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The optimum dietary indispensable amino acid pattern for growing Atlantic salmon (*Salmo salar* L.) fry

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To determine the optimum indispensable (I) amino acid (AA) balance in Atlantic salmon (*Salmo salar* L.) fry, a single protocol established for the pig was adapted. The balance was calculated from the reduction in N gain after replacing about 45 % of a single IAA by a mixture of dispensable AA in isonitrogenous diets. We confirmed that the mixture of AA simulating the AA pattern of cod-meal protein and gelatine (46:3, w/w) was used with the same efficiency as cod-meal protein and gelatine. From the deletion experiment an optimum balance between the IAA was derived. Expressed relative to lysine = 100, the optimal balance was: arginine 76 (SE 0·2), histidine 28 (SE 2·2), methionine + cystine 64 (SE 1·7), phenylalanine + tyrosine 105 (SE 1·6), threonine 51 (SE 2·4), tryptophan 14 (SE 0·7), valine 59 (SE 1·7). No estimates were made for isoleucine and leucine. Expressed as g/16 g N, the optimal balance was: arginine 4·0 (SE 0·0), histidine 1·5 (SE 0·1), lysine 5·3 (SE 0·2), methionine + cystine 3·4 (SE 0·1), phenylaline + tyrosine 5·6 (SE 0·1), threonine 2·7 (SE 0·1), tryptophan 0·7 (SE 0·0), valine 3·1 (SE 0·1). This AA composition is close to that of the Atlantic salmon whole-body, but using it as an estimation of the IAA requirements may lead to an overestimation of the branched-chain AA requirements and an underestimation of aromatic and S-containing AA requirements. The results are discussed in accordance with the key assumptions associated with the model used (broken-line model, IAA efficiencies and maintenance requirements).

Amino acids: Ideal protein: Protein quality: Atlantic salmon

In fish as in other animals, the quality of dietary protein is determined by its amino acid (AA) composition and by their digestibility and availability. Quality can be considered as the degree to which the composition of the absorbed AA mixture accords with the balance required by the animal (Wang & Fuller, 1989). Although fish have higher protein needs than other livestock vertebrates (because they use AA more extensively for energy), the same ten AA are indispensable (I) for fish and higher vertebrates. However, there are more than 20 000 species of fish, and the profiles of IAA requirements have only been established for five species (Mambrini & Guillaume, 2002).

The evaluation of the IAA requirements of an unknown species, as well as an assessment of the quality of any dietary protein, is based on the AA pattern of the 'ideal reference' protein. In fish, different reference proteins have been proposed: hen whole-egg protein (Halver, 1957; Harding *et al.* 1977; Wilson *et al.* 1978; Robinson *et al.* 1981; Kim *et al.* 1983), fish ovarian tissue (Ketola, 1982), fish egg (Ketola, 1983), fish muscle (Cowey & Luquet, 1983) or whole-fish carcass (Rumsey & Ketola, 1975; Arai, 1981; Ogata *et al.* 1983). The whole-fish carcass protein best mimics the requirements, but is not completely satisfactory (Wilson & Cowey, 1985; Mambrini & Kaushik, 1995*a*; Wilson, 2002).

The requirements have usually been assessed by dose–response experiments (Wilson, 2003), a method that is time-consuming and expensive to carry out. It also has inherent methodological problems (Cowey & Luquet, 1983; Cowey & Tacon, 1983; Cowey, 1988, 1994; Wilson, 1989; Dabrowski & Guderley, 2002), which are responsible for large variations observed in the IAA requirement values (Tacon & Cowey, 1985; Cowey, 1994; Kaushik, 1995; Fournier *et al.* 2002). These variations could be attributed to factors such as differences in basal diet composition, size and age of fish, genetic strain, feeding rate and culture conditions, which may affect the overall growth and requirements value. The authors of these papers, however, do not agree on the

Abbreviations: AA, amino acid; I, indispensable.

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choice of response criterion or the modelling of the dose–response curves, and thus on the method to estimate requirements (Kim *et al.* 1992; Cowey, 1994; Mambrini & Kaushik, 1995*a*; Mambrini & Guillaume, 2002).

Other techniques have been employed to estimate the quantitative IAA requirements of fish more easily. They are based on the analysis of IAA in the body. The requirements have been estimated from the daily increases in the specific IAA in the body protein of fed fish (Ogino, 1980; Jauncey *et al.* 1983; Spiridakis, 1989; Kaushik *et al.* 1991; Ng & Hung, 1995) or from whole-body A:E ratios ((IAA content/total IAA content including cysteine and tyrosine) × 1000) when only one IAA requirement was known (Moon & Gatlin, 1991; Wilson, 1994; Brown, 1995). This last procedure is similar to the one based on the ideal protein concept (Wilson, 2003). The reliability of such estimations is hard to assess in the absence of any experimental measurement of the ideal protein composition. For this measurement, a method has been established for piglets (Fuller *et al.* 1989; Wang & Fuller, 1989). The method is based on the concept that the reduction of a non-limiting AA has no effect on N gain. Conversely, when a single AA is limiting in the diet, the rate of body protein accretion is directly related to that one AA. The changes in N gain measured on removal of a proportion of each IAA in turn were used to calculate a dietary AA pattern in which all IAA were equally limiting. To our knowledge, this approach has never been applied to fish, while none of the particularities of fish protein metabolism appears to invalidate the application of those concepts to fish (Cowey, 1994). If the validity of this approach were confirmed, then we would benefit from a rapid and accurate technique to assess the IAA requirements of different species of fish, and to analyse their variations in growth rates.

Atlantic salmon (*Salmo salar* L.) is the most highly cultured species in Europe. Surprisingly however, the requirements for only three IAA have been published for this species (Anderson *et al.* 1993; Lall *et al.* 1994; Rollin *et al.* 1994; Berge *et al.* 1997, 1998). These results have been determined mostly for post-smolts in seawater, but there is no evidence that they are applicable to fry nutrition in freshwater. Indeed, growth rate is usually much higher in the latter than in the former (Austreng *et al.* 1987).

In the present paper, we adapted the technique developed by Wang & Fuller (1989) for fish, with the ultimate objective of determining the ideal protein composition for Atlantic salmon fry grown in freshwater.

Materials and methods

One N balance trial was performed on Atlantic salmon fry using thirteen experimental diets.

Experimental diets

Except for the protein-free diet (diet 13), the diets were formulated to contain 235 g lipid and 72 g N/kg, supplied by cod meal, gelatine and a mixture of crystalline L-AA (Tables 1 and 2). The proximate and AA compositions of the diets are shown in Tables 1 and 3 respectively. Digestible energy was calculated from protein, fat and carbohydrate concentrations, representing 43, 39 and 18 % of the total digestible energy of 20·2 MJ/kg DM respectively (Berge *et al.* 1998). The dietary N level was below the optimum requirement for growth of Atlantic salmon fry (88 g/kg; Grisdale-Helland & Helland, 1997) to ensure maximum utilisation of the limiting AA.

Two control diets were formulated (Table 1). In the first control diet (diet 1, HC1), almost all the AA came from a high-quality fish-protein source, cod meal (C-0271; Toro Food Division, Rieber & Søn a/s, Bergen, Norway). The AA composition of this diet was very close to the whole-body AA pattern of salmon fry (r^2 0·964). The second control diet (diet 12, HC2) was obtained by replacing 50 % of the cod meal-N by a mixture of crystalline AA (Ajinomoto Ltd, Tokyo, Japan), balanced to mimic the cod-protein AA profile. The chosen levels of IAA in the HC2 diet were (g/kg DM): arginine 28·0, histidine 9·0, isoleucine 21·5, leucine 32·0, lysine 36·6, methionine + cystine 18·4, phenylalanine + tyrosine 31·3, threonine 16·8, tryptophan 4·6, valine 22·4. The HC2 control diet was the basis for the experimental diets formulated to estimate the IAA profile of the requirements, as described by Wang & Fuller (1989). In each of the ten experimental diets, the amount of a specific IAA was reduced by about 45 % and the total dietary N content was maintained by adding a mixture of dispensable AA (g/kg mixture): L-proline 12·6, L-alanine 157·4, glycine 54·1, L-glutamate 275·6, L-serine 212·7, L-aspartate 287·7 (Kim *et al.* 1991). The crystalline AA mixture was coated with 1 % agar, as described by Mambrini & Kaushik (1994), to delay its digestive absorption and optimise its use for protein accretion. The pH of the diets was not controlled, because adjusting the pH of the diets containing high levels of crystalline AA does not seem necessary in fish species, such as salmonids, that have a stomach (Fournier *et al.* 2002).

The dry diet components were ground, mixed and homogenised by a Kenwood electric mixer (Kenwood Ltd, Havant, Hants., UK) before and after oil addition, then after water addition, and finally extruded in an electric meat grinder (model HI 22; Simplex, Paris, France). The experimental diets were then freeze-dried and stored at −20°C until feeding or analysis.

Animals

The Atlantic salmon fry used in this experiment came from a batch of 10 000 eyed (embryonic) eggs from a commercial US fast-growing stock (Troutlodge, Inc., Spring Garden, WA, USA) of domestic origin. Fry were reared in our laboratory hatchery (M. Huet Fish Culture Laboratory, Université catholique de Louvain) from eggs to the beginning of the experiment, according to Rollin *et al.* (2003). The experiment was conducted in two consecutive phases: a pre-experimental phase necessary for fish to adapt to their experimental diets and the experiment itself.

The pre-experimental phase consisted of a 1-month period of adjustment for the fry to diet 12 (HC2) rich in crystalline AA (423 g/kg total N in the diet). During this period, the fry were kept in a tank and were continuously

Table 1. Composition of the experimental diets used for the determination of the optimum balance between indispensable amino acids in Atlantic salmon (*Salmo salar* L.) fry*

No. ...	(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)	(11)	(12)	(13)
Diets ...	HC1	ARG	HIS	ILE	LEU	LYS	MET + CYS	PHE + TYR	THR	TRP	VAL	HC2	PF
Components (g/kg dry diet)													
Cod meal†	460	230	230	230	230	230	230	230	230	230	230	230	0
L-Amino acid mixture‡	0·0	256·0	241·2	235·5	234·7	242·2	235	231·8	236·3	236·9	236·5	236·5	0
Glucose§	34·5	0·4	15·2	20·9	21·7	14·2	21·4	24·6	20·1	19·5	19·9	19·9	150
Cod-liver oil‖	182·5	190·6	190·6	190·6	190·6	190·6	190·6	190·6	190·6	190·6	190·6	190·6	198·6
Gelatine¶	30	30	30	30	30	30	30	30	30	30	30	30	0
Modified starch**	108·0	108·0	108·0	108·0	108·0	108·0	108·0	108·0	108·0	108·0	108·0	108·0	436·4
Sucrose‡‡	20	20	20	20	20	20	20	20	20	20	20	20	50
Soyabean lecithin††	40	40	40	40	40	40	40	40	40	40	40	40	40
Vitamin mix‡‡	10	10	10	10	10	10	10	10	10	10	10	10	10
Mineral mix§§	65	65	65	65	65	65	65	65	65	65	65	65	65
Agar¶	10	10	10	10	10	10	10	10	10	10	10	10	10
Carboxymethylcellulose¶	20	20	20	20	20	20	20	20	20	20	20	20	20
α-Cellulose¶	20	20	20	20	20	20	20	20	20	20	20	20	20
Chemical composition													
DM (g/kg diet)	952·3	947·6	950·2	951·0	943·4	953·2	947·8	949·8	952·5	949·9	956·7	945·9	939·4
N (g/kg DM)	72·3	72·5	71·7	73·4	72·7	71·2	71·1	70·4	72·5	73·5	73·4	73·7	2·2
Fat (g/kg DM)	234·5	232·4	231·7	235·6	237·9	240·1	236·7	231·1	238·9	234·4	234·4	233·7	235·0
Ash (g/kg DM)	88·9	75·6	74·5	75·4	76·5	73·4	75·4	76·2	74·7	74·8	75·8	74·4	52·0
Energy (kJ/g DM)	22·76	22·17	22·43	22·52	22·54	22·41	22·53	22·59	22·51	22·50	22·51	22·51	20·29

* Diet (1), HC1, high-control, cod-protein and gelatine-protein only; diet (12), HC2, high-control in which half of the cod-protein present in HC1 was replaced by a mixture of amino acids of similar composition; diet (13), PF, protein-free diet; diet (2), ARG, low-arginine diet; diet (3), HIS, low-histidine diet; diet (4), ILE, low-isoleucine diet; diet (5), LEU, low-leucine diet; diet (6), LYS, low-lysine diet; diet (7), MET + CYS, low-methionine and -cystine diet; diet (8), PHE + TYR, low-phenylalanine and -tyrosine diet; diet (9), THR, low-threonine diet; diet (10), TRP, low-tryptophan diet; diet (11), VAL, low-valine diet.

† C-0271; Toro Food Division, Rieber & Søn, Bergen, Norway.

‡ For the composition of the L-amino acid mixtures used in the diets, see Table 2.

§ Fluka Chemica, Buchs, Switzerland.

‖ Federa, Brussels, Belgium.

¶ Gelatin: G-6144, Sigma, St Louis, MO, USA; agar: A-5306, Sigma; carboxymethylcellulose: C-4888, Sigma; α-cellulose: C-8002, Sigma.

** Merigum; Amylum, Alost, Belgium.

†† Cereal, Beerzel, Belgium.

‡‡ Supplied the following (g/kg mixture, except as noted): retinyl acetate 0·67, ascorbic acid 120, cholecalciferol 0·1, tocopheryl acetate 34·2, menadione 2·2, thiamin 5·6, riboflavin 12, pyridoxine 4·5, calcium pantothenate 14·1, *p*-aminobenzoic acid 40, cyanocobalamin 0·03, niacin 30, biotin 0·1, choline chloride 350, folic acid 1·5, inositol 50, canthaxanthin 5, astaxanthin 5, butylated hydroxytoluene 1·5, butylated hydroxyanisole 1·5, α-cellulose 325.

§§ Supplied the following (g/kg mixture, except as noted): $CaHPO_4{\cdot}2H_2O$ 295·5, $Ca(H_2PO_4)_2{\cdot}H_2O$ 217, $NaHCO_3$ 94·5, $Na_2SeO_3{\cdot}5H_2O$ 11 mg, KCl 100, NaCl 172·4, KI 0·2, $MgCl_2$ 63·7, $MgSO_4$ 34·3, $MnSO_4{\cdot}4H_2O$ 2, $FeSO_4{\cdot}4H_2O$ 10, $CuSO_4{\cdot}5H_2O$ 0·4, $ZnSO_4{\cdot}7H_2O$ 10.

Table 2. Composition of L-amino acid mixtures (g/kg dry diet) used in the experimental diets*†‡

No. ...	(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)	(11)	(12)
Diets ...	HC1	ARG	HIS	ILE	LEU	LYS	MET + CYS	PHE + TYR	THR	TRP	VAL	HC2
Arginine	0·0	0·0	11·8	11·8	11·8	11·8	11·8	11·8	11·8	11·8	11·8	11·8
Histidine	0·0	3·8	0·0	3·8	3·8	3·8	3·8	3·8	3·8	3·8	3·8	3·8
Isoleucine	0·0	8·8	8·8	0·0	8·8	8·8	8·8	8·8	8·8	8·8	8·8	8·8
Leucine	0·0	15·4	15·4	15·4	0·0	15·4	15·4	15·4	15·4	15·4	15·4	15·4
Lysine, hydrochloride	0·0	21·6	21·6	21·6	21·6	0·0	21·6	21·6	21·6	21·6	21·6	21·6
Methionine	0·0	6·3	6·3	6·3	6·3	6·3	0·0	6·3	6·3	6·3	6·3	6·3
Cystine	0·0	2·5	2·5	2·5	2·5	2·5	0·0	2·5	2·5	2·5	2·5	2·5
Phenylalanine	0·0	7·8	7·8	7·8	7·8	7·8	7·8	0·0	7·8	7·8	7·8	7·8
Tyrosine	0·0	6·7	6·7	6·7	6·7	6·7	6·7	0·0	6·7	6·7	6·7	6·7
Threonine	0·0	8·6	8·6	8·6	8·6	8·6	8·6	8·6	0·0	8·6	8·6	8·6
Tryptophan	0·0	2·5	2·5	2·5	2·5	2·5	2·5	2·5	2·5	0·0	2·5	2·5
Valine	0·0	9·0	9·0	9·0	9·0	9·0	9·0	9·0	9·0	9·0	0·0	9·0
Alanine	0·0	23·3	19·7	19·6	20·5	22·7	19·5	19·8	19·7	18·8	19·8	18·4
Aspartic acid	0·0	30·8	24·3	24·1	25·7	29·7	23·9	24·6	24·2	22·7	24·4	21·8
Asparagine·H_2O	0·0	11·1	11·1	11·1	11·1	11·1	11·1	11·1	11·1	11·1	11·1	11·1
Glutamic acid	0·0	35·1	28·9	28·7	30·3	34·0	28·5	29·2	28·8	27·3	29·0	26·5
Glutamine	0·0	15·0	15·0	15·0	15·0	15·0	15·0	15·0	15·0	15·0	15·0	15·0
Glycine	0·0	14·7	13·4	13·4	13·7	14·4	13·4	13·5	13·4	13·1	13·4	13·0
Proline	0·0	8·1	7·8	7·8	7·9	8·1	7·8	7·9	7·8	7·8	7·8	7·7
Serine	0·0	24·8	20·0	19·8	21·1	24·0	19·7	20·3	20·0	18·8	20·1	18·2
Σ	0·0	255·9	241·2	235·5	234·7	242·2	234·9	231·8	236·3	236·9	236·5	236·5

* Diet (1), HC1, high-control, cod-protein and gelatine-protein only; diet (12), HC2, high-control in which half of the cod-protein present in HC1 was replaced by a mixture of amino acids of similar composition; diet (2), ARG, low-arginine diet; diet (3), HIS, low-histidine diet; diet (4), ILE, low-isoleucine diet; diet (5), LEU, low-leucine diet; diet (6), LYS, low-lysine diet; diet (7), MET + CYS, low-methionine and -cystine diet; diet (8), PHE + TYR, low-phenylalanine and -tyrosine diet; diet (9), THR, low-threonine diet; diet (10), TRP, low-tryptophan diet; diet (11), VAL, low-valine diet.
† All amino acids were provided by Ajinomoto Ltd, Tokyo, Japan.
‡ For details of diets, see Table 1.

fed a commercial diet (Trouvit 1; Trouw Products, S.A., Ghent, Belgium) to satiation by an automatic feeder. Fish were adapted over a 2-week period to the experimental diet, the commercial diet being progressively replaced by HC2. During this pre-experimental phase, fish were fed to excess manually twice per d (09.00 and 17.00 hours), 6 d per week and were neither weighed nor handled. The daily mortality rate was always $<0{\cdot}1$ %.

After 36 h of food deprivation, immediately before the experimental phase, the salmon fry were weighed (mean initial body weight (W_i) 1·39 (SD 0·02) g) and randomly distributed amongst thirty-nine indoor aquaria ($0{\cdot}40 \times 0{\cdot}24 \times 0{\cdot}20$ m) of 15 litres. Each test diet was randomly allocated to aquaria (three aquaria per diet). Three more aquaria were each filled with ninety fish, which were killed (excess ethylene glycol monophenyl

Table 3. Amino acid composition of the experimental diets (g/kg protein)*†

No. ...	(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)	(11)	(12)
Diets ...	HCl	ARG	HIS	ILE	LEU	LYS	MET + CYS	PHE + TYR	THR	TRP	VAL	HC2
Arginine	65	36	60	59	62	59	60	62	62	62	65	61
Histidine	23	19	12	19	19	19	20	22	21	23	20	20
Isoleucine	53	37	44	23	43	40	45	42	51	41	41	43
Leucine	72	68	67	73	42	67	69	67	67	70	68	68
Lysine	82	76	83	80	81	43	75	74	80	83	78	87
Methionine	29	29	28	29	30	29	15	30	30	31	28	29
Cystine	12	10	11	09	12	10	06	10	11	10	11	09
Phenylalanine	34	36	37	35	36	36	36	20	34	36	37	42
Tyrosine	34	31	31	34	31	31	31	16	30	34	31	35
Threonine	44	38	42	41	41	41	41	41	21	41	41	39
Tryptophan	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
Valine	60	48	50	53	52	50	52	58	51	50	33	50
Alanine	50	67	63	63	65	68	63	62	58	60	62	59
Aspartic acid	86	122	112	117	120	124	111	117	105	109	114	101
Glutamic acid	158	180	162	165	165	180	173	162	156	157	173	151
Glycine	51	51	51	53	55	53	50	53	51	51	52	49
Proline	45	39	38	37	43	38	37	55	50	44	40	41
Serine	44	58	52	51	44	53	50	41	40	43	45	45

nd, not detectable by analytical method used (see p. 866).
* Diet (1), HC1, high-control, cod-protein and gelatine-protein only; diet (12), HC2, high-control in which half of the cod-protein present in HC1 was replaced by a mixture of amino acids of similar composition; diet (2), ARG, low-arginine diet; diet (3), HIS, low-histidine diet; diet (4), ILE, low-isoleucine diet; diet (5), LEU, low-leucine diet; diet (6), LYS, low-lysine diet; diet (7), MET + CYS, low-methionine and -cystine diet; diet (8), PHE + TYR, low-phenylalanine and -tyrosine diet; diet (9), THR, low-threonine diet; diet (10), TRP, low-tryptophan diet; diet (11), VAL, low-valine diet.
† For details of diets, see Table 1.

ether) at the beginning of the experiment, and kept frozen (−20°C) pending the chemical analyses. Biomass density (ninety fish per tank) was in accordance with optimal growth conditions for that species. Water temperature was optimal (16·5 ± 0·5°C; Dwyer & Piper, 1987; Peterson & Martin-Robichaud, 1989). Water quality and water flow rate (1·8 l/min) were maintained in order to supply sufficient O_2 (>8 mg/l) and to avoid a build-up of NH_3-N (<0·1 mg/l) and nitrite-N (<0·001 mg/l). The fish were exposed to continuous light at an intensity of about 100 lx (measured at the water surface). Mortality, if any, was recorded daily. Fish were weighed by group and counted every 12 d after 36 h of food deprivation to estimate the daily ration. At the end of the 32 d experimental period (twenty-five feeding days), and after 36 h of food deprivation, the groups were weighed, counted and the individual final mean body weight was calculated. Growth rate was estimated as daily growth coefficient (%; Iwama, 1996): $100 \times ((W_f^{1/3} - W_i^{1/3})/\text{no. of feeding days})$. All fish were then killed (excess ethylene glycol monophenyl ether) and kept frozen (−20°C) for further determination of the final carcass composition.

Feeding

During the experimental phase, the diets were given manually 6 d per week three times per d (09.00, 13.00 and 17.00 hours) in equal amounts per meal. The quantities distributed were calculated to ensure equal N intake in all aquaria (324 mg N/kg metabolic body weight per d). The variation of the biomass in the aquaria was estimated to determine the daily ration. For this purpose, the fish in each aquarium were weighed and counted every 12 d during the experimental period. The weighing was always preceded by 36 h of fasting. This time period was proven to be sufficient to ensure an empty digestive tract in fry at the experimental temperature. Between weighing times, the variation of the biomass was estimated as follows for a given aquarium:

$$W_{j+1} = W_j + (D_j \times FE),$$

where W_{j+1} and W_j are the biomass (g) on days j + 1 and j respectively, D_j is the dry feed intake (g DM) on day j and FE is the feed efficiency (g wet weight gain/g dry feed intake) determined during the previous 12 d period (or presumed to be 1·25 g/g DM for the first period).

Sampling and chemical analysis

Initial and final fish carcasses were freeze-dried (Unitop 400L; Virtis, Gardiner, NY, USA), pulverised (particle diameter <1 mm) and homogenised (Grindomix GM 200; Retsch, Haan, Germany), and finally kept frozen (−20°C) until analysis.

The diets were analysed for DM, crude protein (N × 6·25), AA, crude fat, crude ash and gross energy contents. Whole-body fish were analysed for DM, crude protein and AA contents. Proximate analyses of samples were conducted using the following conventional procedures (Association of Official Analytical Chemists, 1995): DM by drying at 105°C for 24 h, ash by incineration at 550°C for 12 h, crude protein (N × 6·25) by the Kjeldhal method after acid digestion, crude fat by Soxhlet extraction with diethyl ether. The gross energy of the diets was determined with an IKA-C-400 adiabatic bomb calorimeter (Ika-Werk, Breisgau, Germany). Daily N gain was calculated on the basis of whole-body composition analysis. The AA compositions of cod meal, gelatine, diets and fish samples were measured by ion-exchange chromatography (Spackman *et al.* 1958). The samples were first treated with performic acid to oxidise methionine and cystine to methionine sulfone and cysteic acid respectively (Moore, 1963). These oxidised samples, as well as unoxidised samples, were hydrolysed *in vacuo* in 6 M-HCl for 22 h at 110°C. After evaporation (SpeedVac; Labconco, Kansas City, MO, USA), the samples were recovered in sodium citrate buffer (0·2 M, pH 2·2) and analysed in a single-column automatic AA analyser (4151 Alpha Plus Amino Acid Analyser; Pharmacia LKB Biochrom Ltd, Cambridge, UK) using a Sodium 4151 High Performance Column (Pharmacia LKB Biochrom Ltd) and Na buffer system. The AA were post-column derivatised with ninhydrin and quantified at 570 nm for primary AA and 440 nm for secondary AA using an integrator (C-R5A; Shimadzu, Kyoto, Japan). Norleucine was used as an internal standard. Tryptophan could not be measured by this procedure.

Calculations

The following criteria were used to evaluate fish feed intake and nutrient utilisation:

$$\text{N intake} = \frac{D_i \times N_d}{\frac{1}{2}((W_f/1000)^{0.75} + (W_i/1000)^{0.75}) \times \frac{1}{2}(n_f + n_i) \times \Delta t},$$

$$\text{feed efficiency} = \frac{W_f - W_i}{D_i},$$

$$\text{protein efficiency ratio} = \frac{(W_f - W_i)}{D_i \times N_d \times 6{\cdot}25} \times 100,$$

$$\text{N gain} = \frac{(W_f \times N_f - W_i \times N_i)}{\frac{1}{2}((W_f/1000)^{0.75} + (W_i/1000)^{0.75}) \times \Delta t},$$

$$\text{N retention efficiency} = \frac{NG}{NI} \times 100,$$

$$\text{IAA retention efficiency} = \frac{(W_f \times N_f \times (IAA)_f - W_i \times N_i \times (IAA)_i)}{D_i \times N_d \times (IAA)_d} \times 100,$$

and

$$\text{net protein utilisation} = \frac{(W_f \times N_f - W_i \times N_i) - (W_{0f} \times N_{0f} - W_{0i} \times N_{0i})}{\frac{1}{2}((W_f/1000)^{0.75}) + (W_i/1000)^{0.75}) \times \Delta t},$$

where W_f and W_i are the mean final and initial fresh body mass (g), W_{0f} and W_{0i} are the mean final and initial fresh

body mass of fry fed on the protein-free diet respectively (g), Δt is the duration of the feeding period (d), D_i is the dry diet intake during the experimental period (g DM), n_f and n_i are the number of fish per aquarium at the end and at the beginning of the experiment respectively, N_f and N_i are the mean N contents of the whole-body fish at the end and at the beginning of the experimental period respectively (g/g), NG is N gain, NI is N intake, N_{0f} and N_{0i} are the mean N contents of the whole-body fish fed on the protein-free diet at the end and at the beginning of the experimental period respectively (g/g), N_d is the N content of the experimental diets (g/g DM), $(IAA)_d$ is the IAA content of the experimental diets (g/g N), and $(IAA)_f$ and $(IAA)_i$ are the mean IAA contents of the whole-body fish at the end and at the beginning of the experiment respectively (g/g N).

Data analysis

All data were analysed by one-way ANOVA. Significant differences between treatments were tested using the Tukey's multiple range test and values of $P<0{\cdot}05$ were deemed statistically significant. The relationship between N gain and AA intake was tested using regression analysis on data obtained with the different diets specifically deficient in each AA and with the HC2 control diet. For this purpose, we used a simple linear model as proposed by Wang & Fuller (1989). In this model, it is assumed that: (1) the removal of the first limiting AA would reduce N gain to the greatest extent; (2) if the removal of an AA does not reduce N gain at all, then the quantity removed is in excess relative to the first limiting AA; (3) if the removal of an AA results in a moderate reduction of N gain, then the proportion that could be removed without reducing N gain can be interpolated proportionately.

In practice, we proposed to calculate the IAA requirement values (g/kg DM) for a given IAA as follows:

$$\text{requirement} = (IAA)_{HC2} \times \left(2 - DEL - \left(\frac{NG_{IAA}}{NG_{HC2}}\right)\right),$$

where $(IAA)_{HC2}$ is the concentration of the considered IAA in the HC2 control diet (g/kg DM), DEL is the deletion rate of the IAA in the deficient diet compared with the HC2 control diet, NG_{IAA} is the N gain (mg N/kg metabolic body weight per d) corresponding to the IAA diet and NG_{HC2} is the N gain observed on the HC2 control diet (mg N/kg metabolic body weight per d). An optimum balance between the IAA was derived by dividing the estimated requirement for each IAA by the estimated requirements for lysine (base lysine = 100). All statistics were performed as described in Sokal & Rohlf (1995), using a Systat statistical package (version 5.2 Systat Inc., Evanston, IL, USA).

Results

Body-weight gain and feed efficiency

Mortality. Mortality was low (<2 %) and unaffected by dietary treatments. No external pathological signs were observed.

Growth performance. The highest growth rates were observed with the high-control diets (Table 4); the replacement of fish protein by the mixture of crystalline AA (HC1 to HC2) did not significantly affect growth or the protein efficiency ratio. Partial deletion of individual IAA tended to reduce daily growth coefficient and protein efficiency ratio in all cases, but the differences were not significant for those diets where the leucine, isoleucine, lysine, threonine or valine contents were reduced.

Table 4. Initial and final body weight, daily growth coefficient (DGC), feed efficiency (FE; wet weight gain/dry feed intake) and protein efficiency ratio (PER; wet weight gain/protein intake) of Atlantic salmon (*Salmo salar* L.) fry given diets with reduction of individual amino acids for 25 d*†‡

(Mean values with their standard errors for three groups of ninety fish)

		Initial weight (g)		Final weight (g)		DGC ($\times 10$, $g^{1/3}$ /d)		FE (g/g DM)		PER (g/g)	
No.	Diets	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
(1)	HC1	1·38	0·01	$2{\cdot}90^{a}$	0·06	$12{\cdot}53^{a}$	0·17	$1{\cdot}34^{a}$	0·06	$3{\cdot}01^{a}$	0·07
(2)	ARG	1·38	0·00	$2{\cdot}61^{cdef}$	0·03	$10{\cdot}58^{cd}$	0·18	$1{\cdot}14^{bc}$	0·03	$2{\cdot}60^{bcd}$	0·04
(3)	HIS	1·39	0·00	$2{\cdot}61^{cdef}$	0·02	$10{\cdot}45^{cd}$	0·18	$1{\cdot}12^{bc}$	0·02	$2{\cdot}56^{cde}$	0·04
(4)	ILE	1·41	0·01	$2{\cdot}79^{ab}$	0·03	$11{\cdot}41^{bc}$	0·19	$1{\cdot}25^{ab}$	0·01	$2{\cdot}78^{abc}$	0·03
(5)	LEU	1·37	0·00	$2{\cdot}68^{bcd}$	0·03	$11{\cdot}17^{bc}$	0·18	$1{\cdot}21^{abc}$	0·02	$2{\cdot}72^{bc}$	0·05
(6)	LYS	1·38	0·00	$2{\cdot}65^{bcde}$	0·03	$10{\cdot}74^{bc}$	0·27	$1{\cdot}14^{bc}$	0·03	$2{\cdot}62^{bcd}$	0·08
(7)	MET + CYS	1·39	0·00	$2{\cdot}46^{f}$	0·02	$9{\cdot}39^{e}$	0·14	$1{\cdot}05^{c}$	0·03	$2{\cdot}32^{e}$	0·04
(8)	PHE + TYR	1·39	0·01	$2{\cdot}49^{ef}$	0·03	$9{\cdot}60^{e}$	0·23	$1{\cdot}06^{c}$	0·03	$2{\cdot}37^{de}$	0·06
(9)	THR	1·40	0·01	$2{\cdot}72^{bc}$	0·05	$11{\cdot}10^{bc}$	0·26	$1{\cdot}17^{abc}$	0·03	$2{\cdot}70^{bc}$	0·08
(10)	TRP	1·39	0·00	$2{\cdot}53^{def}$	0·02	$9{\cdot}84^{de}$	0·05	$1{\cdot}07^{bc}$	0·01	$2{\cdot}38^{de}$	0·03
(11)	VAL	1·41	0·01	$2{\cdot}65^{bcde}$	0·03	$10{\cdot}53^{cd}$	0·22	$1{\cdot}14^{bc}$	0·06	$2{\cdot}55^{cde}$	0·06
(12)	HC2	1·37	0·00	$2{\cdot}76^{ab}$	0·02	$11{\cdot}64^{ab}$	0·16	$1{\cdot}22^{abc}$	0·00	$2{\cdot}86^{ab}$	0·05
(13)	PF	1·45	0·01	$1{\cdot}26^{g}$	0·01	$-2{\cdot}07^{f}$	0·05	–	–	–	–

^{a,b,c,d,e,f,g} Mean values within a column with unlike superscript letters were significantly different (one-way ANOVA and Tukey's multiple range test; $P<0{\cdot}05$).

* For details of diets and procedures, see Tables 1–3 and p. 866.

† Diet (1), HC1, high-control, cod-protein and gelatine-protein only; diet (12), HC2, high-control in which half of the cod-protein present in HC1 was replaced by a mixture of amino acids of similar composition; diet (2), ARG, low-arginine diet; diet (3), HIS, low-histidine diet; diet (4), ILE, low-isoleucine diet; diet (5), LEU, low-leucine diet; diet (6), LYS, low-lysine diet; diet (7), MET + CYS, low-methionine and -cystine diet; diet (8), PHE + TYR, low-phenylalanine and -tyrosine diet; diet (9), THR, low-threonine diet; diet (10), TRP, low-tryptophan diet; diet (11), VAL, low-valine diet.

‡ The initial body weight of the fry was 1·39 (SD 0·02) g; the fish were kept at a temperature of 16·5 ± 0·5°C.

Table 5. Nitrogen and amino acid intakes and gains of Atlantic salmon (*Salmo salmar* L.) fry fed diets with reduction of individual amino acids for 25 d*†‡§

(Mean values with their standard errors for three groups of ninety fish)

No.	Diets	N intake (mg N/kg MBW per d)		N gain (mg N/kg MBW per d)		N gain/N intake (% intake)		NPU (% intake)	
		Mean	SE	Mean	SE	Mean	SE	Mean	SE
(1)	HC1	329	4	146^{a}	2	$45{\cdot}1^{a}$	0·7	$54{\cdot}6^{a}$	0·7
(2)	ARG	325	1	120^{bc}	0	$37{\cdot}0^{bc}$	0·1	$46{\cdot}5^{bc}$	0·1
(3)	HIS	326	1	107^{cd}	8	$33{\cdot}0^{cd}$	2·4	$42{\cdot}6^{cd}$	2·4
(4)	ILE	326	2	131^{abc}	5	$40{\cdot}6^{abc}$	1·6	$50{\cdot}1^{abc}$	1·6
(5)	LEU	328	2	131^{ab}	2	$40{\cdot}4^{ab}$	0·8	$49{\cdot}9^{ab}$	0·8
(6)	LYS	327	2	119^{bc}	3	$36{\cdot}9^{bc}$	0·9	$46{\cdot}4^{bc}$	0·9
(7)	MET + CYS	325	2	95^{d}	3	$29{\cdot}4^{d}$	0·9	$39{\cdot}0^{d}$	0·9
(8)	PHE + TYR	325	3	99^{d}	2	$30{\cdot}7^{d}$	0·5	$40{\cdot}2^{d}$	0·5
(9)	THR	327	2	111^{cd}	5	$34{\cdot}2^{cd}$	1·4	$43{\cdot}8^{cd}$	1·4
(10)	TRP	331	3	105^{cd}	5	$34{\cdot}3^{cd}$	1·5	$43{\cdot}8^{cd}$	1·5
(11)	VAL	329	2	122^{bc}	2	$37{\cdot}9^{bc}$	0·7	$47{\cdot}4^{bc}$	0·7
(12)	HC2	324	2	133^{ab}	3	$41{\cdot}1^{ab}$	1·0	$50{\cdot}6^{ab}$	1·1

MBW, metabolic body weight ((initial body weight (kg)$^{0{\cdot}75}$ + final body weight (kg)$^{0{\cdot}75}$)/2); NPU, net protein utilisation.

[a,b,c,d] Mean values within a column with unlike superscript letters were significantly different (one-way ANOVA and Tukey's multiple range test; $P<0{\cdot}05$).

* For details of diets and procedures, see Tables 1–3 and p. 866.

† Diet (1), HC1, high-control, cod-protein and gelatine-protein only; diet (12), HC2, high-control in which half of the cod-protein present in HC1 was replaced by a mixture of amino acids of similar composition; diet (2), ARG, low-arginine diet; diet (3), HIS, low-histidine diet; diet (4), ILE, low-isoleucine diet; diet (5), LEU, low-leucine diet; diet (6), LYS, low-lysine diet; diet (7), MET + CYS, low-methionine and -cystine diet; diet (8), PHE + TYR, low-phenylalanine and -tyrosine diet; diet (9), THR, low-threonine diet; diet (10), TRP, low-tryptophan diet; diet (11), VAL, low-valine diet.

‡ The initial body weight of the fry was 1·39 (SD 0·02) g; the fish were kept at a temperature of 16·5 ± 0·5°C.

§ Mean values were adjusted for the effect of N intake according to Wang & Fuller (1989).

Feed intake. During the feeding trial, all diets were well accepted by the fish. Even though some slight differences in N intake occurred between aquaria, N intake was similar ($P=0{\cdot}51$) between dietary treatments (Table 5).

Nitrogen gain and optimum indispensable amino acid pattern

N gain, protein efficiency ratio and net protein utilisation were affected by the dietary treatment (Table 5). The N loss in fry given the protein-free diet was 30·8 (SE 0·6) mg/kg metabolic body weight per d. When N gain was expressed as a proportion of N intake (N gain/N intake) or, with correction for endogenous N loss on the protein-free diet, as net protein utilisation, the results demonstrated that the crystalline AA mixture with the AA composition of cod-meal protein could replace cod-meal protein without a significant difference in N utilisation.

Fig. 1 illustrates the responses of N gain to reductions in the daily AA intake. Compared with HC2, the low-methionine+-cystine diet caused the greatest reduction in N gain. For each IAA, except two branched-chain AA (isoleucine, leucine), a 45 % reduction was sufficient to make it in turn the first limiting AA in the diet. It was, therefore, possible to calculate a pattern in which each IAA would be equally limiting. The optimum balance amongst the IAA was (relative to lysine = 100): arginine 76 (SE 0·2), histidine 28 (SE 2·2), methionine+cystine 64 (SE 1·7), phenylalanine + tyrosine 105 (SE 1·6), threonine 51 (SE 2·4), tryptophan 14 (SE 0·7), valine 59 (SE 1·7). No estimates were made for isoleucine and leucine.

There was a good correlation (r^2 0·964, n 8, $P<0{\cdot}0001$) between the IAA composition of the 'experimental ideal protein' determined in the present study (relative to lysine = 100, except isoleucine and leucine) and the IAA composition of the whole-body protein (relative to lysine = 100) of Atlantic salmon fry. This correlation was better than that observed with the IAA composition of the reference protein (cod meal, r^2 0·928, n 8, $P<0{\cdot}001$) and that of the control HC2 diet (r^2 0·942, n 8, $P<0{\cdot}0001$). In addition, neither the nature of the experimental diet nor the sampling time affected the IAA composition of the body. Mean values were (g/16 g N, n 36): arginine 6·9 (SE 0·32), histidine 2·5 (SE 0·06), isoleucine 4·6 (SE 0·07), leucine 7·6 (SE 0·11), lysine 8·5 (SE 0·11), methionine 2·7 (SE 0·15), cystine 1·0 (SE 0·05), phenylalanine 4·8 (SE 0·13), tyrosine 3·7 (SE 0·07), threonine

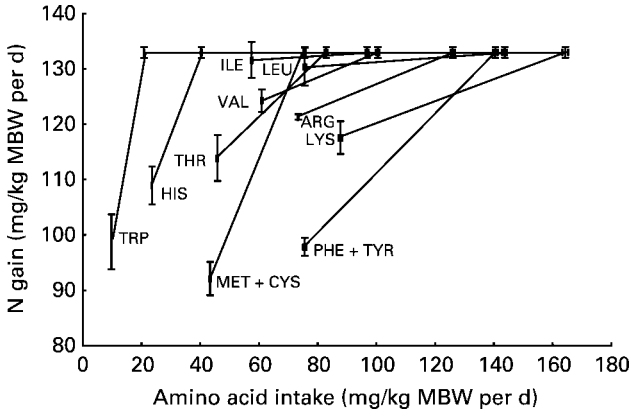

Fig. 1. The effects of reducing each indispensable amino acid by 45 % on N gain in Atlantic salmon (*Salmo salar* L.) fry. MBW, metabolic body weight ((initial body weight$^{0{\cdot}75}$+final body weight$^{0{\cdot}75}$)/2); Trp, tryptophan; His, histidine; Met, methionine; Cys, cystine; Thr, threonine; Ile, isoleucine; Leu, leucine; Val, valine; Arg, arginine; Lys, lysine; Phe, phenylalanine; Tyr, tyrosine. Values are means for three tanks with their standard errors represented by vertical bars for N gain and horizontal bars for amino acid intake. For details of diets and procedures, see Tables 1–3 and p. 866.

4·6 (SE 0·15), valine 5·8 (SE 0·12). The IAA retention efficiencies did not vary between the two control diets (Table 6). For the deficient diets, the IAA retention efficiency was significantly higher for the partially deleted AA. The IAA profile established in the present study and the IAA profile estimated from the AA pattern of the Atlantic salmon whole-body fry protein differ mainly in the amounts of methionine + cystine, phenylalanine + tyrosine, threonine and valine. The concentrations of aromatic and S-containing AA were lower in the whole-body protein (−17 and −8 % respectively), whereas valine and threonine were higher in this protein (+12 and +7 % respectively) compared with the optimum pattern established in the present study.

As the amount of each IAA above the IAA concentrations present in the HC2 diet were known, we were able to calculate the requirements for the following IAA in salmon fry (g/kg DM): arginine 18·2 (SE 0·0), histidine 6·7 (SE 0·5), lysine 23·9 (SE 0·8), methionine + cystine 15·4 (SE 0·4), phenylalanine + tyrosine 25·1 (SE 0·4), threonine 12·1 (SE 0·6), tryptophan 3·3 (SE 0·2), valine 14·1 (SE 0·4). When expressed as g/16 g N, this gave the following values: arginine 4·0 (SE 0·0), histidine 1·5 (SE 0·1), lysine 5·3 (SE 0·2), methionine + cystine 3·4 (SE 0·1), phenylalanine + tyrosine 5·6 (SE 0·1), threonine 2·7 (SE 0·1), tryptophan 0·7 (SE 0·0), valine 3·1 (SE 0·1).

Discussion

Following the general method outlined by Wang & Fuller (1989), the diets were formulated to contain two levels of each IAA at the same N and energy level. Non-protein energy was generously supplied to prevent any energy deficiency. Protein retention was 30–45 % for most diets, values usually found in this species (Grisdale-Helland & Helland, 1997; Peng *et al.* 2003). The AA profile in all diets simulated the fish whole-body dispensable AA and IAA composition.

The IAA-deficient diet, which led to the lowest N gain, was that where the amount of methionine + cystine was reduced. This suggests that methionine and its semi-IAA counterpart were the first limiting AA in cod-meal protein. Based on the N gain results, the second limiting AA should have been phenylalanine + tyrosine. Reducing the amount of S-containing AA by 45 % led to a 29 % reduction in N gain. This means that either oxidation of this AA has been reduced or that the animal has modified its body AA composition (Wang & Fuller, 1989). In the present experiment, we did not observe any significant difference in AA composition in fish fed the different diets, neither between initial nor final fish. This could be due to the relatively short duration of the present experiment or related to the highly conserved IAA profile of carcass proteins irrespective of factors such as feed quality and fish size (Mambrini & Kaushik, 1995*a*; Wilson, 2003). Moreover, the retention of the deficient IAA was increased, indicating a better efficiency in utilisation of the deficient IAA for protein synthesis. This 'sparing' has already been illustrated in rainbow trout (*Oncorhynchus mykiss*; Rodehutscord *et al.* 1995*a*,*b*, 1997). Thus, our results suggest, according to the assumptions

Table 6. Final indispensable amino acid retention of Atlantic salmon (*Salmo salar* L.) fry fed diets with reduction of individual amino acids for 25 d*†‡
(Mean values with their standard errors for three groups of ninety fish)

No. . . .	(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)	(11)	(12)	
Diets. . .	HC1	ARG	HIS	ILE	LEU	LYS	MET + CYS	PHE + TYR	THR	TRP	VAL	HC2	
Amino acids													Pooled SE
Arginine	$57{\cdot}3^{ab}$	$68{\cdot}2^{a}$	$35{\cdot}4^{bc}$	$41{\cdot}1^{bc}$	$50{\cdot}8^{abc}$	$43{\cdot}5^{bc}$	$32{\cdot}6^{c}$	$32{\cdot}3^{c}$	$39{\cdot}1^{bc}$	$32{\cdot}6^{c}$	$35{\cdot}4^{bc}$	$41{\cdot}6^{bc}$	1·27
Histidine	$53{\cdot}9^{bcd}$	$48{\cdot}9^{bcd}$	$66{\cdot}6^{a}$	$47{\cdot}3^{bcde}$	$55{\cdot}4^{bc}$	$43{\cdot}8^{def}$	$32{\cdot}4^{g}$	$34{\cdot}5^{fg}$	$45{\cdot}4^{cde}$	$38{\cdot}2^{efg}$	$46{\cdot}7^{bcde}$	$56{\cdot}3^{a}$	1·59
Isoleucine	$38{\cdot}5^{b}$	$34{\cdot}1^{bd}$	$29{\cdot}6^{de}$	$52{\cdot}1^{a}$	$36{\cdot}0^{b}$	$31{\cdot}3^{ce}$	$22{\cdot}8^{g}$	$23{\cdot}9^{fg}$	$28{\cdot}3^{ef}$	$23{\cdot}3^{g}$	$34{\cdot}5^{bc}$	$36{\cdot}6^{b}$	1·18
Leucine	$46{\cdot}3^{b}$	$38{\cdot}7^{cd}$	$35{\cdot}0^{de}$	$40{\cdot}1^{cd}$	$78{\cdot}3^{a}$	$37{\cdot}9^{cd}$	$29{\cdot}7^{e}$	$30{\cdot}8^{e}$	$38{\cdot}1^{cd}$	$30{\cdot}9^{e}$	$41{\cdot}0^{bc}$	$41{\cdot}4^{bc}$	1·30
Lysine	$47{\cdot}8^{b}$	$38{\cdot}7^{de}$	$35{\cdot}6^{ef}$	$41{\cdot}2^{d}$	$42{\cdot}1^{cd}$	$73{\cdot}3^{a}$	$26{\cdot}4^{h}$	$29{\cdot}4^{gh}$	$38{\cdot}5^{de}$	$31{\cdot}6^{fg}$	$39{\cdot}4^{de}$	$45{\cdot}4^{bc}$	1·28
Methionine + cystine	$46{\cdot}7^{ab}$	$41{\cdot}5^{abcd}$	$33{\cdot}4^{cd}$	$35{\cdot}4^{bcd}$	$47{\cdot}2^{ab}$	$38{\cdot}7^{bcd}$	$53{\cdot}7^{a}$	$28{\cdot}3^{d}$	$32{\cdot}2^{cd}$	$29{\cdot}3^{d}$	$44{\cdot}2^{abc}$	$48{\cdot}6^{ab}$	1·38
Phenylalanine + tyrosine	$55{\cdot}2^{bc}$	$46{\cdot}4^{def}$	$44{\cdot}7^{ef}$	$48{\cdot}0^{cde}$	$46{\cdot}4^{def}$	$45{\cdot}0^{ef}$	$39{\cdot}9^{fg}$	$68{\cdot}6^{a}$	$42{\cdot}8^{efg}$	$37{\cdot}3^{g}$	$52{\cdot}7^{bcd}$	$55{\cdot}9^{b}$	1·43
Threonine	$53{\cdot}3^{b}$	$32{\cdot}6^{c}$	$40{\cdot}2^{b}$	$46{\cdot}7^{b}$	$45{\cdot}1^{b}$	$46{\cdot}0^{b}$	$34{\cdot}2^{b}$	$35{\cdot}5^{b}$	$74{\cdot}7^{a}$	$35{\cdot}2^{b}$	$47{\cdot}9^{b}$	$45{\cdot}2^{b}$	1·52
Valine	$51{\cdot}4^{b}$	$43{\cdot}1^{cde}$	$44{\cdot}5^{bcd}$	$49{\cdot}4^{bc}$	$47{\cdot}1^{bcd}$	$45{\cdot}4^{bcd}$	$36{\cdot}0^{e}$	$40{\cdot}3^{de}$	$41{\cdot}8^{de}$	$41{\cdot}5^{de}$	$75{\cdot}8^{a}$	$50{\cdot}4^{b}$	1·39

a,b,c,dMean values within a row with unlike superscript letters were significantly different (one-way ANOVA and Tukey's multiple range test; $P < 0{\cdot}05$).

*For details of diets and procedures, see Tables 1–3 and p. 866.

†Diet (1), HC1, high-control, cod-protein and gelatine-protein only; diet (12), HC2, high-control in which half of the cod-protein present in HC1 was replaced by a mixture of amino acids of similar composition; diet (2), ARG, low-arginine diet; diet (3), HIS, low-histidine diet; diet (4), ILE, low-isoleucine diet; diet (5), LEU, low-leucine diet; diet (6), LYS, low-lysine diet; diet (7), MET + CYS, low-methionine and -cystine diet; diet (8), PHE + TYR, low-phenylalanine and -tyrosine diet; diet (9), THR, low-threonine diet; diet (10), TRP, low-tryptophan diet; diet (11), VAL, low-valine diet.

‡Indispensable amino acid retention (% intake) = indispensable amino acid gain/indispensable amino acid intake.

of Jürss & Bastrop (1995), that a mechanism exists to reduce the oxidation of a deficient IAA in fish.

The retention of the deficient IAA increased by at least 10 % compared with the retention of the same IAA in the balanced controlled diet. The values recorded (66·6–78·3 %, except for the S-containing AA (52·1 %) and isoleucine (53·7 %)) are close to the maximal efficiencies established in the rat for most IAA (65–85 %) including the S-containing AA (55 %), with the exception of histidine (>100 %) (Heger & Frydrych, 1985). Fish, although they oxidise larger amounts of dietary AA for energy production and exhibit a lower protein turnover rate in the muscle than mammals (Fauconneau & Arnal, 1985), are able to use dietary AA for protein synthesis with a high degree of efficiency. The present results highlight that this efficiency is increased close to maximum when a specific IAA is moderately deficient.

In the present experiment, histidine deficiency led to an intermediate N loss compared with other IAA. This is in contrast with single-stomached land vertebrates, where histidine is usually the IAA whose deficiency leads to the lowest N loss (Heger & Frydrych, 1985; Wang & Fuller, 1987; 1989). It may be that in a situation of histidine deficiency, the nature of the protein synthesised changes to those with lower histidine concentrations (like collagen and keratin) and that the acceleration of the muscle protein turnover due to this deficiency is then sufficient to supply the amount of histidine needed (Heger & Frydrych, 1985). It was not our intention to measure the nature of the protein synthesised, and we do not know whether fish use the same pathways for correcting histidine deficiency. However, we did not observe a significant change in histidine concentration in the fry fed on the histidine-deficient diet. Thus, the synthesis of collagen and/or keratin may not have the same quantitative importance in fish (Phleger, 1991; Houlihan *et al.* 1995).

Unlike Wang & Fuller (1989), we did not obtain improvements in N gain when specific IAA were reduced. This may be because our reduction rate was much greater (45 %) than the one that they used (20 %). The reduction in isoleucine, leucine and valine had little effect on N gain. This demonstrates that the branched-chain AA were much in excess in the high-control diets HC2, i.e. in the cod-meal protein, but also relative to other AA in whole-body salmon fry and, above all, in the egg of this species (Ketola, 1982). On the contrary, the aromatic and S-containing AA seem deficient in these proteins. Using a multivariate analytical procedure, Mambrini & Kaushik (1995*a*) reported that the whole-body carcass protein IAA profile best reflects the requirement pattern, and should be preferred as reference protein to egg or muscle proteins, but can lead to a slight underestimation of phenylalanine and methionine requirements. These results are in accordance with our experimental results. Taken together, they fully demonstrate the limitations in using the carcass AA pattern of the species studied as the only basis for estimating the IAA requirements in fish (Mambrini & Kaushik, 1995*a*; Rodehutscord *et al.* 1997).

The present methodology implies equal N intake in all aquaria. For this purpose, we slightly restricted the feeding of fry by about 5–10 %. Therefore, IAA requirement values reported in the present study may be slightly overestimated (Tacon & Cowey, 1985). However, as a ratio, the optimum IAA pattern is less likely to be influenced by feed intake (Rodehutscord & Pack, 1999). Thus, the optimum IAA pattern established in the present study can be applied more generally than concentrations of single IAA.

Crystalline AA were used to monitor rigorously the AA composition of the experimental diets. Results in the literature show that the efficiency of utilisation of free AA by animals including salmon is not always satisfactory (Batterham, 1979; Espe & Njaa, 1991; Dabrowski & Guderley, 2002) and can be affected by the frequency of feeding (Barroso *et al.* 1999). Several authors have suggested that the use of free AA in experimental diets designed to estimate IAA requirements might lead to overestimation of the requirements, especially when animals are fed only once per d (Batterham, 1979; Rollin, 1999). However, the errors arising from the use of free AA in the present experiment are thought to be small because: (1) compared with other protein sources, cod-meal protein is one of the most fully and rapidly digested (Espe *et al.* 1992) and absorbed (Espe, 1993); (2) not only one, but all the IAA were used in each experimental diet to allow a balanced mixture to be supplied; (3) the AA composition of cod-meal protein is well balanced since it is closer to the ideal pattern compared with other proteins such as casein; (4) the animals were fed three times per d at regular intervals instead of once per d; (5) all fish were accustomed to the high-control diet HC2 containing the free AA mixture over a period of 1 month, which considerably improves the efficiency of N utilisation in the diets (Rollin, 1999); (6) the dietary free AA mixtures were coated with agar, which has been shown to give good growth performances in different fish species (Cho *et al.* 1992; Mambrini & Kaushik, 1994; Fournier *et al.* 2002). Indeed, growth of Atlantic salmon fry was as high when fish were fed the fish-meal control diet as when they were fed the crystalline-AA control diet.

In the present study, the N balance was calculated from the N analysis of carcasses at the beginning and at the end of the experiment. This method has already been shown to be quite feasible for small rainbow trout, selected for uniformity of body weight, in 3-week experiments to assay differences in protein quality of feedstuffs (March *et al.* 1985). The present experiment lasted for twenty-five feeding days and the fry were sorted before the start of the experiment to minimise the inter-aquaria variations in mean body weight. Compared with other indirect techniques, the differential N carcass analysis has an additional advantage of not overestimating the N gain due to potential unrecorded N losses (Heger & Frydrych, 1985) and of allowing the IAA composition of the carcasses to be measured to identify a possible body mobilisation of some IAA.

The 'requirement' equation used in the present study relies on two key assumptions. First, the equation assumes that response to IAA is well described by the 'broken-line' regression approach. The difference in curvilinearity seen in published results is striking (Fuller & Garthwaite, 1993). For example, in mammals, the results of some authors (Heger & Frydrych, 1985; Finke *et al.* 1989; Gahl *et al.*

1991; 1994) have shown a continuously diminishing approach to a maximum, whereas other researchers (Dunkin *et al.* 1986; Campbell *et al.* 1984; 1985) describe the responses of their animals as rectilinear models. In fish, most of the requirement values that have been reported within the last 10 years have been estimated based on the broken-line model (Wilson, 2003). Curvilinear models have also been proposed (Robbins *et al.* 1979; Rodehutscord *et al.* 1997), but the broken-line approach does not generally give a worse fit than the non-linear models with regard to the standard deviation of the residuals (Rodehutscord & Pack, 1999; Rollin, 1999). In addition, inflection points of best-fit broken-lines are objectively established and predict minimal requirement values, and this is viewed as desirable for calculating IAA ratios (Baker, 2003). Second, the equation assumes that IAA are utilised with similar efficiencies and that the maintenance requirements for all IAA are similar. According to Fuller (1994), the results of some authors (Heger & Frydrych, 1985; Gahl *et al.* 1991; Fuller, 1994) suggest that most IAA are used with a similar efficiency in pig and rat. However, it is possible that different IAA are not utilised with the same efficiency in fish, as suggested from our present IAA retention results (Table 6) and some other results found in the piscine literature (Rodehutscord *et al.* 1995*a*,*b*). In addition, the maintenance requirement of different IAA may be different in fish, as suggested by the very few results available in this field (Mambrini & Seudre, 1995; Mambrini & Kaushik, 1995*b*; Rodehutscord *et al.* 1997; Fournier *et al.* 2002). Thus, the IAA pattern of maintenance requirement may be different from the IAA profile needed for protein accretion in fish, according to what is known in mammals (Said & Hegsted, 1970; Fuller *et al.* 1989). The overall IAA requirement must therefore depend on the relative contributions to total needs for maintenance and tissue protein accretion; this must surely vary with the age of the animal. Even though fish never stop growing, their growth rate becomes slower with age. Clearly, research is still needed in these fields in order to clarify the potential limitations of the present methodology.

This present study shows that the ideal protein pattern can be determined experimentally in fish by a technique adapted from that for mammals. It can be used as the reference pattern for evaluating the quality of dietary proteins and practical diet formulation. It also allows the determination of the total IAA requirements in one set of experiments. However, the methodology used relies on some assumptions that still need to be verified in Atlantic salmon fry and in other fish species. Finally, the results were obtained on rapidly growing fish, for which protein accretion represents most of the IAA needs. For other performance levels, another experimental determination of the 'ideal protein' may be necessary.

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