FISHMEAL REPLACEMENT IN AQUACULTURE FEEDS FOR BARRAMUNDI: (i) NUTRITIVE VALUE OF CRYSTALLINE AMINO ACIDS (ii) POTENTIAL OF MEAT MEAL TO REPLACE FISHMEAL

PROJECT 95/069

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FINAL REPORT TO
FISHERIES RESEARCH AND DEVELOPMENT CORPORATION

FISHERIES RESEARCH & DEVELOPMENT CORPORATION

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1. PROJECT

Project Title: **Fishmeal Replacement in Aquaculture Feeds for Barramundi:**
(i) Nutritive value of crystalline amino acids; and
(ii) Potential of meat meal to replace fishmeal in diets for barramundi:- Commercial Farm evaluation

Project No.: 95/069

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2. NON-TECHNICAL SUMMARY

95/069	**Fishmeal Replacement in Aquaculture Feeds for Barramundi:** **(i) Nutritive value of crystalline amino acids; and** **(ii) Potential of meat meal to replace fishmeal:- Commercial Farm Studies**

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OBJECTIVES:

Crystalline amino acid investigations

Primary

1. Determine the efficacy of crystalline amino acids (C-AAs) as dietary supplements for barramundi
2. Improve the nutritional quality of fishmeal alternatives using C-AAs

Secondary (contingent on C-AAs shown **not** to have high efficacy)

3. Improve the nutritional quality of fishmeal alternatives using complementary intact protein sources

Potential of meat meal to replace fishmeal investigations

1. Demonstrate on commercial barramundi farms the suitability of meat meal based diets for rearing fingerling fish to market size (400 to 500 g).

2. Using trained taste panels, compare the sensory characteristics of barramundi when reared on diets based on either meat meal or fishmeal.

NON-TECHNICAL SUMMARY

Australia has an abundant supply of terrestrial animal and vegetable protein feeds which has the potential to at least partly if not fully replace the fishmeal presently used in compounded aquaculture diets. 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 (Met; 20 to 80), lysine (Lys; 20 to 85) and threonine (Thr; 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 (C-AAs) are a proven and cost-effective way of restoring the dietary amino acid balance. However, the efficacy of C-AAs in aquaculture diets is quite equivocal. A clear understanding of the essential amino acid requirements of barramundi and the extent to which C-AA can improve the nutritive value of terrestrial protein feed ingredients is pivotal to further diet development for this species.

Crystalline amino acid investigations

The primary objective of this one-year study was to assess the efficacy of C-AAs as supplements in diets for barramundi. Three growth experiments were carried out. The first (AA1) examined the effectiveness of C-AAs for restoring the amino acid balance of a low protein, high meat meal-containing diet when food intake was controlled to ensure equivalent energy intake. The second (AA2) and third (AA3) experiments directly compared the efficacy of C-AA and protein-bound amino acids (protein-AA) for amino acid enrichment of diets in which the protein content was either high (54%, DM) or low (39%, DM) and the fish were fed once daily to satiety.

The research supports the following conclusions:

- The response to amino acid enrichment was relatively more marked at low compared to high dietary protein. And at low dietary protein, C-AA were equally as effective as protein-AAs. However, efficacy of C-AAs may be inferior to that of protein-AAs when fish are provided with high protein diets and absolute amino acid intake is high. This effect may be due as much to altered efficiency of energy metabolism as to amino acid supply *per se*.
- The importance of the essential amino acid balance of the dietary protein as a factor influencing fish productivity increases inversely with absolute dietary protein (amino acid) intake. 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 (Experiment AA2), the response to improved amino acid balance of the dietary protein was small and in this situation, protein-AA was a more effective supplement than C-AA. Further, when the amino acid quality of the dietary protein was the same, the absolute productivity of the fish was much better for high compared to low protein diets which illustrates the extent to which dietary protein is used for meeting the animal's energy requirements.
- Substitution of fishmeal by terrestrial protein feeds and the attendant deterioration in the essential amino acid profile of the dietary protein is unlikely to adversely affect fish productivity provided the protein content of the diet is maintained above about 50% (DM) and fish are fed liberally.

- The dietary essential amino acid requirements (% DM) of juvenile barramundi were estimated as: Arg, 2.29; His, 0.69; Ile, 1.33; Leu, 2.46; Lys, 2.67; Met + Cysh, 1.20; Phe + Tyr, 2.49; Thr, 1.52; Try, 0.40; and Val, 1.55.

Potential of meat meal to replace fishmeal

Two on-farm experiments (Expts MRC1 and MRC2) were carried out to compare the growth performance and taste characteristics of juvenile barramundi fed one of four diets, a high fishmeal (control) diet, two experimental diets where most or all of the fishmeal was replaced by meat meal and a commercial barramundi diet. Both experiments were carried out using caged fish (400 per $2m^2$ cage) in an aerated freshwater pond. The experimental fish were managed as for other fish on the farm, being fed to satiety once daily except on the weekend when fish were fed only on one of the days. A third growth assay in the Walkamin laboratory (Expt MRC3) was carried out using the same diets as fed in Expt MRC2 to validate the results of that on-farm experiment. In each of the 10-week on-farm studies, a 4x4 randomised block design was employed. For the 6-week laboratory experiment, the number of replicates was increased to 6 and fish were stocked at 8 fish/tank (180 L). At the conclusion of the two on-farm experiments, fish from each cage were sampled for sensory evaluation.

Conclusions from the research were:

- Diets based on meat meal and containing no fishmeal were as palatable to barramundi and supported equivalent or superior fish productivity as those where fishmeal was the predominant protein source.
- Fish reared on diets containing high inclusions of meat meal, with or without some fishmeal but supplemented with fish oil, was found by trained taste panel assessment to be liked as well or better than fish reared on a diet formulated with a high fishmeal content.
- The meat meal based experimental diets were equal to, or better than, a commercial barramundi diet in supporting fish growth and in producing fish with flesh of high sensory value.
- Using conventional high-ash meat meal as a partial or full replacement of fishmeal in nutritionally complete diets resulted in an appreciable reduction in the ingredient cost of the diet and a 16 to 27% lowering of the ingredient cost of the food per unit fish weight increase.
- Other than for potential environmental benefits, there was no advantage in using low-ash meat meal over that of conventional high-ash product.
- These results demonstrate unequivocally the suitability of meat meal as a partial or complete replacement of fishmeal protein in grow-out diets for barramundi.

KEYWORDS: Amino acids, Amino acid efficacy, Nutrient retention, Meat meal, Sensory evaluation, Fishmeal replacement

3. BACKGROUND

Research carried out in Fisheries Research & Development Corporation (FRDC) Project "Dietary requirements and optimal feeding practices for barramundi" (FRDC 92/63) at QDPI's Walkamin Freshwater Fisheries and Aquaculture Centre established feeding strategies and base-line information on the nutrient requirements of grow-out barramundi. The fish's requirements for essential lipids and protein, relationships between protein and energy and strategies to optimise feeding were elucidated. An understanding of such nutrient response relationships in barramundi was seen to be but the first step in the development of cost-effective diets for this species.

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 a project on amino-acid supplementation (this was a technology audit specifically designed to examine the situation with C-AAs and the potential to bio-engineer dipeptides or oligopeptides for aquaculture use).

The barramundi component (93/120-04) of the Fishmeal Replacement Subprogram sought to evaluate the suitability of locally available feed ingredients as alternatives to fishmeal in diets for barramundi. The research was coordinated by QDPI's Bribie Island Aquaculture Research Centre and additionally involved research staff from the University of Queensland (Dr Neil McMeniman) and the Queensland University of Technology (Dr Alex Anderson). The research focused on quantifying the nutritive value of terrestrial protein feed ingredients by determining their apparent digestibility and their effectiveness as substitutes for fishmeal.

Although the Walkamin and Bribie Island Projects were tackling different issues, namely nutrient requirements of the fish on the one hand and nutritive value of alternative dietary feed sources on the other respectively, there was also considerable commonality between the two. So that all of the FRDC aquaculture diet development research was effectively coordinated and benefited from the assembled expertise, the work being done at Walkamin was included as a peripheral project of Dr Allan's Fishmeal Replacement Subprogram. To bring the Walkamin and Bribie Island Projects into the same time alignment, at the conclusion of FRDC 92/63 in 1995, a one-year linking project (FRDC 95/069) was instigated to examine the efficacy of C-AAs as dietary supplements for barramundi.

One of the most noticeable difference between marine and terrestrial protein feed sources is the poor essential amino acid balance of the protein for the latter class of feed ingredients. Crystalline amino acids have been used extensively in diets for terrestrial animals both for elucidating the animal's requirements for essential amino acids and for overcoming dietary amino acid deficiencies (SCA, 1987a, b) but their application in aquaculture diets is a matter of much controversy (Grieve, 1994). However, a clear understanding of the essential amino acid requirements of barramundi is pivotal to further diet development for this species and the

proposed C-AA work was a logical progression of the FRDC 92/63 Walkamin Project. Similarly, an understanding of the extent to which C-AAs can improve the nutritive value of terrestrial protein feed ingredients is important if the full potential of terrestrial protein feed sources as replacements of fishmeal in aquaculture diets is to be realised. Hence, this C-AA Project (FRDC 95/069) provided the means of linking and unifying the barramundi diet development work being done at Walkamin and Bribie Island.

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4. NEED

Aquaculture is the fastest expanding food producing sector in the world, growing at a rate of at least 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 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 prices to rise. As higher 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 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. A major difference between marine and terrestrial protein sources is the marked difference in the amino acid make up of the protein. Compared to an index value of 100 for fishmeal, terrestrial plant protein sources score very low particularly for methionine (Met; 20 to 80), lysine (Lys; 20 to 85) and threonine (Thr; 55 to 85) and while terrestrial animal protein sources tend to score higher than plant proteins, the same three essential amino acids are present at values usually lower than for fishmeal (Tacon, 1994). An imbalanced essential amino acid content of the protein markedly reduces the nutritive value of the diet for terrestrial monogastric animals such as pigs and poultry (Cole, 1980; Campbell, 1988; SCA, 1987a, b; Fuller and Wang, 1990). Supplementation of the diet with C-AAs to correct an otherwise amino acid balance is a proven and cost-effective practice for these species (SCA, 1987a, b). As the efficacy of C-AA supplementation of diets for aquatic animals is equivocal (see Grieve, 1994), research is needed

to determine if C-AAs can be used to improve the nutritive value of protein sources low in one or more of the essential amino acids. This project addresses this need.

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5. OBJECTIVES

Crystalline amino acid investigations

Primary

1. Determine the efficacy of C-AAs as dietary supplements for juvenile barramundi
2. Improve the nutritional quality of fishmeal alternatives using C-AAs

Secondary (contingent on C-AAs shown **not** to have high efficacy)

3. Improve the nutritional quality of fishmeal alternatives using complementary intact protein sources

Potential of meat meal to replace fishmeal investigations

1. Demonstrate on commercial barramundi farms the suitability of meat meal based diets for rearing fingerling fish to market size (400 to 500 g).

2. Using trained taste panels, compare the sensory characteristics of barramundi when reared on diets based on either meat meal or fishmeal.

6. TECHNICAL REPORT - DETAILED RESULTS

6.1 Crystalline amino acid enrichment of meat meal

6.1.1 Introduction

Meat meal and other animal protein meals produced by the rendering of abattoir livestock and poultry wastes appear to have considerable potential as alternatives to fishmeal in diets for carnivorous fish such as barramundi. Aquarium studies with barramundi, prawns and silver perch have shown meat meal to be as well digested as fishmeal and diets containing high inclusions of meat meal were well accepted by these species (Williams and Barlow 1996; Williams et al., 1998). In the five years to 1994/95, Australia's annual production of rendered meat meal averaged 470,000 t with most being used in the preparation of pig and poultry diets but a significant amount – up to 138,000 t in 1994/95 – was surplus to domestic requirements and was exported (D. Roberts, Meat Research Council, pers comm). Typically, the meat meal contains from 48 to 52% protein, 7 to 14% lipid and up to 35% ash; selected lines higher in protein and lower in fat and ash are increasingly becoming available as renderers respond to the need to produce meals more suitable for use as aquaculture feeds (MRC, 1997; Williams et al. 1998). There is also a significant economic benefit if meat meal can substitute for fishmeal as the cost of supplying protein from the former is only half that of the latter (AUD$0.90 vs AUD$1.85 per kg protein, respectively).

One factor that may reduce the suitability of meat meal as an alternative to fishmeal in aquaculture feeds is its relatively low content of critical essential amino acids (Tacon, 1994), particularly Met and Lys. The well established low biological availability of Lys resulting from over-heating and maillard reactions during rendering of meat meal (Carpenter, 1973; Erbersdobler, 1986; Batterham et al., 1986) may further reduce the nutritive value of its protein. However, both Lys and Met are readily and inexpensively available as feed-grade C-AAs and these are extensively used as economical supplements in overcoming deficiencies of these amino acids in pig and poultry diets (SCA, 1987a,b). If barramundi obtain the same benefit from C-AAs as pigs and poultry, enrichment of meat meal with the free amino acid/s may improve nutritive value and increase the potential of meat meal as a fishmeal substitute.

An experiment (AA1) with juvenile barramundi was carried out to examine the suitability of meat meal as an alternative to fishmeal and the extent to which the lower essential amino acid content of meat meal could be corrected through C-AA supplementation.

6.1.2 Materials and Methods

6.1.2.1 Overview of experiment methodology

The objectives of the experiment were three fold. Firstly, to examine the consequences on fish productivity of replacing a significant proportion of the protein in a high fishmeal control diet with an isonitrogenous amount of meat meal. Secondly, to see the effect on fish productivity of serial reduction in the protein content of the meat meal-substituted diet, primarily through lowered inclusions of the meat meal. And thirdly, to see if supplementation of the lowest protein diet with sequential additions of crystalline Lys, Met, Thr and arginine (Arg) to restore dietary contents equivalent to that of the control diet would overcome the expected reduced

productivity of fish fed that diet. All diets were maintained isoenergetic by judicious adjustment of starch and oil and the inclusion of fishmeal was held constant at 15% for all diets other than for the control. To ensure that fish were unable to compensate for any nutritional deficiency of the diet by voluntarily increasing appetite, daily food allowance was controlled to a scale set by the weight of the fish, which was measured fortnightly. The controlled feeding rate was equivalent to 75 to 80% of satiety intake of a well balanced diet of similar energy content.

6.1.2.2 Diets and feeding procedures

The effect of meat meal substitution of fishmeal and sequential C-AA enrichment of the low protein meat meal-based diet was examined using the diets detailed in Table 2.

Table 2 Formulation (g/kg, air dry) of the diets examined in Experiment AA1

Ingredient	Diet designation							
	#1 Ctl	#2 H-mm	#3 M-mm	#4 L-mm	#5 #4 + Lys	#6 #5 + Met	#7 #6 + Thr	#8 #7 + Arg
Wheat (gelled)	150	150	150	150	150	150	150	150
Fishmeal (Tas)	300	150	150	150	150	150	150	150
Meat meal	250	550	400	250	250	250	250	250
Gluten	50	50	50	50	50	50	50	50
Soybean (sol)	100	0	100	100	100	100	100	100
Fish oil	20	40	40	40	40	40	40	40
Soy oil	25	20	20	20	20	20	20	20
Wheat starch	85	20	70	220	210	205	200	195
l-Lysine HCl	0	0	0	0	10	10	10	10
d/l Methionine	0	0	0	0	0	5	5	5
l-Threonine	0	0	0	0	0	0	5	5
l-Arginine	0	0	0	0	0	0	0	5
Micronutrient[1]	20	20	20	20	20	20	20	20

[1] Provided in the final diet (mg/kg): Retinol (Vit A), 1.8; ascorbic acid (coated Vit C), 2,000 cholecalciferol (Vit D3), 1,275; menadione (Vit K3), 6; d/l a-tocopherol (Vit E), 225; choline, 1,000; inositol, 250; para-amino-benzoic acid, 50; thiamine (Vit B1), 15; riboflavine (Vit B2), 20; pyridoxine (Vit B6), 15; pantothenic acid, 50; nicotinic acid, 75; biotin, 0.6; cyanocobalamin (Vit B12), 0.06; folic acid, 4; ethoxyquin, 125; citric acid, 6,000; Al (as AlCl3.6H2O), 0.5: Co (as CoCl2.6H2O), 0.5; Cu (as CuSO4.5H2O), 5; Fe (as FeSO4.7H2O), 40; I (as KI), 4; Cr (as KCr.2SO4), 0.5; Mg (as MgSO4.H2O), 25; Se (as NaSeO3), 0.1; and Zn (as ZnSO4.7H2O), 100.

Diets were prepared using a Hobart dough mixer (Model A200) to mix the respective ingredients into a moist dough, which was layered 10 mm, into trays and steamed for 10 min. in an autoclave at atmospheric pressure. The steamed dough was re-mixed and the dough extruded through a mincer attachment as spaghetti-like strands of 4 mm diameter. The food was dried overnight in a force-draught oven at 40°C, the strands broken into lengths of approximately 10 mm and stored at -20°C until immediately prior to feeding. The daily amount of food offered was scaled to the mean liveweight of the fish in each tank and this scale was expected to provide about 75 to 80% of satiety for fish held at 26°C. The daily allowance (DA, g) was determined according to the expression:

$$DA = \{(26 \times Wt^{0.44})/100\};$$

where Wt was the mean weight (g) of the fish in the tank. The feeding scale was derived from previous experimentation with barramundi where fish of prescribed size ranging from 50 to 300 g were held at water temperatures varying incrementally between 20 and 29°C and fed a high nutrient dry food pellet (Williams and Barlow, 1996). This daily food allowance, as a percentage of fish biomass, declined from 1.6 to 1.2% as fish weight increased from 150 to 250 g. The daily food allowance was fed as two equal portions nominally at 0900 and 1500 h other than on the fortnightly weighing day when all food was withheld. Any uneaten food was recovered quantitatively within 1 h of feeding and the dry weight, adjusted to take into account measured weight losses due to water instability over the feeding period, used to calculate actual food intake.

6.1.2.3 Fish and management

The experiment was carried out at QDPI's Bribie Island Aquaculture Centre with fish reared in flow-through seawater aquaria. Fish were selected from a stock pool and blocked by weight into three groups of 72, 72 and 64 of mean (± sem) weight of 133.6, 147.9 and 164.3 (±1.745) g, respectively. Fish were subjected to a prophylactic salinity change to freshwater over 24 h just prior to being distributed to the experimental aquaria. Within experimental blocks, fish were randomly and equally distributed to eight individually aerated aquaria, each of 200 L capacity (stocked at 9, 9 or 8 fish/aquaria, respectively). The aquaria were situated in an enclosed laboratory and provided with temperature-controlled (27 ±0.5°C) and 20 µ-filtered flow-through (1 L/min) seawater of 34 to 35‰ salinity. Photoperiod was maintained at a 12:12 light-dark cycle and incorporated a 30 minute fade-in, fade-out of lighting to simulate a natural environment. An Horiba U10 water check meter was used daily to measure temperature and water quality (dissolved oxygen, salinity and pH) for a random selection of 4 to 5 tanks. Fish were acclimatised to the experimental conditions for 14 d prior to commencement of the experiment. Fish were individually weighed at the start of the experiment and weighed fortnightly thereafter until the experiment concluded at 42 d. Stress during weighing was minimised by lightly anaesthetising the fish (placed in an aerated tub containing 200 ppm of 2-phenoxyethanol). Dietary treatments were randomly allocated to aquaria within replication blocks.

6.1.2.4 Chemical and statistical analyses

Samples of finely ground raw ingredients or diets were analysed in duplicate by standard laboratory methods essentially in accordance with AOAC (1990) recommendations at QDPI's 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). 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 (Cysh) was measured as cysteic acid, and Met as methionine sulfone after

performic acid oxidation. Tryptophan (Tryp) 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.

The chemical composition of the experimental diets is shown in Table 3.

Table 3 Determined chemical composition of the diets fed in Experiment AA1

Analyte	Diet designation							
	#1 Ctl	#2 H-mm	#3 M-mm	#4 L-mm	#5 #4 + Lys	#6 #5 + Met	#7 #6 + Thr	#8 #7 + Arg
	Composition (%, DM basis)[1]							
DM (of fed diet)	92.6	92.8	92.3	92.7	91.4	92.6	93.0	92.9
C-protein	49.6	50.0	46.7	38.7	39.6	39.9	40.1	40.8
C-fat	11.0	13.7	12.5	11.0	11.1	11.1	11.4	11.4
Ash	13.9	20.7	16.7	11.9	12.0	12.0	12.0	11.9
Ca	4.06	6.61	5.32	3.58	3.61	3.59	3.63	3.53
P	2.34	3.53	2.83	1.97	2.02	1.89	2.01	1.95
Amino acids[2]								
Arg	**2.75**	**2.94**	**3.03**	**2.18**	**2.17**	**2.17**	**2.14**	**2.80**
His	1.40	1.26	1.07	1.06	1.05	1.11	1.05	1.07
Ile	1.70	1.41	1.41	1.23	1.24	1.24	1.25	1.23
Leu	3.32	3.27	3.09	2.49	2.53	2.55	2.53	2.51
Lys	**2.89**	**2.64**	**2.36**	**1.88**	**2.61**	**2.63**	**2.68**	**2.67**
Met	**0.73**	**0.81**	**0.78**	**0.66**	**0.63**	**1.07**	**1.11**	**1.10**
Cysh	0.55	0.51	0.52	0.41	0.42	0.44	0.46	0.45
Phe	1.98	1.88	1.78	1.52	1.54	1.53	1.52	1.54
Tyr	1.35	1.20	1.13	0.98	1.00	0.99	1.00	1.00
Thr	**1.74**	**1.62**	**1.57**	**1.25**	**1.27**	**1.29**	**1.74**	**1.74**
Tryp	0.48	0.41	0.44	0.37	0.37	0.37	0.38	0.38
Val	2.12	2.13	1.97	1.61	1.63	1.65	1.63	1.59
C18:2n-6	1.31	1.11	1.09	1.07	1.07	1.07	1.07	1.07
C18:3n-3	0.19	0.16	0.16	0.16	0.16	0.16	0.16	0.16
C20:5n-3	0.40	0.58	0.58	0.58	0.58	0.58	0.58	0.58
C22:6n-3	0.51	0.49	0.49	0.49	0.49	0.49	0.49	0.49
GE (kJ/g)	20.1	19.5	19.8	19.8	19.9	19.9	20.0	20.0

[1] Determined from analyses of the prepared diet other than for the fatty acids, which were calculated from analysis of individual ingredients.

[2] Those amino acids added to the diet Amino acids.

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). Growth rate was determined as the difference between end (W_e) and start (W_0) weights divided by the number of days on experiment and specific growth rate (SGR, % per d) was calculated as: 100 x (ln W_e - ln W_0)/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. Differences between treatment effects were examined *a-posteriorly* using Fischer's protected 't' test (Snedecor and Cochran, 1967) wherein

differences between means were examined only where the 'F' test of the ANOVA was significant ($P < 0.05$).

6.1.3 Results and discussion

The health of the fish remained excellent throughout the experiment and no losses occurred. Except for some small food refusals during the first 14 d period, all of the allocated food was consumed by the fish. The productivity responses of the fish are detailed in Table 4.

Table 4 Productivity responses of fish in Experiment AA1

Response trait	Diet designation								±sem
	#1 Ctl	#2 H-mm	#3 M-mm	#4 L-mm	#5 #4 + Lys	#6 #5 + Met	#7 #6 + Thr	#8 #7 + Arg	
Weight (g)									
Start	147^{A}	147^{A}	150^{A}	146^{A}	150^{A}	150^{A}	148^{A}	151^{A}	2.8
End	245^{A}	244^{A}	238^{AB}	216^{D}	225^{CD}	231^{BC}	229^{BC}	231^{BC}	3.9
DFI (g DM)	2.11^{A}	2.17^{A}	2.18^{A}	2.13^{A}	2.15^{A}	2.18^{A}	2.19^{A}	2.17^{A}	0.026
ADG (g)	2.34^{A}	2.32^{A}	2.10^{B}	1.65^{D}	1.79^{CD}	1.92^{BC}	1.93^{BC}	1.92^{BC}	0.063
SGR (%/d)	1.04^{A}	1.03^{A}	0.86^{B}	0.70^{C}	0.75^{C}	0.89^{B}	0.89^{B}	0.89^{B}	0.022
FCR (g DM:g)	0.90^{A}	0.93^{A}	1.05^{B}	1.28^{D}	1.21^{CD}	1.15^{BC}	1.14^{BC}	1.14^{BC}	0.036

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

Due to the imposed controlled feeding and high acceptance by the fish for the dispensed food, daily food intake of the fish was similar ($P>0.05$) for all dietary treatments. Increasing the inclusion of meat meal so that it supplied almost 60% of the total protein content of the H-mm diet – at the expense of all of the soybean meal and half of the fishmeal of the Ctl diet – resulted in no significant change in growth rate or food conversion of the fish. Reducing the DM protein content of the diet from 50 to 39%, primarily by decreasing the inclusion content of meat meal in the H-mm diet, resulted in a concomitant and marked deterioration ($P<0.05$) in both growth rate and food conversion of the fish. Addition of both Lys and Met supplements to the low protein (L-mm) diet but not Lys alone, brought about a significant ($P<0.05$) improvement in fish performance which was lower but not significantly worse than that observed for the mid-protein diet (diet M-mm). No further improvement in fish productivity occurred with additional Thr and Arg enrichment of the low protein diet.

These clear-cut results raise a number of interesting and somewhat conflicting issues concerning the dietary amino acid requirements of juvenile barramundi. The consistent improvement in the performance of fish fed the Lys- and Met-enriched low protein diets can only be interpreted as a response by the fish to correction of a dietary deficiency of one or both of these essential amino acids. However, the performance of the fish on these amino acid supplemented diets was still markedly inferior to that seen on the higher protein H-mm diet. Thus, C-AA supplementation was shown to improve the nutritive value of the low protein L-mm diet but this supplementation failed to restore fish performance to the level achieved on the higher protein H-mm diet containing equivalent or lower concentrations of those amino acids for which a response had been elicited. This could indicate that C-AAs were not being utilised by the fish as effectively as those from intact protein or that the low protein L-mm diets contained too low a concentration of protein – rather than amino acid content *per se* – to support better fish performance.

Supplemental C-AAs have often been shown to be less effectively utilised than equivalent amounts provided as intact protein with fish (Andrews et al., 1977; Poston et al., 1977; Cowey and Sargent, 1972; Murai et al., 1981, 1986; Cowey and Luquet, 1983) and prawns (Meyers and Zein-Eldin, 1972; Goldblatt et al., 1980). Rapid leaching of the C-AA from the food prior to its ingestion has been advanced as one reason for this lower efficacy (Akiyama, 1986; Wilson, 1994). This could be important with prawns where food may remain in contact with the water for several hours prior to ingestion (Millamena et al., 1996). However, loss of amino acids through leaching is likely to be far less important and probably negligible with barramundi because of the aggressive feeding behaviour of this fish.

The more rapid absorption of C-AAs as compared to those released from intact protein upon digestion has been widely advocated to explain the apparent lower efficacy of C-AAs. Encapsulation of C-AAs using agar, glycerol and other coating agents so as to slow their absorption has been shown to be more beneficial than equivalent supplements of non-encapsultaed amino acids (Cho et al., 1989; Chen et al., 1992). However, differences between species in effectiveness of amino acid encapsulation has also been reported (Murai et al., 1982a, b). The rapid absorption of crystalline Met and its degradation to methionine sulfoxide was suggested by Thebault and colleagues (Thebault, 1985; Hidalgo et al., 1987) as a reason for the poor efficacy of crystalline Met supplementation in sea bass. The requirement that all amino acids are simultaneously present and in balanced proportions at the sites of protein synthesis is well recognised and regarded as a fundamental tenet of protein synthesis in all eukaryotic organisms (Munro, 1976). Non-synchrous absorption of dietary amino acids resulting from the more rapid uptake of C-AAs has been shown to lower amino acid utilisation in rats (Geiger, 1947; Howe and Dooley, 1963), poultry (Vohra and Kratzer, 1957) and pigs (Ostrowski et al., 1972; Batterham, 1974; Rerat and Bourdon, 1975; Batterham and O'Neill, 1978; Williams and Dunkin, 1980). A similar effect could be expected to occur in fish. Murai (1992) suggested that the absorption rate for amino acids might decrease with decreasing water temperature and this would explain why C-AAs are more effectively utilised by trout than carp. An increased urinary excretion of Met has also been observed in carp following the feeding of diets with crystalline Met (Murai et al., 1984).

An alternative reason for the diminished performance of the barramundi fed the amino acid supplemented L-mm diet was that the protein and or energy content of the diet was insufficient to support a higher rate of growth. With fish, typically about 60% and as little as 30% of the energy content of dietary protein is retained in the form of deposited protein with the balance being deaminated and used for energy (Walton, 1985; Steffens, 1989a,b; Kaushik and Cowey, 1991). Consequently, protein retention efficiencies have typically been found to be 20 to 30% for European sea bass (Hidalgo and Alliot, 1988; Ballestrazzi et al., 1994), 15 to 40% for salmonids (Pieper and Pfeffer, 1980a,b; Pfeffer, 1982; Kim and Kaushik, 1992; Arzel et al., 1995) and 10 to 20% for carp (Das et al., 1991). However, using high amounts of lipid to increase the non-protein energy content of the diet has enabled dietary protein to be spared and for protein retention efficiency to increase with values as high as 57% being reported for salmonids (Pfeffer, 1982; Lanari et al., 1995). Conversely, if dietary energy supply is inadequate, perhaps because of a sub-optimal energy concentration of the diet and/or the result of food restriction, increasingly more of the dietary protein will be used to meet the animal's demand for metabolic energy rather than as an amino acid source for protein synthesis (Millikin, 1982; Tacon and Cowey, 1985). In the present study, the low protein content of the amino acid supplemented L-mm diet, together with the imposed food restriction may well have limited the fish's capacity to either respond fully to the supplemented C-AAs or grow to the

same capacity as fish on higher protein diets. Although all diets in this experiment had a similar gross energy content (Table 3), the digestible energy content of the diets undoubtably would have reduced with decreasing protein content as the high inclusion of starch in the amino acid-supplemented L-mm diets (about 20% starch) would have significantly reduced the digestible energy content of these diets compared to the low starch (2%) H-mm diet. Recent findings have shown that fully gelatinised starch is less than 30% digestible in barramundi (N.P. McMeniman, pers comm) and thus the digestible energy content of the amino acid supplemented L-mm diets would have been up to 0.8 kJ/g lower than for the H-mm diet. This, in conjunction with the imposed controlled feeding schedule, may have contributed to the lower fish productivity of the amino acid supplemented diets. The work reported in the following Section was carried out to determine if C-AAs differed from protein-AAs in their efficacy in overcoming amino acid deficiencies of diets for barramundi.

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6.2 *Comparison of the efficacy of crystalline and protein-bound amino acids*

6.2.1 *Introduction*

There is universal acceptance that protein synthesis in all eukaryotic organisms proceeds as a progressive and sequential addition of amino acids to form the polypeptide chain that characterises the particular protein and that the order of amino acid incorporation is determined by translation of information stored in the sequences of bases in the DNA of the cell nucleus (Munro, 1976). It follows that if protein synthesis (and growth) of the organism is to occur at the highest possible (genetic) rate, all of the constituent amino acids of the protein being synthetised must be available simultaneously and in amounts and balances that best enable this synthesis to proceed uninhibited. Although each of the 20 primary amino acids from which protein is constituted can be synthetised *de novo* in the animal, either from ammonia and carbohydrate or from amino acid precursors by transamination processes (Cantarow and Schepartz, 1957; Prosser and Brown, 1961), there are at least 10 amino acids that universally cannot be synthetised by eukaryotic organisms at rates sufficient to meet requirements for growth and development. These amino acids are referred to as 'essential' or 'indispensable' and must be present in the consumed food for the animal to survive and grow. The essential amino acids for which a dietary need has been demonstrated are Arg, histidine (His), isoleucine (Ile), leucine (Leu), Lys, Met, phenylalanine (Phe), Thr, Tryp and valine (Val). Because Cysh and tyrosine (Tyr) can spare for Met and Phe respectively, it is usual when considering the animal's dietary requirements for Met and Phe to express these in terms of the total dietary supply of Met+Cysh and Phe+Tyr. In addition to the aforementioned amino acids, glycine (Gly) is reservedly considered to be a dietary essential amino acid for young birds (Calet, 1976) and possibly for other species (Grimble et al., 1992; Ikejima et al., 1996; Plath et al., 1996).

The protein or amino acid adequacy of the diet is determined by the extent to which the consumed food permits or limits the animal's potential for protein synthesis and thus, for growth. In studies with terrestrial monogastric mammalian and avian animals, the amino acid composition of dietary protein that best supports maximal animal growth closely resembles that of the amino acid composition of the animal's whole body protein.

In Section 6.1, C-AA supplementation of a low protein diet based on meat meal was shown to improve fish performance but not to the same extent as those fed on a diet higher in protein but of equivalent essential amino acid content. In that experiment, food allocation was strictly controlled to ensure that fish could not compensate for any nutritional deficiency of the diet by voluntarily increasing intake. However, this methodology may have limited the capacity of the fish to respond to the supplemental amino acids because of either their low utilisation as a result of a too rapid absorption (Thebault, 1985; Cho et al., 1989; Murai, 1992; Chen et al., 1992), protein content of the diet being sub-optimal (Catacutan and Coloso, 1995) or the amino acid supplemented diets having a lower digestible energy content compared to the higher protein diet.

Reducing the frequency of feeding of diets containing C-AA or increasing the separation time between consumption of an amino acid deficient diet and repletion with the respective C-AAs have been shown to impair amino acid utilisation in rats (Geiger, 1947; Howe and Dooley, 1963), poultry (Vohra and Kratzer, 1957) and pigs (Ostrowski et al., 1972; Batterham, 1974; Rerat and Bourdon, 1975; Batterham and O'Neill, 1978; Williams and Dunkin, 1980). Such

feeding strategies exasperate differences in absorption rate of amino acids ingested in crystalline or protein-bound forms and consequently result in proportionally more of the C-AA being used for purposes other than for protein synthesis.
Reported herein are two feeding experiments that were designed to see if C-AAs and protein-AAs (protein-AAs) differed in their efficacy when used to correct an anticipated dietary amino acid deficiency In the first experiment (AA2), the efficacy of the different sources of amino acids was compared when they were incorporated in isoenergetic diets of high protein content (~ 54% DM). The second experiment (AA3) was identical in design to the first except that the efficacy of the C-AA and protein-AA was compared in diets of low protein content (~39% DM).

6.2.2 Materials and methods

6.2.2.1 Experiment overview

The objective of each experiment was to see if barramundi used C-AA equally as well as protein-AA in overcoming a dietary amino acid deficiency. The two experiments differed essentially only in the concentration of protein in the diet that the efficacy of the two forms of amino acid enrichment was compared. In Experiment AA2, a protein content typical of that presently used in barramundi diets was used whereas in Experiment AA3, a substantially lower protein content was used. Thus, the balance of amino acids in the protein was similar between experiments but the absolute concentrations of the amino acids were lower in the second compared to the first experiment. This was done in Experiment AA3 to accentuate the response to amino acid enrichment. To increase the likelihood of eliciting a different metabolic response between the alternative amino acid sources, barramundi were fed a single daily meal to appetite over a period of approximately one-hour. The once daily feeding strategy was expected to accentuate differences in the rate of amino acid absorption between ingested C-AA and protein-AA.

6.2.2.2 Experiment AA2 diets

A diet expected to be markedly deficient in Lys and marginally limiting in Arg, Tryp and Thr was formulated using gluten as the major source of protein. The Lys content of the protein of this 'basal' supplemented diet was subsequently determined to be 3.5 g/16gN, ie. 70% of the recommended Lys specification for channel catfish of 5.1 g/16gN (Tacon and Cowey, 1985; Wilson, 1994). The Arg, Tryp and Thr content of this basal diet was marginally limiting with respect to the specifications advocated for channel catfish. Two diet series were formulated wherein the amino acid content of the basal diet was increased incrementally in five steps (diets), one using a mixture of C-AAs and the other by manipulating a mixture of intact protein (protein-AAs). In the C-AA diet series, crystalline Lys, Tryp, Arg and Thr were together incrementally added at the expense of starch to increase the dietary concentrations of these amino acids to values at least 10% above the specifications recommended for channel catfish. In the protein-AA diet series, casein (a rich source of Lys and most of the other essential amino acids) was incrementally substituted for gluten to increase the dietary amino acid concentrations to values similar to those in the C-AA diet series. To avoid other nutrients interfering in the amino acid efficacy comparison, the inclusion content of all ingredients was otherwise held constant. Since the protein contents of casein and gluten were almost identical and as each was almost 100% digestible (see FRDC 93/120-04), substitution of one for the other enabled the amino acid composition of the diet to be varied with minimal change to the protein or (digestible) energy contents of the diet. Likewise, the addition of the C-AAs at the

expense of starch would have had a negligible effect on the energy and protein content of the diet. To ensure good palatability, a high quality fishmeal was included at a constant 20% of diets in both diet series. An additional diet, based on fishmeal, casein and gluten, was included as a positive control (Ctl). The dietary formulations are shown in Table 5.

Table 5 Formulation (%, air dry) of the diets examined in Experiment AA2

Ingredient	Diet designation											
	Basal	Crystalline amino acid supplementation					Protein-bound amino acid supplementation					Ctl
		HC1	HC2	HC3	HC4	HC5	HP1	HP2	HP3	HP4	HP5	
Casein	0	0	0	0	0	0	4	8.0	12.0	16.0	20.0	24.1
Wheat gluten	40.0	40.0	40.0	40.0	40.0	40.0	36.0	32.0	28.0	24.0	20.0	14.0
l-Lysine HCl	0	0.3	0.6	0.9	1.2	1.5	0	0	0	0	0	0.25
l-Arginine	0	0.05	0.1	0.15	0.2	0.25	0	0	0	0	0	0
l-Threonine	0	0.05	0.1	0.15	0.2	0.25	0	0	0	0	0	0.15
l-Tryptophan	0	0.05	0.1	0.15	0.2	0.25	0	0	0	0	0	0.05
d/l-Methionine	0	0	0	0	0	0	0	0	0	0	0	0.3
Wheat starch (gelled)	23.75	23.3	22.85	22.4	21.95	21.5	23.75	23.75	23.75	23.75	23.75	26.85
Fishmeal (Tasmanian)	20.0	20.0	20.0	20.0	20.0	20.0	20.0	20.0	20.0	20.0	20.0	16.8
Fish oil	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.25
Soybean oil	0	0	0	0	0	0	0	0	0	0	0	1.0
Diatomaceous earth	7.5	7.5	7.5	7.5	7.5	7.5	7.5	7.5	7.5	7.5	7.5	7.5
Dicalcium phosphate	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0
NaCl	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Micronutrient[1]	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5

[1] Provided in the final diet (mg/kg): Retinol (Vit A), 1.8; ascorbic acid (coated Vit C), 2,000 cholecalciferol (Vit D3), 1,275; menadione (Vit K3), 6; d/l a-tocopherol (Vit E), 225; choline, 1,000; inositol, 250; para-amino-benzoic acid, 50; thiamine (Vit B1), 15; riboflavine (Vit B2), 20; pyridoxine (Vit B6), 15; pantothenic acid, 50; nicotinic acid, 75; biotin, 0.6; cyanocobalamin (Vit B12), 0.06; folic acid, 4; ethoxyquin, 125; citric acid, 6,000; Al (as AlCl3.6H2O), 0.5: Co (as CoCl2.6H2O), 0.5; Cu (as CuSO4.5H2O), 5; Fe (as FeSO4.7H2O), 40; I (as KI), 4; Cr (as KCr.2SO4), 0.5; Mg (as MgSO4.H2O), 25; Se (as NaSeO3), 0.1; and Zn (as ZnSO4.7H2O), 100.

6.2.2.3 Experiment AA3 diets

The diet arrangement and format of amino acid enrichment were as for Experiment AA2 except that the DM crude protein content of the diets was reduced from 54 to 39% by increasing the inclusion rate of starch at the expense of the protein-rich feed ingredients. Additionally, the composition of the basal mixture of protein-rich ingredients was altered by the inclusion of squid meal and blood meal in lieu of fishmeal to enhance the palatability of these low protein diets. The Lys content of the protein of the 'basal' diet was subsequently determined to be 3.1 g/16gN, ie. 60% of the recommended Lys specification for channel catfish of 5.1 g/16gN. The Arg, Tryp, Thr and Ile content of the basal diet was marginally limiting with respect to the specifications advocated for channel catfish. As for Experiment AA2, the amino acid content of the basal diet was increased incrementally in five steps (diets), one using a mixture of C-AAs (Lys, Arg, Tryp, Thr and Ile) and the other by manipulation of casein and gluten inclusions. The 12th diet was a control diet prepared from the same base ingredients and to the same formulation as for Experiment AA2. Details of the formulation of the diets are shown in Table 6.

6.2.2.4 Diet manufacture

In both experiments, the required amounts of C-AAs and micronutrients were premixed with a portion of the gelatinised starch and then thoroughly mixed with the other finely ground ingredients using an Hobart dough mixer. The dry mixture was pelleted using a small industrial pellet press fitted with a steam injection-conditioning unit. Diets were pressed through a 4 mm diameter die plate and cut to a uniform length of 8 mm; pellets were rapidly cooled and dried by means of a vibrating screen fitted with an air blower. Upon arrival at Walkamin, diets were stored at -20°C until immediately prior to feeding.

6.2.2.5 Fish and management

The experiment was carried out at QDPI's Freshwater Fisheries and Aquaculture Centre, Walkamin with fish held in tanks situated within an environment-controlled laboratory which was supplied with underground fresh (<0.05,) water. The experimental system comprised 48 fibreglass tanks (180 L; 0.3m^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 12 replicate tanks. Flows through the system were maintained using air lifts and pumps, with turn-over rate in the tanks being once every 0.5 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 28°C; 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. Fish of a single spawning cohort and numbering several thousand were sorted according to weight and freedom of physical abnormalities into a group of approximately 800 and 600 uniform animals for Experiments AA2 and AA3, respectively.

Table 6 Formulation (%, air dry) of the diets examined in Experiment AA3

Ingredient	Diet designation											
	Basal	Crystalline amino acid supplementation					Protein-bound amino acid supplementation					Ctl
		LC1	LC2	LC3	LC4	LC5	LP1	LP2	LP3	LP4	LP5	
Casein	0	0	0	0	0	0	2	4.0	6.0	8.0	10.0	24.1
Wheat gluten	30.0	30.0	30.0	30.0	30.0	30.0	28.0	26.0	24.0	22.0	20.0	14.0
l-Lysine HCl	0	0.15	0.3	0.45	0.6	0.75	0	0	0	0	0	0.25
l-Arginine	0	0.02	0.04	0.06	0.08	0.1	0	0	0	0	0	0
l-Threonine	0	0.04	0.08	0.12	0.16	0.2	0	0	0	0	0	0.15
l-Tryptophan	0	0.02	0.04	0.06	0.08	0.1	0	0	0	0	0	0.05
d/l-Methionine	0	0	0	0	0	0	0	0	0	0	0	0.3
l-Isoleucine	0	0.025	0.05	0.075	0.1	0.125	0	0	0	0	0	0
Wheat starch (gelled)	40	39.74	39.48	39.22	38.96	38.7	40.0	40.0	40.0	40.0	40.0	26.85
Fishmeal (Tasmanian)	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	16.8
Blood meal	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	0
Squid meal	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	0
Fish oil	6.0	6.0	6.0	6.0	6.0	6.0	6.0	6.0	6.0	6.0	6.0	4.25
Soybean oil	0	0	0	0	0	0	0	0	0	0	0	1.0
Diatomaceous earth	6.75	6.75	6.75	6.75	6.75	6.75	6.75	6.75	6.75	6.75	6.75	7.5
Dicalcium phosphate	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0
NaCl	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Micronutrient[1]	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5

[1] As detailed in Table 5.

For each experiment, fish from these selected groups were randomly and equally distributed into the experimental system at a stocking rate of either 15 or 10 fish/tank respectively, and diets similarly allocated at random within each of the four recirculation systems (replicate blocks). Fish commenced the experiment at a mean (±SD) weight of 74.7±2.37 g and 252.2±13.01 g respectively, after an acclimatisation period of two weeks during which a prophylactic salt bath (1.2% NaCl for 2 h) against ectoparasites was administered. Sixteen of the fish remaining from the original group were euthanased (immersion in an ice slurry) and retained (-20°C) as a pre-experimental group for determination of chemical composition. Fish in each tank were bulk-weighed fortnightly thereafter and individually when the experiment was terminated at six weeks. Stress at weighing was minimised by light sedation of the fish using the aquatic anaesthetic 2-phenoxyethanol provided in an aerated water bath at 200 mg/L. A prophylactic 1 h salt bath (10, NaCl) against ectoparasites was carried out on the same day of weighing. Fish were offered their respective diets to satiety once daily except on the day of weighing when no food was fed. At each feeding, a weighed amount of food was offered to excess on 3 to 4 occasions during a feeding period of 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 for each diet. At the termination of Experiment AA2, four representative fish from each tank were euthanased and retained for determination of chemical composition as for the pre-experimental fish.

6.2.2.6 Chemical analyses

For determination of the chemical composition of the fish, weighed whole fish were placed into a 2 L wide-mouth glass jar (either 4 fish from each experimental tank per jar or individual fish for the pre-experimental group) and autoclaved at 126°C for 4 h as described by Williams et al. (1995). The autoclaved samples were homogenised in situ using 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 diets and homogenised fish were analysed in duplicate at QDPI's Animal Research Institute, Yeerongpilly, by standard laboratory methods essentially in accordance with AOAC (1990). 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). 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. Cysh was measured as cysteic acid, and Met as methionine sulfone after performic acid oxidation. Trypt 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. The chemical composition of the diets in Experiments AA2 and AA3 is shown in Tables 7 and 8, respectively.

Table 7 Determined chemical composition of the diets examined in Experiment AA2

Ingredient	Diet designation											
	Basal	Crystalline amino acid supplementation					Intact-protein manipulation					Ctl
		HC1	HC2	HC3	HC4	HC5	HP1	HP2	HP3	HP4	HP5	
	Composition (DM basis)											
DM of fed diet (%)	92.0	90.6	91.1	91.7	91.3	91.3	90.9	91.0	90.7	91.8	91.0	90.3
Crude protein (%)	53.1	53.8	53.1	53.1	55.0	55.6	53.1	54.4	53.8	54.4	53.8	51.3
Crude fat (%)	7.3	7.2	7.3	7.1	7.4	7.3	7.2	7.0	6.9	6.8	6.9	7.7
Ash (%)	15.2	14.9	15.1	17.5	15.1	15.0	15.0	14.9	14.9	14.6	14.7	13.9
Ca (%)[1]	1.49	1.49	1.50	1.50	1.51	1.51	1.47	1.47	1.47	1.47	1.47	1.28
P (%)[1]	1.57	1.57	1.56	1.56	1.55	1.55	1.42	1.42	1.41	1.41	1.40	1.26
Amino acids (g/kg)												
Alanine	19.9	19.6	19.9	19.9	12.0	20.5	20.2	20.9	20.9	21.2	21.0	19.6
Arginine	**22.3**	**22.7**	**22.7**	**23.5**	**23.8**	**25.9**	**22.6**	**23.7**	**23.7**	**24.3**	**23.8**	**21.6**
Aspartic acid	27.3	27.2	27.1	27.1	27.6	27.8	29.0	31.2	32.1	34.6	34.7	34.3
Cystine	6.3	6.2	6.3	6.4	6.2	6.3	6.1	5.6	5.2	4.7	4.3	3.6
Glutamic acid	166.8	164.2	166.7	168.1	166.5	169.2	163.1	160.1	154.8	148.3	141.4	127.6
Glycine	24.0	23.6	23.9	23.9	24.1	24.9	23.7	23.6	22.7	22.0	21.3	18.5
Histidine	13.3	14.1	14.3	13.5	14.2	14.1	14.5	15.3	15.9	15.4	15.9	14.0
Isoleucine	20.8	20.6	20.7	20.8	21.1	21.2	21.9	22.5	23.1	24.2	23.9	23.6
Leucine	37.8	37.8	37.8	37.9	38.3	38.6	39.6	41.1	41.9	43.6	43.2	42.2
Lysine	**18.4**	**20.9**	**22.6**	**26.3**	**28.6**	**31.6**	**21.8**	**25.1**	**26.7**	**30.1**	**30.6**	**34.2**
Methionine	7.1	7.0	6.5	7.0	7.0	7.1	7.7	7.9	8.2	8.8	8.9	10.6
Phenylalanine	26.3	25.9	25.8	26.2	26.7	27.1	26.7	26.6	27.1	27.5	26.0	25.7
Proline	56.1	54.7	55.4	56.8	54.9	56.5	56.2	56.6	54.7	55.7	54.2	50.9
Serine	25.6	25.6	25.5	25.5	25.8	26.1	26.2	27.3	27.6	28.4	27.9	27.1
Threonine	**16.5**	**16.9**	**17.3**	**17.7**	**18.5**	**19.2**	**17.5**	**18.5**	**19.1**	**20.1**	**20.0**	**21.4**
Tryptophan	**4.9**	**5.2**	**5.8**	**6.4**	**6.9**	**7.4**	**5.4**	**5.4**	**5.5**	**5.9**	**6.1**	**6.2**
Tyrosine	16.9	16.8	16.6	16.9	17.3	17.4	18.0	18.5	19.6	20.8	20.6	20.9
Valine	22.7	22.6	22.7	22.6	23.1	23.5	24.1	25.8	26.6	28.1	28.1	28.3
Fatty acids (g/kg)[1]												
C18:2n-6	12.1	12.1	12.0	12.0	11.9	11.9	10.7	9.7	8.7	7.7	6.7	9.7
C18:3n-3	0.7	0.7	0.7	0.7	0.7	0.7	0.7	0.7	0.6	0.5	0.5	1.1
C20:5n-3	6.9	6.9	6.8	6.8	6.7	6.7	6.8	6.8	6.8	6.7	6.7	6.9
C22:6n-3	6.2	6.2	6.1	6.1	6.0	6.0	6.0	6.0	6.0	6.0	6.0	5.7
Gross energy (kJ/g)[1]	19.7	19.7	19.7	19.8	19.8	19.8	19.7	19.7	19.8	19.8	19.8	20.0

[1] Values for Diets HC1 to HC4 and HP2 to HP4 were calculated by interpolation between analyses of extreme diets for each of the respective supplementation series.

6.2.2.7 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). Growth rate was determined as the difference between end (W_e) and start (W_0) weights divided by the number of days on experiment and specific growth rate (SGR, % per d) was calculated as: 100 x (ln W_e - ln W_0)/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. Dietary nutrient and energy retention (R) data were calculated as the absolute chemical gain of the fish during the experiment and expressed as a percentage of dietary intake according to the equation:

$$R = [\{(Wt_E \times N_E/100) - (Wt_S \times N_S/100)\} / (FC \times N_D/100)];$$

where Wt_E and Wt_S are the weights (g) of the fish at the end and start of the experiment respectively, N_E and N_S are the nutrient (or energy) contents (% DM bodyweight) of the fish at the end and pre-experiment sampling respectively, FC is the average net food intake (g DM) of the fish and N_D is the nutrient (or energy) concentration (% or kJ/g DM) of the diet. Differences between treatment effects were examined *a-posteriorly* using Fischer's protected 't' test (Snedecor and Cochran, 1967) wherein differences between means were examined only where the 'F' test of the ANOVA was significant ($P < 0.05$). Regression analysis was used to examine relationships between dietary nutrient supply and fish response within each diet series. The homogeneity of the data for derived regressions between diet series was tested and differences between regression slopes and intercepts were tested using standard procedures (Snedecor and Cochran, 1967).

6.2.3 Results and discussion

6.2.3.1 Experiment AA2

Chemical analyses confirmed that the protein and energy contents of the diets in both of the diet series were very similar and that the dietary essential amino acid contents increased incrementally with amino acid enrichment in each series (Table 7). Table 9 details the essential amino acid content (as % of dietary protein) of the basal and highest supplemented diets of each series and compares these with requirement values recommended for two other warm water carnivorous fish, channel catfish and Japanese eel. This comparison shows that the basal diet was deficient in Lys and Arg for channel catfish and additionally low to marginally limiting in Thr, Tryp and Ile for Japanese eel. At the highest enrichment, all essential amino acids exceeded the requirement value recommended for channel catfish in both diet series but was sub-optimal for Japanese eel with respect to Ile, Thr and Met plus Cysh in one or both of the diet series.

The health of the fish was good throughout the experiment and no losses occurred. Growth rate and food conversion of the fish was excellent on all diets with specific growth rates exceeding 2 %/d and food conversions of 1.15 or better on an air-dry basis; dry matter food conversion was 1.04 or better. However, there were productivity differences between diets as detailed in Table 10. Food intake did not vary significantly ($P>0.05$) between diets but food conversion and average and specific growth rates improved with increasing C-AA and protein-AA enrichment.

Table 8 Chemical composition of the diets examined in Experiment AA3

Ingredient	Diet designation											
	Basal	Crystalline amino acid supplementation					Intact-protein manipulation					Ctl
		LC1	LC2	LC3	LC4	LC5	LP1	LP2	LP3	LP4	LP5	
							Composition (DM basis)					
DM of fed diet (%)	91.2	91.2	91.2	91.3	91.3	91.3	91.2	91.2	91.2	91.2	91.2	91.5
Crude protein (%)	38.7	39.0	39.2	39.5	39.8	40.0	38.9	39.0	39.1	39.2	39.3	50.5
Crude fat (%)	8.0	8.0	8.0	8.0	8.0	8.0	7.9	7.9	7.8	7.8	7.8	7.9
Ash (%)	12.8	12.8	12.8	12.8	12.8	12.8	12.8	12.8	12.8	12.8	12.8	14.8
Ca (%)	0.83	0.83	0.83	0.83	0.83	0.83	0.83	0.83	0.83	0.83	0.82	1.06
P (%)	0.88	0.88	0.87	0.87	0.87	0.87	0.86	0.85	0.84	0.83	0.82	1.06
Amino acids (g/kg) [1]												
Alanine	12.6	12.6	12.6	12.6	12.6	12.6	12.8	13.0	13.1	13.3	13.5	17.6
Arginine	**14.1**	**14.3**	**14.6**	**14.8**	**15.0**	**15.2**	**14.3**	**14.4**	**14.6**	**14.7**	**14.8**	**21.8**
Aspartic acid	16.9	16.9	16.9	16.9	16.9	16.9	17.8	18.6	19.4	20.2	21.1	31.2
Cystine	6.6	6.6	6.6	6.6	6.6	6.6	6.3	6.1	5.8	5.5	5.2	4.8
Glutamic acid	99.1	99.1	99.1	99.1	99.1	99.1	97.4	95.7	94.0	92.2	90.5	107.6
Glycine	13.4	13.4	13.4	13.4	13.4	13.4	13.2	13.0	12.8	12.6	12.3	15.7
Histidine	9.6	9.6	9.6	9.6	9.6	9.6	9.8	10.0	10.3	10.5	10.7	13.6
Isoleucine	**11.9**	**12.1**	**12.4**	**12.6**	**12.9**	**13.1**	**12.2**	**12.6**	**13.0**	**13.4**	**13.7**	**21.0**
Leucine	26.0	26.0	26.0	26.0	26.0	26.0	26.7	27.3	28.0	28.6	29.3	38.6
Lysine	**11.9**	**13.1**	**14.3**	**15.5**	**16.7**	**17.9**	**13.2**	**14.4**	**15.6**	**16.9**	**18.1**	**32.2**
Methionine	5.5	5.5	5.5	5.5	5.5	5.5	5.8	6.2	6.5	6.8	7.2	14.9
Phenylalanine	17.5	17.5	17.5	17.5	17.5	17.5	17.7	17.9	18.0	18.2	18.4	22.7
Proline	35.5	35.5	35.5	35.5	35.5	35.5	35.5	35.5	35.5	35.5	35.5	45.2
Serine	16.3	16.3	16.3	16.3	16.3	16.3	16.6	16.9	17.2	17.5	17.8	24.1
Threonine	**10.7**	**11.1**	**11.5**	**11.9**	**12.3**	**12.7**	**11.1**	**11.5**	**11.9**	**12.3**	**12.7**	**18.2**
Tryptophan	**4.7**	**4.9**	**5.1**	**5.3**	**5.5**	**5.7**	**4.8**	**4.9**	**5.0**	**5.1**	**5.2**	**7.2**
Tyrosine	11.9	11.9	11.9	11.9	11.8	11.8	12.4	12.9	13.4	13.9	14.4	21.3
Valine	15.3	15.3	15.3	15.3	15.3	15.3	15.9	16.5	17.1	17.7	18.3	25.3
Fatty acids (g/kg)[1]												
C18:2n-6	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	6.2
C18:3n-3	0.8	0.8	0.8	0.8	0.8	0.8	0.8	0.8	0.8	0.8	0.8	1.4
C20:5n-3	6.2	6.2	6.2	6.2	6.2	6.2	6.2	6.2	6.2	6.2	6.2	4.5
C22:6n-3	9.0	9.0	9.0	9.0	9.0	9.0	9.0	9.0	9.0	9.0	9.0	8.1
Gross energy (kJ/g)[1]	19.4	19.41	19.42	19.43	19.43	19.44	19.43	19.45	19.47	19.49	19.52	20.02

[1] Calculated from the analysis of the individual feed ingredients.

Table 9 The essential amino acid content of the protein of selected diets in Experiment AA2 compared to recommended values for channel catfish and Japanese eel

Amino acid	Experimental diets[1]			Recommended requirement	
	Basal	HC5	HP5	Channel catfish[2]	Japanese eel[3]
			g/16 g N		
Arginine	4.06	4.51	4.52	4.3	4.5
Histidine	2.52	2.55	3.02	1.52	2.1
Isoleucine	3.81	3.80	4.53	2.59	4.0
Leucine	6.95	6.91	8.19	4.0	5.3
Lysine	3.42	5.64	5.89	5.1	5.3
Meth + cystine	2.45	2.39	2.47	2.32	3.2
Phe + tyrosine	7.88	7.94	8.90	5.0	5.8
Threonine	3.03	3.42	3.80	2.21	4.0
Tryptophan	0.90	1.33	1.13	0.5	1.1
Valine	4.17	4.12	5.35	2.98	4.0

[1] Refer to Table 5 for full description of diets.

[2] Mean of values reported by Tacon and Cowey (1985) and Wilson (1994).

[3] Reported by Wilson (1994).

Table 10 Productivity of fish fed a control (Ctl) or a basal diet when enriched by crystalline amino acid supplementation (HC) or intact protein manipulation (HP) in Experiment AA2

Diet	Productivity trait					
	Start wt (g)	End wt (g)	Daily gain[1] (g/d)	SGR (%/d)	Food intake[1] (As-fed g /d)	FCR (As-fed g :g gain)
Basal	74.0^{A}	174.6^{A}	2.43^{C}	2.05^{C}	2.78^{A}	1.15^{D}
HC1	75.0^{A}	189.1^{A}	2.71^{AB}	2.20^{AB}	2.86^{A}	1.09^{C}
HC2	74.4^{A}	185.6^{A}	2.67^{AB}	2.18^{AB}	2.84^{A}	1.07^{BC}
HC3	76.1^{A}	188.3^{A}	2.61^{BC}	2.16^{B}	2.83^{A}	1.09^{C}
HC4	75.5^{A}	184.6^{A}	2.56^{BC}	2.13^{BC}	2.77^{A}	1.08^{C}
HC5	74.3^{A}	182.4^{A}	2.60^{BC}	2.14^{BC}	2.76^{A}	1.07^{BC}
HP1	73.4^{A}	181.7^{A}	2.64^{AB}	2.16^{B}	2.81^{A}	1.07^{BC}
HP2	74.8^{A}	186.0^{A}	2.64^{AB}	2.16^{B}	2.70^{A}	1.04^{AB}
HP3	74.4^{A}	189.1^{A}	2.75^{AB}	2.22^{AB}	2.78^{A}	1.02^{A}
HP4	75.2^{A}	195.0^{A}	2.83^{A}	2.27^{A}	2.86^{A}	1.01^{A}
HP5	74.8^{A}	188.7^{A}	2.71^{AB}	2.20^{AB}	2.75^{A}	1.02^{A}
Ctl	74.9^{A}	188.3^{A}	2.69^{AB}	2.19^{AB}	2.93^{A}	1.09^{C}
± sem	1.19	4.44	0.066	0.035	0.062	0.015

[1] Covariance adjusted to remove effects of differences in start weight.

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

Amino acid enrichment of the Basal diet resulted in a significant ($P<0.05$) quadratic improvement in each of the measured productivity traits for both the C-AA and protein-AA diet series. As the dietary contents of each amino acid in the protein-AA series and for each of

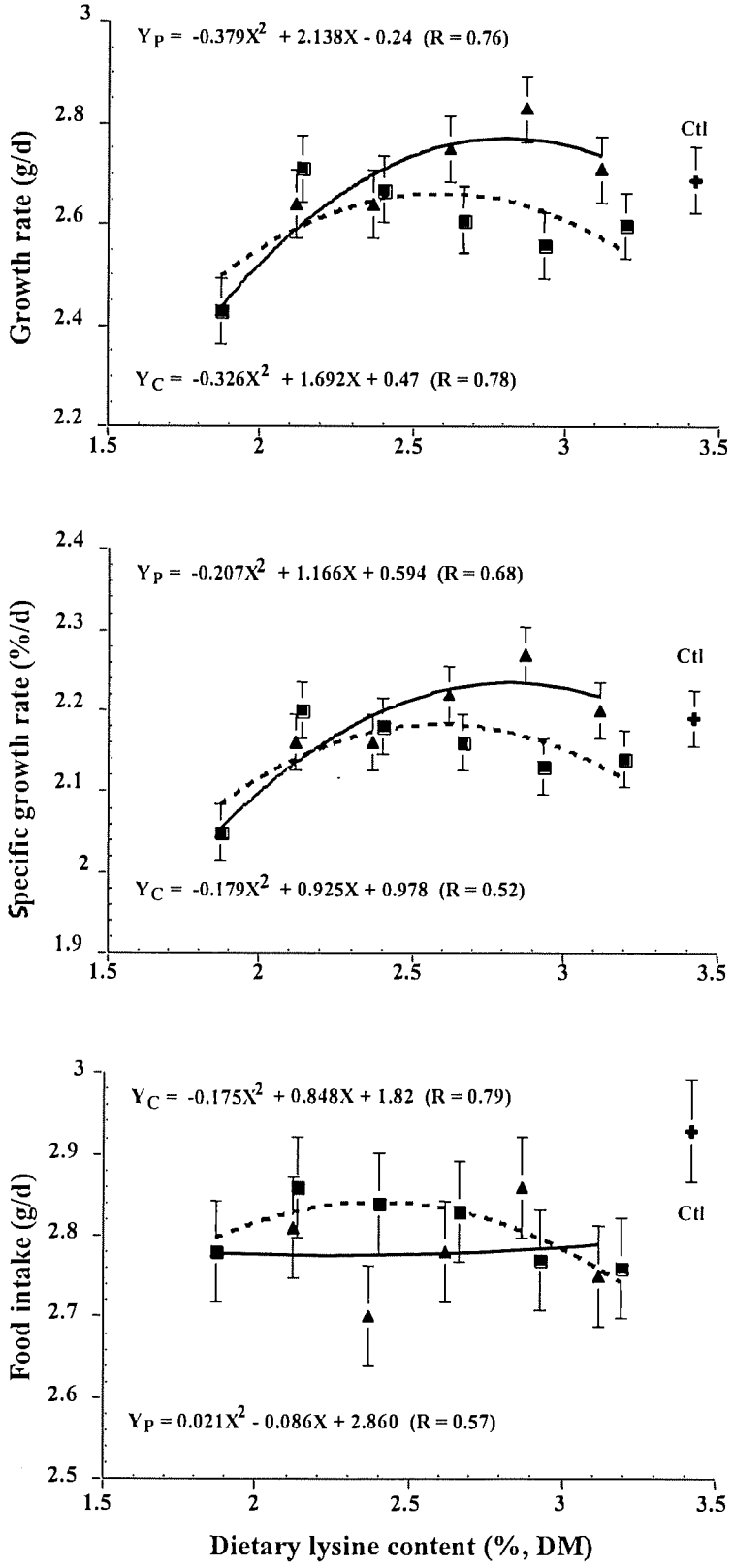

Fig. 1 Relationship between dietary lysine content and average daily gain (Fig A), specific growth rate (Fig B) and daily food intake (Fig C) responses of fish to diets enriched by crystalline amino acid supplementation (■) or intact protein manipulation (▲) in Experiment: Control (Ctl) diet response shown for comparative purposes.

In the case of daily growth rate, specific growth rate and daily food intake, the slope and intercept statistics of the derived regressions did not differ significantly ($P>0.05$) between the diet series; these are depicted in Figure 1. For food conversion, the slope and intercept statistics of the derived response relationship differed ($P<0.05$) between diet series. In the case of the C-AA series, variation in the response to amino acid enrichment was explained equally as well by fitting either a bent-stick or second order polynomial relationship as illustrated in Figure 2. The bent-stick model indicated that food conversion improved with increasing supplementation until a plateau response of 1.08 was observed for dietary DM Lys contents of 2.28% and above.

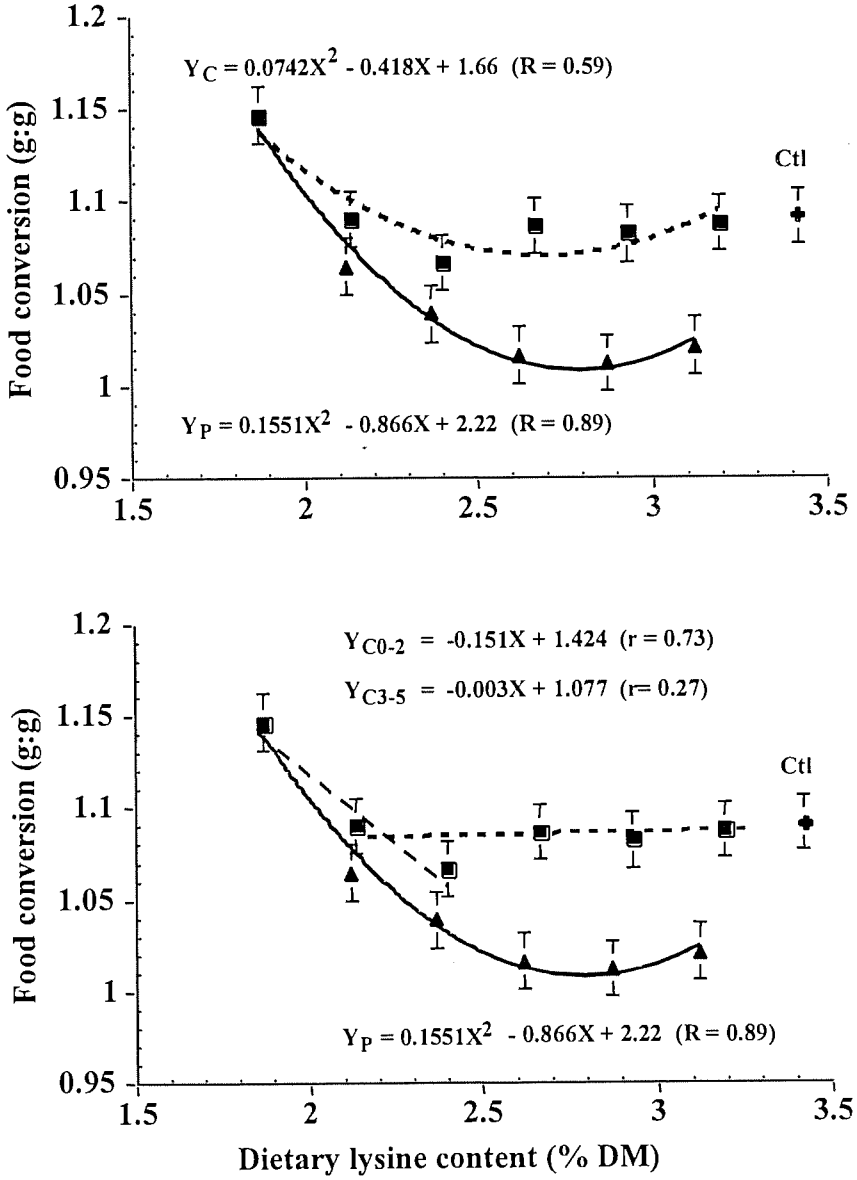

Fig. 2 Quadratic (Fig. A) and bent-stick (Fig. B) relationships between dietary lysine content and food conversion ratio response of fish to diets enriched by crystalline amino acid supplementation (■) or intact protein manipulation (▲) in Experiment AA2: Control (Ctl) diet response shown for comparative purposes.

The polynomial model described a similar maximum food conversion response of 1.07 but at a dietary DM Lys content of 2.80%. In contrast, amino acid enrichment with protein-AAs was best explained as a second order polynomial equation with an asymptotic value of 1.01 being obtained at a dietary DM Lys content of 2.80%.

Irrespective of the mathematical model chosen to describe the effect of C-AA supplementation, the data show clearly that the protein-AAs were used more effectively than C-AAs in improving the efficiency with which food was converted into fish weight gain. Relative differences in supplementation efficacy can be quantified if certain assumptions are made: (i) that the response to supplementation was specifically due to Lys enrichment; and (ii) that the failure of the C-AA supplementation to deliver the same magnitude of improvement in food conversion as seen with protein-AA enrichment was due entirely to loss of efficacy of the crystalline Lys. Based on these assumptions, the bent-stick model predicted that crystalline Lys was used at an efficacy of 70% of that of the protein-bound Lys as supplementation increased the dietary DM Lys content from 1.87 to 2.28%; thereafter, additional supplementation caused the relative efficacy of the C-AA to reduce to 47% at a dietary DM Lys content of 2.80%. By comparison, the polynomial response model predicted that the relative efficacy of the C-AA supplement was 54% at all levels of supplementation up to a dietary DM Lys content of 2.80%.

The proximate chemical and energy contents of the fish sampled before and after being fed the experimental diets in Experiment AA2 are shown in Table 11. The most noticeable changes in the chemical composition of the fish over the course of the experiment were increases in fat and energy contents and decreases in ash and N contents. However, examination of the fat-free body showed that the altered composition was predominantly due to increased fat deposition rather than to any marked alteration in ash or N deposition. There were also however, small but significant ($P<0.05$) changes in the chemical composition of the whole and fat-free body due to dietary treatment. Fish fed the basal diet had the highest fat and ash and lowest N contents. These effects were reversed by amino acid enrichment and were more pronounced for intact protein as compared to C-AA.

Table 11 Mean proximate chemical and energy contents (DM basis) of the whole and fat-free body of fish sampled before (pre-expt) or after (post-expt) being fed the experimental diets in Experiment AA2

Sample &	Whole Body					Fat-free body	
diet	DM (%)	Ash (%)	N (%)	Fat (%)	GE (kJ/g)	Ash (%)	N (%)
Pre-expt[1]	32.8±1.24	14.2±0.63	10.62±0.25	17.8±1.88	22.8±0.42	17.3±0.54	12.9±0.21
Post-expt[2]							
Basal	32.5^{A}	13.8^{A}	9.43^{E}	25.3^{A}	23.8^{CD}	18.5^{A}	12.62^{F}
HC1	32.8^{A}	13.3^{CD}	9.60^{D}	25.1^{A}	24.0^{AB}	17.7^{BC}	12.82^{DE}
HC2	32.3^{A}	13.3^{BCD}	9.73^{CD}	24.4^{A}	23.9^{BC}	17.7^{BC}	12.87^{CD}
HC3	32.6^{A}	13.3^{CD}	9.67^{CD}	24.9^{A}	24.0^{ABC}	17.7^{BC}	12.87^{CD}
HC4	32.4^{A}	13.2^{D}	9.71^{CD}	24.3^{AB}	23.8^{CD}	17.5^{CD}	12.82^{DE}
HC5	32.5^{A}	13.3^{CD}	9.74^{CD}	24.4^{A}	23.9^{BC}	17.6^{CD}	12.88^{BCD}
HP1	32.6^{A}	13.6^{ABC}	9.59^{D}	24.6^{A}	23.8^{BCD}	18.0^{B}	12.71^{EF}
HP2	32.5^{A}	13.7^{AB}	9.91^{AB}	23.0^{C}	23.6^{E}	17.7^{BC}	12.87^{CD}
HP3	33.2^{A}	13.3^{BCD}	9.92^{AB}	23.3^{BC}	23.7^{DE}	17.4^{CDE}	12.93^{ABCD}
HP4	32.8^{A}	13.3^{CD}	10.01^{A}	23.2^{C}	23.7^{DE}	17.3^{DE}	13.04^{A}
HP5	32.8^{A}	13.1^{D}	9.96^{A}	23.2^{C}	23.8^{CD}	17.0^{EF}	12.96^{ABC}
Ctl	32.8^{A}	12.7^{E}	9.78^{BC}	24.8^{A}	24.1^{A}	16.9^{F}	13.01^{AB}
± sem	0.28	0.12	0.054	0.36	0.07	0.13	0.043
Mean	32.6±0.54	13.3±0.35	9.75±0.19	24.2±1.01	23.8±0.19	17.6±0.49	12.90±0.14

[1] Mean (±SD) based on the analysis of 16 individual fish.

[2] Mean based on the analysis of 4 pooled fish from each of the 4 experimental blocks (replicates).

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

The amino acid composition of the whole body before and after feeding of the experimental diets in Experiment AA2 is shown in Table 12.

Table 12 Mean (±SD)[1] amino acid content of the whole fish sampled before (pre-expt) or after (post-expt) being fed the experimental diets in Experiment AA2

Diet/treat	ALA	ARG	ASP	CYS	GLU	GLY	HIS	ILE	LEU	LYS	MET	PHE	PRO	SER	THR	TRY	TYR	VAL
	(g/kg DM)																	
Pre-expt	38.5	34.4	52.0	4.1	76.3	48.8	10.3	21.2	38.8	42.8	16.1	21.9	30.8	22.4	24.1	6.3	16.4	23.9
±SD	(0.82)	(0.99)	(1.07)	(0.12)	(2.34)	(2.48)	(0.43)	(0.49)	(0.86)	(0.91)	(0.38)	(0.73)	(1.47)	(0.58)	(0.42)	(0.14)	(0.50)	(0.38)
Post-expt																		
Basal	35.6	31.9	47.9	3.3	69.8	46.8	9.3	18.4	33.6	36.2	13.6	19.7	35.2	19.7	21.0	6.0	14.3	21.5
±SD	(0.08)	(0.20)	(0.84)	(0.31)	(1.05)	(0.13)	(0.39)	(0.22)	(0.18)	(0.31)	(0.75)	(0.20)	(0.13)	(0.20)	(0.32)	(0.60)	(0.14)	(0.16)
HC1	35.8	32.9	49.1	3.4	71.9	46.5	9.7	19.2	34.8	38.0	13.3	20.2	35.3	20.2	21.7	5.2	15.1	22.0
±SD	(0.36)	(1.36)	(1.64)	(0.15)	(1.58)	(4.05)	(0.49)	(0.12)	(0.92)	(0.58)	(0.23)	(1.05)	(1.36)	(0.39)	(0.34)	(0.78)	(0.76)	(0.23)
HC2	35.4	33.0	48.8	3.4	71.6	46.4	9.9	18.9	34.6	37.8	13.0	20.0	34.7	20.2	21.6	5.1	15.0	21.8
±SD	(0.54)	(0.93)	(0.70)	(0.12)	(1.05)	(2.17)	(0.74)	(0.22)	(0.51)	(0.29)	(0.67)	(0.49)	(0.01)	(0.10)	(0.21)	(0.64)	(0.53)	(0.34)
HC3	36.2	33.7	49.9	3.5	72.9	46.9	9.8	19.3	35.5	38.9	13.6	20.9	34.3	20.5	21.9	5.3	15.4	22.3
±SD	(1.06)	(0.12)	(1.03)	(0.01)	(0.81)	(0.40)	(0.25)	(0.52)	(0.53)	(0.21)	(0.12)	(0.44)	(0.30)	(0.10)	(0.15)	(0.52)	(0.56)	(0.72)
HC4	36.3	33.3	50.3	3.5	72.9	46.5	10.2	19.6	35.4	38.4	13.7	20.5	34.6	20.5	22.1	6.6	15.2	23.0
±SD	(0.33)	(0.40)	(0.14)	(0.11)	(0.13)	(1.81)	(0.42)	(0.76)	(0.03)	(0.36)	(0.37)	(0.33)	(0.91)	(0.17)	(0.14)	(1.14)	(0.07)	(0.70)
HC5	36.9	33.6	50.7	3.5	73.7	47.3	10.4	19.8	35.7	38.8	13.6	20.9	34.8	20.7	22.3	5.5	15.7	22.9
±SD	(0.97)	(1.03)	(1.66)	(0.15)	(2.70)	(4.00)	(1.14)	(0.02)	(1.43)	(1.26)	(0.23)	(1.12)	(2.83)	(0.66)	(0.83)	(0.80)	(0.57)	(0.04)
HP1	36.4	32.8	49.6	3.5	72.9	46.7	9.7	19.4	35.2	38.1	13.5	20.1	35.2	20.6	21.9	5.8	15.2	22.5
±SD	(0.83)	(0.64)	(0.17)	(0.27)	(1.24)	(2.28)	(0.38)	(0.99)	(0.29)	(0.26)	(0.61)	(0.58)	(1.17)	(0.00)	(0.01)	(0.04)	(0.33)	(1.13)
HP2	39.9	36.4	55.5	3.5	80.3	50.9	10.9	21.5	39.1	42.4	13.8	22.4	38.8	22.5	24.3	6.9	16.9	24.7
±SD	(4.85)	(5.66)	(7.27)	(0.09)	(10.43)	(8.02)	(1.71)	(2.33)	(5.18)	(5.77)	(0.25)	(2.89)	(5.58)	(2.81)	(3.03)	(1.33)	(2.21)	(2.92)
HP3	36.5	34.2	51.5	3.6	74.6	47.8	10.4	19.8	36.4	39.1	14.1	21.0	35.5	20.9	22.4	5.7	15.9	22.8
±SD	(0.69)	(0.54)	(0.55)	(0.17)	(1.48)	(0.26)	(0.14)	(0.50)	(0.52)	(0.56)	(0.42)	(0.24)	(0.67)	(0.04)	(0.11)	(0.22)	(0.18)	(0.47)
HP4	39.5	34.1	51.6	3.6	74.4	47.0	10.7	20.0	36.6	40.0	14.4	21.3	35.8	21.0	22.6	5.6	15.8	23.2
±SD	(4.75)	(0.07)	(0.44)	(0.36)	(0.54)	(1.76)	(0.02)	(0.40)	(0.19)	(0.31)	(0.78)	(0.43)	(0.03)	(0.01)	(0.01)	(1.09)	(0.13)	(0.83)
HP5	37.2	34.7	51.8	3.8	75.1	47.8	10.9	20.2	37.0	40.0	12.6	21.7	36.7	21.1	22.9	5.0	16.0	23.1
±SD	(0.24)	(0.75)	(1.38)	(0.23)	(1.44)	(2.23)	(0.19)	(0.03)	(0.69)	(0.80)	2.05)	(0.49)	(0.61)	(0.19)	(0.21)	(0.45)	(0.50)	(0.33)
Ctl	36.3	33.2	50.4	4.2	73.0	45.8	10.4	19.8	35.8	38.8	14.9	20.9	34.4	20.6	22.5	5.6	15.5	22.8
±SD	(1.23)	(0.14)	(0.81)	(0.03)	(0.03)	(0.87)	(0.53)	(0.80)	(0.06)	(0.36)	(1.25)	(0.44)	(0.21)	(0.36)	(0.30)	(0.83)	(0.32)	(0.84)
	(g/16 g N)																	
Pre-expt	**5.94**	**5.30**	**8.03**	**0.64**	**11.77**	**7.52**	**1.58**	**3.27**	**5.99**	**6.61**	**2.49**	**3.38**	**4.75**	**3.46**	**3.73**	**0.97**	**2.54**	**3.69**
±SD	0.075	0.106	0.129	0.019	0.171	0.283	0.037	0.032	0.059	0.065	0.021	0.054	0.164	0.075	0.091	0.023	0.032	0.036
Post-expt	**6.02**	**5.50**	**8.27**	**0.58**	**12.03**	**7.71**	**1.67**	**3.21**	**5.85**	**6.35**	**2.24**	**3.40**	**5.79**	**3.38**	**3.64**	**0.93**	**2.53**	**3.71**
±SD	0.276	0.232	0.335	0.042	0.467	0.401	0.098	0.130	0.235	0.260	0.139	0.138	0.281	0.127	0.141	0.131	0.109	0.155
All	**6.01**	**5.47**	**8.23**	**0.59**	**11.99**	**7.68**	**1.65**	**3.22**	**5.87**	**6.39**	**2.27**	**3.39**	**5.64**	**3.39**	**3.65**	**0.94**	**2.53**	**3.71**
±SD	0.257	0.228	0.324	0.044	0.444	0.388	0.09	0.123	0.223	0.258	0.156	0.129	0.457	0.123	0.137	0.122	0.101	0.144

[1] Mean based on analysis of 4 and 2 samples (Experimental blocks 'B' and 'C') for the pre- and post-experiment groups, respectively.

When expressed as a percentage of protein (as g/16 g N), the before and after amino acid composition of the fish was remarkably similar. For most amino acids, the coefficient of variation amongst the samples (n = 28) was typically 1 to 3% and exceeded 6% only for Met (6.9%), Cysh (7.5%), proline (8.1%) and Tryp (13.0%). The more difficult analytical procedure for Met, Cysh and Tryp was probably the reason for the observed increased variability of these amino acids rather than indicating a genuine difference in the amino acid composition of the protein.

The accretions (retentions) of dietary N, energy and individual amino acids in the fish during the experiment were calculated from the whole body chemical composition and food intake data. Presented in Table 13 are the derived N, energy and selected amino acid retentions for each of the dietary treatments examined in Experiment AA2. Retentions of N and energy were lowest ($P<0.05$) for the Basal diet and increased with amino acid enrichment, and most markedly for the intact protein diet series. Individual amino acid retentions varied both between amino acid type and between the two methods of amino acid enrichment.

Linear and quadratic regression analyses were used to examine for relationships between the amino acid content of the diet and the observed dietary retentions of N and energy by the fish. Presented in Tables 14 and 15 for N and energy respectively, are the derived quadratic relationships for those amino acids that were added as supplements in the C-AA diet series and additionally for Met which was the next most variable essential amino acid in the intact protein diet series.

For the C-AA supplemented diet series, quadratic relationships for N retention ($P<0.05$) were found with Lys, Thr, Tryp but not for Arg nor any other amino acid.

In the case of energy retention, no significant ($P>0.05$) relationships were found for any of the amino acids. For the intact protein diet series, all amino acids exhibited significant ($P<0.05$) quadratic relationships for N retention and similarly for energy, except alanine, Phe and proline. The latter three exhibited the smallest amount of between-diet variation in amino acid concentration (<3.5%) and this may explain why a significant relationship was not evident.

The presence of a significant relationship between dietary amino acid content and the retention of dietary N and/or energy is strong evidence of a causal effect for the C-AA diet series as these respective amino acids were added as specific supplements with minimal effect on the dietary supply of other nutrients. However, since each of the C-AAs was added together, it was not possible to distinguish whether the retention response was due to one or all of the added C-AAs. Similarly, the relationships between dietary amino acid content and retention of dietary N and energy for the intact protein diet series can be attributed generally to the associated change in amino acid composition of the dietary protein, but not to any particular amino acid.

Table 13 Retention of dietary N, energy and essential amino acids[1] by fish in Experiment AA2

Diet fed	Retention (% of dietary intake)[2]														
	N	Energy	Arg	Cys	Gly	His	Ile	Leu	Lys	Met	Phe	Thr	Tryp	Tyr	Val
Basal	32.1^{I}	38.7^{F}	42.3^{D}	13.5^{F}	59.0^{D}	20.5	24.8	24.9^{C}	54.1^{BC}	53.1	21.5^{D}	35.8	37.4^{BC}	23.9^{D}	27.4^{CD}
HC1	36.6^{DEF}	43.4^{ABCD}	49.3^{B}	17.1^{DE}	67.2^{BCD}	23.4	30.8	30.3^{B}	59.2^{A}	58.5	26.1^{BC}	42.2	31.5^{CDEF}	30.0^{A}	32.4^{A}
HC2	36.5^{EFG}	41.6^{DE}	48.5^{BC}	16.2^{EF}	64.0^{D}	23.0	29.0	29.1^{AB}	52.6^{BC}	59.0	25.0^{BC}	39.8	25.6^{EF}	29.1^{AB}	30.8^{AB}
HC3	35.7^{FGH}	41.2^{E}	47.7^{BC}	16.2^{EF}	64.3^{D}	23.9	29.6	29.8^{AB}	46.8^{DE}	58.8	26.1^{BC}	39.3	25.3^{F}	29.4^{AB}	31.8^{A}
HC4	34.6^{H}	40.7^{E}	44.8^{CD}	16.7^{DE}	61.2^{D}	23.3	28.9	28.5^{AB}	40.9^{FG}	57.8	24.0^{CD}	36.9	31.6^{CDEF}	27.3^{ABC}	31.5^{A}
HC5	34.8^{GH}	41.4^{E}	43.2^{D}	16.9^{DE}	63.1^{D}	25.1	30.1	29.6^{AB}	38.9^{GH}	57.7	25.3^{BC}	37.3	45.2^{AB}	29.5^{AB}	31.9^{A}
HP1	36.2^{FGH}	42.2^{BCDE}	47.8^{BC}	17.2^{DE}	65.3^{CD}	21.9	28.4	28.4^{ABC}	55.1^{AB}	53.6	24.2^{CD}	40.2	34.7^{CD}	27.1^{ABC}	30.5^{AB}
HP2	38.2^{CDE}	42.4^{BCDE}	47.5^{BCD}	19.3^{D}	67.8^{BCD}	22.1	29.4	29.2^{AB}	50.5^{BCD}	55.4	26.6^{BC}	40.5	47.0^{A}	28.7^{AB}	29.6^{AB}
HP3	39.6^{ABC}	43.4^{ABC}	51.8^{AB}	22.3^{C}	74.7^{AB}	23.6	29.6	30.1^{AB}	49.9^{CD}	57.3	27.1^{B}	40.5	35.5^{CD}	28.4^{AB}	30.0^{AB}
HP4	39.9^{AB}	43.7^{AB}	49.1^{BC}	25.2^{C}	73.8^{ABC}	25.0	28.1	28.6^{AB}	45.0^{EF}	54.1	26.8^{BC}	38.3	31.7^{CDE}	26.3^{BCD}	28.6^{BC}
HP5	40.4^{A}	44.3^{A}	54.6^{A}	31.7^{B}	82.9^{A}	26.2	30.6	31.1^{A}	47.0^{DE}	45.4	30.9^{A}	41.5	26.4^{EF}	28.6^{AB}	30.1^{AB}
Ctl	38.3^{BCD}	41.7^{CDE}	51.0^{AB}	40.5^{A}	81.0^{A}	25.4	27.2	27.3^{BC}	36.3^{H}	45.1	26.8^{BC}	34.0	28.5^{DEF}	24.4^{CD}	26.6^{D}
± sem	0.60	0.63	1.47	0.95	3.12	1.63	0.78	0.92	1.40	3.42	0.95	1.25	2.71	1.07	0.81

[1] Includes those amino acids for which inter-conversion can spare requirement (viz. Cysh for Met; Tyr for Phe) and Gly for which essentiality is equivocal.

[2] Means for N and energy based on 4 replicates whereas those of the amino acids based on 2 replicates.

^{A,B,C,D,E,F,G,H,I} Within each analysis, diet means without a common superscript letter differ ($P<0.05$).

Table 14 Selected relationships[1] between dietary amino acid content (X) and dietary retention of N (Y_N; % decimal) for diets enriched by crystalline amino acid supplementation or intact protein manipulation in Experiment AA2

Supplementation series	Equation	Significance test statistics[2] F-test	RSD	R
	X = Arginine (g/kg DM)			
Crystalline AA	$Y_N = -2.525 + 0.2387X - 0.00494X^2$	1.23	0.019121	0.32
Intact protein	$Y_N = -12.205 + 1.0480X - 0.02178X^2$	43.0***	0.014445	0.90
	X = Lysine (g/kg DM)			
Crystalline AA	$Y_N = -0.054 + 0.0329X - 0.00065X^2$	5.79**	0.016223	0.60
Intact protein	$Y_N = -0.097 + 0.0325X - 0.00053X^2$	76.5***	0.011323	0.94
	X = Threonine (g/kg DM)			
Crystalline AA	$Y_N = -3.740 + 0.4577X - 0.01277X^2$	3.93*	0.017239	0.52
Intact protein	$Y_N = -1.977 + 0.2362X - 0.00587X^2$	76.0***	0.011358	0.94
	X = Tryptophan (g/kg DM)			
Crystalline AA	$Y_N = -0.222 + 0.1892X - 0.01529X^2$	3.97*	0.017216	0.52
Intact protein	$Y_N = -1.598 + 0.6546X - 0.05351X^2$	43.0***	0.014440	0.90
	X = Methionine (g/kg DM)			
Crystalline AA	$Y_N = 0.384 + 0.0266X - 0.00452X^2$	1.48	0.018913	0.35
Intact protein	$Y_N = -1.660 + 0.4682X - 0.02657X^2$	74.8***	0.011432	0.94

[1] Relationships are presented for those amino acids added as supplements in the C- AA diet series and additionally for Met, which was the next most variable essential amino acid.

[2] F-test, Test of the mean square of the quadratic regression (d.f. = 2) over the mean square of the residual error variance (d.f. = 21) and significance denoted as: * = P<0.05, ** = P<0.01, *** = P<0.001; RSD, Residual standard deviation; R, Correlation Coefficient of Determination.

Table 15 Selected relationships[1] between dietary amino acid content (X) and dietary retention of energy (Y_E; % decimal) for diets enriched by crystalline amino acid supplementation or intact protein manipulation in Experiment AA2

Supplementation series	Equation	Significance test statistics[1] F-test	RSD	R
	X = Arginine (g/kg DM)			
Crystalline AA	$Y_E = -1.235 + 0.1355X - 0.00277X^2$	0.53	0.018764	0.22
Intact protein	$Y_E = -4.180 + 0.3794X - 0.00780X^2$	11.0**	0.017533	0.57
	X = Lysine (g/kg DM)			
Crystalline AA	$Y_E = 0.170 + 0.01952X - 0.00038X^2$	1.77	0.017785	0.38
Intact protein	$Y_E = 0.120 + 0.0211X - 0.00035X^2$	25.0***	0.012345	0.84
	X = Threonine (g/kg DM)			
Crystalline AA	$Y_E = -1.693 + 0.2346X - 0.00652X^2$	0.99	0.018385	0.29
Intact protein	$Y^E = -1.149 + 0.1590X - 0.00398X^2$	24.3***	0.012470	0.84
	X = Tryptophan (g/kg DM)			
Crystalline AA	$Y_E = 0.150 + 0.0859X - 0.00691X^2$	0.73	0.018596	0.25
Intact protein	$Y_E = -0.899 + 0.4391X - 0.03596X^2$	28.3***	0.011798	0.85
	X = Methionine (g/kg DM)			
Crystalline AA	$Y_E = 3.480 - 0.8949X + 0.06520X^2$	0.15	0.019095	0.12
Intact protein	$Y_E = -0.902 + 0.3056X - 0.01739X^2$	28.8***	0.011722	0.86

[1] As for Table 14.

To gain further insight into the way that dietary amino acid supply affected nutrient retention in the fish, relationships between dietary amino acid content and amino acid retention were examined by linear and quadratic regression analyses. For the C-AA series, significant relationships were found only in respect of Lys (P<0.01 for both linear and quadratic expressions); a tendency (P = 0.07) for a linear relationship was observed for Tryp. For the intact protein series, the quadratic term resulted in the best overall fit of the data with significant (P<0.05) relationships being found for each amino acid other than Met, Phe, Tryp

and Val. Derived relationships for a representative selection of amino acids are shown in Table 16 and illustrated in Fig. 3 to 5.

Table 16 Selected relationships between dietary amino acid content (X) and dietary retention of amino acid (Y_A; g/kg DM) for diets enriched by crystalline amino acid supplementation or intact protein manipulation in Experiment AA2

Supplementation series	Equation	Significance test statistics[1] F-test	RSD	R
	X = Alanine (g/kg DM)			
Crystalline AA	$Y_A = 100.537 - 9.9339X + 0.24675X^2$	1.89	0.032385	0.54
Intact protein	$Y_A = -1.0321 + 0.0659X - 0.00065X^2$	6.16*	0.041440	0.78
	X = Arginine (g/kg DM)			
Crystalline AA	$Y_A = -4.766 + 0.4426X - 0.00935X^2$	1.13	0.034847	0.45
Intact protein	$Y_A = -30.164 + 2.6044X - 0.05526X^2$	9.54*	0.026008	0.84
	X = Glutamic acid (g/kg DM)			
Crystalline AA	$Y_A = 52.874 - 0.6322X + 0.001895X^2$	1.79	0.009664	0.53
Intact protein	$Y_A = -0.983 + 0.0176X - 0.00007X^2$	63.0***	0.007000	0.97
	X = Glycine (g/kg DM)			
Crystalline AA	$Y_A = 73.126 - 5.9555X + 0.12226X^2$	0.63	0.061662	0.35
Intact protein	$Y_A = -3.829 + 0.4818X - 0.01238X^2$	19.6***	0.031746	0.91
	X = Lysine (g/kg DM)			
Crystalline AA	$Y_A = 0.852 - 0.01475X$	42.2***	0.035623	0.90
Intact protein	$Y_A = 0.696 - 0.00760X$	47.2***	0.016610	0.92
	X = Lysine (g/kg DM)			
Crystalline AA	$Y_A = 0.461 + 0.0177X - 0.00065X^2$	22.0***	0.035344	0.91
Intact protein	$Y_A = 0.375 + 0.0193X - 0.00055X^2$	32.1***	0.014663	0.94
	X = Methionine (g/kg DM)			
Crystalline AA	$Y_A = -5.753 + 1.9060X - 0.14315X^2$	0.32	0.034180	0.26
Intact protein	$Y_A = -3.527 + 1.0414X - 0.06630X^2$	1.40	0.060675	0.49
	X = Cystine (g/kg DM)			
Crystalline AA	$Y_A = 113.686 - 36.077X + 2.86601X^2$	1.22	0.015447	0.46
Intact protein	$Y_A = 1.011 - 0.2186X + 0.012906X^2$	40.7***	0.022077	0.95
	X = Proline (g/kg DM)			
Crystalline AA	$Y_A = 23.570 - 0.8299X + 0.00737X^2$	1.08	0.016920	0.44
Intact protein	$Y_A = 27.953 - 0.9812X + 0.00868X^2$	12.1**	0.012341	0.87
	X = Threonine (g/kg DM)			
Crystalline AA	$Y_A = -3.547 + 0.4480X - 0.01272X^2$	1.03	0.027124	0.43
Intact protein	$Y_A = -2.831 + 0.3450X - 0.00092X^2$	7.66*	0.014046	0.81
	X = Tryptophan (g/kg DM)			
Crystalline AA	$Y_A = 1.364 - 0.3190X + 0.02292X^2$	2.28	0.061656	0.58
Intact protein	$Y_A = -4.225 + 1.7766X - 0.17066X^2$	2.46	0.077191	0.59

[1] As for Table 14 except that the d.f. of the residual error variance was 10 (linear) or 9 (quadratic).

In the case of Lys (Fig. 3) where significant relationships existed for both the C-AA and intact protein series, comparison of the derived regressions revealed that they had significantly different ($P<0.05$) slopes and intercepts (for linear regression). In both diet series, Lys retention declined with increasing dietary Lys concentration but the rate was greater ($P<0.05$) for the C-AA series.

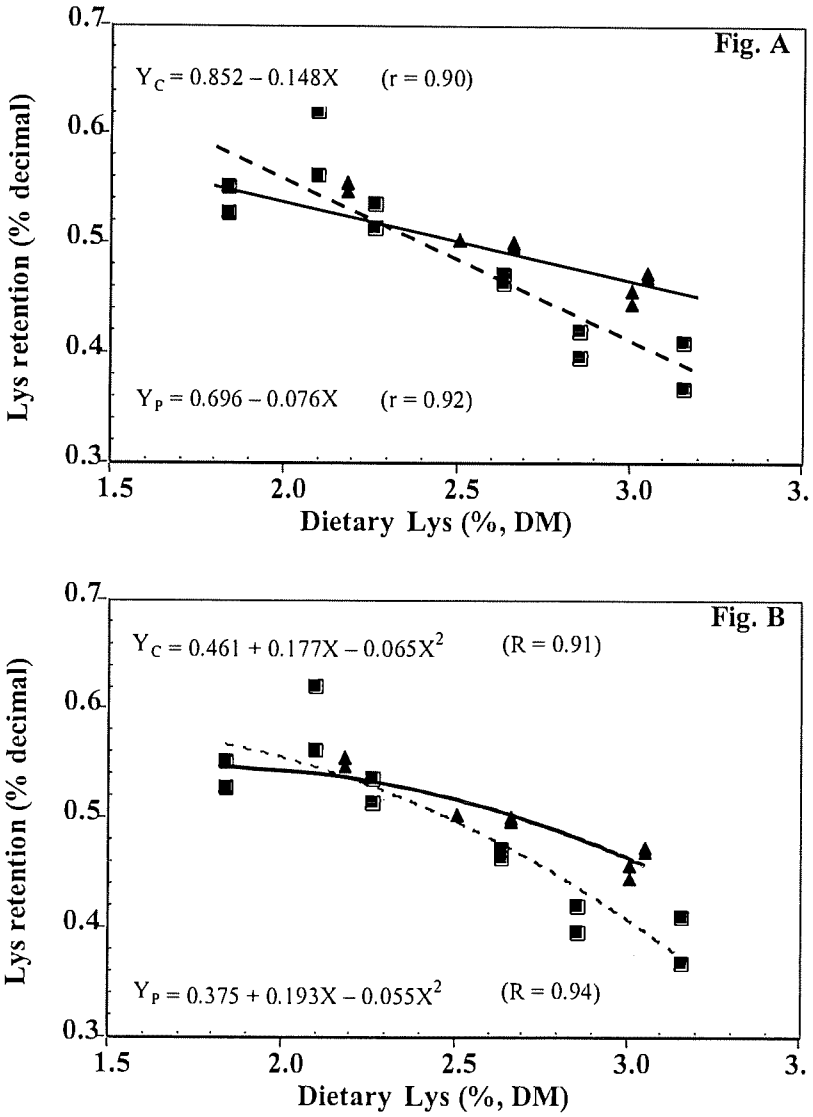

Fig. 3 Linear (Fig. A) and quadratic (Fig. B) regressions between dietary lysine (Lys,) content and lysine retention of fish fed diets enriched using crystalline amino acids (Y_C; ■) or intact protein manipulation (Y_P; ▲) in Experiment AA2.

On the basis of the universally held premise that the most limiting amino acid will be conserved at maximum metabolic efficiency (Munro, 1969, 1976; Harper et al., 1970; Clemens and Pain, 1976), changes in amino acid retention induced by amino acid enrichment of a diet should provide information on the dietary adequacy of each amino acid and also on the efficacy of the supplemental amino acid source. For the Basal diet, retention of the individual indispensable amino acids was higher than that for N as a whole (32.1%) in the case of Thr (35.8%), Arg (42.3%), Met (53.1%) and Lys (54.1%). Retention of Gly was also high (59.0%) but the dietary essentiality of this amino acid is not well defined. Gly is generally, but not unequivocally, thought to be an indispensable amino acid for birds (Calet, 1976; Taylor et al., 1994; ARC, 1995). It has been shown in rats to have beneficial effects against endotoxins (Grimble et al., 1992; Ikejima et al., 1996) and there is some evidence of its dietary importance for human infants (Plath et al., 1996).

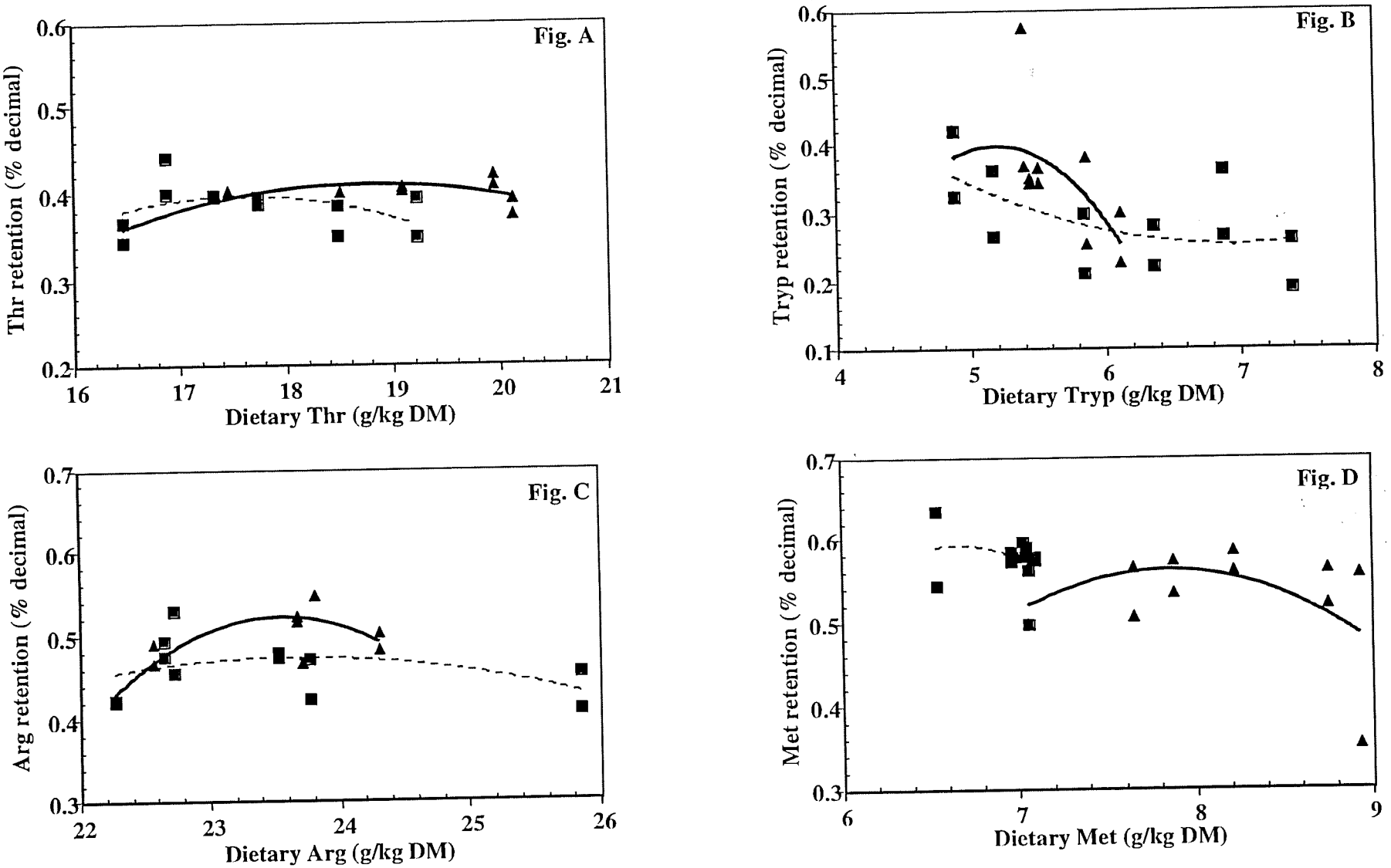

Fig. 4 Quadratic regressions between dietary threonine (Thr, Fig. A) and tryptophan (Tryp, Fig. B), arginine (Arg, Fig. C) and methionine (Met, Fig. D) content and amino acid retention for fish fed diets enriched by crystalline amino acid supplementation (■) or intact protein manipulation (▲) in Experiment AA2.

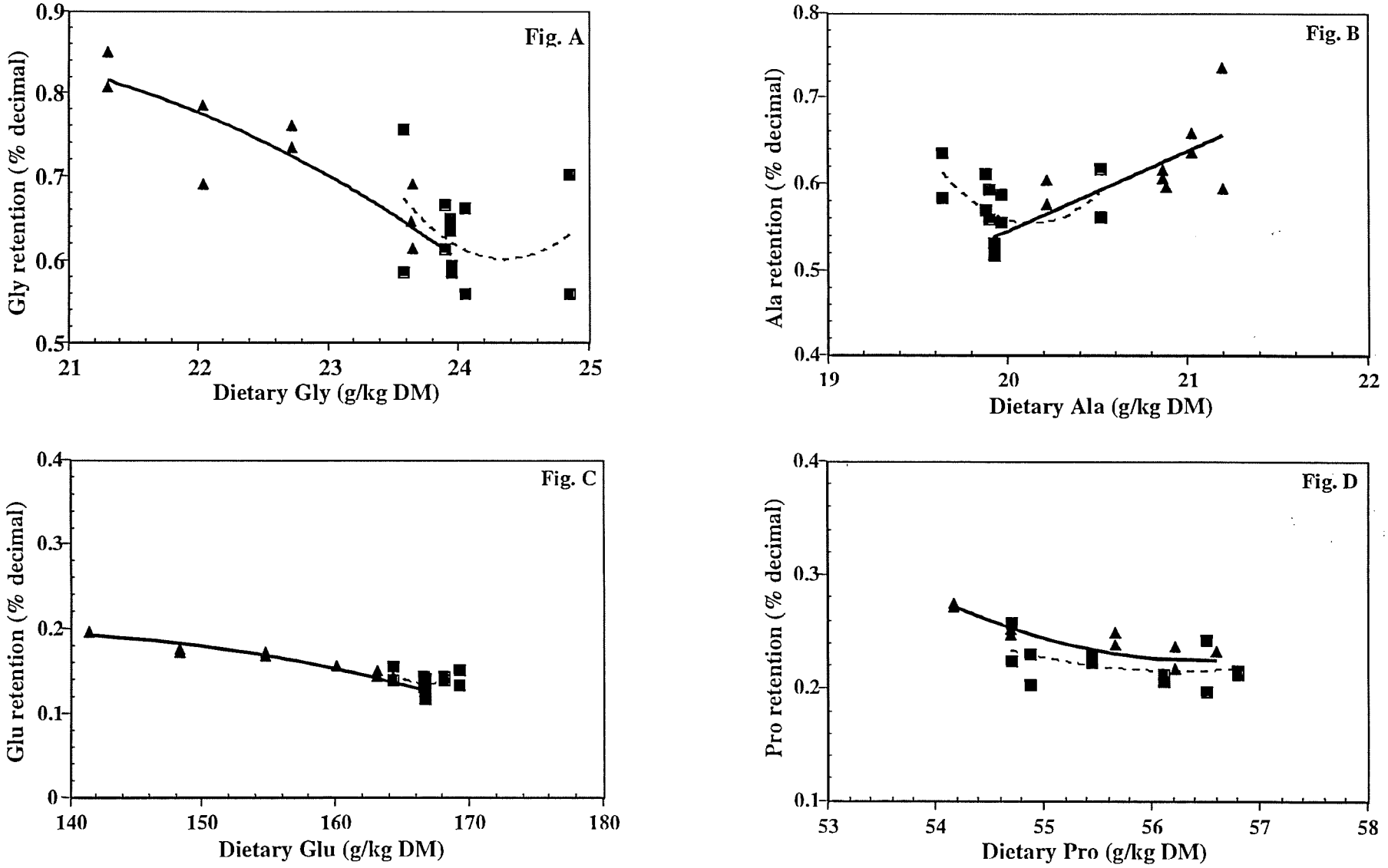

Fig. 5 Quadratic regressions between dietary glycine (Gly, Fig. A), alanine (Ala, Fig. B), glutamic acid (Glu, Fig. C) and proline (Pro, Fig. D) content and amino acid retention for fish fed diets enriched by crystalline amino acid supplementation (■) or intact protein manipulation (▲) in Experiment AA2.

Although Gly is the simplest of all amino acids, its metabolic pathways in eukaryotic cells are fairly unique, being formed by reversible cleavage between the α and β carbon atoms of serine and as the end product of de-methylation of betaine; it also can be synthesised from Thr (Cantarow and Schepartz, 1957). One of the primary functions of Gly is as a precursor of choline and in turn, is involved in the formation of betaine. Indirectly because of betaine role as a methyl-donor, Gly can have a sparing effect on Met. Thus, the essentiality or otherwise of Gly, at least in birds, is dependent on dietary supply of Met and Thr (Calet, 1976; ARC, 1995). Even though the dietary essentiality of Gly for fish is unknown, a similar dependency on the dietary supply of Met and Thr would not be unexpected.

For the above reasons, the amino acids of the Basal diet that are most likely to be rate-limiting protein synthesis are Gly, Lys, Met, Thr and Arg. However, some caution needs to be exercised in assuming that the amino acid with the highest retention is necessarily the most limiting amino acid. The metabolic efficiency of amino acid retention is likely to vary between individual amino acids, being dependent on the particular metabolic pathways involved, and particular care is warranted in the case of Gly where its essentiality is very much in question. A more useful criterion is to examine the change in amino acid retention following amino acid enrichment of the Basal diet.

As evident from Table 13, addition to the Basal diet of the lowest amount of crystalline Arg, Lys and Tryp supplementation resulted in a significantly ($P<0.05$) increased retention of Arg (42.3 to 49.3%), Cysh (13.5 to 17.1%) Leu (24.9 to 30.3), Lys (54.1 to 59.2%), Phe (21.5 to 26.1%), Tyr (23.9 to 30.0%), and Val (27.4 to 32.4%); although not significant, a similar effect occurred for Gly (59.0 to 67.2%), Thr (35.8 to 42.2%) and Met (53.1 to 58.5%). In the case of Tryp, there was a tendency for its retention to decline (37.4 to 31.5%). Further C-AA supplementation of the Basal diet brought about little if any change in amino acid retention for most of the amino acids. The exceptions were Lys, Arg and Gly where retentions progressively decreased, and Tryp where the response was variable but tended also to decline progressively with increased supplementation. These responses suggest that the Basal diet was most deficient for Lys and Arg and possibly also Gly but not Tryp. However, Met was likely to be the next most limiting amino acid as can be deduced from its high and maintained conservation at all rates of C-AA supplementation. Conservation of Cysh also increased with addition of the C-AAs which adds support to the contention that sulphur amino acids were next most limiting in the Basal diet after Lys and/or Arg and/or Gly. This would also explain why there was very little improvement in fish growth beyond the first level of C-AA supplementation (Table 10, Fig. 2).

Amino acid enrichment of the Basal diet by intact protein manipulation resulted in amino acid retention effects fairly similar to those seen for C-AA supplementation. Low enrichment resulted in significantly higher ($P<0.05$) retentions of Arg, Cysh, Leu, Tryp, Tyr and Val. As for the C-AA series, retentions of Tryp progressively declined with increasing amino acid enrichment. One noticeable difference was that retention of Lys did not initially increase but instead, decreased progressively with increasing amino acid enrichment. Two other differences are worthy of comment. Firstly, retention of Gly increased with progressive amino acid enrichment and attained a retention of 82.9% at the highest rate of intact protein manipulation. It should be noted however, that the dietary content of Gly declined as intact protein manipulation increased. Secondly, the retention of Cysh progressively increased ($P<0.01$) with increasing intact protein manipulation (Tables 13 and 16). Although the Met response was not significant ($P>0.05$), there was a strong tendency for the retention of this amino acid to increase

up to the third highest manipulation (Diet HP3) whereafter its retention declined with further intact-protein manipulation (Table 13, Fig. 4D). These responses are interpreted as indicating that in the intact protein manipulation diet series, Arg and the sulphur amino acids (Met and Cysh) were likely to be the most limiting amino acids and perhaps, even more limiting than either Lys or Tryp. Moreover, as the metabolic capacity of the fish to conserve Gly is quite high as demonstrated for the intact-protein manipulation diet series, it implies that a dietary deficiency of Gly was unlikely to have limited the benefit of the supplemental C-AAs in that diet series. Thus, the lower productivity of the fish given the C-AA supplemented diets as compared to those fed the intact protein manipulated diets may have been due to a dietary insufficiency of sulphur amino acids. However, the total dietary content of Met plus Cysh was almost identical in each of the amino acid enrichment series (mean analyses of 13.2 and 13.5 g/kg DM for crystalline AA and intact protein series, respectively; Table 7) and lower than that of the control diet (14.2 g/kg DM) where fish performance was no better and compared to the intact protein series, significantly worse ($P<0.05$) in the case of FCR (Table 10; Fig. 2). Thus, differences in fish productivity between the two forms of amino acid enrichment in Experiment AA2 are concluded as being due either to the C-AAs being utilised less effectively than those provided in protein-bound form or that the overall amino acid supply from the intact protein series was more optimal for barramundi growth and development than those provided by the C-AA series.

6.2.3.2 Experiment AA3

The amino acid adequacy of the protein (g/16gN) of the basal and highest amino acid-enriched diets is detailed in Table 17 and compared with requirement values recommended for channel catfish and Japanese eel. This comparison shows that the basal diet was markedly deficient in Lys and Arg for channel catfish and additionally low to marginally limiting in Thr and Ile for Japanese eel. At the highest rate of supplementation, the content of Lys and Arg was sub-optimal to the requirement value recommended for channel catfish and Japanese eel in both diet series and also sub-optimal with respect to Ile, Thr, Val and Met plus Cysh for Japanese eel in one or both of the diet series.

Table 17 The essential amino acid content of the protein of selected diets in Experiment AA3 compared to recommended requirement values for channel catfish and Japanese eel

Amino acid	Experimental diets[1]			Recommended requirement	
	Basal	LC5	LP5	Channel catfish[2]	Japanese eel[3]
			g/16 g N		
Arginine	3.64	3.80	3.77	4.3	4.5
Histidine	2.48	2.40	2.72	1.52	2.1
Isoleucine	3.07	3.28	3.49	2.59	4.0
Leucine	6.72	6.50	7.46	4.0	5.3
Lysine	3.07	4.88	4.61	5.1	5.3
Meth + cystine	3.12	3.03	3.15	2.32	3.2
Phe + tyrosine	7.59	7.33	8.34	5.0	5.8
Threonine	2.76	3.18	3.23	2.21	4.0
Tryptophan	1.21	1.43	1.32	0.5	1.1
Valine	3.95	3.83	4.66	2.98	4.0

[1] Refer to Table 5 for full description of diets.

[2] Mean of values reported by Tacon and Cowey (1985) and Wilson (1994).

[3] Reported by Wilson (1994).

The experiment progressed well until the forty first day when one of the four independent recirculation systems (B system) collapsed, resulting in a pronounced bacterial bloom and deterioration in the quality of water in that system. Despite three water exchanges and the withholding of food on day 41 for fish in the B system, a large number of the fish appeared stressed and it was decided to prematurely weigh and terminate the fish in that system. All of the fish originally placed in the B system were present and alive at the final weighing on day 41. During the course of the experiment, one fish from each of three tanks (2 in System D and 1 in System C, and each from different dietary treatments) were removed at the 28 d weighing because they had either lost or made negligible weight gain since placement on experiment. The removed fish showed no obvious abnormality and it was assumed that dominance by other fish was the reason for their poor growth. Fish in Systems A, C and D were terminated on the due date after 42 days experimentation. During the final weigh and measure of fish in the D system, the weight data of five of the tanks were lost due to a failure of the electronic weight recorder. Because of these problems, it was decided to dispense with the analysis of the chemical composition of the fish and to confine the treatment comparisons to growth productivity responses alone.

The productivity response data were analysed in two periods, from 0 to 4 weeks and from 0 to 6 weeks, to see which was the most robust before further statistical analyses were conducted.. Responses to dietary treatments for these two periods are shown in Table 18. Despite the loss of some of the final weight data from System D and the collapse on day 41 of System B, the response trends to dietary treatments were very similar for each period but the standard errors were appreciably smaller for the 0 to 6-week period. Accordingly, the 0 to 6-week data set was subsequently used to examine the efficacy of the alternative supplementary amino acid sources.

Table 18 Productivity of fish during periods 0 to 4-weeks and 0 to 6-weeks when fed low protein diets in Experiment AA3

Treat/ diet	Productivity trait							
	Daily gain[1] (g/d)		Specific growth[1] (%/d)		Food intake[1] (g/d)		FCR (As-fed g :g gain)	
	0-4 Wk	0-6 Wk	0-4 Wk	0-6 Wk	0-4 Wk	0-6 Wk	0-4 Wk	0-6 Wk
Basal	1.66^{DE}	1.61^{E}	0.60^{DE}	0.56^{D}	3.50	3.55	2.13^{DEF}	2.23^{E}
LC1	1.85^{BCD}	1.91^{CD}	0.67^{BCD}	0.66^{BCD}	3.60	3.63	1.95^{BCDE}	1.91^{BCD}
LC2	1.74^{CDE}	1.86^{CDE}	0.63^{CDE}	0.65^{CDE}	3.56	3.60	2.07^{CDEF}	1.95^{CD}
LC3	1.96^{BCD}	2.11^{BC}	0.70^{BCD}	0.72^{BC}	3.61	3.64	1.84^{BCD}	1.73^{BC}
LC4	1.90^{BCD}	2.12^{BC}	0.68^{BCD}	0.72^{BC}	3.61	3.65	1.91^{BCDE}	1.72^{BC}
LC5	2.10^{B}	2.19^{B}	0.75^{B}	0.74^{B}	3.59	3.64	1.74^{B}	1.68^{B}
LP1	1.72^{CDE}	1.78^{DE}	0.62^{CDE}	0.62^{CD}	3.51	3.60	2.15^{EF}	2.05^{DE}
LP2	1.54^{E}	1.78^{DE}	0.56^{E}	0.62^{CD}	3.45	3.59	2.26^{F}	2.05^{DE}
LP3	1.83^{BCDE}	1.94^{BCD}	0.66^{BCDE}	0.67^{BCD}	3.56	3.60	1.96^{BCDE}	1.86^{BCD}
LP4	1.79^{CDE}	1.95^{BCD}	0.65^{BCDE}	0.67^{BCD}	3.55	3.58	2.01^{BCDEF}	1.84^{BCD}
LP5	1.96^{BC}	2.04^{BCD}	0.71^{BC}	0.70^{BCD}	3.59	3.61	1.83^{BC}	1.76^{BC}
Ctl	2.98^{A}	3.04^{A}	1.02^{A}	0.98^{A}	3.64	3.69	1.22^{A}	1.22^{A}
± sem	0.106	0.099	0.036	0.018	0.050	0.037	0.059	0.090

[1] Covariance adjusted to remove effects of differences in start weight.

[A,B,C,D,E] Within productivity trait comparisons, means without a common letter differ (P<0.05).

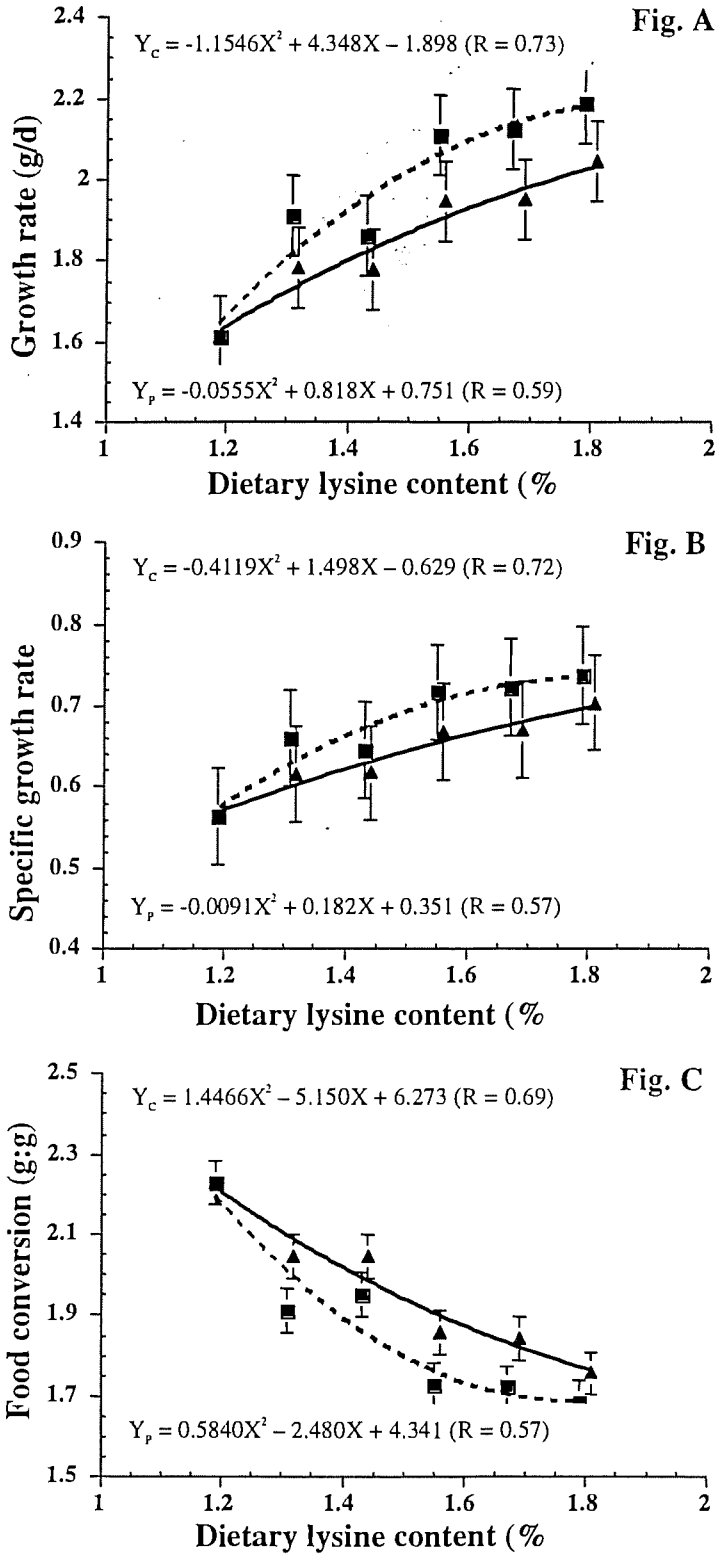

Fig. 6 **Relationship between dietary lysine content and average daily gain (Fig A), specific growth rate (Fig B) and food conversion (Fig C) responses of fish to supplementation with either crystalline amino acids (■) or intact protein manipulation (▲) in Experiment AA3.**

Growth rate of the fish was markedly better ($P<0.05$) on the control diet compared to all other diets and this was due to the food being used more efficiently for production as daily food intake was similar for all diets. Other than for daily food intake where dietary treatment was without effect, supplementation of the basal diet resulted in a significant ($P<0.05$) quadratic improvement in productivity for both the C-AA and intact protein manipulated diet series. Since the dietary contents of each amino acid in the intact protein series and for each of the added C-AAs in the crystalline series were co-correlated within the respective diet series because of the imposed incremental supplementation, only relationships based on dietary Lys content – the amino acid considered to be the most limiting in the diet – are described. For each of the examined response traits, the slope and intercept statistics of the derived regressions did not differ significantly ($P>0.05$) between the diet series and are depicted in Figure 6. Although the derived response relationships did not differ significantly between diet series, the response pattern for each trait was consistently better for the C-AA supplementation. However, in the absence of statistical significance between the two diet series, the data can only be interpreted as indicating that the supplementary amino acids were used with equal efficacy irrespective of whether supplied in crystalline or protein-bound form.

6.2.4 Integrating discussion and conclusions

The primary objective of the experiments reported in this Section was to determine if free (crystalline) amino acids were used equally as efficiently as those consumed as protein-bound in meeting the dietary amino acid requirements of juvenile barramundi. The chosen approach was to develop a basal diet expected to be markedly deficient in one or more essential amino acids and then to examine the efficacy of amino acid enrichment using either serial additions of C-AAs or serial manipulation of two protein meals of identical protein content but which differed markedly in amino acid profile. To ensure that energy supply did not confound the interpretation of the results, the diets were held both isocaloric and isonitrogenous within experiments and were fed to satiety as a single daily meal. In the expectation that absolute amino acid supply might be as important as amino acid balance of the protein in determining the amino acid adequacy of the diet, experiments were carried out using diets that were either moderately high (viz 540 g/kg DM) or low (viz 390 g/kg DM) in protein content.

Inherent in the adopted approach were assumptions that the basal diet was deficient in one or more of the essential amino acids and that the enrichment process, using C-AAs or by protein manipulation, enabled these amino acid inadequacies to be corrected. Addition of C-AAs (Lys, Arg, Thr and Tryp) to the high protein basal diet in Experiment AA2 resulted in a curvilinear improvement in growth rate and FCR. However, the response was characterised by almost all of the improvement occurring at the first level of C-AA supplementation with little, if any improvement with subsequent supplementation. In contrast (Fig. 1 and 2), amino acid enrichment by intact protein manipulation resulted in a sequential and more enhanced improvement in fish productivity. For these response differences to be interpreted as being due to efficacy differences between crystalline and protein-bound amino acids, it must be assumed that the protein-AA enrichment did not additionally provide secondary- or tertiary-limiting amino acids of the basal diet that were not also provided in the C-AAs diet series. Inspection of the individual amino acid retention efficiencies of the fish (Table 13) provided supporting evidence that the basal diet was most deficient in Lys and that Met and perhaps Gly were the next most limiting. Since neither Met nor Gly was added as supplements to the C-AA diet series, it must be questioned whether a deficiency of these amino acids acted to curtail the responsiveness of the C-AAs. However, this is thought unlikely for two reasons. Firstly, the dietary Met plus Cysh content of both the crystalline and protein-bound series was almost

identical (13.2 vs 13.4 g/kg DM, respectively) and thus any dietary sulphur amino acid deficiency would impact similarly on each of the diet series. Secondly, the Gly content of the protein-bound diet series declined with increasing protein manipulation and thus any dietary Gly deficiency would have more disadvantaged the protein-bound rather than the C-AA diet series. For the above reasons, the observed differences in fish productivity between the crystalline and protein-AA diet series in Experiment AA2 are attributed to differences in efficacy between the two amino acid forms. Depending on the mathematical model chosen (Fig. 2), the efficacy of the C-AAs was only from 54 to 70% of that of the protein-AAs.

When the protein content of the diet was low as in Experiment AA3, amino acid enrichment was equally efficacious whether provided as crystalline or protein-AAs (Table 18; Fig. 6). This contrasted markedly with what was observed in the higher protein diets of Experiment AA2 where C-AAs appeared to be used with lower efficacy than those provided in protein-bound form. It was unfortunate that nutrient retention data were not obtained in Experiment AA3 as this may have provided some further insight into the amino acid adequacy of the diet and perhaps suggest a reason for the difference in fish productivity response between the two experiments. Nonetheless, some interesting conclusions can be drawn from a detailed examination of the results of the two experiments. The efficiency of food conversion by the fish fed the control diet was similar in both experiments (FCR of 1.09 vs 1.22 in Experiments AA2 and AA3, respectively) and any difference as such was likely to be due to the respective differences in the size of the fish (start weights of 75 vs 252 g, respectively). In contrast, the productivity of the respective basal diets differed markedly (FCR of 1.15 vs 2.23 for Experiments AA2 and AA3, respectively) although in each experiment the performance of the fish on the basal diet was significantly ($P<0.05$) inferior to that of the control diet. Moreover, the improvement in FCR brought about by amino acid enrichment of the basal diet was comparatively small in the case of Experiment AA2 as compared to that observed in Experiment AA3 (11 vs 25%, respectively) and this was particularly the case with C-AA supplementation where the improvement was even smaller with the high protein (AA2) compared to the low protein (AA3) diets (7 vs 25%, respectively). Because the response to the control diet in each experiment was comparatively similar, the differences between experiments in the magnitude of the response to amino acid enrichment is interpreted as an effect of differences in absolute rather than relative deficiencies of dietary amino acid supply. Thus, in considering the amino acid adequacy of the diet, as much attention must be given to the absolute amount of the amino acid (ie intake x percentage content) supplied as to the amino acid composition of the protein (ie g/16 g N) as a whole. As the protein content of the diet that is fed to the fish is reduced, the amino acid composition of the protein will assume increasing importance in determining the amino acid adequacy of the diet. Conversely, as the protein content of the diet is increased, such that an increasing amount of the protein is used for energy rather than as a source of amino acids for protein synthesis, the amino acid composition of the dietary protein will assume lesser importance in determining the amino acid adequacy of the diet.

Although the aim of Experiments AA2 and AA3 was to test differences in efficacy between crystalline and protein-AAs, the data enable certain inferences to be drawn about the dietary essential amino acid requirements of barramundi. Firstly, the improvement in fish productivity brought about by amino acid enrichment of the basal diet was remarkably small. If it is assumed that Lys was the most limiting essential amino acid in the basal diet and that casein was a highly available source of Lys, a 60% increase in Lys concentration of the basal diet in Experiment AA2 (from 1.87 to 2.92% of diet or from 3.42 to 5.43% of protein) resulted in only a 6 % improvement in growth rate and an 11% improvement in food conversion (Fig. 2). This

implies that the essential amino acid content of the diet can vary quite considerably with only minimal effects on fish productivity. Inspection of Fig. 2 shows that there was virtually no benefit to amino acid enrichment beyond diet HP3 in the intact protein manipulation series. If Lys was the most limiting amino acid, it is reasonable to suggest that the Lys needs of juvenile barramundi in the experiment were satisfied with a diet containing 2.67% Lys (DM). In the absence of any better estimate for essential amino acid requirements, the amino acid balance of the animal's whole body protein can be accepted to be a reasonable guide of the requirement and especially in the case of the young animal where requirements for somatic growth would far outweigh those for maintenance (Wilson, 1994). Assuming a dietary Lys content of 2.67% (DM) is adequate for juvenile barramundi, dietary specifications for other essential amino acids can be estimated as detailed in Table 19.

Table 19 Recommended dietary essential amino acid specification for juvenile barramundi

Essential amino acid	Barramundi whole body composition[1]		Recommended diet specification (%, DM)
	% of protein (g/16g N)	Amino acid balance (Lys = 100%)	
Arginine	5.47	86	2.29
Histidine	1.65	26	0.69
Isoleucine	3.22	50	1.33
Leucine	5.87	92	2.46
Lysine	**6.39**	**100**	**2.67**
Methionine	2.27	36	0.96
Methionine + cystine	2.86	45	1.20
Phenylalanine	3.39	53	1.42
Phenylalanine + tyrosine	5.92	93	2.49
Threonine	3.65	57	1.52
Tryptophan	0.94	15	0.40
Valine	3.71	58	1.55

[1] From Table 12.

Conclusions drawn from these amino acid studies are summarised as:

- The response to amino acid enrichment was relatively more marked at low compared to high dietary protein. And at low dietary protein, C-AAs were equally as effective as protein-AAs. However, efficacy of C-AAs may be inferior to that of protein-AA when fish are provided with high protein diets and absolute amino acid intake is high. This effect may be due as much to altered efficiency of energy metabolism as to amino acid supply *per se.*
- The importance of the essential amino acid balance of the dietary protein as a factor influencing fish productivity increases inversely with absolute dietary protein (amino acid) intake. 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-AA was a more effective supplement than C-AA. Further, when the amino acid quality of the dietary protein was the same, the absolute productivity of the fish was much better for high compared to low protein diets which illustrates the extent to which dietary protein is used for meeting the animal's energy requirements.

- Substitution of fishmeal by terrestrial protein feeds and the attendant deterioration in the essential amino acid profile of the dietary protein is unlikely to adversely affect fish productivity provided the protein content of the diet is maintained above about 50% (DM) and fish are fed liberally.
- The dietary essential amino acid requirements (% DM) of juvenile barramundi were estimated as: Arg, 2.29; His, 0.69; Ile, 1.33; Leu, 2.46; Lys, 2.67; Met + Cysh, 1.20; Phe + Tyr, 2.49; Thr, 1.52; Try, 0.40; and Val, 1.55.

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6.3 *Potential of meat meal to replace fishmeal investigations*

6.3.1 *Introduction*

Global production of cultured carnivorous fish is predicted to increase from 1.5 million tonnes (mt) in 1992 to 2.2 mt by Year 2000, an increase of 46% (Csavas, 1994). One third of the current production comes from Asia. The main species of fish cultured are salmonids, eels and European sea bass in temperate regions and barramundi, grouper and redfishes in more tropical regions. Being carnivorous, these fish are reared on trash fish (in developing countries) or high protein (40 to 45%) artificial dry diets (in Australia) in which fishmeal is the sole or predominant source of protein. However, supplies of trash fish and fishmeal are becoming less plentiful as demand from aquaculture increases while wild fishery catches are either at their maximum sustainable yields or yields are already declining (New and Wijkstrom, 1990; New, 1991; Tacon, 1996). FAO estimates of the aquafeed industry's requirements for fishmeal for the next decade are 1.5 to 2.2 mt accounting for 25 to 30% of total fishmeal supplies (Tacon 1996). If aquaculture is to become a net contributor to human food supplies, cost-effective alternatives to fishmeal must be found. For carnivorous fish, meat by-product meals appear to have the greatest potential as cost-effective replacements for fishmeal.

In Australia, barramundi farming commenced in 1986 with production rapidly increasing to ~400 t in 1994 and projected by QDPI (J Gillespie, pers comm) to reach 1,000 t by Year 2000 and 2,000 t by Year 2005. In Australia as elsewhere in the world, major impediments to profitable fish farming are the high cost of the diet (food comprises up to 60% of on-farm operating costs) and the dependence of the industry on imported fishmeal. Successful replacement of fishmeal by meat by-product meals would ensure continued profitability of the Australian industry and provide the meat industry with a valuable alternative outlet, both in Australia and as export to supply the Asian aquafeed market which is estimated to exceed 2.6 mt annually (Akiyama, 1993).

Aquarium studies with barramundi undertaken as part of FRDC's Replacement of Fishmeal Subprogram have shown meat meal to be highly digestible and equal to that of fishmeal and diets with high inclusions of meat meal (up to 70% content) have been very well accepted by the fish (Williams and Barlow, 1996). The work demonstrated the potential of meat meal to replace a large proportion of the fishmeal currently included in diets formulated for barramundi.

The aim of the experiments reported in this Section was to demonstrate the potential of meat meal to replace fish meal in diets for grow-out barramundi under commercial conditions.

6.3.2 *Material and methods*

6.3.2.1 Experiments and fish management

Two on-farm experiments (Expt MRC1 and MRC2) were carried out to compare the growth performance and taste characteristics of juvenile barramundi fed either a commercial barramundi diet or experimental diets containing varying proportions of fish meal and meat meal. Both experiments were carried out on the property of Mark Fantin (Sugarland Barramundi, Edmonton) with fish held in cages suspended in an aerated freshwater pond. The

experimental fish were managed in the same way as for other fish on the farm, being fed to satiety once daily except on the weekend when fish were fed only on one of the days. Water quality measurements (min and max water temperature, turbidity, dissolved oxygen and pH) were taken regularly throughout the experiment. A third growth assay (Expt MRC3) was carried out in the laboratory using the same diets as fed in Expt MRC2 to validate the results of the second on-farm experiment.

In each of the on-farm studies, four diets were evaluated using a 4x4-randomised block design. The same experimental control diet (Ctl) containing a high content of fish meal and a commercial barramundi grow-out diet manufactured by Ridley Agriproducts, Narangba (Com1 or Com2 - different consignments for Expt MRC1 or Expts MRC2 and MRC3, respectively) were used in both experiments.

In Expt MRC1, the aim was to compare two sources of meat meal - a conventional 52% crude protein product procured from the Casino Abattoir, (Cas) and a low ash, 60% crude protein product procured from MIDCO, Macksville (Mco) - when each was used as the major source of protein in the diet. The inclusion content of fishmeal was held constant at 10% to ensure high palatability of the diets and to preserve a desirable flavour in the produced fish. The three experimental diets were formulated to be isoenergetic and isonitrogenous. The formulation and chemical composition of the diets are detailed in Table 1. For the experiment, approximately 8,000 fish were size graded into 16 groups of 400 fish with each group then being placed into a 2 m^2 cage to which diets were randomly assigned within positional banks of cages (blocks). The starting weight (± SD) of the fish was 280 ±13.4 g. The experiment commenced on 10 November 1995 and continued for 66 days until 15 January 1996. At the end of the experiment, fish in each cage were bulk weighed and six fish taken at random from each cage for determination of dressing-out percentage and subsequent sensory evaluation.

In Expt MRC2, the primary aim was to examine the response of juvenile barramundi to diets containing no fishmeal and when most of the protein was supplied as Cas meat meal. A secondary aim was to examine the effect of increasing the estimated digestible energy (DE) content of the diet from 15 to 16 kJ/g while holding the protein to DE specification to ~29 mg/kJ. Formulation and chemical composition of the diets are given in Table 20. Approximately 8,000 fish were size graded into 16 groups of 400 fish and randomly allocated as for Experiment MRC1. The fish commenced experiment at an initial weight of 226 ±16.3 g on 29 March 1996. At the end of the 66 day experiment on 3 June 1996, fish were bulked weighed, and sampled for dressing-out and sensory evaluation as for Expt MRC1.

Expt MRC3 was carried out at QDPI's Freshwater Fisheries and Aquaculture Centre, Walkamin with fish held in freshwater aquaria situated within an environment-controlled laboratory. Aquaria were arranged as two independent sets of recirculation systems, each consisting of an up-flowing biological filter (120 L of fine gravel), reservoir (2,000 L) and 12 replicate fibreglass tanks (180 L; 0.3m^2 surface area). Flows through the system were maintained using air lifts and pumps, with turn-over rate in the tanks being once every 0.5 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 28°C; 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. From a stock group of approximately 400 fish, 192 fish were selected on size/weight uniformity to form six blocks each of 32 fish. Within blocks, fish were distributed equally to 4 aquaria (8 fish/aquaria) with diet treatments being randomly assigned within blocks. Three complete blocks were assigned to each of the two recirculation systems. Following an acclimatisation period of two weeks during which a prophylactic salt bath (1.2% NaCl for 2 h) against ectoparasites was administered, fish were individually weighed and commenced experiment (initial weight 223.0 ± 25.1g) on 19 September 1996. Fish in each aquarium were bulk-weighed fortnightly thereafter and individually at the last weighing on 30 October 1996 (after six weeks). Stress at weighing was minimised by light sedation of the fish using the aquatic anaesthetic 2-phenoxyethanol (200 ppm). A prophylactic salt bath against ectoparasites was carried out on the same day of weighing. Fish were offered their respective diets to satiety once daily except on day of weighing when no food was fed. At each feeding, a weighed amount of food was offered to excess on 3 to 4 occasions during a feeding period of 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 for each diet.

Table 20 Formulation and nutrient composition of the diets fed in Experiments MRC1, MRC2 and MRC3

Attribute	Diet description and formulation						
	Ctl	M1	M2	M3	M4	Com1[1]	Com2[1]
Expt MRC	1, 2 & 3	1	1	2 & 3	2 & 3	1	2 & 3
	Formulation (%)						
Wheat	30.4	18.1	29.9	16.1	10.4		
Fish meal (Chile)	35.0	10.0	10.0	0	0		
Meat meal (Cas)	0	45.0	0	50.0	50.0		
Meat meal (Mco)	10.0	0	40.0	0	0		
Blood meal (ring)	0	0	0	7.0	9.0		
Soybean (full-fat)	16.0	16.0	5.0	15.0	10.0		
Soybean (solvent)	0	0	5.0	0	0		
Gluten	5.0	5.0	5.0	5.0	10.0		
l-lysine HCl	0	0.6	0.6	0.65	0.7.5		
d/l Methionine	0.15	0.3	0.3	0.3	0.35		
Fish oil (Chile)	2.5	4.0	3.25	5.0	6.0		
Tallow	0	0	0	0	2.5		
Salt	0.25	0.25	0.25	0.25	0.25		
Choline	0.1	0.1	0.1	0.1	0.1		
Vit C (coated)	0.125	0.125	0.125	0.125	0.125		
Vitamin premix	0.25	0.25	0.25	0.25	0.25		
Ca Proprinate	0.25	0.25	0.25	0.25	0.25		
Ingredient cost ($/t)[1]	884	650	1129	621	678	---	---
	Nutrient analysis (air-dry basis)						
Gross energy (kJ/g)	19.4	19.2	20.3	19.0	20.1	20.0	20.3
Est DE (kJ/g)[2]	15.0	15.0	15.2	15.0	16.2	15.0	15.0
CP (g/kg)	438	430	430	425	478	543	501
Fat (g/kg)	94	127	126	153	128	69	96
Ash (g/kg)	95	149	95	146	141	93	76
Calcium (g/kg)	24	47	26	46	44	21	19
Phosphorus (g/kg)	15	25	16	20	20	14	10
Arginine (g/kg)	25.9	28.8	27.3	23.2	25.0	29.7	25.5
Histidine (g/kg)	11.7	8.5	8.6	11.1	13.5	21.2	17.9
Isoleucine (g/kg)	16.6	15.1	15.7	10.3	12.4	18.6	16.3
Leucine (g/kg)	32.4	28.1	29.8	31.6	36.0	47.2	42.7
Lysine (g/kg)	28.3	26.7	27.4	27.7	31.6	46.1	41.1
Meth + Cyst (g/kg)	10.7	8.9	9.2	10.1	11.7	10.8	13.5
P+T (g/kg)	30.6	27.3	29.1	28.5	32.5	42.7	37.9
Threonine (g/kg)	17.0	14.7	15.6	14.5	16.5	23.9	21.5
Tryptophan (g/kg)	4.2	3.5	4.0	5.2	5.0	5.2	7.7
C18:2 n-6 (g/kg)	17.3	17.4	11.9	13.5	11.2	4.7	5.0
C20:5 n-3 (g/kg)	5.0	5.1	4.7	9.9	6.3	5.0	10.8
C22:6 n-3 (g/kg)	8.4	7.5	7.2	5.0	4.0	9.1	10.5

[1] Two batches of extruded barramundi grower diet manufactured by Ridley Agriproducts Pty Ltd. The composition and ingredient cost of the diets are commercial-in-confidence.

[2] Estimated digestible energy (DE) values based on either derived digestibility of similar protein concentrate feed ingredients or assumed digestibility for non-protein feed ingredients. Values for the Com diets are those stated by the manufacturer (P. Krogh - pers comm).

6.3.2.2 Dressing-out and sensory evaluation of fish

Dressing-out percentage of fish in Expts MRC1 and MRC2 was determined as the weight proportion of the fish remaining after evisceration and removal of gills. Fish were stunned by immersion in ice slurry before weighing and dressing out.

Pending sensory evaluation at QDPI's Centre of Food Technology, Brisbane, fish were weight ranked, heaviest to lightest, within each diet treatment according to cage and held at -18°C. 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 20 g) were cut from the central portion of each fillet. These samples were placed in individual foil dishes, covered with a foil lid and held at 5°C until 1 h prior to cooking when they were allowed to equilibrate at room temperature. Cooking was achieved by placing the foil dishes on trays in a fan forced electric oven at 200°C for six minutes. After cooking, samples were held in a holding oven at 75°C for up to 30 minutes prior to tasting.

A panel of experienced tasters (13 males and 3 females for Expt MRC1 samples and 8 males and 2 females for Expt MRC2 samples) assessed four samples (one from each dietary treatment) at each of 4 or 3 sessions respectively, using a standard rating test (SAA, 1988). Order of tasting of treatments was balanced across the panel. Samples were served to tasters in individual booths illuminated with white light (daylight equivalent). Purified water was freely available for palate cleansing prior to and during tasting. Tasters identified and rated colour of internal flesh, and odour, flavour and texture characteristics on unstructured graphic line scales. Overall acceptability of the flesh was also rated, and tasters were given the opportunity to record additional descriptors and add any general comments about the samples.

6.3.2.3 Diet manufacture and storage

All diets were manufactured to formulation by Ridley Agriproducts Pty Ltd at its Narangba, Queensland Aquafeeds feedmill. The experimental diets were prepared as 1 t batches and extruded using a single-screw, interrupted-flight, eight inch Anderson extruder. Extrusion conditions were set to produce a 6 mm diameter semi-float pellet. The two Ridley commercial diets (Com1 and Com2) were sourced from production runs shortly before the start of each on-farm experiment. All diets were held at -15°C after being received with quantities being transferred as required to an on-site refrigerated cool room (4°C) for subsequent feeding.

6.3.2.4 Chemical and statistical analyses

Water samples were analysed for dissolved oxygen and pH using a T.P.S. E90 water Checker and ammonia N and nitrite by colorimetric procedures using a Hach CEL/700 Portable Laboratory test kit. Turbidity of farm water was measured using a Secchi disc.

Nutrient and energy analyses were done on representative samples of the diets using Association of Official Analytical Chemists (AOAC, 1990) methods except as otherwise specified. Dry matter was determined by oven drying at 105°C to constant weight, ash by ignition at 600°C for 2 h, crude fat by soxhlet extraction with petroleum ether (bp 40-60°C) and N by a macro-Kjeldahl technique on a Kjel Foss automatic analyser using mercury in the digestion (crude protein was calculated as N x 6.25). 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). A hydrochloric acid extract of the ash was used to determine Ca by absorption spectroscopy, and P by colorimetric procedures (AOAC, 1990). Gross energy was determined by isothermal bomb calorimetry 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; Tryp by the

method of Allred and MacDonald (1988) after hydrolysis of samples with 4.2_M NaOH at 110°C. Cysteine and Met were measured as cysteic acid and methionine sulfone respectively, after performic acid oxidation.

Sensory evaluation data were collected directly into computers using an integrated software package (Compusense 5.1, Compusense Inc., Canada) and 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).

Fish response data of each experiment were subjected to an analysis of variance appropriate to the experimental design using prepared statistical programs (Siegel, 1992). Due to inevitable differences in the starting weight of the fish between treatments as a result of acclimatisation or sampling effects, responses were adjusted by covariance analysis to remove any effect of differences in fish start weight. Specific growth rate (SGR; % per day) was calculated from the equation: $SGR = 100 \times (\ln W_f - \ln W_i) / T$; where W is the average weight (g) of the fish at the initial (W_i) and at the final (W_f) weighing, ln is the natural logarithm and T is the number of days between weight measurements. Arcsine transformation was applied to all percent data (Sokal and Rohlf, 1981). In all experiments, the cage or aquaria was considered to be the experimental unit. Comparison of treatment means was done using the protected l.s.d. procedure in which significance testing was applied only where the "F" value in the ANOVA was significant at the five percent level of probability (Snedecor and Cochran, 1967).

6.3.3 Results

6.3.3.1 Experiment MRC1

The pond water was of good quality throughout the course of the experiment with Secchi clarity of >35 cm, pH cycled diurnally between low and highs of 6.3-6.5 and 6.8-8.8 respectively and dissolved oxygen cycled diurnally from >7 mg/L at 1500 h to minimum values of =3 mg/L at 0700 to 0800 h. Concentrations of nitrite (<0.015 mg/L), ammonia N (<0.25 mg/L), phosphate (<0.2 mg/L) and hardness (<50 mg Ca equivalents/L) were low. Apart from normal diurnal variation, water temperature was fairly constant throughout the experiment with minimums and maximums typically in the range respectively of 29-30°C and 31-32°C (Fig. 7).

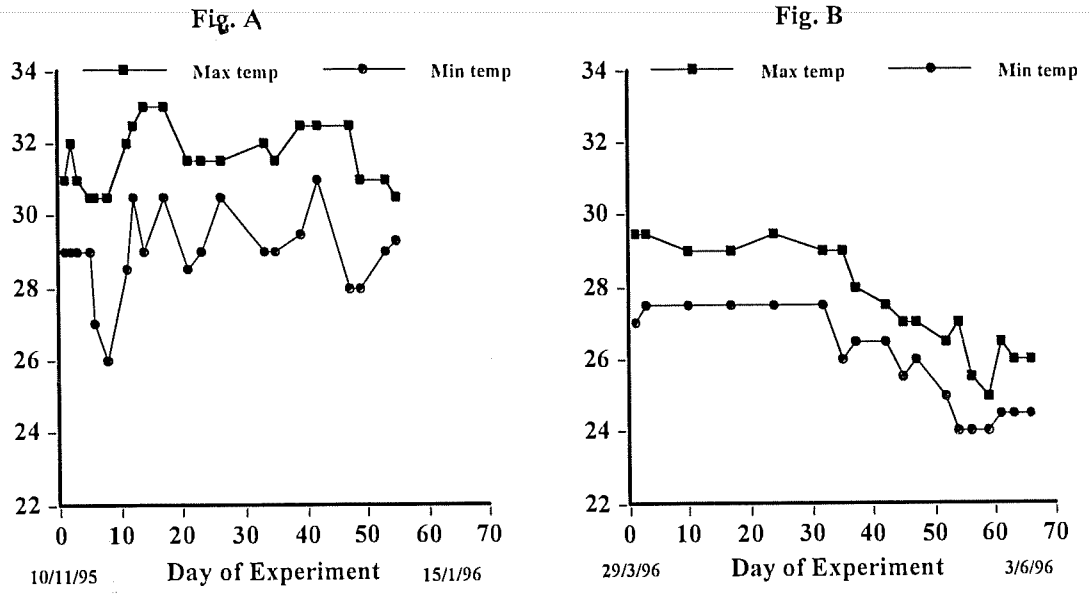

Fig. 7 Monitored minimum and maximum temperatures of the pond water during Expt MRC1 (Fig. A) and Expt MRC2 (Fig. B).

No signs of illness were apparent in the fish during the study with only an occasional mortality being observed. More than 97% of the fish originally placed into the cages were recovered at the end of the experiment with most of these losses occurring as a result of fish escaping during routine net exchange.

There were no significant ($P>0.05$) differences between diets for any of the production responses of the fish although fish on the Com1 diet grew the best and had the most efficient food conversion (Table 21). The statistical power (Searcy-Bernal, 1994) of the experiment was low (20 to 30%) due to an unexpectedly high variability in growth of the fish. The ingredient cost to produce a given weight of fish was lowest for the M1 diet and highest ($P<0.05$) for the M2 diet. Dressing-out percentage averaged 89.6 ±0.49%.

Table 21 Production responses and dressing-out percentage of fish in Expt MRC1

Attribute	Diet				
	Ctl	M1	M2	Com1	± sem
Weight (g)					
Start	275.0	276.8	284.8	282.5	6.72
End	440.6	439.5	459.1	485.8	21.17
Food issued (g/week)	25.0	24.0	25.4	25.9	1.25
Gain (g/week)	18.1	17.6	17.9	21.2	1.75
SGR (%/d)	0.72	0.70	0.72	0.81	0.054
Food conversion (g:g)	1.44	1.43	1.47	1.25	0.070
Fish recovered (Arc °)[1]	76.4	78.2	75.3	79.0	1.96
Dressing-out (Arc °)[1]	63.6	63.8	63.3	63.8	0.32
Food cost ($/kg gain)[2]	1.27[B]	0.93[A]	1.65[C]	---[AB]	0.060

[1] Presented as arcsine-transformed values.

[2] Food cost calculated on basis of prevailing ingredient cost without allowance for processing. Information on the Com1diet is commercial-in-confidence.

[A,B,C] Means without a common superscript letter differ ($P<0.05$).

Sensory evaluation of the fish (Table 22) revealed stronger fishy and sweet flavours for those fed M1 and M2 diets than those fed Com1 diets ($P<0.05$); those fed the Ctl diet were of intermediate intensity. Fish fed the Ctl diet were softer in texture ($P<0.05$) than those fed any of the other diets.

Table 22 Representative data for sensory evaluation of fish from Expt MRC1

Attribute[1]		Diet				
		Ctl	M1	M2	Com1	±sem
Appearance	Greyish	23.8	24.6	25.5	24.3	0.98
	Yellowish	8.6	9.4	8.7	6.9	0.75
Odour	Fishy	34.6	34.5	34.1	33.9	1.66
	Meaty	33.9	34.0	33.9	32.6	1.41
	Muddy	8.3	8.6	9.8	8.1	1.07
Flavour	Fishy	35.2^{AB}	37.3^{A}	36.8^{A}	33.0^{B}	1.30
	Meaty	33.1	31.9	31.6	35.0	1.47
	Sweet	19.2^{B}	21.7^{A}	22.4^{A}	18.1^{B}	1.28
	Muddy	16.1	18.2	14.8	17.7	1.57
Texture	Firm	31.5^{B}	36.1^{A}	35.9^{A}	37.1^{A}	1.71
	Moist	47.4	48.0	48.3	47.0	1.58
	Fibrous	21.1	18.7	18.5	20.5	1.34
Overall liking		58.4	62.1	62.8	59.3	1.23

[1] All scores were scaled from zero (none) to 100 (very).
[A,B] Means without a common superscript letter differ ($P<0.05$).

6.3.3.2 Experiment MRC2

Pond water quality was good during the experiment: Secchi clarity was 40 cm; and pH and dissolved oxygen at 1445 h ranged from 5.8 to 7.3 and 7.4 to 10.6 mg/L, respectively. Maximum and minimum water temperatures fell steadily during the experiment from 30°C to 26°C and 27°C to 24°C, respectively (Fig. 7).

No signs of illness were observed in the fish during the experiment. Net damage to two cages resulted in the escape of a large number of the fish, necessitating production data from these cages being excluded from the analysis. Apart from these mass escapes, fish loss from the cages was low with 98.2% of those initially stocked being present at the end of the experiment.

Production responses of the Expt MRC2 fish are given in Table 23. Fish fed the M4 diet performed the best overall, growing faster ($P<0.05$) than those fed either Ctl or Com2 diets. Fish fed the Ctl diet had the lowest food issued ($P<0.05$) and the best food conversion which was better ($P<0.05$) than either M3 or Com2 diets.

The ingredient cost to produce a given weight of fish for the M3 and M4 diets was lower ($P<0.05$) than for either the Ctl or Com2 diets which did not differ significantly from each other. Dressing-out percentage was unaffected ($P>0.05$) by diet and averaged 89.2 ±0.54%.

Representative data for the sensory evaluation of the fish are shown in Table 24. Differences between diets for all sensory characteristics were not significant ($P>0.05$) with all fish being well liked and with low scores for all undesirable appearance, odour, flavour and texture characteristics.

Table 23 Production responses and dressing-out percentage of fish in Expt MRC2

Attribute	Diet				
	Ctl	M3	M4	Com2	± sem
Weight (g)					
Start	224.4	213.7	232.3	225.2	6.72
End	420.8^{B}	418.1^{B}	449.4^{A}	416.8^{B}	21.17
Food issued (g/week)	25.3^{C}	30.3^{A}	30.3^{A}	27.6^{B}	1.27
Gain (g/week)	20.8^{B}	21.4^{AB}	23.2^{A}	20.3^{B}	1.75
SGR (%/d)	0.96^{B}	0.99^{AB}	1.05^{A}	0.94^{B}	0.054
Food conversion (g:g)	1.22^{A}	1.44^{B}	1.31^{AB}	1.37^{B}	0.070
Fish recovered (Arc °)[1]	82.8^{A}	83.5^{A}	80.5^{A}	80.5^{A}	1.96
Dressing-out (Arc °)[1]	64.0^{A}	62.4^{A}	62.6^{A}	63.3^{A}	0.32
Food cost ($/kg gain)[1]	1.08^{B}	0.89^{A}	0.88^{A}	--- ^{B}	0.038

[1] Presented as arcsine-transformed values.

[2] Food cost calculated on basis of prevailing ingredient cost without allowance for processing. Information on the Com2 diet is commercial-in-confidence.

[A,B,C] Means without a common superscript letter differ ($P<0.05$).

Table 24 Representative data for sensory evaluation of fish from Expt MRC2

Attribute[1]		Diet				
		Ctl	M3	M4	Com2	±sem
Appearance	Greyish	10.5	9.7	10.5	9.5	1.18
	Yellowish	6.9	9.1	8.8	7.6	1.26
Odour	Fishy	46.8	49.9	46.1	50.8	1.85
	Meaty	38.7	35.5	37.2	38.7	2.01
	Muddy	11.3	13.6	12.2	12.3	1.41
Flavour	Fishy	49.0	45.5	47.3	46.8	1.29
	Meaty	47.1	47.5	47.9	47.5	1.57
	Sweet	29.9	27.5	29.9	28.9	1.50
	Muddy	14.8	15.9	13.9	16.6	1.55
Texture	Firm	46.5	44.3	46.9	47.3	1.72
	Moist	44.2	42.7	43.9	47.4	1.82
	Fibrous	31.7	29.1	33.7	33.7	1.88
Overall liking		60.0	61.2	64.3	63.5	1.65

[1] All scores were scaled from zero (none) to 100 (very). There were no significant ($P>0.05$) differences between any of the diets fed for each of the sensory characteristics examined.

6.3.3.3 Experiment MRC3

Excellent water quality was maintained in both recirculation systems throughout the experiment with measurements of free ammonia-N and nitrite not exceeding 0.1 and 0.025 mg/L respectively and pH was consistently 7.5 to 8.0; water temperature ranged from 27.2 to 28.5°C across all aquaria and systems.

There was no apparent illness in the fish. One fish died as a result of jumping from its aquaria and a total of six fish were removed at the second weighing because of feeding timidness. There was no obvious treatment bias for these removals and all aquaria had at least seven fish at the end of the experiment. Production responses of the fish are provided in Table 25.

Table 25 Production responses of fish in Expt MRC3

Attribute[1]	Diet				
	Ctl	M3	M4	Com2	± sem
Weight (g)					
Start	229.0	229.6	230.4	229.2	11.56
End	340.3^{B}	343.5^{B}	372.1^{A}	341.5^{B}	17.87
Food intake (g/week)	20.5^{C}	23.1^{AB}	24.5^{A}	21.6^{BC}	0.77
Gain (g/week)	18.6^{B}	19.0^{B}	23.6^{A}	18.8^{B}	0.89
SGR (%/d)	0.95^{B}	0.96^{B}	1.14^{A}	0.95^{B}	0.033
Food conversion (g:g)	1.10^{B}	1.22^{C}	1.04^{A}	1.16^{B}	0.018
Food cost ($/kg gain)[2]	0.98^{C}	0.75^{B}	0.71^{A}	---^{D}	0.015

[1] Means without a common superscript letter differ (P<0.05).

[2] Food cost calculated on basis of prevailing ingredient cost without allowance for processing. Information on the Com2 diet is confidential.

Growth and food conversion were best for fish on M4 diet and significantly better (P<0.05) than either the Ctl or Com2 diets. Fish on the M3 diet grew at a similar rate (P>0.05) to those on either the Ctl or Com2 diets but food conversion was inferior (P<0.05) to that of all other diets. The ingredient cost to produce a given weight of fish differed significantly (P<0.05) between each diet in the order: M4 < M3 < Ctl < Com2.

6.3.4 Discussion

Considerable variability was observed in fish feeding behaviour during the experiments with fish being less willing to take floating than sinking food pellets. This was thought to be due to the good clarity of the pond water causing the fish to be shy to feed from the surface. Although the same extruder was used to manufacture the 6 mm diameter food pellets, marked differences in pellet buoyancy were found between the diets as illustrated in Fig. 8.

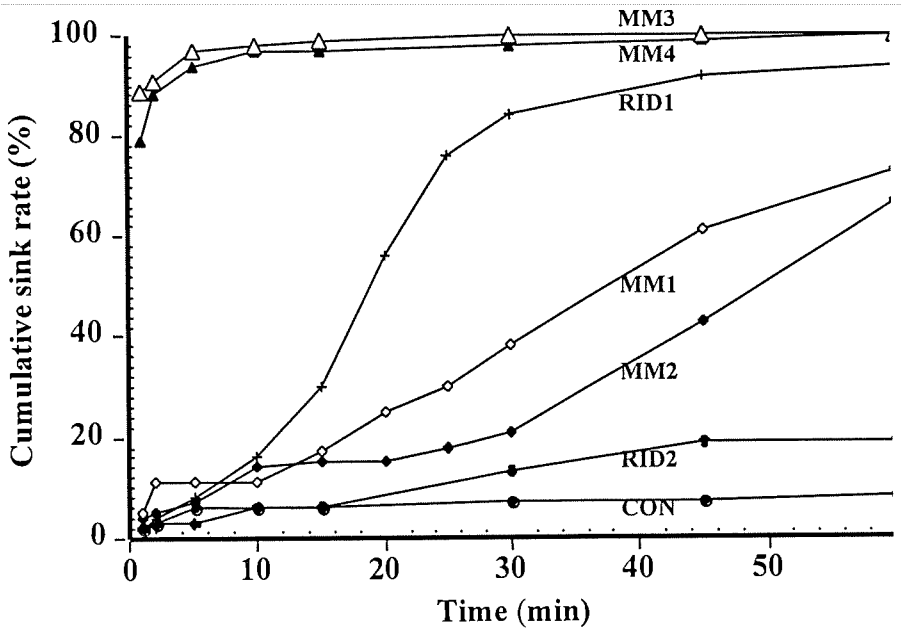

Fig. 8 Cumulative pellet sink rate for each of the diets.

M3 and M4 diets had the fastest sink rate with more than 90% of the pellets sinking within 2 minutes. The most buoyant pellets were the Ctl and Com2 diets where fewer than 20% of the pellets had sunk after 60 minutes contact with the water. The Com1, M1 and M2 pellets were of intermediate sink rate, taking about 20, 40 and 50 minutes respectively, for half of the pellets to sink.

It is difficult to assess whether these differences in pellet sink rates affected the productivity of the fish on the different diets. For the on-farm experiments, shy feeding by the fish fed the buoyant pellets could cause food intake to be suppressed and consequently, for fish growth to be similarly curtailed. Although escape of floating pellets from the cage was prevented, thereby avoiding any gross discrepancy between food issued and its presumed consumption, it is not known whether the nutritional quality of highly buoyant diets was affected by delayed ingestion of the pellet. Some nutrient loss due to leaching following water ingress could be expected (Brown and Robinson, 1989) but quantifying such changes would have been very difficult under these on-farm conditions. Conversely, a rapidly sinking pellet could result in fish being fed unintentionally to excess of satiety, leading to poor food conversion but enabling fish to grow to the full potential of the diet. It was primarily for this reason that the same diets as used in the second on-farm experiment (Expt MRC2) were fed in the laboratory study (Expt MRC3). This enabled the nutritional quality of diets exhibiting marked differences in sink rate to be directly assessed when fed to barramundi either in a highly controlled laboratory study or in a less well controlled on-farm study. For the most part, rank order between diet treatments for all productivity traits was similar for Expts MRC2 and MRC3 (Tables 23 and 25) although there was a heightened differentiation between the diets for food conversion in the laboratory experiment. The food conversion of the farmed fish in Expt MRC2 was nevertheless very good and only about 15% worse than that achieved by fish in the laboratory study (average across all diets of 1.34 vs 1.13, respectively). The similarity in performance and the excellent overall productivity achieved by the fish in Expts MRC2 and MRC3 suggests that pellet sink rate was unlikely to have confounded responses of fish to nutritional differences between diets.

One of the primary objectives of the study was to determine the extent to which meat meal could replace fishmeal in diets for barramundi when the fish were grown under commercial farm conditions. In Expt MRC1, diets comprising high inclusions of meat meal (contributing approximately 55% of the dietary protein) enabled barramundi to be farm-reared as successfully as those fed on either the high fishmeal content Ctl diet or the commercial Com1 diet. However, the meat meal diets used in Expt MRC1 did contain a small amount of fishmeal (contributing 15% of the dietary protein) which was included to ensure good palatability of the diets and their ready acceptance by the barramundi. Exclusion of fishmeal from the experimental meat meal based diets fed in Expt MRC2 had no deleterious effect on barramundi productivity with growth rate of the fish being equal to, or superior to both the high fishmeal Ctl diet and the commercial barramundi diet. Meat meal, either alone or in combination with other terrestrial protein concentrates such as soybean meal and gluten meal, has been successfully used for the partial replacement of fishmeal in diets for cultured carnivorous fish including channel catfish *Ictalurus punctatus* (Mohsen and Lovell, 1990; Lovell, 1992) yellowtail *Seriola quinqueradiata* (Shimeno et al., 1993a,b), rainbow trout *Oncorhynchus mykiss* (Watanabe et al., 1993; Yamamoto et al., 1995), sea bream *Sparus aurata* (Davies et al., 1991) and European sea bass *Dicentrarchus labrax* (Langar and Metailler, 1989). In these cited studies, meat meal was used to replace from 30 to 91% of the protein contributed by fishmeal without any adverse effect on fish growth. Higher levels of fishmeal replacement were not examined possibly for fear of the diets not being palatable to the fish. The only known report where meat meal has been used as a replacement of fishmeal in diets for barramundi is the French study of Aquacop et al. (1993). Including greaves meal (a rendered high fat meat meal product) at 22% of the diet as a partial substitute of fishmeal was found to have no adverse effect on diet digestibility or on fish performance. In our work, we observed no reluctance on the part of barramundi to consume the fishmeal-free meat meal-based diets and feed consumption on these diets in both Expts MRC2 and MRC3 were significantly higher ($P<0.05$) than for the Ctl diet which had a high (35%) fishmeal content (Tables 23 and 25, respectively).

Use of a low-ash, 60% protein Mco meat meal (sourced from Midco Abattoir) conferred no nutritional advantage over that of a conventional 52% protein Cas meat meal (sourced from Casino Abattoir) when each was included to provide similar protein contributions in diets formulated to be isoenergetic and isonitrogenous (Expt MRC1 - Table 22). Lowering the total ash content of the diet fed to the fish through the use of low-ash meat meals such as the Mco product used in this study may however, have important environmental benefits by reducing sediment loading in farm effluent discharges. This is of particular concern for estuary-based fish farms where effluent is continually discharged into the waterway. For land-based pond or recirculation farms where solids can be more easily collected and disposed of in accordance with good environmental practices, it may not be so important that fish diets are low in ash. Unless there is an environmental incentive favouring the use of low-ash diets, the choice of what meat meal product to use in the diet will be dictated by the relative cost of alternative products in meeting prescribed dietary nutrient specifications. At the prevailing 1995/6 prices of $1,700/t and $430/t for the Midco and Cas meat meals respectively, using the low-ash Mco meat meal increased the ingredient cost of the diet by 75% over that of using the higher-ash Cas meat meal ($1,129/t vs $650/t, respectively - Table 20). Ingredient cost of the Mco meat meal and Cas meat meal diets would be similar if the price of the former was reduced from $1,700/t to $500/t. In the prevailing economic climate and in the absence of an environmental incentive, a low-ash meat meal could be expected to attract a price premium of about 15% above that of the high-ash product. This premium is primarily a function of the relative difference in protein content between the alternative products.

In all of the on-farm and laboratory experiments, the ingredient cost of diets formulated with the Cas meat meal was appreciably less than that for all other diets. Moreover, the productivity cost of the diet (expressed as food cost per unit weight increase of fish) for diets containing Cas meat meal was from 73 to 84% lower than for diets containing predominantly fishmeal. In Expts MRC2 and MRC3, increasing the estimated digestible energy content of the diet from 15.0 to 16.2 kJ/g (with a concomitant increase in protein content to maintain a constant protein to energy ratio) caused an almost 10% increase in its ingredient cost (Table 20). However, this increased ingredient cost was offset by improved fish performance such that the productivity cost of the two Cas meat meal diets was either similar (Expt MRC2) or less (Expt MRC3) for the higher energy diet.

The second objective of the work was to assess the impact of feeding high meat meal content diets to barramundi on the sensory characteristics of the flesh. Trained taste panels were used to assess the flesh of farm-reared flesh from each of the diets fed in Expts MRC1 and MRC2. Differences in sensory scores between the diets were few and confined to Expt MRC1 where fish fed the meat meal based diets had higher scores for "fishy" and "sweet" flavours and "firm" texture than those fed the high fishmeal content Ctl diet. Importantly, strong and undesirable taints such as "muddy", "weedy" or "metallic" which might otherwise mask more subtle differences in taste of the flesh were only occasionally detected. The overall liking of the fish was high in both farm experiments and no difference was detected between any of the diets fed.

The strong liking by taste panellists for fish fed the high meat meal content diets indicates that fishmeal can be completely replaced in the diet of barramundi without reducing consumer acceptance. However, particular attention was taken in the present work to ensure that all experimental diets were supplemented with sufficient fish oil to satisfy a minimum dietary eicosapentaenoic acid (EPA, C20:5*n*-3) plus docosahexaenoic acid (DHA, C22:6*n*-3) specification of =12 g/kg. Such supplementation, besides ensuring that the fish's requirement for essential omega-3 fatty acids was met (Boonyaratpalin, 1989; Barlow et al., 1996) would also have assisted in maintaining a desirable "fishy" flavour in the flesh. There is ample evidence showing that the composition of body lipid in cultured fish not only can be modified by diet but increasingly mimics that of the dietary lipid upon prolonged feeding (Watanabe, 1982; Santha and Gatlin, 1991; Borlongan and Parazo, 1991; Fair et al., 1993). Adding fish oil to a purified diet fed to channel catfish (*I. punctatus*) was shown by Dupree et al. (1979) to increase the "fishy" flavour of the flesh although fish fed the same purified diet but with added corn oil was rated more acceptable by the taste panellists. Similarly, substitution of herring oil with either menhaden oil, soybean oil or tallow in diets for Atlantic salmon (*Salmo salar*) in a 23 week experiment did not evoke differences in sensory quality of the flesh but its fatty acid composition reflected that of the diet fed (Hardy et al., 1987).

As terrestrial protein sources such as meat meal become more widely used to replace fishmeal in fish diets, it will be become increasingly important to ensure that these diets are adequately enriched with fish oil or other sources of highly unsaturated omega-3 fatty acids (HUFA). This will be required not only to ensure that the diet satisfies the fish's requirement for these essential HUFA but also that sufficient amounts of these fatty acids are present in the fish. There is growing consumer awareness of the health benefits ascribed to a high dietary intake of omega-3 (fish) oils (Lees and Karel, 1990; Uauy and Valenzuela, 1992; Howe, 1995) and even a perception of lowered HUFA content in cultured fish could have serious marketing implications. There is already concern being expressed about the lowered HUFA content of cultured fish as compared to the same wild-caught fish (Hardy et al., 1987; Pigott, 1989;

Ohaus, 1989) and the importance of maintaining an adequate HUFA content of diets fed to cultured fish.

6.3.5 Conclusions

- Diets based on meat meal and containing no fishmeal were as palatable to the fish and supported equivalent or superior fish productivity as those where fishmeal was the predominant protein source.

- Fish reared on diets containing high inclusions of meat meal, with or without some fishmeal but supplemented with fish oil, was found by trained taste panel assessment to be liked as well or better than fish reared on a diet formulated with a high fishmeal content.

- The meat meal based experimental diets were equal to or better than a commercial barramundi diet in supporting fish growth and in producing fish with flesh of high sensory value.

- Using conventional high-ash meat meal as a partial or full replacement of fishmeal in nutritionally complete diets resulted in an appreciable reduction in the ingredient cost of the diet and a 16 to 27% lowering of the ingredient cost of the food per unit fish weight increase.

- Other than for potential environmental benefits, there was no advantage and a strong economic disincentive in using low-ash meat meal over that of conventional high-ash product.

- These results demonstrate unequivocally the suitability of meat meal to be used for the partial or complete replacement of fishmeal protein in grow-out diets for barramundi.

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7. SUMMARY

Crystalline amino acid investigations

The primary objective of this one-year study was to assess the efficacy of crystalline amino acids (C-AAs) as supplements in diets for barramundi. Three growth experiments were carried out. The first (AA1) examined the effectiveness of C-AAs for restoring the amino acid balance of a low protein, high meat meal containing diet when food intake was controlled to ensure equivalent energy intake. The second (AA2) and third (AA3) experiments directly compared the efficacy of C-AAs and protein-bound amino acids (protein-AAs) as amino acid supplements under conditions of either high (54%, DM) or low (39%, DM) dietary protein respectively, and when fish were fed once daily to satiety.

In Experiment AA1, reducing the fishmeal content of the high protein (50% DM) control diet by increasing the inclusion content of meat meal until it supplied 60% of the total protein resulted in no significant change in growth rate or food conversion (FCR) of the fish. Reducing the DM protein content of the diet from 50 to 39%, primarily by reducing the meat meal content, caused a 30% reduction in growth rate and a 38% worsening of FCR. Addition of crystalline lysine (Lys) and methionine (Met) to the lowest protein diet, but not Lys alone nor further additions of threonine (Thr) and arginine (Arg), brought about a significant ($P<0.05$), but marginal improvement in growth rate and FCR.

In Experiments AA2 and AA3, a high gluten basal-diet markedly deficient in Lys was incrementally supplemented with either a mixture of C-AA (including Lys) or Protein-AA (as casein) to restore the amino acid profile of the protein to a balance similar to that recommended for channel catfish diets. In Experiments AA2 and AA3, the Lys content of the basal diet was 3.5 and 3.1% of protein, respectively. Thus, the amino acid balance of the dietary protein was similar in each experiment but the absolute concentrations of the amino acids were much higher in AA2 compared to AA3. In both experiments, the amino acid balance of the basal diet was serially improved without altering the dietary protein or energy contents by the addition of either a mixture of C-AA or the isonitrogenous substitution of gluten by casein (protein-AA). Increasing the amino acid content of the diet in Experiment AA2 (eg for Lys, from 1.87% to 3.2%), resulted in a significant ($P<0.05$) quadratic improvement in average and specific growth rates and FCR for both the C-AA and protein-AA diet series. The maximum response to amino acid enrichment occurred at a dietary Lys content of about 2.8% (5.2% of protein) for both types of amino acid supplements but the response was slightly better with the protein-AA compared to the C-AA supplement. However, a statistical difference between the two types of amino acid supplements was observed only for FCR where C-AA was only about 50% as effective as protein-AA in eliciting the response. In Experiment AA3, growth rate and FCR improved quadratically with increasing amino acid content of the diet (eg for Lys, from 1.19% to 1.8%) as for Experiment AA2 except that a clear plateau response was not achieved at the highest rate of supplementation. Moreover, no significant difference in efficacy was observed between C-AA and Protein-AA supplements although the former tended to be slightly more effective than the latter, ie an opposite trend to that seen with the high protein diets (AA2).

This research supports the following conclusions:

- The response to amino acid enrichment was relatively more marked at low compared to high dietary protein. And at low dietary protein, C-AAs were equally as effective as protein-AA. However, efficacy of C-AAs may be inferior to that of protein-AAs when fish

are provided with high protein diets and absolute amino acid intake is high. This effect may be due as much to altered efficiency of energy metabolism as to amino acid supply *per se*.

- The importance of the essential amino acid balance of the dietary protein as a factor influencing fish productivity increases inversely with absolute dietary protein (amino acid) intake. 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-AA was a more effective supplement than C-AA. Further, when the amino acid quality of the dietary protein was the same, the absolute productivity of the fish was much better for high compared to low protein diets which illustrates the extent to which dietary protein is used for meeting the animal's energy requirements.
- Substitution of fishmeal by terrestrial protein feeds and the attendant deterioration in the essential amino acid profile of the dietary protein is unlikely to adversely affect fish productivity provided the protein content of the diet is maintained above about 50% (DM) and fish are fed liberally.
- The dietary essential amino acid requirements (% DM) of juvenile barramundi were estimated as: Arg, 2.29; His, 0.69; Ile, 1.33; Leu, 2.46; Lys, 2.67; Met + Cysh, 1.20; Phe + Tyr, 2.49; Thr, 1.52; Try, 0.40; and Val, 1.55..

Potential of meat meal to replace fishmeal

Two on-farm experiments (Expts MRC1 and MRC2) were carried out to compare the growth performance and taste characteristics of juvenile barramundi fed one of four diets, a high fishmeal (control) diet, two experimental diets where most or all of the fishmeal was replaced by meat meal and a commercial barramundi diet. Both experiments were carried out using caged fish (400 per $2m^2$ cage) in an aerated freshwater pond. The experimental fish were managed as for other fish on the farm, being fed to satiety once daily except on the weekend when fish were fed only on one of the days. A third growth assay in the Walkamin laboratory (Expt MRC3) was carried out using the same diets as fed in Expt MRC2 to validate the results of that on-farm experiment. In each of the 10-week on-farm studies, a 4x4-randomised block design was employed. For the 6-week laboratory experiment, the number of replicates was increased to 6 and fish were stocked at 8 fish/tank (180 l). At the conclusion of the two on-farm experiments, fish from each cage were sampled for sensory evaluation.

In Expt MRC1, diets comprising high inclusions of meat meal (contributing approximately 55% of the dietary protein) enabled barramundi to be farm-reared as successfully as those fed on either the high fishmeal control diet or the commercial diet. However, the meat meal diets used in Expt MRC1 did contain a small amount of fishmeal (contributing 15% of the dietary protein) which was included to ensure good palatability of the diets and their ready acceptance by the barramundi. Exclusion of fishmeal from the experimental meat meal based diets fed in Expt MRC2 had no deleterious effect on barramundi productivity with growth rate of the fish being equal, or superior, to both the high fishmeal control diet and the commercial barramundi diet. Rank order between diet treatments for all productivity traits was similar for Expts MRC2 and MRC3 although there was a heightened differentiation between the diets for food conversion in the laboratory experiment. The food conversion of the farmed fish in Expt MRC2 was nevertheless very good and only about 15% worse than that achieved by fish in Expt

MRC3 (average across all diets of 1.34 vs 1.13, respectively). Use of a low-ash, 60% protein meat meal conferred no nutritional advantage over that of a conventional 52% protein meat meal when each was included to provide similar protein contributions in diets formulated to be isoenergetic and isonitrogenous. In all of the on-farm and laboratory experiments, the ingredient cost of diets formulated with the 52% protein meat meal was appreciably less than that for all other diets. Moreover, the productivity cost of the diet (expressed as food cost per unit weight increase of fish) for diets containing 52% protein meat meal was from 73 to 84% lower than for diets containing predominantly fishmeal. In Expts MRC2 and MRC3, increasing the estimated digestible energy content of the diet from 15.0 to 16.2 kJ/g (with a concomitant increase in protein content to maintain a constant protein to energy ratio) caused an almost 10% increase in its ingredient cost. However, this increased ingredient cost was offset by improved fish performance such that the productivity cost of the two meat meal diets was either similar (Expt MRC2) or less (Expt MRC3) for the higher energy diet.

Trained taste panels were used to assess the flesh of farm-reared fish from each of the diets fed in Expts MRC1 and MRC2. Differences in sensory scores between the diets were few and confined to Expt MRC1 where fish fed the meat meal based diets had higher scores for "fishy" and "sweet" flavours and "firm" texture than those fed the high fishmeal control diet. Importantly, strong and undesirable taints such as "muddy", "weedy" or "metallic" which might otherwise mask more subtle differences in taste of the flesh were only occasionally detected. The strong liking by taste panellists for fish fed the high meat meal content diets indicates that fishmeal can be completely replaced in the diet of barramundi without reducing consumer acceptance. However, particular attention was taken in the present work to ensure that all experimental diets were supplemented with sufficient fish oil to satisfy the fish's dietary requirement for highly unsaturated fatty acids.

Conclusions from the research were:

- Diets based on meat meal and containing no fishmeal were as palatable to barramundi and supported equivalent or superior fish productivity as those where fishmeal was the predominant protein source.
- Fish reared on diets containing high inclusions of meat meal, with or without some fishmeal but supplemented with fish oil, was found by trained taste panel assessment to be liked as well or better than fish reared on a diet formulated with a high fishmeal content.
- The meat meal based experimental diets were equal to or better than a commercial barramundi diet in supporting fish growth and in producing fish with flesh of high sensory value.
- Using conventional high-ash meat meal as a partial or full replacement of fishmeal in nutritionally complete diets resulted in an appreciable reduction in the ingredient cost of the diet and a 16 to 27% lowering of the ingredient cost of the food per unit fish weight increase.
- Other than for potential environmental benefits, there was no advantage in using low-ash meat meal over that of conventional high-ash product.
- These results demonstrate unequivocally the suitability of meat meal to be used for the partial or complete replacement of fishmeal protein in grow-out diets for barramundi.

KEYWORDS: Amino acids, crystalline amino acids, nutrient retention, Meat meal, Sensory, Fishmeal replacement

8. BENEFITS

Australia will benefit in four major ways from this project. Firstly, the research will lead to much better, cheaper diets for aquaculture. This will improve the economic viability of aquaculture, and hopefully lead to reduced prices for aquaculture products. Moreover, by demonstrating the effectiveness of terrestrial protein meals as fishmeal substitutes, the work will assist in reducing Australia's dependence on imported fishmeal. Secondly, 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 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. Research into cost-effective methods of increasing the value of agricultural products in aquaculture diets, through processing or the addition of enzymes or amino acids will further improve the marketing potential of these products Thirdly, Australian feed manufacturers will benefit from this research as they will be able to use the results to manufacture better diets. The possibility of selling diets in Asia offers major marketing opportunities for dynamic Australian feed manufacturers. Finally, Australian aquaculture research workers will benefit from close interaction with scientists from other disciplines who have much to contribute to this research topic.

Benefits will flow to the aquaculture industries in Australia and overseas, the Australian agriculture industry, including both plant and animal industries, and the feed manufacturing industry. The aquaculture industry in Australia has the most to gain initially although if a foothold in the Asian or even world aquaculture feed market is achieved, the Australian agriculture and feed manufacturing industries have even more to gain. Benefits are estimated as Australian aquaculture 40%, feed manufacturing industries 20%, Australian agriculture 40%.

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9. PAPERS AND ARTICLES ARISING FROM THE PROJECT

Williams, K.C. and Barlow, C., 1996. Nutritional research in Australia to improve pelleted diets for grow-out barramundi *Lates calcarifer* (Bloch). In: H. Kongkeo and A.S. Cabanban (Editors), Aquaculture of Coral Fishes and Sustainable Reef Fisheries. Network Aquaculture Centres Asia and Pacific, Bangkok, In press.

Williams, K.C., Barlow, C., Rodgers, L., Ruscoe, I., Hockings, I. and Agcopra, C., 1997. Continuing the development of improved grow-out diets for barramundi.. In: M.J. Wingfield and C.G. Barlow (Editors), Proceedings Australian Barramundi Farming Workshop, 15-16 August 1997. QDPI Freshwater Fisheries and Aquaculture Centre, Walkamin, Qld. pp. 10-13.

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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.

10. 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.

11. 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.

Fishmeal is still the protein source of choice for most intensively cultured fish and prawns but unfortunately the situation with fishmeal has deteriorated even faster than predicted. The current status is:

1 Global fishmeal production currently requires more than 30 Mt (over 30%) of the total catch of fish. Production was predicted to decline slowly (Barlow, 1989) but abnormal fishing off the coast of Ireland and disappointing South American catches have led to real dangers of a greater shortfall which is already pushing prices to record levels (Lewis, 1995).

2 The Australian production of high quality fishmeal is based on the Jack Mackerel fishery in Tasmania. However, quotas for this fishery have been reduced, catch effort slashed to less than half previous levels and production will be far less than the previous 7 000 t/yr maximum.

3 Concerns about importation of fishmeal and aquaculture feeds into Australia are mounting and are clearly identified as being potential factors for disease introductions (Humphrey, 1995). Recommendations for heat processing to reduce this risk (Nunn, 1995) will seriously reduce the nutritional value of fishmeal and imported feeds

4 As higher quality fishmeal is generally required for aquaculture feeds, species of fish currently used for human consumption are increasingly being targeted by fishmeal producers. In Malaysia, much of the cheap fish used to produce salted fish for human consumption is now used as aquaculture feed instead (New, 1991).

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.

For silver perch, digestibility coefficients for over 60 different ingredients (including some processed in different ways) have been determined and results used to select ingredients for evaluation with barramundi, prawns and salmon. For these other species digestibility of 8-10 "high priority" ingredients have been determined. For silver perch, barramundi and prawns a number of the most promising ingredients have been further evaluated in growth studies including summit-dilution comparative slaughter experiments.

High priority ingredients include meat meals, especially low ash meals, oilseeds, grain legumes, especially dehulled and processed lupins and field peas and modified wheat gluten products. Additional research on ingredient evaluation of some of these products is required for barramundi and prawns and with wheat gluten for all species. Laboratory-scale processing has indicated that wheat gluten without the strong agglutinating characteristics of traditional gluten can be produced at a 10-20% lower cost. If preliminary results with silver perch are confirmed in more detailed experiments, this protein source could have outstanding potential for domestic and global aquaculture feeds. For some ingredients, effects of processing and supplements, eg enzymes, will improve their potential. Research into utilisation of carbohydrates is needed to ensure the maximum use can be made of Australian grains.

Results from this project are critically important for two related applications on Aquaculture Diet Development; Nutrient Requirements and Diet Validation and Feeding Strategies. Armed with comprehensive data on ingredient digestibility and growth effects, it is possible to determine the cost of providing different nutrient specifications in formulated diets made from a range of ingredients. This analysis has clearly shown that digestible Lys and Met plus Cysh are the first limiting amino acids and that meeting published requirements for fatty acids is also expensive. Defining these requirements precisely is critical to ensure maximum use can be made of cheaper ingredients. Diets are a major component of feed costs but feeding practices need to be optimised to lower operating costs. Optimum feeding frequency is also affected by physical characteristics of the diet and, to some extent, by composition.

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.

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12. STAFF

CSIRO

Name	Qualification	Position	FTE on Project
K.C. Williams	QDAH, BVSc, PhD	Principal Nutritionist	0.05

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	0.1
C. Palmer	DipLabTech	Snr. Lab. Technician	0.15

13. APPENDICES

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

2 Fishmeal replacement in aquaculture diets using rendered protein meals.

3 Continuing the development of improved grow-out diets for barramundi.

4 Larval penaeid and grow-out finfish nutritional research in Australia.

Proc. Aquaculture of Coral Fishes & Sustainable Reef Fisheries, Sabah, Malaysia, 4-8 December 1996, (Eds H. Kongkeo and A.S. Cabanban).

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.

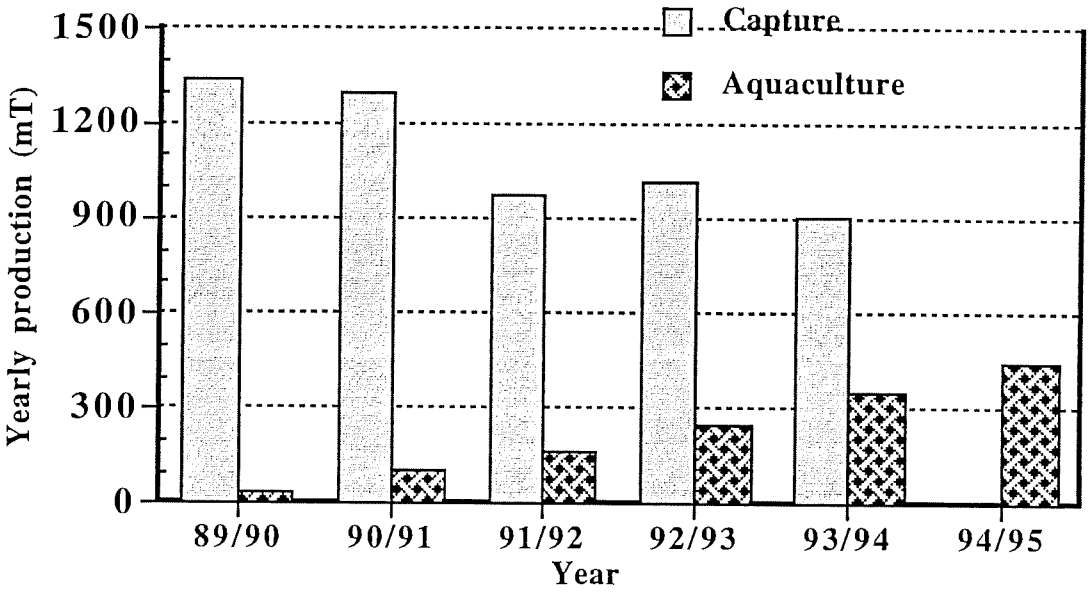
Under laboratory and commercial farm conditions, diets formulated entirely from terrestrial feed ingredients (except for a low inclusion of fish oil to provide essential n-3 fatty acids) have resulted in as good if not better barramundi productivity as conventional diets based on fishmeal. Using trained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

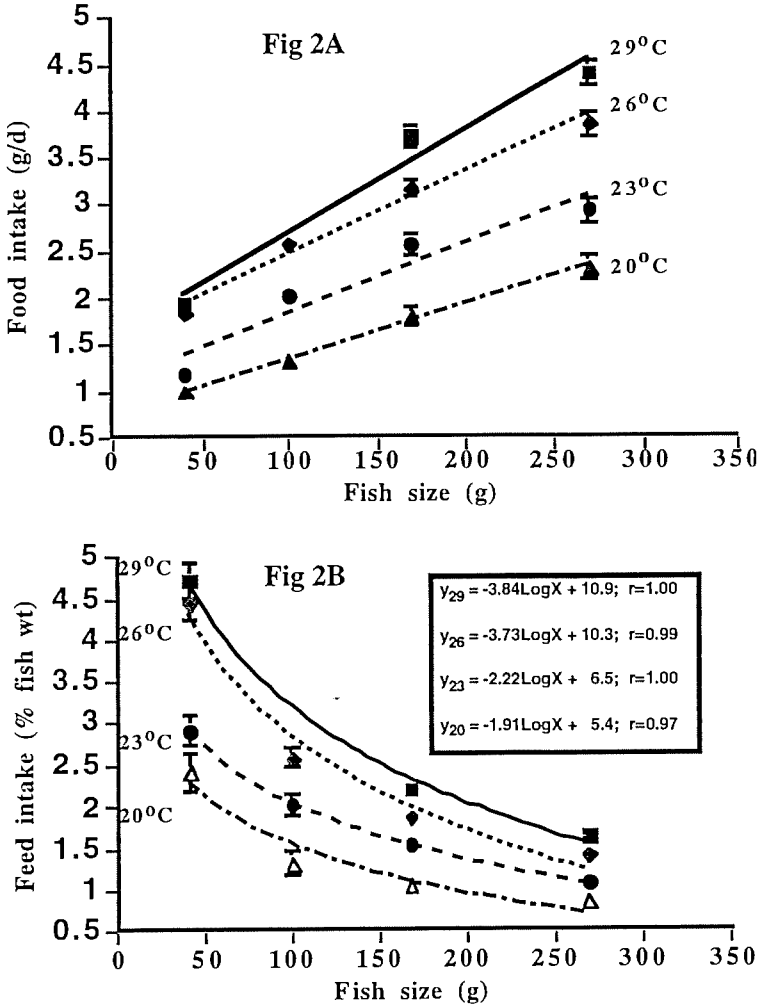
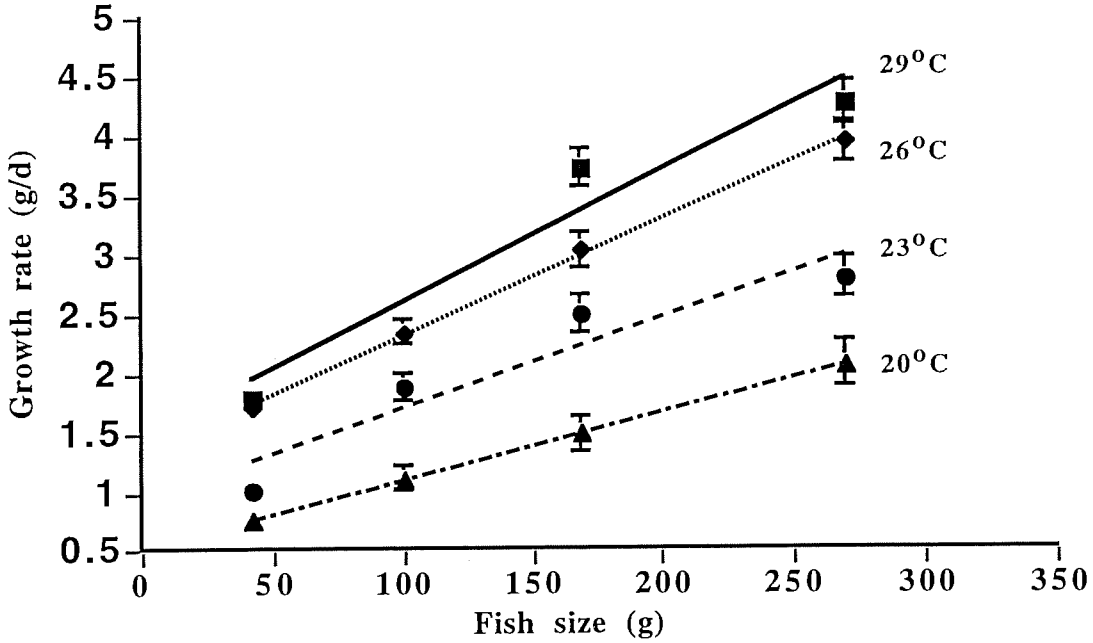


Figure 3. Effect of water temperature and fish size on growth rate of barramundi



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: $\ln DFI = -7.285 + 0.478 \ln W + 0.391T - 0.0065T^2 + 0.074F \quad (R^2 = 0.97)$ 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
	Crude protein	Gross energy	(kJ/g)
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

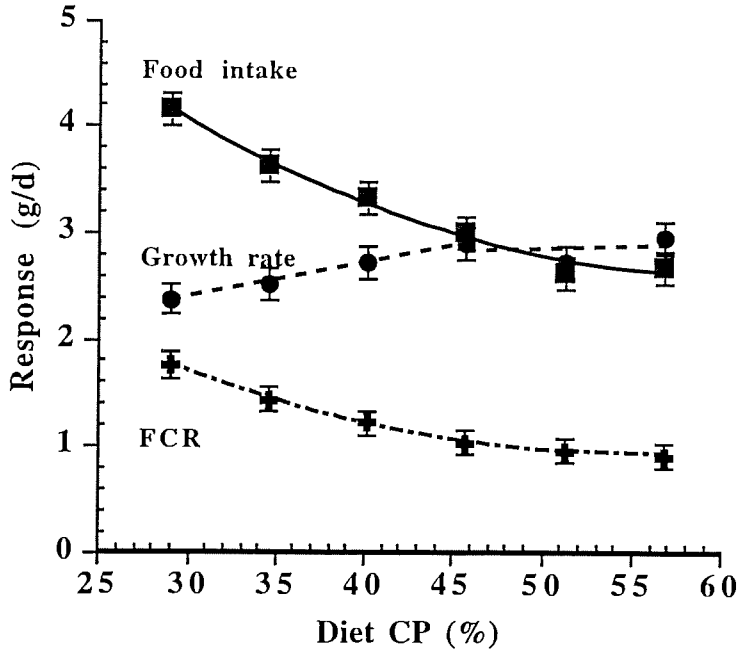
[1] Formulation (g/kg) of the reference protein was: Casein, 430; Fishmeal (Peruvian), 300; Gluten, 250; Lysine HCl, 5; d/l Methionine, 5.5; l Threonine, 2.5; l Tryptophan, 1; and $NaHCO_3$, 6.

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

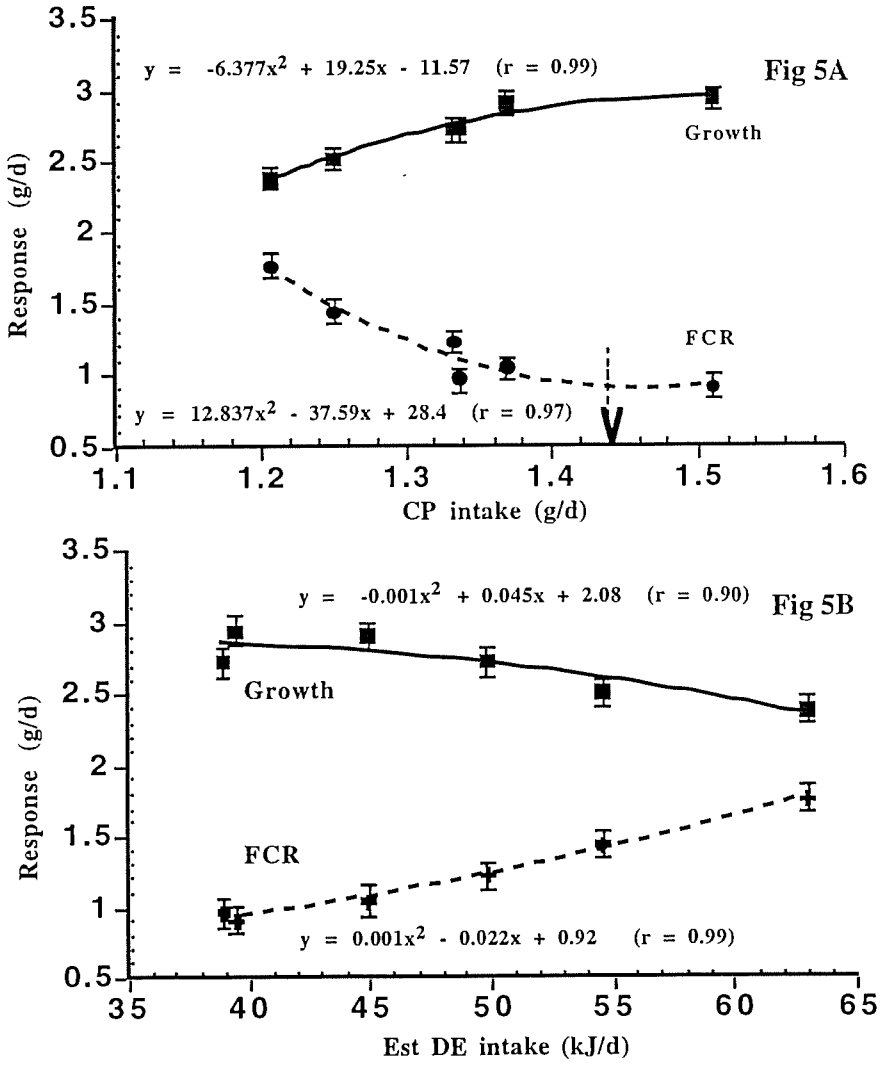
^{A,B,C,D} - Means without a common superscript letter differ ($P<0.05$).

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

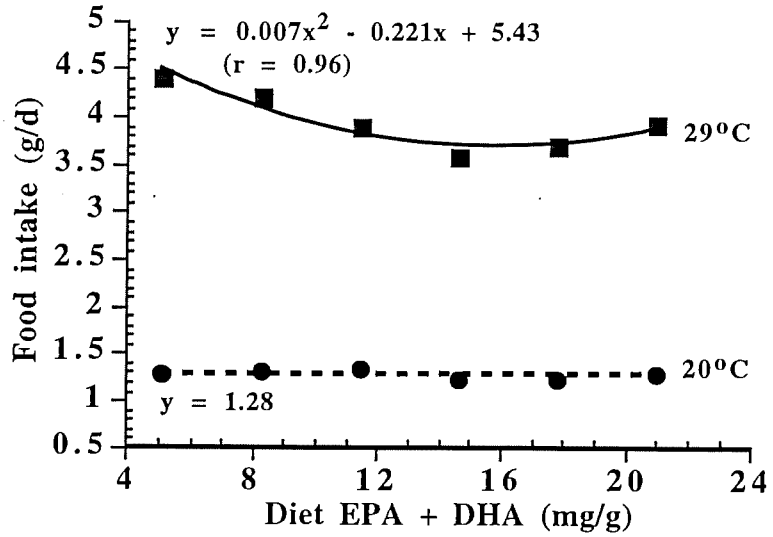
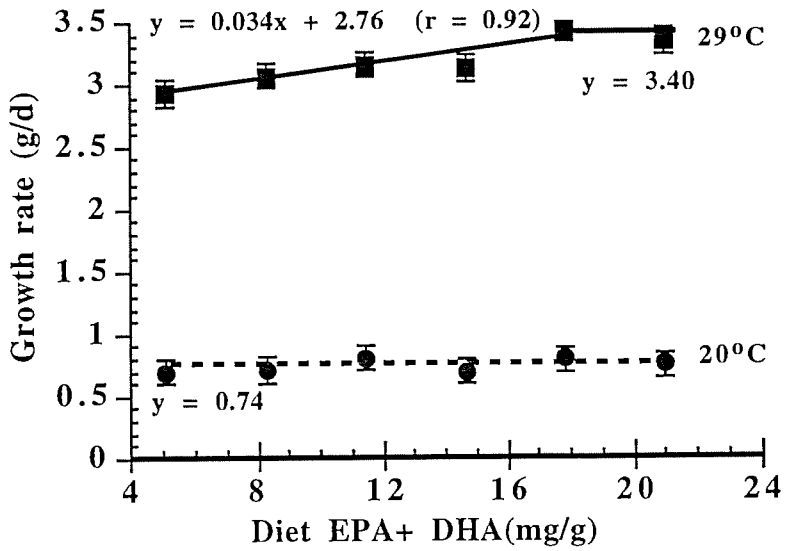


Figure 7. Effect of fatty acid content of the diet and water temperature on growth rate of juvenile barramundi



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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Fishmeal replacement in aquaculture diets using rendered protein meals

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Abstract

Preliminary studies with conventional (50–52% protein) and premium (60% protein) meat meals show them to be well digested by prawns (*Penaeus monodon*), silver perch (*Bidyanus bidyanus*) and barramundi (*Lates calcarifer*) with the protein of the low-ash meat meal being as well digested as that of fish meal. These derived digestibilities were used to formulate meat meal- and fish meal-based diets of specified nutrient digestibility for subsequent growth comparisons. Under both laboratory and field conditions, meat meal was able to replace at least two-thirds (prawn and silver perch) and up to all (barramundi) of the fish meal protein in the diet without any adverse effect on production. Moreover, high dietary inclusions (≥30%) of meat meal did not detract from the taste of the product. In the case of barramundi, this applied even when all of the fish meal was replaced in the diet. Based on these findings, meat meal has the potential to become a major protein source for aquaculture diets. At a conservative dietary inclusion of 20%, the Asian aquafeed market alone would absorb 500,000 t of meat meal – Australia's total annual production – and the amount required is expected to double if not treble by the year 2025 if the predicted expansion of aquaculture in the region is realised. However, for Australian renderers to successfully supply this market, meat meals low in ash (<20%) and fat (<7%) and high in protein (≥60%) are required and at protein-equivalent prices (or at a small premium) to the high-ash, 50 to 52% protein product currently produced.

Introduction

As in any animal farming system, aquaculture species require a dependable supply of nutritious food that must be provided at a cost that is sufficiently low to enable the farming operation to be profitable. Many of these cultured finfish and crustaceans are carnivorous or omnivorous in feeding habit and in most farming systems are reliant on externally supplied aquafeed. Fishery product, either in the form of low-value 'trash' fish and fishery waste or rendered as fish meal, is currently the principal source of protein for these animals and may constitute up to 70% by weight of the diet (Tacon 1995). Concerns that reliance of aquaculture on these fishery products is not sustainable in the long term are now focusing attention on finding cost-effective, non-marine, alternative sources of protein for use in aquafeed.

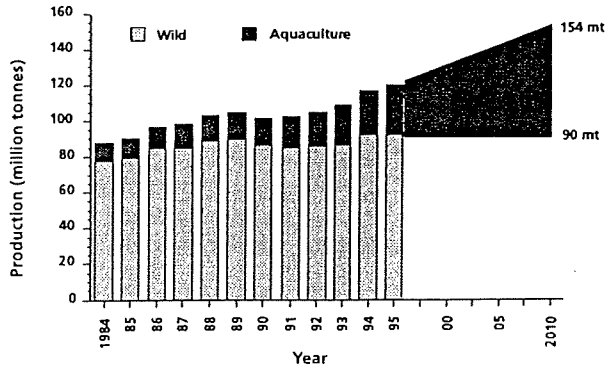
Responding to the need for sustainable non-marine protein sources, the Australian Fisheries Research and Development Corporation (FRDC) funded a nationally coordinated research program in 1993 to find suitable alternatives to fishery product for four Australian 'template' species – a marine, predominantly carnivorous prawn (giant tiger prawn, *Penaeus monodon*), a warm-water carnivorous euryhaline fish (barramundi, *Lates calcarifer*), a freshwater omnivorus fish (silver perch, *Bidyanus bidyanus*) and a cold-water diadromous carnivorous fish (Atlantic salmon, *Salmo salar*). This research was extended through funding from the Meat Research Corporation to examine rendered protein meals as replacements of fish meal in diets for the giant tiger prawn, barramundi and silver perch. This paper reviews the expanding role of aquaculture globally and the Australian research that has been done to determine the suitability of locally available meat meals as cheaper alternatives to fish meal in manufactured aquafeed.

Aquaculture expansion and aquafeed requirements

Aquaculture is the fastest expanding food producing sector in the world, growing at a rate of almost 10% pa. since 1984 to 27.8 million tonne (Mt) worth US$42.3 billion (B) in 1995 (Figure 1). By comparison over the same period, livestock meat increased by 2.8% while that for capture fisheries by just 1.6% (Tacon 1996; Rana 1997). As fish supplies from traditional marine and inland capture fisheries are unlikely to increase substantially because they are already 'being exploited at or beyond the maximum sustainable yield' (Mace 1997), aquaculture production must at least double if not treble by the year 2025 if current per capita 'fish' consumption of 19 kg is to be met (Csavas 1994; Gjedrem 1997).

Fig1

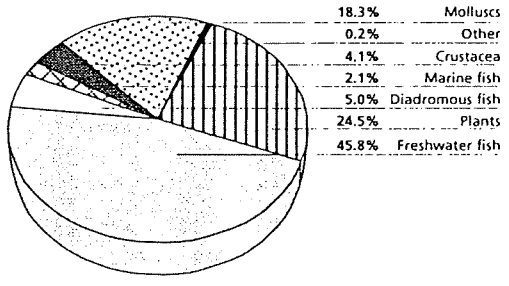
World fisheries and aquaculture production and forecast aquaculture requirement to year 2010



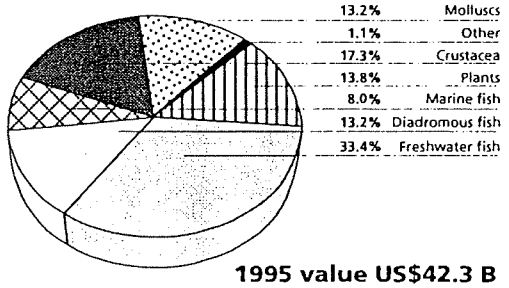
Although more than 250 aquatic species are currently being cultured world wide (Rana 1997), 50% of the 1995 production by weight was derived from just seven species (kelp, four carp species, yesso scallop and pacific oyster) with all of the animal species being either inland freshwater herbivorous fish or marine filter feeders. However, crustaceans (predominantly the giant tiger prawn, *Penaeus monodon*), diadromous fish (predominantly the salmonids rainbow trout, *Oncorhynchus mykiss* and Atlantic salmon, *S. salar*) and marine fish (predominantly Japanese or red seabream, *Pagrus major*) comprise almost 40% (US$16.3 B) of the total (Figure 2). Species in this group are all strict or essentially carnivorous in feeding habit and all rely on compounded aquafeeds. Within developed countries, it is this group of cultured animals that has shown the greatest increases in production (Tacon 1996) and in turn, stimulated a massive expansion in manufactured aquafeed.

Fig 2

World production and value of aquaculture for major culture groups in 1995 (after Rana 1997)



1995 prod 27.8 mt



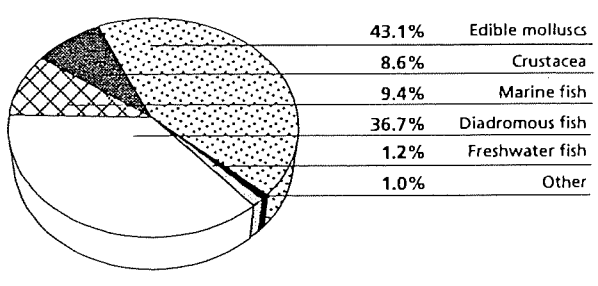
1995 value US$42.3 B

Estimates of global compounded aquafeed production range from 3.34 Mt in 1992 (New and Csavas 1995) to 4.25 Mt in 1994 (Smith and Guerin 1995). Those of Smith and Guerin (1995) are probably the most reliable. Carnivorous fish (40%) and marine prawns (25%) are the main consumers and based on average costs of US$1000 and $500 per tonne for prawn and finfish respectively, global aquafeed production is estimated to be worth US$2.1 to 4.2 B (Tacon 1996). Projections for aquafeed production by the year 2000 range from 4.5 to 7.5 Mt (Meggison 1990; Chamberlain 1993; New and Csavas 1995; Tacon 1996), representing an increase of up to 80% on Smith and Guerin's 1994 estimate of 4.25 Mt.

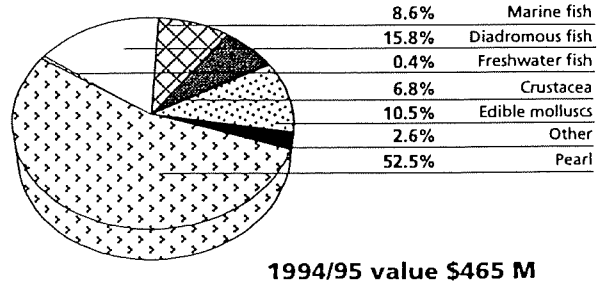
In Australia, aquaculture is the fastest growing primary industry with about 40,000 t of product worth around AUD$500 M being produced in 1994/95, an increase of almost 50% on its 1993/94 value (O'Sullivan and Kiley 1996). Pearl production is by far the most valuable sector ($250 M) with salmonids ($73 M), edible oysters ($48 M), southern bluefin tuna ($38 M) and marine prawns ($28 M) being the other important groups; barramundi ($3.5 M) is a small but expanding industry and silver perch ($1.6 M) has the potential to be a significant fresh water aquaculture industry (Figure 3). Australian requirements for manufactured aquafeed are estimated currently to be about 22,000 t worth $32 M and exclusive of southern bluefin tuna where a satisfactory compounded diet is awaiting development. As stated by the Australian Cooperative Research Centre for Aquaculture (Anon 1996), 'Australian aquaculture is a sustainable and low-impact industry that currently enjoys a high international reputation for quality and contaminant-free product'. Australia is well positioned to be a major supplier of high quality, terrestrially-based aquafeed to the vast Asian market.

Fig 3

Australian production and value of aquaculture for major culture groups in 1994/95 (after O'Sullivan and Kiley 1996)



1994/95 prod 23,867 t



1994/95 value $465 M

Need for fish meal alternatives

Compounded feeds for carnivorous fish and prawns presently contain from 50 to 70% by weight of fishery product. Such foods are preferred by aquafeed manufacturers not only because they are perceived to be 'natural' dietary constituents but also because they are highly palatable to the cultured animal. Moreover, they are concentrated sources of highly digestible (apparent dry matter digestibility generally exceeding 85%) and good quality protein (>65% crude protein of an excellent amino acid profile) and their low ash content (≤15% in rendered dry fish meal) minimises environmental impacts that can result from the discharge of nutrient-rich effluents (Cho et al. 1994; New 1996; Lawrence and Lee 1997; Tacon and Akiyama 1997).

World production of fish meal in 1993 was 6.26 Mt and has been in a state of declining or at best static production since 1989, essentially mirroring the static yield of the capture fishery (Starkey 1994 and T. Starkey pers comm). Aquaculture is a significant and rapidly increasing user of fish meal. FAO estimates of fish meal consumption by aquaculture were 1.1 Mt in 1995 with salmonids (437,000 t) and prawns (260,000 t) being the primary consumers (Tacon 1996). Since only a little over half of world fish meal production is available for export, the global usage of fish meal for aquafeeds represents about 30% of the export-available product (Starkey 1994). It is evident from these statistics that continued expansion of aquaculture will not be possible if fish meal is relied upon as the main source of protein in aquafeed. Increased production of fish meal seems most unlikely as the capture fishery is already fully exploited. Moreover, demand for fishery product from other high profit sectors such as the pet food industry will force fish meal prices up until usage in aquafeeds will become uneconomical. In any event, if aquaculture is to become a net and increasing contributor to human food supplies, it is critical that aquafeeds become less reliant on fishery products which will mean finding suitable and cost-effective terrestrial alternatives.

Nationally-coordinated research on fish meal replacement

In Australia, where domestic fish meal production was just 7,000 t compared to an importation of 63,000 t of fish meal and other non-edible marine product in 1995/96 (ABARE 1996), it is even more critical that aquafeeds are based on terrestrial proteins. Fortunately, Australia has an abundant supply of terrestrial animal and vegetable protein feeds which are potential ingredients for replacement of fish meal in aquaculture diets. In 1993, FRDC created a sub-program to coordinate national research on fish meal replacement in aquafeeds (Allan 1997). Additional funding was provided by the Australian Centre for International Agricultural Research, the Australian Grains' Research and Development Corporation, the Australian Meat Research Corporation, the Australian Academy of Grain Technology and considerable in-kind support was provided by aquaculturists and feed manufacturers. This sub-program formed a collaborative team uniting 11 aquaculture nutrition research groups in Australia for the purpose of finding suitable and cost-effective alternatives to fish meal in aquaculture diets. The approach adopted in addressing this objective was to:

- identify and evaluate alternative terrestrial protein sources;
- develop and evaluate processing methods to improve ingredient utilisation;
- evaluate methods to increase terrestrial feed usage through amino acid and enzyme supplementation;
- examine the role of attractants to increase diet palatability; and
- define requirements of target species for key nutrients.

This research is being continued in a subsequent FRDC 'Aquaculture diet development' sub-program where the emphasis is on continued evaluation of potentially useful energy and protein feeds, a more intensive examination of nutrient requirements of existing and emerging Australian aquaculture species and the development and commercialisation of improved feeds.

Nutritive value of rendered protein (meat) meals

Crucial to the nutritive evaluation of any food is basic information on its chemical composition, apparent digestibility and subsequent assimilation by the animal. Table 1 summarises the dry matter, protein and energy content of various protein concentrate feed ingredients available in Australia.

Composition of the rendered meat meals varied considerably: protein from 46.7 to 80.9%; ash from 9.4 to 38.6%; and lipid from 7.4 to 14.5%. By comparison, the fish meal sources were all high in protein (>70%), low to moderate in ash (<15.0%) and low to moderate in lipid (<12.9%), The vegetable protein meals were all low in ash and lipid and of moderate protein content. On a cost per unit protein basis, the meat meal products were similar to the vegetable proteins and about 40% less expensive than the Australian and Peruvian fish meal.

Table 1

Analysed dry matter (DM), ash, protein, lipid and energy composition for various protein concentrate feeds available in Australia

Ingredient	*DM (%)*	*Ash (%DM)*	*Protein (%DM)*	*Lipid (%DM)*	*Energy (kj/gDM)*	*Cost ($/kgCP)*
Meat meal						
AMH (mixed)	94.2	32.6	55.9	10.4	16.8	0.84
Aspen)Provine)	94.3	9.4	80.9	13.0	25.0	1.02
Beef city (beef)	97.0	38.6	46.7	7.4	13.9	0.98
Fletcher (lamb)	97.2	34.5	53.4	7.4	15.7	0.87
Midco (mixed)	97.7	12.1	60.6	14.5	23.5	0.85
Fishmeal						
Tasmanian	93.5	14.2	75.8	9.9	21.4	1.41
Danish	93.1	13.0	73.2	11.4	21.9	1.76
Peruvian	90.1	15.0	70.5	12.9	21.8	1.42
Soybean (solv ext)	89.1	7.3	52.9	1.6	19.9	1.05
Canola	91.7	6.8	40.9	3.1	19.7	0.87
Lupin (de-hulled)	90.5	3.5	44.8	7.1	20.6	0.86
Wheat gluten	94.0	1.5	76.9	0.5	23.1	1.04

Table 2

Apparent dry matter, protein and energy digestibility coefficients of various protein concentrates determined with prawns (P), silver perch (SP) and barramundi (B)[1]

Ingredient	*Apparent digestibility coefficient (%)*								
	Dry matter			*Protein*			*Energy*		
	P	*SP*	*B*	*P*	*SP*	*B*	*P*	*SP*	*B*
Meat meal									
AMH (mixed)	–	–	43	–	–	64	–	–	67
Aspen)Provine)	78	89	–	83	84	–	64	95	–
Beef city (beef)	60	43	–	77	66	–	61	73	–
Fletcher (lamb)	57	55	–	74	69	–	55	82	–
Midco (mixed)	–	76	–	–	83	–	–	85	–
Fishmeal									
Tasmanian	86	77	–	93	96	–	89	93	–
Danish	–	91	90	–	99	89	–	100	99
Peruvian	–	74	–	–	89	–	–	90	–
Soybean (solv ext)	67	73	56	92	95	86	71	82	69
Canola	49	50	49	79	83	81	53	57	56
Lupin (de-hulled)	67	68	61	94	100	98	68	74	62
Wheat gluten	100	97	100	100	100	100	100	100	99

[1] Most of the digestibility data for barramundi were produced by Dr N. McMeniman (University of Queensland).

The apparent nutrient and energy digestibilities of these feed ingredients as determined with prawns, silver perch and barramundi are detailed in Table 2.

The apparent digestibility of the meat meals was generally lower than for the fish meals with all three aquatic species and particularly so for energy. Interestingly, gluten was highly digestible in all species while that of soybean meal was as well digested as the meat meals. Apparent digestibility values with barramundi were generally lower than with either prawns or silver perch for all feed ingredients other than Danish fish meal and gluten, both of which were highly digestible in all species. The more carnivorous barramundi may be less capable of digesting terrestrial feeds. The digestibility values for meat meals compare favourably with estimates derived with rainbow trout (Asgard 1988; Alexis et al. 1988).

The apparent essential amino acid digestibility of fish meal and various meat meals as determined with prawns is shown in Table 3. Each of the essential amino acids was digested equally as well but tended to be slightly lower than that for

Table 3

The essential amino acid content (Con; g/100g DM) of fish meal and various meat meals (MM) and their apparent digestibility (AD; %) for prawns

Amino acid	*Protein concentrate*							
	MM (Aspen)		*MM (Beef)*		*MM (Fletcher)*		*Fishmeal (Tas)*	
	Con	*AD*	*Con*	*AD*	*Con*	*AD*	*Con*	*AD*
Arginine	6.49	65	3.51	45	3.89	30	4.82	93
Histidine	1.77	61	0.81	59	0.76	56	2.68	93
Isoleucine	3.54	55	1.27	56	1.27	48	3.41	90
Leucine	5.69	54	2.66	55	2.73	43	5.27	91
Lysine	5.00	62	2.52	62	2.44	47	5.95	95
Methionine	1.67	60	0.71	64	0.62	58	2.20	93
Cystine	0.93	50	0.47	35	0.40	27	0.84	85
Phenylalanine	3.96	57	1.64	56	1.67	46	3.36	90
Tyrosine	2.68	59	0.85	74	0.86	56	2.61	100
Threonine	3.65	58	1.63	52	1.54	40	3.49	91
Valine	4.38	57	1.88	53	1.98	42	4.04	91

Table 4

The apparent digestibility (AD) and cost of protein (P) and lysine (Lys) of alternative protein feed ingredients as determined for silver perch

Feed ingredient	*ADP*	*ADLys*	*Cost*		
	(%0	*(%)*	*$/t*	*S/kg ADP*	*S/kg ADLys)*
Meat meal					
Beef city (beef)	33.8	1.91	445	1.32	23.3
Fletcher (lamb)	38.1	2.74	453	1.19	16.5
Midco (mixed)	49.4	2.85	500	1.01	17.5
Aspen (Provine)	67.8	3.53	775	1.14	22.0
Fishmeal					
Danish	68.1	5.23	1200	1.76	22.9
Peruvian	62.0	5.23	850	1.37	16.2
Soybean meal	47.6	2.82	440	0.92	15.6
Peanut meal	39.4	1.56	380	0.96	24.4
Canola meal	40.3	2.25	330	0.82	14.7
Cottonseed	41.6	1.49	370	0.89	24.8
Lupin (hull-on)	30.8	1.58	298	0.97	18.9

the overall protein; digestibility of the fish meal was high and similar to that of the overall protein. With silver perch, the apparent digestibilities of the individual essential amino acids were similar both to one another and to that for the whole protein except for arginine which was from 10 to 20% lower than the other amino acids.

A summary of the apparent digestibility and cost of protein and lysine for a range of feed ingredients determined in silver perch is presented in Table 4.

Based on the cost per unit of digestible protein, the vegetable protein meals are the least expensive with the meat meals being slightly more expensive but cheaper than the fish meals. On a cost per unit of digestible lysine basis, the meat meals and fish meals were similar; the vegetable protein meals ranged from being the cheapest (soybean meal) to the most expensive (cottonseed meal).

Nutrient requirements

It is beyond the scope of this paper to review in any detail the nutrient requirements of the three aquatic species – giant tiger prawn, silver perch and barramundi – that were used to examine the comparative value of rendered protein meals. For many of the micro-nutrients including vitamins, essential fatty acids and essential amino acids, requirements have not been well defined and even optimum protein (amino acid) to energy relationships are at best working guesstimates rather than precise quantitative requirements. However, a broad

Table 5

Formulation, ingredient cost and key nutrient specifications of typical high fish meal diets for giant tiger prawn, silver perch and barramundi

Attribute	*Prawn*	*Silver perch*	*Barramundi*
	Formulation (%)		
Fishmeal (65P)	40 - 45	30 - 35	40 - 45
Marine invertebrate	5 - 10	–	–
Vegetable protein	5 - 15	20 - 25	5 - 15
Marine oil	1 - 2	1 - 2	3 - 5
Grain product	20 - 30	30 - 35	20 - 30
Other	5 - 8	5 - 8	5 - 8
Ingredient cost ($/t)	1050	750	900
Digestible protein (%)	>35	>33	>40
Digestible energy (kj/g)	13 - 14	13 - 14	15 - 16
Lipid (%)	8 - 10	8 - 10	10 - 15
Ash (%)	<12	<12	<12
EPA + DHA (%) [1]	>1.75	<0.8 (?)	>1.2

[1] Essential omega-3 fatty acids: EPA - Eicosapentaenoic acid (20:5n-3); DHA - Docosahexaenoic acid (22:6n-3).

awareness of the gross requirements of the different species is necessary to evaluate the extent to which alternative protein meals might be able to substitute for fish meal. Table 5 provides information on 'typical' high fish meal-based formulations, ingredient cost of the diets and key dietary specifications for each of the three target species. For more specific information on nutrient requirements, the reader is referred to Allan and Rowland (1992) for silver perch, D'Abramo et al. (1997) for prawns and Williams and Barlow (1996) for barramundi.

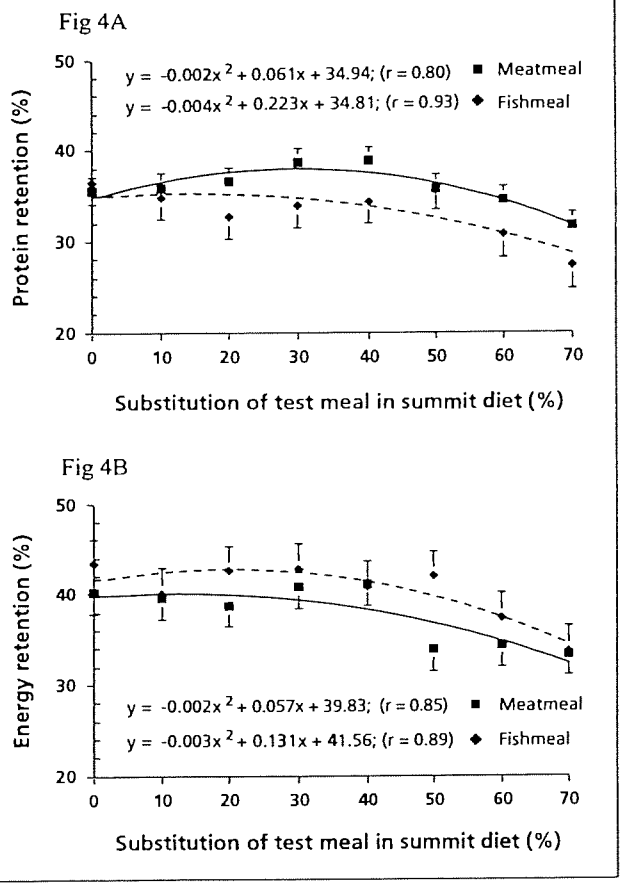
Nutrient assimilation of rendered meat meal

As has been amply demonstrated in pigs (Batterham et al. 1990), a considerable proportion of amino acids from heat-damaged proteins can be absorbed in a form that is not utilised, leading to their poor assimilation (utilisation) by the animal. Lysine, because of the reactivity of its epsilon amino group (malilard reactivity; Erbersdobler 1986), is most vulnerable to this type of damage with meat meals and high temperature processed vegetable protein meals such as cottonseed often suffering from this fate. Hence, digestibility alone may not be a reliable guide to the nutritive value of the meal. To address this issue, experiments were carried out with barramundi and silver perch to determine nutrient retention following the feeding of diets containing serial increments of meat meal. An aquarium study with prawns was also carried out to examine the effect of meat meal substitution of fish meal.

In the barramundi studies, the summit dilution method of Fisher and Morris (1970) was applied in combination with comparative slaughter procedures to quantify protein and energy retention. Barramundi were scale-fed by weight a series of diets wherein the test protein meal was substituted into a nutrient-complete (summit) diet at 10% increments up to 70%. Immediately prior to, and upon termination of the feeding experiment, representative samples of fish were taken for chemical analysis. Nutrient retention was determined as the difference between the pre- and post-feeding samples and expressed as a proportion of nutrient intake. The feeding period varied between 42 and 49 days for the various test ingredients. Presented in Figure 4 is a comparison between AMH-meat meal and Peruvian fish meal for protein (Fig 4A) and energy (Fig 4B) retention.

Fig 4

Effect of serial substitution of AMH-meat meal or Peruvian fish meal on protein (Fig 4A) and energy (Fig 4B) retention in barramundi



As is evident from Figure 4, net protein retention of the meat meal series diets was similar to, if not better than that for fish meal whereas the reverse was the case for net energy retention. However, the overall retention of protein was low (viz. <38% as compared to >50% for pigs; Williams et al. 1993) but similar to that of European bass (*Dicentrarchus labrax*) where retention of dietary protein has been reported to vary from as low as 16 to 18% (Hidalgo and Alliot 1988; Tibaldi et al. 1994) to more typical values of 40 to 46% (Ballestrazzi et al. 1994). Energy retention has similarly been

Table 6

Main constituents and critical nutrient content of experimental diets evaluating meat meal for silver perch at PSRC

Attribute	*Diet*				
	1	*2*	*3*	*4*	*5*
Fishmeal (Danish)	27.0	13.0	6.0	0	0
Meat meal (Fletcher)	0	6.3	7.8	8.9	8.9
Meat meal (Provine)	0	9.1	14.7	18.1	18.9
Blood meal	2.0	3.4	3.0	3.9	3.9
Soybean meal	20.0	20.0	20.0	20.0	20.0
Gluten (corn)	4.0	6.0	6.0	6.0	6.0
Grain product	39.3	35.1	34.9	35.0	35.2
Other[1]	7.1	7.1	7.6	8.1	7.1
Dig. protein (%)	32.1	34.0	34.1	34.1	34.0
Dig. energy (kj/g)	13.0	13.4	13.3	13.2	13.3
Lipid (%)	6.4	6.2	6.0	5.7	5.8
Ash (%)	10.0	11.2	11.2	11.2	11.2
Dig. lysine (%)	2.1	2.2	2.0	2.0	1.8
Dig. meth + cystine (%)	1.4	1.4	1.4	1.4	0.9
Dig. threonine (%)	1.4	1.4	1.4	1.4	1.3

[1] Vitamin, mineral, fish oil and crystalline amino acid supplements except for Diet 5 which contained no crystalline amino acids.

found to vary from 20% to >60% (Cho et al. 1982; Knights 1985; Steffens 1989) and to be affected particularly by the protein to energy (lipid) ratio of the diet. Given their carnivorous feeding habit and dependence on dietary protein for metabolic energy, it is not surprising that retention of dietary protein by barramundi is low. This is the most likely explanation why the relatively poorer essential amino acid composition of meat meal protein did not adversely affect protein deposition (somatic growth) in the barramundi. As a comparatively large proportion of the absorbed amino acids would have been used for energy rather than protein synthesis metabolism, the least abundant essential amino acids would presumably therefore be preferentially conserved for protein synthesis by the animal. Thus, the essential amino acid composition of the dietary protein appears to be much less important in barramundi (and almost certainly in other carnivorous aquatic animals where metabolic energy is derived mainly from dietary protein) than terrestrial monogastrics (pigs and poultry) where dietary protein is used predominantly as a source of amino acids for protein synthesis with carbohydrate being the primary source of metabolic energy.

The barramundi summit studies show protein and energy retentions did not decline until the dietary inclusion of AMH-meat meal (52% protein and 31% ash, air dry) exceeded 40% as also was found with fish meal. These results are conclusive evidence that meat meal is well assimilated by barramundi.

The effect of dietary substitution of fish meal by meat meal on silver perch productivity and nutrient retention was examined in a 65 d growth experiment. In the study, five diets (Table 6) were assigned in triplicate to 10 000 L tanks each stocked with 85 fingerlings (12 g initial weight).

Diets were formulated to examine the effect of serial replacement of the fish meal in a reference diet formulation while the essential amino acid composition of the diet was maintained using crystalline amino acids; the fifth diet examined the effect of full replacement of the fish meal but in the absence of any additional crystalline amino acids. A blend of Fletcher (lamb) and Aspen (Provine) meat meal was used to replace the fish meal.

In the study, growth rate declined when silver perch were fed diets containing less than 13% fish meal (Table 7). However, neither food conversion, protein efficiency nor protein retention was affected by fish meal replacement, including the treatment where all fish meal in the diet was replaced without additional crystalline amino acid supplementation.

Possible reasons for the reduced growth of the silver perch on the low fish meal diets are: a), insufficient essential nutrients; b), reduced attractiveness of the high meat meal diets; and/or c), some growth-reducing effect of the meat meal or conversely, some growth-enhancing effect of the fish meal. As digestible protein and digestible energy contents were similar for all diets, differences in protein to energy ratio do not explain the differences in growth rate. Similarly, essential fatty acid contents were equalised through the addition of fish oil and therefore would seem not to be the reason. Although the essential amino acid composition of meat meal was lower than for fish meal, this also was compensated for by crystalline amino acid supplementation. Had amino acids limited growth, a more marked difference between the amino acid supplemented (diet 4) and the non-supplemented (diet 5) diets would have been expected. While

Table 7

Performance of silver perch fed diets reducing in fish meal content in the tank experiment at PSRC

Attribute	*Diet and % fish meal inclusion*				
	1 27%	*2* 13%	*3* 6%	*4* 0%	*5* 0%
Growth rate (g/d)	0.93^{A}	0.93^{A}	0.83^{AB}	0.80^{B}	0.77^{B}
Food conversion (g:g)	1.5^{A}	1.4^{A}	1.5^{A}	1.5^{A}	1.5^{A}
Protein efficiency (g:g)	2.1^{A}	2.0^{AB}	2.0^{AB}	2.0^{B}	2.0^{AB}
Protein retention (%)	36.7^{A}	35.2^{A}	35.0^{A}	32.9^{A}	36.0^{A}

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

Table 8

Ingredient composition of diets[1] with increasing replacement of fish meal using two meat meal sources trialed with prawns in aquaria

Ingredient	*Diet designation*									
	Base	*Provine*				*Fletcher*				
		20%	*30%*	*40%*	*50%*	*20%*	*30%*	*40%*	*50%*	*60%*
Fishmeal (Tas)	38.9	23.3	15.5	7.7	0	27.1	21.2	15.4	9.5	3.6
Meat meal (Provine)	0	20.0	30.0	40.0	50.0	0	0	0	0	0
Meat meal (Fletcher)	0	0	0	0	0	20.0	30.0	40.0	50.0	60.0
Squid mince (dried)	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0
Gluten	6.0	6.0	6.0	6.0	6.0	6.0	6.0	6.0	6.0	6.0
Starch	20.0	20.0	20.0	20.0	20.0	20.0	20.0	20.0	20.0	20.0
Wheat	23.8	20.4	18.3	15.4	12.4	15.3	11.0	6.8	2.5	0
Other[2]	6.3	5.3	5.2	5.9	6.6	6.6	6.8	6.8	7.0	5.4

[1] Formulated to constant digestible protein content of 35.0%.
[2] Comprised vitamin, squid oil and essential lipid supplements.

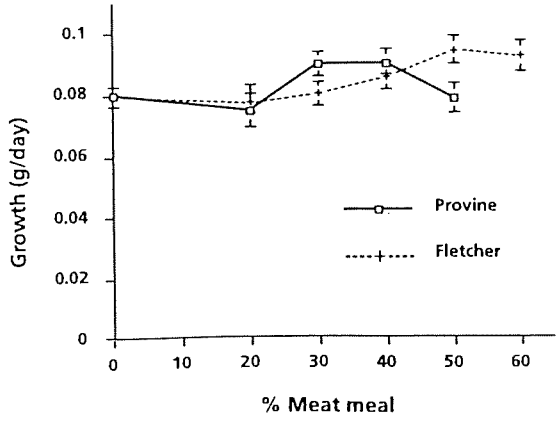
there is some conjecture about the efficacy of crystalline amino acids in aquatic animals (Lovell 1989; Cowey 1992), essential amino acid concentrations in all diets (including the non-supplemented diet 5) were above published requirements for channel catfish (NRC 1993) and moreover, protein retention was unaffected by diet. Food consumption of the fish on the high meat meal diets was lower than for those on the high fish meal diets which suggests a palatability effect. This is surprising as meat meal has been found to be highly attractive to other fish, including barramundi (Moshen and Lovell 1990; Watanabe et al. 1993; Williams and Barlow 1996). The increased concentration of saturated fatty acids in the high meat meal diets may have contributed to the apparent reduced palatability of these diets.

In the prawn aquarium study, Aspen (Provine) and Fletcher (lamb) meat meals were compared when each was used to serially replace fish meal while holding the digestible protein content of the diet at a constant 35%. The experiment was carried out over eight weeks with four replicates of aquaria (each with 6 prawns; 4.6 g initial weight) assigned to test each of the 10 dietary treatments (Table 8).

For the Base and Aspen diets, digestible energy content was held constant at 15 kJ/g whereas the higher ash content of the Fletcher meat meal resulted in digestible energy content progressively falling from 15 to 11.2 kJ/g as meat meal inclusion in the diet increased from 20 to 60%. The effect of meat meal substitution on prawn growth is depicted in Figure 5.

Fig 5

Average daily growth of juvenile prawns fed diets with increasing replacement of fish meal using two meat meal sources trialed with prawns in aquaria at CSIRO



For the Fletcher meat meal series, a significant ($P<0.05$) linear and curvilinear improvement in prawn growth accompanied the increased replacement of fish meal. The regression suggested that prawn growth was maximised with the diet containing 50% Fletcher meat meal. For the Aspen meat meal, the response trend line was not significant ($P>0.05$) but prawn growth reduced significantly ($P<0.05$) beyond 40% inclusion of the meat meal. Because of difficulty with prawn experiments in obtaining accurate food consumption data, it is unknown whether the observed prawn growth responses to fish meal substitution were manifested through altered food intake (palatability) or due to nutritional differences between the diets. The data, however, show that meat meals can be used to replace at least two-thirds of the protein in prawn diets containing 40% crude protein (35% digestible protein) without adversely affecting prawn growth.

Suitability and economics of meat meal under farm conditions

The partial or complete replacement of fish meal in diets for silver perch and barramundi diets has been examined under farm conditions. In the silver perch work, two least-cost diets (LC1 and LC2) were formulated to be of equivalent nutrient specification to the reference diet (SP35) when the fish meal was constrained to inclusions of either 10 or 5 % (Table 9). The LC1 and LC2 diets were 18 and 25% less expensive than the reference diet, respectively and contained up to ≈37% meat meal. All three diets were manufactured commercially and fed at 3% bodyweight/d to fish stocked in 0.1 ha ponds (1,500 fish/pond; 28 g initial weight) at the Grafton Research Centre (GRC). Three replicate ponds were assigned to each diet and the fish were cultured to a marketable weight of ≥400 g.

At the conclusion of the 6-month growing period, representative samples of the fish from each pond were taken for determination of chemical composition and organoleptic quality (sensory evaluation) using trained taste panels at QDPI's Centre for Food Technology. The production responses, chemical composition of the fish and sensory scores for indicative characteristics are detailed in Table 10.

Fish survival was excellent (>96%) and was unaffected by diet. Growth rate, food conversion and total pond productivity for the two least-cost diets were significantly better ($P<0.05$) than for the reference diet. The productivity cost expressed as the ingredient cost of the diet per kg fish weight gain decreased with increasing substitution of the fish meal, being approximately 70 and 65% lower for diets LC1 and LC2 respectively, compared to the reference diet. The chemical composition of the fish was unaffected by diet. The sensory evaluation of the fish showed only minor differences between diets with LC2 being significantly ($P<0.05$) less yellow coloured than for other diets. The texture of LC1 fish was more ($P<0.05$) flaky than for other diets. The overall liking of the fish was exceedingly high (score >66) and unaffected by dietary treatment. These results demonstrate unequivocally the cost-effectiveness of using meat meal (in combination with vegetable protein meals) to replace almost all of the fish meal in the diet.

Table 9

Main constituents and critical nutrient content of the Reference and least-cost diets trialed with silver perch in pond experiment at GRC

	Diet designation		
	SP35	*LC1*	*LC2*
	Formulation and cost (%)		
Fishmeal (Danish)	27.0	10.0	5.0
Meat meal (Fletcher)	–	21.7	36.9
Blood meal (ring)	2.0	2.1	–
Soybean meal (solv)	20.0	–	–
Gluten meal (corn)	4.0	3.8	5.2
Canola meal	–	–	5.0
Peanut meal	–	–	5.0
Field pea	–	14.9	10.4
Lupin (dehul)	–	25.5	7.4
Grain products	39.9	14.7	17.7
Other[1]	7.1	7.3	7.4
Ingredient cost ($/t)	756	619	566
	Composition (air-dry)		
Dig. protein (%)	33.0	33.7	31.9
Dig. energy (kj/g)	12.55	13.44	13.13
Lipid (%)	6.4	8.5	8.5
Dig. lysine (%)	2.05	1.94	1.85
Dig. meth + cystine (%)	1.51	1.42	1.35
Dig. threonine (%)	1.44	1.42	1.30

[1] Vitamin, mineral, fish oil and crystalline methionine supplements

In the barramundi work, a high fish meal control (Ctl) diet was evaluated against a commercial grow-out barramundi diet (C1 or C2 in Experiments B1 or B2, respectively) and two diets containing high inclusions of meat meal (Table 11). In Experiment B1, two sources of meat meal were compared, one being a conventional high-ash product from the Casino abattoir with a crude protein content of 52% while the other was a low-ash product from the Midco abattoir with a crude protein content of 60%. These two meat meals were compared when each contributed 55% of the total dietary crude protein content and diets (M1 and M2 respectively) were formulated to be isonitrogenous and isoenergetic with the Ctl diet. Diets M1 and M2 both contained 10% fish meal (contributing 15% of the total dietary protein content). In Experiment B2, the Casino meat meal was used as the major source of dietary protein in two

Table 10
Performance, fish composition and sensory evaluation of silver perch fed the reference and least-cost diets in the GRC pond experiment

Attribute	*Diet designation*			
	SP35	*LC1*	*LC2*	*Pooled sem*
Growth rate (g/d)	2.23[B]	2.53[A]	2.53[A]	0.06
Food conversion (g:g)	2.23[B]	1.97[A]	1.93[A]	0.05
Survival (%)	97.5[A]	97.0[A]	96.8[A]	0.7
Production (t/ha pond)	5.78[B]	6.28[A]	6.45[A]	0.11
Productivity cost ($/kg gain)	1.96[C]	1.22[B]	1.09[A]	0.04
Fish composition				
Dry matter (DM;%)	41.2[A]	43.1[A]	42.9[A]	0.7
Nitrogen (%DM)	6.18[A]	5.39[A]	5.68[A]	0.08
Ash (%DM)	7.9[A]	6.9[A]	7.7[A]	0.3
Fat (%DM)	50.9[A]	55.0[A]	54.4[A]	0.7
Energy (kj/g DM)	29.72[A]	30.55[A]	30.12[A]	0.21
Sensory evaluation[1]				LSD
Yellow appearance	7.3[A]	6.8[A]	3.6[B]	2.5
Fishy flavour	41.7	42.8	41.6	ns
Muddy flavour	7.9	10.4	8.4	ns
Flakey texture	21.6[B]	27.3[A]	22.3[B]	4.6
Overall liking	66.7	68.5	67.8	ns

[A,B,C] Within row comparisons, means without a common superscript letter differ (P<0.05); LSD, (P<0.05).
[1] All scores were scaled from zero (none) to 100 (very).

diets (M3 and M4) at the total exclusion of fish meal. Diet M3 was formulated to be isonitrogenous and isoenergetic with the Ctl diet while diet M4 was formulated to have a higher digestible energy content (15 vs 16.2 kJ/g, respectively) but with the same protein to energy ratio of 29 mg/kJ. All diets were commercially manufactured.

With the exception of diet M2 where a high-priced and specialised line of Midco meat meal was used, replacement of fish meal resulted in a 25 to 30% reduction in the ingredient cost of the diet.

Each 66 d feeding study was carried out on a north Queensland commercial barramundi farm with fish held in 2 m^2 cages (400 fish/cage; 280 g and 226 g start weight for Experiments B1 and B2, respectively) in an aerated freshwater pond. Four cages were assigned to each of the four diets in each experiment. The experimental fish were managed in the same way as for other fish on the farm, being fed to satiety once daily except on the weekend when fish were fed only on one of the days. At the conclusion of the feeding experiment, representative samples of the fish were taken for determination of dressing percentage and sensory evaluation at the QDPI's Centre of Food Technology.

There were no significant (P>0.05) differences between diets for fish productivity in Experiment B1 while in Experiment B2, fish fed the M4 diet performed best overall, growing faster (P<0.05) than those fed either Ctl or C2 diets (Table 12). Fish fed the Ctl diet in Experiment B2 had the lowest food issued and the best food conversion which was better than either M3 or C2 diets (P<0.05).

Using the low-ash, 60% protein Midco meat meal conferred no nutritional advantage over that of the conventional 52% protein Casino meat meal when each was included to provide similar protein contributions in diets formulated to be isoenergetic and isonitrogenous. However, increasing the estimated digestible energy content of the diet from 15.0 to 16.2 kJ/g (with a concomitant increase in protein content to maintain a constant protein to energy ratio) in Experiment B2 did improve fish growth rate and food conversion.

Replacing fish meal with Casino meat meal reduced the ingredient cost of the diet by 30% and also the food productivity cost ($ food cost/kg fish gain) by 18 to 23%. In the prevailing economic climate and in the absence of an environmental incentive, a low-ash meat meal could be expected to attract a price premium of about 15% above that of a high-ash product. This premium is primarily a function of the relative difference in protein content between the alternative products. Increasing the digestible energy content of the diet in Experiment B2 from 15 to 16.2 kJ/g caused a 10% increase in the ingredient cost of the diet (from $621 to $678/t) but this was offset by increased fish productivity such

Table 11

Formulation and critical nutrient composition of the low-fish meal diets fed to barramundi in two (B1 and B2) on-farm cage experiments

Attribute	*Diet description and experiment*						
	Ctl	*M1*	*M2*	*M3*	*M4*	*C1*[1]	*C2*[1]
Expt	*B1, B2*	*B1*	*B1*	*B2*	*B2*	*B1*	*B2*
			Formulation (%) and cost				
Wheat	30.4	18.1	29.9	16.1	10.4		
Fish meal (Chile)	35.0	10.0	10.0	0	0		
Meat meal (Casino)	0	45.0	0	50.0	50.0		
Meat meal (Midco)	10.0	0	40.0	0	0		
Blood meal (ring)	0	0	0	7.0	9.0		
Soybean (fullfat)	16.0	16.0	5.0	15.0	10.0		
Soybean (solvent)	0	0	5.0	0	0		
Gluten	5.0	5.0	5.0	5.0	10.0		
Fish oil	2.5	4.0	3.3	5.0	6.0		
Vit & min premix	1.1	1.9	1.8	1.9	4.6		
Ingredient cost ($/t)	884	650	1129	621	678	–	–
			Composition (air-dry basis)				
Dig. energy[2] (kj/g)	15.0	15.0	15.2	15.0	16.2	15.0	15.0
Protein (%)	43.8	43.0	43.0	42.5	47.8	54.3	50.1
Ash (%)	9.5	14.9	9.5	14.6	14.1	9.3	7.6
Lysine (%)	2.83	2.67	2.74	2.77	3.16	4.61	4.11
Meth + Cyst (%)	1.07	0.89	0.92	1.01	1.17	1.08	1.35
Threonine (%)	1.70	1.47	1.56	1.45	1.65	2.39	2.15

1 Two batches of commercial extruded grow-out barramundi diets. The composition and ingredient cost of the diets are commercial-in-confidence.

2 Estimated digestible energy (DE) values based on either derived digestibility of similar protein concentrate feed ingredients or assumed digestibility for non-protein feed ingredients. Values for the C diets are those stated by the manufacturer.

Table 12

Production responses and dressing-out percentage of barramundi fed low-fish meal diets in two (B1 and B2) on-farm cage experiments

Attribute[1]	*Diet*						
	Ctl	*M1*	*M2*	*M3*	*M4*	*C1/C2*	*± sem*
				Experiment B1			
Growth (g/week)	18.1^{A}	17.6^{A}	17.9^{A}			21.2^{A}	1.75
Food conversion (g:g)	1.44^{A}	1.43^{A}	1.47^{A}			1.25^{A}	0.070
Fish recovered (%)	97.6^{A}	96.6^{A}	96.8^{A}			98.3^{A}	0.85
Dressing-out (%)	88.8^{A}	89.5^{A}	89.6^{A}			88.6^{A}	0.30
Food cost ($/kg gain)[2]	1.27^{B}	0.93^{A}	1.65^{C}			–	0.060
				Experiment B2			
Growth (g/week)	20.8^{B}			$21.4A^{B}$	23.2^{A}	20.3^{B}	1.75
Food conversion (g:g)	1.22^{A}			1.44^{B}	$1.31A^{B}$	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 cost without allowance for processing. Information on the C diets is commercial-in-confidence.

Table 13
Indicative sensory evaluation data for barramundi fed low-fish meal diets in two (B1 and B2) on-farm experiments

Attribute[1]	*Diet*						
	Ctl	*M1*	*M2*	*M3*	*M4*	*C1/C2*	*± sem*
				Experiment B1			
Appearance							
Greyish	23.8	24.6	25.5			24.3	0.98
Yellow	8.6	9.4	8.7			6.9	0.75
Flavour							
Fishy	35.2^{AB}	37.3^{A}	36.8^{A}			33.0^{B}	1.30
Sweet	19.2^{B}	21.7^{A}	22.4^{A}			18.1^{B}	1.28
Muddy	16.1	18.2	14.8			17.7	1.57
Texture (firm)	31.5^{B}	36.1^{A}	35.9^{A}			37.1^{A}	1.71
Overall liking	58.4	62.1	62.8			59.3	1.23
				Experiment B2			
Appearance							
Greyish	10.5			9.7	10.5	9.5	1.18
Yellow	6.9			9.1	8.8	7.6	1.26
Flavour							
Fishy	49.0			45.5	47.3	46.8	1.29
Sweet	29.9			27.5	29.9	28.9	1.50
Muddy	14.8			15.9	13.9	16.6	1.55
Texture (firm)	46.5			44.3	46.9	47.3	1.72
Overall liking	60.0			61.2	64.3	63.5	1.65

[1] All scores were scaled from zero (none) to 100 (very). Within row compasrisons, means without a common superscript letter differ (P<0.05).

that the food productivity cost was essentially the same (0.89 vs 0.88 $/kg gain).

Differences between diets in sensory scores (Table 13) were evident only in the flesh of fish from Expt B1 where stronger (P<0.05) fishy and sweet flavours were found for the M1 and M2 diets as compared to the C1 diet; fish fed the Ctl diet were softer in texture than those fed other diets. Irrespective of diet, the overall liking of the flesh of the fish was very high (scores of >58) and with scores for all undesirable taints such as 'muddy', 'weedy' or 'metallic' being very low (≤ 18).

The results of these experiments demonstrate the suitability of meat meal as a partial or complete replacement of fish meal in diets for on-growing barramundi and without reducing consumer acceptance. However, particular attention was taken in the present work to ensure that all experimental diets were supplemented with sufficient fish oil to satisfy the fish's requirement for omega-3 fatty acids. Such supplementation would have ensured not only that a desirable 'fishy' flavour was maintained in the flesh but also that high amounts of omega-3 fatty acids were present to satisfy a growing consumer awareness of their health benefits.

Meat meal, either alone or in combination with other terrestrial protein concentrates such as soybean meal and gluten meal, has been successfully used for the partial replacement of fish meal in diets for cultured carnivorous fish including channel catfish *Ictalurus punctatus* (Mohsen and Lovell, 1990; Lovell, 1992) yellowtail *Seriola quinqueradiata* (Shimeno et al., 1993a,b), rainbow trout *O. mykiss* (Watanabe et al., 1993; Yamamoto et al., 1995). sea bream *S. aurata* (Davies et al., 1991) and European sea bass *D. labrax* (Langar and Metailler, 1989). In these cited studies, meat meal was used to replace from 30 to 91% of the protein contributed by fish meal without any adverse effect on fish growth. Higher levels of fish meal replacement were not examined possibly for fear of the diets not being palatable to the fish. The only known report where meat meal has been used as a replacement of fish meal in diets for barramundi is the French study of Aquacop et al. (1991). They found including greaves meal (a rendered high fat meat meal product) at 22% of the diet as a partial substitute of fish meal to have no adverse effect on its digestibility nor did it cause a decrease in the performance of the fish. In more herbivorous/omnivorous species, meat meal has similarly been found to be suitable for the partial replacement of fish meal in tilapia *Oreochromis mossambicus* (Davies et al. 1989) and prawns (Tacon 1993).

In our work, we observed no reluctance on the part of barramundi and prawns to consume high meat meal-based diets. There was some indication from the silver perch tank study that diets containing less than 13% fish meal (and >25% meat product) may have been less palatable than those of higher fish meal content.

Conclusions

Meat meal was shown in this work to be suitable to replace at least two-thirds (prawns and silver perch) and up to all (barramundi) of the fish meal protein in the diet without any adverse effect on 'fish' productivity. Moreover, high dietary inclusions (≥30%) of meat meal did not detract from the taste of the produced prawns and fish, and with barramundi, even when all of the fish meal was replaced in the diet. Based on these findings, meat meal has the potential to become a major protein source for aquaculture diets. At a conservative dietary inclusion of 20%, the Asian aquafeed market alone could absorb 500,000 t of meat meal – Australia's total annual production – and the amount required is expected to double if not treble by the year 2025 if the predicted expansion of aquaculture in the region is realised.

Under the prevailing economic conditions in Australia, the substantial replacement of fish meal by meat meal would result in an appreciable reduction in the ingredient cost of the diet: from 10 % for prawns to at least 25% for silver perch and barramundi. Other than for potential environmental benefits, there was no advantage in using low-ash meat meal over that of the more conventional high-ash product. However, the potential pollution impacts of aquafeed can only increase environmental concerns and for the long-term sustainability of aquaculture it is imperative that only highly digestible and nutrient dense aquafeeds be used. Thus, the increased use of meat meal products in aquafeeds can only be advocated if low-ash products are available and competitively priced with alternative high quality vegetable protein meals.

To facilitate the use of meat meal in aquaculture diets it is recommended that meat meal manufacturers be encouraged to produce meat meals that are high in protein (>60%) and low in both ash (<20%) and fat (<7%). To be price competitive, meat meals with less than 55% crude protein need to be no more expensive on a per unit digestible protein basis than high quality vegetable protein meals such as soybean meal. Meat meals containing above about 60% crude protein could attract a price premium of from 15 to 20% (on a per unit of digestible protein basis) but only if the fat content is kept below about 7 to 8%.

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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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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.

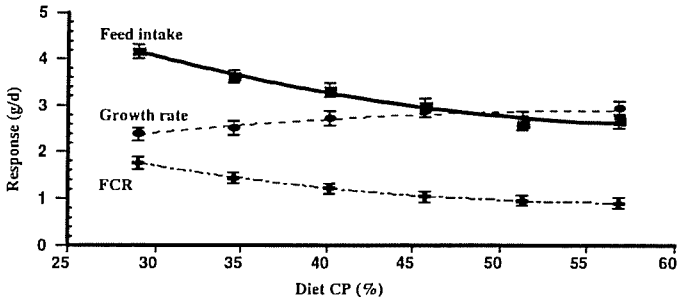
The palatability and nutritive value of ring-dried blood meal for barramundi was examined by growth assay. Inclusion of blood meal in the diet (from 0 to 22.5% at 7.5% increments) at the expense of isonitrogenous amounts of gluten had no adverse effect on the apparent palatability of the diet but FCR 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

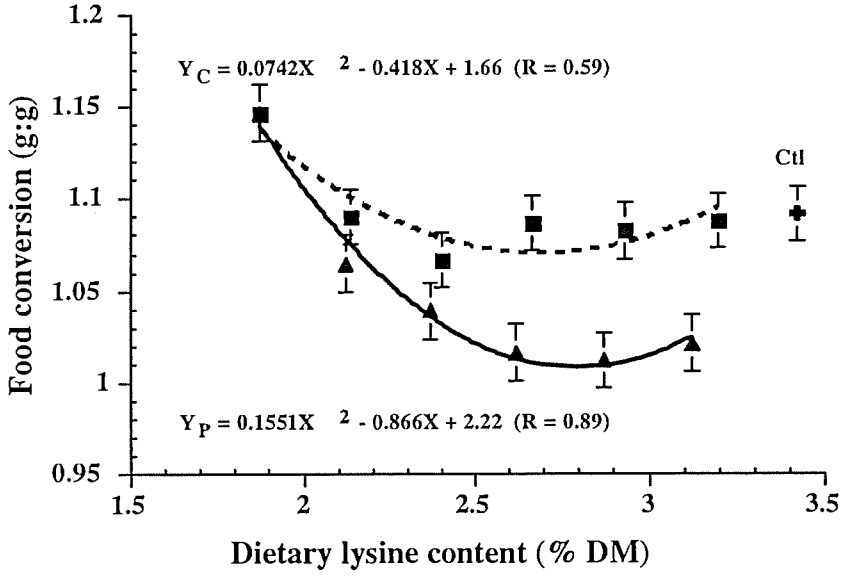
A major difference between marine and terrestrial protein sources is the marked difference in the amino acid make up of the protein. Compared to fishmeal with an amino acid index of 100, terrestrial plant protein sources are very low in methionine (20 to 80), lysine (20 to 85) and threonine (55 to 85). 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.

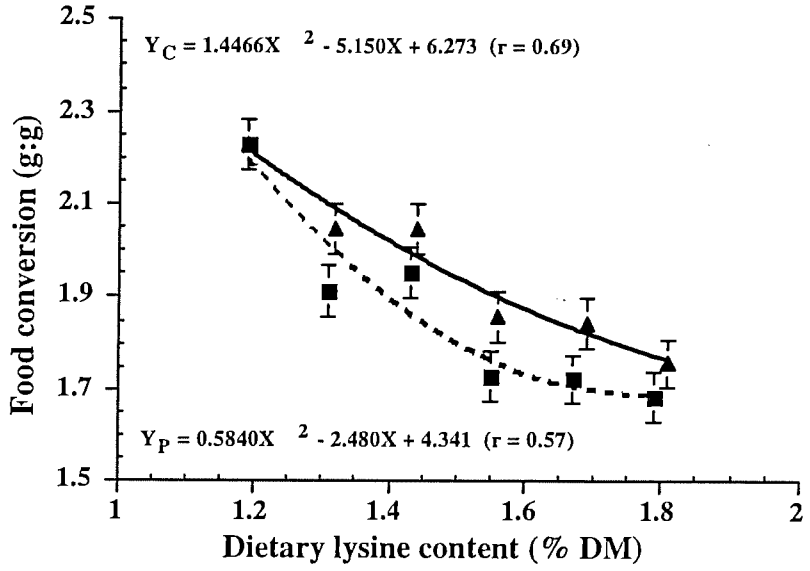
In the 54% DM protein experiment, increasing the lysine content of the diet from 1.87% (3.4% of protein) to 3.2% (5.6% of protein) resulted in a significant ($P<0.05$) quadratic improvement in average and specific growth rates and FCR for both the crystalline and protein-bound amino acid diet series; 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

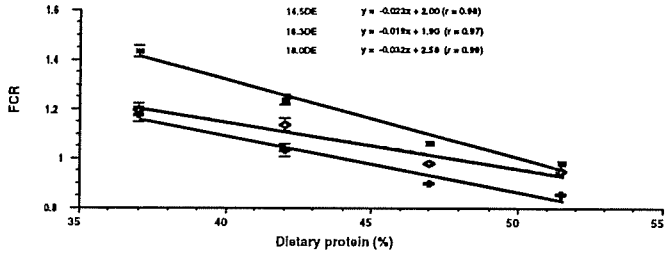
Intensive farming of barramundi in closed (recirculation) systems is becoming more important in Australia in order to gain better control of the production system. Invariably, such intensive systems have a high capital investment and their profitability depends on how successful the higher investment 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.

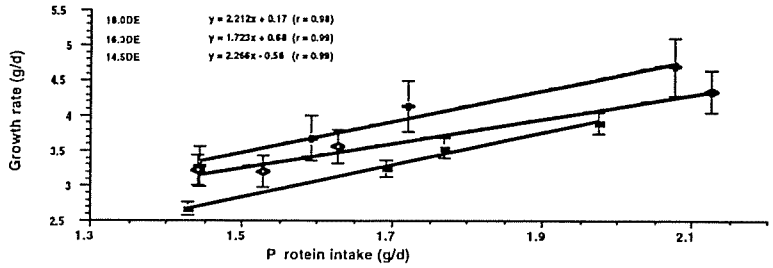
FCR improved linearly as the protein concentration of the diet increased from 37.4 to 51.7% and also was better at each increase in energy concentration (Fig. 4). FCRs were excellent and at the highest protein level were 0.99:1 and 0.85:1 for the lowest and highest energy concentrations, respectively.

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.