AN APPROACH TO STUDY THE NUTRITIONAL REQUIREMENTS OF THE BLUEFIN TUNA (Thunnus thynnus thynnus, L.)

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SUMMARY

The research required in relation to the nutrition of bluefin tuna (BFT) is great. In previous trials in the domestication of large scombrids and in current capture-fattening schemes, nutritional studies have been few. Therefore, virtually nothing is presently known about the nutritional requirements for these species. Nutritional factors are important in any domestication programme in a variety of areas. Correct nutrition is a vital factor in the production of a successful broodstock with high fecundity and fertility producing large numbers of high quality eggs. Reproductive control itself is affected by nutritional factors such as lipid/energy content which can influence sexual maturation in other fish species. Successful larval rearing of marime fish is highly dependent upon suitable diets, whether live prey species or artificial, and their precise composition particularly in relation to fatty acids is an area that still demands much research for all marine species. Clues to the nutritional requirements of any animal can be obtained by looking at the natural food for that animal, in this instance, the natural prey species of the BFT and also by determining the composition of wild caught animals, both prey and predator. The latter certainly being a source of information in terms of lipids and fatty acid requirements. The few data available from previous trials can also add to the overall view. In this section we will briefly review the literature on what is known about the nutrition of the large scombrids and also, importantly, place the problems of their feeding and nutrition in a global perspective.

NATURAL FOOD

Prey species. There are many reports on the prey species of adult BFT based on the examination of gut contents. The stomach contents of BFT in the Mediterranean (Ligurian Sea) were dominated by anchovies although mesopelagic fish and crustaceans and ommastrephid cephalopods were also present (Orsi-Relini et al. 1995). Immature BFT in the Bay of Biscay consumed fish (anchovy)> crustaceans (euphausids)>cephalopods by frequency of occurrence, and crustaceans> fish >cephalopods by numerical frequency with anchovy the most important prey species (Ortiz de Zarate and Cort 1986). The food spectrum for the tuna changes during ontogeny. Thus, BFT larvae in the northern Pacific feed generally on zooplankton, mainly copepod nauplii, calanoids, cyclopoids, cladocerans and corycaeids (Uotani et al. 1981, 1990; Young and Davis 1990).

Composition of wild tuna. The composition of wild BFT may give some indication of possible dietary requirements, at least in respect of lipid content and fatty acid composition. Clearly, there can be great variation in carcass fat levels reflecting condition factors that are almost certainly related to season. In contrast, the protein composition was observed to be less variable. The very strong inverse relationship between body fat and water in BFT indicated that the fish obtain energy for their migrations from muscle lipid reserves (Clay 1988). The fact that flesh lipid levels can vary so widely has important consequences for

farming. Clearly the level of fat in the flesh will be highly dependent upon dietary fat levels but seasonal factors affecting the metabolism of lipids in the fish may also be important. The potential benefits of high fat diets such as rapid growth may have to be balanced with potential deliterious effects such as reduced product quality and consumer acceptance.

The polyunsaturated fatty acid (PUFA) composition of most marine fish are dominated by the n-3 highly unsaturated fatty acids (HUFA), eicosapentaenoic acid (EPA: 20:5n-3) and docosahexaenoic acid (DHA; 22:6n-3) (Sargent et al. 1989). However, the fatty acid compositions of tuna species appear unique in that they are characterised by relatively high levels of the DHA and, especially, a very high DHA:EPA ratio (Sawada et al. 1993). In general, the DHA:EPA ratio of the lipid of northern hemisphere marine fish seldom exceeds 2. In the southern hemisphere, the lipids of marine fish generally show higher EPA levels and consequently even lower DHA:EPA ratios (Ackman 1980). In contrast, Pacific BFT showed flesh DHA levels of between 25% and 36% with DHA:EPA ratios of 3.4 – 5.8, whereas the stomach contents showed a DHA:EPA ratio of just 3.2 (Ishihara and Saito 1996). Muscle phospholipids of Atlantic BFT displayed DHA:EPA ratios of up to 7.4 (Medina et al. 1995). Therefore, the relatively high level of DHA and the high DHA:EPA ratio in tuna appears to be an essential characteristic of tuna species that would probably be required to be reproduced in farmed fish to preserve the qualities that the consumer would expect.

DEFINITION OF BASIC NUTRITIONAL REQUIREMENTS OF THE BFT

Energy. Energy is not a nutrient itself, but is present in the chemical bonds that hold the molecules in the nutrients together. The amount of energy in the various nutrients that make up a feed is of great importance as well as the capacity of different species to utilize the energy contained in the different nutrients. Fish, like most animals, eat to satify energy needs. Nutrients should be balanced so that the fish will have enough of the essential nutrients for optimum growth when energy needs are satisfied. All energy acquired through the ingestion of food is ultimately lost as wastes in faeces or by excretion, used in metabolic processes or deposited as new body tissues (growth or energy gain, maturation). Bioenergetics is concerned with the study of rates of energy intake and transformation within the organism, providing the physiological framework for the study of the relationships between feeding rates and growth rates or maturation of fish subjected to diffrent environmental conditions. In consequence, the energetic requirements of BFT for maintenance, growth and maturation, and its capacity to utilize the energy contained in the food must be primarily considered. Some studies have been done with skipjack and YFT (Kitchell et al 1978) and SBT (Davis 1997), but very little is known about the bioenergetics of BFT. BFT is a pelagic fish having a swim bladder but without respiratory pump, retention of metabolic heat and a respiratory rate, possibly, independent of ambient temperature and the allometric effect of weight. So, the study of the energy budget at different levels is crucial.

Protein. Dietary protein serves two main purposes in fish diets; firstly, as a source of amino acids required for the synthesis of new proteins both for growth and reproduction and also as replacements for existing protein in the process of turnover; and secondly, any protein in excess to the above requirements will be utilized for energy. The optimal dietary protein level for fish, as well as other animals, is influenced by an optimal dietary protein to enrgy balance, the amino acid composition and digestibility of the dietary protein(s), and the amount of the non protein energy sources in the diet. Dietary protein requirements can be broken down into three main categories, gross protein requirements and qualitative and quantitative amino acid requirements. Nothing is known about any of these requirements for BFT but some general assumptions can be made based upon existing knowledge of other species.

The estimated gross protein requirements of fish can vary from around 30% up to about 55% of the diet. The lower protein requirements are usually associated with warm freshwater species that can also utilize carbohydrate to a greater extent than most other fish. As marine fish and also top predators, the large scombrid species are likely to be nearer to the high end of this range and it is probably safe to assume that their gross protein requirement is unlikely to be below 40%. Obviously stage of development, and size of fish are likely to affect the gross protein requirement as, generally speaking, it decreases with both age and size (Wilson 1989). Water temperature is another factor that could affect the protein requirement.

Determining the qualitative requirements for amino acids is a tedious and time-consuming process but it has been carried out for many species. The same 10 amino acids (namely arginine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan and valine) that have been shown to be essential for most animals have also been found to be required by all the finfish studied to date (Wilson 1989). Therefore, it is most likely that the same amino acids will also be required by BFT. The quantitative requirements for all these amino acids have been determined in several species and so can be estimated for BFT using the values obtained for other carnivorous marine fish such as gilthead sea bream or salmon. However, with diets utilizing fish meal as the sole protein source these requirements should be fully met and so particular attention to the essential amino acids may only be required if diets utilizing other sources of proteins are being used.

Lipids and fatty acids. Fish species which migrate have a higher average fat content and greater variation in fat content than those which do not, indicating that fat is used as an energy source during migration. The large percentage of body weight that is muscle tissue allows a large amount of energy to be stored in tuna if depot fats are laid down in both red and white muscle. This is a significant source of energy which may be used for movements between areas of food abundance in a habitat where such areas can be widely separated. As with protein above, dietary lipids also serve two main purposes in fish diets; firstly, as a source of fatty acids required for the synthesis of new lipid for growth and reproduction and also for turnover of existing lipid; and secondly, excess lipid is a major energy source. Again lipid requirements can be broken down into three main categories, gross lipid requirement, and qualitative and quantitative essential fatty acid (EFA) requirements. Virtually nothing is known about any of these requirements for BFT but again some general assumtions can be made based on existing knowledge of other species.

Certainly a major consideration in the diet of BFT will be the gross lipid level of the diet. Research will be required to identify the ideal lipid levels at different life stages of the fish, particularly in relation to season and final grow out to ensure optimal growth without compromising quality. Therefore, the dietary protein sparing effect of lipids which allows protein to be used optimally for growth without depositing excess lipid in the flesh has to beexamined in BFT. It has been concluded that, in general, fish diets containing between 10 to 20% lipid gave optimal protein utilization and growth rates while minimising undesirable alterations in carcass composition (Cowey and Sargent 1979). Considering that the lipid content of the fish used in previous grow out trials with BFT and southern bluefin tuna (see below) will generally have been in this range, this may be a good starting point with the BFT. However, in a market which will probably include export to Japan for the very high value products, sushi and sashimi, and where flesh quality is paramount and so highly prized (high quality fish can realise 4 times the price for lower quality fish), fat content of the diet and feeding regimes will be an area of nutrition requiring close attention.

However, another vital area in lipid nutrition is the provision of sufficient amounts of the correct essential fatty acids (EFA). The EFA requirement of fish varies both in qualitative

and quantitative terms. In freshwater fish, including salmonids such as trout and salmon, the EFA requirements can be met by the shorter chain PUFA, a-linolenic (LNA; 18:3n-3) and/or linoleic (LA; 18:2n-6) acids. LNA and LA are converted to HUFA through a series of alternating desaturations and chain elongations mediated by microsomal fatty acid desaturation and elongation systems (Sargent et al., 1989). Freshwater fish, including salmonids, possess both the D6 and D5 fatty acid desaturases required for the production of EPA and DHA from LNA, and arachidonic acid (AA; 20:4n-6) from LA. In contrast, all marine fish studied to date have only very limited ability to produce the biologically active HUFA from LNA and LA and so have an absolute dietary requirement for the preformed HUFA. The relatively high level of DHA in tuna and the high DHA:EPA ratio may have consequences regarding the formulation of artificial diets as the DHA:EPA ratio seldom exceeds 2 in most commercially available fish oils (Ackman 1980). For instance, marine fish generally may have a limited capacity for the conversion of EPA to DHA (Sargent et al. 1993,1995). Therefore, the lipid biochemistry underpinning the high level of DHA and the high DHA:EPA ratio in tuna is unclear, but it has generally been assumed that tuna must selectively accumulate and retain DHA in their tissues (Saito et al. 1996; Ishihara and Saito 1996). Metabolic studies in addition to nutritional trials are required to fully elucidate this area. In the first instance it appears that it would be advisable that the oil used in experimental formulated diets be a high quality marine fish oil containing high total n-3HUFA with as high a DHA:EPA ratio as possible.

Carbohydrate. The capacity of most fish to effectively utilize dietary carbohydrate for energy is limited, particularly in the case of marine fish (Cowey 1988). Indeed the digestive physiology of BFT will probably mean that dietary carbohydrate cannot be utilized as it is possible that carbohydrate metabolising enzymes in the gut are absent or, at least, very low, as in southern bluefins. Therefore, in the first instance it is unlikely that carbohydrate will feature in the diets of BFT in a nutritional capacity.

Vitamins and minerals. It is fairly safe to assume that BFT will require the same range of water-soluble and fat-soluble vitamins that have been identified as being required in the diets of all fish studied to date (Halver 1989). The quantitative requirements for each vitamin varies between species and so theoretically will have to be determined in BFT. However, a generalised vitamin premix for marine fish is an appropriate starting point for initial trials and may be sufficient to satisfy the BFT requirements and prevent any vitamin deficiency symptoms. One possible aspect of a putative experimental diet that may have consequences regarding vitamin requirements for BFT is the high level of n-3HUFA and the high DHA:EPA ratio that may be required. Particular attention should be paid to the level of the vitamins that possess antioxidant functions, especially vitamin E (tocopherol) and vitamin C (ascorbate). The possibility that intact phospholipids, particularly phosphatidylcholine and phosphatidylinositol, in addition to the vitamins choline and inositol, may also have a growth promoting effect in larval diets as they appear to have in other marine fish species is an aspect that will require study at some point.

BFT BROODSTOCK NUTRITION

There are a number of aspects of fish reproduction which may be affected by nutritional status: the time to first maturity, the number of eggs produced (fecundity), egg size and egg quality as measured by chemical composition, hatchability and larval survivorship. Energy is partitioned by fish between each of the various physiological processes involved in maintenance, growth and reproduction. The maintenance requirements

of fish are met first and then excess energy is divided between growth and reproduction. The relative partitioning of energy between growth and reproduction varies both between species and between strains of individual species.

There are very few studies of the particular reproductive effort and nutrient requirements of aquatic animals for gonadal development and reproduction success, but those that have been undertaken indicate great species variability. Most work has concentrated on essential fatty acid requirements and fat soluble vitamin requirements, and it has been generally assumed that the amino acid requirements of broodstock are similar to those for optimal growth. There must be an optimal protein level for BFT reproductive success, and dietary protein will have to be carefully evaluated for the effects on reproduction if their use for broodstock is to be undertaken with confidence. In general, there is a considerable need for further research in this area, and particularly for BFT.

BFT LARVAL NUTRITION

The analysis of the variations of energy and nutrient contents during embryogenesis and yolk-sac larvae period can give a very uselful information of the nutritional requirements during the early larval stages of BFT (Takii et al 1997). Aspects such as the environmental effects, feeding behaviour, digestive capacity during ontogeny, consumption and assimilation rates and nutritional requirements from first feeding larvae to metamorphosis needs to be investigated for reliable aquaculture of BFT.

CURRENT FEEDS

Whole fish. Although there have been a number of attempts at BFT culture including both complete aquaculture and grow out/fattening programmes, there are few nutritional data pertaining to those studies (Buchanan 1977; Vincent 1981; Aiken 1984; Okamoto et al. 1984; Belle 1994; Doumenge 1996). The southern bluefin tuna has also been the subject of grow out/fattening trials in South Australia over the last 8-9 years (Jeffriess 1993; Carter et al. 1998; Lee 1998). The necessity to formulate artificial diets has generally been avoided in the above programmes by the use of whole fish derived from local or other commercially available fisheries including trash fisheries. In one of the earliest trials at Kinki University in Japan, wild-caught BFT showed very good growth rates, averaging 20 kg/year, on a diet consisting of fishes including mackerel, anchovy and sand eel (Harada et al. 1971). Subsequent trials elsewhere have used ground trash fish (Vincent 1981) and a mixed diet of local mackerel, herring and butterfish for BFT, and locally caught and frozen pilchards/sardines with southern bluefins (Fitz-Gerald and Bremner 1998).

Artificial feeds. The Kinki University experiments included some comparative dietary trials where juvenile BFT were fed four test diets comprising three single species fish diets (sand eel, anchovy and mackerel) and a mixed diet of minced mackerel with a dry commercial diet for yellowtail (Harada et al. 1983). The BFT on both the mackerel diet and the mixture diet showed high survival and had body compositions similar to wild fish. However, whereas the fish on the mackerel diet had the best growth rate, the mixture diet had a relatively poor growth rate. Perhaps surprisingly, the fish on the anchovy diet had the lowest survival, growth rate and fat content (Harada et al. 1983). In more recent sudies with the southern bluefin tuna in South Australia, pellets have been trialled. The fish did not accept dry pellets but moist pellets (sausage) containing 40% moisture and based on fish meal and fish oil were readily accepted. The pellets were 40% protein with lipid levels varying between 12.5% and

7.5%. Growth on the pellets (in combination with bait fish) was similar to that on bait fish (sardines) alone alone with feed conversions slightly better for pellets (Smart 1995).

Broodstock feeding. Atlantic mackerel (*Scomber scombrus*), Atlantic horse mackerel (*Trachurus trachurus*), and shortfin squid (*Illex coindetti*) have been used as feed for BFT broodstock. The preference and feeding quantity on kind of feed is relating on physiological condition of BFT, especially on maturation (Fushimi et al 1996).

BFT NUTRITION – THE GLOBAL PERSPECTIVE

As indicated above, the primary goal of nutrition research will be to define the precise nutritional requirements of BFT with the output being the formulation of ideal artificial diets. This will remove the dependence of the prospective tuna industry on whole fish feeds based on local trash fish or individual species fisheries, such as anchovies or pilchards, which can be subject to very great seasonal and environmental variations (Thorpe et al 1997). However, it is very important to note that the projected artificial pellet/sausage diets will still be heavily dependent upon current commercial marine fisheries that provide the global supplies of fish meal and fish oil.

Capture fisheries and aquaculture. Wild fish capture fisheries are finite resources which, although renewable, are highly vulnerable so that their sustainability is in question. Global capture fisheries have shown little growth over the last 15 years and currently yield less than 100 million tonnes per annum. Over exploitation (fishing) of individual fish species, whether for direct human consumption or reduction to fish meal and fish oil, has caused the collapse or near collapse of some valuable fisheries. Environmentalists are exerting pressure to further reduce fishing effort and catches by introducing tighter regulatory measures. The realisation that global warming and natural climatic events such as El Niño can profoundly affect major fisheries, especially the anchovy fishery, highlights the inherent vulnerability of global fisheries. Projected stagnation and, perhaps, even declining yield from global fisheries predicts that future demand for fisheries products may exceed supply leading to price increases. Indeed, the cost of fish oil increased substantially from 1997 to 1998 to exceed that of soya oil. Against this background, fish production from aquaculture has increased substantially over the last decade or more (double in the past 15 years). Aquaculture has been the world's fastest growing food production sector for over a decade and it is projected to at least double over the next decade or more (Naylor et al 2000).

Fish meal and fish oil. Erratic global fisheries and rapidly increasing aquaculture as described above have to be considered against the background that fish meal and fish oil are major feed stocks for cultured marine fish. The dietary requirement of farmed marine fish for high quality protein, rich in essential amino acids, can probably be met by sources other than fish meal. However, the primary role of marine fish oils in aquaculture is as a dietary source of the HUFA which together can satisfy the essential fatty acid (EFA) requirements of all fish species. As described above, marine fish in particular have an absolute dietary requirement for the preformed HUFA. Currently there is no feasible, alternative source to fish oil for these nutrients in marine fish feeds. Plant seed (vegetable) oils, rich in LA and LNA may be a partial substitute for HUFA in freshwater and salmonid fish feeds but this is an unknown area with marine fish. The effects of partial substitution of fish oil with plant oils in marine species including tuna would have consequences for growth and health of the fish as well as being potentially undesirable in terms of both consumer acceptance and human nutrition because of dilution of the health promoting effects of fish-derived EPA and DHA. Although

this may not be an immediate problem it is certainly one that is projected to have major consequences for aquaculture in the future and it would be highly advisable that the plans for a prospective European tuna farming industry include the necessary steps to address this approaching problem at this early stage.

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