DIETARY REQUIREMENT AND OPTIMAL FEEDING PRACTICES FOR BARRAMUNDI (*Lates calcarifer*)

PROJECT 92/63

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FINAL REPORT
TO
FISHERIES RESEARCH AND DEVELOPMENT CORPORATION

FISHERIES RESEARCH & DEVELOPMENT CORPORATION

QUEENSLAND GOVERNMENT

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1. PROJECT

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2. NON-TECHNICAL SUMMARY

93/63	**Dietary requirements and optimal feeding practices for barramundi (*Lates calcarifer*)**

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OBJECTIVE:

To develop feeding strategies and diets for periods of fast growth (summer) and slow growth (winter) which optimise food conversion and growth rate.

NON-TECHNICAL SUMMARY

Feed is by far the single largest cost component of barramundi farming and accounts for about 35% of on-farm operating costs. Reducing feed costs by better tailoring dietary specifications to the nutrient requirements of the fish, by providing these nutrients at least cost and by adopting feeding practices that optimise productivity will greatly assist farm profitability. In Australia, barramundi are pond-reared over latitudes from 5°S to 22°S and this results in large seasonal variations in water temperature and consequently, in growth rate of the fish. The research conducted in this project sought primarily to characterise the effect of water temperature on fish productivity and how modification of dietary nutrient specifications and/or feeding practices could assist in improving farming profitability. This entailed research to define the fish's response to changes in dietary supply of critical nutrients over a range of water temperatures. Research examining the efficacy of crystalline amino acids in diets for barramundi was carried out in a supplementary project (FRDC 95/69); research on the nutritive value of terrestrial feed ingredients and their suitability as replacements of fishmeal in diets for barramundi is reported in FRDC 93/120-04.

Effect of water temperature and food manipulation on barramundi performance
Five short-term satietal feeding and two long-term restrictively fed growth assays were carried out to investigate the effects of water temperature, feeding frequency and fish size (weight) on food intake and fish growth in order to define optimal feeding practices for juvenile (~30 to 300 g) barramundi. For each fish size, intake of an extruded dry pellet of acclimatised fish increased essentially linearly as water temperature rose from 20 to 29°C. The 'as fed' chemical composition of the extruded food pellet was: dry matter (DM), 95%; digestible crude protein

(DCP), 40%; and estimated digestible energy (DE), 15.5 kJ/g. Increasing water temperature from 20 to 29°C caused food intake (expressed as % of biomass) to increase from 1.9 to 3.7% for a ~50 g fish and from 0.7 to 1.3% for a ~300 g fish, respectively. Absolute growth rate increased linearly with water temperature and size while food conversion (FCR) improved slightly with water temperature and worsened slightly with size. Growth rates of ~50 g fish ranged from 0.75 to 1.85 g/d at 20 and 29°C, respectively and increased to 2.07 and 4.27 g/d for ~300 g fish respectively. Varying feeding frequency from 1 to 3 times daily increased food intake of small fish (<100 g), but the extra food did not significantly improve growth rate. It is recommended that fingerlings from about 40 and up to 100 g should be fed twice daily but thereafter once daily is adequate. For fish above 300 g, skipping a day's feeding at the weekend (a common industry practice) had no deleterious effect on either FCR or growth rate. The effects of water temperature and food restriction on growth and body composition of barramundi were examined in juvenile fish (130 to 260 g). At high water temperatures (26 to 29°C), increasing daily food allowance from 65-70% of satiety up to satiety resulted in a small improvement in FCR whereas at low water temperatures (20 to 23°C), FCR was unaffected by food consumption. However, FCR improved as water temperature increased, irrespective of the amount of food consumed. Growth rates paralleled the changes in FCR.

Dietary protein and energy requirements of juvenile barramundi

Two comparative slaughter growth assays and an on-farm study were carried out to define the dietary protein and protein to energy requirements of juvenile barramundi. The on-farm study sought to examine the effects on growth and eating characteristics of fish fed on pelleted diets differing in protein and energy contents (~32 % DCP at either of 13.9 or 16.4 kJ/g DE and 37% DCP and 14.1 kJ/g DE). The two laboratory studies were carried out to see if growth (and protein deposition) in barramundi was similar to that of terrestrial monogastric animals, exhibiting both dependency and non-dependency to protein and energy intake. The first of these laboratory experiments examined the effect of satietal feeding of six semi-purified diets in which the DCP content varied incrementally from approximately 27 to 52%. The protein was a blend of fishmeal, casein, gluten and crystalline amino acids that was formulated to mimic the essential amino acid composition of the protein of barramundi and to approximate what might be regarded as 'ideal' protein. The object of the second laboratory experiment was to examine the effect of feeding barramundi diets considered from Experiment Wlk 6 to be either protein-limiting (i.e. 37% DCP and 15.35 kJ/g DE) or energy-limiting (i.e., 47% DCP and 16.23 kJ/g DE) at either of four controlled feeding rates (varying from approximately 45-50 to 85-90% of satiety). Increasing the amount of protein in the diet significantly improved FCR but food intake decreased so that the growth of the fish was improved only slightly at dietary DCP concentrations above about 42%. These results suggest that with a 15.5 kJ/g DE diet, growth rate and FCR were optimised at a DCP:DE ratio of 26.5 mg/kJ. Increasing the DE content of the diet by 30% (from 14 to 18 kJ/g) resulted in a commensurate but small (18%) improvement in fish growth rate at 29°C and a 68% improvement of growth rate at 20°C, ie, dietary energy was used more efficiently at low than at high water temperature. The efficiency of protein utilisation also improved with increasing dietary DCP concentration up to a maximum response at about 40%. However, in contrast to energy, protein utilisation efficiency was better at high than at low water temperature. Thus protein deposition in barramundi appeared to be more dependent on dietary energy rather than protein intake and distinctly different to terrestrial monogastrics where protein deposition is regulated by the intake of both protein and energy. However, this is not to conclude that protein deposition in barramundi is solely dependent on energy intake. Rather that the diets fed contained an excess of protein such that amino acid requirements for protein synthesis were being fully met. Since an energy insufficiency appeared to be the major factor influencing the rate of protein deposition, the enhanced N accretion in barramundi fed the 47% DCP diet was most likely due to the 'excess' dietary protein being a more available source of metabolic energy than the 37% DCP diet. This would explain why the efficiency of protein retention was higher for the 47% than for the 37%

DCP diet even though the composition and intake of protein were identical for each diet. For the above reasons, it would be unwise to conclude that a dietary DCP content of 42% and a DCP:DE ratio of 26.5 mg/kJ as suggested from the data of the first laboratory experiment are optimal dietary specifications for juvenile barramundi. Further work is needed to characterise the response of barramundi to protein intake when diets of much higher energy content are fed.

Essential fatty acid (EFA) requirements

Two comparative slaughter growth assays were carried out to define the requirement of juvenile barramundi (~60 to 175 g) for EFAs. The first experiment examined the effect of varying both the energy (~14, 16 or 18 kJ/g DE) and the amounts and proportions of n-3 and n-6 fatty acids in the diet (ratios of either ~1.0 or 1.5:1) when the fish were satietal fed and reared at either cool (20°C) or warm (29°C) water temperatures. In the second experiment, the amounts of n-3 and n-6 fatty acids in the diet were varied serially to provide dietary n-3:n-6 ratios ranging from ~0.6 to 2.2:1 when dietary energy (17 kJ/g DE), protein (39% DCP) and lipid (15.5%) contents were held constant and the fish were satietal fed and reared at either 20 or 29°C. Increasing the dietary concentration of n-3 highly unsaturated fatty acids (HUFAs) from ~1.0 to 1.2 and 1.4% at a n-3:n-6 ratio of 1.0:1 or from 1.4 to 1.8 and 2.0% at a n-3:n-6 ratio of 1.5:1 had only minimal effects on fish productivity. Growth rate and FCR tended to be better for diets having the higher n-3:n-6 ratio but these effects were not significant. Significant interactions were observed between dietary n-3:n-6 ratio and water temperature for key productivity responses of the fish. Varying the dietary n-3:n-6 ratio from ~ 0.6 to 2.2:1 had no effect on DFI or growth rate and only slight effects on FCR and protein retention rate of fish at 20°C. At 29°C, DFI decreased curvilinearly and FCR and protein deposition rate improved curvilinearly and growth rate increased linearly. The dietary n-3:n-6 ratio corresponding to the asymptote value for fish at 29°C was 1.57, 1.69 and 1.78:1 for DFI, FCR and DCP retention respectively, and 1.67 and 1.94:1 for FCR and DCP retention respectively, for fish at 20°C. At water temperatures of either 20 or 29°C, FCR and protein retention responses were optimised at dietary concentrations for linolenic (18:3n-3), eicosapentaenoic (20:5n-3) and docosahexaenoic (22:6n-3) acids of about 0.45, 0.75 and 1.15%, respectively. However, for fish at 20°C, growth rate did not benefit from 20:5n-3 and 22:6n-3 concentrations greater than 0.18 and 0.32% when the 18:3n-3 content was at least 1.0%. On the other hand, fish at 29°C required dietary 20:5n-3 and 22:6n-3 concentrations greater than 0.8 and 1.2% respectively to maximise growth rate. The results of these two experiments suggest that barramundi diets should contain not less than 1.5% of n-3 HUFA and not less than 2% of total n-3 fatty acids. The balance of n-3 to n-6 fatty acids was found to be important in determining voluntary food consumption and economy of food conversion and subsequently, growth rate and nutrient retention. A n-3 to n-6 ratio of about 1.5-1.7:1 is suggested as being optimal for juvenile barramundi but more work is needed to unravel the complexity between absolute requirement for individual EFAs and the interactive effects between the balance of individual EFAs.

The research has shown that growth rate of barramundi increases linearly with increasing water temperature over the range of 20 to 29°C. For optimal growth rate and FCR, barramundi held at high water temperature (26 to 29°C) require diets high in protein and energy (>42% DCP and DCP:DE of ~27 mg/kJ) and not less than 1.5% of n-3 HUFA. At lower water temperatures (<24°C), barramundi growth is more dependent on the energy than on either the protein or EFA contents of the diet. High DE diets (>16 kJ/g) are advocated as a management strategy to minimise the slow growth rate of fish held at low water temperatures.

Extension activities

Extension of information produced within the project has been a prominent activity of all Project staff. Since 1993, research results from the FRDC barramundi Project have been presented at 14 industry workshops and seminars organised by the QDPI in association with the Australian Barramundi Farmers Association. In addition to these forums, other extension

activities including on-farm trials, field days and direct consultation with aquafeed manufacturers have been carried out to publicise the research results. Additionally, a "Barramundi Farming Information Kit" has been developed and provides information on all aspects of barramundi farming, including nutrition. In 1999 a "BarraProfit" CD was produced with additional financial support from QDPI, NT Fisheries and Fisheries WA. The CD has two major components; an economics programs for pond and closed-system farms, and an expanded version of the "Barramundi Farming Information Kit". A separate section on "Feeds and Feeding" is on the CD.

KEYWORDS: Water temperature, Feeding, Nutrient requirements, Protein, Fatty acids, Energy, Barramundi extension

3. BACKGROUND

The first commercial barramundi hatchery and farm in Australia commenced in Queensland in 1986 following the successful development by QDPI Fisheries staff and private enterprise biologists of techniques to breed and rear the species. In Queensland, production of farmed barramundi has increased rapidly from 22 tonnes in 1988 (Lobegeiger and Barlow, 1991) to 328 tonnes in 1995/96 (Bennet and Brown, 1997) and projected (J Gillespie, pers comm) to reach 1,000 t by Year 2000 and 2,000 t by Year 2005. Production is now occurring in all states and territories other than Tasmania, Victoria and ACT.

There are three different methods of farming barramundi. The system currently with the greatest volume of barramundi production is cage (or occasionally free-) culture in either brackish or fresh water purpose-built ponds. The second and similar system is cage-culture in estuarine waters where site availability is the major factor limiting this form of production. The thermal requirements of barramundi limit the aforementioned production systems to the tropics. The third and expanding production system is intensive confinement in an indoors, controlled environment building, using bore water and a high degree of recirculation through biological filters. Although establishment and operational costs of the intensive indoors system are high, it enables barramundi to be farmed anywhere that suitable water is available, regardless of the latitude.

Farmed barramundi are marketed typically as fresh (chilled) product of a size from plate-size (about 300 to 450 g) to fish of 2 to 3 kg; there is also a small market for live fish. Interest in growing-out barramundi to fish of >2 kg size is increasing as a means of gaining morre marketing opportunities as the fresh live and plate-size product are suited only for domestic consumption which is fairly limited in Australia.

In Australia as elsewhere in the world, major impediments to profitable fish farming are the high cost of feeding and sensitivity to price received for the fish (Johnston, 1997). Cost of the feed is by far the largest single component and amounts to 30 to 35% of total operating costs for a typical Australian barramundi farm (Treadwell et al., 1991; Johnston, 1997). Reducing the cost of the feed (by not over-specifying nutrients or through the use of cheaper alternative feed ingredients) and improving FCR (by better nutrient optimisation of the diet and adopting improved feeding practices to limit food wastage) would reduce the impact of feed costs for barramundi farmers. The research carried out in this project sought to address these issues by determining the requirements of the fish for critically important nutrients and by developing feeding practices that optimised productivity.

Recognising that the development of cost-effective and low fishmeal diets was an urgent priority of Australian and world aquaculture, FRDC in 1993 established a subprogram (93/120) – "Fishmeal Replacement in Aquaculture Diets Subprogram" – to coordinate national research on fishmeal replacement. Dr Geoff Allan of NSW Fisheries was appointed to administer the Subprogram, which involved research staff from 13 institutions, including State and Commonwealth government research institutions, universities and private companies. Four key species were chosen; prawn, *Penaeus monodon*, barramundi, *Lates calcarifer*, silver perch, *Bidyanus bidyanus*, and Atlantic salmon, *Salmo salar*. Research was coordinated through six projects, one on each species, one on feed processing technology and one which carried out a technology audit on crystalline amino acid supplementation.

So that all of the FRDC aquaculture diet development research was effectively coordinated and benefited from the assembled expertise, this barramundi project on dietary requirements and

feeding practices (FRDC 92/63) was included as a peripheral project of Dr Allan's Fishmeal Replacement Subprogram.

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4. NEED

Aquaculture is the fastest expanding food producing sector in the world, growing at a rate in excess of 10% p.a. since 1984 to 27.8 mt worth US$42.3 B in 1995. By comparison livestock meat production grew at a rate of just 2.6% over the same period (Tacon, 1996; Gjedrem, 1997). As production from the wild fishery has been static at ~100 mt for the last decade and unlikely to increase further, aquaculture production must at least double by the year 2025 if current per capita seafood consumption of 19 kg is to be met (Chamberlain, 1993; Csavas, 1994; Smith and Guerin, 1995; Gjedrem, 1997). Although much of this aquaculture expansion will have to come from inland water culture of herbivorous fish such as carp and tilapia, there will also be greater demand for high-value species that are grown on artificially-provided food. Global aquafeed requirements are estimated currently at between 3 and 4 mt p.a. and are predicted to double by the year 2000 with Asia alone expected to consume at least 2.6 mt (New and Csavas, 1995; Smith and Guerin, 1995; Tacon, 1996). Compounded feeds for carnivorous fish and prawns presently contain from 50 to 70% by weight of fishery product (fishmeal, other fishery wastes and marine oils). Almost 30% of fishmeal available globally for export is being consumed by the aquaculture sector (Starkey, 1994; Tacon, 1996). It is evident from these statistics that continued expansion of aquaculture will be curtailed unless suitable alternatives to fishmeal are found.

The development of cost-effective diets, with reduced contents of fish- and other aquatic-meals is an urgent priority for most fish and crustacean aquaculture industries. The major protein source currently used in most aquaculture diets is fishmeal (Lovell, 1989, 1992). There are however, some major problems with supplies of fishmeal. Fishmeal and fish oil production is declining (Barlow, 1989) and the aquaculture feed industry currently uses more than 3 mt of the global fisheries catch (New and Wijkstrom, 1990) excluding 'trash fish' fed directly to aquaculture species. As aquaculture production increases, demand for fishmeal will also increase, inevitably forcing up prices. As high quality fishmeal is generally required for aquaculture feeds, species of fish currently used for human consumption will increasingly be targeted by fishmeal manufacturers. In Malaysia, much of the cheap fish previously used to produce salted fish for human consumption is instead now used for aquaculture (New, 1991). While aquaculture remains dependant to this extent upon capture fisheries it will not be a net contributor to human food supplies.

Australia is particularly vulnerable to any world shortage of fishmeal because of our reliance on imported fishmeal and other non-edible marine product. However, Australia has an abundant supply of terrestrial animal and vegetable protein feeds which have the potential to at least partly if not fully replace the fishmeal presently used in compounded aquaculture diets. To gain the maximum benefit of terrestrial feeds as alternatives to fishmeal in diets for barramundi, research must be carried out firstly to define the nutrient requirements of the fish for optimal growth and FCR and secondly, to determine the nutritive value for barramundi of alternative feed ingredients. Determining the nutrient requirements of barramundi was one of the primary objectives of the present project while assessment of the nutritive value of alternative feed ingredients was addressed in the complementary barramundi Fishmeal Replacement Project (FRDC 93/120-04).

Although it is known that growth of barramundi varies considerably according to water temperature, feeding strategies that optimise growth and FCR in different seasons have not been developed. Data from one large commercial farm showed that FCR varied from more than 5:1 during winter to better than 2:1 in summer. These figures compare unfavourably with conversion values of 1.7:1 reported for barramundi farmed overseas (Trendall and Fielder, 1991). Moreover, fish held over the winter period are observed to have a higher visceral fat

content (and thus a lower dressed weight) than those marketed prior to winter. This demonstrates the need for basic information on feeding behaviour and nutritional requirements of barramundi in the range of water temperatures experienced in northern Australia. This project addresses these needs.

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5. OBJECTIVES

The original objectives as stated in the original Project documentation were:

1. To develop feeding strategies and diets for periods of fast growth (summer) and slow growth (winter) which optimise FCR and growth rate.

2. To determine the appropriateness of an extruded, floating pellet for feeding barramundi under commercial conditions.

3 To investigate the potential for sparing of fishmeal in barramundi grow-out diets using synthetic amino acids and cheaper sources of supplementary protein.

With FRDC approval, the 2nd objective was abandoned after industry consultation indicated that this aspect was no longer an issue as the feeding of extruded pellets had become a standard practice in the industry. The work addressing the 3rd objective was carried out and reported as a supplementary one-year Project (FRDC Project 95/069). Consequently, the feeding strategies work in the present project was expanded to focus on the fish's requirements for key nutrients and the application of this knowledge in the development of more cost-effective grow-out diets for barramundi.

6. TECHNICAL REPORT - DETAILED RESULTS

6.1 General Materials and Methods

6.1.1 Fish and management

Barramundi fry of approximately 20 mm total length were obtained from a marine hatchery and transferred to QDPI's Freshwater Fisheries and Aquaculture Centre, Walkamin for on-growing until required for experimentation. During this on-growing stage, fish were housed in indoor tanks supplied with flow-through underground water (salinity, <0.05 ‰; temperature, 25 ±0.5°C) and fed to satiety on a commercially-available pelleted feed. Fish were size sorted at 1 to 2 week intervals until they attained a body length of about 100 mm and occasionally thereafter to reduce stocking density and to maintain fish of a similar size in each tank. For each experiment, fish of a single spawning cohort and numbering several thousand were sorted according to weight and freedom of physical abnormalities into a group of at least twice the total number of animals required for the experiment. Within this selected group, fish were further graded by weight into replicate blocks of fish from which animals were randomly allocated to treatments.

All tank experiments were carried out within an environment-controlled laboratory which was supplied with underground fresh (<0.05‰) water. The experimental system comprised 24 fibreglass tanks (800 L; 1.2 m^2 surface area) which were arranged as four independent recirculation systems, each consisting of an up-flowing biological filter (120 L of fine gravel), reservoir (2,000 L) and 6 replicate tanks. Flows through the system were maintained using air lifts and pumps, with turn-over rate in the tanks being once every 2 h. Filters were back-washed every second day and water exchange was less than 10% per day (to replace water discarded during cleaning and filter back-flushing). Control over water temperature was maintained by lowering ambient temperature in the laboratory and heating the water in the reservoirs to the desired temperature; diurnal variation in water temperature in each recirculation system was no more than ± 0.5°C. The water in each recirculation system was monitored daily for temperature and pH and periodically (2-3/week) for ammonia and nitrite. Photoperiod was held to a constant 12:12 h cycle.

A cage experiment was carried out on the barramundi farm of Cris Phillips (Barramundi Waters, Innisfail) with fish held in cages suspended in an aerated freshwater pond. The experimental fish were managed as for other fish on the farm, being held in mesh-cages of 2 x 2 x 2 m (L x B x D) and fed to satiety once daily except on the weekend when fish were fed only on one of the days. Cages were each stocked with 500 fish and weighed at commencement and termination of the experiment. Water quality measurements (min. and max. water temperature, turbidity, dissolved oxygen and pH) were taken regularly throughout the experiment. At the conclusion of the experiment, 10 fish from each cage were stunned by immersion in ice slurry and dressed by gut evisceration and gill removal. Fish were consigned on ice to QDPI's Centre for Food Technology for sensory evaluation.

6.1.2 Diet manufacture

Feed ingredients of an aquaculture grade were purchased from provender millers and finely ground to pass through a 400 µ screen using a water-cooled hammer mill. Other than for experiments where a commercial feed was used, experimental diets were uniformly mixed into a dry mash and steam-pressed pelleted using a semi-commercial pelleter at Ridley's aquafeed mill at Narangba. For most experiments, diets were pelleted through a die of 4 mm diameter and the length adjusted to suit the size of the fish intended to be fed. Diets were road or rail freighted to Walkamin and stored at -20°C until immediately prior to feeding.

6.1.3 Chemical analyses

For determination of the chemical composition of the fish, weighed whole fish were placed into a 2 L wide-mouth glass jar (usually 4 fish per jar with from 4 to 6 replicates for the pre-experimental group and from each tank at the conclusion of the experiment) and autoclaved at 126°C for 4 h as described by Williams et al. (1995). The autoclaved samples were homogenised *in situ* in a high-speed laboratory blender and the contents transferred to trays for freeze drying. All changes in weight of the sample during autoclaving were attributed to water exchange and the chemical composition expressed relative to the original weight of the fish. Samples of finely ground feeds and homogenised fish were analysed in duplicate by standard laboratory methods essentially in accordance with AOAC (1990) recommendations at QDPI's biochemical laboratory at the Animal Research Institute, Yeerongpilly. Dry matter (DM) was determined by oven drying at 105°C to constant weight, ash by ignition at 600°C for 2 h , N by a macro-Kjeldahl technique on a Kjel Foss automatic analyser using mercury in the digestion and crude fat (C-fat) by soxhlet extraction with petroleum ether (bp 40 to 60°C) for 16 h. Crude protein (CP) was calculated by using the conversion factor of 6.25 irrespective of the nature of the N. Total lipid was determined after a Bligh and Dyer (1959) extraction as modified by Christie (1982) and fatty acids as the methyl ester by capillary gas chromatography. A hydrochloric acid extract of the ash was used to determine calcium by atomic absorption spectroscopy, and phosphorus by colorimetric procedures (AOAC 1990). Crude fibre was determined by the method of Moir and Connor (1977). Gross energy (GE) was determined by isothermal bomb calorimeter using a microprocessor-controlled Lecco AC 200 automatic bomb calorimeter. Amino acid composition was determined by ion-exchange chromatography using Waters' HPLC following hydrolysis of samples with 6 M HCl at 110°C under an atmosphere of N_2 for 18 h. Cystine was measured as cysteic acid, and Methionine as methionine sulfone after performic acid oxidation. Tryptophan was determined by the method of Allred and MacDonald (1988) with 4.2 M NaOH at 110°C under an atmosphere of N_2 for 20 h.

Water samples were analysed for dissolved oxygen and pH using a Horiba U10 Water Checker (Horiba Ltd., Kyoto, Japan) and ammonia N and nitrite N by colorimetric procedures using a Hach CEL/700 Portable Laboratory test kit (Hach Coy., Loveland, Colorado, USA).

6.1.4 Estimation of apparent digestibility of experimental diets

Facilities available at Walkamin at the time did not enable the digestibility of diets to be determined by direct measurement. However, diets used in Experiment Wlk 2 were assayed for apparent digestibility as part of work carried out in FRDC Project 93/120-04. This experiment was carried out at QDPI's Bribie Island laboratory, and the results have been reported in the Final Report of FRDC Project 93/120-04 (see pp. 17-21). The diet used in Experiments WS 1 to WS 5 and Wlk 1 inclusive was made essentially to the same formulation as Diet 1 of Experiment Wlk 2 except that the source of fishmeal was changed from Tasmanian to Chilean. Because this change in ingredient source was unlikely to have markedly altered the apparent digestibility of the diet, the digestibility values determined for Diet 1 of Experiment Wlk 2 have been assumed also to apply to the diet used in Experiments WS 1 to WS 5 and Wlk 1, inclusive. For all other experiments, the apparent digestibility of the diet was estimated from apparent digestibility coefficients determined for individual feed ingredients as part of FRDC Project 93/120-04. These apparent digestibility coefficients are detailed in Table 6.1.1.

Table 6.1.1 Apparent digestibility coefficients[1] of feed ingredients used in the calculation of the estimated apparent digestibility of experimental diets

Feed ingredient	Apparent digestibility coefficient (%)		
	Dry matter	Crude protein	Gross energy
Fishmeal (Tasmanian, Chilean or Peruvian; 67% CP)	86	94	95
Casein	90	90	90
Meat & bone meal (50% CP)	43	64	67
Soybean meal (solvent extracted)	56	86	69
Soybean meal (full-fat)	69	85	76
Wheat gluten (80% CP)	100	100	99
Wheat flour (gelled, 12% CP)[2]	55	65	50
Starch (gelled; <30% of diet)	40	78	36
Mill run[2]	35	60	30
Yeast (Torula)[2]	60	85	75
Poultry offal meal	43	35	62
Fish or vegetable oil	95	na	95

[1] Compiled from data generated from FRDC 93/120-04 and FRDC 96/391.
[2] Estimated with reference to digestibility data reported in FRDC 93/120-04.

6.1.5 Statistical analyses

Fish response data were subjected to an analysis of variance in accordance with the randomised block design of the experiment using prepared statistical programs (Siegel 1992). Where appropriate, the data were subjected to regression analysis and the derived relationships statistically tested for homogeneity, using Bartlett's test for residual variances and differences in slopes and intercepts compared using standard procedures (Snedecor and Cochran, 1989). Growth rate was determined as the difference between end (W_e) and start (W_s) weights divided by the number of days on experiment and specific growth rate (SGR, % per d) was calculated as: $100 \times (\ln W_e - \ln W_s)/d$. Due to inevitable weight changes of the fish during the acclimatisation period, response data were adjusted by covariance analysis to isolate any effect of initial weight disparity on treatment response. Arcsine transformation was applied to all percent data (Sokal and Rohlf, 1981). In all experiments, the cage or tank was considered to be the experimental unit. Differences between treatment effects were examined *a-posteriorly* using Fischer's protected 't' test (Snedecor and Cochran, 1989) wherein differences between means were examined only where the 'F' test of the ANOVA was significant ($P < 0.05$).

6.1.6 Sensory analysis of fish

After being received at QDPI's Centre for Food Technology, fish were weight-ranked, heaviest to lightest, within each diet treatment according to cage. Five fish from one weight-matched cage of each diet were defrosted overnight at 5°C and filleted. Fillets were rinsed under cold tap water and two samples (average weight of 20 g) were cut from the central portion of each fillet. Fillet samples were placed in individual covered foil dishes and held at 5°C until removed to room temperature (24°C) to equilibrate for 1 h prior to cooking. Samples were placed on trays and cooked in a fan-forced electric oven at 200°C for 6 min. and transferred to a holding oven at 75°C for up to 0.5 h prior to tasting.

Trained sensory panelists assessed a sample from each of the dietary treatment at each of three or more tasting sessions. Order of tasting of treatments was balanced across the panel and all treatments were sampled in individual booths illuminated with white light (daylight equivalent). Distilled water was freely available for palate cleansing prior to, and during tasting.

Panelists identified and rated the colour of internal flesh, and odour, flavour and texture characteristics on structured graphic line scales (where 0 = none or disliked intensely and 100 = very or liked extremely) in accordance with a standard rating test (SAA 1988). Overall liking of the flesh was also rated and tasters were given the opportunity to record additional descriptors and comments. Sensory evaluation data were collected directly into computers using an integrated software package (Compusense 5.1, Compusense Inc., Canada) where it was averaged over all tasters within dietary treatments and taste sessions. The accumulated data were analysed by ANOVA with differences between means tested for significance at 5% probability using the range simultaneous test procedure of Tukey (Sokal and Rohlf, 1981).

6.1.7 References

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6.2 Effects of water temperature and feeding frequency

6.2.1 Introduction

Barramundi or Asian seabass *Lates calcarifer* (Bloch) is a catadromous fish that is widely distributed in coastal and fresh water throughout the Indo-west pacific region (Grey, 1987). The species is extensively cultured in South East Asia where moist food, consisting of a large proportion of trash fish or low-value marine product, is used as the predominant source of nourishment for on-growing of fingerlings (Boonyaratapalin, 1990, 1997; New and Csavas, 1993). In contrast to these practices, on-growing of barramundi in Australia relies exclusively on the feeding of formulated dry diets. In Australia, barramundi are grown-out typically in cages suspended in estuarine water or in fresh-brackish water in earthen ponds where water temperature varies seasonally between 20°C and 29-30°C. Because water temperature is known to have a profound effect on food intake of aquatic animals (Braaten, 1978; Steffens, 1989; Talbot, 1993) and food is the largest on-farm operating cost for barramundi farming in Australia (Treadwell et al., 1991; Johnston, 1997) a study was made of the effects water temperature, feeding frequency and fish size (weight) have on food intake and fish productivity.

6.2.2 Methods

6.2.2.1 Experimentation

General methodology has been described earlier in Section 6.1.

Four growth assays (WS 1 to WS 4) were carried out with fish of mean (±SD) initial liveweight of 41±1.6, 100±3.1, 169±1.4 and 270±6.0 g respectively, to examine the effects of water temperature and feeding frequency on food intake and accompanying growth. In each of the assays, water temperatures of 20, 23, 26 and 29°C were compared factorially with a number of feeding frequencies. In Experiments WS 1 and WS 2, comparisons were made between two- and three-times daily feeding (2/d and 3/d, respectively) whereas in Experiments WS 3 and WS 4, food was provided once-daily (1/d), either AM or PM, or 2/d. In a fifth experiment (WS 5), fish of mean initial liveweight of 220±29.0 g were used to contrast the effects of three feeding frequencies (1/d, 2/d or 2/d but for 6 of every 7 d) when held at a water temperature of 28°C. A new batch of fish was used for each experiment. Fish were stocked equally into 24 (WS 1 to WS 4) or 12 (WS 5) tanks of 800 L capacity at densities of 30 fish in Experiment WS 1 and 25 fish in Experiments WS 2 to WS 5. Details of the treatment comparisons in each of the experiments are summarised in Table 6.2.1.

Table 6.2.1 Details of treatment comparisons for studies examining the effects of water temperature and feeding frequency on appetite and growth of juvenile barramundi

Experiment	Initial fish weight (g)	Treatment comparisons		Number replicates (weight blocks)
		Water temperature (°C)	Feeding frequency	
WS 1	41	20 *vs* 23 *vs* 26 *vs* 29	2/d *vs* 3/d	3
WS 2	100	20 *vs* 23 *vs* 26 *vs* 29	2/d *vs* 3/d	3
WS 3	169	20 *vs* 23 *vs* 26 *vs* 29	AM *vs* PM *vs* 2/d	2
WS 4	270	20 *vs* 23 *vs* 26 *vs* 29	AM *vs* PM *vs* 2/d	2
WS 5	220	28	1/d *vs* 2/d *vs* 2/d (6/7)	4

In the experiments, the diet (Table 6.2.2) was extruded commercially by Aquafeed Products, Narangba as a semi-floating 4 mm diameter pellet with the fish oil being applied as a post-pellet application. Feeding times were 0830 h (for 1/d, AM and first feed of 3/d treatments), 1230 h (for midday feeding of 3/d treatment) and 1630 h (for PM and last feed of 3/d treatments). At each meal, a weighed amount of food was offered to excess on 3 to 4 occasions

during a feeding period lasting about 1 h. All uneaten food was collected, the number of pellets counted and the weight of this food calculated by reference to the determined average weight of the food pellet.

Table 6.2.2 Formulation and chemical content of the diet used in Experiments WS 1 to WS 5

Diet formulation (%)		*Chemical composition (as fed)*	
Feed ingredient	Inclusion rate	Analysis	Content
Fishmeal (Tasmanian)	35.0	Dry matter (%)	93.7
Casein	4.0	Ash (%)	7.8
Soybean meal (full-fat)	3.5	Crude protein (%)	44.2
Soybean meal (solv-ext)	8.0	Estimated dig. protein (%)	40.2
Wheat gluten	6.0	Crude fat (%)	11.8
Poultry offal meal	2.0	Crude fibre (%)	6.2
Yeast (torula)	2.5	Calcium (%)	1.55
Wheat flour	17.4	Phosphorus (%)	1.32
Wheal mill run	15.0	Amino acids (%)	
Soybean oil	0.5	Lysine	2.95
Fish oil (red tobis)	3.5	Methionine + cystine	1.60
Salt	0.2	Threonine	1.80
Mineral mix[1]	0.5	Arginine	2.40
Vitamin mix[2]	1.5	Fatty acids (%)	
Amino acid mix[3]	0.4	18:3n-3	0.19
		20:5n-3	0.54
Total	100	22:6n-3	1.01
		Gross energy (kJ/g)	20.64
		Estimated dig. energy (kJ/g)	15.5

[1] The mineral mix provided in the final diet (mg/kg): Al (as $AlCl_3.6H_2O$), 0.5: Co (as $CoCl_2.6H_2O$), 0.5; Cu (as $CuSO_4.5H_2O$), 5; Fe (as $FeSO_4.7H_2O$), 40; I (as KI), 4; Cr (as $KCr.2SO_4$), 0.5; Mg (as $MgSO_4.H_2O$), 300; Mn (as $MnSO_4.H_2O$), 25; Se (as $NaSeO_3$), 0.1; and Zn (as $ZnSO_4.7H_2O$), 100.

[2] The vitamin premix provided in the final diet (mg/kg): Retinol (Vit A), 2.25; ascorbic acid (coated Vit C), 750 cholecalciferol (Vit D3), 1,500; menadione (Vit K3), 15; d/l a-tocopherol (Vit E), 300; choline, 1,500; inositol, 375; para-amino-benzoic acid, 75; thiamine (Vit B1), 22.5; riboflavine (Vit B2), 30; pyridoxine (Vit B6), 22.5; pantothenic acid, 75; nicotinic acid, 110; biotin, 0.75; cyanocobalamin (Vit B12), 0.075; folic acid, 6; ethoxyquin, 190; and citric acid, 7,500.

[3] The amino acid mix provided in the final diet (%): l-lysine HCl, 0.2; dl-methionine, 0.1; and l-threonine, 0.1.

Fish were acclimatised to the experimental conditions for 2 to 3 weeks prior to the start of the experiment. In the week preceding the start, fish were given a prophylactic salt bath (1.2% NaCl for 2 h) against ectoparasites. In Experiments WS 1 to WS 4, fish were weighed, but not fed, on day 0 and then fed in accordance with the treatment schedule for a continuous 10 d period and the fish again weighed. In Experiment WS 5, procedures were similar except that the duration of the experiment was 28 d with the fish being weighed, but not fed, on days 0, 14 and 28.

Data of each experiment were subjected to an analysis of variance appropriate to the experimental design using prepared statistical programs (Siegel, 1992). For Experiments WS 1 to WS 4 inclusive, relationships between fish response and water temperature were examined using orthogonal polynomials (Snedecor and Cochran, 1989). To examine how daily food intake (DFI) was affected by fish weight (W; measured as the arithmetic mean of weights at the start and end of experimentation), water temperature (T) and feeding frequency (FF), the homogeneity of the data across experiments was first examined using Bartlett's test for residual variances and differences in slopes and intercepts compared using standard procedures

(Snedecor and Cochran, 1989). As the coefficient of determination (R^2) of the equation for the pooled data was high (>0.86) for each of the temperature data sub-sets, testing was carried out at the 1% probability level to avoid a false (Type 2 error) rejection (Snedecor and Cochran, 1989). This analysis showed all temperature data sub-sets other than for 29°C to be poolable across experiments. Inspection of the 29°C data sub-set showed significant ($P<0.001$) slope and intercept differences which could be attributed to atypical values for Experiment WS 2. Exclusion of the Experiment WS 2 data sub-set enabled the 29°C data for the other three experiments to be pooled with an improvement in the R^2 of the pooled equation from 0.86 to 0.95. An analysis of the combined data for Experiments WS 1 to WS 4 inclusive but exclusive of the 29°C data of Experiment WS 2, was undertaken to test for slope and intercept differences between water temperatures. Following these analyses, step-wise multiple regression analysis was used to examine the pooled data when fitted to the general allometric equation:

$DFI = \alpha.W^{\gamma} + c.T + d.FF$

or in its linear form:

$\ln DFI = (\alpha + c.T + d.FF) + \gamma.\ln W$

where 'ln' is the natural logarithm, 'α' is a parameter defining the intercept of the regression line (i.e. the amount of food required by the animal when W = 1), 'γ' is the weight exponent (i.e. the slope of the logarithmic equation) and 'c' and 'd' are constants defining the rates of change caused by variations in T and FF, respectively.

6.2.3 Results

6.2.3.1 Water quality and fish health

Water quality remained excellent in each experiment with DO remaining above 90% saturation and maximum NH_3 and NO_2 measurements each being <0.01 mg/L. One fish was lost from each of 3 tanks in the 29°C system in Experiment WS 1 and from 1 tank in the 23 and 26°C systems in Experiment WS 2 as a result of fish escaping over the tank lip. No losses occurred in Experiments WS 3 and 4 while one fish in the 28°C system was lost in WS 5. In Experiment WS 2, all fish in the 29°C system had poor appetite, irrespective of feeding frequency. Water quality in the 29°C system was similar to the other systems and examination of the fish at the end of the experiment showed negligible gill and skin parasites and no evidence of clinical disease. No explanation can be given for the low appetite of the fish.

6.2.3.2 Experiments WS 1 and WS 2

The effects of water temperature and feeding frequency on food intake and productivity of the fish are shown in Tables 6.2.3 and 6.2.4 for Experiments WS 1 and WS 2, respectively.

Feeding frequency had little effect on fish response other than for a minor interaction with water temperature wherein feeding 3/d generally enhanced the effects of water temperature compared with that of feeding 2/d. In both experiments, water temperature markedly affected all response traits measured. In Experiment WS 1, DFI and FCR improved linearly and average growth rate (ADG) and specific growth rate (SGR) increased curvilinearly with temperature ($P<0.05$). In Experiment WS 2, DFI, ADG and SGR increased ($P<0.05$) quadratically with temperature: performance at 29°C was significantly inferior ($P<0.05$) to that at 26°C (Table 6.2.4). FCR was essentially unaffected by water temperature in Experiment WS 2.

Table 6.2.3 Effect of feeding frequency and water temperature on food intake and growth responses [1] of small (~40 g) barramundi in Experiment WS 1

Feed frequency	Water temperature (°C) 20	23	26	29	Mean	±sem[2]
			Average daily gain (g/d)[3]			
2/d	0.75^{f}	1.01^{e}	1.71^{b}	1.79^{ab}	1.32	
3/d	0.77^{f}	1.24^{d}	1.58^{c}	1.85^{a}	1.36	0.040
Mean (LC)[4]	0.76^{S}	1.13^{R}	1.65^{Q}	1.82^{P}		
			Specific growth rate (%/d)[3]			
2/d	1.54^{f}	2.17^{e}	3.41^{b}	3.56^{ab}	2.67	
3/d	1.57^{f}	2.61^{d}	3.19^{c}	3.60^{a}	2.74	0.068
Mean (LQ)	1.55^{S}	2.39^{R}	3.30^{Q}	3.58^{P}		
			Food conversion ratio (g as fed food: g wet fish gain)[3]			
2/d	1.32	1.18	1.04	1.08	1.15	
3/d	1.39	1.18	1.00	0.98	1.14	0.040
Mean (L)	1.35^{R}	1.18^{Q}	1.02^{P}	1.03^{P}		
			Daily food intake (g/d)[3]			
2/d	0.99^{f}	1.19^{e}	1.82^{b}	1.93^{a}	1.48	
3/d	1.06^{f}	1.46^{d}	1.61^{c}	1.86^{ab}	1.50	0.036
Mean (L)	1.03^{S}	1.33^{R}	1.72^{Q}	1.89^{P}		

[1] Means adjusted by covariance analysis to remove effect on trait of differences in fish start weight.
[2] Standard error of mean for feed frequency x water temperature interaction term.
[3] $^{a,b,c,d,e,f;\ P,Q,R,S}$ Within interaction and main effect comparisons, means without a common letter differ (P<0.05).
[4] Significant (P<0.05) linear (L), quadratic (Q) and/or cubic effects of water temperature.

Table 6.2.4 Effect of feeding frequency and water temperature on food intake and growth responses [1] of juvenile (~100 g) barramundi in Experiment WS 2

Feed frequency	Water temperature (°C) 20	23	26	29	Mean	±sem[2]
			Average daily gain (g/d)[3]			
2/d	1.11^{d}	1.87^{b}	2.34^{a}	1.84^{b}	1.79	
3/d	1.41^{c}	1.47^{c}	2.23^{a}	1.88^{b}	1.75	0.074
Mean (LQC)[4]	1.26^{S}	1.67^{R}	2.28^{P}	1.86^{Q}		
			Specific growth rate (%/d)[3]			
2/d	1.03^{d}	1.70^{b}	2.07^{a}	1.67^{b}	1.62	
3/d	1.26^{c}	1.37^{c}	1.97^{a}	1.71^{b}	1.58	0.074
Mean (LQC)	1.14^{S}	1.54^{R}	2.02^{P}	1.69^{Q}		
			Food conversion ratio (g as fed food: g wet fish gain)[3]			
2/d	1.20^{b}	1.08^{a}	1.11^{ab}	1.21^{b}	1.15	
3/d	1.13^{ab}	1.32^{c}	1.15^{ab}	1.16^{ab}	1.19	0.039
Mean	1.17	1.19	1.13	1.18		
			Daily food intake (g/d)[3]			
2/d	1.32	2.02	2.58	2.22	2.03	
3/d	1.56	1.95	2.55	2.19	2.06	0.082
Mean (LQ)	1.44^{S}	1.98^{R}	2.57^{P}	2.20^{Q}		

[1,2,3,4] As for Table 6.2.3.

6.2.3.3 Experiments WS 3 and WS 4

The effects of water temperature and feeding frequency on food intake and productivity of the fish are shown in Tables 6.2.5 and 6.2.6 for Experiments WS 3 and WS 4, respectively. Interaction effects between feeding frequency and water temperature were not significant (P>0.05) except for DFI in Experiment WS 3 where the effects of water temperature were less pronounced for the PM feeding.

Table 6.2.5 Effect of feeding frequency and water temperature on food intake and growth responses [1] of juvenile (~170 g) barramundi in Experiment WS 3

Feed frequency	Water temperature (°C)				Mean	±sem[2]
	20	23	26	29		
			Average daily gain (g/d) [3]			
AM	1.93	2.27	2.85	3.34	2.59	
PM	1.83	2.78	2.90	3.55	2.77	0.219
2/d	1.48	2.49	3.03	3.72	2.68	
Mean (L)[4]	1.74^{R}	2.51^{Q}	2.92^{Q}	3.54^{P}		
			Specific growth rate (%/d) [3]			
AM	1.07	1.25	1.55	1.79	1.41	
PM	1.02	1.51	1.55	1.90	1.50	0.109
2/d	0.85	1.36	1.62	1.97	1.45	
Mean (L)	0.98^{R}	1.37^{Q}	1.57^{Q}	1.89^{P}		
			Food conversion ratio (g as fed food: g wet fish gain) [3]			
AM	0.98	1.07	0.96	0.97	1.00^{XY}	
PM	0.90	0.91	1.01	0.90	0.93^{Y}	0.034
2/d	1.19	1.04	1.06	1.00	1.07^{X}	
Mean	1.02	1.01	1.01	0.96		
			Daily food intake (g/d) [3]			
AM	1.88^{f}	2.42^{e}	2.78^{d}	3.27^{b}	2.59^{XY}	
PM	1.66^{f}	2.54^{de}	2.83^{cd}	3.24^{b}	2.57^{Y}	0.108
2/d	1.79^{f}	2.57^{de}	3.17^{bc}	3.72^{a}	2.81^{X}	
Mean (L)	1.78^{S}	2.51^{R}	2.93^{Q}	3.41^{P}		

[1,2,3,4] As for Table 6.2.3.

Table 6.2.6 Effect of feeding frequency and water temperature on food intake and growth responses [1] of plate-size (~270 g) barramundi in Experiment WS 4

Feed frequency	Water temperature (°C)				Mean	±sem[2]
	20	23	26	29		
			Average daily gain (g/d) [3]			
AM	1.88	2.41	3.02	3.13	2.61^{Y}	
PM	1.96	2.27	2.92	3.73	2.72^{Y}	0.223
2/d	2.07	2.80	3.94	4.27	3.27^{X}	
Mean (L)[4]	1.97^{S}	2.49^{R}	3.29^{Q}	3.71^{P}		
			Specific growth rate (%/d) [3]			
AM	0.66	0.85	1.06	1.09	0.91^{Y}	
PM	0.69	0.81	1.02	1.29	0.95^{Y}	0.072
2/d	0.74	0.98	1.35	1.45	1.13^{X}	
Mean (L)	0.70^{S}	0.88^{R}	1.14^{Q}	1.28^{P}		
			Food conversion ratio (g as fed food: g wet fish gain) [3]			
AM	1.17	1.09	1.09	1.06	1.10	
PM	1.12	1.16	1.07	0.97	1.08	0.055
2/d	1.12	1.05	0.98	1.02	1.04	
Mean (L)	1.14	1.10	1.05	1.02		
			Daily food intake (g/d) [3]			
AM	2.16	2.60	3.28	3.34	2.85^{Y}	
PM	2.18	2.65	3.12	3.60	2.89^{Y}	
2/d	2.31	2.93	3.86	4.40	3.37^{X}	
Mean (L)	2.21^{S}	2.73^{R}	3.42^{Q}	3.78^{P}		

[1,2,3,4] As for Table 6.2.3.

In both experiments, fish fed 2/d and AM had the highest and lowest DFI respectively ($P<0.05$). In Experiment WS 4, ADG and SGR responses mirrored those of DFI. In Experiment

WS 3, FCR was improved by PM feeding which resulted in ADG and SGR being unaffected (P>0.05) by feeding frequency. In both experiments, DFI, ADG and SGR improved linearly (P<0.05) as water temperature increased; FCR was unaffected by water temperature.

6.2.3.4 Experiments WS 5

Results are summarised in Table 6.2.7. Feeding fish twice daily resulted in higher food consumption and faster growth rates compared to those where food was provided only as a single daily meal. For fish that were fed a single daily meal, skipping one day's feeding every week had no effect on fish performance. FCR was unaffected by feeding frequency.

Table 6.2.7 Effect of feeding frequency on food intake and growth responses [1] of plate-size (~220 g) barramundi in Experiment WS 5

Response trait	Feed frequency			±sem
	2/d	1/d (each d)	1/d (6 d in 7)	
Average daily gain (g/d)	3.80[a]	3.30[b]	3.39[b]	0.081
Specific growth rate (%/d)	1.41[a]	1.26[b]	1.29[b]	0.021
Food conversion (g as fed food:g fish gain)	1.14	1.13	1.18	0.027
Daily food intake (g/d)	4.32[a]	3.75[b]	3.99[b]	0.066

[1] Means adjusted by covariance analysis to remove effect on trait of differences in fish start weight.
[2] a,b, Within trait comparisons, means without a common letter differ (P<0.05).

6.2.3.4 Food intake relationships

At each water temperature, DFI varied allometrically with fish weight (Figure 6.2.1).

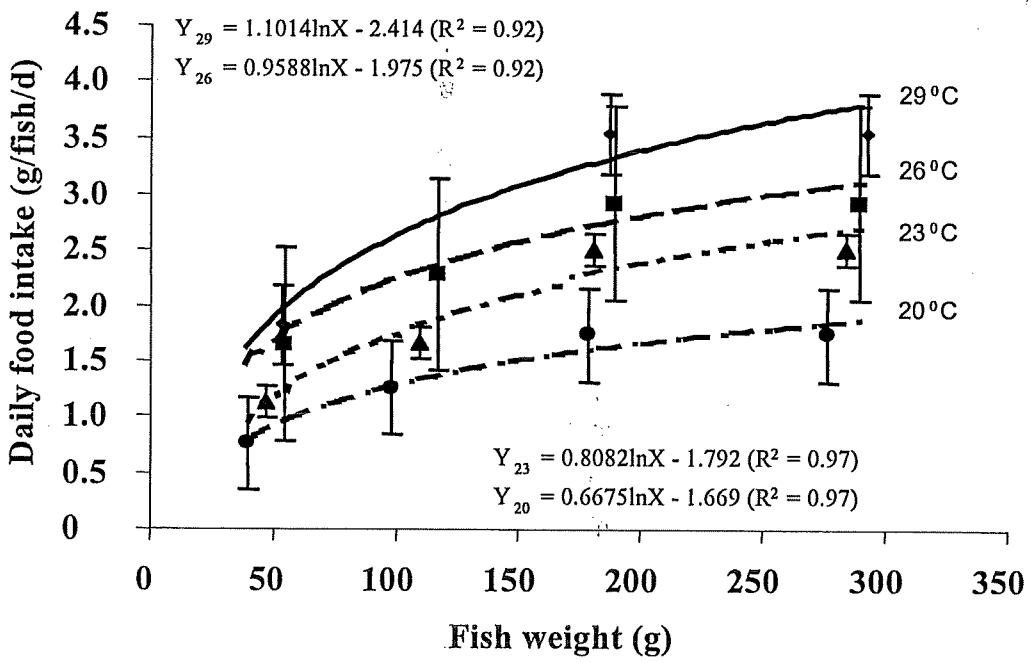

Fig. 6.2.1 Relationships between daily food intake (Y, g/fish/d) and size (X, g) of fish held at water temperatures of either 20 (●), 23 (▲), 26 (■) or 29 (◆) °C. Data from Experiments WS 1 to WS 4 pooled and exclusive of feeding frequency. Error bars are ± residual standard deviation of the derived equation.

Water temperature had a marked effect on DFI and the relationship was improved (P<0.05) by including feeding frequency in the regression. Intercepts (α) were different (P<0.01) at each

water temperature but the weight exponents (γ) were not significantly different (P>0.05). A combined analysis of the data showed that the effect of water temperature on DFI was predominantly linear (P<0.001) with 97% of the variation being explained by the derived relationship; inclusion of a second order component of water temperature enabled a further 1% of the variation to be explained (Table 6.2.8).

Table 6.2.8 Allometric relationships between daily food intake (DFI; g/fish/d) and fish weight (W; g) and influences of feeding frequency (FF; number/d) and water temperature (T; °C) for Experiments WS 1 to WS 4 inclusive

Condition	For general equation: lnDFI = α + γ.lnW + c.FF + d.T								R^2
	α	$\pm SE_\alpha$	γ	$\pm SE_\gamma$	c	$\pm SE_c$	d	$\pm SE_d$	
	Pooled data for individual water temperatures								
20°C	-2.282	0.1223	0.528	0.0198	0.063	0.0190			0.98
23°C	-1.744	0.1689	0.477	0.0277	0.077	0.0244			0.95
26°C	-1.191	0.2269	0.419	0.0373	0.057	0.0307			0.90
29°C	-1.424	0.2219	0.468	0.0353	0.102	0.0348			0.95
	Combined data for all experiments and temperatures[1]								
All data[2]	-3.543	0.1392	0.486	0.0197	0.074	0.0031	0.083	0.0178	0.97

[1] Prediction of DFI was further improved (P<0.05; R^2 = 0.98) by including a second order component of T: lnDFI = $-7.285_{(\pm 0.5234)} + 0.478_{(\pm 0.0155)}$ lnW + $0.391_{(\pm 0.0435)}$T - $0.007_{(\pm 0.0009)}$T2 + $0.074_{(\pm 0.0141)}$FF.

[2] Data for 29°C treatment in Experiment WS 2 excluded.

6.2.4 Discussion

6.2.4.1 Feeding frequency

A major objective of the study was to determine how frequently juvenile barramundi need to be fed to optimise growth rate and FCR. Increasing the feeding frequency from 2/d to 3/d for small fish (viz < 100 g; Experiments WS 1 and WS 2) had minimal effects on DFI and FCR such that daily and specific growth rates were largely unaffected. For larger fish (~ 170 to 270g; Experiments WS 3 and WS 5), feeding 2/d generally resulted in higher DFI than when fed 1/d but this did not necessarily result in better growth rates. In Experiment WS 3, the lower DFI observed with fish fed 1/d was compensated for by improved FCR so that growth rates were similar irrespective of the feeding schedule. Tucker et al. (1988) reported an almost identical response when barramundi of ~ 70 to 150 g were fed either 1/d or 2/d. Similar effects have been observed with other species. Singh and Srivastava (1984) found that increasing feeding frequency above twice daily enabled higher DFI to be achieved with the siluroid catfish *Heteropneustes fossilis* but at the expense of a worsening FCR. Tsevis et al. (1992) found an inverse relationship between frequency of feeding and efficiency of energy conversion for European seabass, *Dicentrarchus labrax*. However, growth rate was not optimised unless the fish were fed at least 2/d.

Twice daily feeding was also found to be optimal for cage-reared channel *catfish Ictalurus punctatus* (Webster et al., 1982) and laboratory-reared Arctic charr *Salvelinus alpinus* (Jobling, 1983). However, in studies where food had been given at a predetermined percentage of body weight, more frequent feeding resulted in better FCR and growth rate with juvenile African catfish *Heterobranchus longifilis* (Kerdchuen and Legendre, 1991), rainbow trout *Oncorhynchus mykiss* (Holm et al., 1990) and milkfish *Chanos chanos* (Chiu et al., 1987). Such improvements in FCR and growth rate associated with frequent feeding may be due to a change in digestive/metabolic efficiency or to reduced food wastage (Talbot, 1993).
The results of the present studies show that barramundi larger than 40 g need not be fed more frequently than 2/d for FCR and growth rate to be optimised. However, there was a slight interaction between feeding frequency and water temperature wherein feeding 3/d generally

enhanced the effects of water temperature compared with that of 2/d feeding. A much greater interaction between water temperature and feeding frequency was reported by Seymour (1989) for the eel *Anguilla anguilla* where DFI increased with frequent feeding at high but not at low water temperatures. Where management practices favour feeding only 1/d, FCR and growth rates may be better if barramundi are fed in the evening rather than in the morning. African catfish *H. longifilis* have also been shown to grow faster when fed during the night than during the day (Kerdchuen and Legendre, 1991). Skipping one day's feeding during the week was shown in Experiment WS 5 with fish of ~ 220 g to have minimal effect on resultant growth rates and FCR as compared to those fed once daily on every day of the week This management practice is widely adopted on Australian farms to reduce the high cost of labour on weekends. Our results indicate that this practice is unlikely to adversely affect the performance of the fish although better DFI and growth rates were seen when the fish were fed 2/d. The effect of feeding juvenile rainbow trout *O. mykiss* to satiation either 1/d every day, 1/d every other day or 1/d every third day was investigated by Lyndon and Wood (1997). They found total food consumption and growth rate to be higher in fish fed every day compared to the other feeding regimens. However, the mean size of the meal consumed by the fish was highest for those fed every third day, indicating some capacity of the fish to compensate for reduced feeding frequency by increasing voluntarily food consumption. This compensation was however insufficient to enable growth rate to be sustained at the same level as for fish fed every day and moreover FCR progressively worsened with less frequent feeding. A somewhat similar result was observed by Cho (1992) with rainbow trout of about 600 g fed once daily either every day, 6 days in every 7 days or 5 days in every 7 days. Skipping one day's feeding resulted in the same growth rate as fish fed every day but skipping feeding on two consecutive days resulted in a profound reduction in growth rate. However, efficiency of FCR was unaffected by feeding practice.

Apart from the slight interactive effect between water temperature and DFI mentioned above, varying feeding from once to three times daily in the present work had little effect on FCR. Based on these results and in consideration of the high labour cost of feeding, our results support a recommendation that fish below a size of about 100 g should be fed at least 2/d whereas 1/d feeding is adequate for fish above 100 g. Further for fish more than about 200 g, skipping a feed on the weekend is unlikely to depress overall fish performance compared to feeding every day and any loss in productivity would be compensated by reduced feeding cost.

6.2.4.2 Water temperature and fish size

DFI increased allometrically with increasing fish size and curvilinearly with increasing water temperature (Figure 6.2.1; Table 6.2.8). For all fish species studied, food consumption has been found to be functionally related to water temperature and animal weight (eg Braaten, 1979; Elliott, 1982; Steffens, 1989; Talbot, 1993). However, for most species, the rate of change (γ) of growth (and food consumption) with fish size, ie the weight exponent, has typically been reported to lie between 0.7 and 0.85 (see reviews by Braaten, 1979; Jobling, 1985). In the present study, γ decreased from a value of 0.53±0.02 for fish at 20°C to values of 0.48±0.03 and 0.42±0.04 at water temperatures of 23 and 26°C respectively, while a value of 0.47±0.04 was found for fish at 29°C (Table 6.2.8). Such low values of γ imply that barramundi have a high maintenance requirement relative to its capacity for growth as compared to cool water species such as salmonids where the value of γ is typically between 0.7 and 0.85 for actively growing fish or close to 1.0 at resting states in small (<4 g) fish (Elliott, 1982). However, values for γ varying between 0.35 and 1.0 have been reported in fish (see Paloheimo and Dickie, 1966; Soofiani and Hawkins, 1985). In general, warm water species such as the Californian pupfish *Cyprinodon macularius* have lower γ values than cold water species such as salmonids, while low values have also been reported to be associated with low metabolic rates, increased age (size) of fish and season-temperature interactions (Paloheimo

and Dickie, 1966). We have not been able to find any other barramundi data to confirm the low and somewhat anomalous weight exponent found in the present work.

The effect of water temperature on metabolic activity in fish has been widely studied and excellent reviews have been provided by Paloheimo and Dickie (1966), Elliott (1976), Weatherley and Gill (1987) and Talbot (1993). As poikilotherms, fish respond to changes in water temperature by altering metabolic activity: within the thermal tolerance range of the animal, metabolic rate increases by 1.65 to 2.7 fold for every 10°C rise in water temperature (Talbot, 1993). In the present barramundi study, increasing the water temperature by 9°C resulted in the voluntary food intake of the fish increasing by 1.8 fold for large fish (from 2.08 to 3.77 g/d for fish of ~ 275 g) and by 2.1 fold for small fish (from 0.79 to 1.65 g/d for fish of ~40 g). Moreover, over the range of water temperatures examined in this study, namely 20 to 29°C, the food intake (and growth rate) response was predominantly linear with water temperature although including a quadratic component of water temperature did very slightly improve the derived relationship (Table 6.2.8). That there was some curvilinearity in the response at the highest water temperature implies that a water temperature of about 29°C is close to the upper thermal tolerance of these fish. It should be noted though, that in the present experiments barramundi were held at constant water temperature. Under natural conditions, the temperature would vary diurnally and the upper threshold temperature before fish productivity would be adversely affected could therefore be expected to be considerably higher than 29°C.

The effect of altered water temperature on efficiency of food use was comparatively small over the range of water temperatures examined in this work. Significant effects of water temperature were observed only with the smallest fish (~40 g; Table 6.2.3) where FCR was observed to improve as water temperature increased up to 26°C. However for fish of 100g and above, FCR was not significantly affected by water temperature although there was a tendency for FCR of fish held at the lower water temperatures to be worse than at higher temperatures. Similar effects have been noted by other researchers across a wide range of fish species, eg salmonids (Elliott, 1976; 1982), cyprinids (Cui and Wootton, 1988) and striped hybrid bass (Keembiyehetty and Wilson, 1998). Thus, as water temperature increases within the thermal tolerance of the animal, food intake and the efficiency with which the food is used for growth also increases. These responses indicate that barramundi productivity will be maximised when held in water temperatures close to their upper threshold.

In Australia where barramundi are farmed in earthen ponds or estuaries across latitudes ranging from 15 to 23° S, water temperatures commonly range from less than 20 to more than 30°C. To assist farmers in estimating the amount of food to be fed to fish, a feeding chart taking into account fish size and water temperature has been prepared as a feeding guide (Table 6.2.9). However, the feeding activity of the fish themselves will be the best guide as to the amount of food to be fed since growth rate and FCR efficiency appear to be optimised at close to full satiation feeding.

Table 6.2.9 Feeding guide for juvenile barramundi fed twice daily (up to 100 g body weight) or once daily (from 100 g bodyweight) a dry extruded pellet (90% DM) of estimated apparent digestible energy content of 15.5 MJ/kg

Fish size (g)	Water temperature (°C)									
	20	21	22	23	24	25	26	27	28	29
	Daily food intake for fish fed twice daily (g food/fish/d)									
40	**0.86**	**0.97**	**1.09**	**1.20**	**1.31**	**1.40**	**1.49**	**1.56**	**1.61**	**1.65**
50	0.95	1.08	1.21	1.33	1.45	1.56	1.66	1.74	1.79	1.83
60	**1.04**	**1.18**	**1.32**	**1.45**	**1.58**	**1.70**	**1.81**	**1.89**	**1.96**	**2.00**
70	1.12	1.27	1.42	1.57	1.71	1.83	1.95	2.04	2.11	2.15
80	**1.19**	**1.35**	**1.51**	**1.67**	**1.82**	**1.95**	**2.07**	**2.17**	**2.25**	**2.29**
90	1.26	1.43	1.60	1.77	1.92	2.07	2.19	2.30	2.38	2.43
100	**1.33**	**1.50**	**1.68**	**1.86**	**2.02**	**2.17**	**2.31**	**2.42**	**2.50**	**2.55**
	Daily food intake for fish fed once daily (g food/fish/d)									
120	**1.35**	**1.52**	**1.70**	**1.88**	**2.05**	**2.20**	**2.34**	**2.45**	**2.53**	**2.59**
140	1.45	1.64	1.84	2.03	2.21	2.37	2.52	2.64	2.73	2.78
160	**1.54**	**1.75**	**1.96**	**2.16**	**2.35**	**2.53**	**2.68**	**2.81**	**2.91**	**2.97**
180	1.63	1.85	2.07	2.28	2.49	2.67	2.84	2.97	3.07	3.14
200	**1.72**	**1.95**	**2.18**	**2.40**	**2.62**	**2.81**	**2.98**	**3.13**	**3.23**	**3.30**
220	1.80	2.04	2.28	2.51	2.74	2.94	3.12	3.27	3.38	3.45
240	**1.88**	**2.12**	**2.37**	**2.62**	**2.85**	**3.07**	**3.26**	**3.41**	**3.53**	**3.60**
260	1.95	2.21	2.47	2.72	2.97	3.19	3.38	3.54	3.67	3.74
280	**2.02**	**2.29**	**2.56**	**2.82**	**3.07**	**3.30**	**3.51**	**3.67**	**3.80**	**3.88**
300	2.09	2.36	2.64	2.92	3.18	3.41	3.62	3.80	3.93	4.01
320	**2.15**	**2.44**	**2.72**	**3.01**	**3.27**	**3.52**	**3.74**	**3.91**	**4.05**	**4.13**
340	2.21	2.51	2.80	3.09	3.37	3.62	3.85	4.03	4.17	4.25
360	**2.28**	**2.58**	**2.88**	**3.18**	**3.46**	**3.72**	**3.95**	**4.14**	**4.28**	**4.37**
380	2.34	2.65	2.96	3.26	3.56	3.82	4.06	4.25	4.39	4.49
400	**2.39**	**2.71**	**3.03**	**3.34**	**3.64**	**3.92**	**4.16**	**4.36**	**4.50**	**4.60**
420	2.45	2.78	3.10	3.42	3.73	4.01	4.26	4.46	4.61	4.71
440	**2.51**	**2.84**	**3.17**	**3.50**	**3.81**	**4.10**	**4.35**	**4.56**	**4.71**	**4.81**
460	2.56	2.90	3.24	3.58	3.90	4.19	4.44	4.66	4.81	4.91
480	**2.61**	**2.96**	**3.31**	**3.65**	**3.98**	**4.27**	**4.54**	**4.75**	**4.91**	**5.02**
500	2.66	3.02	3.37	3.72	4.05	4.36	4.63	4.85	5.01	5.11

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6.3 Effects of food restriction on growth and body composition

6.3.1 Introduction

Barramundi farming in Australia) entails a high degree of risk but equally it has a good potential for high profitability if certain technological problems can be solved (Treadwell et al., 1991; Johnston, 1997, 1998). Amongst these, the most pressing is the high cost of feeding since this comprises up to 35% of on-farm operation costs. This high feeding cost is due both to the expensiveness of the diet and the poor FCR achieved by farmers under commercial conditions. The expensiveness of the diet is due both to an imprecise knowledge of the nutrients requirements by the animal which results in diets that are sub-optimal in nutrient specification being used (Trendall and Fielder, 1991) and a reliance on expensive fishmeal as the primary protein source instead of cheaper terrestrial alternatives. Work to be described in later sections of this Report addresses nutrient requirements of barramundi while the suitability of terrestrial protein meals as replacements of fishmeal has been addressed in FRDC Project 93/120-04. Under commercial farming conditions, FCRs are seldom better than 2:1 and sometimes are worse than 3:1. The poor FCR could be caused by a less than optimum balance of nutrients in the diet but more likely it is due to gross wastage of food that result from inappropriate feeding strategies. On farms, FCRs are particularly poor during the winter where low water temperatures curtail feeding intensity and the propensity of the farmer to over feed.

An associated problem has been the reduced dressing percentage and increased body fatness of plate-size fish that are marketed over the winter period compared to autumn-marketed fish. Appropriate feeding strategies are needed for fish approaching market size in order to improve food economy and to lessen the production of excessively fat fish. Although feeding behaviour of barramundi is widely known to be greatly affected by water temperature as documented in the previous section, the interactive effects of water temperature and restricted food intake on growth, food economy and body composition appear not to have been previously investigated. Accordingly, two experiments were carried out to see if growth and body composition of juvenile barramundi were altered by protein to energy content of the diet, dietary restriction or water temperature.

6.3.2 Methods

6.3.2.1 Experimentation

Two comparative slaughter growth assays were carried out by methods as detailed earlier in Section 6.1. Details for each of the experiments are provided under.

6.3.2.1.1 Experiment Wlk 1

A 10-week growth assay was carried out with fish of mean (±SD) initial liveweight of 134±1.9 g to investigate the way growth and body composition were altered by dietary restriction and water temperature. At each of four water temperatures (20, 23, 26 and 29°C) a high quality extruded diet (Tables 6.2.2 and 6.2.3) was fed according to a scale intended to provide food at intakes corresponding to either 65-70, 80-85 or 95-100% of satiety. To achieve these feeding rates, food intake data from Experiments WS 1 and WS 2 were analysed to estimate satietal intake of twice-daily fed fish at water temperatures of either 20, 23, 26 or 29°C. The predicted satietal intake, expressed as a percentage of fish liveweight (W), for each of these water temperatures was calculated to be approximately 15, 22, 29 and 25% of $W^{0.44}$. At the time, the abnormality of the 29°C data of Experiment WS 2 was not realised and instead the result was attributed to the water temperature being above that of the fish's thermal tolerance. Accordingly, the devised feeding scale as used in the experiment is summarised in Table 6.3.1. Thus, at each of the four water temperatures, three rates of feeding were applied with the intention of applying a similar degree of food restriction at each of the water temperatures. Unfortunately, fish at 29°C were unintentionally subjected to a greater degree of food

restriction than intended on account of satietal intake being erroneously underestimated. Fish were weighed fortnightly and this weight was used to set the food allowance for the next fortnight.

Table 6.3.1 Intended feeding scale for Experiment Wlk 1

Water temperature (°C)	Feeding scale (% fish weight$^{0.44}$)	Daily food allowance (g/d) of fish at weight (g)			
		125	150	175	200
20	**8.5**	**0.71**	**0.77**	**0.82**	**0.87**
20	**11.75**	**0.98**	**1.07**	**1.14**	**1.21**
20	**15**	**1.26**	**1.36**	**1.46**	**1.54**
23	15	1.26	1.36	1.46	1.54
23	18.25	1.53	1.65	1.77	1.88
23	21.5	1.80	1.95	2.09	2.21
26	**21.5**	**1.80**	**1.95**	**2.09**	**2.21**
26	**24.75**	**2.07**	**2.24**	**2.40**	**2.55**
26	**28**	**2.34**	**2.54**	**2.72**	**2.88**
29	18.25	1.53	1.65	1.77	1.88
29	21.5	1.80	1.95	2.09	2.21
29	24.75	2.07	2.24	2.40	2.55

The experiment involved a total of 740 fish which had been acclimated to the laboratory conditions for two weeks before commencement. Twenty fish were used as a pre-experimental slaughter group to determine the initial chemical composition of the fish and the remaining 720 fish were ranked by weight into two blocks and within these blocks then randomly assigned to treatment tanks. Fish were stocked equally into 24 tanks of 800 L capacity at an initial density of 30 fish. In the week preceding the start, fish were given a prophylactic salt bath (1.2% NaCl for 2 h) against ectoparasites. Body composition was assessed both at 4 weeks and at 10 weeks by sampling 5 fish/tank on each occasion. Accordingly, fish productivity and nutrient retention responses were analysed for periods of start to 4 weeks and 5 to 10 weeks. Procedures for chemical and statistical analyses have been described earlier (Sections 6.1.3 and 6.1.5).

6.3.2.1.2 Experiment Wlk 2

An 8-week growth assay was carried out with fish of mean (±SD) initial liveweight of 158±2.2 g to investigate the effects on growth and body composition of fish when they were fed either one of three diets (Table 6.3.2) that varied in protein and energy content and when held at either of one of four water temperatures (20, 23, 26 and 29°C). The diets were intended to provide decreasing amounts of protein at similar energy concentrations in order to provide a range in protein to energy ratios; diet W2-1 was ostensibly the same as the diet fed in Experiment Wlk 1 although the source of fishmeal used was Chilean instead of Tasmanian. At the time of formulation, information on the low apparent digestibility of gelled wheat and starch for barramundi was not known (Williams et al., 1998) and the intended differences in DE content of the diets were not achieved. Thus, the digestible crude protein (DCP) content of the diets varied from 40.7 to 33.4 and 31.5%, and the digestible energy (DE) content decreased from 14.8 to 13.6 and 12.7 kJ/g, such that the DCP:DE ratios of the diets varied from 27.5 to 24.5 and 24.7 mg:kJ for diets W2-1 to W2-3, respectively. The chemical composition of the diets is detailed in Table 6.3.3. The diets were extruded manufactured (Aquafeed Pty Ltd, Narangba) and were fed to a restricted scale intended to provide food at intakes corresponding to about 95% of satiety. To achieve these feeding rates, fish held at 20, 23, 26 and 29°C were offered food once daily at rates corresponding to 15.5, 22, 28.5 and 28.5% of $W^{0.44}$. Thus, the daily amount of food offered increased from 1.40 g/d for a 150 g fish to 1.90 g/d for a 300 g at 20°C and respectively from 2.00 to 2.70 g/d at 23°C and from 2.60 to 3.50 g/d at 26 and 29°C. Fish were weighed fortnightly and this weight was used to set the food allowance for the next fortnight.

Table 6.3.2 Formulation of diets fed in Experiment Wlk 2

Feed ingredient	Diets		
	W2-1	W2-2	W2-3
	Formulation (%)		
Fishmeal (Chilean)	35.0	28.0	21.0
Casein	4.0	3.2	2.4
Soybean meal (full-fat)	3.5	2.8	2.1
Soybean meal (solv-ext)	8.0	6.4	4.8
Wheat gluten	6.0	6.0	6.0
Poultry offal meal	2.0	1.6	2.0
Yeast (torula)	2.5	2.5	2.5
Wheat flour	16.5	19.8	23.1
Wheal mill run	15.0	18.0	21.0
Soybean oil	0.5	2.5	4.0
Fish oil (Chilean)	3.5	4.0	5.0
Starch	0.8	2.58	4.36
l-Lysine HCl	0.2	0.16	0.12
d/l Methionine	0.1	0.08	0.06
l-Threonine	0.1	0.08	0.06
Salt	0.2	0.2	0.2
Titanium dioxide	0.1	0.1	0.1
Mineral premix[1]	0.5	0.5	0.5
Vitamin premix[1]	1.5	1.5	1.5

[1] As detailed for Table 6.2.2.

Table 6.3.3 Determined chemical composition (air-dry basis) of the diets fed in Experiment Wlk 2

Analysis	Diet		
	W2-1	W2-2	W2-3
Dry matter (%)	92.4	90.3	93.0
Ash (%)	9.3	7.4	6.7
Crude protein (%)	44.8	39.1	36.3
Digestible protein (%)[1]	40.7	33.4	31.5
Crude fat (%)	8.8	10.5	11.4
Crude fibre (%)	1.8	3.6	4.6
Calcium (%)	1.8	1.3	1.0
Phosphorus (%)	1.5	1.2	1.1
Amino acids (%)			
Lysine	2.84	2.31	2.05
Methionine + cystine	1.64	1.40	1.33
Threonine	1.81	1.52	1.39
Arginine	2.54	2.15	2.01
Fatty acids (%)			
18:3n-3	0.11	0.20	0.31
20:5n-3	0.38	0.33	0.33
22:6n-3	0.79	0.69	0.68
Σ n3 fatty acids	1.48	1.44	1.54
Σ n6 fatty acids	0.79	0.98	1.33
Gross energy (kJ/g)	19.60	19.52	18.57
Digestible energy (kJ/g)[1]	14.82	13.61	12.74
Protein:energy (mg:kJ)			
CP:GE	22.9	20.0	19.6
DCP:DE	27.5	24.5	24.7

[1] Mean of estimates derived using either intestinal dissection or 3-h sedimentation procedures (Williams et al., 1998).

The experiment involved a total of 620 fish that had been selected from a pool of about 1500 fish from the same spawning. Fish were acclimatised to the laboratory conditions and water temperature for two weeks before commencement of the experiment. Twenty fish were used as a pre-experimental slaughter group to determine the initial chemical composition of the fish and the remaining 600 fish were ranked by weight into two blocks and within these blocks then randomly assigned to treatment tanks. Fish were stocked equally into 24 tanks of 800 L capacity at a density of 25 fish. In the week preceding the start, fish were given a prophylactic salt bath (1.2% NaCl for 2 h) against ectoparasites. Body composition was assessed at the conclusion of the 8-week experiment by sampling 5 fish/tank. The same fish that were sampled for body composition determination were also used to determine dressing-out percentage. After gilling and gutting, the eviscerated organs and blood were added back to the carcase for determination of chemical composition of the whole fish. Procedures for chemical and statistical analyses have been described earlier (Sections 6.1.3 and 6.1.5).

6.3.3 Results

6.3.3.1 Water quality, fish health and food intake

Water quality remained excellent during the experiment with DO remaining above 90% saturation and maximum NH_3 and NO_2 measurements each not exceeding 0.01 mg/L. Losses of fish were negligible in the experiments (4 fish or 0.6% up to four weeks and 5 fish or 0.8% between 4 and 10 weeks in Experiment Wlk 1 and 2 fish in Experiment Wlk 2) with most deaths being associated with weighing. In Experiment Wlk 2, one of the losses was due to a septicaemia of unknown origin. Actual food intake was lower than intended and more variable than expected in Experiment Wlk 1 (Table 6.3.4) due to the fish not always consuming their food allocation in the allowed feeding period. Food consumption was better in Experiment Wlk 2 although some food refusal occurred at the lowest water temperatures.

Table 6.3.4 Weight and food consumption of fish in Experiment Wlk 1

Water temp (°C)	Feeding scale (% Weight$^{0.44}$)	Fish weight (g)			Food intake (g/d)	
		Start	End 4-Week	End 10 week	0-4 week	5-10 week
20	8.5	135.2	152.5	178.1	0.88	0.94
20	11.75	135.8	161.4	192.4	1.25	1.23
20	15	133.4	161.9	188.7	1.42	1.14
23	15	136.0	164.7	212.4	1.45	1.51
23	18.25	134.1	165.5	216.4	1.61	1.58
23	21.5	136.4	167.0	219.0	1.64	1.56
26	21.5	136.7	203.2	280.6	2.36	2.32
26	24.75	132.1	200.0	289.1	2.56	2.66
26	28	132.7	203.6	302.4	2.58	2.81
29	18.25	134.4	176.3	245.9	1.74	1.84
29	21.5	133.2	175.3	263.5	1.78	2.29
29	24.75	132.4	164.8	270.1	1.58	2.66
± sem		1.33	3.89	3.66	0.077	0.058

However, Experiment Wlk 1was successful in achieving a range of food restrictions at each water temperature. Similarly in Experiment Wlk 2, success was achieved in restricting food intake on each of the diets to a similar amount within each of the four water temperatures.

6.3.3.2 Fish productivity and relationships to food intake: Experiment Wlk 1

Productivity responses of the fish in Experiment Wlk 1 for periods 0 to 4 weeks and 5 to 10 weeks are summarised in Table 6.3.5. Daily (ADG) and specific (SGR) growth rates varied directly with DFI as did FCR.

Table 6.3.5 Food intake (DFI), growth rate (ADG), specific growth rate (SGR), food conversion rate (FCR) and dressing percentage of fish in Experiment Wlk 1

Water temp	Period 0 to 4 weeks					Period 5 to 10 weeks				
	DFI	ADG	SGR	FCR	Dress	DFI	ADG	SGR	FCR	Dress
(°C)	(g/d)	(g/d)	(%/d)	(g:g)	(%)	(g/d)	(g/d)	(%/d)	(g:g)	(%)
20	0.88	0.62^{D}	0.43^{D}	1.44^{BC}	86.8^{A}	0.94	0.78^{F}	0.36^{E}	1.55^{D}	85.5^{EF}
20	1.25	0.96^{CD}	0.64^{CD}	1.33^{BC}	85.3^{D}	1.23	0.78^{F}	0.41^{E}	1.67^{D}	85.6^{EF}
20	1.42	1.05^{BCD}	0.72^{C}	1.35^{BC}	85.7^{CD}	1.14	0.68^{F}	0.37^{E}	1.81^{E}	85.0^{F}
23	1.45	1.05^{BCD}	0.70^{C}	1.41^{BC}	86.0^{BC}	1.51	1.17^{E}	0.59^{D}	1.33^{C}	85.7^{EF}
23	1.61	1.13^{BC}	0.76^{BC}	1.42^{BC}	86.0^{BC}	1.58	1.24^{E}	0.64^{D}	1.30^{C}	86.3^{DE}
23	1.64	1.12^{BC}	0.74^{BC}	1.49^{C}	86.0^{BC}	1.56	1.25^{E}	0.63^{D}	1.27^{C}	86.1^{E}
26	2.36	2.34^{A}	1.40^{A}	1.03^{A}	86.3^{AB}	2.32	1.75^{CD}	0.75^{C}	1.26^{C}	87.6^{BC}
26	2.56	2.41^{A}	1.48^{A}	1.05^{A}	85.9^{BC}	2.66	2.05^{BC}	0.91^{B}	1.25^{C}	87.3^{CD}
26	2.58	2.52^{A}	1.53^{A}	1.02^{A}	85.9^{BC}	2.81	2.26^{AB}	0.95^{B}	1.20^{BC}	88.4^{AB}
29	1.74	1.42^{B}	0.94^{B}	1.23^{B}	86.1^{BC}	1.84	1.65^{D}	0.79^{C}	1.11^{AB}	88.9^{A}
29	1.78	1.42^{B}	0.95^{B}	1.27^{B}	86.1^{BC}	2.29	2.09^{BC}	0.98^{B}	1.09^{AB}	88.5^{AB}
29	1.58	1.11^{BC}	0.76^{BC}	1.39^{BC}	86.4^{AB}	2.66	2.55^{A}	1.20^{A}	1.06^{A}	87.8^{BC}
± sem		0.141	0.072	0.070	1.14		0.110	0.032	0.043	2.19

^{A,B,C,D,E,F} Within trait comparisons, means without a common letter differ ($P<0.05$).

Relationships between growth rate and food intake of the fish across all water temperatures for the 0 to 4 week and 5 to 10 week periods are illustrated in Figures 6.3.1 and 6.3.2, respectively. At each water temperature, a linear relationship explained most of the variation in growth rate when absolute DFI was regressed against growth rate (Fig A of Figures 6.3.1 and 6.3.2) and these relationships were significant ($P<0.05$) for most water temperatures and in both periods. However, the narrow range of food intake at some water temperatures reduced the significance of the relationship (eg at 23°C in the 5 to10 week period; Fig A of Figure 6.3.2). Using the power weight exponent of 0.8 as a reasonable estimate of metabolic weight (Paloheimo and Dickie, 1966; Braaten, 1979; Cho et al., 1982; Jobling, 1985; Steffens, 1989; Kaushik and Medule, 1994), expressing DFI as a function of metabolic weight showed similar linear relationships with growth rate (Fig B of Figures 6.3.1 and 6.3.2 for periods 0 to 4 and 5 to 10 weeks, respectively).

Although this form of expression further reduced the range in food intakes at each of the water temperatures, significant relationships were observed in both periods. Pooling of the data across all temperatures showed that almost all of the variation in growth rate (R^2 of 0.88 and 0.94 for periods 0 to 4 and 5 to 10 weeks, respectively) could be accounted for by a simple linear model. Extrapolation of the linear relationship to zero weight gain gave estimates for the maintenance requirement of these fish as 12.0 and 11.0 g food/kg fish weight$^{0.8}$/d for periods 0 to 4 and 5 to 10 weeks, respectively. In the experiment where mean fish weight of the fish for the respective periods was 154 and 206 g, the maintenance requirement corresponded to a DFI intake of 0.68 and 0.78 g; the corresponding DE maintenance requirements were 10.54 and 12.09 kJ/d.

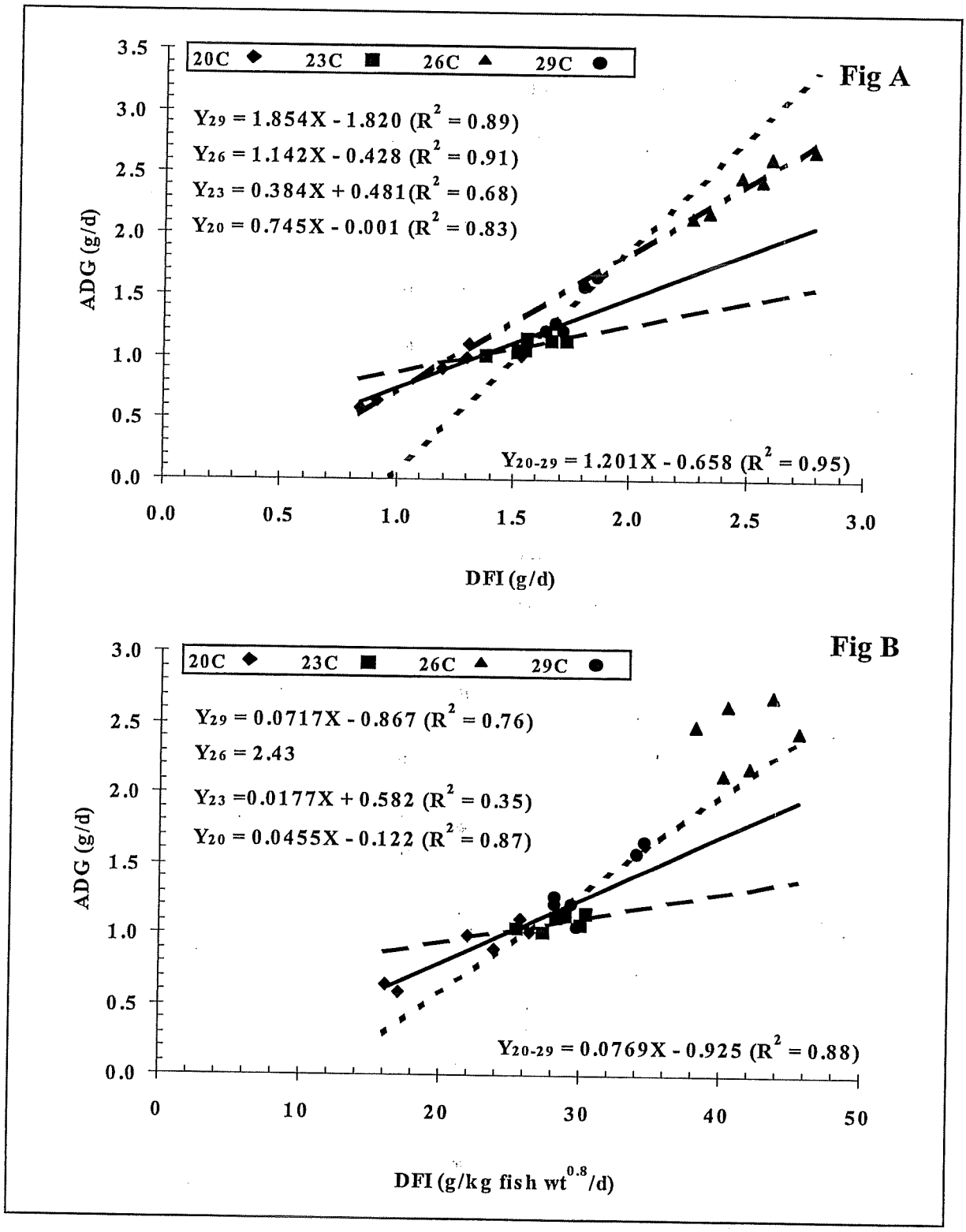

Fig 6.3.1 Relationships between growth rate and food intake (DFI) of fish for the 0-4 week period of Experiment Wlk 1: Fig A, DFI expressed as g/d; Fig B, DFI expressed as g/d per kg fish weight$^{0.8}$.

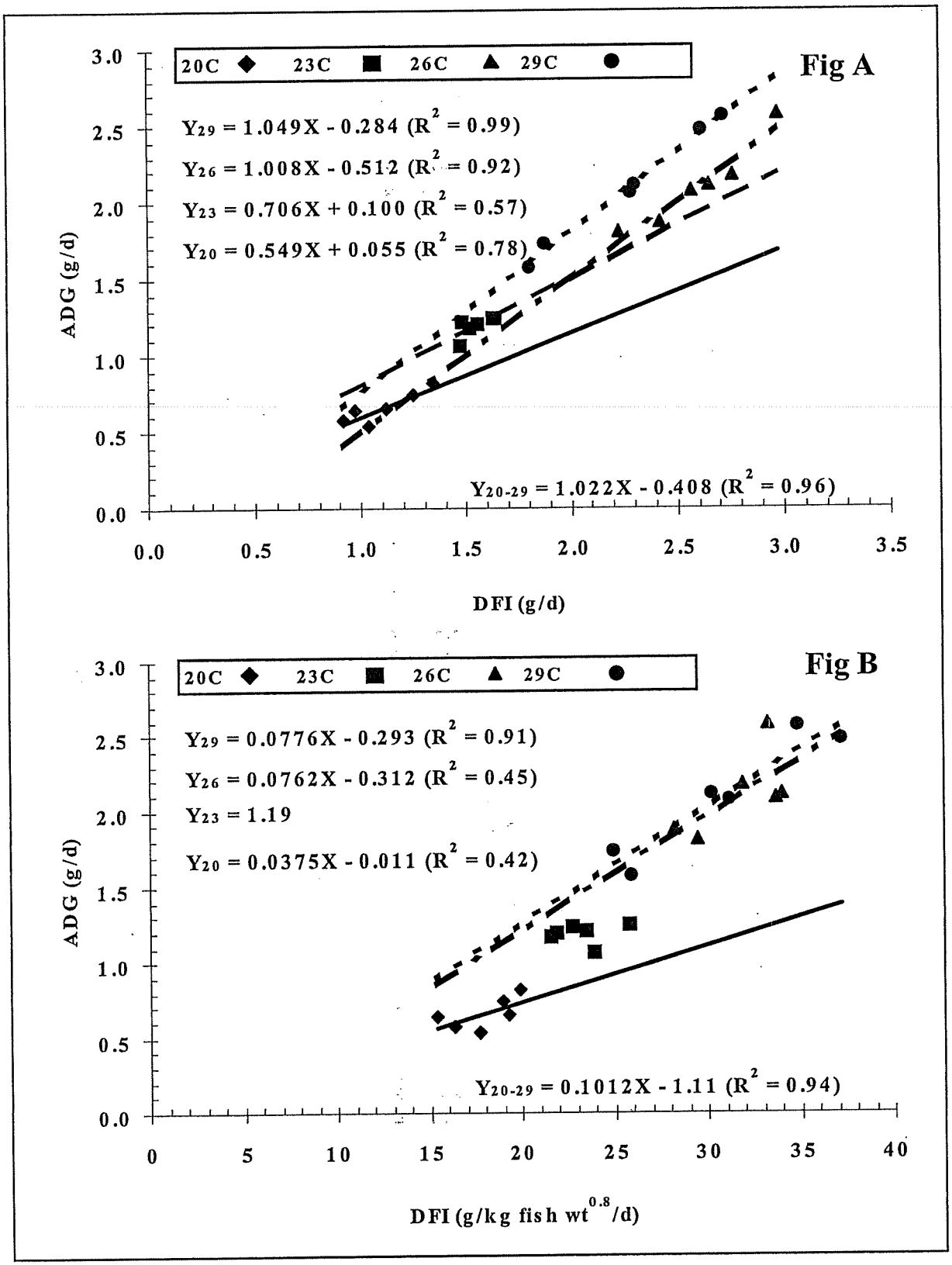

Fig 6.3.2 **Relationships between growth rate and food intake (DFI) of fish for the 5-10 week period of Experiment Wlk 1: Fig A, DFI expressed as g/d; Fig B, DFI expressed as g/d per kg fish weight$^{0.8}$.**

6.3.3.3 Fish productivity and relationships to food intake: Experiment Wlk 2

Productivity responses of the fish in Experiment Wlk 2 are shown in Table 6.3.6. There was a small but significant ($P<0.05$) interaction between water temperature and diet for DFI wherein consumption of the 40.7% DCP diet by fish at 23°C was significantly lower than for the other diets at that temperature and consumption of the 31.5% DCP diet by fish at 26°C was significantly lower than for the 33.4% DCP diet at the same water temperature. There was also a significant ($P<0.05$) main effects interaction for ADG which partially followed the interactive effect observed for DFI but differed in that:

a) ADG of fish at 29°C was lower when fed the 31.5% DCP diet than the other diets; and

b) the differences between diets in DFI at 23°C was not seen for ADG.

Table 6.3.6 Effect of diet and water temperature on productivity responses of barramundi fed to scale in Experiment Wlk 2

Diet (% DCP)	Water temperature (°C)				Mean	±sem[1]
	20	23	26	29		
	Average daily gain (g/d)[2]					
W2-1 (40.7)	1.01^{e}	1.76^{d}	2.61^{a}	2.69^{a}	2.02^{X}	
W2-2 (33.4)	1.00^{e}	1.81^{d}	2.65^{a}	2.61^{a}	2.02^{X}	
W2-3 (31.5)	0.98^{e}	1.66^{d}	2.23^{c}	2.38^{b}	1.82^{Y}	0.045
Mean	1.00^{R}	1.74^{Q}	2.50^{P}	2.56^{P}		
	Specific growth rate (%/d)[2]					
W2-1 (40.7)	0.55	0.89	1.16	1.19	0.95^{X}	
W2-2 (33.4)	0.56	0.89	1.17	1.16	0.94^{X}	
W2-3 (31.5)	0.53	0.85	1.03	1.08	0.88^{Y}	0.020
Mean	0.55^{R}	0.88^{Q}	1.12^{P}	1.14^{P}		
	Food conversion ratio (g as fed food: g wet fish gain)[2]					
W2-1 (40.7)	1.32	1.10	1.08	1.05	1.14^{X}	
W2-2 (33.4)	1.36	1.15	1.10	1.11	1.18^{Y}	
W2-3 (31.5)	1.46	1.27	1.23	1.22	1.30^{Z}	0.023
Mean	1.38^{R}	1.18^{Q}	1.13^{P}	1.13^{P}		
	Daily food intake (g/d)[2]					
W2-1 (40.7)	1.32^{E}	1.90^{D}	2.80^{PQ}	2.80^{PQ}	2.21^{X}	
W2-2 (33.4)	1.34^{E}	2.08^{R}	2.91^{P}	2.91^{P}	2.31^{Y}	
W2-3 (31.5)	1.41^{E}	2.11^{R}	2.74^{Q}	2.90^{P}	2.29^{Y}	0.036
Mean	1.36^{R}	2.03^{Q}	2.82^{P}	2.87^{P}		

[1] Standard error of mean for feed frequency x water temperature interaction term.

[2] $^{a,b,c,d,e;\ P,Q,R;\ X,Y,Z}$ Within interaction and main effect comparisons, means without a common letter differ ($P<0.05$).

Apart from the above minor interactions, the expected profound effect of water temperature on all production attributes was seen with growth (ADG and SGR) and FCR improving ($P<0.05$) with increasing water temperature up to 26°C. Decreasing the protein (and energy) content of the diet caused a worsening of FCR at each step and a worsening of growth rate with the lowest protein diet.

Relationships between growth rate and food intake of the fish across all water temperatures for each of the diets are illustrated in Figure 6.3.3. For each diet, a linear relationship explained most of the variation in growth rate when absolute DFI was regressed against growth rate (Fig A of Figure 6.3.3) or when expressed as a function of metabolic weight (Fig B of Figure 6.3.3). The derived linear relationship for each of the diets differed significantly ($P<0.05$) from each other in their slopes and intercepts although quantitatively these differences were quite small (eg slopes varying from 0.106 to 0.086 and intercepts from -1.12 to -0.91 for DFI expressed as

a function of metabolic weight). Extrapolation of the linear relationship to zero weight gain gave estimates for the maintenance requirement of these fish as 10.6, 11.3 and 10.5 g food/kg fish weight$^{0.8}$/d for diets W2-1, W2-2 and W2-3, respectively. In the experiment where mean fish weight of the fish was 214, 215 and 209 g for diets W2-1, W2-2 and W2-3, the calculated maintenance requirement corresponded to a DFI intake of 0.78, 0.82 and 0.76 g, respectively.

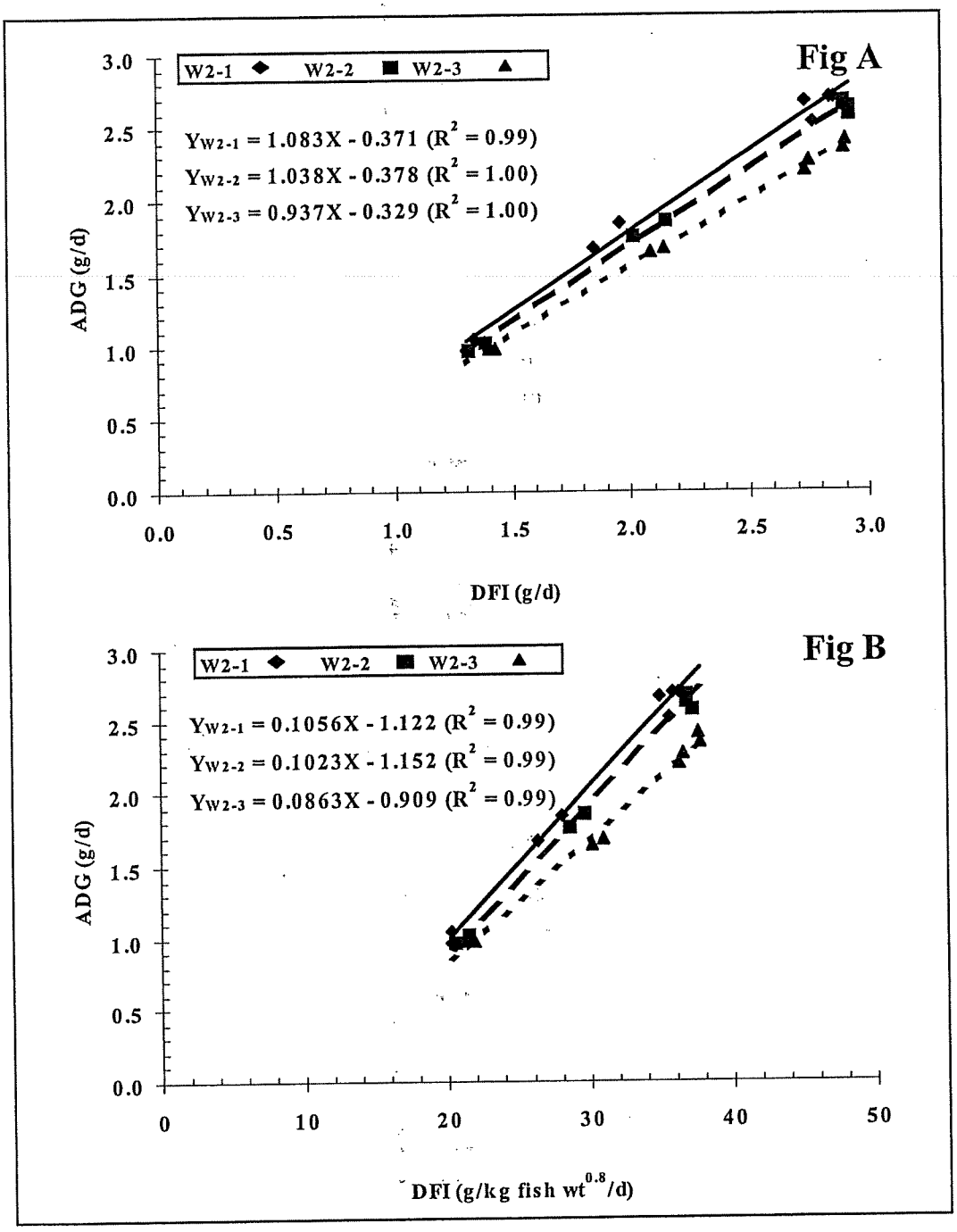

Fig 6.3.3 **Relationships between growth rate and food intake (DFI) of fish in Experiment Wlk 2: Fig A, DFI expressed as g/d; Fig B, DFI expressed as g/d per kg fish weight$^{0.8}$.**

6.3.3.4 Body composition and nutrient retention: Experiment Wlk 1

The chemical composition of the fish at the start and after 4-weeks of feeding is detailed in Table 6.3.7 and that of the fish after10 weeks is shown in Table 6.3.8.

Table 6.3.7 Chemical composition of the fish at initial (±SD) and after 4-weeks of feeding in Experiment Wlk 1

Water temp (°C)	DFI 0-4 Wk (g/d)	DM % wet fish	Fat (% DM)	GE kJ/g DM	Ash (fat free) (% DM)	N (fat free) (% DM)
Initial		32.1±0.38	31.1±1.01	24.82±0.701	18.8±0.33	13.24±0.080
20	0.88	33.2	30.8^{BC}	24.27	19.4	13.28
20	1.25	33.5	33.0^{A}	25.04	18.9	13.26
20	1.42	33.3	31.6^{ABC}	24.70	18.6	13.37
23	1.45	33.5	31.3^{ABC}	24.91	20.1	13.24
23	1.61	33.1	31.4^{ABC}	24.50	19.2	13.37
23	1.64	33.7	31.7^{AB}	24.10	19.8	13.32
26	2.36	32.9	29.9^{C}	24.46	19.1	13.31
26	2.56	32.9	30.2^{BC}	23.80	19.2	13.32
26	2.58	33.2	31.1^{BC}	24.88	19.0	13.40
29	1.74	33.7	31.1^{BC}	23.79	20.4	13.22
29	1.78	33.0	30.1^{BC}	24.19	20.2	13.25
29	1.58	32.8	29.9^{C}	23.76	19.0	13.32
± sem		0.25	0.55	0.360	0.40	0.062

^{A,B,C} Within trait comparisons, means without a common letter differ (P<0.05).

Although food intake had some effect on body composition after 4 weeks of feeding, changes were more apparent after 10 weeks. DM content of the fish increased with increasing DFI while an opposite effect was observed for fat (and energy content). The relationship between DFI and DM fat content of the fish after 10 weeks of feeding is illustrated in Figure 6.3.3. DFI had little effect on the composition of the fat-free body although some small but significant differences (P<0.05) in ash content were apparent at 10 weeks.

Table 6.3.8 Chemical composition of the fish at initial (±SD; n=6) and after 10-weeks of feeding in Experiment Wlk 1

Water temp (°C)	DFI 0-10 Wk (g/d)	DM % wet fish	Fat (% DM)	GE kJ/g DM	Ash (fat free) (% DM)	N (fat free) (% DM)
Initial		32.1±0.38	31.1±1.01	24.82±0.701	18.8±0.33	13.24±0.080
20	0.88	33.8^{ABCD}	32.5^{AB}	25.49^{AB}	19.0^{CD}	13.38
20	1.25	34.1^{ABC}	32.7^{AB}	25.66^{A}	18.4^{E}	13.49
20	1.42	34.2^{ABC}	33.4^{A}	25.73^{A}	18.7^{DE}	13.50
23	1.45	34.3^{AB}	31.6^{BCD}	25.18^{BCD}	19.6^{AB}	13.32
23	1.61	34.1^{ABC}	31.5^{BCDE}	25.12^{BCDE}	19.3^{BC}	13.26
23	1.64	34.6^{A}	32.4^{ABC}	25.41^{ABC}	19.5^{BC}	13.33
26	2.36	34.2^{AB}	30.4^{DEF}	25.03^{CDE}	19.7^{AB}	13.22
26	2.56	34.4^{AB}	30.8^{CDEF}	24.91^{DEF}	20.1^{A}	13.29
26	2.58	34.1^{ABC}	29.7^{F}	24.74^{EF}	19.7^{AB}	13.21
29	1.74	33.3^{CD}	29.9^{EF}	24.60^{F}	19.8^{AB}	13.44
29	1.78	33.2^{D}	29.4^{F}	24.54^{F}	19.9^{AB}	13.41
29	1.58	33.6^{BCD}	29.6^{F}	24.83^{DEF}	19.5^{BC}	13.29
± sem		0.28	0.52	0.13	0.19	0.110

^{A,B,C,D,E,F} Within trait comparisons, means without a common letter differ (P<0.05).

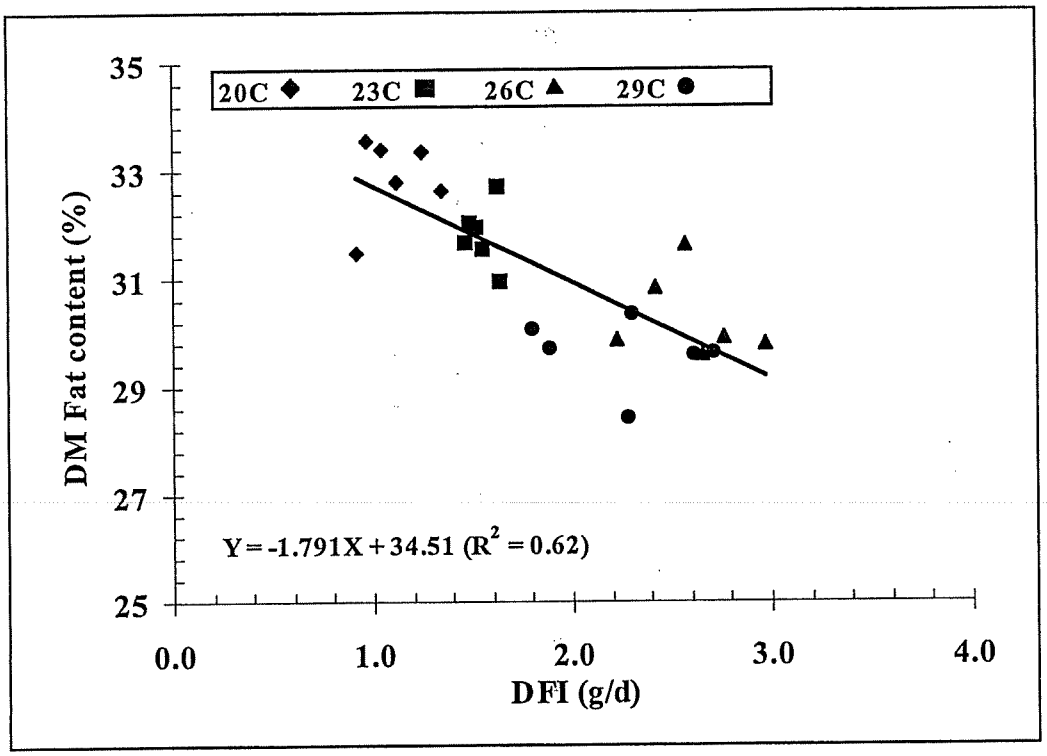

Fig. 6.3.4 **Relationship between the DM fat content of the fish after 10 weeks of feeding and food intake in Experiment Wlk 1.**

The nutrient retention efficiencies of the fish during the periods 0 to 4 weeks and 5 to 10 weeks are summarised in Tables 6.3.9 and 6.3.10, respectively.

Table 6.3.9 **Nutrient retention of dry matter (DM), crude protein (CP), digestible protein (DCP), gross energy (GE) and digestible energy (DE) of fish during the 0-4 week period of feeding in Experiment Wlk 1**

Water temp (°C)	DFI 0-4 Wk (g/d)	Nutrient retention efficiency (%) DM	CP	DCP	GE	DE
20	0.88	30.6^{CDE}	38.4^{BC}	42.2^{BC}	29.3^{CD}	39.0^{CD}
20	1.25	32.5^{ABCD}	32.9^{C}	36.1^{C}	38.4^{AB}	51.1^{AB}
20	1.42	31.0^{BCDE}	37.5^{BC}	41.3^{BC}	34.0^{ABC}	45.2^{ABC}
23	1.45	30.1^{CDE}	35.6^{C}	39.2^{C}	34.6^{ABC}	46.1^{ABC}
23	1.61	27.9^{DE}	34.2^{C}	37.6^{C}	29.8^{CD}	39.7^{CD}
23	1.64	28.9^{DE}	34.3^{C}	37.7^{C}	28.4^{CD}	37.9^{CD}
26	2.36	35.9^{AB}	46.3^{A}	50.9^{A}	38.8^{AB}	51.7^{AB}
26	2.56	34.8^{ABC}	44.2^{AB}	48.6^{AB}	34.8^{ABC}	46.4^{ABC}
26	2.58	36.8^{A}	45.9^{A}	50.4^{A}	41.8^{A}	55.7^{A}
29	1.74	34.0^{ABC}	40.7^{ABC}	44.8^{ABC}	32.4^{BCD}	43.1^{BCD}
29	1.78	30.8^{CDE}	39.1^{ABC}	42.9^{ABC}	31.6^{BCD}	42.0^{BCD}
29	1.58	27.2^{E}	36.2^{BC}	39.8^{BC}	24.5^{D}	32.7^{D}
± sem		1.61	2.42	2.66	2.70	3.60

^{A,B,C,D,E} Within trait comparisons, means without a common letter differ ($P<0.05$).

In both periods and for the entire 10-week period, there was a weak trend (R^2 values of the relationship ranging from 0.2 to 0.5) for nutrient retention efficiencies to improve linearly with increasing DFI. In the 0 to 4 week period, nutrient retention efficiency appeared to be optimised at a water temperature of 26°C, coinciding with maximum daily food consumption

of the fish. In the 5 to 10 week period, nutrient retention efficiency tended to improve with increasing water temperature up to 29°C which was at variance with maximum daily food consumption which was higher in fish held at 26°C.

Table 6.3.10 Nutrient retention of dry matter (DM), crude protein (CP), digestible protein (DCP), gross energy (GE) and digestible energy (DE) of fish during the 5-10 week period of feeding in Experiment Wlk 1

Water temp (°C)	DFI 5-10 Wk (g/d)	Nutrient retention efficiency (%)				
		DM	CP	DCP	GE	DE
20	0.94	25.8^{CD}	28.1^{D}	30.8^{D}	37.4^{ABC}	49.7^{ABC}
20	1.23	23.6^{D}	36.5^{BC}	40.2^{BC}	30.6^{C}	40.8^{C}
20	1.14	23.3^{D}	25.4^{D}	28.0^{D}	33.0^{BC}	43.9^{BC}
23	1.51	29.6^{BC}	36.0^{C}	39.6^{C}	35.0^{ABC}	46.6^{ABC}
23	1.58	30.8^{AB}	36.3^{BC}	39.9^{BC}	37.2^{ABC}	49.6^{ABC}
23	1.56	32.0^{AB}	37.2^{ABC}	40.9^{ABC}	42.3^{A}	56.3^{A}
26	2.32	31.9^{AB}	37.6^{ABC}	41.4^{ABC}	38.2^{ABC}	50.8^{ABC}
26	2.66	32.1^{AB}	38.4^{ABC}	42.2^{ABC}	39.1^{AB}	52.1^{AB}
26	2.81	32.0^{AB}	39.7^{ABC}	43.7^{ABC}	35.5^{ABC}	47.3^{ABC}
29	1.84	31.0^{AB}	42.0^{ABC}	46.2^{ABC}	37.7^{ABC}	50.2^{ABC}
29	2.29	32.7^{AB}	42.9^{AB}	47.1^{AB}	37.4^{ABC}	49.8^{ABC}
29	2.66	35.0^{A}	43.6^{A}	48.0^{A}	41.9^{A}	55.8^{A}
± sem		1.41	2.14	2.36	2.64	3.52

^{A,B,C,D} Within trait comparisons, means without a common letter differ ($P<0.05$).

Relationships between dietary intakes of digestible N and digestible energy (DE) and their respective accretions in the fish during the experiment are shown in Figures 6.3.5 and 6.3.6 for periods of 0 to 4 and 5 to 10 week, respectively. In all instances, a simple linear model relating accretion to intake was as good in explaining the variation in accretion as more complex quadratic models. The derived relationships were similar in each period but the amount of the variability explained was slightly higher for the 5 to 10 week period (R^2 ranging from 0.82 to 0.96) than for the 0 to 4 week period (R^2 ranging from 0.76 to 0.93). Although relationships were derived for each of the water temperatures, the narrow range of intakes within each of the respective water temperature groups precluded any meaningful results on the effects of water temperature being obtained. The efficiencies with which digestible N and DE were retained by the fish decreased between the successive periods but they were very similar within each respective period (viz. 0.61 cv 0.62 for N and energy respectively in the 0 to 4 week period and 0.52 cv 0.54 for the 5 to 10 week period; Figures 6.3.5 and 6.3.6). The DE maintenance requirements (i.e. at zero energy accretion) predicted from the derived relationships were 142 and 134 kJ/kg fish weight$^{0.8}$/d) for periods 0 to 4 and 5 to 10 weeks, respectively. For mean fish weights of 154 and 206 g in the two respective periods, the DE maintenance requirement was 8.0 and 9.5 kJ/d. The digestible N maintenance requirement (at zero N accretion) was similarly predicted to be 0.740 and 0.674 g/ kg fish weight$^{0.8}$/d (equivalent to 4.63 and 4.21 g digestible protein/ kg fish weight$^{0.8}$/d) for periods 0 to 4 and 5 to 10 weeks, respectively. These corresponded to maintenance digestible N requirements of 0.042 and 0.048 g /d for the fish in periods 0 to 4 and 5 to 10 weeks, respectively (0.26 and 0.30 g digestible protein/d, respectively).

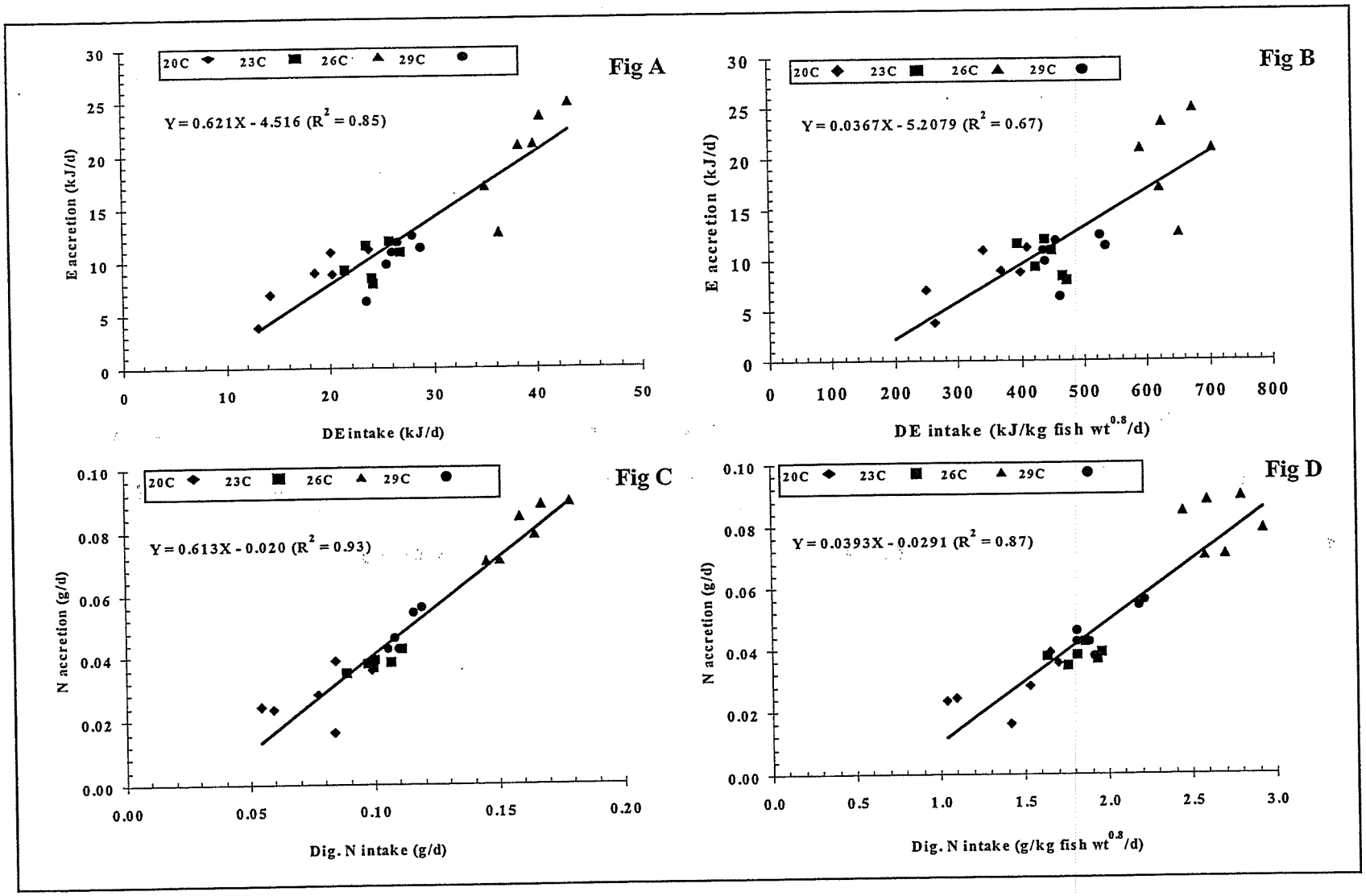

Fig. 6.3.5 **Relationships between dietary intake and accretion in fish during the 0 to 4 week period in Experiment Wlk 1: daily intake of digestible energy expressed either as absolute intake (Fig A) or as function of fish weight$^{0.8}$ (Fig B) and daily intake of digestible N expressed as absolute intake (Fig C) or as function of fish weight$^{0.8}$ (Fig D).**

6.3.3.5 Body composition and nutrient retention: Experiment Wlk 2

The chemical composition of the fish at the start and after 8-weeks of feeding the three diets is detailed in Table 6.3.11 .

Table 6.3.11 Chemical composition of the fish at the start (±SD) and after 8-weeks of being fed to scale in Experiment Wlk 2

Diet (% DCP)	Water temperature (°C)				Mean	±sem[1]
	20	23	26	29		
			DM content (%)[2]			
Initial					33.6±0.24	
W2-1 (40.7)	34.8	32.9	34.1	33.8	33.9	
W2-2 (33.4)	34.3	33.1	34.3	34.3	34.0	
W2-3 (31.5)	34.9	33.8	34.3	34.9	34.4	0.52
Mean	34.7^{P}	33.2^{Q}	34.2^{P}	34.3^{P}		
			DM fat content (%/)[2]			
Initial					25.5±0.67	
W2-1 (40.7)	28.4^{abc}	24.7^{g}	24.9^{fg}	22.5^{h}	25.1^{X}	
W2-2 (33.4)	27.0^{bcd}	25.3^{defg}	26.6^{cdef}	25.0^{efg}	25.9^{X}	
W2-3 (31.5)	29.0^{a}	27.8^{abc}	26.8^{bcde}	27.2^{bc}	27.7^{Y}	0.58
Mean	28.1^{P}	25.9^{QR}	26.1^{Q}	24.9^{R}		
			Gross energy content (kJ/g)[2]			
Initial					23.96±0.289	
W2-1 (40.7)	24.78	24.73	24.05	22.50	24.02^{X}	
W2-2 (33.4)	24.47	24.75	25.25	25.00	24.87^{XY}	
W2-3 (31.5)	24.82	26.25	25.81	27.15	26.01^{Y}	0.738
Mean	24.69	25.24	25.03	24.88		
			DM ash fat-free content (%)[2]			
Initial					20.4±0.29	
W2-1 (40.7)	19.3	18.9	19.8	20.3	19.6	
W2-2 (33.4)	19.6	19.5	19.7	19.9	19.7	
W2-3 (31.5)	20.0	19.7	20.4	19.8	20.0	0.34
Mean	19.7	19.4	20.0	20.0		
			DM crude protein fat-free content (%)[2]			
Initial					76.8±1.41	
W2-1 (40.7)	80.9	79.3	80.0	81.2	80.4^{X}	
W2-2 (33.4)	79.3	78.7	78.2	79.0	78.8^{Y}	
W2-3 (31.5)	79.7	79.5	79.5	78.2	79.2^{XY}	0.74
Mean	80.0	79.2	79.2	79.4		

[1] Standard error of mean for feed frequency x water temperature interaction term.

[2] $^{a,b,c,d,e,f,g,h;\ P,Q,R;\ X,Y}$ Within interaction and main effects, means without a common letter differ (P<0.05).

There was a significant (P<0.05) interaction between diet and water temperature for the DM fat content of the fish wherein differences between the diets were enhanced as water temperature increased. Other effects of diets and water temperature on the chemical composition of the fish were small with energy content increasing (P<0.05) and CP content of the fat-free body decreasing (P<0.05) as the protein content of the diet decreased. The DM content of fish held at 23°C was lower (P<0.05) than for fish at other water temperatures but the biological significance of this observation is questionable.

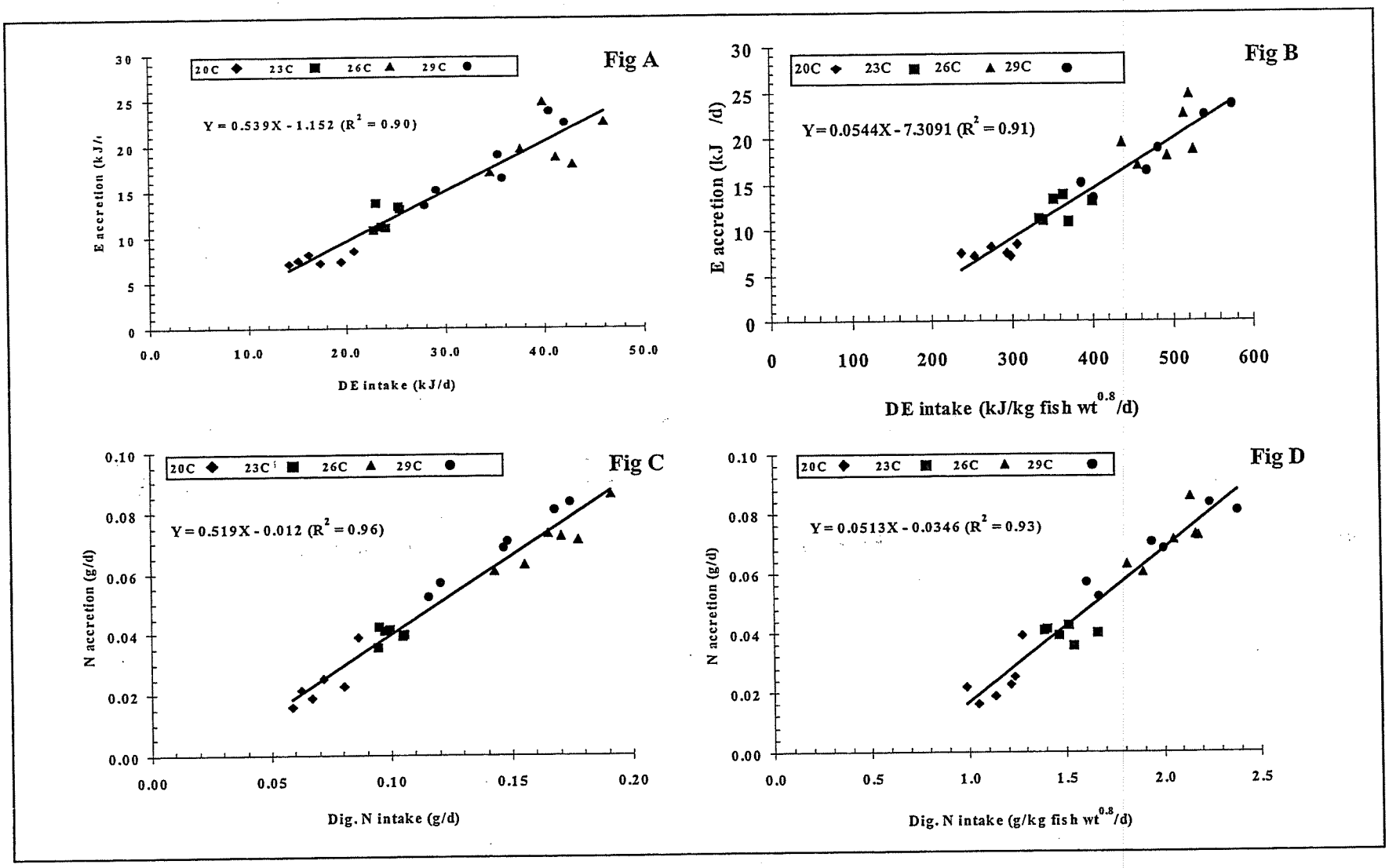

Fig. 6.3.6 **Relationships between dietary intake and accretion in fish during the 5 to 10 week period in Experiment Wlk 1: daily intake of digestible energy expressed either as absolute intake (Fig A) or as function of fish weight$^{0.8}$ (Fig B) and daily intake of digestible N expressed as absolute intake (Fig C) or as function of fish weight$^{0.8}$ (Fig D).**

The effects of diet and water temperature on dressing percentage and efficiencies of dietary protein and energy retentions are shown in Table 6.3.12. Reducing the protein content of the diet improved (P<0.05) the efficiency of DE retention and reduced dressing out percentage with these effects being most marked for the diet containing the lowest protein content. CP and DCP retention efficiencies and dressing out percentage increased (P<0.05) as water temperature increased.

Table 6.3.12 Effect of diet and water temperature on dressing out percentage and efficiencies of dietary protein and energy retentions in Experiment Wlk 2

Diet (% DCP)	Water temperature (°C)				Mean	±sem[1]
	20	23	26	29		
			Dressing-out (%)[2]			
W2-1 (40.7)	86.5	87.1	89.0	90.4	88.2^{X}	
W2-2 (33.4)	87.0	87.6	88.9	89.7	88.3^{X}	
W2-3 (31.5)	86.1	87.1	88.5	88.5	87.5^{Y}	0.33
Mean	86.5^{P}	87.3^{Q}	88.8^{R}	89.5^{S}		
			Crude protein (%)			
W2-1 (40.7)	38.6	41.4	45.1	50.0	43.8	
W2-2 (33.4)	40.4	43.7	46.7	49.2	45.0	
W2-3 (31.5)	40.1	42.3	46.5	46.4	43.8	2.44
Mean	39.7^{P}	42.5^{PQ}	46.1^{QR}	48.5^{R}		
			Digestible protein (%)[2]			
W2-1 (40.7)	42.5	45.6	49.7	55.0	48.2	
W2-2 (33.4)	47.3	51.2	54.7	57.6	52.7	
W2-3 (31.5)	46.2	48.8	53.6	53.5	50.5	2.76
Mean	45.3^{P}	48.5^{PQ}	52.6^{QR}	55.4^{R}		
			Gross energy (%)[2]			
W2-1 (40.7)	40.1	38.8	39.7	35.0	38.4	
W2-2 (33.4)	35.5	37.5	43.4	42.2	39.6	
W2-3 (31.5)	39.1	43.5	43.2	49.5	43.8	3.91
Mean	38.2	39.9	42.1	42.2		
			Digestible energy (%)[2]			
W2-1 (40.7)	53.0	51.3	52.5	46.3	50.8^{X}	
W2-2 (33.4)	50.9	53.8	62.3	60.5	56.9^{XY}	
W2-3 (31.5)	56.9	63.4	63.0	72.2	63.9^{Y}	5.61
Mean	53.6	56.2	59.3	59.6		

[1] Standard error of mean for feed frequency x water temperature interaction term.
[2] $^{P,Q,R;\ X,Y}$ Within interaction and main effect comparisons, means without a common letter differ (P<0.05).

Relationships between dietary intakes of digestible N and DE and their respective accretions in the fish are shown in Figure 6.3.7. In all instances, a simple linear model relating accretion to intake satisfactorily explained the relationship with R^2 values of ≥ 0.84 and generally 0.94 to 0.98. The derived relationships for the individual diets were not poolable because of significantly (P<0.05) different intercepts and slopes except for the relationship between N accretion and DE intake (Fig C of Figure 6.3.7) where each of the regression statistics were not significantly different. The efficiencies with which digestible N and DE were retained by the fish were similar for the two higher protein diets and higher than for the 31.5% DCP diet (0.80 and 0.72 cv 0.46 for energy and 0.60 and 0.63 cv 0.56 for nitrogen, respectively). The DE maintenance requirements (i.e. at zero energy accretion) predicted from the derived relationships were 77, 163 and 142 kJ/kg fish weight$^{0.8}$/d) for the 40.7, 33.4 and 31.5 % DCP diets, respectively. The DCP maintenance requirement (at zero N accretion and expressed as digestible N) was similarly predicted to be 0.76, 0.61 and 0.53 g/ kg fish weight$^{0.8}$/d, respectively.

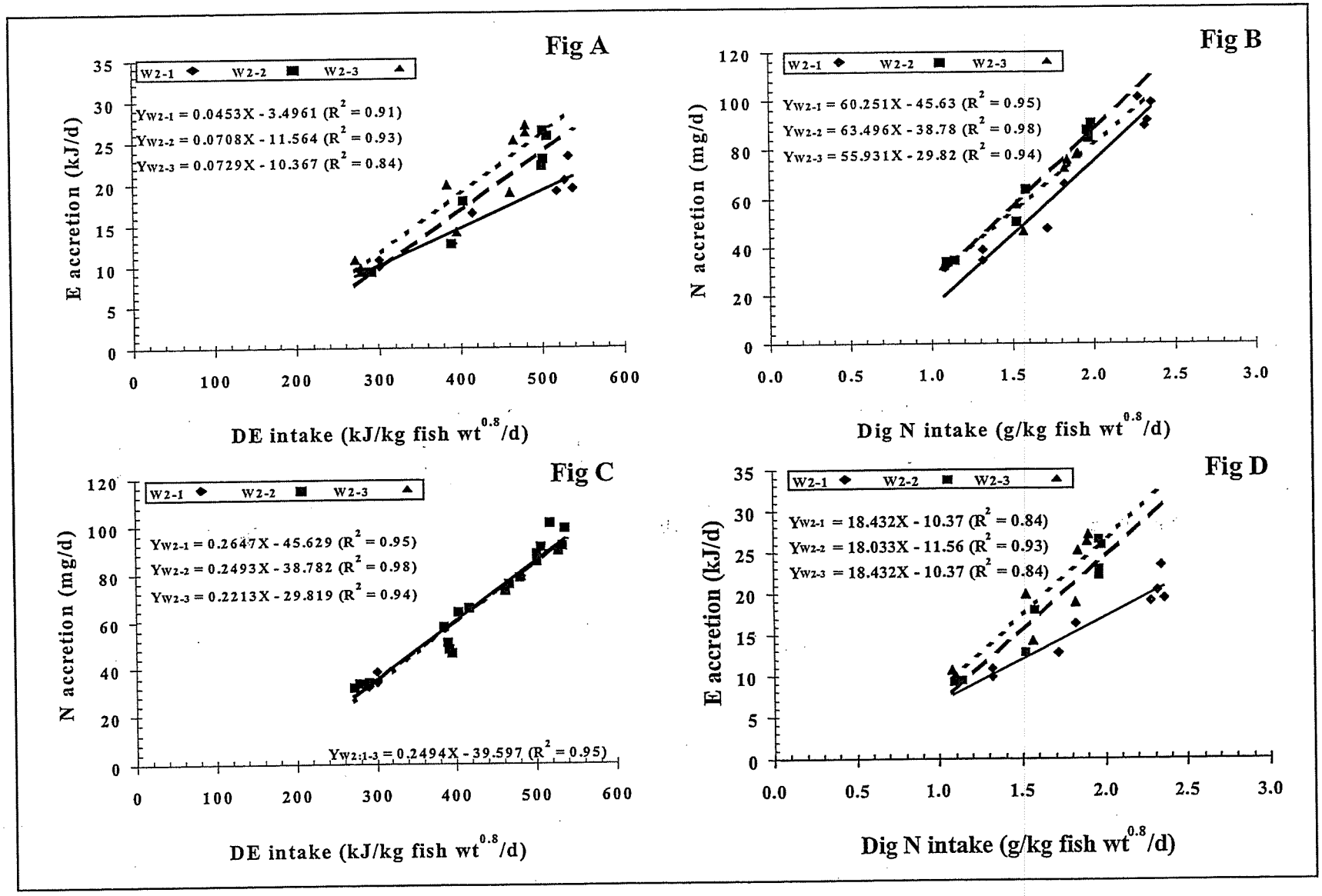

Fig 6.3.7 Relationships between intake (expressed as function of fish weight$^{0.8}$) of dietary digestible energy (DE) or dietary digestible nitrogen (Dig. N) and accretions of nitrogen (N) or energy (E) of fish in Experiment Wlk 2.

6.3.4 Discussion

Increasing the feeding rate in Experiment Wlk 1had the expected productivity benefits of higher growth rates, better FCRs and higher dressing out percentages. Somewhat unexpected was the finding that the DM fat content of the fish decreased with increasing food consumption and this response was significant ($P<0.01$) over the 10-week experimental period (Figure 6.3.4). Studies with salmonids (Elliott, 1976), European sea bass (Hidalgo et al., 1987) and carp (Zeitler et al., 1984) have shown that the fat content of the fish increases directly with food intake. However, in recent work with sunshine bass (*Morone chryops* x *Morone saxatilis*), Keembiyehetty and Wilson (1998) have shown that the nutritional balance of the diet is as important in determining the chemical composition of the fish as that of absolute food intake. In their work, sunshine bass fingerlings held at water temperatures of 27 or 32°C were fed to apparent satiation twice daily for eight weeks either of six diets that varied in protein to energy ratio from 38.7 to 22.2 g/MJ. Voluntary food intake decreased directly with decreasing protein to energy ratio (from 5.3 to 3.2 and 3.9 to 2.1 g/week for water temperatures of 27 and 32°C, respectively) but fat content of the fish increased dramatically (from 7.8-8.9 to 11.4-12.8 % wet weight) as the protein to energy ratio of the diet decreased to 25.9 or below. Although water temperature had a marked effect on voluntary food intake, it had no profound effect on the fat content of the fish although fish at the lower water temperature, and consuming more food, tended to have slightly higher body fat content on each of the diets. In Experiment Wlk 2 where diets were control-fed, the fat content of the fish increased with decreasing dietary DCP and this effect was more pronounced at high than at low water temperatures (Table 6.3.11). These results illustrate the interactive effects between dietary nutrient supply and chemical composition of the accreted tissue. Similar effects have been well documented in terrestrial animals such as pigs (SCA, 1990). The results of the present study wherein body fat composition decreased slightly with increasing food intake, paralleling the findings of Keembiyehetty and Wilson (1998), suggests that the DCP:DE ratio of the diet fed in Experiment Wlk 1 (i.e. 26 g:MJ) and the two highest protein diets in Experiment Wlk 2 (i.e. 27.5 and 24.5 g:MJ) were adequate in protein and not limiting protein accretion. This was confirmed by the N accretion responses where relationships between N accretion and digestible N intake for the two highest protein diets were almost identical and producing higher N retention than for the lowest protein diet (Fig B of Figure 6.3.7).

Numerous studies have explored the influence water temperature has on DFI and the concomitant effect this has on fish productivity (see Section 6.2.4.2). Most studies have been carried out to understand metabolic processes in the fish to generate data required for developing fish growth models. Fundamental to such models has been the need to quantify maintenance requirement and the energetic and nutrient costs of growth and how these are affected by culture conditions. Water temperature, along with size of the fish, has been found to be one of the most critical non-nutritional parameters affecting the maintenance requirement of the fish (see Brett and Groves, 1979; Elliott, 1982; Weatherley and Gill, 1987; Steffens, 1989; Talbot, 1993). As summarised some three decades earlier by Paloheimo and Dickie (1966), increasing water temperature alone causes the metabolic rate to rise, partly in response to the enhancement by temperature of biochemical processes within the animal and in part to the demand induced by greater activity (locomotion) of the fish. If food is readily available as for example under most culture conditions, these effects stimulate an increased consumption of food which results in higher rates of growth and/or reproductive development. If however, food availability should be manipulated so that food consumption is not allowed to increase with water temperature, the higher metabolic rate associated with higher temperature could be expected to be expressed as a reduced rate of growth and development. To these effects of temperature alone must be considered the concomitant effects a higher food intake has on increasing metabolic demand. Thus, the effect of temperature on metabolic rate will be greater when food consumption is allowed to respond to the animal's demand than when food

consumption is restricted.

In Experiment Wlk 1 where food intake was restricted, water temperature was seen to affect the growth of the fish much as described above (Figures 6.3.1 and 6.3.2). Although the limited range in DFI achieved in the experiment prevented significant relationships being found at all temperatures, extrapolation of the equation to zero weight change (maintenance requirement) showed that metabolic rate fell with reducing water temperature while the rate at which food supply above maintenance was used to support growth increased with temperature as relatively more of the consumed food was available for nutrient accretion. Since activity level of the fish will have a more profound effect on energy demand than true basal metabolism (Brett and Groves, 1979; Cho et al., 1982; Talbot, 1993) and because of the limited range in DFI achieved in the experiment at each of the water temperatures, not a lot of confidence should be placed on the derived mathematical relationships for the respective temperatures. However, the excellent fit of the fish's response, whether expressed as growth rate (Figure 6.3.1 and 6.3.2) or as accretions of energy or N (Figures 6.3.5 and 6.3.6) to DFI across all water temperatures provides useful practical information on the response of barramundi to nutrient supply. Maintenance requirements of the fish during the 0 to 4 and 5 to 10 week periods (at mean mid fish weights of 154 and 206 g, respectively) were predicted to be 0.012 and 0.011 g food /kg fish weight$^{0.8}$/d, respectively which corresponded to actual intakes of 0.68 and 0.78 g food/d or 10.54 and 12.09 kJ DE/d. Similarly, the predicted DE requirements for zero energy accretion were predicted to be 8.0 and 9.5 kJ/d for the two periods. The slightly lower estimates when based on energy accretion rather than body weight are explained as being due to differences in the chemical composition of the body at each of these two 'zero' states. Expressed as a function of metabolic weight (/kg weight$^{0.8}$), the DE maintenance requirement was 142 and 134 kJ/d for periods 0 to 4 and 5 to 10 weeks, respectively.

In Experiment Wlk 2, the DE requirement for zero energy accretion was predicted to be 77, 163 and 142 kJ/kg fish weight$^{0.8}$/d for the 40.7, 33.4 and 31.5% DCP diets, respectively. Statistically, the estimate of 77 kJ/d for the 40.7% DCP diet was lower ($P<0.05$) than for the two lower protein diets, indicating that the former diet was a more efficient source of dietary energy than the latter two diets. Interestingly, the DE intake required to meet maintenance N requirement did not differ between the three diets and was predicted to be 159 kJ/ kg fish weight$^{0.8}$/d (Fig C of Figure 6.3.7).

These values for the DE requirement of barramundi can be compared to estimates (all expressed as kJ/kg weight$^{0.8}$/d) of 40 to 305 (and typically 50 to 70) for salmonids (Cho et al., 1982; Steffens, 1989; Cho, 1992; Talbot, 1993; Kaushik and Medale, 1994), 30 to 103 (and typically 40 to 50) for carp (Meyer-Burgdorff et al., 1989; Steffens, 1989) and 25 to 38 for catfish (Brown et al., 1990; Hassan and Jafri, 1994). The large variability of these estimates even within species, is undoubtedly due to different states of metabolism at which the estimates were derived with level of activity, water temperature, feeding rate and fish size being amongst the most important contributing factors – the latter reflecting the less than precise relationship of the weight exponent of 0.8 to true metabolic weight.

The predicted digestible N requirement for maintenance of the barramundi in Experiment Wlk 1 was 0.042 and 0.048 g/d (or 0.26 and 0.30 g DCP/d) for periods of 0 to 4 and 5 to 10 weeks respectively. Expressed as g/ kg fish weight$^{0.8}$/d, the corresponding digestible N and DCP requirements were 0.74 and 0.67 g and 4.63 and 4.21 g, respectively. Estimates in Experiment Wlk 2 decreased from 0.76 to 0.61 and 0.53 g/kg fish weight$^{0.8}$/d for digestible N and 4.75, 3.81 and 3.31 g/kg fish weight$^{0.8}$/d for DCP as the protein content of the diet decreased from 40.7 to 33.4 and 31.5% DCP, respectively. Since as much as 50% of the fish's energy requirements are met from dietary protein even when they are fed high lipid (20%) diets (Cho, 1992), and is likely to be influenced by the amino acid balance of the protein (Tacon and Cowey, 1985;

Murai, 1992; Wilson, 1994), the notion of maintenance N requirements is far less important than energy. Similarly, the efficiency with which dietary N is retained by the fish will be influenced more by the protein to energy balance of the diet than its absolute N (protein) content. However in Experiment Wlk 2, the efficiency with which DCP was retained by the fish was not significantly affected by diet (Table 6.3.12) and neither were the regression slopes relating N accretion to digestible N intake different between the diets (Fig B of Figure 6.3.7). There was however, evidence that efficiency of N accretion was improved with increasing water temperature in both Experiment Wlk 1 (Tables 6.3.9 and 6.3.10) and Experiment Wlk 2 (Table 6.3.12), although the confounding influence of food ration can not easily be separated from that directly attributable to that of temperature in Experiment Wlk 1. The inadvertent low feeding rate allowed for fish at 29°C compared to fish held at 26°C in Experiment Wlk 1, provides some interesting insights. Efficiency of N retention was highest where food intake was highest, irrespective of temperature, which implies that food intake rather than temperature was the primary factor affecting efficiency of N utilisation. This agrees with the conclusions of Hidalgo et al. (1987) reviewing work across a wide range of both carnivorous and herbivorous fish. In contrast, data of Experiment Wlk 2 for fish control-fed the diets showed clearly that retention of DCP improved incrementally from 0.45 to 0.55 as water temperature increased from 20 to 29°C (Table 6.3.12).

As comprehensively discussed by Cho et al. (1982), Cho (1992) and Kaushik and Medale (1994) in their reviews of salmonid bioenergetics, energy is expended by the animal during digestion and absorption, in the transformation and interconversion of the substrates for maintenance and growth of the animal and in the formation and excretion of metabolic wastes. Collectively, these metabolic energetic costs are variously referred to as the heat increment of feeding, specific dynamic effect, calorigenic effect or dietary thermogenesis (Cho et al., 1982; Steffens, 1989) and their magnitude will depend primarily, but not exclusively, on the balance of dietary nutrients and plane of nutrition. When averaged over all temperatures in Experiment Wlk 1, the efficiency with which dietary DE was retained in the fish increased with increasing DFI at rates of 0.62 and 0.54 for periods of 0 to 4 and 5 to 10 weeks, respectively. These values are consistent with DE efficiencies of 0.4 to 0.6 as reported for salmonids and other carnivorous fish (Brett and Groves, 1979; Elliott,1982; Cho et al., 1982; Weatherley and Gill, 1987; Steffens, 1989).

The experiments have confirmed not unexpectedly that barramundi are similar to cold water carnivorous fish in the way metabolic processes respond to altered food rationing and sub-optimal water temperature conditions. At near optimal water temperatures (26 to 29°C), FCR improved curvilinearly with increasing food consumption whereas FCR was relatively unaffected by food consumption at low water temperatures (20 to 23°C). Moreover, FCR improved with increasing water temperature irrespective of the amount of food consumed. These effects could be explained as the combined effects of an improvement in energy utilisation that accompanied high rates of feeding at high water temperatures and higher metabolic costs due to increased activity and maintenance requirements associated with high water temperatures. The net effect in barramundi is an improved metabolic efficiency and energy retention as water temperature increases up to at least 29°C which is observed in the form of increased growth rate and an improvement in FCR. To these effects must be added those due to dietary nutrient supply. Reducing the DCP content of the diet significantly impaired FCR in Experiment Wlk 2 when the DCP content of the diet fell below 33.4%.

6.3.5 References

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6.4 Dietary protein and protein to energy responses of barramundi

6.4.1 Introduction

In defining the nutrient requirements of finfish, more has been written about protein than any other nutrient and this plethora of literature is matched only by the diversity of methodology that has been advocated for its determination (Steffens, 1989; Murai, 1992; NRC, 1993; Arzel et al., 1995). It is well recognised that fish, like all other vertebrates, have a dietary requirement for at least 10 amino acids – the so called essential amino acids – if somatic growth of the animal is not to be checked. These essential amino acids are arginine (Arg), histidine (His), isoleucine (Ile), leucine (Leu), lysine (Lys), methionine (Met), phenylalanine (Phe), threonine (Thr), tryptophan (Try) and valine (Val); cystine (Cys) and tyrosine (Tyr) can spare for part of the Met and Phe requirement respectively, and thus are often considered together when detailing essential amino acid requirements (Ketola, 1982; Millikin, 1982; Steffens, 1989; Wilson, 1994). The essentiality of glycine (Gly) for fish is unknown but it is regarded as being indispensable for young birds (Calet, 1976; Taylor et al., 1994; ARC, 1975) and it has been shown to have beneficial effects in rats (Grimble et al., 1992; Ikejima et al., 1996) and for human infants (Plath et al., 1996).

For terrestrial monogastric animals such as pigs and poultry, dietary protein requirements are increasingly being expressed in terms of the requirement for essential amino acids, singly and together, and as a function of dietary energy consumption. Consequently, the concept of 'ideal' protein as espoused by Cole (1980) has been widely adopted for expression of amino acid requirements (see SCA, 1987; Stranks et al., 1988; Fuller and Wang, 1990). An 'ideal' protein is one that is perfectly balanced in terms of its amino acid composition, exactly matching the animal's physiological need for amino acids as required to meet demands for maintenance, growth and/or reproduction. By definition, such an 'ideal' protein has the highest possible biological value, permitting the greatest efficiency of conversion of dietary protein into deposited protein, i.e. the highest possible productive protein value (PPV). Since protein deposition is energy dependent, it is not surprising that there should be an interdependency between dietary supply of amino acids and energy. Such an interdependence between amino acid and energy supply was admirably demonstrated with pigs in the pioneering work of Campbell (see Campbell, 1985, 1987; Campbell and Taverner, 1988) and further elaborated on by Bikker (Bikker et al., 1995, 1996a,b). These studies showed that protein deposition increased as the dietary supply of all required amino acids were increased up to a point where further protein deposition was limited by an insufficiency of dietary energy. Increasing the supply of dietary energy enabled protein deposition to increase further provided the supply of amino acids was adequate. These functional relationships between protein deposition and amino acid and energy supplies are illustrated in Fig. 6.4.1.

The importance of amino acid balance and protein to energy interdependence is increasingly being recognised in fish nutrition. Numerous reviews on the protein requirements of fish have stressed the need for essential amino acids to be provided in the diet as an optimal balance (eg Millikin, 1982; Ketola, 1982; Steffens, 1989; Arzel et al., 1995) and Wilson (1994) has recently advocated adoption of the 'ideal' protein concept. Similarly, in specifying the protein requirements of fish, greater recognition is being given to its functionality with energy and requirements are increasingly being stated in terms of protein to energy (Zeitler et al., 1984; Reis et al., 1989; Santiago and Laron, 1991; Das et al., 1991; Lanari et al., 1995; Catacutan and Coloso, 1995).

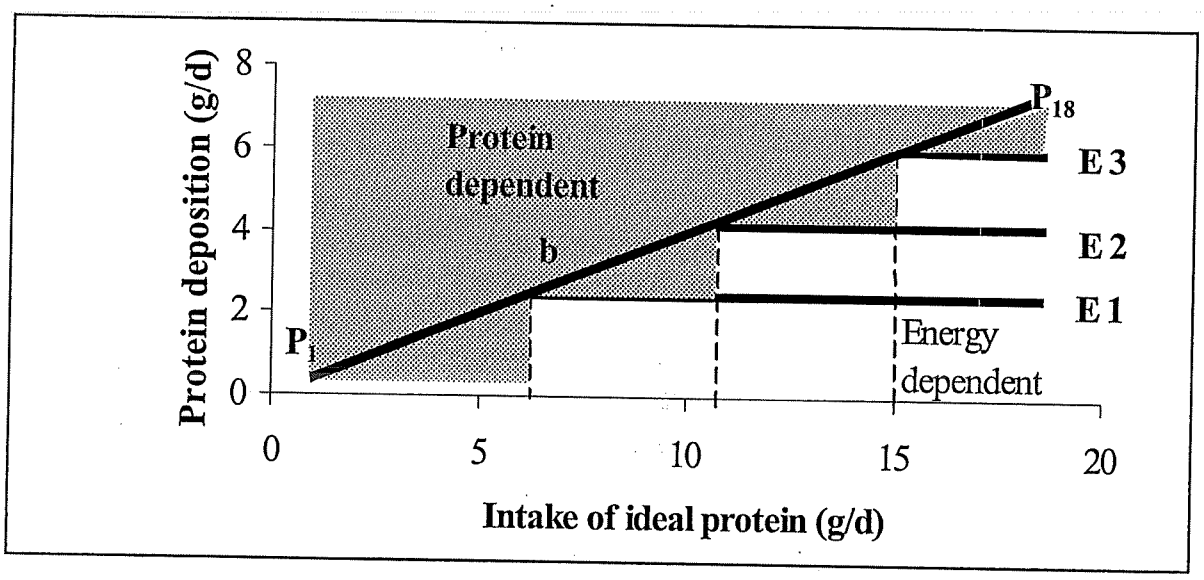

Fig 6.4.1 **Schematic portrayal of the mammalian model for the interdependency of dietary intake of protein (continuous increase of 'ideal' protein as indicated by the line P_1-P_{18}) and of energy (E1, E2, E3 etc) as developed in pigs by Campbell (1988). The slope (b) of the line P_1-P_{18} describes the productive protein value of the 'ideal' protein while the amount of available energy (E) sets the upper limit of protein deposition. For each energy threshold, protein deposition is maximised at the same dietary protein to energy ratio**

Some work has been reported on the protein and lipid requirements of Asian seabass/barramundi (Cuzon and Fuchs, 1988; Tucker et al., 1988; Sakaras et al., 1988, 1989; Wong and Chou, 1989; Boonyaratpalin, 1991). These studies showed growth of seabass was optimised with diets containing 45 to 55% protein and 6 to 18% lipid and that protein requirements decreased slightly as fish grew from fry to grow-out. SEAFDEC (1994) recommended a lower dietary protein requirement of 43% for juvenile fish and also advocated that 50% of the dietary energy should come from the protein. A recent review of the nutrient requirements of Asian seabass has been made by Boonyaratpalin (1997) who recommended that the dietary protein specifications for fry and grow-out stages be set respectively, at about 50% and between 40 and 45%. The effects on growth and body composition of barramundi of varying the protein to energy ratio of the diet have been reported by Catacutan and Coloso (1994). In a 3 x 3 factorial comparison of protein (35, 42.5 and 50%) and energy (lipid levels of 5, 10 and 15%), they found fish growth was highest for the diet containing 50% protein and 15% lipid (a crude protein to gross energy ratio of ~30 mg/kJ). However, the diet containing 42.5% protein and 10% lipid (~ 31mg/kJ) resulted in comparable fish growth and better protein efficiency and apparent protein retention while fish fed the 35% protein diets showed very poor growth. Based on these results, they recommended a dietary specification of 42.5% protein and 10% lipid – a crude protein to gross energy ratio of ~ 31 mg/kJ.

In order to further define the dietary protein and dietary protein to energy requirements of juvenile barramundi, an on-farm cage study and two laboratory experiments were carried out. The on-farm experiment (Wlk 4) sought to examine the effect on fish growth and eating characteristics of juvenile barramundi that were fed on dry pelleted diets differing markedly in protein to energy ratios. The two laboratory experiments were carried out to see if growth (and protein deposition) in fish was similar to that of terrestrial monogastric animals, exhibiting both dependency to, and non-dependency of, both protein and energy intake (see Figure 6.4.1). The first laboratory experiment (Wlk 6) examined the effect on growth and nutrient retention of barramundi fed semi-purified dry pelleted diets in which the protein content varied from approximately 30 to 55% while the energy content was held constant. The protein was a blend

of fishmeal, casein and crystalline amino acids that was formulated to mimic the amino acid composition of barramundi protein and to approximate what might be thought of as 'ideal' protein. The object of the second laboratory experiment (Wlk 7) was to examine the effect on growth and nutrient retention of barramundi when energy intake was controlled and the fish offered diets that were either protein-limiting or energy-limiting.

6.4.2 Methods

The general methods were as described in Section 6.1. Specific details for each of the experiments are enumerated below.

6.4.2.1 On-farm cage experiment Wlk 4

Three practical diets were formulated (Table 6.4.1) to see if varying the protein to energy ratio of the diet affected either the growth or eating characteristics of barramundi reared under typical farm conditions. The three diets comprised an incomplete 2 x 2 factorial arrangement of two protein concentrations (~37.5 and 43% crude protein; ~32 and 37% digestible protein) and two energy concentrations (~18.5 and 21 kJ gross energy/g; ~14 and 16.4 kJ digestible energy/g) with the high protein, high energy diet being omitted because of the unavailability of sufficient cages for the study. Protein content was varied by manipulation of fishmeal, soybean meal and meat meal inclusions while energy content differences were achieved essentially by substituting de-fatted soybean meal for full-fat soybean meal. The determined chemical composition of the three experimental diets is shown in Table 6.4.2.

Table 6.4.1 Formulation of diets fed in the on-farm cage Experiment Wlk 4

Feed ingredient	Diets		
	W4-1	W4-2	W4-3
		Formulation (%)	
Fishmeal (Chilean)	25.0	25.0	35.0
Soybean meal (full-fat)	0	22.0	0
Soybean meal (solv-ext)	15.0	0	10.0
Wheat gluten	5.0	5.0	5.0
Meat meal	10.0	10.0	15.0
Wheat flour	38.6	25.6	31.4
Fish oil (Chilean)	5.0	5.5	2.5
Tallow	0	5.5	0
l-Lysine HCl	0.2	0.2	0
d/l Methionine	0.1	0.1	0
Vitamin premix[1]	1.1	1.1	1.1

[1] The vitamin premix was a proprietary formulation supplied by Aquafeed P/L and included at 0.5% of the diet. It was stated to supply vitamins in type and amounts similar to that detailed in Table 6.4.1. Additional inclusions (% of diet) were: choline chloride, 0.1; vitamin C (coated), 0.25; and calcium proprinate, 0.25.

Table 6.4.2 Chemical composition of the diets fed in Experiment Wlk 4

Analysis	Diet		
	W4-1	W4-2	W4-3
		As fed basis	
Dry matter (%)	91.8	92.4	91.6
Ash (%)	8.3	8.3	10.7
Crude protein (CP; %)	37.6	37.2	43.1
Digestible protein (DCP; %)[1]	32.2	32.1	37.0
Crude fat (%)	9.9	19.6	8.9
Calcium (%)	1.8	1.8	2.5
Phosphorus (%)	1.1	1.2	1.6
Amino acids (%)			
Lysine	2.16	2.18	2.47
Methionine + cystine	1.22	1.20	1.28
Threonine	1.31	1.31	1.55
Arginine	2.15	2.17	2.51
Fatty acids (%)			
18:3n-3	0.07	0.35	0.06
20:5n-3	1.11	1.18	0.90
22:6n-3	0.48	0.51	0.38
Σ n3 fatty acids	2.00	1.99	1.58
Σ n6 fatty acids	0.48	2.48	0.39
Gross energy (GE; kJ/g)	18.66	20.98	18.29
Digestible energy (DE; kJ/g)[1]	13.88	16.44	14.05
CP:GE (mg/kJ)	20.2	17.7	23.6
DCP:DE (mg/kJ)	23.2	19.5	26.3

[1] Estimated using apparent digestibility coefficients of individual feed ingredients as detailed in Table 6.1.1.

Six thousand juvenile barramundi of mean (±SD) initial weight of 296±21.0 g were selected on the basis of weight uniformity and freedom from obvious abnormality from the available pool of fish on Mr Cris Phillips' Barramundi Waters farm at Innisfail. These fish were allocated equally (500 fish/cage) to 12 net cages (2 x 2 m surface x 2 m deep; 4 cages per diet treatment) in an aerated freshwater pond; the cages were serviced by a walkway and diets randomly allocated to the cages. Fish fed the experimental diets were managed as for other fish on the farm. They were fed to satiety once daily (except on weekends when fish were fed only on one of the days) from buckets containing pre-weighed allocations of the diet. No attempt was made to collect any apparent uneaten food. At the conclusion of the 13-week experiment, 100 fish from each cage were mass-weighed and an accurate count taken of all fish. A representative sample of 10 fish from each experimental cage was taken for sensory analysis; fish from a similar number of adjacent cages that had been fed on a commercial barra grower pellet were also sampled for comparative taste testing. Sensory analysis of the fish was carried out by trained taste panelists at QDPI's Centre for Food Technology by procedures described in Section 6.1.6.

6.4.2.2 Laboratory Experiment Wlk 6

The aim of the experiment was to measure the effect of feeding barramundi on diets that varied incrementally in protein content. A 6 x 4 randomised block design was used to test 6 diets in which the inclusion of a reference protein was serially incremented at the expense of gelled wheat starch, with concomitant adjustment of the diatomaceous earth and fish oil inclusions to maintain constancy of ash and lipid (Table 6.4.3). The diets were manufactured in a small-scale commercial steam-press pelleter.

Table 6.4.3 Formulation (% as mixed) of the diets fed in Experiment Wlk 6

Feed ingredient	Diet					
	W6-1	W6-2	W6-3	W6-4	W6-5	W6-6
Reference protein[1]	35.0	42.0	49.0	56.0	63.0	70.0
Starch (gelled)	47.5	40.0	32.5	25.0	17.5	10.0
Diatomaceous earth	3.5	5.3	7.1	8.9	10.7	12.5
Soybean oil	2.0	1.8	1.6	1.4	1.2	1.0
Fish oil	8.0	7.2	6.4	5.6	4.8	4.0
Dicalcium PO_4	1.5	1.2	0.9	0.6	0.3	0
Salt	0.5	0.5	0.5	0.5	0.5	0.5
Vit & min premix[2]	1.5	1.5	1.5	1.5	1.5	1.5

[1] Composition (g/kg) was: Casein, 430; Fishmeal (Peruvian), 300; Gluten, 250; Lysine HCl, 5; d/l Methionine, 5.5; l Threonine, 2.5; l Tryptophan, 1; and $NaHCO_3$, 6.

[2] As detailed in Table 6.4.1.

The reference protein was the only source of protein in the diet (excepting for negligible amount contributed by the vitamin premix) and was intended to closely match the essential amino acid composition of barramundi protein. A comparison of the amino acid composition of the reference protein and barramundi protein (Williams and Barlow, 1998) is shown in Table 6.4.4. The chemical composition of the diets is shown in Table 6.4.5.

Table 6.4.4 Comparison of the amino acid composition (g/16 g N) of the reference protein and that of the protein of barramundi

Source	Essential amino acids									
	ARG	HIS	ILE	LEU	LYS	MET	PHE	THR	TRY	VAL
Ref. protein	3.74	2.44	4.75	8.46	6.57	3.06	5.00	4.15	1.33	5.70
Barramundi	5.47	1.65	3.22	5.87	6.39	2.27	3.39	3.65	0.94	3.71
	Non-essential amino acids									
	ALA	ASP	CYS	GLU	GLY	PRO	SER	TYR		
Ref. protein	3.69	6.41	0.93	24.28	3.17	10.17	5.10	4.25		
Barramundi	6.01	8.23	0.59	11.99	7.68	5.64	3.39	2.53		

Table 6.4.5 Chemical composition (air-dry basis) of the diets in Experiment Wlk 6

Analysis	Diet					
	W6-1	W6-2	W6-3	W6-4	W6-5	W6-6
Dry matter (%)	92.3	91.8	92.6	92.9	92.9	92.4
Ash (%)	7.7	9.4	11.4	12.5	14.4	17.1
Crude protein (%)	29.0	34.2	40.0	45.7	53.0	55.5
Est. dig. protein (%)	26.8	31.9	37.0	42.1	47.2	52.4
Crude fat (%)	11.7	11.1	10.1	9.5	8.3	8.0
Amino acids (%)						
Arginine	1.21	1.12	1.53	1.69	2.10	1.94
Lysine	1.90	2.20	2.70	3.00	3.53	3.56
Met + cystine	1.20	1.39	1.65	1.79	2.02	2.14
Threonine	1.21	1.40	1.71	1.91	2.21	2.24
Fatty acids (%)[1]						
18:3n-3	0.24	0.22	0.20	0.17	0.15	0.13
20:5n-3	0.62	0.58	0.54	0.51	0.47	0.43
22:6n-3	1.02	0.97	0.92	0.86	0.81	0.76
Σ n3 fatty acids	2.06	1.94	1.82	1.69	1.57	1.45
Σ n6 fatty acids	1.16	1.05	0.93	0.82	0.70	0.59
Gross energy (kJ/g)	18.92	18.82	18.80	18.67	18.86	18.39
Est. dig. energy (kJ/g)	14.47	14.91	15.35	15.79	16.23	16.67

[1] Calculated from fatty acid analysis of the fishmeal, soybean oil and fish oil used in the diets.

A total of 720 fish of mean (±SD) initial weight of 76±2.9 g were selected from the same spawning cohort on the basis of weight uniformity and freedom from obvious abnormality from a pool of several thousand fish. A further 20 fish of a similar size were sampled for determination of the chemical composition of the fish at the start of the experiment. The experimental fish were distributed equally (30 fish/tank) to the 800 L tanks in the environment-controlled laboratory at Walkamin (Section 6.1.1) where the water temperature was maintained at 28°C. Experimental diets were randomly allocated as a block to each of the 4 independent recirculation units, constituting four complete replications. Fish started on experiment after 14 d acclimatisation and during this time a prophylactic salt water bath was administered. Fish were fed twice daily to satiety (except on day of fortnightly weighing) with all uneaten food being accounted for. The growth assay was intended to extend for six-weeks but was reduced to 28 d for fish on the four lowest protein diets and to 35 d for fish on the two highest protein diets because of unexpectedly high rates of food consumption.

6.4.2.3 Laboratory Experiment Wlk 7

The aim of this growth assay experiment was two-fold: to determine the biological value of the reference protein used in Experiment Wlk 6; and to see if fish exhibited similar protein and energy interdependencies as described for terrestrial monogastric animals (Figure 6.4.1). To test these effects, diets (see Tables 6.4.3 and 6.4.5) found in Experiment Wlk 6 to be either protein-limiting (diet W6-3) or energy-limiting (diet W6-5) were fed to barramundi at either one of four feeding rates. For the protein-limiting diet (37% DCP), the designated feeding rates were 15, 20, 25 and 30% of fish weight$^{0.48}$. The highest rate was equivalent to 85% of satiety as observed in Experiment Wlk 6 for that particular diet. For the energy-limiting diet (47.2% DCP), the feeding rates were as for the protein-limiting series except that the daily ration was reduced by a factor of 0.78 (i.e. 37/47.2). This factor reduction ensured that the daily protein intake of fish fed the energy-limiting diet was identical to that of fish fed the protein-limiting diet at the same feeding rate. Thus, the effect on barramundi performance of diets differing in protein to energy ratios could be evaluated at different absolute energy intakes but at identical absolute protein intakes (within a given feeding rate). The experiment was arranged as a factorial comparison of 2 diets and 4 feeding rates, with 3 replicates. Fish on the two highest feeding rates remained on experiment for 42 d while those on the two lowest feeding rates remained on experiment for 56 d. This staggered ending was designed to ensure all fish grew to approximately the same termination weight.

A total of 600 selected fish of mean (±SD) initial weight of 222±12.4 g were equally allocated to 800 L tanks (25 fish/tank) as three blocks formed by weight sorting of the fish. Since each of the four independent recirculation units in the laboratory serviced only 6 tanks, the treatments were arranged so that each recirculation system contained an equal number of the two diets with feeding rate then being randomly assigned amongst 8 adjacent tanks. Thus all treatments comprising an experimental block were contained within two recirculation systems. Lighting was standardised to a 12:12 cycle, water temperature was held at 28°C and fish were fed twice daily other than on the day of fortnightly weighing. During a 14 d acclimatisation period, fish were given a prophylactic salt water bath. A sample of 20 fish from the same cohort as the experimental group was taken for determination of the initial chemical composition of the fish. At termination, a sample of 5 fish from each tank was taken for chemical analysis.

6.4.3 Results

6.4.3.1 On-farm Experiment Wlk 4

Min-max water temperature (measured 0.75 m below the surface) was generally 20-22°C for the first 20 d, then increased rapidly to 23-26°C by d 30 and to 24-27°C by d 40. Temperatures continued to increase to 25-27°C by d 50 and thereafter generally remained between 27-30°C. The mean (±SD) minimum and maximum water temperature during the experiment was

24.6±2.83 and 26.8±3.10°C, respectively. Water chemistry was checked weekly at 0900 h and critical data were: total ammonia, <0.8 ppm; nitrite, <0.2ppm and pH, >7.6 and <8.5. Fish mortality in the experiment was relatively low (3.3%) with most mortalities occurring within the first fortnight when water temperatures were low. Production responses of fish fed the three experimental diets are tabulated in Table 6.4.6.

Table 6.4.6 Production responses[1] of on-farm caged barramundi fed diets varying in protein and digestible energy content in Wlk 4

Attribute	Diets			±sem
	W4-1	W4-2	W4-3	
DCP (%)	32.3	32.1	37.0	
DCP:DE (mg/kJ)	23.2	19.5	26.3	
Survival (%)	97.8	96.7	95.6	1.37
Growth rate (g/d)	1.46	1.59	1.56	0.181
Specific growth rate (%/d)	0.41	0.44	0.43	0.049
Food intake (g/d)	2.49	2.50	2.41	0.038
FCR (g:g)	1.77	1.67	1.62	0.191
Food costs ($/kg gain)[2]	1.08	1.09	1.05	0.122

[1] No significant differences in production responses were observed in the experiment (P>0.05).

[2] The cost of the diet is for ingredients only with no manufacturing cost included.

There were no significant (P>0.05) differences between diets for any of the production traits. However, the statistical power of the experiment was low (0.15 to 0.17) because of the small number of cages available to the experiment. Thus the apparent trend for growth rate and FCR to improve as protein or energy content of the diet increased may be important biologically even if not statistically different. Similarly, the food cost per unit of fish weight gain was lowest for fish fed the highest protein diet (W4-3). Sensory scores of the fish are presented in Table 6.4.7.

Table 6.4.7 Sensory scores (0 = none or dislike; 100 = all or like) for barramundi fed either the three experimental diets in Wlk 4 or a commercial diet

Sensory attribute	Diet fed				'F' value
	W4-1	W4-2	W4-3	Commercial	
Odour					
Fishy	34.2	33.5	32.9	37.6	1.24
Weedy	6.8	10.1	7.9	10.5	1.78
Muddy	7.9	8.4	8.5	8.4	0.07
Meaty	28.0	25.8	27.0	31.2	1.59
Colour					
Grey	17.4^{AB}	16.5^{AB}	14.0^{B}	20.7^{A}	4.32
Yellow	10.4^{AB}	9.0^{B}	8.7^{B}	13.8^{A}	4.34
Flavour					
Fishy	37.6	36.8	37.9	37.1	0.08
Weedy	8.2	8.1	7.5	7.8	0.08
Muddy	16.0	19.4	17.1	14.2	1.96
Stale	13.8^{AB}	17.8^{A}	12.1^{B}	17.4^{AB}	3.34
Texture					
Firm	43.2	43.2	43.5	45.7	0.63
Moist	50.1	49.1	51.3	52.5	0.64
Sticky[1]	24.8	24.9	21.8	18.9	3.05
Overall liking	61.0	56.9	62.2	58.7	1.62

[A,B] Means without a common letter differ (P<0.05; Tukey's LSD). Significant (P = 0.05) $F_{3, 144}$ value = 2.64.

[1] Significant (P = 0.03) 'F' value but Tukey's LSD (6.08) not significant (P>0.05).

Significant differences between the diets were confined to colouration and flavour of the flesh. Fish fed the commercial diet had darker yellow and grey colouration than those fed the highest protein diet ($P<0.05$) while the fish fed the other two diets were not significantly different from all other diets. Fish fed the highest protein diet had significantly higher ($P<0.05$) scores for stale flavour than for the high-energy, low-protein diet (W4-2) while all other diets were not different from each other. There was a tendency for the flesh of fish fed the commercial diet to have a less sticky texture than for other diets but this difference was not significant by Tukey's LSD. The overall liking of the fish from all diets was high and scores for undesirable characteristics such as earthy odours and tastes were low and generally less than 10.

6.4.3.2 Laboratory Experiment Wlk 6

No mortalities occurred and the fish remained in excellent health during the experiment. Productivity of the fish are detailed in Table 6.4.8 and illustrated graphically in Fig. 6.4.2. Food intake declined and FCR improved curvilinearly ($P<0.05$) with increasing protein content of the diet. As a result, ADG and specific growth rate initially increased as the protein content of the diet increased up to Diet W6-4 and plateaued thereafter. The overall relationship was best described as two straight lines intersecting at 41.0% DCP (44.4% CP).

Table 6.4.8 Effect of varying dietary protein content on productivity responses[1] of barramundi in Experiment Wlk 6

Diet (%DCP)	Growth rate (g/d)	Specific growth rate (%/d)	Food conversion (g as-fed food:g fish gain)	Food intake (g/d)
6-1 (26.8)	2.38^{C}	2.45^{AB}	1.76^{E}	4.16^{A}
6-2 (31.9)	2.51^{BC}	2.38^{BC}	1.44^{D}	3.62^{B}
6-3 (37.0)	2.72^{AB}	2.45^{AB}	1.22^{C}	3.32^{C}
6-4 (42.1)	2.90^{A}	2.59^{A}	1.04^{B}	3.00^{D}
6-5 (47.2)	2.72^{AB}	2.24^{C}	1.00^{AB}	2.59^{E}
6-6 (52.4)	2.94^{A}	2.37^{BC}	0.95^{A}	2.65^{E}
± sem	0.072	0.058	0.027	0.061

[1] $^{A,B,C,D,E;}$ Within main effect comparisons, means without a common letter differ ($P<0.05$).

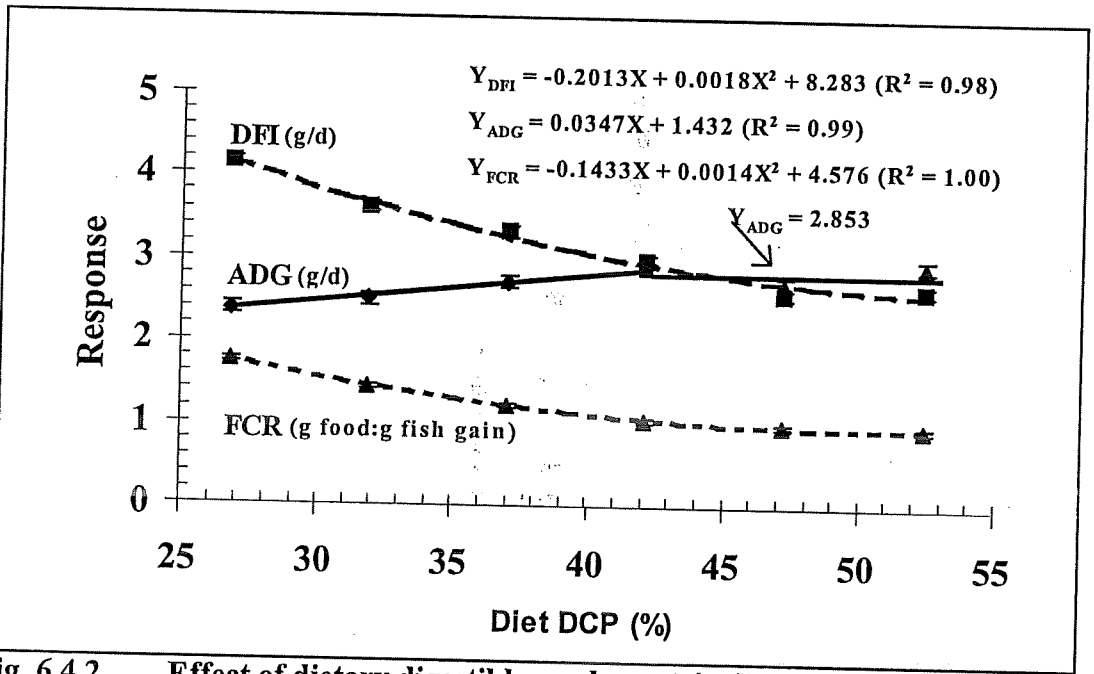

Fig. 6.4.2 Effect of dietary digestible crude protein (DCP) on daily food intake (DFI), average daily gain (ADG) and food conversion (FCR) responses of fish in Experiment Wlk 6.

The chemical composition of the fish at the start and at the conclusion of the experiment is shown in Table 6.4.9.

Table 6.4.9 Effect of varying dietary protein content on the chemical composition[1] of the fish at the start and end of Experiment Wlk 6

Diet (%DCP)	DM (%)	Fat (% DM)	GE (kJ/g DM)	Ash (fat-free) (% DM)	CP (fat-free) (% DM)
Initial	34.5±0.97	31.5±4.48	24.28±0.479	18.2±0.79	76.1±1.39
6-1 (26.8)	32.4	30.4^{B}	24.75^{AB}	18.2^{AB}	78.6^{A}
6-2 (31.9)	33.2	31.5^{A}	24.98^{A}	18.4^{A}	80.1^{B}
6-3 (37.0)	32.4	29.8^{B}	24.75^{AB}	17.9^{BC}	80.1^{B}
6-4 (42.1)	33.5	28.4^{C}	24.65^{B}	17.5^{D}	80.9^{C}
6-5 (47.2)	32.3	27.5^{D}	24.20^{C}	17.7^{CD}	81.1^{C}
6-6 (52.4)	31.1	27.1^{D}	24.18^{C}	17.7^{CD}	81.2^{C}
± sem	0.54	0.30	0.078	0.09	0.24

[1] $^{A,B,C,D,E;}$ Within main effect comparisons, means without a common letter differ ($P<0.05$).

The most noticeable effect of increasing the protein content of the diet was a marked decrease ($P<0.05$) in the fat content of the fish and a smaller but significant decrease and increase respectively in the fat-free contents of ash and protein. Compared to the chemical composition of the initial sampled fish, at the end of the experiment, fish appeared to be leaner and to contain a higher content of fat-free protein. Retention of dietary protein and energy during the experiment are presented in Table 6.4.10.

Table 6.4.10 Effect of varying dietary protein content on dietary protein and energy retentions[1] in Experiment Wlk 6

Diet (%DCP)	CP (%)	DCP (%)	GE (%)	DE (%)
6-1 (26.8)	32.4	30.4^{B}	24.75^{AB}	18.2^{AB}
6-2 (31.9)	33.2	31.5^{A}	24.98^{A}	18.4^{A}
6-3 (37.0)	32.4	29.8^{B}	24.75^{AB}	17.9^{BC}
6-4 (42.1)	33.5	28.4^{C}	24.65^{B}	17.5^{D}
6-5 (47.2)	32.3	27.5^{D}	24.20^{C}	17.7^{CD}
6-6 (52.4)	31.1	27.1^{D}	24.18^{C}	17.7^{CD}
± sem	0.54	0.30	0.078	0.09

[1] $^{A,B,C,D,E;}$ Within main effect comparisons, means without a common letter differ ($P<0.05$).

Relationships between dietary intake of either protein or energy and their respective retention are illustrated graphically in Figure 6.4.3 and compared with effects on fat composition of the fish. Daily retention of dietary DCP and DE declined with increasing protein content of the diet. Since DFI decreased markedly with increasing dietary protein content (Table 6.4.8; Fig. 6.4.2), relating daily retention to actual daily intake was a more useful way of viewing the data. As depicted in Figure 6.4.3, nitrogen retention increased quadratically with increasing DCP intake and declined quadratically with increasing DE intake; DE retention increased linearly with DCP intake and quadratically with DE intake ($P<0.05$). Fat content of the fish declined linearly with increasing DCP intake and increased quadratically with increasing DE intake ($P<0.05$).

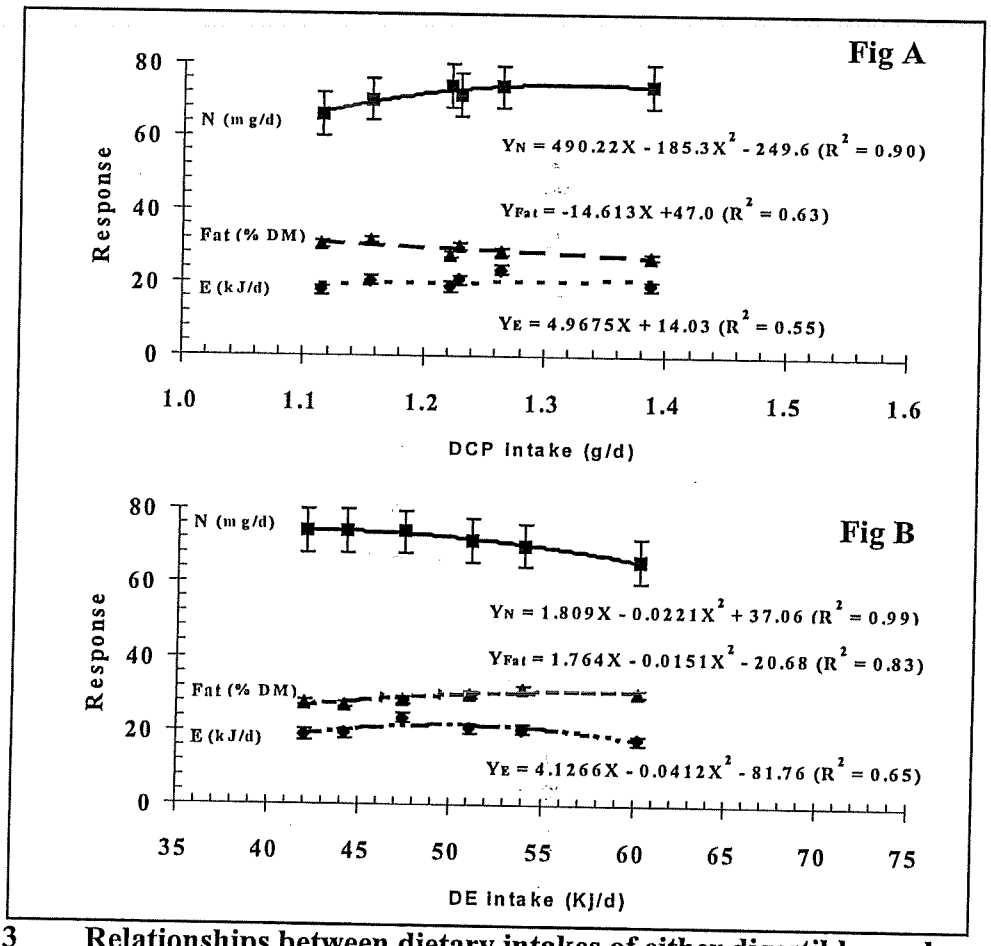

Fig. 6.4.3 **Relationships between dietary intakes of either digestible crude protein (DCP; Fig. A) or digestible energy (DE; Fig B) and nitrogen and energy retentions and fat composition of the fish in Experiment Wlk 6.**

6.4.3.3 Laboratory Experiment Wlk 7

No fish were lost during the experiment and almost all of the presented food was avidly consumed by the fish. Table 6.4.11 shows the productivity responses of the fish.

Table 6.4.11 Productivity responses of barramundi in Experiment Wlk 7

Diet (DCP)	Feed rate (% fish $wt^{0.48}$)				Mean	±sem
	15	20	25	30		(Diet x Rate)
			Average daily gain (g/d)			
W6-3 (37.0)	1.22	1.86	2.34	2.92	2.08	
W6-5 (47.2)	1.19	1.72	2.26	2.90	2.02	0.054
Mean	1.20^{P}	1.79^{Q}	2.30^{R}	2.91^{S}		
			Specific growth rate (%/d)			
W6-3 (37.0)	0.48	0.68	0.84	1.06	0.76	
W6-5 (47.2)	0.46	0.67	0.87	1.03	0.76	0.020
Mean	0.47^{P}	0.67^{Q}	0.86^{R}	1.05^{S}		
			Food conversion ratio (g as fed food: g wet fish gain)			
W6-3 (37.0)	1.60	1.43	1.42	1.35	1.45^{X}	
W6-5 (47.2)	1.28	1.16	1.11	1.07	1.16^{Y}	0.037
Mean	1.44^{P}	1.30^{Q}	1.26^{QR}	1.21^{R}		
			Daily food intake (g/d)			
W6-3 (37.0)	1.93	2.67	3.31	3.95	2.96^{X}	
W6-5 (47.2)	1.52	2.00	2.49	3.10	2.28^{Y}	0.37
Mean	1.72^{P}	2.33^{Q}	2.90^{R}	3.52^{S}		

$^{P,Q,R;\ S,\ X,Y}$ Within interaction and main effect comparisons, means without a common letter differ (P<0.05).

DFI increased in accordance with the planned feeding scale and the proportional difference in intake between diets W6-5 and W6-3 at each of the feeding rates was maintained close to the intended 0.78 (mean and range of 0.77 and 0.75 to 0.79, respectively). Growth rate of fish progressively increased with higher feeding rate but there was no difference between the two diets. However, FCR of the energy-limiting diet (W6-5) was markedly better than for the protein-limiting diet (W6-3) and for each diet, FCR progressively improved with increasing food allowance. Relationships between growth rate and intakes of either DCP or DE are shown in Figure 6.4.4.

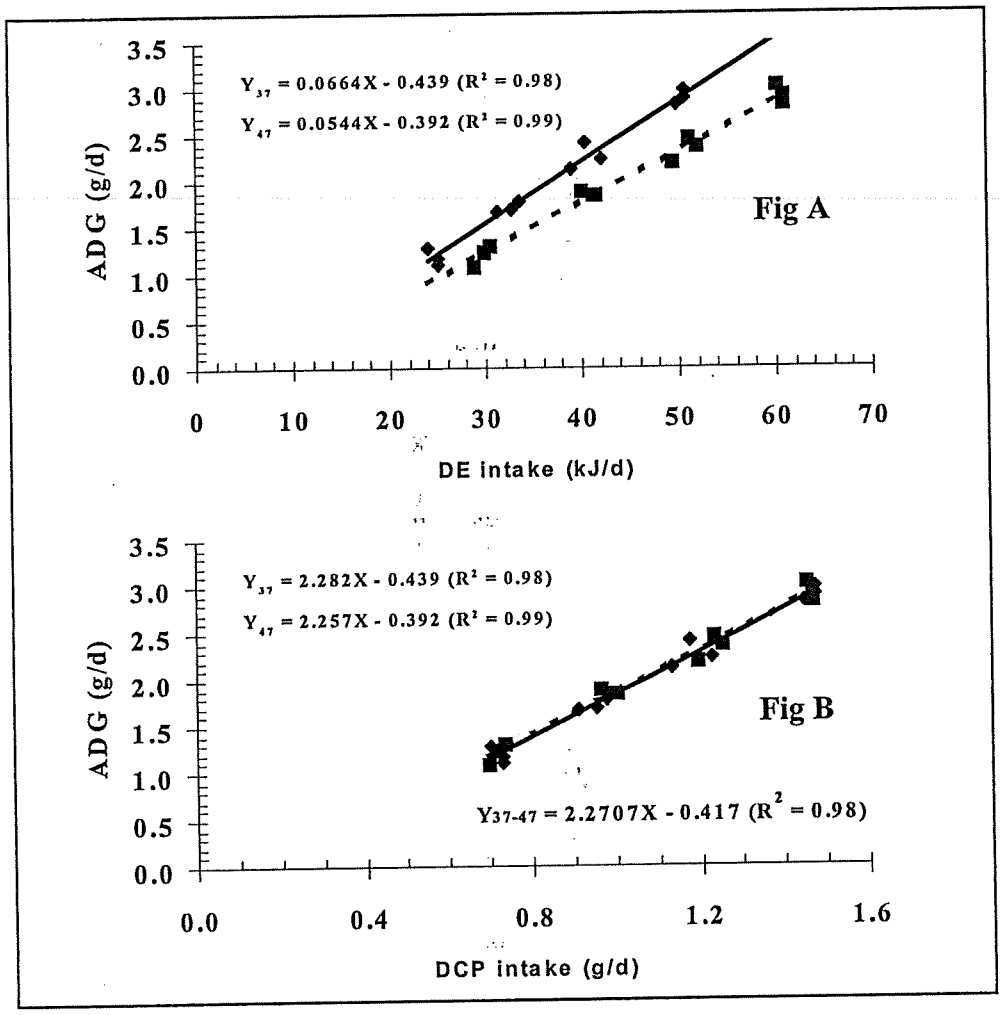

Fig. 6.4.4 **Relationships between growth rate and intakes of either DE (Fig A) or DCP (Fig B) for diets that were either protein-limiting (Diet W6-3; Y_{37}; ◆) or energy-limiting (Diet W6-5; Y_{47}; ■) and fed at prescribed feeding rates in Experiment Wlk 7.**

For each of the diets, growth rate increased linearly with increasing intake of DCP or DE. Relationships for the two diets were coincident ($P>0.05$) for DCP intake but differed significantly in their intercepts and slopes ($P<0.05$) for DE intake.

The chemical composition of the fish at the start and at the conclusion of the experiment is shown in Table 6.4.12. Fat and energy content of the fish increased and fat-free ash content decreased with feeding rate ($P<0.05$). Even at the lowest rate of feeding, the fat content of the fish at the end of the experiment was greater than that of fish sampled at the start. Differences between the two dietary treatments were confined to fat content which was greater ($P<0.05$) for fish fed on the protein-limiting diet (W6-3).

Table 6.4.12 Chemical composition of the fish at the end and start of Experiment Wlk 7

Diet (DCP)	*Feed rate (% fish $wt^{0.48}$)*						Initial fish (±SD)
	15	20	25	30	Mean	±sem[1]	
			DM content (%) [2]				
W6-3 (37.0)	32.2	33.0	31.9	33.4	32.6		29.7±0.65
W6-5 (47.2)	32.7	32.2	31.6	32.5	32.2	0.71	
Mean	32.4	32.6	31.8	33.0			
			DM fat content (%) [2]				
W6-3 (37.0)	21.6	25.9	26.5	28.0	25.5^{X}		20.5±1.13
W6-5 (47.2)	22.9	22.4	24.9	24.5	23.7^{Y}	0.99	
Mean	22.3^{P}	24.2^{PQ}	25.7^{Q}	26.3^{Q}			
			Gross energy content (kJ/g) [2]				
W6-3 (37.0)	22.90	23.77	23.33	24.00	23.5		22.10±0.141
W6-5 (47.2)	23.10	23.20	23.33	23.47	23.3	0.234	
Mean	23.00^{P}	23.48^{PQ}	23.33^{PQ}	23.73^{Q}			
			DM ash fat-free content (%) [2]				
W6-3 (37.0)	19.4	19.4	18.9	18.6	19.1		20.9±0.41
W6-5 (47.2)	19.1	18.8	18.9	18.2	18.7	0.29	
Mean	19.3^{P}	19.1^{P}	18.9^{PQ}	18.4^{Q}			
			DM crude protein fat-free content (%) [2]				
W6-3 (37.0)	79.0	79.2	77.1	78.8	78.5		77.4±1.23
W6-5 (47.2)	78.8	78.8	79.5	80.3	79.3	0.68	
Mean	78.9	79.0	78.3	79.5			

[1] Standard error of mean for feed frequency x water temperature interaction term.
[2] ^{P,Q,X,Y} Within interaction and main effect comparisons, means without a common letter differ (P<0.05).

The derived protein and energy retention efficiencies are given in Table 6.4.13 and relationships between intake and nutrient accretion are illustrated in Fig. 6.4.5.

Table 6.4.13 Effect of diet and feeding rate on efficiencies of dietary protein and energy retentions in Experiment Wlk 7

Diet (DCP)	Feed rate (% fish $wt^{0.48}$)				Mean	±sem[1]
	15	20	25	30		
			Crude protein (%)			
W6-3 (37.0)	39.6	37.7	30.9	37.1	36.4	
W6-5 (47.2)	36.9	36.7	34.5	39.3	36.9	2.74
Mean	38.3	37.2	32.7	38.2		
			Digestible protein (%) [2]			
W6-3 (37.0)	42.9	40.8	33.4	40.1	39.3	
W6-5 (47.2)	41.4	41.3	38.8	44.1	41.4	3.03
Mean	42.1	41.0	36.1	42.1		
			Gross energy (%) [2]			
W6-3 (37.0)	33.4	39.4	35.6	41.8	37.5^{X}	
W6-5 (47.2)	45.0	43.1	44.3	47.5	44.9^{Y}	2.60
Mean	39.2	41.2	39.9	44.6		
			Digestible energy (%) [2]			
W6-3 (37.0)	40.9	48.3	43.6	51.1	46.0^{X}	
W6-5 (47.2)	52.3	50.0	51.4	55.1	52.2^{Y}	3.13
Mean	46.6	49.1	47.5	53.1		

[1] Standard error of mean for feed frequency x water temperature interaction term.
[2] ^{X,Y} Within interaction and main effect comparisons, means without a common letter differ (P<0.05).

Feeding rate did not significantly (P>0.05) affect the efficiency with which protein or energy was retained by the fish. Similarly, diet had no affect on the efficiency of protein retention but the efficiency of energy retention was better (P<0.05) for the energy-limiting diet (W6-5) than for the protein-limiting diet (W6-3). The rate at which dietary N (as DCP) was accreted was significantly better (P<0.05) for the energy-limiting diet than for the protein-limiting diet with the derived biological value of the protein in these diets being 0.46 and 0.34 respectively (Fig A of Fig. 6.4.5). The rate of N accretion was also significantly affected by DE intake with a higher rate (P<0.05) being observed for the energy-limiting diet. The rate at which dietary energy (as DE) was accreted was similar (P>0.05) for the two diets ('b' values each of 0.58) but absolute accretion was higher (P<0.05) for the energy-limiting diet (Fig D of Fig. 6.4.5). No differences were seen between the two diets in the way DCP intake affected energy accretion.

6.4.4 Discussion

Increasing the DE (from 13.9 to 16.4 kJ/g) or increasing the DCP (from 27 to 34%) content of the diet in the on-farm cage experiment (Wlk 4) had little effect on either growth performance or sensory characteristics of the barramundi. However, this result must be interpreted carefully because of the low statistical power of the experiment and the relatively low water temperatures during a large part of the experiment. Both of these effects would reduce the likelihood of finding significant differences between the diets. There was some indication that increasing either the energy or the protein content of the diet resulted in a favourable enhancement of growth and food conversion.

Under controlled laboratory conditions, increasing the amount of a balanced high-quality protein in the diet in Experiment Wlk 6 had a marked effect on growth performance and nutrient retention of the barramundi. As the protein content of the diet increased, voluntary food intake progressively declined while FCR similarly improved such that growth rate increased to an asymptote at a dietary protein content of about 41-42% DCP (= 44-45% CP) and similar to that of Diet W6-4. Interestingly, body accretion of N continued to increase curvilinearly with increasing DCP intake but the efficiency of N retention remained constant at 0.37 until an intake of 1.25 g/d was attained (by fish fed diet W6-4) whereupon efficiency steadily declined to reach 0.33 at an intake of 1.4 g/d (Fig. 6.4.3). This result suggests that for the type of formulations used in Experiment Wlk 6, diets with DCP concentrations lower than about 42% were protein-limiting while those with higher protein concentrations were energy-limiting. The absolute retention of dietary energy also declined at the same point (see Fig B of Fig. 6.4.3) which appears to confirm that the change between protein dependency and energy dependency occurred in the diet series at diet W6-4. The DCP:DE ratio of diet W6-4 was 26.7 mg/kJ or in terms of gross units, equal to 24.5 mg/kJ. This was not greatly different to that of Catacutan and Coloso (1995) who found growth and FCR of barramundi to be optimised with diets that contained either 50% protein and 15% lipid (CP:GE ratio of 23.3 mg/kJ) or 42.5% protein and 10% lipid (CP:GE ratio of 20.9 mg/kJ). These values agree reasonably well with the estimate of 25.8 mg/kJ (CP:GE) for sea bass (barramundi) reported by Tubongbanua (1987).

However, the data of Catacutan and Coloso (1995) showed that DFI increased with increasing dietary protein concentration which contrasted to the decline we observed in Experiment Wlk 7. Moreover, their data revealed an apparent interaction between dietary protein and lipid with DFI being reduced by high lipid content at high but not at low dietary protein contents. This implies that the fish were regulating food consumption to satisfy an energy requirement. The reduced DFI seen in Experiment Wlk 6 may similarly have been due to energy intake being satisfied rather than a response to increasing dietary protein concentration *per se*. Examination of the daily nutrient retention data of Experiment Wlk 7, shows that N retention progressively

declined whereas that of energy remained relatively constant (viz 17 to 21 kJ/d) as DE intake increased from 42 to 62 kJ/d (Fig B of Fig. 6.4.3). This suggests that the fish's capacity to utilise energy either for growth or fat deposition was quite finite. Clearly, there was some capacity for surplus food energy to be deposited as fat since body fat composition increased as a function of DE intake but equally apparent was the ability of the fish to curtail food intake when sufficient protein was consumed to meet requirements for somatic growth.

The interdependency of dietary protein and energy intake was more closely examined in Experiment Wlk 7. The objective of the experiment was to see if protein and energy utilisation in barramundi was similar to, or differed to, that of the terrestrial mammalian model developed in pigs by Campbell (see Campbell, 1988; Fig. 6.4.1). This was done by feeding barramundi either of two diets from the Experiment Wlk 6 series which were considered to be either protein-limiting (Diet W6-3) or energy-limiting (Diet W6-5) based on the observed growth and protein deposition responses of the fish in that experiment. These two diets were fed at either of four controlled feeding rates but the rates for each diet were varied to ensure that the daily intake of protein, but not energy, was the same for each diet. Since the DCP concentration of the two diets differed markedly from each other (viz 37 vs 47%) it meant that the daily food allocation for Diet W6-5 was set at 78% (i.e 37/47) of that of Diet W6-3 at each of the four feeding rates. Even though the DE content of Diet W6-5 was slightly higher than that of Diet W6-3 (viz.16.23 vs 15.35 kJ/g, respectively), the daily DE intake of fish on the energy-limiting diet (W6-5) was only about 80% of that of fish fed the protein-limiting diet (W6-3).

If barramundi responded similarly to terrestrial mammals, it would be expected that the efficiency of protein retention would be identical for each of the diets. Furthermore, protein deposition would increase linearly with increasing DCP intake for the protein-limiting diet but for the energy-limiting diet, protein deposition would be limited by energy, rather than protein intake. Instead, the efficiency of protein retention of DCP differed between the diets, being significantly higher for the higher protein (47% DCP) 'energy-limiting' diet (W6-5) than for the lower protein (37% DCP) 'protein-limiting' diet (W6-3) (0.46 vs 0.34, respectively; Fig A of Fig. 6.4.5). Moreover, at the same daily intake of DE, N accretion was significantly greater ($P<0.05$) for Diet W6-5 compared to Diet W6-3 and this difference widened with increasing DE intake (Fig C of Fig. 6.4.5). Thus, protein deposition in barramundi was more dependent on dietary energy intake than on dietary protein intake and this contrasts with that seen in the pig where protein deposition is regulated by dietary intake of both protein and energy. However, this is not to say that protein deposition in barramundi is solely dependent on energy intake. Rather, it is interpreted as demonstrating that both diets fed in Experiment Wlk 7 contained an excess of protein such that amino acid requirements for protein synthesis were being fully met. Since an energy insufficiency appeared to be the major factor influencing rate of protein deposition, the enhanced N accretion observed in barramundi fed the 47% DCP diet (W6-5) was most likely due to the excess dietary protein in that diet being a more available source of metabolic energy than the 37% DCP diet (W6-3). This would explain why the efficiency of protein retention was higher for diet W6-5 than for diet W6-3 even though the composition and intake of the protein were identical for both diets.

Further evidence for the assertion that dietary energy and not protein was the key factor determining protein deposition in barramundi is provided by the poor protein retention rates for the reference protein (highest in Diet W6-5 where DCP retention was 0.46). A protein of similar amino acid composition would be expected to result in a DCP retention rate of ~0.85 in pigs and lambs (Stranks et al., 1988; Campbell, 1988). It might be argued that the low efficiency of dietary protein retention of the reference protein seen in Experiment Wlk 7 was due to the amino acid composition of the protein being suboptimal for barramundi. However, this seems unlikely. Not only were the constituents of the reference protein all highly digestible but its amino acid composition closely mimicked that of barramundi protein (Table

6.4.4) and thus was expected to have a very high biological value. Even if the amino acid composition of the reference protein was suboptimal for barramundi, increasing the concentration of the reference protein in the diet could not be expected to improve its biological value. Since protein retention of the reference protein was observed to increase in Experiment Wlk 7, it must be interpreted as an effect of energy and not protein supply. Thus, dietary protein in excess of that needed to provide amino acids for body protein synthesis will be catabolised and the energy made available to support other metabolic functions, including an enhanced rate of protein deposition which otherwise was being constrained by an insufficiency of energy. Barramundi appear capable of using the excess protein for energetic functions very efficiently (viz. 0.58; Fig D of Fig 6.4.5) and more effectively than other dietary energy sources such as carbohydrate. This would explain both the greater energy retention and the better N retention of fish fed Diet W6-5 compared to those fed Diet W6-3.

It is interesting to speculate what would happen if the protein content of the diet was substantially reduced so that dietary amino acid supply more closely matched that required only for protein synthesis. One would anticipate that the efficiency of protein retention would improve as more and more of the dietary protein was retained as body protein and less catabolised for energy. At some point, protein supply would genuinely limit the supply of amino acids available for protein synthesis and thus, a dependency of protein synthesis on dietary protein (amino acids) would be exhibited. An indication of such an effect was seen in Experiment Wlk 6 where retention of dietary DCP increased with decreasing dietary protein concentration (Table 6.4.10). However, the confounding of energy and protein intakes in the experiment because fish were able to compensate for the nutrient composition of the diet by voluntarily varying food intake, makes it difficult to fully interpret the retention data. An improvement in retention of dietary protein with decreasing dietary protein concentration has also been observed with both carnivorous (Sabaut and Luquet, 1973; Pieper and Pfeffer, 1980; Arzel et al., 1995) and herbivorous fish (Ogino and Saito, 1970; Das et al., 1991) while an opposite effect was reported with European sea bass by Hidalgo and Alliot (1988).

The experiments failed to adequately define the dietary protein specification that would maximise productivity (in terms of growth rate and economy of food conversion) of barramundi. Nor was it possible to accurately define an optimal dietary protein to energy ratio. When barramundi were fed semi-purified diets providing serially incremented concentrations of a high quality reference protein in Experiment Wlk 6, growth rate, FCR and N accretion were optimised with a diet that contained about 42% DCP and 15.8 kJ/g DE, suggesting an optimum DCP:DE ratio of about 26 mg/kJ. However, these fish were fed twice daily to appetite and DFI was observed to decline markedly with increasing protein content of the diet. Examination of the N and energy retention data of the experiment led to the notion that energy and not protein was limiting the growth rate of barramundi. In Experiment Wlk 7, controlled feeding of two diets from the Experiment Wlk 6 diet series – one above and one below the apparent optimum of 42% DCP – confirmed that energy and not protein intake was regulating growth rate and N deposition of the barramundi. Barramundi fed the highest protein diets in Experiment Wlk 6 voluntarily reduced food intake to two-thirds of that eaten by fish fed the lowest protein diets, indicating that the fish's physical gut capacity was apparently not the factor limiting energy consumption of the high protein diets. It was initially thought that the reduced food consumption of fish fed these high protein diets was a response to excessive protein intake. However, this appears not to be the case since retention data from Experiment Wlk 7 show that excess protein was efficiently, and perhaps preferentially utilised for energy. In light of this finding, it must now be concluded that the marked decline in voluntary food intake of fish in Experiment Wlk 6 was not due to accompanying increased protein content. Rather, it would seem that other factors were responsible and perhaps the unpalatability induced by increasing inclusions of casein and/or diatomaceous earth was the reason. Subsequent work (Williams et al., 1998) has shown that casein, while highly digestible, is

disliked by barramundi and that diatomaceous earth can similarly restrict intake. For these reasons, it would be unwise to conclude that a dietary DCP content of 42% and a DCP:DE ratio of 26.5 mg/kJ as suggested from the data of Experiment Wlk 6 are optimal dietary specifications for juvenile barramundi. However, the experiments have demonstrated the importance of maximising dietary energy intake as a means of maximising rates of protein deposition. In this regard, the response of barramundi is not unlike that exhibited by terrestrial mammals. Further research is needed to characterise the response of barramundi to protein intake in diets of higher energy content.

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6.5 Essential lipid (fatty acid) requirements of barramundi

6.5.1 Background

The inclusion of lipid (or fat) in the diet has two primary functions – a non-specific role as a source of metabolic and storage energy and a specific role as a source of essential nutrients. Lipid is the most dense source of energy of all food classes, typically containing about 40 kJ/g DM of gross energy as compared to that of protein and carbohydrate which contain only 20-23 and 16-18 kJ/g DM, respectively. While this aspect has far-reaching implications on dietary formulation and productivity/profitability of fish farming, it is the role of lipid as a source of essential nutrients that will be the primary focus of the work to be reported in this section. And specifically, attention will focus on lipid as a source of essential fatty acids (EFA) rather than other essential constituents such as fat-soluble vitamins, phospholipids, sterols, carotenoids, etc.

A fatty acid is an elongated chain of methyl units (CH_3) that are covalently joined together with a single terminal carboxyl (COOH) group. Fatty acid chains vary both in the number of methyl units present and the number and position of double bonds (unsaturated bonds) they contain. In this report, the nomenclature used to describe fatty acids will follow that recommended by The British Journal of Nutrition and which in turn, is based on the International Union of Pure and Applied Chemistry – International Union of Biochemistry (IUPAC-IUB) except that numbering of the carbon atoms is from the terminal methyl group rather than from the carboxyl end. Hence, linolenic acid with 18 carbon atoms and three double bonds (situated between the 3rd and 4th, 6th and 7th and 9th and 10th carbon atoms from the methyl terminus) is abbreviated as 18:3n-3 where the '18' refers to the number of carbon atoms in the chain, the ':3' refers to the number of double bonds in the chain and the 'n-3' refers to the position of the first double bond from the methyl terminus. Fatty acids that contain no double bonds are collectively referred to as saturated fatty acids (SFAs) while those containing either one, two and three or four and more double bonds are respectively referred to as mono-unsaturated (MUFAs), poly-unsaturated (PUFAs) or highly-unsaturated (HUFAs) fatty acids. Additionally, those unsaturated fatty acids that have the first double bond from the methyl terminus in the n-3 position are collectively referred to as the n-3 or α-linolenic series fatty acids, and those in the n-6 position are referred to as the n-6 or linoleic series fatty acids.

In addition to their non-specific energy role, fatty acids have important functions in regulating the fluidity and permeability of cell membranes (as constituents of phospholipids and lipoproteins), in transport of lipid and protein, the activation of enzymes, and as constituents of many other metabolically active compounds such as steroid and eicosanoid (prostaglandin) hormones etc (Tinoco, 1982; Crawford, 1992). Importantly, these functions are dependent on the presence of PUFAs and HUFAs for their activity. All living organisms have the capacity to synthesize fatty acids *de novo* by the sequential addition or removal of 2-carbon methyl units and to synthesize 16:1n-7 and 18:1n-9 MUFAs by desaturation of their respective saturated forms (Sargent, 1976; Tinoco, 1982; Smith et al., 1983; Rawn, 1989). However, the ability to further desaturate MUFAs to form PUFAs and HUFAs and to synthesize the n-3 series fatty acids differs considerably between and within the plant and animal kingdoms (see Tinoco, 1982 for a comprehensive review). Photosynthetic processes enable algae and lower plant forms such as mosses, liverwort and ferns to synthesize n-3 and n-6 HUFAs whereas higher plants are limited in not being able to synthesize HUFAs but can synthesize 18:3n-3, primarily from 18:2n-6 but in some plants also from 16:3n-3. In vertebrate animals, the n-3 and n-6 fatty acid series can not be synthesized *de novo* but must come from the diet or as precursors that are present in the food. Thus for these animals, the n-3 and n-6 fatty acids or their precursors are clearly essential nutrients that must be supplied in the diet to meet the animal's requirement. However, the efficiency with which the precursors 18:2n-6 and 18:3n-3 can be desaturated and

elongated to their respective C-20 and C-22 HUFAs varies greatly between phyla and even within taxonomically similar classes. For example in the fishes, rainbow trout *Salmo gairdneri*, carp *Cyprinus carpio* and coho salmon *Oncorhynchus kisutch* can efficiently desaturate and elongate n-3 and n-6 fatty acids whereas channel catfish *Ictalurus punctatus*, turbot *Scophthalamus maximus*, chum salmon *O.* keta, red sea bream *Chrysophrys major*, gourami *Trichogaster cosby*, and eel *Anguilla japonica*, all have limited ability to produce the longer chain HUFAs (Tinoco, 1982, Watanabe, 1982; Millikin, 1982). Thus the latter group of fishes can be said to have an essential dietary requirement for individual HUFAs whereas the former group of fishes can be thought to have an essential dietary requirement for only the precursor fatty acids, 18:2n-6 and 18:3n-3. As a generalization, marine fish have a more limited ability to desaturate and elongate fatty acids than freshwater fishes but clearly there are exceptions to this general rule such as for channel catfish and coho salmon. Equally, the essentiality for n-3 fatty acids is not universal since tilapia *Tilapia zillii*, has a requirement for n-6 rather than for n-3 acids (Kanazawa et al., 1980).

6.5.2 Introduction

There are marked interspecies differences between fishes in their dietary essentiality for n-3 and n-6 fatty acids and with startling differences even between closely related fish. For example, for optimal survival and growth, chum salmon (*O. keta*) required 1% of both 18:2n-6 and 18:3n-3 in the diet (Takeuchi et al., 1979), coho salmon (*O. kisutch*) required 1 to 2.5% of 18:3n-3 but more than 1% of 18:2n-6 was detrimental (Yu and Sinnhuber, 1979) and rainbow trout (*S. gairdneri*) required 0.8 to 1.6% of 18:3n-3 but with no demonstrated requirement for n-6 fatty acids (Castell et al., 1972a,b,c; Watanabe et al., 1974a,b).

In addition to the essentiality for individual EFAs, there is mounting evidence that dietary requirements are affected by the amount and types of other fatty acids contained in the diet. Since HUFAs have critical metabolic functions, it is not surprising that supplying these preformed in the diet would be more advantageous to the animal than in the form of their C-18 precursors. Thus as reported by Watanabe and Takeuchi (Watanabe and Takeuchi, 1976; Takeuchi and Watanabe, 1976, 1977, 1978), diets containing 0.25% of 22:6n-3 or 0.25% of a mixture of equal parts of 22:6n-3 and 20:5n-3 were as effective for rainbow trout as one containing twice as much of 18:3n-3. Similarly, a dietary supply of 22:6n-3 was shown to be superior to 20:5n-3 in promoting survival and growth of marine fish larvae such as red sea bream (Watanabe et al., 1989), yellowtail (Furuita et al., 1996), striped jack (Takeuchi et al. 1996) and cod (Zheng et al., 1996) but their efficacy was similar for Japanese flounder (Furuita et al., 1998, 1999). Conversely, 20:5n-3 was more effective than 22:6n-3 for plaice larvae (Dickey-Collas and Geffen, 1992). Providing an excess amount of n-3 HUFA in the diet can also have adverse effects on growth and food utilization. Increasing the dietary content of 18:3n-3, 20:5n-3 or 22:6n-3 to 4-times the requirement level resulted in poor growth and FCRs and signs of EFA-deficiency in rainbow trout (Takeuchi and Watanabe, 1976). Increasing the dietary content of 18:2n-6 above 1% or high amounts of n-3 fatty acids was found by Yu and Sinnhuber (1976, 1979) to be detrimental to the growth of rainbow trout and coho salmon.

As is the case with essential amino acids, the balance between the individual EFAs and particularly the ratio of n-3 to n-6 fatty acids may be as important in determining the animal's requirements as the actual amount of each EFA present in the diet. This interaction between EFAs has most clearly been shown by Glencross and Smith (1999) working with the tiger shrimp *Penaeus monodon*. They fed prawns on semi-purified diets in which the only sources of n-3 or n-6 fatty acids were 18:3n-3 or 18:2n-6 respectively. In a 5x5 factorial comparison, each of these fatty acids was incrementally increased from 0 to 32% of the total neutral lipid fatty acid content (6%) of the diet. The study showed that the requirement for the respective EFA varied as a function of the amount of the other present in the diet. Including only 18:2n-6 or

18:3n-3 in the diet resulted in prawn growth being optimised when each was present at the 16% level; when added into the diet together, growth was significantly better and was highest at dietary inclusions of 8% of 18:2n-6 and 24% of 18:3n-3, i.e. at a n-3 to n-6 ratio of 3:1. As the n-3 to n-6 ratio of the diet varied from 3:1, prawn growth decreased with this effect being more pronounced as 18:2n-6 became more abundant. The importance of the n-3 to n-6 ratio as a determinant of EFA requirement of fish had earlier been noted by Yu and Sinnhuber (1979), Watanabe (1982) and Henderson and Tocher (1987).

There have been few reported studies on the EFA requirement of barramundi. Boonyaratpalin (1991, 1997) cites work of Buranapanidgit et al. (1988, 1989) that was done at the National Institute of Coastal Aquaculture, Songkhla, Thailand, where the dietary requirement for n-3 HUFA was found to be between 1.0 and 1.7% for a diet that contained about 13% total lipid. For 25-day old fry, a 1 to 1 ratio of cod liver oil and soybean oil at a total dietary inclusion of 9% resulted in better growth of the fish than those fed equivalent amounts of either of the oils alone or a 1:1 blend of each with coconut oil. The n-3 and n-6 contents of the cod liver oil and soybean oil diet were each 2.0% (1:1 n-3 to n-6 ratio) while those of the cod liver oil-only diet were 2.6 and 0.65% (4:1 n-3 to n-6 ratio), respectively.

Two experiments were carried out in this Project to further define the EFA requirement of juvenile barramundi. The first experiment (Wlk 3) examined the effect of varying both the energy density and the amounts and proportions of n-3 and n-6 fatty acids in the diet when juvenile barramundi were reared at either cool or warm water temperatures. In the second experiment (Wlk 5), the amounts of n-3 and n-6 fatty acids in the diet were varied serially to provide a wide range of n-3 to n-6 ratios but when dietary energy, protein and lipid contents were held constant and the fish again reared at either cool or warm water temperatures.

6.5.3 Methods

The general methods for laboratory growth assays have been described in Section 6.1. Specific details for each experiment are set out below.

6.5.3.1 Experiment Wlk 3

Six practical diets (Table 6.5.1) were formulated to compare three dietary DE densities (~14, 16 or 18 kJ/g) at either of two n-3:n-6 ratios (~1:1 or 1.5:1). Energy differences between the diets were achieved by varying the dietary inclusion of lipid while keeping the DCP:DE ratio of the diet approximately the same through the concomitant adjustment of dietary protein (casein); these changes were made at the expense of wheat offal. The n-3 to n-6 ratio was varied by manipulating the proportions of fish and soybean oils used in the diet. Since the total amount of the oil used in the diet was increased to achieve the desired increases in dietary DE density, the absolute amounts of fatty acids also increased with energy density. The proportions of the two oils used in the diets were intended to result in n-3 to n-6 fatty acid ratios that were either 1:1 or 1.5:1 for each of the three formulated dietary DE densities. However, as can be seen from the determined chemical composition of the diets (Table 6.5.2), this intended ratios were only poorly approximated.

Table 6.5.1 Formulation (% as used) of diets fed in Experiment Wlk 3

Feed ingredient	Diet					
	W3-1	W3-2	W3-3	W3-4	W3-5	W3-6
Fishmeal (Danish)	30.0	30.0	30.0	30.0	30.0	30.0
Casein	4.0	11.0	18.0	4.0	11.0	18.0
Soybean meal (full-fat)	3.5	3.5	3.5	3.5	3.5	3.5
Wheat gluten	6.0	6.0	6.0	6.0	6.0	6.0
Wheat flour	18.0	18.0	18.0	18.0	18.0	18.0
Wheal offal	20.0	12.75	5.5	20.0	12.75	5.5
Soybean oil	4.0	6.0	8.0	1.5	2.75	4.0
Fish oil (Chilean)	2.0	3.75	5.5	4.5	7.0	9.5
Starch	7.1	3.7	0.3	7.1	3.7	0.3
l-Lysine HCl	0.2	0.1	0	0.2	0.1	0
Salt	0.2	0.2	0.2	0.2	0.2	0.2
Mineral premix[1]	0.5	0.5	0.5	0.5	0.5	0.5
Vitamin premix[1]	3.0	3.0	3.0	3.0	3.0	3.0

[1] Reformulated to supply active vitamins and trace minerals at dietary inclusions as detailed in Table 6.2.2.

Table 6.5.2 Determined chemical composition (air-dry basis) of the diets fed in Experiment Wlk 3

Analysis	Diet					
	W3-1	W3-2	W3-3	W3-4	W3-5	W3-6
Dry matter (%)	89.3	88.5	89.3	89.8	89.9	90.2
Ash (%)	5.8	5.5	5.2	5.6	5.4	5.1
Crude protein (%)	38.5	42.8	46.7	39.1	42.9	47.5
Digestible protein (%)[1]	34.4	38.5	42.4	34.9	38.6	43.1
Crude fat (%)	11.8	14.7	15.9	12.0	14.7	17.7
Crude fibre (%)	2.7	1.9	1.8	nd	nd	nd
Calcium (%)	1.5	1.5	1.5	1.5	1.5	1.5
Phosphorus (%)	0.9	0.9	0.9	0.9	0.9	0.9
Amino acids (%)						
Lysine	2.52	2.33	2.25	nd	nd	nd
Methionine + cystine	1.32	1.44	1.56	nd	nd	nd
Threonine	1.57	1.45	1.43	nd	nd	nd
Arginine	2.00	1.74	1.60	nd	nd	nd
Fatty acids (%)						
18:2n-6	1.95	2.07	2.22	1.58	1.63	1.56
18:3n-3	0.47	0.59	0.71	0.31	0.41	0.47
20:5n-3	0.38	0.48	0.58	0.55	0.72	0.84
22:6n-3	0.58	0.74	0.86	0.83	1.05	1.20
Σ n-3 fatty acids	1.63	2.12	2.45	1.96	2.55	2.95
Σ n-6 fatty acids	2.01	2.15	2.29	1.64	1.72	1.68
n-3: n-6 ratio	0.8	1.0	1.1	1.2	1.5	1.8
Gross energy (kJ/g)	19.47	20.27	21.80	19.49	20.50	21.56
Digestible energy (kJ/g)[1]	14.05	15.94	18.30	14.06	16.12	18.10
Protein:energy (mg:kJ)						
CP:GE	19.8	21.1	21.4	20.1	20.9	22.0
DCP:DE	24.5	24.2	23.2	24.8	23.9	23.8

[1] Mean of estimates derived using either intestinal dissection or 3-h sedimentation procedures (Williams, 1998).
nd Not determined.

The six diets were fed to 720 barramundi fingerlings of mean (± SD) initial weight of 59 ± 1.8 g which were stocked equally into 24 tanks (800 L capacity; 30 fish/tank) arranged as four independent freshwater recirculation systems at QDPI's Walkamin laboratory. A representative

sample of 15 fish from the same cohort as used in the experiment were taken for chemical analysis immediately prior to the start of the experiment. Two of the recirculation systems were maintained at 20°C and the other two at 29°C . Thus, the experiment comprised a 3x2x2 factorial comparison of six diets (3 DE densities x 2 n-3 to n-6 ratios) and 2 water temperatures with 2 complete replications. The fish were acclimatized to the conditions for 14 d prior to the start and during this time a prophylactic salt bath (12 ppt for 2 h) was administered. During the experiment, fish were fed twice daily (at 0830 and 1630 h) to apparent satiety except on the day of the fortnightly weighing when the fish were not fed. Photoperiod in the laboratory was 12:12. Fish held at 29°C water temperature remained on experiment for 8 weeks while those held at 20°C were continued for a further 2 weeks to increase the overall weight gain of these fish. At termination, a representative sample of 5 fish was taken from each tank for chemical analysis.

6.5.3.2 Experiment Wlk 5

Six diets were formulated to examine the effect dietary n-3 to n-6 fatty acid ratio has on the productivity of fish that were held at water temperatures of either 20 or 29°C. By incrementally varying the proportions of soybean oil and fish oil but not the total amount of oil in the diet, the dietary n-3 to n-6 ratio was varied from 0.5:1 to 2.2:1 while all other constituents of the diet were held constant (Table 6.5.3). The chemical composition of the diets is given in Table 6.5.4.

Table 6.5.3 Formulation of diets fed in Experiment Wlk 5

Feed ingredient	Diet					
	W5-1	W5-2	W5-3	W5-4	W5-5	W5-6
			Formulation (%)			
Fish (Chilean)	20.0		All	diets		20.0
Soybean meal (full fat)	4.0		"	"		4.0
Wheat gluten	7.0		"	"		7.0
Casein	18.0		"	"		18.0
Yeast - torula	2.5		"	"		2.5
Wheat flour (gelled)	30.0		"	"		30.0
Starch	2.5		"	"		2.5
l-Lysine HCl	0.1		"	"		0.1
d/l-Methionine	0.1		"	"		0.1
l-Threonine	0.1		"	"		0.1
Soybean oil	1.5	3.5	5.5	7.5	9.5	11.5
Fish oil - Chile	10.5	8.5	6.5	4.5	2.5	0.5
Salt	0.2		All	diets		0.2
Trace mineral premix[1]	0.5		"	"		0.5
Vitamin premix[1]	3.0		"	"		3.0

[1] Provided active vitamins and trace minerals at amounts as detailed in Table 6.2.2.

Table 6.5.4 Determined chemical composition (air-dry basis) of the diets fed in Experiment Wlk 5

Analysis	Diet					
	W5-1	W5-2	W5-3	W5-4	W5-5	W5-6
Dry matter (%)	88.8	91.8	91.0	90.4	90.7	90.6
Ash (%)	4.0	4.2	4.1	4.0	4.2	4.2
Crude protein (%)	43.0	43.8	43.4	43.8	43.9	43.4
Digestible protein (%)[1]	38.6	39.3	39.0	39.4	39.4	39.0
Crude fat (%)	15.5	15.3	15.7	15.4	15.4	15.9
Crude fibre (%)	1.5	0.9	1.1	1.2	1.5	1.4
Amino acids (%)						
Lysine	2.23	2.55	2.39	2.78	2.75	2.71
Methionine + cystine	1.31	1.28	1.24	1.27	1.30	1.34
Threonine	1.64	1.76	1.73	1.77	1.76	1.75
Arginine	1.72	1.93	1.86	1.85	1.89	1.93
Fatty acids (%)						
18:2n-6	1.12	1.52	1.77	2.12	2.18	2.72
18:3n-3	0.29	0.44	0.56	0.70	0.73	0.96
20:5n-3	0.78	0.75	0.59	0.47	0.29	0.18
22:6n-3	1.19	1.15	0.91	0.73	0.46	0.32
Σ n-3 fatty acids	2.82	2.87	2.48	2.24	1.68	1.57
Σ n-6 fatty acids	1.27	1.69	1.90	2.22	2.23	2.75
Σ n-3:Σ n-6	2.22	1.70	1.30	1.0	0.75	0.57
Gross energy (kJ/g)	21.21	21.39	21.29	21.06	21.13	21.20
Digestible energy (kJ/g)[1]	17.24	17.38	17.30	17.12	17.17	17.23
Protein:energy (mg:kJ)						
CP:GE	20.3	20.5	20.4	20.8	20.8	20.5
DCP:DE	22.4	22.6	22.5	23.0	22.9	22.6

[1] Mean of estimates derived using either intestinal dissection or 3-h sedimentation procedures (Williams, 1998).
nd Not determined.

The six diets were fed to 600 barramundi juveniles of mean (± SD) initial weight of 176 ± 3.3 g which were stocked equally into 24 tanks (800 L capacity; 25 fish/tank) arranged as four independent freshwater recirculation systems at QDPI's Walkamin laboratory. A representative sample of 15 fish from the same cohort as used in the experiment was taken for chemical analysis immediately prior to the start of the experiment. Two of the recirculation systems were maintained at 20°C and the other two at 29°C . Thus, the experiment comprised a 6x2 factorial comparison of six diets and 2 water temperatures with 2 complete replications. The fish were acclimatised to the conditions for 14 d prior to the start and during this time a prophylactic salt bath (12 ppt for 2 h) was administered. During the experiment, fish were fed twice daily (at 0830 and 1630 h) to apparent satiety except on the day of the fortnightly weighing when the fish were not fed. Photoperiod in the laboratory was 12:12. Fish held at 29°C water temperature remained on experiment for 4 weeks while those held at 20°C were continued for a further 2 weeks to reduce the otherwise greater disparity in termination weight between these two temperature groups. At termination, a representative sample of 5 fish was taken from each tank for chemical analysis.

6.5.4 Results

6.5.4.1 Experiment Wlk 3

There was no evidence of any disease in the fish or water quality problems in any of the recirculation systems. Five fish 'fainted' and died during handling for weighing. All of these losses occurred in the two high water temperature (29°C) systems and by chance or otherwise

occurred mostly on diets W3-2 (2 deaths) and W3-5 (2 deaths); the other death occurred on diet W3-3. A further loss (diet W3-2 at 29°C) occurred in the last fortnight of the experiment due to one fish escaping from its tank. A low percentage (<1%) of runt fish was observed in a number of tanks: 3 on diet W3-1 and 1 each on diets W3-5 and W3-6 with all of these fish being in the 29°C systems.

Significant ($P<0.05$) interactions were seen between main effects of water temperature, DE density of the diet and dietary n-3:n-6 ratio for a number of productivity and body composition responses (Table 6.5.5). Most of these interactions were between water temperature and DE density of the diet where the response to increasing DE density was less at 20 than at 29°C except for FCR where an opposite effect was apparent. The only interaction involving dietary n-3:n-6 ratio was with DE density where the depression of daily food intake resulting from increases in DE density were less pronounced at 20 than at 29°C.

Table 6.5.5 Interaction effects between water temperature, n-3:n-6 ratio and energy density of the diet for food conversion (FCR), food intake (DFI) and average daily gain (ADG) responses[1] and for dry matter (DM) and fat composition[1] of fish in Experiment Wlk 3

Water temperature (°C)	Energy density of diet (kJ/g) 14	16	18	±sem
	Food conversion ratio (g as fed-food:g fish gain)			
20	2.01^{A}	1.56^{B}	1.19^{C}	
29	1.32^{C}	1.19^{C}	0.97^{D}	0.046
	Daily food intake (g/d)			
20	0.68^{C}	0.66^{C}	0.67^{C}	
29	3.07^{A}	2.98^{A}	2.67^{B}	0.073
	Average daily gain (g/d)			
20	0.34^{C}	0.43^{C}	0.57^{C}	
29	2.33^{B}	2.52^{AB}	2.76^{A}	0.079
	DM composition of fish at end (%)			
20	35.0^{B}	35.4^{B}	36.7^{A}	
29	32.7^{D}	34.2^{C}	35.6^{B}	0.24
	Fat composition of fish at end (% DM)			
20	32.4^{C}	34.3^{B}	38.2^{A}	
29	29.3^{D}	33.8^{BC}	37.7^{A}	0.49
n-3:n-6 ratio	***Daily food intake (g/d)***			
1.0	1.76^{BC}	1.90^{AB}	1.70^{BC}	
1.5	2.00^{A}	1.74^{BC}	1.64^{C}	0.073

[1] $^{A,B,C,D;}$ Within interaction effects, means without a common letter differ ($P<0.05$).

The chemical composition of the fish at the commencement and end of the experiment is detailed in Table 6.5.6. The most marked change in chemical composition over the course of the experiment was an apparent increase in the dry matter and fat content of the fish. Varying the dietary n-3:n-6 ratio had no effect ($P>0.05$) on body composition of the fish. The chemical composition of fish held at 20°C differed ($P<0.05$) from those held at 29°C in having higher contents of dry matter, fat and fat-free ash and lower contents of energy and fat-free protein. Increasing the DE density of the diet resulted in a progressively higher contents of dry matter, fat and energy with these differences being significant ($P<0.05$) between each energy density.

Table 6.5.6 Main effects of water temperature, n-3:n-6 ratio and energy density of the diet on chemical composition[1] of the fish at the start (±SD) and end in Experiment Wlk 3

Diet (%DCP)	DM (%)	Fat (% DM)	GE (kJ/g DM)	Ash (fat-free) (% DM)	CP (fat-free) (% DM)
Initial	31.9±0.39	27.0±1.59	24.87±0.361	18.4±0.24	79.1±0.52
			Water temperature		
20°C	35.7^{A}	35.0^{A}	25.87^{X}	19.2^{A}	81.3^{A}
29°C	34.2^{B}	33.6^{B}	26.26^{Y}	18.4^{B}	82.1^{B}
±sem	0.14	0.28	0.059	0.10	0.24
			n-3:n-6 ratio		
1.0:1	35.0	34.4	26.08	18.9	81.8
1.5:1	34.9	34.2	26.05	18.6	81.6
±sem	0.14	0.28	0.059	0.10	0.24
			Digestible energy density		
14	33.9^{X}	30.8^{X}	25.45^{X}	18.9	81.2
16	34.8^{Y}	34.0^{Y}	26.07^{Y}	18.8	81.6
18	36.1^{Z}	37.0^{Z}	26.67^{Z}	18.7	82.3
±sem	0.17	0.35	0.073	0.12	0.29

[1] A,B; X,Y,Z; Within main effect comparisons, means without a common letter differ (P<0.05).

Productivity and DCP and DE retention responses of the fish to the main treatment effects are shown in Table 6.5.7. No differences were observed between the two n-3:n-6 fatty acid diet series. In addition to the interactive effects shown in Table 6.5.5, daily and specific growth rates were higher (P<0.05) at 29°C

Table 6.5.7 Main effects of water temperature, n-3:n-6 ratio and energy density of the diet on growth rate (ADG), specific growth rate (SGR), food conversion (FCR), food intake (DFI), digestible crude protein retention (DCPR) and digestible energy retention (DER) responses[1] of fish in Experiment Wlk 3

Treatment	ADG (g/d)	SGR (%/d)	FCR (g :g)	DFI (g/d)	DCPR (%)	DER (%)
			Water temperature effect			
20°C	0.45^{A}	0.63^{A}	1.59^{A}	0.67^{A}	32.3^{A}	46.1
29°C	2.54^{B}	2.12^{B}	1.16^{B}	2.91^{B}	42.3^{B}	51.3
±sem	0.046	0.047	0.027	0.042	2.42	2.83
			n-3:n-6 ratio effect			
1.0:1	1.45	1.40	1.41	1.79	35.6	46.6
1.5:1	1.53	1.41	1.33	1.79	39.0	50.8
±sem	0.046	0.047	0.027	0.042	2.42	2.83
			Digestible energy density effect			
14	1.34^{X}	1.27^{X}	1.66^{X}	1.88^{X}	37.5	44.5
16	1.47^{X}	1.36^{XY}	1.37^{Y}	1.82^{XY}	35.3	46.2
18	1.66^{Y}	1.51^{Y}	1.08^{Z}	1.67^{Y}	39.1	55.5
±sem	0.056	0.057	0.033	0.051	2.96	3.46

[1] A,B; X,Y,Z; Within main effect comparisons, means without a common letter differ (P<0.05).

6.5.4.1 Experiment Wlk 5

Three fish deaths occurred in the experiment, all from the 29°C recirculation systems. Two fish (on diets W5-2 and W5-6) 'fainted' and died during weighing handling while the other loss (diet W5-5) was due to an escape from the tank. There were no apparent runt fish at the end of the experiment.

There was a significant ANOVA interaction between the main effects of water temperature and n-3:n-6 ratio for DFI (Table 6.5.8). As the dietary n-3:n-6 ratio was reduced from 2.2:1 to 0.6:1, food consumption increased for fish held at 29°C whereas no such increase in DFI occurred with those held at 20°C.

Table 6.5.8 Interaction effects between water temperature and n-3:n-6 ratio of the diet for daily food intake responses[1] of fish in Experiment Wlk 5

Water temperature (°C)	n3:n6 ratio of diet (kJ/g)						
	2.2	1.7	1.3	1.0	0.8	0.6	±sem
	Daily food intake (g/d)						
20	1.25^{E}	1.19^{E}	1.19^{E}	1.34^{E}	1.31^{E}	1.29^{E}	
29	3.93^{BC}	3.69^{CD}	3.58^{D}	3.88^{C}	4.21^{AB}	4.41^{A}	0.091

[1] $^{A,B,C,D,E;}$ Means without a common letter differ (P<0.05).

Productivity and protein and energy retention responses of the fish to the main effects are given in Table 6.5.9. Fish held at 29°C grew faster, converted food more efficiently and had a higher retention of DCP than those at 20°C; retention of energy appeared to be higher for fish held at 29°C compared to those at 20°C but this was not significant (P>0.05). Decreasing the n-3:n-6 fatty acid ratio from 2.2:1 to 0.6:1 caused a worsening (P<0.05) of specific growth rate, FCR and retention of DCP and similar but not significant (P>0.05) effects for ADG and retention of energy.

Table 6.5.9 Main effects of water temperature and n-3:n-6 ratio of the diet on growth rate (ADG), specific growth rate (SGR), food conversion (FCR), food intake (DFI), digestible crude protein retention (DCPR) and digestible energy retention (DER) responses[1] of fish in Experiment Wlk 5

Treatment	ADG (g/d)	SGR (%/d)	FCR (g :g)	DFI (g/d)	DCPR (%)	DER (%)
			Water temperature effect			
20°C	0.74^{X}	0.40^{X}	1.70^{X}	1.26^{X}	39.7^{X}	51.4
29°C	3.18^{Y}	1.44^{Y}	1.25^{Y}	3.95^{Y}	42.9^{Y}	52.4
±sem	0.042	0.013	0.031	0.037	0.93	1.49
			n-3:n-6 ratio effect			
2.2	2.05	0.96^{AB}	1.43^{A}	2.59^{BC}	43.8^{AB}	52.0
1.7	2.11	0.97^{A}	1.30^{A}	2.44^{CD}	47.0^{A}	56.4
1.3	1.91	0.90^{BCD}	1.42^{A}	2.38^{D}	42.7^{AB}	53.3
1.0	1.98	0.95^{ABC}	1.44^{AB}	2.61^{BC}	40.7^{BC}	54.0
0.8	1.89	0.88^{CD}	1.60^{BC}	2.76^{AB}	37.6^{C}	49.3
0.6	1.82	0.86^{D}	1.67^{C}	2.85^{A}	36.0^{C}	46.5
±sem	0.073	0.022	0.055	0.064	1.60	2.58

[1] $^{A,B,C,D;\ X,Y;}$ Within main effect comparisons, means without a common letter differ (P<0.05).

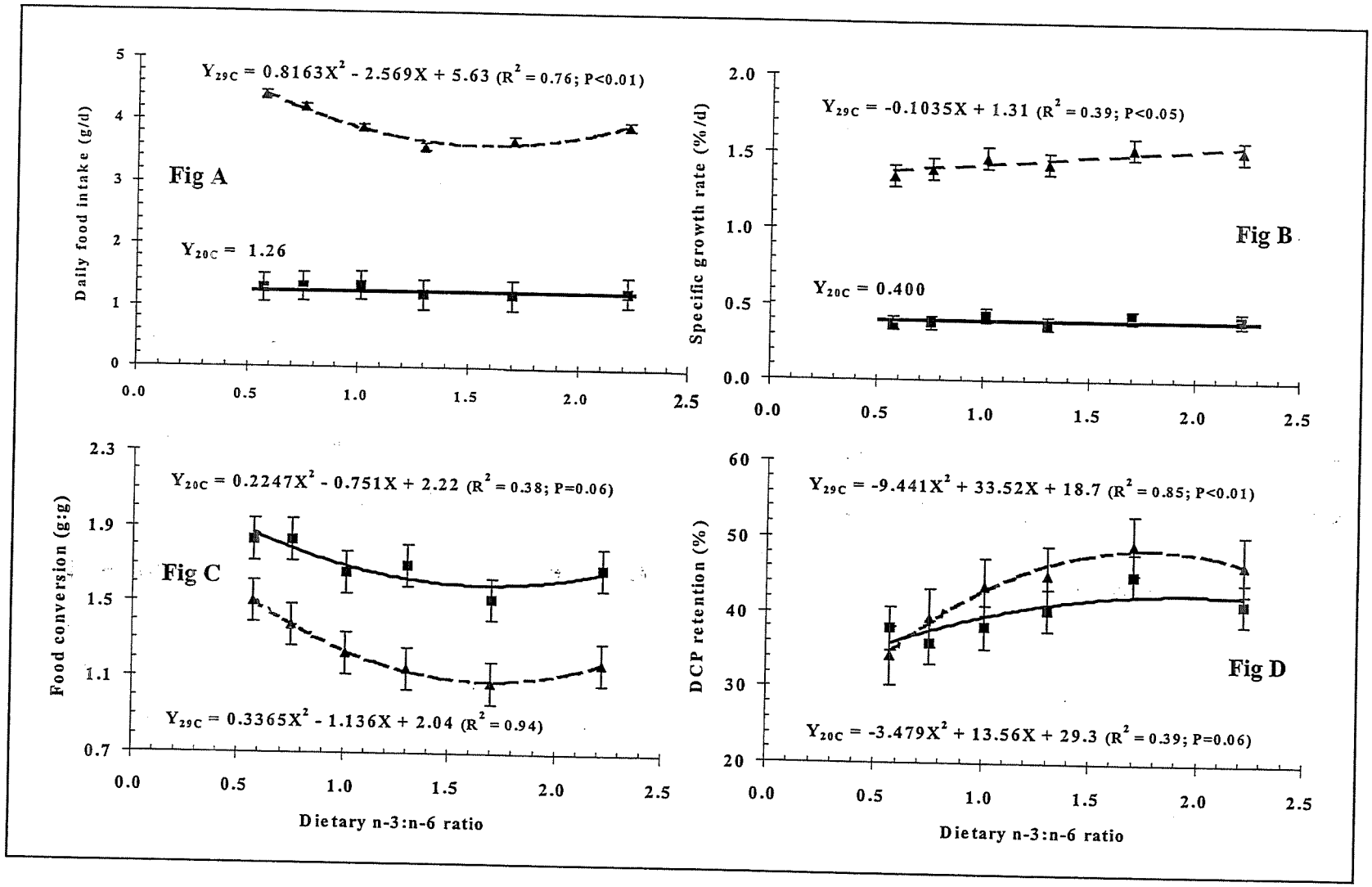

Fig. 6.5.1 Effect of varying the dietary n-3 to n-6 fatty acid ratio on daily food intake (Fig A) specific growth rate (Fig. B), food conversion (Fig. C) and retention of digestible crude protein (Fig D) of fish held at water temperatures of either 20 (■) or 29°C (▲) in Experiment Wlk 5.

A graphic illustration of these effects for DFI, SGR, FCR and DCP retention is shown in Fig. 6.5.1. This revealed significant linear and curvilinear responses to dietary n-3:n-6 ratio and differences in these relationships between water temperatures. For fish held at 29°C, DFI, FCR and DCP retention improved ($P<0.05$) and SGR tended ($P=0.06$) to increase with increasing dietary n-3:n-6 ratio. With fish held at 20°C, the response to n-3:n-6 ratio was either non-existent as for DFI and SGR or in the case of FCR and DCP retention, some slight improvement ($P=0.06$) was seen similar to that at 29°C.

The chemical composition of the fish at the start and end of the experiment is shown in Table 6.5.10. Over the course of the experiment, there was an apparent increase in the dry matter and fat content of the fish which was modified by the applied treatments. Compared to fish held at 29°C, those held at 20°C had significantly ($P<0.05$) lower contents of fat, energy and fat-free protein but higher contents of dry matter and fat-free ash. No significant ($P>0.05$) changes in the gross chemical composition of the fish were seen in response to the examined dietary n-3:n-6 fatty acid ratios.

Table 6.5.10 Main effects of water temperature and n-3:n-6 ratio of the diet on chemical composition of the fish in Experiment Wlk 5

Treatment	DM (%)	Fat (% DM)	GE (kJ/g DM)	Ash (fat-free) (% DM)	CP (fat-free) (% DM)
Initial	32.6±0.43	27.3±1.10	24.00±0.316	19.7±0.48	79.3±2.44
			Water temperature		
20°C	35.7^{A}	30.8^{A}	24.98^{A}	19.1^{A}	80.6^{A}
29°C	35.0^{B}	32.6^{B}	25.50^{B}	18.6^{B}	82.3^{B}
±sem	0.13	0.33	0.109	0.12	0.21
			n-3:n-6 ratio		
2.2	35.6	31.3	24.90	18.1	81.0
1.7	35.3	31.4	25.15	18.8	81.5
1.3	35.2	31.9	25.20	19.1	81.6
1.0	35.4	32.1	25.43	18.6	81.6
0.8	35.3	31.8	25.48	19.0	81.9
0.6	35.3	31.7	25.32	19.6	81.3
±sem	0.23	0.58	0.188	0.21	0.36

$^{A,B;}$ Within main effect comparisons, means without a common letter differ ($P<0.05$).

The effects of water temperature and dietary n-3:n-6 fatty acid ratio on the fatty acid composition of the body lipid of the fish at the start and end of the experiment are shown in Table 6.5.11.

The fatty acid composition of the lipid of fish held at 20°C most closely resembled that of fish at the commencement of the experiment, having greater proportions of n-3 than n-6 fatty acids as compared to fish at 29°C. As the dietary n-3 to n-6 ratio was decreased from 2.2 to 0.6, there was a variable but significant ($P<0.05$) pattern in which the proportions of 18:2n-6 and 18:3n-3 fatty acids in the body lipid increased while that of 22:6n-3 decreased; the relative abundance of n-3 to n-6 fatty acids in the body lipid also reduced as the dietary n-3:n-6 ratio decreased.

Table 6.5.11 Main effects[1] of water temperature and dietary n-3:n-6 ratio on the composition (mg/kg) of body lipid for selected fatty acids of fish in Experiment Wlk 5

Treatment	18:2n-6	18:3n-3	20:5n-3	22:6n-3	∑n-3	∑n-6	n-3:n-6
Initial	15.4±2.38	3.1±0.84	11.6±0.21	23.7±0.32	47.1±1.86	16.7±3.19	2.88±0.427
				Water temperature			
20°C	22.8^{X}	5.4^{X}	11.7^{X}	22.9^{X}	49.9	23.9^{X}	2.13^{X}
29°C	30.9^{Y}	8.6^{Y}	10.0^{Y}	20.7^{Y}	47.9	32.2^{Y}	1.52^{Y}
±sem	0.91	0.34	0.23	0.38	1.00	1.10	0.060
				n-3:n-6 ratio			
2.2	22.6^{B}	5.5^{B}	11.8	23.9^{A}	50.1	23.5	2.24^{A}
1.7	26.8^{B}	6.8^{B}	10.8	20.2^{B}	47.1	28.3	1.73^{A}
1.3	24.5^{B}	6.3^{B}	11.6	23.9^{A}	52.1	26.3	2.00^{A}
1.0	28.1^{AB}	7.4^{AB}	9.8	19.7^{B}	44.9	28.9	1.58^{AB}
0.8	25.2^{B}	6.7^{B}	11.2	23.5^{A}	51.1	27.0	1.90^{AB}
0.6	33.7^{A}	9.4^{A}	9.9	19.8^{B}	48.2	34.4	1.47^{B}
±sem	1.58	0.58	0.40	0.66	1.73	1.90	0.103

[1] Fatty acid analyses were done on only two complete replicate blocks; the interaction term was used as the estimate of error in the ANOVA.

$^{A,B;\ X,Y;}$ Within main effect comparisons, means without a common letter differ ($P<0.05$).

6.5.5 Discussion

As expected, water temperature had a profound effect on productivity responses of the fish in both experiments (see Sections 6.2 and 6.3). However, the poor productivity of the fish at 20°C was lessened when more energy dense diets were fed in Experiment Wlk 3. This interaction was evident for FCR where increasing the DE density of the diet from 14.1 to16.1 and 18.2 kJ/g resulted in a 41% improvement with fish at 20°C compared to only a 27% improvement with fish at 29°C (2.01 to 1.56 and 1.19 and 1.32 to 1.19 and 0.97, respectively). However, increasing the DE density of the diet caused a concomitant reduction in the voluntary food intake of fish at 29°C (from 3.07 to 2.98 and 2.67 g/d, respectively) but no such effect on appetite was seen with fish at 20°C (from 0.68 to 0.66 and 0.67 g/d, respectively). As a consequence of these interactive effects between water temperature and dietary energy density on FCR and DFI, feeding of the highest DE diet resulted in a 68% improvement in growth rate of fish at 20°C but only an 18% improvement for those held at 29°C.

These differences in growth rate of the fish at 20 and 29°C to increasing DE density can easily be explained in terms of the net energy available to the fish for growth after maintenance requirements had been satisfied. If the daily DE requirement of the fish for maintenance is assumed to be about 130 kJ/kg fish weight$^{0.8}$ (range of 77 to 163 depending on diet and environmental conditions; see Section 6.3.4), the maintenance DE demand of the fish at mid experiment weight (75 and 130 g for fish at 20 and 29°C, respectively) is calculated to be 4.1 and 6.4 kJ/d, respectively. The corresponding net DE available to support fish growth (i.e. total DE intake - maintenance DE) can be calculated to be 5.45, 6.5 and 8.1 kJ/d for fish at 20°C as compared to 36.8, 41.4 and 42.2 kJ/d for fish held at 29°C for diets containing 14.1, 16.1 and 18.2 kJ DE/g, respectively. Based on these derived estimates, increasing the DE density of the diet from 14.1 to 18.2 kJ/g resulted in the net DE available to the fish for growth increasing by 48% for fish at 20°C compared to 15% for fish at 29°C. These values agree quite well with the corresponding observed growth rate improvements of 68 and 18%, respectively.

The observed water temperature x dietary DE density interaction has a number of implications for practical barramundi farming. In Australia, barramundi are most commonly farmed using cage confinement of the fish in earthen dams where water temperature varies with season over

a range of 20 to 30°C annually. An increasing number of fish are being held over winter, either to be grown to heavier weights of 2 to 3 kg in the following year or because they have been late spawnings which have not yet grown to a suitable marketing size prior to the onset of winter. The results of Experiment Wlk 3 indicate that there would be substantial benefits of feeding high DE diets to fish during the winter-spring season when water temperatures are coolest. Not only would the high DE diets enable the fish to use the food more efficiently, but it would also enable the fish to grow faster and maximising growth rate has been demonstrated by Johnston (1997, 1998) to be as important, if not more important than FCR, in determining overall farm profitability. The results of Experiment Wlk 3, indicate that barramundi were eating to satisfy an energy demand and not to gut fill since voluntary food intake progressively declined as the DE density of the diet was increased. This finding may thus limit the extent to which growth rate of barramundi can be improved by feeding the fish on high energy diets. If this is confirmed, it would mean that barramundi respond quite differently to salmon and other cool-water species where high energy diets containing more than 32% lipid are being used, both as a means of protein-sparing and to maximise growth rate (Hardy et al., 1987; Sargent, 1995; Einen and Roem, 1997; Hillestad et al., 1998; Helland and Grisdale-Helland, 1988). Although the response of barramundi appears to be different to that of salmonids, some qualification needs to be made because of the effect dietary protein has been shown to have on voluntary food intake as discussed in Section 6.44. In Experiment Wlk 3, the DCP content of the diet was increased in line with DE density in order to hold constant the DCP:DE ratio of the diet. However, it is not known whether this manipulation was beneficial to, or adversely affected the appetite of the fish. In absolute energy terms, there was a small net DE intake benefit from using the higher DE density diet and this was translated into improved rates of growth and more efficient food utilisation (Table 6.5.5). Clearly, further work is needed to clarify the extent to which high energy diets can be used by barramundi either to spare for protein or to improve growth rates.

Increasing the absolute EFA concentration or the ratio of n-3 to n-6 fatty acids in the diet in Experiment Wlk 3 had only minor effects on barramundi productivity responses with these being limited to a significant interaction between dietary n-3:n-6 ratio and energy density for DFI. Increasing the energy density of the diet resulted in a progressive decrease in DFI of fish fed diets with a n-3:n-6 ratio of 1.5:1 whereas no significant change in intake occurred with diets containing a ratio of 1:1 (Table 6.5.5). Growth rate and FCR were better for diets having the higher n-3:n-6 ratio but these differences were not significant ($P>0.05$). Neither were there significant differences between the two n-3:n-6 ratio diet series for the retention of dietary protein or energy which confirms that these differences in absolute or ratio amounts of these fatty acids had no significant effect on metabolic processes in the fish (Table 6.5.7).

Interestingly, a small but unusually high number of fish in Experiment Wlk 3 died during handling for weighing with these fish exhibiting signs of fainting or shock. Death following a fainting reaction to handling and an apparent increased sensitivity of the fish to stressful situations are typical signs of EFA deficiency in fish (Castell et al., 1972b, c; Watanabe, 1982; Millikin, 1982). Similar signs of a shock syndrome were reported to occur in barramundi fed diets containing low amounts of n-3 HUFAs (0.46 or 0.88%) but other signs of EFA deficiency such as reddening of the fins and skin were also observed (Wanakowat et al., 1991; Boonyaratpalin,1991, 1997). All of the fish in Experiment Wlk 3 that showed a shock reaction and died (five fish) were being held at 29°C. Four of the deaths occurred in fish being fed the intermediate energy diet (16 kJ/g DE) and an equal number were from each of the two n-3:n-6 ratio treatments. Other signs of EFA deficiency such as reddening of the fins and poor appetite were not seen in the fish. Therefore, it is questionable whether the fish losses and the atypically high number of runt fishes (not restricted to any particular diet treatment) that occurred in the experiment can be attributed to an EFA deficiency.

The data of Experiment Wlk 5 provide a clearer picture of the responsiveness of barramundi to dietary EFA and n-3:n-6 fatty acid ratio. Firstly, there were marked differences in the response to dietary EFA manipulation between fish held at the two different water temperatures. This is readily apparent from the graphed data presented in Fig. 6.5.1 where changes to the dietary n-3:n-6 ratio brought about a much greater response in fish at 29°C compared to those at 20°C. Thus, varying the dietary n-3:n-6 ratio from ~0.6:1 to 2.2:1 had no effect on growth rate or DFI and only small effects on FCR and protein retention with fish at 20°C. At 29°C, DFI decreased curvilinearly, FCR and protein retention improved curvilinearly and growth rate improved linearly. The dietary n-3:n-6 ratio corresponding to the asymptote value was 1.57, 1.69 and 1.78:1 for DFI, FCR and DCP retention respectively, for fish at 29°C and 1.67 and 1.94:1 for FCR and DCP retention respectively, for fish at 20°C.

Because of the interdependence between the absolute amounts of the each of the fatty acids in the diet, it is not possible from the experimental data to determine an exact requirement of the fish for any particular fatty acid. Nonetheless, it is useful to determine the amount of each of the n-3 fatty acids present in the diet at the derived optimal n-3:n-6 ratio to gauge some indication of the fish's requirement for these EFAs under the imposed experimental conditions. Irrespective of the water temperature, a dietary concentration for 18:3n-3, 20:5n-3 and 22:6n-3 of about 0.45, 0.75 and 1.15% respectively, optimised FCR and protein retention. However, for fish at 20°C, growth rate did not benefit from 20:5n-3 and 22:6n-3 specifications greater than 0.18 and 0.32% when the 18:3n-3 content was at least 1.0%. On the other hand, fish at 29°C required dietary 20:5n-3 and 22:6n-3 concentrations greater than 0.8 and 1.2% respectively to maximise growth rate. These n-3 HUFA requirement estimates are similar to those of Buranapanidgit et al. (1988, 1989) cited by Boonyaratpalin (1991, 1997) where a dietary n-3 HUFA content of 1.0 to 1.7% n-3 HUFA was deemed adequate to support good growth rates and an absence of EFA deficiency signs in barramundi. Wanakowat et al. (1991) report similar n-3 HUFA requirement estimates for barramundi. Borlongan and Parazo (1991) investigated the suitability of cod liver, soybean and coconut oils when used either singularly or in combinations at a total inclusion of 9% in diets for barramundi fry reared at 28°C. Although their study was not examining EFA requirements *per se*, they found a 1 to 1 mixture of cod liver and soybean oils to result in no signs of EFA deficiency and to produce the best fish growth. The n-3:n-6 ratio of the cod liver/soybean diet was 1.0:1 and the contents of 18:3n-3, 20:5n-3 and 22:6n-3 were 0.69, 0.44 and 0.48%, respectively. These EFA estimates of Borlongan and Parazo (1991) are somewhat lower than those suggested from the present work and those reported by Boonyaratpalin (1991, 1997). This is a little surprising since the barramundi used in the study of Borlongan and Parazo (1991) were fry of only 28 mg and dietary EFA requirements are expected to reduce with advancing age/size (Watanabe, 1982; Millikin, 1982).

Varying the dietary n-3 to n-6 fatty acid ratio in Experiment Wlk 5 had no significant effect on the gross chemical composition of the fish (Table 6.5.10). However, the fatty acid composition of the fish's body lipid paralleled the fatty acid composition of the diet for fish held at 29°C but little change from the initial profile was seen for fish held at 20°C (Table 6.5.11). This response was not unexpected. Food consumption by fish at 20°C was very low and only sufficient to meet maintenance requirements and a very low rate of fish growth (~0.45 g/d). Since the duration of feeding was only six weeks for fish at 20°C, this, together with the poor appetite of the fish, would have limited the likelihood of differences in dietary fatty acid content being expressed in the body lipid. However, for fish at 29°C where DFI and growth rate of the fish were on average 5 to 6 times higher (2.54 g/d), even a short exposure to the diets of four weeks was sufficient time to induce significant changes in the fatty acid composition of body lipid. Numerous studies with salmonids (Hardy et al., 1987; Kennish et al., 1992), bass (Fair et al., 1993; Fowler et al., 1994), gilthead bream (Kalogeropoulos et al., 1993), channel catfish (Santha and Gatlin, 1991; Morris et al., 1995), white sturgeon (Xu et al., 1996) and

barramundi (Borlongan and Parazo, 1991) have shown that the fatty acid composition of body lipid increasingly mimics that of the diet with prolonged feeding of the diet. However, in the present barramundi study, the change in the fatty acid composition of the body lipid in response to the dietary supply of each of the n-3 and n-6 fatty acids was greatest for 18:2n-6 and least for both 20:5n-3 and 22:6n-3. Thus, as the absolute concentration of dietary n-3 HUFAs decreased, their abundance in the body lipid diminished but only slightly in contrast to the much greater prevalence of 18:2n-6 that accompanied an increased dietary supply of that fatty acid. This response wherein the n-3 HUFAs were conserved and the18:2n-6 and to a lesser extent thel 8:3n-3 fatty acids were accumulated, is highly indicative of a greater dietary essentiality of barramundi for the former compared to the latter fatty acids.

The dietary requirement of salmonids and other cold water fish for n-3 HUFAs has been reported to range from 1 to 2.5% depending on species and experimental conditions and n-6 fatty acids in excess of 1% depress growth (see reviews of Watanabe, 1982; Millikin, 1982). Even higher requirements for n-3 HUFAs have been reported for larvae with estimates of 3-3.5% being advocated for Japanese flounder, *Paralichthys olivaceus* (Izquierdo et al., 1992; Furuita et al., 1999). In comparison, the dietary n-3 HUFA requirement of warm water fish, including that of barramundi, appear to be much lower. Whether or not these differences in dietary n-3 HUFA requirement between cold and warm water fishes are due to the latter species having lower needs for cell membrane permeability functions or a greater capacity to synthesise longer chain unsaturated fatty acids from their C-18 precursors is equivocal. Differences in n-3 HUFA synthesis have been demonstrated between both similar and dissimilar fish species and no clear distinction can be made between either temperature or salinity habitat (see Section 6.5.2). The presents results confirm the need for barramundi diets to contain not less than 1.5% of n-3 HUFA and not less than 2% of total n-3 fatty acids. Our data demonstrate that the balance of n-3 to n-6 fatty acids is also important. A n-3 to n-6 ratio of about 1.5-1.7:1 is suggested as being optimal for juvenile barramundi but more work is needed to unravel the complexity between absolute requirement for individual EFAs and the interactive effects between the balance of individual EFAs.

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7. SUMMARY

The research carried out in this project was aimed at better understanding the dietary nutrient requirements of juvenile barramundi and the extent to which different water temperatures and feeding frequencies affected these requirements. Understanding these processes was considered to be of vital importance in the development of appropriate dietary specifications and feeding practices that would enable barramundi to be farmed most profitably. The research addressed four key issues: (i) the influence water temperature and feeding frequency have on the feeding (and consequent productivity) response of different size barramundi; (ii) what effect food restriction had on growth and body composition of barramundi; (iii) specification of the dietary protein requirements of barramundi and the interrelationship with dietary energy supply; and (iv) examination of the essential fatty acid requirements of barramundi. For each of these issues, a number of discrete experiments was carried out during the course of the three-year project. Most of the experiments were carried out under closely controlled laboratory conditions at QDPI's Freshwater Fisheries and Aquaculture Centre at Walkamin using four independent freshwater recirculation systems each consisting of a 2,000 L header tank and six experimental tanks of 800 L capacity. A 12:12 night/day photoperiod cycle was maintained in the air-conditioned laboratory and water temperature was finely controlled by heating of the recirculated water above a lower ambient air temperature. One on-farm experiment was also carried out to extend the laboratory findings to the field. A summary of the work and key findings are provided below.

(i) **Influence of water temperature and feeding frequency on appetite of different size fish**
Five short-term growth assays were carried out to investigate the effects feeding frequency and water temperature have on appetite and growth responses of different size barramundi. When fish were fed a commercially-extruded dry pellet containing 40% digestible crude protein (DCP) and 15.5 kJ/g digestible energy (DE), daily food intake (DFI) increased allometrically with increasing fish size and almost linearly with increasing water temperature. Over the range of 20 to 29°C, appetite increased 1.8 fold for large fish fed 1/d (from 2.14 to 3.87 g/d for fish of ~ 300 g) and by 2 fold for small fish fed 2/d (from 0.94 to 1.89 g/d for fish of ~ 50 g). The slight curvilinearity of the response at the highest temperature implies that a water temperature of 29°C is close to the fish's upper thermal tolerance when held at constant water temperature. The effect of water temperature on food conversion ratio (FCR) was comparatively small except with small fish (~ 50 g) where it progressively improved with increasing temperatures until the response plateaued at 26°C. However even with larger fish, there were consistent trends between the experiments for FCR to be worst at low water temperatures (23°C or lower).

Increasing the feeding frequency from 2/d to 3/d for small fish (<100g) had little to no effect on either DFI or FCR such that growth rate was unaffected. For larger fish (~170 to 270 g), there was no benefit in feeding more frequently than 1/d. However, there was some evidence that a PM feeding was better than an AM feeding where fish were fed only 1/d. Compared to feeding 1/d every day, skipping one day's feeding during the week had no deleterious effect on either FCR (1.13 cv 1.18, respectively) or growth rate (3.30 cv 3.39 g/d, respectively) with fish of ~ 220 g. Based on these findings it is recommended that fish between 40 and 100 g be fed 2/d but this rate can be reduced to 1/d after 100g and further reduced by skipping one of the feeds on the weekend for fish >300 g. A feeding chart taking into account fish size, water temperature and recommended feeding frequency has been developed as a farming guide. However, the feeding activity of the fish is the best guide as to the amount of food to be fed since growth rate and FCR appeared to be optimised at close to full satiation feeding.

(ii) Effect of food restriction on growth and body composition

Two comparative slaughter growth assays of 8 to 10 week duration were carried out to investigate the effects dietary restriction, water temperature and dietary protein to energy ratio have on growth and body composition of juvenile barramundi. In Experiment Wlk 1, fish held at water temperatures of either 20, 23, 26 or 29°C were fed the same diet as in (i) above at controlled rates approximately corresponding to either 65-70, 80-85, 95-100 or 95-100% of satiety. Growth rate was linearly related to DFI at each water temperature and FCR significantly improved as water temperature increased but was not greatly affected by feeding rate. When pooled across all water temperatures, almost all of the variation in growth rate (R^2 of 0.88 to 0.94) was explained by a linear function of DFI. This enabled the daily DE maintenance requirements of the fish at zero growth to be calculated to be 171 to 186 kJ/kg fish weight$^{0.8}$ /d. The dry matter (DM) fat content of the whole fish decreased with increasing DFI and the efficiency of retention of dietary DCP and DE tended to improve linearly with DFI; nutrient retention efficiencies appeared to be optimised at a water temperature of 26°C, coinciding with maximum daily food consumption. The DE requirement for maintenance at zero energy accretion was predicted from the derived accretion to intake relationships to be 134 to 142 kJ/kg fish weight$^{0.8}$ /d. The higher DE requirement for maintenance based on zero growth rate (i.e., 171 to 186 kJ/kg fish weight$^{0.8}$ /d) was thought to be due to differences in body composition occurring at zero growth as compared to at zero energy accretion.

In Experiment Wlk 2, fish held at water temperatures of either 20, 23, 26 or 29°C were controlled fed to 95% of satiety either of three diets that differed in DCP (from 40.7 to 33.4 and 31.5%) and DE (14.8, 13.6 and 12.7 kJ/g, respectively) such that the DCP:DE ratio varied from 27.5 to 24.5 and 24.7 mg/kJ respectively. Decreasing the DCP (and DE) content of the diet caused a progressive worsening of FCR and growth rate with these effects being more marked at the lower water temperatures. When pooled across water temperatures, daily growth rate was found to be linearly related to DFI but the relationships for each of the three diets were significantly different although quantitatively these differences were quite small (eg slopes varying from 0.106 to 0.086 and intercepts from -1.12 to -0.91). Extrapolation of the derived linear relationships to zero weight gain gave estimates for the DE requirement for maintenance of 157, 154 and 133 kJ/kg fish weight$^{0.8}$ /d for the three diets respectively. The predominant effect of diet on body composition of the fish was a decreased DM fat content that accompanied an increased dietary DCP (and DE) content with this effect again being more pronounced at the higher water temperatures. Accretions of DCP and DE were linearly related to their respective intakes but the relationships were different for each diet. Extrapolation of these relationships to zero energy accretion gave estimates for the DE requirement for maintenance of 77, 163 and 142 kJ/kg fish weight$^{0.8}$ /d for diets containing DE contents of 14.8, 13.6 and 12.7 kJ/g, respectively. The corresponding estimates for the DCP requirement for maintenance at zero N accretion were 0.76, 0.61 and 0.53 g/ kJ/kg fish weight$^{0.8}$ /d for DCP contents of 40.7, 33.4 and 31.5%, respectively.

These experiments have shown that barramundi are similar to cold water carnivorous fish in the way metabolic processes respond to altered food rationing and sub-optimal water temperature conditions. At near optimal water temperatures (26 to 29°C), FCR improved curvilinearly with increasing food consumption whereas it was relatively unaffected by food consumption at low water temperatures (20 to 23°C). Moreover, FCR improved with increasing water temperature irrespective of the amount of food eaten. These responses could be explained as the combined effects of an improvement in energy utilisation accompanying high rates of feeding at high water temperatures and higher metabolic costs due to increased locomotor activity and maintenance requirements associated with high water temperatures. The net effect is an improved metabolic efficiency and energy retention as water temperature increases up to at least 29°C which is manifested in the form of increased growth rate and improved FCR. To

these effects must be added those due to dietary nutrient supply. Reducing the dietary DCP content below 33.4% in Experiment Wlk 2 was observed to significantly impair FCR.

(iii) Dietary protein and protein to energy responses

Two comparative slaughter growth assays and an on-farm experiment were carried out to define the dietary protein and protein to energy requirements of juvenile barramundi. The on-farm study sought to examine the effects on growth and eating characteristics of fish fed on pelleted diets differing in protein and energy contents (~32 %DCP at either of 13.9 or 16.4 kJ/g DE and 37% DCP and 14.1 kJ/g DE). The two laboratory studies were carried out to see if growth (and protein deposition) in barramundi was similar to that of terrestrial monogastric animals, exhibiting both dependency and non-dependency to protein and energy intake. The first of these laboratory experiments (Wlk 6) examined the effect of satietal feeding of six semi-purified diets in which the DCP content varied incrementally from approximately 27 to 52%. The protein was a blend of fishmeal, casein, gluten and crystalline amino acids that was formulated to mimic the essential amino acid composition of the protein of barramundi and to approximate what might be regarded as 'ideal' protein. The object of the second laboratory experiment (Wlk 7) was to examine the effect of feeding barramundi diets considered from Experiment Wlk 6 to be either protein-limiting (i.e. 37% DCP and 15.35 kJ/g DE) or energy-limiting (i.e., 47% DCP and 16.23 kJ/g DE) at either of four controlled feeding rates (varying from approximately 45-50 to 85-90% of satiety). To ensure that fish fed the two different diets had identical DCP intakes at each of the respective feeding rates, the daily food allocation of the diet containing the higher DCP content was further restricted by a factor of 37/47.

The 13-week on-farm study entailed 6,000 fish of initial weight of 296 g being confined in cages of $4m^2$ surface area (500 fish/cage) and managed similar to other fish on the farm. The study was conducted over the spring – summer period where daily min/max water temperatures increased during the first seven weeks from 20/22 to 25/27 and thereafter remained around 27/30°C. Increasing the DE from 13.9 to 16.4 kJ/g or increasing the DCP from 27 to 32% had no significant effect on fish productivity responses and only minimal effects on sensory characteristics of the fish. The low statistical power of the experiment require that these results are treated with a degree of caution.

Under controlled laboratory conditions, altering the dietary concentration of a high quality 'ideal' protein in Experiment Wlk 6 profoundly affected both growth and nutrient retention responses of juvenile (~ 80 g initial weight) barramundi. As the dietary DCP content increased, voluntary food intake progressively declined and FCR improved such that growth rate increased to an asymptote at about 41-42% DCP. Accretion of N continued to increase curvilinearly with increasing DCP intake but the efficiency of N retention remained constant at 0.37 until an intake of 1.25 g/d was attained (by fish fed the 42% DCP diet) whereupon efficiency steadily declined to reach 0.33 at an intake of 1.4 g/d. This response suggested that dietary DCP concentrations lower than about 42% were protein-limiting while those with higher concentrations were energy-limiting. The absolute retention of dietary energy also declined at the same break point, suggesting a change between protein-dependency and energy-dependency was occurring with the diet containing 41% DCP. The interdependency of dietary protein and energy intake was more closely examined in Experiment Wlk 7. If barramundi exhibited similar protein and energy interdependencies as for terrestrial mammals, the efficiency of protein retention would be identical for each of the diets. Moreover, protein deposition would increase linearly with increasing DCP intake for the 'protein-limiting' diet but for the 'energy-limiting' diet, protein deposition would be limited by energy, rather than by protein intake. Instead, the efficiency of protein retention was higher for the 47% DCP 'energy-limiting' diet than for the 37% DCP 'protein-limiting' diet (rates of 0.46 vs 0.34, respectively). Furthermore, at the same daily DE intake, N accretion was significantly greater for the higher compared to the lower DCP diet and this difference widened with increasing DE intake. Thus

protein deposition in barramundi appeared to be more dependent on dietary energy rather than protein intake and distinctly different to terrestrial monogastrics where protein deposition is regulated by the intake of both protein and energy. However, this is not to conclude that protein deposition in barramundi is solely dependent on energy intake. Rather it is concluded that both of the diets fed in Experiment Wlk 7 contained an excess of protein such that amino acid requirements for protein synthesis were being fully met. Since an energy insufficiency appeared to be the major factor influencing the rate of protein deposition, the enhanced N accretion in barramundi fed the 47% DCP diet was most likely due to the 'excess' dietary protein being a more available source of metabolic energy than the 37% DCP diet. This would explain why the efficiency of protein retention was higher for the 47% than for the 37% DCP diet even though the composition and intake of protein were identical for each diet. For the above reasons, it would be unwise to conclude that a dietary DCP content of 42% and a DCP:DE ratio of 26.5 mg/kJ as suggested from the data of Experiment Wlk 6 are optimal dietary specifications for juvenile barramundi. Further work is needed to characterise the response of barramundi to protein intake when diets of much higher energy content are fed.

(iv) Essential fatty acid requirements of barramundi

Two comparative slaughter growth assays were carried out to define the requirement of juvenile barramundi (~60 to 175 g) for essential fatty acids (EFAs). The first experiment (Wlk 3) examined the effect of varying both the energy (~14, 16 or 18 kJ/g DE) and the amounts and proportions of n-3 and n-6 fatty acids in the diet (ratios of either ~1.0 or 1.5:1) when the fish were satietal fed and reared at either cool (20°C) or warm (29°C) water temperatures. In the second experiment (Wlk 5), the amounts of n-3 and n-6 fatty acids in the diet were varied serially to provide dietary n-3:n-6 ratios ranging from ~0.6 to 2.2:1 when dietary energy (17 kJ/g DE), protein (39% DCP) and lipid (15.5%) contents were held constant and the fish were satietal fed and reared at either 20 or 29°C.

As expected, water temperature had a marked effect on all productivity responses of the fish. However, increasing the dietary DE content in Experiment Wlk 3 from 14 to 16 and 18 kJ/g caused a relatively greater growth rate improvement in fish at 20 than at 29°C (68 vs 18%, respectively). This could be explained as resulting from a reduction in voluntary food intake of fish at 29°C (from 3.07 to 2.98 and 2.67 g/d, respectively) and relatively more energy being available to the fish at 20°C after requirements for maintenance had been satisfied. Under typical open systems of farming barramundi in Australia where water temperature varies seasonally between 20 and 30°C, the use of high energy diets during the winter-spring period when water temperatures are coolest is recommended as a means of improving FCR and growth rates of the fish at these times. Increasing the dietary concentration of n-3 highly unsaturated fatty acids (HUFAs) from ~1.0 to 1.2 and 1.4% at a n-3:n-6 ratio of 1.0:1 or from 1.4 to 1.8 and 2.0% at a n-3:n-6 ratio of 1.5:1 in Experiment Wlk 3 had only minimal effects on fish productivity. Growth rate and FCR tended to be better for diets having the higher n-3:n-6 ratio but these effects were not significant. Retention of protein and energy were also not affected by either the dietary concentration of the fatty acids or the n-3:n-6 ratio. In Experiment Wlk 5, significant interactions were observed between dietary n-3:n-6 ratio and water temperature for key productivity responses of the fish. Varying the dietary n-3:n-6 ratio from ~ 0.6 to 2.2:1 had no effect on DFI or growth rate and slightly curvilinearly improved FCR and protein retention rate of fish at 20°C. At 29°C, DFI decreased curvilinearly and FCR and protein deposition rate improved curvilinearly and growth rate increased linearly. The dietary n-3:n-6 ratio corresponding to the asymptote value for fish at 29°C was 1.57, 1.69 and 1.78:1 for DFI, FCR and DCP retention respectively, and 1.67 and 1.94:1 for FCR and DCP retention respectively, for fish at 20°C. The fatty acid composition of the body lipid of the fish paralleled the fatty acid composition of the diet the fish were fed. These effects were far more marked for fish at 29°C compared to those at 20°C and this was attributed to the large differences in the absolute food consumption of the fish at these two water temperatures. Because of the

interdependence between the absolute amounts of the each of the fatty acids in the diet, it was not possible from the experimental data to determine an exact requirement of the fish for any particular fatty acid. However, some indication of possible requirements can be gauged from the concentrations of the individual n-3 fatty acids present in the diet at the derived optimal n-3:n-6 ratio. Irrespective of water temperature, dietary concentrations for linolenic (18:3n-3), eicosapentaenoic (20:5n-3) and docosahexaenoic (22:6n-3) acids of about 0.45, 0.75 and 1.15% respectively, optimised FCR and protein retention. However, for fish at 20°C, growth rate did not benefit from 20:5n-3 and 22:6n-3 concentrations greater than 0.18 and 0.32% when the 18:3n-3 content was at least 1.0%. On the other hand, fish at 29°C required dietary 20:5n-3 and 22:6n-3 concentrations greater than 0.8 and 1.2% respectively to maximise growth rate. The results of these two experiments suggest that barramundi diets should contain not less than 1.5% of n-3 HUFA and not less than 2% of total n-3 fatty acids. Our data demonstrate that the balance of n-3 to n-6 fatty acids is important in determining voluntary food consumption and economy of food conversion and subsequent effects on growth rate and nutrient retention. A n-3 to n-6 ratio of about 1.5-1.7:1 is suggested as being optimal for juvenile barramundi but more work is needed to unravel the complexity between absolute requirement for individual EFAs and the interactive effects between the balance of individual EFAs.

The research has shown that growth rate of barramundi increases linearly with increasing water temperature over the range of 20 to 29°C. For optimal growth rate and FCR, barramundi held at high water temperature (26 to 29°C) require diets high in protein and energy (>42% DCP and DCP:DE of ~27 mg/kJ) and not less than 1.5% of n-3 HUFA. At lower water temperatures (<24°C), barramundi growth is more dependent on the energy than on either the protein or EFA contents of the diet. High DE diets (>16 kJ/g) are advocated as a management strategy to minimise the slow growth rate of fish held at low water temperatures.

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9. BENEFITS

The research carried out in this project will have direct benefits to Australia in several ways. Firstly, the research will assist feed manufacturers in formulating more cost effective barramundi diets by providing nutrients at concentrations that optimise barramundi productivity. Secondly, barramundi farmers will benefit directly from the knowledge gained on how to best manage feeding of barramundi and indirectly from cheaper and higher performance diets being available. As a result of these effects, the economic viability of aquaculture will be enhanced, and hopefully this may lead to reduced prices for aquaculture products. Thirdly, marketing opportunities for Australian agriculture products will be substantially increased, both from an increase in production of aquaculture feeds for the growing Australian industry and as ingredients for aquaculture feeds produced in Asia. The research has shown how Australian feed ingredients can be economically used for the manufacture of high-performing pelleted dry diets. The global market for aquaculture feeds is enormous. In Asia, the region where aquaculture is growing most rapidly, the feed's market was estimated at around 26 mt in 1990 and this market grew more than four fold between 1986 and 1990 (Akiyama, 1991). There is a great potential to market Australian agriculture products, including oilseed, grain legumes, other cereal crops and animal protein sources like blood meal and meat meal as ingredients in aquaculture diets. Finally, Australian aquaculture research workers will benefit from close interaction with scientists from other disciplines who have much to contribute to this research topic.

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10. PAPERS AND ARTICLES ARISING FROM THE PROJECT

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Williams, K., Barlow, C. and D'Souza, F., 1998. Larval penaeid and grow-out finfish nutritional research in Australia. In: M.Rimmer and K.C. Williams (Editors), Proceedings of ACIAR-NACA Grouper Aquaculture Workshop, Bangkok, 7-8 April 1998. In press.

11. INTELLECTUAL PROPERTY

The focus of the work was to conduct public domain research so all stakeholders can benefit.

Results will be published and disseminated widely. It is not anticipated that any patents or commercial intellectual property will arise from this project.

12. FURTHER DEVELOPMENTS

A new Sub-Program (Aquaculture Diet Development 96/391, 96/392 and 96/393) commenced in July 1996 to build on the results of the Fishmeal Replacement Sub-Program which successfully identified high priority Australian ingredients and evaluated them for silver perch, barramundi, prawns and salmon. The new Sub-Program will conduct research to identify and improve Australian ingredients for use in aquaculture diets with the major focus on protein ingredients to replace expensive, imported fishmeal.

In Australia, aquaculture will not develop beyond a small scale unless aquaculturists can purchase cheap, efficient feeds. We will not have the luxury of using cheap fishmeal to produce these feeds and so must develop viable alternatives. Fortunately, Australia has abundant sources of cheap agricultural proteins and results from the Replacement of Fishmeal in Aquaculture Diets Sub-Program have been excellent. Scientists involved with the Sub-Program have developed and validated techniques to determine diet and ingredient digestibility for silver perch, prawns, barramundi and salmon.

On-going diet development needs to incorporate all four aspects; ingredient evaluation, determination of limiting nutrient requirements, diet validation and determination of optimum feeding strategies.

13. STAFF

QDPI

Name	Qualification	Position	FTE on Project
K.C. Williams	QDAH, BVSc, PhD	Snr. Prin. Scientist	0.3
C. Barlow	BSc, MSc	Senior Fish. Biologist	0.3
L Rodgers	DipLabTech	Senior Fish Techician	0.5
I Hockings	BSc	Fish. Technician	1.0
C. Agcopra	BSc	Fish Technician	1.0
I. Ruscoe	BSc	Fish Technician	0.25
J. Rose	BSc	Fish. Technician	0.05
B. Kelly	BAppSc	Fish. Technician	0.05
H. Thaggard	CAppSc	Fish. Technician	0.05
I. Brock	DipLabTech	Snr. Lab. Technician	0.25
C. Palmer	DipLabTech	Snr. Lab. Technician	0.25

14. APPENDICES

1. Effect of dietary restriction and water temperature on the growth and body composition of grow-out barramundi.
2. Effects of water temperature and feeding frequency on food intake and growth of juvenile barramundi.
3. Barramundi nutrition research 1994.
4 Use of autoclaving in the preparation of homogenates for determining the proximate chemical and fatty acid composition of fish.
5. Barramundi nutrition research 1995.
6. Nutritional research in Australia to improve pelleted diets for grow-out barramundi *Lates calcarifer* (Bloch).
7. Continuing the development of improved grow-out diets for barramundi.
8. Barramundi feeding and nutritional requirements..
9. Larval penaeid and grow-out finfish nutritional research in Australia.

EFFECT OF DIETARY RESTRICTION AND WATER TEMPERATURE ON THE GROWTH AND BODY COMPOSITION OF GROW-OUT BARRAMUNDI

K C Williams[1], C Barlow[2], L Rodgers[2], I Hockings[2] and C Agcopra[2]

As part of a series of experiments at Walkamin studying the nutritional management of grow-out barramundi (*Lates calcarifer*), an experiment was undertaken to investigate the way growth and body composition are altered by dietary restriction and water temperature. At each of the four water temperatures investigated (20, 23, 26 or 29 °C), the same high quality extruded diet (% composition: fat, 11; crude protein, 44; lysine, 3.0; methionine and cystine, 1.6; PUFA's, 0.45; and estimated metabolisable energy, 15 kJ/g) was fed according to a scale intended to provide intakes corresponding to either 65-70, 80-85 or 95-100% of satiety. The experiment involved a total of 740 fish with 20 being used as a pre-experimental slaughter group for determining the initial body composition and 720 being used in the feeding study. Fish were distributed equally amongst 24 tanks (each 800 L) which were arranged as four independent recirculation systems for each of the specified water temperatures. The initial weight of the fish (± SD) was 134 ±1.9 g and the experiment continued for 10 weeks. Body composition was assessed after four weeks and at the end of the experiment (sub samples of 5 fish/tank being taken each time).

Although actual food intakes were 10-20% below what was intended, the achieved intakes represented a wide range of diet restrictions for the fish across all water temperatures. The results of the main effect treatments for the 10 week period are tabled below. Analytical results for the body composition of the fish are not yet available.

Trait/ Treatment	Start Wt (g)	End Wt (g)	Food intake (g/fish/d)	Growth rate (g/fish/d)	FCR (g food:g gain)
Water temp					
20	135^{A}	186^{A}	1.14^{A}	0.75^{A}	1.52^{A}
23	136^{A}	216^{B}	1.55^{B}	1.15^{B}	1.35^{B}
26	134^{A}	291^{D}	2.56^{D}	2.24^{D}	1.15^{C}
29	133^{A}	260^{C}	2.03^{C}	1.79^{C}	1.14^{C}
± s.e.m.	0.8	3.0	0.031	0.039	0.016
Feeding rate					
Low	136^{X}	229^{X}	1.63^{X}	1.32^{X}	1.29^{X}
Medium	134^{X}	240^{Y}	1.88^{Y}	1.52^{Y}	1.29^{X}
High	134^{X}	245^{Y}	1.94^{Y}	1.59^{Y}	1.29^{X}
± s.e.m.	0.7	2.6	0.027	0.034	0.014

$^{A,B,C,D;X,Y}$ - Within comparisons, means without a common superscript letter differ ($P<0.05$).

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Dressing percentage increased linearly ($P<0.05$) with increasing water temperature (85.4, 86.0, 87.7 and 88.4% respectively) but was not affected by feeding rate. Condition factor decreased linearly ($P<0.05$) with increasing water temperature (1.52, 1.41, 1.38 and 1.31 respectively) and was least ($P<0.05$) with the lowest rate of feeding (1.38, 1.41 and 1.42 respectively).

The experiment has shown:

- The daily food intake needed by the fish for maintenance purposes is about 7% of liveweight ($x10^{0.44}$) and 1.05 kJ of metabolisable energy/liveweight ($x10^{0.44}$) if the ME content of the diet is assumed to be 15 kJ/g.

- FCR improves with increasing food intake up to at least 80% of satiety and this effect is relatively independent of water temperature (i.e. severe food restriction even at high water temperatures will cause a worsening of FCR).

- Condition of the fish declines with increasing water temperature but high rates of feeding counters this effect at high water temperatures and has an opposite effect at low water temperatures. Conversely, dressing percentage improves with increasing water temperature.

EFFECTS OF WATER TEMPERATURE AND FEEDING FREQUENCY ON FOOD INTAKE AND GROWTH OF JUVENILE BARRAMUNDI

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The Fisheries Research and Development Corporation has funded a three year project at the Freshwater Fisheries and Aquaculture Centre, Walkamin on the dietary requirements and optimal feeding practices for barramundi.

As part of this project, we have conducted three experiments testing the effect of water temperature and feeding frequency on food intake, growth and food conversion. The three experiments were designed as follows:

Experiment 1: 2 versus 3 feeds/day, starting weight of fish approximately 40g.
Experiment 2: 2 versus 3 feeds/day, starting weight of fish approximately 100g.
Experiment 3: 1 morning versus 1 evening versus 2 feeds/day, starting weight of fish approximately 160g.

All experiments were conducted at four temperatures, namely 20°, 23°, 26° and 29°C.

The results of the experiments showed that there was effectively no difference in food intake, growth rate or food conversion when fish of 40g or 100g were fed two or three times per day.

However, there were different responses between the treatments in experiment 3. At 20° and 23°, the daily food intake was the same for all feeding regimes. At 26° and 29° the amount of food eaten with 2 feeds per day was greater than that with one feed per day, either morning or evening. Growth rates at all temperatures tended to be slightly better for afternoon and 2 feeds per day than for morning feed. Food conversion ratio tended to be slightly better with afternoon feed only, and worse with 2 feeds per day, compared with morning feed.

The data from the experiments show clearly that 2 feeds per day is ample for fish 40-160g, and one evening feed is best for fish 160g. It is also clear from the results and other research conducted at Walkamin that utilisation of food is better at night than in the day, hence the desirability of feeding in the evening. The experiments however, did not define the minimum fish sizes at which 2 and 1 feeds per day is adequate for good growth.

The data have enabled us to make a comparison of the respective growth rates at different temperatures. This is presented in the table below, in which the growth rates are expressed as a percentage of that obtained at 29°C (nominally taken as 100%). Is apparent that growth rates of 90% or better can be achieved at temperatures greater than 26°C, and that growth rates decline markedly at temperatures below about 25-26°C.

Table: Percentage of growth rate at 20°, 23° and 26° with respect to growth rate at 29°C, which is nominally taken as 100% for the purposes of this comparison.

Fish Weight	Temperature 20°	23°	26°	29°
40	35	60	92	100
100	42	65	92	100
100	50	70	88	100

BARRAMUNDI FEEDING GUIDE (Preliminary)

Fish Wt g	WATER TEMPERATURE (°C)												Fi
	19	20	21	22	23	24	25	26	27	28	29	30	
	DAILY FOOD ALLOWANCE (KG) PER 1 000 FISH												
50	0.50	0.72	0.94	1.13	1.31	1.47	1.62	1.74	1.85	1.94	2.02	2.08	
60	0.64	0.86	1.07	1.27	1.45	1.61	1.75	1.88	1.99	2.08	2.16	2.21	
70	0.76	0.99	1.20	1.40	1.58	1.74	1.88	2.01	2.12	2.21	2.28	2.34	
80	0.88	1.10	1.32	1.51	1.69	1.85	2.00	2.12	2.23	2.32	2.40	2.46	
90	0.99	1.21	1.42	1.62	1.80	1.96	2.10	2.23	2.34	2.43	2.51	2.56	
100	1.09	1.31	1.53	1.72	1.90	2.06	2.21	2.33	2.44	2.53	2.61	2.67	10
110	1.18	1.41	1.62	1.82	2.00	2.16	2.30	2.43	2.54	2.63	2.70	2.76	11
120	1.27	1.50	1.71	1.91	2.09	2.25	2.39	2.52	2.63	2.72	2.79	2.85	12
130	1.36	1.59	1.80	2.00	2.18	2.34	2.48	2.61	2.72	2.81	2.88	2.94	13
140	1.44	1.67	1.88	2.08	2.26	2.42	2.56	2.69	2.80	2.89	2.96	3.02	14
150	1.52	1.75	1.96	2.16	2.34	2.50	2.64	2.77	2.88	2.97	3.04	3.10	15
160	1.60	1.83	2.04	2.24	2.42	2.58	2.72	2.85	2.96	3.05	3.12	3.18	16
170	1.68	1.90	2.11	2.31	2.49	2.65	2.79	2.92	3.03	3.12	3.20	3.25	17
180	1.75	1.98	2.19	2.38	2.56	2.72	2.87	2.99	3.10	3.19	3.27	3.33	18
190	1.82	2.05	2.26	2.45	2.63	2.79	2.94	3.06	3.17	3.26	3.34	3.40	19
200	1.89	2.11	2.32	2.52	2.70	2.86	3.00	3.13	3.24	3.33	3.41	3.46	20
210	1.95	2.18	2.39	2.59	2.77	2.93	3.07	3.20	3.21	3.40	3.47	3.53	21
220	2.02	2.24	2.45	2.65	2.83	2.99	3.13	3.26	3.37	3.46	3.54	3.59	220
230	2.08	2.31	2.52	2.72	2.89	3.05	3.20	3.32	3.43	3.53	3.60	3.66	230
240	2.14	2.37	2.58	2.78	2.95	3.12	3.26	3.39	3.49	3.59	3.66	3.72	240
250	2.20	2.43	2.64	2.84	3.01	3.18	3.32	3.45	3.55	3.65	3.72	3.78	250
260	2.26	2.48	2.70	2.89	3.07	3.23	3.38	3.50	3.61	3.70	3.78	3.84	260
270	2.32	2.54	2.75	2.95	3.13	3.29	3.43	3.56	3.67	3.76	3.84	3.89	270
280	2.37	2.60	2.81	3.01	3.19	3.35	3.49	3.62	3.73	3.82	3.89	3.95	280
290	2.43	2.65	2.86	3.06	3.24	3.40	3.54	3.67	3.78	3.87	3.95	4.00	290
300	2.48	2.71	2.92	3.12	3.29	3.46	3.60	3.73	3.83	3.93	4.00	4.06	300
320	2.58	2.81	3.02	3.22	3.40	3.56	3.70	3.83	3.94	4.03	4.10	4.16	320
340	2.69	2.91	3.12	3.32	3.50	3.66	3.80	3.93	4.04	4.13	4.21	4.26	340
360	2.78	3.01	3.22	3.42	3.60	3.76	3.90	4.03	4.14	4.23	4.30	4.36	360
380	2.88	3.10	3.31	3.51	3.69	3.85	3.99	4.12	4.23	4.32	4.40	4.45	380
400	2.97	3.20	3.41	3.61	3.78	3.95	4.09	4.22	4.32	4.42	4.49	4.55	400

Table is for a high quality extruded diet (15 MJ metabolisable energy/kg) and is based on the derived relationship of:

. DFI = -9.019 + 0.295$Wt^{0.44}$ + 0.564T - 0.0086T^2 + 0.121F; [r = 0.97] - where DFI is daily feed intake, g/fish/day; Wt is the exponential ($^{0.44}$) weight of the fish, g; T is water temperature, °C; and F is number of feeds/day

BARRAMUNDI NUTRITION RESEARCH

K.C. Williams[1], C. Barlow[2], L. Rodgers[2], I. Hockings[2] and C. Agcopra[2]

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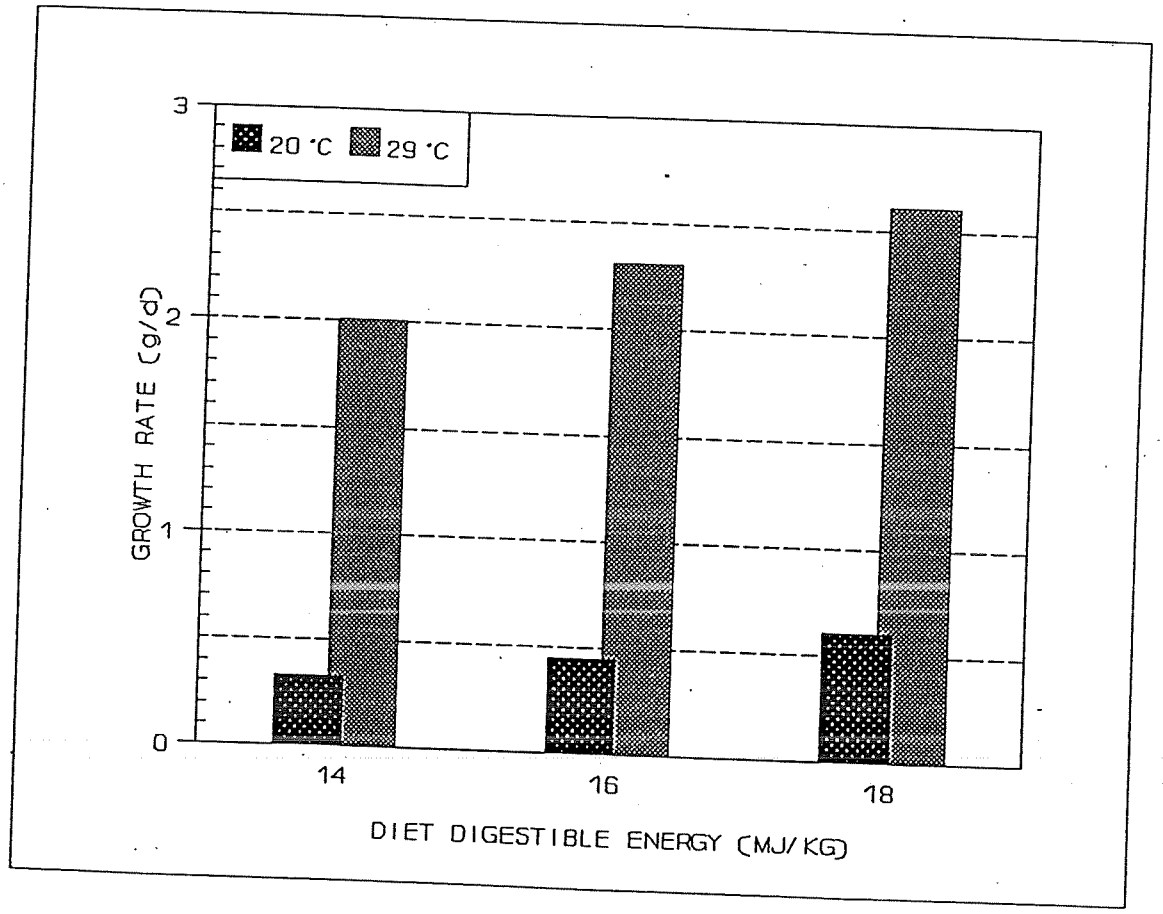
[2] Freshwater Fisheries and Aquaculture Centre, QDPI, Walkamin.

The Fisheries Research and Development Corporation is funding two QDPI research projects on the nutrition of grow-out barramundi. At Walkamin, research is targeting the nutritional requirements of barramundi. This data is essential for designing the best feeding strategies for growing barramundi. The work at Bribie is assessing the feeding value of locally available foods as alternatives to imported fishmeal. This will identify how well these cheaper food ingredients can be used to lower the ingredient cost of the diet. The combined approach - determining what nutrients barramundi require and how these can be obtained from cheap, locally available foods - will ensure that our ultimate goal of developing cost-effective diets and optimum feeding strategies for barramundi will be realised.

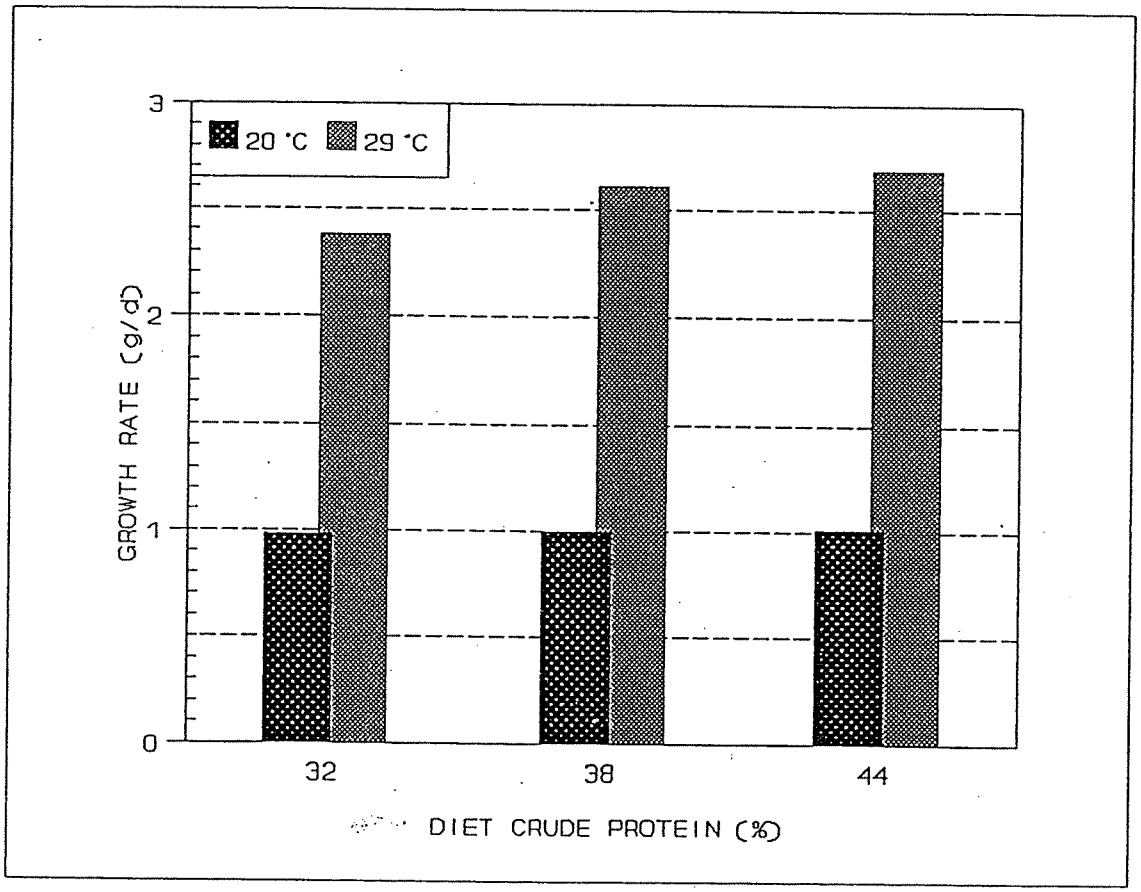
The effects of water temperature and feeding frequency on food intake and growth of barramundi were discussed at last year's workshop. More recent work has examined how fish growth is altered by changes in the dietary content of energy (Fig. 1) and protein (Fig. 2) at water temperatures simulating winter or summer conditions.

Fig. 1 Effect of dietary digestible energy content on growth of barramundi in water at 20° and 29°C



Increasing the dietary digestible energy (DE) content by 30% (from 14 to 18 MJ/kg) doubled growth rate of barramundi in water at 20°C as compared to a 30% improvement at 29°C. More importantly, the efficiency with which the energy was used for growth also improved as diet DE increased and this effect was better in water at 20°C than at 29°C. On the other hand, barramundi growth in 20°C water was not improved by increasing the crude protein (CP) content of the diet. However, increasing diet CP was beneficial with barramundi in water of 29°C. These findings apply to diets in which good quality fishmeal is the main protein source and essential amino acids are provided in balanced amounts.

Fig. 2 Effect of dietary crude protein content on growth of barramundi in water at 20° and 29°C



Using inferior protein meals is likely to be detrimental unless they are provided at high inclusion levels or are adequately supplemented with synthetic amino acids.

Based on these findings, there is a strong argument for using diets of different specifications for summer and winter feeding as recommended in Table 1.

Table 1 Recommended dietary energy and protein specifications for optimal growth of barramundi in summer and winter

Season/water temperature	Diet digestible energy (MJ/kg)	Diet crude protein (%)
Summer (>26°C)	Low-Medium (14-15MJ/kg)	High (≈40-42%)
Winter (<23°C)	High (17-18MJ/kg)	Low (≈33-35%)

The ingredient cost of the "summer" and "winter" diets should be almost the same as the added expense of meeting a high DE specification for winter should be compensated for by its lower CP content. However, some additional manufacturing cost could be involved because of the increased difficulty of producing good pellets with diets of high fat content. Manufacturers may be reluctant to supply a range of barramundi diets varying in nutrient specification as overall costs are increased and tonnages are low which makes it unattractive for the feed miller. One way to overcome this difficulty is to feed a "salmon diet" (having a total crude fat content of 18-20%) during winter and a "barra diet" during summer. This approach should maximise barramundi growth while minimising food costs per unit of production.

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Use of Autoclaving in the Preparation of Homogenates for Determining the Proximate Chemical and Fatty Acid Composition of Fish*

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Abstract: A comparative study was made of the proximate chemical and fatty acid composition of barramundi fish (*Lates calcarifer*, Bloch) processed by repeated mincing either without (M) or with autoclaving for 4 h at 126°C (M + A). M + A processing caused complete disintegration of the tissue, enabling easy homogenisation by blending. The eviscerated carcase and pooled gill/gut of 12 plate-sized barramundi were individually processed by M or M + A procedures and the resultant freeze-dried product analysed. Processing method did not alter ($P > 0.05$) the analysed ash, nitrogen or fat content of the sample. An additional two samples of pooled gill/gut were M or M + A processed and analyzed both before or after freeze-drying. Freeze-drying caused a 16 and 19% depression ($P < 0.05$) of eicosapentaenoic acid (C20 : 5ω3) and docosahexaenoic acid (C22 : 6ω3) respectively for samples processed by M but not by M + A. It is speculated that M + A processing inactivates endogenous lipolytic enzymes which otherwise cause fatty acid losses during freeze-drying. The present study demonstrates the suitability of autoclave processing for determining the proximate chemical or fatty acid composition of fish tissue.

Key words: barramundi, fish, autoclaving, chemical composition, fatty acids, freeze-drying.

INTRODUCTION

Changes in the chemical composition of the body accompanying growth provide a valuable indication of an animal's nutritional status and is useful for assessing the feeding value of a diet. There is however, a reluctance on the part of researchers to use chemical composition as a response criterion because of difficulties (and potential errors) in the homogenisation of the animal. Conventional (mincer) processing methods involve separating the animal into components of similar structural consistency (eg soft tissue, skin/appendages and bone) with these being homogenised by repeated passage through mincing and blending equipment (Morris and Moir 1964; Clayton *et al* 1974). Such a process is labour intensive, and handling errors and losses may ensue.

An alternative and simple processing procedure is autoclaving followed by blending. Autoclaving reduces the structural integrity of the tissue, including that of bone and keratin, producing a product able to be easily blended into an homogeneous mixture suitable for analysis immediately or after freeze-drying. This procedure has been used for poultry by Sibbald and colleagues (Sibbald and Fortin 1982; Sibbald and Wolynetz 1984) who found no apparent change in the proximate chemical composition of the animal following autoclaving for >8 h. This paper reports the results of a study examining the effects of autoclaving on the proximate chemical and fatty acid composition of barramundi (Asian seabass) *Lates calcarifer* (Bloch).

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EXPERIMENTAL

Experimental outline

The primary objective of the work was to evaluate the effect of autoclaving on the proximate chemical and fatty acid analysis of eviscerated body (carcase) and gill/gut samples of fish. This was achieved by repeatedly grinding individual samples of frozen fish tissue into a mince so that representative portions of the whole sample could then either be processed without (M) or with autoclaving (M + A). This ensured that any difference in chemical composition between companion M and M + A samples could confidently be ascribed to the effect of autoclaving *per se*. Initially, the carcases of 12 barramundi (mean ± SD of 321 ± 70·2 g) and one sample of pooled gill/gut (505 g) were individually subjected to M or M + A procedures and proximate chemical analyses done on the freeze dried material. The fatty acid content of the freeze-dried product of one carcase and that of the pooled gill/gut was also determined. Because of apparent differences in fatty acid composition between M and M + A processing, a further two samples of pooled gill/gut (260 and 295 g) were each M or M + A processed and fatty acid analyses done on the product both before (WET) and after (DRY) freeze-drying. This enabled the effects of processing and freeze-drying to be simultaneously compared.

Sample preparation

Prior to processing, samples were held frozen (−20°C) in individual lots. Samples were individually sawn into small pieces using a butcher's bandsaw and while frozen were passed three times through a 2·4 mm die plate of a Hobart meat mincer. The mince was hand-mixed between each mincing and thoroughly mixed after the last mincing whereupon it was divided into two approximately equal parts which were designated either M or M + A. The M part was immediately re-frozen (−20°C) and held until the companion M + A part had been autoclaved. The M + A part was weighed into an aluminium tin of known weight to which a small amount of water (25–50 g) was added, the tin covered with aluminium foil and autoclaved at 150 kPa above atmospheric pressure (126°C) for 4 h. After cooling, the tin minus the foil top was weighed (all weight changes being attributed to water exchange) and the contents quantitatively transferred to a Waring blender for homogenisation and immediate transfer to trays for freeze-drying. The M part was partially thawed to allow homogenisation in a Waring blender and immediately transferred to trays for freeze-drying. Companion M and M + A samples were simultaneously freeze-dried; the maximum time between initial mincing and freeze-drying of the sample was 7 days. After freeze-drying, M and M + A samples were similarly homogenised in a Waring blender before chemical analysis. Analyses were also made on the wet homogenised product immediately prior to freeze-drying. Subsequently, the M + A method was refined by autoclaving the sample without water addition and in a large mouth glass jars so that the sample could be homogenised *in situ*, thereby eliminating a transfer step.

Chemical analyses

All analyses were done in duplicate on either wet or freeze dried samples using Association of Official Analytical Chemists (AOAC 1990) methods except as otherwise specified. Dry matter (DM) was determined by oven drying at 105°C to constant weight, ash by ignition at 600°C for 2 h (AOAC: 942·05), nitrogen by a macro-Kjeldahl technique on a Kjel Foss automatic analyser using mercury in the digestion (AOAC: 976·05) and crude fat by Soxhlet extraction with petroleum ether (bp 40–60°C) for 16 h (AOAC: 960·39). Fatty acid content was determined as the fatty acid methyl ester by capillary gas chromatography following extraction procedures as described by Bligh and Dyer (1959) with modifications of Christie (1982).

Statistical analysis

Data were analysed using prepared statistical programs (Siegel 1992). The effects of processing method (M versus M + A) on the proximate chemical composition of the three gill/gut and 12 carcase samples were examined by paired '*t*'-test comparisons after determining that variances of the two sample groups were both independent (χ^2) and homogeneous (Bartlett's-test). Relationships between processing methods for each of the proximate chemical analytes were further examined using linear regression procedures. The effects of processing method (M versus M + A) and sample type (WET versus DRY) on the total and individual fatty acid contents of two samples of gill/gut were analysed as independent 2 × 2 factorial ANOVA.

RESULTS

The effect of M or M + A processing on the proximate chemical composition of the freeze dried gill/gut and carcase is shown in Table 1. Differences between pro-

TABLE 1
Proximate chemical composition (g kg^{-1})[a] of freeze dried homogenates of gill/gut and carcase prepared either without (M) or with (M + A) autoclaving

Analyte	*Gill/gut samples (n = 3)*			*Carcase samples (n = 12)*		
	M	*M + A*	*±SEM*	*M*	*M + A*	*±SEM*
Moisture	651a	630a	5·5	706a	716b	2·8
Ash (DM)	140a	134a	9·5	179a	172a	3·6
Nitrogen (DM)	69a	68a	1·5	106a	107a	1·0
Crude fat (DM)	408a	403a	14·7	134a	134a	5·3

[a] Within tissue type and analyte, means with different following letters differ significantly ($P < 0·05$).

cessing methods were confined to moisture content of the carcase where M + A resulted in higher ($P < 0·05$) values.

The effects of processing method and sample type on the fatty acid content of gill/gut is shown in Table 2. Interactions between processing method and sample type were significant ($P < 0·05$) for total fatty acid, eicosapentaenoic acid (EPA; C20 : 5ω3), decosapentaenoic acid (DPA, C22 : 5ω3) and docosahexaenoic acid (DHA; C22 : 6ω3), while strong trends ($P \leqslant 0·10$) were

TABLE 2
Interaction and main effects of processing (M and M + A)[a] and sample type (WET and DRY)[b] on the total and individual fatty acid content (mg g^{-1} DM) of gill gut

Analysis	*Interaction effect*[c]				*±SEM (n = 2)*	*Significance of main effect*[d]	
	M		*M + A*			*Process*	*Sample*
	WET	*DRY*	*WET*	*DRY*			
Total FA	272·4ab	269·6b	273·6ab	281·3a	2·04	0·07	NS
C14	10·1	10·3	10·2	10·0	0·22	NS	NS
C16	45·0	45·4	45·8	44·3	0·75	NS	NS
C16 : 1ω7	12·8	12·9	13·0	12·6	0·19	NS	NS
C18	12·5	12·8	12·5	12·6	0·17	NS	NS
C18 : 1ω9	46·2	47·0	46·8	46·6	1·03	NS	NS
C18 : 1ω7	6·9	7·0	6·9	6·9	0·05	NS	NS
C18 : 2ω6	34·8	33·7	35·4	34·5	0·77	NS	NS
C18 : 3ω3	4·4	4·0	4·3	4·4	0·10	NS	NS
C18 : 4ω3	4·1	3·7	4·2	4·3	0·11	0·05	NS
C20 : 1ω11	1·4	1·6	1·3	1·6	0·08	NS	NS
C20 : 1ω9	10·5	10·8	10·5	10·7	0·26	NS	NS
C20 : 1ω7	0·7	0·8	0·7	0·9	0·08	NS	NS
C20 : 2ω6	0·8	1·1	0·7	1·1	0·12	NS	0·06
C20 : 4ω6	1·6	1·8	1·6	2·1	0·08	NS	0·03
C20 : 5ω3	12·9a	10·9b	13·0a	13·6a	0·27	0·02	0·09
C22 : 1ω11	10·4	11·6	10·3	11·2	0·42	NS	0·09
C22 : 1ω9	1·3	1·7	1·4	1·6	0·16	NS	NS
C22 : 1ω7	0·3	1·0	0·3	0·8	0·20	NS	0·06
C22 : 1ω6	0·1	0	0	0	0·02	NS	NS
C22 : 1ω3	0·1	0	0	0	0·02	NS	NS
C22 : 4ω6	0	0·2	0	0·3	0·13	NS	NS
C22 : 5ω3	6·7ab	6·0b	6·7ab	7·2a	0·18	0·05	NS
C22 : 6ω3	23·8b	19·4c	24·1ab	25·3a	0·29	<0·01	0·01

[a] Processed by mincing either without (M), or with (M + A), autoclaving.
[b] Analysis done on homogenates either prior to (WET), or after (DRY), freeze-drying.
[c] Within rows, means without a common letter differ significantly ($P < 0·05$).
[d] NS, $P > 0·10$.

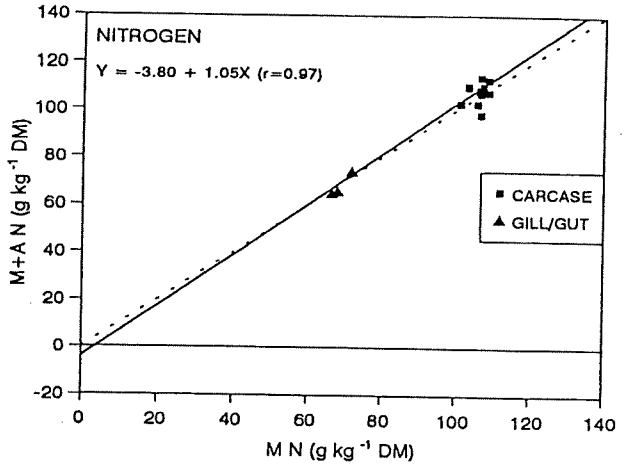
evident for linolenic acid (C18 : 3ω3), octadecatetraenoic acid (ODA: C18 : 4ω3) and arachidonic acid (AA; C20 : 4ω6). For each of these analytes except AA, analysis of the DRY sample following M processing resulted in the lowest content while differences between WET and DRY samples were small when M + A processed. In the case of AA which was present at only low amounts ($\leqslant$2·1 mg g^{-1} DM), an opposite trend was apparent in which the M + A processed WET sample had the lowest content. Exclusive of interaction effects, differences between main effects were confined to ODA where M processing resulted in higher ($P = 0{\cdot}05$) values than M + A processing and for AA where values for DRY were higher ($P < 0{\cdot}05$) than for WET (Table 2).

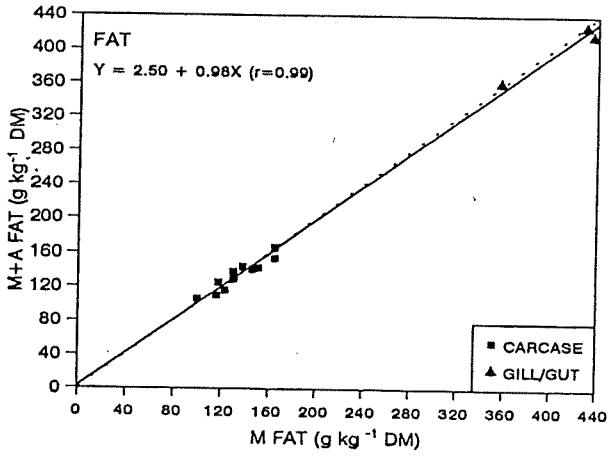
DISCUSSION

The observed difference between processing methods in moisture content of eviscerated carcase samples was small (1% in absolute value) and was most likely due to handling procedures adopted with M + A processing. Water was deliberately added to the M + A sample immediately prior to autoclaving and when rinsing the autoclave container during transfer of the product to the blender for homogenisation. Although sample weight was recorded at each handling stage, all changes in weight were ascribed solely to the addition or loss of moisture. This is possibly an incorrect assumption. Moreover, because of this added water, the final homogenised product of the M + A sample was very fluid which made subsequent freeze drying more difficult. Our current practice is not to add any water to the sample and to use large mouth glass jars so that the material can be blended *in situ* after autoclaving. This avoids any loss of material prior to homogenisation.

Processing method had no effect on the derived dry matter contents of ash, nitrogen or fat for samples of either eviscerated carcase or gill/gut. This agrees with the findings of Sibbald (Sibbald and Fortin 1982; Sibbald and Wolynetz 1984) with chickens. While the agreement between M and M + A processing was excellent for fat and nitrogen, it was not as good for ash (Fig 1). It is likely that the increased variability in ash analysis was caused by the uneven distribution of bone fragments in the minced product, despite the repeated mincing and mixing practised during its preparation. Morris and Moir (1964) also observed a high variation in ash between duplicate samples of minced sheep and cattle carcases with this being attributed to sampling errors due to uneven distribution of bone fragments.

One of the most significant findings of this study was the effect processing conditions had on the analysed fatty acid composition of the tissue. Freeze-drying reduced ($P < 0{\cdot}05$) the amounts of total fatty acid, EPA,





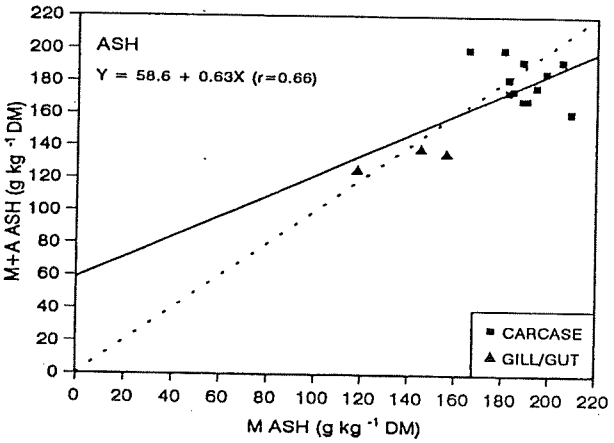


Fig 1. Comparison of the nitrogen, fat and ash content of carcase ($n = 12$; ■) and gill/gut ($n = 3$; ▲) samples processed either without (M) or with (M + A) autoclaving: dotted line denotes equality.

DPA and DHA in the case of M processed samples but no such effect was seen with companion samples that were M + A processed (Table 2). Although an opposite trend was seen for AA, the relevance of this observation is questionable since this fatty acid was present at only

very low concentrations ($\leqslant 2{\cdot}1$ mg g^{-1} DM). In the case of M + A processed samples, the contents of total and individual fatty acids were similar ($P > 0{\cdot}05$) irrespective of whether analysed WET and DRY. When these processing effects were examined across different classes of fatty acids (Fig 2), it is clear that changes were greatest for long-chain poly unsaturated fatty acids (PUFA) and least for the saturated fatty acids.

Lipids and especially PUFA are very vulnerable to autoxidation decomposition even when samples are held at temperatures of −15°C (Johnson and Davenport 1971; Ke *et al* 1977; Gray 1978). Because of the time that samples remain wet prior to drying, it is possible that fatty acid loss occurs during freeze-drying, particularly highly unsaturated members such as DHA and EPA. Such an effect is consistent with autoxidative changes where decomposition increases exponentially with increasing degree of unsaturation (Johnson and Davenport 1971; Enser 1984).

Since oxidative decomposition of fats is generally accelerated by heating and retarded by cooling (Gray 1978; Ke *et al* 1978), it was surprising that M + A processing lessened rather than increased fatty acid loss. Although purely speculative, the probable reason for this effect was that autoclaving denatured and thus inactivated endogenous lipolytic enzymes. A similar mechanism has been postulated for the increased shelf life of animal tissue following brief immersion in boiling water or dilute acetic acid (Christie 1982). Lipolytic enzymes appear to play a central role in the overall autoxidation process rather than merely initiating the primary peroxidation chain reaction (Johnson and Davenport 1971). The present studies support this view since autoclaving is unlikely to reduce the activity of other autoxidation catalysts such as pro-oxidants like metal ions and metal complexes and other agents including visible or UV light and high energy radiation. The absence of any effect of processing method on the analysed crude fat content (Table 1), is not surprising. The conjugated diene hydroperoxides produced in early autoxidative decomposition reactions and many of the resultant secondary products including aldehydes, glycerides and chain fragments (Johnson and Davenport 1971) would be soluble in petroleum ether and thus contribute to the analysed content of crude fat.

CONCLUSIONS

The present study has demonstrated the suitability of autoclaving as a procedure for preparing fish homogenates for the determination of proximate chemical or fatty acid composition. For routine use, whole fish or parts there of, can be processed by autoclaving without any need for prior mincing. The size of the autoclave appears to be the only constraint for this methodology. Autoclaving is preferred over that of conventional mincing procedures because a truly homogenised

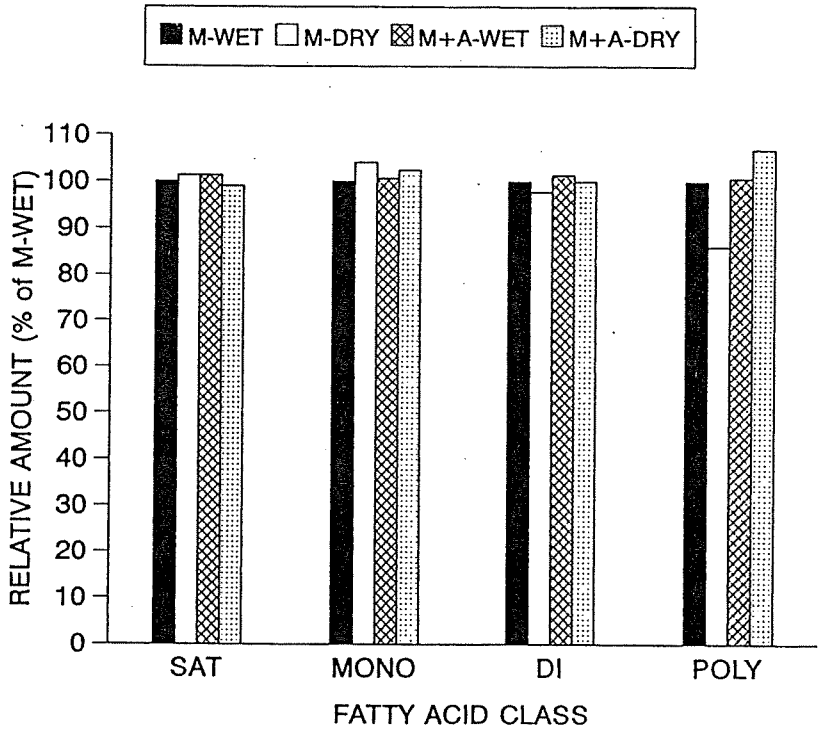


Fig 2. Relative change in fatty acid content of gill/gut samples following processing either without (M) or with (M + A) autoclaving and analysis before (WET) or after (DRY) freeze-drying.

product suitable for analysis is more easily achieved and the methodology is far less demanding of skilled labour. Autoclaving is eminently suitable for fatty acid analysis especially where samples need to be dried or have to be stored for an extended period of time before analysis.

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BARRAMUNDI NUTRITION RESEARCH

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The Fisheries Research and Development Corporation is supporting research at Walkamin and Bribie Island which is defining the essential nutrient requirements of juvenile barramundi and assessing the suitability of locally available protein meals as substitutes for imported fishmeal. The ultimate goal of the research is to develop cost-effective diets which are manufactured predominantly from feedstuffs available locally.

Work reported at previous workshops has discussed how food intake and growth are affected by water temperature and feeding frequency and how dietary specifications should be tailored according to season. Table 1 shows the dietary specifications currently recommended.

Table 1 Recommended dietary specifications for optimal growth of barramundi in summer and winter

Season (water temperature)	Crude fat (%)	Crude protein (%)	EPA+DHA[1] (%)	Dig. energy (MJ/kg)
Summer (above 26°C)	7-10	≈40-42	1.2	14-15
Winter (below 23°C)	18-22	≈33-35	0.5	17-18

[1] Sum of the omega-3 polyunsaturated fatty acids, eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA).

An on-farm trial was carried out to evaluate the effects of altering dietary energy and protein specifications on growth and taste of barramundi. The trial was intended to be done during winter when water temperatures were expected to be below 22°C. However, the 13 week trial was done in late winter/spring when daily minimum and maximum water temperatures averaged 24.6 and 26.8°C respectively. Twelve cages (2x2x2 m) were each stocked with 500 barramundi of approximately 300 g initial weight.

Fish were fed one of three diets:

(i) LP/LE - low protein (38%); low energy (14.5 MJ/kg)
(ii) LP/HE - low protein (38%); high energy (17 MJ/kg)
(iii) HP/LE - high protein (44%); low energy (14.5 MJ/kg)

The performance of the fish on each of the diets is shown in Table 2. Because of high variability between cages, growth performance of the fish was not significantly ($P<0.05$) different between diets. There was however, a tendency for the LP/LE diet to deliver the worst fish performance. The similar growth performance of fish fed the LP/HE and HP/LE diets is not surprising since water temperatures during the trial were about 25-27°C.

Table 2. Production responses of juvenile barramundi fed diets of varying protein and digestible energy content

Response criterion	Diets[A]			±sem[B]
	LP/LE	LP/HE	HP/LE	
Growth rate (g/d)	1.46	1.59	1.56	0.181
FCR (g:g)	1.77	1.67	1.62	0.191
Survival %	97.8	96.7	95.6	1.37
Food costs ($/kg gain)	1.08	1.09	1.05	0.122

[A] Costs are for ingredients only with no manufacturing cost included.
[B] Differences between treatments were not significant (P>0.05).

The eating quality of the fish from the on-farm trial was assessed at the International Food Institute of Queensland and compared with similar fish taken from cages fed a commercial barramundi diet. Some of the main findings are shown in Table 3.

Table 3. Sensory evaluation of fish fed either a commercial barra diet or experimental diets varying in protein and energy (fat) content

Characteristic	Diet			
	LP/LE	LP/HE	HP/LE	Commercial
Odour				
Weedy[1]	6.8	10.1	7.9	10.5
Muddy[1]	7.9	8.4	8.5	8.4
Colour				
Grey[1]	17.4^{AB}	16.5^{AB}	14.0^{B}	20.7^{A}
Yellow[1]	10.4^{AB}	9.0^{B}	8.7^{B}	13.8^{A}
Overall[2]	61.0	56.9	62.2	58.7
Flavour				
Muddy[1]	16.0	19.4	17.1	14.2
Stale[1]	13.8^{AB}	17.8^{A}	12.1^{B}	17.4^{AB}
Texture				
Firm[1]	43.2	43.2	43.5	45.7
Moist[1]	50.1	49.1	51.3	52.5
Sticky[1]	24.8	24.9	21.8	18.9

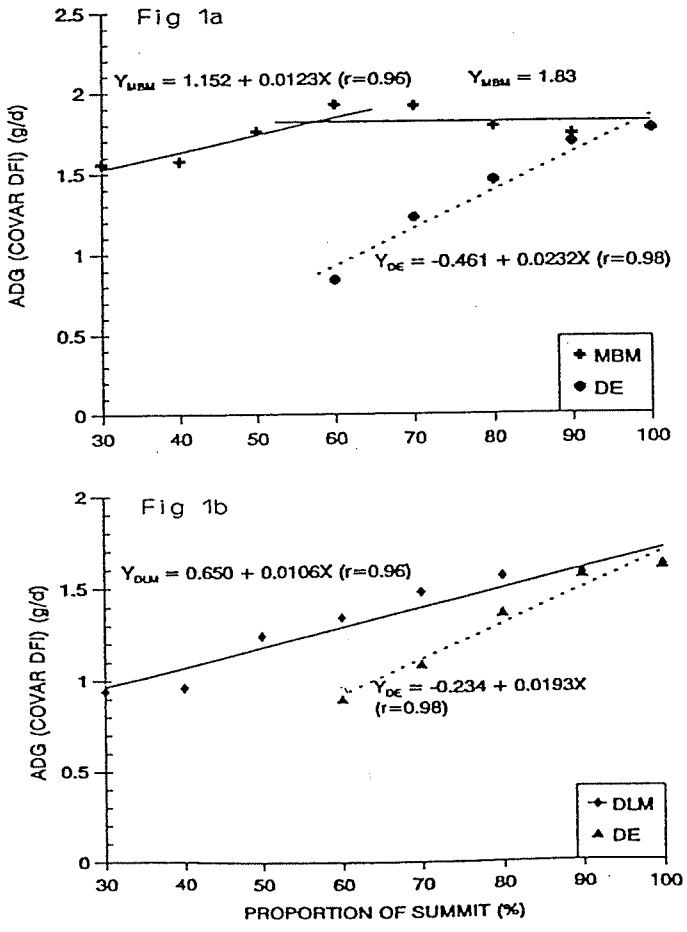
[A,B] Means not followed by a common letter are significantly different (P<0.05; Tukey's LSD).
[1] 0 = none; 100 = very .....
[2] 0 = dislike extremely; 100 = like extremely.

The eating characteristics of the fish on all diets was excellent with off-flavour scores being low and the overall rating being high. Significant effects (P<0.05) between the diets were confined to colour and flavour. Fish on the commercial diets had the highest grey and yellow discolouration scores while staleness was found to be highest for both the commercial and the LP/HE diets. The high fat content of the LP/HE diet (18.6%) may be the reason for the higher stale score as the fat content of the other experimental diets was only 7.7%.

Less than half of the protein in the diets fed in the on-farm trial was derived from fishmeal; most of the dietary protein came from meat meal and soybean meal. This trial has demonstrated the suitability of low fishmeal diets to deliver good barramundi growth and to result in fish of high eating quality.

The suitability of locally available protein meals as substitutes for imported fishmeal in diets for growout barramundi is continuing at the Bribie Island Centre. Previous work has determined the apparent digestibility of a variety of feed sources. While this is useful for feed formulation purposes, it provides only limited information of how well the feeds are ultimately utilised by barramundi for growth and development. One of the best ways of evaluating the nutritional value of a protein meal is to feed it in a growth assay. The procedure we are using is to compare the growth and protein deposition responses of barramundi when fed a highly nutritious "summit" diet that is diluted with a number of levels of either an inert diluent (such as diatomaceous earth) or the test protein meal. The difference in response between the test protein series and the diluent series of diets can then be interpreted as being due solely to the nutrients being provided by the test protein meal. Results for studies on meat & bone meal and dehulled lupin meal are illustrated in Figure 1.

Figure 1. Effect of dilution of a summit diet with either diatomaceous earth (DE) or a test protein meal on barramundi growth rate:- Meat & bone meal (MBM; Fig 1a) or dehulled lupin meal (DLP; Fig 1b)



The difference in growth response of the fish to the two test protein meals was quite striking. With meat & bone meal, dilution of the summit diet had no adverse effect on fish growth until the substitution level exceeded 40%; thereafter a steady decline was apparent but at a rate only about half that seen with diatomaceous earth. In the case of dehulled lupin meal, fish growth declined immediately it was substituted in the summit diet with the rate again being about half that seen with diatomaceous earth.

The results of these studies indicate that both meat and bone meal and dehulled lupin meal are reasonably well utilised by barramundi but not as well as that of the protein in the summit diet which was primarily of fishmeal origin. The steady decline in growth performance seen with both meat & bone meal (at substitution levels above 40%) and dehulled lupin meal is typical of either a simple amino acid deficiency in the test protein or a low energy availability of the test meal. Since growth did not decline with meat & bone meal until the substitution level exceeded 40%, an amino acid deficiency is the most likely explanation as a reduced energy availability would have been expected to have been expressed immediately.

A similar explanation appears likely for dehulled lupin meal since vegetable proteins are known to be particularly low in the essential amino acids, lysine and methionine. Results of the pending chemical analysis of the fish will tell us whether it is an amino acid or energy problem. A similar study with solvent extracted soybean meal showed an almost identical growth response to that of dehulled lupin meal.

Future studies at Walkamin will quantify the essential amino acid requirements of growout barramundi. Work at Bribie Island will further evaluate ways to improve the nutritive value of locally available protein meals, with priority being given to animal by-product meals and high protein oil-seed meals.

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Nutritional research in Australia to improve pelleted diets for grow-out barramundi *Lates calcarifer* (Bloch)

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Abstract

Farming of Asian seabass or barramundi *Lates calcarifer* is an emerging aquaculture industry in Australia with 1995/96 production expected to exceed 500 t. In Australia, barramundi are fed exclusively on extruded dry diets. Since 1992, an intense research program supported by the Australian Fisheries Research and Development Corporation has examined the nutritional requirements of grow-out barramundi and assessed the nutritive value of locally available protein meals. Optimal feeding practices have been defined for juvenile barramundi held at water temperatures varying incrementally from 20 to 29°C (the range normally experienced on Australian farms). Evaluation of alternative feed ingredients has shown that animal by-product meals such as meat meal and poultry offal meal are as well digested as fishmeal and are highly palatable to barramundi. Vegetable protein meals such as soybean, canola and lupin are less well digested and not well liked but can be used cost effectively for the partial replacement of fishmeal.

Increasing the dietary concentration of a reference protein incrementally from 29 to 57% crude protein (CP) caused food intake and food conversion to decrease and improve curvilinearly respectively such that growth rate exhibited a bent-stick response, increasing linearly up to about 46% CP. From these studies, the optimum protein to digestible energy (DE) ratio of the diet was estimated to be about 24-25 mg CP/kJDE. The essential fatty acid requirements (as the sum of eicosapentaenoic and docosahexaenoic acids) were found to vary with water temperature from ≈5 mg/g at 20°C to 18 mg/g at 29°C.

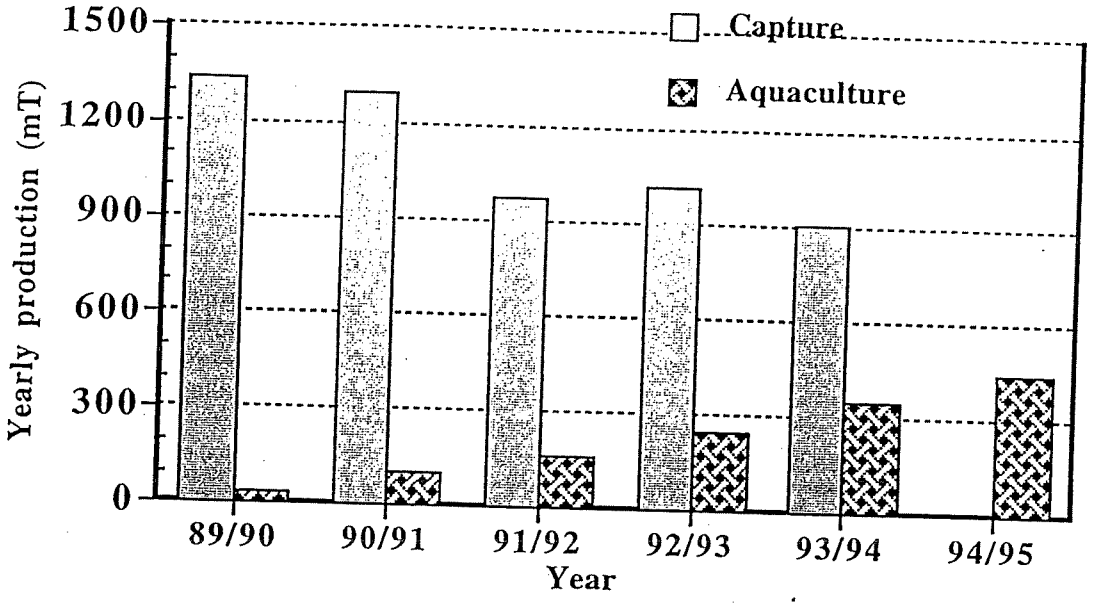
Under laboratory and commercial farm conditions, diets formulated entirely from terrestrial feed ingredients (except for a low inclusion of fish oil to provide essential n-3 fatty acids) have resulted in as good if not better barramundi productivity as conventional diets based on fishmeal. Using trained taste panels, the eating quality of the fish reared on these nil-fishmeal diets has been the same as for conventional diets.

Introduction

In Australia, Asian seabass or barramundi *Lates calcarifer* is a highly prised recreational and capture fishery and an emerging aquaculture industry expected to produce more than 500 t of fish in 1995/96, worth AUD$5 M (Figure 1). Most farmed barramundi are sold as plate size (400 to 500 g) whole fish destined for the restaurant trade although there is some interest in growing fish to a larger size (2 to 3 kg) for the fillet trade.

A major impediment to continued expansion of barramundi farming is the high cost of feeding since food comprises 40-50% of on-farm costs. In Australia, all farmed barramundi are grown out on pelleted (extruded) dry diets which are expensive (AUD$1200 to $1500/t). Feed cost is high as diets currently contain large amounts of imported fishmeal which is expensive and because a lack of information on the fish's nutrient requirements hinders the development of cost-effective feeds and feeding strategies.

Figure 1. Production of barramundi from capture fisheries and aquaculture in Australia



Research to define the nutrient requirements of grow-out fish and shrimp and to assess the suitability of terrestrial protein sources as cheaper alternatives to fishmeal is a major priority for Australian aquaculture. This is being addressed in a nationally co-ordinated research program administered by the Australian Fisheries Research and Development Corporation. A large team of aquaculturists from Commonwealth, State and University research institutions and private industry is working collaboratively to develop improved and more cost effective grow-out diets for barramundi, shrimp (*Penaeus monodon*), silver perch (*Bidyanus bidyanus*) and Atlantic salmon (*Salmo salar*).

This paper reviews our work with barramundi to determine their requirements for critically important nutrients and to assess the suitability of locally available terrestrial feedstuffs as cheaper alternatives to fishmeal in manufactured diets.

Effect of water temperature on food intake and growth

In Australia, barramundi are grown-out typically in cages suspended in estuarine water or in fresh-brackish water in earthen ponds. In areas where barramundi are farmed, water temperature varies seasonally between 20°C and 29-30°C. Because water temperature is known to have a profound effect on food intake of aquatic animals (Braaten, 1978; Steffens, 1989; Talbot, 1993), studies to define optimal feeding practices for juvenile (≈30 to 300 g) barramundi examined the effects of water temperature, feeding frequency and fish size (weight). Food intake (of a dry pellet containing: dry matter, 95%; crude protein, 44%; and estimated digestible energy, 15 kJ/g) of acclimatised fish increased essentially linearly with water temperature (over the range of 20 to 29°C) and fish size (Figure 2A); expressed as a function of fish biomass, food intake declined allometrically with fish size (Figure 2B).

Absolute growth rate increased linearly with fish size at each water temperature (Figure 3).

Figure 2. Effect of water temperature and fish size on intake of dry food pellet by juvenile barramundi: A, daily food intake; B, percent of fish biomass

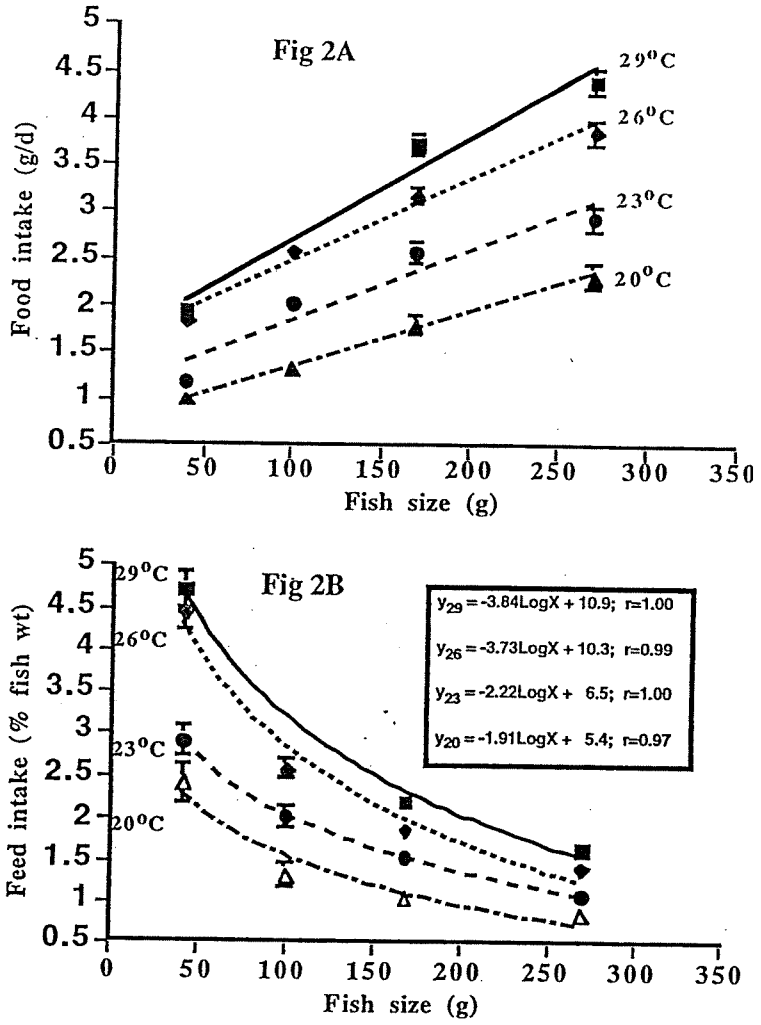
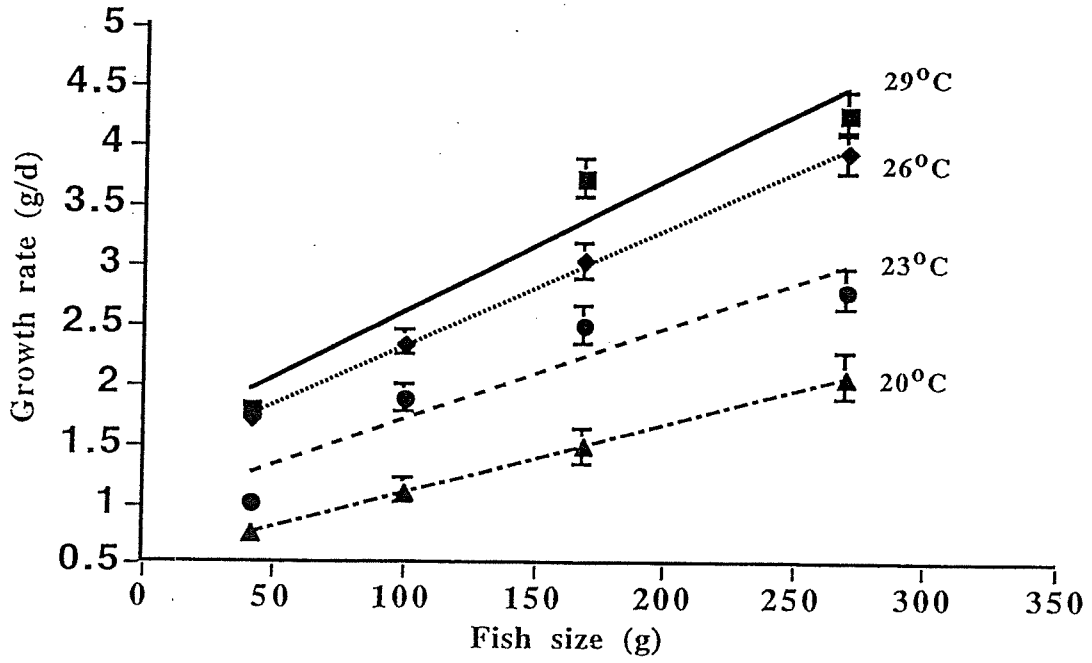


Figure 3. Effect of water temperature and fish size on growth rate of barramundi



Increasing the feeding frequency from 1 to 3 times daily increased food intake, slightly especially for smaller fish (<100 g), but the extra food did not result in

better growth rate. A similar observation had earlier been made by Tucker et al. (1988). Analysis of the data generated the following food intake prediction equation: $\ln DFI = -7.285 + 0.478 \ln W + 0.391T - 0.0065T^2 + 0.074F$ ($R^2 = 0.97$) where ln is the natural logarithm, DFI is daily food intake (g/fish/d), W is weight (g) of the fish, T is water temperature (°C) and F, the number of feeds/d.

Assessment of nutritive value of feed ingredients

Measurement of the apparent digestibility of a feedstuff is essential if diets are to be formulated to meet prescribed nutrient specifications at least cost. Because of the difficulty if not impossibility of collecting the total daily faecal output of an aquatic animal, apparent digestibility is typically measured using indirect procedures employing digestibility markers. Differences in the concentrations of the marker and of the particular nutrient in the food and in representative samples of faeces allows digestibility to be derived from the equation:

$$AD_{Nut} = 100 * [\, 1 - \{(M_{FI}/M_{FO}) * (Nut_{FO}/Nut_{FI})\}]$$

where AD is apparent digestibility (%); M and Nut are the concentrations (% dry matter) of the marker and nutrient respectively in the food (FI) and faeces (FO).

We have found Ytterbium acetate (at 0.05 to 0.1% of diet) to be a more reliable digestibility marker than chromic oxide. Its analysis however, requires a mass spectrophotometer. Apparent digestibility measurements were made using substitution procedures with the test ingredient being substituted in a basal diet at amounts of not less than 30%. We found faecal samples collected by sedimentation resulted in an over- and under-estimation of the apparent digestibility of protein and lipid respectively, because of the rapid leaching of soluble N which was almost half of the total N in the faeces (Windell et al., 1978; Smith et al., 1980; Williams et al., 1996). While intestinal dissection is the preferred method for faecal collection, it is not ideal for routine digestibility measurements since large fish have to be sacrificed and the procedure is very labour intensive. However, stripping of lightly anaesthetised fish has proved to be a reliable and efficient method for collecting faecal samples.

Presented in Table 1 are the crude protein and energy apparent digestibility values for a number of dry feed ingredients commonly available in Australia.

Table 1. The apparent digestibility of air-dry feed ingredients for barramundi

Feed ingredient	Apparent digestibility (%)		Digestible energy (kJ/g)
	Crude protein	Gross energy	
Fishmeal (Danish)[1]	88.7	99.2	20.0
Fishmeal (tuna)[1]	92.3	68.1	11.2
Meat meal (55% CP)[2]	75.1	76.3	13.4
Meat meal (50% CP)[2]	60.4	63.5	12.0
Poultry offal meal[2]	75.8	73.6	15.8
Soybean meal (full-fat)[2]	82.3	72.2	15.7
Soybean meal (solv)[2]	80.8	59.3	11.8
Canola meal[2]	80.0	54.2	10.7
sem (range)	0.9-10.2	1.9-8.4	0.4-1.8

[1] Determined by intestinal dissection (Williams et al., 1996).

[2] Determined by stripping of fish (McMeniman et al., 1996).

It is apparent from this data that barramundi are capable of digesting the protein from a wide variety of animal and plant feedstuffs but that they are less well able to digest the energy contained in terrestrial animal and plant food sources.

Protein requirement of juvenile barramundi

Many different approaches have been used with terrestrial and aquatic animals to define essential amino acid requirements. The most widely used (traditional) methodology involves feeding graded levels of one amino acid at a time in a test diet containing either all crystalline amino acids or a mixture of pure proteins and crystalline amino acids. Disadvantages of this methodology are (i) it is a slow process to evaluate each of the 10 or so essential amino acids; (ii) absolute response to diets comprised mostly of crystalline amino acids is usually inferior to that seen with diets based on intact proteins; and (iii) the derived dietary amino acid level that maximises fish response will be specific to the experimental conditions, particularly energy intake and the adequacy of all of the other essential amino acids.

An alternative methodology which has gained considerable support over the last decade is the "ideal protein" concept as espoused by Cole (1980). 'Ideal' protein is defined as one that is perfectly balanced in terms of its amino acid content for the type of production required (viz for growth, maintenance, reproduction). Such a protein would have the highest possible biological value, i.e. the greatest efficiency of conversion of dietary protein into deposited protein. Once this is determined, dietary specifications can easily be tailored for any given rate of growth (strictly speaking, for a given rate of protein deposition) of the fish. If growth in fish mirrors that seen in terrestrial monogastric animals such as pigs and poultry, growth is expected to exhibit dependency and independency to both protein and energy intake. The optimum dietary protein to energy ratio can be determined by feeding increasing amounts of protein (of constant quality) in conjunction with a constant amount of energy. The slope of the response line (ie biological value) will indicate how close the amino acid composition of this protein is to the ideal pattern.

This approach was tested by formulating a semi-purified diet in which all of the protein (of an amino acid composition closely matching that of barramundi protein) was provided as a protein mixture (reference protein). Protein content of the diet was varied incrementally from 29 to 57% by adding the reference protein at the expense of non-protein ingredients manipulated to maintain the desired energy content (Table 2). Fish were fed to satiety twice daily and held in water at 28°C for an experimental period of 28 d. Production responses are tabulated in Table 3 and Figure 4.

As the inclusion content of the reference protein increased, there was a marked curvilinear reduction in food intake and a corresponding although less marked improvement in food conversion ($P<0.05$). These effects caused growth rate to exhibit a bent stick response, increasing linearly up to a dietary protein content of about 46%. This is similar to the recommendation of Boonyaratpalin (1989) that the dietary crude protein content of grow-out Asian sea bass should be 45 to 50% (supplied predominantly from fishmeal).

Table 2. Composition of the diets in the protein requirement experiment

Feed source	Diet					
	1	2	3	4	5	6
			Formulation (%)			
Reference protein[1]	35.0	42.0	49.0	56.0	63.0	70.0
Starch (autoclaved)	47.5	40.0	32.5	25.0	17.5	10.0
Diatomaceous earth	3.5	5.3	7.1	8.9	10.7	12.5
Soybean oil	2.0	1.8	1.6	1.4	1.2	1.0
Fish oil	8.0	7.2	6.4	5.6	4.8	4.0
Vit + Min premix	4.0	4.0	4.0	4.0	4.0	4.0
			Chemical analysis			
Crude protein (%)	29.0	34.6	40.1	45.7	51.2	56.8
Gross energy (kJ/g)	18.93	18.85	18.78	18.70	18.63	18.55
Est dig. energy (kJ/g)	15.0	15.0	15.0	15.0	15.0	15.0
Crude fat (%)	11.7	11.0	10.2	9.5	8.7	8.0

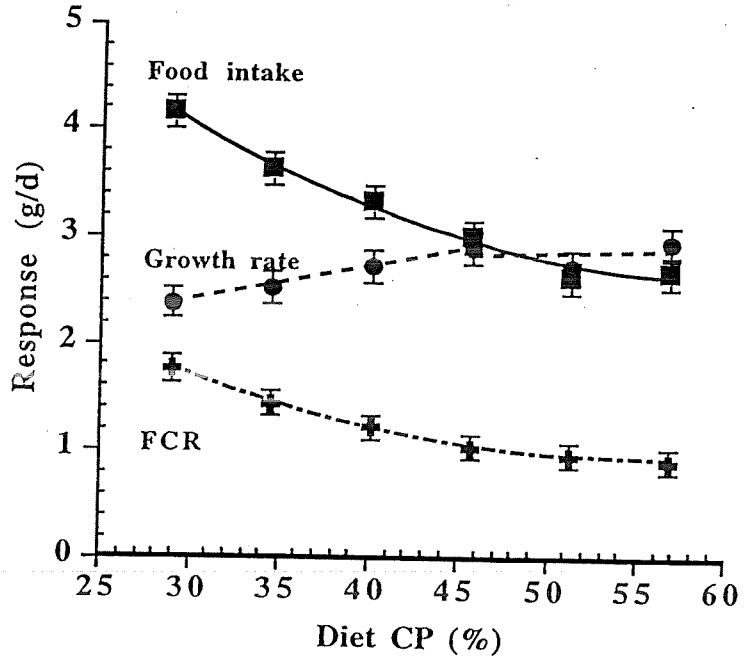
[1] Formulation (g/kg) of the reference protein was: Casein, 430; Fishmeal (Peruvian), 300; Gluten, 250; Lysine HCl, 5; d/l Methionine, 5.5; l Threonine, 2.5; l Tryptophan, 1; and $NaHCO_3$, 6.

Table 3. Production responses of barramundi to diets varying in protein content

Attribute	Treatment (diet CP%)						±sem
	29.0	34.6	40.1	45.7	51.2	56.8	
Start weight(g)	74.9	75.2	78.6	76.3	75.8	76.2	1.47
End weight (g)	123.6^{C}	144.2^{B}	153.5^{A}	157.5^{A}	152.1^{A}	158.5^{A}	2.14
Food intake (g/d)	4.16^{A}	3.62^{B}	3.32^{C}	3.00^{D}	2.61^{E}	2.66^{E}	0.058
Growth (g/d)	2.38^{C}	2.51^{BC}	2.72^{AB}	2.90^{A}	2.72^{AB}	2.94^{A}	0.072
FCR (g:g)	1.76^{E}	1.44^{D}	1.22^{C}	1.04^{B}	0.96^{AB}	0.91^{A}	0.026

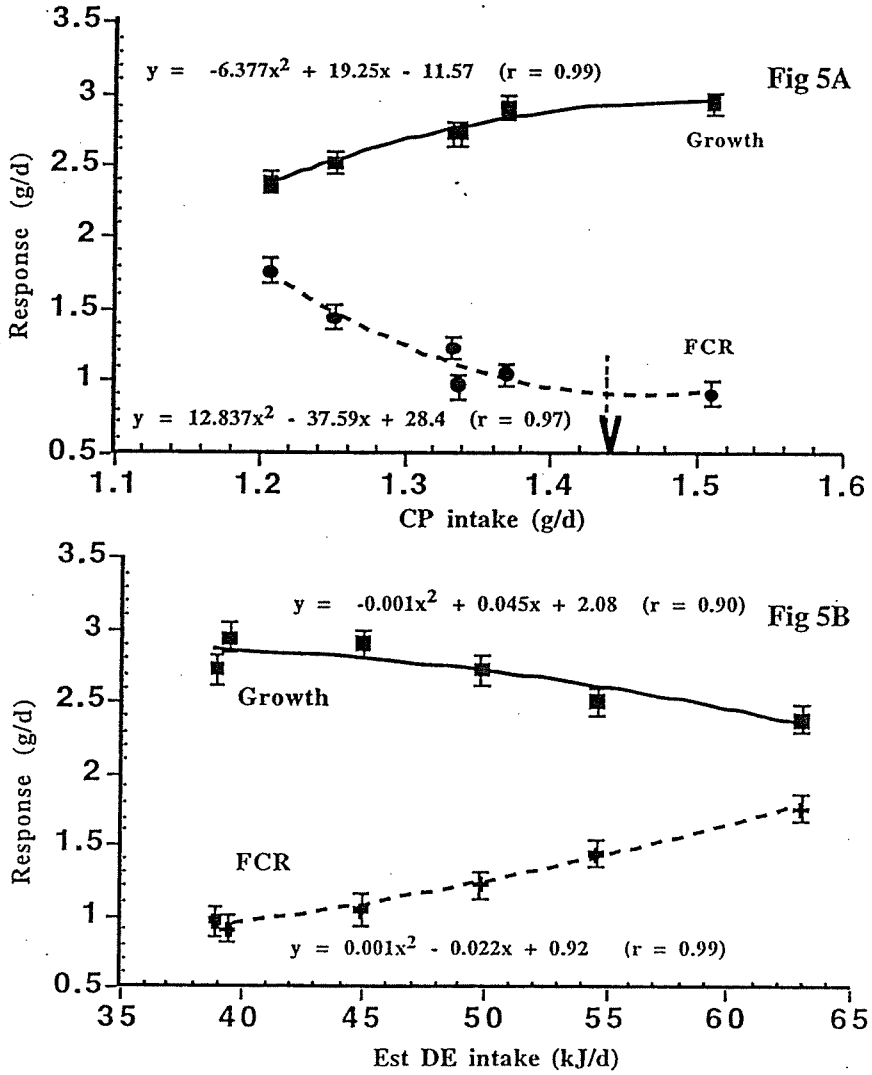
^{A,B,C,D} - Means without a common superscript letter differ ($P<0.05$).

Figure 4. Production responses of juvenile barramundi to isoenergetic diets providing incremental inclusions of a reference protein



When expressed as a function of absolute intake, growth and food conversion improved curvilinearly ($P<0.05$) with increasing protein intake with the response reaching an asymptote value at an intake of 1.44 g protein/fish/d (Figure 5A). In contrast, growth rate and food conversion deteriorated with increasing digestible energy consumption (Figure 5B), indicating that the response was clearly that of a simple protein dependency. Based on this result, the dietary protein to digestible energy ratio (P:DE) of juvenile barramundi was calculated to be no greater than 24 mg /kJ. This P:DE value is considerably lower than the 30 to 40 values tabulated by Tucker (1992) for carnivorous marine fish including barramundi. The lower value found by us may be due to the reference protein having a more optimal ("ideal") amino acid profile than the protein used in the other cited studies. Further work is being done to confirm this result and to define requirements for essential amino acids.

Figure 5. Responses of barramundi to absolute intake of protein (Fig 5A) or digestible energy (Fig 5B)



Essential fatty acid requirements

Varying the amounts of n-3 fatty acids in the diet have been investigated when juvenile barramundi were held at water temperatures of either 20 or 29°C (Barlow et al. 1966). Diets containing the desired content of n-3 fatty acids were formulated by serially varying the inclusion rate of soybean oil and fish oil such

that all other food ingredients remained constant. The assayed content of the diets (mg/g air dry) of total n-6 fatty acids, total n-3 fatty acids, eicosapentaenoic acid (C20:5n-3; EPA) and docosahexaenoic acid (C22:6n-3; DHA) varied respectively from 26.8 to 13.4, 15.6 to 30.0, 1.9 to 8.3 and 3.2 to 12.7. Fish (176 ± 3.3 g) were acclimatised to the desired water temperature for 21 days and fed their respective diets twice daily to satiety. The experiment continued for either 4 or 6 weeks for fish held at water temperatures of 29 or 20°C respectively.

The effect of the fatty acid content of the diet on the fish's response varied according to the water temperature (Table 4).

Table 4. Effect of water temperature (WT) and essential fatty acid content (EFA) of the diet on the growth performance of juvenile barramundi

WT (°C)	EFA(EPA + DHA) content (mg/g) 5.1	8.3	11.5	14.6	17.8	21.0	Response[1] (WT x EFA)	±sem
	Food intake (g/d)							
20	1.29	1.31	1.34	1.19	1.19	1.25	ns	
29	4.41	4.21	3.89	3.58	3.69	3.93	L;Q	0.091
	Growth rate (g/d)							
20	0.70	0.72	0.81	0.70	0.79	0.75	ns	
29	2.93	3.07	3.16	3.13	3.44	3.24	L;Q	0.103
	Food conversion (g:g)							
20	1.83	1.83	1.66	1.70	1.52	1.68	Q	
29	1.50	1.37	1.23	1.14	1.07	1.18	Q	0.077

[1] Response to diet at each water temperature: ns, not significant (P>0.05); L, Linear (P<0.05); Q, Quadratic (P<0.05).

At low water temperature, fish response was unaffected by dietary fatty acid content whereas at high water temperature food intake declined curvilinearly with increasing n-3 fatty acid content (Figure 6); because of a concomitant improvement in food conversion, growth rate improved linearly up to a total EPA and DHA content of 17.8 mg/g (Figure 7).

Figure 6. Effect of dietary fatty acid content and water temperature on food intake of juvenile barramundi

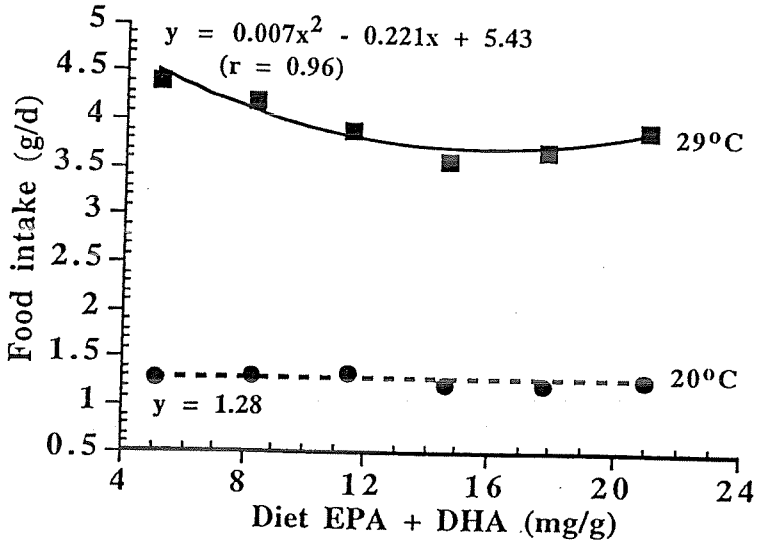
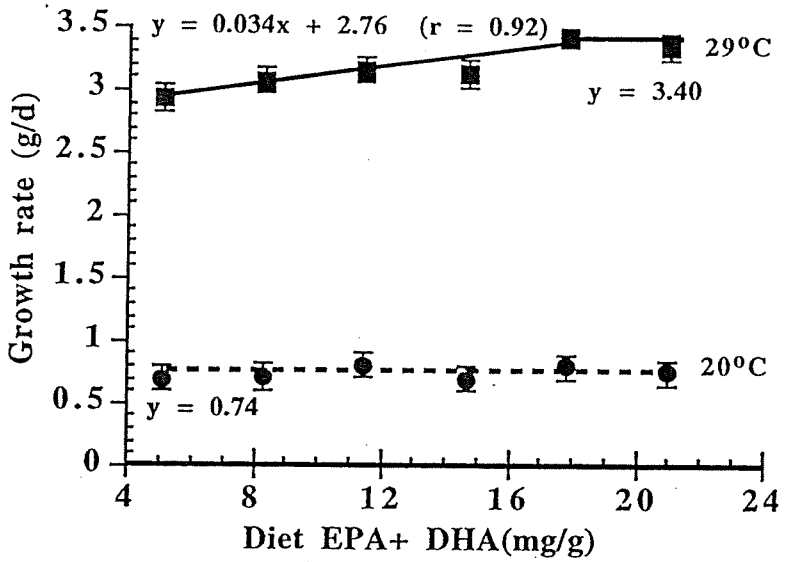


Figure 7. **Effect of fatty acid content of the diet and water temperature on growth rate of juvenile barramundi**



The observed curvilinear response of food intake to dietary fatty acid content at high water temperature was an unexpected result. The increased food intake on the diets containing the lowest n-3 fatty acid content could be interpreted as an attempt by the fish to increase its intake of critical n-3 fatty acids. This is plausible since food conversion also showed a marked deterioration for diets containing the lowest n-3 fatty acid content. The lack of response to dietary fatty acid content by fish held at low water temperature was probably due to the reduced growth and thus, a minimal requirement for n-3 fatty acids.

These results indicate that the optimal dietary n-3 to n-6 fatty acid content should be not less than 1.6:1 (equivalent to an EPA + DHA content of 17.8 mg/g) for rapidly growing fish at high water temperature whereas at low water temperature the ratio need not be greater than 0.6:1 (EPA + DHA content of 5 mg/g). In reviewing the essential fatty acid requirements of marine fishes, Tucker (1992) concluded that a dietary EPA + DHA concentration of 20 mg/g was a reasonable specification for the young of most species but this could be reduced to 14 mg/g for older fish . Tucker (1992) stressed the essentiality of DHA and advocated that it comprise at least half of the n-3 fatty acid content of the diet. Boonyaratpalin (1989) recommended that the total n-3 fatty acid content of the diet for juvenile Asian sea bass should be 10 to 15 mg/g.

Commercial trailing of nil-fishmeal grow-out diets for barramundi

The primary objective of the research program was to develop improved and cheaper barramundi grow-out diets with a reduced dependency on fishmeal. Information on the nutritive value of alternative feedstuffs and the fish's requirements for key nutrients was used to formulate practical diets for commercial evaluation. Several laboratory and on-farm trials have been done to demonstrate the suitability of these new generation diets. The results of a study comparing diets formulated without any fishmeal with either a proprietary barramundi diet or a high fishmeal experimental control diet are discussed to illustrate the progress that has been made.

A 4x4 randomised block design was used to compare three experimental diets (two containing no fishmeal) with a proprietary barramundi diet, all being commercially extruded as dry floating pellets (Table 5). The ingredient cost of the nil-fishmeal diets was 15 to 20% cheaper than that of the proprietary diet.

Table 5. Composition of the diets fed in the on-farm trial

Attribute	Diet description and formulation			
	Diet 1 (Control)	Diet 2	Diet 3	Diet 4 (Proprietary[1])
	Formulation (g/kg)			
Wheat	304	105	161	
Chile fishmeal (65% CP)	350	0	0	
Meat meal (52% CP)	0	500	500	
Meat meal (60% CP)	100	0	0	
Blood meal (ring)	0	90	70	
Soybean (fullfat)	160	100	150	
Gluten (90% CP)	50	100	50	
l-lysine HCl	0	7.5	6.5	
d/l Methionine	1.5	3	3	
Fish oil (Chile)	25	60	50	
Tallow	0	25	0	
Vit & min mixture	9.5	9.5	9.5	
	Chemical analysis			
Gross energy (kJ/g)	19.2	21.0	19.6	20.0
Est DE (kJ/g)	15.0	16.4	15.3	nd
CP (g/kg)	436	470	440	543
Fat (g/kg)	87	138	116	69
Arginine (g/kg)	27.4	29.4	28.8	29.7
Lysine (g/kg)	28.7	30.2	28.5	46.1
Meth + Cyst (g/kg)	9.2	8.1	7.3	10.8
Threonine (g/kg)	17.4	16.0	15.2	23.9
C20:5 n-3 (g/kg)	4.3	6.7	5.5	5.0
C22:6 n-3 (g/kg)	7.5	8.9	7.4	9.1

[1] Formulation of the proprietary diet is confidential. nd, not determined.

Cages ($2m^2$) were stocked with 300 fish (initially 226 ±16.3 g) and suspended in an aerated freshwater pond. Fish were fed once daily to satiety and reared on the diets for 10 weeks. At the conclusion of the feeding period, all fish were weighed and samples taken toassess eating quality using taste panel procedures.

Table 6. Effect of diet on performance of barramundi reared under commercial farm conditions

Response attribute	Diets				±sem
	Diet 1	Diet 2	Diet 3	Diet 4	
Food supply (kg/wk/cage)	7.6^{C}	9.1^{A}	9.1^{A}	8.3^{B}	0.10
Growth rate (kg/wk/cage)	6.2^{B}	7.0^{A}	6.4^{AB}	6.1^{B}	0.18
Farm food conversion	1.22^{A}	1.31^{AB}	1.44^{B}	1.37^{B}	0.041

[A,B] Within response attributes, means without a common letter differ ($P<0.05$).

There were significant ($P<0.05$) differences in fish growth performance between the diets (Table 6). Food intake of fish on both of the nil-fishmeal diets (diets 2 and 3) was higher than on each of the other diets, indicating high acceptability by the fish for the nil-fishmeal diets. Food conversion and growth rate on the high energy nil-fishmeal diet (diet 2) were as good if not better ($P<0.05$) than all of the other diets. Food conversion was best on the fishmeal control diet (diet 1) but not significantly better ($P>0.05$) than that for the high energy nil-fishmeal diet).

Assessment of the eating quality of the fish using trained taste panels at the Queensland Government's Centre for Food Technology showed similar scores for all diets (Table 7). Scores for undesirable off-colours and flavours were very low and the overall acceptance of the fish on all diets was very high.

Table 7. Effect of diet on eating quality scores (0 = low; 100 = high) of fish reared under commercial farm conditions

Response attribute[1]	Diets			
	Diet 1	Diet 2	Diet 3	Diet 4
Colour				
Yellow	6.9	8.8	9.1	7.6
Grey	10.5	10.5	9.7	9.5
Flavour				
Sweet	19.2	22.4	21.7	18.1
Fishy	49.0	47.3	45.5	46.8
Muddy	14.8	13.9	15.9	16.6
Texture				
Firm	46.5	46.9	44.3	47.3
Moist	44.2	43.9	42.7	47.4
Overall acceptability	60.0	64.3	61.2	63.5

[1] Differences between diets for all attributes were not significant ($P>0.05$).

These results demonstrate that appropriately formulated and cheaper diets without fishmeal (but containing some fish oil as a source of n-3 fatty acids) are able to grow barramundi as well as those fed on conventional high fishmeal diets. Equally important, the eating quality of the fish reared on nil-fishmeal diets was indistinguishable from fish fed on high fishmeal diets. Further studies are continuing to specify requirements of grow-out barramundi for critical essential amino acids and the role of high energy diets for the commercial production of the fish..

Acknowledgments

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CONTINUING THE DEVELOPMENT OF IMPROVED GROW-OUT DIETS FOR BARRAMUNDI

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Introduction

As reported at previous workshops and recently summarised (Williams and Barlow 1996), considerable research has been carried out over the past four years at Walkamin and Bribie to develop more cost-effective grow-out diets for barramundi. This work has been part of a FRDC nationally-coordinated diet development program seeking to reduce Australia's dependency on fishmeal in diets for prawns and fish. The barramundi research has involved close cooperation between researchers, a feed manufacturer and barramundi growers who have together collaborated in trailing new diets under farm conditions. The first three on-farm studies were reported at the 1996 Workshop. Presented at this Workshop are the results of a further two on-farm studies and a laboratory study which were carried out as extensions of the earlier work demonstrating the potential of terrestrial protein sources to replace fishmeal in grow-out diets for barramundi.

Farm Trials

All three studies examined the same three commercially-produced and extruded diets – (i) the standard Ridley® (15MJ) diet; (ii) a low-fishmeal (Wlk1) diet of equivalent nutrient specification as the 15MJ; and (iii) a low-fishmeal, low-protein/high energy (Wlk2) diet. All three diets were sourced at the same time (August 1996) and stored in a cool room (4 to 7°C) for the duration of the work. Each of the on-farm studies comprised a 10-week feeding period with five (5) cages of fish assigned to each of the three diets. Both of the on-farm studies were carried out during the period September to December 1996. The laboratory study was carried out to confirm the on-farm results under standardised water temperature and management conditions but duplicating the differences between the two farms in water salinity (<0.5 versus 30‰). The 8-week laboratory feeding experiment was carried out during the period March to May 1997 and involved 8 tanks (500 L) of fish assigned to each of the three diets.

The performance of the fish in the on-farm studies are summarised in Table 1.

Table 1. Performance of fish fed test diets on two farms

Criterion	Farm and diet fed						
	Farm A			Farm B			
	15MJ	WLK1	WLK2	15MJ	WLK1	WLK2	=sem
Start weight (g)	159	164	164	119	119	120	3.6
End weight (g)	234^{A}	232^{A}	203^{B}	232^{A}	233^{A}	135^{C}	7.2
Growth (g/week)[1]	7.9^{B}	7.2^{B}	4.2^{C}	10.9^{A}	11.1^{A}	1.1^{D}	1.12
Adj farm FCR[1]	1.40^{A}	1.39^{A}	2.83^{A}	1.62^{A}	1.89^{A}	15.2^{B}	1.90
Survival %	98.7	97.8	88.3	98.7	98.6	91.2	1.18
Gill/gut %	11.5	10.9	12.4	11.8	11.6	12.0	0.38

A,B,C,D For each criterion, means with a common letter do not differ significantly (P>0.05).

1 Data covariance adjusted to negate effects of differences in start weight and fish mortality.

The absolute growth rate of the fish was higher on Farm B than on Farm A and this could have been due to any number of differences between the farms including culture conditions (eg water temperature, water quality, salinity etc), fish stock, management etc. However, the response to the three diets was remarkably similar on each farm with the 15MJ and Wlk1 diets producing similar fish productivity while the Wlk2 diet resulted in demonstrably inferior performance. The similarity in fish performance for the 15MJ and Wlk1 diets confirms previous laboratory and on-farm work showing the suitability of low fishmeal diets for growing barramundi. The poor performance of fish on the Wlk2 diet indicates however, that barramundi may not be able to use high inclusions of lipids (fats or oils) as a way of reducing the amount of protein in the diet. This contrasts with findings in salmonids where research over the past two decades has resulted in dietary levels of protein declining from 55 to 38% and lipid increasing from 8 to 33% (Smith and Guerin, 1995). Although barramundi may not have the same capacity to use lipid as salmonids, there may be productivity benefits of using diets high in both energy and protein. Work to address this issue will shortly commence at Walkamin.

At the conclusion of the on-farm studies, fish were taken from all cages for taste evaluation at QDPI's Centre for Food Technology. In this assessment, trained taste panels evaluated the fish by scoring them for appearance, odour, flavour and texture. Differences between diets for fish from Farm A were confined to flavour and texture where both Wlk1 and Wlk2 diets resulted in sweeter and more fishy flavours and had a firmer texture than the 15MJ diet. On Farm B, differences between diets were observed for all sensory characteristics. Fish fed the Wlk1 and Wlk2 diets had a slightly meaty odour, were darker in flesh colour, sweeter and more fishy flavours and firmer in texture to those fed the 15MJ diet. Overall acceptability of the fish was rated highly irrespective of farm or diet and scores for undesirable characteristics eg weedy, muddy, stale and metallic tastes were all less than 12 (out of 100). These results indicate that the flesh of barramundi fed on similar low-fishmeal diets is unlikely to be distinguishable by appearance or taste from those fed high fishmeal diets.

Laboratory Trial

The results of the laboratory study comparing the same diets under fresh and simulated estuarine water conditions are shown in Table 2.

Table 2. Performance of fish fed test diets in the laboratory study

Criterion	Salinity and diet fed						
	<0.5 ‰			30 ‰			
	15MJ	WLK1	WLK2	15MJ	WLK1	WLK2	±sem
Start weight (g)	273	275	256	260	263	256	5.0
End weight (g)	507^{A}	446^{B}	354^{D}	453^{B}	407^{C}	337^{D}	13.1
Growth (g/week)	28.8^{A}	20.9^{BC}	12.7^{D}	24.3^{AB}	18.0^{C}	10.4^{D}	1.58
FCR	1.06^{A}	1.30^{A}	1.68^{A}	1.17^{A}	1.57^{A}	2.44^{B}	0.24
Food (g/week)	30.5^{A}	27.2^{AB}	21.0^{B}	28.6^{A}	28.2^{A}	20.7^{B}	1.86

^{A,B,C,D} Within each criterion, means with a common letter do not differ significantly (P>0.05).

Under the controlled conditions in the laboratory, fish productivity was slightly better on the fresh compared to the saline water. An examination of the growth and FCR on a fortnightly basis showed that the performance of the fish in freshwater remained constant through the trial, but for the fish in saltwater the performance gradually improved as the trial progressed, and in fact equalled the freshwater system during the final fortnight of the trial. This indicates that it may take up to eight weeks for barramundi to become fully acclimated to salt water. Hence, no conclusions should be drawn from this work on relative growth rates of barramundi in fresh and salt water.

As for the farm studies, the response of the fish on the three diets was very similar irrespective of the water salinity. However, in contrast to the on-farm work, there was a clear ranking between all three diets in fish performance with the 15MJ being the best, the Wlk2 the worst and the Wlk1 intermediate between the former two diets. This was an unexpected finding as previous companion on-farm and laboratory experiments have always given complementary results. Compared to previous companion studies, the striking difference in the present work was the extended time between the companion experiments. For instance, the diets used on the farms were just a few weeks old at the start of the experiment whereas at the start of the laboratory study the diets were then seven months old. Even though the diets had been stored under favourable cold-room conditions (low humidity, 4 to 7°C), the most likely explanation for the observed reduced fish performance on the Wlk1 diet is that the prolonged storage caused a deterioration in its nutrient content. The reason why the 15MJ diet did not show a similar deterioration in nutrient quality although stored identically to the other diets is unclear but may be the result of differences in 'shelf life' of the various ingredients used in the respective diets. These aspects are currently under investigation.

Conclusions

The present studies have provided some very interesting findings. On the one hand, the on-farm studies build on previous research demonstrating that nutritionally balanced diets based predominantly on terrestrial protein sources are more cost-effective and produce equivalent fish productivity as those having a high fishmeal content. Similarly, the eating characteristics of the fish fed on low-fishmeal diets are equal to, or better than, those fed on high-fishmeal diets. On the other hand, the companion laboratory study provides a timely warning that diets should be turned over quickly (preferably within one month of purchase) and stored under the coldest practical conditions to preserve their nutritional vitality. Further research is planned to enhance the 'shelf life' of low-fishmeal diets but gains made in this direction should never be used to justify improper or prolonged storage of the food.

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BARRAMUNDI FEEDING AND NUTRITIONAL REQUIREMENTS

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INTRODUCTION

Nutritional research to develop better and cheaper grow-out diets for barramundi has been a major research focus at Walkamin and Bribie since 1993. This work has been part of a coordinated national effort funded by the Fisheries Research and Development Corporation (FRDC) to develop more cost-effective diets for prawns and finfish. Additional funding support for the work has come from other R&D corporations and from the feed's industry. A particular focus of the research has been to lessen aquaculture's heavy reliance on fishmeal as the basis of aquafeeds by finding more sustainable and renewable terrestrial alternatives. Coupled with this work has been research to better understand the animals requirements for particular nutrients and to develop improved feeding strategies.

Progressive results of the barramundi research have been reported at the Australian Barramundi Farming Workshops which have been run by QDPI every year since 1993. This paper will summarise some of the key findings from this earlier research and outline the studies now being done to develop high energy diets which will particularly suit recirculation systems of farming.

Effect of water temperature on food intake and growth

The effects of water temperature, feeding frequency and fish size (weight) on food intake and fish growth were examined in order to define optimal feeding practices for juvenile (30 to 300 g) barramundi. Food intake of acclimatised fish increased as water temperature increased from 20 to 29°C for each size class of fish (Figure 1). Growth rate also increased with water temperature and fish size (Figure 2).

Figure 1. Food intake of barramundi at different water temperatures

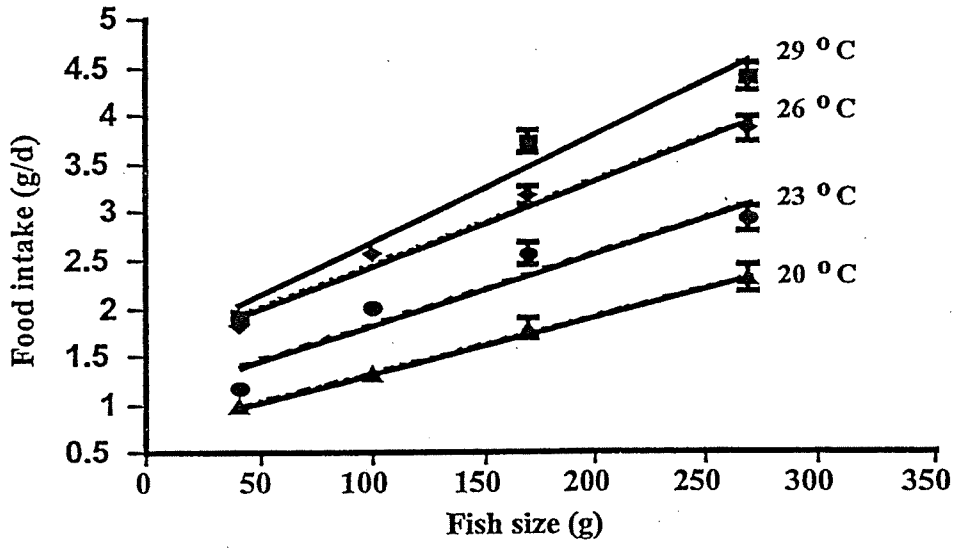
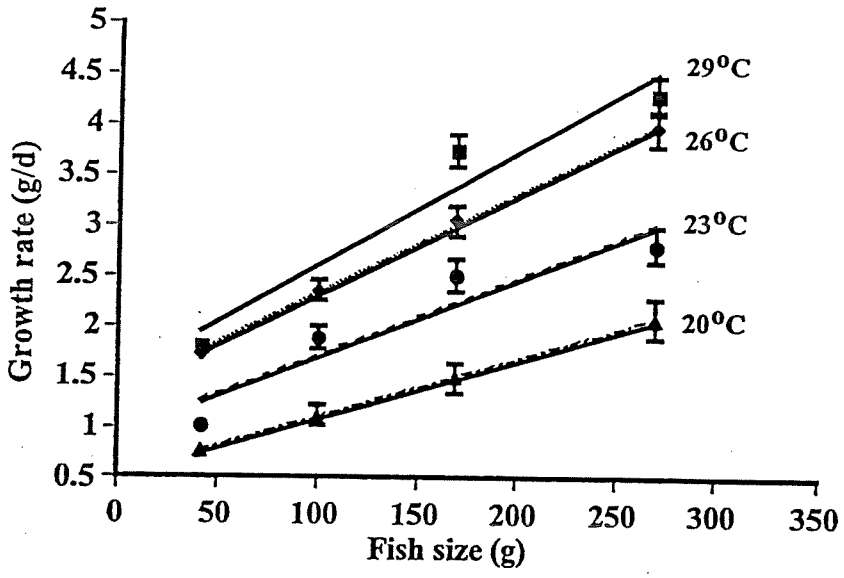


Figure 2. Growth of barramundi at different water temperatures



Increasing the feeding frequency from 1 to 3 times daily increased food intake, slightly especially for smaller fish (<100 g), but the extra food did not result in significantly better growth rate. However, as a recommendation, fingerlings should be fed twice daily up to about 100 g, whereas for bigger fish once daily feeding is adequate. For fish above 300 g, skipping a day's feeding on the weekend is a common industry practice but our research has shown that this will result in some diminished growth of the fish.

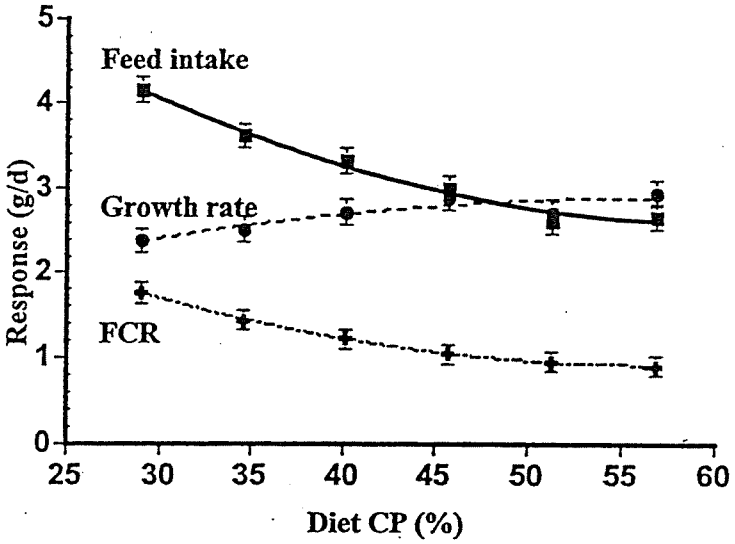
Assessment of nutritive value of feed ingredients

Crucial to the nutritive evaluation of any food is basic information on its chemical composition, apparent digestibility and subsequent utilisation by the animal. Digestibility and assimilation studies have been done for most of the terrestrial protein feeds considered to have potential as alternatives to fishmeal in barramundi diets. Animal by-product meals such as meat meal were as well digested as fishmeal and were found to be highly palatable to barramundi. Vegetable protein meals such as soybean, canola and lupin were less well digested and not well liked but could be used cost effectively for the partial replacement of fishmeal. Based on the cost per unit of digestible protein, the vegetable protein meals tend to be the least expensive with meat meal being slightly more expensive and far more cheaper than fish meal.

Dietary protein requirement of juvenile barramundi

We have examined the response of barramundi to diets varying in protein concentration over the range of 29 to 57%. The protein used was a high quality mixture of fishmeal and casein (with an amino acid profile similar to that of the protein in barramundi) and the digestible energy content of the diet was held constant at 15 MJ/kg. The fish were held in a recirculation freshwater system maintained at 28°C and were fed twice daily to satiety. Production responses of the fish are shown in Figure 3.

Figure 3. Production responses of juvenile barramundi to isoenergetic diets providing incremental inclusions of a reference protein



Increasing the amount of protein in the diet significantly improved FCR but food intake showed an even greater reduction so that the growth of the fish was improved only slightly by high protein. These results suggest that fish growth and FCR were optimised for a 15 MJ digestible energy diet when the protein content was about 45%.

Essential fatty acid requirements

The amount of the critical EPA and DHA omega-3 fatty acids required in the diet of barramundi has been investigated with fish held at water temperatures of either 20 or 29°C. Fish were fed twice daily to satiety on diets where the EPA+DHA content was varied serially from 0.5 to 2.1%.

At the low water temperature, fish response was unaffected by the amount of EPA+DHA in the diet. However, at the high water temperature, fish responded to increasing dietary EPA+DHA much in the same way as for increasing protein. Namely, food intake declined, FCR improved and growth increased slightly, reaching a plateau when the diet contained more than 1.8% EPA+DHA. While fish in low temperature water to have a lower requirement for these omega-3 fatty acids than those at higher temperatures, it is recommended that the diet should contain not less than 1.5% of EPA+DHA.

Commercial trialing of low-fishmeal grow-out diets for barramundi

Four experiments have been done on farms to demonstrate the extent to which meat meal can replace fishmeal in grow-out barramundi diets. In one of the 10 week feeding studies, a high fishmeal (control) diet was compared with two experimental diets containing no fishmeal and these evaluated against a commercial barramundi diet (Table 1). The ingredient cost of the zero-fishmeal diets (M3 and M4) was 15 to 20% lower than for the high fishmeal control diet. The diets were fed to caged fish in an aerated freshwater pond and managed as for other fish on the barramundi farm. At the conclusion of the feeding period, samples of the fish were taken for taste panel assessment. Results are shown in Table 1.

Table 1. Formulation and nutrient characteristics of the diet and the resulting productivity of barramundi reared under commercial farm conditions

Attribute[1]	Diet Description				
	Control	M3	M4	Commercial	± sem
	Formulation & nutrient composition				
Fishmeal (% in diet)	35	0	0	?	
Meatmeal (% in diet)	10	50	50	?	
Soybean (f-fat)(% in diet)	16	15	10	?	
Fish Oil (% in diet)	2.5	5	6	?	
Diet C-protein (%)	43.8	42.5	47.8	50.1	
Diet digest. energy (kJ/g)	15.0	15	16.2	15.0	
	Fish performance				
Growth (g/week)	20.8^{B}	21.4^{AB}	23.2^{A}	20.3^{B}	1.75
Food conversion (g:g)	1.22^{A}	1.44^{B}	1.31^{AB}	1.37^{B}	0.070
Fish recovered (%)	94.6^{A}	97.8^{A}	97.9^{A}	99.2^{A}	2.96
Dressing–out (%)	89.9^{A}	88.7^{A}	88.6^{A}	89.4^{A}	0.30
Food cost ($/kg gain)[2]	1.08^{B}	0.89^{A}	0.88^{A}	---B	0.038

1 Within rows comparisons, means without a common superscript letter differ (P<0.05)
2 Food cost calculated on basis of prevailing ingredient prices without any allowance for processing. Information on the commercial diets is not available.

Fish fed the higher energy zero-fishmeal diet (M4) performed best overall, growing faster than those fed either the high fishmeal control diet or the commercial diet. FCR of fish on the lower energy zero-fishmeal diet (M3) was slightly worse than the control but not significantly different to the other diets.

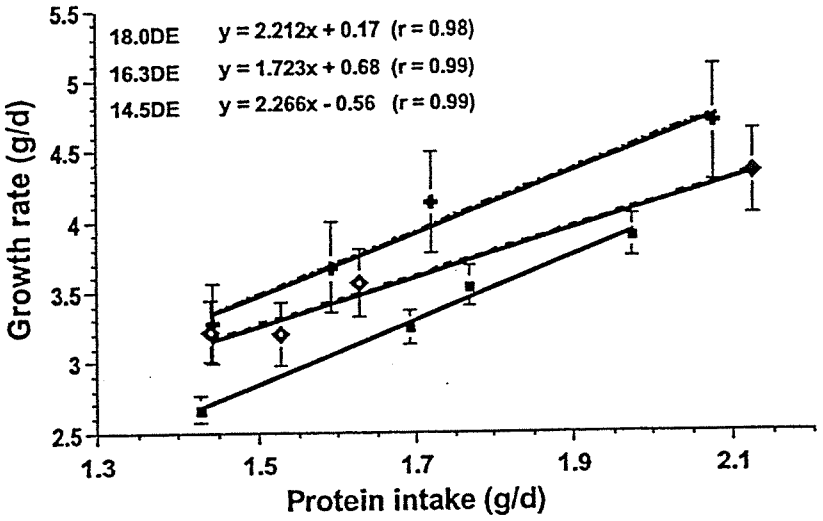
Assessment of the eating quality of the fish using trained taste panels at the Queensland Government's Centre for Food Technology showed similar scores for all diets. The overall acceptance of the fish on all diets was very high.

These results demonstrate that appropriately formulated diets without fishmeal are as good as and less expensive than conventional high fishmeal diets for barramundi. Equally important, the eating quality of the fish reared on zero-fishmeal diets was indistinguishable from those fed on high fishmeal diets.

Current research: High energy diets for intensive barramundi farming

Intensive farming of barramundi in closed (recirculation) systems is becoming more popular as a way of better controlling and increasing fish production. Invariably, these systems have a high capital investment and whether or not profits are better than for other less intensive farming systems depends on how successful these higher investment costs can be offset against an increased rate of production.

Figure 5. Effect of protein intake and digestible energy concentration of the diet on growth rate of barramundi



Growth rate showed a similar response as for FCR except that the response was more curved with the 16.3 kJ/g energy diets because the food intake of fish on the two intermediate protein levels was unexpectedly low. However, plotting growth against actual protein intake (Figure 5) rather than dietary protein concentration showed clearly that the responses at each energy level were indeed linear. Again growth improved with each increase in energy concentration of the diet. Growth rates also were excellent with growth rates of 27 g/week for the lowest energy and increasing to 33 g/week for the highest energy concentration.

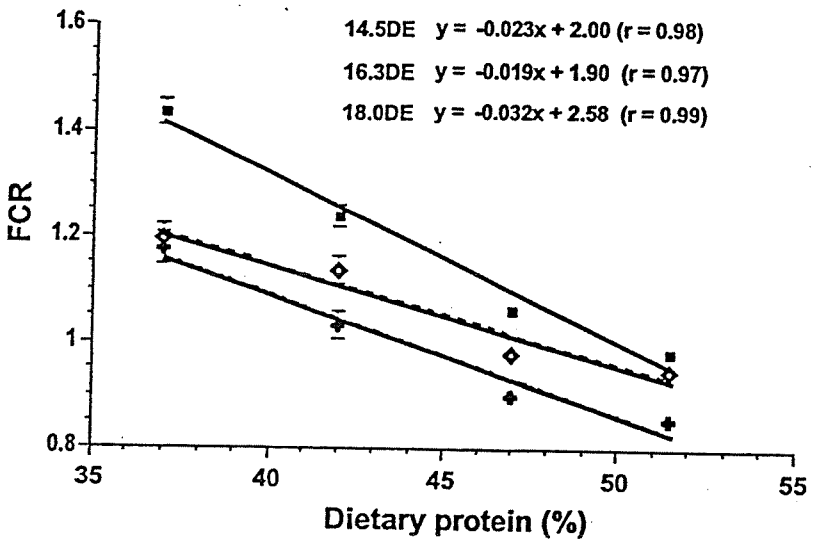
These results show that barramundi growth can be markedly improved by increasing the protein and energy concentrations of the diet. The observed response suggests that further gains are possible with even higher concentrations and this will be the subject of further research. While these more dense diets will require higher and therefor more expensive inclusions of oil and protein feeds, the cost to meet these specifications can be minimised if less expensive feed ingredients are used which previous research has shown to be very successful.

Simply, this means growing the fish at their maximum rate to increase throughput or achieve higher harvest weights in the same time.

Our nutrition research is now focusing on how we can grow barramundi faster and more economically by using higher density diets to deliver more nutrients in the same amount of food. These diets should deliver higher rates of fish production in both cage and recirculation systems but in the case of the latter system, they will have the additional advantage of producing less organic wastes.

A Walkamin laboratory experiment to test the optimum protein concentration for high energy diets is almost completed. In the study, four protein levels (37.4, 42.2, 46.9 and 51.7%) are being examined at each of three digestible energy levels (14.5, 16.3 and 18.0 kJ/g). The 12 diets are being fed once daily to satiety; barramundi started at an average weight of 230 g. Results after 6 weeks are shown below.

Figure 4. Effect of dietary protein and digestible energy content on FCR



FCR improved linearly as the protein concentration of the diet increased from 37.4 to 51.7% and also was better with each increase in energy concentration (Figure 4). FCRs were very good and at the highest protein level was 0.99:1 and 0.85:1 at the lowest and highest energy concentrations, respectively.

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Larval penaeid and grow-out finfish nutritional research in Australia

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Introduction

Research to define the nutritional requirements of penaeid prawns and barramundi and developing more cost effective feeds for their culture has been a major and collaborative research focus of the Commonwealth Scientific and Industrial Research Organisation (CSIRO) and Queensland Department of Primary Industries (QDPI) since the early 1990's. The nutritional methodology used in the penaeid larvae and grow-out barramundi research could equally be applied for the development of improved feed's technology for the culture of groupers. The approach used and key findings of the research are summarised in this paper.

Nutritional requirements of penaeid larvae

Microalgae are essential for the herbivorous larval stages of penaeid prawns during development from nauplii to mysis (protozoeal phase). The nutritional value of microalgae for prawn larvae varies markedly with species but little is known about what causes this variation. To address this need, penaeid naupli (*Penaeus japonicus*, *P. monodon* and *P. semisulcatus*) were either starved or fed four species of microalgae (*Chaetoceros muelleri*, *Tetraselmis suecica*, Tahitian *Isochrysis* (T-iso) *spp* and *Dunaliella tertiolecta*) as either monospecific or mixed cultures using an automated 100-vessel culture system (larvatron). On the basis of survival and development of the larvae, the microalgae were ranked *C. muelleri* • T. *suecica* > T-iso >*D. tertiolecta*; a mixed diet of *C. muelleri* and *T. suecica* (2:3 by dry weight) was equivalent to, or better than, *C. muelleri* and better than *T. suecica*. The 'gross' biochemical composition of the microalgae had little or no effect on either the biochemical composition of the larvae or their performance. However, larvae that performed the best (i.e. fed *C. muelleri*) had significantly more lipid and polysaccharide than those which performed the worst (ie fed *D. tertiolecta*). Comparison of starved and fed larvae showed that lipid was the major energy source for the larvae during metamorphosis. Monounsaturated fatty acids (MUFAs) were the primary sources of this energy and during starvation, these and the polyunsaturated fatty acids (PUFAs) were metabolised while the highly unsaturated fatty acids (HUFAs) were conserved; conservation of eicosapentaenoic acid (C20:5n-3; EPA) and arachidonic acid (C20:4n-6; ARA) was more pronounced than for other HUFAs. Increasing the carbohydrate content of the microalga at the expense of protein (i.e. reducing the protein to carbohydrate ratio from 0.3-0.4:1 to 0.1-0.2:1) by lowering the nitrate concentration in the culture media delayed development but not survival of the larvae. Discriminant analysis was used to relate the survival and growth development of the larvae to the variability of the microalgae in their composition of 31 fatty acids. This analysis showed that the nutritional value of the microalgae for penaeid protozoeal larvae depended critically on the amount of EPA and ARA but not docosahexaenoic acid (C22:6n-2; DHA) that was present. These studies demonstrated that the requirement of penaeid larvae for linoleic acid (C18:2n-6; LOA), linolenic acid (C18:3n-3; LNA) and DHA does not exceed a dietary concentration of 0.1% DM. However, the requirement for LOA and LNA appears to be modulated by the presence of HUFAs which may be saturated and/or chain shortened to produce these two PUFAs. Research is continuing to quantify the ARA and EPA requirements of penaeid larvae and to characterise the essentiality of sterols in growth and development of penaeid larvae.

Nutritional studies with grow-out barramundi (Asian sea bass)

As part of a coordinated Australian effort to develop more cost-effective diets for prawns and finfish, research with barramundi has concentrated on optimising the use of terrestrial feedstuffs as alternatives to fishmeal in pelleted dry diets. In order to do this, research was needed to define the animals requirements for key nutrients and to develop feeding strategies appropriate for Australian farming conditions. Funding for this research was provided by the Australian Fisheries Research and Development Corporation (FRDC), other R&D statutory councils, ACIAR and industry. The following sections highlight the major findings from this research.

Effect of water temperature on food intake and growth

The effects of water temperature, feeding frequency and fish size (weight) on food intake and fish growth were examined in order to define optimal feeding practices for juvenile (• 30 to 300 g) barramundi. For each fish size, intake of an extruded dry pellet (DM, 95%; CP, 44%; and estimated digestible energy, 15 kJ/g) of acclimatised fish increased essentially linearly as water temperature increased from 20 to 29°C. As a percent of biomass, intake of a 50 g fish ranged from 2.15 to 4.4% at 20 and 29°C respectively whereas the respective values for a 300 g fish were 2.15 and 0.67%. Absolute growth rate also increased linearly with water temperature and size while food conversion (FCR) improved slightly with water temperature and deteriorated slightly with size. Growth rates of 50 g fish ranged from 0.7 to 2.0 g/d at 20 and 29°C, respectively and increased to 2.05 and 4.5 g/d for 300 g fish respectively. Varying the feeding frequency from 1 to 3 times daily increased food intake of small fish (<100 g), but the extra food did not result in significantly better growth rate. As a recommendation, fingerlings should be fed twice daily up to about 100 g, whereas once daily feeding is adequate for fish above 100g. Analysis of the data generated the following food intake prediction equation:

$$\ln DFI = -7.285 + 0.478 \ln W + 0.391T + 0.074F \qquad (R^2 = 0.97);$$

where ln is the natural logarithm, DFI is daily food intake (g/fish/d), W is the weight (g) of the fish, T is water temperature (°C) and F is the number of feeds/d. For fish above 300 g, skipping a day's feeding on the weekend is a common industry practice in Australia but our research has shown that this will result in a commensurate decrease in fish growth.

Assessment of nutritive value of alternative 'terrestrial' feed ingredients

Feedstuffs identified to have potential as fishmeal replacements were evaluated by determining their apparent digestibility and subsequent nutrient utilisation using nutrient retention and growth assay experimentation. The apparent digestibility of two fishmeals (Danish and tuna), three terrestrial abattoir meals (poultry offal meal and two meat meals) and six plant protein meals (solvent- and full-fat soybean, peanut, canola, dehulled-lupin and wheat gluten) was determined. The derived apparent digestibility coefficients of protein and energy respectively, for the meals (mean ±sem) were: Danish fishmeal, 87.9±0.98 and 83.3±1.27; tuna fishmeal, 92.3±0.98 and 69.3±1.27; poultry offal meal, 78.8±3.5 and 76.7±5.6; meat meal 'A', 53.9±3.9 and 58.2±6.5; meat meal 'B', 63.5±3.4 and 66.5±3.4; solvent soybean meal, 86.0±0.8 and 69.4±1.7; full-fat soybean meal, 84.8±3.8 and 75.9±7.8; peanut meal, 91.9±8.0 and 68.7±5.0; canola, 81.0±2.3 and 56.1±3.0; dehulled lupin, 98.1±1.3 and 61.5±1.8; and wheat gluten, 101.9±1.6 and 98.8±3.1. Protein and energy apparent digestibilities were high for all meals although the animal feeds were slightly better digested than the plant feeds other than for wheat gluten which was completely digestible. The digestibility of meat meal was variable and lower than for fish meal and this was attributed to meat meal's high ash content. These results demonstrate the similarity of barramundi to other carnivorous fish in being able to digest protein and energy from a wide variety of different terrestrial feeds.

The efficiency with which protein and energy was utilised by juvenile barramundi when provided from six different protein meals (meat, Peruvian fish, casein, solvent- and full fat-soybean and dehulled lupin) was characterised using nutrient retention and summit dilution procedures. Each

of the test protein meals was substituted for a high fishmeal summit diet at inclusions incremented up to 70%. In all experiments, dietary accretion of N and energy was linear, indicating that the fish were responsive to dietary N and energy intake over the entire range of summit diet dilutions examined. Pooled across experiments, the efficiency with which N and energy of the summit diet was retained by barramundi was determined to be 37.8 and 50.6% respectively. Dilution of the summit diet with the respective test feed ingredient resulted in either a 'bent-stick' or a linear change in nutrient retention efficiency. For N retention, the response was a bent-stick relationship for each ingredient with the plateau value being similar to that of the summit diet, namely varying from 34.5 to 41.7%. However, the extent to which the summit diet could be diluted before N retention efficiency was affected differed between the test ingredients. The effect of substitution rate on retention efficiency was greatest for soybean meal, casein and dehulled lupin and least for fish meal and meat meal. Moreover, the rate of change in N retention efficiency with progressive test ingredient substitution was fastest for soybean meal, dehulled lupin and casein and slowest for meat meal and fish meal. For energy, a similar bent-stick response pattern was observed with each of the animal feed ingredients although the 'break-point' occurred at a lower substitution rate than for N with meat meal and fishmeal but at a higher substitution rate with casein. A different pattern was seen in the case of both of the plant protein sources where retention efficiency declined linearly with increasing substitution of the summit diet. These differences in nutrient retention efficiency response patterns between the various feed ingredients clearly indicate the superiority of animal over that of plant feed ingredients. In terms of fish weight gain and nutrient retention, the three animal protein meals – Peruvian fishmeal, casein and meat meal – were clearly superior as substitutes for the summit diet than the dehulled lupin or soybean meals which were of similar nutritive value. The lower nutritive value of the soybean and lupin meals was thought to be due to their lower digestible energy content and an apparently higher energy requirement for their metabolism. The results provide evidence that the inferiority of the plant proteins was the result of low available energy content rather than an effect of poor amino acid balance of the protein.

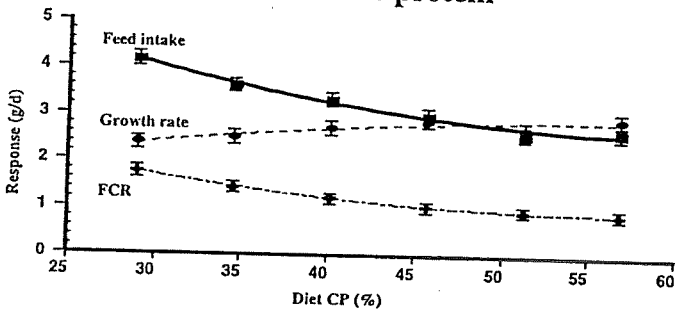
The palatability and nutritive value of ring-dried blood meal for barramundi was examined by growth assay. Inclusion of blood meal in the diet (from 0 to 22.5% at 7.5% increments) at the expense of isonitrogenous amounts of gluten had no adverse effect on the apparent palatability of the diet but FCR deteriorated slightly (viz. 0.91, 0.92, 0.97 and 1.05 g DM:g gain for increasing blood meal inclusion). The results showed that barramundi readily accept diets containing high inclusions of blood meal and that blood meal may be a useful attractant to improve the unpalatability of other dietary constituents such as casein and plant protein meals.

This work has shown that terrestrial animal protein sources such as meat meal have considerable potential as dietary feed ingredients for barramundi and could be used to replace most, if not all of the fishmeal. Plant protein meals such as soybean, canola and lupin were less well digested and not well liked but could be used cost effectively for the partial replacement of fishmeal. Based on the cost per unit of digestible protein, the plant protein meals were the least expensive; meat meals were only slightly more expensive but much cheaper than fish meal.

Dietary protein requirement of juvenile barramundi

We have examined the response of barramundi to diets varying in protein concentration over the range of 29 to 57%. The protein used was a high quality mixture of fishmeal and casein (with an amino acid profile similar to that of the protein in barramundi) and the digestible energy content of the diet was held constant at 15 MJ/kg. The fish were held in a recirculation freshwater system maintained at 28°C and fed twice daily to satiety. Production responses of the fish are shown in Fig. 1.

Fig. 1 Production responses of juvenile barramundi to isoenergetic diets providing incremental inclusions of a reference protein



Increasing the amount of protein in the diet significantly improved FCR but food intake showed a pronounced reduction such that the growth of the fish was improved only slightly at dietary protein contents above about 45%. These results suggest that with a 15 MJ/kg digestible energy diet, growth rate and FCR were optimised at a protein content of about 45%, i.e at a protein to digestible energy ratio of 30 g/MJ.

Essential fatty acid requirements

The amount of the critical EPA and DHA n-3 fatty acids required in the diet of barramundi was investigated with fish held at water temperatures of either 20 or 29°C. Fish were fed twice daily to satiety on diets where the EPA+DHA content was varied serially from 0.5 to 2.1%. At low water temperature, the amount of dietary EPA + DHA had no effect on fish response but at high water temperature, fish responded to increasing dietary EPA+DHA much in the same way as for increasing protein. Namely, food intake declined, FCR improved and growth increased slightly, reaching a plateau when the dietary EPA+DHA content exceeded 1.8%. It is recommended that diets for barramundi contain not less than 1.5% of EPA+DHA.

Efficacy of synthetic (crystalline) amino acids as dietary supplements

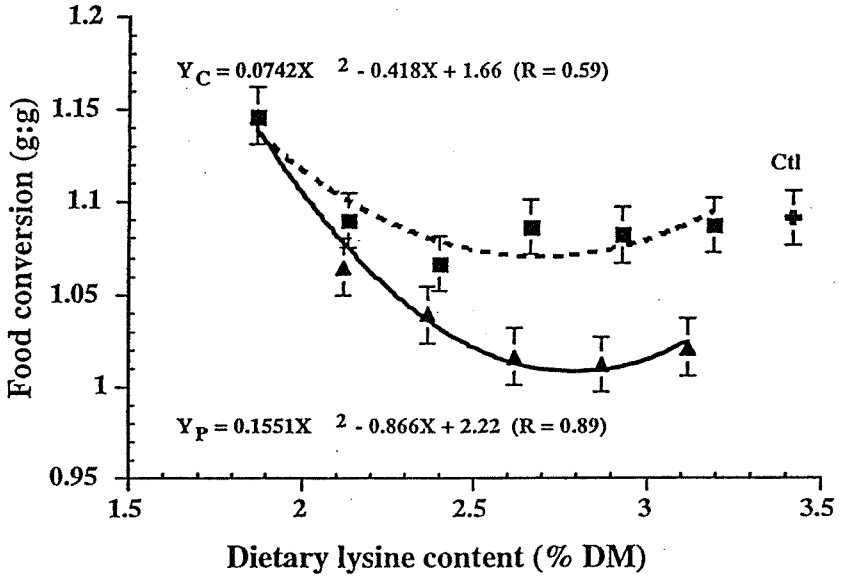
A major difference between marine and terrestrial protein sources is the marked difference in the amino acid make up of the protein. Compared to fishmeal with an amino acid index of 100, terrestrial plant protein sources are very low in methionine (20 to 80), lysine (20 to 85) and threonine (55 to 85). Terrestrial animal protein sources score higher but the same three essential amino acids are often deficient. An imbalanced essential amino acid profile of the protein markedly reduces the nutritive value of the diet for terrestrial monogastric animals such as pigs and poultry. In these species, crystalline amino acids are a proven and cost-effective way of restoring the dietary amino acid balance. However with aquatic animals, there is considerable uncertainty as to whether or not crystalline amino acids are used effectively for overcoming dietary amino acid deficiencies.

Two feeding experiments were carried out to see if protein-bound amino acids (as casein) were more effective in overcoming a dietary amino acid deficiency than crystalline amino acids. A high gluten diet markedly deficient in lysine was incrementally supplemented with either a mixture of crystalline amino acids (including lysine) or casein to restore the amino acid profile of the protein to a balance similar to that recommended for channel catfish diets. Diets were

held isonitrogenous and isoenergetic by adding the crystalline amino acids at the expense of starch, and casein at the expense of gluten. In the first experiment, the DM protein content of the diets was 54% which was higher than that typically used for commercial barramundi diets. In the second experiment, the dietary DM protein content was reduced to 39% to accentuate the response to amino acid supplementation. Thus, the amino acid balance of the dietary protein was similar in each experiment but the absolute concentrations of the amino acids were much lower in the second compared to the first experiment. Barramundi were fed once daily to appetite so as to accentuate differences in the rate of absorption of amino acids from the two types of amino acid supplementation. This was expected to increase the likelihood of eliciting different metabolic responses between the alternative amino acid sources.

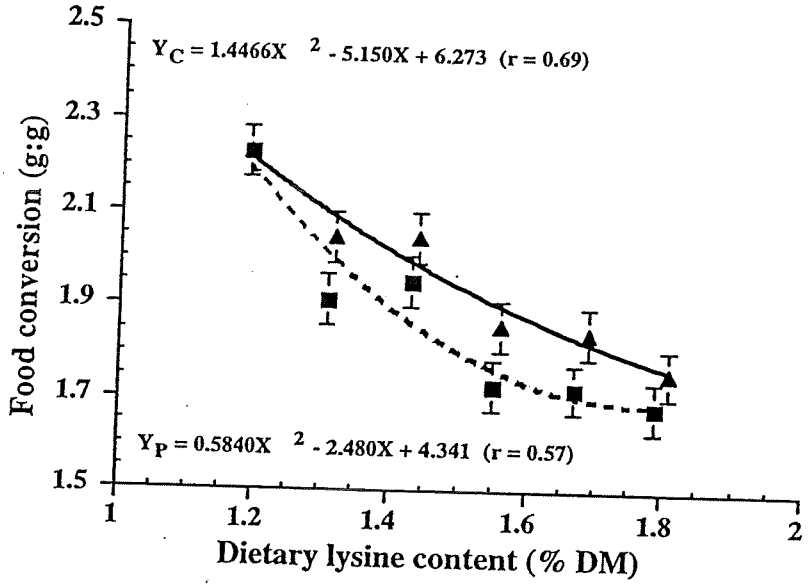
In the 54% DM protein experiment, increasing the lysine content of the diet from 1.87% (3.4% of protein) to 3.2% (5.6% of protein) resulted in a significant ($P<0.05$) quadratic improvement in average and specific growth rates and FCR for both the crystalline and protein-bound amino acid diet series; daily food intake showed no consistent change with supplementation. The maximum response to amino acid enrichment occurred at a dietary lysine content of about 2.8% lysine (5.2% of protein) for both types of amino acid supplements but the response was better with the protein-bound compared to the crystalline amino acid supplement. However, a statistical difference between the two types of amino acid supplements was observed only for FCR where crystalline amino acids were only about 50% as effective as protein-bound amino acids in eliciting a response (Fig. 2).

Fig. 2 Quadratic relationships between dietary lysine content and food conversion ratio response of fish to supplementation with either crystalline (Y_C; ■) or protein-bound (Y_P; ▲) amino acids in diets containing 54% DM crude protein.



In the 39% DM protein diet experiment, increasing the lysine content of the diet from 1.19% (3.1% of protein) to 1.8% (4.55% of protein) resulted in a significant ($P<0.05$) quadratic improvement in average and specific growth rates and FCR for both the crystalline and protein-bound amino acid diet series. As in the high protein diet experiment, daily food intake showed no consistent change with supplementation. However, unlike the high protein diet experiment, a clear plateau response was not achieved at the highest rate of supplementation. Neither was a significant difference observed in the response between types of amino acid supplements although there was a tendency for the crystalline amino acid supplements to be used more effectively than the protein-bound amino acids as illustrated for FCR in Fig. 3.

Fig. 3 **Relationship between dietary lysine content and food conversion response of fish to supplementation with either crystalline (Y_C; ■) or protein-bound (Y_P; ▲) amino acids in diets containing 39% DM crude protein.**



Not unexpectedly, the response to amino acid enrichment was relatively more marked with the low protein diets than with the high protein diets. And at low dietary protein, crystalline amino acids were equally as effective as protein-bound amino acids. Thus, where there is a critical shortage of an essential amino acid, barramundi will conserve the limiting amino acid, irrespective of whether supplied as free or protein-bound form, and will show the greatest benefit to amino acid enrichment. However, where the absolute deficiency of the essential amino acid is not so critical as was the case with the high protein diets, the response to improved amino acid balance of the dietary protein was small and in this situation, protein-bound amino acids were more effective as supplements than crystalline amino acids. Further, when the amino acid quality of the dietary protein was the same, the absolute productivity of the fish was much better with the high protein diets than for the low protein diets which illustrates the extent to which dietary protein is used for meeting the animal's energy requirements.

Commercial trialing of zero-fishmeal grow-out diets for barramundi

Four experiments have been carried out on commercial farms to demonstrate the extent to which meat meal can replace fishmeal in grow-out barramundi diets. In a 10 week feeding study, a high fishmeal (control) diet was compared with two experimental diets that contained no fishmeal and these were compared against a commercial barramundi diet (Table 1). The ingredient cost of the zero-fishmeal diets (M3 and M4) was 15 to 20% lower than for the high fishmeal control diet. The diets were fed to caged fish in an aerated freshwater pond and managed as for other fish on the farm. At the conclusion of the feeding period, samples of the fish were taken for taste panel assessment. Details of the diets and productivity responses of the fish are shown in Table 1.

Table 1. Formulation and nutrient characteristics of the diet and the resulting productivity of barramundi (initial weight 220 g) reared for 10 weeks under commercial farm conditions

Attribute[1]	Diet description				
	Control	M3	M4	Commercial	± sem
	Diet formulation (%)				
Fishmeal (65% CP)	35	0	0	?	
Meatmeal (50% CP)	10	50	50	?	
Soybean (full fat, 38% CP)	16	15	10	?	
Fish oil (Chilean)	2.5	5	6	?	
Blood meal (spray dry)	0	7	9	?	
Gluten	5	5	5	?	
Wheat	30.4	16.1	10.4	?	
Vitamin & other	1.1	1.9	4.6	?	
	Critical nutrient composition				
Crude protein (%)	43.8	42.5	47.8	50.1	
Digest. energy (kJ/g)	15.0	15.0	16.2	15.0	
Lysine (%)	2.83	2.77	3.16	4.11	
C20:5n-3 (%)	0.5	0.63	0.99	1.08	
C22:6n-3 (%)	0.84	0.40	0.50	1.05	
	Fish performance				
Growth (g/week)	20.8^{b}	21.4^{ab}	23.2^{a}	20.3^{b}	1.75
Food conversion (g:g)	1.22^{a}	1.44^{b}	1.31^{ab}	1.37^{b}	0.070
Fish recovered (%)	94.6^{a}	97.8^{a}	97.9^{a}	99.2^{a}	2.96
Dressing-out (%)	89.9^{a}	88.7^{a}	88.6^{a}	89.4^{a}	0.30
Food cost ($/kg gain)[2]	1.08^{b}	0.89^{a}	0.88^{a}		0.038

1 Within row comparisons, means without a common superscript letter differ (P<0.05).

2 Food cost calculated on basis of prevailing ingredient prices without any allowance for processing. Information on the commercial diet is not available.

Fish fed the higher energy zero-fishmeal diet (M4) performed best overall, growing faster than those fed either the high fishmeal control diet or the commercial diet. FCR of fish on the lower energy zero-fishmeal diet (M3) was slightly worse than the control but not significantly different to the other diets. Assessment of the eating quality of the fish using trained taste panels at the Queensland Government's Centre for Food Technology showed similar scores for all diets. The overall acceptance of the fish on all diets was very high. These results demonstrate that appropriately formulated diets without fishmeal are as good as, and less expensive, than conventional high fishmeal diets for barramundi. Equally important, the eating quality of the fish reared on zero-fishmeal diets was indistinguishable from those fed on high fishmeal diets.

Current research: High energy diets for intensive barramundi farming

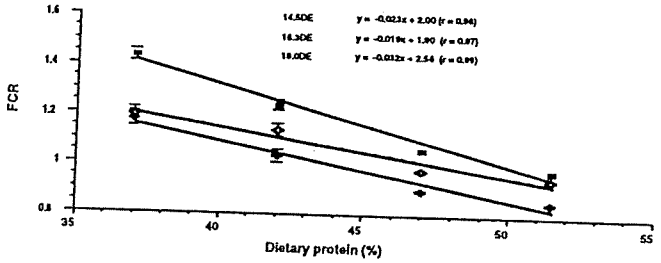
Intensive farming of barramundi in closed (recirculation) systems is becoming more important in Australia in order to gain better control of the production system. Invariably, such intensive systems have a high capital investment and their profitability depends on how successful the higher investment costs are able to be offset by an increased rate of production. This means rearing the fish at their maximum growth rate so as to increase throughput or achieve higher harvest weights in the same growing period.

The extent to which barramundi will respond to diets containing nutrients at high density is a current research focus. In one study, four protein levels (37.4, 42.2, 46.9 and 51.7%) were

examined at each of three digestible energy levels (14.5, 16.3 and 18.0 kJ/g). Barramundi of 230 g initial weight were fed the 12 diets once daily to satiety during an experiment of six weeks.

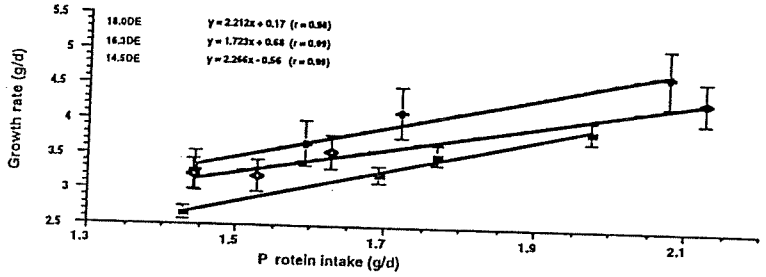
FCR improved linearly as the protein concentration of the diet increased from 37.4 to 51.7% and also was better at each increase in energy concentration (Fig. 4). FCRs were excellent and at the highest protein level were 0.99:1 and 0.85:1 for the lowest and highest energy concentrations, respectively.

Figure 4. Effect of dietary protein and digestible energy content on FCR



Growth rate improved similarly to FCR except that the response was more curved with the 16.3 kJ/g energy diets because food intake of the fish on the two intermediate protein levels was unexpectedly low. However, plotting growth against actual protein intake (Fig. 5) rather than dietary protein concentration showed clearly that the responses at each energy level were indeed linear, increasing from 3.9 g to 4.7 g/d at the highest dietary protein concentration.

Figure 5. Effect of protein intake and digestible energy concentration of the diet on growth rate of barramundi



These results show that barramundi growth can be markedly improved by increasing the protein and energy concentrations of the diet. However, as shown in this experiment and observed previously, barramundi have limited ability to use high lipid and other high energy nutrients to spare for dietary protein and in this regard they differ from salmonids and channel catfish. The present work suggests that further growth improvement is possible with barramundi using even higher dietary protein and energy concentrations. While such high density diets will require higher and therefore more expensive inclusions of oil and protein feeds, the cost of meeting these specifications can be minimised if terrestrial protein feeds are used instead of fishmeal.

The nutritional approach as outlined for penaeid larvae and barramundi could equally be adapted and applied for advancing the culture technology of groupers. Already, the results of nutritional studies with groupers are being reported from laboratories throughout SE Asia and these will provide a strong base for making rapid advances in the development of improved and more cost effective feeds for groupers.