Post Prandial Plasma Free Arginine Concentrations Increase in Rainbow Trout Fed Arginine-deficient Diets

Gunjun Park, Sungchul C. Bai*, Im-ho Ok, Kyungmin Han, Silas S. O. Hung[1]
Quinton R. Rogers[2] and Taesun Min[3]
Department of Aquaculture, Pukyong National University, Busan 608-737, Korea

ABSTRACT : Three experiments were conducted to determine the effects of dietary arginine concentrations on plasma free amino acid (PAA) concentrations in rainbow trout, *Oncorhynchus mykiss* (Walbaum). The first experiment was conducted to determine appropriate post-prandial and food deprivation sampling times in dorsal aorta cannulated rainbow trout averaging 519±9.5 g (mean±SD) at 16°C. Blood samples were taken at 0, 2, 3, 4, 5, 6 and 24 h after feeding (0 and 24 h blood samples were taken from the same group of fish). PAA concentrations increased by 2 h post-feeding and the concentration of all essential amino acids except histidine peaked at 5 h and returned to 0 time values by 24 h. In the second experiment dorsal aorta cannulated rainbow trout averaging 528±11.3 g (mean±SD) were divided into 6 groups of 4 fish to study the effect of dietary arginine levels on PAA. After 24 h food deprivation, each group of fish was fed one of six L-amino acid diets containing graded levels of arginine (0.48, 1.08, 1.38, 1.68, 1.98 or 2.58%) by intubation. Blood samples were taken at 0, 5 and 24 h after feeding. Post-prandial (5 h after feeding) plasma-free arginine concentrations (PParg) showed a breakpoint at 1.03% arginine in the diet and post-absorptive (24 h after feeding) plasma free-arginine concentrations (PAarg) showed a breakpoint at 1.38% arginine. PAarg increased linearly from fish fed diets containing arginine between 0.48% and 1.38%, and the concentrations remained constant from fish fed diets containing arginine at or above 1.38%, but were all below PParg at all time points. Results of the third experiment confirm the results that PParg concentrations from fish fed arginine deficient diets were higher than PAarg (0 or 24 h values). Thus, in contrast to mammals and birds, the PParg when arginine is present in the diet as the most limiting amino acid such that it severely limits growth, increases in plasma rather than decreases. ***(Asian-Aust. J. Anim. Sci. 2005. Vol 18, No. 3 : 396-402)***

Key Words : Arginine, Rainbow Trout, Dorsal Aorta Cannulation, Plasma Free Amino Acids

INTRODUCTION

Evaluation of plasma free amino acids concentrations in mammals and birds has led to discoveries involving the genetic defects of amino acid metabolism, the secondary perturbations 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). Factors influencing plasma free amino acids (PAA) concentrations in growing animals have been studied extensively. Although assay procedures used in PAA studies have varied considerably, the results obtained have been quite consistent in demonstrating that dietary amino acid deficiencies result in reduced plasma concentration of that amino acid, post-prandially (Hill and Olsen, 1963), whereas dietary amino acid excesses have resulted in increase of that amino acid in the plasma (Richardson et al., 1953).

Studies in the chick (Richardson et al., 1953; Hill and Olsen, 1963), rat (Swendseid et al., 1963; Young and Zamora, 1968), pig (Puchal et al., 1962), human (Longenecker and Hause, 1961; Snyderman et al., 1964) and fish (Thebault, 1985) have clearly established that a reduced concentration of an essential amino acid (EAA) in plasma reflects a deficient level of that amino acid in the diet. Others (Munro, 1970; Young and Scrimshaw, 1970; Young et al., 1971) showed that the pattern of amino acids and the level of a specific EAA in plasma correlate with the ability of the dietary protein to support growth. The relationships between the concentration of PAA and dietary amino acid intake have been the subject of reviews (Leathem, 1968; McLaughlan and Morrison, 1968; Munro, 1970; Young and Scrimshaw, 1970; Zicker and Rogers, 1990).

Although the effects of dietary protein sources and amino acid mixtures on plasma free essential amino acid concentrations in sea bass (Thebault, 1985) and in rainbow trout (Schuhmacher et al., 1997; Vermeirssen et al., 1997) have been reported, the complete dose-response relationships for arginine have not been investigated. Therefore, the objectives of the present study were to

* Corresponding Author: Sungchul C. Bai. Tel: +82-51-620-6137, Fax: +82-51-628-6873, E-mail: scbai@mail.pknu.ac.kr
[1] Department of Animal Science, College of Agriculture and Environmental Sciences, University of California, Davis, CA 95616 USA.
[2] Department of Molecular Biosciences, School of Veterinary Medicine, University of California, Davis, CA 95616 USA.
[3] Korea Science and Engineering Foundation, Gajeong-dong, Yuseong-Gu, Daejeon 305-350, Korea.
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Table 1. Composition of the basal diet (% of dry matter)[1]

Ingredients	%
EAA[2]	17.27
NEAA[3]	12.36
Casein[4]	5.00
Gelatin[4]	2.00
Dextrin[4]	27.97
Dextrose[4]	5.00
α-cellulose[4]	8.20
Fish oil[5]	10.00
Carboxymethyl cellulose[4]	1.00
$Ca(H_2PO_4)_2H_2O$	3.00
Choline bitartrate[4]	1.20
Vitamin mixture[6]	3.00
Mineral mixture[7]	4.00

[1] Diets were neutralized with NaOH to give a final pH 6.6.
[2] EAA: Essential amino acids, Ajinomoto, Tokyo, Japan.
[3] NEAA: Non-Essential amino acids, Ajinomoto, Tokyo, Japan.
[4] United States Biochemical (USB), Cleveland, Ohio.
[5] Ewha Oil Company, Pusan, Korea.
[6] Vitamin mixture (mg/kg feed unless indicated otherwise): vit. A, 3,000 IU; vit. D_3, 2.400 IU; vit. E, 120 IU; menadione sodium bisulfate, 6; vit. B_1-HCl, 15; vit. B_2, 30; vit. B_6-HCl, 15; vit. B_{12}, 0.06; vit. C, 300; calcium pantothenate, 150; nicotinamide, 150; inositol, 150; d-biotin, 1.5; choline chloride, 3.000; pancreatin, 12.5. Vitamin mixture prepare by our laboratory and the individual vitamins purchased from USB, Cleveland, Ohio, USA.
[7] Mineral mixture (mg/kg feed): $MnSO_4$, 320; $ZnSO_4$, 270; $FeSO_4$, 750; $CuSO_4$, 60; $CoSO_4$, 7; $MgSO_4$, 17.3; K_2SO_4, 212; NaCl, 519; K_2HPO_4, 136; $NaSeO_3$, 0.01; KI, 0.15. Mineral mixture was prepared by our laboratory and the individual minerals purchased from Junsei Chemical, Tokyo, Japan.

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

Amino acids	From casein +gelatin	From crystalline amino acids	Total[1]
EAA			
Arginine	0.353	1.924[2]	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	1.030	1.182
Cystine	0.019	0.172	0.191
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
NEAA			
Alanine	0.345	1.741	2.086
Aspartic acid	0.483	3.280	3.763
Glycine	0.538	0.758	1.296
Glutamic acid	1.298	3.616	4.914
Proline	0.790	0.568	1.358
Serine	0.374	2.398	2.772

[1] The amino acid profile simulated that of 35% whole chicken egg protein (Robinson et al., 1981).
[2] Six experimental diets were formulated to have graded levels of arginine (0.48, 1.08, 1.38, 1.68, 1.98 or 2.58%): equal amounts of aspartic acid and glutamic acid by weight were substituted by arginine in the basal diet.

determine the effects of the different dietary arginine levels on PAA concentrations and to estimate the dietary arginine requirement by using surgically modified young growing rainbow trout (*Oncorhynchus mykiss*).

MATERIALS AND METHODS

Animals and husbandry

Rainbow trout averaging 519±9.5 g (Experiment I), 528±11.3 g (Experiment II) and 521±13.1 g (Experiment III) were obtained from Ewhajung Trout Farm in Sang Joo, Korea. For all experiments, net cages (1.3 m×1.3 m×1.3 m) were placed in a flow-through raceway with a water flow of 60 L/min. Supplemental aeration was also provided to maintain the dissolved oxygen near 7.2±0.4 mg/L. Water temperature was maintained at 16±0.2°C.

Dorsal aorta cannulation and intubation

The trout were anesthetized with 200 mg/L 3-aminobenzoic acid ethyl ester methansulfonate (MS 222, Sigma Chemical Company, St. Louis, MO) for 3 to 5 minutes, placed on a V-shape table and gills were continuously irrigated with 16°C water containing 100 mg/L of MS 222 during the operation. A 50 cm-long cannula (Clay Adams PE 50 tubing, Parsippany, NJ) with a bubble about 5-6 cm from one end was washed with 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 heparin 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 (I-H Ok et al., 2001; Bai et al., 2003).

Experimental design and diets

Experiment I was conducted to determine the appropriate post-prandial and post-absorptive time for blood sampling in dorsal aorta cannulated rainbow trout (I-H Ok et al., 2001; Bai et al., 2003). After dorsal aorta cannulation, the trout were divided into 6 groups (4 fish per group) in each net cage and were fed a commercial rainbow

trout diet (Woosung Feed Co. Ltd., Taejon-Si. Korea) for 3 days until the fish recovered from the operation of dorsal aorta cannulation. After 24 h food deprivation, these fish were intubated with the L-amino acid based diet at 1% body weight (dry-matter), anesthetized with MS222 and blood was sampled at 0, 2, 3, 4, 5, 6 and 24 h thereafter (0 and 24 h blood samples were taken from the same group of fish). The basal diet was formulated by the modification of Kim (1997) and contained a 29.6% crystalline amino acid mixture, 5% casein and 2% gelatin. Ingredients 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 basal diet was prepared by adding fish oil (10% of diet) and water (0.4 part of distilled water:diet, w/w) before intubation.

Experiment II was conducted to determine the effects of different dietary arginine levels on post-prandial (5 h after feeding, PParg) and post absorptive (24 h after feeding, PAarg) plasma free arginine concentrations in rainbow trout. Rainbow trout were divided into 6 groups of 4 fish each in net cage and fed a commercial diet (Woosung Feed Co. Ltd., Taejon-Si. Korea) for 3 days until the fish recovered from the operation of dorsal aorta cannulation. After 24 h food deprivation, these fish were intubated with 1% body weight (dry-matter) of the experimental diets. Six diets were formulated to contain 0.48, 1.08, 1.38, 1.68, 1.98 or 2.58% of arginine. Equal amounts of aspartic acid and glutamic acid by weight were substituted for the proper amounts of arginine in the diets. Each group of fish was anesthetized and blood was sampled from each fish within a group at 0, 5 and 24 h after intubating the experimental diets (0.4 parts of distilled water:diet, w/w) by using a 3 ml syringe.

Experiment III was conducted to confirm the results from Experiment II in which PParg were higher than PAarg from fish fed the arginine deficient diet (0.48%). Four rainbow trout were fed a basal diet for 3 days until the fish recovered from the operation of dorsal aorta cannulation. After 24 h food deprivation, these fish were intubated at 1% body weight (dry-matter) of the 0.48 or 2.58% arginine diet. Two consecutive dietary periods were used: the first period, 3 days of the 2.58% arginine diet: the second period, 3 days of the 0.48% arginine diet. In the first period, 0 h post feeding blood samples were taken at the beginning of day 1 and 24 h later (24 h after feeding). During the second period, 0 h and postprandial (5 h after feeding) blood samples were taken for 3 days (day 1, 2 and 3). The post-absorptive (0 h) blood samples were taken on day 4 (24 h after feeding fish on day 3).

Sample collection and analysis

Fish were anesthetized with 200 mg/l MS222 and 300 μl blood were sampled from each fish. Plasma samples were prepared by centrifugation at 3,000×g for 10 min. For deproteinization, the plasma samples were mixed with 10%

Table 3. Plasma free amino acid concentrations (nmol/ml) after feeding the basal diet (Experiment I)[1]

Amino acids	Time (h) after feeding							Pooled SEM
	0	2	3	4	5	6	24	
EAA								
Arginine	79^{c}	131^{b}	119^{b}	128^{b}	228^{a}	78^{c}	131^{b}	9
Histidine	114^{d}	244^{a}	186^{b}	143^{c}	185^{b}	96^{d}	113^{d}	10
Isoleucine	84^{e}	200^{d}	260^{c}	346^{b}	488^{a}	367^{b}	110^{e}	26
Leucine	146^{f}	307^{e}	392^{d}	516^{c}	784^{a}	581^{b}	166^{f}	41
Lysine	108^{e}	270^{d}	354^{c}	422^{b}	515^{a}	237^{d}	248^{d}	24
Methionine	49^{d}	149^{c}	155^{c}	222^{b}	321^{a}	235^{b}	62^{d}	17
Phenylalanine	93^{e}	178^{d}	225^{c}	426^{b}	714^{a}	239^{c}	98^{d}	40
Threonine	123^{e}	432^{c}	532^{b}	450^{c}	793^{a}	518^{b}	165^{d}	41
Tryptophan	10^{d}	23^{ab}	26^{a}	25^{a}	20^{bc}	18^{c}	10^{d}	1
Valine	222^{c}	530^{b}	594^{b}	560^{b}	783^{a}	579^{b}	238^{c}	37
Total	1,028^{e}	2,464^{d}	2,843^{c}	3,238^{b}	4,831^{a}	2,948^{c}	1,341^{e}	181
NEAA								
Alanine	603de	1086^{b}	1248^{a}	715^{cd}	845^{c}	671^{d}	465^{e}	52
Aspartic acid	474^{e}	627^{d}	694^{c}	795^{b}	976^{a}	892^{ab}	512^{e}	36
Asparagine	103^{c}	165^{b}	185^{a}	153^{b}	156^{b}	98^{c}	115^{c}	8
Citrulline	38^{bc}	45^{b}	40^{b}	49^{a}	37^{c}	31^{d}	18^{e}	2
Glycine	329^{b}	446^{a}	241^{c}	143^{d}	140^{d}	242^{c}	396^{ab}	23
Glutamic acid	272^{d}	463^{c}	614^{b}	454^{c}	1,092^{a}	614^{b}	261^{d}	52
Ornithine	111^{b}	105^{b}	126^{a}	131^{a}	118^{ab}	70^{c}	48^{d}	8
Serine	112^{c}	253^{b}	255^{b}	282^{b}	377^{a}	415^{a}	134^{c}	21
Tyrosine	39^{e}	88^{d}	136^{c}	195^{b}	300^{a}	175^{b}	57^{e}	16
1-Methylhistidine	33^{e}	76^{c}	88^{c}	111^{b}	127^{a}	87^{c}	53^{d}	6
3-Methylhistidine	31^{d}	54^{c}	72^{c}	119^{a}	115^{a}	101^{b}	39^{d}	5

[1] Values are means of four fish where the means in each row with different superscripts are significantly different ($p<0.05$).

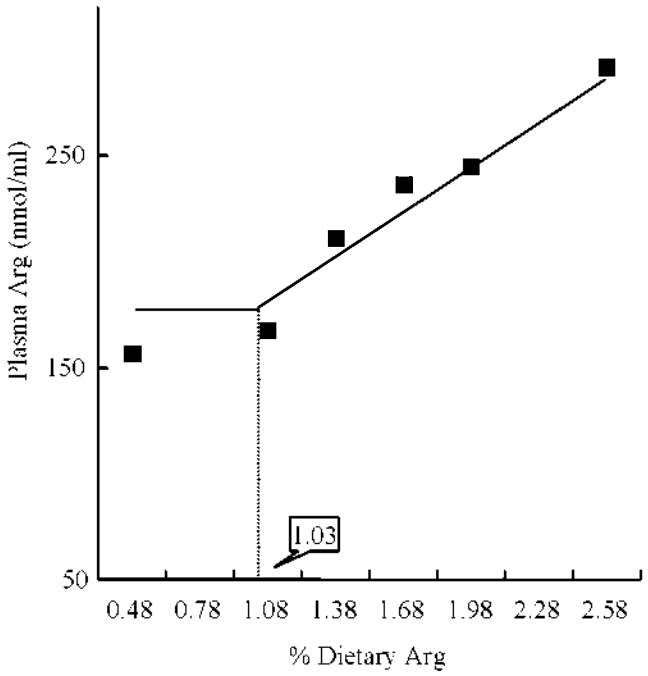

Figure 1. Plasma free arginine concentrations (nmol/ml) at 5 h (post-prandial arginine) after feeding in fish fed graded levels of dietary arginine (Experiment II). Y=175.1-33.2 (R-X), R=1.03± 0.299 (SE).

Table 4. Post-prandial and post-absorptive plasma free arginine concentrations (nmol/ml) of rainbow trout fed graded levels of dietary arginine (Experiment II)[1]

Level of arginine (%)						Pooled
0.48	1.08	1.38	1.68	1.98	2.58	SEM[2]
Post-prandial values						
157[c]	168[c]	211[b]	236[b]	245[b]	292[a]	1.98
Post-absorptive values						
66[d]	93[c]	117[ab]	113[b]	121[ab]	132[a]	0.82

[1] Values are means (n=5) and means with different superscripts are significantly different ($p<0.05$).

[2] Pooled standard error of mean: $SD/\sqrt{n}$.

5-sulfosalicylic acid in the ratio of four to one, cooled on ice for 30 min and centrifuged. The protein-free supernatant was diluted in pH 2.2 lithium citrate sample dilution buffer in the ratio of one to one and the samples were stored at -80°C until analysis. The plasma free amino acids were separated and quantified using a S433 amino acid analyzer (Sykam, Germany) using the ninhydrin method.

Statistical analysis

Data were subjected to analysis of variance test by 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 both PParg and PAarg were estimated by using the broken line model of Robbins et al. (1979).

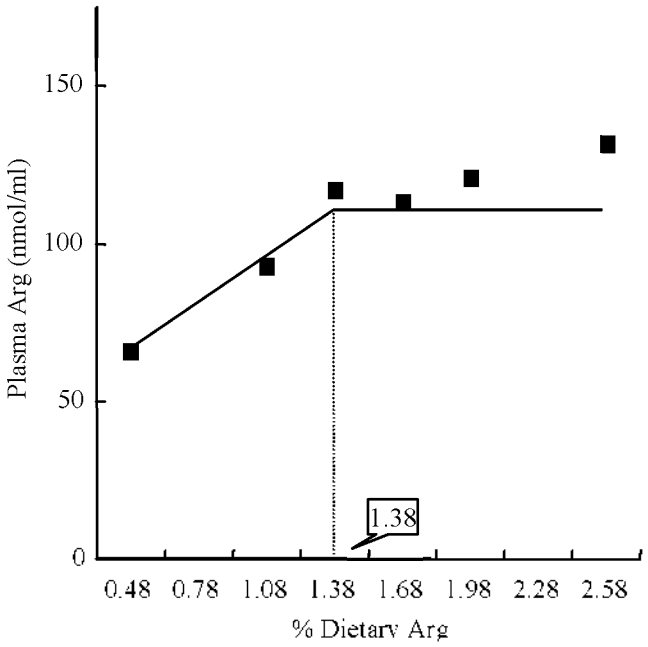

Figure 2. Plasma free arginine concentrations (nmol/ml) at 24 h (post-absorptive arginine) after feeding in fish fed graded levels of dietary arginine (Experiment II). Y=175.1-33.2 (R-X) R=1.03± 0.299 (SE).

RESULTS

Experiment I

Plasma free amino acid concentrations from fish force-fed the basal diet are shown in Table 3. Plasma free essential amino acid concentrations, with exception of histidine, lysine and tryptophan, began to increase at 2 h, peaked at 5 h and returned to near basal level at 24 h. Plasma free histidine concentration peaked at 2 h and returned to the basal level at 6 h. Plasma free lysine concentration peaked at 5 h and did not return to the basal level by 24 h. Plasma free tryptophan concentrations peaked at 2 h, remained constant between 2 h and 4 h and returned to the basal level at 24 h. Total plasma free essential amino acid concentrations peaked at 5 h and returned to near basal level at 24 h. Plasma dispensable amino acid concentrations peaked between 2 h and 6 h and returned to near basal levels at 24 h.

Experiment II

Post-prandial plasma free arginine concentrations (PParg) and post-absorptive plasma free arginine concentrations (PAarg) of fish fed graded levels of arginine are shown in Figure 1 and 2. PParg concentrations declined with decreasing dietary arginine. PParg from fish fed 0.48 and 1.08% arginine diets were not significantly different ($p>0.05$); however, PParg increased with dietary arginine levels among fish fed diets containing from 1.08 to 2.58%. PAarg significantly increased with dietary arginine levels

Table 5. Plasma free arginine concentrations (nmol/ml) from fish fed diets containing 0.48 and 2.58% dietary arginine (Experiment III)[1]

Dietary arginine (%)	Time (h) after feeding				
	0 h[2]	5 h (d1)	5 h (d 2)	5 h (d 3)	24 h (d 4)
0.48		153±25[a]	158±21[a]	168±34[a]	87±18[b]
2.58	143±27[a]				135±33[a]

[1] Values are means±SD from four fish and means with different superscripts are significantly different ($p<0.05$).

[2] 5 h postprandial blood samples were taken daily for 3 days (d1, 2 and 3) and 24 h post-absorptive blood samples were taken on day 4 (at 24 h after feeding fish on d 3). 0 h post-prandial blood samples were taken at the beginning of day 1 and feeding fish once a day, 24 h post-absorptive feeding blood samples were taken on day 4 (at 24 h after feeding fish on day 3).

from 0.48% to 1.38%, while it increased at a lower rate beyond 1.38%. The breakpoints, using the broken-line model, were 1.03 and 1.38% for PParg and PAarg, respectively.

Experiment III

There were no significant differences between PParg from fish fed 0.48% arginine diet and PAarg from fish fed 2.58% arginine diet. However, the fourth day PAarg from fish fed 0.48% arginine diet was significantly lower than PParg from fish fed the same diet and the PAarg from fish fed 2.58% arginine diet ($p<0.05$).

DISCUSSION

Experiment I demonstrated that most amino acid concentrations peaked at 5 h and returned to near basal level at 24 h after feeding. From the 6 h plasma amino acid concentrations it would appear that post-absorptive concentrations might have been reached long before 24 h. In mammals, including rats (Swendseid et al., 1963; Young and Zamora, 1968), pigs (Puchal et al., 1962), dogs (Longenecker and Hause, 1959) and humans (Young and Scrimshaw, 1970) severe essential amino acid deficiency causes a decrease in the limiting amino acid during the absorptive phase, with a return toward normal 12-24 h after meal. Murai et al. (1987) and Schuhmacher et al. (1997) reported similar results that the plasma concentrations of arginine, leucine, isoleucine, valine, phenylalanine and threonine from fish force-fed crystalline amino acids at 1% body weight (dry-matter) peaked at 6-9 h and returned to baseline by 24-32 h post feeding in rainbow trout.

Post-absorptive (24 h after feeding) plasma free arginine concentrations (PAarg) of the trout increased as dietary protein increased, with somewhat of a plateau occurring at about 1.38% of dietary arginine. Perhaps this is the concentration of dietary arginine that results in "regulation" (either reutilization for protein synthesis or oxidation of excess of that mobilized) of body arginine during the post-absorptive phase.

Table 6. 0 h and 24 h post feeding plasma free arginine concentrations (nmol/ml) from fish fed the various levels of dietary arginine (Experiment I, II and III)[1]

	0 h post feeding value	24 h post feeding value
Exp. I	79±11 (1.12%)[2]	131±13 (2.28%)[3]
Exp. II		66±5 (0.48%)
		132±15 (2.58%)
Exp. III	143±27 (2.28%)[4]	87±18 (0.48%)
		135±33 (2.58%)

[1] Values are means±SD from four fish where the means in each row with different superscripts are significantly different ($p<0.05$).

[2] 0 h post feeding value is 24 h post feeding value from fish fed commercial diet containing 1.21% arginine.

[3] 24 h post feeding value from fish fed experimental diet (dietary arginine levels).

[4] 0 h post feeding value is 24 h post feeding value from fish fed basal diet containing 2.28% arginine.

In Experiment II the effects of alterations of dietary arginine intake on post-prandial (5 h after feeding) plasma free arginine concentrations (PParg) were dependent upon the relative adequacy of the dietary arginine supply. PParg from fish fed 0.48 and 1.08% arginine diets were not significantly different; however, PParg increased with increasing dietary arginine from 1.08 to 2.58%. In the chicks, Zimmerman and Scott (1967) found that the dose-response curves for lysine, valine and arginine in the plasma remained almost flat initially and then increased at the point when the intake of each amino acid just exceeded the level required for maximum growth. In rats, McLaughlan and Illman (1967) found that the dietary level of each essential amino acid that supported the concentration of the amino acids after overnight food deprivation, was the same as that published for the requirements for each amino acid.

If the breakpoint was taken as the requirement of arginine for the trout in the present experiment, the requirement would be 1.03% of diet on the basis of PParg, considerably lower than that shown for the dose-response curve using maximum growth (Ogino, 1980; Walton et al., 1986). PAarg increased with dietary arginine from 0.48% to 1.38%, then showed a breakpoint with a slight continued slope. If this breakpoint were used as the arginine requirement the arginine requirement of trout would be 1.38% dietary arginine. The latter breakpoint is close to the requirement as determined by Ogino (1980) who reported that the arginine requirement of rainbow trout was 1.4% of diet. Kim et al. (1992) estimated the arginine requirement of rainbow trout as 1.41% of diet based on the growth data when L-amino acid mixture, casein and gelatin were used as protein source. Other reported estimates of the arginine requirement of trout ranged between 1.2-1.8% of the diet (Kaushik, 1979; Walton et al., 1986). Since the breakpoint of post-prandial plasma essential amino acids has not been consistently found at the requirement for all essential amino

acids in other species, more work needs to be done before the breakpoint of post-prandial plasma essential amino acids should be taken as the requirement in trout or other fish.

Experiment II showed that PParg (157±22 nmol/ml) was higher than PAarg (66±22 nmol/ml) for fish fed 0.48% arginine diet (less than half of the estimated requirement). This response indicates a basic difference between rainbow trout and mammals and birds in the metabolic response to a dietary deficiency of arginine. Perhaps arginine is catabolized more slowly and thus is available for protein synthesis and gluconeogenesis over a longer period of time after a given meal in rainbow trout. This is not true for all amino acids in fish since for methionine in sea bass (Thebault, 1985) and lysine in rainbow trout (Schuhmacher et al., 1997) dietary deficiencies of these amino acids cause a decrease in their concentrations in post-prandial plasma.

Experiment III confirmed the results from Experiment II that PParg from fish fed the arginine deficient diet were higher than PAarg from fish fed either the arginine deficient diet (0.48%) or the arginine adequate diets (1.68-2.58%). This experiment shows that the response of PAarg pattern in trout is not similar to those of mammals and birds. PAarg from fish fed the arginine deficient diet was lower than that from fish fed the arginine adequate diet (Table 5). This might indicate that PAarg concentrations are dependent upon the previous arginine intake.

In conclusion, these results show that post-prandial plasma arginine concentrations are responsive to dietary arginine level. Feeding a diet severely deficient in arginine to trout results in a postprandial rise, not fall in plasma arginine concentration, in contrast to decreases found in birds and mammals. Breakpoint analysis using PParg resulted in a breakpoint at 1.03% dietary arginine, whereas using PAarg it resulted in a breakpoint at 1.38% dietary arginine. However, validity of using these breakpoints to estimate arginine requirement needs further study.

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