

## Protein and arginine requirements for maintenance and nitrogen gain in four teleosts

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(Received 20 February 2001 – Revised 7 January 2002 – Accepted 14 January 2002)

Besides being an indispensable amino acid for protein synthesis, arginine (Arg) is also involved in a number of other physiological functions. Available data on the quantitative requirement for Arg in different teleosts appear to show much variability. So far, there are very limited data on the maintenance requirements of indispensable amino acids (IAA) in fish. In the present study, we compared N and Arg requirements for maintenance and growth of four finfish species: rainbow trout (*Oncorhynchus mykiss*), turbot (*Psetta maxima*), gilthead seabream (*Sparus aurata*) and European seabass (*Dicentrarchus labrax*). Groups of fish having an initial body weight close to 5–7 g were fed semi-purified diets containing graded levels of N (0 to 8% DM) and Arg (0 to 3% DM) over 4 to 6 weeks. For each species, N and Arg requirements for maintenance and for growth were calculated regressing daily N gain against daily N or Arg intakes. N requirement for maintenance was estimated to be 37.8, 127.3, 84.7 and 45.1 mg/kg metabolic body weight per d and 2.3, 2.2, 2.6 and 2.5 g for 1 g N accretion, in rainbow trout, turbot, gilthead seabream and European seabass respectively. The four species studied appear to have very low or no dietary Arg requirements for maintenance. Arg requirement for g N accretion was calculated to be 0.86 g in rainbow trout and between 1.04–1.11 g in the three marine species. Turbot required more N for maintenance than the other three species, possibly explaining its reputedly high overall dietary protein requirement. Data suggest a small but sufficient endogenous Arg synthesis to maintain whole body N balance and differences between freshwater and marine species as regards Arg requirement. It is worth verifying this tendency with other IAA.

### Requirement: Maintenance: Growth: Nitrogen: Arginine: Teleosts

One common feature of most aquatic animals is the high dietary protein requirement (30–65% DM; Nutrition Research Council, 1993) making teleosts appear as distinct from other animals cultivated for human food. Significant inter-species differences with regard to protein requirements also appear to exist in fish. Given the increase in aquaculture production and the implications of poor protein utilisation on nitrogenous losses in the effluents, there is an increasing need for optimising protein and indispensable

amino acid (IAA) supply. Further, given the variety of species under cultivation, there is also a need for knowledge on possible differences between the various fish species.

A critical analysis of existing data on amino acid (AA) requirements of different species of fishes shows similarities as well as differences (Tacon & Cowey, 1985; Cowey, 1994; Kaushik, 1995). The variations in the IAA requirement of fishes can be attributed to a number of

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**Abbreviations:** AA, amino acid; Arg, arginine; DAA, dispensable amino acid; DGC, daily growth coefficient; FBW, final body weight; FE, feed efficiency; HA<sup>-</sup>, arginine-free high-protein diet; HP, high-protein diet; IAA, indispensable amino acid; IBW, initial body weight; LA<sup>-</sup>, arginine-free low-protein diet; LP, low-protein diet; MA<sup>-</sup>, arginine-free medium-protein diet; MBW, metabolic body weight; MP, medium-protein diet; OUC, ornithine-urea cycle; PER, protein efficiency ratio; PF, protein-free diet; SAA, sulfur amino acid; VFI, voluntary feed intake.

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factors such as differences in basal diet composition, size and age of fish, genetic differences, feeding rate and culture conditions, all of which affect overall growth rate besides experimental design and choice of response criterion (Cowey, 1994; Mambrini & Kaushik, 1995a).

While data on protein requirements for maintenance are available for a few freshwater (Kaushik *et al.* 1981; Kaushik & Luquet, 1984) and marine species (Lupatsch *et al.* 1998), data on the maintenance requirements for IAA are scarce. For estimating the AA requirements for maintenance of pigs, Fuller *et al.* (1989) proposed a linear model based on the relationship between AA intake and N gain. This method involves regression of daily N gain (protein accretion) *v.* daily AA intake, where the *x*-intercept of the regression represents the amount of the given IAA required for maintaining N balance and where the reciprocal of each regression coefficient represents the amount of AA needed to increase protein accretion by 1 g. Using the foresaid approach, Mambrini & Kaushik (1995b) estimated the maintenance requirement for sulfur AA (SAA) of rainbow trout and found that to be about two-fold higher than those of terrestrial omnivores, possibly indicating a different basal protein metabolism in fish. More recently, Rodehutschord *et al.* (1997) have estimated the maintenance requirement of rainbow trout for several IAA, through intrapolation of dose–response data to zero growth.

Arginine (Arg) is an IAA necessary for optimal growth of young fish (Wilson, 1989) and terrestrial animals (Visek, 1986). Of the IAA, data on Arg requirement show a greater degree of variation than others (Wilson, 1989; Cho *et al.* 1992), possibly due to its involvement in several metabolic pathways. Besides protein synthesis, Arg is related to urea-genesis, synthesis of proline, creatine, polyamine and nitric oxide. The versatile nature of Arg is also subject to much speculation as regards its role as a potent secretagogue affecting plasma insulin levels as well as growth hormone and somatomedin release in mammals as well as in fishes (Swanson, 1990; Mommsen, 2001).

The purpose of the present study was to evaluate N and Arg requirements for maintenance and for growth between four species: one euryhaline species, rainbow trout (*Oncorhynchus mykiss*) grown in freshwater; three marine species, i.e. turbot (*Psetta maxima*), gilthead seabream (*Sparus aurata*) and European seabass (*Dicentrarchus labrax*).

## Materials and methods

### Experimental diets

Diets for all the four species were identical and were manufactured in the same laboratory (IFREMER, Brest, France), using the same batch of ingredients. Seven semi-purified diets were formulated (Table 1) to contain

**Table 1.** Composition of the semi-purified experimental diets used for the determination of maintenance and growth requirements for nitrogen and for arginine (Arg) in the four species

Diets...	PF	LP	MP	HP	LA <sup>-</sup>	MA <sup>-</sup>	HA <sup>-</sup>
N level (% DM)	0	2.4	4.8	8.0	2.4	4.8	8.0
Arg content (% DM)	0	1.0	2.0	3.0	0	1.5	2.5
Components (g/kg diet)							
Basal casein mixture*	0	0	150	250	0	150	250
L-amino acids mixture	0	163.9	190.2	264.8	177.9	196.1	273.5
Dextrin†	718.9	555.0	378.7	204.1	541.0	372.8	195.4
Cod-liver oil‡	90	90	90	90	90	90	90
Soya lecithin§	20	20	20	20	20	20	20
Vitamin mix	50	50	50	50	50	50	50
Mineral mix¶	50	50	50	50	50	50	50
α-Cellulose**	50	50	50	50	50	50	50
Agar§	10	10	10	10	10	10	10
Fish-protein concentrate††	10	10	10	10	10	10	10
Ethoxyquin†	0.15	0.15	0.15	0.15	0.15	0.15	0.15
Chemical composition							
DM (% diet)	97.91	91.50	91.00	93.19	92.33	92.45	93.53
N (% DM)	0.23	2.54	5.49	8.13	2.43	5.37	8.09
Fat (% DM)	10.61	10.53	11.61	11.85	10.88	10.40	11.57
Ash (% DM)	4.69	4.87	4.98	5.31	4.94	5.10	5.27
Energy (kJ/g DM)	19.15	20.14	21.11	21.43	19.89	20.88	21.39

PF, protein-free diet; LP, low-protein diet; MP, medium-protein diet; LA<sup>-</sup>, Arg-free low-protein diet; MA<sup>-</sup>, Arg-free medium-protein diet; HA<sup>-</sup>, Arg-free high-protein diet.

\* Basal casein mixture contained: 70% casein, vitamin-free (ICN Biochemicals, Orsay, France; no. 904798); 20%.

† Dextrin, Sigma (St Quentin Fallavier, France) D2256; ethoxyquin, Sigma E8260.

‡ La lorientaise des produits de la pêche, Lorient, France.

§ Louis François, St Maur, France.

|| Supplied the following according to National Research Council (1993) (to provide g/kg mixture, except as noted): retinyl acetate (500 000 IU/g), 0.5; ascorbyl phosphate (25%), 20; cholecalciferol (500 000 IU/g), 0.5; tocopheryl acetate (500 IU/g), 10; menadione, 1; thiamin, 0.1; riboflavin, 0.4; pyridoxine, 0.3; calcium-pantothenate, 2; vitamin B<sub>12</sub>, 1; niacin, 1; biotin (2%), 1; folic acid, 0.1; choline (50%), 200; inositol, 30.

¶ Supplied the following (to provide g/kg mixture, except as noted): magnesium carbonate, 124; calcium carbonate, 215; potassium chloride, 90; sodium chloride, 40; potassium iodide, 40 mg; copper sulfate, 3; cobalt sulfate, 20 mg; ferric sulfate, 20; manganese sulfate, 3; zinc sulfate, 4; dibasic calcium phosphate, 500; sodium fluoride, 1.

\*\* Arbocel BOO, J. Rettenmaier & Söhne, Ellwangen Hotzmühle, Deutschland.

†† CPSP, Sopropêche, Lorient, France.

**Table 2.** Composition of the L-amino acids\* mixtures (g/kg diet) used in the semi-purified diets†

Diets...	LP	MP	HP	LA <sup>-</sup>	MA <sup>-</sup>	HA <sup>-</sup>
Arginine	9.4	15.7	23.2	0	11.8	17.4
Histidine	3.9	4.0	5.4	3.9	4.0	5.4
Isoleucine	6.6	6.7	9.1	6.6	6.7	9.1
Leucine	11.0	9.3	12.1	11.0	9.3	12.1
Lysine	11.3	13.3	18.6	11.3	13.3	18.6
DL-Methionine	4.0	3.3	4.2	4.0	3.3	4.2
Cystine	1.5	2.5	3.7	1.5	2.5	3.7
Phenylalanine	6.6	6.0	7.8	6.6	6.0	7.8
Tyrosine	4.5	1.1	0.4	4.5	1.1	0.4
Threonine	6.8	8.2	11.5	6.8	8.2	11.5
Tryptophan	1.5	1.6	2.2	1.5	1.6	2.2
Valine	7.2	5.8	7.5	7.2	5.8	7.5
Alanine	12.3	21.1	31.2	15.6	22.4	33.2
Aspartic acid	18.4	27.7	40.3	23.2	29.7	43.2
Glutamic acid	28.6	27.5	36.7	36.0	30.6	41.3
Glycine	13.4	24.9	37.4	16.9	26.4	39.5
Proline	8.7	2.7	1.8	11.0	3.7	3.2
Serine	8.1	8.6	11.8	10.3	9.5	13.1
Sum	163.9	190.2	264.8	177.9	196.1	273.5

LP, low-protein diet; MP, medium-protein diet; HP, high-protein diet; LA<sup>-</sup>, arginine-free low-protein diet; MA<sup>-</sup>, arginine-free medium-protein diet; HA<sup>-</sup>, arginine-free high-protein diet.

\* All amino acids were provided by Eurolysine, Amiens, France.

† For details of diets and procedures, see Table 1 and p. 460.

graded levels of N and Arg, supplied by raw materials and a balanced mixture of L-amino acids (Table 2). The AA composition of the diets (Table 3) was based on the AA composition of fishmeal taken as reference for both IAA and dispensable amino acid (DAA) composition of the diets and met the IAA requirements of fish according to National Research Council (1993). Protein-free (PF), low-protein (LP), medium-protein (MP) and high-protein (HP) diets contained 0, 2.4, 4.8 and 8% respectively of

N and were used to determine the protein requirements for maintenance and growth. Three further diets (LA<sup>-</sup>, MA<sup>-</sup>, and HA<sup>-</sup>) were formulated to describe the relationship between Arg intake and N gain. Diet LA<sup>-</sup> corresponded to diet LP but without Arg. Diets MA<sup>-</sup> and HA<sup>-</sup> corresponded to diets MP and HP but with reduced Arg levels.

In order to ensure an adequate feed intake of the semi-purified diets, a fish protein soluble concentrate was included (10 g/kg) in the diets. In the case of turbot, inosine, known to be a potent feeding stimulant (Mackie & Adron, 1978), was also included (1 g/kg diet). The AA mixtures were coated with agar (Mambrini & Kaushik, 1995a) before mixing with the other ingredients. Diets were pelleted, dried for 10 min at 80°C then at room temperature for 10 min in a fluid-bed drier, and sieved to 1.6–2 mm diameter and kept at 4°C. Ingredient and chemical composition of the experimental diets are summarised in Table 1.

### Fish

**Rainbow trout.** Triplicate groups of eighty juvenile rainbow trout originating from an INRA stock, having an initial mean body weight (IBW) of  $6.7 \pm 0.1$  g were reared in 1 m<sup>3</sup> circular glass fibre tanks (water volume: 300 litres; flow rate: 50 l/min) for 4 weeks at a constant water temperature of  $17 \pm 1^\circ\text{C}$ .

**Turbot.** Triplicate groups of sixty juvenile turbot (IBW:  $5.0 \pm 0.1$  g) obtained from a private fish farm (France turbot, Noirmoutier, France) and acclimatised to the experimental conditions were each reared in tanks of 50 litre capacity for 4 weeks in thermoregulated ( $17 \pm 1^\circ\text{C}$ ) seawater (salinity 35 parts per thousand).

**Table 3.** Amino acid composition of the experimental diets, along with data on requirements for salmonids according to National Research Council (1993)\*

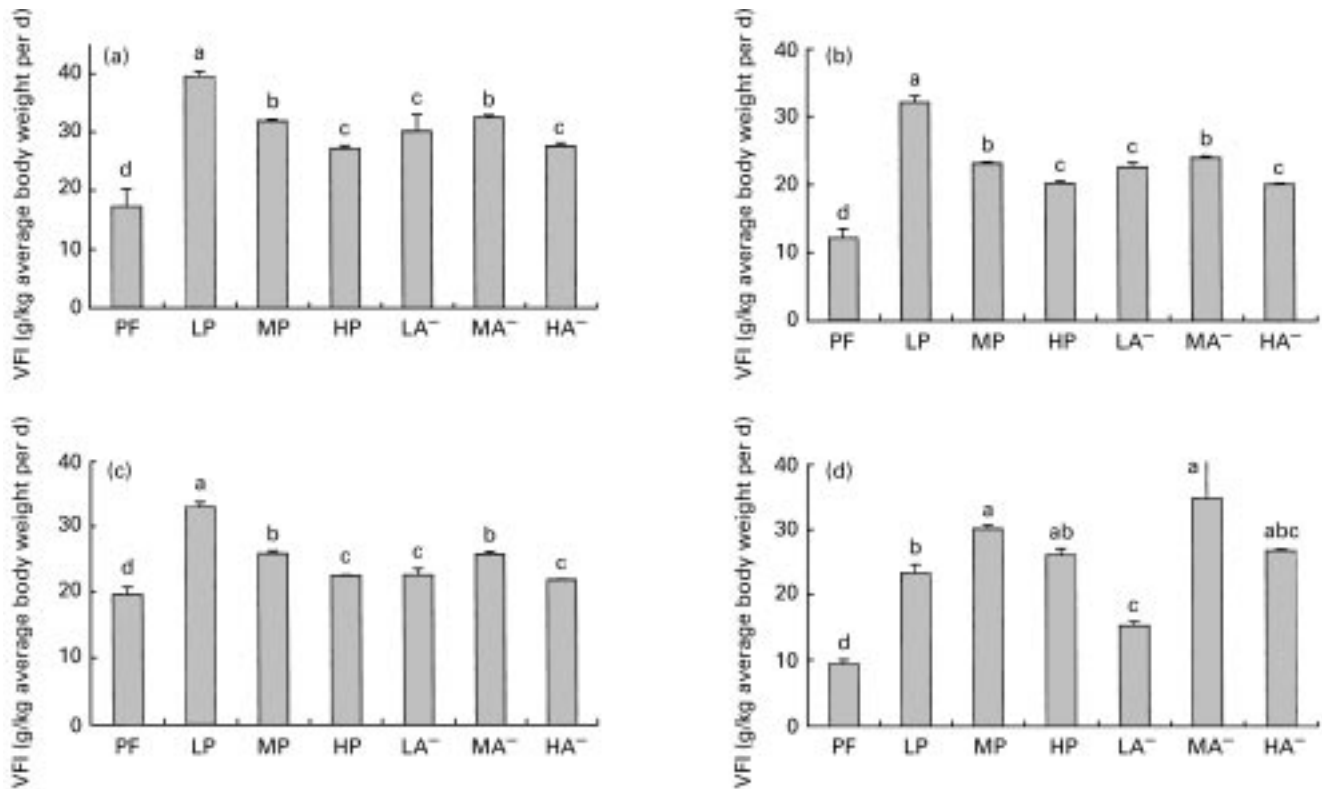
Diets...	(g/kg diet)						Requirement
	LP	MP	HP	LA <sup>-</sup>	MA <sup>-</sup>	HA <sup>-</sup>	
Arginine	11	26	33	1	22	29	15
Histidine	10	11	11	11	11	14	7
Isoleucine	8	14	14	5	9	20	9
Leucine	8	22	24	7	16	21	14
Lysine	13	29	35	13	29	42	18
Methionine	3	6	6	4	6	6	10†
Cystine	3	8	8	3	6	9	
Phenylalanine	15	13	14	4	13	16	18‡
Tyrosine	15	13	14	4	13	16	
Threonine	7	13	19	6	17	17	8
Tryptophan	nd	nd	nd	nd	nd	nd	2
Valine	7	18	17	8	12	33	12
Alanine	12	28	47	16	26	31	
Aspartic acid	19	39	41	16	39	55	
Glutamic acid	30	62	59	27	61	71	
Glycine	10	23	31	6	34	43	
Serine	7	16	25	11	17	21	

LP, low-protein diet; MP, medium-protein diet; HP, high-protein diet; LA<sup>-</sup>, arginine-free low-protein diet; MA<sup>-</sup>, arginine-free medium-protein diet; HA<sup>-</sup>, arginine-free high-protein diet; nd, not determined.

\* For details of diets and procedures, see Table 1 and p. 460.

† Methionine + cystine.

‡ Phenylalanine+tyrosine.



**Fig. 1.** Voluntary feed intake (VFI) in the four species as affected by dietary protein and arginine levels: (a) rainbow trout, 17°C, initial body weight (IBW) 6.8 g; (b) turbot, 17°C, IBW 5.0 g; (c) European seabass, 24°C, IBW 5.0 g; (d) gilthead seabream, 24°C, IBW 7.4 g. Values are mean values of three tanks and standard errors are represented by vertical bars. Mean values with unlike letters were significantly different,  $P < 0.05$ . PF, protein-free diet; LP, low-protein diet; MP, medium-protein diet; HP, high-protein diet; LA<sup>-</sup>, arginine-free low-protein diet; MA<sup>-</sup>, arginine-free medium-protein diet; HA<sup>-</sup>, arginine-free high-protein diet.

*European seabass.* Fish originating from a private fish farm (Panittica pugliese, Torre Canne, Italy) were acclimatised to the experimental facilities. Triplicate groups of sixty juvenile seabass (IBW:  $5.0 \pm 0.1$  g) were each reared in glass fibre tanks of 200 litre capacity for 6 weeks in recirculating, thermoregulated ( $24 \pm 1^\circ\text{C}$ ) seawater (salinity: 35 parts per thousand).

*Gilthead seabream.* Fish were obtained from a private fish farm and acclimatised to the experimental conditions. Duplicate groups of forty juvenile seabream (IBW:  $7.4 \pm 0.2$  g) were each reared in tanks of 250 litre capacity for 6 weeks in a recirculating and thermoregulated ( $24 \pm 1^\circ\text{C}$ ) seawater (salinity: 32 parts per thousand) system.

In all trials, fish were acclimatised to the experimental conditions over at least 2 weeks before the start of the feeding trials. Density of biomass was in tune with optimal growth conditions in the respective experimental culture facilities. Water temperature was set at the thermal preferendum allowing maximum growth rates of each species. Water quality and flow rates were maintained in order to supply sufficient  $\text{O}_2$  (above 8 parts per million) and to avoid build up of ammonia N (below 0.1 parts per million). The tanks were allocated at random to each test diet. The diets were manually distributed two to four times a day to visual satiety (visual observation of first feed refusal to ensure intake of all feed that was supplied). Mortality, if any, was recorded daily. At the end of the study, the fish were kept unfed for a 24 h period and then weighed

in groups. Growth rate was estimated as daily growth coefficient (DGC, %) as follows:  $100 \times ((\text{FBW}^{1/3} - \text{IBW}^{1/3}) / \text{number of days})$  where FBW and IBW represent final and initial body weight respectively. Mean metabolic body weight (MBW) was calculated as follows:  $\text{MBW} = ((\text{IBW}^{0.75}) + (\text{FBW}^{0.75})) / 2$ .

For each species, a pooled sample of ten fish at the beginning and pools of ten fish per tank at the end of the feeding trial were withdrawn for whole body analysis. All the fish were killed by an excess of anaesthetic (ethylene glycol monophenyl ether) or cervical section. Although all fish had empty digestive tracts, they were checked and rinsed to avoid any possible contamination. Samples were kept frozen ( $-20^\circ\text{C}$ ) until analysis.

#### Body composition analysis

Samples from each species were analysed using the same methodologies. Whole fish samples were ground and a sample was analysed for DM, before being freeze-dried and kept for further analyses. For both diets, as well as freeze-dried whole body samples, analyses were performed following usual laboratory procedures (Association of Official Analytical Chemists, 1984): DM ( $110^\circ\text{C}$ , 24 h), ash ( $800^\circ\text{C}$ , 24 h), fats (extraction with dichloroethane) and gross energy by adiabatic calorimetry. Crude protein ( $\text{N} \times 6.25$ ) was determined using either the Dumas elemental analyser or the Kjeldahl method after acid digestion.

Based on whole body composition analysis, daily N gains were calculated for all species. Dietary AA were analysed after hydrolysis (6 M HCl, 110°C, 24 h, *in vacuo*) of diet samples followed by evaporation (SpeedVac Labconco, Kansas City, USA), recovery in Li buffer and separation and post-column derivatisation with ninhydrine in an HPLC system (Waters 2690). Calculations were made using Millennium software version 3.0, St Quentin en Yvelines, France with norleucine as an internal standard.

### Data analysis

All data were analysed by one-way ANOVA followed by the Newman–Keuls test, using the STAT-ITCF (1991) computing package. Differences between means were considered significant at  $P < 0.05$ . For determination of maintenance requirements for N and Arg, two methods were used. Based on the method described by Fuller *et al.* (1989), the requirement for maintenance and growth were calculated using the coefficients  $a$  and  $b$  after a linear regression analysis ( $y = a + bx$ ) of data relating N or Arg intake to N gain: maintenance as  $-a/b$  and N requirement for unit N gain as  $1/b$ , where  $x = N$  or Arg intake/kg MBW per d and  $y = N$  gain/kg MBW per d. The relationship between Arg intake and N gain was also analysed using

a four-parameter model described by Mercer (1982):

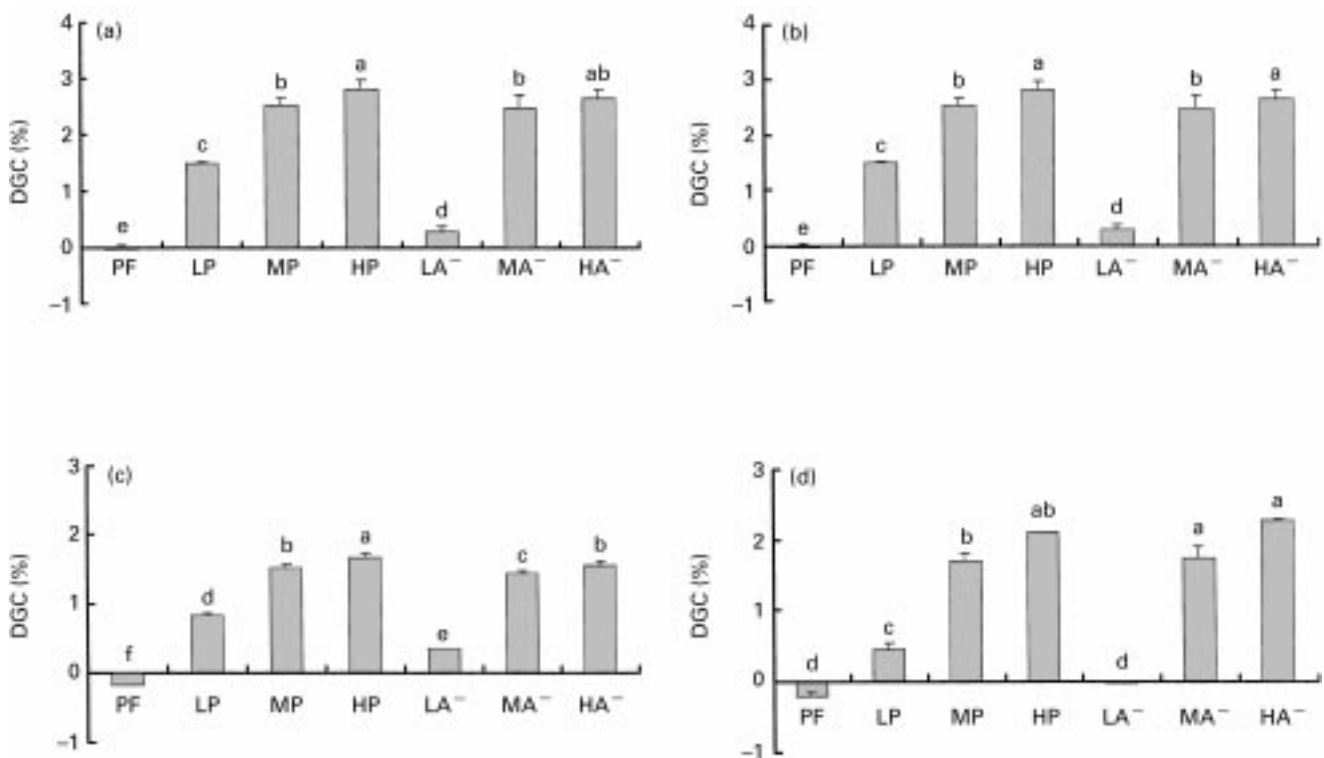
$$Y = \frac{b(K_{0.5})^n + R_{\max}(X)^n}{(K_{0.5})^n + (X)^n}$$

where  $Y$  is N gain (mg/kg MBW per d),  $X$  is Arg intake (mg/kg MBW per d),  $b$  is the intercept on the  $Y$  axis,  $R_{\max}$  is the maximal response,  $K_{0.5}$  is the value of  $X$  for  $\frac{1}{2}(R_{\max} + b)$  and  $n$  the apparent kinetic order. Graphical presentations and best-fits were made using a commercial PC software (GraphPad Prism Inc, San Diego, CA, USA).

## Results

### Body-weight gain and feed efficiency

**Feed intake.** In all the four species, mortality was very low ( $< 1\%$ ) and unaffected by dietary treatments and no external pathological signs were observed even in fish fed the PF or  $LA^-$  diets. During the feeding trial, all diets were well accepted by the fish fed even diet PF and diet  $LA^-$  (Fig. 1), except in turbot where, after 3 weeks, feed intake was reduced in fish fed diets  $LA^-$  and PF. Voluntary feed intake (VFI, expressed as g/kg average body weight per d) was high in fish fed the LP diet and decreased significantly ( $P < 0.05$ ) with increasing dietary crude protein levels in rainbow trout, turbot and European seabass whereas in the gilthead seabream, VFI was not sig-



**Fig. 2.** Daily growth coefficient (DGC) in the four species as affected by dietary protein and arginine levels: (a) rainbow trout, 17°C, initial body weight (IBW) 6.8 g; (b) turbot, 17°C, IBW 5.0 g; (c) European seabass, 24°C, IBW 5.0 g; (d) gilthead seabream, 24°C, IBW 7.4 g. Values are mean values of three tanks and standard errors are represented by vertical bars. Mean values with unlike letters were significantly different,  $P < 0.05$ . PF, protein-free diet; LP, low-protein diet; MP, medium-protein diet; HP, high-protein diet;  $LA^-$ , arginine-free low-protein diet;  $MA^-$ , arginine-free medium-protein diet;  $HA^-$ , arginine-free high-protein diet. DGC was calculated as:  $100 \times ((FBW^{1/3} - IBW^{1/3})/\text{number of d})$  where FBW and IBW represent final body weight and initial body weight respectively.

**Table 4.** Feed efficiency (FE; wet weight gain/dry feed intake) and protein efficiency ratio (PER; wet weight gain/protein intake) for the four species fed experimental diets\*  
(Mean values and standard errors of the mean)

Diets	FE						PER									
	Rainbow trout (n 3)		Turbot (n 3)		Gilthead seabream (n 2)		European seabass (n 3)		Rainbow trout (n 3)		Turbot (n 3)		Gilthead seabream (n 2)		European seabass (n 3)	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
LP	0.50 <sup>d</sup>	0.00	0.35 <sup>c</sup>	0.02	0.28 <sup>c</sup>	0.03	0.34 <sup>c</sup>	0.02	3.14 <sup>a</sup>	0.02	2.16 <sup>c</sup>	0.14	1.78 <sup>a</sup>	0.18	2.37 <sup>a</sup>	0.10
MP	0.95 <sup>c</sup>	0.02	0.93 <sup>b</sup>	0.01	0.73 <sup>b</sup>	0.02	0.72 <sup>b</sup>	0.02	2.74 <sup>b</sup>	0.05	2.60 <sup>ab</sup>	0.04	2.14 <sup>a</sup>	0.07	2.31 <sup>a</sup>	0.06
HP	1.20 <sup>a</sup>	0.03	1.17 <sup>a</sup>	0.00	0.97 <sup>a</sup>	0.02	0.93 <sup>a</sup>	0.01	2.33 <sup>c</sup>	0.06	2.31 <sup>bc</sup>	0.01	1.94 <sup>a</sup>	0.04	1.95 <sup>ab</sup>	0.02
LA <sup>-</sup>	0.14 <sup>e</sup>	0.01	0.17 <sup>d</sup>	0.02	-0.00 <sup>d</sup>	0.00	0.21 <sup>d</sup>	0.01	0.92 <sup>d</sup>	0.07	1.08 <sup>d</sup>	0.11	-0.02 <sup>b</sup>	0.02	1.53 <sup>b</sup>	0.07
MA <sup>-</sup>	0.92 <sup>c</sup>	0.03	0.91 <sup>b</sup>	0.01	0.66 <sup>b</sup>	0.13	0.72 <sup>b</sup>	0.03	2.73 <sup>b</sup>	0.08	2.66 <sup>a</sup>	0.02	1.97 <sup>a</sup>	0.39	2.34 <sup>a</sup>	0.08
HA <sup>-</sup>	1.13 <sup>b</sup>	0.03	1.20 <sup>a</sup>	0.03	1.01 <sup>a</sup>	0.01	0.92 <sup>a</sup>	0.03	2.24 <sup>c</sup>	0.06	2.38 <sup>abc</sup>	0.05	2.00 <sup>a</sup>	0.02	1.91 <sup>ab</sup>	0.05

LP, low-protein diet; MP, medium-protein diet; HP, high-protein diet; LA<sup>-</sup>, arginine-free low-protein diet; MA<sup>-</sup>, arginine-free medium-protein diet; HA<sup>-</sup>, arginine-free high-protein diet.

<sup>a,b,c,d,e</sup>Mean values within a column with unlike superscript letters were significantly different ( $P < 0.05$ ).

\* For details of diets and procedures, see Table 1 and p. 460.

nificantly ( $P > 0.05$ ) affected by dietary N level. In all the species, comparison of data between those fed MP or HP diets, and those fed diets with reduced Arg supply (MA<sup>-</sup> and HA<sup>-</sup>) showed that a partial reduction in dietary Arg levels did not have any significant effect ( $P > 0.05$ ) on VFI whereas the total suppression of Arg (LA<sup>-</sup> v. LP) led to a significant ( $P < 0.05$ ) decrease of VFI.

**Weight gain.** Although fish fed diet PF showed a normal feeding behaviour during the trials, as expected, they showed significant body-weight loss. In all the four species, DGC were significantly ( $P < 0.05$ ) improved with increasing dietary protein and Arg levels (Fig. 2). At the low-protein level, the total absence of Arg (diet LA<sup>-</sup>) induced a reduction in or a total lack of body-weight gain. But at higher protein levels, a partial decrease in dietary Arg level did not lead to any significant reduction in weight gain except in seabass (Fig. 2).

**Feed efficiency.** In all species, feed efficiency (FE: wet weight gain/dry feed intake) increased significantly ( $P < 0.05$ ) with an increase in the dietary N and Arg level (Table 4). Suppression of Arg led to a significant reduction of FE in all species ( $P < 0.05$ ). A decrease in dietary Arg (HA<sup>-</sup> v. HP and MA<sup>-</sup> v. MP) also had no effect on FE except in trout where there was a significant reduction in FE in fish fed HP compared with fish fed HA<sup>-</sup>.

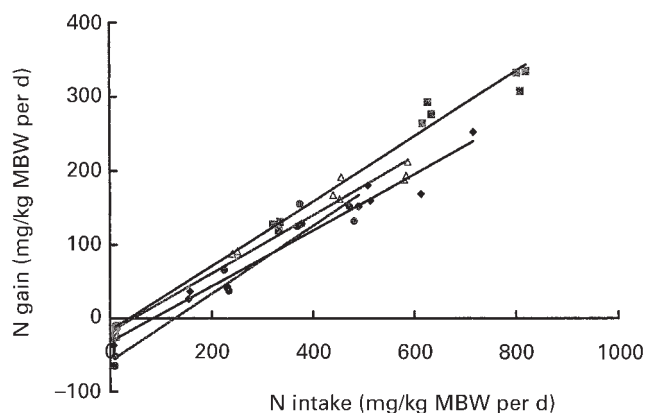
Protein efficiency ratio (PER; wet weight gain/protein intake) was the highest in rainbow trout fed the LP diet (2.4% N, 1% Arg) and was significantly affected by increasing dietary N level ( $P < 0.05$ ). In turbot, gilthead seabream and European seabass, protein level did not significantly ( $P > 0.05$ ) affect the PER value. In all species, PER decreased significantly with the suppression of Arg with a very strong effect for gilthead seabream ( $P < 0.05$ ). But at medium and high dietary N levels, a decrease in dietary Arg (MA<sup>-</sup> and HA<sup>-</sup>) had no significant effect on PER irrespective of the species ( $P > 0.05$ ).

**Protein requirement.** The relationship between N intake and N gain for the four species fed PF, LP, MP and HP diets was analysed using both models. Given that the best fits were obtained with the linear model, the relationship was retained and illustrated in Fig. 3.

The linear relationship between daily N intake and daily N gain showed a very high degree of correlation for each of the species studied. Based on the linear regression model parameters, N requirements for maintenance and for growth were calculated for the four species (Table 5).

N requirement for maintenance was in the same range for rainbow trout and seabass (38 and 45 mg/kg MBW per d respectively) whereas it was two-fold higher in seabream (85 mg/kg MBW per d) and three-fold higher in turbot (127 mg/kg MBW per d). N requirement for unit N gain was lower for turbot and rainbow trout (2.16 and 2.28 g/g N gain respectively) than for European seabass and gilthead seabream (2.53 and 2.64 g/g N gain respectively).

**Arginine requirement.** Fig. 4 illustrates the relationship between Arg intake and N gain established by fitting the data obtained for the four species fed all diets except the PF diet. Both the linear model ( $y = ax + b$ ) and the non-linear model (Mercer, 1982) for each species are depicted in Fig. 4.



**Fig. 3.** Relationship between nitrogen intake and nitrogen gain in the four species. (■), rainbow trout:  $y = -16.581 + 0.4388x$ ,  $R^2$  0.9874; (●), turbot:  $y = -58.84 + 0.4622x$ ,  $R^2$  0.9508; (Δ) seabass:  $y = -17.866 + 0.3958x$ ,  $R^2$  0.9723; (◆) seabream:  $y = -32.101 + 0.3791x$ ,  $R^2$  0.9798. Each point represents the mean nitrogen gain of the fish per tank. MBW, metabolic body weight  $((IBW^{0.75} + FBW^{0.75})/2)$  where FBW and IBW represent final body weight and initial body weight respectively.

Based on the linear relationship between daily Arg intake and daily N gain, Arg requirement for maintenance and for unit N gain were calculated according to Fuller *et al.* (1989) and the data are summarised in Table 6.

Data indicate either no (rainbow trout, turbot and European seabass) or very low (gilthead seabream) Arg requirement for maintenance. Arg requirement per unit protein accretion was very similar between the three marine species (166–178 mg) and slightly lower for the freshwater-grown rainbow trout (138 mg).

## Discussion

Following the general method as outlined by Fuller *et al.* (1989), the diets were formulated to contain two levels of Arg at each total N level. The AA profiles in the LP and LA<sup>-</sup> diets were chosen in order to simulate more or less the fish whole body AA composition, including those of DAA. Minor differences in the DAA profiles of diets are due to the fact that the partial or total suppression of Arg in the diets (LA<sup>-</sup>, MA<sup>-</sup> and HA<sup>-</sup>) was compensated by a supply of DAA on an equivalent N basis of diets LP, MP and HP.

The good feed acceptance and excellent growth performance of the four species indicate that the semi-purified diets

used in the present study were appropriate to determine nutrient requirements. Good performances with diets containing crystalline AA properly coated with agar have already been shown in rainbow trout (Cho *et al.* 1992; Rodehutsord *et al.* 1994) and in seabass (Tibaldi *et al.* 1994). An adjustment of the pH of diets containing high levels of crystalline AA has been found necessary for stomachless fish such as the common carp, whereas in all the four species studied here, preliminary studies had shown that this was not necessary.

The inverse relationship between VFI and dietary N level suggests that fish were able to partially compensate for low dietary protein supply by increasing their voluntary feed intake. Besides, rainbow trout fed the low-protein and low-Arg diet (LP) showed a two-fold increase in body weight with a very good PER value (3.14). These results indicate that this species has a low N and Arg requirement for maintenance and for growth. Low growth in all fish fed the diet devoid of Arg (LA<sup>-</sup>) indicate a clear Arg deficiency after the 4 or 6 weeks of the growth trial. The lack of growth in seabream suggests a higher Arg requirement for maintenance in this species compared with the other three species.

The reliability of the linear model as proposed by Fuller *et al.* (1989) for estimating maintenance requirements is based on high growth and positive N balance and necessarily involves short duration of the trials. Each species of fish had maximum growth rates very much similar to those obtained with practical diets based on fishmeal and the DGC were comparable to those reported earlier (Kaushik, 1998). In an earlier study on Arg requirement for growth of rainbow trout, Cho *et al.* (1992) have already used the four-parameter nutrient dose-response analysis to estimate the requirements. A comparison of regressions according to the four-parameter nutrient kinetics model of Mercer (1982) and the simple linear regression model as proposed by Fuller *et al.* (1989) was also made for describing the relationship between N intake and N gain as well as for the relationship between Arg intake and N gain (Fig. 4). As regards N intake and N gain, the responses were better expressed and the calculations for both maintenance and per unit N gain were better determined using the linear regression model (best-fit model,  $P < 0.05$ ). Besides, given that only four dietary groups (PF, LP, MP and HP) were used in analyses, the linear regression model was more suitable than the four-parameter model.

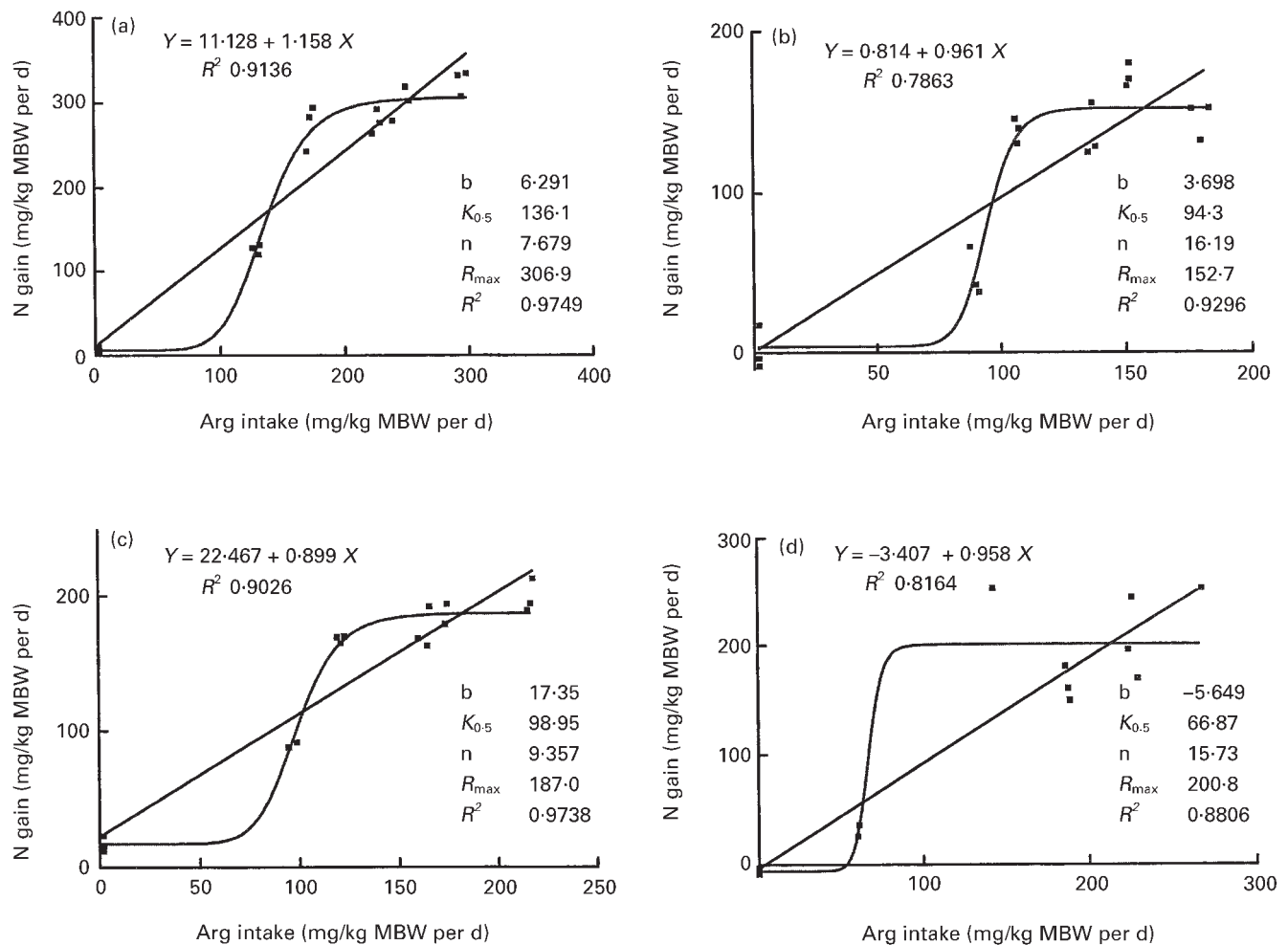
In an earlier study on Arg requirement for growth of

**Table 5.** Nitrogen requirements for nitrogen balance and protein accretion in the four species calculated based on the method described by Fuller *et al.* (1989)\*

N requirement for...	N balance (mg N/kg MBW per d)	Unit gain (g N/g N gain)	Growth needs	
			g N/g protein gain	As times of maintenance
Rainbow trout	37.79	2.28	0.365	60.31
Turbot	127.30	2.16	0.346	16.99
Gilthead seabream	84.68	2.64	0.422	31.15
European seabass	45.14	2.53	0.404	55.97

MBW, metabolic body weight  $((IBW^{0.75} + FBW^{0.75})/2)$ ; IBW, initial body weight (kg); FBW, final body weight (kg).

\* For details of procedures, see p. 461, and for details of the calculation, see Fig. 3 and p. 462.



**Fig. 4.** Relationship between arginine (Arg) intake and nitrogen gain in the four species: (a) rainbow trout; (b) turbot; (c) seabass; (d) sea-bream. Both linear and the multivariate curve fittings (Mercer, 1982) for each set of data are provided along with the values for each parameter with the corresponding equations and the goodness ( $R^2$ ) for each model are provided. Each point represents the mean nitrogen gain of the fish per tank. MBW, metabolic body weight  $((IBW^{0.75} + FBW^{0.75})/2)$  where FBW and IBW represent final body weight and initial body weight respectively.

rainbow trout, Cho *et al.* (1992) have already used the four-parameter nutrient dose–response analysis to estimate the requirements. The relationship between Arg intake and N gain is in most occasions best described by the non-linear model, as was already shown by Mambriani & Kaushik (1995b) when estimating the SAA requirements of rainbow trout. Indeed, the strict application of the linear model as proposed by Fuller *et al.* (1989) for

estimating the IAA requirements for maintenance in pigs would have been more reliable had we used diets ( $MA^-$  and  $HA^-$ ) with a greater degree of reduction in the IAA levels of the MP and HP diets. Irrespective of the model chosen, given that in trout, turbot and seabass, the intercept on the y axis was positive, there is a clear indication of either nil or very low Arg requirements for maintenance in these species.

**Table 6.** Arginine (Arg) requirements for maintenance and protein growth in the four species calculated based on the method described by Fuller *et al.* (1989)\*

Arg requirement for...	N balance (mg Arg/kg MBW per d)	Unit gain	
		mg Arg/g N gain	mg Arg/g protein gain
Rainbow trout	0	863	138
Turbot	0	1041	167
Gilthead seabream	4.39	1040	166
European seabass	0	1112	178

MBW, metabolic body weight  $((IBW^{0.75} + FBW^{0.75})/2)$ ; IBW, initial body weight (kg); FBW, final body weight (kg).

\* For details of procedures, see p. 461, and for details of the calculation, see Fig. 4 and p. 462.



Turbot require more N for maintenance (127 mg N/kg MBW per d) than the other fish species. This value is even superior to that pointed out for mammals (100 mg N/kg MBW per d; Reeds, 1988). Such high maintenance needs might explain the overall reputedly higher protein requirement of turbot. Available data suggest that dietary protein needs of flatfish such as turbot, sole (*Solea vulgaris*) and plaice (*Pleuronectes platessa*) are in the range of 55–65% diet (Cowey *et al.* 1972; Cadena-Roa, 1983; Caceres-Martinez *et al.* 1984). N requirement for maintenance of juvenile rainbow trout grown at 17°C (38 mg/kg MBW per d) is comparable with data of Mambrini & Kaushik (1995b): 52 mg N/kg MBW per d and 26 mg N/kg MBW per d in fish reared at 16°C or 8°C respectively. On the contrary, data on maintenance needs of gilthead seabream in the present study (85 mg N/kg MBW per d) is lower than the 137.6 mg N/kg BW<sup>0.70</sup> per d estimated by Lupatsch *et al.* (1998). For turbot, recalculation of N requirement for maintenance expressed as per average body weight per d was 470 mg/kg average body weight/d ( $y = -226.73 + 0.4823x$ ), a value considerably higher than that of 250 mg N/kg per d, reported for plaice, another flatfish, by Cowey *et al.* (1972) and of other data on endogenous N excretion levels measured by Birkett (1969) and Jobling (1981). Further investigations need to be undertaken to explain the high N need for maintenance in turbot, especially since the basal energy requirements do not appear to be different between turbot and other pelagic fish (Kaushik, 1998). N requirement for unit N gain of rainbow trout and turbot are similar. The slope of the curve relating N intake and N gain was 0.46 in turbot and 0.44 in rainbow trout. This observation confirms the higher N retention efficiency in turbot compared with other farmed fish as already suggested by Caceres-Martinez *et al.* (1984), Dreanno (1994) and Dosdat *et al.* (1995).

Although N needs for maintenance of rainbow trout and seabass were similar, the higher N requirement for unit protein accretion of seabass (0.404 v. 0.365 g/g protein gain) could explain its higher dietary protein needs (Hidalgo & Alliot, 1988; Dias *et al.* 1998; Peres & Oliva-Teles, 1999). Data obtained here on the protein requirements of gilthead seabream are in accordance with those reported by Santinha *et al.* (1996).

The four species studied appear to have no or very low dietary Arg requirements for maintenance. Such observations have been made in ureotelic mammals such as man, pig and rat while in the uricotelic fowl, an amount of 81 mg Arg/kg MBW per d was needed to maintain N equilibrium (Fuller *et al.* 1989). This remains an extremely intriguing observation for ammoniotelic species. Whether this would apply to all stages of the life cycle needs to be verified. In the juvenile stages studied here, whole body protein breakdown or Arg biosynthesis seems to provide enough Arg to meet maintenance requirement. Based on his data, Rodehutsord *et al.* (1995) suggested already that rainbow trout should be able to synthesise Arg when fed Arg-deficient diets. Studies underway to analyse the ornithine-urea cycle (OUC) enzyme activities should throw more light on this hypothesis of endogenous Arg synthesis. Huggins

*et al.* (1969) detected all enzymes of the OUC in the liver of a number of teleosts. Dépêche *et al.* (1979) showed urea production by the OUC in juvenile trout but not in bigger fish. Cowey & Walton (1989) detected only low activities of the OUC enzymes. Chiu *et al.* (1986) showed that Arg synthesis was functional in juvenile rainbow trout only under conditions of starvation. Servais (1994) detected ornithine carbamoyltransferase activity in the liver of rainbow trout, turbot, European seabass and gilthead seabream.

In mammals, Arg synthesis from glutamic acid via the intestinal–renal axis is known to occur (Wu & Morris, 1998). In channel catfish (*Ictalurus punctatus*), dietary glutamic acid leads to increased plasma citrulline and to a lesser extent that of Arg, with possible reduction of dietary Arg requirement (Buentello & Gatlin, 2000). In a subsequent study, the same authors (Buentello & Gatlin, 2001) showed that there is endogenous synthesis of citrulline and suggested that this may serve as a possible *de novo* source of Arg. Whether similar Arg synthesis occurs in the four species studied here requires confirmation. In formulating the diets, care was taken not to modify the level of a single DAA such as glutamic acid to a great extent. Assessment of the activities of the OUC enzymes in the intestinal–renal axis in the species under study here might throw further light.

The comparison of Arg requirement for unit protein gain between the four species indicates a variation of about 20% between the four species studied. It is worth verifying this tendency with other IAA. As already mentioned by Mambrini & Kaushik (1995a) and Cowey (1995), much variability has been recorded as regards Arg requirements of teleosts (4–6 g Arg/16 g N). Data for rainbow trout (Cho *et al.* 1992; Rodehutsord *et al.* 1995) and for European seabass (Tibaldi *et al.* 1994) suggest values of around 3.8–4 g/16 g N. Luquet & Sabaut (1974) suggested that the Arg requirement for gilthead seabream would be below 4 g/16 g N. Currently, no quantitative data on Arg needs of turbot are available. But, based on data obtained here, a dietary level of 5 g/16 g N would appear to be a reasonable estimate for turbot. Turbot fed diet HP showed significantly ( $P < 0.05$ ) lower N gain compared with fish fed HA (150 (SE 7) v. 170 (SE 5) mg N/kg MBW per d respectively; see Fig. 4). Arg is known to play a major role in mediating responses in the somatotrophic axis (Swanson, 1990) and is also considered to be a potent growth promoter even in fish (Mommensen, 2001). But, a dietary excess of Arg does not seem to affect neither plasma insulin nor growth hormone levels in the Atlantic salmon (*Salmo salar*) (Lall *et al.* 1994). Considering the fact that high dietary Arg levels do not impair growth in the three other species, the adverse effect of excess Arg on growth of turbot requires further analysis.

Greater understanding of the metabolic pathway of Arg biosynthesis is also warranted to explain the apparent lack of Arg requirement for maintenance in at least three out of four species studied. Data obtained here correspond to juveniles under fast growing conditions. Further insight is needed on whether bigger fish or those subjected to a long-term Arg deficiency would react in a similar manner.

### Acknowledgements

This work was funded by the European Union under the FAIR programme (CT 98–3408). The authors gratefully acknowledge the technical assistance of H. Le Delliou and E. Rancati for biochemical analysis, and E. Desbruyères, C. Huelvan and J. Moriceau for diet preparation. V. Fournier acknowledges the receipt of a joint scholarship from INRA and Conseil Régional d'Aquitaine.

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