *Oncorhynchus mykiss*

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ABSTRACT : This study was designed to re-evaluate the dietary methionine requirement by means of the plasma methionine and ammonia concentrations in surgically modified rainbow trout, *Oncorhynchus mykiss*. A total of 35 rainbow trout averaging 505±6.5 g (initial body weight, mean±SD) were randomly distributed into seven groups with five fish in each group. After 48 h of feed deprivation, each group of fish was fed one of seven L-amino acid based diets containing 0.5% cystine and graded levels of methionine (0.25, 0.40, 0.50, 0.60, 0.70, 0.80 or 0.95% of diet, dry matter bases) by intubation at 1% body weight on dry matter basis. Blood samples were taken at 0, 5 and 24 h after intubation. Post-prandial plasma free methionine concentrations (PPmet, 5 h after intubation) and post-absorptive plasma free methionine concentrations (PAmet, 24 h after intubation) of fish fed diets containing 0.60% or higher methionine were significantly ($p<0.05$) higher than those of fish fed diets containing 0.50% or lower methionine. PPmet and PAmet in fish fed diets containing 0.60% or higher methionine were not significantly different except PPmet of fish fed diet containing 0.95% methionine. Post-prandial plasma ammonia concentrations (PPA, 5 h after intubation) of fish fed diets containing 0.70% or higher methionine were significantly higher than those of fish fed diets containing 0.60% or lower methionine, and PPA of fish fed diets containing 0.25 and up to 0.60% methionine were not significantly different from each other. Broken-line model analyses on PPmet, PAmet, and PPA indicated that the dietary methionine requirement of rainbow trout was between 0.59 (1.69) and 0.67 (1.91) % of diets (% dietary protein bases) when the diets contained 0.5% cystine. (**Key Words :** Methionine, Rainbow trout, Requirement, Intubation, Plasma, Ammonia)

INTRODUCTION

Methionine is a sulfur containing and an essential amino acid (EAA) required by fish as well as terrestrial vertebrates for normal growth and metabolic functions. This sulfur containing EAA, in the form of *S*-adenosyl-methionine serves as a principal donor of methyl group, a major contributor to the whole body pool of one carbon units that are required for trans-methylations, the biosynthesis of choline, thymidine, polyamines and creatine (Alam et al., 2000; Mai et al., 2006). Methionine deficiency resulted in reduced growth and feed efficiency, as well as cataract in rainbow trout (Poston et al., 1977; Walton et al., 1982) and hybrid striped bass (Keembiyehetty and Gatlin, 1993). Therefore it is very important to determine the methionine requirement of cultured fish for normal growth and feed utilization. Dietary methionine requirements have been estimated ranging from 1.8 to 4.0% of dietary protein for commonly cultured species of fish (Wilson, 2002). Several researchers have reported the quantitative methionine requirements of rainbow trout ranging between 0.5 (1.0) % and 0.8 (2.35) % of diets (% of dietary protein bases) (Walton et al., 1982; Rumsey et al., 1983; Cowey et al., 1992; Kim et al., 1992; Rodehutscord et al., 1995).

The above requirements were determined by feeding trials. Feeding trials are expensive and last for several weeks or months before appreciable responses are observed. Determination of amino acid requirements by measuring plasma amino acid and ammonia concentrations on the other hand lasts for just a week, with insignificant labor and material costs. This method has been employed in several

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species and results are similar to those obtained by feeding trials (Cowey, 1995; Ok et al., 2002). Hence, it is advisable that this procedure be confirmed in important species so that they could be employed in determination of yet undetermined amino acid or other nutrient requirements in those species.

Plasma free amino acids (PFAAs) concentrations were used to investigate amino acids metabolism and to evaluate the quality of dietary protein in rainbow trout (Nose, 1972; Walton and Wilson, 1986; Schuhmacher et al., 1997), common carp, *Cyprinus carpio* (Plakas et al., 1980; Dabrowski, 1982) and Atlantic salmon, *salar* (Espe et al., 1993; Sunde et al., 2003). Evaluation of PFAAs concentrations has led to the discovery of genetic defects of amino acid metabolism as a result of primary renal or liver disease and the effects of amino acid deficiencies, imbalances and toxicities on amino acid metabolism (Zicker and Rogers, 1990). The relationships between the concentration of PFAAs and intake of dietary amino acids have been investigated by several researchers (Plakas et al., 1980; Murai et al., 1987; Bai et al., 2003). Although the effects of dietary protein or amino acid mixtures on PFAAs concentrations in rainbow trout (Murai et al., 1987; Schuhmacher et al., 1997; Vermeirssen et al., 1997) and sea bass, *Dicentrarchus labrax* (Thebault, 1985) have been reported, the complete dose response relationships for amino acids have not been investigated. In our previous studies, dietary essential amino acids such as arginine, lysine, valine, and threonine requirements for rainbow trout were obtained by using the surgically modified methods in our laboratory (Ok et al., 2001; Ok, 2002; Bai et al., 2003; Park et al., 2005). As a part of our studies on essential amino acid requirements of rainbow trout, the purpose of the present study was to estimate the dietary methionine requirement based on the plasma free methionine and ammonia concentrations in dorsal aorta cannulated rainbow trout.

MATERIALS AND METHODS

Experimental fish

Rainbow trout, *O. mykiss* averaging 505±6.5 g (initial, mean±SD) were obtained from Ewhajung trout farm in Sangju, Korea. In the present experiment, seven net cages (1.3×1.3×1.3 m) were placed in a flow-through concrete raceway with a water flow of 60 L min^{-1}. Fish were kept in the net cages and fed a commercial rainbow trout diet (Woo-sung Feed Co. Ltd., Daejon, Korea) for 72 h until the fish recovered from the operation of dorsal aorta cannulation. Supplemental aeration was also provided to maintain the dissolved oxygen at 6.7±0.5 mg L^{-1}, and water temperature was maintained at 15±0.3°C.

Experimental diets

A basal diet was formulated by modification of Kim (1997) and it contained 36.6% crude protein (29.6% crystalline amino acids mixture, 5% casein and 2% gelatin). Seven experimental diets were formulated based on the basal diet and they contained graded levels of methionine (0.25, 0.40, 0.50, 0.60, 0.70, 0.80 or 0.95% of diet, on a dry matter base) and all diets contained 0.50% cystine. Equal amount of aspartic acid and glutamic acid by weight were substituted for the graded amounts of methionine in the basal diet to maintain the isonitrogenous of the seven experimental diets. Formulation and amino acid composition of the basal diet are shown in Table 1 and 2, respectively. The ingredient mixtures without oil were stored at -80°C until used and the diets were prepared by adding fish oil (10% of diets) and water (4 parts of distilled water: 10 parts of diet, w/w) before intubation.

Dorsal aorta cannulation and intubation (force-feeding)

Rainbow trout were anaesthetized with 200 mg L^{-1} 3-aminobenzoic acid ethyl ester methansulfonate (MS-222, Sigma Chemical Company, St. Louis, MO) for 3 to 5 min, placed on a V-shape table, and gills were irrigated continuously with 16°C water containing 100 mg L^{-1} of MS-222 during the operation. A 50 cm-long cannula (Clay

Table 1. Composition of the basal diet[1]

Ingredients	% of dry matter
EAA[2]	16.55
NEAA[3]	5.47
Casein[4]	5.00
Gelatine[4]	2.00
Dextrin[4]	27.97
Dextrose[4]	5.00
α-Cellulose[4]	8.20
Fish oil[5]	10.00
Other ingredients[6]	12.20
L-methionine[7]	0.10
Aspartic acid[7]	3.59
Glutamic acid[7]	3.93

[1] Diets were neutralized with NaOH to give a final pH of 6.6.
[2] EAA, essential amino acids (g/100 g diet): arginine, 1.924; histidine, 0.725; isoleucine, 1.674; leucine, 2.702; lysine, 1.904; cystine, 0.481; phenylalanine, 1.742; tyrosine, 1.335; threonine, 1.601; tryptophan,0.462; valine, 1.999 (Ajinomoto, Tokyo, Japan).
[3] NEEA, Non-essential amino acids (g/100 g diet): alanine, 1.741; glycine, 0.758; proline, 0.568; serine, 2.398 (Ajinomoto, Tokyo, Japan).
[4] United States Biochemical (USB), Cleveland, Ohio, USA.
[5] Ewha Oil Company, Busan, Korea.
[6] Other ingredients include: carboxymethyl cellulose, 1.00% (United States Biochemical, Cleveland, Ohio, USA); $Ca(H_2PO_4)_2H_2O$, 3.00%; choline bitartrate, 1.20% (United States Biochemical, Cleveland, Ohio, USA), vitamin mixture, 3.00% and mineral mixture, 4.00% (Park et al., 2005).
[7] Ajinomoto, Tokyo, Japan.

Table 2. Amino acid composition of the basal diet (% of dry matter base)

Amino acids	From casein and gelatin	From crystalline amino acids	Total[1]
Essential amino acids			
Arginine	0.353	1.924	2.277
Histidine	0.194	0.725	0.919
Isoleucine	0.252	1.674	1.926
Leucine	0.493	2.702	3.195
Lysine	0.502	1.904	2.406
Methionine	0.152	0.098[2]	0.250
Cystine	0.019	0.481	0.500
Phenylalanine	0.271	1.742	2.013
Tyrosine	0.270	1.335	1.605
Threonine	0.221	1.601	1.822
Tryptophan	0.065	0.462	0.527
Valine	0.350	1.999	2.349
Non-essential amino acids			
Alanine	0.345	1.741	2.086
Aspartic acid	0.483	3.592	4.075
Glycine	0.538	0.758	1.296
Glutamic acid	1.298	3.928	5.226
Proline	0.790	0.568	1.358
Serine	0.374	2.398	2.772

[1] The amino acid profile was simulated with that of 35% whole chicken egg protein (Robinson et al., 1981).

[2] Seven experimental diets were formulated to have graded levels of methionine (0.25, 0.40, 0.50, 0.60, 0.70, 0.80 or 0.95%); equal amount of aspartic acid and glutamic acid by weight were substituted for the proper amounts of methionine in the basal diet.

Adams PE 50 tubing, Parsippany, NJ, USA) with a bubble of about 5-6 cm from one end was washed with the heparinized Cortland saline solution (Houston, 1990), and a 13-gauge needle was used to pierce a hole on the right nostrum (ventral side up) for the cannula to exit. A 19-gauge needle was used to bore a small hole in the roof of the mouth at the mid-line behind the third gill arc at a 30° angle, and a piano wire was inserted into the PE 50 tubing as a guide. The proper insertion was verified by the observation of a slow blood flow after the wire was withdrawn from the cannula. A 3 ml syringe with a 23-gauge needle was used to remove air and blood clot and the cannula was flushed with the heparinized saline solution. The cannula was sutured behind the bubble on the roof of the mouth, led out from the right nostrum, plugged with a color head pin, and sutured at the dorsal fin (Ok et al., 2001; Bai et al., 2003; Park et al., 2005). Thirty five dorsal aorta cannulated rainbow trout were randomly distributed into seven groups with five fish per group and they were fed one of the seven experimental diets per group by intubation at 1% body weight on dry matter basis. After 24 h feed deprivation, these fish were anesthetized with 200 mg L^{-1} of MS-222 and fed the experimental diet by the stomach intubation method (diet plus 0.4 parts of distilled water per diet) using a 3 ml syringe.

Sample collection and analysis

Five fish per group were anesthetized with 200 mg L^{-1} MS-222 and blood was sampled using a 3 ml syringe from each fish at 0, 5 and 24 h after intubation of the experimental diets. Plasma samples were prepared by centrifugation at 3,000 g for 10 min at room temperature. For deproteinization, plasma samples were mixed with a 10% 5-sulphosalicylic acid solution in the ratio of 4:1 (v/v), cooled on ice for 30 min and re-centrifuged. The protein-free supernatant was dissolved in pH 2.2 lithium citrate sample dilution buffer in the ratio of 1:1 (v/v), and the samples were stored at -80°C until analysis. Plasma free amino acids were quantified using a S433 amino acid analyzer (Sykam, Gilching, Germany) using the ninhydrin method. Plasma ammonia concentrations were analyzed using the Berthelot reaction (Sigma).

Statistical analysis

Data were subjected to one way analysis of variance test using Statistix 3.1 (Analytical Software, St Paul, MN, USA). When a significant treatment effect was observed, a Least Significant Difference test was used to compare means. Treatment effects were considered significant at $p<0.05$. The breakpoints for PPmet, PAmet, and PPA were estimated by using the broken line model of Robbins et al. (1979).

RESULTS

Plasma free methionine concentrations

Results of post-prandial plasma free methionine concentrations (PPmet, 5 h after intubation) and post-absorptive plasma free methionine concentrations (PAmet, 24 h after intubation) in dorsal aorta cannulated rainbow trout force-fed with diets containing seven graded levels of methionine are shown in Figure 1. PPmet of fish fed diets containing 0.60% or higher methionine were significantly ($p<0.05$) higher than those of fish fed diets containing 0.50% or lower methionine. PPmet of fish fed diet containing 0.95% methionine was significantly higher than those of fish fed the other diets ($p<0.05$). PPmet of fish fed diets containing 0.60, 0.70 and 0.80 (%) methionine were not significantly ($p>0.05$) different from each other. Broken-line model analyses on the basis of PPmet indicated that the dietary methionine requirements of rainbow trout were 0.60 (1.71) % of diets (% of dietary protein on a dry matter base) (Figure 1). PAmet of fish fed diets containing 0.60% or higher methionine were significantly ($p<0.05$) higher than those of fish fed diets containing 0.50%

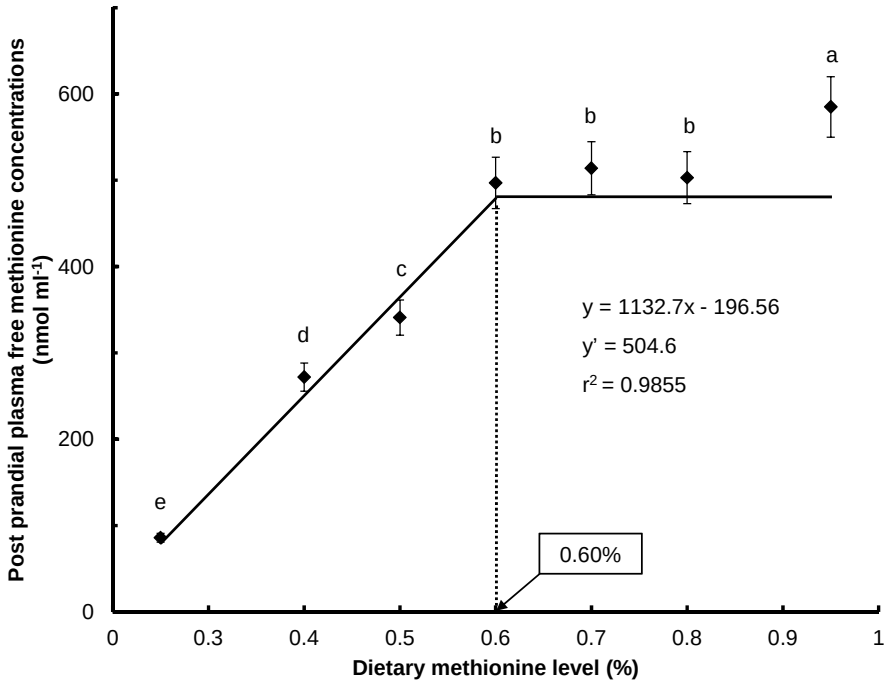

Figure 1. Broken line analysis of post-prandial plasma methionine concentrations (nmol ml^{-1}) in rainbow trout fed graded levels of dietary methionine. Values of the X-axis are the methionine levels in the experimental diets. Values of the Y-axis are post-prandial plasma free methionine concentrations. Values are means±SD of 5 replicates.

methionine. PAmet of fish fed diets containing 0.60% or higher methionine in the diets were not significantly ($p>0.05$) different from each other. Broken-line model analyses on the basis of PAmet indicated that the dietary methionine requirements of trout could be 0.67 (1.91) % of diets (% of dietary protein on a dry matter base) (Figure 2).

Plasma ammonia concentrations

Post-prandial plasma ammonia concentrations (PPA, 5 h after intubation) and post-absorptive plasma ammonia concentrations (PAA, 24 h after intubation) in rainbow trout force-fed graded levels of dietary methionine are shown in Figure 3. Post-prandial plasma ammonia concentration from fish fed diets containing methionine from 0.25% (168±5 nmol ml^{-1}) to 0.60% (156±12 nmol ml^{-1}) were not significantly different from each other. However, PPA were significantly increased with dietary methionine levels from 0.70 (195±8 nmol ml^{-1}) to 0.95% (251±11 nmol ml^{-1}). PAA in the present study were not significantly ($p>0.05$) different among the groups. Broken-line model analyses on the basis of PPA indicated that the dietary methionine requirement of rainbow trout could be 0.59 (1.69) % of diets (% of dietary protein on a dry matter base) (Figure 3).

DISCUSSION

In the present study, the dietary methionine requirements for the rainbow trout based on the plasma free methionine concentrations (PPmet and PAmet) could be between 0.60 (1.71) and 0.67 (1.91) % of diets (% of dietary protein bases) if the diet contained 0.5% cystine. In the previous studies, several researchers have reported the quantitative methionine requirements of rainbow trout with range between 0.50 (1.00) % and 0.80 (2.35) % of diets (% of dietary protein bases) (Walton et al., 1982; Rumsey et al., 1983; Cowey et al., 1992; Kim et al., 1992; Rodehutscord et al., 1995). These results are similar to those in other fresh water fish species, such as channel catfish, *Ictalurus punctatus* (0.56%, Harding et al., 1977), Nile tilapia, *Oreochromis niloticus* (0.75%, Santiago and Lovell, 1988), and common carp, *C. carpio* (0.86% of diet, Schwarz et al., 1998). Furthermore, the methionine requirements of rainbow trout was lower than those of major marine finfish species, such as hybrid striped bass, *Morone chrysops*×*M. saxatilis* (1.00%, Keembiyehetty and Gatlin, 1993), red drum, *Sciaenops ocellatus* (1.06%, Moon and Gatlin, 1991), yellowtail, *Seriola quinqueradiata* (1.11%, Ruchimat et al., 1997), grouper, *Epinephelus coioides* (1.31%, Luo et al., 2005), cobia, *Rachycentron canadum* (1.19%, Zhou et al., 2006), and yellow croaker, *Pseudosciaena crocea* (1.44%, Mai et al., 2006). The variations of methionine requirement may have resulted from fish size and age, feed ingredients, palatability of diets, feeding regime, and culture conditions among the different experiments (Tacon and Cowey, 1985; Rodehutscord et al., 1997).

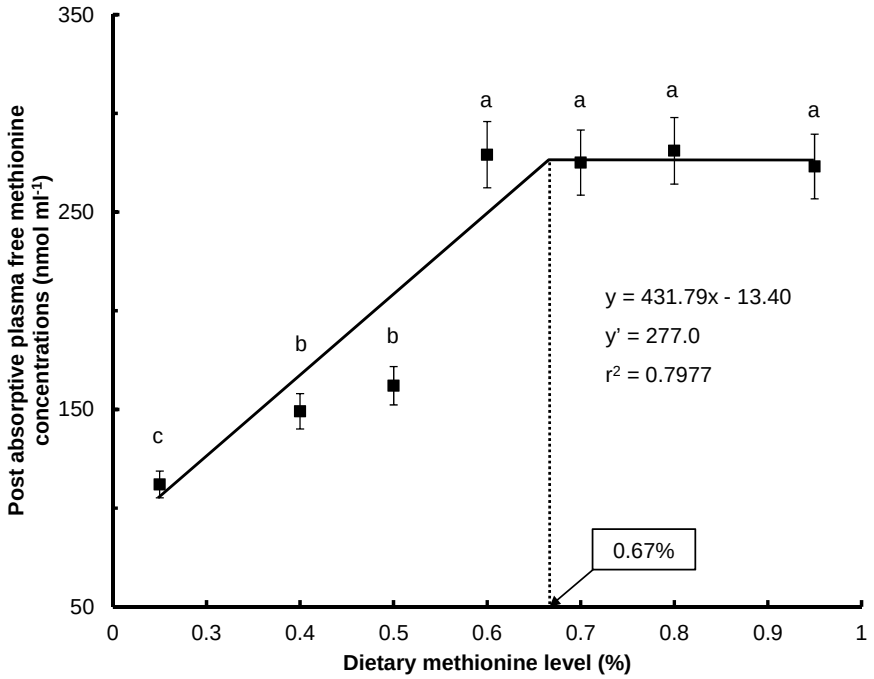

Figure 2. Broken line analysis of post-absorptive plasma methionine concentrations (nmol ml^{-1}) in rainbow trout fed graded levels of dietary methionine. Values of the X-axis are the methionine levels in the experimental diets. Values of the Y-axis are post-absorptive plasma free methionine concentrations. Values are means±SD of 5 replicates.

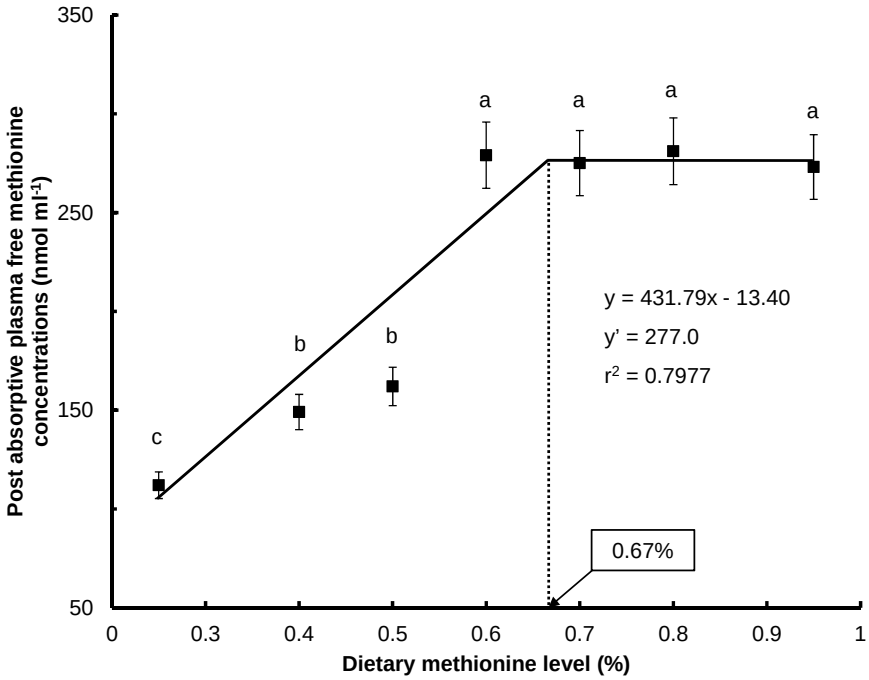

Figure 3. Broken line analysis of post-prandial plasma ammonia concentrations (nmol ml^{-1}) in rainbow trout fed graded levels of dietary methionine. Values of the X-axis are the methionine levels in the experimental diets. Values of the Y-axis are post-prandial plasma ammonia concentrations. Values are means±SD of 5 replicates.

The effects of dietary methionine intake on PPmet and PAmet were dependent upon the relative adequacy of the dietary methionine supply. Similar results have shown that dietary methionine levels affected plasma free amino acids in several fish species such as sea bass, *Dicentrarchus labrax* (Hidalgo et al., 1987), common carp, *C. carpio* (Schwarz et al., 1998), and yellow perch, *Perca flavescens* (Twibell et al., 2000). Murai et al. (1987) and Schuhmacher et al. (1997) reported that the plasma concentrations of arginine, leucine, isoleucine, valine, phenylalanine and threonine in rainbow trout force-fed crystalline amino acids at 1% body weight (dry matter) peaked at 6-9 h and returned to the basal level at 24-32 h post feeding. Ok et al. (2001) demonstrated that most of the plasma free amino acids peaked approximately 4 h after feeding, and returned to near basal level in 24 h in rainbow trout that force-fed the experimental diets after. In chicks, Zimmerman and Scott (1967) found that lysine, valine, and arginine concentrations in the plasma increased in a dose response manner when the intake of the dietary amino acid just exceeds the level required for maximum growth. In the present study, PPmet and PAmet increased with dietary methionine increased from 0.25% to 0.60% of diets (dry matter base) and they only increased slightly thereafter. In another words, PPmet and PAmet increased with increasing dietary methionine when their level is below requirement. Their concentrations remained constant when dietary methionine is higher than requirement values.

Post-prandial plasma ammonia concentrations were affected by dietary methionine levels whereas post-absorptive plasma ammonia concentrations were not. Similar observations were reported that PPA was significantly affected by dietary arginine, valine, lysine, and threonine level in rainbow trout (Ok, 2002). This has also been reported in studies of methionine requirements in rainbow trout (Kim et al., 1992) and yellowtail (Ruchimat et al., 1997). Much of the excess amino acids beyond the requirement were taken up by the liver and degraded. The liver of fish is the main site of ammonia production and where most enzymes of amino acid catabolism are concentrated (Walton and Wilson, 1986). Judging from a large increase of ammonia excretion in rainbow trout after being fed a high protein diet, Brett and Zala (1975) suggested that the excess dietary amino acid was rapidly catabolized by the liver.

The patterns of most plasma free amino acid concentrations from force-fed rainbow trout with and without dorsal aorta cannulation were similar, and the dorsal aorta cannulation did not affect the pattern of plasma free amino acid concentrations in rainbow trout (Ok et al., 2001). These results indicate that force-fed rainbow trout might recover from the stress of dorsal aorta cannulation within 48 h of the operation, and the dorsal aorta cannulation would allow repeated sampling on the same individual fish to study nutrient metabolism in the blood circulation. Plasma amino acid concentrations may be useful in determining the optimum blood sampling time and evaluating protein quality and essential amino acid requirements. Furthermore, this technique can be used in pharmaceutical and toxicological, as well as in nutritional research in rainbow trout. Since results in this and other studies involving measurement of plasma free amino acids and ammonia are similar to values obtained by feeding trial, it could be suggested that this technique could be employed in estimating other amino acid requirements in rainbow trout.

In conclusion, quantitative estimation of the methionine requirement using the response of broken line model with plasma free methionine or ammonia concentrations should be an acceptable method for determining essential amino acid requirements of other fish species. Broken-line model analyses on the basis of PPmet, PAmet, and PPA concentrations indicated that the dietary methionine requirements of rainbow trout could be between 0.59 (1.69) % and 0.67 (1.91) % of diets (dietary protein) when the diet contained 0.5% cystine.

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REFERENCES

Alam, M. D., S. I. Teshima, M. Ishikawa and S. Koshio. 2000. Methionine requirement of juvenile Japanese flounder, *Paralichthys olivaceus*. J. World Aquac. Soc. 31:618-626.

Bai, S. C., I. H. Ok, G. J. Park, K. W. Kim and S. M. Choi. 2003. Development of modeling system for assessing essential amino acid requirements using surgically modified rainbow trout. J. Aquacult. 16(1):1-7.

Brett, J. R. and C. A. Zala. 1975. Daily pattern of excretion and consumption of sockeye salmon under controlled conditions. J. Fish. Res. Board Can. 33:2479-2486.

Cowey, C. B. 1995. Protein and amino acid requirements: a critique of methods. J. Appl. Ichthyol. 11:199-204.

Cowey, C. B., C. Y. Cho, J. G. Sivak, J. A. Weerheim and D. D. Stuart. 1992. Methionine intake in rainbow trout, *Oncorhynchus mykiss*, relationship to cataract formation and the metabolism of methionine. J. Nutr. 122:1154-1163.

Dabrowski, K. 1982. Postprandial distribution of free amino acids between plasma and erythrocytes of common carp, *Cyprinus carpio*. Comp. Biochem. Physiol. 72A:753-763.

Espe, M., E. Lied and K. R. Torrissen. 1993. Changes in plasma and muscle free amino acids in Atlantic salmon, *Salmo salar* during absorption of diets containing different amounts of

hydrolysed cod muscle protein. Comp. Biochem. Physiol. 105A:555-562.

Harding, D. C., O. W. Allen and R. P. Wilson. 1977. Sulfur amino acid requirements of channel catfish, L-methionine and L-cystine. J. Nutr. 107:2031-2035.

Hidalgo, F., E. Alliot and H. Thebault. 1987. Methionine and cystine supplemented diets for juvenile sea bass, *Dicentrarchus labrax*. Aquaculture 64:209-217.

Huston, A. H. 1990. Blood and circulation. In: Methods for Fish Biology (Ed. C. B. Schreck and P. B. Moyle). American Fisheries Society, New York, USA. pp. 273-343.

Keembiyehetty, C. N. and D. M. Gatlin III. 1993. Total sulphur amino acid requirement of juvenile hybrid striped bass, *Morone chrysops×M. saxatilis*. Aquaculture 110:331-339.

Kim, K. I. 1997. Reevaluation of protein and amino acid requirements of rainbow trout *Oncorhynchus mykiss*. Aquaculture 151:3-7.

Kim, K. I., B. K. Terrence and H. A. Clyde. 1992. Requirements for sulfur amino acids and utilization of D-methionine by rainbow trout, *Oncorhynchus mykiss*. Aquaculture 101:95-103.

Luo, Z., Y. J., Liu, K. S. Mai, L. X. Tian, H. J. Yang, X. Y. Tan and D. H. Liu. 2005. Dietary l-methionine requirement of juvenile grouper, *Epinephelus coioides* at a constant dietary cystine level. Aquaculture 249:409-418.

Mai, K., J. Wan, Q. Ai, W. Xu, Z. Liufu, L. Zhang, C. Zhang and H. Li. 2006. Dietary methionine requirement of large yellow croaker, *Pseudosciaena crocea*. Aquaculture 253:564-572.

Moon, H. Y. and D. M. Gatlin III. 1991. Total sulfur amino acid requirement of juvenile red drum, *Sciaenops ocellatus*. Aquaculture 95:97-106.

Murai, T., H. Ogata, Y. Hirasawa, T. Akiyama and T. Nose. 1987. Portal absorption and hepatic uptake of amino acids in rainbow trout force fed complete diets containing casein or crystalline amino acids. Nippon Suisan Gakkaishi 53(10):1847-1859.

Nose, T. 1972. Changes in patterns of free plasma amino acids in rainbow trout after feeding. Bull. Fresh. Fish. Res. Lab. Tokyo 22:137-144.

Ok, I. H., S. C. Bai, G. J. Park, S.M. Choi and K. W. Kim. 2001. The patterns of plasma free amino acids after force-feeding in rainbow trout, *Oncorhynchus mykiss* with and without dorsal aorta cannulation. Aquac. Res. 32:70-75.

Ok, I. H. 2002. Determination of essential amino acid requirements by using plasma free amino acid concentrations in rainbow trout, *Oncorhynchus mykiss*. Ph.D. Thesis, Pukyong National University, Busan, Korea.

Park, G. J., S. C. Bai, I. H. Ok, K. M. Han, S. O. Hung, Q. R. Rogers and T. S. Min. 2005. Post prandial plasma free arginine concentrations increase in rainbow trout fed arginine deficient diets. Asian-Aust. J. Anim. Sci. 18(3):396-402.

Plakas, S. M., T. Katayama, Y. Tanaka and O. Deshimaru. 1980. Changes in the levels of circulating plasma free amino acids of carp, *Cyprinus carpio* after feeding a protein and an amino acid diet of similar composition. Aquaculture 21:307-322.

Poston, H. A., R. C. Riis, G. L. Rumsey and H. G. Ketola. 1977. The effect of supplemental dietary amino acids, minerals and vitamins on salmonids fed cataractogenic diets. Cornell Vet. 67:472-509.

Robbins, K. R., H. W. Norton and D. H. Baker. 1979. Estimation of nutrient requirements from growth data. J. Nutr. 109:1710-1714.

Robinson, E. H., R. P. Wilson and W. E. Poe. 1981. Arginine requirement and apparent absence of a lysine arginine antagonist in fingerling channel catfish. J. Nutr. 111:46-52.

Rodehutscord, M., J. Stephan, P. Michael and P. Ernst. 1995. Growing from 50 to 150 g to supplements of DL-methionine in a semipurified diet containing low or high levels of cystine. J. Nutr. 125:964-969.

Rodehutscord, M., A. Becker, M. Pack and E. Pfeffer. 1997. Response of rainbow trout, *Oncorhynchus mykiss* to supplements of individual essential amino acids in a semipurified diet, including an estimate of the maintenance requirement of essential amino acids. J. Nutr. 126:1166-1175.

Ruchimat, T., T. Masumoto, H. Hosokawa and S. Shimeno. 1997. Quantitative methionine requirement of yellowtail, *Seriola quinqueradiata*. Aquaculture 150:113-122.

Rumsey, G. L., J. W. Page and M. L. Scott. 1983. Methionine and cystine requirements of rainbow trout. Progressive Fish-Culturist 45:139-143.

Santiago, C. B. and R. T. Lovell. 1988. Amino acid requirements for growth of Nile tilapia. J. Nutr. 118:1540-1546.

Schuhmacher, A., C. Wax and J. M. Gropp. 1997. Plasma amino acids in rainbow trout, *Oncorhynchus mykiss* fed intact protein or a crystalline amino acid. Aquaculture 151:15-28.

Schwarz, E. J., M. Kirchgessner and U. Deuringer. 1998. Studies on the methionine requirement of carp, *Cyprinus carpio*. Aquaculture 161:121-129.

Sunde, J., A. Kiessling, D. Higgs, J. Opstvedt, G. Venturini and K. R. Torrissen. 2003. Evaluation of feed protein quality by measuring plasma free amino acids in Atlantic salmon, *Salmo salar* after dorsal aorta cannulation. Aquacult. Nutr. 9:351-360.

Tacon, A. G. and C. B. Cowey. 1985. Protein and amino acid requirements. In: Fish Energetics and New Perspectives (Ed. P. Tytler and P. Calow). Croom-Helm, London. pp. 155-183.

Thebault, H. 1985. Plasma essential amino acid changes in sea bass, *Dicentrarchus labrax* after feeding diets deficient and supplemented in L-Met. Comp. Biochem. Physiol. 82A:233-237.

Twibell, R. G., K. A. Wilson and P. B. Brown. 2000. Dietary sulfur amino acid requirement of juvenile yellow perch fed the maximum cystine replacement value for methionine. J. Nutr. 130:612-616.

Vermeirssen, E. L. M., A. P. Scott and N. R. Liley. 1997. Female rainbow trout urine contains a pheromone which causes a rapid rise in plasma 17α, 20β-dihydroxy-4-pregnen-3-one levels and milt amounts in males. J. Fish Biol. 50:107-119.

Walton, M. J., C. B. Cowey and J. W. Adron. 1982. Methionine metabolism in rainbow trout fed diets of differing methionine and cystine content. J. Nutr. 112:1525-1555.

Walton, M. J. and R. Wilson. 1986. Postprandial changes in plasma and liver free amino acids of rainbow trout fed complete diets containing casein. Aquaculture 51:105-115.

Wilson, R. P. 2002. Amino acids and proteins. In: Fish Nutrition, 3rd Ed. (Ed. J. E. Halver and R. W. Hardy). Academic Press, Inc., New York, USA. pp. 143-179.

Zhou, Q. C., Z. E. Wu, B. P. Tan, S. Y. Chi and Q. H. Yang. 2006. Optimal dietary methionine requirement for juvenile cobia, *Rachycentron canadum*. Aquaculture 258:551-557.

Zicker, S. C. and Q. R. Rogers. 1990. Use of plasma amino acid concentration in the diagnosis of nutritional and metabolic diseases in veterinary medicine. In: Proceeding of 6th Congress of the International Society for Animal Clinical Biochemistry (Ed. J. J. Kanedo). Davis, California, USA. pp. 107-212.

Zimmerman, R. A. and H. M. Scott. 1967. Effect of fasting and of feeding a nonprotein diet on plasma amino acid levels in the chick. J. Nutr. 91:507-508.