Nutritional requirements of fish

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The impetus for accurate information on the nutrient requirements of fish derives very largely from the development, in many parts of the world, of an aquaculture industry that is dependent on artificial feeds. At the same time such information can provide the basis for comparative nutrition, whereby features of the nutrition of cold-blooded, water-breathing and mainly, carnivorous vertebrates which differ from the pattern largely common to omnivorous mammals are identified. Since this topic was last addressed (Cowey, 1988) the aquaculture industry has continued to grow, fuelled both by the continuing overexploitation of the marine environment, resulting in declining yields, and by the high quality of aquaculture products.

The first serious studies on nutritional requirements of fish were made in the 1950s. Since then much has been learned concerning fish husbandry, water quality, pellet quality (water stability) of fish diets and so on. Consequently, general methodological standards in nutritional experiments have improved greatly and some of the early values for nutrient requirements need to be revised, usually downward.

VITAMINS

Many of the earlier measurements, particularly of the vitamin B requirements of salmonids, indicated requirement levels much higher than those of mammals and birds. More recent studies on rainbow trout (*Oncorhynchus mykiss*) have demonstrated much lower requirements in line with those of omnivorous mammals.

In Table 1 the requirements of rainbow trout for certain B vitamins, obtained in recent experiments on rapidly growing young fish given a semi-purified, steam-pelleted diet are

Table 1. *Some vitamin B requirements (mg/kg air-dry diet) for maximal weight gain of young rainbow trout* (Oncorhynchus mykiss) *in comparison with (US) National Research Council (1981) recommended levels and with those of chick and piglet*

	Trout*	National Research Council (1981)	Chick[†]	Piglet[‡]
Riboflavin	3·6	20	3·6	3·0
Pyridoxine	2·0	10	3·0	1·5
Biotin	0·1	10	0·15	0·1
Pantothenic acid	12·1	40	10·0	13·0
Thiamin	1·0	10	1·8	1·3
Vitamin B_{12}	0·007	0·02	0·009	0·02
Folic acid	0·56	5·0	0·55	0·30

* Woodward (1989).
† National Research Council (1984).
‡ National Research Council (1988).

compared with the recommended levels of cold-water fishes based on earlier estimates from experiments on salmonids (National Research Council, 1981). The more recent measurements are several-fold lower (in some cases an order of magnitude lower) than the earlier measurements. Moreover, there is, as would be expected on metabolic grounds, close agreement between the requirements of cold-blooded carnivorous fish and omnivorous mammals.

Another matter of interest is the vitamin E requirement of fish. As a lipid-soluble antioxidant the vitamin E requirement is related to the intake of polyunsaturated fatty acids. From experiments carried out at a water temperature of 15°, Cowey et al. (1981) demonstrated a vitamin E requirement of 30 mg/kg diet (containing 10 g linolenic acid/kg) for rainbow trout; with 100 g fish oil/kg diet the requirement was 50 mg/kg diet (Cowey et al. 1983). The high-energy diets currently used in salmonid cultivation, with up to 300 g fish oil/kg, probably necessitate greater supplementation with vitamin E, possibly of the order of 100 mg/kg. A complicating factor is that water temperatures may fall to very low levels, 5° or lower, and there are indications that vitamin E requirement may increase as water temperature decreases (Cowey et al. 1984). The critical experiments remain to be done.

Nutritional interactions between vitamins A and E may also occur. Frigg & Broz (1984) observed that α-tocopherol levels in chick plasma were markedly decreased by high levels of dietary vitamin A supplementation (15 v. 3 mg retinol equivalent/kg). The interaction has not yet been examined in fish, but with very high levels of fish oil currently being added to salmon (*Salmo salar*) diets, very high concentrations of vitamin A might occur more or less adventitiously.

MINERALS

Mineral nutrition is complicated because fish are able to obtain appreciable quantities of essential cations from the environmental water. This is especially true of marine fish. Sakamoto & Yone (1979) showed that a purified diet for red sea bream (*Chrysophrys major*) containing only ferric citrate and Na_3PO_4 as a mineral supplement was as effective in maintaining growth and freedom from pathology as a similar diet containing a complete mineral supplement. Consequently, a stated cation requirement for freshwater fish should specify the concentration of that cation in the water in which the measurement was made. While the role of the environment as a source of cations applies to most metals, it has been most closely studied for Ca and this will be used by way of illustration of the role of the environment as a source of cations; it may be noted that a quantitative dietary Ca requirement has not been demonstrated in salmonids. In other species a requirement has been demonstrated only under somewhat unusual conditions, i.e. the use of water, free of Ca (Wilson, 1991).

About 95% of fish body Ca is contained in the acellular bone (bone without enclosed osteocytes) and scales but these tissues seem to play only a minor role in Ca dynamics. Thus, the skeleton does not serve as a reservoir of Ca as in mammals; the environmental water might be seen as fulfilling this role. During periods of dietary Ca deprivation fish may rely entirely on the environment for Ca.

Ca concentrations are generally less homeostatic in plasma and tissues of fish than in those of other vertebrates. In freshwater fish, at least, the gills may be regarded as the main Ca-regulating organ because of the facility with which they transport Ca from

environmental water directly to the blood. Ca can be transported from environmental concentrations as low as 0·015 mM into blood where normal Ca concentrations are 2–3 mM (Fontaine et al. 1972). Ca transport across the gills is thought to involve apical cAMP-regulated channels and active extrusion across the basolateral membrane (Fenwick, 1989). A Ca-activated ATPase (EC 3.6.1.38) in these cells increases in activity in response to transfer to water low in Ca (Flik & Perry, 1989) and activity decreases during acclimation to seawater.

While a dietary requirement for cholecalciferol has been demonstrated in rainbow trout, fish deficient in vitamin D showed no change in Ca concentration in plasma and other tissues (Barnett et al. 1982a). In addition, inclusion of cholecalciferol or 25-hydroxycholecalciferol or 1,25-dihydroxycholecalciferol in rainbow trout diets did not lead to detectable levels of 25-hydroxycholecalciferol in the plasma of the fish (Barnett et al. 1982b). Hormonal interactions involved in Ca homeostasis in fish remain to be clarified. Fish have no parathyroid glands and so lack parathormone. The function of calcitonin (formed in the ultimobranchial glands) has not been established; injection of calcitonin has had inconsistent effects on plasma Ca concentration. The principal hormone involved in lowering plasma Ca appears to be stanniocalcin from the corpuscles of Stannius (located at the posterior end of the kidney). In many species of fish prolactin is a hypercalcaemic hormone, but it has not yet been shown to be effective in inducing hypercalcaemia in freshwater trout.

CARBOHYDRATES

The capacity of most fish to assimilate and metabolize dietary carbohydrate is limited (Cowey, 1988). Given this caveat, warm-water fish in general appear better able to use dietary carbohydrate than do cold-water and marine fish. As noted earlier (Cowey, 1988) gelatinization of starch leads to its more efficient use. There is some species variation in utilization of mono- and disaccharides. Fructose, galactose and lactose are not well used by any species and in channel catfish (Ictalurus punctatus) this also holds true for maltose and glucose (Wilson & Poe, 1987). By contrast, these latter sugars, when included (272 g/kg) in diets for white sturgeon (Acipenser transmontanus), gave better growth rates than did starch or dextrin used at the same dietary concentration (Hung et al. 1989). They were also lipogenic; sturgeon given diets containing glucose or maltose were hyperlipidaemic, and the activities of glucose-6-phosphate dehydrogenase (EC 1.1.1.49), malic enzyme (EC 1.1.1.38, 1.1.1.40) and isocitrate dehydrogenase (EC 1.1.1.41) in their livers were significantly enhanced.

Blood glucose concentration is not closely controlled in fish despite the fact that plasma insulin levels are comparable with those of omnivorous mammals (Hilton et al. 1987). Fish may be said, therefore, to resemble mammals with non-insulin-dependent diabetes. Several recent studies examined the binding of insulin to receptors in skeletal muscle. That of Gutierrez et al. (1991) showed trout muscles to have 10–30-fold fewer insulin receptors per μg protein than the red and white skeletal muscle of the rat. Over all, insulin-receptor binding in trout is lower than that reported for mammals. From experiments with diets of different carbohydrate content (70 or 140 g dextrin/kg) Gutierrez et al. (1991) concluded that the higher glycaemic levels observed in the trout given the high-carbohydrate diet were not a consequence of impaired binding of insulin to its receptors in skeletal muscles. Assays on trout livers from this experiment showed

specific binding of insulin to be lower in the fish given the high-carbohydrate diet. It appears that the phenomenon of down regulation of receptor numbers by circulating levels of insulin may, under some so far unspecified circumstances, be applicable to fish liver.

A sluggish and uneven response to dietary carbohydrate was seen in recent experiments by Mazur *et al.* (1992). A suggested explanation was that the pancreatic D-cells, producing somatostatins, are sensitive to glucose, and as piscine somatostatin inhibits insulin release (Sheridan *et al.* 1987) it may limit insulin levels in the plasma when carbohydrate is administered. This conflicts directly with the findings of Hilton *et al.* (1987), that a diet containing highly digestible carbohydrate given to rainbow trout gave rise to high levels of plasma insulin 3 h after feeding. No resolution of these findings has yet been forthcoming.

LIPIDS

Diets for cold-water fish usually contain 150–250 g fish oil/kg, although the amounts needed to meet essential fatty acid (EFA) requirements are very small (Cowey, 1988). Diets for warm-water fish usually contain 50–100 g lipid/kg.

The experiments of Castell *et al.* (1972) demonstrated that rainbow trout have appreciable capacity to metabolically modify dietary fatty acids. Dietary 18:1(n-9), 18:2(n-6) and 18:3(n-3) gave rise respectively to 20:3(n-9), 20:4(n-6) and to 22:6(n-3) in the liver and other tissues. The pathways operating in these desaturation and elongation reactions have not been demonstrated in rainbow trout and other fish, but were assumed to occur by a series of reactions similar to those occurring in omnivorous mammals. Thus, docosahexaenoic acid would be formed from linolenic acid by the sequential actions of $\triangle$6-, $\triangle$5-, and $\triangle$4-desaturases, docosahexaenoate, 22:6(n-3) being formed from 22:5(n-3) by the action of a $\triangle$4-desaturase. Recent work in mammals (Voss *et al.* 1991) has shown that 22:6(n-3) is indeed formed from 22:5(n-3), but by a pathway independent of a $\triangle$4-desaturase. The results of Voss *et al.* (1991) indicate that rat liver microsomes do not possess $\triangle$4-desaturase activity. The pathway from 22:5(n-3) to 22:6(n-3) in rat hepatocytes involves microsomal chain elongation of 22:5(n-3) to 24:5(n-3) followed by its desaturation to 24:6(n-3); this microsomal product is then metabolized, via β-oxidation, to 22:6(n-3).

None of the desaturases present in fish liver microsomes have been purified and characterized. However, if an analogy is drawn between pathways of fatty acid desaturation and elongation in mammals and those in fish, it seems highly unlikely that $\triangle$4-desaturase activity is present in liver microsomes of trout or of other fish.

Although a dietary supply of linolenic acid is said to meet the EFA requirements of rainbow trout, it continues to be the case that a small, but significant requirement for linoleic acid, or in the case of marine fish, of 20:4(n-6) cannot be ruled out (Cowey, 1988). In compositional studies on cultured cells from rainbow trout, Tocher (1990), emphasized that the high level of 20:4(n-6) in phosphatidyl inositol is indicative of the presence of 'precise and tightly controlled' mechanisms for the maintenance of 20:4(n-6) in fish cells where it would be required to fulfil a role in eicosanoid synthesis. Similar findings were made in studies by Tocher & Mackinlay (1990) on cultured cells of turbot (*Scophthalmus maximus*). As yet the critical growth experiments, needed to establish a dietary (n-6) requirement in fish, remain to be carried out.

ENERGY REQUIREMENT

Food intake tends to be controlled by the energy need of the animal and, accordingly, energy requirement will regulate within certain limits the intake of nutrients. Therefore, it is important that the energy content of the diet be adjusted so as to promote the desired intake of all nutrients.

A feature of energy requirement in poikilotherms is that it is affected by environmental temperature. This controls metabolic rate and in addition the maintenance energy requirement of cold-water fish is especially influenced by a rise in water temperature from low levels up to and above their (generalized) optimum. Thus, the maintenance requirement of sockeye salmon (*Oncorhynchus nerka*) at 20° is seven times its value at 1° (Brett *et al.* 1969). Maintenance energy requirement is approximated by fasting heat production (kJ/d) which is given by:

$$(-1{\cdot}04+3{\cdot}26T-0{\cdot}05T^2)\ W^{0{\cdot}824},$$

where T is temperature (°), and W is weight (kg) (Cho, 1992). Fasting heat production generally accounts for about 30% of digestible energy (DE) intake depending on temperature. Of the remaining energy, approximately 20% is lost in the form of heat increment of feeding and non-faecal losses.

The dietary energy required for the production of 1 kg biomass of salmonids is, in the case of rapidly growing fingerlings, approximately 12 MJ DE. For larger fish (1–2 kg live weight) requirements are higher, about 20 MJ DE being needed. In line with this, diets presently used in salmonid production have energy densities in the range 17–22 MJ DE/kg. The protein in the fingerling diets amounts to 25 g digestible protein (DP)/MJ DE and in diets for larger fish 20 g DP/MJ DE. Energy retention in salmonid fish is of the order of 45–55% DE, appreciably greater than is the case in mammals.

PROTEINS AND AMINO ACIDS

Amino acid requirements. In most of the test diets used to measure amino acid requirements, the N component has consisted of both a crystalline amino acid mixture (making up about 50% of the N component) and some whole protein. Such diets gave growth rates that were inferior, often markedly so, to diets of similar amino acid composition in which the N component was entirely protein (Cowey, 1988). Recently Kim *et al.* (1992*a,b*) obtained high growth rates with rainbow trout given diets containing up to 270 g crystalline amino acid mixture/kg plus 90 g protein/kg as the N component. No special measures were taken to prevent rapid absorption of crystalline amino acids in the gastrointestinal tract, which might lead to elevated concentrations of amino acids in tissues and enhanced catabolism of those amino acids; it is not obvious why similar diets failed to provide such high rates of growth in other laboratories.

In these experiments Kim *et al.* (1992*a*) demonstrated a total S amino acid requirement of 8 g/kg diet (23 g/kg dietary protein); they also showed that D-methionine is used as efficiently as L-methionine for weight gain and that, on a molar S basis, cysteine will replace 42% of the methionine requirement. The lysine and arginine requirements reported by Kim *et al.* (1992*b*) were 13 and 14·1 g/kg diet (37·1 and 40·3 g/kg dietary protein) respectively. Kim *et al.* (1992*b*) also demonstrated that there are no interactions between dietary arginine and lysine levels. These values are in line with other recent measurements (total S amino acid requirement of 9·6 g/kg diet or 24 g/kg dietary protein

Cowey *et al.* (1992), arginine requirement 14 g/kg diet or 42 g/kg dietary protein (Cho *et al.* 1992)). These requirement values, for all three amino acids are very much at the low end of previously reported requirements for rainbow trout.

Non-essential amino acids as a preferred energy source. In another recent study Kim *et al.* (1991) re-examined the requirement of fingerling rainbow trout for dietary protein. They obtained a value, to achieve maximal growth, of not more than 300 g protein/kg diet (the reference protein being casein, a highly digestible protein, supplemented with methionine and arginine in a diet containing 100 g lipid/kg), a value lower than currently accepted values (National Research Council, 1981). It was also shown that some of this dietary protein could be replaced with alanine or a non-essential amino acid mixture, but could not be replaced with dextrin on an equal DE basis. The requirement of the trout for essential amino acids was met by a diet containing 240 g casein (supplemented with arginine and methionine)/kg, the remaining 60 g/kg of supplemented casein was seen as meeting energy needs. Kim *et al.* (1991) regard a diet containing 250 g whole protein/kg together with 100 g non-essential amino acids/kg (Table 2 of Kim *et al.* (1991)) as having only 250 g crude protein ($N \times 6 \cdot 25$)/kg. They conclude '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'. It is a peculiar concept that alanine, or a non-essential amino acid mixture, when included in a diet, should not be regarded as part of the crude protein content.

We have worked along similar lines to Kim and his colleagues (Kim *et al.* 1991, 1992*a,b*), supplying in the diet a concentration of whole protein that would just meet the essential amino acid requirements of the fish and attempting to achieve maximal growth rates by supplementing this diet with different non-essential or essential amino acids or mixtures of amino acids. Diet 1 (Table 2) which meets the essential amino acid requirements of rainbow trout contained 251 g protein/kg and had a DE content of 15 MJ/kg, an appreciable part of which was supplied by lipid (161 g/kg). Energy densities of diets 1–6 were 15–16 MJ/kg, diet 7 contained 18 MJ/kg. As a proportion of DE, DP levels ranged from 16·7 g/MJ (diet 1) to about 22 g/MJ (diets 2–7). It can be seen from Table 3 that supplementation of the basal diet with either alanine, a mixture of glycine and glutamine, or a complete mixture of non-essential amino acids did not enhance growth rate over that obtained with the basal diet. By contrast substitution with an essential amino acid mixture (diet 5) led to a significant increase in growth rate to a value comparable with that of a high fishmeal diet (diet 6); further increase in growth rate occurred when nutrient density was again increased (diet 7). The second experiment (Tables 2 and 4) was carried out mainly to determine whether any essential amino acids might possibly be limiting in the diets supplying only 251 g DP/kg or 251 g DP together with one or more non-essential amino acids (i.e. diets 1–4, Expt 1). To this end diet 1 (Expt 2) served as a control diet with an energy density equal to that of the other diets, diet 2 was the control diet together with a non-essential amino acid mixture, diet 3 was the control diet with alanine (shown by Kim *et al.* (1991) to substitute for protein on an equal ME basis), the remaining diets had added to them essential amino acids that might possibly be limiting. However, these essential amino acids were sensibly without effect on growth rate (diets 3–7) and only when a complete essential amino acid mixture was added to the diet was growth rate enhanced (diet 8). Even so, it was inferior to that of a high-fishmeal diet (diet 9).

Table 2. *Compositions of diets used to examine the effect on growth of rainbow trout (Oncorhynchus mykiss) of supplementation with non-essential amino acids (NEAA) of a basal diet formulated to meet their essential amino acid (EAA) requirements at a minimal concentration of dietary protein (g/kg feed)**

Diet no.	1	2	3	4	5	6	7	8	9
Expt 1:									
Basal mix†	615	615	615	615	615	615	870		
Ala	—	102	43	—	—	—	—		
Gly + Gln	—	—	42	—	—	—	—		
NEAA‡	—	—	—	124	—	—	—		
EAA§	—	—	—	—	124	—	—		
Fishmeal	—	—	—	—	—	137	—		
Starch, maize	245	188	205	166	166	168	—		
Fish oil	140	95	95	95	95	80	130		
Digestible protein (g/kg)‖	251	349	349	349	349	349	402		
Digestible fat (g/kg)	161	116	116	116	116	115	168		
Digestible energy (MJ/kg)	15	16	15	16	16	15	18		
Expt 2:									
Basal mix†	615	615	615	615	615	615	615	615	615
Ala	—	—	115·5	—	—	—	—	—	—
Met	—	—	8·5	8·5	8·5	8·5	8·5	—	—
Arg	—	—	—	—	14·6	14·6	14·6	—	—
Lys	—	—	—	—	—	12·4	12·4	—	—
Trp	—	—	—	—	—	—	2	—	—
NEAA‡	—	124	—	115·5	100·9	88·5	86·5	—	—
EAA§	—	—	—	—	—	—	—	124	—
Fishmeal	—	—	—	—	—	—	—	—	184
Starch, maize	225	171	171	171	171	171	171	171	121
Fish oil	160	90	90	90	90	90	90	90	80
Digestible protein (g/kg)‖	251	372	372	372	372	372	372	372	372
Digestible fat (g/kg)	181	111	111	111	111	111	111	111	119
Digestible energy (MJ/kg)	16	16	16	16	16	16	16	16	16

* All crystalline amino acids were coated with gelatinized starch.

† Supplied (g/kg basal mix): fishmeal 150, maize-gluten meal 120, soya-bean meal 60, wheat middlings 100, dried whey 100, gelatin 30 and vitamin–mineral premixes.

‡ NEAA supplied (g/kg): Ala 15·9, Asp 25, Glu 50·6, Gly 11·3, Pro 9·7, Ser 11·5.

§ EAA supplied (g/kg): Met 5·8, Cys 3, Lys 12·6, Trp 2·1, Thr 8·6, Ile 9·5, Val 13·6, Leu 21·6, Arg 15, Phe 10·2, Ala 22.

‖ Digestible N × 6·25.

The contrasting findings of Kim *et al.* (1991) and ourselves are not easy to explain. Growth rates in the experiments from the two laboratories appear somewhat similar. It has been held that, in warm-blooded animals, there is a constant relationship between essential amino acid requirement and protein intake up to the level of protein required for maximum growth (Almquist, 1972). For this reason amino acid requirements of fish are usually expressed as a proportion of dietary protein as well as on a dry matter basis. If this relationship does indeed hold true for fish also, then an increase in dietary protein

Table 3. *Expt 1. Live-weight gain and feed efficiency of rainbow trout* (Oncorhynchus mykiss) *fed on either a basal diet or a basal diet supplemented with amino acids for 12 weeks*

(Mean initial body wt was 7 g/fish; each 60 litre fibreglass tank contained sixty fish; there were three replicate tanks per diet. Water temperature was 15°)

Diet	Digestible protein (g/kg)		Live-wt gain (g/fish)	Feed efficiency (wt gain/feed intake)
1 Basal, high-fat	251		44·91[a]	0·79[a]
2 Alanine	349		45·57[a]	0·87[b]
3 Gly + Gln	349		44·96[a]	0·81[a]
4 NEAA	349		44·35[a]	0·83[ab]
5 EAA	349		51·69[b]	0·95[c]
6 Fishmeal	341		53·17[b]	0·96[c]
7 High nutrient density	402		65·89[c]	1·18[d]
		SEM (12 df)	0·60	0·01
		HSD	2·97	0·04

HSD, Tukey's honestly significant difference ($P \le 0.05$).
[a,b,c,d] Within the same column values with unlike superscript letters were significantly different.

Table 4. *Expt 2. Live-weight gain and feed efficiency of rainbow trout* (Oncorhynchus mykiss) *fed on either a basal diet or a basal diet supplemented with amino acids for 12 weeks*

(Mean initial body wt was 5·8 g/fish; each 60 litre fibreglass tank contained eighty-three fish; there were three replicate tanks per diet. Water temperature was 15°)

Diet	Digestible protein (g/kg)		Live-wt gain (g/fish)	Feed efficiency (wt gain/feed intake)
1 Basal, high-fat	251		34.42[a]	0·84[a]
2 NEAA	372		35·56[ab]	0·87[ab]
3 Ala + Met	372		35·60[ab]	0·88[ab]
4 NEAA + Met	372		35·26[a]	0·88[ab]
5 NEAA + Met + Arg	372		36·68[ab]	0·90[ab]
6 NEAA + Met + Arg + Lys	372		36·74[ab]	0·90[b]
7 NEAA + Met + Arg + Lys + Trp	372		37·96[b]	0·93[b]
8 EAA	372		41·92[c]	1·03[c]
9 High fishmeal	372		45·44[d]	1·11[d]
		SEM (16 df)	0·498	0·01
		HSD	2·504	0·06

HSD, Tukey's honestly significant difference ($P \le 0.05$).
[a,b,c,d] Within the same column values with unlike superscript letters were significantly different.

content brought about by adding non-essential amino acids to a diet, would lower essential amino acid level as a proportion of dietary protein.

The addition of non-essential amino acids to a diet containing 251 g protein/kg (Expt 1) was without effect on the activities of certain liver enzymes involved in amino acid

catabolism. Glutamate dehydrogenase (EC 1.4.1.2), mitochondrial aspartate amino-transferase (EC 2.6.1.1) and glutaminase (EC 3.5.1.2) activities (expressed as units in the liver of a fish of 100 g) did not differ significantly in any of the treatments in Expt 1. Hepatic D-amino acid oxidase (EC 1.4.3.3) activity was also measured in trout from Expt 1. This enzyme, a flavin-dependent enzyme, serves no obvious metabolic purpose and its activity is not conserved under conditions of riboflavin deficiency (Woodward, 1983). It seemed feasible that, if there are adaptations at the enzyme level to dietary protein restriction, D-amino acid oxidase activity would not be conserved. However, no significant changes in its activity were found.

REFERENCES

Almquist, H. J. (1972). *Proteins and Amino Acids in Animal Nutrition*, 5th ed. New York: S. B. Penick.

Barnett, B. J., Cho, C. Y. & Slinger, S. J. (1982*a*). Relative bipotency of dietary ergocalciferol and cholecalciferol and the role of and requirement for vitamin D in rainbow trout (*Salmo gairdneri*). *Journal of Nutrition* **112**, 2011–2019.

Barnett, B. J., Jones, G., Cho, C. Y. & Slinger, S. J. (1982*b*). The biological activity of 25-hydroxy-cholecalciferol and 1,25-dihydroxycholecalciferol for rainbow trout (*Salmo gairdneri*). *Journal of Nutrition* **112**, 2020–2026.

Brett, J. R., Shelbourne, J. E. & Shoop, C. T. (1969). Growth rate and body composition of fingerling sockeye salmon, *Oncorhynchus nerka*, in relation to temperature and ration size. *Journal of the Fisheries Research Board of Canada* **26**, 2363–2394.

Castell, J. D., Lee, D. J. & Sinnhuber, R. O. (1972). Essential fatty acids in the diet of rainbow trout (*Salmo gairdneri*): lipid metabolism and fatty acid composition. *Journal of Nutrition* **102**, 93–100.

Cho, C. Y. (1992). Feeding systems for rainbow trout and other salmonids with reference to current estimates of energy and protein requirements. *Aquaculture* **100**, 107–123.

Cho, C. Y., Kaushik, S. J. & Woodward, B. (1992). Dietary arginine requirement of young rainbow trout (*Oncorhynchus mykiss*). *Comparative Biochemistry and Physiology* **102A**, 211–216.

Cowey, C. B. (1988). The nutrition of fish: the developing scene. *Nutrition Research Reviews* **1**, 255–280.

Cowey, C. B., Adron, J. W., Walton, M. J., Murray, J., Youngson, A. & Knox, D. (1981). Tissue distribution, uptake, and requirement for α-tocopherol of rainbow trout (*Salmo gairdneri*) fed diets with a minimal content of unsaturated fatty acids. *Journal of Nutrition* **111**, 1556–1567.

Cowey, C. B., Adron, J. W. & Youngson, A. (1983). The vitamin E requirement of rainbow trout (*Salmo gairdneri*) given diets containing polyunsaturated fatty acids from fish oil. *Aquaculture* **30**, 85–93.

Cowey, C. B., Cho, C. Y., Sivak, J. G., Weerheim, J. A. & Stuart, D. D. (1992). Methionine intake in rainbow trout (*Oncorhynchus mykiss*), relationship to cataract formation and the metabolism of methionine. *Journal of Nutrition* **122**, 1154–1163.

Cowey, C. B., Degener, E., Tacon, A. G. J., Youngson, A. & Bell, J. G. (1984). The effect of vitamin E and oxidized fish oil on the nutrition of rainbow trout (*Salmo gairdneri*) grown at natural, varying water temperatures. *British Journal of Nutrition* **51**, 443–451.

Fenwick, J. C. (1989). Calcium exchange across fish gills. In *Vertebrate Endocrinology: Fundamentals and Biomedical Implications*, vol. 3, pp. 319–342 [P. K. T. Pang and M. P. Schriebman, editors]. New York: Academic Press Inc.

Flik, G. & Perry, S. F. (1989). Cortisol stimulates whole body calcium uptake and the branchial calcium pump in freshwater rainbow trout. *Journal of Endocrinology* **120**, 75–82.

Fontaine, M. M., Delerue, N., Martelly, E., Marchekidon, J. & Milet, C. (1972). Role des corpuscles de Stannius dans les echanges de calcium d'un poisson teleosteen, l'Anguilla, avec le milieu ambiant (The role of Stannius' corpuscles in the calcium exchange of a teleost fish, the eel, with its surroundings). *Comptes Rendus Hebdomadaires Seances Academie Sciences*, series D **275**, 1523–1528.

Frigg, M. & Broz, J. (1984). Relationships between vitamin A and vitamin E in the chick. *International Journal of Vitamin and Nutrition Research* **54**, 125–134.

Gutierrez, J., Asgard, T., Fabbri, E. & Plisetskaya, E. M. (1991). Insulin receptor binding in skeletal muscle of trout. *Fish Physiology and Biochemistry* **9**, 351–360.

Hilton, J. W., Plisetskaya, E. M. & Leatherland, J. F. (1987). Does oral 3,5,3'-triiodo-L-thyronine affect dietary glucose utilization and plasma insulin levels in rainbow trout (*Salmo gairdneri*)? *Fish Physiology and Biochemistry* **4**, 113–120.

Hung, S. S. O., Fynn-Aikins, F. K., Lutes, P. B. & Xu, R. (1989). Ability of juvenile white sturgeon (*Acipenser transmontanus*) to utilize different carbohydrate sources. *Journal of Nutrition* **110**, 727–733.

Kim, K.-I., Kayes, T. B. & 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. & Amundson, C. H. (1992*a*). 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. & Amundson, C. H. (1992*b*). Requirements for lysine and arginine by rainbow trout (*Oncorhynchus mykiss*). *Aquaculture* **106**, 333–344.

Mazur, G. N., Higgs, D. A., Plisetskaya, E. & March, B. E. (1992). Utilization of dietary starch and glucose tolerance in juvenile chinook salmon (*Oncorhynchus tshawytscha*) of different strains in seawater. *Fish Physiology and Biochemistry* **10**, 303–313.

National Research Council (1981). *Nutrient Requirements of Domestic Animals*, no. 16. *Nutrition Requirements of Coldwater Fishes*. Washington, DC: National Research Council.

National Research Council (1984). *Nutrient Requirements of Domestic Animals. Nutrient Requirements of Poultry*. 8th revised ed. Washington, DC: National Research Council.

National Research Council (1988). *Nutrient Requirements of Domestic Animals. Nutrient Requirements of Swine*. 9th revised ed. Washington, DC: National Research Council.

Sakamoto, S. & Yone, Y. (1979). Mineral mixture in purified diet for red sea bream. *Nippon Suisan Gakkaishi* **45**, 873–877.

Sheridan, M. A., Plisetskaya, E. M., Bern, H. A. & Gorbman, A. (1987). Effects of somatostatin-25 and urotensin II on lipid and carbohydrate metabolism of coho salmon, *Oncorhynchus kisutch*. *General and Comparative Endocrinology* **66**, 365–372.

Tocher, D. R. (1990). Incorporation and metabolism of (*n*-3) and (*n*-6) polyunsaturated fatty acids in phospholipid classes in cultured rainbow trout (*Salmo gairdneri*) cells. *Fish Physiology and Biochemistry* **8**, 239–249.

Tocher, D. R. & Mackinlay, E. M. (1990). Incorporation and metabolism of (*n*-3) and (*n*-6) polyunsaturated fatty acids in phospholipid classes in cultured turbot (*Scophthalmus maximus*) cells. *Fish Biochemistry and Physiology* **8**, 251–260.

Voss, A., Reinhart, M., Sankarappa, S. & Sprecher, H. (1991). The metabolism of 7,10,13,16,19-docosa-pentaenoic acid to 4,7,10,13,16,19-docosahexaenoic acid in rat liver is independent of a 4-desaturase. *Journal of Biological Chemistry* **266**, 19995–20000.

Wilson, R. P. (1991). *Handbook of Nutrient Requirements of Fish*. Boca Raton, Florida: CRC Press, Inc.

Wilson, R. P. & Poe, W. E. (1987). Apparent inability of channel catfish to utilize dietary mono- and di-saccharides as energy sources. *Journal of Nutrition* **117**, 280–285.

Woodward, B. (1983). Sensitivity of hepatic D-amino acid oxidase and glutathione reductase to the riboflavin status of the rainbow trout (*Salmo gairdneri*). *Aquaculture* **34**, 193–201.

Woodward, B. (1989). Dietary requirements of some water soluble vitamins for young rainbow trout. *Proceedings of the Twenty-fifth Annual Nutrition Conference for Feed Manufacturers*, pp. 187–195. Ottawa, Canada: Canadian Feed Industry Association.

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