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Protein and lysine requirements for maintenance and for tissue accretion in Atlantic salmon (*Salmo salar*) fry

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Abstract

Available data on the quantitative requirement for lysine (Lys) in different salmonids show much variability. So far, there are very limited data on the maintenance requirements of indispensable (I) amino acids (AA) in fish. In the present study, we determined simultaneously the Lys requirements for maintenance and for protein accretion in Atlantic salmon fry by adapting a protocol established for the piglet. Groups of fish having an initial body weight of 1.5 g were fed for 28 days on isoenergetic diets with increasing nitrogen (N) content supplied by cod meal and a mixture of crystalline AAs (50% of total dietary N). Except the protein-free diet (PF; 0.2% dry matter (DM)), the N content of the other diets was either low (2.9% DM), medium (6.2% DM) or high (8.5% DM). Two types of diets with the same N content were formulated. The AA pattern of three control diets was based on the AA pattern of the cod meal protein. For the other three diets, Lys·HCl was totally omitted from the low-N diet (LPD), and 50% of Lys·HCl was removed from the medium- and high-N diets. After a 28-day feeding trial, carcass N and Lys gains were estimated. N and Lys requirements for maintenance and for growth were calculated regressing daily N or Lys gain against N or Lys intakes. The daily N requirement for growth above maintenance was 3.05 g per g protein gain and for maintenance it was 54 mg kg body weight$^{-0.75}$. From the regression between protein and Lys intake, we calculated that for the accretion of 1 g body protein, the dietary Lys requirement was 152 mg, and that the Lys maintenance requirement for zero N gain was 20 mg kg body weight$^{-0.75}$/day. This last value is higher than the previous estimations obtained for rainbow trout and could be explained by the lower body weight or age of the fish used here.

Keywords: Requirement; Maintenance; Growth; Lysine; Nitrogen; Atlantic salmon fry

1. Introduction

Lysine (Lys) is considered to be the first limiting amino acid (AA) in fish as in higher vertebrates. Precise estimations of the lysine requirement are needed for evaluating the quality of the dietary proteins supplied. This is tremendously important for aquaculture where fish meal, which makes up the bulk of the dietary protein supply, has and needs to be replaced by plant proteins which often show deficiencies in some indispensable amino acids (IAA) (Hardy and Barrows, 2002; Wilson, 2002).

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Considerable variation in estimates of the quantitative dietary Lys requirements for fish expressed either as a percentage of the diet or as percentage of the dietary protein has been reported in the piscine literature (Mambrini and Kaushik, 1995a; Hauler and Carter, 2001a; Wilson, 2003). Variations in Lys estimates in dose-response experiments are typically regarded as the result of inherent methodological problems (Cowey and Luquet, 1983; Cowey and Tacon, 1983; Cowey, 1988, 1994; Wilson, 1989; Dabrowski and Guderley, 2002). Given that in dose-response experiments growth is measured against dietary Lys concentration, it remains unknown as to what degree of growth is the result of different metabolic efficiencies linked to variable Lys intake or to the variations in Lys intake per se (Hauler and Carter, 2001b). Therefore, some authors have suggested that partitioning IAA requirements between maintenance and protein accretion would give more insight into the metabolic use of these IAAs (Hurwitz and Bornstein, 1973; Hurwitz et al., 1978, 1983; Cook, 1991; Shearer, 1995; Fournier et al., 2002).

While data on protein requirements for maintenance are available for a few freshwater (Kaushik et al., 1981, 1991, 1995; Kaushik and Luquet, 1984; Gatlin et al., 1986; Kaushik and Gomes, 1988; Ng and Hung, 1995; Mambrini, 1996) and marine species (Birkett, 1969; Lupatsh et al., 1998; McGoogan and Gatlin, 1998; Fournier et al., 2002), data on the maintenance requirements for Lys are scarce and only available for rainbow trout (Rodehutscord et al., 1997). The maintenance requirement is defined as the amount of an AA to be ingested by fish to maintain its N equilibrium, which

Table 1
Composition of the experimental diets fed to Atlantic salmon fry

Diets[a]	PF	LP	LPD	MP	MPD	HP	HPD
Components (g kg^{-1} diet)							
Cod meal[b]	0	82	82	177	177	268	268
L-amino acid mixture[cd]	0	83	85	179	181	272	275
Modified starch[e]	436	220	220	160	160	102.	102
Glucose[f]	150	205.	203	102	100	4	0
Sucrose[f]	50	50	50	25	25	0	0
Cod liver oil[g]	199	196	196	192	192	189	189
Soya lecithin[h]	40	40	40	40	40	40	40
Vitamin mix[i]	10	10	10	10	10	10	10
Mineral mix[j]	65	65	65	65	65	65	65
Bacteriological agar[k]	10	10	10	10	10	10	10
Carboxymethylcellulose[k]	20	20	20	20	20	20	20
α-cellulose[k]	20	20	20	20	20	20	20
Chemical composition (g kg^{-1} diet)							
Moisture	6.06	13.06	11.97	15.37	14.05	8.74	5.27
Crude protein	1.40	17.86	18.61	38.77	38.23	53.37	54.11
Crude fat	23.50	23.5	23.5	23.5	23.5	23.5	23.5
Ash	5.20	6.02	6.37	7.20	7.32	6.98	7.48
Gross energy (MJ kg^{-1} DM)	20.29	21.04	21.0	21.91	21.90	22.75	22.70

DM, dry matter.
[a]PF protein-free diet; LP low protein diet, LPD low protein deleted diet; MP medium protein diet; MPD medium protein deleted diet; HP high protein diet, HPD high protein deleted diet.
[b]91.5% crude protein Toro Food Division, Rieber and Søn (Bergen, Norway) C-0271.
[c]All amino acids were provided by Ajinomoto Ltd., Tokyo, Japan.
[d]For the composition of L-amino acid mixtures see Table 2.
[e]Merigum, Amylum, Alost, Belgium.
[f]Fluka Chemica, Buchs, Switzerland.
[g]Federa, Brussels, Belgium.
[h]Cereal, Beerzel, Belgium.
[i]mg kg^{-1} dry diet : vitamin A acetate (1500 IU mg^{-1}): 10,000 IU, vitamin D3 (40,000 IU mg^{-1}): 4000 IU; vitamin E (1 IU mg^{-1}): 342 IU; vitamin K1: 22 mg; vitamin B1 56 mg; vitamin B2: 120 mg; vitamin B6: 45 mg; vitamin B12: 0.3 mg; niacine: 300 mg; biotin: 1 mg; folic acid: 15 mg; myo-inositol: 500 mg; D-panthotenic acid : 141 mg; choline chloride: 3000 mg; vitamin C: 1200 mg; canthaxantin (10%): 70 mg; butylated hydroxytoluene (BHT): 15 mg; butylated hydroxyanisole (BHA): 15 mg; 4-amino-benzoic acid: 400 mg; alpha-cellulose: 3238 mg.
[j]g kg^{-1}: $CaHPO_4 \cdot 2H_2O$: 295.56 g; $Ca(H_2PO_4)2 \cdot H_2O$: 217 g; $NaHCO_3$: 94.5 g; $Na_2SeO_3 \cdot 5H_2O$: 0.011 g; KCl: 100 g; NaCl: 172.37 g; KI: 0.2 g; $MgCl_2$: 63.7 g; $MgSO_4$: 34.3 g; $MnSO_4 \cdot 4H_2O$: 2 g; $FeSO_4 \cdot 4H_2O$:10 g; $CuSO_4 \cdot 5H_2O$: 0.4 g; $ZnSO_4 \cdot 7H_2O$: 10 g.
[k]Agar:A-5306, Sigma, St Louis, MO, USA; carboxymethylcellulose: C-4888, Sigma; α-cellulose, Sigma: C-8002.

means that no net synthesis or net breakdown of body protein takes place. In practice, it is interpolated from the relationship between N gain and IAA intake (*x*-intercept). The relationship is described after measuring the N gain of animals fed diets supplying increasing levels of the IAA tested, assuming that when a single dietary AA is limiting, the rate of body protein accretion is related to the supply of that AA. Two types of diets may be formulated. For example, the approach used by Rodehutscord et al. (1997) consisted in a classical empirical dose-response relationship where the limiting AA is gradually increased in a basal *iso*-nitrogenous diet. However, a deficiency of one AA does not always lead to a weight lost, as observed with pigs for arginine, histidine or leucine (Baker et al., 1966; Baker and Allee, 1970) or with fish for arginine (Buentello and Gatlin, 2000; Fournier et al., 2002). In the presence of a well balanced amount of the other AAs, AA endogenous biosynthesis (in the case of arginine) or even a weak lysis of body proteins may supply an amount of deficient AA which is enough to ensure a compensatory protein synthesis. A second approach, called IAA deletion method, has been developed in pigs (Fuller et al., 1989). It consists of deleting individual AA from a mixture of all AAs, assuming a linear dose-response relation between the dietary supply of the limiting AA and the N retention of the animal. This approach has been successfully adapted to fish (Mambrini and Kaushik, 1995b; Mambrini and Seudre, 1995; Fournier et al., 2002; Rollin et al., 2006). It allows the simultaneous determination of protein and IAA requirements. Mambrini and Kaushik (1995b) estimated that the maintenance requirement for sulphur AAs of rainbow trout was about two-fold higher than those of terrestrial omnivores. More recently, Fournier et al. (2002) have estimated that the arginine maintenance requirement was very low in four species of teleosts and suggested that fish were able to synthesize endogenous arginine to maintain whole-body N balance. The factorial approach treats a dietary AA requirement as the sum of its physiological compounds (D'Mello, 2003).The application of this approach to estimate lys requirements for fish necessitates quantitative data on the Lys maintenance requirements. This protocol has been applied to 10 to 100 g fish but has never been tested in fry. Fish growth is extremely high in the early life stages. It can be inferred that the partitioning of the requirement between maintenance and growth is rapidly changing with the age of the fish.

The purpose of the present study was to evaluate protein and Lys requirements of Atlantic salmon at an early developmental stage for maintenance and for tissue protein accretion by adapting the IAA deletion method.

2. Materials and methods

2.1. Experimental diets

Following the general method of Fuller et al. (1989), seven semi-purified diets were formulated: one protein-free diet (PF) and diets with three graded N levels each with two Lys levels (Table 1). Non-protein energy was supplied to prevent any energy limitation. The diets were based on cod meal, digestible carbohydrates, fish oil, vitamins and minerals (Table 1) and a mixture of crystalline AAs (Table 2),. The N content of the other diets was either low (L, 2.9% DM), medium (M, 6.2% DM) or high (H, 8.5% DM). Two types of diets with the same N content were formulated. The AA composition of three diets was balanced (LP, MP, HP), patterned (g 16 g^{-1} N) following the AA composition of the cod meal protein which reflects the salmon AA requirements (Rollin et al., 2003a; Wilson, 2003; Tables 2 and 3). For the other three diets, Lys was specifically removed. Lys·HCl was totally omitted from the low N diet (LPD), and 50% of Lys·HCl was removed from the medium (MPD) and high (HPD) N diets (Tables 2 and 3). N content of these diets was corrected by adding a mixture of dispensable

Table 2
Composition of the L-amino acid[a] mixtures (g kg^{-1} diet) used in the semi-purified diets containing graded lysine levels (g kg^{-1} dry diet)

Diets[b]	PF	LP	LPD	MP	MPD	HP	HPD
Lysine level...	0.1	13.8	7.1	32.2	22.5	49.5	32.2
L-arginine	0	4.72	4.72	10.23	10.23	15.54	15.54
L-histidine	0	1.61	1.61	3.49	3.49	5.30	5.30
L-isoleucine	0	3.54	3.54	7.66	7.66	11.64	11.64
L-leucine	0	6.12	6.12	13.27	13.72	20.16	20.16
L-lysine·HCl	0	8.65	0	18.74	9.37	28.47	14.23
L-methionine	0	2.73	2.73	5.92	5.92	8.99	8.99
L-cystine	0	0.84	0.84	1.81	1.81	2.75	2.75
L-phenylalanine	0	3.08	3.08	6.67	6.67	10.14	10.14
L-tyrosine	0	2.71	2.71	5.87	5.87	8.91	8.91
L-threonine	0	3.43	3.43	7.43	7.43	11.29	11.29
L-tryptophan	0	0.90	0.90	1.96	1.96	2.97	2.97
L-valine	0	3.85	3.85	8.34	8.34	12.67	12.67
L-alanine	0	5.64	7.36	12.23	14.10	18.57	21.41
L-aspartic acid	0	5.66	8.81	12.27	15.68	18.64	23.82
L-asparagine·H_2O	0	3.94	3.94	8.54	8.54	12.97	12.97
L-glutamic acid	0	7.32	10.34	15.88	19.14	24.12	29.09
L-glutamine	0	5.33	5.33	11.56	11.56	17.56	17.56
Glycine	0	4.7	5.29	10.18	10.82	15.47	16.44
L-proline	0	2.93	3.06	6.33	6.48	9.63	9.85
L-serine	0	4.79	7.12	10.39	12.91	15.79	19.62

[a]All amino acids were provided by Ajinomoto Ltd., Tokyo, Japan.
[b]PF protein-free diet; LP low protein diet, LPD low protein deleted diet; MP medium protein diet; MPD medium protein deleted diet; HP high protein diet, HPD high protein deleted diet.

amino acids (DAA) in the same proportion as in the whole-body protein, since large amounts of a single dispensable amino acid reduce the performance of fish (Mambrini and Kaushik, 1994). The diets were manufactured as described in Rollin et al. (2003b); they were extruded, freeze-dried and stored at −20 °C until use. In such diets, absorption efficiencies of AAs can be assumed to be 100% (Espe et al., 1992; Espe, 1993).

2.2. Animals

This experiment was approved by the Belgian Experimental and Welfare Committee according to the EC Directive applied to vertebrate animals. Anadromous Atlantic salmon (*Salmo salar* L.) eyed (embryonic) eggs, of partially domesticated origin, were supplied to our laboratory hatchery (M. Huet Fish Culture Laboratory, Université catholique de Louvain) by a commercial fish farm (Saumons et truites des Monts d'Arrée, Huelgoat, Finistère, France). The size of the eggs (mean diameter) was 6 mm just before hatching. Fry were reared in our laboratory hatchery from eggs to the beginning of the experiment according to Rollin et al. (2003b) and weighed 170 mg at first feeding (40 days after hatching).

We conducted a pre-experimental phase to adapt the fish to semi-synthetic diets. It consisted of a three-week period where the fry were fed the HP diet. During this pre-experimental period, the fry were kept in a single tank and were continuously fed to a slight excess by an automatic feeder 7 days a week. The daily mortality was below 0.1%.

At the end of the pre-experimental phase and after 36 h of food deprivation, the groups of salmon fry were weighed, counted and the individual initial average body weight was calculated (1.46±0.01 g, (W_i)±S.E.), They were then sorted according to their body weight (March et al., 1985) and randomly distributed amongst 21 indoor aquaria (0.40×0.24×0.20 m) of 15 l (65 fish per tank). Each test diet was randomly allocated to three aquaria. Three more aquaria were each filled with 65 fish, which were killed (excess ethylene glycol mono-phenylether) at the beginning of the experiment, and kept frozen (−20 °C) for chemical analyses. Biomass density was in accordance with optimal growth conditions for that species. Water temperature was set near the optimum (14.7±0.3 °C). Water quality, water flow rate

Table 3
Amino acid composition of the experimental diets (g kg^{-1} diet)

Diets[a]	PF	LP	LPD	MP	MPD	HP	HPD	Requirement
Lysine level[b]...	0.1	13.8	7.1	32.2	22.5	49.5	32.2	
Arginine	0.17	9.36	10.03	23.15	21.92	32.77	29.51	18.2
Histidine	0.05	3.17	3.45	7.59	7.09	10.66	10.96	6.7
Isoleucine	0.12	6.07	7.00	14.39	13.89	22.16	20.57	9[c]
Leucine	0.24	11.10	11.54	23.95	22.87	36.74	32.66	14[c]
Lysine·HCl	0.15	13.74	7.08	32.20	22.48	49.46	32.07	23.9
Methionine	0.07	5.13	5.41	12.08	11.51	17.48	16.37	15.4[d]
Cystine	<0.01	1.08	1.22	3.05	2.75	3.32	3.60	
Phenylalanine	0.14	5.82	6.19	12.61	12.39	18.87	17.82	25.1[e]
Tyrosine	0.11	5.34	5.78	12.28	11.86	17.62	16.09	
Threonine	0.11	7.09	7.37	16.27	15.60	22.85	21.67	12.1
Tryptophan	nd	nd	nd	nd	nd	nd	nd	3.3
Valine	0.15	7.05	7.75	16.59	15.22	24.61	23.15	12[c]
Alanine	0.20	11.09	12.05	22.30	20.71	31.38	33.57	
Aspartic acid	0.26	17.13	21.63	41.85	39.93	55.27	56.80	
Glutamic acid	0.44	26.37	30.30	60.27	61.97	86.28	81.72	
Glycine	0.22	9.24	10.27	21.15	20.88	29.87	28.64	
Proline	0.17	5.74	6.12	13.30	13.01	18.80	17.55	
Serine	0.13	8.53	10.71	18.92	21.15	27.48	29.18	

nd, not detectable by analytical method used.
[a]PF protein-free diet; LP low protein diet, LPD low protein deleted diet; MP medium protein diet; MPD medium protein deleted diet; HP high protein diet, HPD high protein deleted diet.
[b]g kg^{-1} dry diet.
[c]According to NRC (1993).
[d]Methionine+cystine.
[e]Phenylalanine+tyrosine.

and light regime were as previously described (Rollin et al., 2003a). Mortality, if any, was recorded daily. At the end of the 28-day feeding trial, and after 36 h of food deprivation, the groups were weighed, counted and the individual final average body weight was calculated. All fish were then killed (excess ethylene glycol monophenylether) and kept frozen (−20 °C) for determination of the final carcass chemical composition.

2.3. Feeding

During the feeding trial, the diets were fed manually 6 days per week two times per day (0900 and 1700 h). Fish were fed to visual satiation and feed intake was recorded after each meal.

2.4. Sampling and chemical analysis

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

The diets were analysed for dry matter (DM), crude protein (N × 6.25), crude fat, ash and gross energy (Table 1) and AA contents (Table 3). Fish carcasses were analysed for DM, crude protein, ash and AA contents. Proximate analyses of samples were conducted using standard methods (AOAC, 1995): DM by drying at 105 °C for 24 h, ash by incineration at 550 °C for 12 h, crude protein by the Kjeldahl method after acid digestion, crude fat by a simple 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 composition analysis multiplied by 6.25.

We analysed total AAs in diets and carcasses. Samples were hydrolysed by boiling 500 mg of homogeneous sample in 370 ml of azeotropic 6 M-HCl for 20 h under reflux and under a continuous nitrogen flow. The hydrolysate was made up to 500 ml and filtered through a sintered glass filter. An aliquot of the filtrate was evaporated to dryness at 40 °C in a rotavapor system (Büchi Rotavapor R-114; Flawil, Switzerland). Twenty-five ml of a lithium acetate injection buffer solution (pH 2.2) was added to redissolve the residue. After ultrafiltration (0.22 μm) 50 μl of sample was injected into the analyser (Biotronik LC3000 AA). The technique is based on the separation of the AAs using cation exchange chromatography (using 5 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-AAs and at 440 nm for the imino acids (Ooghe, 1983). Tryptophan cannot be measured with this procedure.

2.5. Calculations

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

Feed efficiency (FE) = $(W_f - W_i)/D_i$;

Live weight gain (g fish^{-1}) = $W_f - W_i$;

Feed intake (FI, g DM fish^{-1}) = $D_i/[1/2 \times (n_f + n_i)]$;

Protein efficiency ratio (PER) = $100 \times [(W_f - W_i)/(D_i \times N_d \times 6.25)]$;

Daily growth coefficient (DGC, %) = $100 \times [(W_f^{1/3} - W_i^{1/3})/\Delta t]$;

Nitrogen (or Lys) intake (mg fish^{-1}) = $1000 \times [FI \times N_d$ (or $Lys_d)]$;

Table 4
Mean initial and final body weight, daily growth coefficient (DGC), feed efficiency (FE) and protein efficiency ratio (PER) of Atlantic salmon fry fed on graded levels of lysine for 28 d[a]

Diets[b]	Dietary lys (g kg^{-1} DM)	Initial weight (g)	Final weight[c] (g)	DGC[c] (%)	FE[c] (g/g DM)	PER[c]
PF	0.1	1.45±0.01	1.26±0.01^{z}	–	–	–
LP	13.8	1.46±0.01	1.83±0.02^{y}	0.32±0.02^{z}	0.49±0.03^{y}	2.75±0.17^{xz}
LPD	7.1	1.45±0.01	1.69±0.04^{y}	0.21±0.02^{z}	0.32±0.03^{z}	1.74±0.18^{y}
MP	32.2	1.47±0.01	2.24±0.00^{x}	0.62±0.01^{y}	0.86±0.01^{x}	2.21±0.04^{xyz}
MPD	22.5	1.48±0.01	2.25±0.05^{x}	0.61±0.03^{y}	0.85±0.01^{x}	2.22±0.03^{xyz}
HP	49.5	1.49±0.00	2.60±0.10^{v}	0.83±0.06^{x}	1.01±0.06^{vx}	1.87±0.12^{y}
HPD	32.1	1.46±0.01	2.66±0.05^{v}	0.90±0.04^{x}	1.12±0.04^{v}	2.07±0.07^{y}

[a]Means±SE of three replicates and values within the same column with different superscripts are significantly different ($P<0.05$).
[b]PF protein-free diet; LP low protein diet, LPD low protein deleted diet; MP medium protein diet; MPD medium protein deleted diet; HP high protein diet, HPD high protein deleted diet.
[c]Linear ($P<0.01$) response.

Table 5
Body weight, protein, lysine (Lys) accretion and nitrogen retention in Atlantic salmon fry fed on graded levels of Lys for 28 days[a]

Diets[b]	Dietary lys	Intake		Accretion			Efficiency	
	Level	Dry diet[c]	Lys[c]	Body weight[c]	Nitrogen[c]	Lys[c]	NRE[d]	LYSRE[d]
	(g kg^{-1} DM)	(g DM fish^{-1})	(mg fish^{-1})	(g fish^{-1})	(mg fish^{-1})	(mg fish^{-1})	(%)	(%)
PF	0.1	0.41±0.00^{z}	0.06±0.00^{z}	−0.19±0.02^{z}	−5.71±0.08^{z}	−4.31±0.05^{x}	–	–
LP	13.8	0.76±0.01^{y}	10.47±0.12^{x}	0.37±0.02^{y}	5.74±0.81^{y}	2.78±0.46^{y}	26±3.87^{x}	26±4.16^{x}
LPD	7.1	0.74±0.02^{y}	5.22±0.12^{y}	0.24±0.03^{y}	3.19±0.54^{y}	0.17±0.28^{y}	14±2.41^{y}	3±5.48^{z}
MP	32.2	0.91±0.00^{x}	29.18±0.12^{u}	0.78±0.01^{x}	17.32±0.75^{x}	8.44±0.42^{vx}	31±1.27^{x}	29±1.34^{x}
MPD	22.5	0.91±0.05^{x}	20.39±1.04^{v}	0.77±0.04^{x}	16.79±0.76^{x}	8.23±0.41^{x}	30±0.80^{x}	40±0.82^{x}
HP	49.5	1.11±0.03^{v}	54.98±1.56^{s}	1.11±0.10^{v}	25.77±2.82^{v}	13.90±1.58^{u}	27±2.33^{x}	25±2.18^{y}
HPD	32.2	1.07±0.02^{v}	34.33±0.80^{t}	1.20±0.06^{v}	25.39±1.10^{v}	11.55±0.34^{uv}	27±0.90^{x}	34±1.71^{xy}

[a]Means±SE of three replicates and values within the same column with different superscripts are significantly different ($P<0.05$).
[b]PF protein-free diet; LP low protein diet, LPD low protein deleted diet; MP medium protein diet; MPD medium protein deleted diet; HP high protein diet, HPD high protein deleted diet.
[c]Linear ($P<0.01$) response.
[d]NRE, nitrogen retention efficiency (100× nitrogen gain/nitrogen intake); LYSRE, lysine retention efficiency (100× lysine gain/lysine intake).

Nitrogen (or Lys) gain (mg fish^{-1}) = $1000\times[W_f\times N_f$ (or $Lys_f) - W_i\times N_i$ (or $Lys_i)$];

Nitrogen (or Lys) retention efficiency (NRE, LYSRE, %) = 100 × (nitrogen (or Lys) gain / nitrogen (or Lys) intake).

Where: W_f and W_i are the average final and initial fresh body mass (g); Δt is the duration of the feeding period (days); D_i, is the dry diet distributed 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; N_f (or Lys_f) and N_i (or Lys_i) are the nitrogen (or Lys) contents of the carcass at the end and at the beginning of the experimental period (g g^{-1}), N_d and Lys_d, are the nitrogen and Lys contents of the experimental diets (g g^{-1} DM).

2.6. 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. The relationship

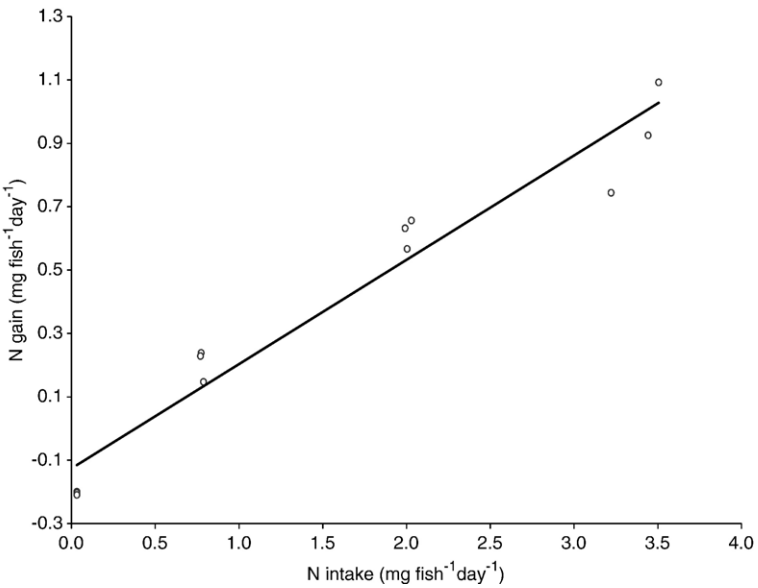

Fig. 1. Best-fit regression of nitrogen gain (mg fish^{-1} day^{-1}) vs. nitrogen intake (mg fish^{-1} day^{-1}) was $Y=-0.123$ (SE 0.05) + (0.328 (SE 0.025, $n=12$))X ($R^2=0.95$) for Atlantic salmon fry fed on graded levels of lysine. Each data point represents the mean gain of 65 fish per aquarium during a 28-day feeding period.

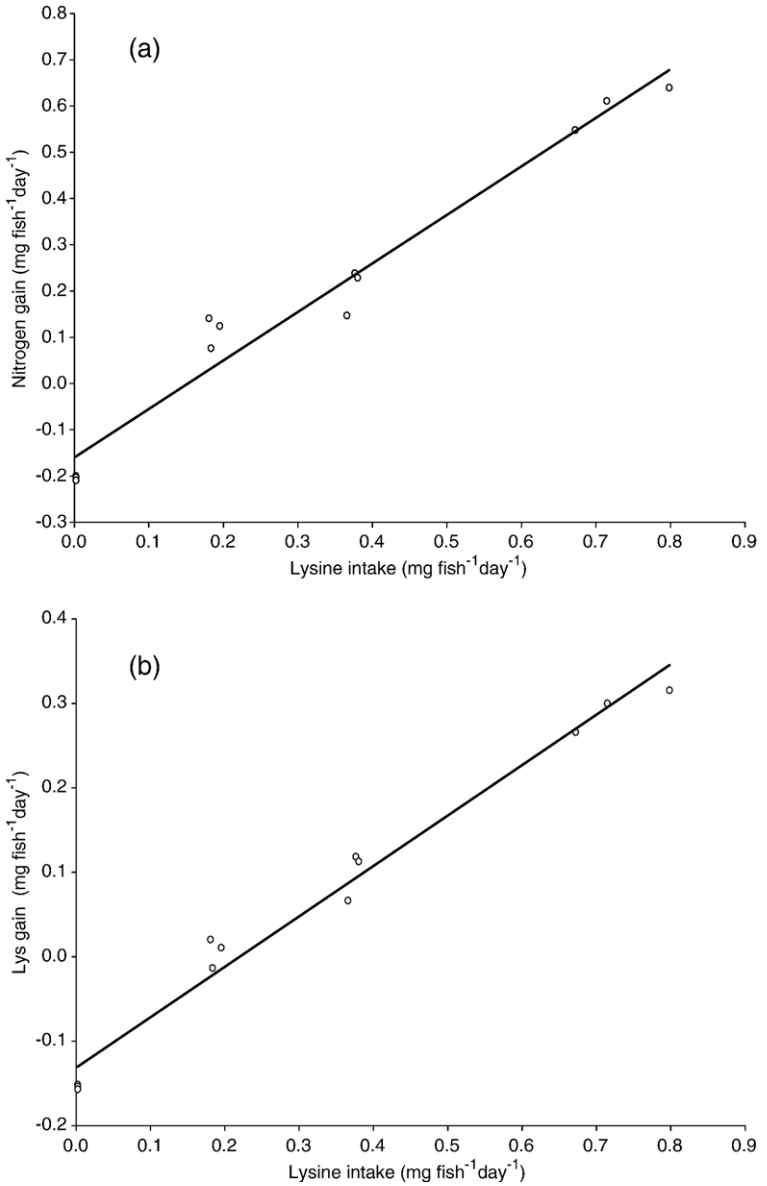

Fig. 2. Best-fit regression of (a) nitrogen gain (Y; mg fish^{-1}day^{-1}) vs. lysine intake (X; mg fish^{-1}day^{-1}) was described well by a straight-line fit: $Y=-0.159$ (SE 0.026)+(1.049 (SE 0.062; $n=12$))X ($R^2=0.97$) and (b) of lysine (Lys) gain (Y; mg fish^{-1}day^{-1}) as a function of lysine intake (X; mg fish^{-1} day^{-1}) was described well by a straight-line fit: $Y=-0.131$(SE 0.011)+(0.60 (SE 0.026; $n=12$))X ($R^2=0.98$) for Atlantic salmon fry fed on graded levels of lysine. Each data point represents the mean gain of 65 fish per aquarium during a 28-day feeding period.

between daily protein deposition and daily Lys or N intake was analysed using regression analysis ($Y=a+bX$). Maintenance was calculated as $-a/b$ and requirement per unit protein or N gain was $1/b$. Standard errors were computed for each regression analysis. Statistical analyses were performed using the Systat statistical package (version 5.2 Systat Inc. Evanson, IL, USA). Requirements for N were estimated from the regression obtained with PF, LP, MP and HP diets. For estimating the requirements for Lys, data obtained on the deficient diets (LPD, MPD, LP and PF diets) were used.

3. Results

3.1. Body weight gain and feed efficiency

Mortality was low (<1%) and unaffected by dietary treatments. No external pathological signs were observed, even in fish fed the PF diet.

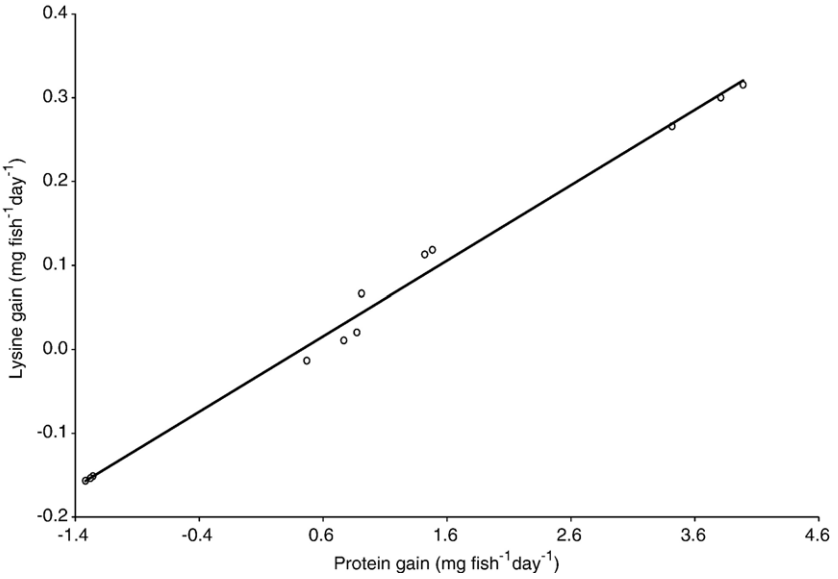

Fig. 3. Lysine gain (Y; mg fish^{-1} day^{-1}) versus protein gain (X; mg fish^{-1} day^{-1}). The linear-regression equation, $Y=-0.038$ (SE 0.006)+0.090 (SE 0.003)X ($R^2=0.99$) for Atlantic salmon fry fed on graded increments of lysine. Each data point represents the mean gain of 65 fish per aquarium during a 28-day feeding period.

During the feeding trial, all diets were well accepted by the fish, even for the PF and LPD diets (Tables 4 and 5). Feed intake, body weight (BW) gain and daily growth coefficient increased ($P<0.01$) with increasing dietary N level. Lys·HCl deletion within a protein level (50% in HP and MP diets, 100% in LP diet) did not significantly reduce feed intake in salmon fry. Although fish fed the PF diet showed a normal feeding behaviour during the trial, they significantly lost weight.

Feed efficiency (FE) increased significantly ($P<0.05$) with dietary N level (Table 4). At the low protein level, total deletion of L-Lys·HCl led to a significant reduction of FE ($P<0.05$). However, in medium and high N diet, the 50% reduction of the Lys content did not affect significantly FE ($P>0.05$).

Protein efficiency ratio (PER) was the highest in fry fed the LP diet and decreased significantly when dietary N content was higher ($P<0.05$). PER decreased significantly with the suppression of L-Lys·HCl ($P<0.05$) in the LPD diet compared to the LP diet. But at medium and high dietary N levels, such a decrease was not observed ($P>0.05$).

3.2. Protein requirement

The N loss in fry fed the PF diet was 0.20 (SE 0.003) mg fish^{-1} day^{-1} or 28.8 (SE 0.3) mg kg BW$^{-0.75}$ day^{-1}. The coefficient of the regression of N gain (Y; mg fish^{-1} day^{-1}) vs. N intake (X; mg fish^{-1} day^{-1}) was: $Y=-0.123$ (SE 0.05)+(0.328 (SE 0.025)X ($n=12$; $R^2=0.95$; Fig. 1). The slope value from best-fit linear-regression equation indicated that 33% of the N intake above maintenance was recovered as N in the whole-body N gain. The daily N requirement for zero N gain was calculated to be 0.375 mg fish^{-1} (or 54 mg kg BW$^{-0.75}$ day^{-1}) and the daily N requirement for growth was estimated to be 3.05 g N intake for 1 g N deposition.

N retention efficiencies were roughly the same for all diets, except for the LPD diet which showed a significantly reduced value compared to the LP diet (Table 5).

3.3. Lysine requirement

The partial reduction of dietary Lys level at medium and high protein levels did not lead to any significant reduction in N gain (Table 5), indicating that a 50% reduction in the Lys·HCl supply of MP and HP diets was not sufficient to impact growth. Following the recommendations of Fuller et al. (1989), we have estimated Lys requirements for maintenance and growth from the data obtained with LPD, MPD; LP and PF diets.

Accretion of whole-body N increased linearly ($P<0.05$) as dietary Lys levels increased (Fig. 2a). The regression of whole-body N gain (Y; mg fish^{-1} day^{-1}) vs. Lys intake (X; mg fish^{-1} day^{-1}) was well described ($n=12$; $R^2=0.97$) by a straight line fit: $Y=-0.159$ (SE 0.026)+1.049 (SE 0.062)X (Fig. 2a). The inverse value of the slope from best-fit linear-regression equation indicated that 953 mg Lys intake is required for 1 g N

Table 6
Nitrogen content (N, g 100 g^{-1} fresh fish) and amino acid composition of whole-body protein (AA, g 100 g^{-1} amino acids) in Atlantic salmon fry fed on different experimental diets containing graded levels of lysine (g kg^{-1} DM)

Diets[a]	PF	LP	LPD	MP	MPD	HP	HPD	SEM[b]
Lysine level...	0.1	13.8	7.1	32.2	22.5	49.5	32.2	
Nitrogen[c,d]	1.97	1.99	2.0	2.15	2.13	2.19	2.11	0.00
Amino acids[e]								
Arginine	6.99	6.62	6.67	6.64	6.79	6.84	6.87	0.05
Histidine	2.34	2.35	2.39	2.47	2.51	2.59	2.61	0.01
Isoleucine	4.16	4.00	3.06	3.37	3.84	4.26	4.35	0.10
Leucine	7.68	7.46	7.40	7.51	7.59	7.66	7.66	0.10
Lysine	8.60	9.01	8.45	8.73	8.75	8.97	8.79	0.02
Methionine	3.29	3.23	3.25	3.28	3.28	3.24	3.21	0.01
Cystine	0.65	0.61	0.66	0.67	0.63	0.64	0.61	0.01
Phenylalanine	4.25	4.18	4.15	4.24	4.28	4.33	4.33	0.01
Tyrosine	3.12	3.03	3.10	3.09	3.08	3.08	3.12	0.00
Threonine	4.59	4.61	4.85	4.94	4.84	4.79	4.78	0.02
Tryptophan	nd	nd	nd	nd	nd	nd	nd	
Valine	4.73	4.51	3.60	4.00	4.39	4.78	4.88	0.09
Alanine	6.42	6.30	6.84	6.64	6.55	6.38	6.38	0.03
Aspartic acid	9.98	9.95	10.58	10.50	10.22	9.97	9.90	0.06
Glutamic acid	15.37	15.32	16.02	15.90	15.54	15.24	15.26	0.02
Glycine	7.56	7.11	7.86	7.53	7.49	7.40	7.49	0.09
Proline	4.34	3.99	4.50	4.26	4.21	4.15	4.12	0.12
Serine	4.59	4.59	5.21	5.04	4.80	4.59	4.51	0.05

DM, dry matter.
nd, not detectable by analytical method used.
[a]PF protein-free diet; LP low protein diet, LPD low protein deleted diet; MP medium protein diet; MPD medium protein deleted diet; HP high protein diet, HPD high protein deleted diet.
[b]Standard errors of means calculated from residual mean squares in the analyses of variance.
[c]Mean values within a row with any superscript letters were not significantly different (one-way ANOVA and Tukey's multiple range test; $P>0.05$).
[d]N content of initial Atlantic salmon fry was 2.11(g/100 g fresh fish) and values are the mean of three replicates of 65 fish each.
[e]AA composition (g 16 g^{-1} N) of initial Atlantic salmon fry was as follow: arg 6.98, his 2.47, iso 4.28, leu 7.63, lys 9.24, met 3.25, cys 0.69, phe 4.31, tyr 3.18, thr 4.71, val 4.82, ala 6.2, asp 10, glu 15.37, gly 7.09, pro 4.14, ser 4.57. Values are the mean of duplicated samples obtained by pooling three replicates of 65 fish each.

gain (or 152 mg Lys intake for 1 g protein gain) in salmon fry. The parameters of the regression of Lys gain (Y; mg $fish^{-1}$ day^{-1}) vs. Lys intake (X; mg $fish^{-1}$ day^{-1}) were: $Y=-0.13$ (SE 0.01)+(0.60 (SE 0.03)X ($n=12$; $R^2=0.98$; Fig. 2b). The slope value indicates that the efficiency of Lys utilisation above maintenance in our conditions was 60% (SE 3). Extrapolating the linear-regression equation of N or Lys gain to the Y intercept indicated that zero Lys intake resulted in a net daily loss per fish of 123 μg whole-body N (or 23 mg N kg $BW^{-0.75}$ day^{-1}) and of 131 μg Lys (or 19 mg Lys kg $BW^{-0.75}$ day^{-1}). Daily Lys intake required for daily zero N gain was calculated to be 152 μg $fish^{-1}$ (or 20 mg Lys kg $BW^{-0.75}$ day^{-1}) and for daily zero Lys gain to be 217 μg $fish^{-1}$ (or 29 mg Lys kg $BW^{-0.75}$ day^{-1}). From these results, the maintenance Lys requirement, expressed as a proportion of the protein maintenance requirement, was calculated to be 6 g 16 g^{-1} N. Finally, from the linear-regression between live weight gain (Y; mg $fish^{-1}$ day^{-1}) and Lys intake (X; mg $fish^{-1}$ day^{-1}) ($Y=-3.66$ (SE 1.33)+44.41(SE 3.16)X; $n=12$; $R^2=0.95$), we estimated the overall Lys requirement per kg live weight gain (LWG, inverse value of the slope×1000) to be 22.5 (SE 0.32) g kg^{-1} LWG.

Fig. 3 shows that Lys gain (Y; mg $fish^{-1}$ day^{-1}) was a straight line function of protein gain (X; mg $fish^{-1}$ day^{-1}). The linear-regression equation, $Y=-0.038$ (SE 0.006)+0.090 (SE 0.003)X ($R^2=0.99$), showed that for each 1 g increase in protein gain, Lys gain increased by 90 mg. This suggests that Lys concentration in the whole-body protein accreted was a constant 9% at all levels of Lys intake. N content and AA composition of whole-body protein were not significantly affected ($P>0.05$) by the dietary Lys level (Table 6).

4. Discussion

The aim of the present work was to determine simultaneously the N and the Lys requirements for maintenance and for tissue growth in Atlantic salmon fry using an adaptation of the method proposed by Fuller et al.

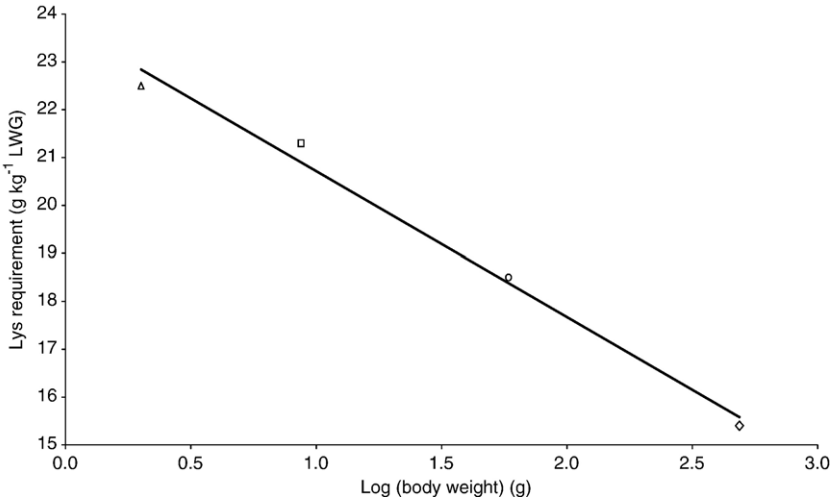

Fig. 4. Lysine (Lys) requirement (g kg^{-1} live weight gain) of Atlantic salmon to body weight (log (body weight) from the present study (△), Anderson et al., 1993 (□), Hauler and Carter, 2001 (○), Berge et al., 1998 (◇). The negative correlation between Lys requirement (g kg^{-1} LWG) and Log (body weight) (g) is described by the equation: $Y=23.75$ (SE 0.38) − (3.04 (SE 0.23, $n=4$))X ($R^2=0.99$).

(1989) for piglets. One of our major concerns was to ensure that fish fry will efficiently used semi-synthetic diets. In the present study, nitrogen retention efficiency was 33%, a value in the range of the data reported in Atlantic salmon fry (Peng et al., 2003; Rollin et al., 2003a,b). Crystalline AAs were used to monitor rigorously the AA composition of the experimental diets. Results in the literature show that the efficiency of utilisation of dietary crystalline AAs by fish including salmon is not always satisfactory (Espe and Njaa, 1991; Kim et al., 1991; Dabrowski and Guderley, 2002). However, this limitation may not affect all species of fish in the same way (Aoe et al., 1970). Rollin (1999) and Rollin et al. (2003a) demonstrated that crystalline AA mixtures with the AA composition of fish meal protein could replace fish meal protein without a significant difference in growth and N utilization efficiency in Atlantic salmon fry provided that fish were previously accustomized to a crystalline AA rich diet (423 g kg^{-1} crude protein). The growth performance of the fry observed in this study indicates that the semi-purified diets and the accustomization strategy of the present study were appropriate to determine nutrient requirements.

The 50% reduction of Lys·HCl in medium and high N diets did not have any significant adverse effect neither on voluntary dry matter intake nor on N gain between balanced and deleted diets. If we accept the concept that the reduction of a non-limiting IAA has no effect on N gain (Wang and Fuller, 1989), then we should conclude that the dietary Lys content was equal or in excess of the total Lys requirement of the fry in those diets and that the total Lys requirement of salmon fry is equal or less than the Lys concentration of the MPD diet, i.e. 5.89 g/16 g N. From a deletion experiment with *iso*-nitrogenous diets (445 g crude protein kg^{-1} DM) in salmon fry of similar size (mean body weight, 2.01 g^{-1} fish), we recently reported the optimum dietary Lys concentration for growing Atlantic salmon fry (Rollin et al., 2003a), i.e. 5.37 g/16 g N. Although this value is applicable only to the reported growth performance of the fry in that specific study, the similarity of growth performance between the two fry stocks used in these two experiments allows a direct comparison of these requirement values and thus confirms that the total Lys level in the MPD diet was equal or near the requirement of the fry in the present study. Furthermore, the application of the linear model as proposed by Fuller et al. (1989) for estimating the IAA requirements for maintenance in pigs would have been more reliable had we used diets (MPD and HPD) with a greater degree of reduction in the Lys·HCl levels of the MP and HP diets. This should be possible without increasing the proportion of crystalline AAs in the experimental diets.

The reliability of the linear model for estimating maintenance AA requirements is based on high growth and positive N balance. The period of measurement must be long enough to measure significant differences in protein growth but short enough to ensure that maintenance requirements will not vary during their evaluation. In the present experiment, the fry consumed all diets offered, almost doubled their initial weight and their growth performances were similar to the ones usually obtained in our laboratory (Peng et al., 2003;

Rollin et al., 2003a,b). Furthermore, N balance was calculated by comparative slaughter technique. This method has been shown to be feasible for small rainbow trout, selected for uniformity of body weight to limit inter-tank discrepancies, in 3-week experiments to assay differences in protein quality of feedstuffs (March et al., 1985). The current experiment lasted for 28 feeding days and the fry were sorted before the start of the experiment to minimize the inter-aquaria variations. Compared with the indirect estimations of N balance, the differential N carcass analysis has an additional advantage of not overestimating the N gain due to potential unrecorded N losses (Heger and Frydrych, 1985; Rollin et al., 2003a). For these reasons, we believe that the duration of our experiment was long enough to measure accurately N balance in salmon fry.

With longer measurement periods (>1 month), a decline of N excretion has been reported for fingerlings (Brett and Zala, 1975; Kaushik and Luquet, 1977; Kaushik, 1980), but also for Atlantic salmon and brown trout (*Salmo trutta*) fry (Rollin, 1999; Dumonceau, 2004), which may reflect metabolic adaptation and modified maintenance conditions (Beck and Gropp, 1995). Therefore, we believe that the duration of the experiments should not be longer than 1 month for the determination of IAA maintenance requirements in salmon fry. However, further studies are necessary to determine the influence of the duration of the experiments or fish size on the IAA requirements for maintenance in fish.

The N requirement for maintenance of Atlantic salmon fry grown at 14.7 °C determined in the current study (54 mg kg $BW^{-0.75}$ day^{-1}) is similar to the data reported in rainbow trout juveniles at a similar temperature (52 mg kg $BW^{-0.75}$ day^{-1} at 16 °C; Mambrini and Kaushik, 1995b) and superior to the value obtained at 17 °C by Fournier et al. (2002) (38 mg kg $BW^{-0.75}$ day^{-1}). Interspecies differences in N requirement for maintenance have been suggested in fish (Fournier et al., 2002). In the present study, dietary N was utilised for maintenance with 53% efficiency, indicating that a large part of the dietary supply for maintenance is oxidized.

According to Hauler and Carter (2001a), 33 dose-response Lys requirement experiments have been reported for fish, 20 species and 12 families. Twelve Lys requirement experiments have been conducted with salmonids, among which three with Atlantic salmon (Anderson et al., 1993; Berge et al., 1998; Hauler and Carter, 2001b) and six with rainbow trout (Kim and Kayes, 1982; Ketola, 1983; Walton et al., 1984; Lanari et al., 1991; Pfeffer et al., 1992; Rodehutscord et al., 1997). For these two species, dose-response dietary Lys requirements have shown considerable interspecies variation, which may not fully be accounted for by methodological discrepancies between studies (Hauler and Carter, 2001a). Alternatively, Hauler and Carter (2001a) suggest that Lys requirements of fish are not dissimilar when expressed relative to LWG. Indeed, expressed in this way, Lys requirements ranged between 15.4 and 21.3 g Lys kg^{-1} LWG for Atlantic salmon (Berge et al., 1998; Anderson et al., 1993, respectively) and between 15.7 and 21.1 g Lys kg^{-1} LWG for rainbow trout (Kim and Kayes, 1982; Lanari et al., 1991, respectively). In the present study, we reported a slightly higher value for Atlantic salmon fry (22.5 g Lys kg^{-1} LWG). This difference could be due to the smaller size of our fish (2 g vs. 8.7 g in Anderson et al., 1993; 58.5 g in Hauler and Carter, 2001b; 487 g in Berge et al., 1998). By gathering all the results obtained with Atlantic salmon so far, we have found that Lys requirement (g Lys kg^{-1} LWG) was negatively correlated to the body weight (Fig. 4), indicating that Lys requirement for fry is certainly higher than in older fish.

The approach proposed by Fuller et al. (1989) also assumes that the dietary supply of the tested IAA is limiting the animal performance. In our experiment, total Lys contents were clearly not limiting in diets HP, HPD and MP. Therefore, the N gain data for those diets were not included in the linear-regression analysis. In the present study we reported that 152 g Lys intake is required for 1 kg protein deposition in salmon fry, a value higher than the earlier results reported in our laboratory (118–129 g Lys kg^{-1} protein gain, Rollin et al., 2003a). This difference could be related with the fry stock origin. Recently, Peng et al. (2003) reported some inter-strains differences of growth rate, feed efficiency and N retention efficiency in Atlantic salmon fry. Another factor that could partly explain the differences obtained is the possible effect of the dietary protein level on efficiency of utilisation of the first limiting AA in the diet. In this context, in pigs, Langer and Fuller (1996) found that the efficiency of Lys utilisation was unaffected by dietary crude protein concentration. In fish, Cowey and Cho (1993) suggested that the efficiency of utilisation of the first limiting dietary IAA decreases with increasing dietary protein level. This conclusion is not supported by the present finding. Indeed, we observed in our study a slight increase of Lys retention efficiency (expressed as Lys gained. Lys $intake^{-1}$, Table 5) between the MP and HPD diets that contain the same concentration of Lys (32.2 g kg^{-1} DM). Although, the difference was not significant ($P>0.05$), this was an indication towards a better utilisation efficiency of Lys with higher protein level, also reported in rainbow trout

by Rodehutscord et al. (2000). Therefore, the higher N gain observed with the HPD diet compared to the MP diet is mostly explained by the higher feed intake observed with the high protein diet. In view of the current discrepancy on the effect of dietary protein level on the efficiency of Lys utilisation, it would be of great interest with regard to practical diet formulation to focus on this aspect with further dose-response studies, which cover a wider range in lysine concentration at different crude protein levels.

Reported values of Lys requirement for protein tissue accretion seem much lower in pigs (68 g kg^{-1} protein deposition, Fuller et al., 1989; 78 g kg^{-1} protein deposition, Heger et al., 2002) and chicks (83 g kg^{-1} protein deposition, Edwards et al., 1999) than the ones found in the current study. This could be partly related to the higher Lys content of carcass (+35%) found in Atlantic salmon (8.8 to 9.3 g 100 g^{-1} protein present study, Rollin, 1999; Wilson, 2003) compared to pig or chick carcass (6.5 g 100 g^{-1} protein Fuller, 1994; Edwards et al., 1999). Lys utilisation efficiency above maintenance obtained in the present study is also lower than the reported values for pigs (86%, Batterham et al., 1990; 96%, Fuller, 1994; Heger et al., 2002) and chicks (79%, Edwards et al., 1999). Then a lower Lys utilisation efficiency above maintenance in fish compared to mammals may also explain their relative higher requirement for protein accretion.

In the current experiment and based on N accretion, the Lys requirement for maintenance of Atlantic salmon fry grown at 14.7 °C was much higher (20 mg Lys kg $BW^{-0.75}$ day^{-1}) than the reported value for rainbow trout juveniles (11.2 mg Lys kg $BW^{-0.75}$ day^{-1}, Rodehutscord et al., 1997). With the current estimate of Lys maintenance requirement together with our recent estimate of the total requirement for digestible Lys of 2.39% of diet (106 mg kg $BW^{-0.75}$ day^{-1}; Rollin et al., 2003a), one can calculate that the maintenance requirement represented 19% of the total digestible Lys requirement. This value is higher than the values reported by Rodehutscord et al. (1997) and Pfeffer et al. (1992) in rainbow trout juveniles (4 and 10% of the total needs, respectively). It is also higher than the values published for pig (7% Fuller et al., 1989; 1.7%–4.5% Benevenga et al., 1994; 6% Heger et al., 2002). These differences may be explained by the strategies employed between studies or by the differences of fish species, size or age. In the approach chosen by Rodehutscord et al. (1997) the maintenance requirement was estimated from data obtained with *iso*-nitrogenous near optimum protein diets, where even a weak lysis of body proteins can supply the deficient AA, particularly Lys which concentration is high in fish whole-body proteins (9%, Table 6; Pfeffer et al., 1994; Kaushik and Cuzon, 1999). Then the growth reduction observed with such deficient diets may not fully reflect the metabolic rate of the specific AA tested. This may finally lead to the underestimation of the requirement for maintenance. This discrepancy can also be explained by interspecies differences and by the age of the fish used (100 g in the study of Rodehutscord et al., 1997).

It may be that, unlike what is commonly reported for higher vertebrates, maintenance requirements are higher for early stage fish and decreases with the age. This may be due to the particular use of AAs by fish, especially in early life stages (Conceicão et al., 1993; Rønnestad and Fyhn, 1993; Rønnestad and Naas, 1993; Sivaloganathan et al., 1998; Rønnestad et al., 1999; Finn et al., 2002). AAs are a major source of energy in fish (Van Waarde, 1983; Cowey and Walton, 1989), whatever their developmental stage. The importance of N requirement for maintenance noted in the present study (as high as for older fish) argues in favour of an intense utilisation of AAs for energy in fry. Early stage of fish has high potential rate of growth (Houde, 1989; Kamler, 1992; Conceiçào et al., 1997; Otterlei et al., 1999). Whole-body protein turnover is higher in fry than in older animals (Houlihan et al., 1986), but fry also require an abundant supply of dietary AAs for energetic purposes (Rønnestad and Naas, 1993; Finn et al., 1995, 2002). Furthermore, a higher AA catabolism was observed in early stage fish compared to older fish (Conceiçào et al., 2002). These results could possibly explain the higher maintenance Lys requirement value obtained for fry in the present study compared to larger fish (Rodehutscord et al., 1997) as well as the higher total Lys requirement observed for Atlantic salmon fry compared to salmon parr and post-smolts (Fig. 4). The evolution of the maintenance requirement for AAs with fish age needs further insights. This may have important implications on the dietary composition if the pattern of AA requirement for maintenance is different from the one for protein accretion.

In the present study, the proportion of the protein requirement for maintenance covered by Lys was estimated to be 6%. This value is close to the that published for sturgeon based on AA composition and N retention values of the whole fish (5 to 8%, Kaushik et al., 1991; Ng and Hung, 1995). If the AA pattern of the maintenance requirement may be comparable among fish species (Mambrini and Kaushik, 1995b), this pattern could differ from the one of the requirements for protein accretion in fish (Ng and Hung, 1995; Rodehutscord et al., 1997) as in other vertebrate species (Fuller et al., 1989; Benevenga et al., 1994). In the present study, Lys

contribution to the requirements for protein accretion was more than double than Lys contribution to the maintenance requirement. This suggests that estimates of AA requirements based solely on the AA pattern of body protein or based on the assumption that the maintenance requirements makes up only a negligible proportion of total requirement may lead to inadequate interpretations (Rodehutscord et al., 1997). This may have particular negative implications for fry.

In conclusion, the present experiment highlights that 1) 152 g Lys intake is required for 1 kg protein deposition in salmon fry; 2) Lys requirement for maintenance of Atlantic salmon fry is 20 or 29 mg Lys kg $BW^{-0.75}$ day^{-1} for zero N gain or zero Lys accretion, respectively; 3) the Lys pattern for maintenance (6 g 16 g^{-1} N) clearly differs from the Lys pattern of requirements for protein tissue gain (15 g 16 g^{-1} N); 4) N loss from fry given the PF diet suggest the dietary N was utilised for maintenance with 53% efficiency. Further studies are needed to determine for salmon fry a) the relationship between fish growth, fish size or life stage and Lys requirement for maintenance, b) the whole AA profile for maintenance and c) the effect of dietary crude protein level on IAA utilisation efficiency above maintenance.

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