The effect of dietary lysine levels on growth and metabolism of rainbow trout (*Salmo gairdneri*)

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1. Groups of rainbow trout (*Salmo gairdneri*; mean weight 5 g) were given diets containing 10, 12, 14, 17, 21, 24 and 26 g lysine/kg diet for 12 weeks.

2. By analysis of the growth values the dietary requirement of lysine in this experiment was found to be 19 g/kg diet. A similar requirement value was obtained from a dose-response curve of expired $^{14}CO_2$ (following an intraperitoneal injection of L-[U-^{14}C]lysine) *v.* dietary lysine concentration.

3. Liver concentrations of total lipid and carnitine and activities of lysine-α-ketoglutarate reductase (saccharopine dehydrogenase (NADP$^+$, lysine-forming), *EC* 1.5.1.8) in the liver were not significantly different in fish from the different dietary treatments. Hepatosomatic index, however, was higher in those fish given low levels of dietary lysine.

Lysine is an essential dietary amino acid for all fish species so far studied (see Millikin, 1982). The quantitative requirement of several species for lysine has been determined by dose-response (growth) curves (see review by Ketola, 1982); e.g. chinook salmon (*Oncorhynchus tschawytscha*) requires 20 g lysine/kg diet containing 400 g crude protein (nitrogen $\times$ 6·25)/kg (Halver *et al.* 1958) and channel catfish (*Ictalurus punctatus*) requires 15 g/kg diet containing 300 g crude protein/kg (Robinson *et al.* 1980). By measurement of the retention of lysine in the bodies of rainbow trout (*Salmo gairdneri*) given diets containing different proteins of high biological value, Ogino (1980) inferred the requirement to be 21 g/kg diet containing 400 g protein/kg.

When the present experiment was begun there were no other reports in the literature of the lysine requirements of rainbow trout, but recently values of 29 g/kg diet containing 470 g crude protein/kg and 13 g/kg diet containing 350 g crude protein/kg have been reported by Ketola (1983) and Kim & Hayes (1983) respectively. Thus wide variations exist in the literature on the requirement level for lysine whether expressed on a whole-diet basis or a dietary-protein basis. In several mammalian species, dietary lysine–arginine antagonistic effects have been noted (e.g. Chi & Speers, 1976) but none were observed in studies on channel catfish (Robinson *et al.* 1981).

As well as poor growth in lysine-deficient trout, Ketola (1983) also observed fin erosion and increased mortality. In rats, lysine (together with methionine) is a precursor of carnitine which has an important role in the intramitochondrial transport of long-chain fatty-acyl groups for β-oxidation (Tanphaichitr *et al.* 1971). When rats were given diets deficient in lysine, there were disturbances in fatty acid oxidation together with increased levels of total lipid and triglyceride in the liver (Khan & Bamji, 1979).

The purpose of the present paper was to quantify the dietary requirement of rainbow trout for lysine and to examine the effects of variations in lysine intake on the activities of hepatic lysine-α-ketoglutarate reductase (saccharopine dehydrogenase (NADP$^+$, lysine-forming), *EC* 1.5.1.8), levels of hepatic lipid and carnitine and oxidation of a tracer dose of L-[U-^{14}C]lysine.

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Table 1. *Amino acid composition (g/kg complete diet) of dietary basal mix*

Amino acid	From 120 g white fish meal	From 300 g gluten	Total from protein	Free amino acid added	Amount (g) in 450 g cod-flesh protein
Alanine	5·4	5·9	11·3	—*	32·4
Arginine	4·8	7·5	12·3	18	30·3
Aspartic acid	7·5	7·4	14·9	—*	47·9
Cystine	0·6	5·1	5·7	0·7	6·4
Glutamic acid	11·1	81·5	92·6	0	74·6
Glycine	6·6	7·7	14·3	—*	22·9
Histidine	2·1	4·9	7·0	8·6	15·6
Isoleucine	3·5	7·6	11·1	10·8	21·9
Leucine	6·1	14·6	20·7	21·2	41·9
Lysine	6·2	4·2	10·4	—*	46·4
Methionine	2·6	3·8	6·4	2·9	9·3
Phenylalanine	3·3	10·8	14·1	6·9	21·2
Proline	3·8	28·1	31·9	0	18·9
Serine	4·0	10·8	14·8	—*	24·2
Threonine	3·7	5·6	9·3	14·4	23·7
Tryptophan	0·8	1·0	1·8	4·1	5·9
Tyrosine	2·8	8·1	10·9	7·3	18·2
Valine	3·8	9·2	13·0	13·1	26·1

* Variable amounts added, as indicated in Table 2.

EXPERIMENTAL

Fish and diets

Rainbow trout of approximately 5 g mean weight, obtained from the Highlands & Islands Development Board, Inverness, were randomly distributed (forty fish per tank) amongst fourteen circular glass-fibre tanks of diameter 1 m, depth 0·6 m and each containing 500 litres water (a mixture of 2 vol. fresh-water and 1 vol. sea-water). The water from the tanks was mainly (95%) recirculated with about 5% of a new water-mixture being continuously bled into the system (which contained large biological filters and faecal traps) and a similar amount being removed; tank-water temperature was $15 \pm 3°$. The fish, which had previously been fed on a commercial hatchery diet, were weaned on to an experimental diet (diet 7, see Table 2, p. 117) for 7 d before commencement of the experiment.

The experimental diets were prepared so that the amino acid composition, apart from lysine content, simulated the amino acid composition of hydrolysed white muscle protein from cod (Connell & Howgate, 1959) when present at a dietary protein level of 450 g/kg (Table 1). The full compositions of the seven experimental diets are shown in Table 2. Each diet was given to duplicate tanks of fish, the fish being fed to satiation twice daily for 6 d each week. The diets were prepared as moist pellets which were then freeze-dried and stored at $-20°$ until required. The fish were weighed individually every 4 weeks for the 12-week duration of the growth experiment and they were then given the same diets for a further 3 weeks to allow the other assays to be performed.

Amino-acid analyses

Samples of diets and dietary components were hydrolysed in 5·7 M-hydrochloric acid according to the procedure of Roach *et al.* (1967). Amino acid analyses were performed

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Table 2. *Composition (g/kg dry diet) of experimental diets*

Component Diet no. ...	1	2	3	4	5	6	7
Basal mix*	947·7	947·7	947·7	947·7	947·7	947·7	947·7
α-Cellulose	3·9	3·5	3·0	2·25	1·5	0·75	0
Non-essential amino acid mix†	48·4	46·8	44·8	41·8	38·8	35·8	32·8
Lysine hydrochloride	0	2·0	4·5	8·25	12·0	15·75	19·5
Total lysine	10·4	12	14	17	20	23	26

* Basal mix supplied (g/kg dry weight of complete diet): white fish meal 120, gluten 300, starch 200, cod-liver oil 150, vitamin mix 28, mineral mix 40, butylated hydroxyanisole 0·5, ascorbyl palmitate 0·4, essential amino acid mix 108·8. For details of the composition of the mineral and vitamin mixes, see Cowey *et al.* (1981). The composition of the essential amino acid mix was as shown in Table 1.

† The non-essential amino acid mix was prepared by mixing together (g): 231 aspartic acid, 65·8 serine, 602 glycine, 147·7 alanine.

using a Jeol amino-acid analyser, model JLG-6AH, and peak areas were automatically calculated by a Shimadzu CRI-A peak-height integrator.

Lipid and carnitine analyses

Total lipid in liver samples was measured gravimetrically by the method of Folch *et al.* (1957). Total carnitine was extracted from several combined livers for each determination and any acyl esters present were hydrolysed by the method of Tanphaichitr & Broquist (1973). Carnitine in the extract was then assayed by the DTNB method described by Pearson *et al.* (1974).

Lysine-α-ketoglutarate reductase assay

Trout were killed by a sharp blow to the head; the liver was removed and homogenized in 4 vol. ice-cold 0·25 M-sucrose, 10 mM HEPES (pH 7·4), 1 mM-EDTA, 1 mM-2-mercaptoethanol. Nuclei and cell debris were removed by centrifugation at 1000 ***g*** for 10 min. Portions (2 ml) of supernatant fraction were then centrifuged at 12000 ***g*** for 10 min and the subsequent supernatant fraction discarded. The pellet was washed twice by suspension in homogenizing medium and recentrifuging. The mitochondrial pellet was finally suspended in 0·1 M-potassium phosphate buffer (pH 7·6), 1 mM-EDTA, 1 mM-2-mercaptoethanol to give a final volume of 2 ml. Immediately before assay the suspension was subjected to three 15 s bursts of sonication. The enzyme was assayed by incubating 0·1 ml enzyme solution with 0·8 ml 50 mM-potassium phosphate buffer (pH 7·6), 1 mM-EDTA, 1 mM-2-mercaptoethanol, 10 mM-α-ketoglutarate, 0·2 mM-NADPH (final concentration) for 5 min at 25°. L-lysine (0·4 M; 0·1 ml) was then added and the change in absorbance at 340 nm recorded. A blank reading was obtained by adding 0·1 ml water instead of lysine (Hutzler & Dancis, 1975; Noda & Ichihara, 1978). When mammalian preparations are assayed a high blank value is obtained if the enzyme is not dialysed, due to mitochondrial glutamate dehydrogenase (*EC* 1 . 4 . 1 . 2) reacting with the α-ketoglutarate, NADPH and endogenous ammonia. However, with the trout-enzyme preparations high blank values were not observed, presumably because trout liver glutamate dehydrogenase, unlike its mammalian equivalent, has much higher affinities for NAD and NADH than for NADP and NADPH (Walton & Cowey, 1977).

For the preliminary and kinetic experiments on the enzyme, several livers from fish fed on a high-protein diet (Cowey *et al.* 1977) were combined and a mitochondrial fraction

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Table 3. *Growth values of rainbow trout* (Salmo gairdneri) *given diets containing different levels of lysine* for 12 weeks*

Diet no. ...	1	2	3	4	5	6	7
Dietary lysine (g/kg)	10·4	12	14	17	20	23	26
Mean initial wt (g)†	5·15	5·24	5·19	5·13	5·29	5·01	5·33
Mean final wt (g)†	28·54	35·32	41·14	51·17	57·52	53·18	55·08
Mean wt gain (%)	454	574	693	897	987	961	933
Specific growth rate‡	2·04	2·27	2·46	2·71	2·84	2·81	2·78
Feed conversion ratio§	1·94	1·57	1·32	1·07	0·97	1·06	0·99
Mortalities	7	0	0	0	3	2	0

* For details of diets, see Table 2.

† Values are the average of the mean values obtained from each of the two tanks of fish per treatment (forty fish per tank).

‡ 100 (ln (final weight) − ln (initial weight))/84 d.

§ Dry weight feed/wet weight gain (mean values for each pair of tanks per diet treatment).

obtained as described previously. After sonication, the suspension was centrifuged at 100000 ***g*** for 30 min and the supernatant fraction used for the enzyme assays.

Oxidation of L-[*U*-^{14}*C*]*lysine*

L-[U-^{14}C]lysine (340 μCi/μmol) was obtained from Amersham International, Amersham, Bucks. A solution of this isotope was prepared in 0·15 M-sodium chloride such that 100 μl contained 1 μCi. A 1 μCi portion was injected intraperitoneally into a fish at 13.00 hours and the carbon dioxide respired between then and 09.00 hours the following day collected by the procedure described previously (Walton *et al.* 1984). During the incubation period the fish was kept in 3 litres water (2 parts fresh-water, 1 part sea-water) containing 150 mg penicillin G, 100 mg streptomycin and 10 mM-HEPES (pH 7.0) at 15°. After collection of the CO_2, 10 ml portions of the water were taken to dryness and radioactivity in the residue measured. In no case did the total radioactivity in the water amount to more than 2% of the injected dose.

Statistics

Results were subjected to analysis of variance and, if appropriate, Duncan's multiple-range test (Steel & Torrie, 1980) was applied to determine if differences between means were statistically significant.

RESULTS

Mean weight gains, food conversion ratios, specific growth rates and mortalities of trout given the seven experimental diets are shown in Table 3. Values obtained for each of these indices from the duplicate tanks were sensibly the same. When the mean weight gains were plotted against the dietary level of lysine (see Fig. 1) a break-point occurred at 19·5 g lysine/kg diet (equivalent to 43 g/kg dietary protein) and this value was taken to represent the dietary requirement level. Some evidence of fin erosion was noted in fish fed on diets 1 and 2 as observed by Ketola (1983) but otherwise no other gross pathological symptoms were seen and high mortalities did not occur in those fish given low-lysine diets.

Feed conversion ratios were high in diets containing low concentrations of lysine but decreased sharply as dietary lysine level increased. At a dietary lysine level of 20 g/kg the feed conversion ratio was 0·97. There was little further decrease in feed conversion ratio as dietary lysine level was further increased.

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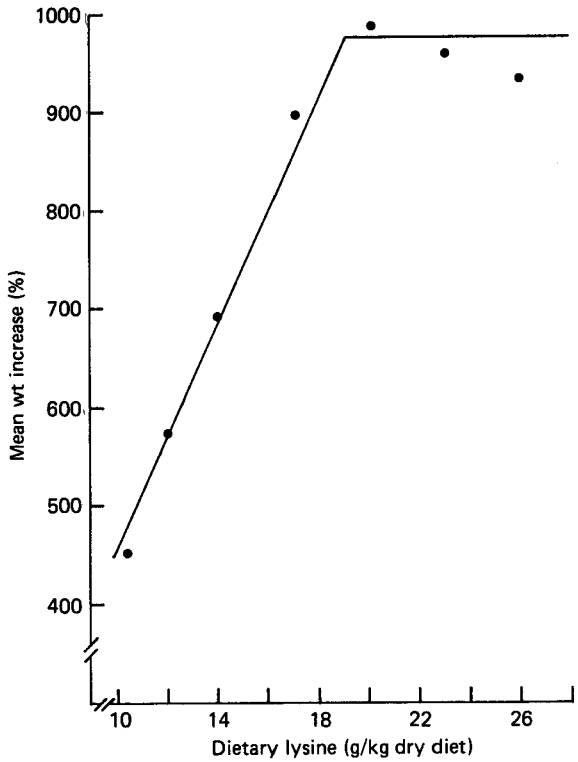

Fig. 1. Mean weight gain (%) of rainbow trout (*Salmo gairdneri*; mean initial weight 5 g) given seven different levels of dietary lysine for 12 weeks. Points are the means obtained from two duplicate tanks (forty fish per tank).

The effects of the different dietary treatments on several liver components are shown in Table 4. Values for the hepatosomatic index tended to be higher in those fish given diets 1 and 2 than in those fish given the other five diets. No significant differences, however, were found among treatments in the context of total lipid or carnitine or in the activities of lysine-α-ketoglutarate reductase. Carnitine content tended to be lower and enzyme activity tended to be higher in those fish given a lysine-adequate diet (diet nos. 5–7) than those given a deficient diet but the differences were slight.

Before the growth experiment some properties of trout-liver lysine-α-ketoglutarate reductase were studied. The enzyme had a mitochondrial location, a pH optimum of 7·6 and NADPH could not be replaced by NADH. The enzyme was rather unstable and a considerable (80%) decrease in activity was observed if the enzyme preparation was dialysed overnight as described by Hutzler & Dancis (1975). The values for the Michaelis constant, K_m, for L-lysine and α-ketoglutarate were 7·3 mM and 0·5 mM respectively (determined at 15°) which compares to values (determined at 37°) of 2·2 mM and 1·4 mM for the rat enzyme (Noda & Ichihara, 1978) and 1·5 mM and 1 mM for the human enzyme (Hutzler & Dancis, 1975).

Fig. 2 shows the effect of dietary lysine levels on the oxidation of an injected tracer dose of L-[U-^{14}C]lysine over a 20 h period. The level of oxidation was very low in those fish given diet nos. 1 and 2; was somewhat higher in fish given diet nos. 3, 4 and 5 and was even higher in those fish given diet nos. 6 and 7. The break-point on the plotted values indicated a dietary

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Table 4. *Hepatosomatic index (HSI), levels of total lipid and carnitine and activities of lysine-α-ketoglutarate reductase (saccharopine dehydrogenase (NADP+, lysine-forming), EC 1.5.1.8) in liver of rainbow trout* (Salmo gairdneri) *given diets containing different levels of lysine**

(Values in parentheses are the no. of individuals for each mean value in the row. For HSI five fish were used from one of the duplicate tanks, six fish from the other; for lysine-α-ketoglutarate reductase and total lipid, equal nos. of fish were taken from each of the duplicate tanks)

Diet no. ...		1	2	3	4	5	6	7	SEM
Dietary lysine (g/kg)		10·4	12	14	17	20	23	26	
HSI†	(11)	2·15[a]	2·21[a]	1·99[ab]	1·75[bc]	1·62[bc]	1·65[bc]	1·57[bc]	0·09
Total lipid (g/kg)	(6)	28·3	28·9	27·8	24·7	31·2	28·5	28·9	1·6
Carnitine (μmol/kg)	(3)	91	97	115	97	79	80	79	13
Lysine-α-ketoglutarate reductase (nmol/min per g liver wt)	(4)	155	158	162	182	182	170	180	31

[a, b, c] Mean values, within a horizontal row, not sharing a common superscript letter were significantly different ($P < 0.05$).

* For details of diets, see Table 2.

† HSI = (100 × liver weight)/body-weight.

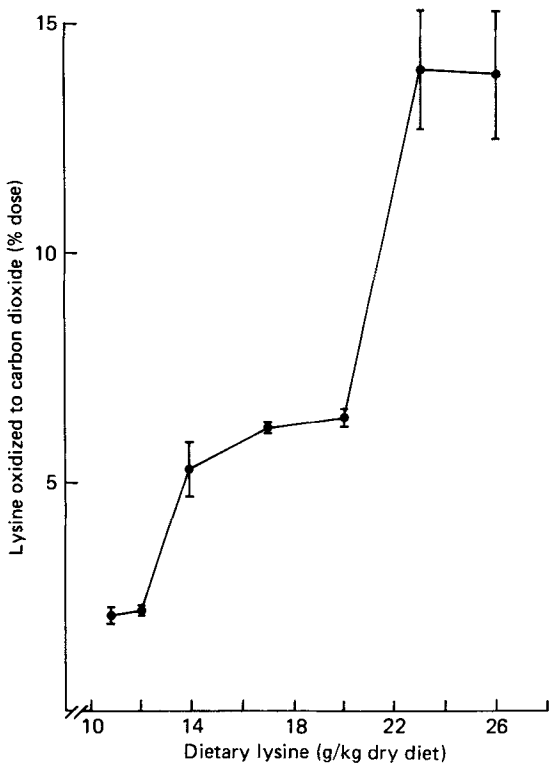

Fig. 2. Oxidation of intraperitoneally-injected L-[U-^{14}C]lysine (% dose) over 20 h in rainbow trout (*Salmo gairdneri*; three fish per value) given seven different levels of dietary lysine. Points are mean values with their standard errors represented by vertical bars.

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requirement level of 20 g/kg diet which is in close agreement to the value obtained from the growth data (see Fig. 1).

DISCUSSION

The dietary requirement of rainbow trout for lysine calculated from the growth values in the present study was 19 g/kg diet (equivalent to 43 g/kg dietary crude protein). Other values recently reported in the literature are 13 g/kg diet (37 g/kg dietary protein) by Kim & Hayes (1983); 29 g/kg diet (61 g/kg dietary protein) by Ketola (1983); and 21 g/kg diet (50 g/kg dietary protein) by Ogino (1980).

Complete agreement between independent estimates for a given nutrient would not necessarily be expected at this stage in the study of fish nutrition because of the variability of techniques used. In the present instance, factors other than dietary lysine, such as the amount of the second-limiting essential amino acid (or other nutrient) in the diet, growth rate and initial body size may have influenced performance and affected the determined requirement. Nevertheless, these independent estimates of lysine requirement are large. The variability cannot be explained by the differences in environmental conditions used. For example, our water temperature was 15°, that of Ketola (1983) 9·4°, and that of Ogino (1980) 15–18° but there is no convincing evidence that changes in water temperature affect the dietary protein requirement of rainbow trout (Cowey & Luquet, 1983). Again our experiments differed from those of other workers in that one-third strength sea-water (a salinity of about 10‰) was used instead of fresh-water. This was done to prevent the possible occurrence of pathogens (such as the fungus *Saprolegnia* and the protozoan *Ichthyophthirus multifilius* which causes white spot) in the water. As a salinity of 10‰ is approximately isotonic with the tissues of the fish it might marginally reduce expenditure of metabolic energy on osmoregulation. In fact, however, with coho, sockeye or chinook salmon, salinities of this level did not affect growth rate relative to that in fresh-water (Clarke *et al.* 1981).

To amplify and add confidence to the lysine requirement determined by weight gain, we attempted to assess the adequacy of the value obtained by using an additional method, namely lysine oxidation. Another method sometimes employed is the measurement of plasma and tissue levels of the free amino acid, but this method, when applied in fish, has met with only limited success (e.g. see Robinson *et al.* 1980; Walton *et al.* 1984). The oxidation technique has been applied to the determination of lysine requirement of growing rats (Brookes *et al.* 1972) and it was shown that the amount of lysine oxidized by rats given graded levels of lysine provided a means of estimating the lysine level optimal for growth. When the dietary supply of amino acids exceeds the demands for protein synthesis the surplus of C residues enters alternative pathways (lipogenesis, gluconeogenesis, direct oxidation) and the amount oxidized increases with dietary intake once the requirement value has been exceeded. The technique has recently been applied to determining the tryptophan requirement of rainbow trout (Walton *et al.* 1984). In the present instance production of $^{14}CO_2$ from an injected tracer dose of [U-^{14}C]lysine increased sharply when the dietary lysine value exceeded 20 g/kg. Agreement with requirement value from growth values was therefore close.

Although trout given low-lysine diets (nos. 1 and 2) tended to have higher hepatosomatic indices than those given the other five diets, no significant differences were seen in liver lipid or carnitine concentration (Table 4). In rats, lysine deficiency had little effect on liver carnitine concentration but did cause increased lipid levels (Khan & Bamji, 1979). Thus, in the present experiment, the trout would not seem to be suffering any adverse effects of carnitine deficiency as a consequence of a low-lysine diet.

The hepatic enzyme lysine-α-ketoglutarate reductase which is assumed to be the main

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degradative enzyme for lysine did not adapt to different dietary levels of lysine. This contrasts with the situation in chickens (Wang *et al.* 1973) and rats (Chu & Hegsted, 1976) in which enzyme activity increased with dietary lysine level. However, it was recently shown that in rainbow trout tryptophan pyrrolase (*EC* 1 . 13 . 11 . 11) was unaffected by the dietary level of tryptophan (Walton *et al.* 1984). In fact few amino-acid-deaminating enzymes of trout (other than those such as serine–pyruvate aminotransferase (*EC* 2 . 6 . 1 . 51) which are functionally gluconeogenic) have reduced activity levels in response to reduced protein or amino acid intake (Cowey & Luquet, 1983).

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