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OXIDATION OF PHENYLALANINE AND THREONINE IN RESPONSE TO DIETARY ARGININE SUPPLY IN RAINBOW TROUT (*SALMO GAIRDNERI* R.)

B. FAUCONNEAU,* ANNE BASSERES and S. J. KAUSHIK

*Fish Physiology Unit, INRA Campus de Beaulieu, 35042 Rennes Cedex, France. Telephone: 9928-5002; Fish Nutrition Unit, INRA, B.P. 3, 64310 St Pee Sur Nivelle, France

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Abstract—1. The effect of increasing amounts of dietary arginine on oxidation of other amino acids was tested. Two experiments were conducted in rainbow trout fed on diets containing grading amounts of arginine from 0.55 to 2.55%.

2. The growth of fish and the efficiency of food conversion were measured and there were no significant differences between the different diets tested.

3. After an oral administration of either L-[U-^{14}C]phenylalanine or L-[U-^{14}C]threonine, the rate of excretion of $^{14}CO_2$ over a period of 3 hr and the radioactivity in the free pool and the protein pool of liver at the end of that period were measured.

4. The rate of oxidation of phenylalanine and threonine was assessed using their respective specific activities in the free pool of liver.

5. The rate of oxidation of phenylalanine increased from 246 ± 101 nmol/g body weight/3 hr at 0.55% dietary arginine supply to 679 ± 85 nmol/g body weight/3 hr at 2.00% arginine then it decreased for higher arginine supply.

6. The oxidation rate of threonine increased from 50 ± 22 nmol/100 g body weight/3 hr up to 774 ± 195 nmol/100 g body weight/3 hr for a dietary arginine supply of 2.45% then it decreased.

7. It was concluded that oxidation of phenylalanine and threonine could not be used as an indirect indicator of arginine status.

INTRODUCTION

Arginine is involved in many metabolic pathways: protein synthesis, urea cycle, production of creatine phosphate, synthesis of polyamines (Kaushik *et al.*, 1988). The requirements for these different pathways are generally covered in adult mammals by the arginine production through the urea cycle. It is known that in young growing mammals in which the requirement for protein deposition and tissue development are high, a dietary supply of arginine is required (Visek, 1986). In fish, which are ammoniotelic, production of urea and thus possible supply of arginine from this source are limited so a dietary supply of arginine is required for growth. The relation between arginine supply by urea cycle and requirement for a dietary supply needs to be analysed further.

The dietary arginine requirement have been recently reevaluated in salmonids using various biochemical indices such as plasma and tissue levels of arginine and urea, rate of oxidation of arginine and rate of oxidation of arginine precursors such as citrulline and ornithine (Woodward and Cho, 1985; Chiu *et al.*, 1986; Walton *et al.*, 1986; Kaushik *et al.*, 1988). There is a large disparity for the dietary requirement of arginine: from 0.8 to 2.8% according to different authors. Furthermore, the measurement of arginine requirement based on assessment of arginine status gives conflicting patterns depending on the arginine pathways analysed (Kaushik *et al.*, 1988). Thus, more information and implementation of new methods are required.

A method of assessment of amino acid requirement developed in mammals has been using measurement of oxidation of another amino acid as an index (Kim *et al.*, 1983). The oxidation rate of the "indicator" amino acid is high when animals are fed on amino acid deficient or imbalanced diets and it is stabilized to a low level if the deficient amino acid is supplied above its requirement. A model dose response has been drawn which allows measurement of individual amino acid requirement. It has been used for the assessment of tryptophane requirements of pig and rat using phenylalanine oxidation which is mainly catabolized in liver (Ball and Bayley, 1984).

The aim of this work was to analyse, in growing trout, the changes in the rate of oxidation of two essential amino acids, phenylalanine and threonine catabolized mainly in liver, in response to changes in the dietary supply of arginine.

MATERIALS AND METHODS

Fish and diet

Two experiments were realized in rainbow trout (120 g and 100 g mean body weight, respectively). In each experiment, eight groups of 10 fish were constituted. Fish of each group were reared in 60 l cylindrico-conical tanks supplied by thermoregulated (16°C) recirculated water at a flow rate of 2.5 l/min. They were fed experimental diets based on skimmed milk and wheat middlings used as protein sources

deficient in arginine (Table 1). The diet contained 94.5% dry matter and 30.5 g protein ($N \times 6.25$)/100 g dry matter. This basal diet was supplemented with graded amounts of arginine to provide from 0.55 to 3.25% dry matter of total arginine by steps of 0.45 or 0.50%. A casein/gelatin based diet which contained 33% crude protein and 6.13 g/16 g N of arginine was used as a control diet.

The fish were fed at a feeding level of 2.5% body weight in two meals per day. The amount of food distributed was adjusted weekly. Growth of fish was controlled by weighing every week. Growth experiment stopped at 18 days for the first experiment and at 21 days for the second experiment. Fish were used for metabolic study after the growth experiment, so that fish were fed on experimental diet for at least 3 weeks.

Manipulation of fish

For metabolic studies, the fish were anesthesized (mono phenyl ether ethylene glycol 0.4 p. 1000). A catheter was inserted in the urinary bladder for urine collection. Fish were force-fed pellets of a labelled experimental diet (0.5 g/fish) by gastric tubing. The labelled diet provided 0.21 MBq/g (5.7 μCi/g) of L-[U-^{14}C]phenylalanine or 0.15 MBq/g (3.9 μCi/g) of L-[U-^{14}C]threonine (CEA, Service Molécules Marquées, France). The fish were placed in individual tanks supplied with free running thermoregulated water. The fish were force-fed again 2 hr later with the same amount of labelled diet. Then they were left for 2 further hr in the individual tanks.

The fish were placed in a tightly closed "metabolic chamber" filled with 2 l of water. The chamber was continuously aerated with CO_2-free air. The carbon dioxide and the soluble labelled compounds excreted by fish in the air, in the water and in the urine were collected during a 3 hr period (for more detail see Fauconneau and Arnal, 1985). A sample of water and urine was stored for ammonia and urea analysis.

At the end of that period the fish were anesthesized and a sample of blood was withdrawn, centrifuged, the plasma collected and stored at −20°C until analysis. The fish were killed and the liver was rapidly removed, rinsed in cold saline (NaCl 9 g/l) solution, dried on absorbent paper, weighed, frozen in liquid nitrogen and stored until analysis. Other tissues—kidney, digestive tract and carcass remains—were dissected and weighed.

Tissue treatments

The liver (1 g) was homogenized in 10 vol. of cold trichloroacetic acid (TCA 10% w/v), and centrifuged (5000 *g*, 15 min). The supernatant which contained the free amino acids was collected. The procedure (homogenization, centrifugation) was repeated four times on the residue and the supernatants, called acid soluble fraction, were combined. The same procedure was applied on the labelled diet and the radioactivity was measured directly on the acid soluble fraction.

Table 1. Composition of the basal experimental diet

Ingredients	g/kg
Milk powder	250
Wheat middlings (60% CP)	150
Starch	170
Fish oil	150
Vitamin mix*	30
Mineral mix†	60
Cellulose	30
Amino acid mix‡	160

*†See Woodward and Cho (1985).

‡Amino acid mix which provided in the L-form and in g/kg diet, essential amino acids: cystine 3, histidine 4, isoleucine 2, leucine 2, methionine 7, phenylalanine 3, threonine 3, tryptophane 2, tyrosine 2, valine 4; non essential amino acid: aspartic acid 10, glycine 10, proline 25, serine 7 and glutamic acid 33 to 61 by substitution of arginine 0 to 28.

The TCA was removed from the combined supernatant by three successive extractions with diethyl ether. The aqueous solution was concentrated by evaporation under vacuum and collected with distilled water to reach a final volume of 2 ml. The radioactivity of that amino acid extract was measured.

The residue was neutralized and washed with sodium acetate/methanol solution (185 g/l). Then the lipids were removed from the residue by two successive extractions with a methanol/dichloromethane solution (2 vol./1 vol.). The residue was washed with ethanol and dried with diethyl ether. An aliquot of the residue which was called protein extract was hydrolysed (NaOH, 0.3 N, 30°C, 20 hr). The radioactivity of that hydrolysate was measured.

Analysis

The free amino acid analysis was performed after derivatization of amino acid with Ortho-Phthalaldehyde (OPA) on an HPLC system according to Hogan *et al.* (1982). Amino acids were transformed as thioisoindol with OPA solution (50 mg/l in sodium borate buffer 0.4 M, pH 10) using 2-mercaptoethanol as a reducing agent. Then the amino acid derivatives were separated by HPLC (Vista 5000, Varian) on a C18 column (Micropack, Varian) with a gradient of methanol (from 20 to 80%) in sodium acetate buffer (0.05 M, pH 4.5). OPA derivates of amino acids were detected by fluorescence and quantified using gamma-butyric acid as an internal standard. The column eluate was collected and fractionated and the radioactivity corresponding to either phenylalanine or threonine was measured. Then the specific activity of threonine was calculated by the ratio between the radioactivity and the amount of amino acid in their respective fractions. The amino acid analyses were realized on pooled samples of liver free amino acid extract. The measurements of radioactivity in fractions collected after phenylalanine separation were not very accurate, so the specific activity of phenylalanine was assessed by the ratio between the total radioactivity measured in the free amino acid extract of liver and the amount of phenylalanine detected in that extract.

Plasma arginine was measured directly using the automated method of Bacchus and London (1971). Plasma glucose level was measured directly using a glucose oxidase probe (Glucose analyser, Beckmann).

Ammonia and urea were measured in samples of water and urine according to Kaushik (1980). Creatine levels in urine were measured according to Zender and Falbriard (1965).

Calculations

The rate of oxidation of phenylalanine and threonine was assessed from the ratio between the rate of production of $^{14}CO_2$ (in dpm/hr) and the specific activity of the free amino acid in the liver. A rough estimate of the rate of incorporation of phenylalanine and threonine into the liver protein was calculated from the ratio between the radioactivity incorporated into the protein extract of liver and the specific activity of the free amino acid in the liver.

The significance of the difference observed were tested either after a one way analysis of variance or directly using the Student's *t*-test.

RESULTS

Growth and feed efficiency

In the first experiment (Table 2), the growth of fish fed the experimental diet and the control commercial diet were not different. A decrease in growth rate

Table 2. Growth performance of trout fed for 18 days on experimental diets containing graded amount of arginine (from 0.55 to 3.25%) as compared to a casein/gelatin control diet in the first experiment (labelled phenylalanine experiment)

Diet	I	II	III	IV	V	VI	VII	Control
Arginine content (%)	0.55	1.00	1.55	2.00	2.45	2.90	3.25	2.0
Arginine consumption (mg/kg BW/day)	75	133	216	272	307	369	455	257
Mean initial body weight (g)	123	126	123	122	128	117	125	122
Mean final body weight (g)	142	140	139	133	138	130	147	146
Daily weight gain (g/day)	1.1	0.80	0.93	0.65	0.55	0.80	1.28	1.38
Feed conversion ratio	1.64	2.21	2.10	2.7	3.29	2.13	1.56	1.20
Protein efficiency ratio	2.0	1.4	1.6	1.2	1.0	1.5	2.0	2.4

BW: Body weight.
Protein efficiency ratio: growth rate (g/day)/protein consumption (g/day).

associated with an increase in the feed conversion ratio was observed when the dietary supply of arginine increased from 0.55 to 1.55% of the diet. In the second experiment (Table 3), growth and feed efficiency ratios of fish fed the diet containing low amount of arginine (from 0.55 to 1.5%) were, respectively, lower and higher than those of fish fed and other experimental diet and the control diet.

The hepatosomatic indices (HSI) of fish fed the 0.55 and 3.25% arginine diets and the control diet (HSI: 2.5–2.9%) were significantly higher ($P < 0.01$) than those of fish fed other diets (HSI: 2.0%). The composition of the carcass (eviscerated carcass) measured on five fish per group in the first experiment was not significantly affected by the diet: dry matter content 25.5–26.5% of fresh tissue; crude protein 64.5–67.5% of dry matter and energy 22.9–23.9 KJ/g.

Plasma and liver metabolites (Table 4)

The arginine and urea levels in the plasma increased slightly when the dietary supply of arginine increased. A correlation was observed between plasma arginine and plasma urea concentrations (slope 0.3, $r = 0.80$, $N = 14$). No significant changes in the free arginine content of the liver were found. Finally, the increase in the dietary supply of arginine did not affect the plasma glucose level, the lowest level (95 mg/100 ml) was observed in fish fed either diets deficient (0.55%) or highly supplemented (2.5–3.5%) or the control diet and the highest level (130 mg/100 ml) was observed in fish fed the diet containing 2.0% of arginine.

Urea and ammonia excretion

The excretion of ammonia and urea in the water through the gill was higher in fish fed the experimental diet than in fish fed the control commercial diets but no significant changes were observed within the experimental diets (Figs 1 and 2).

In the first experiment the urinary excretion of urea seemed to decrease when the dietary arginine supply increased but this was not observed in the second experiment. No clear patterns in the urinary excretion of urea and creatine were found in the two experiments.

Radioactivity collected in different fractions of the liver and in $^{14}CO_2$ excreted by fish

In fish fed a trace dose of labelled phenylalanine, the ratioactivity recovered in the acid soluble and in the protein fractions of liver were in the same range in the different groups (1.5–2.5% of the dose per liver) (Fig. 3) with no apparent effect of the level of arginine in the diet. The $^{14}CO_2$ excreted during 3 hr was significantly lower in fish fed on 0.55% arginine diet than in that fed other experimental diets and no other significant differences were observed. A small amount of radioactivity (0.4–0.8% of the dose) was excreted in other compounds than $^{14}CO_2$. In fish fed the control commercial diet the radioactivity collected in the different fractions of liver and in $^{14}CO_2$ were lower than in fish fed the experimental diet.

In fish fed a trace dose of labelled threonine (Fig. 4), the radioactivity recovered in the protein of liver (2.7–4.6% of the dose) was twice that recovered in the acid soluble fraction (1.3–2.2% of the dose). The radioactivities recovered in these fractions increased when the dietary arginine supply increased. The radioactivity measured in the $^{14}CO_2$ excreted by fish increased slightly when the dietary arginine increased but none of the differences observed were significant. A significant amount of radioactivity was also measured in compounds other than CO_2 in the water (0.2–0.4% of the dose within 3 hr).

The radioactivity collected in $^{14}CO_2$ was lower in fish fed the labelled threonine diet (1.2–2.2% of the dose/3 hr) than in fish fed the labelled phenylalanine diet (1.5–2.7% of the dose/3 hr).

Table 3. Growth performance of trout fed for 21 days on experimental diets containing graded amount of arginine (from 0.55 to 3.25%) as compared to a casein/gelatin control diet in the second experiment (labelled threonine experiment)

Diet	I	II	III	IV	V	VI	VII	Control
Arginine content (%)	0.55	1.00	1.55	2.00	2.45	2.90	3.25	2.0
Arginine consumption (mg/kg BW/day)	69	130	199	246	299	362	417	264
Mean initial body weight (g)	99	105	100	97	101	98	99	96
Mean final body weight (g)	120	127	121	119	127	124	126	128
Daily weight gain (g/day)	1.01	1.03	1.00	1.04	1.23	1.21	1.28	1.50
Feed conversion ratio	1.36	1.43	1.42	1.27	1.13	1.14	1.09	0.95
Protein efficiency ratio	2.41	2.24	2.26	2.45	2.70	2.59	2.26	3.04

BW: Body weight.
Protein efficiency ratio: growth rate (g/day)/protein consumption (g/day).

Table 4. Plasma and liver free arginine levels (μmol/ml and nmol/g respectively) and plasma urea levels (μmol/ml) in trout fed for 3 weeks on experimental diets containing graded amounts of arginine (from 0.55 to 3.25%) as compared to a casein/gelatin based control diet in two separate experiments. Results are expressed as mean (SD) ($N = 6$ fish)

Diet	I	II	III	IV	V	VI	VII	Control
Arginine content (%)	0.55	1.00	1.55	2.00	2.45	2.90	3.25	2.0
Experiment I								
Plasma arginine	0.8	0.9	0.8	0.9	0.9	1.1	0.9	1.2
	(0.2)	(0.3)	(0.3)	(0.4)	(0.3)	(0.3)	(0.4)	(0.3)
Plasma urea	0.3	0.5	0.6	0.5	0.5	0.4	0.4	0.2
	(0.1)	(0.2)	(0.2)	(0.2)	(0.3)	(0.2)	(0.1)	(0.1)
Liver arginine (nmol/g)	55	60	69	76	64	60	52	44
	(11)	(10)	(23)	(12)	(24)	(06)	(06)	(14)
Experiment II								
Plasma arginine	0.7	0.7	0.9	0.9	1.1	0.9	1.2	n.m.
	(0.2)	(0.1)	(0.2)	(0.4)	(0.2)	(0.3)	(0.2)	
Plasma urea	0.6	0.8	0.7	0.7	0.7	1.0	0.9	n.m.
	(0.1)	(0.2)	(0.2)	(0.2)	(0.2)	(0.1)	(0.2)	
Liver arginine* (nmol/g)	14	19	48	31	47	36	30	n.m.

n.m.: not measured.
*Data of a pooled sample of six livers.

Phenylalanine and threonine oxidation (Table 5)

The specific activity of phenylalanine in the liver and the rate of oxidation of phenylalanine, 7 hr after the first administration of labelled phenylalanine, decreased and increased respectively from fish fed the 0.55% arginine diet to fish fed the 2.9% arginine diet.

The specific activity of threonine in the liver was almost 4 times lower than that of phenylalanine. It was very high in fish fed the diet containing 0.55% arginine. The specific activity of threonine in the liver and the rate of oxidation decreased and increased from 0.55% arginine diet to 2.45% arginine diet, respectively but then increased and decreased respectively.

DISCUSSION

Method of an amino acid indicator in fish

When animals are fed an amino acid deficient diet supplemented gradually, the tissue level of that amino acid is low and does not change until its level of dietary requirement is reached. Above that threshold there is a parallel accumulation in the tissue of that amino acid and an increase in its rate of oxidation. In mammals, such model response depends on the amino acid (Young *et al.* 1985). In fish, a clear dose response has been observed for many amino acids (Cowey and Luquet, 1983; Walton *et al.*, 1986), but not in all cases for arginine. The response of both tissue arginine accumulation and arginine oxidation (Kaushik *et al.*, 1988; Walton *et al.*, 1986) are not always clear. In the present experiment, no clear accumulation of liver arginine occurs with increasing dietary arginine.

It is observed that when the tissue level of an amino acid is low due to its dietary deficiency, there is an accumulation of the other amino acids (Pion *et al.*,

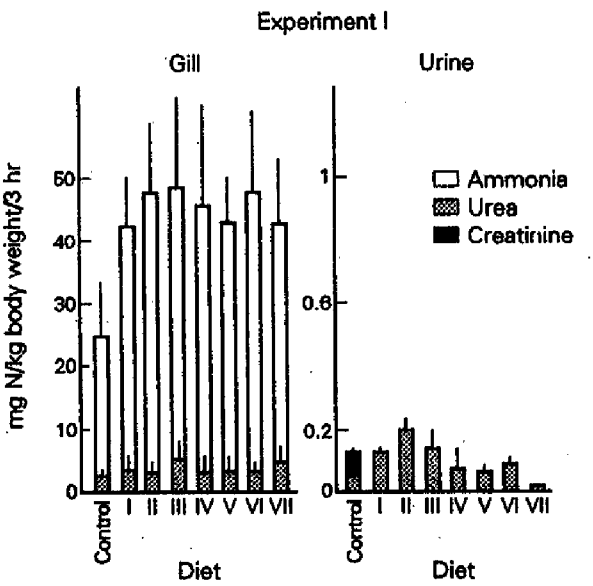

Fig. 1. Ammonia, urea and creatine excreted in urine and in water through the gills (in mg N/kg body weight/3 hr) by trout fed for 3 weeks experimental diets containing graded amounts of arginine (from 0.55 to 3.25%) as compared to a casein/gelatin based control diet. Data of Experiment I (fish fed labelled phenylalanine). Results are the mean of five fish.

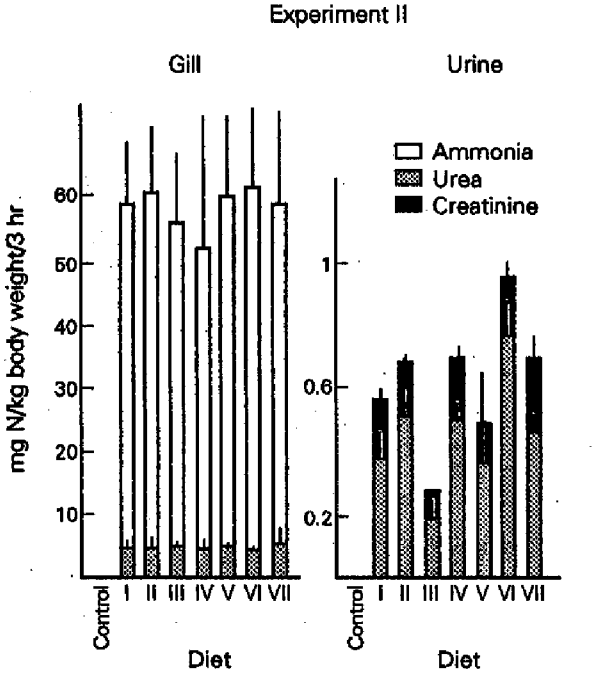

Fig. 2. Ammonia, urea and creatine excreted in urine and in water through the gills (in mg N/kg body weight/3 hr) by trout fed for 3 weeks experimental diets containing graded amounts of arginine (from 0.55 to 3.25%) as compared to a casein/gelatin based control diet. Data of Experiment II (fish fed labelled threonine). Results are the mean of five fish.

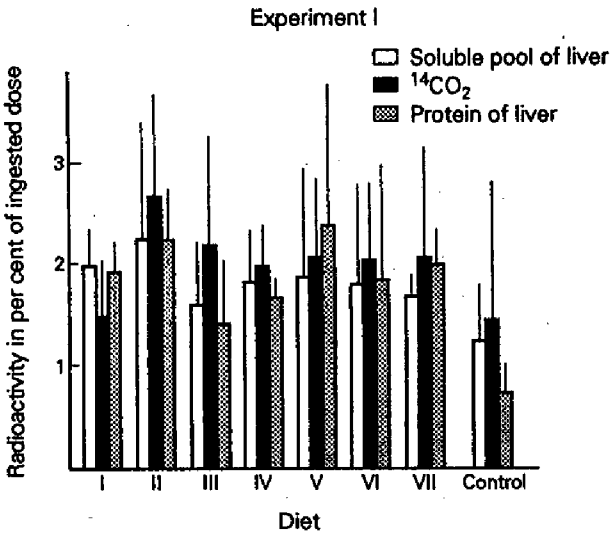

Fig. 3. Radioactivity excreted during 3 hr in $^{14}CO_2$, and recovered at the end of that period in the soluble pool and in the protein pool of liver 4 hr after oral administration of [^{14}C]phenylalanine in two successive meals. Trout were fed for 3 weeks experimental diets containing graded amounts of arginine (from 0.55 to 3.25%) or a casein/gelatin based control diet. Results were expressed as per cent of radioactivity ingested.

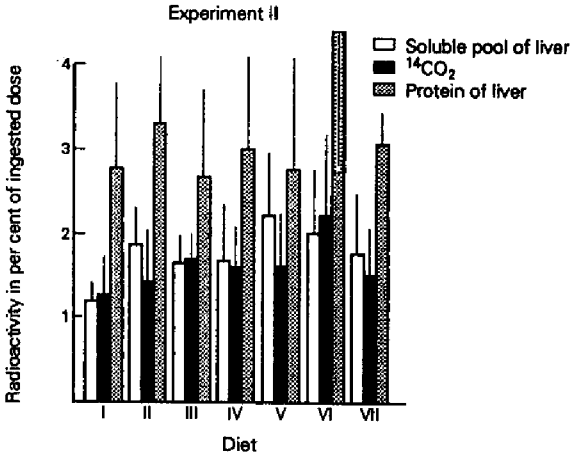

Fig. 4. Radioactivity excreted during 3 hr in $^{14}CO_2$, and recovered at the end of that period in the soluble pool and in the protein pool of liver 4 hr after oral administration of [^{14}C]threonine in two successive meals. Trout were fed for 3 weeks experimental diets containing graded amounts of arginine (from 0.55 to 3.25%) or a casein/gelatin based control diet. Results were expressed as per cent of radioactivity ingested.

1978) and this has been observed in fish fed an arginine deficient diet (Kaushik *et al.*, 1988). When the normal level of the deficient amino acid is restored, the tissue levels of the other amino acids decrease. Thus, it has been suggested that the tissue level of other amino acids or their rate of metabolism could be used as indexes of the status of the deficient amino acid. This is the basis of the method developed by Kim *et al.* (1983) which analysed the changes of the rate of catabolism of an amino acid used as an index to assess the level of requirement of an amino acid. Some conditions are needed both for the choice of the amino acid indicator and for the measurement of its rate of catabolism.

The method is rationalized by using an amino acid which is oxidized mainly in one tissue (at least for its first step of catabolism) to assess more easily the oxidation rate of that amino acid (Kim *et al.*, 1983). For that purpose, phenylalanine, whose level in liver is high and which is catabolized in the liver (Waterlow and Fern, 1982) is recommended, but threonine could also be used according to such criteria. Lysine could not be used because the pathways of its catabolism are much more complex than that of phenylalanine and there is an antagonism between lysine and arginine in mammals and also in fish (Kaushik and Fauconneau, 1984). Thus, we have tested phenylalanine and threonine as amino acid indicators.

Some conditions have to be reached to measure accurately the rate of oxidation of these labelled amino acids. First, the administration of the label has to be regular so that the specific activity of the amino acid in the free pool of the liver is stabilized during the period of measurement of the excretion of labelled end-product compounds or catabolites. Then, the rate of catabolism is directly proportional to the rate of excretion of the catabolites. The continuous infusion of the labelled amino acid could be used but the oral administration by several small meals has also been used in mammals (Kim *et al.*, 1983). In fish, the rate of intestinal transit Fauconneau *et al.*, 1983) is low compared to that of mammals, and the maximal post prandial level of phenylalanine and threonine is observed 2 to 3 hr after the meal in the liver (Medale *et al.*, 1986). Thus, the labelled amino acid was given in two successive meals within 2 hr.

Table 5. Specific activities (SA) of free phenylalanine and free threonine in liver and rate of oxidation of phenylalanine and threonine in trout fed for 3 weeks on an experimental diet containing graded amounts of arginine (0.55–3.25%) as compared to a casein/gelatin control diet and fed on the day of the experiment with two successive meals of a diet containing a labelled amino acid ([^{14}C]phenylalanine in experiment I and [^{14}C]threonine in experiment II). Results are expressed as mean (SD) ($N = 6$)

Diet	I	II	III	IV	V	VI	VII	Control
Arginine content (%)	0.55	1.00	1.55	2.00	2.45	2.90	3.25	2.0
Experiment I (Phenylalanine)								
PHE SA (dpm/nmol)	753	968	502	664	544	540	475	n.m.
	(270)	(377)	(225)	(161)	(367)	(294)	(72)	
PHE Oxidation (nmol/100 g/3 hr)	246	432	590	679	472	476	540	n.m.
	(101)	(240)	(368)	(85)	(165)	(256)	(237)	
Experiment II (Threonine)								
THREO SA (dpm/nmol)	560	154	98	51	43	129	127	n.m.
	(111)	(89)	(41)	(20)	(11)	(56)	(34)	
THREO Oxidation (nmol/100 g/3 hr)	50	202	362	647	774	361	245	n.m.
	(22)	(88)	(67)	(202)	(195)	(157)	(87)	

n.m.: not measured.

The measurement of the excretion of CO_2 started 4 hr after the first labelled meal (and 2 hr after the second labelled meal) and lasted 3 hr.

Secondly, the kind of labelling of amino acids is important. Using ^{14}C-amino acid labelled only on the carbon of the carboxyl group gives the possibility to measure only the first step of degradation of that amino acid. Unfortunately, such amino acids are not commonly available. Furthermore, it has been shown in trout that the relative rate of oxidation of uniformly and carboxy-labelled branched chain amino acids during long periods of time (6–12 hr) are similar (Zebian and Creach, 1979). It is also supposed that during short periods of time, it is mainly the first step which is measured. In fact, in that experiment, the rate of oxidation of phenylalanine and threonine were in the same range as that observed for other amino acids (leucine, lysine, arginine) using other method of administration (Fauconneau and Arnal, 1985; Fauconneau *et al.*, 1986; Kaushik *et al.*, 1988; Fauconneau and Tesseraud, 1990).

The results obtained with phenylalanine and threonine which showed no clear dose response of their oxidation rates to arginine supply were not conclusive. It could be emphasized that the period administration of the labelled amino acid was not well chosen and that oxidation of phenylalanine and threonine reflect rather overall utilization of amino acid than a specific response of oxidation to arginine supply. Thus, it suggests that utilization of amino acid increase up to an arginine supply of 2.00–2.45% which could be an optimal level of arginine supply. This did not demonstrate that such a method could not be used in fish to assess amino acid requirement.

Responses to the dietary supply of arginine

There is no evidence that some of the experimental diets were deficient in arginine. The poor growth rate and feed conversion ratio observed with medium arginine supply (2.00 and 2.45%) compared to that of low and high arginine supply and control diet in the first experiment suggest that a second amino acid could be limiting. Furthermore, it could be concluded also that arginine dietary requirement is lower than 0.55% of the diet. Some authors suggested also that the level of dietary arginine requirement is very low in trout (Kaushik, 1977; Woodward and Cho, 1985; Walton *et al.*, 1986). The same conclusion has been drawn, using various biochemical indicators which do not demonstrate a clear relationship with arginine supply above the level of 1% in the diet (Kaushik *et al.*, 1988). However, some authors find a high dietary requirement of arginine in trout fry (Ketol *et al.*, 1976; Walton *et al.*, 1986) but this could be related to changes in arginine requirement with aging (or growth rate).

The recent work of Chiu *et al.* (1986) demonstrated that, although the activities of the enzyme of the urea cycle are very low (Vellas, 1975), a part of the urea cycle is active in fish. It could be proposed that the activity of the urea cycle is partially controlled by the dietary supply of arginine. The functioning of urea cycle could counterbalance the dietary supply of arginine so that at low level, of arginine in the diet the activity of the urea cycle is high. Evidence of such an adaptation could be found in previous results (Kaushik *et al.*, 1988) and in the results of urea excretion in the present work, but this could be confirmed by the measurement of the flux rate of metabolites throughout the urea cycle, depending on the dietary supply of arginine.

Finally, with high levels of arginine supply in the diet, most of the parameters analysed reached the values observed with low levels of dietary arginine. A possible activation of amino acid uptake in peripheral tissues including muscle through a stimulation of insuline secretion by arginine could be suggested. In fact, the glucose level in the plasma was not significantly affected by the dietary supply of arginine and no increase in free amino acids of muscle is observed with high levels of arginine in the diet (Kaushik *et al.*, 1988), but such a possibility has to be investigated.

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