Essential fatty acid and lipid requirements of farmed aquatic animals – sourcing the good oils

BD Glencross

Mariculture Research and Advisory Group, Fisheries Western Australia, c/-Fremantle
Maritime Centre, Fleet St, Fremantle, WA 6160, Australia

Summary

Lipids are a critically important nutrient in the diets of farmed aquatic species as they provide not only a key energy source for these animals, but also essential fatty acids. Lipid requirements in fish vary primarily on the basis of whether they are carnivorous, omnivorous or herbivorous species. Essential fatty acid requirements in aquatic animals are a complex response to the proportion of particular fatty acids in the lipid of the diet, the ratio of the n-3 to n-6 fatty acids and the actual amount of lipid in the diet. Furthermore, environmental aspects also play an important role in defining what types of fatty acids have more value for particular species. Because of the retention of the long-chain highly-unsaturated fatty acids (HUFA), farmed fish and crustaceans provide one the most reliable sources of the n-3 HUFA for human consumption. In general the n-3 HUFA levels of farmed fish are higher than any other available meat source.

Introduction

Lipids play an important role in the nutrition of most aquatic animals (1, 2). Unlike most terrestrial animals, aquatic animals generally have a comparatively poor capacity to deal with dietary carbohydrates. As a consequence many aquatic animals have evolved a considerable capacity to utilise and/or metabolise dietary lipids as a key energy and nutrient source (2). Fish are particular in this regard, with some species showing a capacity to deal with lipid levels up to 50% of the their dietary intake. However, not all aquatic animals can deal with high dietary lipid intake, but almost all have a clearly defined requirement for either or both the n-3 and n-6 series of fatty acids (2, 3, 4). This paper provides a brief review of the basis of lipid utilisation by farmed aquatic animals. A more in depth examination is made of the requirements for dietary fatty acids by growing juvenile animals and the resultant impacts that these differences have on the nutritional value of various farmed fish to the consumer.

Dietary lipid requirements

The actual amount of lipid in the diet that optimises fish growth performance varies primarily on a generic basis. The requirement of fish for lipids appears to follow from whether they are carnivores, omnivores or herbivores (Table 1).

Carnivorous species

Carnivorous fish have an optimal lipid requirement typically higher than that of most other species. Latest research supports that Atlantic salmon (*Salmo salar*) grow best on diets with lipid levels in excess of 50% lipid (5). Similarly, both Rainbow trout (*Oncorhynchus mykiss*) and Barramundi (*Lates calcarifer*) also show superior performance when fed diets with higher lipid levels, providing that the diets also had adequate protein to support growth (1,6). Some species, like red seabream (*Pagrus auratus*) that may almost be regarded as omnivorous, have difficulty dealing with dietary lipid levels greater than 20% (7). The basis for the high levels of dietary lipid being required in the diet of carnivorous fish stems from the primary need for maximising dietary energy intake. Traditionally this entailed diets with high protein levels and moderate lipid levels (1, 7). However recent research has shown that by maximising the lipid content considerable reductions to the protein inclusion can be made (3, 5, 6). However, both the technical feasibility of feed engineering and the fish's capacity to actually utilise dietary lipids can restrict the inclusion level of lipids.

Omnivorous species

Distinctly omnivorous freshwater species like silver perch (*Bidyanus bidyanus*) have difficulty dealing with lipid levels greater than 10%. Generally this is reflected by poorer growth performance by fish fed diets with higher lipid levels (8). Prawns are also similar in this regard, showing limited capacity to be able to deal with dietary lipid levels greater than 9% (9). Conversely, these species have often compensated for this by being able to deal with dietary carbohydrates more effectively than the carnivorous species.

Herbivorous species

Few farmed aquatic animals (other than filter feeding molluscs) are truly herbivorous. Typically though those regarded as herbivorous aquatic animals have a very poor capacity to deal with dietary lipid. An example of this is the greenlip abalone (*Haliotis laevigata*). A study by van Barneveld et al. (10) clearly showed that as the level of dietary lipid increased above 3%, the digestion of key amino acids was compromised. Consequently most modern compound abalone feeds typically have fat levels no greater than 5%. The ability of this group of animals to handle dietary carbohydrates is close to rivaling that of some terrestrial animals.

Essential fatty acid requirements

Apart from the energetic value of lipids in the diet, the provision of essential fatty acids is also of particular importance to most aquatic species. Essential fatty acids differ from many of the other fatty acids in that they have different pathways of metabolism and in many cases are preferentially saved from catabolism. Fatty acid requirements of aquatic animals are a complex response to the proportion of particular fatty acids in the neutral lipid of the diet, the balance of the n-3 to n-6 fatty acids and the actual amount of lipid in the diet (1, 2, 9)

Qualitative and quantitative requirements

Discrepancies reported in fatty acid composition and fatty acid metabolism between fish and crustacean species, suggest that the precise dietary fatty acid requirements may also differ between species (1, 11, 12). A preliminary approach to the determination of fatty acid requirements in many animal species has been to analyse the native fatty acid composition of the animal. However, in fish and crustaceans the characteristic fatty acid composition of each species is a complex response to food availability, water salinity, water temperature, stage of development, nutritional condition, or reproductive condition (1, 3, 13, 14).

The specific fatty acid content of the dietary lipid has been shown to be important to its nutritive value to fish and crustaceans (1) (Table 1). Certain fatty acids have been observed to support an enhanced level of growth. Indeed, some of these fatty acids are so critical for growth that they are deemed as essential. As such they are often referred to as the essential fatty acids (EFA). The requirement for these nutrients may be based on needs to supplement inadequate or non-existent *de novo* synthesis to sustain normal growth in these animals (12).

Notable are the differences in fatty acid class requirements between marine, estuarine/diadromous and freshwater species (1). Desaturation of mono- or saturated fatty acids to make linoleic (LOA; 18:2n-6) or linolenic (LNA; 18:3n-3) acids has not been detected in any farmed aquatic species. However some aquatic animals have shown ability to bioconvert LOA and/or LNA to long-chain unsaturated fatty acids (12). Indeed the dietary value of the essential fatty acids appears to be linked with the animal's own biosynthetic capacity. Diadromous fish such as the rainbow trout (*O. mykiss*) showed improvement in growth with addition of eicosapentaenoic (EPA; 20:5n-3) or docosahexaenoic acid (DHA; 22:6n-3), though were able to grow to the same extent when provided only with LNA, albeit at higher inclusion levels (1). Considerable capacity to elongate and desaturate LNA to long-chain unsaturated fatty acids has been demonstrated in this species (12). In contrast, marine fish such as the red seabream (*Pagrus auratus*) and the tiger puffer (*Takifugu rugripes*) have been shown to have essentially no capacity for synthesis of long-chain unsaturated fatty acids from the precursors of LOA and LNA. Estuarine species such as the giant tiger prawn (*Penaeus monodon)*, showed some capacity for synthesis of the long-chain unsaturated fatty acids from precursors of LOA and LNA (11, 12). Considerable growth promoting effects have been observed in this and other species when fed arachidonic acid (ARA; 20:4n-6), or EPA or DHA. This suggested that these fatty acids were also essential in the diet, particularly when $[^{14}C]$-acetate studies with *P. monodon* identified low level synthesis of each of these HUFA. It was suggested that to alleviate this low biosynthetic capacity, prawns also have a dietary requirement for these particular fatty acids (11, 12, 15, 16).

Table 1. Lipid and EFA requirements of some farmed aquatic species (g/kg dry diet)

Species	Diet	*Optimal lipid level (g/kg)	LOA (g/kg)	LNA (g/kg)	ARA (g/kg)	EPA (g/kg)	DHA (g/kg)	n-3 : n-6
Atlantic salmon	C	470 (5)	n/a	n/a	n/a	n/a	n/a	n/a
Rainbow trout	C	250 (3)	0	10 (17)	0	3 (18)	3 (18)	n/a
Barramundi	C	200 (6)	14 (6)	5 (6)	n/a	8 (6)	12 (6)	1.7 : 1 (6)
Red seabream	O	150 (7)	0 (7)	0 (7)	0 (7)	15 (7))	15 (7)	n/a
Tilapia	O	100 (4)	10 (4)	0 (4)	10 (4)	0 (4)	0 (4)	n/a
Common carp[A]	O	90 (8)	10 (4)	10 (4)	0 (4)	0 (4)	0 (4)	n/a
Silver perch	O	90 (8)	27 (19)	18 (19)	n/a	n/a	n/a	0.66 : 1 (19)
Kuruma prawn[B]	C	70 (20)	10 (15)	10 (15)	n/a	10 (16)	20 (20)	n/a
Giant tiger prawn	O	60 (9)	10 (21)	15 (21)	0 (9)	3 (22)	3 (22)	2.3 : 1 (9)
Freshwater prawn[C]	O	60 (23)	0 (23)	0 (23)	R (23)	0 (23)	R (23)	0.08 : 1 (23)
Greenlip abalone	H	30 (10)	n/a	n/a	n/a	n/a	n/a	n/a

Numerical superscripts denote source of information. Diet; C : carnivore, O : omnivore, H : herbivore. n/a : not available. R : required though amount not defined.* As defined by maximum growth rate on a N retention and/or feed efficiency basis. Not necessarily the same lipid content under which optimal fatty acid requirements were determined. [A]Cyprinus carpio, [B]Penaeus japonicus, [C]Macrobrachium rosenbergii

Though there have been a number of studies to define qualitative and quanitative essential fatty acid requirements of farmed fish species (7,9,15,16,17,21,22,24) (Table 1), these requirements are still generally regarded as poorly defined (2). A fuller understanding of the importance of these nutrients in the diets of many species is required, particularly in light of the increasing need for resources of EPA and DHA rich fish oils.

Studies on the quantitative fatty acid requirements of two key aquaculture species has found that the fatty acid requirement is one where the requirement is related to the amount of lipid in the diet; the higher the lipid content, the higher the demand for EFA. Watanabe (1) found that the requirement for LNA by rainbow trout (*O. mykiss*) increased as the amount of lipid in the diet increased. Castell et al. (17) and Watanabe et al. (18) consequently suggested that the essential fatty acid requirement should be expressed as a percentage of the dietary lipids, rather than an absolute dietary amount. Another study examining the relationship of essential fatty acid proportionality and absolute levels in farmed tiger prawns *(Penaeus monodon)* clearly identified that essential fatty acids were also required by this species on a proportional basis of the total lipid in the diet (9). While some of this effect may be related to the differences in digestion of the various types of fatty acids, a clear effect of the proportionality was also evident in this study, that was not shown from earlier studies (1).

The linolenic fatty acid series (n-3) have generally been observed to have a greater essential fatty acid value in marine animals than the linoleic series (n-6). Conversely, freshwater species have been observed to have a greater requirement for the linoleic series (n-6), (1, 3, 4, 24). Crustaceans demonstrate this effect well. For example, LNA included singly at a level of 1% of the total diet was found to be optimal for growth of the marine Kuruma prawn (*P. japonicus).* Similarly a 1% inclusion level of LOA acid was also found to be optimal for this fatty acid (15), though at these inclusion levels LNA supported better growth. In the estuarine giant tiger prawn (*P. monodon*), a slightly different scenario was reported, with LOA supporting better growth than LNA when the requirements for each were examined singly. However, when these two fatty acids were provided in combination, their requirements for optimal growth as a percentage of the diet changed from 1% and 1.5%, to that of 1.5% LNA and 1% LOA respectively (21). In comparison, the freshwater prawn (*M. rosenbergii*) showed no significant weight gains when purified LOA or LNA were added to an otherwise EFA-free diet, although this species did show specific requirements for some HUFA (23, 25).

The HUFA derived from LNA (EPA and DHA) have been reported to possess even greater value as essential fatty acids than the shorter-chain polyunsaturated fatty acids (PUFA) (1, 2). Several studies have examined the effect of varying the dietary levels of PUFA and HUFA in species such as *P. japonicus* (16, 20), the estuarine Chinese prawn, *Penaeus chinensis* (26, 27), *P. monodon* (9, 22) and several fish species (1, 2, 7, 18, 24, 28, 29). Inclusion levels of EPA at 1% of the diet were established as optimal for growth in *P. japonicus* (16). DHA was found to be required at a 2% inclusion level, although diets with higher content than 2% were not examined. A proposed nutritional ranking of essential fatty acid in *P. japonicus* was: EPA > DHA > LNA > LOA > 18:1n-9.

The requirements of the n-6 HUFA, ARA, are still largely unclear for many aquatic species. Work by Xu et al. (26, 27) focusing on the requirements of *P. chinensis*, determined that ARA had greater essential fatty acid value than either LOA or LNA, but less than both EPA and DHA. It was also observed that ARA provided no additional benefit to the growth of the estuarine prawn *P. monodon* when LOA and LNA, or LOA, LNA, EPA and DHA were provided at predetermined optimal levels (9). Further examination of factors that may have influenced this finding suggested that the balance between the dietary n-3 and n-6 fatty acids might have been important (9). However, while the freshwater prawn (*M. rosenbergii*) was not observed to respond to inclusion of either LOA or LNA in the diet, it was reported to respond to inclusion of either ARA or DHA in the diet, and that growth was superior when the n-6 to n-3 ratio was about 12:1 (23, 25). No specific effect was attributable to EPA inclusion (25). The marked differences between this species' requirements and those of the marine and estuarine species suggest an influence of water salinity on an animal's EFA requirements.

Need for balance between dietary n-3 and n-6 fatty acids

The combination of LOA (n-6) and LNA (n-3) in the diet has considerable influence on the growth of many aquatic animals (2, 3, 9, 26, 28, 29). Studies examining the requirements for n-6 (LOA) and n-3 (LNA) by the Coho salmon (*Oncorhynchus kisutch*), identified that these animals had a very dominant n-3 (LNA) requirement, but that a minimal amount of n-6 (LOA) in the diet gave added growth performance (29). Differences observed between several prawn species, with respect to their metabolism of [^{14}C] acetate and/or palmitate (11, 12, 20), indicate that the quantitative essential fatty acid requirements for each species may also be quite different. A study examining the inter-relationships of two or more of these essential fatty acids in the diet of the farmed tiger prawn (*P. monodon*) has clearly identified that the essential fatty acids not only interact with each other to influence an animal's growth, but that the balance of n-3 to n-6 fatty acids was also critically important (9). Work with the coho salmon also indicated that the requirement for a particular fatty acid is a complex function of the amount and type of other fatty acids present (29). There have been several studies that show that the balance of particular dietary fatty acid classes, the n-3 and n-6, are as important as the actual types of fatty acids in the diet of aquatic animals (2, 9, 26, 28, 29). Furthermore, it has been suggested that, although the n-3 and n-6 balance hypothesis does have some basis, it is still perhaps a simplistic model. It was suggested that the affinity heirachy of the EFA for the desaturase enzyme system may be one of the influencing points in this process and as such the balance is perhaps more accurately reflected not just on an n-3 and n-6 basis, but also on a chain length and level of unsaturation basis (2, 9).

Essential fatty acid synthesis

Biosynthesis of fatty acids longer than 16:0 and with unsaturated bonds, involves both a mitochondrial and a microsomal enzyme systems as opposed to the cytosolic enzyme system used to make 16:0 (30). The elongation process of the longer-chain fatty acids is similar to that of the cytosolic fatty acid synthetase system, in that it also increases chain length by addition of two-carbon acetyl CoA units to the chain (30). The microsomal system in particular has great physiological importance, in that it is the one required to carry out chain elongation from 18:0 to 24:0 (30). The introduction of an unsaturated bond requires an oxygen-dependant desaturase enzyme system (30). However, the synthesis of certain fatty acids by the desaturase enzyme system is not possible, these being 18:2n-6 (linoleic acid) or 18:3n-3 (linolenic acid). This limitation is why they are essential dietary nutrients (1, 2). The desaturase enzyme system is one of great importance to the synthesis of the other essential fatty acids. Two desaturase enzyme systems (Δ^6 and Δ^5) have been identified and a third was suggested (Δ^4) (2, 3, 31).

The Δ^6 desaturase enzyme system has the capacity to work with a variety of substrates (12). However, the substrates have been reported to exhibit a competitive affinity hierarchy for the access to the Δ^6 desaturase enzyme system. The affinity of the fatty acids for the Δ^6 desaturase enzyme system increases with chain length and level of desaturation, such that 18:3n-3 > 18:2n-6 > 18:1n-9 > 16:1n-7 (2, 3). It was suggested that the Δ^4 reactions were actually carried out by Δ^6 following an additional elongation step (2). A study by Morente and Tocher (31) supported this proposal of Δ^6 desaturation to make 22:6n-3 (DHA) from 20:5n-3 (EPA). Increased levels of [1-^{14}C] 24:5n-3 and [1-^{14}C] 24:6n-3 were observed, consistent with an increase in [1-^{14}C] 22:6n-3 production from fish injected with [1-^{14}C] 20:5n-3.

Most aquatic animals, particularly marine ones, lack the Δ^6-desaturase system required to desaturate 18-carbon fatty acid chains. Some diadromus species (notably the salmonids) have shown a defined ability to elongate and desaturate either 18:2n-6 or 18:3n-3 to the longer and more highly-unsaturated fatty acids. Indeed studies with rainbow trout have shown that about 70% of an injected dose of [1-^{14}C] 18:3n-3 ended up in the total lipid 22:6n-3 (1, 12), compared with the marine turbot (*Scophthalamus maximus*), in which only 3 to 15% was found elongated and further desaturated. Other species, like some of the estuarine prawns, have shown a biosynthetic capacity in between that exhibited by the rainbow trout and the distinctly marine species (12).

Table 2 Flesh lipid and essential fatty acid content of selected Australian fish species, ranked by total n-3 and n-6 HUFA content

Species	Total lipid (% wet weight)	ARA (20:4n-6) (mg/100g flesh)	*EPA (20:5n-3)* (mg/100g flesh)	DHA (22:6n-3) (mg/100g flesh)
Southern bluefin tuna (farmed)	7.0	58	350	1380
Swordfish	7.7	423	371	541
Atlantic salmon (farmed)	2.7	71	171	378
Smooth oreo	3.0	57	124	286
Blue groper	3.6	165	145	140
Spanish mackerel	3.0	66	75	281
Spikey oreo	1.7	73	76	273
Snapper (farmed)	1.8	9	160	190
Mussel (farmed)	1.7	13	153	166
Tailor	1.3	29	49	251

All data derived from Nichols et al. (32), Yearsley et al. (33) and Glencross, unpublished.

Seafood the good food

The fatty acid composition, and in particular the levels of the n-3 highly unsaturated fatty acids have, of recent, gained a lot of publicity over their health benefits to humans (34). Seafood is notable by its relatively high levels of the beneficial n-3 HUFA, EPA and DHA (32, 33). The recent release of two books, "Seafood the good food" (33) and the "Australian Seafood Handbook: Domestic Species" (34) have both clearly documented the fatty acid content, particularly the HUFA (both n-3 and n-6) of most Australian seafoods, including some key aquaculture species (Table 2). Assessment of the fish species with the highest levels (on a mg/100g fresh fish basis) of these beneficial fatty acids (both n-3 and n-6) reveals that of the top ten species, at least 40% are those from aquaculture (Table 2). When this is considered with the fact that aquaculture production accounts for less than 10% of the volume of fish product produced in Australia it provides good testament to the beneficial value of farmed fish to the health of Australians. Similarly, farmed fish also tend to have higher fat levels than their wild counterparts, this in part is the reason behind these higher HUFA levels. This suggests that not only is fish farming a possible answer to satisfying the world's increasing demands for seafood, but that in many cases it could possibly be healthier for you too.

References

1. Watanabe, T. Lipid nutrition in fish. Comparative Biochemistry and Physiology, 1982;73B: 3-15.
2. Sargent, J. R., Bell, J.G., Bell, M.V., Henderson, R.J. and Tocher, D.R. The metabolism of phospholipids and polyunsaturated fatty acids in fish. In: B. Callou and P. Vittelo (Eds), Coastal and Estuarine Studies - Aquaculture: fundamental and applied research., American Geophysical Union, Washington, DC, USA, 1993; pp103-124.
3. Henderson, R J. and Tocher, D.R.. The lipid composition and biochemistry of freshwater fish., Progress in Lipid Research 1987;26: 281-347.
4. Stickney, R.R. and Hardy, R.W. Lipid requirements of some warmwater species. Aquaculture 1989;79: 145-156.
5. Lie, O. Recent developments in nutrition of Atlantic salmon. World Aquaculture Society Annual Meeting, Sydney, Australia, 26th April – 2nd May 1999. 1999; pp 449.
6. Williams, K.C. and Barlow, C.Fishmeal replacement in aquaculture diets for barramundi. Fisheries Research and Development Corporation. Final Report. FRDC, Canberra. 1997;114pp.
7. Takeuchi, T., Shiina, Y. and Watanabe, T. Suitable levels of n-3 highly-unsaturated fatty acids in the diet for fingerlings of red seabream. Nippon Suisan Gakkaishi 1992;58(3) 509-514.
8. Allan, G.L., Rowland, S.J., Mifsud, C., Glendenning, D., Stone, D.A.J. and Ford, A. Replacement of fish meal in diets for Australian silver perch *Bidyanus bidyanus*: Least-cost formulation of practical diets. Aquaculture 2000;186: 327-340.
9. Glencross, B.D. Optimisation of the dietary essential fatty acid requirements for the prawn, *Penaeus monodon*. Doctoral Dissertation, Department of Zoology, The University of Queensland. 1998; pp 234.
10. van Barneveld, R.J., Fleming, A.E., Vandepeer, M.E., Kruk, J.A. and Hone, P.W. Influence of dietary oil type and inclusion level in manufactured feeds on the digestibility of nutrients by juvenile greenlip abalone (*Haliotis laevigata*). Journal of Shellfish Research 1998;17(3): 649-655.
11. Kanazawa, A., Teshima, S., Ono, K. and Chalayodeja, K. Biosynthesis of fatty acids from acetate in the prawn, *Penaeus monodon* and *Penaeus merguinensis*. Memoirs of the Faculty of Fisheries, Kagoshima University, Japan 1979a;28: 21-26.
12. Kanazawa, A., Teshima, S.I., Ono, K. Relationship between essential fatty acid requirements of aquatic animals and the capacity for bioconversion of linolenic acid to highly unsaturated fatty acids. Comparative Biochemistry and Physiology 1979b;63B: 295-298.

13. Bottino, N.R., Gennity, J., Lilly, M.L., Simmons, E. and Finne, G. Seasonal and nutritional effects on the fatty acids of three species of shrimp, *Penaeus setiferus, P. aztecus* and *P. duorarum.* Aquaculture 1980;19: 139-148.
14. Chandumpai, A., Dall, W. and Smith, D.M. Lipid-class composition of organs and tissues of the tiger prawn *Penaeus esculentus* during the moulting cycle and during starvation. Marine Biology 1991;108: 235-245.
15. Kanazawa, A., Tokiwa, S., Kayama, M. and Hirata, M.. Essential fatty acids in the diet of prawn - I : Effects of linoleic and linolenic acids on growth. Bulletin Japanese Society of Scientific Fisheries 1977;43: 1111-1114.
16. Kanazawa, A., Teshima, S., Endo, M. and Kayama, M.. Effects of eicosapentaenoic acid on growth and fatty acid composition of the prawn, *Penaeus japonicus*. Memoirs of the Faculty of Fisheries, Kagoshima University, Japan 1978;21: 35-40.
17. Castell, J.D., Sinnhuber, R.O., Wales, J.H. and Lee, J.D. Essential fatty acids in the diet of the rainbow trout (*Salmo gairdneri*): Growth, feed conversion and some gross deficiency symptoms. Journal of Nutrition 1972;102: 77-86.
18. Watanabe, T., Ogino, C., Koshiishi, Y. and Matsunaga, T. Requirement of rainbow trout for essential fatty acids. Bulletin of Japanese Sciences and Scientific Fisheries 1974;40: 263-269.
19. Hunter, B.L, Allan, G.L., Booth, M., Glencross, B.D., Smith, D.M. and Roberts, D.C.K. The dietary requirement of linoleic and linolenic acid for the silver perch (*Bidyanus bidyanus*) fingerlings: effect on growth and fatty acid composition. Aquaculture Nutrition 2000; IN PRESS
20. Kanazawa, A. Nutrition of penaeid prawns and shrimps. In: SEAFDEC Aquaculture Department (Eds), Proceedings of the The First International Conference on the Culture of Penaeid Prawns/Shrimps, Iloilo City, Philippines. 1984; pp 124-130.
21. Glencross, B.D. and Smith, D.M. The linoleic and linolenic acids requirements of the prawn, *Penaeus monodon.* Aquaculture Nutrition 1999;5(1): 53-64.
22. Glencross, B.D. and Smith, D.M. Optimising the essential fatty acids, eicosapentaenoic and docosahexaenoic acid in the diet of the prawn, *Penaeus monodon.*; Aquaculture Nutrition 2000;6(4): IN PRESS
23. D'Abramo, L.R. and Conklin, D.G. New developments in the understanding of the nutrition of penaeid and caridean species of shrimp. In: C.L. Boyd and J.S. Hopkins (Eds), Proceedings of the Aquaculture '95., World Aquaculture Society, Baton Rouge, LA, USA. 1995; pp 95-107.
24. Shikata, T. and Shimeno, S. Metabolic response to dietary stearic acid, linoleic acid, and highly unsaturated fatty acid in Carp. Fisheries Science 1994;60: 735-739.
25. D'Abramo, L.R.. Triacylglycerols and fatty acids. In: L. R. D'Abramo D.E. Conklin and D.M. Akiyama (Eds), Advances in World Aquaculture - Crustacean Nutrition. World Aquaculture Society, Louisianna, MO, USA, 1997; pp 587.
26. Xu, X.L, Ji, W., Castell, J.D. and O'Dor, R.K. The nutritional value of dietary n-3 and n-6 fatty acid for the chinese prawn (*Penaeus chinensis*). Aquaculture 1993;118: 277-285.
27. Xu, X. L., Ji, W., Castell, J.D. and O'Dor, R.K. Essential fatty acid requirements of the Chinese prawn, *Penaeus chinensis.* Aquaculture 1994;127: 29-40.
28. Castell, J.D., Bell, J.G., Tocher, D.R. and Sargent, J.R. Effects of purified diets containing different combinations of arachidonic and docosahexaenoic acid on survival, growth and fatty acid composition of juvenile turbot (*Scophthalmus maximus*)., Aquaculture 1994;128: 315-333.
29. Yu, T.C. and Sinnhuber, R.O. Effect of dietary ω3 and ω6 fatty acids on growth and feed conversion efficiency of coho salmon (*Oncorhynchus kisutch*). Aquaculture 1979;16: 31-38.

30. Rawn, J.D. Biochemistry. Neil Patterson Publishers, Burlington, NC, USA. 1989; pp 1105.
31. Mourente, G. and Tocher, D.R.. In vivo metabolism of [1-^{14}C] linolenic acid (18:3(n-3)) and [1-^{14}C] eicosapentaenoic acid (20:5(n-3)) in a marine fish : Time-course of the desaturation/elongation pathway. Biochemica et Biophysica Acta 1994;1212: 109-118.
32. Nichols, P.D. Virtue, P., Mooney, B.D., Elliot, N.G. and Yeardsley, G.K. Seafood the good food: the oil (fat) content and composition of Australian commercial fishes, shellfishes and crustaceans. CSIRO Division of Marine Research, Hobart: 1998;201 pp.
33. Yearsley, G.K., Last, P.R. and Ward, R.D. Australian Seafood Handbook: Domestic Species. CSIRO Division of Marine Research, Hobart, 1999; 461 pp.
34. Sinclair, A.J. and Anderson, T.A. Health implications of farmed fish. Proceedings of the Nutrition Society of Australia 1994;18: 88-97.