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Effects of fish size and diet adaptation on growth performances and nitrogen utilization of rainbow trout (*Oncorhynchus mykiss* W.) juveniles given diets based on free and/or protein-bound amino acids

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ABSTRACT

The quality of dietary protein is an important factor influencing fish growth. It is usually assessed by amino acid (AA) composition, protein digestibility and protein utilization efficiency. Here it was investigated with rainbow trout (*Oncorhynchus mykiss* W.) juveniles 1) if the molecular form of the ingested nitrogen (free (F) AA, peptides or proteins) may also affect the dietary protein quality; 2) if this possible influence may be affected by juvenile size and adaptation to the diet; and 3) what is the optimum synthetic FAA to protein ratio. Two experiments were carried out at 15 °C (3 tanks/treatment) in which 1050 small juveniles (0.70 g) and 450 large juveniles (2.85 g) were randomly assigned to fifteen 15 l-tanks (70 fish/tank) and to fifteen 45 l-tanks (30 fish/tank), respectively. In both experiments, fish were fed twice a day to satiation for 9, 17 and 25 feeding days (3 periods) five isoenergetic and isonitrogenous (412 g crude protein/kg dry matter (DM)) diets containing graded levels of coated FAA, replacing 0, 25, 50, 75 or 100% (D0–D100) of the cod muscle meal, an intact protein. Compared to D0, growth rate (DGC, % per day) and feed intake (g DM/kg$^{0.75}$ per day) were significantly reduced only for small juveniles and large juveniles fed diets containing at least 50 and 75% of FAA, respectively. Protein deposition (g/kg$^{0.75}$ per day) was not significantly reduced for juveniles fed diets D25–D50, but well for juveniles fed diets D75–D100. The decrease of growth rate and protein deposition at FAA inclusion rate below 75% was largely explained by a reduction of voluntary feed intake while nitrogen retention efficiency was significantly reduced only for diet D100. The maximum FAA level that still ensured reasonable growth (85% of maximum) was 65%, at both juvenile sizes, after an adaptation period of 17 days. Larger juveniles tolerated higher FAA dietary levels than smaller juveniles, indicating ontogeny-related changes in FAA utilization efficiencies. Finally fish accustomed to FAA-rich diets were able to tolerate higher FAA dietary levels but the maximum FAA inclusion level was modulated by fish size. In conclusion, here we show that the dietary protein quality is dependent of the molecular form of the ingested nitrogen in rainbow trout juveniles and that this quality is modulated by the ratio of synthetic FAA to protein (or FAA inclusion rate), the adaptation of the fish to the diet and the fish size.

1. Introduction

The quality of dietary protein is an important factor influencing the growth performance of a fish. Dietary protein quality is usually assessed by amino acid (AA) composition, protein digestibility and protein utilization efficiency. Some observations have shown that the molecular form of the ingested nitrogen (free (F) AA, peptides or proteins) affects growth rate, AA absorption kinetics, the degree of whole-body AA oxidation and utilization for protein synthesis in fish as well as in mammals (Collin-Vidal et al., 1994; Dabrowski et al., 2003; Daenzer et al., 2001; Metges et al., 2000; Terjesen et al., 2006; Zhang et al., 2006), raising the question as to whether this variable may also affect protein quality. This question is of great economical and environmental importance in the context of fishmeal shortage, particularly when fishmeal is replaced by AA-deficient proteins that need to be supplemented with free (F) indispensable (I) AA, in order to meet the requirements of the targeted fish species (Cheng et al., 2003; Davies and Morris, 1997; Nang Thu et al., 2007, 2009; NRC, 2011; Watanabe et al., 1997).

Several authors have attempted to define the AA requirements for different fish species (Wilson, 2003). However, in many instances, the observed differences between studies exceeded credibility (Dabrowski and Guderley, 2002). For instance, tryptophane requirements of rainbow trout (*Oncorhynchus mykiss*) ranged from 0.5 to 1.4% and methionine requirements in salmonids were estimated from 2.2% in

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rainbow trout to 4.0% in chinook salmon (*O. tshawytscha*) (Akiyama et al., 1997). Several factors have been proposed to explain these large variations, being either nutritional or methodological (Bodin et al., 2007, 2009; Rollin et al., 2003a). According to Dabrowski and Guderley (2002), the questionable formulation of test diets to secure acceptability and maximize growth of fish is the single, most important factor resulting in discrepancies observed in published IAA requirements in fish. Diet formulation for IAA requirement are based entirely, or in variable proportions, on synthetic FAA (Bureau and Encarnação, 2006; Dabrowski and Guderley, 2002). This approach, presuming that these FAA are able to sustain close to optimal growth and are utilized with the same efficiency as the AA derived from dietary proteins, has been questioned by several authors (Dabrowski and Guderley, 2002; Nolles et al., 2008; Rollin, 1999). Therefore, it seems that more studies are required to test this hypothesis.

In 1970, Aoe and colleagues started a still ongoing discussion as to whether the nutritional value of FAA and protein of similar AA composition in the diet of rainbow trout is equivalent (Abboudi et al., 2006; Aoe et al., 1970; Barroso et al., 1999; Cowey, 1992, 1994; Cowey and Luquet, 1983; Cowey and Walton, 1988; Dabrowski and Guderley, 2002; Dabrowski et al., 2003; Espe and Njaa, 1991; Kim et al., 1991; Rodehutscord et al., 1995; Rollin et al., 2003a, 2006; Terjesen et al., 2006). Some authors concluded that FAA can replace protein-bound AA in diets of rainbow trout (Murai, 1992; Rodehutscord et al., 1995) while other obtained sometimes dramatically reduced growth performances when intact protein sources were substituted by FAA mixtures equivalent to their AA composition (Barroso et al., 1999; Cowey and Luquet, 1983; Cowey and Walton, 1988; Dabrowski et al., 2003; Terjesen et al., 2006). These contrasting results could possibly be related to differences of dietary FAA level, fish size and development stage as well as diet adaptation to these FAA-rich diets. Here we propose to investigate the effect of those three variables and to optimize the ratio of FAA to protein in experimental diets aimed at examining requirements for individual AA in rainbow trout juveniles.

The aim of this work was to study the effect of total or partial replacement of cod muscle meal protein, essentially composed of protein-bound AA, by mixtures of FAA of equivalent AA composition on growth performances and nitrogen deposition and utilization in rainbow trout at two juvenile sizes during three feeding periods. The maximum substitution levels that still support reasonable growth or nitrogen utilization efficiency was evaluated for both juvenile sizes at each period.

2. Materials and methods

2.1. Experimental diets

Five isonitrogenous (64 g N/kg DM) and isoenergetic (±18.75 MJ kg/DM) diets (Table 1) were formulated. The dietary N was supplied by gelatin and by cod muscle meal and/or FAA. The cod muscle meal, obtained from a commercial source (Toro Food Division, Rieber and Søn AS, Bergen, Norway), was obtained by freeze-drying muscle of Atlantic cod (*Gadus morhua*). Its nitrogen content is high (14.7% DM) and was reported earlier to be highly digestible (apparent digestibility of 94.5%) and to provoke high growth rates in salmonids (Abboudi et al., 2009; Rollin et al., 2003a). It contains mostly intact protein, FAA and peptide AA representing only 0.3% and 4.3% of the total AA, respectively (Espe, 1993). Diet D0 was the control diet and all the AA were in the form of cod muscle meal (40,5% DM) and gelatin (3% DM). The cod muscle meal was replaced at levels of 25, 50, 75, and 100% by a mixture of crystalline FAA (Table 2) of similar AA composition to produce diets D25, D50, D75 and D100. Since this substitution led to a progressive decrease of the dietary N content, the diets were made isonitrogenous by the addition of some more crystalline dispensable L-AA in the AA mixtures (2.1, 4.2, 6.3 and 8.3% DM in diets D25, D50, D75 and D100, respectively) in the following proportions (modified from Kim et al., 1991; g/kg): aspartic acid 191.7, asparagine 95.9, alanine 157.4, glutamic acid 183.7, glutamine 91.9, glycine 54.1, proline 12.6 and serine 212.7. The analytical indispensable AA composition of the five diets was equivalent among diets (Table 3). Sodium bicarbonate was added in the FAA mixtures at a similar proportion (about 3% by weight of the FAA mixture) with

Table 1
The substitution of cod muscle meal by a mixture of crystalline free amino acids of similar composition for rainbow trout (*Oncorhynchus mykiss*) juveniles: ingredients and chemical composition (g/kg dry matter $(DM)^{-1}$) for the five experimental diets.

Diet	D0	D25	D50	D75	D100
Components (g/kg DM)					
Cod muscle meal [a]	405.2	303.9	202.6	101.3	0.0
Free amino acids mixture [b]	0.0	104.7	209.4	314.3	419.0
Gelatin [c]	30.0	30.0	30.0	30.0	30.0
Cod liver oil [d]	169.4	173.0	176.5	180.1	183.6
Modified starch [e]	215.4	205.4	195.4	185.4	175.4
Saccharose [f]	30.0	30.0	30.0	30.0	30.0
Soya lecithin [g]	40.0	40.0	40.0	40.0	40.0
Vitamin premix [f,h]	10.0	10.0	10.0	10.0	10.0
Mineral premix [f,i]	40.0	40.0	40.0	40.0	40.0
$CaHPO_4$ [f]	10.0	10.0	10.0	10.0	10.0
Agar [c]	10.0	10.0	10.0	10.0	10.0
$NaHCO_3$ [f]	0.0	3.0	6.0	9.0	12.0
Carboxymethylcellulose [j]	40.0	40.0	40.0	40.0	40.0
Chemical composition					
Gross energy (MJ/kg DM)	18.7	18.8	18.7	18.7	18.8
Crude fat (%)	22.4	22.2	22.3	22.5	22.1
Crude protein (%)	41.0	41.7	42.2	40.4	41.4
pH	4.59	5.01	5.59	6.09	6.46

[a] Toro Food Division, Rieber and Søn AS (Bergen, Norway).
[b] Ajinomoto Ltd., Tokyo, Japan. See Table 2 for amino acids mixtures composition.
[c] Gelatin Sigma (St. Louis, MO, USA), G-6144; agar, Sigma A-5306.
[d] Federa, Brussels, Belgium.
[e] Merigum, AMYLUM, Alost, Belgium.
[f] Fluka Chemika, Buchs, Switzerland.
[g] Cereal, Beerzel, Belgium.
[h] The vitamin complex (per kg dry matter): vit. A 10,000 IU; vit. D3 4000 IU; vit. E 342 IU; vit. K3 22 mg; vit. B1 56 mg; vit. B2 120 mg; vit. B6 45 mg; vit. B12 0.3 mg; niacine 300 mg; biotine 1 mg; folic acid 15 mg; inositol 500 mg; pantotenic acid 141 mg; choline chloride 3000 mg; vit. C 1000 mg; canthaxantin 100 mg; butylated hydroxytoluene (BHT) 15 mg; α-cellulose 3238 mg.
[i] The mineral complex (g/kg mixture): $AlKO_8S_2 \cdot (1.5)\ H_2O$ 1.39, $CaCO_3$ 144.89; $Ca(H_2PO_4)_2 \cdot H_2O$ 385.43, $CoCl_2 \cdot 6\ H_2O$ 1.04, $CuSO_4 \cdot 5H_2O$ 0.60, KCl 132.50, KI 0.11, $MgSO_4 \cdot 7H_2O$ 48.89; $MnSO_4 \cdot H_2O$ 0.40; HNa_2O_4P 274.73, $Na_2O_3Se \cdot 5H_2O$ 0.05, $ZnCO_3$ 1.54, $C_6H_5FeO_7 \cdot H_2O$ 8.43.
[j] Carboxymethylcellulose, Merck (Darmstadt, Germany) 7687.5000.

Table 2
Amino acids supplied by increasing amounts of the free L-amino acid mixture in diets of small and large rainbow trout juveniles (g/kg diet).[a]

Diet	D0	D25	D50	D75	D100
Arginine	0.00	5.19	10.38	15.57	20.76
Histidine	0.00	1.67	3.34	5.01	6.67
Isoleucine	0.00	3.89	7.79	11.68	15.57
Leucine	0.00	6.77	13.53	20.30	27.07
Lysine·HCl	0.00	7.60	15.20	22.80	30.40
Methionine	0.00	2.78	5.56	8.34	11.12
Cystine	0.00	1.11	2.22	3.34	4.45
Phenylalanine	0.00	3.43	6.86	10.29	13.72
Tyrosine	0.00	2.97	5.93	8.90	11.86
Threonine	0.00	3.80	7.60	11.40	15.20
Tryptophane	0.00	1.11	2.22	3.34	4.45
Valine	0.00	3.99	7.97	11.96	15.94
Alanine	0.00	8.46	16.93	25.41	33.88
Aspartic acid	0.00	10.29	20.59	30.91	41.21
Asparagine·H_2O	0.00	4.90	9.79	14.69	19.59
Glutamic acid	0.00	12.36	24.72	37.11	49.47
Glutamine	0.00	6.63	13.26	19.88	26.51
Glycine	0.00	5.85	11.71	17.56	23.42
Proline	0.00	3.41	6.82	10.24	13.65
Serine	0.00	8.50	17.01	25.53	34.03
Σ	0.00	104.72	209.44	314.26	418.98

[a] For the details of diets and procedures, see Table 1.

that reported by Espe and Lied (1994). The experimental diets were produced (pellets of 1 and 1.6 mm) as previously reported (Rollin et al., 2003a) and more specifically, the crystalline-AA mixtures were coated with 1% agar, to hinder leakage, delay their digestive absorption and optimize their use for protein gain (Mambrini and Kaushik, 1994). After freeze-drying, the diets were stored at −20 °C until feeding or analysis.

2.2. Animals and feeding

The experiment was approved by the Animal Welfare Commission of the Université catholique de Louvain in accordance with the EU Directive concerning vertebrate laboratory animals. Rainbow trout (*Oncorhynchus mykiss*) of domestic origin were supplied to our laboratory hatchery (M. Huet Fish Culture Laboratory, Université catholique de Louvain, Louvain-la-Neuve, Belgium) as eyed diploid eggs by a commercial fish farm (La Fontaine aux Truites, Gerouville, Belgium). The juveniles were reared in our laboratory hatchery, from eggs to the beginning of the experiment, according to Rollin et al. (2003b). They were fed the Trouvit 000, 00, and 0 commercial diets (Trouw France SA, Fontaine-les-Vervins, France) up to the start of the experiment.

Fifty-one days after hatching, 1050 small rainbow trout juveniles (initial mean bodyweight ± standard deviation (SD): 0.70 ± 0.01 g) were randomly distributed to groups of 70 fish in 15 aquaria (0.4 × 0.24 × 0.2 m) of 15 l each. For the large juveniles, 94 days after hatching, 450 rainbow trout juveniles (initial mean bodyweight ± SD: 2.86 ± 0.02 g) were chosen. They were randomly distributed to groups of 30 fish in 15 aquaria of (0.6 × 0.3 × 0.3 m) 45 l each. For both sizes, temperature and water flow were maintained constant during the experiment, and were respectively 15 ± 1 (SD)°C and 2 l min^{-1}. An aeration system maintained the dissolved oxygen levels close to saturation (± 10 mg l^{-1}) and the light intensity was constant (± 150 lux). Each test diet was randomly allocated to the aquaria (three aquaria per diet and per size). In addition, three aquaria per size served as initial samples. These initial juveniles were weighed, killed with excess ethylene glycol monophenyl ether, and kept frozen (−20 °C) until chemical analysis.

The experiment was of twenty-five feeding days for the small juveniles and the large juveniles. Each group was fed manually twice a day (1000 h and 1800 h) to apparent satiation. The amount of feed distributed to each aquarium was recorded after each meal. During the meals, it was ensured that the pellets were eaten by the juveniles within a maximum of 15 s of contact with the water in order to minimize the leaching of crystalline AA into the water. Mortality was recorded daily. After 36 h of fasting, the juveniles were weighed by groups and counted every nine, eight and eight feeding days corresponding to three successive experimental periods.

At the end of the experiment, and after 72 h of fasting, the fish were weighed by groups and counted. The final mean bodyweight was calculated. All fish were killed with excess ethylene glycol monophenyl and kept frozen (−20 °C) for further determination of the final whole body chemical composition.

Table 3
Indispensable amino acid composition of the experimental diets (g/kg dry matter; mean ± SEM (n = 5)), along with data on relative indispensable amino acid requirements for optimum growth of rainbow trout according to the National Research Council (2011).

Diets	D0–D100	Requirement
Arginine	22.49 ± 0.33	15
Histidine	7.40 ± 0.22	8
Isoleucine	16.74 ± 0.51	11
Leucine	29.17 ± 0.30	15
Lysine	32.74 ± 0.49	24
Methionine	11.71 ± 0.33	11[b]
Cystine	4.45 ± 0.14	
Phenylalanine	15.21 ± 0.25	18 [c]
Tyrosine	12.13 ± 0.17	
Threonine	16.81 ± 0.21	11
Tryptophane	4.45[a]	3
Valine	16.59 ± 0.29	12
Σ	189.89	

[a] Value calculated on the basis of ingredient amino acid composition (Rollin, 1999).
[b] Methionine + cystine.
[c] Phenylalanine + tyrosine.

2.3. Sampling and chemical analysis

Initial and final fish whole bodies were freeze-dried, pulverized (particle diameter < 1 mm) and homogenized (Grindomix GM 200, Retsch, Haan, Germany), and finally kept frozen (−20 °C) until chemical analysis. The diets were analyzed for pH, DM, crude protein (N × 6.25), crude lipid, gross energy and the amino acid contents. The juveniles whole bodies were analyzed for the amount of DM, crude protein (N × 6.25) and crude ash. Proximate analyses of samples were conducted using the following conventional procedures (AOAC, 2005): dry matter 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 lipid using the acid-hydrolysis Soxhlet method. The gross energy of the diets was determined with an IKA-C-400 adiabatic calorimeter (Ika-Werk, Breisgau, Germany). The amino acid analysis of diets and fish samples was carried out using a Biotronik LC3000 amino acid analyzer as previously reported (Rollin et al., 2006). Tryptophan cannot be measured with this procedure, but was estimated on the basis of the amino acid composition of the ingredients (Rollin, 1999).

2.4. Calculations

The variables that were obtained or measured directly during and after the experiment were the following:

DI is the dry diet intake per fish (g DM/fish) during the experimental period;
NI is the N intake per fish (g N/fish);
n is the mean number of fish per aquarium, obtained by summing the number of fish per aquarium at the beginning and at the end of the experiment and then dividing by 2;
W_f and W_i are the average final and initial fresh body weights of fish (g/fish);
$feeding_d$ is the number of feeding days;
N_f and N_i are the mean N contents of the whole body juveniles at the end and at the beginning of the experimental period (g N/g).

Mean metabolic body weight (MBW in kg/fish) was calculated as $((W_f/1000)^{0.75} + W_i/1000)^{0.75})/2$.

The following response criteria were used to evaluate fish growth, feed intake and nutrient utilization:

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

$$\text{Daily growth coefficient, DGC (\%per d)} = 100 \times \left(W_f^{1/3} - W_i^{1/3}\right) \Big/ \text{feeding}_d;$$

$$\text{Protein gain (g/kg MBW per day)} = 6.25 \times (W_f \times N_f - W_i \times N_i)/(\text{MBW} \times \text{feeding}_d);$$

$$\text{Fat gain (g/kg MBW per day)} = (W_f \times \text{LIP}_f - W_i \times \text{LIP}_i)/(\text{MBW} \times \text{feeding}_d),$$

where

LIP is the lipid content in g/g body weight and it was calculated as: 1 − water content (g/g body weight) − ash (g/g body weight) − protein content (g N × 6.25/g body weight);

Voluntary feed intake, VFI (g DM/kg MBW per day) $= DI/(MBW \times feeding_d)$;

Nitrogen intake (mg/kg MBW per day) $= NI/(MBW \times feeding_d)$;

Protein in weight gain (g/kg) $= (1000 \times \text{protein gain}) \times (MBW \times feeding_d)/(W_f - W_i)$;

Fat in weight gain (g/kg) $= (1000 \times \text{fat gain}) \times (MBW \times feeding_d)/(W_f - W_i)$;

Feed efficiency, FE (g/g DM) $= (W_f - W_i)/DI$;

Protein efficiency ratio, PER (g/g protein) $= (W_f - W_i)/(NI \times 6.25)$;

Protein productive value, PPV (%) $= 100 \times (W_f N_f - W_i N_i)/NI$;

Fat productive value, FPV (%) $= 100 \times (W_f LIP_f - W_i LIP_i)/FI$,

where

FI is the lipid intake per fish (g fat/fish).

2.5. Data analysis

All data were subjected to one-way analysis of variance. Significant differences between treatments were tested using the Tukey's multiple range test and results of $P<0.05$ were deemed statistically significant. The following full ANOVA model was used to analyze simultaneously the results for the three periods (diet adaptation or time effect), the two initial juvenile sizes and the five substitution levels (diets): response parameter = adaptation effect + juvenile size effect + diet effect + (adaptation × juvenile size) + (adaptation × diet) + (juvenile size × diet) + (adaptation × juvenile size × diet) + aquarium [nested to (juvenile size × diet)] + aquarium [nested to (juvenile size × diet)] × adaptation + ε, where ε is the error term. Significant effects were tested with the Fisher F-test. The following simple ANOVA model was applied to each diet separately to analyze further the effects of the adaptation and juvenile size on the response criteria: adaptation effect + juvenile size effect + (adaptation × juvenile size) + ε. To determine the suitable substitution level and adaptation period for reasonable growth, a second degree polynomial model was fit to the data for each juvenile size and each period. Next, the maximum value of the response criterion of these polynomial curves, 85% of this value, and then the corresponding x values, were calculated. The highest of these x values gave an estimation of the substitution level that should allow 85% of the maximum of the chosen response criterion. The parameters of the polynomial models were estimated by the least-squares method for each response criterion. All calculations were carried out with the SAS/JMP statistical software (SAS Institute, Cary, NC, USA).

3. Results

3.1. Growth performances and voluntary feed intake

During the feeding trial, all diets were well accepted by the fish. Mean mortality rate was less than 0.02% per day and was not related to dietary treatments.

Fig. 1A shows the responses for the whole experimental duration of the daily growth coefficient (DGC, % per day) to dietary treatments. Significant differences were observed between diets D0 and D50, D50 and D75 for the larger juveniles and between diets D75 and D100 for both sizes of juvenile. Body weight gain (g/fish) was reduced by 71% and 48% from diets D0 to D100 ($P<0.05$), but only by 8% and 6% from diets D0 to D50 ($P>0.05$) for the smaller juveniles and the larger juveniles, respectively (Table 4, Fig. 1A). The dramatic reduction of growth in the smaller juveniles fed on D100 (−71%) was partly explained by a reduction of voluntary feed intake (−34%) and by a decrease of feed efficiency (−35%). By contrast, the reduction of growth observed in the larger juveniles fed on D100 (−48%) was mostly explained by a decrease of feed efficiency (−31%; $P<0.05$) and a moderate reduction of voluntary feed intake (−12%; $P>0.05$; Table 4).

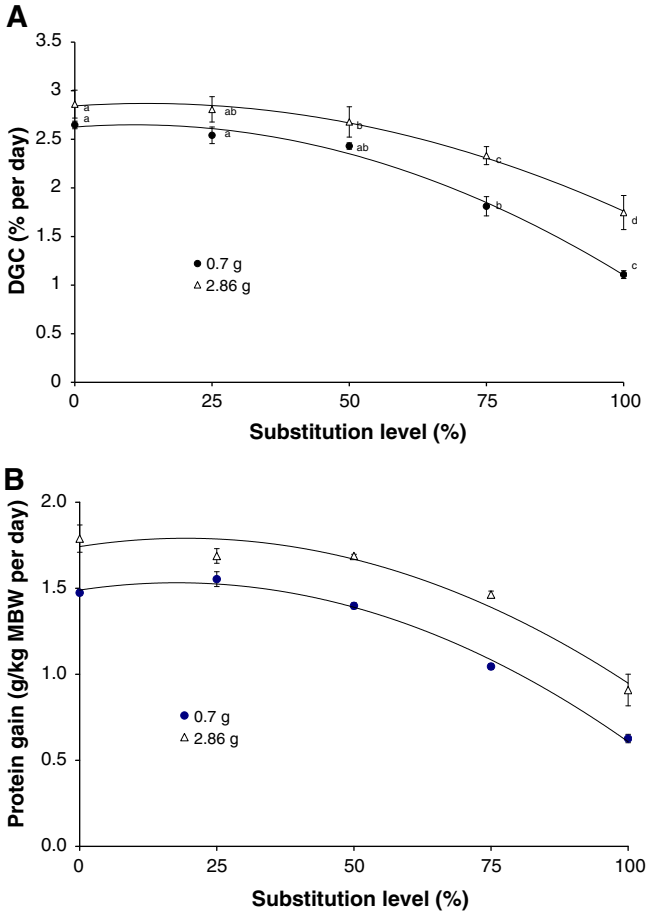

Fig. 1. (A) Daily growth coefficient (DGC) or protein gain (B) in rainbow trout of two different initial body weights (0.70 and 2.86 g), fed five diets (40.5% crude protein) with increasing levels of substitution of cod muscle meal by crystalline amino acids of a similar composition (25, 50 75 and 100%). Bars represent means ± standard errors (n = 3). Within a size, means with different letters are significantly different ($P<0.05$).

When the data was analyzed with the full ANOVA model, taking into account the DGC performances for the three periods ($r^2 = 0.94$), the only significant effect was the 'diet' effect ($P<0.001$), as well as the 'diet × adaptation' interaction ($P = 0.05$). This meant that for both juvenile sizes, the substitution level of intact cod muscle meal by FAA had an effect on their growth, and that this diet effect significantly changed with time (adaptation effect). A simple ANOVA model was used to analyze further the effect of the adaptation period on DGC (% per day). The three effects studied were 'adaptation time', 'juvenile size' and 'adaptation time × juvenile size' and the model was applied separately for each diet (Table 5). 'Adaptation time' was significant for diets D75 and D100. Indeed, the growth performances of juveniles fed D75 and D100 improved as the experiment progressed. 'Juvenile size' was significant for all diets, from D0 to D100, meaning that for all diets, the growth performance of the smaller juveniles differed from that of the larger juveniles. Finally, the 'adaptation time × juvenile size' interaction was significant only for D0 and D25, meaning that the evolution of juveniles' growth performance as the

Table 4
Initial and final body weight, body weight gain, daily growth coefficient (DGC; $g^{1/3}$ per day), voluntary feed intake (mg/kg metabolic body weight (MBW) per day), feed efficiency (FE; wet weight gain/dry feed intake), nitrogen intake (mg/kg MBW per day), protein efficiency ratio (PER; wet weight gain/protein intake), fat and protein (N × 6.25) depositions (g/kg MBW per day) and retention efficiencies (fat retention efficiency (FRE), fat gain/fat intake; nitrogen retention efficiency (NRE), nitrogen gain/nitrogen intake) of rainbow trout juveniles fed diets with increasing substitution of cod muscle meal by crystalline amino acids of similar composition showing mean values of three groups of 30 (2.86 g) or 70 (0.70 g) fish (SE, standard errors).[e].

Diets	Initial fish size (g)	D0		D25		D50		D75		D100	
		mean	SE	mean	SE	mean	SE	mean	SE	mean	SE
Final weight[f] (g/fish)	0.70	$3.73^{a,y}$	(0.07)	$3.52^{ab,y}$	(0.13)	$3.33^{b,y}$	(0.05)	$2.40^{c,y}$	(0.13)	$1.57^{d,y}$	(0.03)
	2.86	$9.73^{a,x}$	(0.48)	$9.56^{a,x}$	(0.45)	$9.12^{ab,x}$	(0.50)	$8.03^{b,x}$	(0.28)	$6.41^{c,x}$	(0.48)
Body weight gain[g] (g/fish)	0.70	$3.03^{a,y}$	(0.07)	$2.83^{ab,y}$	(0.14)	$2.63^{b,y}$	(0.04)	$1.71^{c,y}$	(0.12)	$0.87^{d,y}$	(0.04)
	2.86	$6.89^{a,x}$	(0.46)	$6.69^{a,x}$	(0.50)	$6.22^{ab,x}$	(0.49)	$5.13^{b,x}$	(0.25)	$3.57^{c,x}$	(0.42)
DGC[f] (% per day)	0.70	$2.65^{a,x}$	(0.04)	$2.54^{ab,y}$	(0.09)	$2.43^{b,x}$	(0.03)	$1.81^{c,y}$	(0.10)	$1.11^{d,y}$	(0.04)
	2.86	$2.86^{a,x}$	(0.14)	$2.81^{a,x}$	(0.13)	$2.68^{ab,x}$	(0.16)	$2.33^{b,x}$	(0.09)	$1.75^{c,x}$	(0.18)
Protein deposition[h] (g/kg MBW per day)	0.70	$1.38^{a,y}$	(0.15)	$1.55^{a,x}$	(0.07)	$1.40^{a,y}$	(0.03)	$1.04^{b,y}$	(0.03)	$0.63^{c,y}$	(0.04)
	2.86	$1.79^{a,x}$	(0.14)	$1.69^{ab,x}$	(0.07)	$1.69^{ab,x}$	(0.02)	$1.46^{b,x}$	(0.04)	$0.91^{c,x}$	(0.16)
Fat deposition[i] (g/kg MBW per day)	0.70	$1.44^{b,y}$	(0.05)	$1.60^{a,x}$	(0.05)	$1.45^{b,x}$	(0.00)	$1.09^{c,y}$	(0.09)	$0.62^{d,y}$	(0.04)
	2.86	$1.66^{a,x}$	(0.11)	$1.58^{ab,x}$	(0.06)	$1.53^{ab,x}$	(0.12)	$1.34^{bc,x}$	(0.11)	$1.10^{c,x}$	(0.06)
Feed intake[i] (g/kg MBW per day)	0.70	$9.38^{a,y}$	(0.36)	$8.78^{ab,y}$	(0.07)	$8.30^{b,y}$	(0.01)	$7.29^{c,y}$	(0.39)	$6.24^{d,y}$	(0.13)
	2.86	$11.12^{ab,x}$	(0.08)	$11.89^{a,x}$	(0.45)	$10.88^{ab,x}$	(0.98)	$9.37^{c,x}$	(0.31)	$9.82^{bc,x}$	(0.30)
Nitrogen intake[i] (mg/kg MBW per day)	0.70	$616.42^{a,y}$	(23.56)	$585.28^{ab,y}$	(4.53)	$560.29^{b,y}$	(0.60)	$471.62^{c,y}$	(25.18)	$413.16^{d,y}$	(8.70)
	2.86	$730.14^{ab,x}$	(5.06)	$792.81^{a,x}$	(29.83)	$734.01^{ab,x}$	(65.83)	$605.72^{c,x}$	(19.75)	$649.75^{bc,x}$	(19.98)
Protein in weight gain[j] (g/kg)	0.70	$110.40^{a,y}$	(13.08)	$128.47^{ab,x}$	(5.55)	$120.35^{ab,y}$	(2.16)	$115.99^{ab,y}$	(2.72)	$109.17^{b,x}$	(6.36)
	2.86	$140.59^{a,x}$	(6.04)	$135.70^{a,x}$	(5.43)	$142.20^{a,x}$	(6.24)	$139.61^{a,x}$	(1.58)	$111.13^{b,x}$	(10.90)
Fat in weight gain[i] (g/kg)	0.70	$115.09^{bc,x}$	(4.46)	$132.01^{a,x}$	(2.39)	$124.58^{ab,x}$	(1.34)	$120.76^{b,x}$	(4.40)	$108.62^{c,y}$	(4.40)
	2.86	$130.43^{a,x}$	(11.10)	$127.32^{a,x}$	(2.78)	$128.73^{a,x}$	(5.93)	$127.50^{a,x}$	(6.50)	$135.18^{a,x}$	(6.55)
FE[i] (g/g dry matter)	0.70	$1.36^{a,x}$	(0.04)	$1.39^{a,x}$	(0.04)	$1.41^{a,x}$	(0.02)	$1.23^{b,x}$	(0.02)	$0.89^{c,x}$	(0.04)
	2.86	$1.21^{a,y}$	(0.06)	$1.11^{a,y}$	(0.06)	$1.13^{a,y}$	(0.13)	$1.13^{a,y}$	(0.04)	$0.84^{b,x}$	(0.06)
PER[i] (wet weight gain/crude protein intake)	0.70	$3.25^{ab,x}$	(0.10)	$3.30^{a,x}$	(0.07)	$3.32^{a,x}$	(0.04)	$3.06^{b,x}$	(0.06)	$2.22^{c,x}$	(0.11)
	2.86	$2.79^{a,y}$	(0.14)	$2.51^{a,y}$	(0.14)	$2.61^{a,y}$	(0.30)	$2.77^{a,y}$	(0.03)	$2.00^{b,x}$	(0.11)
NRE[k] (%)	0.70	$35.92^{a,x}$	(5.05)	$42.45^{a,x}$	(1.78)	$39.92^{a,x}$	(0.92)	$35.49^{a,y}$	(1.50)	$24.30^{b,x}$	(2.12)
	2.86	$39.21^{a,x}$	(3.27)	$34.12^{a,y}$	(2.51)	$37.00^{a,x}$	(3.54)	$38.65^{a,x}$	(0.33)	$22.31^{b,x}$	(3.25)
FRE[k] (%)	0.70	$69.73^{b,x}$	(1.97)	$82.65^{a,x}$	(2.52)	$79.23^{a,x}$	(0.19)	$67.85^{b,x}$	(2.51)	$45.42^{c,x}$	(3.20)
	2.86	$67.68^{a,x}$	(4.37)	$60.58^{ab,y}$	(2.11)	$64.23^{a,y}$	(6.18)	$64.84^{a,x}$	(3.29)	$50.78^{b,x}$	(1.18)

[a,b,c,d]Mean values within a row with different superscript letters were significantly different (one-way ANOVA and Tukey's multiple range test; $P<0.05$).
[e]For the details of diets and procedures, see Table 1.
[f]ANOVA test (diet, size, diet × size): diet and size effects were highly significant ($P<0.01$), and diet × size effect was significant ($P<0.05$).
[g]ANOVA test (diet, size, diet × size): diet and diet × size effects were highly significant ($P<0.01$), and size effect was significant ($P<0.05$).
[h]ANOVA test (diet, size, diet × size): diet and size effects were highly significant ($P<0.01$), and diet × size effect was not significant ($P=0.08$).
[i]ANOVA test (diet, size, diet × size): all effects were highly significant ($P<0.01$).
[j]ANOVA test (diet, size, diet × size): size effect was highly significant ($P<0.01$), and diet and diet × size effects were significant ($P<0.05$).
[k]ANOVA test (diet, size, diet × size): diet and diet × size effects were highly significant ($P<0.01$), and size effect was not significant ($P>0.05$).
[x,y]Mean values within a column (between the two sizes for a given response criterion), with different superscript letters were significantly different (one-way ANOVA and Tukey's multiple range test; $P<0.05$).

experiment progressed was not the same for the small juveniles as for the large juveniles for those diets. A similar pattern was observed for protein deposition as response criterion (Table 5).

When ANOVA type III model was applied to feed intake expressed in g DM/kg MBW per day ($r^2=0.98$) the 'diet' effect ($P=0.002$) was the only significant effect. Taking into account the whole experimental duration fish ingested relatively similar amounts of diets D0 and D25 for the smaller juveniles and diets D0 to D50 for the larger juveniles ($P>0.05$), but lower quantities of the diets D50 to D100 for the smaller juveniles and D75 and D100 for the larger juveniles (Table 4). When voluntary feed intake was expressed in percent of feed intake observed for the protein-bound AA-diet D0 (=100) (Fig. 2), a significant 'adaptation time' effect was observed ($P<0.05$) for the large juveniles fed diets D75 and D100. Indeed, contrary to the large juveniles fed the other diets, large juveniles fed diets R75 and 100 increased their voluntary feed intake as the experiment progressed. This phenomenon was not observed in the smaller juveniles (Fig. 2).

When ANOVA type III model was applied to FE ($r^2=0.92$) the 'diet' effect ($P=0.004$) was also significant. In addition for FE, the 'diet × adaptation' effect was also significant ($P=0.0005$) indicating that adaptation time to the diet had an effect on FE but that this effect was different from diet to diet (Fig. 3). In particular, FE significantly decreased with time in juveniles fed diets D0–D50 as the experiment progressed in both juvenile size. In juveniles fed D75 and D100, FE increased significantly with time in the smaller juveniles, but not in the larger juveniles.

Table 5
P-values of the two-factor, crossed ANOVA model (see Fig. 1) for the daily growth coefficient (DGC, % per day), voluntary feed intake (VFI, % VFI of the control-diet, D0) and protein gain (PG; g/kg metabolic body weight per day). Significant values ($P<0.05$) are in bold.

Effect	Adaptation time			Initial size			Adaptation time × initial size		
	DGC	VFI	PG	DGC	VFI	PG	DGC	VFI	PG
Substitution level									
0%	**0.026**	1.000	<**0.001**	**0.017**	1.000	0.067	**0.001**	1.000	<**0.001**
25%	0.198	0.959	0.095	**0.034**	<**0.001**	0.618	**0.041**	0.105	**0.033**
50%	0.191	0.227	0.149	**0.004**	0.052	**0.006**	0.071	**0.023**	**0.008**
75%	**0.001**	**0.001**	<**0.001**	<**0.001**	0.954	<**0.001**	0.070	<**0.001**	**0.014**
100%	**0.009**	<**0.001**	**0.002**	<**0.001**	<**0.001**	**0.023**	0.332	<**0.001**	<**0.001**

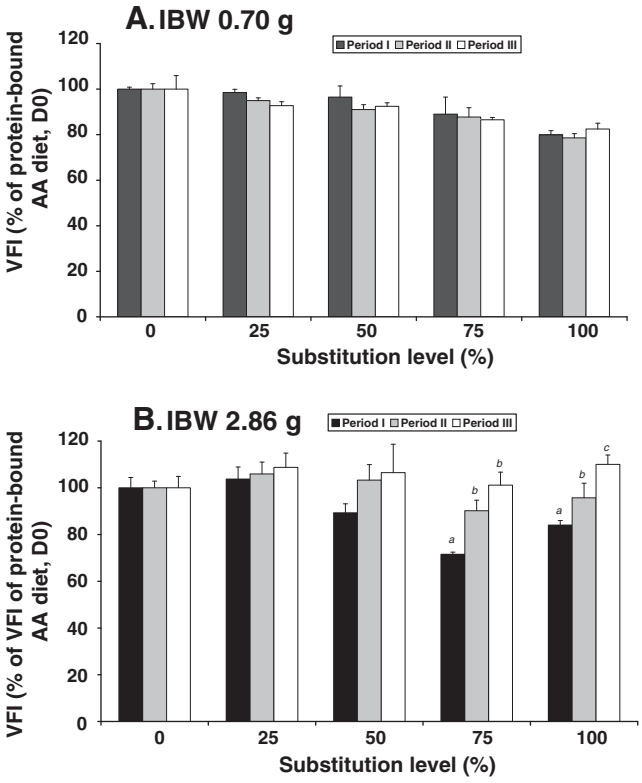

Fig. 2. Voluntary feed intake (VFI, % of the VFI observed with the cod muscle meal protein-bound amino acid (AA)-based diet (0% substitution level, D0)) in rainbow trout of two different initial body weights (A. 0.70 g and B. 2.86 g). Five diets (40.5% crude protein) were fed under the form of cod muscle meal (0% substitution level) or increasing levels of substitution of cod muscle meal by crystalline amino acids of a similar composition (25, 50, 75 and 100%).

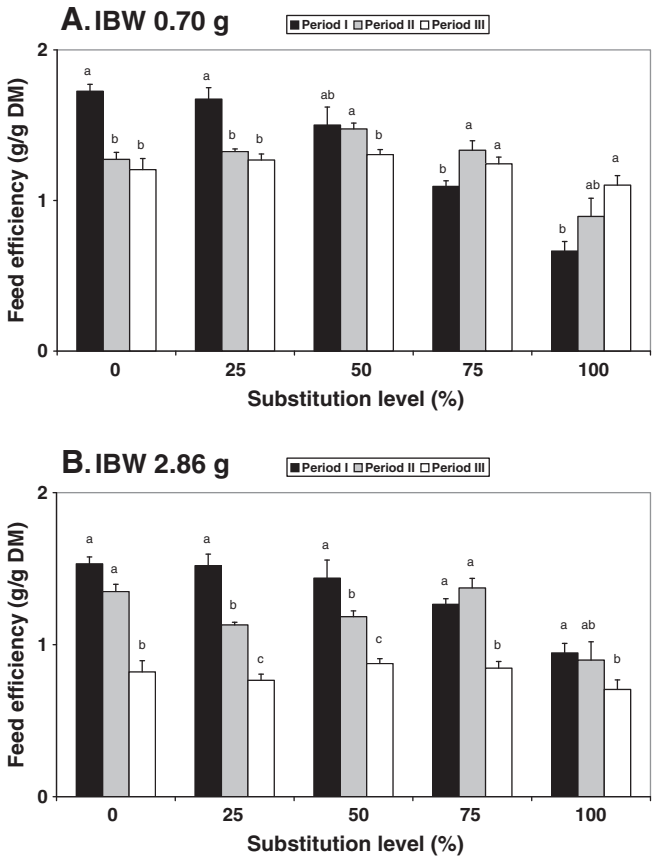

Fig. 3. Feed efficiency (g fresh body weight gain/g dry diet intake) observed with the cod muscle meal protein-bound amino acid (AA)-based diet (0% substitution level, D0) in rainbow trout of two different initial body weights (A. 0.70 g and B. 2.86 g). Five diets (40.5% crude protein) were fed under the form of cod muscle meal (0% substitution level) or increasing levels of substitution of cod muscle meal by crystalline amino acids of a similar composition (25, 50, 75 and 100%).

3.2. Protein deposition and utilization efficiency

Compared to the D0 control diet, protein gain (g/kg MBW per day) was not significantly reduced for juveniles of both sizes fed diets D25 and D50 (Fig. 1B). The reduction of protein deposition in juveniles fed on diet D75 (−25%, $P<0.05$, and −18%, $P>0.05$, compared to D0 in the small juveniles and the large juveniles, respectively), was entirely explained by a decrease of nitrogen intake (−24% and −17%, respectively) since no significant reduction of nitrogen utilization efficiency (PPV) was observed for this treatment. By contrast, the significant reduction of protein gain in juveniles fed D100 (−54% and −49%, respectively) was explained by both a reduction of nitrogen intake (−33%, $P<0.05$, and −11%, $P>0.05$, respectively) and nitrogen utilization efficiency (PPV; −32% and −43%, respectively; $P<0.05$). Fat gain followed a similar pattern as protein gain (Table 4).

Fig. 4 illustrates the effect of 'adaptation time' on protein deposition expressed in percent of protein deposition observed with the protein-bound AA-diet D0. It can be seen that the effect tends to increase with the dietary FAA level in both juvenile size, being small ($P>0.05$) for diet D25, intermediary ($P>0.05$) for D50 and high ($P<0.05$) for diets D75 and D100 (Table 5). The 'size effect' was significant for diets D50 to D100 (Table 5), protein deposition being significantly higher in the large juveniles compared to the smaller ones for these diets (Table 4). Finally, the 'adaptation time × size' effect was significant for all diets, meaning that the adaptation effect was juvenile size-dependent (Table 5).

3.3. Substitution level and adaptation period for reasonable growth

When the two initial juvenile sizes were studied separately and over each period, small differences in the influence of the FAA substitution level were observed. Six polynomial models were fit to the DGC and the substitution level that should allow for 85% of the maximum DGC or of the maximum nitrogen retention efficiency was estimated. The resulting substitution values were represented graphically for each initial juvenile size and each period (Fig. 5). After 17 feeding days (after period two), the substitution level that allowed for 85% of the maximum DGC for both initial juvenile sizes was of about 65%. Fig. 5A shows that the juveniles of both sizes needed 17 days to adapt to the diets containing between 50% and 75% of FAA. It also shows that diets with a higher FAA content required a longer adaptation period of at least 25 days to reach 85% of the maximum DGC performance. Very similar results were obtained for protein deposition (g/kg MBW per day, Table 6). Fig. 5B showed that the substitution level that allowed 85% of the maximum nitrogen retention efficiency increased with adaptation time to the diet, reaching 100% after 25 feeding days of adaptation. It can also be seen that the larger juveniles need much less adaptation to the diet for a given performance than the smaller ones and that a substitution level of 100% can be reached for both juvenile sizes after 25 feeding days of adaptation for this specific response criterion.

4. Discussion

The nutritional value of FAA as compared to protein-bound AA is still controversial in fish nutrition for many species (for a review, see Dabrowski and Guderley, 2002) and rainbow trout is not an exception. Some authors concluded that FAA can replace protein-bound AA in experimental diets of rainbow trout (Kim, 1997; Kim

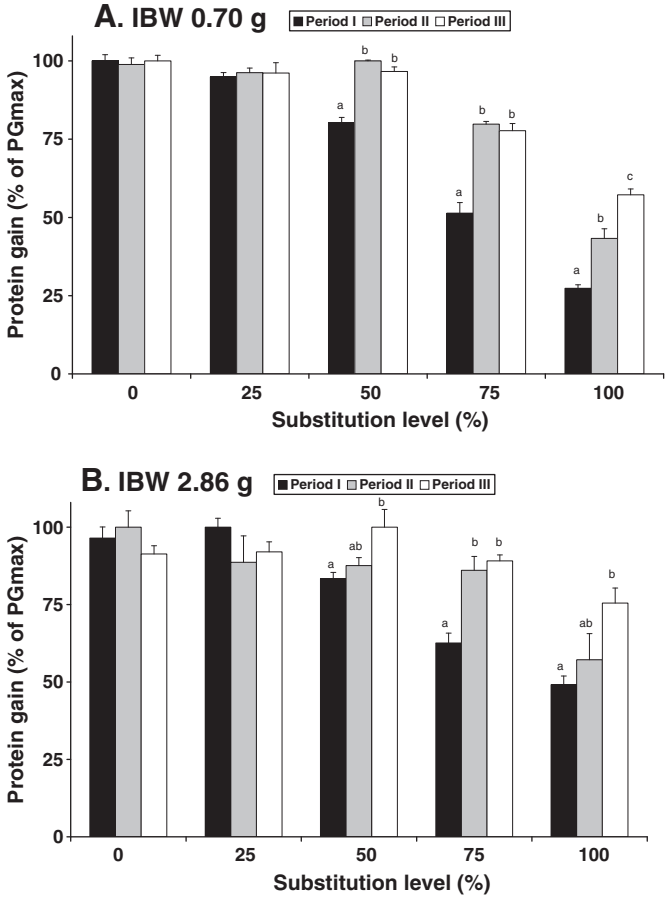

Fig. 4. Protein deposition (% of the maximum protein deposition (PGmax) observed with the cod muscle meal protein-bound amino acid (AA)-based diet (0% substitution level, D0)) in rainbow trout of two different initial body weights (A. 0.70 g and B. 2.86 g) Five diets (40.5% crude protein) were fed under the form of cod muscle meal (0% substitution level) or increasing levels of substitution of cod muscle meal by crystalline amino acids of a similar composition (25, 50, 75 and 100%).

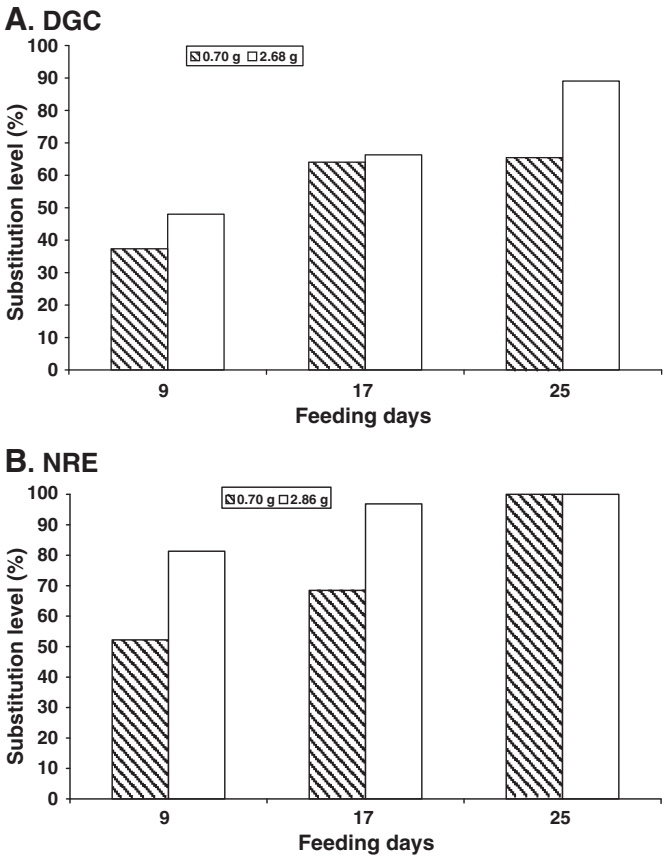

Fig. 5. Estimated substitution rates allowing for 85% of the maximum daily growth coefficient (A. DGC, % per day) or of the maximum nitrogen retention efficiency (B. NRE, %) for two sizes of juvenile, 0.70 g and 2.86 g, in rainbow trout. See Section 2.5 (Data analysis) for explanations.

et al., 1991; Rodehutscord et al., 1995) while others concluded to sometimes dramatically inferior performances for FAA-rich diets (Aoe et al., 1970; Barroso et al., 1999; Cowey and Luquet, 1983; Cowey and Walton, 1988; Dabrowski et al., 2003; Zhang et al., 2006). These contrasting results need clarification. Here we studied (1) the effects of dietary FAA level, fish size and diet adaptation to these FAA-rich diets in rainbow trout juveniles and (2) the optimum ratio of FAA to cod muscle meal, a muscle protein essentially devoid of FAA, in experimental diets aimed at examining requirements for individual AA in rainbow trout juveniles.

In the present study, increased substitution of cod muscle meal by mixtures of FAA of similar AA composition led to a significant decrease in growth, protein deposition and feed intake when substitution rates were higher than 50% (D75 and D100). From polynomial regressions (Table 6) we evaluated that a substitution rate of 65% of a cod meal intact protein by an equivalent FAA mixture allowed for 85% of the maximum DGC for both juvenile sizes after 17 days of adaptation to the diet. A higher FAA inclusion rate decreased dramatically the growth performances, except if a longer adaptation period was applied in the larger juveniles only.

The dietary FAA level seems to be a major factor able to explain some differences between studies in salmonids. Halver (1957) first reported on the growth of juvenile salmonid fed FAA-based diets. The growth rates were said to be very slow because the fish were reared at a very low water temperature (8 °C) (Dabrowski et al., 2003), but probably also because of the high dietary FAA level in the diets. Later, Aoe et al. (1970) concluded that rainbow trout (IBW 8.3 g) reared at 15 °C exhibited 'a normal or only slightly retarded growth' when fed on a FAA-based diet. In fact, body weight gain obtained with the FAA-based diet was 67% of that of the casein/gelatin-based diet (no replicates), and the maximum observed growth rates for both diets were rather low (recalculated DGC of 1.1–1.5% per day) compared to the optimal (recalculated) rates published for rainbow trout of similar size and raised at similar temperature (Austreng et al., 1987). On their side, Rodehutscord et al. (1995) obtained similar weight gains in 55 g-trout reared at 16.5 °C and fed diets either containing 15% wheat gluten plus 22% FAA or 27% wheat gluten plus 6.9% FAA, and concluded that dietary FAA do not negatively affect the efficiency of utilization of AA for growth in comparison to protein-bound AA. However, according to Dabrowski and Guderley (2002) their conclusions do not seem to be warranted because of the low growth rates observed (specific growth rate of 1.7%

Table 6
Parameters of the polynomial models ($Y = ax^2 + bx + c$) fit to the daily growth coefficient (DGC, % per day) or the protein gain (g/kg metabolic body weight per day) as a function of substitution level (x, %) and x-value at 85% of the maximum value of the response criterion.

Response criterion	Parameter	Initial weight 0.70 g	Initial weight 2.86 g
DGC	a	−0.0002	−0.0002
	b	0.0042	0.0038
	c	2.6265	2.8439
	x-value at 85%	56	67
Protein gain	a	−0.0001	−0.0001
	b	0.0048	0.0050
	c	1.4865	1.7423
	x-value at 85%	58	64

versus 4.1% in normal conditions) and low dietary protein content. Our view is that the results of Rodehutscord et al. (1995) can be explained by a combination of factors, i.e. the relatively low maximum dietary FAA level tested (about 63% by weight), the relatively long duration of the study (61 feeding days) and the rather high trout size used (see the diet 'adaptation effect' and the 'size effect' discussed below).

In the present study, the dietary protein level was considered to be optimal for rainbow trout (Satia, 1974) and the DGC values of 2.65–2.86% per day obtained in the present study for the juveniles fed D0 are close to the optimal (recalculated) rates (2.95–3.15% per day) published for rainbow trout juveniles of similar size and raised at similar temperatures (Austreng et al., 1987). Therefore, the conclusions of the present study can be generalized, at least for rainbow trout at the juvenile stage, and are useful in experimental diet formulations.

Several explanations have been proposed to explain the inferior performances of fish (and other animals) fed a FAA-based diet compared to a diet containing protein-bound AA. The most popular one is based on the fact that FAA do not have to be released from protein by intestinal digestion and can therefore be immediately absorbed in the first part of the intestine (Cowey and Walton, 1988) and only by AA transporters (Espe and Lied, 1994). By contrast, protein-bound AA have to be digested first and thus will become available later for absorption, at a lower rate, by a wider variety of transporters (peptides, FAA, proteins) and in a more distal part of the intestine. After absorption, as the size of the FAA pool remains relatively constant during the postprandial phase, the protein synthesis capacity may be exceeded by the high AA appearance rate in the FAA pool of fish fed FAA-rich diets. In this case, the surplus in FAA, potentially toxic for the body at high levels (Nolles et al., 2008), is removed by hepatic oxidation (Bos et al., 2003; Morens et al., 2000, 2003; Nolles et al., 2009; Tome and Bos, 2000), leading to an imbalanced AA profile at the tissue level (Espe and Njaa, 1991) and provoking a higher ammonia and/or urea excretion (Dabrowski et al., 2003; Kaushik and Dabrowski, 1983; Schuhmacher et al., 1997), or by direct AA excretion (Murai et al., 1984; Ng et al., 1996) or fecal egestion (Dabrowski and Dabrowska, 1981; Kaushik and Dabrowski, 1983). Surplus AA may also be metabolized into fat (Mambrini and Kaushik, 1994; Yamamoto et al., 2002) and glycogen (Kaushik and Dabrowski, 1983; Walton et al., 1986). All these process lead to an inferior nitrogen utilization efficiency.

Dabrowski et al. (2003) observed marked increased deamination of dietary FAA in rainbow trout alevins compared with fish fed a casein-based diet, but not in juveniles. Furthermore, urea excretion was two-fold higher and continued for more than 24 h post-feeding in trout juveniles fed an FAA-based diet, compared to the faster-growing juveniles fed a casein-based diet, leading to inferior growth in the former. These observations confirms the higher postprandial oxidative losses observed in rats and humans fed FAA compared to protein-bound AA (Daenzer et al., 2001; Metges et al., 2000; Nolles et al., 2009). Recent results of Nolles et al. (2009) further suggested that postprandial AA oxidations of free and protein-derived AA are independent of each other, in accordance with the idea that AA absorption in the intestine is also separated in time and place. No indication for an interaction between the oxidation of protein-bound AA and FAA was observed, even when these two different sources were given together in the same diet. In the present study, we gave FAA and protein-bound AA alone (D100 and D0) or in various combinations (D25–D75). We observed higher relative losses of nitrogen (= 100-NRE, % of nitrogen intake) in juveniles of both sizes fed the FAA-based diet compared to the protein-bound AA-based diet, supporting the previous observations. However, it is difficult to make a safe comparison between treatments because nitrogen intake were not the same in all groups. Overall, these observations indicate major differences in digestive physiology and metabolism of FAA versus protein-bound AA and in their development between the alevin and juvenile stages in trout fed a FAA-based diet (Dabrowski et al., 2003).

The present study shows that rainbow trout juveniles fed a diet in which the AA source is entirely supplied in the form of crystalline FAA (D100 diet) has a positive body weight gain, equivalent to 29–52% (0.70 and 2.86 g IBW, respectively) of the one of juveniles fed a high-quality protein-based control diet (D0 diet). Moreover there was a size effect, growth rate being significantly higher with the former diet in the larger juveniles compared to the smaller ones (+58%). This is in agreement with some authors that obtained positive growth rate and high survival in rainbow trout juveniles (0.8 g IBW) after 2 weeks when fed a FAA-based diet (Dabrowski et al., 2003), but no growth and low survival in first feeding alevins (Dabrowski et al., 2003; Terjesen et al., 2006). This possible 'size effect' on FAA utilization needs a further analysis of the literature. Therefore, we compiled our data with some data available in the rainbow trout literature. Keeping in mind that these data were not obtained in the same conditions (intact protein source used as control, temperature, etc.), which can possibly lead to invalid extrapolation, we obtained an interesting logarithmic relationship (Fig. 6, $Y = 16.67 \times LnX + 32.59$, $n = 7$, $r^2 = 0.99$) between the relative body weight gain obtained in rainbow trout fed a FAA-based diet (Y, expressed in percent of the body weight gain obtained in a protein-bound AA-based diet of equivalent AA composition) and the trout initial body weight (X, g/fish), confirming the suggested size effect on FAA utilization for trout growth. From this equation, it can be predicted that a trout of about 57 g should perform as well with a FAA-based diet than with a protein-bound AA diet. Interestingly Rodehutscord et al. (1995) used rainbow trout of 55 g (IBW) in an experiment from which they concluded that 'FAA can replace protein-bound AA in test diets for studies in rainbow trout'. Probably, this relatively high fish IBW may have contributed to this ability. Overall, these results suggest that the utilization of FAA for growth in salmonids is size-dependent or age-specific at the juvenile stage, alevins being unable to utilize FAA for growth at first feeding, but acquiring progressively this ability in a logarithmic way in their further development. This 'size effect' can probably explain a part of the variation of performances reported in the piscine literature with rainbow trout fed a FAA-rich diet. Further studies will have to analyze if this factor resulted in discrepancies observed in published IAA requirements.

Ontogeny involves the formation and development of systems and functions (Dabrowski, 1986), and in fish, ontogeny concerns mostly the larval life stages and somewhat the juvenile life stages. For example, marine fish larvae initially assimilated simple forms of AA (FAA) more efficiently and 3.5 times faster than complex forms (protein-bound) (Ronnestad et al., 2000) but these differences in assimilation rates decreased as the larvae grew (Rust, 1995). Though the digestive

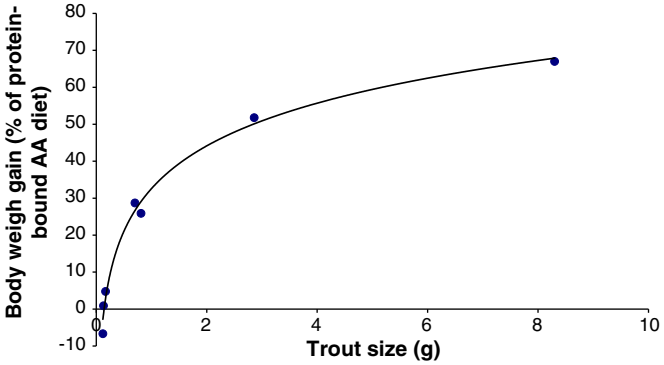

Fig. 6. Logarithmic relationship ($Y = 16.67 \times LnX + 32.59$, $n = 7$, $R^2 = 0.99$) between the relative body weight gain obtained in rainbow trout fed a free amino acid (AA)-based diet (Y, expressed in percent of the body weight gain obtained in a protein-bound AA-based diet of equivalent AA composition) and the trout initial body weight (X, g/fish). Data sources: Aoe et al. (1970); Dabrowski et al. (2003); Terjesen et al. (2006); and present study.

tract of salmonids already resembles the adult form at the beginning of feeding (Dabrowski, 1986), it is probable that all of the digestive and absorptive functions are not yet fully operational. For instance, in rainbow trout, protease activities in the stomach and intestine reached their highest levels only at a size of around 90 g live weight (Kitamikado and Tachino, 1960). Also in rainbow trout alevin, dipeptide transporters and hydrolyzing enzymes may have limited the utilization for growth of a diet containing a 100% substitution level of intact protein by synthetic dipeptides (Terjesen et al., 2006). In the present study it is suggested that the inferior growth performances observed in smaller juveniles with high FAA-rich diets (≥75% inclusion rate) compared to the larger juveniles was related to the inferior capability of the digestive tract of the former to absorb (not enough AA transporters) and/or regulate absorption of this high flux of AA, leading to a higher FAA catabolism and a lower feed intake. Further studies will have to test this hypothesis.

In this study, the 'size effect' showed some limitations in trout juveniles. Indeed, when the juveniles of both sizes were fed a FAA-based diet (D100), nitrogen utilization efficiency was impaired (about one third) contrary to all other diets (D0–D75), suggesting an intrinsic inadequacy of this type of diet in salmonids. However, since voluntary feed intake was markedly lower in fish fed D100 compared to the others, it is not possible to conclude from the present results the inability of rainbow trout juveniles to utilize efficiently FAA-based diets.

The reduction of protein deposition in juveniles fed on diets D75 and D100 was largely explained by a decrease of voluntary feed intake. According to Harper and Rogers (1965), a reduction in feed intake with such diets could be explained by an AA imbalance. Indeed, feeding animals dietary FAA levels would lead to an imbalanced pattern of plasma FAA and to a higher hepatic uptake for FAA. This would, in turn, lead to a change in the FAA plasma levels and would induce a metabolic signal to an appetite-regulating center, which would negatively impact feed intake. This type of response has been referred to as the neurological pathway response (Dabrowski et al., 2007). Another possible feed intake regulation mechanism is the olfactory response pathway (Dabrowski et al., 2007; Kasumyan and Doving, 2003), or palatability. Taste aversion can cause reduced voluntary feed intake and growth (Gatlin et al., 2007; Glencross et al., 2006; Opstvedt et al., 2000) while attractants can improve feed intake (Tiril et al., 2008). Poor diet palatability has been evoked as a possible reason for reduced growth in FAA-rich diets (Peres and Oliva-Teles, 2007). In the present study, the reduced feed intake observed for diets D75 and D100 for both juvenile sizes can be explained by either the olfactory or the neurological pathway response hypotheses, but this is certainly a size-dependent dynamic process. Indeed, the time effect was significant in the larger juveniles but not in the smaller one for voluntary feed intake, indicating a differential adaptation of juveniles to FAA-rich diets.

Marked adaptation to FAA-rich diets was observed in the present study for growth rate and protein deposition in both juvenile sizes, but also for voluntary feed intake in the larger juveniles and for FE for the smaller juveniles. This time effect was already reported with similar diets in both rainbow trout alevins and juveniles (Dabrowski et al., 2003), but also in other fish species (Gaye-Siessegger et al., 2007). Recently, Nolles et al. (2009) observed in rats that the higher oxidation losses of dietary FAA observed during the postprandial phase after 5 days of adaptation decreased with time. After an adaptation of 20 days, the maximal oxidation rate was lower and the peak value of oxidation was delayed for 2–3 h, suggesting that the handling of FAA was slowed down, most probably in the gastrointestinal tract, thus reducing the appearance rate of the FAA in the body pool and the oxidation losses. In the present study, nitrogen retention efficiency was observed to increase with adaptation time to the diet (Fig. 5B), suggesting that a certain capacity of the body to adapt to the presence of FAA in the diet does also exist in rainbow trout juveniles. Furthermore, this capacity may be dependent of the developmental stage. Indeed, the juveniles' adaptation seemed dependent of their size (Fig. 5). In particular, larger juveniles were able to continue to adapt (>17 days) to higher substitution levels (going from 65% to almost 90%) whilst smaller juveniles, despite additional exposure time (25 days total) were not able to adapt to higher levels and stagnated around a 65% substitution level, for the growth response (DGC).

Another possible reason of reduced AA utilization for growth in FAA-rich diets in this study is leaching. With larval micro-diets, the leaching of soluble nutrients such as FAA is a major problem because they are composed of much smaller particles (50–250 μm) than juvenile fish diets (several millimeters in diameter), and the surface to volume ratio is very high in comparison (Lopezalvarado et al., 1994). In the present study, particle size was of 1 and 1.6 mm, an intermediate size between larval microdiets and juvenile fish diets. Furthermore, the FAA were coated with agar to prevent leaching. Coating or encapsulating the FAA has been shown to reduce solubility and absorption rates of FAA thereby improving the efficiency of utilization of these AA (Chen et al., 1992; Segovia-Quintero and Reigh, 2004; Teshima et al., 1992; Zhou et al., 2007). Cho et al. (1992) were the first to coat the FAA component of the diet with a solution of aqueous agar prior to mixing it with the other dietary components. Later, Mambrini and Kaushik (1994) added the step of heating the agar and the water to 100 °C and then adding the FAA only when the mixture cooled to 40 °C. Many subsequent studies used this agar coating method for FAA and obtained reasonable growth rates (Abboudi et al., 2007; Nang Thu et al., 2007, 2009; Peres and Oliva-Teles, 2005; Rollin et al., 2003a). Finally, in the present study, during feeding phase, it was ensured that the juveniles ate the particles within 15 s of contact with the water. These precautions were believed to reduce FAA leaching to a minimum in the present study and to warrant our conclusions.

In conclusion, these trials showed that the dietary protein quality depends on the molecular form of the ingested nitrogen (at least for rainbow trout juveniles) and that this quality is modulated by the ratio of synthetic FAA to protein, the adaptation to the diet and juvenile size. In particular, our results showed that 1) the growth performances of juveniles fed increasing dietary FAA levels follow a quadratic function of FAA inclusion rate, being maximum at 0–50% and then decreasing rapidly; 2) a maximum FAA level that still ensures reasonable growth (85% of maximum) after an adaptation period of 17 days minimum is 65% at both juvenile sizes; 3) the decrease of growth rate and protein deposition at FAA inclusion rate below 75% is largely explained by a reduction of voluntary feed intake while nitrogen utilization efficiency remains essentially unchanged in this range; 4) larger juveniles tolerate higher FAA dietary levels than smaller juveniles indicating ontogeny-related changes in FAA utilization efficiencies; and 5) fish accustomed to FAA-rich diets before the start of the experiment can tolerate higher FAA dietary levels but the maximum substitution level is modulated by fish size. Further investigations are needed, though, in order to determine if the low AA utilization efficiencies of very high FAA substituted diets (>75%) in juveniles are a result of decreased voluntary feed intake, inadequate intestinal absorption, increased AA oxidation, or other factors, and if the fish body shows a capacity to adapt to the presence of FAA in the diet and if this capacity is dependent of the developmental stage, as suggested by the present study.

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