Response of Rainbow Trout (*Oncorhynchus mykiss*) to Supplements of Individual Essential Amino Acids in a Semipurified Diet, Including an Estimate of the Maintenance Requirement for Essential Amino Acids[1,2,3]

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ABSTRACT We studied the effects of increasing dietary concentrations of each of the following amino acids on growth, feed intake, feed conversion ratio and composition of gain in rainbow trout in six dose-response experiments: L-lysine, L-tryptophan, L-histidine, L-valine, L-leucine and L-isoleucine. Semipurified diets containing 20.1 MJ digestible energy/kg dry matter, with wheat gluten and crystalline amino acids as sole sources of amino acids, were fed to rainbow trout [initial mean body weight (BW) 40–51 g, depending on the amino acid studied]. In one series of 24 diets, lysine concentration ranged from 4.5 to 58.0 g/kg dry matter; in five further series of 12 diets each, concentrations ranged from (in g/kg dry matter): tryptophan, 1.3 to 5.6; histidine, 2.6 to 13.5; valine, 6.2 to 34.2; leucine, 10.0 to 42.0 and isoleucine, 5.0 to 15.3. Each diet was fed to a group of 20 fish for 53–64 d, depending on the amino acid studied. Dry matter intake, weight gain, feed conversion ratio, protein concentration of gain and total protein deposition followed exponential response functions. To achieve 95% of the maximum protein deposition, dietary concentrations of 27.7 g lysine, 2.0 g tryptophan, 5.8 g histidine, 15.7 g valine, 13.6 g leucine and 13.7 g isoleucine/kg dry matter were required. Maintenance requirements, estimated from exponential functions for protein deposition, were [in mg/(100 g BW · d)]: lysine, 1.93; tryptophan, 1.05; histidine, 1.07; valine, 2.92; leucine, 8.26 and isoleucine, 0.91. This corresponds to 4% of the requirement for protein deposition for lysine and isoleucine but 32% for leucine, with the other amino acids being intermediate. Therefore, different dietary amino acid requirement patterns were derived from protein deposition data depending on the chosen level of performance. J. Nutr. 126: 1166–1175, 1997

KEY WORDS: • *requirement* • *rainbow trout* • *essential amino acids* • *protein deposition* • *maintenance requirement*

Studies on the requirements of heavier rainbow trout for histidine, valine, leucine and isoleucine have not been published to date. Values reported for the lysine requirement vary from 13.0 to 28.7 g/kg diet (Ketola 1983, Kim et al. 1992, Lanari et al. 1991, Pfeffer et al. 1992, Walton et al. 1984b) and for tryptophan from 2.0 to 2.5 g/kg (Kim et al. 1987, Poston and Rumsey 1983, Walton et al. 1984a). Several factors such as size of the fish, dietary energy concentration, dietary ingredients used, feeding regimen, growth rate achieved, response criteria and mathematical model used may influence recommendations derived from dose-response experiments and thus make it difficult to compare or combine results from different laboratories. Thus far, nothing is known about the amount of essential amino acids required to cover maintenance requirements.

To overcome this lack of consistent requirement estimates, we developed a high energy, semipurified diet that fosters a high growth rate in rainbow trout (Rodehutscord et al. 1995c). This diet can be used for dose-response studies on requirements in trout; it has been used for experiments on requirements for almost all essential amino acids, with the exception of phenylalanine, in an attempt to standardize experimental conditions as far as possible. Results for methionine and cystine, arginine, and threonine have already been published (Rodehutscord et al. 1995a and 1995b). In this paper, results of the dose-response experiments on lysine, tryptophan, histidine, valine, leucine and isoleucine are presented.

Based on the results presented here in earlier papers, an attempt to estimate the maintenance requirements for individual amino acids based on protein retention data is made.

MATERIALS AND METHODS

Six dose-response experiments were performed, each investigating one individual essential amino acid. Semipurified diets were fed with wheat gluten and crystalline amino acids as the only sources of amino acids (**Table 1**). All basal diets were calculated to contain equal amounts of digestible energy (DE),[5] N and essential

[1] Presented in part at the Annual Meeting of the Society of Nutrition Physiology, February 27–29, 1996, Göttingen, Germany [Becker, A., Rodehutscord, M. & Pfeffer, E. (1996) Dosis-Wirkungs-Beziehungen zwischen Leistungskenngrößen und der Versorgung mit Valin, Leucin und Isoleucin bei der Regenbogenforelle. Proc. Soc. Nutr. Physiol. 5: 54 (abs.)].

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[5] Abbreviations used: BW, body weight; DE, digestible energy; MSE, mean square error; NEAA, nonessential amino acids.

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TABLE 1

Composition of the basal diets used in six dose-response experiments with rainbow trout

	Amino acid under test					
	Lysine	Tryptophan	Histidine	Valine	Leucine	Isoleucine
	g/kg dry matter					
Basal components[1]	550	550	550	550	550	550
Wheat starch, gelatinized	185	209	198	199	201	214
DL-Methionine	9.7	9.5	9.7	9.3	9.3	9.7
L-Arginine	16.3	16.4	16.6	16.5	16.5	16.4
L-Threonine	12.1	11.9	12.1	11.9	11.9	11.9
L-Phenylalanine	8.2	8.4	8.6	8.8	8.8	8.4
L-Lysine · HCl	—	29.4	31.6	31.0	31.0	29.3
L-Lysine · H_2O	2	—	—	—	—	—
L-Tryptophan	3.3	—	3.2	3.2	3.2	3.2
L-Histidine · HCl · H_2O	7.4	7.5	—	8.1	8.1	7.8
L-Valine	13.6	13.4	13.5	—	13.6	13.4
L-Leucine	18.9	18.9	18.8	19.0	—	18.9
L-Isoleucine	11.2	11.2	11.2	11.3	11.3	—
Nonessential amino acid mixture[2]	162.3	114.4	126.7	131.9	135.3	117.0
Analyzed						
N, *g/kg dry matter*	54	53	54	55	55	53
Lipids, *g/kg dry matter*	279	276	284	280	275	280

[1] Basal components (in g/kg dry diet); wheat gluten, 150; fish oil, 100; sunflower oil, 88; beef tallow, 87; binder (Sipernat[TM]50S, Degussa AG, Frankfurt, Germany), 60; minerals, 45; vitamins, 10; choline chloride (50%), 10. For composition of mineral and vitamin premixes see Rodehutscord et al. (1995c). Wheat gluten contained (in g/kg dry matter, average from analyses of five different batches used): N, 134; lysine, 14.2; methionine, 12.6; cystine, 18.0; arginine, 29.7; threonine, 21.2; tryptophan, 7.4; histidine, 17.2; valine, 32.8; leucine, 58.7; isoleucine, 30.5; phenylalanine, 44.3; glutamic acid, 266; aspartic acid, 29.3; alanine, 22.5.

[2] Contained (in g/100 g): L-glutamic acid, 85; L-aspartic acid, 9; L-alanine, 6 (corresponding to the pattern determined in wheat gluten protein).

amino acids with the exception of the respective amino acid under test. Therefore, the proportions of gelatinized wheat starch and crystalline amino acids differed slightly, depending on the concentration of amino acids determined in the different batches of wheat gluten and on the N concentration in the respective amino acid under test. As a further source of nitrogen, a mixture of L-glutamic acid, L-aspartic acid and L-alanine was used, reflecting the ratios among these amino acids analyzed in wheat gluten protein. Analyzed concentrations of total N and lipids in the basal diets varied between 53 and 55 g/kg dry matter and between 275 and 284 g/kg dry matter, respectively. Concentrations of digestible energy in a diet very similar to these diets was determined previously to be 20.1 MJ/kg dry matter (Rodehutscord et al. 1995a), and this DE is assumed for all diets used here. A very similar diet containing extruded corn instead of gelatinized wheat starch had previously been developed especially for dose-response studies in trout and was shown to support performance of trout in a manner comparable to diets based on fish meal as the main source of amino acids (Rodehutscord et al. 1995c).

In each experiment, graded levels of the amino acid under test were added to the respective basal diet. A total of 24 diets were used in Experiment Lysine and twelve in each of the other experiments. L-Isomers of the amino acids replaced the corresponding amount of the mixture of nonessential amino acids (NEAA) in Experiment Lysine and of L-glutamic acid in each of the other experiments. The following concentrations of the amino acid under test were used in the experiments (in g/kg dry matter): lysine 4.5, 5.5, 7.0, 8.5, 10.0, 11.5, 13.0, 14.5, 16.0, 17.5, 19.0, 22.0, 25.0, 28.0, 31.0, 34.0, 37.0, 40.0, 43.0, 46.0, 49.0, 52.0, 55.0 and 58.0; tryptophan 1.3, 1.5, 1.8, 2.0, 2.3, 2.6, 2.8, 3.1, 3.7, 4.3, 5.0 and 5.6; histidine 2.6, 3.3, 4.1,

TABLE 2

Number of diets, range in concentration of individual amino acids, initial body mass of fish, experimental period and water temperature in six experiments with rainbow trout

	Amino acid under test					
	Lysine	Tryptophan	Histidine	Valine	Leucine	Isoleucine
Number of diets	24	12	12	12	12	12
Range in amino acid concentration, *g/kg dry matter*	4.5–58.0	1.3–5.6	2.6–13.5	6.2–34.2	10.0–42.0	5.0–15.3
Supplement used	L-Lysine · H_2O	L-Tryptophan	L-Histidine · HCl · H_2O	L-Valine	L-Leucine	L-Isoleucine
Replacement against	Mixture of NEAA[1]	L-Glutamic acid	L-Glutamic acid	L-Glutamic acid	L-Glutamic acid	L-Glutamic acid
Initial body mass[2], *g/trout*	51 ± 0.8	50 ± 0.5	40 ± 0.7	49 ± 1.0	49 ± 0.6	47 ± 0.5
Feeding days	55	64	53	53	53	59
Mean water temperature (°C)	15.7	15.5	17.0	15.7	15.7	16.0

[1] Mixture of nonessential amino acids (NEAA) as given in Table 1.

[2] Mean ± SEM, $n = 24$ and 12, respectively, depending on the amino acid studied.

TABLE 3

Parameters estimated by fitting the experimental data to an exponential curve[1]

	a	*b*	*c*	*d*	r^2	Root MSE
Lysine[2]						
Dry matter intake, *g/fish*	125.6	0.143	1.753	—	0.72	9.32
Weight gain, *g/fish*	122.3	0.154	3.728	—	0.67	12.9
Feed conversion ratio, *g gain/g dry matter*	0.976	0.181	−2.210	—	0.41	0.05
Protein concentration of weight gain, *g/kg*	147.4	0.105	−5.855	—	0.68	7.30
Lipid concentration of weight gain, *g/kg*	206.8	0.138	—	327.5	0.87	12.5
Protein deposition, *g/fish*	17.93	0.130	4.661	—	0.86	1.40
Tryptophan[3]						
Dry matter intake, *g/fish*	120.7	5.581	1.113	—	0.79	7.19
Weight gain, *g/fish*	117.8	3.789	1.146	—	0.84	9.69
Feed conversion ratio, *g gain/g dry matter*	0.974	3.546	0.981	—	0.91	0.03
Protein concentration of weight gain, *g/kg*	143.4	5.185	1.029	—	0.87	4.36
Lipid concentration of weight gain, *g/kg*	208.5	3.008	—	4908	0.90	10.97
Protein deposition, *g/fish*	16.96	3.588	1.186	—	0.87	1.44
Histidine[4]						
Dry matter intake, *g/fish*	119.9	0.719	0.294	—	0.64	5.92
Weight gain, *g/fish*	113.9	0.721	1.038	—	0.82	6.05
Feed conversion ratio, *g gain/g dry matter*	0.951	0.855	0.517	—	0.89	0.02
Protein concentration of weight gain, *g/kg*	145.8	0.564	−1.237	—	0.81	2.82
Protein deposition, *g/fish*	16.61	0.663	1.234	—	0.87	0.91
Valine[5]						
Dry matter intake, *g/fish*	109.6	0.283	5.183	—	0.91	9.81
Weight gain, *g/fish*	118.1	0.303	5.639	—	0.93	10.6
Feed conversion ratio, *g gain/g dry matter*	1.074	1.165	5.266	—	0.94	0.03
Protein concentration of weight gain, *g/kg*	137.8	0.507	4.009	—	0.96	3.39
Lipid concentration of weight gain, *g/kg*	228.2	0.195	−1.678	—	0.80	9.46
Protein deposition, *g/fish*	16.23	0.307	5.970	—	0.92	1.64
Leucine[6]						
Dry matter intake, *g/fish*	116.8	0.635	8.500	—	0.83	7.10
Weight gain, *g/fish*	126.0	0.648	8.766	—	0.81	9.37
Feed conversion ratio, *g gain/g dry matter*	1.080	0.729	6.787	—	0.62	0.03
Protein concentration of weight gain, *g/kg*	140.0	0.608	6.072	—	0.35	6.02
Lipid concentration of weight gain, *g/kg*	224.2	0.708	—	25,297	0.45	8.08
Protein deposition, *g/fish*	17.60	0.641	8.935	—	0.74	1.79
Isoleucine[7]						
Dry matter intake, *g/fish*	98.62	0.332	2.176	—	0.61	10.8
Weight gain, *g/fish*	100.8	0.268	2.893	—	0.71	12.3
Feed conversion ratio, *g gain/g dry matter*	1.001	0.317	0.967	—	0.87	0.04
Protein concentration of weight gain, *g/kg*	139.6	0.490	1.760	—	0.73	6.16
Lipid concentration of weight gain, *g/kg*	247.3	0.655	—	1563	0.74	12.4
Protein deposition, *g/fish*	13.85	0.297	3.611	—	0.76	1.77

[1] Abbreviations used: *a*, *b*, *c* and *d*, estimated parameters; MSE, mean square error.
[2] Initial body mass 51 g/trout, 55 feeding days.
[3] Initial body mass 50 g/trout, 64 feeding days.
[4] Initial body mass 40 g/trout, 53 feeding days. Lipid concentration in weight gain of fish fed the basal diet without supplemental L-histidine·HCl·H_2O was higher than in weight gain of fish fed any of the other histidine concentrations, but no clear dose-response relationship was detectable for this trait.
[5] Initial body mass 49 g/trout, 53 feeding days.
[6] Initial body mass 49 g/trout, 53 feeding days.
[7] Initial body mass 47 g/trout, 59 feeding days.

4.9, 5.7, 6.5, 7.5, 8.5, 9.5, 10.5, 12.0 and 13.5; valine 6.2, 7.2, 8.2, 10.2, 12.2, 14.2, 16.2, 18.2, 22.2, 26.2, 30.2 and 34.2; leucine 10.0, 11.0, 12.0, 14.0, 16.0, 18.0, 22.0, 26.0, 30.0, 34.0, 38.0 and 42.0; isoleucine 5.0, 5.5, 6.0, 6.5, 7.0, 7.5, 8.0, 9.0, 10.0, 11.3, 13.3 and 15.3. Fish oil, sunflower oil and warmed beef tallow were stirred with the binder before being added to the other components in a drum mixer. Mixtures were moistened in a cutter to a sticky paste which was pelleted by forcing it through 3-mm screens of a mincer. The moist pellets were stored at −18°C until feeding.

For each experiment, trout (mean initial body mass given in **Table 2**) were taken from homogenous populations either reared in our department (Experiments Lysine, Histidine, Valine and Leucine) or obtained from a commercial fish farmer (Fischzucht Mohnen, Stollberg, Germany) 3 wk before starting the experiments (Experiments Tryptophan and Isoleucine). Up to the beginning of the experiments, all trout had been fed a commercial diet (Aminoforte, Rheinkrone, Wesel, Germany). Groups of 20 trout each were placed in 24 (Experiment Lysine) or 12 (all other experiments) 250-L round plastic tanks that were part of a circulatory system and continuously supplied with water in parallel, with ~70% of the outflowing water recirculated after clarification and aeration. The water supply to each tank was ~4.5 L/min. Water could be heated during winter but could not be cooled during summer; therefore, the mean water temperature ranged among experiments from 15.5 to 17.0°C (Table 2), but within each experiment only by a maximum of ±1°C. Experiments were performed in a room illuminated for 16 h/d.

Body masses of each group of fish were recorded at the beginning of the experiments after the fish were anesthetized in a solution of 1,1,1-trichloro-2-methyl-2-propanol-hemihydrate, and at the end of the experiments after the fish had been killed in 4-ethyl-aminobenzoate. Feed was withheld 36 h before killing. One additional representative group of 20 trout was killed for base-line measurements at the beginning of each experiment.

After being frozen at −18°C, all fish (experimental and base-line

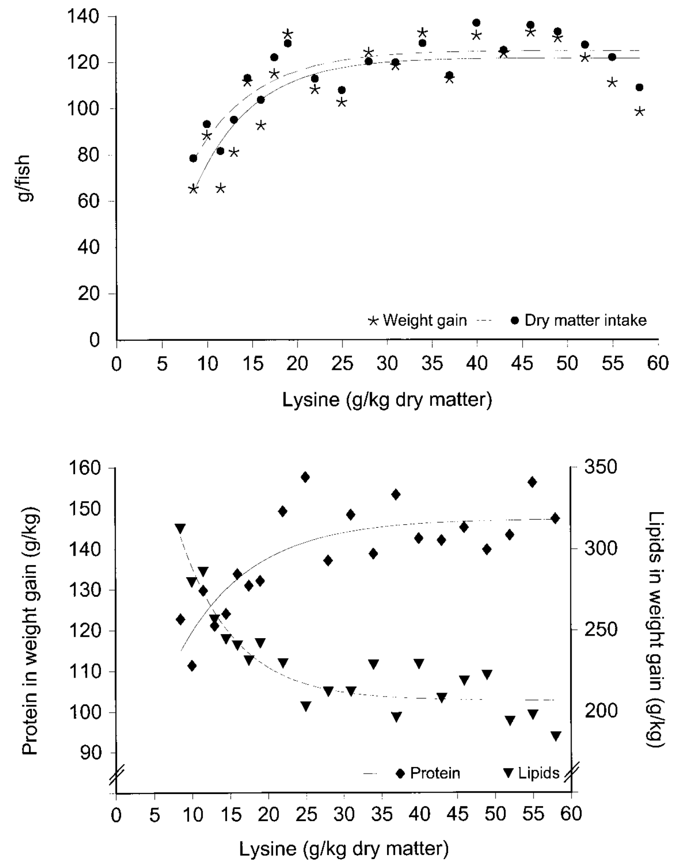

FIGURE 1 Effect of dietary lysine on dry matter intake, weight gain and composition of weight gain of trout achieved during the 55 d of the experiment (initial body mass 51 g/fish). Each point represents a group of fish. The parameters of equations are summarized in Table 3.

groups) were cut into small pieces with a ribbon saw, forced repeatedly through a mincer, homogenized in a cutter and freeze-dried. The concentrations of protein and lipids in the weight gain of experimental groups were calculated from differences between the experimental and base-line groups as described previously (Rodehutscord et al. 1995c).

In each experiment, each group was fed one of the experimental diets for a period given in Table 2. Trout were fed to near satiation twice daily during the week and once daily on weekends. Thawed feeds were offered by hand until pellets were first seen to sink to the bottom of the tank. Thus, feed losses could be avoided almost completely. General care, handling and maintenance of trout followed the procedures approved by the Animal Welfare Commissioner of the University of Bonn in accordance with the German Animal Welfare Law.

In diets and body homogenates, dry matter (105°C), ash (550°C), total N and lipids (petroleum ether extract after HCl treatment) were determined according to the official methods (Naumann and Bassler 1976). Amino acid content of all basal diets was determined by ion exchange chromatography (Llames and Fontaine 1994). Tryptophan was determined by HPLC after alkaline hydrolysis. Supplemented levels of crystalline amino acids were checked separately after extraction with diluted HCl. Similarly, added tryptophan was determined by HPLC after hydrolysis with barium hydroxide solution and autoclaving under vacuum (J. Fontaine, Degussa AG, Germany, personal communication).

Exponential functions were calculated to fit the experimental data because the response to a limiting dietary component is nonlinear. For traits that increased with increasing dietary concentration of the amino acid under test, the equation employed for describing the respective response was

$$y = a\,(1 - e^{-b(x-c)}) \qquad (1)$$

Lipid concentration in weight gain, on the contrary, responded negatively to increasing dietary amino acid concentration in most of the experiments. Therefore, the equation employed was

$$y = a + de^{-bx} \qquad (2)$$

where x = dietary concentration of the amino acid under test (g/kg dry matter), a = plateau value of the respective curve, b = parameter characterizing the steepness of the curve, c = dietary concentration of the amino acid under test at y = 0, and d = maximum response to supplemented amino acid.

Model parameters were estimated by the least-squares principle (Rawlings 1988, Seber and Wild 1989). The resulting nonlinear equations were solved iteratively by the Levenberg-Marquard method (Press et al. 1992). Calculations were performed using the program BFIT (H. P. Helfrich, Seminar of Mathematics, Faculty of Agriculture, University of Bonn), which implements this method.

RESULTS

Half of the trout fed the three diets containing the lowest lysine concentrations (4.5, 5.5 and 7.0 g/kg dry matter) died within the first 19, 23 and 34 d, respectively, of feeding, and the complete groups were excluded from further evaluation. Within the experiment, nine fish of the group receiving the diet with 8.5 g lysine/kg dry matter died and two or three fish in each group receiving the following four lysine concentrations (10.0, 11.5, 13.0 and 14.5 g/kg dry matter). Among all fish of the remaining groups, only one died within the feeding period.

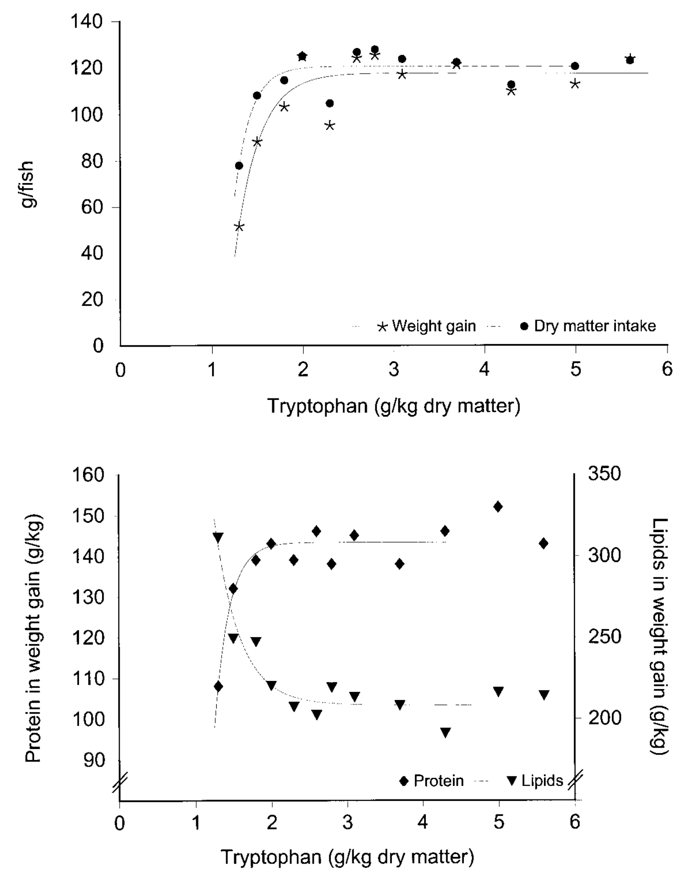

FIGURE 2 Effect of dietary tryptophan on dry matter intake, weight gain and composition of weight gain of trout achieved during the 64 d of the experiment (initial body mass 50 g/fish). Each point represents a group of fish. The parameters of equations are summarized in Table 3.

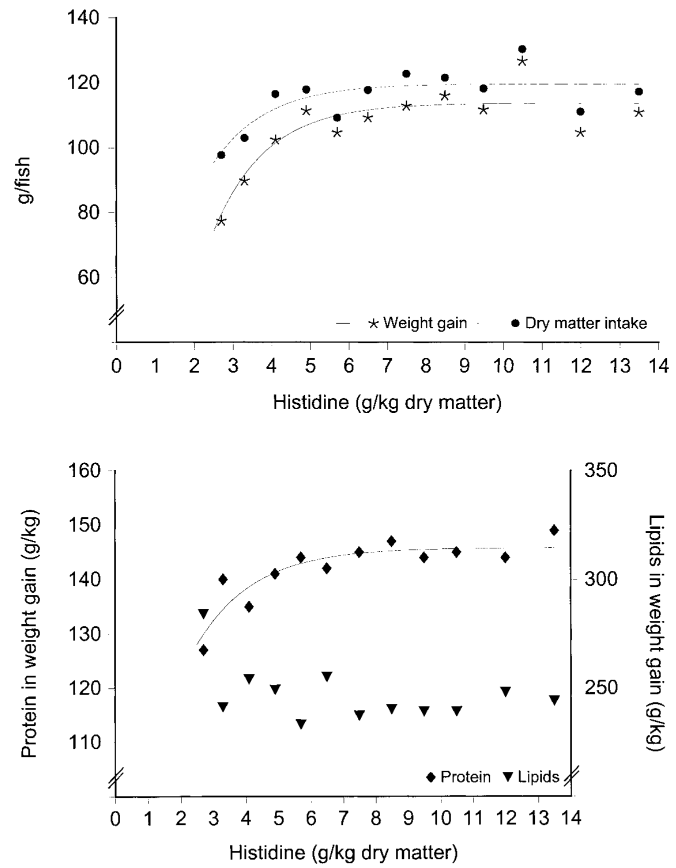

FIGURE 3 Effect of dietary histidine on dry matter intake, weight gain and composition of weight gain of trout achieved during the 53 d of the experiment (initial body mass 40 g/fish). Each point represents a group of fish. The parameters of equations are summarized in Table 3.

The lowest dietary lysine concentration resulting in any growth of the fish to the end of the experiment was, therefore, 8.5 g/kg dry matter. In Experiment Valine, six of the trout fed the basal diet without supplemental L-valine died within the last 18 d of the experimental period. In Experiments Isoleucine, Tryptophan and Histidine, from the total of 240 fish each, three, two and one fish, respectively, died without a clear relation to the dietary concentration of the respective amino acid under test. No fish was lost in Experiment Leucine.

Parameters of all estimated regression equations are summarized in **Table 3.** Additionally, responses of trout in weight gain, dry matter intake and in composition of the weight gain to increasing dietary concentration of individual amino acids are shown in **Figures 1** (Lysine), **2** (Tryptophan), **3** (Histidin), **4** (Valine), **5** (Leucine) and **6** (Isoleucine). Dry matter intake, weight gain, gain per feed ratio and protein concentration of weight gain increased with increasing dietary concentrations of the respective amino acid under test in all experiments, and the rate of increase decreased with increasing dietary concentration. Concentration of lipids in weight gain, however, did not respond as uniformly to the increase in dietary concentrations of individual amino acids. In Experiments Lysine, Tryptophan, Leucine and Isoleucine, lipid concentration in weight gain decreased with increasing concentration of the respective amino acid (Fig. 1, 2, 5 and 6) whereas it increased with increasing dietary concentration of valine (Fig. 4). No clear dose-response relationship was detectable between dietary concentration of histidine and lipid concentration in weight gain (Fig. 3).

Concentrations of essential amino acids were determined in body homogenates of trout fed the diets containing either the lowest or the highest level of the respective amino acid in each experiment. Results are shown in **Table 4.**

DISCUSSION

Nonlinear equations were used for evaluating the results of the experiments described in this work. Previous papers noted that these equations are regarded as most appropriate for evaluating results from dose-response experiments because the response to improved dietary concentrations of a limiting nutrient is not linear (Cowey 1992, Fuller and Garthwaite 1993, Mercer 1989, Schutte and Pack 1995, Rodehutscord 1996, Rodehutscord et al. 1995a and 1995b). The efficiency of supplemented individual amino acids decreased with increasing dietary concentration of the respective amino acid, resulting in plateaus that could be described by exponential functions. Reduction in either absorption rate or intermediary utilization of absorbed amino acids or both must be the reason for plateaus in performance.

Each dietary treatment comprised only one group of 20 trout as one data point, and no repeated measurements were done for the individual dietary concentrations. This would be completely unsatisfactory if different individual treatments were to be compared. Evaluation of monofactorial dose-re-

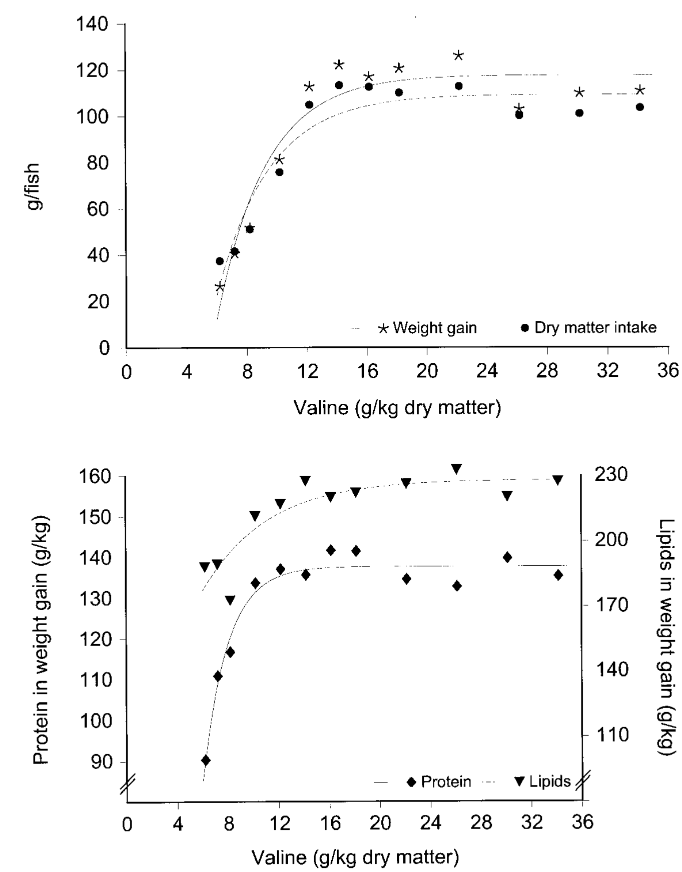

FIGURE 4 Effect of dietary valine on dry matter intake, weight gain and composition of weight gain of trout achieved during the 53 d of the experiment (initial body mass 49 g/fish). Each point represents a group of fish. The parameters of equations are summarized in Table 3.

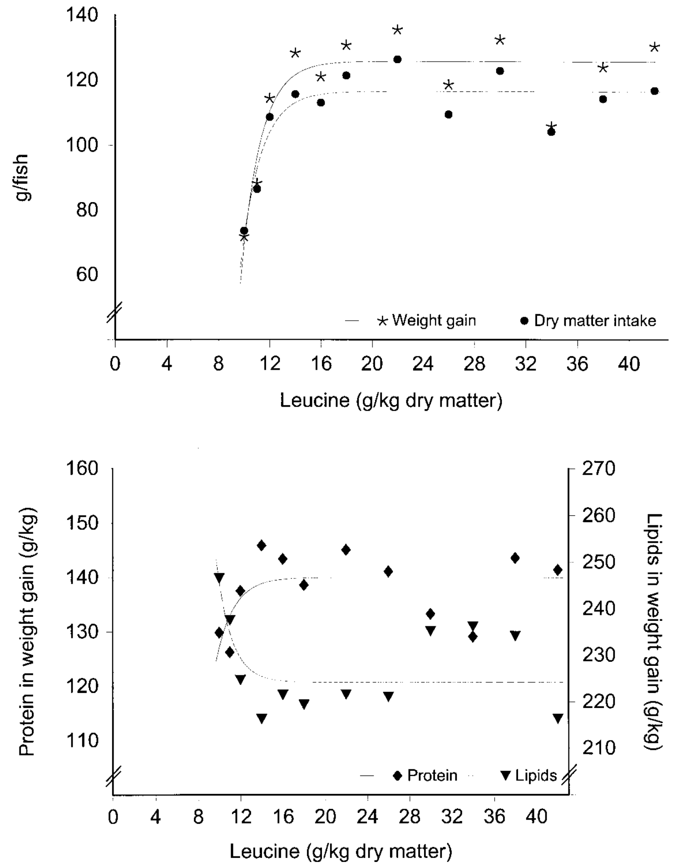

FIGURE 5 Effect of dietary leucine on dry matter intake, weight gain and composition of weight gain of trout achieved during the 53 d of the experiment (initial body mass 49 g/fish). Each point represents a group of fish. The parameters of equations are summarized in Table 3.

sponse experiments by regression analysis, however, always uses the complete set of data for deriving one function. Parameters of the function are estimated with the aim of minimizing the sum of squared deviations. Maximizing the number of finely graded dietary levels at the expense of replications on the levels chosen seems to us to be the preferred way of conducting these experiments. All of our conclusions concerning response to varying supply or to requirements are based on derived functions and never on individual measuring points. Values for r^2 and mean square error (MSE) calculated for the regressions in this study (Table 3) indicate that the responses to changes in dietary amino acid concentrations are well described by the calculated functions within the ranges of concentrations studied. In previous studies (Rodehutscord et al. 1995c), using four repeated measurements per treatment (with one tank of 20 trout as one replicate), we found that the pooled SEM in body weight gain was between 2 and 5% of the mean, depending on the experiment. This gives an indication of the between-tank variability in our tank system.

Choo et al. (1991) studied effects of dietary excesses of leucine on growth and body composition of trout fry initially weighing 2 g. Body protein concentration appeared to be reduced when dietary leucine was increased from 11 to 35 g/kg but was unaffected by further increases in dietary leucine up to 134 g/kg, whereas body concentration of lipids tended to increase with increasing dietary leucine. A similar response was observed in lake trout (*Salvelinus namaycush*) fry when dietary leucine concentration was increased from 5.2 to 16.0 g/kg (Hughes and Rumsey 1983). However, in the present experiment, increasing dietary leucine up to ~12 g/kg dry matter increased concentration of protein in gain and decreased concentration of lipids. At present, no clear explanation can be given for this discrepancy between our results and those quoted, but we assume that the composition of the experimental diets (in particular lipid concentration) may account at least for part of this. Choo et al. (1991) assume, referring to experiments of Tischler and Goldberg (1980), that dietary leucine was used to synthesize triglycerides in adipose tissue and that this may account for increased body lipid seen in fish fed high dietary leucine. We used a diet containing 280 g lipid/kg dry matter, whereas a lipid concentration of about 100 g/kg can be recalculated for the diets used by Choo et al. (1991). It has frequently been shown that lipid concentration in the fish body increases with increasing dietary lipid concentration (Rodehutscord et al. 1995c, Storebakken and Austreng 1987), and any effect of leucine on the synthesis of triglycerides in adipose tissue was probably overlapped by the high amount of fatty acids directly transferred to this tissue. As long as dietary leucine limits protein retention when dietary leucine concentration is increased, an increased proportion of ingested digestible energy can be used for body protein synthesis and does not have to be deposited in adipose tissue; this may explain the decrease in body lipid concentration observed when dietary leucine was elevated to 12 g/kg dry matter.

We cannot explain why body lipid concentration increased with dietary valine concentration whereas it decreased when

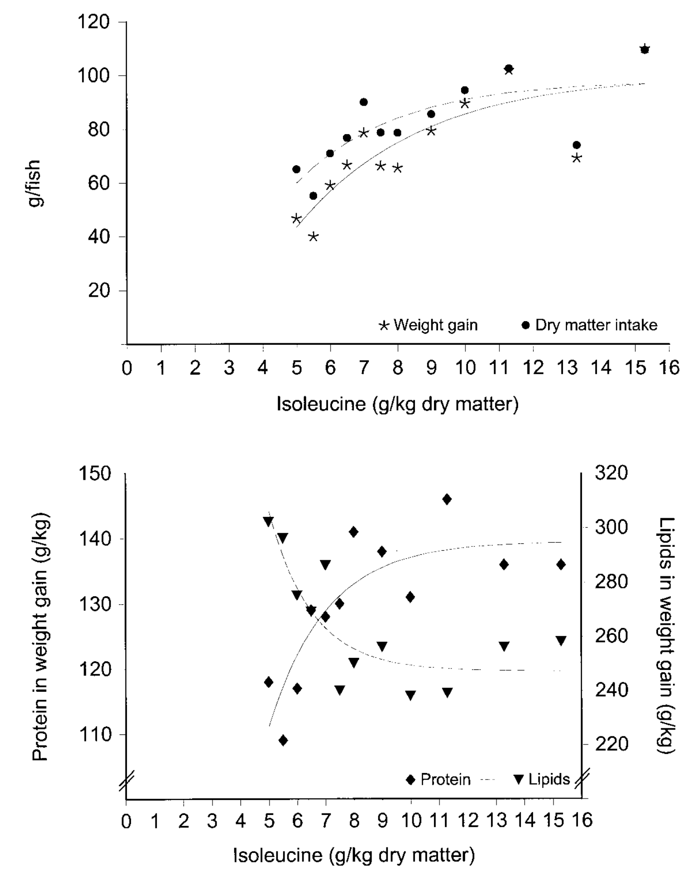

FIGURE 6 Effect of dietary isoleucine on dry matter intake, weight gain and composition of weight gain of trout achieved during the 59 d of the experiment (initial body mass 47 g/fish). Each point represents a group of fish. The parameters of equations are summarized in Table 3.

TABLE 4

Essential amino acids in whole-body protein of trout fed the diets with the lowest or the highest level of individual supplemental amino acids, determined at the end of the experiments[1]

	Dietary amino acid levels (g/kg dry matter)											
	Lysine		Tryptophan		Histidine		Valine		Leucine		Isoleucine	
	8.5	58.0	1.3	5.6	2.6	13.5	6.2	34.2	10.0	42.0	5.0	15.3
	g/16 g N											
Lysine	7.1	7.8	7.2	7.8	8.0	7.8	7.9	7.6	7.2	7.6	7.4	7.4
Tryptophan	ND[2]	ND	0.8	0.9	ND	ND	ND	ND	ND	ND	0.9	0.9
Histidine	2.2	3.3	2.3	2.6	3.5	4.7	3.2	3.6	3.2	3.6	3.3	3.5
Valine	4.4	4.7	4.4	4.8	5.0	4.9	4.8	4.7	4.5	4.7	4.6	4.8
Leucine	6.6	7.0	6.8	7.2	7.1	6.9	7.0	6.8	7.0	6.9	6.8	6.7
Isoleucine	3.8	4.1	3.7	4.0	4.2	4.1	4.1	3.9	3.6	3.9	4.0	4.0
Methionine	2.7	2.9	2.8	2.9	3.0	3.0	2.9	2.8	2.7	2.8	2.7	2.7
Cystine	0.9	1.0	0.9	1.0	1.0	1.0	1.0	1.1	1.1	1.1	1.0	0.9
Arginine	6.0	5.9	6.1	6.0	6.3	6.1	5.9	5.9	5.6	6.0	5.9	5.9
Threonine	4.0	4.3	4.1	4.3	4.4	4.2	4.2	4.1	4.1	4.3	3.9	4.1
Phenylalanine	4.2	3.6	4.2	4.5	4.2	4.3	3.9	3.8	3.8	3.9	3.8	4.2

[1] One homogenate of 20 trout per treatment.
[2] ND, not determined.

dietary concentrations of all other amino acids studied here increased.

Dietary excess of leucine may depress growth and feed conversion when concentrations > 92 g/kg are used in rainbow trout (Choo et al. 1991) or >50 g/kg in lake trout (Hughes et al. 1984). Accordingly, in the present study, increasing dietary leucine to a concentration that was about threefold higher than that required for high protein deposition (42 g/kg dry matter) did not influence either the amount or the composition of weight gain. However, studying the effects of dietary excesses of amino acids has not been the objective of our experiments.

For each of the traits evaluated with exponential functions, plateau values that describe a theoretical maximum or minimum that can be approached by continuously increasing the dietary concentration of the individual amino acids under test were calculated (parameter a in Table 3). The exponential functions describe the changes in efficiency of supplemented amino acids with increasing dietary concentrations of the respective amino acid and, therefore, leave space for interpretation regarding the "optimum" concentration required in the diet. With the following transformations of Equations (1) and (2), the dietary concentration of an amino acid x_z (g/kg dry matter) required to reach z% of the respective plateau value can be calculated as follows:

$$x_z = c + 1n\ [100/(100 - z)]/b$$

for traits evaluated with Equation (1) and

$$x_z = (1n\ \{d/[(100 - z)\ 0.01\ a]\})/b$$

for traits evaluated with Equation (2)

where a, b, c and d are the respective parameters summarized in Table 3. **Table 5,** as an example, summarizes those dietary concentrations of all essential amino acids studied here that were required to reach 95% of the calculated plateau values for each trait. We decided more or less arbitrarily to use this 95% level for further conclusions, but data given in Table 5 can be recalculated for any level differing from 95% using the original data given in Table 3. Data shown in Table 5 indicate that, among the traits monitored, protein deposition is the most sensitive indicator of a suboptimal supply of an amino

TABLE 5

Dietary amino acid concentrations required to reach 95% of plateau value of performance traits in six experiments with rainbow trout

	Amino acid under test					
	Lysine	Tryptophan	Histidine	Valine	Leucine	Isoleucine
	g/kg dry matter					
Dry matter intake	22.7	1.7	4.5	15.8	13.2	11.2
Weight gain	23.2	1.9	5.2	15.5	13.4	14.1
Feed conversion ratio (gain/feed)	14.3	1.8	4.0	7.8	10.9	10.4
Protein concentration of weight gain	22.7	1.6	4.1	10.0	11.0	7.9
Lipid concentration of weight gain	25.0	2.0	NE[1]	13.7	10.9	7.4
Protein deposition	27.7	2.0	5.8	15.7	13.6	13.7

[1] NE, no estimate.

TABLE 6

Recommended dietary amino acid concentrations

	Lysine	Methionine[1]	Arginine[2]	Threonine[2]	Tryptophan	Histidine	Valine	Leucine	Isoleucine
Own results[3]									
dry matter, *g/kg*	27.7	8.0	11.5	10.3	2.0	5.8	15.7	13.6	13.7
digestible energy, *g/MJ*	1.38	0.40	0.57	0.51	0.10	0.29	0.78	0.68	0.68
NRC (1993)									
digestible energy,[4] *g/MJ*	1.19	0.66[5]	0.99	0.53	0.13	0.46	0.80	0.93	0.60

1 Results from Rodehutscord et al. (1995a).
2 Results from Rodehutscord et al. (1995b).
3 Concentrations required to reach 95% of plateau in protein deposition.
4 Recalculated values.
5 Methionine + Cystine.

acid. This conclusion could also be drawn from results found previously for methionine, arginine and threonine (Rodehutscord et al. 1995a and 1995b), and it shows that at least part of the differences found in recommendations for amino acid supply in the literature must be due to different response criteria chosen in individual studies. Our recommendations concerning necessary amino acid concentrations in trout diets are based on protein deposition data.

Lanari et al. (1991) studied the lysine requirement using trout similar in size to those in this study. From their results in body weight gain, they recommended 21.6 g lysine/kg dry matter, which is close to that concentration required for 95% of maximum body weight gain in the present experiment (23.2 g/kg dry matter).

Results presented in this paper and those published previously (Rodehutscord et al. 1995a and 1995b) form the basis to derive recommendations for the dietary supply with nine out of ten essential amino acids, based on a uniform method and almost identical basal diets. These recommendations are summarized in **Table 6.** Concentration of digestible energy grossly determines the gain/feed ratio and, therefore, the amount of amino acids ingested per unit of gain. Because DE concentrations may vary greatly between diets, it is preferable to relate concentrations of nutrients to MJ DE rather than to kilograms of feed. Based on the 20.1 MJ DE/kg dry matter of the diets used here, the recommendations are expressed in Table 6. Recommendations of NRC (1993) were recalculated in a similar way. Recommendations derived from our data exceed those of NRC (1993) for lysine and isoleucine by 16 and 13%, respectively, but they are lower by 3–4% for valine and threonine and by 23–42% for the remaining amino acids. It must be pointed out, however, that the 1993 NRC recommendations are based on a literature survey considering a large number of papers dealing with requirement studies on trout and salmon, most of which were performed with juvenile fish and widely different methodological approaches. Obviously, different response criteria lead to different recommendations (see Table 5), as do differences in growth rate (Cowey 1994), probably because of an increase in the proportion of maintenance requirements with decreasing growth rate. Requirement for any amino acid comprises both retention of the amino acid and maintenance requirement. Assuming that the maintenance requirement depends on the body size of a fish, the proportion of maintenance requirement on total amino acid requirement decreases with increasing retention of the amino acid. When the daily growth coefficient is calculated for the present experiments as a way of standardizing growth rates according to the suggestion of Cowey (1992), the figures which result (at least 2.9) are relatively high (Cowey 1992). This might explain why most of the requirement figures derived from our own results are lower than those collected by NRC (1993).

In this type of growth study, the total amino acid requirement for both maintenance and retention of protein usually is investigated. In fact, little is known about the proportion of the total amino acid requirement that has to be spent to cover the maintenance requirement of trout. Based on the data presented here and in previous studies from our laboratory, an attempt was undertaken to estimate maintenance requirement figures for each of the amino acids studied. When maintenance requirement is defined as the amount of an amino acid to be ingested by the fish to maintain its body protein pool in an equilibrium, which means that no net synthesis or net breakdown of body protein takes place, this amount can be obtained by extrapolating the dose-response curves for protein retention to $y = 0$. However, the equations given in Table 3 cannot be used immediately for this because they are based on the dietary concentration of amino acids as an independent variable and not on the quantitative amino acid intake. Therefore, protein retention data were recalculated using Equation (1), with intake of the respective amino acid (mg/fish) as an independent variable. Results are shown in **Table 7.** The parameter c (the intersection of the curves with the x-axis) in this case describes the amount of the amino acid required to be ingested to avoid any net change in the amount of body protein during the experimental phase. In **Table 8,** these values are presented together with the total amounts required, which were calcu-

TABLE 7

Parameters of the exponential functions relating protein deposition (g/fish) to intake of the respective amino acid under study (mg/fish)[1]

	a	b	c	r^2	Root MSE
Lysine	18.01	0.0009	119.3	0.90	1.17
Methionine[2]	14.24	0.0034	77.65	0.94	1.13
Arginine[3]	14.88	0.0026	144.3	0.85	1.37
Threonine[3]	14.59	0.0032	171.3	0.91	1.08
Tryptophan	17.39	0.0132	72.45	0.91	1.23
Histidine	16.68	0.0046	55.29	0.87	0.89
Valine	16.14	0.0022	166.5	0.95	1.26
Leucine	17.63	0.0027	489.5	0.75	1.77
Isoleucine	16.57	0.0014	51.63	0.90	1.17

1 Equation (1) was used.
2 Results from Rodehutscord et al. (1995a).
3 Results from Rodehutscord et al. (1995b).

TABLE 8

Calculation of the maintenance requirement for amino acids in rainbow trout and the proportion of ingested amino acids spent to cover the maintenance requirement

	Required for 95% of the plateau in protein deposition					
	Dietary concentration	Dry matter intake[1]	Amino acid intake	Maintenance requirement		
	g/kg dry matter	*g/fish*	*mg/fish*	*mg/fish*	*mg/(100 g BW·d)*[2]	*% of total requirement for protein deposition*
Lysine	27.7	123	3407	119	1.93	4
Methionine[3]	8.0	99	792	78	1.61	10
Arginine[4]	11.5	98	1127	144	2.74	13
Threonine[4]	10.3	96	989	171	3.35	17
Tryptophan	2.0	120	240	73	1.05	30
Histidine	5.8	118	684	55	1.07	8
Valine	15.7	104	1633	167	2.92	10
Leucine	13.6	112	1523	490	8.26	32
Isoleucine	13.7	97	1329	52	0.91	4

[1] Calculated from equations for dry matter intake given in Table 3 and the amino acid concentration required for 95% of the plateau in protein deposition.
[2] Mean body weight of fish was calculated as follows: initial body mass (g) + [plateau value for body weight gain (g)/2].
[3] Results from Rodehutscord et al. (1995a).
[4] Results from Rodehutscord et al. (1995b).
Abbreviation used: BW, body weight.

lated by multiplying the dietary concentration necessary for 95% of the plateau value in protein deposition (from Table 5) and the dry matter intake achieved at this concentration (from equations for dry matter intake given in Table 3). It is apparent from Table 8 that the maintenance requirement and the proportion of ingested amino acid spent to cover the maintenance requirement differ between individual amino acids. The proportion of maintenance requirement, calculated in this way, was lowest for lysine and isoleucine (4% of total requirement for protein deposition) but could make up as much as one third of the total requirement for tryptophan and leucine (30 and 32%, respectively). The high value for tryptophan may result from the low requirement for growth purposes resulting from the low tryptophan concentration in body protein. The value for leucine, however, appears surprisingly high. Even expressed in absolute terms, the maintenance requirement for leucine is more than fourfold higher than the average value of all other amino acids.

In the past, different groups have worked on the optimum amino acid pattern required in the diet to achieve high N retention in different animal species (e.g., Kirchgessner et al. 1995, Wang and Fuller 1989). This might be helpful in generalizing results in so far as amino acid pattern rather than amino acid requirements should remain largely unaffected by factors such as body weight, growth rate, dietary energy concentration or environmental factors. The authors determined these patterns by deleting individual amino acids from a mixture of all amino acids, assuming a linear dose-response relation between the dietary supply of the limiting amino acid and the N retention of the animal. On the basis of the experiments presented here, the optimum dietary amino acid pattern can be derived via an alternative method, i.e., by calculating the amino acids required for a certain level of protein retention directly from the dose-response curves. Depending on the level of protein retention chosen, the derived amino acid patterns are different, as shown in **Table 9** for arbitrarily chosen levels of 95, 80 and 65% of the plateau in protein deposition. The lower the desired level of protein retention, the closer the amino acid pattern moves toward that determined for maintenance requirement. This suggests that the assumption of a linear relationship between the supply of a limiting amino acid and retention of body protein is not necessarily valid, at least not in trout. From Table 9 it is also obvious that the amino acid pattern determined in body protein cannot be used as a dietary pattern. Doing so would mean ignoring both differences in the proportion of maintenance requirement and differences in utilization of individual amino acids.

A conclusion from Tables 8 and 9 must be that estimates of amino acid requirements based solely on the amino acid pattern of body protein or based on the assumption that the maintenance requirement makes up only a negligible propor-

TABLE 9

Dietary amino acid patterns (relative to lysine) derived for different levels of protein retention and for maintenance and pattern of body protein in rainbow trout

	% of maximum protein deposition[1]				
	95	80	65	Maintenance requirement	Body protein pattern[2]
				Lysine = 100	
Methionine[3]	29	31	33	46	37
Arginine[4]	42	47	52	83	78
Threonine[4]	37	44	50	92	55
Tryptophan	7	9	12	25	12
Histidine	21	22	22	27	46
Valine	57	66	74	128	62
Leucine	49	67	83	189	90
Isoleucine	50	53	57	78	52

[1] Based on equations for protein deposition given in Table 3.
[2] Based on the mean values of all groups receiving adequate amino acid supply listed in Table 4.
[3] Results from Rodehutscord et al. (1995a).
[4] Results from Rodehutscord et al. (1995b).

tion of total requirement (Ogino 1980) or even is equal for all amino acids, most likely lead to inadequate interpretations.

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