Reprinted from

Aquaculture

Aquaculture 151 (1997) 3-7

Re-evaluation of protein and amino acid requirements of rainbow trout (Oncorhynchus mykiss)

Kyu-Il Kim

Department of Animal Science, Cheju National University, Cheju 690-756, South Korea



Aquaculture

An international journal devoted to research on the exploration, improvement and management of all aguatic food resources, both floristic and faunistic, from freshwater, brackish and marine environments, related directly or indirectly to human consumption.

Section Editors:

- Nutrition: R.P. Wilson, Mississippi State University, Department of Biochemistry and Molecular Biology, P.O. Drawer BB, Mississippi State, MS 39762, USA
- Husbandry and Management: G. Hulata, Agricultural Research Organization, Volcani Center, Aquaculture Research Unit, P.O. Box 6, Bet Dagan 50250, Israel
- Physiology and Endocrinology: N. Bromage, Institute of Aquaculture, University of Stirling, Stirling FK9 4LA, UK
- Genetics and Breeding (+ Economics and Marketing): G.A.E. Gall, Department of Animal Science, University of California, Davis, CA 95616-8521, USA
- Diseases: D.J. Alderman, CEFAS, Weymouth Laboratory, The Nothe, Weymouth, Dorset DT4 8UB, UK

Honorary Editor: S.J. de Groot

Editorial Advisory Board:

B. Austin, Edinburgh, UK P. Bienfang, Honolulu, HI, USA R. Billard, Paris, France C.B. Cowey, Aberdeen, UK C. Crawford, Sandy Bay, Tasmania, Australia L.W. Crim, St. John's, Nfld., Canada L.R. D'Abramo, Mississippi State, MS, USA K. Dabrowski, Columbus, OH, USA R.H. Devlin, West Vancouver, B.C., Canada E.M. Donaldson, West Vancouver, B.C., Canada T.P.T. Evelyn, Nanaimo, B.C., Canada S.-Y. Shiau, Taiwan F.J. Gatesoupe, Plouzane, France D.M. Gatlin, College Station, TX, USA B. Gierde, Ås, Norway E.P. Glenn, Tucson, AZ, USA E.M. Gosling, Galway, Ireland E.M. Hallerman, Blacksburg, VA, USA R.W. Hardy, Seattle, WA, USA T. Hecht, Grahamstown, South Africa W.K. Hershberger, Seattle, WA, USA P.M. Hine, Wellington, New Zealand M. Jobling, Tromsø, Norway Y. Zohar, Baltimore, MD, USA

L. Kelly, Edinburgh, UK T.J. Lam, Singapore, Singapore L. Laubier, Brussels, Belgium O.V. Lindqvist, Kuopio, Finland R. Mann, Gloucester Point, VA, USA G. McBryde, Kingsville, TX, USA L. Orbán, Godollo, Hungary C.J.J. Richter, Wageningen, Netherlands C.J. Rodgers, Palma de Mallorca, Spain C.B. Schreck, Corvallis, OR, USA P. Smith, Galway, Ireland J.E. Stewart, Dartmouth, N.S., Canada T. Storebakken, Sunndalsøra, Norway A. Tandler, Eilat, Israel D. Teichert-Coddington, Auburn, AL, USA P. Thomas, Port Aransas, TX, USA K.T. Wada, Mie, Japan J.F. Wickins, Conwy, UK N.P. Wilkins, Galway, Ireland Z. Yaron, Tel Aviv, Israel

Publication information: Aquaculture (ISSN 0044-8486). For 1997 volumes 149-158 are scheduled for publication. Subscription prices are available upon request from the Publisher. Subscriptions are accepted on a prepaid basis only and are entered on a calendar year basis. Issues are sent by surface mail except to the following countries where air delivery via SAL mail is ensured: Argentina, Australia, Brazil, Canada, Hong Kong, India, Israel, Japan, Malaysia, Mexico, New Zealand, Pakistan, PR China, Singapore, South Africa, South Korea, Taiwan, Thailand, USA. For all other countries airmail rates are available upon request. Claims for missing issues must be made within six months of our publication (mailing) date.

Orders, claims, and product enquiries: please contact the Customer Support Department at the Regional Sales Office nearest you:

New York: Elsevier Science, P.O. Box 945, New York, NY 10159-0945, USA; Tel. (+1) 212-633-3730, [Toll free number for North American Customers: 1-888-4ES-INFO (437-4636)], Fax (+1) 212-633-3680, E-mail: usinfo-f@elsevier.com.

Amsterdam: Elsevier Science, P.O. Box 211, 1000 AE Amsterdam, The Netherlands; Tel. (+31) 20-4853757, Fax (+31) 20-4853432, E-mail: nlinfo-f@elsevier.nl.

Tokyo: Elsevier Science, 9-15, Higashi-Azabu 1-chome, Minato-ku, Tokyo 106, Japan; Tel. (+81) 3-5561-5033, Fax (+81) 3-5561-5047, E-mail: kyf04035@niftyserve.or.jp.

Singapore: Elsevier Science, No. 1 Temasek Avenue, #17-01 Millenia Tower, Singapore 039192; Tel. (+65) 434-3727, Fax: (+65) 337-2230, E-mail: asiainfo@elsevier.com.sg.



Aquaculture 151 (1997) 3-7

Aquaculture

Re-evaluation of protein and amino acid requirements of rainbow trout (Oncorhynchus mykiss)

Kyu-Il Kim

Department of Animal Science, Cheju National University, Cheju 690-756, South Korea

Abstract

Work on protein and amino acid requirements of rainbow trout, previously done at the University of Wisconsin, Aquaculture Research Laboratory, was re-evaluated with the view that the requirements are not much different from those of other rapidly growing farm animals. Most fish species are carnivorous and are adapted to use protein as a preferred energy source over carbohydrate, and thus require high levels of dietary protein (30-60%). Rainbow trout were found to utilize a dispensable amino acid (DAA) mixture or alanine alone as effectively as casein as an energy source. When a DAA mixture was used as a substitute for casein in a diet containing 2% gelatin and casein supplemented with arginine and methionine, the level of dietary protein needed to meet the indispensable amino acid (IDAA) requirements was found to be about 24%. This level is not different from that for baby pigs as recommended by the National Research Council. The levels of IDAA in the 24% protein diet exceeded the IDAA requirements of rainbow trout estimated to date. The estimated requirements (vs the contents in the 24% protein diet) for lysine, sulfur amino acids, tryptophan, aromatic amino acids and arginine were 1.3 (1.9), 0.8 (0.98), 0.2 (0.28), 1.5 (2.5) and 1.4 (1.6)% of diet, respectively. These values are comparable to those recommended for baby pigs except for arginine: 1.4, 0.68, 0.2, 1.1 and 0.6%, respectively. Results indicate that the requirements of rainbow trout for protein and amino acids are not much different from those of other rapidly growing farm animals. © 1997 Elsevier Science B.V.

Keywords: Fish nutrition; Amino acid requirements; Rainbow trout; Oncorhynchus mykiss

1. Introduction

Quantitative amino acid requirements of various fish species have been summarized in a recent National Research Council bulletin (NRC, 1993). A comprehensive review of the amino acid requirements of fish has also been published previously (Wilson, 1989). Wide variations in amino acid requirements within and among fish species are noted in those publications: e.g. lysine requirement of rainbow trout (1.3-2.9%) of diet or 3.71-6.1% of dietary protein) and chinook salmon (2%) or 5%). These variations may be the result of laboratory variances: different laboratories using different basal diets, different feeding levels and environmental conditions, and using fish of different ages, sizes and species (or strains). Although high levels of dietary protein are required by most fish species (NRC, 1993), individual amino acid requirements may not be correspondingly high because fish use a significant portion of dietary protein as an energy source (Kim et al., 1991). After these considerations were taken into account, data on the protein and amino acid requirements of fingerling rainbow trout, reported by the University of Wisconsin, Aquaculture Research Laboratory, were re-evaluated.

2. Procedures

Table 1

4

In an experiment to determine the protein requirement, three groups of 40 10-g rainbow trout (*Oncorhynchus mykiss*) each were fed, for 6 weeks, diets containing 10, 15, 20, 25 or 35% protein (not counting crystalline dispensable amino acids which replaced casein protein to vary the protein level). For studies to determine the amino acid requirements, four groups of 30 fish each (mean initial body weights, 10-15 g) were fed diets containing varying levels of lysine, arginine, tryptophan, sulfur amino acids or aromatic amino acids. The composition of the diet used for the protein study

| Ingredient | Diet | | | | | |
|------------------------------|-------------|-------------|-------------|-------------|-------------|--|
| | 10% protein | 15% protein | 20% protein | 25% protein | 35% protein | |
| Casein | 8.9 | 14.4 | 19.8 | 25.2 | 36.1 | |
| DAA mix ^a | 25 | 20 | 15 | 10 | 0 | |
| Gelatin | 2 | 2 | 2 | 2 | 2 | |
| Arginine | 0.18 | 0.31 | 0.45 | 0.58 | 0.85 | |
| Methionine | 0.13 | 0.18 | 0.23 | 0.28 | 0.38 | |
| Dextrin | 28.83 | 28.20 | 27.58 | 26.96 | 25.71 | |
| Dextrose | 5.00 | 5.00 | 5.00 | 5.00 | 5.00 | |
| Herring oil | 10.00 | 10.00 | 10.00 | 10.00 | 10.00 | |
| α-cellulose | 8.20 | 8.20 | 8.20 | 8.20 | 8.20 | |
| Carboxy methyl cellulose | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | |
| CaHPO ₄ | 2.94 | 2.94 | 2.94 | 2.94 | 2.94 | |
| Mineral mix ^b | 5.06 | 5.06 | 5.06 | 5.06 | 5.06 | |
| Vitamin mix ^b | 2.00 | 2.00 | 2.00 | 2.00 | 2.00 | |
| DL-α-tocopherol ^c | 0.0455 | 0.0455 | 0.0455 | 0.0455 | 0.0455 | |
| Protein level d | 10 | 15 | 20 | 25 | 35 | |

Composition (%) of the diets used for the determination of protein requirement

^a Dispensable amino acids as %: Ala 15.7, Asp 28.8, Gly 5.4, Glu 27.6, Pro 1.3, Ser 21.3.

^b Kim et al. (1991).

^c 1.1 IU mg⁻¹ in acetate form, which was added to herring oil before being mixed into diet.

^d Calculated excluding the nitrogen attributable to the added dispensable amino acid mixture.

| Ingredient | % | Ingredient | % |
|-----------------------|------|--------------------------|--------|
| IDAA ^a | 10.7 | Herring oil | 10.0 |
| DAA ^a | 16.3 | Tocopheryl acetate | 0.0455 |
| Casein (92% protein) | 8.9 | CM cellulose | 1.0 |
| Gelatin (89% protein) | 2.0 | Vitamin mix ^a | 2.0 |
| Dextrin | 27.2 | Mineral mix ^a | 5.1 |
| Dextrose | 5.0 | CaHPO ₁ | 2.9 |
| α-Cellulose | 8.2 | Choline chloride | 0.7 |

Table 2 Composition of the basal diet used for the determination of the amino acid requirements

^a Kim et al. (1992b).

IDAA, indispensable amino acid; DAA, dispensable amino acids.

and that of the basal diet used for the amino acid studies are shown in Tables 1 and 2, respectively. Fish were fed experimental diets three times daily to satiation in tanks containing 100 l of water at 15°C with a flow rate of $2-4 \ 1 \ min^{-1}$. Detailed experimental procedures including conditions, diet preparation and statistical analysis were as previously described (Kim et al., 1991).

3. Results and discussion

Weight gain increased linearly $(r^2 = 0.998)$ with protein levels up to 25% and breakpoint was found at 24% (Fig. 1), indicating that the dietary protein (24%) from casein and gelatin supplemented with arginine and methionine was sufficient to supply



Fig. 1. Relationship of dietary protein levels to weight gain in rainbow trout. Each point is a mean of three replicate groups. A broken-line model was used to find a break point; the slope \pm SE of the ascending line was 1.17 ± 0.11 , protein level at break point was 24.01 ± 1.05 and weight gain at break point was 20.00 ± 0.55 .

| by young pigs | | | | | |
|---------------|------------------|---------------------------|-----------------------|--|--|
| Amino acid | Trout (% diet) | Level in 25% protein diet | Young pig (NRC, 1988) | | |
| Lys | 1.3 ^a | 1.7 | 1.4 | | |
| Arg | 1.4 ^a | 1.6 | 0.6 | | |
| Met + Cys | 0.8 ^b | 0.8 | 0.7 | | |
| Phe + Tyr | 1.5 ^c | 2.6 | 1.1 | | |
| Ттр | 0.2 ^d | 0.38 | 0.2 | | |

Table 3

6

Amino acid levels required by rainbow trout as compared with those in the 25% protein diet or those required by young pigs

^a Kim et al. (1992b).

^b Kim et al. (1992a).

^c Kim (1993).

^d Kim et al. (1987).

all the indispensable amino acids required for optimum growth. This result suggests that the protein level (40%) recommended by the NRC (1993) includes dietary protein (24%) required to meet the indispensable amino acids and that (16%) required for meeting energy needs.

Studies done at the University of Wisconsin, Aquaculture Laboratory, (Smith, 1986) showed that 10% alanine or 10% dispensable amino acid mixture promoted growth as well as a diet containing 35% protein (from 36% casein and 2% gelatin). In those studies, fish fed the control diet containing 35% protein, and diets containing 25% protein and 10% dispensable amino acid mixture, 10% alanine or 10% (above the basal level) dextrin, gained 28.5, 28.4, 27.2 and 25.5 g over a 6-week feeding period, respectively. This result again suggests that the 25% protein from 25% casein and 2% gelatin in the diet was enough to meet the requirements of rainbow trout for the indispensable amino acids, and further suggests that the protein requirement of rainbow trout is not more than 25% when appropriate energy sources that have metabolizable energy (ME) values equivalent to protein are used to substitute for protein.

Requirement values have been reported for rainbow trout for tryptophan (Kim et al., 1987), sulfur amino acids (Kim et al., 1992a) lysine and arginine (Kim et al., 1992b) and aromatic amino acids (Kim, 1993). The requirement values for the respective amino acids were 0.2, 0.8, 1.3, 1.4 and 1.5% of diet (Table 3). The levels of these amino acids in the 25% protein diet used for the above study were 0.28, 0.98, 1.9, 1.6 and 2.5, respectively, and exceeded their respective requirement levels. This comparison confirms the aforementioned contention that the dietary protein level required to meet the requirements for indispensable amino acids by rainbow trout is not more than 25%.

As shown in Table 3, the amino acid requirements of rainbow trout are also comparable to those of young pigs (NRC, 1988) except for arginine (1.4 vs 0.6% of diet): lysine (1.3 vs 1.4), sulfur amino acids (0.8 vs 0.68), aromatic amino acids (1.5 vs 1.1) and tryptophan (0.2 vs 0.2).

Collectively, the present review indicates that energy sources in fish diets influence the protein requirement for optimum growth, and the metabolic partition of the dietary protein between use for protein synthesis and for energy supply. Therefore, the energy value of a diet must be defined in terms of its individual components before any conclusions about the protein requirement of a particular species can be drawn.

As proposed in the previous study (Kim et al., 1991), alanine equivalents of fish feed ingredients as an alternative to ME values may be used by comparing the growth of fish fed a diet containing an energy source of interest with the growth of fish fed a diet containing alanine, because alanine could substitute for protein in trout diet on an equal ME basis. This alanine equivalent method would be more useful than the conventional ME measurements because the ME value of a given dietary ingredient can vary with many factors, such as species and size of fish, environments and other dietary ingredients.

References

- Kim, K.I., 1993. Requirement for phenylalanine and replacement value of tyrosine for phenylalanine in rainbow trout (*oncorhynchus mykiss*). Aquaculture, 113: 243-250.
- Kim, K.I., Kayes, T.B. and Amundson, C.H., 1987. Effects of dietary tryptophan levels on growth, feed/gain, carcass composition and liver glutamate dehydrogenase activity in rainbow trout (*Salmo gairdneri*). Comp. Biochem. Physiol., 88B: 737-741.
- Kim, K.I., Kayes, T.B. and Amundson, C.H., 1991. Purified diet development and re-evaluation of the dietary protein requirement of fingerling rainbow trout (*Oncorhynchus mykiss*). Aquaculture, 96: 57–67.
- Kim, K.I., Kayes, T.B. and Amundson, C.H., 1992a. Requirements for sulfur amino acids and utilization of D-methionine by rainbow trout (*Oncorhynchus mykiss*). Aquaculture, 101: 95-103.
- Kim, K.I., Kayes, T.B. and Amundson, C.H., 1992b. Requirements for lysine and arginine by rainbow trout (*Oncorhynchus mykiss*). Aquaculture, 106: 333-344.
- NRC (National Research Council), 1988. Nutrient Requirements of Swine. 9th edn., National Academy Press, Washington, DC, 93 pp.
- NRC (National Research Council), 1993. Nutrient Requirements of Fish. National Academy Press, Washington, DC.
- Smith, D.A., 1986. Studies on the protein requirement of rainbow trout (Salmo gairdneri). PhD thesis, University of Wisconsin. Madison, WI. 241 pp.
- Wilson, R.P., 1989. Amino acids and proteins. In: J.E. Halver (Editor), Fish Nutrition. 2nd edn., Academic Press, New York, NY, pp. 112-151.

Aquaculture

Note to Contributors

Submission of articles. Manuscripts should be sent (in triplicate) directly to the relevant Section Editor.

Types of papers published in the journal: papers reporting results of original research – review articles – short communications – technical papers – editorials – book reviews – news and announcements.

Electronic manuscripts: Electronic manuscripts have the advantage that there is no need for the rekeying of text, thereby avoiding the possibility of introducing errors and resulting in reliable and fast delivery of proofs. For the initial submission of manuscripts for consideration, hardcopies are sufficient. For the processing of **accepted papers**, electronic versions are preferred. After **final acceptance**, your disk plus two, final and exactly matching printed versions should be submitted together. Double density (DD) or high density (HD) diskettes (3.5 or 5.25 inch) are acceptable. It is important that the file saved is in the native format of the wordprocessor program used. Label the disk with the name of the computer and wordprocessing package used, your name, and the name of the file on the disk. Further information may be obtained from the Publisher.

Authors in Japan please note: Upon request, Elsevier Science Japan will provide authors with a list of people who can check and improve the English of their paper (*before submission*). Please contact our Tokyo office: Elsevier Science Japan, 1-9-15 Higashi-Azabu, Minato-ku, Tokyo 106; Tel. (03)-5561-5032; Fax (03)-5561-5045.

All contributions will be carefully refereed for international relevance and quality. Submission of an article is understood to imply that the article is original and unpublished and is not being considered for publication elsewhere.

Enquiries concerning manuscripts and proofs: questions arising after acceptance of the manuscript, especially those relating to proofs, should be directed to Elsevier Editorial Services, Mayfield House, 256 Banbury Road, Oxford OX2 7DH, UK, Tel: (+44) 1865 314900, Fax (+44) 1865 314990.

Advertising information: Advertising orders and enquiries may be sent to: International: Elsevier Science, Advertising Department, The Boulevard, Langford Lane, Kidlington, Oxford OX5 1GB, UK, Tel. (+44)(0)1865 843565, Fax (+44)(0)1865 843976. USA and Canada: Weston Media Associates, Daniel Lipner, P.O. Box 1110, Greens Farms, CT 06436-1110, USA, Tel. (+1)(203)261-2500, Fax (+1)(203)261-0101. Japan: Elsevier Science Japan, Marketing Services, 1-9-15 Higashi-Azabu, Minato-ku, Tokyo 106, Japan, Tel. (+81)-3-5561-5033, Fax (+81)-3-5561-5047.

US mailing notice – Aquaculture (0044-8486) is published three times per month and four times per month in the months January, April, July and October by Elsevier Science B.V., Molenwerf 1, Postbus 211, 1000 AE Amsterdam. Annual subscription price in the USA is US\$2370.00 (valid in North, Central and South America), including air speed delivery. Second class postage paid at Jamaica, NY 11431.

USA POSTMASTERS: Send address changes to *Aquaculture*, Publications Expediting, Inc., 200 Meacham Avenue, Elmont, NY 11003. **AIRFREIGHT AND MAILING** in the USA by Publications Expediting.

Aquaculture has no page charges

For a full and complete Guide for Authors, please refer to Aquaculture, Vol. 149, Nos. 1–2, pp. 167–172. The guide can also be found on the World Wide Web: access under http://www.elsevier.nl or http://www.elsevier.com.

Copyright © 1997, Elsevier Science B.V. All rights reserved.

0044-8486/1997/\$17.00

 The paper used in this publication meets the requirements of ANSI/NISO Z39.48-1992 (Permanence of Paper).