Maintenance threonine requirement and efficiency of its use for accretion of whole-body threonine and protein in Atlantic salmon (*Salmo salar* L.) fry

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Eighteen groups of seventy Atlantic salmon (*Salmo salar* L.) fry (initial mean body weight 0·8 (SD 0·01) g) were fed on semi-purified diets containing graded levels of L-threonine (Thr) in 15 litres aquaria at a temperature of 14·5 ± 1°C. Doses of Thr represented 1, 31, 41, 51, 62, 72, 83 and 93 % of its ideal level for optimum protein deposition. Indispensable amino acids other than Thr were included in the same proportion (on a g/16 g N basis) as in the Atlantic salmon fry whole-body carcass. Following 36 d of feeding and a 36 h fast, fry were killed for whole-body protein and amino acid analysis. Weight gain (r^2 0·98), protein accretion (r^2 0·97), and Thr accretion (r^2 0·97) were linear ($P<0·01$) functions of Thr intake. Slope of the Thr accretion regression line showed that the efficiency of Thr utilisation above maintenance was 76 %. At zero Thr intake, fry lost 5·4 mg Thr/kg body weight$^{0.75}$ per d. The Thr maintenance requirement was 7·2 mg/kg body weight$^{0.75}$ per d and the Thr requirement for growth was 66 mg for 1 g protein deposition. Increasing doses of Thr resulted in increased ($P<0·05$) concentrations of histidine and lysine, and decreased concentrations of isoleucine in whole-body protein. The maintenance need for Thr represented 13·4 % of the total need for Thr. The data suggest that efficiency of Thr utilisation above maintenance is constant at all levels of Thr intake between 1 and 93 % of the level required for optimum protein deposition.

Threonine: Amino acids: Atlantic salmon fry: Maintenance requirement: Growth requirement

Among the main approaches to estimating amino acid (AA) requirements, the factorial approach has recently gained acceptance in fish (Hauler & Carter, 2001*a*). The method treats a dietary AA requirement as the sum of its physiological components (D'Mello, 2003*a*) and requires accurate information on efficiency of AA absorption, maintenance AA requirements, whole-body AA accretion rates, and efficiency of AA utilisation above maintenance (Edwards *et al.* 1999). The strength of the approach is that it allows requirements to be estimated for animals differing in their productive state (Fuller, 1994) and thus can be applied to a wide range of conditions (Shearer, 1995). This is viewed as particularly appropriate to fish since the productive state of these animals is very susceptible to vary with water temperature, the performance potential of the populations considered or the age of the animal (Rollin *et al.* 2003*a*). However, although there is much information on rates of protein accretion in the fish body, one main limitation in the application of the factorial approach to fish is the paucity of quantitative data concerning the maintenance AA requirements and the efficiency with which AA are utilised in meeting the various components of their requirements (Mambrini & Kaushik, 1995; Mambrini & Seudre, 1995; Rodehutscord *et al.* 1997; Hauler & Carter, 2001*a,b*; Fournier *et al.* 2002).

Two different experimental strategies have been proposed in animals to describe the relationship between the body AA accretion and the AA intake: the so-called 'graded supplementation technique' and the 'diet-dilution procedure' (D'Mello, 2003*a,b*). The first method involves the addition of graded supplements of the crystalline form of the AA under test to a basal diet deficient in that AA (D'Mello, 1982). The second method is based on the sequential dilution of a high-protein 'summit' diet with an isoenergetic protein-free mixture (Gous, 1980; Morris *et al.* 1987). The summit diet is normally formulated to contain a large excess of all indispensable AA (IAA) except the one under test, which is set at a lower level of presumed requirements. Although successive dilutions of the summit diet result in different crude protein concentrations, the AA under test will be first limiting at all levels of dilution and the dietary AA pattern will remain constant throughout the diluted series (D'Mello, 1994*a,b*).

Both strategies have been used for AA requirement estimations in fish. Using the graded supplementation technique, Rodehutscord *et al.* (1997) estimated the maintenance requirement of rainbow trout (*Oncorhynchus mykiss*) for several IAA, through intrapolation of dose–response data to zero growth. According to Hauler & Carter (2001*a*), a perceived limitation of this approach has been the inability of dietary crystalline AA to sustain fish growth rates (Cowey & Luquet, 1983; Tacon & Cowey, 1985; National Research Council, 1993; Cowey, 1994, 1995; Dabrowski & Guderley, 2002; Dabrowski *et al.* 2003). However, this limitation may not affect in the same way all species and all sizes of fish in all conditions. Indeed, Rollin *et al.* (2003*a*) recently demonstrated that a crystalline AA mixture with the AA composition of fishmeal protein could replace fishmeal protein without any significant difference in growth rate and N utilisation efficiency in Atlantic salmon (*Salmo salar* L.) fry when the dietary AA mixtures were coated with agar and when the fry were accustomed to a crystalline AA-rich diet over a period of 1 month before the experiment. Some other authors used the second approach to estimate the efficiency of utilisation (above maintenance) and the maintenance requirement for lysine (Allen *et al.* 1998), arginine (Fournier *et al.* 2002) and sulfur AA (Mambrini & Kaushik, 1995; Mambrini & Seudre, 1995) in some fish species. This method means that the use of crystalline AA can be avoided and offers a means of formulation with graded levels of protein-bound AA. However, doubts as to the validity of the diet-dilution technique have been raised for several years in some animals (D'Mello, 1982, 1983, 1994*a,b*) and it is still today a matter of some debate (D'Mello, 2003*a,b*). For example, the data of Morris *et al.* (1987) showed that distinct and separate growth responses occured to graded supplements of the limiting AA at each protein level in the diluted series, implying reduced utilisation of the first-limiting AA as dietary crude protein increased (D'Mello, 2003*a*). Moreover, this method is limited by some technical difficulties in fish, particularly substituting appropriate non-protein energy in a pelleted form (Hauler & Carter, 2001*a*). Therefore, since the effect of dietary crude protein on the efficiency of AA utilisation above maintenance is controversial in fish (Rodehutscord & Pack, 1999) and since AA mixtures can be used effectively in Atlantic salmon fry in some specific conditions (Rollin, 1999; Rollin *et al.* 2003*a*), the first approach was prefered to the second in the present study.

In dose–response experiments, the assumed form of the response often influences the estimation of AA requirements for growth and for maintenance. In fish, the broken line model has been used to estimate most of the requirement values reported within the last 10 years (Wilson, 2003). Curvilinear models have also been applied to either the graded supplementation approach (Rodehutscord *et al.* 1995*a,b*, 1997) or the diet-dilution procedure (Mambrini & Kaushik, 1995; Mambrini, 1996; Fournier *et al.* 2002), but the broken line method does not generally give a worse fit than the non-linear models with regard to the standard deviation of the residuals (Rodehutscord *et al.* 2000; Rollin, 1999). Recently, using the graded supplementation approach, several authors (Rollin, 1999; Hauler & Carter, 2001*b*) reported that lysine deposition responded linearly to marginal lysine intake in Atlantic salmon juveniles. Therefore, we used the simple linear regression model for presenting our salmon data in the present study.

The purpose of the present study was to evaluate the efficiency of utilisation (above maintenance) and the maintenance requirement of threonine (Thr) in Atlantic salmon fry. These values were determined by the graded supplementation approach using a linear model, as proposed by Fuller *et al.* (1989), based on the relationship between Thr intake and Thr or protein gain. Thr and protein depositions were calculated using whole-body N and AA analysis of fry fed on semi-purified diets in which the absorption efficiency of AA could be assumed to be 100 % (Espe *et al.* 1992; Espe, 1993). Thr was selected for intensive study because: (1) noticeable differences in Thr requirements have been reported among fish species (20–50 g/kg protein), even in salmonids (20–37 g/kg protein; Wilson, 2002, 2003); (2) it is an important limiting AA for poultry and pigs (Saldana *et al.* 1994; Kidd *et al.* 1997), but also in practical fish diets, especially when plant protein sources such as maize or wheat gluten are used to replace substantial amounts of dietary fish meal protein (Tibaldi & Tulli, 1999); (3) it is an AA whose maintenance requirement has been found to be high (relative to lysine) in swine (Baker *et al.* 1966; Fuller, 1994), but also in rainbow trout where it has been shown to account for 174 % of the lysine maintenance requirement (Rodehutscord *et al.* 1997); (4) Thr is a major component of mucosal mucins (Fuller, 1994), a family of large glycoproteins that are important constituents of the mucus that protects not only the gastrointestinal mucosa, but also the skin and the gills in fish (Hara *et al.* 1984; Perry & Laurent, 1993). As mucus is constantly being produced and sloughed in fish, Thr losses from epithelia may have a particular significance in this group of vertebrates. Finally, oxidative pathways of Thr may be specific in fish. In higher vertebrates, Thr oxidation forms mainly glycine (87 % of Thr oxidation in rats; 80 % in pigs), which can be then oxidised or contribute to glucose formation (Bird & Nunn, 1983; Ballèvre *et al.* 1990). Whether this is also true in fish needs further investigation, but this is one more reason for studying its implication for basal metabolism, and thus its importance in maintenance AA requirements in fish.

Materials and methods

Experimental diets

Eight semi-purified diets were formulated (Table 1) to contain graded levels of Thr (1, 31, 41, 51, 62, 72, 83 and 93 % of the optimum Thr requirement for salmon fry protein growth (12·1 g Thr/kg dry diet; Rollin *et al.* 2003*a*)), supplied by raw materials and a balanced mixture of purified L-amino acids (Table 2). The AA composition of the diets (Table 3) was based on the AA composition of Atlantic salmon fry whole-body carcass taken as a reference (on a g/16 g N basis) for both IAA and dispensable AA (DAA) composition of the diets and met the IAA requirements of Atlantic salmon fry according to Rollin *et al.* (2003*a*). Seven graded levels of L-Thr (between 0 and 7·5 g/kg DM) were added to the high-protein basal diet to give diets with 3·75, 5, 6·25, 7·5, 8·75, 10 and 11·25 g Thr/kg DM. The Thr added replaced progressively on an N basis the crystalline DAA mixture

Table 1. Composition of the experimental diets used for the determination of maintenance and growth requirements for threonine in Atlantic salmon (*Salmo salar* L.) fry

Diets...	PF	LP	HP1–7*
Components (g/kg diet)			
Cod meal†	0	0	10
L-Amino acid mixture‡	0	138·5	268
Wheat gluten§	0	0	115
Gelatine§	0	0	40
Cod liver oil‖	215	215	205
Glucose¶	240	150	9
Modified starch**	240	281·5	177
Sucrose¶	150	50	20
Soya lecithin††	40	40	40
Vitamin mix‡‡	10	10	10
Mineral mix§§	65	65	65
Agar§	10	10	10
Carboxymethylcellulose§	20	20	20
α-Cellulose§	10	20	10
Chemical composition			
DM (g/kg diet)	942	820	921
N (g/kg DM)	0·1	21·8	63·6
Fat (g/kg DM)	253	251	251
Ash (g/kg DM)	53	62	62
Energy (kJ/g DM)	21·8	21·3	22·4

PF, protein-free diet; LP, threonine-free low-protein diet.

* HP1, high-protein diet with threonine level of 3·75 g/kg DM; HP2, high-protein diet with threonine level of 5·0 g/kg DM; HP3, high-protein diet with threonine level of 6·25 g/kg DM; HP4, high-protein diet with threonine level of 7·5 g/kg DM; HP5, high-protein diet with threonine level of 8·75 g/kg DM; HP6, high-protein diet with threonine level of 10 g/kg DM; HP7, high-protein diet with threonine level of 11·25 g/kg DM.

† Toro Food Division, Rieber & Søn (Bergen, Norway).

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

§ Wheat gluten, Sigma (St Louis, MO, USA) G-5004; gelatine, Sigma G-6144; agar, Sigma A-5306; α-cellulose, Sigma C-8002; carboxymethylcellulose, Sigma C-4888.

‖ Federa, Brussels, Belgium.

¶ Glucose, Merck (Darmstadt, Germany) 8337.5000; sucrose, Merck 7687.5000.

** Merigum; Amylum SA, Alost, Belgium.

†† Cereal, Beerzel, Belgium.

‡‡ Supplied the following (to provide g/kg mixture): retinyl acetate (516 mg/g), 0·67; ascorbic acid, 120; cholecalciferol (100 mg/g), 0·1; tocopheryl acetate (34·2), 34·2; phylloquinone, 2·2; thiamin, 5·6; riboflavin, 12; pyridoxine, 4·5; calcium pantothenate, 14·1; *p*-aminobenzoic acid, 40; vitamin B_{12}, 0·03; niacin, 30; biotin, 0·1; choline chloride, 300; folic acid, 1·5; inositol, 50; canthaxanthin, 7; butylated hydroxytoluene, 1·5; butylated hydroxyanisole, 1·5; α-cellulose, 323·8.

§§ Supplied the following (to provide g/kg mixture): $CaHPO_4.2H_2O$, 117·28; $CaHPO_4$, 165·28; $Ca(PO_4)_2.H_2O$, 236·03; $NaHCO_3$, 100·44; Na_2SO_3, 0·011; KCl, 108·61; NaCl, 143·49; KI, 0·218; $MgCl_2$, 101·88; $MnSO_4.H_2O$, 1·75; $FeSO_4.7H_2O$, 13·51; $CuSO_4.5H_2O$, 0·435; $ZnSO_4.7H_2O$, 10·88.

(except proline) present in the high-protein basal diet (Table 2). However, the IAA:DAA ratio of the diets varied only modestly (47:53 to 49:51). The diets based on the high-protein basal diet were isonitrogenous (63·6 g/kg DM) and their lipid concentration and gross energy content were 251·4 g/kg DM and 22·4 MJ/kg DM respectively (Table 1). Besides the previous diets, a low-protein diet (21·8 g N/kg DM; Table 1) devoid of Thr (Table 3) was fed with crystalline AA as the only N sources (Fournier *et al.* 2002). Its low protein content assured limited excesses of IAA other than Thr. In the low-protein diet, IAA other than Thr were included in the same proportion (on a g/16 g N basis) as in the high-protein basal diet to avoid one of these IAA to be first limiting in place of Thr. The eight diets, in which Thr could thus be assumed being first limiting, were used to describe the relationship between Thr intake and protein or Thr gain. Finally, a protein-free diet (PF; 0·16 g N/kg DM; Table 1) was also fed to

salmon fry to measure the protein or Thr losses of salmon fry fed a diet devoid of all AA.

The experimental diets were produced as previously reported (Rollin *et al.* 2003*a*). In particular, crystalline AA mixtures were coated with 1 % agar, as described by Mambrini & Kaushik (1994), to delay their digestive absorption and optimise their use for protein accretion. After extrusion, the diets were freeze-dried and stored at −20°C until feeding or analysis.

Animals

The Atlantic salmon fry used in the present experiment came from a batch of 10 000 eyed (embryonic) diploid 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-la-Neuve, Belgium) from eggs to the beginning of the experiment, according to Rollin *et al.* (2003*b*). After hatching, salmon fry were fed a Trouvit 000 commercial diet (Trouw France SA, Fontaine-les-Vervins, France) up to age 80 d, followed by Trouvit 00 (Trouw France SA) up to the start of the pre-experimental phase. The experiment was conducted in two consecutive phases: a pre-experimental phase necessary for fish to adapt to a free AA-rich diet and the experiment itself.

The pre-experimental phase consisted of a 3-week period of adjustment by the fry to the high-protein diet rich in crystalline AA (643 g/kg crude protein in the diet) and Thr (18·75 g/kg DM) according to Rollin *et al.* (2003*a*). During this pre-experimental period, the fry (mean initial body weight (W_i) of 0·41 (SD 0·01) g) were kept in a single tank and were continuously fed to a slight excess by an automatic feeder 7 d per week. The daily mortality rate was always below 0·1 %.

After 36 h of food deprivation, immediately before the experimental phase, the salmon fry were weighed (W_i of 0·80 (SD 0·01) g) and randomly distributed amongst eighteen indoor aquaria (0·40 × 0·24 × 0·20 m) of 15 litres (seventy individuals per aquarium). Each test diet was randomly allocated to aquaria (two aquaria per diet). Two more aquaria were each filled with seventy fish, which were killed (excess ethylene glycol monophenyl ether) at the beginning of the experiment, and kept frozen (−20°C) pending the chemical analyses. Biomass density (seventy fish per tank) was in accordance with optimal growth conditions for that species. Water temperature was set near the optimum (14·5 ± 1°C). Water quality, water flow rate and light regimen were as previously described (Rollin *et al.* 2003*a*). Mortality, if any, was recorded daily. At the end of the 36 d feeding experimental period, and after 36 h of food deprivation, the groups were weighed, counted and the individual final mean body weight was calculated. All fish were then killed (excess ethylene glycol monophenyl ether) and kept frozen (−20°C) for further determination of the final carcass chemical composition.

Feeding

During the experimental phase, the diets were given manually 6 d per week two times per d (10.00 and 20.00 hours). Fish

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

Diets... Threonine level... Amino acid†	LP 0·1	HP1 3·75	HP2 5·0	HP3 6·25	HP4 7·5	HP5 8·75	HP6 10·0	HP7 11·25
Arginine	9·2	21·9	21·9	21·9	21·9	21·9	21·9	21·9
Histidine	3·4	8·4	8·4	8·4	8·4	8·4	8·4	8·4
Isoleucine	6·3	15·4	15·4	15·4	15·4	15·4	15·4	15·4
Leucine	10·5	24·5	24·5	24·5	24·5	24·5	24·5	24·5
Lysine hydrochloride	12·6	36·4	36·4	36·4	36·4	36·4	36·4	36·4
Methionine	5·1	13·9	13·9	13·9	13·9	13·9	13·9	13·9
Cystine	1·3	1·5	1·5	1·5	1·5	1·5	1·5	1·5
Phenylalanine	6·4	14·0	14·0	14·0	14·0	14·0	14·0	14·0
Tyrosine	4·9	12·0	12·0	12·0	12·0	12·0	12·0	12·0
Threonine	0	0	1·25	2·5	3·75	5·0	6·25	7·5
Tryptophan	1·2	3·9	3·9	3·9	3·9	3·9	3·9	3·9
Valine	8·2	20·6	20·6	20·6	20·6	20·6	20·6	20·6
Alanine	9·6	17·8	17·6	17·4	17·2	17·0	16·9	16·7
Aspartic acid	6·9	14·4	14·4	14·3	14·1	14·0	13·8	13·7
Asparagine monohydrate	8·6	18·1	17·9	17·7	17·5	17·3	17·1	16·9
Glutamic acid	9·8	8·6	8·5	8·4	8·3	8·3	8·2	8·1
Glutamine	9·8	8·5	8·4	8·3	8·2	8·1	8·0	8·0
Glycine	12·2	17·8	17·6	17·4	17·2	17·0	16·8	16·7
Proline	6·2	0	0	0	0	0	0	0
Serine	6·2	9·9	9·8	9·7	9·6	9·5	9·4	9·3
Sum	138	268	268	268	268	269	269	269

LP, threonine-free low-protein diet; HP1, high-protein diet with threonine level of 3·75 g/kg DM; HP2, high-protein diet with threonine level of 5·0 g/kg DM; HP3, high-protein diet with threonine level of 6·25 g/kg DM; HP4, high-protein diet with threonine level of 7·5 g/kg DM; HP5, high-protein diet with threonine level of 8·75 g/kg DM; HP6, high-protein diet with threonine level of 10 g/kg DM; HP7, high-protein diet with threonine level of 11·25 g/kg DM.
* For details of diets and procedures, see Table 1 and p. 235.
† All amino acids were provided by Ajinomoto Ltd, Tokyo, Japan.

Table 3. Amino acid composition of the experimental diets, along with data on requirements for Atlantic salmon (*Salmo salar* L.) fry according to Rollin *et al.* (2003*a*) (g/kg diet)*

Diets... Amino acid	PF	LP	HP1–7†	Requirement
Arginine	0·06	11·2	31·4	18·2
Histidine	0·03	3·9	11·3	6·7
Isoleucine	0	6·7	19·1	9‡
Leucine	0·05	9·6	28·8	14‡
Lysine	0·07	11·9	34·5	23·9
Methionine	0	5·1	16·2	15·4§
Cystine	0	1·2	3·8	
Phenylalanine	0	6·8	19·8	25·1||
Tyrosine	0	5·1	16·3	
Threonine	0·05	0·13	3·9–11·6	12·1
Tryptophan	nd	nd	nd	3·3
Valine	0·09	8·9	26·0	12‡
Alanine	0·07	11·0	24·2–22·8	
Aspartic acid	0·13	16·1	36·4–34·2	
Glutamic acid	0·22	23·6	64·6–60·8	
Glycine	0·10	13·6	31·8–30·0	
Proline	0·07	6·8	17·4	
Serine	0·09	6·6	16·6–15·6	

PF, protein-free diet; LP, threonine-free low-protein diet; nd, not detectable by analytical method used (see p. 236).
* For details of diets and procedures, see Table 1 and p. 235.
† HP1, high-protein diet with threonine level of 3·75 g/kg DM; HP2, high-protein diet with threonine level of 5·0 g/kg DM; HP3, high-protein diet with threonine level of 6·25 g/kg DM; HP4, high-protein diet with threonine level of 7·5 g/kg DM; HP5, high-protein diet with threonine level of 8·75 g/kg DM; HP6, high-protein diet with threonine level of 10 g/kg DM; HP7, high-protein diet with threonine level of 11·25 g/kg DM.
‡ According to National Research Council (1993).
§ Methionine + cystine.
\|\| Phenylalanine + tyrosine.

were fed to apparent satiation and feed intake was recorded after each meal.

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), crude fat, crude ash and gross energy contents. Moreover, the AA content was measured for diets PF, low protein (LP), HP1 and HP6 (Table 3). Fry were analysed for DM, crude protein, crude ash 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 calorimeter (Ika-Werk, Breisgau, Germany). Daily protein gain was calculated on the basis of whole-body N content multiplied by 6·25. The AA analysis of diets and fish samples was carried out using a Biotronik LC3000 AA analyser. The technique is based on the separation of the AA using cation exchange chromatography (using five lithium acetate buffer solutions of increasing pH and ionic strength) followed by the ninhydrin colour reaction and photometric detection at 570 nm for the α-AA and at 440 nm for the imino acids (Ooghe, 1983). Samples were

hydrolysed by boiling 500 mg homogeneous sample in 370 ml azeotropic 6 M-HCl for 20 h under reflux and under a continuous N flow. The hydrolysate was made up to 500 ml and filtered through a sintered glass filter. A sample of the filtrate was evaporated to dryness at 40°C in a rotavapor system (Büchi Rotavapor R-114; Flawil, Switzerland). A lithium acetate injection buffer solution of pH 2·2 (25 ml) was added to redissolve the residue. After ultrafiltration (0·22 μm), 50 μl of sample was injected into the analyser. Tryptophan cannot be measured with this procedure.

Calculations

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

$$\text{Feed efficiency} = \frac{(W_f - W_i)}{D_i} \times \frac{1}{2}(n_f + n_i)$$

$$\text{Live-weight gain (g/fish)} = W_f - W_i$$

$$\text{Feed intake (g DM/fish)} = \frac{D_i}{\frac{1}{2}(n_f + n_i)}$$

$$\text{Protein efficiency ratio} = \frac{(W_f - W_i)}{D_i \times N_d \times 6 \cdot 25} \times \frac{1}{2}(n_f + n_i) \times 100$$

$$\text{Protein accretion (g/fish)} = (W_f \times N_f \times 6 \cdot 25 - W_i \times N_i \times 6 \cdot 25)$$

$$\text{Thr gain (g Thr/fish)} = (W_f \times N_f \times N_f \times 6 \cdot 25[Thr]_f - W_i$$
$$\times N_i \times N_i \times 6 \cdot 25[Thr]_i)$$

where W_f and W_i are the mean final and initial individual fresh body weights (g), D_i, the dry diet intake per aquarium during the experimental period (g DM), n_f and n_i, the number of fish per aquarium at the end and at the beginning of the experiment, N_f and N_i, the N contents of the whole-body fish at the end and at the beginning of the experimental period (g N/g), and Thr_f and Thr_i, the Thr contents of the whole-body fish at the end and at the beginning of the experiment (g/g AA).

Data analysis

All data were analysed by one-way ANOVA. Significant differences between treatments were tested using Tukey's multiple range test and values of $P < 0.05$ were deemed statistically significant. Four linear-regression equations were determined: Thr and protein (N × 6·25) gain *v.* Thr intake, live weight gain *v.* Thr intake, and Thr gain *v.* protein gain; the last-mentioned was used to determine whether the protein accreted contained a constant amount of Thr and whether zero protein gain resulted in zero Thr gain (Edwards *et al.* 1997). Standard errors were computed for each regression coefficient. Durbin–Watson values were computed for each of the regression equations to prove lack of autocorrelation among data points (Durbin & Watson, 1951; Draper & Smith, 1981). The Thr requirement for maintenance (zero Thr or protein gain) was calculated by determining Thr intake at zero gain. All statistics were performed as described in Sokal & Rohlf (1995), using a Systat statistical package (version 5.2; Systat Inc., Evanston, IL, USA).

Results

Mortality was very low ($<1\%$), was unaffected by dietary treatments and no external pathological signs were observed even in fish fed the low-Thr diet. Final body weight, daily weight gain, feed efficiency (wet weight gain/dry feed intake) and protein efficiency ratio (wet weight gain/protein intake) increased linearly ($P < 0.01$) with an increase in dietary Thr level (Table 4). Suppression of Thr in the LP diet led to a significant reduction of body-weight gain and negative values for feed efficiency and protein efficiency ratio.

All dosing data points (1 (obtained with the LP diet), 31, 41, 51, 62, 72, 83 and 93 % of the ideal Thr level) produced an excellent description of the linear regression of Thr and protein gain *v.* Thr intake (Table 5; Fig. 1). Diet and Thr intakes increased linearly ($P < 0.01$) as dietary Thr levels increased from 1 to 93 % of ideal levels (Table 5). The correlation of gain (*y*; mg/kg metabolic body weight (MBW) per d) *v.* Thr intake (*x*; mg/kg MBW per d) was well described (r^2 0·97) by a linear relationship: $y = -5 \cdot 44 + (0 \cdot 76 \text{ (SE } 0 \cdot 03) x)$ (*n* 16; Fig. 1 (a)). The slope value from the best-fit linear-regression equation showed that the efficiency of Thr utilisation above maintenance was 76 (SE 3) %. Protein gain (*y*; mg/kg MBW per d) as a function of Thr intake (*x*; mg/kg MBW per d) was also well described (r^2 0·97) by a straight-line fit: $y = -91 \cdot 1 + (15 \cdot 2 \text{ (SE } 0 \cdot 7) x)$ (*n* 16; Fig. 1 (b)). The inverse value of the slope from the best-fit linear-regression equation indicated that 66 mg Thr intake is required for 1 g protein deposition in salmon fry. Extrapolating the linear-regression equations of Thr and protein gain to the *y* intercept showed that zero Thr intake resulted in a net daily loss of 5·4 mg whole-body Thr and 91 mg whole-body protein per kg MBW. These values are not significantly different ($P > 0.05$) compared with the losses observed in salmon fry fed the PF diet (4·9 mg whole-body Thr and 97 mg whole-body protein per kg MBW). Daily Thr intake required for maintenance was calculated to be 7·2 mg/d per kg MBW based on zero Thr accretion and 6 mg/d per kg MBW based on zero protein accretion. Finally, from the linear regression between live weight gain (*y*; mg/fish per d) and Thr intake (*x*; mg/fish per d) ($y = -1 \cdot 06 + (108 \cdot 1 \text{ (SE } 4 \cdot 62) x)$ (*n* 16; r^2 0·98), we estimated the overall Thr requirement per kg live weight gain (inverse value of the slope × 1000), i.e. 9·3 (SE 0·4) g Thr/kg live weight gain.

Fig. 2 shows that Thr gain (*y*; mg/fish) was a straight-line function of protein gain (*x*; g/fish). The linear-regression equation, $y = -0 \cdot 185 + (49 \cdot 87 \text{ (SE } 0 \cdot 48) x)$ (*n* 16; r^2 0·999), showed that for each 1 g increase in protein gain, Thr gain increased by 49·9 mg. This suggests that Thr concentration in the whole-body protein accreted was a constant 5 % at all levels of Thr intake. Some IAA tended to increase or decrease in whole-body protein as Thr was incremented (Table 6). The increase was significant ($P < 0.05$) for histidine and lysine and the decrease was significant ($P < 0.05$) for isoleucine.

Discussion

The objectives of the present study were to determine: (1) the efficiency of Thr utilisation above maintenance; (2) the Thr maintenance requirement; (3) the effects of dietary Thr on the AA composition of protein deposited in Atlantic salmon

Table 4. Mean initial and final body weight, weight gain, 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 fed on graded levels of threonine for 36 d*

(Mean values with their standard errors for two groups of seventy fish)

Diets	Dietary threonine g/kg DM	Dietary threonine % of ideal†	Initial weight (g) Mean	Initial weight (g) SE	Final weight‡ (g) Mean	Final weight‡ (g) SE	Weight gain‡ (mg/fish per d) Mean	Weight gain‡ (mg/fish per d) SE	FE‡ (g/g DM) Mean	FE‡ (g/g DM) SE	PER‡ Mean	PER‡ SE
PF	0·05	0·4	0·80	0·01	0·72[e]	0·01	−2·34[d]	0·10	–	–	–	–
LP	0·13	1	0·80	0·00	0·72[e]	0·01	−2·13[d]	0·21	–	–	–	–
HP1	3·75	31	0·81	0·00	1·05[d,e]	0·00	7·74[c,d]	0·38	0·48[d]	0·01	1·15[d]	0·00
HP2	5·00	41	0·80	0·00	1·13[d,e]	0·00	8·59[c,d]	0·40	0·44[d]	0·01	1·05[d]	0·01
HP3	6·25	51	0·80	0·00	1·34[c,d]	0·06	14·93[b,c]	1·64	0·66[c,d]	0·12	1·62[c,d]	0·27
HP4	7·50	62	0·79	0·01	1·47[b,c,d]	0·14	18·41[b,c]	3·23	0·73[b,c]	0·06	1·85[b,c]	0·14
HP5	8·75	72	0·79	0·00	1·86[a,b,c]	0·02	29·65[a,b]	0·45	1·00[a,b]	0·08	2·53[a,b]	0·22
HP6	10·00	83	0·79	0·00	2·13[a]	0·18	37·36[a]	5·18	1·02[a]	0·05	2·58[a,b]	0·19
HP7	11·25	93	0·79	0·00	2·29[a]	0·03	41·50[a]	1·01	1·14[a]	0·04	2·89[a]	0·13

PF, protein-free diet; LP, threonine-free low-protein diet; HP1, high-protein diet with threonine level of 3·75 g/kg DM; HP2, high-protein diet with threonine level of 5·0 g/kg DM; HP3, high-protein diet with threonine level of 6·25 g/kg DM; HP4, high-protein diet with threonine level of 7·5 g/kg DM; HP5, high-protein diet with threonine level of 8·75 g/kg DM; HP6, high-protein diet with threonine level of 10 g/kg DM; HP7, high-protein diet with threonine level of 11·25 g/kg DM.

[a,b,c,d,e] Mean values within a column with unlike superscript letters were significantly different (one-way ANOVA and Tukey's multiple range test; $P > 0.05$).

* For details of diets and procedures, see Table 1 and p. 235.

† Ideal ratio based on Rollin *et al.* (2003*a*). All amino acids other than threonine (see Tables 1 to 3) were maintained in excess relative to threonine.

‡ Linear ($P < 0.01$) response.

fry. To achieve this, we used the approach of feeding graded levels of the limiting AA (Thr), using a linear model, as proposed by Fuller *et al.* (1989) in pigs or Edwards *et al.* (1997, 1999) in chicks, based on the relationship between Thr intake and Thr or protein gain. Thr and protein depositions were calculated using whole-body N and AA analysis of fry fed on semi-purified diets in which the absorption efficiency of AA could be assumed to be 100 % (Espe *et al.* 1992; Espe, 1993). Except for Thr, the AA profiles in the high-protein diets as well as in the LP diet were chosen in order to simulate the Atlantic salmon whole-body AA composition, including those of DAA. Minor differences in the DAA profiles of diets are due to the fact that progressive suppression of Thr in the diets was compensated by a supply of DAA on an equivalent N basis. Because the requirement for bioavailable

Thr in Atlantic salmon fry is 12·1 g/kg dry diet (Rollin *et al.* 2003*a*), the Thr increments of the present study represented 1, 31, 41, 51, 62, 72, 83 and 93 % of the ideal level for protein accretion in salmon fry.

There are two common approaches to studying the efficiency of utilising AA for protein or AA accretion: (1) feeding graded levels of the limiting AA in diets containing all other AA at a constant (and excess) level – the 'graded supplementation technique'; or (2) feeding graded levels of an AA mixture (or intact protein) that is first limiting in the AA under study – the 'diet-dilution procedure' (D'Mello, 2003*a*,*b*). Both strategies have been used for estimations of fish requirements. In the present study, we used the graded supplementation technique because the effect of dietary crude protein level on the efficiency of AA utilisation above maintenance

Table 5. Accretion of body weight, protein, and threonine in Atlantic salmon (*Salmo salar* L.) fry fed on graded levels of threonine for 36 d*

(Mean values with their standard errors for two groups of seventy fish)

Diets	Dietary threonine Level (g/kg DM)	Dietary threonine % of ideal†	Intake Dry diet‡ (g DM/fish) Mean	Intake Dry diet‡ (g DM/fish) SE	Intake Threonine‡ (mg/fish) Mean	Intake Threonine‡ (mg/fish) SE	Accretion Body weight‡ (g/fish) Mean	Accretion Body weight‡ (g/fish) SE	Protein‡ (g/fish) Mean	Protein‡ (g/fish) SE	Threonine‡ (mg/fish) Mean	Threonine‡ (mg/fish) SE
PF	0·05	0·4	0·29	0·01	0·01	0·00	−0·08[d]	0·01	−0·016[d]	0·000	−0·80[d]	0·02
LP	0·13	1	0·72[b,c]	0·03	0·09[d]	0·00	−0·07[d]	0·01	−0·017[d]	0·000	−0·86[d]	0·02
HP1	3·75	31	0·60[c]	0·03	2·26[c,d]	0·11	0·27[c,d]	0·01	0·025[c,d]	0·000	1·05[c,d]	0·02
HP2	5·00	41	0·74[b,c]	0·01	3·69[c,d]	0·07	0·31[c,d]	0·01	0·031[c,d]	0·001	1·36[c,d]	0·04
HP3	6·25	51	0·83[b,c]	0·05	5·22[c,d]	0·30	0·54[b,c]	0·06	0·058[c]	0·008	2·65[c]	0·37
HP4	7·50	62	0·89[a,b,c]	0·09	6·67[b,c,d]	0·67	0·66[b,c]	0·12	0·078[b,c]	0·016	3·64[b,c]	0·77
HP5	8·75	72	1·07[a,b]	0·07	9·28[a,b,c]	0·65	1·06[a,b]	0·02	0·134[a,b]	0·000	6·46[a,b]	0·00
HP6	10·00	83	1·29[a]	0·08	12·99[a,b]	0·84	1·34[a]	0·19	0·170[a]	0·018	8·19[a]	0·87
HP7	11·25	93	1·29[a]	0·03	14·53[a]	0·30	1·50[a]	0·04	0·192[a]	0·001	9·64[a]	0·05

PF, protein-free diet; LP, threonine-free low-protein diet; HP1, high-protein diet with threonine level of 3·75 g/kg DM; HP2, high-protein diet with threonine level of 5·0 g/kg DM; HP3, high-protein diet with threonine level of 6·25 g/kg DM; HP4, high-protein diet with threonine level of 7·5 g/kg DM; HP5, high-protein diet with threonine level of 8·75 g/kg DM; HP6, high-protein diet with threonine level of 10 g/kg DM; HP7, high-protein diet with threonine level of 11·25 g/kg DM.

[a,b,c,d,e] Mean values within a column with unlike superscript letters were significantly different (one-way ANOVA and Tukey's multiple range test; $P > 0.05$).

* For details of diets and procedures, see Table 1 and p. 235. The initial body weight of the fry was 0·80 (SD 0·01) g. The fish were kept at a temperature of 14·5 ± 1°C.

† Ideal ratio based on Rollin *et al.* (2003*a*). All amino acids other than threonine (see Tables 1 to 3) were maintained in excess relative to threonine.
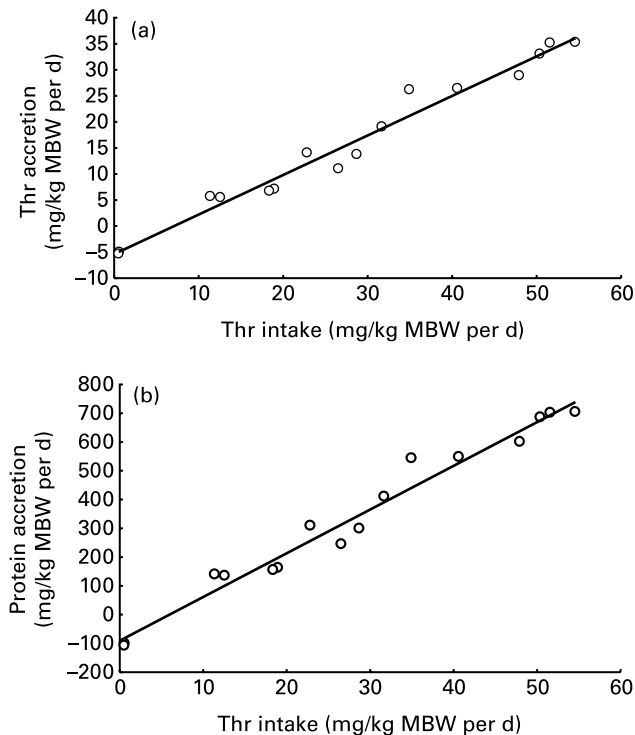
‡ Linear ($P < 0.01$) response.

Fig. 1. Best-fit straight-line plots of (a) whole-body threonine (Thr) accretion (*y*) as a function of Thr intake (*x*; $y = -5.44 + (0.76$ (SE 0.03) x) (*n* 16); r^2 0.97; Durbin–Watson value = 2.18) and (b) whole-body protein accretion (*y*) as a function of Thr intake (*x*; $y = -91.1 + (15.2$ (SE 0.7) x) (*n* 16); r^2 0.97; Durbin–Watson value = 2.22) for Atlantic salmon (*Salmo salar* L.) fry fed on graded increments of L-Thr. MBW, metabolic body weight ((initial body weight$^{0.75}$ + final body weight$^{0.75}$)/2). Each data point represents the mean gain of seventy fish per aquarium during a 36 d feeding period. For details of diets and procedures, see Tables 1–3 and p. 235.

Fig. 2. Best-fit straight-line plots of whole-body threonine (Thr) accretion (*y*) as a function of protein accretion (*x*) for Atlantic salmon (*Salmo salar* L.) fry fed on graded increments of L-Thr ($y = -0.185 + (49.866$ (SE 0.478) x) (*n* 8); r^2 1.00; Durbin–Watson value = 2). Each data point represents the mean gain of 140 fish during a 36 d feeding period. For details of diets and procedures, see Tables 1–3 and p. 235.

is not resolved in fish to date. This raises the question of the validity of the diet-dilution technique in fish. In addition to this '*a contrario*' reason, we recently demonstrated (Rollin, 1999; Rollin *et al.* 2003*a*) that AA mixtures can be used effectively in Atlantic salmon fry under some specific conditions (AA profile simulating the AA composition of fishmeal protein, AA mixtures coated with agar, accustomisation of fry to a crystalline AA-rich diet, etc). Therefore, the reported inability of dietary crystalline AA to sustain fish growth (Cowey & Luquet, 1983; National Research Council, 1993; Cowey, 1994; Dabrowski *et al.* 2003) is no more a limitation in salmon fry and the graded supplementation technique can be performed effectively with this species under these conditions.

In both aforementioned procedures, absolute intake of the limiting AA is a function of both voluntary food intake and concentration of the limiting AA in the diet (Chung & Baker, 1992). The estimation of AA requirements for growth and for maintenance depends on the interpretation of this function, and in particular what form of response is assumed (Fuller, 1994). Several non-linear models as well as the linear broken line approach have been suggested for animals (Pack *et al.* 2003). The question of the model chosen is directly related to the question of whether the efficiency of AA utilisation decreases or remains constant as AA intakes above maintenance increase. Whether diminishing returns
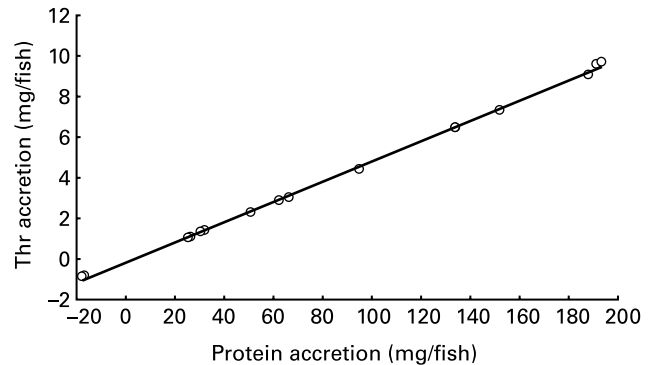
occur in AA utilisation as intakes increase above maintenance has been controversial for many years (Fisher *et al.* 1973; Heger & Frydrych, 1985; Baker, 1991; Fuller & Garthwaite, 1993; Gahl *et al.* 1996; Rodehutscord & Pack, 1999; Baker, 2003; Pack *et al.* 2003). Indeed, the differences in curvilinearity seen in published data are striking (Fuller & Garthwaite, 1993). For example, in mammals and birds, the data of some authors (Campbell *et al.* 1984, 1985; Dunkin *et al.* 1986; Fuller *et al.* 1989; Batterham *et al.* 1990; Baker, 1991; Chung & Baker, 1992; Bikker, 1994; Adeola, 1995; Baker *et al.* 1996; Edwards *et al.* 1997, 1999) represented very well the responses of their animals by straight lines. Gahl *et al.* (1991, 1996) and Heger & Frydrych (1985) interpreted their rat data as suggesting decreasing utilisation of lysine and Thr as graded doses of each AA were increased. However, Baker *et al.* (1996) studied their response curves carefully and concluded that straight-line fits appeared to describe their response curves as well as curvilinear fits if one eliminates AA intakes near and above the requirement. 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 applied to either the graded supplementation approach (Rodehutscord *et al.* 1995*a*,*b*, 1997; Rollin, 1999) or the diet-dilution procedure (Mambrini & Kaushik, 1995; Mambrini, 1996; Fournier *et al.* 2002), 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; Fournier *et al.* 2002). Rollin (1999) reported a comparison of regressions according to the four-parameter nutrient kinetics model of Mercer (1982) and the simple linear regression model proposed by Fuller *et al.* (1989) for describing the relationship between lysine gain and lysine intake in Atlantic salmon fry fed on graded levels of lysine and concluded that the linear model did not give a worst fit than the four-parameter model (r^2 0.967 *v.* r^2 0.968; *n* 31; $P > 0.05$). Also Hauler & Carter (2001*b*) reported that lysine deposition responded linearly to marginal lysine intake in Atlantic salmon parr. In the present study, the linear plots depicted in Fig. 1 resulted in Durbin–Watson values of 2.2, and

Table 6. Nitrogen content (g/100 g fresh fish) and amino acid composition of whole-body protein (g/100 g amino acids) in Atlantic salmon (*Salmo salar* L.) fry fed on different experimental diets containing graded levels of threonine*

(Mean values with their standard errors for two groups of seventy fish)

| Diets… | LP | HP1 | HP2 | HP3 | HP4 | HP5 | HP6 | HP7 | |
Threonine level…	0·13	3·75	5·0	6·25	7·5	8·75	10·0	11·25	SEM
N†	1·96[a,b]	1·95[b]	1·98[a,b]	1·97[a,b]	2·02[a,b]	2·06[a,b]	2·07[a,b]	2·08[a]	0·02
Amino acids									
Arginine	7·09	6·81	7·13	7·17	7·05	7·06	7·11	7·05	0·08
Histidine†	2·39[b]	2·49[a,b]	2·51[a,b]	2·53[a,b]	2·54[a,b]	2·57[a]	2·56[a,b]	2·59[a]	0·03
Isoleucine‡	4·09[a]	3·87[a,b]	3·82[a,b]	3·82[a,b]	3·41[b]	3·74[a,b]	3·64[b]	3·78[a,b]	0·06
Leucine	7·65	7·65	7·57	7·57	7·66	7·64	7·53	7·62	0·06
Lysine†	8·53[b]	8·51[b]	8·50[b]	8·61[a,b]	8·59[a,b]	8·94[a]	8·68[a,b]	8·81[a]	0·07
Methionine	3·29	3·30	3·25	3·24	3·32	3·23	3·30	3·34	0·02
Cystine	0·59	0·38	0·36	0·44	0·41	0·42	0·37	0·43	0·07
Phenylalanine	4·25	4·15	4·20	4·19	4·06	4·19	4·13	4·19	0·02
Tyrosine	3·31	3·34	3·37	3·35	3·36	3·29	3·30	3·36	0·04
Threonine	4·81	4·68	4·73	4·73	4·75	4·83	4·82	4·95	0·09
Tryptophan	nd	nd	nd	nd	nd	nd	nd	nd	–
Valine	4·61	4·55	4·14	3·90	4·09	4·11	4·32	4·45	0·04
Alanine	6·41	6·47	6·57	6·61	6·80	6·57	6·64	6·55	0·03
Aspartic acid	9·87	9·96	9·82	9·90	10·22	10·02	9·81	9·73	0·02
Glutamic acid	15·37	15·61	15·29	15·33	15·85	15·45	15·17	15·00	0·17
Glycine	7·63	7·74	8·00	8·05	8·06	7·57	7·86	7·56	0·10
Proline	4·21	4·41	4·53	4·57	4·46	4·22	4·36	4·24	0·05
Serine	4·82	4·92	4·93	4·86	5·14	4·95	4·94	4·93	0·02

LP, threonine-free low-protein diet; HP1, high-protein diet with threonine level of 3·75 g/kg DM; HP2, high-protein diet with threonine level of 5·0 g/kg DM; HP3, high-protein diet with threonine level of 6·25 g/kg DM; HP4, high-protein diet with threonine level of 7·5 g/kg DM; HP5, high-protein diet with threo-nine level of 8·75 g/kg DM; HP6, high-protein diet with threonine level of 10 g/kg DM; HP7, high-protein diet with threonine level of 11·25 g/kg DM; nd, not detectable by analytical method used (see p. 237).

[a,b] Mean values within a row with unlike superscript letters were significantly different (one-way ANOVA and Tukey's multiple range test; $P > 0·05$).

* For details of diets and procedures, see Table 1 and p. 235.

† Significant ($P < 0·05$) linear increase with increasing dietary threonine level.

‡ Significant ($P < 0·05$) linear decrease with increasing dietary threonine level.

these values together with the high r^2 values provide good evidence that our linear fit represents an acceptable description of our accretion data. Therefore, we believe the present results with Atlantic salmon fry provide clear evidence that Thr util-isation above maintenance (and below the requirement for maintenance + protein accretion) is constant. It can be argued that, if no diminishing return was observed, it may be because Thr intake even at the highest level (93 % of ideal) was lower than the overall needs of salmon fry. How-ever, calculating the overall Thr requirement from our present data, we obtained 11·4 g/kg dry diet or 2·8 g/16 g N, two values very similar to our previously published results (12·1 g/kg diet or 2·7 g/16 g N; Rollin *et al.* 2003*a*) and to the highest Thr level tested in the present experiment (11·75 g/kg dry diet or 3 g/16 g N). In any event, the present results undoubtedly reflect the excellent genetic, age and weight uniformity of salmon fry used in the present experiment.

Very few data on AA maintenance requirements are pre-sently available in the piscine literature (Mambrini & Kaushik, 1995; Mambrini & Seudre, 1995; Rodehutscord *et al.* 1997; Fournier *et al.* 2002). To our knowledge, no such data are available for fish fry and only one estimation has been pub-lished for Thr; this was for rainbow trout (Rodehutscord *et al.* 1997). It is interesting to compare the estimation of Thr requirement for maintenance obtained in the present study to data already reported in fish as well as in higher ver-tebrates. The estimation of Thr maintenance requirement obtained here in salmon fry (about 7 mg/kg MBW per d) seems much lower than the published data for pigs (39 mg/kg MBW per d, Baker *et al.* 1966; 53 mg/kg MBW per d,

Fuller *et al.* 1989), chicks (46 mg/kg MBW per d, Edwards *et al.* 1997), rats (54 mg/kg MBW per d, Gustafson *et al.* 1984) and even in 100 g (average body weight) rainbow trout, i.e. 18·8 mg/kg MBW per d or 17 % of the total Thr requirement for protein accretion (Rodehutscord *et al.* 1995*a,b*, 1997). Atlantic salmon is an ectotherm living in cold water (Elliott, 1991). Compared with homeotherms, this species shows reduced protein growth, synthesis and turnover (Houlihan *et al.* 1995). This probably explains the lower main-tenance AA requirements when compared with homeothermic animals. Moreover, several assumptions can be made to explain the possible lower maintenance Thr requirement of salmon fry compared with 100 g rainbow trout when expressed as per kg MBW per d. Firstly, Rodehutscord *et al.* (1995*a,b*, 1997) estimated the maintenance requirement in rainbow trout through intrapolation of dose–response data to zero growth using an exponential model. However, if their response curve for protein deposition is carefully studied, and if Thr levels above the total Thr requirement are eliminated, straight-line fit appears to describe their response curve as well or even better than the exponential model and would cer-tainly have resulted in a much lower estimation of Thr require-ment for maintenance in that species. The choice of the model has a substantial effect on the estimate of requirement (Fuller, 1994). Second, the average body weight of rainbow trout used by the German researchers was much higher (100 g/fish) than that considered in the present study on Atlantic salmon fry (approximately 1·5 g/fish). It is possible that AA maintenance needs increase as the fish increase in size, as has been reported for pigs (Fuller, 1994), being greater in growing fish than in

fry. Thr is a major component of mucosal mucins, which are important constituents of mucus that protect the mucosa, and it is continuously being produced and sloughed into the gut (Wilson *et al.* 1981; Fuller, 1994), but also over the epidermis (Perry & Laurent, 1993). Maintenance requirements of Thr may be directly related to the Thr turnover rate in the growing gut, the mucus production of the skin and ultimately to the size of the gut and of the skin. After first feeding, the internal organs of salmonids, including the gut and skin, have been reported to grow faster than the body as a whole (Denton & Yousef, 1976; Weatherley & Gill, 1983; Shearer, 1995). This could partly explain a higher Thr maintenance requirement for fry when compared with growers. Further insight is needed on whether Thr maintenance requirement is related to fish size and Thr turnover rate in the growing gut. The influence of mucus production on Thr losses at maintenance, as well as the form of the response of body Thr accretion to dietary Thr supply at low Thr intakes, should also be determined in the future.

With our estimate of the maintenance requirement for absorbed Thr of 7·2 mg/kg MBW per d (assuming an absorption efficiency of 100 % for Thr; Espe *et al.* 1992; Espe, 1993), together with the recent estimate (Rollin *et al.* 2003*a*) of the total requirement for digestible Thr of 1·21 % of diet (54 mg/ kg MBW per d), it can be calculated that the Thr maintenance requirement represents 13·4 % of the total digestible Thr need for Atlantic salmon fry during this early growth phase.

The loss of body N, observed in animals on a diet devoid of one IAA, varied with the type of AA omitted (D'Mello, 2003*c*). In the rat, Said & Hegsted (1970) and Heger & Frydrych (1985) identified Thr-free and isoleucine-free diets together with a diet free of sulfur AA as those that caused the loss of body N comparable to a PF diet. In the present experiment, the whole-body Thr loss observed with the LP diet devoid of Thr was also similar ($P>0.05$) to the Thr loss obtained with the PF diet. It seems that the extent of N loss due to the severe AA deficiency depends on the order of limitation of endogenous AA (Heger & Frydrych, 1985). The importance of Thr in maintenance has been reported in a number of studies in rats, pigs, cats and dogs (Yokogoshi & Yoshida, 1976; Fuller *et al.* 1989; Hendricks *et al.* 1996; Hendricks, 2003), and when supplemented to a PF diet it has been demonstrated to have a significant N-sparing effect in the rat (Yoshida & Moritoki, 1974). Furthemore, our data show that, even in presence of large amounts of the other IAA, the Thr gain is strictly related to Thr intake. Further studies will have to highlight if: (1) Thr is one of the first-limiting AA of endogenous origin under maintenance conditions in salmon fry; (2) some body proteolysis occurs in fry fed diets devoid of Thr to ensure compensatory proteosynthesis in the presence or absence of large amounts of the other IAA.

The exact value for the efficiency term is crucial to the application of the factorial method for the estimation of AA requirements (Fuller, 1994). In the present study, the efficiency of recovery of ingested Thr above maintenance in whole-body protein was 76 (SE 3·4) % and seemed constant at all levels of Thr intake between 1 and 93 % of its requirement for optimum protein deposition. For instance, with Thr at 83 % of its ideal level, 63 % (8·2 mg/fish) of the Thr intake (13 mg/fish) was recovered in whole-body protein (Table 5). Of the 37 % not recovered, 13·4 % can be assigned

to the maintenance need, and the remaining 23·6 % can be assigned to oxidation loss. This last value corresponds well with the 24 % loss (100 minus 76 %) estimated from regression (Fig. 1 (a)). Thus, Thr utilisation above maintenance showed no evidence of diminishing returns (i.e. declining efficiency) as its level in the diet was increased. This finding of constant efficiency (above maintenance) agrees well with previous work in Atlantic salmon fry, in which constant utilisation (above maintenance) was observed for lysine below the requirement for maintenance plus protein accretion (70 %, Rollin, 1999; 71 %, Hauler & Carter, 2001*b*). Unfortunately, there is little published information on fish with which to compare these estimates. Recently, Encarnação *et al.* (2004) reported in rainbow trout that, at marginally deficient lysine intake levels, efficiency of lysine utilisation for body protein deposition (lysine retained/lysine intake) was contained between 65 and 75 % for the high-energy diet (20 MJ digestible energy/kg), these values being in the range of our estimates. Some other estimates of AA utilisation efficiency have also been reported in fish larvae and post-larvae. In post-larval Senegal sole (*Solea senegalensis*), Rønnestad *et al.* (2000, 2001*a,b*) reported assimilation efficiency (label AA retained/total label AA fed) of 87, 82 and 89 % for lysine, arginine and methionine, respectively. High assimilation of methionine (85–90 %) was also found in larval stages of striped bass (*Morone saxatilis*) and Walleye (*Stizostedion vitreum*; Rust, 1995). Estimating a metabolic budget for lysine in fasting herring (*Clupea harengus*) larvae, Conceição *et al.* (2002) reported a 70 % utilisation efficiency in first-feeding larvae and 63 % in 47-d-old (premetamorphosis) larvae. However, since these values have been obtained with larval fish, which have been reported to be morphologically and functionally incomplete (Fyhn, 1989), and/or with completely different methodologies (tube-feeding using ^{14}C-labelled AA or proteins; Rust *et al.* 1993; Rønnestad *et al.* 2001*a,b*), it makes difficult direct comparisons with the present results (number of fish tested, level of intake, composition of the diets used, etc.).

Paradoxically, from a methodological point of view, most of the data available on mammals and birds are easier to compare with our own data. Indeed, in the present study, we draw our inspiration from methodologies mainly applied to single-stomached animals over several years (D'Mello, 1982, 2003*a,b*). Most chick work (Baker, 1991; Baker *et al.* 1996; Edwards *et al.* 1997, 1999) has reported a constant AA utilisation efficiency (above maintenance): Thr (82 %), valine (73 %), lysine (80 %) and isoleucine (61 %). Likewise, similar studies involving graded dosing in pigs also resulted in efficiency estimates above maintenance that were constant at all levels of intake of the limiting AA: 74 % (Bikker, 1994) to 86 % (Batterham *et al.* 1990; Adeola, 1995) for lysine, 72 % (Chung & Baker, 1992) for methionine and only 60 % (Adeola, 1995) for Thr, considerably less than our present estimate for salmon fry and also that reported for chicks (Edwards *et al.* 1999). Batterham (1994) evaluated efficiencies for retaining ileal digestible AA from soyabean meal in growing pigs and reported efficiencies (including maintenance costs) of 75 % for lysine, 64 % for Thr, 45 % for methionine and 38 % for tryptophan. From the studies that have been reported in single-stomached animals, it seems that individual IAA differ in the efficiency with

which absorbed AA are retained in whole-body protein. Our estimate of Thr utilisation above maintenance in Atlantic salmon fry is similar to that observed for lysine in similar experimental conditions (Rollin, 1999). This has also been reported in chicks (Baker, 1991; Edwards *et al.* 1997), but work in pigs and rats has suggested that lysine is retained more efficiently than Thr (Adeola, 1995; Gahl *et al.* 1996). However, it is difficult to make an objective comparison between values reported in different studies and, moreover, in different animal species, different dietary models, etc. For instance, energy concentration and lipid levels in pig and poultry diets are much lower than in salmonid diets. Furthermore, regulation of AA utilisation in fish may be different from pigs and poultry (Encarnação *et al.* 2004), in which AA catabolism appears to play a much smaller role in supplying energy than carbohydrates and lipids. Therefore, caution is warranted when transposing concepts or when comparing AA utilisation efficiency values in other single-stomached animals with fish species.

It has been widely documented that a reduction in feed intake may be regarded as the primary factor responsible for depressed growth in rats, chickens, kittens and beagle dogs fed diets deficient or limiting in Thr (Titchenal *et al.* 1980; Burns & Millner, 1982; Cieslak & Benevenga, 1984; Webel *et al.* 1996). This was also observed in the present study with Atlantic salmon fry as well as in other studies with chum salmon (*Oncorhynchus keta*; Akiyama *et al.* 1985), rainbow trout (Rodehutscord *et al.* 1995*a,b*), common carp (*Cyprinus carpio*; Nose, 1979) and *Catla catla* (Ravi & Devaraj, 1991). However, in other studies carried out to assess the overall Thr requirement of the channel catfish (*Ictalurus punctatus*; Wilson *et al.* 1978), milkfish (*Chanos chanos*; Borlongan, 1991), red drum (*Sciaenops ocellatus*; Boren & Gatlin, 1995), hybrid striped bass (*Morone chrysops* ♀ × *M. saxatilis* ♂; Keembiyehetty & Gatlin, 1997) or European sea bass (*Dicentrachus labrax*; Tibaldi & Tulli, 1999), the dietary deficiency of Thr was coupled with only minor or no effect on feed intake. To date, we do not have any ready explanation for these discrepancies. However, at least in some studies, it could be related to an overestimation of feed intake (Hauler & Carter, 2001*a*).

The Thr and protein accretion data of the present experiment point to at least six important conclusions: (1) efficiency of utilising (retaining) absorbed Thr above maintenance was 76 ± 3 %; (2) efficiency of utilising absorbed Thr above maintenance was constant at Thr intakes between 1 and 93 % of the (ideal) Thr level required for optimal protein deposition; (3) Thr concentration in the whole-body protein accreted was the same at all levels of Thr intake; (4) zero protein accretion resulted in negative Thr accretion, suggesting a higher maintenance Thr requirement for zero Thr accretion than for zero protein gain; (5) the maintenance need for Thr represented 13·4 % of the total need (maintenance + accretion) for Thr; (6) the whole-body Thr loss observed with the LP diet devoid of Thr was similar to the Thr loss obtained with the PF diet.

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