THE NUTRITION OF FISH: THE DEVELOPING SCENE

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INTRODUCTION

Although fish, such as carp (*Cyprinus carpio*), have been farmed since ancient times, scientific study of their nutrition is of recent origin. The first serious studies were made immediately post war and the subject has gathered pace since about the mid-sixties.

Research has concentrated on relatively few species. These species (1) are sufficiently highly esteemed by the public to make them a profitable market commodity when cultivated, (2) are catholic in their tastes, i.e. they will accept food pellets that differ markedly from natural food in taste and texture, (3) produce fry that are large enough to accept artificial food at first feeding. Much of the research has been carried out on rapidly growing juvenile fish.

Wholly marine fish have very small eggs that hatch to very small first-feeding larvae and are generally reared on natural food (rotifers (*Brachionus plicatilis*), brine shrimps (*Artemia salina*)) to begin with. Subsequent later weaning to a partly defined diet for nutritional studies is difficult, so, while some marine fish (yellowtail (*Seriola quinqueradiata*), red sea bream (*Chrysophrys major*)) are successfully farmed, knowledge of their nutritional requirements is limited.

While species of fish have evolved to fill all ecological niches in the natural environment the great majority of them lie near the head of the food pyramid. They are carnivorous and have developed a metabolism to deal with a high intake of dietary protein, so capacity to metabolize carbohydrate is correspondingly limited.

CARBOHYDRATE

UTILIZATION OF DIETARY CARBOHYDRATE

It was evident from some of the early studies in fish nutrition that few species are able to utilize diets containing large amounts of native starches effectively. Digestibility is low and decreases as concentrations of starch in the diet increase. This is reflected in amylase (*EC* 3.2.1.1) activity which is very low in marine fish, although larger activities are found in the more omnivorous species such as carp (Shimeno *et al.* 1977). In line with this Furuichi & Yone (1980) showed that optimal levels of dietary dextrin for carp (300–400 g/kg in the diet) were greater than for red sea bream (approximately 200 g/kg) and for yellowtail (approximately 100 g/kg). In all three species diets containing dextrin led to elevated levels of glycogen in liver, this elevation being less marked in carp than in the other two species.

Pre-gelatinization of starch leads to more efficient use of it at least by rainbow trout (*Salmo gairdneri*). This has been shown by several workers including Bergot & Breque (1983) who found gelatinized starch to be about 90% digested, and by Kaushik & Oliva Teles (1985) who used 300 g gelatinized starch/kg in high-protein–high-energy diets containing 400 g protein/kg. Elevated levels of liver glycogen and high values for relative liver weight (up to 40 g/kg body-weight compared to about 10 g/kg in fish given low-carbohydrate diets) were characteristic of trout given these diets. Whether such diets could be used with impunity over a 14 to 18-month growth period remains to be examined.

The low digestibility of raw starch by rainbow trout is apparently partly due to amylase being adsorbed to raw starch (but not to pre-gelatinized starch) so that hydrolysis of the starch is inhibited (Spannhof & Plantikow, 1983). It is not clear whether this implies some major difference in physico-chemical properties of amylase from trout compared with that from mammals.

By contrast with gelatinized starch, diets containing monosaccharides and disaccharides are not well used by either rainbow trout or channel catfish (*Ictalurus punctatus*); in the former species dietary glucose levels in excess of 140 g/kg were not effectively used (Hilton & Atkinson, 1982). Subsequently Hilton *et al.* (1987) obtained net energy values for glucose and raw starch, for trout, that were 24·6 and 12·6% respectively of their gross energy values (they were present in diets at 250 g dry matter/kg).

Wilson & Poe (1987) demonstrated that channel catfish do not use glucose, fructose, maltose or sucrose as energy sources (330 g/kg in diet). Dextrin proved an excellent energy source and maize starch was almost as well used. Oral carbohydrate tolerance tests with glucose and maltose led to persistent hyperglycaemia, sucrose led to a gradual increase in plasma glucose, while fructose was poorly absorbed with no conversion to glucose. The authors suggest that 'channel catfish appear to have insufficient insulin, which indicates a diabetic-like status'.

METABOLISM OF CARBOHYDRATE

Several lines of evidence serve to show that carbohydrate is not metabolized by carnivorous fish as easily as is the case in omnivorous mammals. These are briefly as follows.

(1) Glucose tolerance tests give rise to prolonged hyperglycaemia; the lack of ability to regulate blood glucose is reminiscent of the diabetic mammal (e.g. Palmer & Ryman, 1972).

(2) During the course of a spawning migration exceeding 1000 km (when they do not feed and the gonads are developed) muscle and liver glycogen concentrations of Pacific salmon (*Oncorhynchus nerka*) change little (French *et al.* 1983); concentrations in both tissues actually increase from gluconeogenesis before spawning.

(3) Glycogenolysis. Liver glycogen concentrations change very slowly under conditions of starvation (Nagai & Ikeda, 1971). In isolated hepatocytes from trout Seibert (1985) showed that the temperature dependence of glucose production is clearly different from that of glycogenolysis. Renaud & Moon (1980) suggest that glycogenolysis is an important source of plasma glucose as a short-term strategy before gluconeogenesis is fully activated.

(4) Glucose phosphorylating capacity of rainbow-trout liver is low (Walton & Cowey, 1982). There is no evidence of a glucokinase (high K_m, V_{max} enzyme) being induced in fish.

(5) Utilization of glucose in the whole animal. Measurement of various indices of glucose metabolism by a variety of methods including isotope dilution, in which [6-^{3}H]glucose and [6-^{14}C]glucose were administered to kelp bass (*Paralabrax* sp.) via an indwelling arterial cannula (Bever *et al.* 1977, 1981), constant infusion of isotopic glucose into American eels (*Anguilla rostrata*) (Cornish & Moon 1985) and a single injection technique in skipjack tuna (*Katsuwonus pelamis*) (Weber, *et al.* 1986), have shown a spread of values for turnover rate of glucose in different species of fish (for a table of values, see Suarez & Mommsen, 1987). Suarez & Mommsen (1987) see the rates of glucose utilization as being species-specific and matched by corresponding rates of production. This matching of rates occurs even under conditions where hepatic and renal glucose concentrations remain comparatively constant and these authors' conclude that 'gluconeogenesis must account for the major part of the glucose produced to satisfy the animal's needs'.

Suarez & Mommsen (1987) note that while blood glucose concentrations of fish vary widely (both within and between species) they do not, even during prolonged starvation, develop severe hypoglycaemia. This latter condition is prevented by an activation of gluconeogenesis, a metabolic depression leading to a decrease in overall rate of glucose utilization, or other cause. Suarez & Mommsen (1987) develop the interesting theory that maintenance of a 'supracritical blood glucose concentration' even though it varies within wide limits, is essential to ensure the continuation of glucose turnover during a prolonged period of fasting.

The effect of dietary glucose concentration (0 or 250 g/kg) on glucose utilization and plasma insulin level has recently been investigated (Hilton *et al.* 1988). In line with expectation rainbow trout given low-carbohydrate diets had better growth rates together with much lower blood glucose and liver glycogen levels than those given high-carbohydrate diets (the diets were of similar energy density). But of considerable interest was the finding that plasma insulin levels (measured with a completely-homologous salmonid radioimmunoassay system) were significantly higher in the fish fed on the high-carbohydrate diet (48·2 ng/ml compared with 27·4 ng/ml in the low (zero)-carbohydrate diet). Thus increased dietary glucose intake stimulated increased secretion of insulin in trout presumably via elevated plasma glucose levels. Of equal interest were the plasma insulin concentrations in these fish after fasting for 18 h; they had fallen to 9.5 (high-

carbohydrate diet) and 5·2 (low-carbohydrate diet) ng/ml. Even assuming that these were true fasting levels (i.e. that they would not fall further with longer fasting) it is clear that feeding the diet lacking carbohydrate stimulated insulin secretion (from 5·2 to 27·4 ng/ml). This stimulation was of similar magnitude to that which occurred when the diet containing 250 g glucose/kg (48·2 ng insulin/ml) was compared with that of a diet lacking glucose (27·4 ng insulin/ml).

Earlier work on the perfused pancreatic gland of eels (*Anguilla anguilla*) (Ince & Thorpe, 1977; Ince, 1979, 1980) demonstrated that lysine, leucine, arginine and phenylalanine were more effective secretagogues than glucose. It remains possible, as with other species, that the presence of one or other of these amino acids is necessary before glucose will function as a secretagogue.

Despite the fact that dietary glucose stimulated increased insulin secretion, Hilton *et al.* (1988) acknowledge that the rainbow trout still is reminiscent of a diabetic mammal. Clearly now, the condition cannot be said to arise from failure of insulin production. Hilton *et al.* (1988) suggest that it may result from tissue insensitivity to the hormone, linked to the receptors in extra-hepatic peripheral target tissues. In this context Ablett *et al.* (1983) showed that while hepatocytes from trout reared on a high-carbohydrate diet showed a significant increase in receptor concentration compared with control fish or fish fed on a high-protein diet, receptor concentrations in skeletal-muscle plasma membranes did not differ between treatments. As a reciprocal relationship was established in mammals between endogenous insulin levels and the insulin-receptor concentration (Kahn, 1979) appreciably more studies are necessary to clarify the position in fish.

PROTEINS AND AMINO ACIDS

AMINO ACID REQUIREMENT

Essential amino acid requirements have been recently reviewed by Wilson (1985) and by Wilson & Halver (1986). An absolute requirement for ten amino acids, arginine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, valine and tryptophan has been demonstrated in all fish species so far examined. Quantitative requirements for all ten essential amino acids are known only for chinook salmon (*Oncorhynchus tshawytscha*), common carp, Japanese eel (*Anguilla japonica*) and channel catfish, although paradoxically a greater number of studies have been made on rainbow trout; most of the measurements have been made on juvenile fish. Table 1 provides most of the values available. There is considerable variation in requirement between species. Stated tryptophan requirement varies from 1·2 to 4 g/kg diet (see Kim *et al.* 1987) and, for rainbow trout alone, reported arginine requirement ranges from 25 to 60 g/kg dietary protein (see Chiu *et al.* 1986). These variations stretch credibility and raise questions over the approaches used or the way in which they are applied.

Measurement of essential amino acid requirement has relied very largely on dose–response (growth) curves. Various types of purified and semi-purified diet have been used to provide graded increments of the amino acid under test. These have included imbalanced proteins, deficient in certain amino acids, as the major source of dietary amino acids, e.g. zein (Dabrowski, 1981) or maize gluten (Halver *et al.* 1958; Ketola 1983); comparatively small amounts of crystalline amino acids are then added to balance the protein component, leaving it deficient in one amino acid only. Doubts over this approach centre on protein digestibility, amino acid availability and the high levels of certain amino acids such as leucine, which may depress the assimilation of other amino acids.

Most studies, however, have used test diets in which the nitrogen component consisted either of amino acids or a mixture of amino acids, casein and gelatin formulated to provide

Table 1. *Essential amino acid requirements (g/kg dry diet) of certain species of fish measured by dose–response curves**

Amino acid	*Cyprinus carpio*	*Ictalurus punctatus*	*Oncorhynchus tshawytscha*	*Anguilla japonica*	*Salmo gairdneri*	*Oncorhynchus kisutch*	*Oreochromis mossambicus*
Arginine	13	10·3	24	17	12–25	24	15·9
Histidine	8	3·7	7	8	–	7	–
Isoleucine	9	6·2	9	15	–	–	–
Leucine	13	8·4	16	20	–	–	–
Lysine	22	15	20	20	13–29	–	16·2
Methionine†	8	3·2	6	9	5–21·4	–	05·3
Phenylalanine‡	13	4·9	17	12	–	–	–
Threonine	15	5·3	9	15	–	–	–
Tryptophan	3	1·2	2	4	2·5	2	–
Valine	14	7·1	13	15	–	–	–

* For further details and original references, see Tacon & Cowey (1985).
† In the presence of cysteine.
‡ In the presence of tyrosine.

an overall amino acid spectrum identical with whole hen's egg (or other reference) protein less the amino acid under test. For many fish species growth rates obtained when diets contain large amounts of free amino acids are inferior, often markedly so, to diets of similar amino acid composition in which the N component is protein (Wilson *et al.* 1978; Robinson *et al.* 1981; Walton *et al.* 1982, 1986). Thus amino acid requirements obtained in this way are based on growth rates below (often well below) the optimum.

Ogino (1980) measured retention of essential amino acids in whole-body protein of carp and rainbow trout and used values for increase in essential amino acid content to estimate requirement. The method appears to assume that maintenance requirements in the young growing animal are low (although it is not easy to reconcile this view with the fact that only 30–40% of dietary N is retained in the growing fish) so that the pattern of amino acids deposited in body-weight gain is the main factor determining patterns of amino acids required. Arai (1981) and Ogata *et al.* (1983), similarly, used the amount of each essential amino acid as a proportion by weight of all essential amino acids in whole-body protein of coho salmon (*Oncorhynchus kisutch*) and of cherry (*Oncorhynchus masou*) and Amago salmon (*Oncorhynchus rhodurus*) respectively to formulate test diets. These diets gave high rates of growth and feed conversion efficiency.

Wilson & Poe (1985) noted that amino acid composition of whole-body protein for three different size groups of channel catfish (30–863 g) did not differ and a significant correlation was obtained when essential amino acid requirement pattern (from dose–response curves) was regressed against whole-body-protein pattern (r 0·96). Findings from all these workers indicate that whole-body essential amino acid pattern provides a valuable index of essential amino acid requirement. Since muscle protein is the principal product being formed during growth, and as amino acid composition of muscle differs little, if at all, between species the variations in estimated essential amino acid requirement of juvenile fish mentioned previously become even more questionable. When the principal product being formed differs from muscle (egg protein) then the amino acid requirements (of broodstock) would be expected to change.

The inferior growth rate of trout given diets containing large amounts of amino acids (compared with controls given protein diets of similar amino acid profile and N content) may be a function of more-rapid absorption in the former case. Yamada *et al.* (1981) observed that after force-feeding an amino acid mixture (of similar composition to casein) most of the free essential amino acids in systemic plasma reached a mean peak concentration at 12 h and returned to the fasting level at 48 h post feeding. By contrast, when casein was force-fed, free amino acid levels did not change appreciably during a 12 h period, but increased thereafter to reach maximum levels 24–36 h after force-feeding, declining to fasting levels at 72–96 h. Higher maximum levels of essential amino acids were observed with fish force-fed the amino acid diet rather than the casein diet. High tissue concentrations of amino acids would lead to more rapid rates of deamination and catabolism with reduced utilization of dietary N.

Recently, however, Murai *et al.* (1987) measured amino acid concentrations in plasma from the portal vein of trout that had been force fed (using a similar method to that employed by Yamada *et al.* (1981)) diets containing either casein or an equivalent mixture of amino acids. For both diets there were steep rises in portal plasma concentrations of amino acids within 3 h of force feeding, 'respective peaks' were said to be reached by 12 h at the latest and returned to fasting levels at 24 h. In fact the values do not show any significant differences in concentration of either total essential amino acids or total non-essential amino acids on either treatment at 3, 6 or 12 h. The levels plateau rather than peak, indeed a peak is evident only when total ninhydrin-reactive substances (presumably including ammonia and taurine) are considered. The time-course for free amino acids in the

systemic plasma (caudal vein–artery complex) obtained by Murai *et al.* (1987) does show concentrations of total essential amino acids reaching a higher peak (6 h) in amino acid-fed fish than from casein-fed fish (peak at 12 h). Again the time-course is much shorter than that obtained by Yamada *et al.* (1981).

The discrepancies between the two sets of values are difficult to explain. Both groups used a binding agent (carboxymethylcellulose) in the meal and neither adapted the fish from their commercial diet. Yamada *et al.* (1981) carried out their experiments at 10° and Murai *et al.* (1987) at 15°, but it seems unlikely that this temperature difference would have altered the time-course so markedly. The results of Murai *et al.* (1987) indicate that rainbow trout can absorb amino acids from either a protein diet or an amino acid diet at similar speed. These authors clearly agree with earlier findings that there is 'less efficient utilization of an amino acid diet than a protein diet even in cold water fish' but offer no explanation for this phenomenon.

By contrast with these findings current work in the University of Guelph (C. Y. Cho, B. Woodward & S. J. Kaushik, personal communication) suggests that dietary free amino acids are well utilized by trout when their rate of uptake is retarded. In experiments to determine their arginine requirement trout were given diets containing the equivalent of 310 g crude protein (N × 6·25)/kg (this was provided in the form of skim milk (providing 82·5 g protein/kg) maize-gluten meal (90 g protein/kg) and an amino acid premix (equivalent to 140 g protein/kg)). The amino acids were coated with thin agar paste before inclusion in the diet 'to avoid massive release of free amino acids in the digestive tract and into the bloodstream'. Very-good growth rates were obtained.

Whether or not di- and tripeptides are important end-products of protein digestion in fish is also germane to the issue. Absorption of amino acids in this form would reduce oxidation of them in the enterocytes. Further experiments, perhaps involving the distribution of radioactivity from a meal containing both ^{3}H-labelled amino acids and ^{14}C-labelled protein may help resolve the question.

Chiu *et al.* (1986) suggested that variations mentioned earlier in arginine requirement of rainbow trout might relate to the presence or absence of a functional urea cycle; the thesis being that conditions which altered the activities of the five enzymes in a functional urea cycle might affect the amount of dietary arginine required for maximal growth. Chiu *et al.* (1986) demonstrated the presence of a functional urea cycle in trout from (1) measurements of the enzymes involved in fish given diets of different protein content (the results were not consistent with a detoxication function for the cycle), (2) the incorporation of L-[1-^{14}C]ornithine and L-[carbamoyl-^{14}C]citrulline, injected intraperitoneally, into tissue arginine and (3) the fact that ornithine and citrulline were able to spare about half the dietary arginine in semi-purified diets. It remains to be shown that net synthesis of arginine occurs via the cycle.

It may be noted here, parenthetically, that the main source of excreted urea is thought to be via the catabolism of purines. In mammals the end-product of purine catabolism is uric acid, but fish possess the necessary enzymes in liver, i.e. uricase (*EC* 1.7.3.3), allantoinase (*EC* 3.5.2.5) and allantoicase (*EC* 3.5.3.4) to convert uric acid to urea.

There is little evidence that urea excretion in fish is closely linked to amino acid catabolism. Brett & Zala (1975) showed that ammonia excretion in sockeye salmon (*Oncorhynchus nerka*) rose to a sharp peak of 35 mg N/kg per h, 4–4·5 h after the start of feeding and fell to a baseline level of 8·2 mg N/kg per h 18–24 h later. Urea excretion remained relatively steady at a mean rate of 2·2 mg N/kg per h throughout the day, showing no diurnal response to feeding. Urea excretion was of a similar order in juvenile carp (3–4 mg N/kg per h) irrespective of whether the diet contained casein or free amino acids (Kaushik & Dabrowski, 1983) lower levels of excretion (0·6–2 mg N/kg per h)

occurred when a protein-free diet was given. These findings do not suggest any causal relationship between disposal of bicarbonate (arising from amino acid oxidation) and urea production as may occur in ureotelic animals. Carbon dioxide excretion and acid–base regulation in salmonids have been discussed comparatively recently by Randall *et al.* (1982).

HEAT INCREMENT

Heat increment of feeding (HI) in mammals has been thought to be associated mainly with the metabolism of protein. At the levels of protein used in fish diets (300–500 g/kg dry matter) the loss of dietary energy could be large if the magnitude of the effect is similar to that in mammals. Assessment of HI in fish presents difficulties and its magnitude is a matter of controversy.

Both direct and indirect calorimetry have been applied to measurement of metabolic heat production. Direct calorimetry presents problems because of the high specific heat of water giving a large heat capacity in the system against which a low heat production by the fish is to be measured (Brett & Groves, 1979). Smith *et al.* (1978), however, claimed that measurements made in an adiabatic calorimeter were accurate (they measured temperature changes of less than 0·1° with Beckman differential thermometers which were readable to 0·001°) and that specific changes in metabolic rate could be detected immediately. In their experiments 24 h-fasted rainbow trout (30–50 g) were first placed in the calorimeter for a period of about 2 h to establish basal heat production. They were then force-fed a measured quantity of protein (casein or casein–gelatin (70:30, w/w)) and heat production again measured until it returned to basal level. Smith *et al.* (1978) obtained a value for HI of 3–5% of metabolizable energy. This value is much lower than those found for terrestrial mammals.

Cho (1982) used a controlled open-circuit, indirect, fish respirometer (Cho *et al.* 1982) to examine effects of diet composition on HI in rainbow trout and demonstrated higher values for HI compared with measurements made by direct calorimetry. Their results (Table 2) showed that diets with a high level of digestible protein (470 g/kg) gave higher values for HI, irrespective of lipid content (up to 160 g/kg) than did a diet of lower (360 g/kg) protein content. Increasing lipid levels (from 60 to 160 g/kg) in the lower-protein diet resulted in an appreciable fall in HI, this was probably due to a fall in the amount of protein catabolized. Increasing lipid levels in the high-protein diet was sensibly without effect on HI. Cho & Kaushik (1985) interpreted this as due to a luxus of protein being supplied in this diet, under these conditions degradation of excess amino acids (i.e. those not needed directly for protein synthesis) would take precedence over the degradation of dietary lipid irrespective of the amounts of dietary lipid supplied (Krebs, 1972).

Indirect calorimetry has involved mainly closed-circuit systems with cylindrical respirometers in which water may be circulated at different temperatures and rates (so obtaining measurements with fish swimming under different conditions). Thus the cost of locomotion may be eliminated from the measurements made. The fish studied by indirect calorimetry have usually been given complete diets. A study of Beamish (1974) on large-mouth bass (*Micropterus salmoides*) gave a value of 14·2% of food energy lost to HI. LeGrow & Beamish (1986) in a factorial study (three lipid levels: 70, 150, 230 g/kg; four protein levels 340, 400, 480, 600 g/kg) on rainbow trout also showed that HI is influenced by diet composition, being positively related to digestible energy in the diet. Beyond a threshold (evident in diets containing 70 and 150 g lipid/kg and below which HI did not decline as digestible energy intake decreased) an increase in dietary protein level (and so of total digestible energy) led to an increase in HI at each of the three lipid levels. For all

Table 2. *Energy utilization by rainbow trout* (Salmo gairdneri) (*Values from Cho, 1982*)

Digestible protein in diet (g/kg)....	360			470		
Lipid (g/kg)....	60	110	160	60	110	160
Energy retained (% of digestible energy)	49	55	61	57	57	61
Heat increment of feeding (% of digestible energy)	14	11	7	15	14	13

dietary lipid levels used HI rose from 18·35 to 23·0% digestible energy as dietary protein was increased from 400 to 600 g/kg. Between 340 and 400 g dietary protein/kg HI decreased from 19·4 to 18·3% digestible energy.

A diet containing 300 g glucose/kg gave significantly higher HI when fed to rainbow trout than a diet of similar protein content and energy density but lacking glucose (it contained more oil). Values were 16·0 and 10·1% of gross energy intake for the high-glucose and high-lipid diets respectively (Beamish *et al.* 1986). It was suggested that the difference might be due to expenditure of energy both in eliminating glucose and in utilizing amino acids as an alternative energy source.

The wide disparity in values for HI obtained by direct and indirect calorimetry needs to be resolved. On the basis of indirect calorimetry studies HI constitutes a substantial proportion of the total energy budget of fish. LeGrow & Beamish (1986) estimate it as the equivalent of 15–24% of digestible energy intake depending on diet composition. The corresponding value of Cho (1982) is 10–15%. The spread of values here is also considerable; a possible explanation may lie in the fact that fish are confined in one of the equipments but not in the other. Cho & Kaushik (1985) point out that as HI is greater than non-faecal energy loss then net energy is a more appropriate measure of the production potential of a complete diet than is metabolizable energy. These authors estimate metabolizable energy as 92–96% of digestible energy; non-faecal energy loss comprises the small amount of energy lost in urea excretion, the heat of combustion of ammonia and losses (mucus) from the body surface.

PROTEIN SYNTHESIS

Methods used to measure rates of protein synthesis in mammals have, in recent years, been applied to fish; these include the constant infusion technique (Garlick & Marshall, 1972), and the use of a single high-dose injection of [^{3}H]phenylalanine to flood the intracellular pools and stabilize precursor specific activity over the course of the measurement (Garlick *et al.* 1980). Protein degradation rates have generally been obtained from the difference between amount of protein synthesized and amount of protein deposited. As protein deposition is measured over a period of weeks while protein synthesis is measured over a period of minutes this is not an especially satisfactory procedure.

Fractional rates of protein synthesis in visceral tissues of mammals are appreciably greater than in skeletal muscle (Reeds & Lobley, 1980). Values so far obtained from fish (Table 3) indicate a similar situation with synthetic rates from gill, liver and other visceral tissues 20-fold or more those in skeletal muscle. However, present indications are that the rate of muscle protein synthesis in trout differs markedly from that in mammals growing at a similar rate. Smith (1981) found that trout with a specific growth rate of 0·25%/d had

Table 3. *Fractional rates of protein synthesis (%/d) in two fish species, rainbow trout* (Salmo gairdneri) *and toadfish* (Opsanus tau)
(Mean values with their standard errors)

	Rainbow trout*, 12°		Toadfish†, 20°	
	Mean	SE	Mean	SE
Liver	17·4	2·6	13·7	4·4
Gill	4·7	1·0	7·7	3·5
Epaxial muscle	0·38	0·17	0·23	0·04
Pectoral muscle		—	0·20	0·07
Head kidney		—	15·2	3·0
Spleen		—	5·0	1·0

* Values from Smith (1981), constant infusion of [^{14}C]tyrosine.
† Values from Pocrnjic *et al.* (1983), [^{14}C]phenylalanine swamping.

a fractional rate of protein synthesis in muscle of 0·38 %/d and an RNA activity of 0·72 g protein /g RNA per/d. Corresponding values for rats growing at 0·29 %/d were 4·9 %/d and 11·5 g protein/g RNA per d (Millward *et al.* 1976). From this sort of comparison Smith (1981) concluded that much more of the muscle protein synthesized by epaxial muscle of fish is retained as growth than is the case in rat gastrocnemius.

Fractional rates of synthesis and degradation were directly correlated and fell, as body size increased, in all tissues of rainbow trout (Houlihan *et al.* 1986). Comparisons between species should therefore only be made for fish in a similar weight range. Values obtained by Houlihan *et al.* (1986) for white and red muscle (0·49 (SE 0·04) and 1·25 (SE 0·09)%/d respectively) were similar to earlier values of Smith (1981) and of Loughna & Goldspink (1985).

The results of Houlihan *et al.* (1986) also confirmed the conclusion of Smith (1981) in showing that white muscle differs from most other tissues of growing fish; 76 % of protein synthesized in white muscle was retained as growth (referred to as efficiency of protein growth) compared with, for example, 4 % for gill (a metabolically-active tissue with a fractional rate of protein synthesis of 9·07 (SE 0·57)).

Starvation appears to have a differential effect on the rates of protein synthesis in red and white muscle of carp (Watt *et al.* 1988). The former tissue appears to be spared the large drop in synthesis that is found in white muscle, this is also the case in rainbow trout (Loughna & Goldspink, 1985). Watt *et al.* (1988) suggest that red muscle is spared because of its continued recruitment during slow cruising as well as for faster swimming.

Differences between white muscle and other tissues were clearly evident in a further study of cod (Houlihan *et al.* 1988). Rates of protein degradation and synthesis in the whole animal both increased linearly with increasing growth rate; the efficiency with which synthesized protein was retained also increased with growth rate. This effect was particularly marked in white muscle and, at a maximum growth rate of 2 %/d, efficiency of protein retention was almost 60 %. Values for gill, intestine and stomach lay between 10 and 30 %. As a consequence, in the whole animal, at this growth rate, over 40 % of the protein synthesized was retained as growth and white muscle accounted for 40 % of the daily total protein accretion.

The relationship between temperature and protein synthesis in fish has been examined by several groups, mainly from the viewpoint of ascertaining 'whether a control over protein synthesis might provide a common basis for the increased levels of enzymes of respiratory

metabolism and other pathways responsible for physiological adaptation to low temperatures' (Haschemeyer, 1969*b*). It is known that Antarctic fish have muscle protein synthesis rates about 3-fold those expected from extrapolation of values from fish from warmer waters (Smith & Haschemeyer, 1980). Earlier Haschemeyer (1969*a*) had demonstrated that the rate of protein biosynthesis is controlled at the stages of polypeptide chain elongation and release and that the rate increase in cold-acclimatized fish was paralleled by elevated levels of the enzyme that promotes binding of amino acyl tRNA units at codon recognition sites (Haschemeyer 1969*a*).

Subsequently Goldspink and his colleagues carried out experiments aimed at elucidating acute temperature effects on muscle protein synthesis and ascertaining to what extent these may be modified by acclimatory compensation. Loughna & Goldspink (1985) measured muscle protein synthesis in carp and trout that had been reared at 15° and then acclimated to higher or lower temperatures for either 1 or 28 d. Values obtained after 1 d were considered as an effect of temperature change *per se* as it is unlikely that any degree of compensation to the new thermal regimen could occur by metabolic re-organization over such a short time-period. In carp the rate of protein synthesis was modified as a result of temperature acclimation; for white muscle at 10° rates were significantly higher after 28 d acclimation than those obtained after 1 d. Inverse effects occurred at 25°; 28 d acclimation at this temperature caused a significant reduction in white-muscle protein synthesis. In trout, rates of protein synthesis at all temperatures studied were not significantly different whether measured after 1 or 28 d.

The higher rate of protein synthesis in cold acclimated carp was later shown to hold true for both red and white skeletal muscle (Watt *et al.* 1988). However temperature acclimation did not significantly alter the slope of the Arrhenius plots and there was no significant difference in activation energy for red (86·7 kJ/mol) and white (78·7 kJ/mol) muscle. Also there was no significant change in the relative amounts of DNA- and RNA-phosphorus as a result of temperature acclimation. The higher rate of protein synthesis in the cold-acclimated fish must therefore have resulted from an increased use of existing RNA.

As the myofibrillar ATPase (*EC* 3.6.1.37) from cold-acclimated cyprinids had a decreased Arrhenius activation energy compared with the enzyme from warm-acclimated fish, Watt *et al.* (1988) infer that changes in protein synthesis rate in response to temperature acclimation are achieved in a different way than is the case with the muscle enzyme.

Exercise training led to an increase of almost 2-fold in protein synthesis rates of white muscle, red muscle and ventricle in rainbow trout over rates found in control fish (Houlihan & Laurent, 1987). Calculated protein degradation rates also increased in the trained animals, increased growth resulting from a proportionately-greater increase in synthesis rate. These findings might be exploited in selecting flow-rates for fish culture sites.

Study of the inter-relationship between protein synthesis and nutrition is at an early stage, but the application of reliable methods for its measurement may provide a means to resolve questions such as the utilization of diets containing large amounts of free amino acids and to quantify effects of variation in dietary protein energy:total energy ratio on protein synthesis.

LIPIDS

FATTY ACIDS IN FISH LIPIDS

Indications that essential fatty acid (EFA) requirements of fish might differ from those of mammals and birds were first evident from compositional studies on fish lipids. The lipids of component members of aquatic ecosystems were shown to contrast markedly with those of their terrestrial counterparts. In particular lipids from wild fish contain comparatively

Table 4. *Component fatty acids (% by weight) in some freshwater and marine fish*

Fatty acid	Perch* (*Perca fluviatilis*)	Brown* trout (*Salmo trutta*)	Salmon† smolt	Haddock* (*Melanogrammus aeglefinus*)	Herring* (*Clupea harengus*)	Cod* (*Gadus morthua*)
14:0	1·0	1·9	1·5	0·7	6·5	4·0
16:0	15·8	22·0	14·2	12·7	9·1	10·5
18:0	6·2	5·8	5·3	5·0	1·0	2·3
16:1	4·9	2·6	5·5	1·9	6·2	8·9
18:1	10·4	14·1	12·6	10·0	9·8	21·0
20:1	—	0·1	0·8	1·2	10·1	12·5
22:1	—	0·4	0·2	4·4	14·8	7·6
18:2(*n*-6)	2·0	2·4	3·1	1·3	2·2	1·5
18:3(*n*-3)	2·0	2·1	2·2	—	—	0·9
20:4(*n*-6)	11·5	8·6	8·0	2·6	0·4	0·7
22:4(*n*-6)	0·6	0·2	1·3	0·3	1·5	0·6
22:5(*n*-6)	1·1	0·4	2·0	1·7	0·6	0·1
20:5(*n*-3)	9·3	9·0	4·6	20·4	11·9	9·9
22:5(*n*-3)	2·6	4·4	3·3	3·4	1·4	1·0
22:6(*n*-3)	27·2	22·8	15·4	24·7	15·7	9·9
(*n*-3):(*n*-6)	2·8	3·3	1·7	8·1	7·7	7·5

* Values from Gunstone *et al.* (1978).
† Values from Ackman & Takeuchi (1986).

high levels of (*n*-3) polyunsaturated fatty acids (PUFA). This is especially so in marine fish; (*n*-3) fatty acids are mainly synthesized in the cellular thylakoid (photosynthetic) membranes of phytoplankton and retained through the food chain. Freshwater fish tend to contain greater amounts of (*n*-6) fatty acids perhaps deriving from freshwater insects that form a large part of their diet. Even so, the overiding fact is that, like their marine brethren, freshwater fish are also rich in (*n*-3) PUFA.

Gunstone *et al.* (1978) analysed fatty acids from the lipids of several species of marine and freshwater fish from the natural environment. They made several points with respect to PUFA including: (1) (*n*-3) fatty acids of which 20:5(*n*-3) and 22:6(*n*-3) are the major components always exceed (*n*-6) fatty acids, (2) (*n*-6) polyenes are higher in freshwater fish (mean 13% by weight in the fish they examined) than in marine fish (5%), (3) the (*n*-3):(*n*-6) ratio was in the range 1–4 for freshwater fish compared with 5–14 for marine fish; this ratio was the most significant value distinguishing freshwater from marine lipids. These points are largely borne out by recent analyses of fatty acids from wild Atlantic salmon (*Salmo salar*) smolts (Ackman & Takeuchi, 1986) from freshwater. The mean (*n*-3):(*n*-6) ratio in these fish was 1·7. Some fatty acid analyses from the lipids of freshwater and marine fish are shown in Table 4.

ESSENTIAL FATTY ACIDS

EFA requirements have generally been identified from growth responses to partially-defined diets; changes in composition of tissue polar lipids in response to specific dietary fatty acids have provided additional information. Some pathologies associated with EFA deficiency have been described; these include a shock syndrome (Castell *et al.* 1972*b*; Watanabe *et al.* 1974) and fin erosion (Castell *et al.* 1972*b*) in rainbow trout, membrane damage to fat cells (Cowey *et al.* 1976*a*) damage to chloride cells in the gills and fin rot (Bell *et al.* 1985) in turbot (*Scophthalmus maximus*), but to what extent these pathologies are reproducible or diagnostic is by no means clear.

As with mammals fish are not able to synthesize either linoleic or linolenic acid *de novo* so that one or other or both must be supplied preformed in the diet for the maintenance of cellular function. Whether or not these acids meet the EFA requirements of fish depends partly on the capacity of the species concerned to chain elongate and further desaturate the parent acid to longer chain, more unsaturated members of the series with full EFA activity. It will be seen that many marine fish are extremely limited in their ability to transform fatty acids in this way, consequently the long-chain, highly-unsaturated acid with full EFA activity must be supplied in the diet.

The EFA requirements of rainbow trout have been studied in more detail than those of any other species. The addition of 10 g linolenic acid/kg to partially-defined diets containing only saturated fat (50 g/kg) restored growth to normal levels (Castell *et al.* 1972*b*). While linoleic acid, added to such diets, did improve growth over that obtained when the lipid components of the diet contained only saturated fat, no combination of linoleic and linolenic acids together (and comprizing 10 g/kg diet in total) gave growth rates better than that occurring when linolenic acid alone was present at 10 g/kg. These results were confirmed by Watanabe *et al.* (1974) who defined the linolenic acid requirements as 8·3–16·6 g/kg diet the total lipid content of which was 50 g/kg. Takeuchi & Watanabe (1977*a*) went on to show that the requirement for linolenic acid increased as dietary lipid increased – with 10–15% purified lipid in the diet some 20% of it should be 18:3(*n*-3). Subsequently (Takeuchi & Watanabe, 1977*b*) 20:5(*n*-3) and 22:6(*n*-3) were shown to be more effective than 18:3(*n*-3) and their effects were additive, 2·5 g/kg of each being sufficient to meet EFA requirement (50 g lipid/kg diet). Thus linolenate was seen as playing a similar role in trout as linoleate in mammals.

Among Pacific salmon, coho resemble rainbow trout in requiring (*n*-3) series fatty acids; linoleate supplied as trilinolein at a level of 10 g/kg depressed growth (Yu & Sinnhuber, 1976, 1979) and its role is far from clear. Chum salmon (*Oncorhynchus keta*) showed optimal growth when both linoleate and linolenate were present at 10 g/kg diet (Takeuchi *et al.* 1979) providing an enigmatic picture for this genus. The herbivorous *Tilapia zilli* grown in freshwater at 27° grew best when either 18:2(*n*-6) or 20:4(*n*-6) were present in the diet (Kanazawa *et al.* 1980) similarly *Tilapia nilotica* requires 18:2(*n*-6) at a minimum level of 5 g/kg (Takeuchi *et al.* 1983).

Other freshwater fish seem less demanding in their EFA requirements. Carp of 2·5 g live weight and kept in freshwater at 20–25° grew well for 22 weeks on a diet entirely lacking fat, without obvious problems (Watanabe *et al.* 1975). Subsequent experiments with carp of 0·65 g (kept on a diet free of fat for 4 months beforehand) revealed a requirement for both 10 g 18:2(*n*-6) and 10 g 18:3(*n*-3)/kg diet. Another warm-water fish, channel catfish, appears similarly undemanding in EFA requirement. Fish have grown well on diets containing beef tallow or menhaden oil (Stickney & Andrews, 1972); more recently better growth rates were obtained with fish given diets containing 18:2(*n*-6) or 18:1 (*n*-9) than with diets containing 18:3(*n*-3).

Wholly marine fish seem especially conservative in their requirement for EFA. All those so far studied have a requirement for (*n*-3) series fatty acids and this can be met only by 20:5(*n*-3) and 22:6(*n*-3). Cowey *et al.* (1976*b*) showed that turbot grew well when given diets containing cod-liver oil but not when given diets containing 18:3(*n*-3), 18:2(*n*-6) and 20:4(*n*-6). Similar results have been reported for red sea bream, black sea bream (*Mylio macrocephalus*), opal eye (*Girella nigricans*) and yellowtail (Yone & Fujii, 1975*a*, *b*; Fujii & Yone, 1976; Yone, 1978).

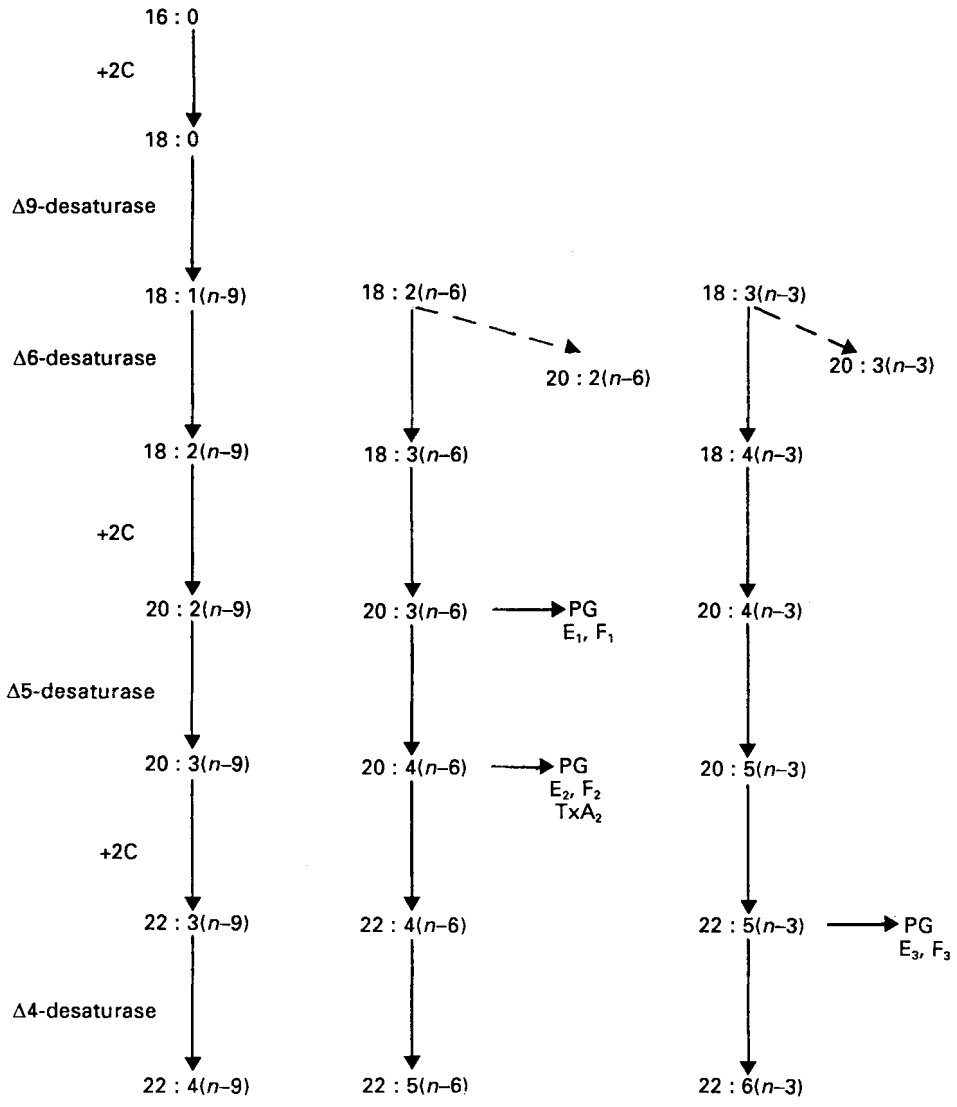

Fig. 1. Probable pathways involved in elongation and desaturation of fatty acids in tissues of rainbow trout (*Salmo gairdneri*). PGE_1-E_3,F_1-F_3, prostaglandins; TxA_2, thromboxane.

DESATURATION AND ELONGATION OF FATTY ACIDS

EFA requirements are a function of the ability of fish to modify and transform dietary fatty acids to meet structural and metabolic needs. The fatty acid synthetase system also gives rise to saturated fatty acids which are substrates for chain elongation and desaturation. Thus most of the radioactivity recovered from the body lipids of *Tilapia zilli* following injection of [^{14}C]acetate was present in 16:0 and 18:0, but appreciable radioactivity was also present in the monoenes 16:1 and 18:1 (Kanazawa *et al.* 1980).

That rainbow trout have appreciable capacity to transform dietary fatty acids was evident from the experiments of Castell *et al.* (1972*a*) referred to previously; 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 22:5(*n*-6) and to 22:6(*n*-3) in the liver and other tissues. These findings have been confirmed in subsequent

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experiments (Takeuchi & Watanabe, 1982). Pathways that are likely to operate in desaturation and elongation in trout tissues are shown schematically in Fig. 1. Apart from knowing that the desaturases are complex systems associated with microsomes, none of them have been purified and characterized in any species of fish. The Δ6-desaturase of mammals has most affinity for 18:3(*n*-3) as substrate and least affinity for 18:1(*n*-9), competition exists between substrates (Brenner & Peluffo, 1966). Thus 18:1(*n*-9) is only a substrate for Δ6-desaturase in the absence of the other C_{18} fatty acids. The analytical findings of Castell *et al.* (1972*a*) suggest that the same may be true of rainbow trout; levels of 20:3(*n*-9) in liver phospholipids only increasing in the absence of dietary 18:2(*n*-6) and 18:3(*n*-3). Other analytical studies (Yu & Sinnhuber 1976, 1979) in which diets containing different proportions of 18:2(*n*-6) and 18:3(*n*-3) were given to salmonids showed that 18:3(*n*-3) is a more-potent inhibitor of 18:2(*n*-6) than the latter is of 18:3(*n*-3). Finally feedback inhibition of 18:2(*n*-6) and 18:3(*n*-3) desaturation is exerted by long-chain (*n*-3) PUFA such as 22:6(*n*-3) (Leger *et al.* 1981).

Hagve *et al.* (1986) have recently examined and compared desaturation and chain elongation in hepatocytes from rat (incubated at 37°) and rainbow trout (incubated at 12°). Chain elongation from 18:3(*n*-6) to 20:3(*n*-6), 18:4(*n*-3) to 20:4(*n*-3) and 20:5(*n*-3) to 22:5(*n*-3) were very similar in the two animals but 20:4(*n*-6) was converted to 22:4(*n*-6) more efficiently by trout hepatocytes (20:3(*n*-6) the substrate here). With 20:3(*n*-6) as substrate no Δ4-desaturase activity could be demonstrated in either animal, but with 20:5(*n*-3) as substrate conversion of 22:5(*n*-3) to 22:6(*n*-3) was 2-fold greater in trout than in rat hepatocytes. Δ6-desaturase activity was similar in the two animals with 18:2(*n*-6) as substrate, for the rat 18:3(*n*-3) was the preferred substrate. By contrast, Δ5-desaturase measured with 20:3(*n*-6) as substrate was 4-fold greater in rat hepatocytes than in those of trout, with 18:3(*n*-3) and 18:2(*n*-6)as substrates differences were less marked but activity in trout was consistently lower.

Hagve *et al.* (1986) also observed that so-called dead-end elongation to fatty acids that would not be further desaturated or elongated (18:2(*n*-6)→20:2(*n*-6); 18:3(*n*-3)→20:3(*n*-3)) occurred to a significant extent in rainbow trout. These fatty acids were thought to be stored preferentially in triacylglycerols and later liberated for retroconversion to substrates of active desaturation and elongation pathways. While there is evidence that retroconversions do occur in rats (22:6(*n*-3)→22:5(*n*-3) and 20:5(*n*-3)) compositional findings (Yu & Sinnhuber, 1972) did not show retroconversion of 22:6(*n*-3) to shorter-chain PUFA in trout.

Hagve *et al.* (1986) also made the important inferences that (1) dietary fatty acid pattern is not the only factor affecting the endogenous fatty acid pattern in liver phospholipids of trout, (2) rates of desaturation and chain elongation, which are basically similar in the two animals, apparently cannot explain the very different patterns of endogenous phospholipid fatty acids in them ((*n*-3):(*n*-6) was 10 for rainbow trout and 0.3 for rats).

Marine fish appear much more limited in their capacity to transform dietary fatty acids than do rainbow trout (and possibly other salmonids). Owen *et al.* (1975) fed pellets containing [1-^{14}C]-labelled oleic, linoleic or linolenic acids to turbot and examined the distribution of radioactivity in tissue fatty acids 1 week later. For all three fatty acids the bulk of the radioactivity recovered was in the parent fatty acid fed (70–90%), there was no evidence of desaturation and chain elongation was limited. By contrast, in trout given pellets containing [1-^{14}C]linolenic acid 70% of the radioactivity recovered from tissues was in 22:6(*n*-3).

Similar methods were applied to other marine fish by Kanazawa *et al.* (1979) and Yamada *et al.* (1980). All the marine fish examined had a very-limited capacity to transform linolenic acid to highly-unsaturated (*n*-3) fatty acids. Consequently linolenic acid is ineffective as an EFA for marine fish. The dependence of marine fish on their diet for long-

Table 5. *Principal polyunsaturated fatty acids esterified to phosphatidylinositol and to other phospholipids in rainbow trout* (Salmo gairdneri) *liver and in microsomes from cod gills* (*% by weight*)

	Rainbow trout liver*						
	Total phospholipid		Phosphatidyl inositol		Microsomal fraction from cod gills†		
Fatty acid	5°	20°	5°	20°	Phosphatidyl choline	Phosphatidyl ethanolamine	Phosphatidyl inositol
20:4(*n*-6)	3·24	2·63	20·41	14·49	2·7	3·7	15·3
20:5(*n*-3)	4·06	2·24	2·36	0·64	9·6	8·5	8·8
22:6(*n*-3)	35·20	33·43	10·61	10·66	16·5	28·5	12·0

* Values from Hazel (1979). † Values from Bell *et al.* (1983).

chain highly-unsaturated fatty acids is probably a reflection of their position at the head of the marine food pyramid with an abundance of C_{20} and C_{22} PUFA in their natural diet.

Other freshwater fish, e.g. ayu (*Plecoglossus altivelis*) and Japanese eel were also more limited than rainbow trout in their capacity to transform linolenic acid to (*n*-3) pentaenes and hexaenes (Kanazawa *et al.* 1979); this capacity may not be very widespread among fish species.

ROLE OF (*n*-6) FATTY ACIDS

Kanazawa (1986) suggested that on the basis of their EFA requirements fish might be divided roughly into three groups. These were referred to as rainbow trout-type, Tilapia-type and red sea bream-type, implying EFA requirements would be met by 18:3(*n*-3), 18:2(*n*-6) or long-chain highly-unsaturated fatty acids respectively.

The thesis that linolenic acid is the only EFA for rainbow trout was tested by Yu *et al.* (1979). They fed a semi-purified diet, containing 10 g linolenate/kg as the only source of EFA, to rainbow trout for 34 months. During this time the fish matured, the eggs produced were hatched and the second generation fry grown on for 3 months. The fry grew normally and no tissue pathologies were evident. These findings were seen as strongly supporting the view that (*n*-3) series fatty acids are the only EFA for trout.

When analysed, the eggs and carcass phospholipids were found to contain a small quantity (0·5% by weight) of 20:4(*n*-6). This was thought to have arisen because of incomplete extraction of diet components such as dextrin, casein and gelatin. Nevertheless it leaves open the possibility that (*n*-6) fatty acids are a significant factor in the well-being of fish.

In this context fatty acid analyses of individual phospholipids from tissues of fish are interesting. While the major phospholipids of marine fish and rainbow trout (phosphatidylcholine and phosphatidylethanolamine) are rich in (*n*-3) PUFA with an (*n*-3):(*n*-6) ratio of 10–15:1 one of the minor phospholipids i.e. phosphatidylinositol (PI) contains large amounts of 20:4(*n*-6). This is the case in tissues from rainbow trout (Hazel, 1979), cod and turbot (Bell *et al.* 1983, 1985) and gonads from a number of marine fish (Tocher & Sargent, 1984). Comparative values for total phospholipids and PI are shown in Table 5.

Thus PI from fish tissues resembles that from mammals, where the fatty acid composition is closely controlled. In mammals PI is extremely active metabolically, being involved in the transduction of hormone signals through biomembranes. Similarly, it is known that in fish

that metabolic activity of salt-transporting epithelia in the gills is affected by the metabolic activity of PI (Girard *et al.* 1977).

Arachidonate is an important precursor of eicosanoids in mammals. The early work of Christ & Van Dorp (1972) demonstrated the capacity of homogenates from gills of tench (*Tinca tinca*) and carp to form prostaglandin PGE_1, from 20:3(*n*-6). There is now considerable evidence that prostaglandins derived from 20:4(*n*-6) are active in fish (Anderson *et al.* 1981; Bandyopadhya *et al.* 1982) where they seem to perform similar functions as in mammals. Arachidonic acid is generally the preferred substrate in vivo for the cyclo-oxygenase pathways, and in blood neutrophils of plaice (*Pleuronectes platessa*), it is the preferred substrate for the 5-lipoxygenase pathway (Tocher & Sargent, 1986).

The high levels of 20:4(*n*-6) esterified in PI, which has a high turnover rate in response to hormone signals, suggest that this phospholipid may serve as a source of the fatty acid for eicosanoid formation.

In any event the accumulating evidence of an important role for 20:4(*n*-6) in eicosanoid metabolism lends weight to the view that there may be a small but significant requirement for 20:4(*n*-6) in marine fish (and rainbow trout). This has been difficult to show by conventional feeding experiments and may require approaches at cellular and subcellular level. In particular the metabolism of C_{18} fatty acids by different cell lines from a number of freshwater and marine species should find a place in future studies on fatty acid nutrition in fish.

TEMPERATURE ADAPTATION

Part of the requirement of fish for highly-unsaturated fatty acids relates to their poikilothermic habit so that they face particular problems in maintaining membranes in a functional state at low temperatures. Membrane properties are closely allied to characteristics of the lipids of which they are composed; fluidity is a function of both the melting points of the component fatty acids and the tightness with which they are packed. Melting points of fatty acids depend not only on the number of double bonds they contain, but also on their location in the acyl chain while the introduction of *cis* double bonds into molecules, by interrupting the fully-extended conformation, reduces closeness of packing. PUFA of both (*n*-6) and (*n*-3) series are fluid at temperatures well below those found in the natural environment and there is no obvious physico-chemical advantage in the presence of (*n*-3) over (*n*-6) fatty acids in fish biomembranes; again membrane fluidity would not appear to be increased by replacing 20:4(*n*-6) or 20:5(*n*-3) with 22:6(*n*-3).

Even so adaptation of fish to low environmental temperatures leads to an increase in unsaturation of lipids, moreover this generally results from an increase in levels of (*n*-3) PUFA. There was a 2-fold increase in 22:6(*n*-3) in phospholipids of the intestine of goldfish (*Carrassius auratus*) adapted to water at 5° compared to those reared at 30° (Miller *et al.* 1976).

The main changes in liver phospholipids of rainbow trout cold-adapted at 5° were an increase in the quantity of PUFA, a reduction in the level of saturated fatty acids and little alteration in the concentration of monoenes and dienes (Hazel, 1979). The increases in PUFA in phosphatidylcholine and phosphatidylethanolamine occurred in the 2-position at the expense of monoenes and dienes, overall the relative amounts of (*n*-3) fatty acids increased while (*n*-9) declined. For phosphatidylcholine the increase was largely in 22:6(*n*-3) and for PI 20:4(*n*-6) (Hazel, 1979). More recently, Hazel & Zerba (1986) also showed that in certain membranes from trout hepatocytes changes in molecular species of phospholipid occurred. Proportions of species such as 16:0/22:6 increased while shorter chain mono- and dienoic species (14:0/16:2, 16:1/18:1) decreased.

Table 6. *Some B vitamin requirements for maximum live-weight gain of rainbow trout* (Salmo gairdneri): *an overview of results and comparison to (US) National Research Council (NRC) (1981) recommended levels**

Source	Vitamin B_2	Vitamin B_6	Biotin	Pantothenic acid
NRC (1981)	20	10	1·0	40
Takeuchi *et al.* (1980)	3–6	—	—	—
Hughes *et al.* (1981)	3	—	—	—
Woodward (1985)	4	—	—	—
B. Woodward†	—	2	0·08	—
C. Y. Cho & B. Woodward†	—	—	—	12

* mg/kg dry diet; all other sources quoted express results as mg/kg diet as fed.
† Unpublished results.

VITAMINS

DIETARY REQUIREMENT

Fish are one of the few classes of animals that have a requirement for ascorbic acid; all species so far examined require the vitamin at least in their juvenile stage, which is a period of rapid growth. The lability of the vitamin is probably a limiting factor in the shelf-life of practical diets and considerable effort has gone into production of a protected form of the vitamin, so far without conspicuous success. Several practical diets appear to contain a considerable excess (overestimated requirement) of the vitamin, at the time of pelleting, as a means of combatting the problem.

The requirements of cold-water fish for some B vitamins have been closely investigated in recent years, the studies, principally on rainbow trout were directed mainly at fry stages (1–2 g live weight). Results from these studies (some of which are shown in Table 6, and in which good growth rates were obtained) indicate that requirements are lower than levels used in commercial practice and recommended by US National Research Council (1981).

Measurements of the activities of those tissue enzymes of which the vitamin is a component or co-factor have been used as an adjunct to growth information in requirement studies. Baker (1986) noted that while maximal growth and feed efficiency are desired in a growing animal the same cannot necessarily be said of maximal enzyme activity. Nevertheless enzyme activity information is often useful as an indication of incipient deficiency. In this context the thesis has been developed (B. Woodward, unpublished results) that the choice of enzyme may relate to how metabolically important the enzyme is. Enzymes that are less-metabolically vital provide a more-sensitive index of requirement than others. White-muscle pyruvate carboxylase (*EC* 6.4.1.1) activity was much more sensitive than the corresponding liver enzymes to dietary biotin level. Aspartate aminotransferase (*EC* 2.6.1.1) is more sensitive than alanine aminotransferase (*EC* 2.6.1.2) to pyridoxine deficiency in white muscle. Likewise hepatic D-amino acid oxidase (*EC* 1.4.3.3) activity is more sensitive than glutathione reductase (*EC* 1.6.4.2) to riboflavin deficiency (Table 7). Maximum D-amino acid oxidase activity in rainbow trout is depressed by frank riboflavin deficiency through a mechanism independent of the metabolic consequences of reduced feed intake (Woodward, 1983). In addition D-amino acid oxidase activity provides a more-sensitive indicator of incipient riboflavin deficiency than any other variables examined; depression in this enzyme activity was detected after only 3 weeks, i.e. before the appearance of other signs of riboflavin deficiency.

Table 7. *Live weights and maximal activities of hepatic* D-*amino acid oxidase (DAAO) and glutathione reductase (GR) in rainbow trout* (Salmo gairdneri) *given diets depleted of, or supplemented with riboflavin for 14 weeks (Values from Woodward, 1983)*

Group no.†	Riboflavin in diet (mg/kg)	Initial wt (g)	Final wt (g)	DAAO (units*/g protein)		GR (units*/g protein)	
				Initial	Final	Initial	Final
1	1·4	11·0	33·7	2·3	0·1	3·9	4·1
2	36·8	10·9	57·1	2·6	2·6	4·1	4·1
3	36·8	10·9	46·3	2·5	2·4	4·1	4·2

* μmol substrate converted/min.

† Groups nos. 1 and 2 were fed to satiation each day, group no. 3 was pair-fed to group no. 1.

ASCORBIC ACID

Ascorbate 2-sulphate, a stable form of ascorbic acid, is said to be as effective as ascorbic acid in equimolar concentrations in meeting the requirements of rainbow trout for vitamin C (Halver *et al.* 1975). It is claimed that dietary ascorbate can be converted into ascorbate 2-sulphate, which is stored in connective tissue throughout the body; complementary enzyme systems (synthetase, sulphohydrolase) apparently act to maintain tissue ascorbate concentrations at appropriate levels (Halver, 1985).

Maximal growth rates of channel catfish were obtained with diets containing approximately 50 mg ascorbic acid/kg, growth response to ascorbate sulphate resembled a Michaelis–Menten-type curve and 200 mg/kg was necessary to achieve maximal growth (Murai *et al.* 1978). No ascorbate sulphate was detected in blood or liver from any of the fish; ascorbic acid concentrations in these tissues were very much higher in fish given diets containing ascorbic acid than when they contained a molar equivalent of ascorbate sulphate. Murai *et al.* (1978) inferred that supplemental ascorbate sulphate is less efficiently utilized by catfish than by young rainbow trout.

Atlantic salmon given diets containing ascorbate 2-sulphate (equivalent in molar terms to 500 mg ascorbic acid/kg) grew at the same rate as control fish (given diets containing ascorbic acid) (Sandnes *et al.* 1988). Liver levels of ascorbic acid in the control fish were 166 g/kg compared with 27 g/kg in those given ascorbate 2-sulphate; corresponding concentrations of ascorbate 2-sulphate were 75 g/kg in control fish and 10 g/kg liver in fish fed on ascorbic 2-sulphate. These findings appear more in line with those on catfish than those on rainbow trout and raise serious questions about the bioavailability of ascorbate 2-sulphate and its metabolism in salmon.

VITAMIN A

Under certain conditions xanthophylls may be converted to β-carotene in rainbow trout and so are potential precursors of vitamin A. Schiedt *et al.* (1985) administered tritiated carotenoids or ^{14}C-labelled retinyl acetate in gelatin or sunflower oil capsules to groups of rainbow trout which had been fed either (1) a vitamin A-depleted diet for 3–12 months or (2) a basal diet supplemented with 2·4 mg vitamin A/kg feed.

All three carotenoids used, astaxanthin, zeaxanthin and canthaxanthin were converted to β-carotene; they were poorly absorbed in young fish, but rapidly taken up by vitamin A-depleted fish nearing sexual maturity and converted to vitamin A. After administering labelled astaxanthin, up to 17% of the radioactivity in the liver was in the vitamin A

fraction and the purified vitamin had a specific activity similar to the precursor. When fish replete with vitamin A were used no significant conversion of astaxanthin to retinol was observed.

Similar results were obtained with radioactive canthaxanthin, in vitamin A-depleted fish 54% of radioactivity was recovered in the vitamin A fraction compared with 7·4% in control trout. Accumulation of vitamin A (fed as retinyl acetate) was not itself affected by the vitamin A status of the fish. An important negative backfeed control appears to operate on the activity of the β-carotene15,15′-oxygenase (*EC* 1.13.11.21) which splits the carotene molecule at the central double bond.

Reduction of xanthophylls in the skin of rainbow trout (their main site of accumulation) proceeds by stepwise removal of the keto groups at C-4, C-4′ but no elimination of hydroxyl groups at C-3, C-3′ occurred. Consequently only canthaxanthin, not a natural xanthophyll, can give rise to β-carotene (Schiedt *et al.* 1985).

The isotope experiments of Schiedt *et al.* (1985) show that specific enzymes must be present in the intestinal mucosa that reduce astaxanthin to β-carotene. The identification and characterization of these enzymes presents a formidable challenge to nutritional biochemists in coming years.

VITAMINS AND RESISTANCE TO INFECTION

Studies on the relationship between certain vitamins and immunity together with non-specific protective factors in fish so far concern ascorbic acid and vitamin E. Li & Lovell (1985) showed that mortality rates of channel catfish experimentally infected with *Edwardsiella ictaluri* (a bacterium causing enteric septicaemia) decreased with increasing dosage of ascorbic acid. Antibody response to *E. ictaluri* antigen and haemolysis of sensitized sheep erythrocytes by serum complement activity were lower in fish given ascorbic acid-deficient diets than when diets contained 30–300 mg ascorbic acid/kg; megadoses significantly enhanced both activities still further. Ascorbic acid deficiency also significantly reduced the number of bacteria engulfed by blood phagocytes.

High dietary levels of ascorbic acid (up to 2000 mg/kg) enhance protective mechanisms in rainbow trout (Navarre & Halver, 1988). When challenged with *Vibrio anguillarum* by injection or immersion, survival was related to ascorbic acid intake. Protection against *V. anguillarum* appeared very quickly in fish that were vaccinated; this is surprising as humoral antibody production has a long inductive phase. Thus antibody titres were detected 2 weeks after vaccination and were still increasing after 12 weeks in trout given diets containing 100–2000 mg ascorbic acid/kg. Antibody titres tended to decrease or stabilize at lower levels in trout fed on diets lacking ascorbic acid.

Evidence that vitamin E enhances protective mechanisms in salmonids is conflicting. Among other effects, Blazer & Wolke (1984) found a depressed immune response to both sheep erythrocytes and the bacterium *Yersinia ruckeri* in rainbow trout fed on a diet depleted of vitamin E for 12–17 weeks. No information was offered to show to what extent the fish actually were deficient. Some non-specific mechanisms (e.g. phagocytosis of latex beads by resident peritoneal macrophages) were also significantly depressed in trout given the diet depleted of vitamin E.

More recently Lall *et al.* (1988) reduced concentrations of vitamin E in tissues of groups of Atlantic salmon to low levels by feeding diets depleted of the vitamin. Other groups of salmon were fed on diets containing up to 250 mg vitamin E/kg. Lall *et al.* (1988) then investigated antibody production, bactericidal activity of the serum complement system and non-specific resistance to the disease furunculosis caused by *Aeromonas salmonicida*. For all diets no effect of the dietary treatments on the non-specific resistance of Atlantic

salmon to furunculosis was observed. Fish from some of the treatments were also vaccinated with formalin-killed *A. salmonicida* cells. At 1 month after vaccination both the humoral response and the complement system were not affected by these dietary levels of vitamin E.

For both nutrients additional evidence must be sought before a specific role in defence against infection can be ascribed to them.

PROSPECTS

Resources and effort for research into the nutritional biochemistry of fish are limited and a rapid resolution of the points raised in this essay is unlikely. Current studies on the composition and role of glucagon-like peptides may help elucidate relationships between tissue glycogen, blood sugar, gluconeogenesis and dietary carbohydrate. Understanding of this system seems also to require work at the receptor level, especially with respect to insulin function, and on the extent to which flux through particular pathways can be affected by the endocrine system.

Investigations on the utilization of diets containing free amino acids should benefit from accurate measurements of protein synthesis, amino acid absorption and possibly excretion. Together with the use, in a single meal, of whole protein and free amino acids labelled with different isotopes quantitative differences in the fate of free amino acids compared with protein-bound amino acids should be identified.

Research on lipid metabolism seems likely to demonstrate an important role for eicosanoids derived from arachidonic acid and thereby identify a requirement for (*n*-6) fatty acids in the food of fish. From a different viewpoint the capacity for certain species to synthesize highly-unsaturated fatty acids (from suitable precursors) of either (*n*-3) or (*n*-6) series may be exploited for human nutrition.

Studies on vitamin C should clarify whether ascorbate sulphate has a metabolic (? storage) function in salmonids or whether it is simply hydrolysed in the gut. Questions of bioavailability should be resolved. Current research should clarify whether any nutrient has a specific role in mechanisms that protect against infection. In particular, whether they can affect any of the non-specific systems that are immediately effective as opposed to immune response with a long induction period.

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