

# **Aquaculture Diet Development Subprogram: Diet Validation and Feeding Strategies**

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**FRDC Project No. 1996/393**

**December 2003**

NSW Fisheries Final Report Series  
No. 60  
ISSN 1440-3544



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**Published By:** NSW Fisheries  
**Postal Address:** PO Box 21, Cronulla NSW 2230  
**Internet:** [www.fisheries.nsw.gov.au](http://www.fisheries.nsw.gov.au)

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ISSN 1440-3544

## TABLE OF CONTENTS

<b>TABLE OF CONTENTS.....</b>	<b>I</b>
<b>ACKNOWLEDGMENTS.....</b>	<b>II</b>
<b>NON-TECHNICAL SUMMARY .....</b>	<b>III</b>
1. BACKGROUND .....	6
2. NEED .....	8
3. OBJECTIVES .....	8
4. RESULTS / DISCUSSION .....	9
4.1. <i>On-farm and laboratory validation of commercial barramundi diets: report commissioned by a commercial feed manufacturer (C1).....</i>	9
4.2. <i>Laboratory validation of experimental and commercial barramundi diets: a report commissioned by a commercial feed manufacturer (C2).....</i>	28
4.3. <i>Laboratory validation of experimental and commercial barramundi diets: a report commissioned by a commercial feed manufacturer (C1).....</i>	37
4.4. <i>Laboratory and farm validation of high specification barramundi diets: a collaborative research project between FRDC Aquaculture Diet Development Subprogram and a commercial feed manufacturer (C2) .....</i>	46
4.5. <i>Efficacy of dehulled lupin meal in diets for the black tiger prawn, Penaeus monodon .....</i>	72
4.6. <i>In-pond evaluation of high meat meal diets for the black tiger prawn: report to Meat &amp; Livestock Australia PRCOP.011 .....</i>	78
4.7. <i>The effect of feeding frequency on water quality and growth of the black tiger shrimp (Penaeus monodon).....</i>	87
4.8. <i>Development of a feeding strategy for silver perch (Bidyanus bidyanus) based on restricted rations .....</i>	98
4.9. <i>Performance and sensory evaluation of silver perch (Bidyanus bidyanus Mitchell) fed soybean or meat meal-based diets in earthen ponds.....</i>	124
5. BENEFITS .....	142
6. FURTHER DEVELOPMENT .....	143
7. CONCLUSION .....	144
8. STAFF .....	145
9. PUBLICATIONS .....	146
10. APPENDICES .....	152
10.1. <i>Sensory Profiling of Barramundi</i>	
10.2. <i>Economic Comparison of Dietary Productivity</i>	
10.3. <i>Guidelines for Feeding Silver Perch</i>	

## **ACKNOWLEDGMENTS**

We would like to thank the following technical staff at all locations for their dedication. At QDPI Freshwater Fisheries and Aquaculture Centre, Walkamin, Les Rodgers, Ian Hockings, Clarita Agcopra, Karina Ryan and Ian Ruscoe; at the NSW Fisheries Grafton Aquaculture Centre, Charlie Mifsud, Mark Nixon, Peter Boyd and Carole Bryant; at the CSIRO Marine Laboratory, Cleveland, Michelle Burford, Simon Tabrett, Margaret Barclay, Leigh Whitlock and Simon Irvin. We would also like to thank the staff at the QDPI Centre for Food Technology and the UNSW Centre for Chemosensory Research for their taste panel evaluation of barramundi and silver perch respectively. This research was funded by the Fisheries Research and Development Corporation, Aquaculture Diet Development Subprogram with additional support from the Grain Research and Development Corporation, Meat and Livestock Australia, Ridley Agriproducts Pty Ltd and Pivot Aquaculture. The assistance of Mrs Helena Heasman in organising meetings, preparing and distributing newsletters and facilitating communication is gratefully acknowledged. Mrs Heasman assisted with the preparation of this report.

## NON-TECHNICAL SUMMARY

96/393                      Aquaculture Diet Development Sub-Program: Diet Validation and Feeding Strategies

**PRINCIPAL INVESTIGATOR:**            Dr Chris Barlow

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### OBJECTIVES:

1. Demonstrate the cost-effectiveness of the diets incorporating 'high priority' Australian ingredients which meet determined or published nutrient requirements for fish/prawns reared in commercially applicable facilities.
2. Identify optimum feeding schedules, based on the combined costs of feeds and labour associated with feeding, for the on-growing of barramundi and silver perch to market size (0.5-3 kg).
3. Determine the potential for compensatory growth in silver perch and barramundi.
4. Determine the organoleptic qualities of fish/prawns fed different diets.
5. Communicate the research findings to industry and the scientific community by means of technical and scientific publications.

### OUTCOMES ACHIEVED

- Validation of previous research with ingredients and nutritional requirements to show fishmeal can be completely replaced in high performance diets for silver perch using inexpensive meat meal and wheat.
- Validation that the majority of fishmeal can be replaced in high performance diets for barramundi (with meat and bone meal).
- Validation that a substantial proportion of fishmeal can be replaced in high performance diets for black tiger prawns with meat meal or dehulled lupins.
- Cost effectiveness of alternative diets to all species was demonstrated. For silver perch, diet ingredient costs have been more than halved compared with initial formulations based on fishmeal and soybean meal.
- For barramundi, the performance and cost-effectiveness of nutrient dense diets, including nutritional dense diets where much of the fishmeal was replaced with meat meal, were validated.
- Optimal feeding schedules were determined experimentally for silver perch and prawns. For silver perch a comprehensive series of eight experiments were conducted allowing compilation of feeding tables for different size fish cultured under different temperatures. For prawns, feeding frequency was studied and results indicated that the number of feeds delivered per day could be reduced to 3 (from up to 6 commonly used) without affecting performance.
- Although daily feeding for silver perch was found to be unnecessary for larger fish during cooler months, no clear evidence for growth compensation was found.

- Organoleptic properties of both barramundi and silver perch were investigated in the recognition that feeding diets based on alternative ingredients to fishmeal might negatively affect taste and this might be unacceptable to farmers. Provided fish oil was provided in diets, totally replacing fishmeal in diets for silver perch or barramundi did not negatively affect taste or other organoleptic properties of this species.
- This research has been commercialised. Barramundi diets developed by researchers or by commercial feed formulators using information supplied by researchers involved with the FRDC ADD sub-program (or the earlier FMR sub-program) are now the most effective barramundi diets sold in Australia or Southeast Asia. All commercially available silver perch diets are based solely on results from the ADD sub-program research (or the earlier FMR sub-program). The latest and best (cheapest, no fishmeal, produces best tasting fish) silver perch diet (GRC3) has yet to be commercially manufactured. The company selling the most silver perch feed were forced to abandon a commercial trial of this diet due to problems at the farms chosen for the trial and, because of the marketing success they have enjoyed with the second last ADD silver perch diet (95LC2), they have been reluctant to change. For prawns all information has been transferred to the main prawn diet manufacturer in Australia. For all species, one of the greatest benefits has been the demonstration that diets containing alternative ingredients to fishmeal can be just as effective as fishmeal-based diets. As the price of fishmeal continues to increase, the value of this research will continue to increase.
- Some of the research for project 96/393 remains commercial-in-confidence. In addition five scientific manuscripts and 60 articles or presentations have been published, submitted or prepared.

The first aim of this project was to ‘validate’ on a large, commercially relevant scale, research on ingredient evaluation and nutritional requirements that indicated that diets based on Australian agricultural ingredients (instead of imported fishmeal) could be successfully used. The second aim was to determine experimentally based feeding strategies in the recognition that inappropriate feeding, either overfeeding or underfeeding can add significantly to the cost of aquaculture.

The first aim was achieved through a series of three separate grow-out experiments with barramundi (fish taken to market size in cages with ponds), two long-term experiments in replicated earthen ponds with silver perch (fish taken to market-size) and two long-term studies with tiger prawns. All these studies showed that ‘new’ diets out-performed earlier diets in terms of fish/prawn performance and/or cost-effectiveness. For barramundi, nutrient dense diets, although found to be more expensive, were more cost-effective. Meat meal was shown to be capable of totally replacing fishmeal in standard formulations and was also capable of replacing the majority of fishmeal in nutrient/dense diets without affecting organoleptic properties, provided fish oil was included.

Soybean meal was used as the primary protein source to replace all but 5% of fishmeal in the first experiment with silver perch. In the second experiment, meat meal and wheat were the primary ingredients. In both experiments, “new” diets were compared with the best previous diets. Fish oil (up to 7%) was used in all diets and fish performance was unaffected even when fishmeal was totally replaced. The best diet, in terms of performance, lowest ingredient cost, and being ranked as the ‘overall best’ in taste panel studies had no fishmeal and was comprised mainly of meat meal and wheat. Silver perch diets are now the cheapest of any aquaculture diets for carnivorous or omnivorous species sold in Australia.

Prawn pellets must be highly water stable as it can be several hours before they are consumed. In addition, prawns macerate their food before consumption. Because of these factors, the effects of new diets on water quality must be assessed. Two experiments with prawns were done one where dehulled lupins were used to replace fishmeal and the other where meat meal was used. During both experiments, water quality was not negatively affected by substitution of fishmeal and



performance not reduced even when diets contained 25% lupins or 50% meat meal. The economic impacts of ingredient substitution with prawns were encouraging.

Research on optimal feeding schedules was done with silver perch and prawns. For silver perch, eight experiments were completed with small fingerlings (initial mean weight 2.0 g), large fingerlings (15-28 g) and large fish (163-511 g) at different water temperatures. Different frequencies and/or feeding rates were compared. This allowed the determination of accurate feeding tables for a range of fish sizes and water temperatures. Recommendations for broodfish and fish at temperatures below those tested were also derived following extensive consultation with farmers.

For prawns, effects of 3, 4, 5 or 6 feeds/day on performance were evaluated. This research showed that there was no advantage in feeding more than 3 times/day, potentially saving on labour costs associated with more frequent feeding.

Results have been extensively reported to farmers, feed manufacturers, ingredient suppliers and other researchers and form the basis for new, high performance diets for barramundi, silver perch and prawns with reduced reliance on fishmeal. New diets do not negatively impact on the taste of the cultured fish/prawns.

## 1. BACKGROUND

This project is the delivery arm of a series of previous and current related studies on the development of cost-effective diets for Australian aquaculture. It has two thrusts: firstly, the validation demonstration on a commercial scale of diets developed within the related studies; and secondly, the identification of optimum feeding schedules for fishes with different dietary or trophic habits.

The FRDC nutrition subprogram has targeted four species (barramundi, silver perch, Atlantic salmon and prawns) as “template” species for the development of aquaculture diets based on Australian feedstuffs and with minimal use of fishmeal. These species were chosen as they are representative of animal groupings with very different feeding habits (i.e. a warm water carnivorous fish, an omnivorous fish, a coldwater carnivorous fish and a crustacean). Thus the information developed for these species should be widely applicable to other aquaculture species. This application is for research on three of these species; barramundi, silver perch and prawns. It is proposed that research on salmon will commence in 1997/98 if results from current research indicate a clear priority for new research on diet validation and feeding strategies for this species.

Considerable data has already been generated on replacement of fishmeal and the suitability of a range of alternative protein and energy sources for diets for the target species. The bulk of the work has, of necessity, been done under highly controlled laboratory conditions. It is now necessary to bring the information together and formulate diets for testing under commercial conditions. Such commercial scale validation of the diets is an essential step in the research and development process. Neither feed millers nor farmers will readily change their diets and feeding practices without firm evidence that the change will have a commercial impact.

Diets are a major component of feed and feeding costs, but feeding practices also need to be optimised to lower operating costs. Despite this, there has been very little work done to evaluate the economics of different feeding strategies. Farmers have consistently pointed out that if they could avoid feeding on just one weekend day, without compromising fish performance, they could save approximately 20-25% of the costs of labour associated with feeding. It has even been suggested that with carnivorous species it may be possible to skip a feed... that is, feed only every second day, and still get the same performance. This conjecture is based on the demonstrated ability of some species for compensatory growth, or ‘catch-up’ growth following a non-feeding period (Quinton & Blake 1990; Sullivan & Smith 1982). Optimal feeding strategies for each of our target species are likely to vary because of the trophic habits – in their natural habitat carnivorous species feed on larger meals less often than omnivorous species, which feed more regularly and on smaller quantities.

Clearly, there is a substantial economic benefit to the farmer in identifying the minimum feeding pattern to produce satisfactory fish growth. The costs of labour associated with feeding are minimized. In addition, the feed conversion will be maximized, which in turn will minimize wastage (both uneaten food and metabolic wastes). The overall effect of minimal wastage will benefit both the farmer (better water quality for the fish) and the downstream environment (less release of nutrients).

## References

- Quinton J.C. & Blake R.W. (1990) The effect of feed cycling and ration level on the compensatory growth response in rainbow trout, *Oncorhynchus mykiss*. J. Fish Biol., 37: 37-41.
- Sullivan K. & Smith K. (1982) Energetics of sablefish, *Anoptopoma fimbria*, under laboratory conditions. Can. J. Fish. Aquatic Sc., 39, 1012-1020.

## 2. NEED

The need for on-farm validation of diets developed under experimental conditions is obvious. Aquaculturists, like all people adopting new technology, need to test the product themselves to be sure of its usefulness. Similarly, feed millers need to know that the diets will be effective on the farms of their clients. We have consistently found that the best way to ensure uptake of research information is via on-farm trials, as the farmers readily integrate the practical demonstration of the research results.

One of the major costs in aquaculture in Australia is labour associated with feeding. Farmers contend that feeding once per day, rather than more often, and not feeding on one or both weekend days, could significantly reduce costs if fish performance was not overly affected. Producer groups from several sectors of the aquaculture industry have identified a need to test different feeding strategies for different sized fish, to establish feeding protocols which will give optimum return of fish for minimal expenditure on feed and labour.

Compensatory growth (growth following periods of starvation allowing fish to 'catch up' to continuously fed fish) has been demonstrated for several species. As many Australian species have evolved in alternating wet and dry regimes where periods of low food availability are common, it is worth investigating if compensatory growth occurs in the species targeted in the Aquaculture Diet Development Subprogram.

## 3. OBJECTIVES

1. Demonstrate the cost-effectiveness of the diets incorporating 'high priority' Australian ingredients which meet determined or published nutrient requirements for fish/prawns reared in commercially applicable facilities.
2. Identify optimum feeding schedules, based on the combined costs of feeds and labour associated with feeding, for the on-growing of barramundi and silver perch to market size (0.5-3 kg).
3. Determine the potential for compensatory growth in silver perch and barramundi.
4. Determine the organoleptic qualities of fish/prawns fed different diets.
5. Communicate the research findings to industry and the scientific community by means of technical and scientific publications.

## 4. RESULTS / DISCUSSION

### 4.1. On-farm and laboratory validation of commercial barramundi diets: report commissioned by a commercial feed manufacturer (C1)

**Christopher G. Barlow<sup>1</sup>, Kevin Williams<sup>2</sup>, Les Rogers, Ian Ruscoe<sup>1</sup>, Ian Hockings<sup>1</sup> and Clarita Agcopra<sup>1</sup>**

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#### Introduction

Since 1993, a research program supported by the Fisheries Research and Development Corporation (FRDC) has been conducted at QDPI's Freshwater Fisheries and Aquaculture Centre (FFAC), Walkamin, to develop more cost-effective grow-out diets for barramundi. The approach has been to define the requirements of juvenile barramundi for key nutrients such as protein (amino acids) and essential fatty acids, to see how seasonal changes in water temperature affect these requirements and then to devise feeding strategies that optimise fish growth under varying seasonal conditions. In parallel with this work at Walkamin, the nutritional value of locally available feed ingredients has been determined for barramundi at QDPI's Bribie Island Aquaculture Research Centre as part of a national FRDC project seeking to reduce Australia's dependency on fishmeal in diets for farmed prawns and fish.

The results of the Walkamin and Bribie Island research led to the development of new grow-out barramundi diets in which the primary protein source was meat meal. In a project funded by the Meat Research Council (MRC) in 1996, two on-farm trials and a laboratory-based trial at the FFAC were conducted to compare two of the new diets, a "control" fishmeal-based diet and a commercial barramundi diet. The results of the trials (reported in detail in Williams & Barlow (1996)) were as follows.

- The meat meal based experimental diets were equal to or better than the commercial barramundi diet in supporting fish growth and in producing fish with a high sensory value.
- Using conventional high-ash meat meal as a partial or complete 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 productivity cost of the food.
- Diets based on the meat meal and containing no fishmeal were as palatable to the fish and supported equivalent or superior fish productivity as one 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, were found by trained taste panel assessment to be liked as well or better than fish reared on a diet formulated on a high fishmeal content.

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

A commercial feed manufacturer (C1) expressed an interest in the new formulations, and funded a further trial involving major barramundi producers in an on-farm evaluation of the best of the new diets. It was considered that involvement of the major producers would facilitate uptake of the results and thus the new dietary specification by industry. The results of this trial form the basis of the second section of the present report.

Subsequent to the on-farm trials, a laboratory-based trial was undertaken using the same test diets as used in the on-farm trial. This was done to further validate the new specifications, and to examine the growth performance of barramundi in fresh and salt water. The results of the laboratory trial, detailed in the third section of the present report, were contrary to that anticipated from the MRC trials and the on-farm trial, in that the meat meal diet resulted in markedly reduced growth response compared with the commercial fishmeal based diet. The reason for the deteriorating performance of the meat meal diet was not clear, although a more rapid ageing of the meat meal diet compared with the fishmeal diet was implicated. The conclusions from the on-farm and laboratory trials, and some considerations for future commercialisation of new barramundi diets, are discussed in the fourth section of the report.

#### ON-FARM VALIDATION OF BARRAMUNDI DIETS

The objective of the trial was to examine the comparative growth performance of barramundi when reared on commercial farms and fed either a commercial fishmeal based diet (C1 15MJ), an experimentally derived meat meal based diet (Walkamin summer), or an industry requested, high energy diet.

Specific aims of the trial were as follows:

1. demonstrate on commercial barramundi farms the suitability of least-cost diets for rearing juvenile barramundi to market size (400–500 g);
2. examine the response of barramundi to a high energy diet formulation based on least-cost feed ingredients;
3. determine the cost effectiveness of the different diet formulations as assessed by food costs, live and dressed weight gains and mortality data; and
4. evaluate by taste panel assessment the eating quality of the reared fish after being fed the diets.

### **Materials and Methods**

#### Experimental design

The trial was conducted on two commercial barramundi farms in northern Queensland, beginning on one farm on the 26<sup>th</sup> September and concluding on the 5<sup>th</sup> December 1996, and beginning and ending on the other one day later. One farm, Bluewater Barramundi, is located within the tidal estuary of the Hinchinbrook Channel, therefore operating under saline conditions. The other farm, Barramundi Waters, is located inland adjacent to Liverpool creek and operates in fresh water.

The experimental design at both sites was 3 treatments with 5 replicates, arranged in a randomised block design. Each cage on both farms was stocked with 200 fish. The experimental units on both farms consisted of 10 mm knotless mesh fabric cages measuring 8 m<sup>3</sup>, suspended from floating 100mm PVC 2\*2 m squares. These cages are typical of those used on commercial farms for rearing juvenile barramundi.

At Bluewater Barramundi (salt water) a grid of 24 cages, (6 \* 4, with only 15 being used for the trial), was allocated as the experimental area. Each block of three cages (1 replicate) was arranged so that each cage was adjacent to another cage within its own block. There was a considerable tidal influence on the farm, so the cages were arranged so as not to advantage or disadvantage any

particular treatment. In an effort to maintain oxygen concentrations in the experimental cages, water from outside, but adjacent to the 24 cage grid, was pumped into each experimental cage. A venturi was made in the pipe so as to oxygenate the water which was then sprayed into the cages from a height of approximately 40 cm.

At Barramundi Waters (fresh water) a small triangular pond of approximately 0.1 hectare was used. The water within the pond was slowly circulated with two diagonally opposed aspirators. The aspirators were not in close proximity to any experimental cages. The experimental units were arranged in blocks along a straight walkway. Each cage was aerated to ensure dissolved oxygen levels were not limiting.

#### Feeds and feeding

The three treatments were the same on both farms and consisted of the commercially available C1 15MJ pellet, the Walkamin summer diet and a high energy diet. The proximate composition is shown in Table 1.

**Table 1.** Proximate composition of 3 experimental diets tested in a farm trial on grow-out of barramundi under commercial conditions.

	C1 15MJ %	Walk Sum %	Hi - E %
DE kJ/g	15.4	14.9	17.9
CP %	47.5	42.3	31.5
Fat %	9.3	11.9	22.8
Ca %	1.9	4.5	2.3
P %	1.4	2.3	1.2

At both farms the experimental cages were subject to the same husbandry and management practices as other fish on the respective farms. However there was a difference in feeding regimes. At Bluewater Barramundi the fish were fed daily between 8 and 9 am until there was no further observed feeding. At Barramundi Waters, due to labour constraints, the fish were fed usually only four times per week between 3 and 4 pm until no further feeding was observed.

Approximately 4 weeks into the trial, significant mortalities were observed at Bluewater Barramundi. The cause was diagnosed as a streptococcal infection. At this time the food was supplemented with the antibiotic Trimethosol. Each day, for the remainder of the trial, the feed for each cage was lightly wetted with a calculated dose of Trimethosol in solution, allowed to dry and was then fed to the cage.

As well as differences in nutrients, the pellets also differed in physical dimensions (even though they were all manufactured to be 4 mm pellets). Consequently, volume and sink rate tests were conducted on 100 randomly selected pellets per diet.

#### Dressing-out and sensory evaluation of fish

At the end of the trial, a sample of 5 fish from each cage was taken for dressing out and sensory evaluation. Dressing out percentage was determined as the weight proportion of fish remaining after evisceration and removal of the gills. These same 5 fish (per cage) were then frozen and transferred to QDPI's Centre for Food Technology for evaluation of colour, flavour, odour and

texture characteristics and for overall palatability of the cooked flesh. Details of the methodology employed for preparing, cooking and evaluating the fish are contained in Appendix 1.

#### Chemical and statistical analysis

Water quality was measured every other day on both farms with a T.P.S. FL90 water checker. The light penetration was measured using a Secchi disc. Nutrient and energy analyses were done on a representative sample of the three diets using recommended methods of the Association of Official Analytical Chemists.

Sensory evaluation data were collected directly onto computers and analysed by ANOVA with differences between means tested for significance at 5% probability using the range simultaneous test procedure of Tukey.

Initial and final weights, growth (average daily gain), survival, food conversion and dressing percentage were compared using analysis of variance. In each case residuals were examined for homogeneity, in all cases data transformations were not necessary. Pair-wise comparison of treatment means were made with the least significant difference test only where the ANOVA F values were significant ( $P < 0.05$ ). All statistical tests were performed at the 5% significance level.

## **Results**

#### Production data

There was a significant interaction ( $P < 0.05$ ) between diet and site for many of the performance attributes. These interactions and means for main treatment effects are listed in Table 2. To simplify presentation, production data for each farm are treated separately below.

The initial and final weights (means and standard errors) of the fish in each treatment on both farms are shown in Figures 1 & 2. There was a significant difference in final weight between treatments on both farms. In both cases the C1 15MJ and Walkamin Summer diet performed equally as well and significantly better than the high energy diet.

Survival also varied between treatments, with the same trend being seen on both farms. Survival for the 15MJ and Walkamin Summer diets exceeded 97.5% on both farms, but for the high energy diet it was 88% on the freshwater farm and 91% on the saltwater farm (Figure 3).

Because of uneven mortality between treatments and poor performance by fish fed the high energy diet, the food conversion ratio was adjusted for 100% survival. There was no significant difference between 15 MJ and Walkamin Summer diet, and these diets had significantly lower FCRs than the high energy diet on both farms (Figure 4).

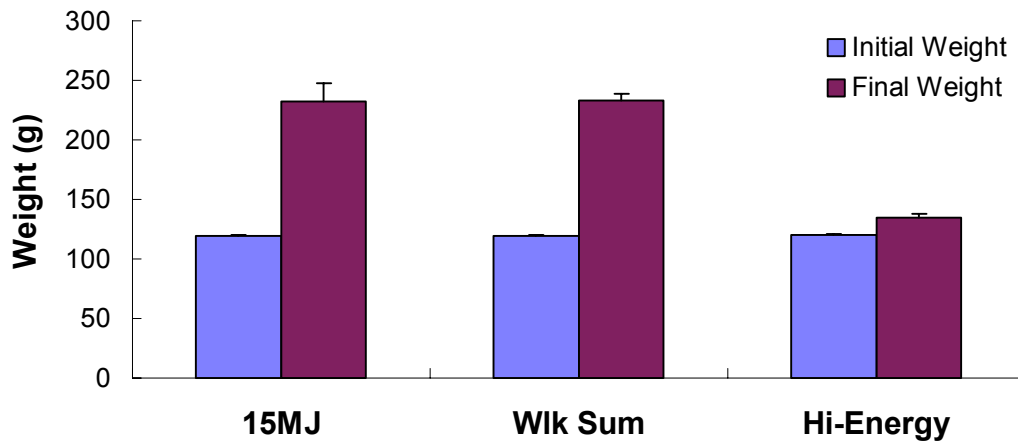


**Table 2.** Production data for the three test diets on each farm.

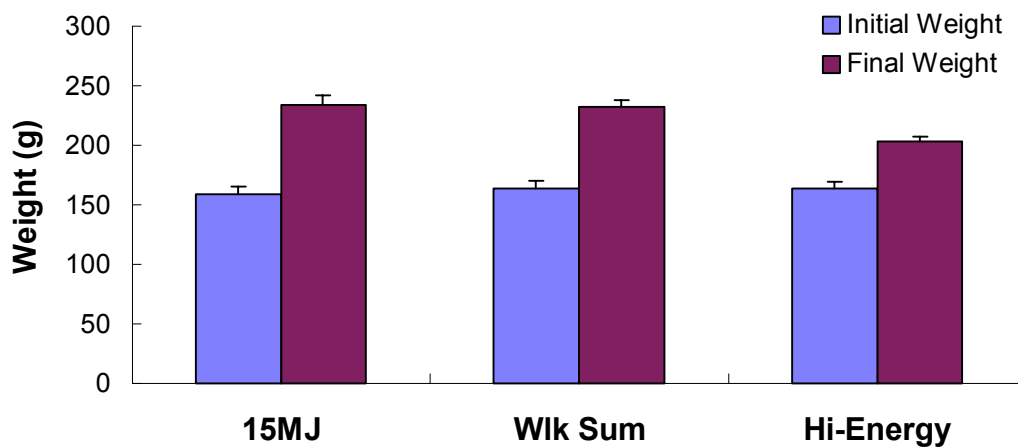
Farm	Diet			Mean (sem)
	Rid -15	Walk-Sum	Hi-E	
<i>Start weight</i>				
Bluewater Barra	159	164	164	162 <sup>X</sup>
Barra Waters	119	119	120	119 <sup>Y</sup>
Mean (sem)	139	141	142	(3.6)
<i>End weight (g)</i>				
Bluewater Barra	234 <sup>a</sup>	232 <sup>a</sup>	203 <sup>b</sup>	223 <sup>X</sup>
Barra Waters	232 <sup>a</sup>	233 <sup>a</sup>	135 <sup>c</sup>	200 <sup>Y</sup>
Mean	233 <sup>A</sup>	232 <sup>A</sup>	169 <sup>B</sup>	(7.2)
<i>Growth rate (g/d)</i>				
Bluewater Barra	0.90 <sup>b</sup>	0.81 <sup>b</sup>	0.46 <sup>C</sup>	0.72 <sup>X</sup>
Barra Waters	1.34 <sup>a</sup>	1.36 <sup>a</sup>	0.17 <sup>d</sup>	0.96 <sup>Y</sup>
Mean	1.21 <sup>A</sup>	1.08 <sup>A</sup>	0.32 <sup>B</sup>	(0.078)
<i>Adjusted Food intake (g/d)</i>				
Bluewater Barra	1.23 <sup>C</sup>	1.07 <sup>C</sup>	1.27 <sup>C</sup>	1.19 <sup>X</sup>
Barra Waters	2.05 <sup>b</sup>	2.55 <sup>a</sup>	2.07 <sup>b</sup>	2.23 <sup>Y</sup>
Mean	1.64	1.81	1.67	(0.093)
<i>FCR (adjusted for mortalities)</i>				
Bluewater Barra	1.40 <sup>a</sup>	1.39 <sup>a</sup>	2.83 <sup>a</sup>	1.87 <sup>X</sup>
Barra Waters	1.62 <sup>a</sup>	1.89 <sup>a</sup>	15.20 <sup>b</sup>	6.23 <sup>Y</sup>
Mean	1.51 <sup>A</sup>	1.64 <sup>A</sup>	9.02 <sup>B</sup>	(1.90)
<i>Dressing %</i>				
Bluewater Barra	11.5	10.9	12.4	11.6
Barra Waters	11.8	11.6	12.0	11.8
Mean	11.7	11.3	12.2	(0.38)
<i>Survival %</i>				
Bluewater Barra	98.7 <sup>a</sup>	97.8 <sup>a</sup>	88.3 <sup>b</sup>	94.9
Barra Waters	98.7 <sup>a</sup>	98.6 <sup>a</sup>	91.2 <sup>b</sup>	96.2
Mean	98.7 <sup>A</sup>	98.2 <sup>A</sup>	89.8 <sup>B</sup>	(1.18)

<sup>a,b,c</sup> etc. For interaction effects and within attributes, means with different superscript letters are significantly different ( $P < 0.05$ ).

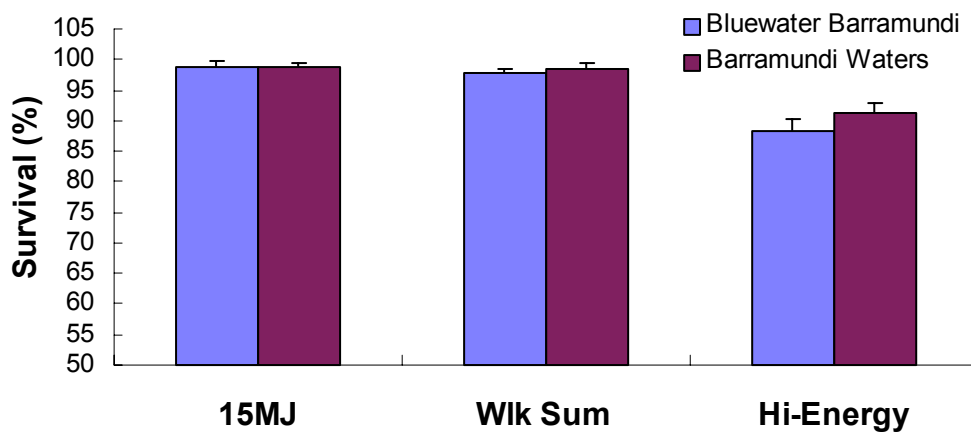
<sup>A,B,X,Y</sup> For main treatment effects and within attributes, means with different superscript letters are significantly different ( $P < 0.05$ ).



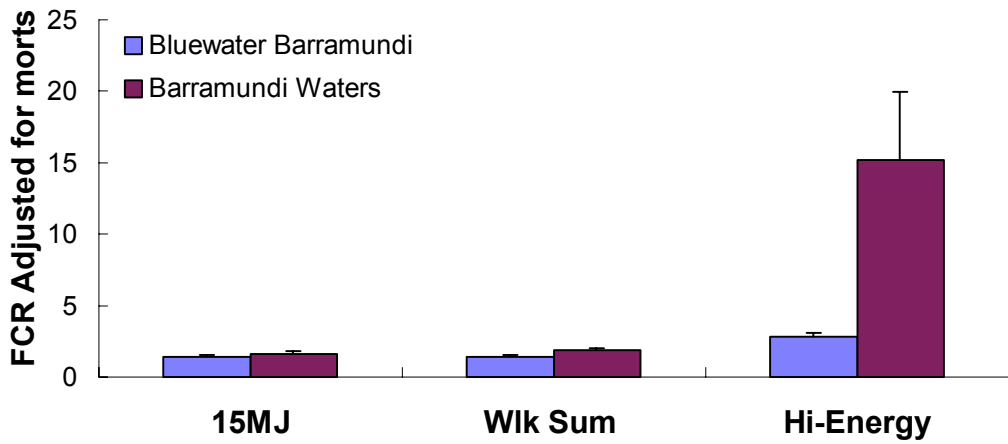
**Figure 1.** Initial and final weights with standard errors of barramundi at Barramundi Waters (fresh water).



**Figure 2.** Initial and final weights with standard errors of barramundi at Bluewater Barramundi (salt water).



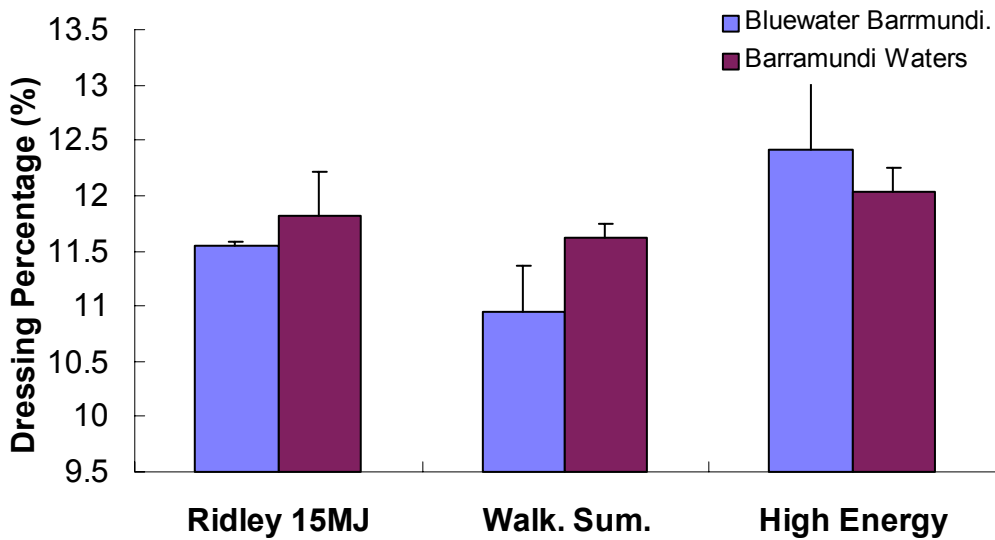
**Figure 3.** Survival with standard errors of barramundi in the three treatments on both farms.



**Figure 4.** Food Conversion Ratios (adjusted for 100% survival) with standard errors for the three treatment diets. (Bluewater Barramundi - closed bars, Barramundi Waters- open bars).

Dressing-out

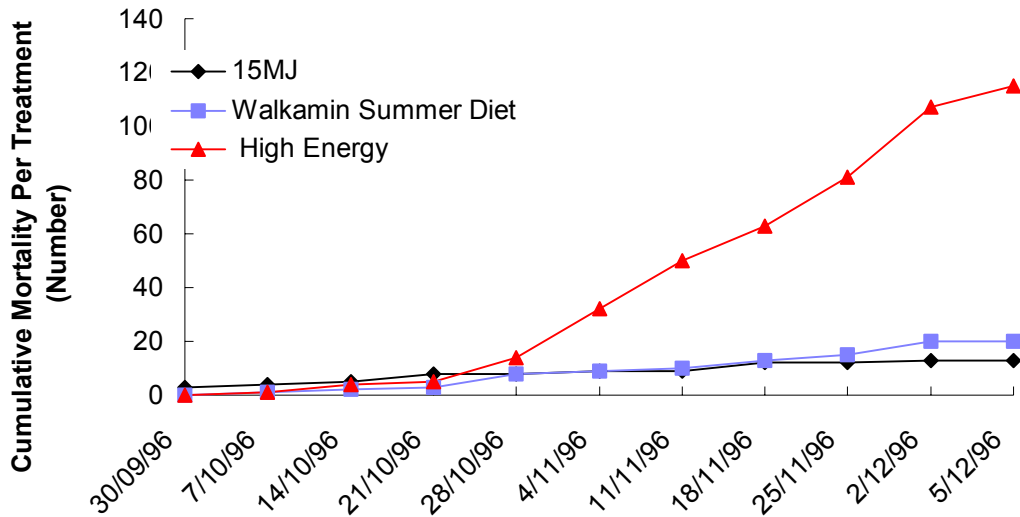
There was no significant difference in dressing-out percentage between any treatments on either farm. Means of dressing-out percentage ranged from 10.94 to 12.42 and are presented in Figure 5.



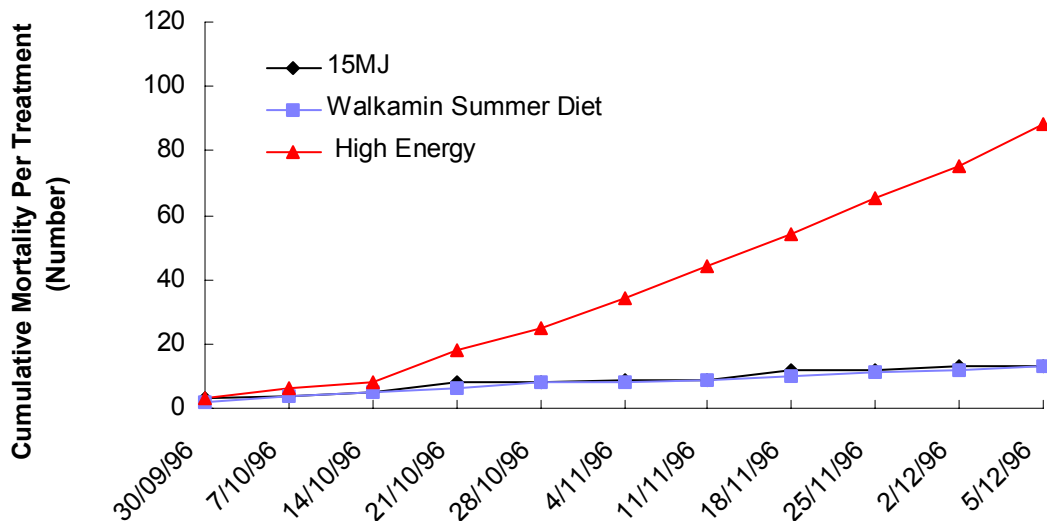
**Figure 5.** Dressing percentages with standard errors for each treatment on each farm.

### Mortality data

Mortality data for both the Walkamin Summer diet and the C1 15MJ diet were similar on both farms and remained below 2.5% over the duration of the trial. However, the fish receiving the high energy diet showed a steadily increasing pattern of mortality in all cages at both farms (Figures 6 & 7). At the end of the trial 115 and 88 fish had died in the high energy treatment at Bluewater Barramundi and Barramundi Waters respectively.



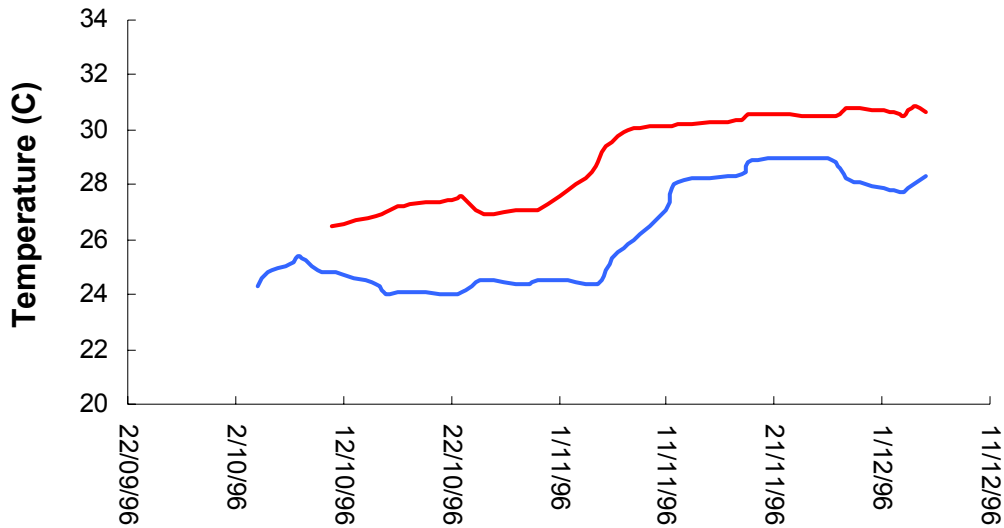
**Figure 6.** Cumulative mortality for the three treatments at Bluewater Barramundi.



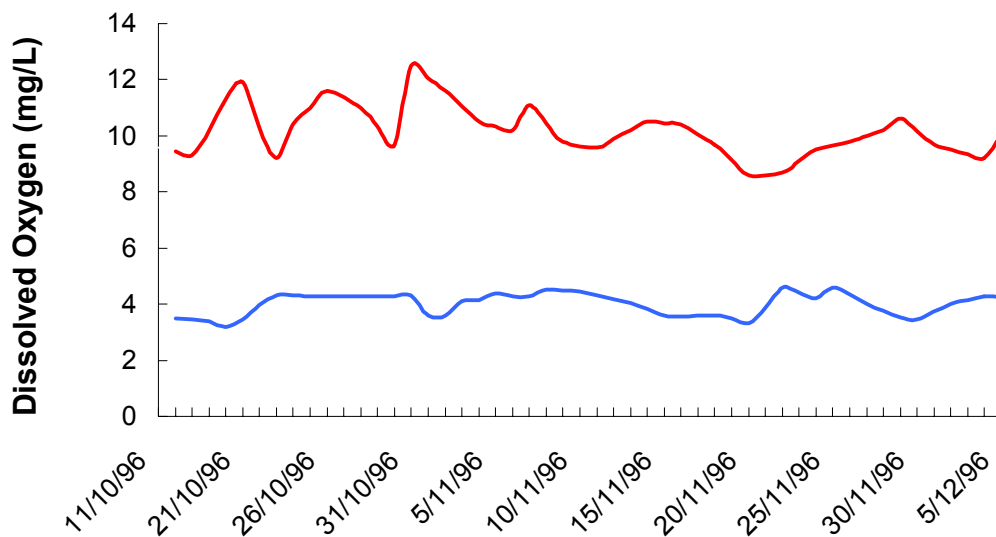
**Figure 7.** Cumulative mortality for the three treatments at Barramundi Waters.

Water quality

Mean daily water temperatures at Barramundi Waters (fresh) was consistently 2 to 3°C warmer than Bluewater Barramundi (Figure 8). Dissolved oxygen levels were consistently higher at Barramundi Waters than at Bluewater Barramundi (Figure 9). The salinity of the Bluewater Barramundi farm was in the range 34.3 to 42.2 ppt, with a mean salinity of 36.7 ppt. Mean Secchi depth was 15 cm at Barramundi Waters and 125 cm at Bluewater Barramundi.



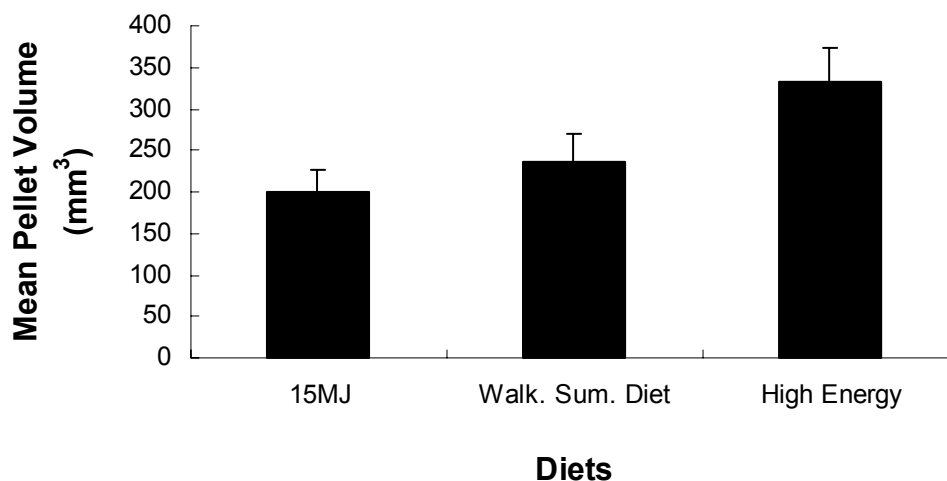
**Figure 8.** Daily mean water temperatures for Barramundi Waters (red) and Bluewater Barramundi (blue).



**Figure 9.** Dissolved Oxygen levels for Barramundi Waters (red) and Bluewater Barramundi (blue).

### Pellet characteristics

The volumes of the three diets calculated after measuring diameters and pellet lengths are shown in Figure 10. There was also a difference noted in pellet density as shown by sink rate tests. The 15MJ and Walkamin summer diets showed a much slower sink rate than did the high energy pellet in fresh and salt water (35 ppt). The high energy diet had no pellets remaining on the surface after 10 seconds while the other diets had between 30 and 60% at the surface.

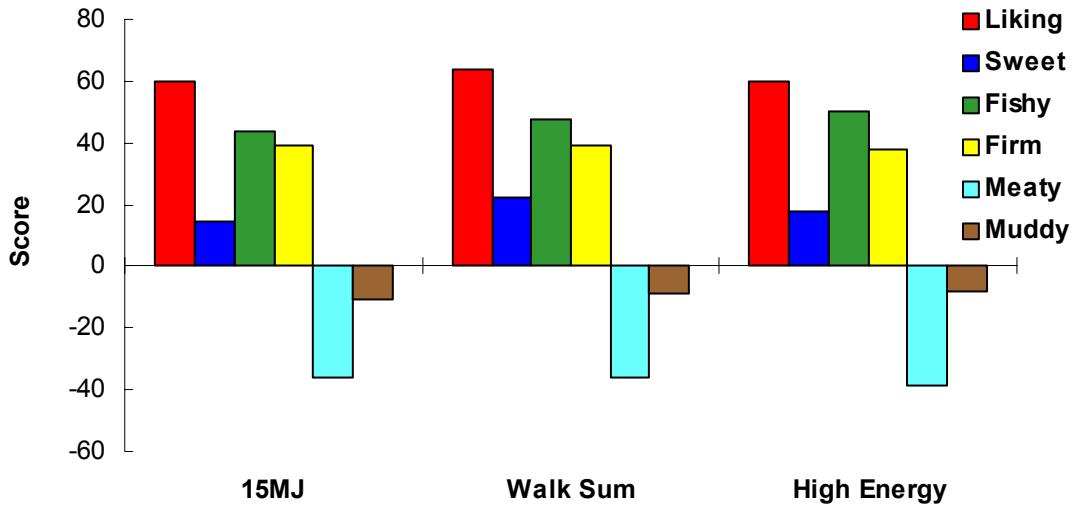


**Figure 10.** Volumes (+ standard errors) of pellets in the experimental diets.

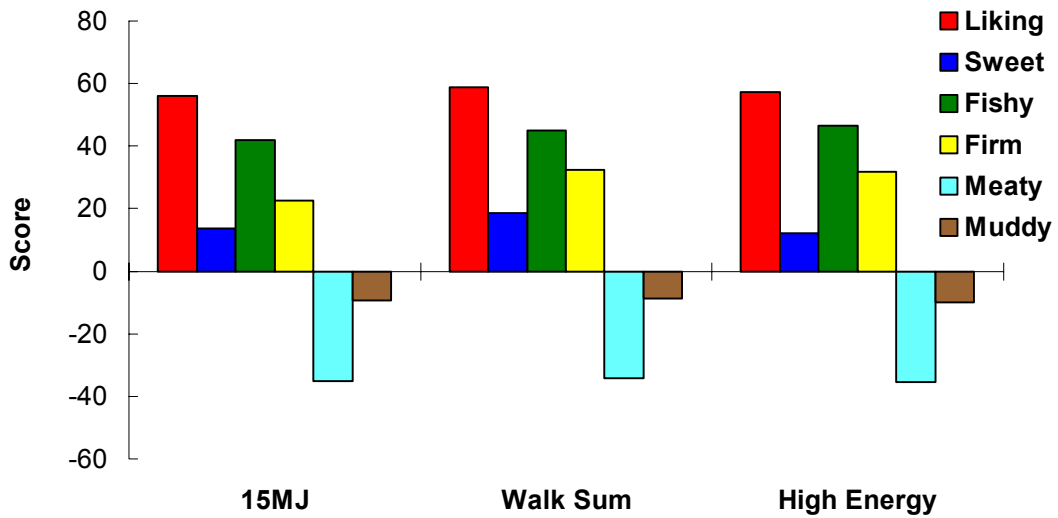
### Sensory evaluation

Full details of the sensory evaluation is provided in Appendix 1. In general, all diets and both farms produced fish which scored highly for positive attributes (those above the horizontal axis in Figures 11 & 12) and low for negative attributes (those below the horizontal axis in Figures 11 & 12). The score for overall liking for the fish from all diets from both the saltwater and freshwater farms was approximately 60 out of a possible score of 100. There was no negative effect on overall taste of the meat meal based diets; interestingly, fish reared on the fishmeal based diet were perceived as “less fishy” than those reared on the meat meal based diets.

Scores for the attributes grouped within the categories odour, colour and flavour were quite similar between farms (Appendix 1). In the case of texture, however, the differences between fish grown on the saltwater and freshwater farms were quite marked. Fish from the salt water were considered more firm and flaky and less fibrous than those from the fresh water.



**Figure 11.** Sensory evaluations for barramundi grown on the three experimental diets at Bluewater Barramundi. (Within an attribute, bars with a different letter are significantly different,  $P < 0.05$ ).



**Figure 12.** Sensory evaluations for barramundi grown on the three experimental diets at Barramundi Waters. (Within an attribute, bars with a different letter are significantly different,  $P < 0.05$ ).

## Conclusions from on-farm validation of barramundi diets

1. The growth response of the fish to each diet was the same on both farms.
2. The performance of the fish between farms was quite different, but because of the many parameters (chemical, physical and environmental) relevant to fish production which varied between farms it was not possible to identify the reasons for the different performance.
3. The Walkamin summer diet, based primarily on meat meal for its protein source, resulted in growth performance equal to the commercial diet.
4. However, on a cost per unit fish production basis, the Walkamin summer diet was superior to the commercial diet.
5. The high energy diet resulted in comparatively poor growth performance and survival. Further investigations are required to determine the response of barramundi to high energy diets.
6. There was no negative impact of the meat meal based diets on the sensory characteristics of the fish.

### LABORATORY VALIDATION OF BARRAMUNDI DIETS

The objective of this trial was to examine the growth performance of barramundi when fed the same three test diets as used in the on-farm evaluation, but on this occasion under controlled laboratory conditions. The laboratory-based assessment eliminates variables present in commercial trials, such as weather, water temperature, water quality, fish origin and health, and management, and thus provides a more precise measure of the efficacy of the diets.

Specific aims of the trial were as follows:

1. determine the effectiveness of the different diet formulations as assessed by growth rate, food conversion ratio and mortality;
2. examine the growth response of the fish when cultured in fresh and salt (30 ppm) water.

## Materials and Methods

### Experimental design

The laboratory evaluation of diets was conducted in the experimental facilities of the Freshwater Fisheries and Aquaculture Centre, Walkamin. Six 180 litre tanks within each of four independent 3080 litre recirculating systems (experimental blocks) were used. The primary treatment consisted of the three diets previously detailed, while the secondary treatment consisted of two salinities (0 ppt and 30 ppt). Two blocks were allocated to each salinity, and the three diets were replicated twice within each experimental block. Temperature was maintained at  $28 \pm 0.5^{\circ}\text{C}$ . Photoperiod was 12L/12D, with lighting supplied by daylight fluorescent tubes. Dietary treatments were assigned randomly within each of the recirculating systems.

The trial commenced on the 18th and 19th March, 1997 after a ten day acclimatisation period and continued for eight weeks with trial completion on the 13th and 14th May, 1997. During the acclimation period, the fish were fed the appropriate experimental diet in order to acclimate them to the different characteristics (sink rate, odour, taste, texture) of each feed type.

Fish were sorted by weight with eight barramundi of approx. 250 g being initially allocated to each tank. A precautionary prophylactic bath of 100 ppm formalin for one hour was administered to fish following initial tank allocation, again on day 1 and thereafter at fortnightly intervals which



coincided with weigh days. Fish were individually weighed on day 1 and bulk weighed every 14 days thereafter, before being individually weighed again at trial completion on day 57.

#### Feeds and feeding

The experimental feeds were selected from the same feed batches used in the on-farm trial. The diets were manufactured by the commercial feed manufacturer during early September 1996, immediately prior to the commencement of farm trials. Subsequently, the diets were stored in a cool room at 4 to 7 °C during the interim period of about 6.5 to 8.5 months between manufacture and completion of the laboratory trial.

Fish were fed to satiation each morning over a 45 to 60 minute period. Uneaten pellets were then removed and counted. The dry weight equivalent of the uneaten pellets was determined from the mean pellet weight of a sample of 1000 pellets of the corresponding diet. Daily feed intake was then calculated for each tank by subtracting the calculated weight of uneaten pellets from the difference in corresponding feed bucket weights, recorded at the beginning and end of each feed.

#### Pellet stability

One pellet characteristic which had not been previously considered was the rapidity of water penetration and associated pellet degradation. This was subjectively assessed by comparing the rate of softening and swelling of pellets in water.

#### Pathology

Liver samples were collected from fish offered the High Energy diet at the trial and sent to DPI's Oonoonba Veterinary Laboratory (Townsville) for histological examination.

#### Dietary analysis

Samples of the C1 15 MJ and Walkamin Summer diets were collected at the completion of the laboratory trial and dispatched for analysis to determine the concentrations of vitamins A, C and E remaining in the stored diets.

#### Statistical analysis

Initial and final weights, growth (average daily gain), survival, food conversion and dressing percentage were compared using analysis of variance. In each case residuals were examined for homogeneity, in all cases data transformations were not necessary. Pair-wise comparison of treatment means were made with the least significant difference test only where the ANOVA F values were significant ( $P < 0.05$ ). All statistical tests were performed at the 5% significance level.

## **Results**

#### Production data

The interaction between system (fresh or salt water) and diets was not significant ( $P > 0.05$ ) for any of the performance attributes. Performance data with respect to diets and system are listed in Table 3.

**Table 3.** Production data and standard errors (sem) for the three test diets in each system.

Attribute	Diets				System		
	Rid 15MJ	Wlk-Std	Hi-Fat	sem	Salt	Fresh	sem
Weights(g)							
Start	266.6 <sup>A</sup>	269.1 <sup>A</sup>	255.7 <sup>B</sup>	3.53	259.5 <sup>Y</sup>	268.1 <sup>X</sup>	2.88
End	480.2 <sup>A</sup>	426.4 <sup>B</sup>	345.5 <sup>C</sup>	9.26	398.8 <sup>Y</sup>	435.9 <sup>X</sup>	7.56
Feed intake(g/d)	4.22 <sup>A</sup>	3.95 <sup>A</sup>	2.98 <sup>B</sup>	0.189	3.69	3.75	0.152
Growth (g/d)	3.80 <sup>A</sup>	2.78 <sup>B</sup>	1.65 <sup>C</sup>	0.144	2.51 <sup>Y</sup>	2.97 <sup>X</sup>	0.129
FCR (g:g)	1.12 <sup>A</sup>	1.44 <sup>A</sup>	2.06 <sup>B</sup>	0.171	1.73	1.34	0.137

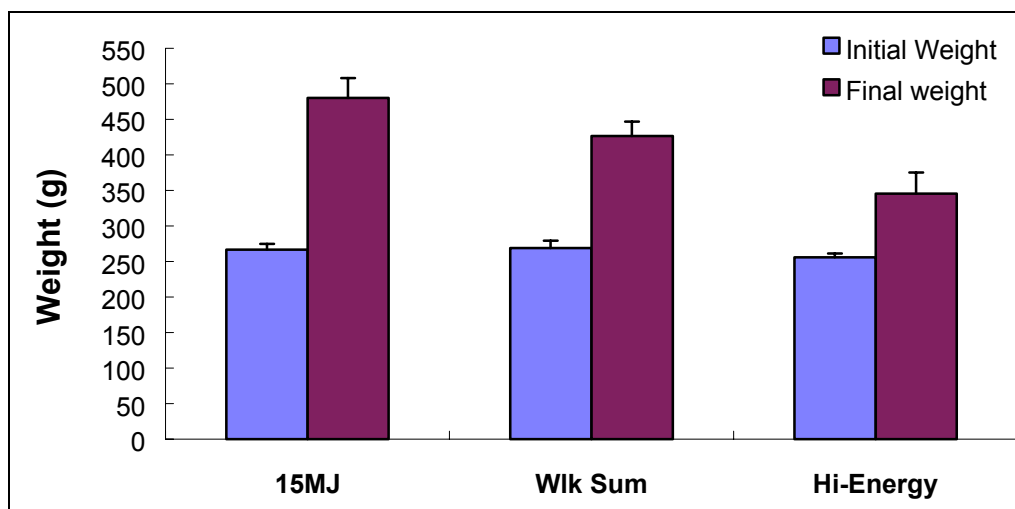
A,B,C, X,Y; Within treatment effects and within attributes, means with different superscript letters are significantly different ( $P < 0.05$ ).

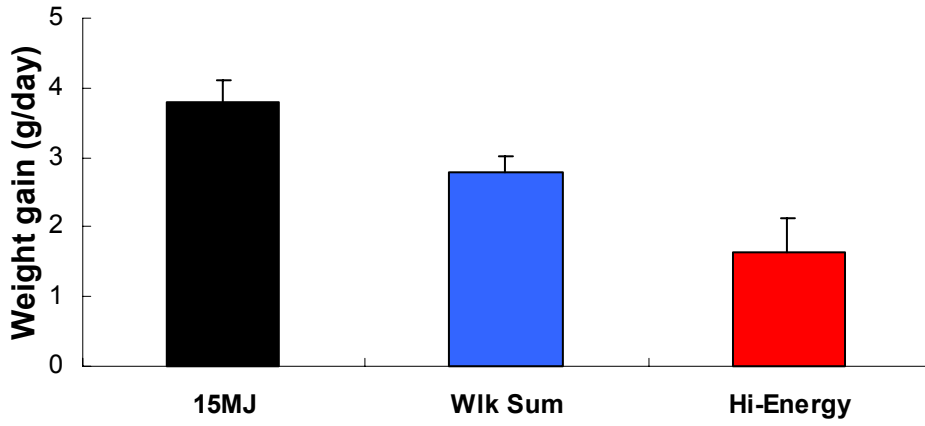
There was a small difference in start weights between treatments primarily due to being fed the different experimental diets during the acclimation period. There was a significant difference in the final weights between all treatments. The growth rate on the 15 MJ diet was significantly better than the Walkamin Summer diet which in turn was superior to the High Energy diet (Figures 13 & 14).

No mortalities were recorded during the trial. However, there were several emaciated fish in the High Energy treatment which would have undoubtedly died if the trial had continued for a longer duration.

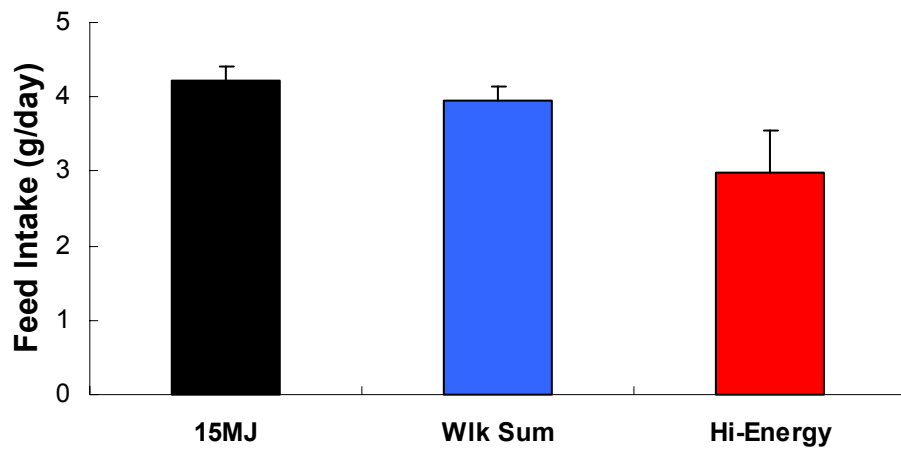
The mean daily feed intakes of fish offered the C1 15MJ and Walkamin Summer diets were not significantly different. However, fish on the High Energy diet ingested significantly less feed than those on the other two diets (Figure 15, Table 3). Pellet ejection in the High Energy treatment was commonly observed during feeding.

Both the C1 15MJ and Walkamin Summer Diets resulted in similar food conversion ratios which were significantly lower than the High Energy treatment. The mean food conversion ratios for each diet are displayed Figure 16.

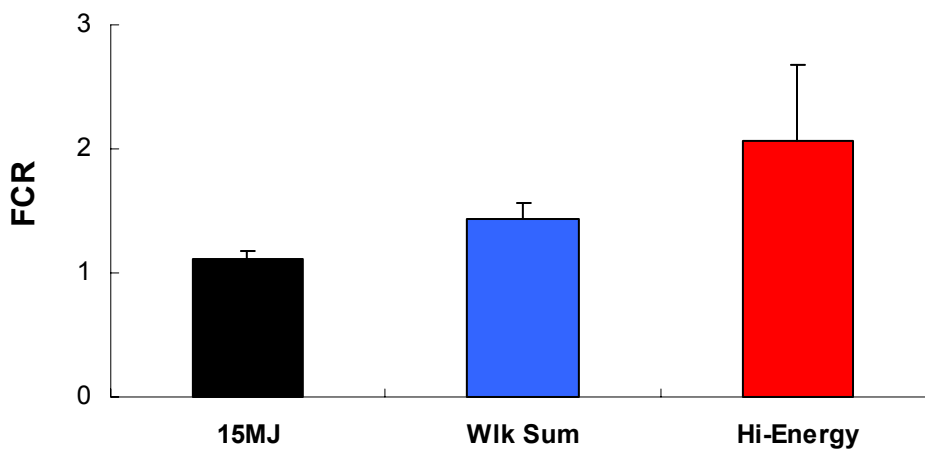
**Figure 13.** Initial and final weights (g) and standard errors for the three diet treatments.



**Figure 14.** Daily weight gain (g/d) and standard errors for the treatment diets.



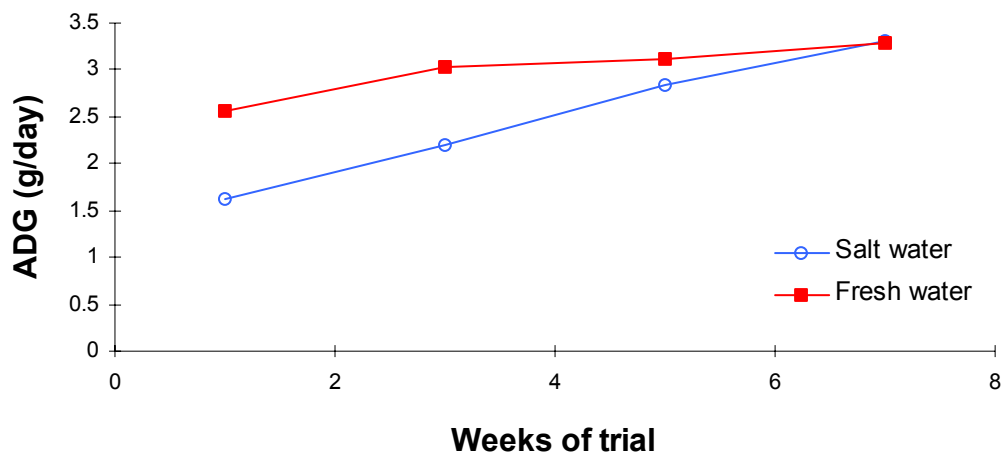
**Figure 15.** Daily feed intake (g/d) for the treatment diets



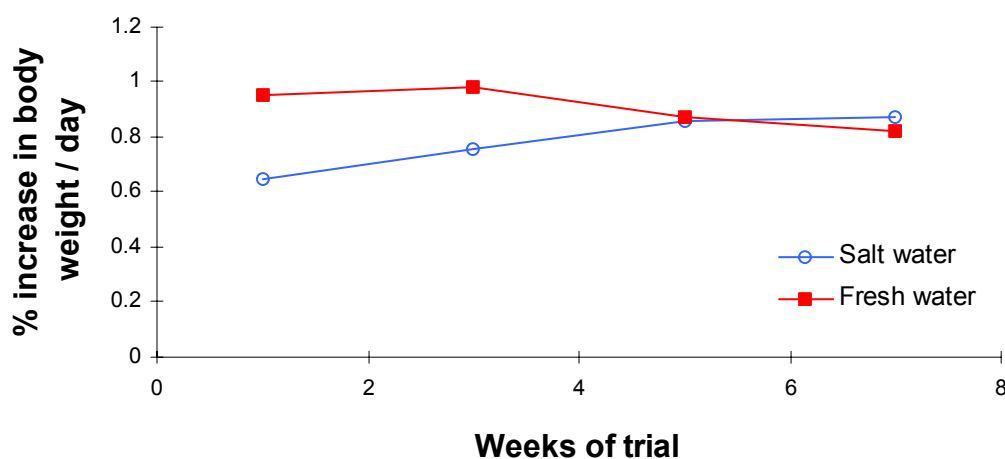
**Figure 16.** Food conversion ratio and standard errors for the treatment diets.

### Fresh and salt water comparison

Fish in the freshwater system grew significantly faster through the course of the 8 week trial than fish in the saltwater system (Table 3), although daily feed intake and food conversion ratio were not significantly different between the two systems. An examination of the growth data on a fortnightly basis indicates a trend of comparatively poor growth in the saltwater system in the early part of the trial, with growth rate progressively improving and in fact surpassing that of the freshwater system during the 7<sup>th</sup> to 8<sup>th</sup> weeks of the trial (Figures 17 & 18). This probably indicates that barramundi initially held in fresh water may take 7 to 8 weeks to fully acclimate to salt water. Consequently, the results of the present trial should not be considered a valid comparison of the comparative growth performance of barramundi in fresh and salt water.



**Figure 17.** Average daily weight gain (g/day) of barramundi in fresh and salt water during the course of an 8 week trial.



**Figure 18.** Daily percentage increase in body weight of barramundi in fresh and salt water during the course of an 8 week trial.

### Pellet stability

The Walkamin Summer diet was characterised by rapid water penetration and swelling to a greatly increased volume over a period of about twenty-five minutes while the C1 15 MJ diet swelled and degraded more slowly.

In contrast, pellets of the High Energy diet displayed negligible water penetration and softening over the entire feeding period, merely becoming slimy to a depth of about 1 to 2 mm and remaining extremely hard below this depth. Observations on weigh days indicated that faeces were still present 24 hours post feeding in fish consuming the High Energy diet but not present in fish in other treatments. This observation accords with the reduced feed intake of fish in the High Energy treatment, but it may also have been associated with a slower passage of food through the digestive system because of delayed moisture penetration into pellets.

### Pathology

A number of fish from the High Energy treatment displayed reduced or negligible feeding during the trial and slowly lost weight. Seven livers from emaciated fish were histologically examined. No evidence of fatty liver disease was found, however most livers showed signs of prolonged anorexia.

### Other observations

All treatments included some experimental fish which lost condition during the course of this trial. This observation is not unusual and is generally confined to small numbers of fish. It may be due to a variety of reasons including aggression between individuals, feeding timidity by subordinate fish in the tank community and dietary influences. In the present study 3 (4.7%), 4 (6.3%) and 12 (18.8%) of fish were observed to be of poor condition for C1 15 MJ, Walkamin Summer and High Energy diets respectively.

A number of fish in all treatments displayed small, shallow epidermal lesions on mouthparts generally associated with abrasion due to feeding off the tank bottom or manipulation of pellets during subsequent ingestion. One (1.6%), four (6.3%) and nine (14.1%) fish were recorded with these lesions for C1 15 MJ, Walkamin Summer and High Energy diets respectively. There did not appear to be any association between the presence of mouth, lip or tongue lesions and the individuals of poor physical condition. The lesions were therefore considered to be of a superficial nature but could be related to pellet characteristics such as hardness or sharp edges.

### Vitamin analysis of diets

The results of vitamin analyses are listed in the following Table 4.

**Table 4.** Vitamin analyses of experimental diets after approximately 9 months of storage at 4–7°C.

Vitamin	Diet		
	C1 15MJ	Walkamin Summer	High Energy
Vitamin A	5.3 iu/g	6.9 iu/g	Not Analysed
Vitamin E	82.3 ppm	74.1 ppm	Not Analysed
Vitamin C	N/A	N/A	Not Analysed

N/A Results of analyses not available at the time of reporting.

### Conclusions from laboratory validation of barramundi diets

1. The performance of the Walkamin Summer diet (a meat meal based diet) was inferior to that of the commercial, fishmeal based diet, as measured by growth rate of the fish.
2. The trial did not yield conclusive results regarding the comparative growth performance of barramundi in fresh and salt water. The results indicated that barramundi may take 7 to 8 weeks to fully acclimate to salt water.

### Discussion

The lower productivity of the fish on the Walkamin Summer diet as compared to the C1 15 MJ diet in the laboratory study was an unexpected result and contrasts with both farm studies where these same diets produced equivalent fish performance. Previous studies testing other meat meal-based diets under both farm and laboratory conditions have shown a strong consistency in findings between the farm and the laboratory. Compared to previous companion farm and laboratory studies, the striking difference in the present work was the extended time between the sets of experiments. For instance in the present work, the diets used for the two farm studies were just a few weeks old at the start of the experiment whereas a period of about 7 months had elapsed between manufacture and commencement of the laboratory experiment. Even though the diets had been stored under favourable cold-room conditions (low humidity, 4 to 7°C), the most likely explanation for the reduced fish performance on the Walkamin Summer diet in the laboratory experiment was that prolonged storage caused a deterioration in its nutrient content.

The reason why the C1 15MJ diet did not show a similar deterioration in nutrient quality although stored identically to the other diets is not known for certain but may be due to differences in 'shelf life' of the various ingredients used in the respective diets. For example, the C1 15 MJ diet had a high fishmeal content whereas the Walkamin Summer diet had a high meat meal component. Because of the well reported vulnerability of fishmeal and fish oil to undergo oxidative rancidity (Kochlar & Rosell 1990; Botta 1995), these products invariably have an antioxidant (such as ethoxyquin, BHA, BHT etc.) added at manufacture. Conversely, because of the great stability of the more saturated fats in terrestrial abattoir by-product protein meals, antioxidants are not routinely added to meat meal at manufacture. Since subsequent investigation has revealed the barramundi vitamin premix used at the Narangba feed mill also did not contain any antioxidant, it is highly likely that the meat meal-based Walkamin Summer diet would have a shorter shelf life to that of the C1 15 MJ diet. Although prolonged storage of any aquafeed would never be advocated, it is strongly recommended that all aquafeed contain an appropriate amount of antioxidant at time of manufacture. This may extend the shelf life of the diet and assist in retaining the vitality of the feed under less than optimal on-farm storage conditions. The use of a multi antioxidant preparation (e.g. Kemin's "Finox Liquid" which is a combination of BHA, ethoxyquin and citric acid) would be advocated.

The laboratory validation trial was conducted at the same time as the commercial feed manufacturer launched a new meat meal based diet, labelled "Barramundi LE" (for low energy). The new diet was generally not well received by the industry, and eventually it was withdrawn from sale. The reasons for the adverse reaction from industry were complex. The first batch of the new diet sank, which is contrary to the requirements of nearly all barramundi farmers. The results of the laboratory trial indicated that poorly stored or aged food could have resulted in reduced growth rates. However, the situation was confounded by negative reports from one farm using a different diet and inexperienced growers using recirculation systems, and positive reports from some experienced farmers.

It is beyond the scope of this report, and not helpful anyway, to further discuss the reasons behind the lack of acceptance of the new diet. The salient point is that there is now some negativity in the industry regarding changes to dietary formulations. Given this situation, it is appropriate to document some factors to be considered in future commercialisation of new diets, based on our recent experience. We emphasise that these are from our perspective, and are only part of a 'commercialisation strategy'.

- Name: any new diet needs an appropriate name, with positive connotations;
- Laboratory evaluation: should precede farm validation, because of the greater precision of laboratory trials;
- Farm validation: farms trials should involve other growers by, for instance,
  - a pre-, mid and post-trial meeting of farmers, millers and researchers, and
  - a field day at the termination of the trial (fish inspection and tasting, data inspection).
- Provision of information regarding ingredients to growers.
- Quality control pre- and post-milling, to ensure good product reaches the fish (i.e. avoid situations such as incorrect pellet buoyancy or on-farm storage conditions).

The present studies have provided some 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 not be used after a period of prolonged storage.

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#### 4.2. Laboratory validation of experimental and commercial barramundi diets: a report commissioned by a commercial feed manufacturer (C2)

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### Executive Summary

A commercial feed manufacturer (C2) commissioned the Freshwater Fisheries and Aquaculture Centre at Walkamin to undertake a laboratory-based trial to test a series of diets. The work was undertaken as part of a larger project currently in progress at Walkamin, FRDC 96/393 - Diet Validation and Feeding Strategies. The objective of the trial was to examine the growth performance of barramundi when fed six diets under controlled laboratory conditions in terms of Average Daily Gain (ADG), Specific Growth Rate (SGR), Daily Feed Intake (DFI) and Food Conversion Ratio (FCR).

The six diets tested in this trial were Diet 1 (C2 45/20), Diet 2 (C2 45/20), Diet 3 (C2 45/20), Diet 4 (C2 salmon grower 45/25), Diet 5 (C2 barra stock 43/15) and Diet 6 (Standard barra diet). There were four replicates tanks for each diet, with each tank containing eight fish of approximately 250 g. The bulk weight of fish in each tank was measured on a fortnightly basis over the eight week trial. No mortalities or stressed fish were observed at any time during the trial.

The performance of diet 3 was generally better than the remaining five diets. The average final weights were significantly higher for diets 3, 2 and 4. Diet 3 had significantly better ADG and SGR. DFI was significantly higher for diets 3 and 2. Diet 4 had the best FCR, however diets 3 and 1 were next in ranking. The worst performing diets were diets 5 (C2 Barra Stock) and 6 (Standard Barra Diet) which had significantly lower average final weights, ADG, SGR, DFI and the worst performing FCR compared to the other diets. The dressed weight of fish was significantly lower for diets 5 and 6.

Appendix 10.2 provides an economic comparison of dietary productivity. Diet 3, which exhibited the most outstanding production values, cost 4% more than the standard barramundi diet to produce an equivalent amount of fish. However, fish on diet 3 grew from 320 grams to 560 grams in 44 days compared to 54 days on the standard barramundi diet. The economic benefit derived from the faster growth rates of fish grown on diet 3 compared with the standard barramundi diet would far outweigh the impact of slightly greater feed costs.

Production time for grow-out of barramundi from 100 to 500 g was determined using three separate trials for improved and standard diets (diets 3 and 5 respectively from the present trial and similar diets from 2 previous trials). Barramundi were estimated to grow from 100 to 500 g in 100 days on the improved diet, compared with 150 days on the standard diet. This variation in growth rate would make an enormous difference to farm productivity.



## Introduction

A commercial feed manufacturer (C2) approached the Freshwater Fisheries and Aquaculture Centre at Walkamin to undertake a laboratory-based trial to test a series of diets with barramundi. This was one step in C2's commercial strategy to market more product into the barramundi industry. The trial was undertaken on a contract basis, as part of a larger project currently in progress at Walkamin, FRDC 96/393 - Diet Validation and Feeding Strategies.

The objective of this trial was to examine the growth performance of barramundi when fed six test diets under controlled laboratory conditions. The laboratory-based assessment eliminates variables present in commercial trials, such as weather, water temperature, water quality, fish origin and health, and management, and thus provides a more precise measure of the efficacy of the diets. Assessment of diet efficacy was based on Average Daily Gain (ADG), Specific Growth Rate (SGR), Daily Feed Intake (DFI) and Food Conversion Ratio (FCR).

## Materials and methods

### *Experiment design*

The laboratory evaluation of diets was conducted in the experimental facilities of the Freshwater Fisheries and Aquaculture Centre, Walkamin. Two independent 3680 litre recirculating systems were used for this trial. Each system was sub-divided, effectively giving two experimental blocks per recirculating system. Within each block there were six 140 litre tanks, thus a total of 24 tanks distributed among 4 blocks were used for the trial.

The treatments were six diets, identified as follows:

- Diet 1 C2's 45/20
- Diet 2 C2's 45/20
- Diet 3 C2's 45/20
- Diet 4 C2's salmon grower 45/25
- Diet 5 C2's barra stock 43/15
- Diet 6 Standard barra diet

There were four replicates of each of the six diets, with treatments allocated randomly within each block (Table 1). Temperature in all blocks was maintained at  $28 \pm 0.5^\circ\text{C}$  and water salinity was maintained below 0.5 ppt. Photoperiod was 12L/12D, with lighting supplied by daylight fluorescent tubes.

**Table 1.** Random allocation of six experimental diets for laboratory-based testing.

System A			System B		
Block 1	Tank A12 - Diet 3	Tank A1 - Diet 2	Block 3	Tank B12 - Diet 2	Tank B1 - Diet 5
	Tank A11 - Diet 5	Tank A2 - Diet 4		Tank B11 - Diet 3	Tank B2 - Diet 6
	Tank A10 - Diet 1	Tank A3 - Diet 6		Tank B10 - Diet 4	Tank B3 - Diet 1
Block 2	Tank A9 - Diet 3	Tank A4 - Diet 1	Block 4	Tank B9 - Diet 5	Tank B4 - Diet 2
	Tank A8 - Diet 2	Tank A5 - Diet 5		Tank B8 - Diet 3	Tank B5 - Diet 6
	Tank A7 - Diet 6	Tank A6 - Diet 4		Tank B7 - Diet 1	Tank B6 - Diet 4

The trial commenced in January 1998, after a 14 day acclimatisation period, and continued for eight weeks with trial completion in March 1998. During the acclimatisation period, the fish were held in the recirculation system at 28°C and fed a standard barra diet in order to standardise the pre-trial condition of the fish.

Fish were sorted by weight with eight barramundi of approximately 250 g being randomly allocated to each tank. A precautionary prophylactic salt bath of 12‰ for one hour was administered to fish following initial tank allocation, again on day 1 and thereafter at fortnightly intervals that coincided with weigh days. Fish were individually weighed on day 1 and bulk weighed every 14 days thereafter, including at the end of the fourth fortnight. Fish were not individually weighed at the completion of the trial.

#### Feeds and feeding

Diets 1, 2 and 3 were dispatched from C2 in December 1997, but held at the seaport in Melbourne until after the New Year. These diets arrived at Walkamin on January 13th 1998. Diets 4 and 5 were consigned separately from C2 in the first week of January 1998. These diets were transported by rail, however the delivery was delayed by floodwater damage to rail links. These diets were unloaded in Cairns and transported to Walkamin on January 27th 1998. Diet 6 was a standard barra diet purchased commercially. Date of manufacture was indicated as December 22nd 1997. All diets were stored at Walkamin in a cool room at 4 to 7 °C during the period between delivery and completion of the trial. Pellets in all diets were approximately 4 mm in diameter and length.

Fish were fed to satiation each morning from 8:30 to 10:30 am over a 45 to 60 minute period. Fish were not fed on day 1 when fish were being allocated to tanks and individually measured. Nor were fish fed on days during which bulk weight measurements were being recorded (at the end of each fortnight).

After feeding, uneaten pellets were removed and counted. The dry weight equivalent of the uneaten pellets was determined from the mean pellet weight of a sample of 100 pellets of the corresponding diet. Daily feed intake was then calculated for each tank by subtracting the calculated weight of uneaten pellets from the difference in corresponding feed bucket weights, recorded at the beginning and end of each feed.

#### Experimental data collected during the trial

The following experimental data were collected during the trial:

- a) Average weight of pellets for each diet;
- b) Food consumption (daily record of weight of feed issued minus uneaten food) for each experimental tank accumulated fortnightly;
- c) Individual weight of fish in each tank on Day 1;
- d) Bulk weight of fish in each tank, taken on Day 15 and each fortnight thereafter;
- e) Daily record of mortalities, water chemistry and temperature; and
- f) Dressing percentage of 12 fish per treatment (i.e. 3 per tank) at end of trial.

#### Calculation of production values

The experimental data outlined in the previous section were used to calculate Average Daily Gain (AGD) in g/fish/day, Specific Growth Rate (SGR) in percentage body weight/fish/day, Daily Feed Intake (DFI) in g/fish/day, Food Conversion Ratio (FCR) in g dry food:g wet weight fish, and Dressed Weight expressed as a percentage of total weight (Dressing %). In the following equations,  $W_{t_i}$  is the average fish weight at the beginning of the experiment ( $t_i$ ) and  $W_{t_f}$  is the average fish weight at the end of the experiment ( $t_f$ ).

$$ADG = \frac{Wt_t - Wt_i}{t_t - t_i}$$

$$SGR = \left[ \frac{\ln(Wt_t) - \ln(Wt_i)}{t_t - t_i} \right] * 100$$

$$DFI = \frac{\text{Total food consumption per tank}}{\text{Number of fish per tank} * (t_t - t_i)}$$

$$FCR = \frac{DFI}{ADG}$$

$$\text{Dressing \%} = \frac{[(\text{Total weight} - (\text{gill} + \text{gut}) \text{ weight}) \div \text{Total weight}] * 100}{\text{Total weight}}$$

#### Pellet sink rates

The sink rate of pellets was assessed by adding 100 pellets to a tank, and recording the number floating at various time intervals up to one hour thereafter.

#### Biochemical analysis of diets

A 500 g sample of each diet was dispatched to the Animal Research Institute for proximate (dry matter, ash, crude protein, total lipid, energy) and fatty acid analyses as soon as the diets were available.

#### Statistical analysis

Initial and final weights, growth (ADG and SGR), survival, FCR and dressing percentage were compared using analysis of variance. Variances were examined for homogeneity and in all cases data transformations were not necessary. Pair-wise comparison of treatment means was made with the least significant difference test applied only where the F value of the ANOVA was significant ( $P < 0.05$ ). All statistical tests were performed at the 5% significance level.

## **Results and Discussion**

#### Production data

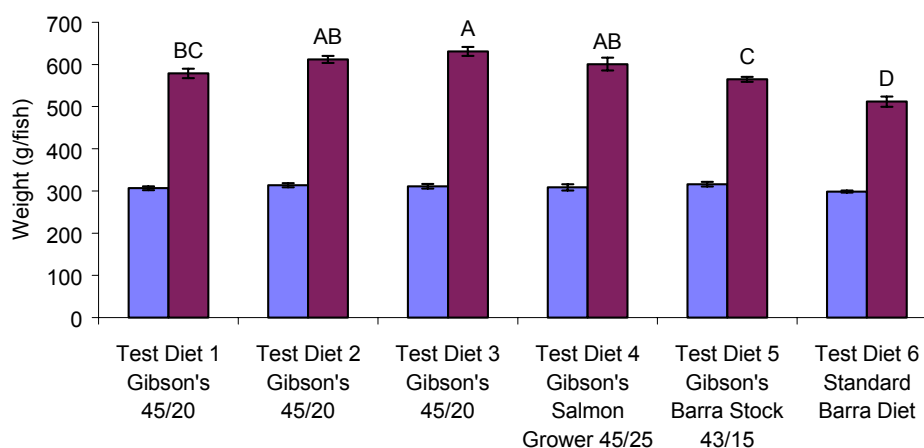
Performance data with respect to diets are listed in Table 2. The respective performance traits are presented graphically and discussed separately below. No mortalities or stressed fish were observed at any time during the trial.

**Table 2.** Production data and standard errors for six diets following an 8 week growth assay with 8 fish per tank. Within trait comparisons, means with a common superscript letter were not significantly different ( $P > 0.05$ ). Values for ADG, SGR and DFI were covariance-adjusted to remove the effect of minor differences in starting weight of the fish.

Trait	Diet Label						± sem
	Diet 1 C2's 45/20	Diet 2 C2's 45/20	Diet 3 C2's 45/20	Diet 4 C2's Salmon Grower	Diet 5 C2's Barra Stock	Diet 6 Standard Barra Diet	
Initial Wt (g)	306.6	313.8	311.3	308.6	316.0	298.5	5.450
Final Wt (g)	578.7 <sup>BC</sup>	611.9 <sup>AB</sup>	631.1 <sup>A</sup>	600.7 <sup>AB</sup>	564.7 <sup>C</sup>	511.8 <sup>D</sup>	11.31
ADG (g/d)	4.86 <sup>B</sup>	5.27 <sup>B</sup>	5.69 <sup>A</sup>	5.22 <sup>B</sup>	4.37 <sup>C</sup>	3.92 <sup>C</sup>	0.139
SGR (%/d)	1.13 <sup>B</sup>	1.20 <sup>AB</sup>	1.26 <sup>A</sup>	1.19 <sup>B</sup>	1.04 <sup>C</sup>	0.95 <sup>D</sup>	0.025
DFI (g/d)	4.97 <sup>B</sup>	5.47 <sup>A</sup>	5.56 <sup>A</sup>	4.83 <sup>B</sup>	4.88 <sup>B</sup>	4.78 <sup>B</sup>	0.135
FCR (g:g)	1.02 <sup>BC</sup>	1.04 <sup>C</sup>	0.98 <sup>B</sup>	0.93 <sup>A</sup>	1.12 <sup>D</sup>	1.22 <sup>E</sup>	0.013
Dress %	89.0 <sup>A</sup>	89.1 <sup>A</sup>	87.7 <sup>B</sup>	87.5 <sup>B</sup>	89.1 <sup>A</sup>	89.0 <sup>A</sup>	0.270

### *Initial and final weight*

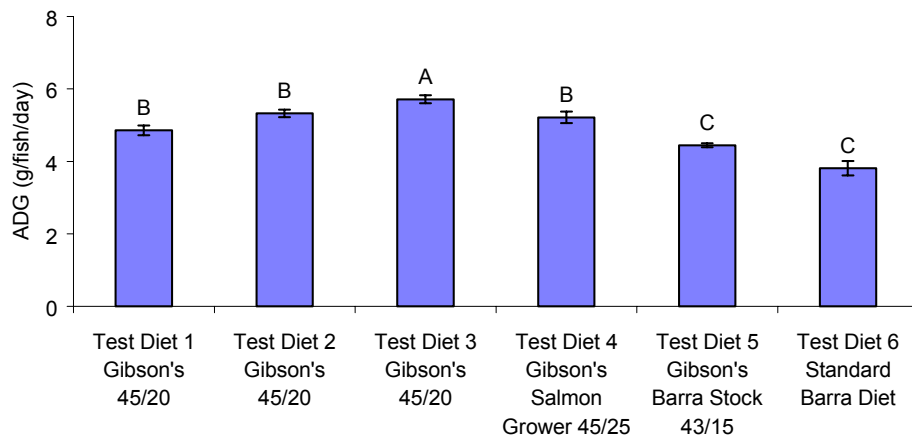
Differences between diets in the start and final weight of the fish are illustrated in Figure 1. Start weights of the fish after the acclimatisation period were found to vary slightly but not significantly ( $P > 0.05$ ) between diets. However, the final weights at completion of the trial were different for the six diets (Figure 1). The highest average final weights were observed for diets 3, 2 and 4 (C2's Salmon Grower). These three diets were not significantly different. Additionally, comparison of means indicated diets 2 and 4 were not significantly different from diet 1. Diet 5 (C2's Barra Stock) and diet 6 (Standard Barra Diet) were significantly different from each other and from the other 4 diets.



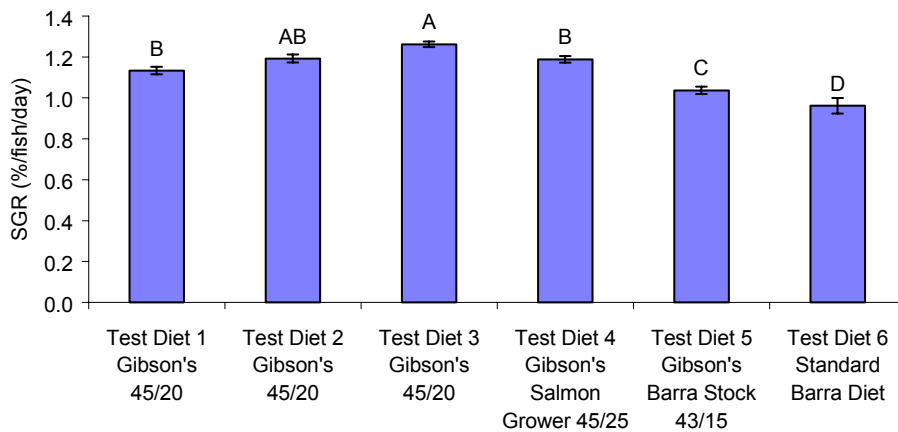
**Figure 1.** Initial and final weights (g) and standard errors for the six diet treatments.

Average daily gain (ADG) and specific growth rate (SGR)

ADG (g/fish/day) differed markedly between the six diets (Figure 2). The best performing diet in terms of ADG was diet 3, which was significantly better ( $P < 0.05$ ) than all other 5 diets. Diets 2, 4 and 1 were similar and significantly better than diets 5 (C2's Barra Stock) and 6 (Standard Barra Diet). The latter two diets did not differ significantly ( $P > 0.05$ ). The trends in differences between SGR (%/fish/day) for the six diets (Figure 3) were identical to the ADG.



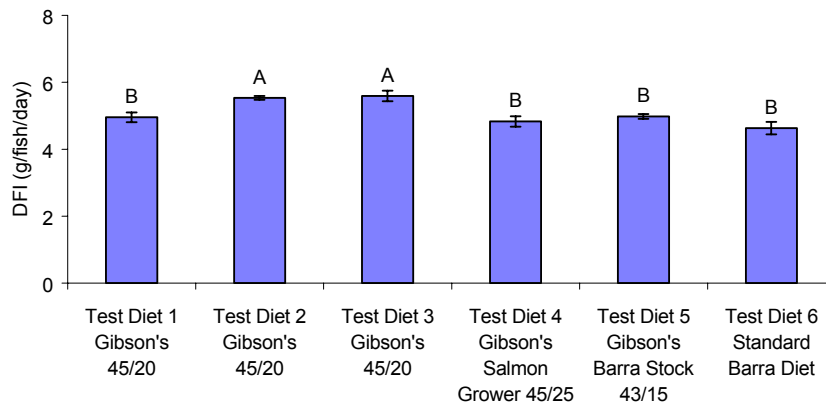
**Figure 2.** Comparison of Average Daily Gain and standard errors among six diets.



**Figure 3.** Comparison of Specific Growth Rate and standard errors among six diets.

### Daily feed intake (DFI)

There was a highly significant difference in DFI (g/fish/day) between the six diets (Figure 4). DFI was greatest for diets 3 and 2, which were significantly different ( $P < 0.05$ ) from the other four diets. The DFI for diets 1, 5, 4 and 6 were similar.

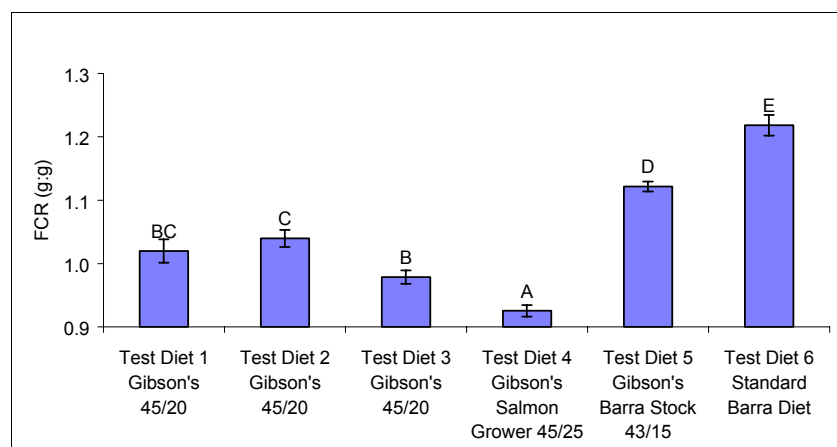


**Figure 4.** Comparison of average Daily Feed Intake and standard errors among six diets.

### Food conversion ratio (FCR)

The results from ANOVA indicate there was a highly significant difference in FCR among diets (Figure 5). The best FCR was with diet 4, which was significantly different from the other 5 diets. Diets 3 and 1 were similar and next in ranking, followed by diet 2. Diets 5 (C2's Barra Stock) and 6 (Standard Barra Diet, FCR = 1.25) both had the worst performing FCR and were significantly different from the other diets, and from each other.

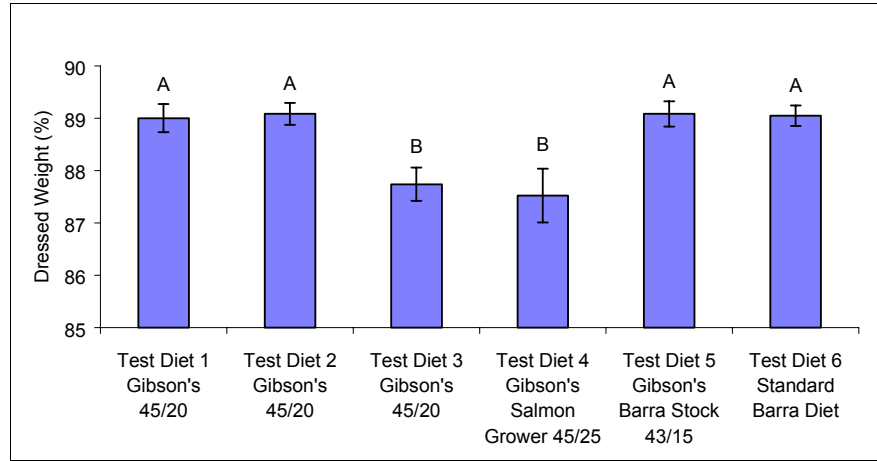
In considering all performance responses to the diets, we see that diet 3 (C2's 45/20) resulted in excellent growth rate and feed intake, but its FCR was slightly inferior to that of Diet 4 (Salmon Grower). The excellent FCR but low daily food intake of the Salmon Grower may be due to either nutritional thresholds being met by this diet or some degree of unpalatability to the fish. Overall, diet 3, and to a slightly lesser extent diet 2, resulted in superior fish productivity compared with the two commercially available barramundi diets (diets 5 and 6).



**Figure 5.** Comparison of average FCR and standard errors among six diets after an eight week.

Dressed weight of fish

Dressing % was significantly lower ( $P < 0.05$ ) for the two existing barra diets (Diets 5 and 6) and best for the other four diets and differences within these two diet groupings were otherwise not significantly different (Figure 6).



**Figure 6.** Average Dressing % of fish and standard errors among six diets after an eight week.

Pellet sink rates

More than 95% of pellets from the three experimental diets (diets 1 to 3), the Salmon Grower (diet 4) and Barra Stock (diet 5) sank within 20 seconds. C2 manufactured all of these diets. In contrast, the standard barramundi diet (diet 6) was very buoyant, with about 75% of pellets still floating after 1 hour (Table 3).

**Table 3.** Cumulative sink rate for pellets from the six diets measured at different time intervals. \* indicates resuspension of some pellets.

Diet	10 s	20 s	1 min	5 mins	15 mins	30 mins	1 hour
1		96 %	96 %	100 %			
2		100 %					
3	100 %						
4		99 %	100 %				
5	97 %	100 %					
6			21 %	25 %	27 %	28 %	25 % *

*Biochemical analysis of diets*

The results of the nutritional analysis are listed in Table 4. The low ash content of diet 6 suggests that very little meat meal was incorporated in its formulation, whereas the high ash content of Diets 1, 2 and 3 suggest a reasonable amount of meat meal may have been used in these diets. The poor daily food intake and reduced growth of diet 4 (C2's Salmon Grower) may be due to a sub-optimal dietary protein to energy (fat) ratio of this diet. Unlike salmon where lipids can be used efficiently to spare for dietary protein, barramundi show much less capacity to use lipid as a primary energy source and the benefit of high energy (fat) diets can only be realised when dietary protein is maintained at a comparatively high specification.

**Table 4.** Nutritional analysis of the six diets used in the trial.

Diet	% DM	% ASH	% N	% FAT	G E MJ/kg
1	91.7	15.0	7.81	20.0	21.7
2	92.1	17.6	8.01	18.8	21.3
3	90.6	14.2	8.14	22.1	22.4
4	91.8	10.8	7.71	27.5	24.2
5	90.4	13.7	7.61	15.7	21.3
6	92.5	9.6	8.67	11.9	22.1



#### 4.3. **Laboratory validation of experimental and commercial barramundi diets: a report commissioned by a commercial feed manufacturer (C1)**

**Christopher G. Barlow<sup>1</sup>, Kevin Williams<sup>2</sup>, Les Rodgers<sup>1</sup>, Ian Hockings<sup>1</sup>, Clarita Agcopra<sup>1</sup> and Karina Ryan<sup>1</sup>**

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#### **Executive Summary**

A commercial feed manufacturer (C1) commissioned the Freshwater Fisheries and Aquaculture Centre at Walkamin to undertake a laboratory-based trial to test a series of diets. The work was undertaken as part of a larger project currently in progress at Walkamin, FRDC 96/393 - Diet Validation and Feeding Strategies. The objective of the trial was to examine the growth performance of barramundi when fed six diets under controlled laboratory conditions in terms of Average Daily Gain (ADG), Specific Growth Rate (SGR), Daily Feed Intake (DFI) and Food Conversion Ratio (FCR).

The six diets tested in this trial were 4 experimental commercial diets (diets 1 to 4), C1 15 MJ (diet 5) and Standard barra diet (diet 6). There were four replicates tanks for each diet, with each tank containing eight fish of approximately 250 g. The bulk weight of fish in each tank was measured on a fortnightly basis over the eight week trial. No mortalities or stressed fish were observed at any time during the trial.

The performance of diet 1 was generally better than the remaining five diets. Diets 1 and 2 had significantly better final weights, ADG, SGR and DFI than the other diets, although diet 2 was not significantly different in most cases from the other experimental diets. All the experimental diets had significantly better FCR. The worst performing diet was C1 15 MJ, which had significantly lower average final weights, ADG, SGR, DFI and the worst performing FCR compared to the other diets.

Appendix 3 provides an economic comparison of dietary productivity. Diet 1, which exhibited the most outstanding production values, cost more than C1 15 MJ to produce an equivalent amount of fish. However, fish on diet 1 grew from 340 to 560 grams in 36 days compared to 47 days on C1 15 MJ. The economic benefit derived from the faster growth rates of fish grown on diet 1 compared with C1 15 MJ would far outweigh the impact of slightly greater feed costs.

Production time for grow-out of barramundi from 100 to 500 g was determined using three separate trials for improved and standard diets (diets 1 and 5 respectively from the present trial and similar diets from 2 previous trials). Barramundi were estimated to grow from 100 to 500 g in approximately 100 days on the improved diet, compared with 150 days on C1 15 MJ. This variation in growth rate would make an enormous difference to farm productivity.

## Introduction

The commercial feed manufacturer approached the Freshwater Fisheries and Aquaculture Centre at Walkamin to undertake a laboratory-based trial to test a series of diets with barramundi. The trial was undertaken on a contract basis, as part of a larger project currently in progress at Walkamin, FRDC 96/393 - Diet Validation and Feeding Strategies.

The objective of the trial was to examine the growth performance of barramundi when fed six test diets under controlled laboratory conditions. The laboratory-based assessment eliminates variables present in commercial trials, such as weather, water temperature, water quality, fish origin and health, and management, and thus provides a more precise measure of the efficacy of the diets. Assessment of diet efficacy was based on Average Daily Gain (ADG), Specific Growth Rate (SGR), Daily Feed Intake (DFI) and Food Conversion Ratio (FCR).

## Materials and Methods

### *Experimental design*

The laboratory evaluation of diets was conducted in the experimental facilities of the Freshwater Fisheries and Aquaculture Centre, Walkamin. Two independent 3680 litre recirculating systems were used for this trial. Each system was sub-divided, effectively giving two experimental blocks per recirculating system. Within each block there were six 140 litre tanks, thus a total of 24 tanks distributed among 4 blocks were used for the trial.

The treatments were six diets, identified as follows:

Diet 1	CFM 1
Diet 2	CFM 2
Diet 3	CFM 3
Diet 4	CFM 4
Diet 5	C1 15 MJ
Diet 6	Standard barra diet

There were four replicates of each of the six diets, with treatments allocated randomly within each block (Table 1). Temperature in all blocks was maintained at  $28 \pm 0.5^\circ\text{C}$  and water salinity was maintained below 0.5‰. Photoperiod was 12L/12D, with lighting supplied by daylight fluorescent tubes.

**Table 1.** Random allocation of six experimental diets for laboratory-based testing.

System C			System D		
Block 1	Tank C6 - Diet 4	Tank C7 - Diet 6	Block 3	Tank D6 - Diet 5	Tank C7 - Diet 3
	Tank C5 - Diet 5	Tank C8 - Diet 2		Tank D5 - Diet 2	Tank C8 - Diet 1
	Tank C4 - Diet 3	Tank C9 - Diet 1		Tank D4 - Diet 6	Tank C9 - Diet 4
Block 2	Tank C3 - Diet 2	Tank C10 - Diet 4	Block 4	Tank D3 - Diet 4	Tank C10 - Diet 2
	Tank C2 - Diet 1	Tank C11 - Diet 5		Tank D2 - Diet 3	Tank C11 - Diet 6
	Tank C1 - Diet 6	Tank C12 - Diet 3		Tank D1 - Diet 1	Tank C12 - Diet 5

The trial commenced in January 1998, after a 14 day acclimatisation period, and continued for eight weeks with trial completion in March 1998. During the acclimatisation period, the fish were held in the recirculation system at 28°C and fed C1 15 MJ, 4 mm pellet manufactured on the 10th January 1998, in order to standardise the pre-trial condition of the fish.

Fish were sorted by weight with eight barramundi of approximately 300 g were randomly allocated to each tank. A precautionary prophylactic salt bath of 12‰ for one hour was administered to fish following initial tank allocation, again on day 1 and thereafter at fortnightly intervals that coincided with weigh days. Fish were individually weighed on day 1 and bulk weighed every 14 days thereafter, including at the end of the fourth fortnight. Fish were not individually weighed at the completion of the trial.

### Feeds and feeding

Diets 1, 2, 3, 4 and 5 arrived at Walkamin on February 5th 1998. The label on diet 5 indicates a date of manufacture of January 9th 1998. Diet 6 was a standard barra diet purchased commercially. Date of manufacture was not indicated on any packages, however this feed arrived at Walkamin on February 9th 1998. All diets were stored at Walkamin in a cool room at 4 to 7°C during the period between delivery and completion of the trial. Pellets in all diets were approximately 5 to 6 mm in diameter and length.

Fish were fed to satiation each morning from 8:30 to 10:30 am over a 45 to 60 minute period. Fish were not fed on day 1 when fish were being allocated to tanks and individually measured. Nor were fish fed on days during which bulk weight measurements were being recorded (at the end of each fortnight).

After feeding, uneaten pellets were removed and counted. The dry weight equivalent of the uneaten pellets was determined from the mean pellet weight of a sample of 1000 pellets of the corresponding diet. Daily feed intake was then calculated for each tank by subtracting the calculated weight of uneaten pellets from the difference in corresponding feed bucket weights, recorded at the beginning and end of each feed.

### Experimental data collected during the trial

The following experimental data were collected during the trial:

- a) Average weight of pellets for each test diet;
- b) Food consumption (daily record of weight of feed issued minus uneaten food) for each experimental tank accumulated fortnightly;
- c) Individual weight of fish in each tank on Day 1;
- d) Bulk weight of fish in each tank, taken on Day 15 and each fortnight thereafter;
- e) Daily record of mortalities, water chemistry and temperature; and
- f) Dressing percentage of 12 fish per treatment (i.e. 3 per tank) at end of trial.

### Calculation of production values

The experimental data outlined in the previous section were used to calculate Average Daily Gain (AGD) in g/fish/day, Specific Growth Rate (SGR) in percentage body weight/fish/day, Daily Feed Intake (DFI) in g/fish/day, Food Conversion Ratio (FCR) in g:g and Dressed Weight expressed as a percentage of total weight (Dressing %). In the following equations,  $W_{t_i}$  is the average fish weight at the beginning of the experiment ( $t_i$ ) and  $W_{t_f}$  is the average fish weight at the end of the experiment ( $t_f$ ).

$$ADG = \frac{Wt_f - Wt_i}{t_f - t_i}$$

$$SGR = \left[ \frac{\ln(Wt_f) - \ln(Wt_i)}{t_f - t_i} \right] * 100$$

$$DFI = \frac{\text{Total food consumption per tank}}{\text{Number of fish per tank} * (t_f - t_i)}$$

$$FCR = \frac{DFI}{ADG}$$

$$\text{Dressing \%} = \frac{[(\text{Total weight} - (\text{gill} + \text{gut}) \text{ weight}) \div \text{Total weight}] * 100}{\text{Total weight}}$$

#### Pellet sink rates

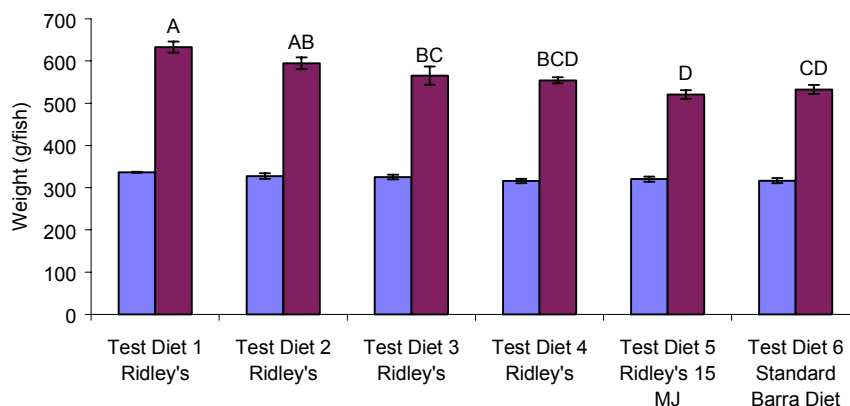
The sink rate of pellets was assessed by adding 100 pellets to a tank, and recording the number floating at various time intervals up to one hour thereafter.

#### Biochemical analysis of diets

A 500 g sample of each diet was dispatched to the Animal Research Institute for proximate (dry matter, ash, crude protein, total lipid, energy) and fatty acid analyses as soon as the diets were available.

#### Statistical analysis

Initial and final weights, growth (ADG and SGR), survival, food conversion and dressing percentage were compared using analysis of variance. Variances were examined for homogeneity and in all cases data transformations were not necessary. Pair-wise comparison of treatment means were made with the least significant difference test applied only where the 'F' value of the ANOVA was significant ( $P < 0.05$ ). All statistical tests were performed at the 5% significance level.



**Figure 1.** Initial and final weights (g) and standard errors for the 6 diet treatments.

## Results and Discussion

### *Production data*

Performance data with respect to diets are listed in Table 2. The respective performance traits are presented graphically and discussed separately below. No mortalities or stressed fish were observed at any time during the trial.

**Table 2.** Production data and standard errors for six test diets following an 8 week growth assay with 8 fish per tank. Within trait comparisons, means with a common superscript letter were significantly different ( $P > 0.05$ ). Values for ADG, SGR and DFI were covariance-adjusted to remove the effect of minor differences in starting weight of the fish.

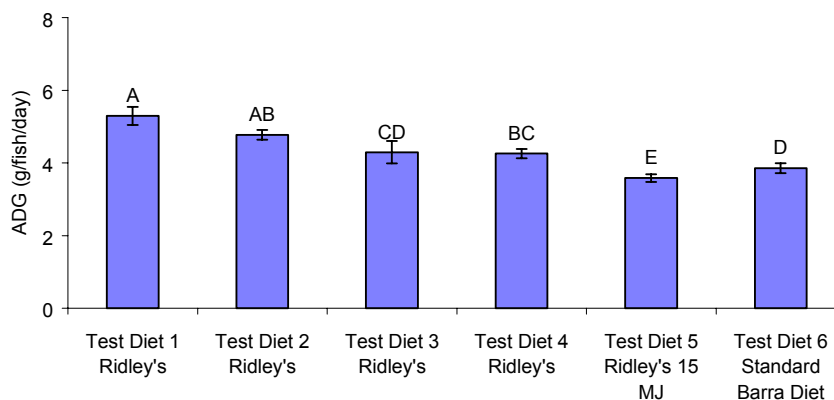
Trait	Diet Label						± sem
	Diet 1 CFM 1	Diet 2 CFM 2	Diet 3 CFM 3	Diet 4 CFM 4	Diet 5 C1 15 MJ	Diet 6 Standard Barra Diet	
Initial Wt (g)	336.5	327.4	325.2	316.4	320.3	316.8	5.30
Final Wt (g)	633.0 <sup>A</sup>	594.7 <sup>AB</sup>	565.7 <sup>B</sup>	554.5 <sup>BCD</sup>	521.0 <sup>D</sup>	532.8 <sup>CD</sup>	13.52
ADG (g/d)	5.01 <sup>A</sup>	4.69 <sup>AB</sup>	4.26 <sup>CD</sup>	4.43 <sup>BC</sup>	3.66 <sup>E</sup>	4.01 <sup>D</sup>	0.121
SGR (%/d)	1.11 <sup>A</sup>	1.06 <sup>AB</sup>	0.98 <sup>BC</sup>	1.02 <sup>B</sup>	0.87 <sup>D</sup>	0.94 <sup>C</sup>	0.020
DFI (g/d)	5.16 <sup>A</sup>	4.78 <sup>AB</sup>	4.44 <sup>BC</sup>	4.62 <sup>BC</sup>	4.29 <sup>C</sup>	4.32 <sup>C</sup>	0.142
FCR (g:g)	1.04 <sup>A</sup>	1.02 <sup>A</sup>	1.04 <sup>AB</sup>	1.04 <sup>A</sup>	1.18 <sup>C</sup>	1.07 <sup>B</sup>	1.011
Dress %	87.3 <sup>C</sup>	89.4 <sup>A</sup>	88.1 <sup>B</sup>	88.8 <sup>A</sup>	88.9 <sup>A</sup>	89.0 <sup>A</sup>	0.22

### *Initial and final weight*

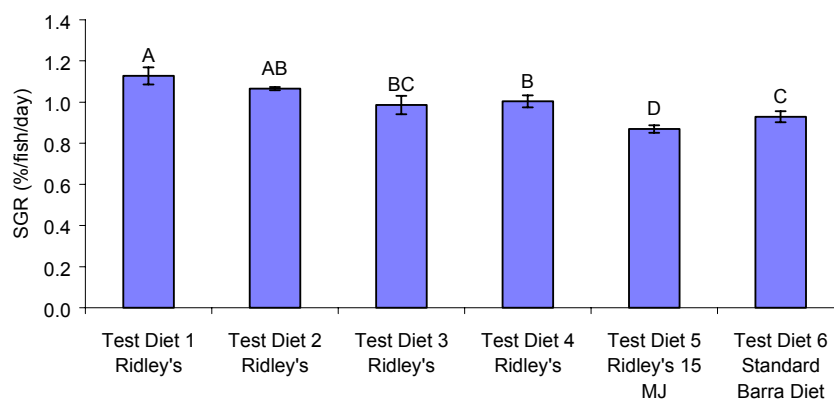
Differences between diets in the start and final weight of the fish are shown in Table 2, Figure 1. Start weights of the fish after the acclimatisation period were found to vary slightly but not significantly ( $P > 0.05$ ) between diets. At the end of the trial, fish were heaviest on diet 1 and significantly heavier than those on all other diets except for diet 2. Diet 5 produced the lightest fish and significantly lighter ( $P < 0.05$ ) than for Diets 1, 2 and 3. Final weight of fish on diet 5 also was low and as for diet 6, was significantly different to Diets 1, 2 and 3. (Figure 1).

Average daily gain (ADG) and specific growth rate (SGR)

The effect of dietary treatment on ADG and SGR were similar but with a slightly clearer treatment differentiation in the case of ADG (Figure 2). The best performing diets in terms of ADG were diets 1 and 2. However, diet 2 was not significantly different from diet 4. Diet 5 (C1 15 MJ) had an ADG significantly less than the other 5 diets. There was a highly significant difference in SGR (%/fish/day) among diets (Figure 3). The best performing diets in terms of SGR were Diets 1 and 2. However, diet 2 was not significantly different from Diets 3 and 4. Diet 5 (C1 15 MJ) had the worst performing SGR, which was significantly different from the other 5 diets.



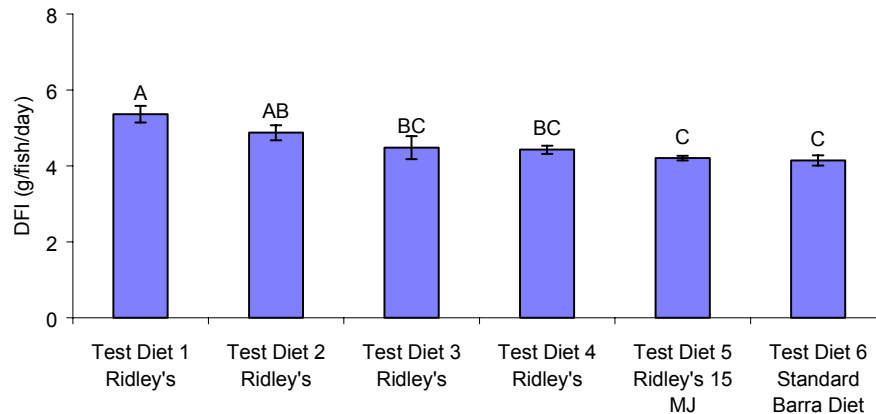
**Figure 2.** Comparison of Average Daily Growth and standard errors among six diet treatments.



**Figure 3.** Comparison of Specific Growth Rate and standard errors among six diet treatments.

### Daily feed intake (DFI)

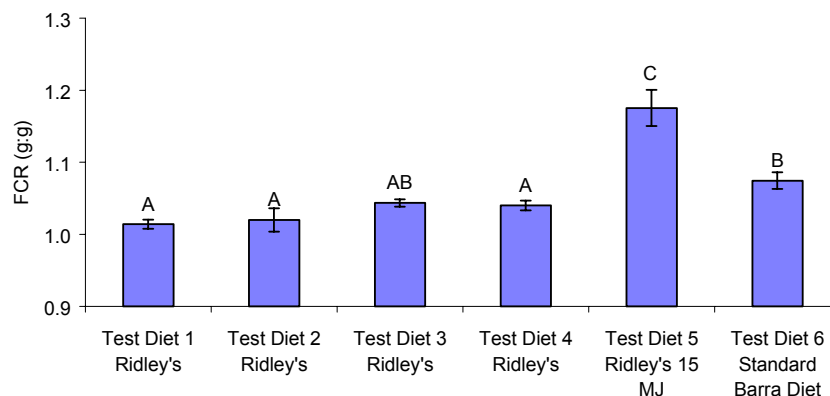
There was a highly significant difference in DFI between diets (Figure 4). DFI was highest for diet 1 and significantly better than all diets other than for diet 2. Diet 2 had a higher ( $P < 0.05$ ) DFI than either of Diets 5 and 6 but differences between diets 3, 4, 5 and 6 were not significant. Examination of the growth and food intake data show that Diets 1 and 2 were clearly superior to all other diets while that of Diets 5 and 6 generally worse than all other diets. That is, Diets 1 and 2 resulted in excellent fish production and were highly palatable (or more correctly acceptable) to the fish.



**Figure 4.** Comparison of Daily Feed Intake and standard errors among six diet treatments.

### Food conversion ratio (FCR)

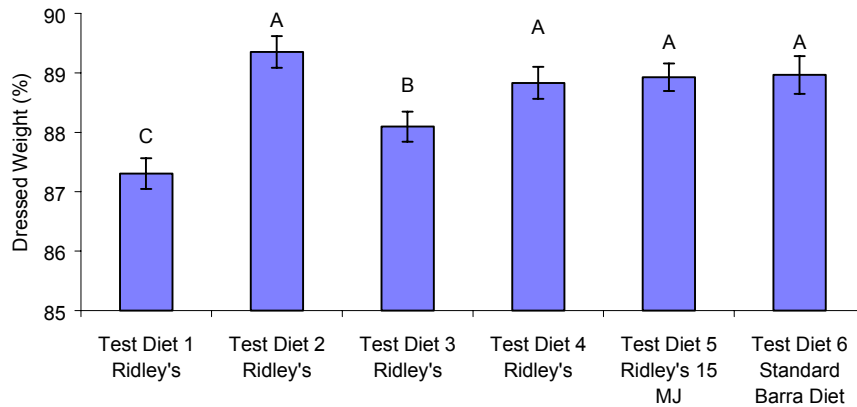
The FCRs of the four experimental diets (Diets 1 to 4) were similar and excellent (Figure 5). Diets 3 and 6 were not significantly different from one another while diet 5 had the worst FCR which was significantly different to all other diets.



**Figure 5.** Comparison of Food Conversion Ratio and standard errors among six diet treatments.

### Dressed weight of fish

Dressing % was best for Diets 2, 4, 5 and 6 with all of these diets being significantly ( $P < 0.05$ ) better than either Diets 3 or 1 with the latter being worst overall ( $P < 0.05$ ) (Figure 6). Nevertheless, the very narrow range (87.3 to 89.4%) for dressing % across all treatments indicates that the commercial impact of the two worst diets (Diets 1 and 3) would be negligible.



**Figure 6.** Average dressing % of fish and standard errors among six diet treatments.

### Pellet sink rates

The four experimental diets sank very rapidly (Table 3). In contrast, C1 15 MJ was very buoyant, with 95% of the pellets still floating after 1 hour. For the standard barra diet (diet 6), more than half of the pellets sank within 1 minute, but the majority of the remaining pellets continued to float for longer than 1 hour.

**Table 3.** Cumulative sink rate for pellets from the six test diets measured at different time intervals.

Diet	10 s	20 s	1 min	5 mins	15 mins	30 mins	1 hour
1	100 %						
2	100 %						
3	100 %						
4	100 %						
5			3 %	4 %	5 %	5 %	5 %
6			63 %	67 %	72 %	74 %	77 %



*Biochemical analysis of diets*

The results of the nutritional analysis are listed in Table 4. The low ash content of Diets 1 to 5 suggests that very little if any meat meal was used in the formulation. In contrast, the high ash content of diet 6 suggests that a reasonable amount of meat meal had been used in the diet.

**Table 4.** Nutritional analysis of the six test diets used in the trial.

Diet	% DM	% ASH	% N	% FAT	G E MJ/kg
1	93.7	9.1	9.36	19.8	23.7
2	93.7	9.3	9.48	15.8	23.2
3	94.1	8.5	8.33	17.5	23.1
4	93.7	8.7	8.67	14.9	22.8
5	94.0	9.4	8.48	14.4	22.6
6	92.6	11.8	7.81	17.5	22.3

#### 4.4. Laboratory and farm validation of high specification barramundi diets: a collaborative research project between FRDC Aquaculture Diet Development Subprogram and a commercial feed manufacturer (C2)

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### Executive Summary

The Freshwater Fisheries and Aquaculture Centre at Walkamin was commissioned by a commercial feed manufacturer (C2) to compare the performance of two, prototype high specification barramundi diets with the currently available industry standard diets. These experiments were undertaken as part of the Fisheries Research and Development Corporation's (FRDC) Aquaculture Diet Development subprogram. The objectives of these trials were to examine the growth performance of barramundi over a wide size range, when fed the specified diets under both laboratory and on-farm conditions.

The three diets tested in these trials were:

- Diet 1 — a 50/50 mixture of two commercially and currently available barra diets - (C1 50/15 MJ and C2's stock 45/15 Barra diets);
- Diet 2 — C2's meat meal based 45/20; and
- Diet 3 — C2's fishmeal based 45/20.

In the laboratory study, all three diets were tested with each diet being fed to four replicate tanks of fish. In the farm study at Sugarland Barramundi, Edmonton, only diets 1 and 3 were used, with each diet being fed to six replicate cages of fish.

Fish were grown to market size in both trials and growth was measured in terms of final weight, average daily gain, specific growth rate, daily feed intake, and food conversion ratio (FCR). The bulk weights of all fish in the laboratory and farm trials were recorded at fortnightly and six weekly intervals respectively. Minimal mortalities occurred in both trials with survivals in excess of 95% in all treatment groups.

In the laboratory study, the final weights of fish fed with Diets 2 and 3 were similar, but were not significantly different to those fed with Diet 1 ( $P = 0.11$ ). However, the low  $P$ -value suggests that, in a biological sense, Diets 2 and 3 were likely to be superior to Diet 1. Diets 2 and 3 resulted in significantly better FCRs than Diet 1 ( $P < 0.05$ ). That is, fish receiving either meat meal or fishmeal based 20MJ diets displayed similar growth rates and FCR. On the negative side, dressing percentages of fish grown on Diets 2 and 3 were significantly worse ( $P < 0.05$ ) than fish grown on Diet 1, by about 1.5 to 2%.

Many trends observed in the laboratory trial were confirmed in the farm trial. Diet 3 resulted in significantly greater production, better FCR and lower dressing percentage than Diet 1. The mean individual weight of fish receiving Diet 3 increased from 106 g to a market size of 500 g in 126

days under farm trial conditions, while fish on the Diet 1 achieved a corresponding final mean weight of 450 g.

The improved performance of Diets 2 and 3 relative to Diet 1 were economically modelled. Diet 3, which resulted in an 11% improvement in FCR relative to Diet 1 under farm trial conditions, was 13.1% more expensive. However, production times were considerably faster with Diet 2 and 3. Analyses using the BarraProfit model showed that Diet 3 is expected to result in a 4.9% increase in averaged annual output compared with Diet 1. This equates to a 31% increase in profitability on a model farm producing an average of 50 tonne of fish per year. Because barramundi preferentially use protein rather than fat for energy metabolism, increasing the protein concentration of the diet to at least 50% would enable even higher rates of production and farm profits.

## **Introduction**

A commercial feed manufacturer (C2) approached the Freshwater Fisheries and Aquaculture Centre at Walkamin to undertake laboratory and farm based evaluations of two new high specification barramundi diets. The development of these diets represented additional progress in the realisation of C2's commercial strategy and commitment to the barramundi farming industry. The trial was undertaken on a contract basis, as part of a larger project currently in progress at Walkamin, namely FRDC 96/393 — Diet Validation and Feeding Strategies. This is one of several major projects comprising FRDC's Aquaculture Diet Development subprogram.

The objectives of these trials were to examine the growth performance of barramundi when fed specified diets under both laboratory and farm conditions. The laboratory and farm trials complement each other. The laboratory-based assessment provides a concise comparison of the efficacy of the diets, as it eliminates variables such as weather, changing water temperatures, variable water quality, cage fouling, risks to fish health and some other management considerations. On the other hand, the farm trial provides an insight into diet performance and productivity, as seen from a practical farming perspective.

Barramundi were grown to market size in both trials and growth was measured in terms of final weight, average daily gain (ADG), specific growth rate (SGR), daily feed intake (DFI) and food conversion ratio (FCR).

## **Materials and Methods**

### Laboratory Experiment

#### *Experimental design and procedures*

The laboratory evaluation of diets was undertaken in the experimental facilities of the Freshwater Fisheries and Aquaculture Centre at Walkamin. Four similar but independent recirculating systems of 6050 litres, with six 800 l tanks (experimental units) in each, were used for the study.

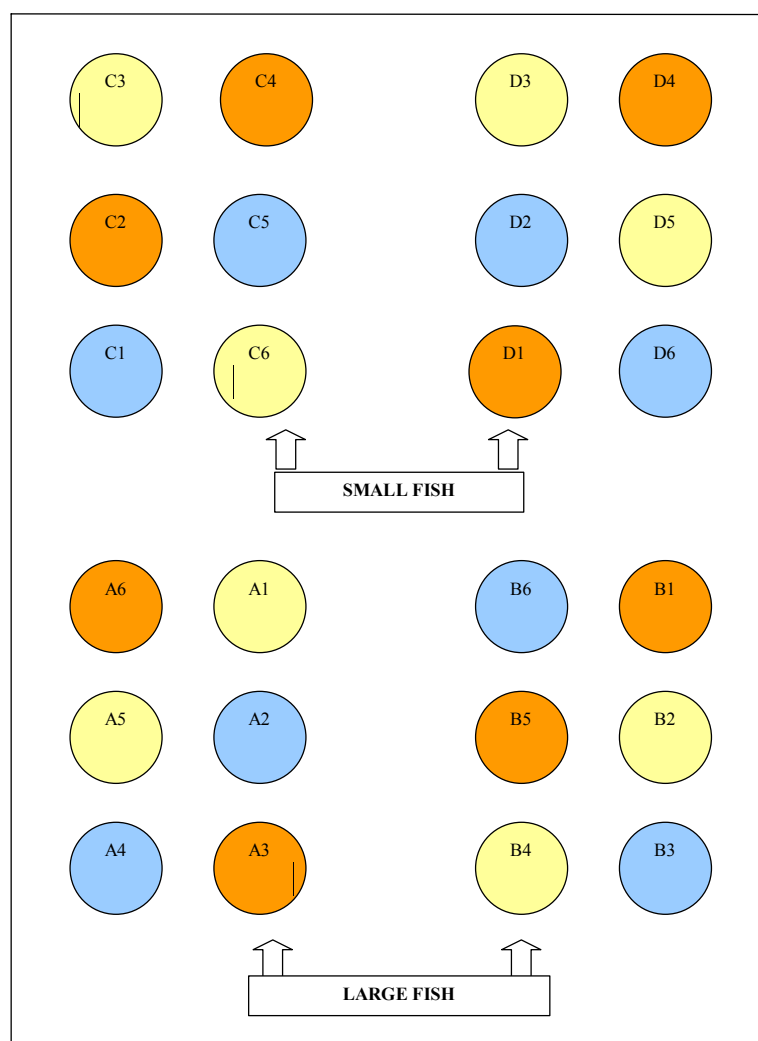
Two recirculating systems (C and D systems), were initially stocked with 12 small barramundi per tank of 62.4 g average start weight, while the tanks of the remaining two systems (A and B systems) were stocked with 8 larger fish of 239.9 g average start weight. Each recirculation system was subdivided into two experimental blocks of three tanks, with each tank receiving a different dietary treatment. That is, the laboratory trial consisted of eight replicates (four large fish, four small fish) for each of three dietary treatments (24 tanks in total, see Figure 1).

The small and large fish groups were then simultaneously grown to about 250 g and 780 g respectively over a 14 week period. That is, the final weight of the small fish group overlapped the start weight of the larger fish. The use of these two size groups allowed the assessment of the growth of fish from about 60 g to 780 g in the experimental period of 14 weeks.

The trial commenced on 28 October 1998, after a 14 day acclimatisation period and continued for fourteen weeks until 4 February 1999. During and prior to the acclimatisation period, the fish were fed on a standard 15MJ commercial barramundi diet, to standardise the pre-trial condition of fish.

Fish were individually weighed on day 1 and bulk weighed every 14 days thereafter, before being individually weighed again at trial completion. A precautionary prophylactic salt bath of 12 g/l was administered to fish for one hour following initial tank allocation, again on day one and thereafter at fortnightly intervals that coincided with fish handling on weigh days.

The temperature in all blocks was maintained at  $28 \pm 0.5^\circ\text{C}$  and water salinity maintained below 0.5ppt. Photoperiod was 12L/12D, with lighting supplied by daylight fluorescent tubes.



**Figure 1.** Experimental layout for laboratory trial consisting of four independent recirculating systems, together with the randomly allocated dietary treatment for each tank of fish.

### Feeds and feeding

The three diets fed to barramundi during the trial were as follows.

- Diet 1 consisted of an equal mixture by weight of 6 mm C1 50/15 MJ diet manufactured on the 8 September and the 25 November 1998, together with 6.5 mm C2's stock barramundi diet manufactured on the 7 September 1998 (production run 628).
- Diet 2 was a 6.5 mm 45% protein/18.5 MJ meat meal based diet manufactured by C2 on the 30 September 1998. An additional 1.5% fish oil by weight was added to the diet prior to feeding, in order to bring the diet to the specified energy level ( $\approx 20$  MJ). The final test diet was 45%/20 MJ.
- Diet 3 was a 6.5 mm 45% protein/20 MJ fishmeal based diet manufactured by C2 during September 1998 (production run 676).

All diets were stored in a cold-room at 4–7°C, however a temporary 24 hr breakdown of cold-room facilities occurred between Christmas and New Year 1998 during which temperatures rose to 30°C for a few hours.

During the trial, all fish within each tank were fed to satiation each morning between 8.30–10.30am, with the exception of the weigh days at the start of each fortnight. Uneaten pellets were removed from each tank after feeding and counted. The dry weight equivalent of the uneaten pellets was determined from the mean pellet weight of a sample of 1000 pellets of the corresponding diet. Daily feed intake (DFI) was then calculated for each tank by subtracting the calculated weight of uneaten pellets from the difference in the corresponding feed bucket weights for each tank, recorded at the beginning and end of each feed.

### Experimental data

The following experimental data were collected during the trial:

- (a) Average weight of pellets for each diet.
- (b) Daily feed intake (DFI). Daily record of weight of feed issued minus uneaten food for each tank.
- (c) Individual weight of each fish in each tank on trial start and completion dates.
- (d) Bulk weight of all fish in each tank recorded on the first day of each fortnight during the interim trial period.
- (e) Daily record of mortalities and temperatures, with the additional monitoring of other water quality parameters on a twice weekly basis.
- (f) Dressing percentage of three fish from each tank (24 fish/treatment) at trial conclusion.

These experimental data were then used to calculate average daily gain (ADG) in g/fish/day; specific growth rate (SGR) in percentage body weight /fish/day; DFI in g/fish/day; food conversion ratio (FCR) in g dry food:g wet weight fish; and dressing percentage (Dressing %) expressed as dressed weight as a percentage of total weight. In the following equations,  $W_{t_i}$  is the average fish weight at the initiation of the trial ( $t_i$ ) and  $W_{t_f}$  is the average fish weight at the termination of the trial ( $t_f$ ).

$$ADG = \frac{W_{t_f} - W_{t_i}}{t_f - t_i}$$

$$SGR = \left[ \frac{\ln(W_{t_f}) - \ln(W_{t_i})}{t_f - t_i} \right] * 100$$

$$DFI = \frac{\text{Total food consumption per tank}}{\text{Number of fish per tank} * (t_i - t_0)}$$

$$FCR = \frac{DFI}{ADG}$$

$$\text{Dressing \%} = \frac{[\text{Total weight} - (\text{gill} + \text{gut weight})]}{\text{Total weight}} * 100$$

### *Statistical analyses*

Initial and final weights, growth (ADG and SGR), survival, FCR and dressing percentage were compared using analysis of variance. Variances were examined for homogeneity and in all cases data transformations were not necessary. Pair-wise comparison of treatment means was made with the least significant difference test applied only when the F value of the ANOVA was significant ( $P < 0.05$ ). All statistical tests were performed at the 5% significance level.

### *Biochemical analyses of diets*

A 500 g sample of each diet was dispatched to the Animal Research Institute for proximate analyses of dry matter, ash, crude protein, total lipid and gross energy.

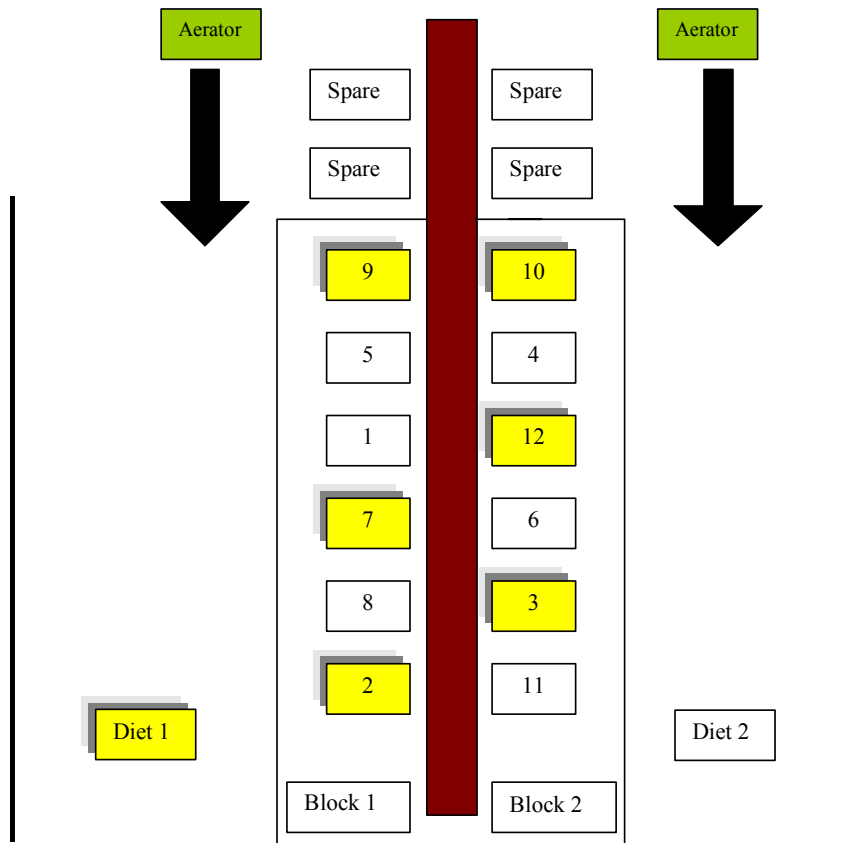
### *Farm Experiment*

#### *Experimental design and procedures*

The field evaluation of diets was conducted at Sugarland Barramundi, Edmonton, which is a barramundi farm located 15 km south of Cairns, owned and operated by Mark Fantin. Twelve commercial type 8 m<sup>3</sup> cages, constructed of approx 12mm mesh with internal 90mm PVC floating collars, were individually stocked with 200 Barramundi of approx 106 g. All fish used in this trial had been previously stocked and maintained in the experimental pond for 6 weeks prior to commencing the experiment.

The 12 cages were positioned in the production pond and divided equally into two experimental blocks of six cages located on either side of a central, floating walkway (Figure 2). Three randomly selected cages within each block were allocated to each of the two experimental dietary treatments. Two aspirator-type aerators provided oxygenation.

The farm study commenced on 27 October 1998 and was completed eighteen weeks later on 2 March 1999. Fish were grown at ambient temperatures in the experimental pond, which was characterised by extensive macrophyte beds, good oxygenation from two mechanical aerators and limited but constant water exchange.



**Figure 2.** Experimental layout for the farm study at Sugarland Barramundi.

### *Feeds and feeding*

The two dietary treatments evaluated in the farm study corresponded to Diets 1 and 3 of the laboratory study. Consequently, these same diet numbers have been used in the farm study for the purposes of clarity of description and ease of understanding the results.

Diet 1 was a 50/50 mixture of the two currently available, industry standard diets (45-50% protein and 15 MJ energy), while Diet 3 was a C2 fishmeal based diet of 45% protein and 20 MJ energy. The batch manufacturing details are provided in Section 3.1.2. All diets were floating and of 6 to 6.5 mm pellet size.

Feeding was conducted between 4pm and 6pm on six days per week (generally excluding Sundays), during which the fish were fed to satiation from individual feed bins for each corresponding cage. Farm operators fed the fish on four days per week, while research staff fed the fish on the remaining two days of each week. Fish were not fed on the days prior to, or on the days of periodic handling and weighing, which was conducted at 6 weekly intervals. The weights of all feed offered to individual cages were recorded throughout the trial.

The only exception to this feeding schedule occurred during Cyclone Rona in February when feeding was disrupted for a 5 day period.

### *Experimental data*

All fish within each cage were captured and anaesthetised with 2-phenoxyethanol (200 ppm) to facilitate bulk weighing and individual counting at 6 weekly intervals. A running record of all feed offered to each cage, which included the actual feed consumed in addition to that wasted, was also kept throughout the trial.

The following experimental data were collected during the trial:

- (a) Average initial start weight of fish in each cage.
- (b) Total weight of feed offered to fish within each cage, for the trial duration.
- (c) Survival of fish as determined at 6 weekly intervals throughout the trial.
- (d) Bulk weight of all fish within each cage recorded at 6 weekly intervals.
- (e) Average finish weight for fish from each cage.
- (f) Dressing percentage of five fish from each cage (30 fish per treatment) at the trial completion.

Production parameters were determined as for the laboratory trial.

### *Statistical analyses*

Statistical analyses were conducted as described in Section 3.1.4. The total increase in fish weight in each cage (biomass gain, kg) was also analysed.

### *Economic analyses*

The purpose of the economic analyses was to compare the profitability of a “model” barramundi farm model using either the existing standard diet (Diet 1) or the high specification experimental barramundi diet (Diet 3), using data derived in the Sugarland Barramundi farm study.

The analyses incorporated impacts of changing on various economic and production components of the farming operation. These included:

- Food conversion ratios (FCR) – the amount of feed required to produce a standard quantity of fish.
- Growth rates – the time required to produce a fish of certain weight.
- Price of feed – a variable operating cost.

The following farm model was created to compare these two diets using the specified physical, biological and economic parameters (Table 1).



**Table 1.** Physical, biological and economic parameters of the model barramundi farm. For the purposes of analyses, all other parameters in the model barramundi farm were assumed to remain constant, except for FCR, growth rate and price of feed.

Physical	
Total farm area	5.0 hectares
Total ponded area	2.5 hectares
Farming method	Freshwater - cages in ponds
Biological	
Biomass of farm	30,541 tonnes
Death rate	40 %
Target weight of fish	500 grams
Economic	
Capital establishment cost	\$476,300
Employees	2 Permanent (including owner)
Marketing method	Whole, plate-size fish
Price received	\$10.00 / kg

## Results

### Laboratory Experiment

#### *Biochemical analyses of diets*

The results of biochemical analyses of all experimental diets are summarised in Table 2. The most noticeable difference between the diets was the comparatively high level of fat in Diets 2 and 3 compared with the commercial diet.

**Table 2.** Biochemical analyses of experimental diets on an 'as-fed' basis.

Diet	% DM	% Ash	%CP	% Fat	G E (MJ/kg)
Diet 1 (a)	92.7	9.5	49.4	12.1	20.5
Diet 1 (b)	94.2	10.8	51.3	12.0	20.6
Diet 1 (c)	90.8	12.2	43.5	12.7	19.3
Diet 2	91.4	14.5	43.8	18.4	20.0
Diet 3	90.0	10.8	44.2	19.8	20.4

(a) commercial barramundi diet manufactured 8 September 1998 by C1.

(b) commercial barramundi diet manufactured 25 November 1998 by C1.

(c) C2's commercial barramundi diet manufactured 7 September 1998 (run 628).

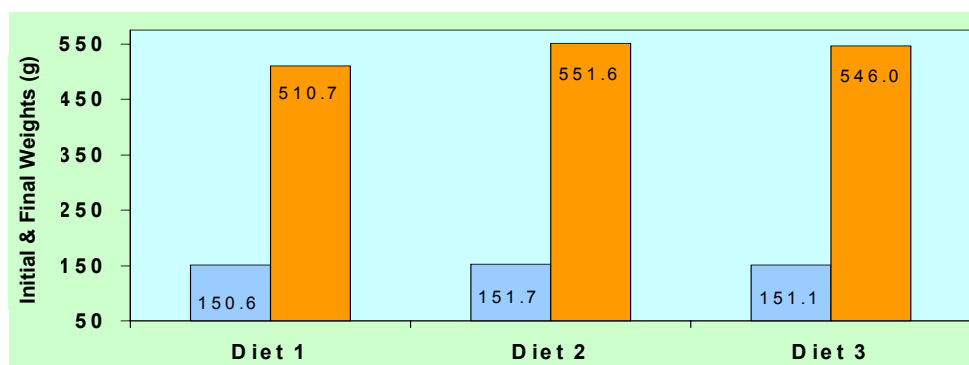
### Initial and final weight

There were no significant differences ( $P > 0.05$ ) in the mean start weights between the treatments for either the small or large fish groups. Analysis of variance of the final weights showed that  $P = 0.11$ , indicating that there were no statistically significant differences in the weights of fish in the 3 treatments at the end of the trial. However, in biological terms, Diets 2 and 3 were equal to each other and gave superior production compared with Diet 1 (Table 3). The better growth recorded with Diets 2 and 3 is depicted in Figure 3, which shows the mean start and finish weights for the fish (small and large groups combined) on the 3 dietary treatments.

Other detailed performance data with respect to these diets are presented in the Table 4 and also graphically in the following figures.

**Table 3.** Initial and final weights for the large and small fish groups, and mean weights for the two groups, in the laboratory trial.

Fish size	Diet 1	Diet 2	Diet 3
<i>Start weight (g)</i>			
Large	238.0	240.6	241.1
Small	63.3	62.7	61.1
Mean	150.6	151.7	151.1
<i>Final (14 week) weight (g)</i>			
Large	691.9	769.2	759.7
Small	329.5	333.9	332.3
Mean	510.7	551.6	546.0



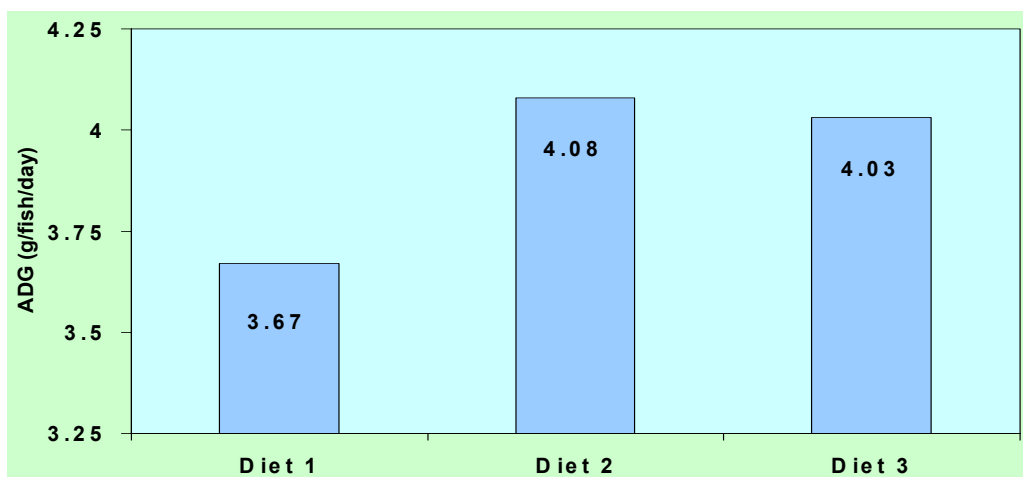
**Figure 3.** Mean initial and final weights across fish sizes for the three treatment groups in the laboratory study.

**Table 4.** Production data for the laboratory study. The initial and final weights are means for the large and small fish combined. Within trait comparisons, means with a common superscript letter were not significantly different ( $P > 0.05$ ).

Trait	Diet 1 1 (C1) : 1 (C2) commercial diets	Diet 2 C2's meat meal 45/20	Diet 3 C2's fishmeal 45/20
<i>Small fish</i>			
ADG (g/d)	2.72	2.77	2.77
SGR (%/d)	1.68	1.70	1.73
DFI (g/d)	2.86	2.58	2.51
FCR (g/g)	1.05 <sup>B</sup>	0.93 <sup>A</sup>	0.91 <sup>A</sup>
Dressing percentage	88.74 <sup>A</sup>	86.86 <sup>B</sup>	86.53 <sup>B</sup>
<i>Large fish</i>			
ADG (g/d)	4.63	5.39	5.29
SGR (%/d)	1.09	1.19	1.17
DFI (g/d)	5.34	5.61	5.45
FCR (g/g)	1.15 <sup>B</sup>	1.04 <sup>A</sup>	1.03 <sup>A</sup>
Dressing percentage	88.18 <sup>A</sup>	87.22 <sup>B</sup>	86.07 <sup>C</sup>

#### *Average daily gain (ADG)*

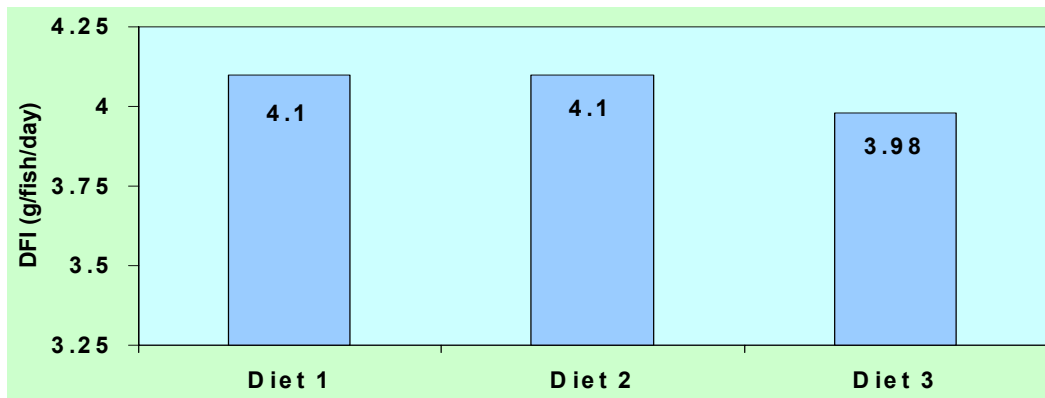
Differences in average daily gain (g/fish/day) between the treatments at the conclusion of the trial were not significant ( $P < 0.05$ ). However, there was a strong trend ( $P = 0.11$ ) for fish fed Diet 1 to grow more slowly than those fed either Diets 2 or 3 and this trend was more apparent with the large compared to the small fish (Table 4).



**Figure 4.** Average daily weight gains across fish sizes for the three treatment groups in the laboratory trial.

### Daily feed intake

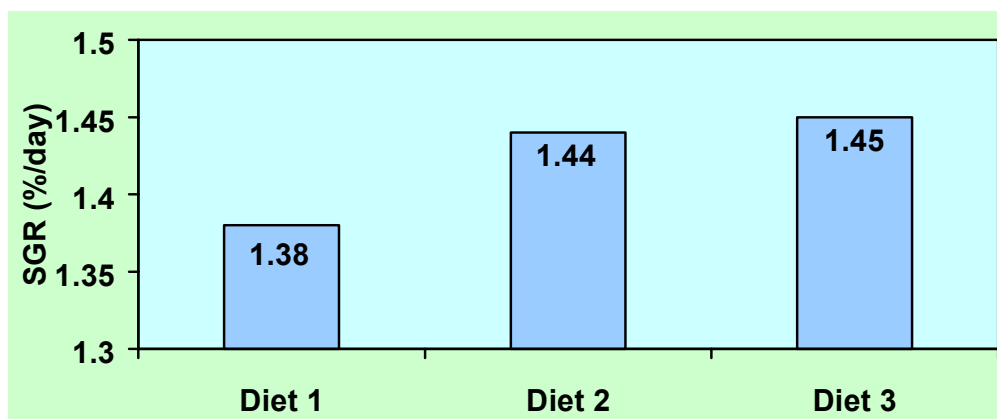
There were no significant differences in daily feed intake (g/fish/day) between the three treatments at the conclusion of the trial (Figure 5).



**Figure 5.** Daily feed intake (g/fish/day) across fish sizes for the three treatment groups in the laboratory trial.

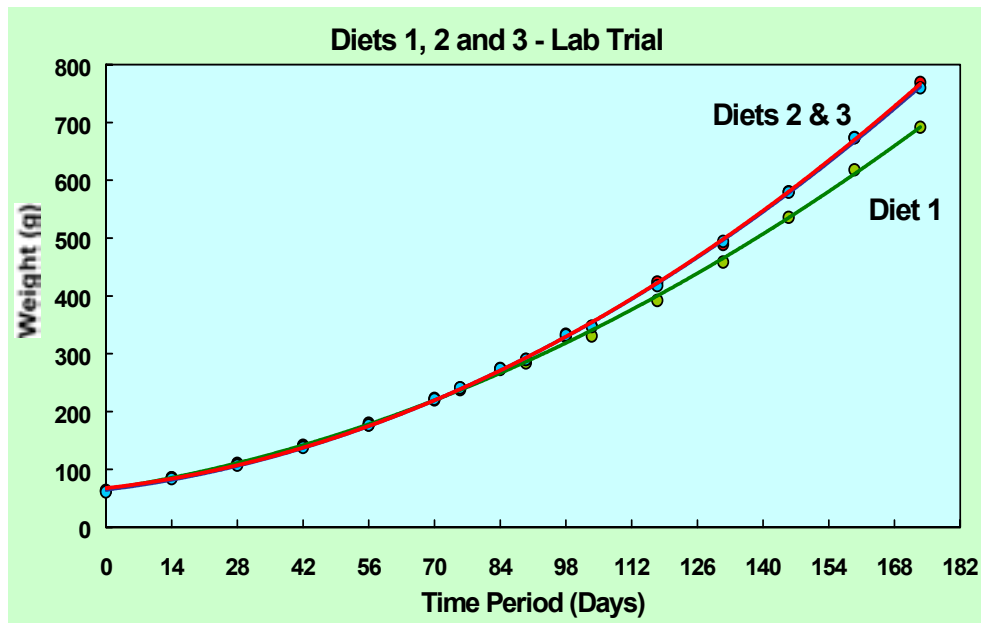
### Specific growth rate (SGR)

There were no significant differences in specific growth rate (% wt/day) between the treatments at the end of the trial (Figure 6).



**Figure 6.** Specific growth rate (% wt/day) across fish sizes for the three treatment groups during the laboratory study.

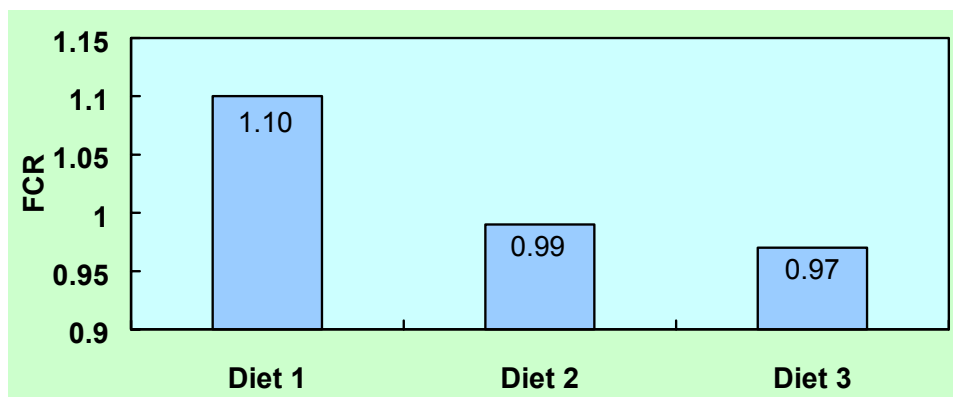
The growth curves of the three experimental fish groups are shown in Figure 7. Fish on Diets 2 and 3 grew at similar rates, and were slightly faster (but not significantly so) than those on Diet 1.



**Figure 7.** Average growth of barramundi fed the three test diets in the laboratory study.

*Food conversion ratio (FCR)*

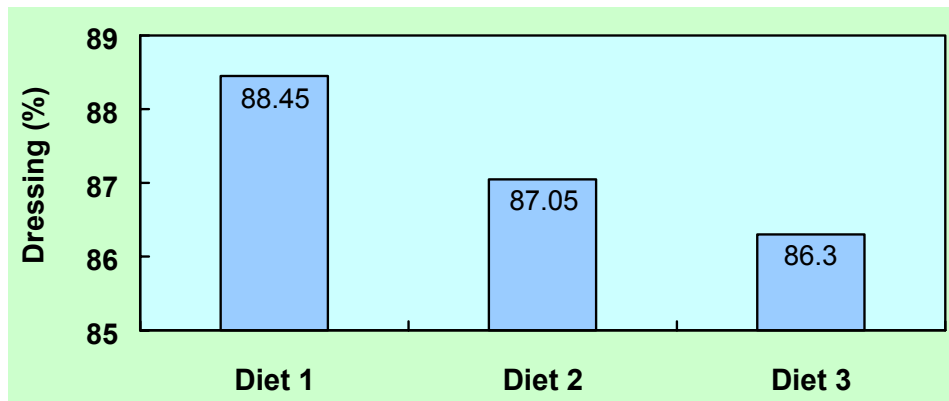
The results from ANOVA indicate that there was a significant difference ( $P < 0.05$ ) in FCR between treatment groups over the trial period (Table 4, Figure 8). Diets 2 and 3 resulted in significantly better FCRs than Diet 1.



**Figure 8.** Food conversion ratios across fish sizes for the three treatment groups in the laboratory trial.

### *Dressing Percentage*

Dressing percentage for fish grown on Diets 2 and 3 was significantly lower ( $P < 0.05$ ) than for fish grown on Diet 1 (Table 4, Figure 9).



**Figure 9.** Dressing percentages across fish sizes for the three treatment groups in the laboratory trial.

### *Survival*

The survival of fish during the laboratory trial was 100%, 96.3% and 95% for diets 1,2,and 3 respectively.

### *Farm Study*

#### *Growth analyses of fish*

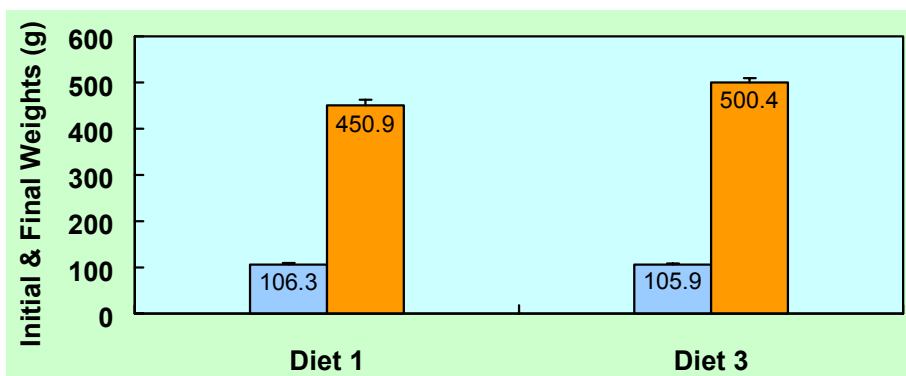
The production performance data for fish receiving the two test diets are listed in Table 5. The respective performance traits are also presented graphically and discussed separately below.

#### *Initial and final weight*

The differences between diets in mean start and final weights of the experimental fish groups are illustrated in Figure 10. There were no significant differences in start weights for the two treatment groups. However, the final weights were significantly different, with Diet 3 fish being significantly heavier than Diet 1 fish (Table 5 & Figure 10).

**Table 5.** Production data and standard errors for the 18-week farm trial. Within trait comparisons, means with a common superscript letter were not significantly different ( $P > 0.05$ ). Values for ADG, SGR, and DFI were covariance-adjusted to remove the effect of minor differences in start weight.

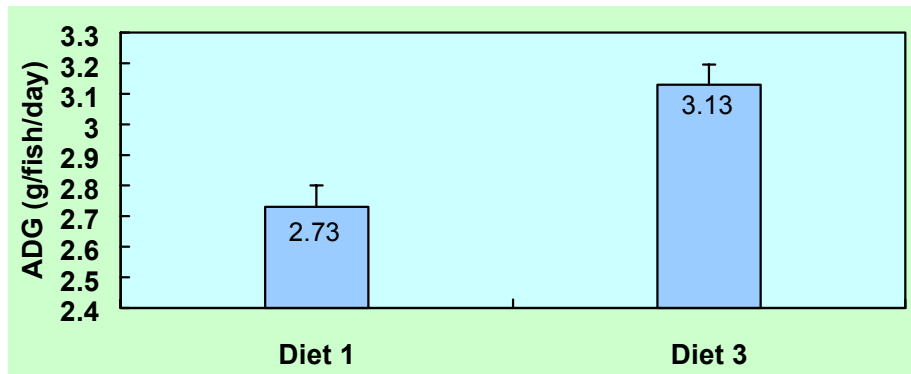
Trait	Diet 1 1 (C1) : 1 (C2) commercial diets	Diet 3 C2's fishmeal 45/20	±sem
Initial wt (g)	106.3	105.9	3.68
Final wt (g)	450.9 <sup>B</sup>	500.4 <sup>A</sup>	8.58
ADG (g/day)	2.73 <sup>B</sup>	3.13 <sup>A</sup>	0.029
SGR (% wt/day)	1.15 <sup>B</sup>	1.23 <sup>A</sup>	0.006
DFI (g/day)	3.30	3.40	0.036
Biomass gain (kg)	66.7 <sup>B</sup>	76.7 <sup>A</sup>	0.99
FCR (g/g)	1.21 <sup>B</sup>	1.09 <sup>A</sup>	0.013
Dressing percentage	88.83 <sup>B</sup>	86.94 <sup>A</sup>	
Survival percentage	97.7	97.8	0.54



**Figure 10.** Mean initial and final weights (and standard errors) for the two treatment groups in the farm trial.

### Average daily gain (ADG)

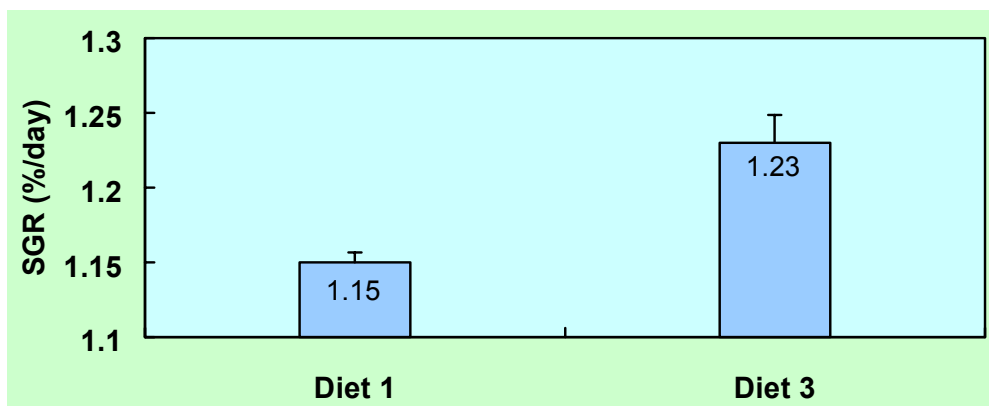
Average daily gain (g/fish/day) differed between the two test diets. Diet 3 resulted in significantly greater daily weight gains ( $P < 0.05$ ) than Diet 1 (Table 5 & Figure 11).



**Figure 11.** Average daily weight gains (and standard errors) for fish grown on Diets 1 and 3 in the farm trial.

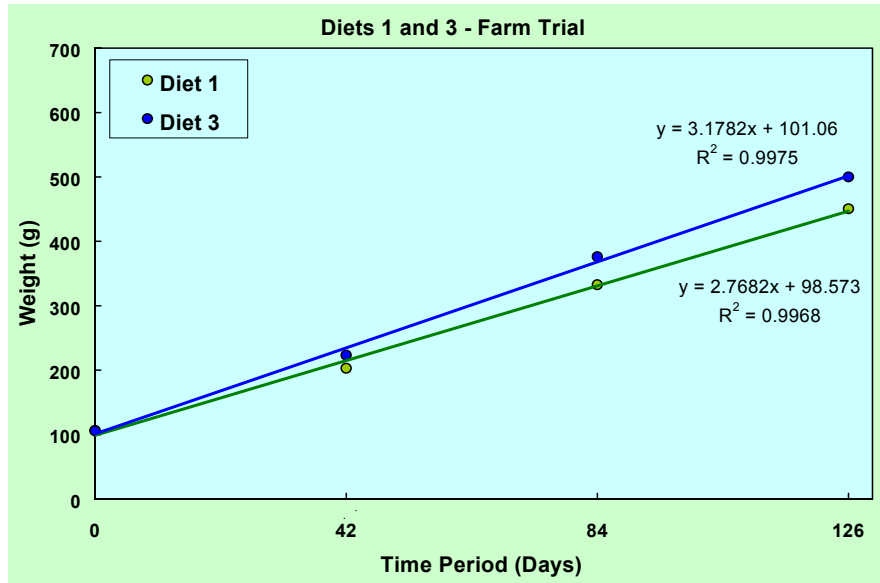
### Specific Growth Rate (SGR)

The specific growth rate of fish on Diet 3 was significantly different ( $P < 0.05$ ) to fish on Diet 1 (Table 5 & Figure 12). Growth curves for the two treatment groups are displayed in Figure 13.



**Figure 12.** Specific growth rates (and standard errors) for fish fed Diets 1 and 3 in the farm trial.

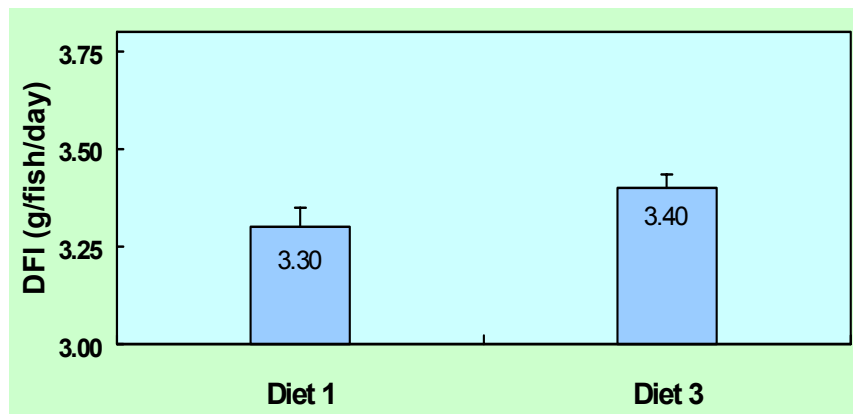




**Figure 13.** Growth of barramundi fed the two test diets during the farm trial.

*Daily feed intake (DFI)*

There was no significant difference in daily food intake of 3.3 g and 3.4 g for Diets 1 and 3 respectively (Figure 14).



**Figure 14.** Daily feed intake (and standard errors) for fish fed Diets 1 and 3 during the farm trial.

### Food conversion ratio (FCR)

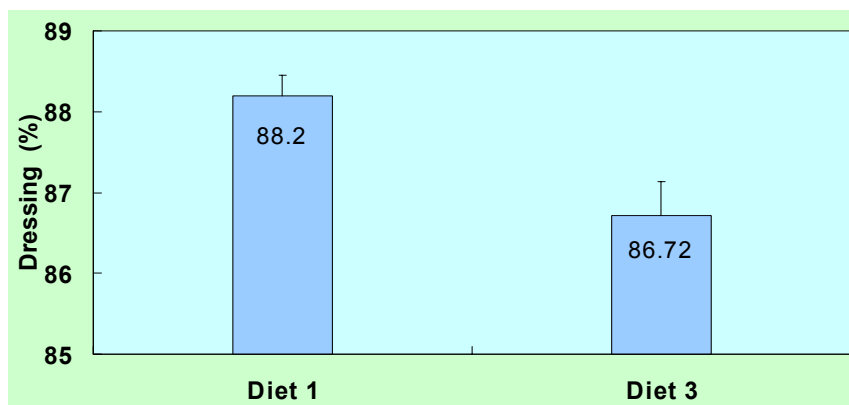
Diet 3 resulted in significantly better FCRs than Diet 1 (Figure 15).



**Figure 15.** Food conversion ratios (and standard errors) for barramundi fed Diets 1 and 3 in the farm trial.

### Dressing percentage

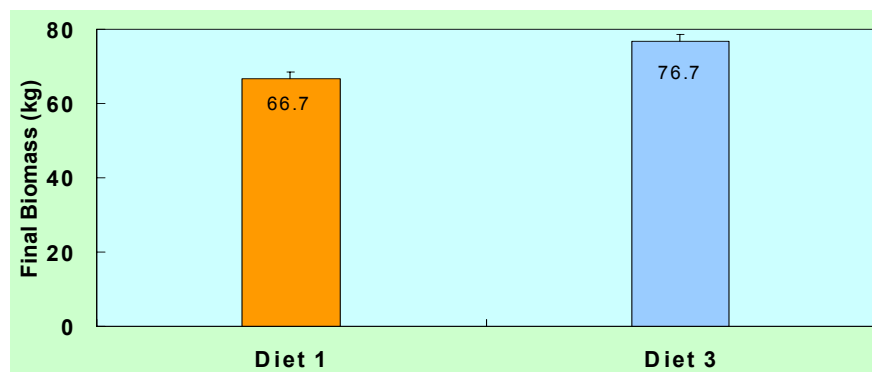
A comparison of live and dressed weights indicated that Diet 3 resulted in significantly lower dressed weights ( $P < 0.05$ ) than Diet 1 (Table 5 & Figure 16).



**Figure 16.** Dressing percentages (and standard errors) for barramundi fed Diets 1 and 3 in the farm trial.

### Survival and biomass gain

There were no significant differences in survival between the treatments, which averaged 98% for both treatment groups. The biomass gain (production) was significantly greater ( $P < 0.05$ ) for fish on Diet 3 than those on Diet 1 (Table 5 & Figure 17).



**Figure 17.** Biomass gains (kg/cage) (and standard errors) for cages of barramundi fed Diets 1 and 3 in the farm trial.

#### *Decreased fish production in third assay period*

Production data recorded at 6-weekly intervals indicated that growth rates decreased in the period 12-18 weeks compared with that in the period 6-12 weeks (Table 6). Similarly, food conversion ratios also were not as good in the 12-18 week period compared to the 0-12 week period (Table 6). The reasons for the drop-off in performance were not clear, although Cyclone Rona (11 February 1999) was implicated (see Discussion).

**Table 6.** Production data (average daily gain, specific growth rate, biomass gain per cage and food conversion ratio) for the farm trial in successive 6-weekly periods during the farm trial. Within trait comparisons, means with a common superscript letter were not significantly different ( $P > 0.05$ ). Values for ADG and SGR were covariance-adjusted to remove effects of minor differences in start weight.

Trait	Diet		± sem
	Diet 1 1 (C1) : 1 (C2) commercial diets	Diet 3 C2's fishmeal 45/20	
<i>Fish productivity (0-6 week)</i>			
ADG (g/d)	2.29 <sup>B</sup>	2.80 <sup>A</sup>	0.049
SGR (%/d)	1.54 <sup>B</sup>	1.77 <sup>A</sup>	0.022
Biomass gain (kg)	18.9 <sup>B</sup>	23.1 <sup>A</sup>	0.64
<i>Fish productivity (7-12 week)</i>			
ADG (g/d)	3.25	3.46	0.116
SGR (%/d)	1.18	1.23	0.032
Biomass gain (kg)	25.3 <sup>B</sup>	29.8 <sup>A</sup>	1.11
FCR (0-12 WK)	1.13 <sup>B</sup>	1.00 <sup>A</sup>	0.015
<i>Fish productivity (13-18 week)</i>			
ADG (g/d)	2.71	3.08	0.093
SGR (%/d)	0.66	0.74	0.021
Biomass gain (kg)	22.5	23.8	0.64
FCR	1.38 <sup>B</sup>	1.28 <sup>A</sup>	0.026

<sup>A,B</sup> Within trait comparisons, means without a common letter differ ( $P < 0.05$ ).

## Economic Analyses

### *Validation components of model*

#### *Costs of feed*

The relative costs of the two diets delivered to Cairns are as follows:

Diet 1	\$1,260 per tonne
Diet 3	\$1,425 per tonne

#### *Food conversion ratio*

The food conversion ratios recorded during the farm trial were:

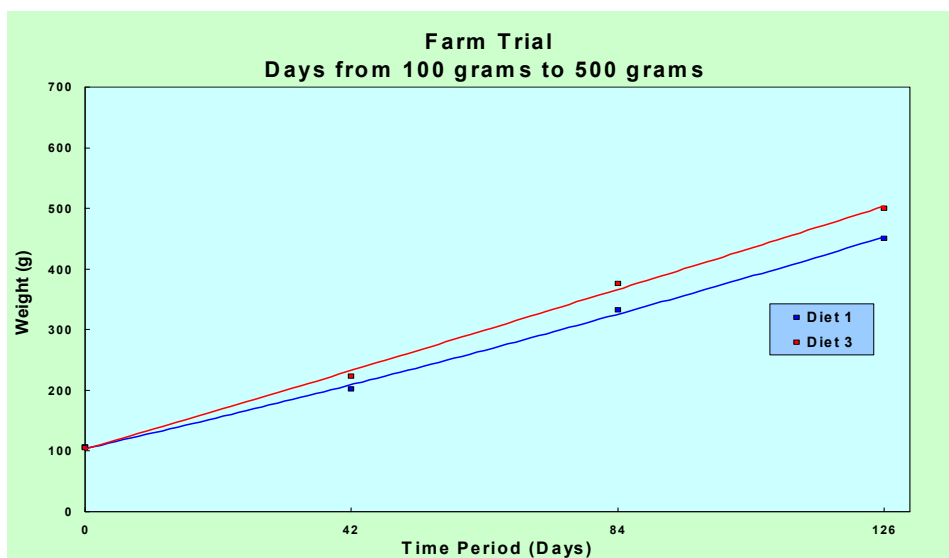
Diet 1	1.21
Diet 3	1.09

#### *Growth*

Comparative production times for fish on each diet were determined from growth curves generated from experimental data. All growth curves were best described by second order polynomial equations:

$$y = ax^2 + bx + c$$

where  $y$  = weight (g),  $a$  and  $b$  are constants, and  $c$  is the  $y$  intercept (initial starting weight of barramundi in the trial) (Figure 18).



**Figure 18.** Growth curves for barramundi grown on Diet 1 and Diet 3 from 100 grams over a 126 day period.

The values for the equations are listed in Table 7. Solving the equations for  $y = 500$  enables determination of the production time for fish grown from 100 grams to 500 grams, which herein is considered to be market size.

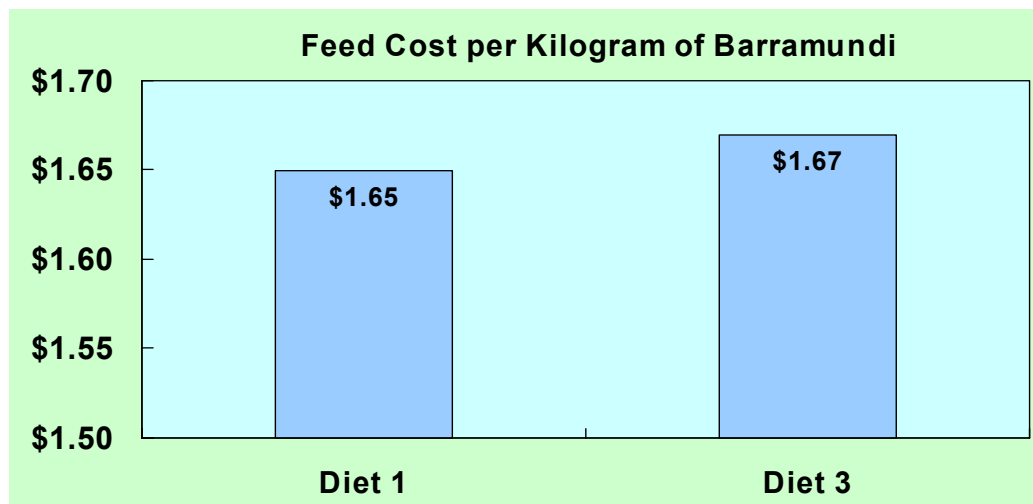
**Table 7.** Values for polynomial equations describing the growth of barramundi on Diets 1 and 3 during the trial period. Production time in days to grow the fish from 100 grams to 500 grams is also tabulated.

	a	b	c	Production time (100 to 500 grams)
Diet 1	0.0031	2.3806	104	140.6 days
Diet 3	0.001	3.0572	102.71	124.8 days

For the purpose of applying these production times to a commercial situation, it was assumed that the barramundi fingerlings, once stocked, took an average of 100 days to achieve a weight of 100 grams (trial start weight). Therefore, total production time for barramundi on Diet 1 was 240.6 days (8 months), and 224.8 days (7.5 months) on Diet 3. By using Diet 3 a reduction in production time of 15.8 days was achieved.

#### *Cost of feed per kilogram of barramundi produced*

The cost of feed can be calculated on a per kilogram basis and is useful in observing the apportionment of feed in production cost. In this instance Diet 3 is more expensive than Diet 1 but Diet 3 had a better FCR, which means less feed is required in the production period. The relative feed cost per kilogram of barramundi is shown in Figure 19.



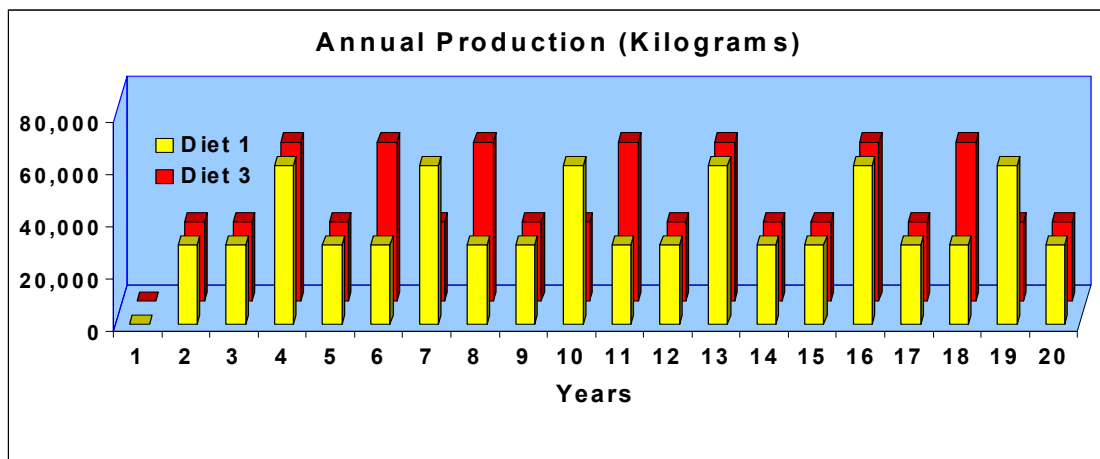
**Figure 19.** Feed cost per kilogram of barramundi produced on Diet 1 and Diet 3.

The cost of feed per kilogram of barramundi is \$0.02 greater if Diet 3 is used rather than Diet 1. Therefore, there is no significant benefit in the use of Diet 3 in regard to feed costs per kilogram of barramundi produced.

### Production time

The time taken to achieve a selected target weight, using the improved feed (Diet 3) as compared to Diet 1, is an important economic consideration. The results indicate that the production time required to produce a 500 gram fish is reduced by 15.8 days when using Diet 3 rather than Diet 1.

This production data was incorporated into the model barramundi farm using production times of 7.5 months and 8.0 months respectively for Diets 3 and 1. The model farm was run over a 20-year period to provide adequate time to show the cumulative benefits of a reduced production time. The comparative annual production of the model farm when barramundi are fed on the two diets is graphed in Figure 20.



**Figure 20.** Annual production of barramundi fed on Diets 1 and 3.

When comparing the annual production figures for the two diets the number of years in which two harvests are achieved is greater when the fish are fed on Diet 3. The average annual farm production of barramundi when Diet 1 is used is 35,870 kilograms as compared to 37,641 kilograms when fish are fed on Diet 3. This equates to an increase in average annual production of 4.9%. The increase is a direct result of the reduction in production time (15.8 days). In addition, there will be savings in labour resulting from reduced feeding and production times. However, the labour saving was not incorporated into the model, as is assumed that the farm would absorb the labour saving in other areas.

### Economic benefits

The economic benefit from the use of Diet 3 is illustrated in the average annual returns of the barramundi farm. The internal rates of return and the increase in average annual returns resulting from the production gain of 4.9% is listed in Table 8. (The IRR represents the maximum rate of interest that could be paid on all capital invested in the project).

**Table 8.** Average annual returns and internal rates of return for the model barramundi farm using Diets 1 and 3.

	Average annual return	IRR	% Increase in average annual returns
Diet 1	\$27,519	13 %	
Diet 3	\$36,029	14 %	30.9 %

Total production cost per kilogram can also be derived from average annual returns. The production cost for barramundi on Diet 1 and Diet 3 is \$9.23 and \$8.60 per kilogram respectively. The saving in production cost arises from the fact that we are able to use the same capital base, and therefore create an economy of scale effect. That is, we are producing more barramundi (production gain of 4.9%) using the same capital resources.

## Discussion

### Laboratory experiment

Data from the laboratory trial indicated that the high-fat meat meal (Diet 2) and fishmeal diets (Diet 3) resulted in marginally better growth than the commercial diet (Diet 1). While the result was not statistically significant, the *P*-value of 0.11 indicates some likelihood that this might be a real treatment effect. That the FCRs for Diets 2 and 3 (0.99 and 0.97, respectively) were significantly ( $P < 0.05$ ) better than for Diet 1 (1.10) provides further evidence of a real dietary treatment effect. Consequently, one can conclude based on the results of the laboratory trial alone that the two high-fat diets were superior to the standard commercial diet mix, and that there was no difference in the performance of the fish between the meat meal and fishmeal based diets. The latter result confirms previous research indicating the flexibility of barramundi in utilising protein from either meat meal or fishmeal with high efficiency.

It is notable that the greatest differentiation in growth in the laboratory trial occurred with the large fish, that is, those initially 240 g and growing to 690–770 g over the 14 weeks of the trial. The three test diets had no discernible differential impact on the growth of the small fish, initially 60 g and growing to 330 g (Table 3). The explanation for the lack of apparent effect with the small fish is not readily obvious. The most likely reason for the differentiated response however, is the different physiological stage of development of the two size groups of fish and the effect this could have on the way nutrients are partitioned in the fish between somatic growth and fat deposition. It is well established with terrestrial animals that the dietary protein requirement decreases with increasing age and physiological development of the animal. Further, the capacity of the animal to conserve ingested dietary energy by depositing surplus energy as fat also increases with advancing physiological development and is greatest after attainment of sexual maturity. Thus for the small fish, it is not unexpected that increasing the dietary fat (energy) and decreasing the dietary protein content of the Diets 2 and 3 (viz approximately 19% fat and 44% CP for Diets 2 and 3 compared to 12.3% fat and 47% CP for Diet 1) did not result in any marked change in growth rate or FCR. Other work at Walkamin with small fish (80 g initial weight) has shown growth rate (and FCR) to improve as the dietary CP content was increased up to 60% (with diets containing from 12 to 23% fat).

### Farm study

The superior performance of Diet 3 compared to the standard commercial diet mix was confirmed in the farm trial. Fish on Diet 3 grew significantly faster, and had a better food conversion ratio, than those on Diet 1. The apparent food conversion ratios (1.09 for Diet 3 and 1.21 for Diet 1) are particularly impressive considering the trial was conducted under fully commercial conditions. The increase in food conversion efficiency of 10% with Diet 3 (see also the laboratory trial result) is a significant decrease in waste production, and would have benefits for management of production ponds and effluent released from the farm.

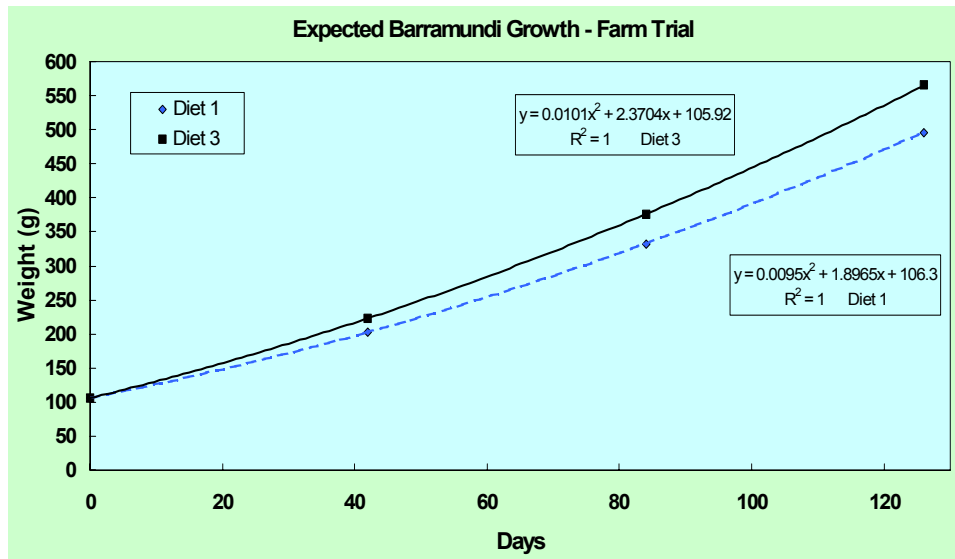
The growth and food conversion efficiency of fish in the farm trial decreased in the third 6-week period (20 Jan to 2 March 1999) compared to the previous two 6-weekly periods (Table 6). The probable cause was Cyclone Rona, which crossed the coast near Cairns on 11 February 1999. Rainfall recorded at Cairns on the 10<sup>th</sup> to 14<sup>th</sup> February inclusive was 29, 276, 98 and 18 mm respectively. However, the associated winds, rain and flooding caused severe disruption to feeding schedules, as the farm could not be accessed for 5 days. In addition, it was obvious that the fish were not feeding actively for an additional period of several days following the cyclonic interruption.

Prior to the cyclone, afternoon pond temperatures (4.30pm) generally ranged between 28°C and 30°C during the preceding two trial periods. On 17 February (6 days after the cyclone crossed the coast) a pond temperature of 26.1°C was recorded. Overcast weather continued but pond temperatures returned to 29.1°C by 22 February. Consequently, we conclude the cyclone resulted in growth rate reduction in both treatments, presumably due to a combination of physical disturbance, interruptions to feeding schedules and reduced pond temperatures.

An indication of the impact of the cyclone on fish production can be seen from an examination of production data in each of the three 6-weekly periods. The rate of weight gain of fish between weighings and change in total biomass should have actually increased during period three, simply because of the increasing size of the fish. There is no quantitative method of determining the “expected” growth through the final 6 weeks, but a growth curve based on data up to 12 weeks can be extrapolated by eye. This has been done in Figure 21, which indicates “expected” final weights of 496 g and 565 g for fish on Diets 1 and 3 respectively, compared with actual final weights of 450 g and 500 g, respectively.

One potentially negative aspect of the high-fat diets, seen in both the laboratory and farm trials, was the reduction in dressing percentage of about 1.5–2% compared with fish raised on the standard commercial diet mix (Figures 9 & 16). The weight difference would have minimal or zero economic impact, as the great majority of barramundi is sold gut-in. However, relative levels of fat in muscle tissue may be of importance in the future, particularly when further product differentiation occurs in the market place.





**Figure 21.** Expected growth of barramundi fed the two test diets during the farm experiment, based on actual data to day 84 and extrapolation to day 126.

#### Protein:Energy ratios in barramundi diets

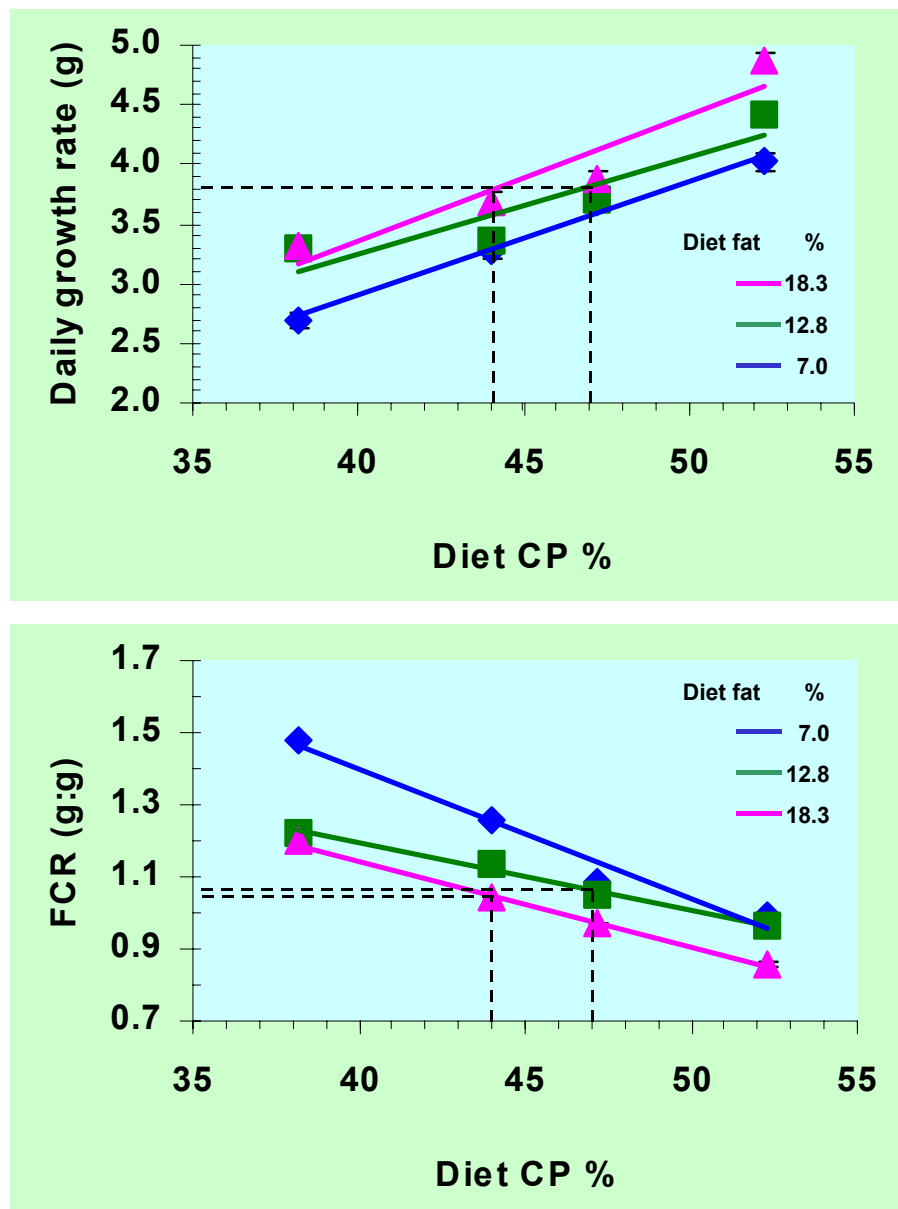
Although the high fat diet (Diet 2 and 3) resulted in better barramundi productivity than the standard commercial diet (Diet 1) under both laboratory and farm conditions, the differences were not huge. In the laboratory experiment, feeding the high fat diets resulted in a 10% improvement in FCR and from 2% (small fish) to 15% (large fish) increase in growth rate. In the farm trial, FCR and growth rate were improved by 10 and 15% respectively.

This result is not surprising in light of earlier studies at Walkamin which examined the interactive response of barramundi to alterations in dietary protein and energy (fat) concentration (Figure 22). In this and other similar studies, barramundi were found to have only a limited capacity to use fat to spare for protein, instead apparently preferring to metabolise protein rather than fat for energy. Thus some improvement in barramundi productivity was seen when the dietary energy (fat) content was increased but the improvement was far greater when the dietary protein concentration was also increased. It is interesting to note from the data of the Walkamin protein to energy study (Figure 22) that reducing dietary CP from 47 to 44% and increasing the fat content from 12.8 to 18.3% – a change consistent with comparing Diets 2 and 3 with Diet 1 – could be expected to have almost no effect on growth rate and only a small improvement on FCR. Thus the results observed in the present laboratory and farm studies are consistent with earlier findings.

In light of these results and depending on the relative economies of increasing the fat or protein concentration of the diet, it might be more advantageous to maintain dietary CP at a concentration of not less than 50% rather than adding more fat and at the expense of protein. Since the supply of individual amino acids is not a critical issue when feeding barramundi on high protein diets, alternative and cheaper sources of digestible protein to fishmeal could be used to advantage. As shown in the laboratory study, a diet containing meat meal was equally as good as one based on fishmeal.

### Economic evaluation

Analyses using the BarraProfit program, retail prices supplied by C2 for Diets 1 and 3, and the production data generated in the farm trial, showed that the cost of feed per kilogram of barramundi produced was \$0.02 greater if Diet 3 was used rather than Diet 1 (Figure 19). However, the faster growth recorded with Diet 3 results in considerably shorter production times (Table 7), and hence a greater average annual production of 4.9% (Figure 20) and financial return of 30.9% (Table 8). These figures equate to a production cost of \$9.23 and \$8.60 per kilogram for Diets 1 and 3 respectively. The figures should not be considered absolute, because conditions vary from farm to farm. Nevertheless, they do provide a tangible indication of the comparative performance of the two diets.



**Figure 22.** Daily growth rate and FCR of barramundi fingerlings fed diets varying in crude protein and fat content at Walkamin.

Our analyses have been based on the actual data generated in the trial. In our opinion they provide a conservative estimate of the relative impacts of the experimental diets compared with the standard commercial diet mix.

- Firstly, production on the farm was sub-optimal during the final 6-week period. Using results shown in Figure 21 would have accentuated the economic difference between the diets.
- Secondly, our analyses were based on growing fish from 100 g to 500 g. If the new diets (or ones slightly higher in protein) were used from fingerling stage (say 5 g) onwards, there would be an expected increased and cumulative improvement in barramundi productivity.
- Thirdly, we are confident that an increase in protein content from 45% to 50%, while keeping the energy level at 20 MJ/kg, would result in faster growth rates and better FCRs.
- Finally, it should be noted that meat meal can be used as a partial, or in fact complete, substitute for fishmeal in barramundi diets. Incorporating at least some meat meal into Diet 3 – particularly the newer lines of high protein, low ash meat meals now being produced by some renderers – would enable the protein content of the diet to be increased without a concomitant increase in cost.

#### 4.5. Efficacy of dehulled lupin meal in diets for the black tiger prawn, *Penaeus monodon*

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##### Summary

The nutritive value of prawn diets that contained 250 g/kg of dehulled lupin meal from the standard form of *Lupinus angustifolius* (var Warrah) and from a high-methionine variety has been compared against the performance of prawns (*Penaeus monodon*) fed a basal diet using diets whose formulations were similar to commercial feeds. The lupin meal replaced part of the fishmeal and wheat flour the basal diet on an isonitrogenous basis. There were no significant differences in the growth rates or survival of prawns fed the test diets. The results demonstrate that dehulled lupin meal derived from *L. angustifolius* can be included at up to 250 g/kg in commercial prawn feeds without adversely affecting performance. In addition, the results indicate that the concentration of the essential amino acid, methionine in the lupin protein does not limit the performance of the prawn diets at this inclusion level, when they contain 40% crude protein.

##### Background

Aquaculture is the fastest expanding food producing sector in the world, growing at a rate of almost 12% p.a. since 1984, with production almost trebling from 13 to 36 million tonnes (Mt) in the last 10 years (Tacon 1999). This rate of expansion will have to be maintained if production is to keep pace with the demand solely due to the increase in world population. However, much of the increase in aquaculture production has been brought about through the adoption of intensive farming practices using formulated feeds. Fishmeal is one of the most important ingredients in formulated aquafeeds, and particularly in prawn feeds where it is typically included at between 200 and 300 g.kg<sup>-1</sup> (Tacon and Akiyama 1997). Its inclusion is primarily for its high quality protein but has the additional benefit of its oil content and associated highly unsaturated fatty acids.

Tacon (1999) estimated that almost 1/3 of aquaculture production was derived from species that were fed formulated feeds which consumed almost 12 Mt of aquafeeds containing 2.3 and 0.7 Mt of fishmeal and fish oil respectively. World fishmeal production was 6.2 Mt in 1997 and has generally fluctuated between 6 and 7 Mt over the past decade and is unlikely to increase. However, it is subject to sharp, periodic declines such as that of 1998 when only 4.75 million tonnes were produced (Tacon 1999).

Therefore, continued expansion of aquaculture will not be possible if fishmeal is relied upon as the main source of protein in aquafeeds. Moreover, demand for fishery product from other sectors, such as the cattle, pig, poultry and pet food industries, will force fishmeal prices up until its usage in aquafeed will become uneconomical. Therefore, 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.

Australia's agricultural industries produce an abundant supply of protein-rich feed ingredients of animal and plant origin that have potential as replacements of fishmeal in aquaculture diets. In

1993, the Australian Fisheries' Research and Development Corporation (FRDC) established a nationally coordinated research program to assess and develop Australian-produced ingredients that had the potential to be used as cost-effective replacements to fishmeal in aquafeeds (Allan 1997). Additional funding was provided by the Grains Research and Development Corporation and the Meat Research Corporation and considerable in-kind support was provided by aquaculturists and feed manufacturers.

## Introduction

In the FRDC-funded Fishmeal Replacement Project (93/120-02), lupins were identified as having significant potential to be used to replace fishmeal in prawn feeds. Factors that were considered were their relatively high crude protein content, their lack of anti-nutritional factors and the large tonnages of lupins that are produced in Australia. Subsequent research demonstrated that the apparent crude protein digestibility of de-hulled lupins was high and comparable to that of soybean meal, its main global competitor (Smith *et al.* 1998). Studies with whole and dehulled lupin meals, derived from both *L. albus*. and *L. angustifolius* have demonstrated that better performance with diets containing *L. angustifolius* than with *L. albus* (Sudaryono *et al.* 1999). The growth of prawns fed de-hulled lupin meal (*L. angustifolius*) in a summit dilution experiment, declined as the inclusion level of lupin meal increased, limiting its maximum practical inclusion level to about 200 g/kg (Sarac *et al.* 1998). It was postulated that this decline in growth could have been due to a deficiency in lupins of the sulphur-containing amino acids, methionine and cystine.

As supplementation of prawn feeds with crystalline essential amino acids (EAA), such as methionine, has not proved to be a reliable method for adjusting the EAA intake of prawns (Deshimaru & Kuroki 1975; Divakaran 1994), a transgenic form of lupin, *L. angustifolius* (variety: Warrah) whose protein contains higher levels of methionine was used to investigate whether the observed reduction in performance was due to methionine deficiency. A growth assay was carried out to compare the performance of prawns fed diets, whose formulations were similar to commercial feeds and which contain 250 g/kg of lupin meal either from the standard form of *L. angustifolius* and from the high-methionine variety.

## Materials and Methods

### Experimental design and diets

The experiment comprised four dietary treatments each with 5 replicates in a fully randomised design that was conducted over 8 weeks. The test diets, which consisted of a basal diet and the two dehulled lupin meal (*L. angustifolius*) diets, were formulated to have the same estimated content of digestible protein and digestible energy (Table 1). The dehulled lupin meal was included in the diets by replacing all the soybean meal and part of the fishmeal in the basal diet. The fourth diet, included as a reference diet, was a commercial diet made for *P. monodon* by a commercial feed manufacturer. The dehulled lupin meal of both varieties was donated to the project by CSIRO Division of Plant Industry. The proximate composition of the standard dehulled lupin meal (*L. angustifolius* var Warrah) was: 95% dry matter, 3.5% ash, 44.1% crude protein, 7.9% total lipid and 0.29% methionine. The proximate composition of the high methionine variety was: 95% dry matter, 3.5% ash, 45.0% crude protein, 9.7% total lipid, and 0.56% methionine. The test diets were prepared by mixing the ingredients, adding enough water to form a stiff dough and extruding through a Hobart mincer. The spaghetti-like strands of extruded diet were steamed for 5 min in a commercial steamer, air dried overnight and broken up into 5–8 mm long pellets. These were stored at -10°C until used.

*Experimental prawns*

Prawns for the experiment were obtained from a single pond at Moreton Bay Prawn Farm and at the start of the experiment weighed between 4 and 4.5 g (mean  $\pm$  SD of  $4.2 \pm 0.05$  g). Fifteen prawns were stocked in each of the 20 polymesh cages (1 x 1 x 1 m, 6 mm mesh) so that each cage contained a similar size range of animals. The cages were set up in two rows in a 4 x 15 x 1 m deep concrete raceway supplied with seawater pumped for 4 h each day from a nearby grow-out pond. The raceway was covered with a polycarbonate dome roof to reduce heat loss at night, the water temperature being monitored continuously with a data logger. Each cage was supplied with supplementary aeration through a single airstone. An automatic feeder that was set on the plywood lid of the cage and a feeding tray (300 mm in diameter, 1 mm mesh) was positioned beneath it so that > 95% of the feed fell on the tray.

**Table 1.** Ingredient composition of diets used to study the effect of using lupin meals in prawn diets. (g/kg 'as used'). L(A) Standard lupin diet; L(T) transgenic lupin diet.

Ingredient	Basal	Lupin (A)	Lupin (T)
Fishmeal	373	214	210
Squid meal	50	50	50
Prawn shell meal	100	100	100
Lupin angustifolius (dehulled)	-	250	-
Lupin (transgenic) (dehulled)	-	-	250
Flour, plain	340	250	250
Gluten (wheat)	60	60	60
Binder	30	30	30
Squid oil	30	30	30
Cholesterol	2	2	2
Lecithin	12	12	12
Antioxidant	0.2	0.2	0.2
Carophyl pink (8%)	0.5	0.5	0.5
Choline chloride (50%)	0.2	0.2	0.2
Vitamin C*	1	1	1
Vitamin premix**	2	2	2
Crude Protein (% as used)	40	40	40
Est. Digest CP (% as used)	35	35	35
Total Lipid (% as used)	8	8	8
Est. DE (MJ/kg as used)	15	15	15

\* Stay C, L-Ascorbyl-2-polyphosphate, Argent Laboratories, Redmond, WA, USA.

\*\* As recommended by Conklin, 1997.

The prawns were placed in the cages 7 d prior to the start of the experiment to allow them to adapt to the conditions. They were then weighed on the starting day and then at 4 weekly intervals. A weighed ration was placed on the autofeeder each day in the late afternoon, distributed so that the feed was given in 5 portions with the largest at sunrise and sunset, two others during the night and one during the day. The feeding tray was inspected each day and the ration size adjusted to ensure that the prawns were being fed ad lib. but also minimising the amount of uneaten food left on the feeding tray. Uneaten food was not recovered and weighed, so feed intake was estimated from the ration weight data.

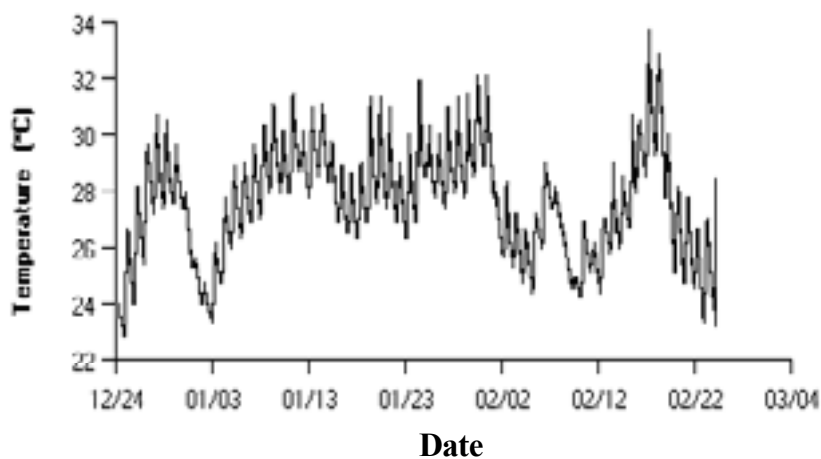
The water stability of the pellets was determined from weighed samples of feed pellets. These were placed on a 1.0 mm mesh nylon screen in jars containing 100 mL of distilled water. The jars were agitated in a shaking water bath (60 rpm) at 28°C for 4 hours. After that period the material retained by the screen was dried at 105°C for 4 h, cooled in a desiccator and weighed. The pellet stability was calculated as the percentage of dry matter (DM) retained on the screen.

## Results

The growth rate of prawns on all dietary treatments was good (~1.4 g/wk) and the mortality rate was also very low (< 10%) (Table 2). In addition, the growth rate of the prawns on the experiment was similar to that of the prawns in adjacent ponds. These factors are indicative that the prawns were of high health status during the experiment and that the environmental conditions were suitable, enabling the diets to be tested on their merits.

Water temperatures in the raceway pond varied between 23 and 33°C during the experiment with the mean temperature being 27.8°C (Figure 1). Diurnal fluctuation in water temperature was in the order of 2.5. During daylight hours the dissolved oxygen was high (> 5.8 ppm) but dropped to a mean ( $\pm$  SD) of  $3.4 \pm 0.93$  ppm at 6.00 am, with a minimum recording of 1.6 ppm). Salinity varied between 24.4 and 34.9 with a mean ( $\pm$  SD) of  $31 \pm 2.7$ .

There were no significant differences in the survival of prawns among the four treatments. The growth rate of prawns fed the diets containing 25% de-hulled lupin meal was not significantly different ( $P > 0.05$ ) from that of the prawns fed the basal diet (Table 2). In addition, there was not a significant difference between the growth rates of prawns fed either of the lupin diets. However, the growth rate of prawns fed the test diets was significantly less than that of the prawns fed the reference diet.



**Figure 1.** Water temperatures in the raceway pond during the lupin diet experiment.

**Table 2.** Means ( $\pm$  s.e) of initial weight, final weight, growth rates, FCR and survival of prawns fed from the four diet-based treatments in the 8-week lupin experiment.

Treatment	Initial Wt (g/prawn)	Final Wt (g/prawn)	Growth Rate (g/prawn/wk)	FCR	Survival (%)
Basal	4.24 ( $\pm$ 0.028)	15.2 ( $\pm$ 0.35)	1.38 ( $\pm$ 0.047)	2.5 ( $\pm$ 0.13)	92 ( $\pm$ 3.0)
LM (A)	4.25 ( $\pm$ 0.019)	15.9 ( $\pm$ 0.34)	1.46 ( $\pm$ 0.043)	2.5 ( $\pm$ 0.16)	99 ( $\pm$ 3.0)
LM (T)	4.23 ( $\pm$ 0.025)	15.6 ( $\pm$ 0.41)	1.42 ( $\pm$ 0.050)	2.5 ( $\pm$ 0.02)	97 ( $\pm$ 3.0)
Reference	4.22 ( $\pm$ 0.022)	17.3 ( $\pm$ 0.27)	1.64 ( $\pm$ 0.035)	2.0 ( $\pm$ 0.12)	97 ( $\pm$ 3.0)

The estimated feed intake of prawns from all treatments was very similar ( $P > 0.05$ ), though the intake of the reference diet appeared marginally lower. As a consequence of the growth and estimated feed intake, the FCR of the test diets were not significantly different from each other, whereas that of the reference diet was significantly lower ( $P < 0.05$ ) (Table 2).

## Discussion

The initial expectation was that the high inclusion level of lupin meal would have resulted in a decrease in performance; this clearly was not the case. The growth and survival of prawns fed the diets containing 25% dehulled lupin meal was not significantly different from that of prawns fed the basal diet. In addition, it does not appear that the methionine content is limiting the performance of the diet containing the standard variety of *L. angustifolius*. However, it is likely that the protein content of these diets, which was 40% on an 'as used' basis, provided more of the essential amino acids than are required for maintenance and growth and that the excess is being catabolized as an energy source (Guillaume 1997). It is worth noting that the best growth rate with *P. monodon* appears to occur with diets containing 40 to 45% crude protein (Alava & Lim 1983; Shiau *et al.* 1991; Chuntapa *et al.* 1999). The need to address the balance of essential amino acids in prawn diets will not be a pressing issue while the higher growth rates of prawns fed 40% crude protein diets is considered economically a better proposition than using diets with lower protein content. This is despite the low protein diets costing less and producing less nitrogenous waste. It is possible that the high methionine lupin may have performed better than the standard strain had higher inclusion levels of lupins been used such that methionine became limiting.

Though there were no significant differences in the performance of the test diets, the growth rate of prawns fed the test diets was significantly different (13% lower) than that of prawns fed the reference diet. The statistical power of this experiment demonstrated the ability to detect as significant ( $P < 0.05$ ), a difference of about 10% growth rates; the largest difference between the test diets was about 5%. In comparing the performance of the test diets and the reference diet, consideration must be given to the differences in manufacturing conditions, particularly the finer grinding of the commercial feed that is likely to have improved its digestibility and performance.

In the lupin diet formulations, dehulled lupin meal had been included at the expense of some of the fishmeal and flour in the basal diet. The similarity in performance of the lupin diets with the basal diet clearly suggests that dehulled lupins (*L. angustifolius*) can be included at 250 g/kg of a diet without adversely affecting the growth rate. This level of inclusion equates to a replacement of 30% of the protein of marine origin in the diet. The use of dehulled lupin at a 250 g/kg inclusion



provides the opportunity for feed manufacturers to formulate a less expensive feed that performs as well as a feed containing conventional aquafeed ingredients.

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#### 4.6. In-pond evaluation of high meat meal diets for the black tiger prawn: report to Meat & Livestock Australia PRCOP.011

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### Executive Summary

Currently fishmeal is a major component of aquaculture diets where it constitutes between 25 and 50% of most diets and is the major protein source. Previous research has demonstrated that meat meal can be used to supply up to two thirds of the crude protein in prawn diets which is equivalent to an inclusion of 500g meat meal/kg of diet. If these findings were taken up by the feed manufacturing industries in Australia and southeast, it would open-up a market to supply meat meal as an ingredient in the 1 million tonnes of prawn feed that are manufactured annually. Discussions with feed manufacturers and the prawn farming industries revealed that they would be more willing to adopt the research findings, which had been obtained in facilities using clear water aquarium tanks, if they had been confirmed in an environment more closely resembling a commercial prawn pond.

Because of the variability in environmental conditions and production rates between commercial prawn ponds, a large number of ponds would be required to carry out a statistically meaningful pond-based comparison of diets. This, coupled with the cost and quantity of feed that would be required, makes it expensive and logistically difficult to carry out the simultaneous testing of a number of diets in commercial ponds. Hence, in this study we have used 20 x 1 m<sup>3</sup> cages which were deployed in a small prawn pond supplied with water drawn from a production pond, as a proxy for commercial ponds.

We investigated the efficacy of a high protein, low ash, low fat meat meal used in diets for *Penaeus monodon*. The meat meal was used at inclusion levels up to 300 g/kg to partially replace fishmeal in a basal diet that had a formulation similar to that of some commercial prawn feeds. Growth rates of prawns fed the meat meal diets (1.14 to 1.16 g/wk) were not significantly different from those obtained with the basal diet (1.14 g/wk) or with a commercial diet (1.17 g/wk) that was used as a benchmark. Average survival was > 92% and not significantly different between treatments. There were also no significant differences in food conversion ratios. One of the problems associated with culturing prawns is an accumulation of waste in the form of sludge on the pond bottom. From visual inspection of the substrate under the cages, there did not appear to be a difference between the amount of sludge under the cages fed the meat meal diets and the basal or commercial diet. This suggests that the meat meal diets are unlikely to create a significant waste problem when used in a pond environment.

### Background

In 1997, the world capture fisheries yielded 94.5 million tonnes of 'seafood' product (Tacon & Dominy 1999). There has been little growth in these fisheries (0.2% since 1996 and only 1.5%/year since 1984) and this is unlikely to change. In contrast, aquaculture has been the fastest growing food production sector for more than a decade. However, the growth in the aquaculture production has been dependent on fishmeal derived from the capture fisheries.

Currently fishmeal is a major component of aquaculture diets where it constitutes between 25 and 50% of most diets and is the major protein source. The global fishmeal production in 1997 was about 6.2 million tonnes of which about 37% were used in aquaculture feeds (Tacon & Dominy 1999). In 1998, the *El Nino* effect on fish stocks resulted in a decrease in fishmeal production to 4.74 million tonnes, leading to a 25% increase in the cost of fishmeal. There continues to be a worldwide increased demand for fishmeal, particularly for the high quality fishmeals used in aquaculture feeds. In 1997, 1.45 million tonnes of feed were used in the production of 942,000 tonnes of farmed prawns (Tacon & Dominy 1999), with most of the production occurring in the east and Southeast Asian region.

The need to replace fishmeal in aquaculture diets is recognised internationally as a high research priority. There are marketing opportunities for protein-rich feed ingredients that can be shown to be viable alternatives to fishmeal in aquaculture feeds. Meat meal has the potential to be used as a major component in aquaculture diets for fish and prawns to replace or partially replace fishmeal (Williams *et al.* 1997). The aquaculture feed market could offer an outlet for tens of thousands of tonnes of Australian meat meal if feed manufacturing companies were to adopt recent research findings. However, the feed manufacturing and prawn farming industries need to be convinced of the cost-effectiveness of using meat meal in the diets in a commercial production environment. They will also have to be satisfied that the meat meal diets will not create markedly more waste in their ponds than is currently produced from conventional diets. Finally, they will have to assess the risk of any negative effect associated with market perceptions of bovine spongiform encephalopathy (BSE) arising from prawns fed diets containing meat meal.

## Introduction

Research funded by the Meat Research Corporation and carried out within the FRDC Fishmeal Replacement Sub-program indicated that there was potential to use meat meals as a partial replacement for fishmeal in commercial prawn diets (Smith 1995, 1997). Results obtained in preliminary growth studies using aquarium tanks, indicated that meat meal could be used to replace at least half of the fishmeal in diets for juvenile prawns. Further research, again with aquarium tanks, demonstrated that meat meal could be effectively used to replace two-thirds of the crude protein in the diets of *Penaeus monodon* provided that the remaining protein was of good quality and of marine origin (Smith 1997). However, the apparent digestibility of dry matter, protein and energy in meat meals was found to be lower than that of fishmeals (Smith 1995). This suggests that where a significant proportion of dietary fishmeal is replaced with meat meal there will be an increase in faecal waste. Neither study could address the issue of the effect that the use of a high meat meal diet would have on pond bottom conditions, or on the performance of prawns in ponds when fed meat meal diets.

There is a great paucity in the scientific literature, of good quality data on the use of meat meals for aquaculture. Lawrence & Castille (1988) reported the use of between 0 and 200 g/kg meat meal in a low protein (27.6%) diet and between 0 and 400 g/kg meat meal in a high protein (40%) diet for *P. vannamei*. In contrast to our observations (Smith 1995, 1997), they found that the inclusion of meat meal resulted in a decrease in growth rate in both series of diets. However, they concluded that meat meals could be cost-effectively used in diets for *P. vannamei* depending on the cost of the meat meal. There does not appear to have been any other systematic studies into the use of meat meals in penaeid prawn diets (reviewed by Tacon & Akiyama 1997).

Because of the variability in environmental conditions and production rates between commercial prawn ponds, a large number of ponds would be required to carry out a statistically meaningful pond-based comparison of diets. This, coupled with the cost and quantity of feed that would be required, makes it expensive and logistically difficult to carry out the simultaneous testing of a

number of diets in commercial ponds. Hence, in this study we have used cages deployed in a small prawn pond, supplied with water drawn from a production pond, as a proxy for a commercial pond.

We investigated the efficacy of a high specification meat and bone meal used in diets for *P. monodon* in a pond environment. The meat meal was used to partially replace fishmeal in a basal diet that had a formulation similar to that of some commercial prawn feeds. The performance of the diets was compared with that obtained with the basal diet and a commercial feed used in the prawn farming industry in Australia. The performance was assessed on the basis of growth rate and survival of the prawns, food conversion ratio (FCR), and the effect that the feed wastes and faeces had on the substrate under the cages.

## Materials and Methods

### Experimental design and diets

The experiment comprised 5 blocks, with each of the four treatment diets being given to one of the 4 cages within a block. The blocks were based on position within the raceway pond and prawns of a particular size range were used to stock cages within a block. Each cage was stocked with 15 prawns; the size ranges being: 1.8–2.0 g; 1.8-2.0 g; 2.0-2.2 g; 2.2-2.4 g; 2.4-2.6 g for blocks A, B,C,D and E respectively.

The test diets, which consisted of a basal diet and two meat meal diets, were formulated to have the same content of digestible protein and digestible energy (Table 1). The fourth diet, included as a reference diet, was a commercial diet made for *P. monodon* by a commercial feed manufacturer. Meat meal used in this study was obtained from Vodusec Meats, Cobram, Victoria. The proximate composition of the meat meal, 'as used' was: moisture, 4.3%; crude protein, 59.0%; total lipid, 10.9% and ash, 21.2%. The test diets were prepared by mixing the ingredients, adding enough water to form a stiff dough and extruding through a Hobart mincer. The spaghetti-like strands of extruded diet were steamed for 5 min in a commercial steamer, air dried overnight and broken up into 5–8 mm long pellets. These were stored at -10°C until used.

The water stability of the pellets was determined from weighed samples of feed pellets. These were placed on a 1.0 mm mesh nylon screen in jars containing 100 mL of distilled water. The jars were agitated in a shaking water bath (60 rpm) at 28°C for 4 hours. After that period the material retained by the screen was dried at 105°C for 4 h, cooled in a desiccator and weighed. The pellet stability was calculated as the percentage of dry matter (DM) retained on the screen.

**Table 1.** Ingredient composition of diets used to study the effect of using meat meal in prawn diets. (g/kg 'as used').

Ingredient	Basal	MM 15	MM 30
Meat & bone meal	-	150.0	300.0
Fishmeal	273.1	187.9	104.5
Soybean meal (45%)	100.0	50.0	-
Squid meal	100.0	100.0	100.0
Shrimp shell meal	100.0	100.0	100.0
Gluten	60.0	60.0	60.0
Flour	289.1	284.4	266.8
Cholesterol	2.0	2.0	2.0
Lecithin	12.0	12.0	12.0
Squid oil	30.0	20.0	21.0
Carophyll pink	0.5	0.5	0.5
Vitamin C	1.0	1.0	1.0
Vitamin Premix	2.0	2.0	2.0
Choline chloride	0.2	0.2	0.2
Binder	30.0	30.0	30.0

### Prawns and management

Juvenile prawns, *Penaeus monodon*, were obtained as post-larvae from Moreton Bay Prawn Farm and reared at the CSIRO laboratories in a 10,000 L tank until used in this experiment. Their growth was at the expected rate and there were no signs of disease or unusual mortality events.

The cages used in the experiment were 1 x 1 x 1 m high, made of Nylex PVC mesh (mesh size 6 mm) strung over a frame made of 35 mm diameter PVC pipe. The top was open but fitted with a lid made from 8 mm exterior grade plywood. An automatic feeder was placed over a 100 mm hole cut in the centre of the lid. Feed fell from the autofeeder onto the feeding tray (300 mm diam.) on the bottom of the cage. Each cage was provided with aeration from a single airstone. Twenty cages were placed in two rows in a 4 x 15 x 1 m deep concrete-lined raceway pond. The bottom of the raceway was covered with a 50 mm layer of sand. The entire raceway was covered with curved, clear acrylic roof. Seawater was pumped into the raceway for 4 hours every 24 hours from a nearby grow-out pond, that was fully stocked with prawns, resulting in a 50% exchange of water in the raceway each day.

After stocking, the prawns were left for one week before the start of the experiment to adapt to the cage conditions. During this period they were fed the reference diet. The prawns were weighed at the start of the experiment, at 4 weeks and at 6 weeks when the experiment was terminated. The automatic feeders were refilled each afternoon with a weighed amount of feed and the food distributed so the prawns were fed five times per day with the largest feeds at dawn and dusk. Each morning the amount of food remaining on the feeding tray was noted and the following day's ration increased or decreased to ensure that the prawns were fed *ad lib* but at the same time minimising the amount of uneaten food. The water temperature in the raceway was recorded every 15 minutes throughout the experiment using a data logger. Salinity, dissolved oxygen and turbidity in the raceway were measured twice daily at about 0600 and 1700 h.

### Chemical and statistical analyses

Proximate composition of the major ingredients and diets was analysed using Association of Official Analytical Chemists (AOAC) procedures. Dry matter was determined by weighing before and after drying at 105°C for 16 h and cooling in a vacuum desiccator; ash by heating a weighed and dried sample at 550°C for 16 h before cooling in a desiccator and re-weighing. Crude protein was determined following Kjeldahl digestion, distillation of the liberated ammonia into 3% boric acid, and titration of the boric acid with hydrochloric acid to an end point at pH 5.0. Total lipid was determined gravimetrically following a chloroform-methanol (2:1) extraction using the method of Folch *et al.* (1957). Gross energy was determined by isothermal bomb calorimetry using a Leco AC200 Bomb Calorimeter.

Differences in growth, apparent food intake and FCR were tested using ANOVA in accordance with the randomised block design of the experiment.

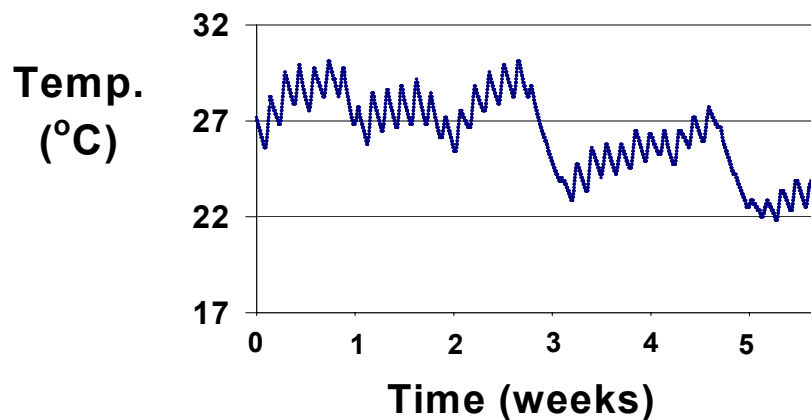
### Results

The 4-h water stability of the diet pellets was high for all the diets (92.8 to 95.7%), with a slight increase in stability as the proportion of meat meal in the diet increased (Table 2). The gross energy of the diets varied between 19.7 and 21.5 MJ/kg DM and crude protein content of the diets was between 45.5 and 48.0% DM. However, the three test diets contained the same amount of estimated digestible energy and estimated digestible crude protein, 15 MJ/kg DM and 35% DM respectively (Table 2).

The average water temperature over the 6 weeks of the experiment was 26.5 ± 2°C (range 32 - 23°C), though the average over the first 4 weeks was 27.1°C and dropped to 24.6°C over the final 2 weeks (Figure 1). The diurnal variation in pond water temperature was about 2°C throughout the experiment. The mean salinity was 34.8 and varied between 33.5 and 36.5 for most of the experiment, except for the last 5 d when it gradually decreased to 32 due to rainfall. The mean dissolved oxygen (DO) level in the morning was 4.56 ppm and in the afternoon was 7.03 ppm. On four occasions the morning DO was below 4.00 ppm and on one occasion was at 2.94 ppm.

**Table 2.** Pellet stability and nutrient composition (dry matter basis) of the test diets and the reference diet used to evaluate the efficacy of meat meal in prawn diets.

Ingredient	Basal	MM 15	MM 30	Reference Diet
Dry Matter (%)	90.5	90.8	90.7	92.2
Stability (%)	92.8	93.6	95.7	94.1
	(dry matter basis)			
Ash (%)	10.2	11.0	12.8	13.7
Gross energy (MJ/kg)	21.5	20.9	20.9	19.7
Est. DE (MJ/kg)	15.0	15.0	15.0	-
Crude Protein (%)	45.5	46.4	47.6	48.0
Est. Digest CP (%)	35.0	35.0	35.0	-
Total Lipid (%)	8.2	8.2	9.3	10.3



**Figure 1.** Pond water temperature (°C) recorded over the 6-week experiment.

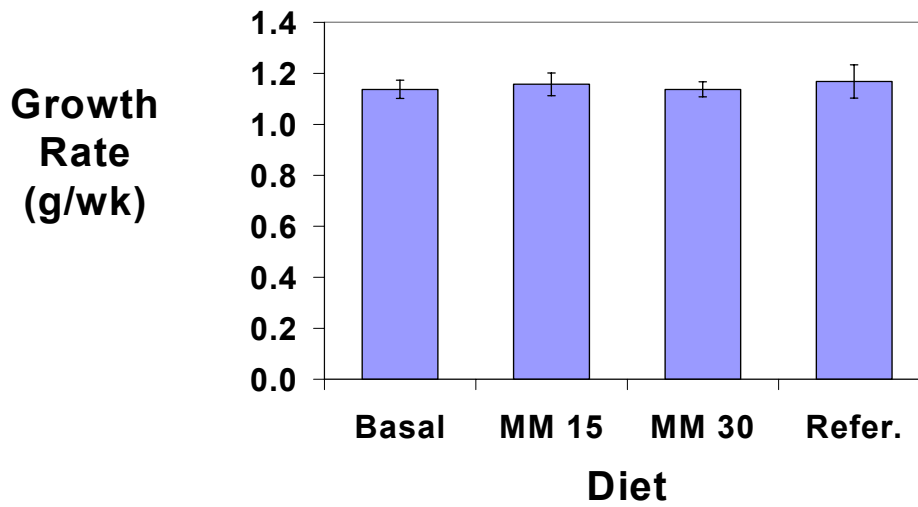
At the start of the experiment the weight of the prawns was very uniform with (< 0.5 g range) within a block and there were no statistical differences between treatments in the average prawn biomass per cage. The survival of the prawns after 6 weeks was greater than 90% in all treatments. The prawns fed the test and reference diets grew at about 1.15 g/week (Table 2, Figure 2) with differences between the means being not significant ( $P > 0.05$ ). Growth during the first 4 weeks was about 1.2 g/wk but this decreased in the final 2 weeks to 1.0 g/wk.

The total amount of food given to prawns in each treatment over the 6 weeks was about 16 g/prawn. The FCRs during the first 4-week period were about 1.85 but this increased during the final 2-week period resulting in the mean FCR over 6 weeks increasing to about 2.3, (Table 2, Figure 3). There were no a significant differences between the FCR means ( $P > 0.05$ ).

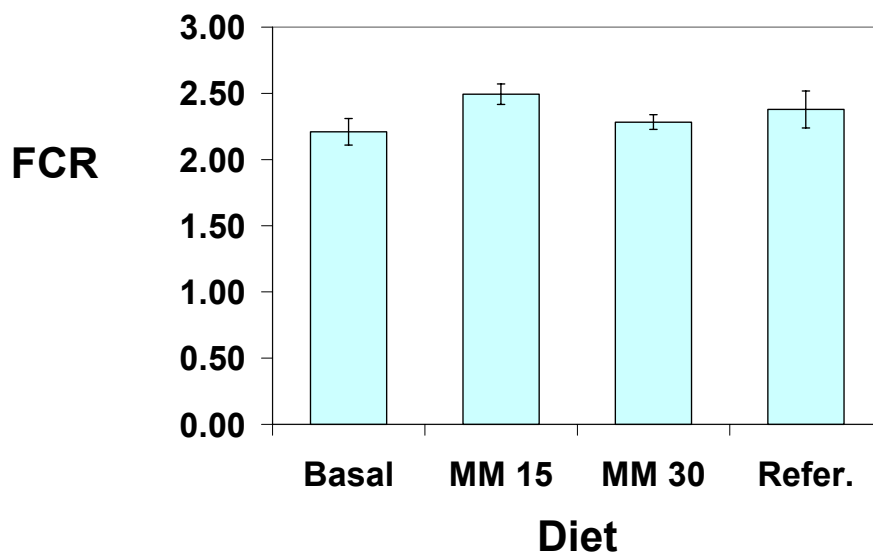
At the end of the experiment there was little anaerobic sludge under the cages from all treatments. From close visual inspection, there appeared to be no differences between the sediment condition under the cages fed the reference and basal diet, and the cages fed the meat meal diets.

**Table 2.** Initial weight, growth rate, FCR and survival of prawns held for 6 weeks in cages and fed the four diets (standard errors in parentheses).

Diet	Initial Wt (g/prawn)	Growth Rate (g/prawn/wk)	FCR	Survival
Basal	2.18 ( $\pm 0.10$ )	1.14 ( $\pm 0.04$ )	2.21 ( $\pm 0.10$ )	92
MM 15	2.19 ( $\pm 0.09$ )	1.16 ( $\pm 0.05$ )	2.49 ( $\pm 0.08$ )	99
MM 30	2.18 ( $\pm 0.11$ )	1.14 ( $\pm 0.03$ )	2.28 ( $\pm 0.06$ )	97
Reference	2.15 ( $\pm 0.10$ )	1.17 ( $\pm 0.07$ )	2.38 ( $\pm 0.14$ )	97



**Figure 2.** Growth rates (g/week) of *P. monodon* juveniles fed the test diets and the reference diet (Refer.) over 6 weeks. Error bars depict the standard error of the mean.



**Figure 3.** Food conversion ratio (FCR) of *P. monodon* juveniles fed the test diets and the reference diet (Refer.) over 6 weeks. Error bars depict the standard error of the mean.



## Discussion

The growth performance of prawns fed the basal diet was high ( $> 1$  g/wk) and not significantly different ( $P < 0.05$ ) from that obtained with the commercially manufactured reference diet. The substitution of some fishmeal and soybean meal in the basal diet with either 150 g/kg or 300 g/kg of meat meal in the test diets also resulted in performance that was not significantly different to that obtained with the basal diet or the reference diet. However, because of the magnitude of the variance of the mean growth rate of each treatment, a difference of greater than 15% of the basal diet growth rate would be required for the detection of a statistically significant difference. The differences between the basal diet and MM15 and MM30 diets were very small, 1.7% and 0.0% respectively while the reference diet resulted in a growth rate that was only slightly higher (2.7%).

The basal diet used in this experiment contained crude protein and estimated digestible energy levels similar to those found in commercial prawn feeds (Akiyama *et al.* 1992). The protein sources used are all recognised ingredients in prawn feeds, with a predominance of marine protein sources (fishmeal, squid meal and shrimp meal) and a lesser proportion of plant protein (soybean meal). As such, the performance obtained with the basal diet was expected and proved to be similar to that of the reference diet.

The meat meal used in this study had high crude protein (59%) and low fat (10.9%) and ash (21.2%) contents. Because of these specifications, it could be used in the diet formulation at 300 g/kg without increasing the amount of dietary ash above an arbitrary limit of 15%. It also could be used at a high dietary inclusion with minimal disturbance to the fatty acid profile of the diets, that otherwise occurs with higher fat meat meals. The arbitrary limit of 15% ash was based on the effect of ash on water stability of the feed pellets and wear of the dies in the steam pellet press used in the commercial manufacture of feed pellets. It is unlikely that a dietary ash content of  $>15\%$  would be considered acceptable by manufacturers using a steam pellet press.

The growth rates of the prawns were higher during the first 4 weeks of the experiment when the water temperatures were generally above 26°C. They then declined markedly as the water temperature dropped to below 26°C in the last 2 weeks of the experiment. The drop in water temperatures resulted in a reduction in the appetite of the prawns. Difficulties were experienced during this period in estimating the amount of feed the prawns would eat each day, which resulted in slight overfeeding. This in turn resulted in an increase in the apparent FCRs for the period. However, the difficulties were experienced across all treatments and so the relative performance of the diets would not have been affected.

The substrate under a cage, left undisturbed in a pond for several weeks, can become anaerobic due to the decomposition of accumulated faeces and feed fragments. The use of feed trays minimises the mass of feed fragments collecting at the bottom of the cage but has little effect on the amount of faecal waste that collects there. There was no information available as to whether the faeces or feed fragments of diets containing high levels of meat meal would have a greater or lesser effect than similar fishmeal-based diets. However, previous research (Smith 1995) showed that the apparent digestibility of dry matter and the apparent digestibility crude protein in several meat meals were lower than in fishmeal. This indicates that there would be more waste produced from high meat meal diets than from similar conventional diets. In this experiment, there was little accumulation of anaerobic sludge under the cages at 4 weeks and at 6 weeks when the prawns were weighed, and no noticeable difference between treatments. Though this experiment cannot represent the situation during grow-out of prawns in a production pond, it does suggest that it is unlikely that the meat meal diets will create particular sludge problem due to the meat meal in feed fragments or partially digested meat meal in the faeces.

## Conclusion

This experiment has demonstrated that a high specification meat meal can be used in commercial diets for prawns, at inclusion levels up to 300 g/kg of diet in a pond environment. The growth rate and FCR of prawns fed the meat meal diets was very similar to and not statistically different from those obtained with the basal diet and the commercially manufactured reference diet. The high meat meal diets did not appear to have created a greater or lesser amount of sludge under cages than conventional diets. With this information, larger scale farm testing of meat meal diets can be undertaken in the knowledge that use of meat meal involves little to no risk to production or to the pond environment. Future work should be directed towards convincing feed manufacturers and prawn farming companies of the economic benefits of using meat meal as a major component in commercial prawn feeds.

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#### 4.7. The effect of feeding frequency on water quality and growth of the black tiger shrimp (*Penaeus monodon*)

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#### Abstract

The feeding strategy used in the commercial culture of shrimp can have a significant impact on pond water quality and hence growth, health and survival of the shrimp, as well as the efficiency of feed utilization. These factors contribute to the profitability of production and to the environmental impact of shrimp farming. The effect of four different feeding frequencies (3, 4, 5 and 6 feedings d<sup>-1</sup>) on the growth and survival of the black tiger shrimp, *Penaeus monodon*, and water quality was studied in an 8-week growth trial. The shrimp were held in 20 x 2,500 l outdoor tanks containing water and sediment from a shrimp pond. The water management and aeration strategies were designed to simulate a shrimp pond system. The shrimp (initial weight of 5.6 g), were stocked at a density of 25 animals m<sup>-2</sup> and fed a widely used, commercial pelleted feed, with all the feed being placed on feeding trays. The uneaten feed on the feeding trays was removed at specific time intervals so that in all treatments the shrimp had access to the feed for 12 h d<sup>-1</sup>. There were no significant ( $P > 0.05$ ) differences due to feeding frequency on growth rate ( $1.4 \pm 0.08$  g week<sup>-1</sup>), feed conversion ratio (FCR) ( $2.0 \pm 0.27$ ) or survival ( $84 \pm 7.6\%$ ) of shrimp. Similarly the water quality parameters (total N, ammonium, nitrate/nitrite, dissolved organic nitrogen, total phosphorus, phosphate, chlorophyll *a*, oxygen, pH, temperature, salinity, turbidity) were not different among treatments. The results suggest that there is no benefit from feeding *P. monodon* more frequently than three times per day when using a feed that is nutritionally adequate and has high water stability. Therefore, it may be possible to reduce feeding frequency in commercial shrimp ponds without adversely affecting water quality, shrimp growth rate and survival, thereby improving farm profitability.

#### Introduction

The costs of formulated feed and labour associated with feeding are a major component of the cost of cultured shrimp production (Lawrence & Lee 1997). Optimizing the feeding strategy is a prime consideration in intensive shrimp pond management, and involves nutrition, processing and feed management. It is well established that the nutrient content of the feed will influence growth, survival and the amount of metabolic and excreted waste products entering the system. However, processing also plays a critical role as it influences stability of the feed and hence availability of the feed over time. These factors have a substantial effect on the amount of waste produced through pellet fragmentation, leaching loss, residual feed and undigested material. Feeding strategies have also been found to influence water quality and shrimp health (Jory 1995; Burford & Williams 2001). Cuzon *et al.* (1982) found that the time of feeding was very important to ensure rapid consumption of the feed by shrimp, thereby minimizing the loss of nutrients and resulting in an improvement in growth rate. In addition, Tacon (1996) recommended that feeding studies be carried out under conditions that are close to, or simulate commercial production conditions to ensure the relevance of the results to the shrimp farming industry.

The shrimp farming industry is facing increasing pressure to lower its environmental impact (Naylor *et al.* 1998). A major concern is the discharge of nutrients from shrimp farms into coastal waters, with the potential to contribute to increased algal blooms, oxygen depletion of bottom waters and reduced biodiversity. Most of the nutrients discharged from intensive shrimp farms originate from the formulated feed (Funge-Smith & Briggs 1998). Therefore, efforts to improve feeding strategies must focus on both optimizing production and minimizing waste.

*Penaeus monodon* is the most widely cultured species in southeast Asia where, during the grow-out phase, it is typically fed five or six times per day (Jory 1995). However, there have been very few studies reported in the scientific literature on the effects of different feeding frequencies with this species (Jory 1995; Josekutty & Jose 1996). Robertson *et al.* (1993) reported that the growth rate of *P. vannamei* [= *Litopenaeus vannamei*], held in pens in an earthen pond, increased progressively as the feeding frequency increased from one to four times per day. However, Velasco *et al.* (1999), using an indoor culture system, found that feeding *L. vannamei* more often than once per day did not significantly increase the growth rate of the shrimp. They also found that the inorganic nitrogen (N) concentration in the culture water was similar across treatments. We found limited information on the impact of feeding frequency on water quality and growth of *P. monodon* juveniles.

Our hypothesis was that more frequent feeding would result in higher nutrient intake and hence higher growth rates of the shrimp. The objective of this study was to quantify the effect of feeding frequencies between three and six times per day on both shrimp growth and survival, as well as water and substrate quality, in tanks designed to simulate pond conditions.

## Methods

### Experimental design and maintenance

The experiment comprised an 8-week growth assay with four treatments: feeding frequencies of 3, 4, 5 and 6 feedings evenly spaced over a 24-h period. A balanced block design was used with five blocks each containing four randomly allocated treatment tanks. The blocked design was used to statistically determine the effect of tank position, and temporal effects resulting from a staggered start of the experiment (see below).

The experiment was conducted in 20 circular outdoor tanks (2,500 l) that were set up and managed to simulate a pond environment. Each tank contained a 50 mm deep layer of sediment taken from the bottom of a shrimp pond. Approximately 2 weeks prior to the start of the experiment, the tanks were filled with water drawn from a single commercial shrimp pond. Airlift pumps were used to aerate the tanks and circulate the water, leading to deposition of wastes in the centre of the tank. To encourage the growth of microalgae, tank water was fertilized with 0.028 mg l<sup>-1</sup> dihydrogen potassium orthophosphate. The tanks were initially stocked with 10 x 10 g shrimp to help establish a benthic community in the sediment. These shrimp were trapped and removed from the tanks just prior to the start of the experiment.

The start and end of the experiment were staggered over 5 successive days, with one block of four of the 2,500 l tanks stocked with shrimp (or harvested) on each of the 5 days. Shrimp (*P. monodon*) were obtained from a commercial shrimp farm and were weighed and distributed so that each tank contained 75 shrimp with a similar mean weight and size distribution (mean weight  $\pm$  SD of 5.6  $\pm$  0.10 g, density of 25 shrimp m<sup>-2</sup>). To prevent shrimp from escaping from the tanks, each tank was covered with a 12 mm mesh of monofilament net.

Shrimp in all tanks were fed the same commercial diet (Charoen Pokphand, # 4004, Thailand), that was designated for shrimp of between 3 and 12 g, the pellets having a diameter of 2.1 mm and mean length of 3.2 mm (range 2.0 to 4.8 mm). The feed comprised of 90.5% dry matter (DM) with

45.7% crude protein (7.31% total N), 10.8% ash and 11.3% total lipid, all expressed on a DM basis. At each feeding, all of the feed for each tank was distributed over two feeding trays (300 mm diam.). The feeding trays were removed from the tanks at specific time intervals after feeding, so that with each treatment, the shrimp had the opportunity to feed for a total time of 12 h, regardless of the number of times that they were fed each day. For example, in the treatment where shrimp were given 3 feeds  $d^{-1}$ , the feeding trays were removed 4 h after each feeding, and those fed 6 times  $d^{-1}$  after 2 h. The amount of feed given to each tank was adjusted daily based on the amount of feed consumed in the tank on the previous day. This was done to minimise the amount of feed remaining on the feeding trays when they were removed from the tanks, and also to minimise the occasions when all allocated feed was consumed. All uneaten feed was recovered from the feed trays in each tank after each feeding period over the 8-week period and stored separately at  $-10^{\circ}C$ .

At the end of the experiment, the collected uneaten feed was dried at  $105^{\circ}C$  for 12 h and weighed. A water stability correction factor was applied to its dry mass to account for leaching loss of DM during the time that the feed was in the water (see section on Nutrient leaching). This corrected value for the mass of uneaten feed was subtracted from the amount of feed given to each tank (on DM basis) to determine apparent feed intake. Apparent feed intake was used to calculate the FCR.

FCR is the total amount of feed consumed by shrimp in a tank relative to the wet weight gain of shrimp biomass, calculated as:

$$FCR = AFI_{DM} / Wt_w$$

Where:  $AFI_{DM}$  = apparent feed intake on DM basis (g)

$Wt_w$  = wet weight gain in shrimp biomass (g)

The experiment was carried out in summer 2000 (mid January to mid March). The diurnal cycle changed from sunrise at 0500 h and sunset at 1900 h to sunrise at 0600 h and sunset at 1800 h over the course of the experiment. Water exchanges were conducted as required to prevent algal blooms from becoming unstable. When water exchanges were found to be required, the same volume of water was exchanged in all 20 tanks. Water samples were taken prior to the exchange to quantify the nutrients removed from the tanks. In the early weeks of the experiment, 10 to 20% of the volume was removed and oceanic seawater was used to refill the tanks about once a week; in the latter part of the experiment this increased to 10% exchanges every 2 or 3 d. The tank walls were cleaned weekly to remove barnacles, filamentous algae and other organisms. Dihydrogen potassium orthophosphate ( $0.028 \text{ g l}^{-1}$ ) was added to each tank on three occasions during the experiment to ensure that phosphate was not limiting microalgal growth. At the end of the experiment, the tanks were drained and the shrimp harvested, counted, lightly dried on a towel and weighed.

#### Water and sediment quality

Water temperature, salinity, pH, dissolved oxygen (DO) and turbidity were measured in all tanks with a data logger (Yeokal, Australia) at 0500 and 1400 h each day. Previous studies have shown that these are the times of maximum and minimum temperature, pH and DO (Burford & Glibert 1999). Profiles were taken through the water column to ensure that there was no vertical stratification of any of the parameters.

Water samples were taken for fluorescence measurements from all tanks at 0900 h each weekday. They were pumped through a field fluorometer (Turner Designs Model 10-AU) and readings taken. A subset of water samples was analysed for chlorophyll *a* concentrations by filtering through glass fibre filters (Whatman, GF/F) and extracting the filters in acetone (Jeffrey & Welshmeyer 1997). Fluorescence was correlated with chlorophyll *a* concentrations to produce an equation for the relationship ( $R^2 = 0.89$ ). Duplicate water samples for nutrient analyses were also taken in each block of four treatments each weekday at 1030 h, totalling  $5 \text{ d week}^{-1}$ . Water samples were filtered

through prefilters (glass fibre) and disposable filters (cellulose acetate, 0.45 µm pore size) within 1 h of collection and frozen until analysed. Ammonium, dissolved N and phosphate concentrations were determined (Parsons *et al.* 1984). Nitrate/nitrite was analysed using the method of Jones (1984). An automated sampling and wet chemistry sensor unit (Aqualab, Greenspan Technology) containing a gas diffusion electrode (Orion) was used to record total ammonium concentrations in four tanks: two tanks with 3 feedings d<sup>-1</sup> and two tanks with 6 feedings d<sup>-1</sup>, every 2 h throughout the experiment. Values obtained were validated with data from manual sampling and analysis of ammonium.

At the end of the experiment, after shrimp had been trapped and removed, the tanks were drained to a depth of 20 cm above the sediment. Sediment cores (37 mm diam.) were taken at 20 random sites in each tank ensuring that disturbance to the sediment was minimized. The water above the cores was decanted off and the cores were then combined and mixed. A sub sample was taken for porewater ammonium and nitrate/nitrite analysis by centrifuging at 10,000 g for 15 min at 4°C, decanting the supernatant and filtering through a cellulose acetate filter (0.45 µm) before freezing. Samples were analysed for ammonium and nitrate/nitrite (Parsons *et al.* 1984; Jones 1984).

#### Nutrient composition of feed and leaching loss

The shrimp feed used in this study was analysed to determine DM, ash, crude protein and total lipid content. DM was determined by weighing before and after drying at 105°C for 16 h and cooling in a vacuum desiccator, and ash by heating a weighed and dried sample at 550°C for 16 h before cooling in a desiccator and re-weighing (method 938.08, AOAC 1999). Total N and crude protein (6.25 x total N) were determined by the Kjeldahl method. Total lipid was determined gravimetrically following a chloroform-methanol (ratio 2:1) extraction (Folch *et al.* 1957).

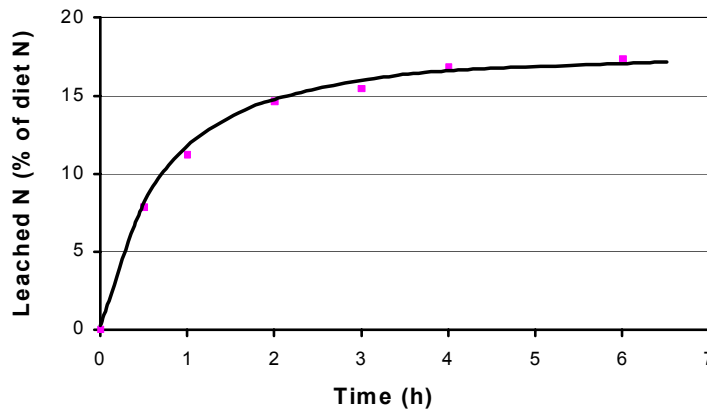
The rate of DM loss in water (and hence stability) and the leaching rate of total N were determined from samples of the shrimp feed. Weighed samples of feed (ca. 1 g) were immersed in jars containing 70 ml of seawater and maintained at 28°C in a shaking water bath (oscillating at 60 rpm) for periods of 0.5, 1, 2, 3, 4 and 6 h. At the end of the immersion period, the samples were removed from the water onto a 1 mm mesh screen, lightly rinsed with distilled water, dried at 105°C overnight, cooled in a vacuum desiccator and weighed to determine the DM loss. The remaining water in each jar was filtered through a glass fibre filter (Whatman GF/C) and analysed to determine N concentration. Dissolved N was determined by Kjeldahl digestion with the same procedure used to determine total N in the diet. The DM and total N leaching loss was expressed as a percentage of the diet, on a DM basis.

#### Statistical analysis

Differences across treatments in growth rate, specific growth rate (SGR), apparent feed intake, FCR survival and water quality parameters were tested using one-way ANOVA ( $P < 0.05$ ) in accordance with the randomized block design of the experiment. Statistical analyses were conducted using SAS statistical software (ProcMixed and Proc GLM procedures, SAS Institute, Cary NC.). Prior to analysis, survival data (%) were arcsine-transformed and water quality data were normalised. Differences between treatment effects were examined *a-posteriorly* using Fischer's protected 't' test (Snedecor & Cochran 1989) wherein differences between means were examined only where the 'F' test of the ANOVA was significant ( $P < 0.05$ ).

## Results

The feed pellets retained their cohesion after 4 h of immersion in water and shrimp were regularly observed feeding on them at this stage. There was a 12% loss of DM after 4 h of immersion. Total N loss from the feed into the water was initially rapid with about 15% of the N in the diet being lost over the first 2 h (Figure 1). Thereafter the leaching rate was much slower with only 2% of the N in the feed being lost over the following 4 h.



**Figure 1.** Loss of N through leaching from feed pellets over time.

The mean water temperatures for all tanks were  $25.1 \pm 0.5^\circ\text{C}$  at 0500 h and  $28.0 \pm 2.3^\circ\text{C}$  at 1400 h over the course of the experiment (Table 1). Morning temperatures (0500 h) of  $< 23^\circ\text{C}$  were recorded on 4 days and afternoon temperatures (1400 h) of  $> 33^\circ\text{C}$  were recorded on 4 days. At temperatures  $< 23^\circ\text{C}$ , the feed intake of *P. monodon* has been shown to decrease significantly while at temperatures  $> 33^\circ\text{C}$  it is possible that the shrimp were stressed (Deering *et al.* 1995).

Mean DO concentrations for all tanks were  $3.9 \pm 0.9 \text{ mg l}^{-1}$  at 0500 h and  $8.2 \pm 1.6 \text{ mg l}^{-1}$  at 1400 h over the course of the experiment (Figure 2, Table 1). There were no significant differences in temperature, salinity, pH, DO or turbidity between treatments. In three of the tanks, on one or two occasions, the DO at 0500 h was below  $2 \text{ mg l}^{-1}$ . However, there did not appear to be a correlation between these low DO concentrations and shrimp growth or survival.

Chlorophyll *a* and ammonium concentrations in water samples that were taken from all tanks each weekday were highly variable between tanks and over time (Mean  $\pm$  SD:  $110 \pm 67 \mu\text{g l}^{-1}$  and  $1.3 \pm 1.1 \text{ mg l}^{-1}$ , respectively) and there were no significant differences among treatments (Table 1). Ammonium concentrations were also monitored in four of the 24 tanks (two tanks with 3 feedings  $\text{d}^{-1}$ , two with 6 feedings  $\text{d}^{-1}$ ) with samples taken and analysed from each tank every 2 h over the course of the experiment. In these tanks concentrations changed rapidly over short time frames and increased over the last 4 weeks of the experiment (Figure 3).

Nitrate, nitrite and phosphate concentrations were relatively low compared with ammonium concentrations (Table 1). Dissolved organic N concentrations (DON) ( $1.2 \pm 0.44 \text{ mg l}^{-1}$ ) and sediment porewater ammonium concentrations ( $24.6 \pm 4.52 \text{ mg l}^{-1}$ ) were also less variable than ammonium concentrations and were not significantly different between treatments.

Frequency of feeding did not change the growth rate or survival of shrimp (Table 2). The starting weight of shrimp in each treatment was the same, shrimp grew at similar rates (1.4 to 1.5 g week<sup>-1</sup>), and they reached similar final weights. Survival rates were the same for each treatment and, as a consequence of similar growth, the final biomass was the same for each treatment. The total mass of feed given to each tank (DM basis) over the 8 weeks and the total mass of uneaten feed (DM basis) removed from each tank were not significantly different among treatments (means and SD: 1500 ± 120 g; 190 ± 43 g). Apparent feed intake and FCR were the same for all treatments (Table 2).

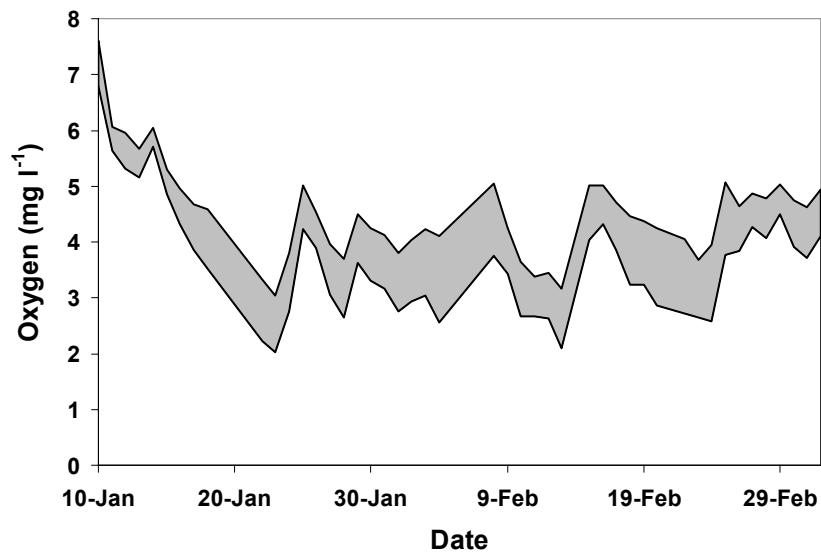
**Table 1.** Mean and standard deviation (SD) of water quality parameters, nutrient concentrations (mg l<sup>-1</sup>) and chlorophyll *a* concentrations (µg l<sup>-1</sup>) for all tanks in which shrimp were cultured for 8 weeks. There were no significant differences among treatments ( $P > 0.05$ ). *n* is number of measurements that were taken across all treatments and times.

Parameter	Sample time	Mean	SD	<i>n</i>
Temperature (°C)	0500 h	25.1	0.55	800
	1400 h	28.0	2.29	1063
Salinity	0500 h	35.1	1.50	884
	1400 h	35.1	1.45	880
pH	0500 h	7.7	0.26	1000
	1400 h	8.0	0.03	1043
Dissolved oxygen (mg l <sup>-1</sup> )	0500 h	3.9	0.87	1035
	1400 h	8.2	1.63	879
Turbidity (ntu)	0500 h	23.8	11.70	840
	1400 h	28.7	11.60	900
Chlorophyll <i>a</i>	0900 h	1134	6	800
Total N	1030 h	4.5	1.4	80
Ammonium	1030 h	1.3	1.1	240
Nitrate/nitrite	1030 h	0.1	0.1	240
Dissolved organic N	1030 h	1.2	0.4	240
Total P	1030 h	0.3	0.0	80
Phosphate	1030 h	0.01	0.009	240
Ammonium in sediment porewater	End of experiment	26.6	4.52	20

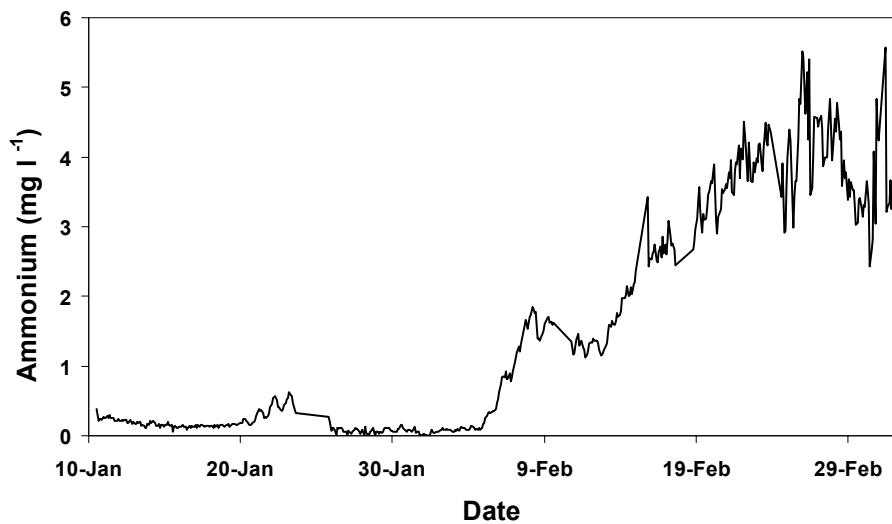
**Table 2.** Mean productivity of *Penaeus monodon* fed at different frequencies for 8 weeks. There were no significant differences among treatments ( $P > 0.05$ ). (AFI - apparent feed intake; FCR - feed conversion ratio)

Treatment (feeds d <sup>-1</sup> )	Initial wt. (g shrimp <sup>-1</sup> )	Final wt (g shrimp <sup>-1</sup> )	Growth rate (g wk <sup>-1</sup> )	Survival (%)	Final biomass (g tank <sup>-1</sup> )	AFI (g tank <sup>-1</sup> )	FCR
3	5.6	16.8	1.4	84	1060	1300	2.1
4	5.5	16.8	1.4	85	1070	1250	1.9
5	5.6	17.2	1.5	84	1080	1280	2.0
6	5.6	16.9	1.4	84	1070	1360	2.1
± SEM	0.05	0.28	0.04	3.4	45	45	0.12





**Figure 2.** Daily mean dissolved oxygen concentrations ( $\text{mg l}^{-1}$ ) of all tanks at 0500 h and 1400 h throughout the course of the experiment. Shaded area shows the daily fluctuation range of the mean dissolved oxygen concentration.



**Figure 3.** Ammonium concentrations ( $\text{mg l}^{-1}$ ) in a representative tank receiving 3 feedings  $\text{d}^{-1}$ .

Measurements were taken every 2 h throughout the 8-week experiment.

## Discussion

These results are contrary to our hypothesis that shrimp would grow faster if they were offered a commercial shrimp diet more frequently. We found that shrimp on all treatments grew at the same rate. Furthermore, none of the other parameters associated with growth and production showed any difference among treatments. The initial and final mean weight of shrimp, survival, final biomass, apparent feed intake and FCR were the same for all treatments. The growth rate of the shrimp was slightly lower (1.4 to 1.5 g week<sup>-1</sup>) than that modelled for farmed *P. monodon* of the same size, grown under the same temperature regimen (1.7 g week<sup>-1</sup>) (Jackson & Wang 1998). Given that the disturbance of shrimp would be greater with our feeding and tank management protocols than in commercial shrimp ponds, the growth rate of the shrimp in this experiment compares favourably.

There appears to be no directly comparable study on *P. monodon* reported in the literature. However, Josekutty & Jose (1996) reported that there was no significant difference in the weight gain of small juvenile *P. monodon* (initial weight of 0.2 g) reared in aquaria when fed 3 or 4 times daily. They also reported that the shrimp grew at a faster rate when fed 3 times d<sup>-1</sup> than they did when fed 1 or 2 times d<sup>-1</sup>. We did not investigate the effects of feeding the shrimp less frequently than 3 times d<sup>-1</sup> so the effect of feeding *P. monodon* juveniles once or twice each day remains to be examined.

Similar feeding frequency studies have been carried out with juvenile *L. vannamei* by Robertson *et al.* (1993), using shrimp (initial weight of 6.7 g) in pens in an earthen pond, and by Velasco *et al.* (1999), using smaller shrimp (initial weight 0.5 to 0.6 g) in a zero-exchange culture tank system. Robertson *et al.* (1993) found that the shrimp growth rate increased progressively as feeding frequency increased from 1 to 4 times d<sup>-1</sup> whereas Velasco *et al.* (1999) found no significant differences in the growth rates of shrimp fed using a range of strategies with feeding frequencies varying from 1 to 15 times d<sup>-1</sup>.

Our experimental system and management protocol more closely matched that of Robertson *et al.* (1993) than Velasco *et al.* (1999), but our results were more consistent with those of Velasco *et al.* (1999). It is possible that the increased growth rates measured by Robertson *et al.* (1993) were related to the period during which the feed remained physically intact or attractive to the shrimp. If there was a relatively rapid decline in the physical stability of the feed or in its attractiveness, it is possible that shrimp fed more frequently would eat more than those fed less frequently, with a consequential effect on shrimp growth rates. In our experiment, the feed remained available to the shrimp for the same total time (12 h) each day irrespective of the treatment. Our observations of the feeding trays showed that the shrimp continued to feed on pellets that had been in the water for up to 4 h (the maximum time that the feed was left on the feeding trays).

Our laboratory study of the commercial diet showed that nutrients leached from the feed pellets into the surrounding water relatively quickly. Most of the leaching loss occurred in the first 2 h, with a relatively small amount lost thereafter. As there was no adverse effect on shrimp growth rate or apparent feed intake, it suggests that for the shrimp, the nutritional value of the feed that had been in the water < 2 h was not different from that of feed that has been in the water for between 2 and 4 h. It also suggests that the nutrients that were lost from the feed (12% of DM, 15% of crude protein) were either not critical for growth or that they were present in excess of the requirements of the shrimp. Though leaching loss of nutrients from the feed was measured, we did not measure microbial degradation of the feed, which is likely to have been a slower process. As there was no difference in the growth rate of shrimp across treatments, it suggests that there was little microbial degradation over this period.

Our experiment showed that different feeding frequencies also did not significantly affect water quality. This result is consistent with the fact that the shrimp in all treatments were issued with and consumed the same amount of feed, and the unconsumed feed was left in the tanks for at least 2 h in all treatments, during which time most of the leaching of nutrients would have occurred. Our results are also consistent with those of Velasco *et al.* (1999) who found no differences in the total N or P concentrations in the water, regardless of feeding frequency.

Our study differed from common practice on commercial shrimp farms in that uneaten feed on the feeding trays was removed from the tanks in order to assess feed intake. About 13% of the total mass of feed fed to each tank of shrimp was removed in this way. However, it is likely that crumbling of feed while the shrimp were feeding, and removal of feed from the feeding trays by the shrimp would have resulted in a proportion of the residual feed being retained in the tank system. Our rationale for removing feed remaining on the feeding trays was due to concerns about leaving a disproportionately high load of residual feed into the tank system compared with a commercial pond system, negatively affecting water and sediment quality, and ultimately shrimp health across all treatments.

Despite deviating from commercial practice by removing uneaten feed from the tanks, the magnitude and variability of the water quality parameters (total N, ammonium, nitrate/nitrite, DON, total phosphorus, phosphate, chlorophyll, DO, pH and salinity) in our experiment were comparable with those in commercial *P. monodon* ponds (Cowan *et al.* 1999; Tookwinas & Songsangjinda 1999). There were also distinct diel patterns in temperature, oxygen and ammonium concentrations of a similar magnitude to those previously described in shrimp ponds (Burford & Glibert 1999). Water exchanges were conducted at a similar frequency and of the same proportion of the total volume as with commercial ponds (Cowan *et al.* 1999; Tookwinas & Songsangjinda 1999). Therefore, it appears that the key biological and chemical processes in our tank system were functioning in a similar way to those in shrimp ponds with high and variable concentrations of dissolved and particulate N, particulate phosphorus and chlorophyll *a*.

Ammonium concentrations in the water increased over the experiment from 0.01 to higher than 5 mg l<sup>-1</sup> as shrimp biomass and N loading to the tanks increased. This is consistent with findings in shrimp ponds (Tookwinas & Songsangjinda 1999). The sediment porewater ammonium concentrations were substantially lower (26.6 ± 4.62 mg l<sup>-1</sup>) than those reported for shrimp ponds late in the growth season (2080 mg l<sup>-1</sup>) (Burford & Longmore, in press). Therefore the contribution of sediment remineralization of organic matter to ammonium levels in the water column is likely to be lower than that for shrimp ponds.

The cost of labour for the feeding of shrimp ponds can be a significant component of the fixed costs (Lawrence & Lee 1997). During the grow-out phase, *P. monodon* are typically fed 5 or 6 times d<sup>-1</sup> (Jory 1995). We have estimated that with a reduction in feeding frequency from 5 times d<sup>-1</sup> to 3 times d<sup>-1</sup>, savings of up to 25% of the cost of the labour associated with feeding could be made.

Our study has shown there could be significant benefits for producers in reducing the frequency of feeding *P. monodon* from 6 to 3 times d<sup>-1</sup>, provided the feed is available to the shrimp for the same period as more frequent feeding, is nutritionally adequate and has high water stability. In addition, less frequent feeding is not likely to negatively affect water quality, and therefore will not increase the nutrient loads in pond discharge water. There is also considerable potential to optimize feeding strategies further using more technologically enhanced feeding strategies as used with salmonids (Alanara 1993), and by improving feed formulations and pellet stability, with the ultimate aim of improving profitability and reducing the environmental impact of shrimp farming.

## Acknowledgments

We wish to thank Noel Herbst from Gold Coast Marine Prawn Farm, Doug Pearson from ProAqua for their input into the planning of this study and constructive criticism of the manuscript. Ian Williams, Kevin Williams and Peter Rothlisberg also provided constructive comments on the manuscript. We thank Gold Coast Marine Prawn Farm for providing the shrimp used in the experiment, Elwyn Truloff of Truloff Prawn Farm for assistance in the logistics of setting up the experiment, Margaret Barclay and Peter Thompson from CSIRO Marine Research for carrying out the chemical analyses, and Quinton Dell, James Belford and David Irvin for their assistance with feeding the shrimp, particularly on the night shifts and weekends.

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#### 4.8. Development of a feeding strategy for silver perch (*Bidyanus bidyanus*) based on restricted rations

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#### Abstract

The Australian freshwater fish silver perch (*Bidyanus bidyanus*) has great potential for aquaculture and an industry based on high-volume, low-cost production is developing. Practical, least-cost diets containing low levels of fishmeal have been formulated, but a feeding strategy for silver perch has not been established. To provide a basis for the development of a strategy, a series of eight consecutive experiments was conducted in 1m<sup>3</sup> cages in an aerated, earthen pond at the Grafton Aquaculture Centre (GAC) to determine the effects of feeding rate (% body weight) and feeding frequency (feeds/day) on the growth and food conversion ratio (FCR) of fingerlings and larger fish under ambient water temperatures over the range 13.8 - 30.6°C. Fish were fed extruded pellets of a commercially-available silver perch diet containing 34% digestible protein (5% fishmeal) and 14 MJ/kg digestible energy. In addition, commercial silver perch farmers were consulted about feeding practices for large fish (> 500 g), and at water temperatures below 12°C, and practices for silver perch broodfish at GAC, and channel catfish (*Ictalurus punctatus*) in winter were used to complete the strategy.

In the feeding experiments, growth and FCR increased with increasing feeding rates to a level, above which only FCR increased. Optimum feeding rates and frequencies were those which resulted in maximum growth, while minimising effort (feeding frequency) and FCR. The highest feeding frequency required for maximum growth, including that of small fingerlings was twice (2x) daily, and the optimum feeding rates varied with water temperature and size of fish. The optimum daily regimes were: small fingerlings (initial mean weight, 2.0 g) 7.5%/2x at a mean temperature of 23.3°C; fingerlings (14.9 - 27.7g) 7.5%/2x at 27.1°C, 5.0%/2x at 23.7°C and 2.0%/1x at 16.8°C; and large silver perch (162.5 - 510.6 g) 0.5%/1x daily or 1.0% on alternate days at 15.6°C, 1.0%/1x at 17.3°C, 3.0%/2x at 24.1°C and 2.0%/2x at 27.9°C. It is suggested that regimes of 0.5%/1x daily for fingerlings (< 50 g) and 0.5%/1x on alternate days for larger fish are used at temperatures of 9° to 12°C, and 0.5% 3 days/week and 0.5% 1 day/week for fingerlings and larger fish respectively, at 6° to 9°C. To ensure the maintenance of good water quality, particularly dissolved oxygen and ammonia, the amount of feed applied should not exceed 150 kg/ha/day in small ponds (< 0.3-ha) or 100 kg/ha/day in larger ponds.

Our research has established an overall feeding strategy for silver perch based on restricted rations. Recommended feeding rates, frequencies and feed sizes for fingerling and larger silver perch, and broodfish at different water temperatures are presented. The performances of silver perch in cages in this study are similar to those reported for the species in aerated earthen ponds, justifying the use of the feeding strategy in pond culture, and suggesting that silver perch has potential for cage culture.

## Introduction

Aquaculture is a relatively new industry in Australia, and most recent advances have been in industries based on luxury products such as Atlantic salmon (*Salmo salar*), southern blue-fin tuna (*Thunnus thunnus*) and shrimp (*Penaeus monodon*). The omnivorous, native, freshwater fish silver perch (*Bidyanus bidyanus* Mitchell) has been identified as a white-fleshed finfish with aquaculture potential (Rowland & Barlow 1991) and research has demonstrated that it is an excellent species for intensive pond culture (Rowland *et al.*, 1995). Techniques for hatchery production and the culture of fingerlings and market-size fish in static, aerated earthen ponds have been established, and a 3-phase production strategy is recommended: I - Hatchery, II - Fingerling, III - Grow-out (Rowland 1995a). Most commercial grow-out farms purchase small fingerlings (0.5 - 5g) from hatcheries in summer and produce market-size fish (> 500g) in 15 to 24 months. An industry based on high-volume, low-cost production is developing, and has the potential to become one of Australia's largest fisheries (Rowland 1999).

Nutrition research conducted at NSW Fisheries facilities since 1995, has resulted in the formulation of a least-cost diet, LC2, based on Australian agricultural products and containing 34% digestible protein (including only 5% fishmeal) and 14 MJ/kg digestible energy (Allan *et al.* 2000). This diet is now available commercially to industry. Feed constitutes around 20% of production costs in silver perch culture and so, as in all aquaculture industries, efficient delivery of diets is necessary for economically viable production (Allan & Rowland 2002). An overall feeding strategy for silver perch has not been developed. Current recommendations to industry (Allan 1995) are based on those used in other warm water finfish industries, particularly the channel catfish (*Ictalurus punctatus*) industry in the USA (Tucker & Robinson 1990) and on the results of the initial silver perch culture experiments conducted at the Grafton Aquaculture Centre, GAC (Rowland *et al.* 1994, 1995; Rowland 1995b).

Feeding strategies vary between and within species and industries, and include feeding *ad libitum*, to satiation, or a restricted ration based on a proportion of body weight (Hepher 1988; Lovell 1989a, b; Tucker & Robinson 1990; El-Sayed & Teshima 1991). Feeding tables and observation of surface feeding activities have traditionally been used to gauge the required daily ration of farmed fish (Hepher 1988; Lovell 1989a; Talbot *et al.* 1999) and more recently, electronic sensors have been used to adjust delivery of feed according to feeding activity in some salmonid industries (Blyth *et al.* 1993).

Many silver perch farmers attempt to feed to satiation by observing the feeding activity of the fish at or near the surface. However, satiation can be very difficult to determine in the characteristically turbid silver perch ponds, where not all fish feed at the surface. Feeding behaviour and activity of silver perch vary with stocking density and temperature (Rowland *et al.* 1995), genetic strain (Stuart Rowland, unpublished data) and are also thought to be influenced by water quality, turbidity, availability of natural food, time of day, cloud cover and wind (GAC staff, personal observations). Consequently it is easy for silver perch farmers to either over-feed or under-feed their fish. Poor feeding practices increase the cost of production because over-feeding wastes feed and adversely affects water quality, while under-feeding results in reduced growth and greater size variation because of increased inter-fish competition for feed; both may increase susceptibility to disease. In addition, feeding to satiation can be time-consuming, and increase labour costs and the overall cost of production. Feeding fish based on surface activities does not guarantee that bottom foraging fish have an opportunity to feed (Ang & Petrell 1998) and the difficulty in determining satiation is a major cause of sub-optimal production efficiency in commercial fish farming (Talbot *et al.* 1999).

The aim of this research project was to develop an overall feeding strategy for silver perch. This was achieved by:

1. Conducting a series of experiments to determine the effects of feeding rate and feeding frequency on the performance of fingerling and larger fish over a range of water temperatures at GAC.
2. Reviewing feeding practices for broodfish at GAC.
3. Consulting commercial silver perch farmers about feeding practices, particularly for large fish (> 500 g) and during winter in regions where water temperatures fall below 12°C.
4. Reviewing winter feeding practices in the channel catfish industry in the United States.

## Materials and Methods

### General

Eight consecutive experiments were conducted using cages in a 0.32-ha earthen pond at GAC. Although feed requirements of some species may vary between culture environments (Tucker & Robertson 1990; Webster *et al.* 1992) the use of cages in the current study enabled the replication of a number of treatments in each experiment. It also subjected the fish in each experiment to the same daily and weekly fluctuations in ambient temperature and water quality.

The square, 1m<sup>3</sup> cages, were hung from a floating pontoon that was attached to a fixed walkway in the pond. Cages in Experiment 1 were made of solid Nyllex with a mesh size of 4 mm, while the cages in other experiments were made of knot-less netting with a bottom lead-line. Cages in the fingerling Experiments 2, 3 and 4 had a stretched mesh size of 21 mm, and the mesh size in cages for larger fish was 40 mm. A 25 cm deep apron of 1 mm gauze was suspended from the top of each cage to prevent food from washing out at the surface. Each cage had a Nyllex lid of 10 mm mesh to prevent predation by birds.

Four experiments with fingerlings (numbers 1 - 4) and four other experiments (5 - 8) with larger fish were run in different seasons, to expose fish to the annual range of ambient water temperatures at GAC of approximately 10° to 30°C (Rowland 1995c). The design and duration of each experiment, the size of fish at stocking and termination, and water temperatures are given in Tables 1 & 2.

The pond was aerated nightly between 00.00h 08.30h using two 1 hp paddlewheel aerators, positioned to provide an even flow through all cages. Water was added periodically to the pond to replace that lost by evaporation and seepage. The pond was drained and dried between each experiment. Prior to each experiment, fish were pooled and quarantined for 7 days in 9,000 L tanks and treated continuously with NaCl at 5 g/L to reduce stress, ensure that they were free of ectoparasites and to prevent fungal infection (Rowland & Ingram 1991). Immediately before stocking, all fish in the tank were anaesthetised using 20 mg/L Benzocaine (ethyl *p*-aminobenzoate), the appropriate number counted, weighed and then placed in cages that had been randomly allocated to treatments and replicates. The initial mean weight and biomass for each cage were determined. A stocking density of 100 fish/m<sup>3</sup> was used in fingerling experiments, and 50 fish/m<sup>3</sup> for experiments involving larger fish. Every 21 days all fish were removed from each cage, anaesthetised, counted, weighed (total weight), and returned to the cage; the biomass in each cage was then determined and the daily ration adjusted accordingly. At termination, fish were counted and weighed, and the survival rate, absolute growth rate (g/d) and food conversion ratio (FCR) determined.



### *Diet and feeding*

Fish in all experiments were fed extruded, slow-sinking pellets of the commercial diet, LC2, containing 34% digestible protein (including 5% fishmeal) and 14 MJ/kg digestible energy (Allan *et al.* 2000). Feeding times were 08.00, 12.00 and 16.00h. In treatments where fish were fed once daily, feed was provided at 16.00h, and those fed twice daily at 08.00h and 16.00h. The daily ration was divided evenly between meals. At each meal, three even “batches” of feed were delivered to each cage over a period of about 30 min.

### *Water quality and disease monitoring*

Routine monitoring of water quality followed Rowland (1995c). An Horiba U-10 meter was used to monitor temperature, dissolved oxygen (DO) and pH. Total ammonia-nitrogen (TAN) was determined using Nessler Reagent; un-ionised ammonia (NH<sub>3</sub>-N) was calculated using temperature and pH data after Trussell (1972) and Boyd (1982). Water quality was monitored at a depth of 1 m from the pond walkway. During each experiment, 100 fingerlings or 50 larger fish were stocked into an extra cage, located adjacent to the experimental cages. Gill and skin tissue were sampled from three fish in this cage each 21 days to monitor fish health.

### *Experiments*

#### Fingerling experiments

In Experiment 1, small fingerlings (initial mean weight, 2.0g) were stocked in early autumn (March), following the production strategy currently used in the commercial industry. Fish were fed at rates of 5.0%, 7.5% or 10.0% body weight/day (bw/d), twice (2x) or three times (3x) daily (Table 1). In Experiment 2, larger fingerlings (initial mean weight, 24.8g), typical of those overwintered on farms, were stocked during winter at temperatures of 13.8° - 18.9°C (mean 16.8°C) (Table 2). Fish were fed 1.0%, 2.0% or 3.0% bw/d, 1x or 2x daily. Experiment 3 was conducted during late spring and summer at temperatures of 20.5° - 28.0°C (mean 23.7°C); the fingerlings (initial mean weight, 14.9g) were fed 2.5%, 5.0% or 7.5% bw/d, 1x, 2x or 3x daily. Experiment 4 was conducted in late summer (25.1° - 29.4°C; mean 27.1°C) and fish (initial mean weight, 27.7g) were fed at 5.0%, 7.5% or 10.0% bw/d, 1x, 2x or 3x daily.

#### Large fish experiments

In Experiment 5, large silver perch (initial mean weight, 458.6 g) were fed at rates of 0.5%, 1.0% and 2.0% bw/d, 1x daily or 1x on alternate days at water temperatures of 14.1° - 16.9°C (15.6°C). In Experiment 6, large silver perch (initial mean weight, 510.6 g) were fed at 0.5%, 1.0% and 2.0% bw/d once daily at temperatures of 15.3° - 20.1°C (17.3°C). In Experiment 7, silver perch (initial mean weight, 162.5 g) were fed 1%, 2%, 3% or 4% bw/d, 1x or 2x daily at temperatures of 20.6° - 27.1°C (24.1°C). These treatments were repeated in Experiment 8 for fish (initial mean weight, 300.7 g) at higher temperatures of 23.9° - 29.6°C (27.9°C).

### *Data and statistical analysis*

Following Hopkins (1992), the use of an absolute growth rate (g/d) is considered appropriate for the description and comparison of growth in this experiment, because there was no significant difference between the mean weights of fish in each treatment at the commencement of each experiment, and the experimental periods were relatively short (21 - 42 days). The weights of fish at stocking in each experiment were compared using one-way analysis of variance. The effects of feeding rate and feeding frequency on the survival, growth and FCR of silver perch were determined using two-way analysis of variance. Homogeneity of variance was assessed using Cochran's Test. Percentage data were arc-sin transformed before analysis. Where there were significant differences the treatment means were compared using Student Newman-Keuls procedure. Means were considered significant at  $P < 0.05$ .

## Results

### *Survival*

Feeding rate or frequency did not significantly affect survival in any experiment. Mean survival rates of fingerlings exceeded 92% in Experiments 1, 2 and 4, but in Experiment 3 means ranged from 77.3% to 93.0% (Table 3). In that experiment, some fish meshed in the cages, and some were killed by bird predation through the side of the cages during the first week. A large net was subsequently placed around all the cages to prevent further predation. The dead fish were replaced within two days. Mean survival rates exceeded 95% in all experiments with large fish. Feeding rate, feeding frequency and the loss of fish in Experiment 3 did not significantly affect survival. In most experiments, fish commenced feeding within two days of initial stocking, and recommenced feeding the day after sampling.

### *FINGERLINGS*

#### *Experiment 1*

Small fingerlings (2.0 g) fed 7.5% and 10.0% grew significantly faster (0.32 - 0.33 g/d) than those fed 5.0% (0.26, 0.27 g/d) (Figure 1). Feeding frequency did not affect growth or FCR which increased with increasing feeding rate from 0.9 to 1.8 (Figure 1). The optimum feeding regime is 7.5%/2x daily for small fingerlings at a mean temperatures of 23.3°C.

#### *Experiment 2*

Fingerlings (24.8 g) fed 2.0% or 3.0% grew significantly faster (0.17 - 0.20 g/d) than fish fed 1.0% daily (0.12, 0.13 g/d) (Figure 2). FCR increased with increasing feed rate from 1.9 to 3.9 and was significantly different at each rate (Figure 2). Feeding frequency did not affect growth or FCR. The optimum regime is 2.0%/1x daily at 16.8°C.

#### *Experiment 3*

Fingerlings (14.9 g) fed 5.0% or 7.5% 2x or 3x daily grew significantly faster (0.81 - 0.90 g/d) than fish fed these rates 1x daily or 2.5% (0.50 - 0.74 g/d) (Figure 3). FCR of fish fed 7.5% was significantly higher than those fed 2.5% or 5.0%, and feeding frequency did not affect FCR (Figure 3). The optimum regime is 5.0%/2x daily for fingerlings at temperatures of 23.7°C.

#### *Experiment 4*

Fingerlings (27.7 g) fed 7.5% or 10.0% grew significantly faster (0.95 - 1.03 g/d) than fish fed these rates 1x daily or 5.0% (0.67 - 0.85 g/d) (Figure 4). FCR was significantly higher at 10.0% (3.1 - 3.5) than at 5.0% or 7.5% (1.6 - 2.5). The optimum regime is 7.5%/2x daily at 27.1°C.

### *LARGE FISH*

#### *Experiment 5*

Fish (458.6 g) fed 0.5% on alternate days grew significantly slower (0.3 g/d) than those fed other regimes (1.0 - 1.3 g/d), and FCR was significantly higher for fish fed 2.0% daily (Figure 5). Similar growth rates and FCRs were achieved with 0.5% daily or 1.0% on alternate days at temperatures of 15.6°C.

### *Experiment 6*

Fish (510.6 g) fed 1.0% or 2.0% daily grew significantly faster (1.6, 1.7 g/d) than fish fed 0.5% daily (1.0 g/d), and feeding rates did not significantly affect FCR (Figure 6). The optimum regime is 1.0%/1x daily at 17.3°C.

### *Experiment 7*

Fish (162.5 g) fed 3.0% and 4.0% grew significantly faster (3.1 - 3.5 g/d) than those fed 1.0 and 2.0% (1.4 - 2.6 g/d) and feeding frequency did not affect growth (Figure 7). FCR at the rate of 4.0% was higher than those at 1.0%, 2.0% or 3.0% (Figure 7) and FCRs of fish fed 3.0% or 4.0% 2x daily were significantly lower than fish fed these rates 1x daily. The optimum feeding regime for silver perch up to about 350g is 3.0%/2x daily at 24.1°C.

### *Experiment 8*

Fish (300.7 g) fed 2.0%, 3.0% or 4.0% grew significantly faster (2.3 - 2.7 g/d) than those fed 1.0% (1.3, 1.7 g/d), and FCR was significantly higher for fish fed 3.0% and 4.0%. FCRs of fish fed 2x daily were lower than fish fed 1x daily (Figure 8). Optimum regime for silver perch over about 300g is 2.0%/2x daily at 27.9°C.

### Water quality and disease

Ranges of the water quality variables DO, pH and TAN, and maximum values of NH<sub>3</sub>-N during each experiment are given in Table 4. DO concentrations were above 3.8 mg/L throughout all experiments. In Experiment 7, DO declined from 10.9 to 3.8 mg/L over 4 days after the application of 30 mg/L formalin to treat a heavy infestation of the parasite *Dactylogyrus* sp. For 4 weeks prior to the infestation and treatment, the fish had been exposed to concentrations of NH<sub>3</sub>-N of 0.10 - 0.69 mg/L and pH up to 10.1 (Table 4). There were no disease outbreaks in other experiments, and the ecto-parasites *Trichodina* sp., *Dactylogyrus* sp. and *Ichthyobodo* sp. were seen only at low levels on gill tissue.

### Feeding strategy

An overall feeding strategy, including recommended feeding rates and frequencies for fingerling, larger silver perch and broodfish over the range of water temperatures from 6° to 30°C is presented in Table 5. Recommended feed particle sizes for silver perch are presented in Table 6. The growth rates, FCRs and production rates of silver perch under the optimum feeding regimes in the current study are presented in Table 7.

## **Discussion**

The delivery of feed is one of the most important practices in aquaculture because it directly influences growth, production and food conversion ratio (FCR, food fed : gain in wet weight of fish) and consequently economic viability. Feed requirements are well known to vary between species and with fish size and age, dietary protein and energy, feeding history and environmental factors, particularly temperature. As with other animals, feeding activities in fishes exhibit “circadian-like” patterns (Noeske & Spieler 1984; Boujard & Leatherland 1992); however, numerous studies have shown that regulated feeding in aquaculture may override to some degree, the various natural rhythms associated with appetite, feeding and digestion (Davis & Bardack 1965; Boujard & Leatherland 1992; Robinson *et al.* 1995; Jarboe & Grant 1996; Koskela *et al.* 1997; Wang *et al.* 1998; Chen *et al.* 1999).

### Feeding rates and frequencies

Growth and FCR of silver perch increased with increasing feeding rates up to a level, above which only FCR increased significantly, and the well known inverse relationship between feeding rate and feed conversion efficiency (Jauncey 1982; Lovell 1989a) was evident in all experiments (Figs 1 - 8). The asymptotic relationship between ration size and growth has been reported for other species (Brett *et al.* 1969; Papoutsoglou & Voutsinos 1988; Hung *et al.* 1989; Qin & Fast 1996; Adebayo *et al.* 2000). With most species, the use of feeding levels that achieve maximum growth rather than lower levels that optimise food conversion is economically desirable (Lovell 1989a). However, high values of FCR reflects a wastage of feed that leads to deterioration of water quality, increased costs and inefficient production, and so the selected optimum feeding regimes for silver perch were combinations of rates and frequencies that resulted in maximum growth, while minimising effort (frequency) and FCR.

The optimum feeding rate for small silver perch fingerlings (2 g) at 23.3°C was 7.5%, confirming the suggestion by Russell *et al.* (1996) that the feeding level required to obtain maximum growth of juvenile silver perch (1.3 g) was between 5% and 10%. At a similar temperature (23.7°C), larger fingerlings required only 5% for maximum growth, but at 27.1°C and 16.8°C, rates of 7.5% and 2.0% respectively were optimal. This relationship between size of fingerlings, feeding requirement and temperature has been reported for other species. Rates up to 24% have been recommended for very small fingerlings of some species including tilapia (Macintosh & De Silva 1984; Siraj *et al.* 1988). Optimum rates for 1g tilapia fry were 4% body weight/day, but only 3% for fry of around 6.5g (de Silva *et al.* 1986). Feeding rates of 5% - 6% have been reported for fingerling snakehead (*Channa striatus*) (Qin & Fast 1996) and channel catfish (Tucker & Robinson 1990). A rate of 3%/day was optimal for hybrid clariid catfish grown to a larger size of around 100g (Adebayo *et al.* 2000). Rates of 1.0% to 2.0% are recommended for fingerlings of various species at temperatures less than 20°C (Hidalgo *et al.* 1987; Tucker & Robinson 1990). In fingerling experiments in the current study, FCR increased with increasing feeding rate (Figures 1-4) as was reported for tilapia (Clark *et al.* 1990). Higher feeding rates were providing excess feed and so subsequent values of FCR were high.

The optimum daily feeding rates of 2% or 3% for larger silver perch (50 - 500 g) at temperatures over 20°C (Table 5) are similar to those reported for many other cultured species (de Silva *et al.* 1986; Merola & de Souza 1988; Papoutsoglou & Voutsinos 1988; Hung *et al.* 1989; Lovell 1989a, b). Feeding rates are temperature dependent, and optimum rates for silver perch below 20°C were 0.5% and 1.0% (Table 5). Rates of 2.0% or lower are usually recommended for warm water species at temperatures under 20°C (Hung *et al.* 1989; Tucker & Robinson 1990; McNulty *et al.* 2000). The optimum rate is also dependent on the protein level of the diet. Harpaz *et al.* (2001) reported similar growth of silver perch fingerlings fed a 40.6% protein diet at 2% body weight, and those fed a 24.8% protein diet at 4% body weight, and Sumagaysay & Borlongan (1995) described a similar relationship in milkfish (*Chanos chanos*).

The highest feeding frequency required for maximum growth of silver perch was twice daily, even for small fingerlings; more feeds did not improve growth significantly. Many species studied in aquaculture show a positive effect of feeding frequency on growth, at least up to 2 or 3 meals per day depending on species (Andrews & Page 1975; Grayton & Beamish 1977; Siraj *et al.* 1988; Wang *et al.* 1998; Lee *et al.* 2000; Thompson *et al.* 2000), although there are reports of much higher frequencies such as up to 6 daily feeds for maximum growth in young red-spotted grouper (*Epinephelus akaara*) (Kayano *et al.* 1993). However, Jarboe & Grant (1996) found no significant differences in survival and growth of channel catfish fed 3% once or 3 times daily in a recirculating raceway system, and Thommasen & Fjæra (1996) found no difference in growth of post-smolt Atlantic salmon fed a restricted ration at frequencies between 3 and 80 times/day. The response to feeding frequency can depend on a number of factors including the nutrient density of the diet (Rouhonen *et al.* 1998; Lee *et al.* 2000) and feeding strategy. de Silva *et al.* (1986) found

that optimum growth of tilapia fed ad libitum was obtained at a frequency of 4 times per day, whereas on a restricted ration of 3% body weight best performance was with 1 or 2 feeds daily.

FCRs were lower with more frequent feeds in larger silver perch at temperatures over 20°C (Figures 7 & 8). Greenland & Gill (1979) also found higher food conversion efficiency with more numerous feeds in channel catfish. Hepher (1988) stated that the effect of feeding frequency on food conversion is usually small, and this is supported by some studies using juvenile fish (e.g. Wang *et al.* 1998). There is a positive correlation between gastric evacuation rate, digestion rate (passage through the entire gut) and temperature, and larger fish digest meals much faster than smaller fish (Fänge & Grove 1979; Ross & Jauncey 1981; Hepher 1988). Consequently, smaller meal sizes, faster digestion rates, and a return of appetite for the second meal may have resulted in more efficient feeding, less pellet wastage and subsequent lower FCRs in the larger silver perch fed twice daily at the higher temperatures.

Optimum feeding regimes for large silver perch were different at the mean temperatures of 24.1° and 27.9°C. This finding may be due to the larger fish and/or higher temperatures in Experiment 8. Larger fish are known to require less feed than smaller fish (Brett 1979; Lovell 1989b). At 24.1°C, the optimum feeding rate was 3%, whereas at 27.9°C the growth of silver perch fed 3% or 4% was slower and FCRs higher than fish fed 2% (Figure 8). This is similar to the finding of Merola & de Souza (1988) who recommended the use of 3% for pacu (*Colossoma mitrei*) up to 180 g and 2% for larger fish. Prolonged exposure (3 - 4 weeks) of silver perch to temperatures around 30°C is thought to adversely affect growth (Rowland 1995a). A decrease in growth rate with increasing feeding levels at relatively high temperatures has also been reported in carp, *Cyprinus carpio* (Huisman 1976; Jauncey 1982). Metabolic rates of fish increase with temperature and as the ration size increases from maintenance to satiation (Paloheimo & Dickie 1966), and so the poorer growth at high temperatures may have been due, in part to lower levels of energy available for growth.

Some commercial growers consider that a regime of 2.0%/1x is sufficient for maximum growth of large silver perch (Bruce Rhoades, Mike Beveridge, personal communication). Results of our study suggest that silver perch (200 - 500 g) perform better if fed 2x daily at temperatures over 20°C, and Rowland *et al.* (1995) reported growth rates over 3 g/d for silver perch > 200 g that were fed 3%/2x at temperatures of 21.1° to 30.0°C. Optimum feeding regimes for fish over 500 g were not determined in our study. Farmers must evaluate the benefits of faster growth, lower FCR and expected better water quality against the additional cost and effort of feeding twice daily. In the channel catfish industry, although feeding twice daily is beneficial, it is virtually impossible to achieve on large farms (Tucker & Robinson 1990).

#### Maximum input of feed to ponds

As fish grow and the pond biomass and daily ration increase, the increasing quantity of feed applied to ponds causes a deterioration of water quality, in particular dissolved oxygen (DO) concentrations decrease and concentrations of total ammonia-nitrogen (TAN), and usually un-ionised ammonia (NH<sub>3</sub>) increase. Maximum feed inputs of 168 and 210 kg/ha/day have been reported to cause low DO (down to 2.2 mg/L) and high TAN in intensive silver perch ponds (Rowland 1995b; Rowland *et al.* 1995). Concentrations of TAN rise rapidly in ponds when feeding rates exceed 70 to 100 kg/ha/day (Cole & Boyd 1986; Rowland 1995b; Rowland *et al.* 1995) and prolonged exposure to concentrations of un-ionised ammonia over 0.1 mg/L are known to reduced growth of silver perch (Rowland, *et al.* 1995; Francis *et al.* 2000). In the channel catfish industry, farmers limit feed inputs to 100 - 150 kg/ha/day to avoid oxygen depletion (Lovell 1989a, b; Tucker & Robinson 1990). To help maintain good water quality, it is recommended that total feed inputs into silver perch ponds do not exceed 150 kg/ha/day in small ponds (< 0.3 ha) and 100 kg/ha/day in larger ponds. Farmers must ensure they have adequate aeration in ponds, i.e. around 10 hp/ha (Rowland 1995c), and the ability to exchange water.

### Feeding at water temperatures of 6° to 12°C

Activity and appetite in warm water fishes decline as temperatures fall below 20°C. Silver perch is a warm water species that is cultured over a wide geographic range in eastern Australia, and some farms are located in regions where water temperatures in winter decline to 5°C. There are benefits to feeding fish in winter. Channel catfish and golden shiners (*Notemigonus crysoleucas*) feed at temperatures as low as 10°C, and feeding in winter can prevent weight loss, achieve small weight gains, and keep fish healthier than those that are not fed (Lovell 1989a; Tucker & Robinson 1990; McNulty *et al.* 2000).

The range of ambient temperatures at GAC is 10° to 30°C (Rowland 1995c); however, during the current study, the lowest temperature recorded in winter was only 13.8°C (Table 2) and so feeding regimes at lower temperatures were not determined. Commercial silver perch farmers in temperate regions use regimes of 0.5%/1x daily for fingerlings and 0.5%/1x on alternate days for large fish at temperatures of 9° to 12°C (Bruce Rhoades, Bruce Malcolm, Ian Charles, personal communication). Recommended rates for channel catfish at 7.0° - 10.0°C are 0.5% 3 days/week for fingerlings, and 0.5% weekly for larger fish (Tucker & Robinson 1990). As a guideline, these rates are recommended for silver perch at temperatures of 6° to 12°C (Table 5).

### Feeding behaviour and use of natural food on cages

Feeding behaviour of silver perch was strongly influenced by temperature and the size of the daily ration. Fish often fed aggressively at and near the surface when temperatures were 20° to 30°C, less aggressively and lower in the water column at 15° to 20°C, and few fish were seen when temperatures fell below 15°C. However, even at high temperatures, it was evident that not all fish in each cage were feeding at the surface, suggesting that there are “more aggressive” fish in each batch. Competition and dominance of aggressive fish during feeding has been reported to adversely affect feed intake, growth, size variation and FCR in some species including Atlantic salmon (*Salmo salar*), Arctic charr (*Salvelinus alpinus*), rainbow trout (*Oncorhynchus mykiss*) and sea bass (*Dicentrarchus labrax*) (Thorpe *et al.* 1990; Alanärä 1992; Brännäs & Alanärä 1992, 1994; Paspatis *et al.* 1999). In addition, the fish in cages receiving the lowest feeding rate in each experiment usually fed aggressively and close to the surface through-out each meal, whereas fish at higher rates weren't visible for the duration of the meal. This is typical behaviour, where the rate of feed ingestion is initially high and then declines as the meal progresses reflecting a decreased level of hunger and number of feeding fish (Talbot *et al.* 1999).

There were growths of filamentous algae (*Cladophora* sp.) and periphyton on the inside of cages that contained fish fed the highest rates. Such growths were not evident where fish were fed low rates, suggesting that these silver perch grazed on the organisms growing on the surface of cages. However, the significantly slower growth rates of fish on the lowest feeding rates in each experiment suggest that the nutritive value of the algae and periphyton was not as high as the formulated diet. Although tilapia graze on the periphyton in cages, there are only small increases in production due to this food source even at low stocking densities in extensive systems (Shrestha & Knud-Hansen 1994; Norberg 1999).

### Broodfish

A silver perch breeding program is undertaken at GAC, and up to 1 million fingerlings are produced annually. Broodfish are induced to spawn using human chorionic gonadotrophin and the larvae are reared extensively in earthen ponds following techniques described by Rowland (1984) and Thurstan & Rowland (1995). Broodfish are fed the commercial silver perch diet LC2 following the strategy in Table 5. A daily regime of 2.0/2x is used prior to the breeding season, when temperatures reach 21°C in spring and early summer in an attempt to provide adequate nutrition for final gonadal maturation. No specific broodfish diet has yet been developed for silver perch, and

the decline in reproductive performance of wild, non-domesticated broodfish in captivity (Stuart Rowland, unpublished data) may be due in part to inadequate nutrition.

### Feeding strategy

This research project has developed an overall feeding strategy based on restricted rations.

Feeding regimes and feed particle sizes for fingerling and large silver perch, and broodfish at different temperatures are presented in Tables 5 & 6. These regimes are recommended as guidelines for silver perch farmers. The effective use of the restricted rations is dependent on the regular sampling of fish to estimate the biomass and determine the daily ration. At GAC, fish are sampled and the ration adjusted each 2 weeks for fingerlings (< 50g) and each 4 weeks for larger fish. In ponds, 100 - 200 fish (usually 5 - 10% of the crop) are sampled using a seine net. During the current study, all fish in each cage were sampled; however, in large cages under commercial conditions a randomly selected sample of around 10% is probably sufficient to estimate biomass. The feeding regimes can also be used as a guideline for farmers wishing to continue feeding to satiation because the recommended daily rations that were close to satiation levels. Studies with some species including tilapia and channel catfish have shown that feeding rates around 90% of satiation result in similar performance to fish fed to satiation (Andrews 1979; Clark *et al.* 1990). Different feeding regimes are likely to give optimum production as long as the correct ration is given per unit of time and biomass (Talbot *et al.* 1999).

### Potential for cage culture

Although the experiments in the current study were of relatively short duration (21 - 42 days), results suggest that silver perch has potential for cage culture. Growth and food conversion (Table 7) were better or similar to those reported by Rowland *et al.* (1994) for small fingerlings in ponds during summer. In addition, survival rates in the cages were high, whereas bird predation can cause significant losses of fingerlings in ponds (Rowland 1995d). The growth rates of large fish (3.5, 2.8 g/d, Table 7) were faster than silver perch grown to around 500g in ponds (Rowland 1995b; Rowland *et al.* 1995). The maximum mean production rate of 27.5 kg/m<sup>3</sup> in a treatment (Table 7) is similar to rates reported for some other species stocked at 100/m<sup>3</sup> and grown to edible size in cages (Merola & de Souza 1988; Hengsawat *et al.* 1997). Further research to establish cage culture techniques for silver perch is warranted.

### **Acknowledgments**

We gratefully acknowledge the funding and support of the project by the Fisheries Research and Development Corporation. Support with the technical work and management of the pond and cages was provided by Joel Crispin, Nat Sewell, Todd Rowland and Glen Trewick. Bruce Rhoades, Bruce Malcolm and Ian Charles provided information on feeding practices in the commercial industry. All feed used in the experiments was manufactured by Select Nutrition, and we thank Andrew Rhodes for his service and support. We thank Phil Read his comments on a draft of the report.

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**Table 1.** Design of silver perch feeding experiments.

Fish	Experiment No.	Feeding rate (% body wt/day)	Feeding frequency <sup>a</sup>	No. of replicates	Duration (days)
<i>Fingerlings</i>	1	5.0, 7.5, 10.0	2x, 3x	3	35
	2	1.0, 2.0, 3.0	1x, 2x	4	42
	3	2.5, 5.0, 7.5	1x, 2x, 3x	3	42
	4	5.0, 7.5, 10.0	1x, 2x, 3x	3	21
<i>Large fish</i>	5	0.5, 1.0, 2.0	1x 1x, alternate days	5	42
	6	0.5, 1.0, 2.0	1x	5	21
	7	1.0, 2.0, 3.0, 4.0	1x, 2x	4	42
	8	1.0, 2.0, 3.0, 4.0	1x, 2x	4	42

<sup>a</sup> once (1x), twice (2x), three times (3x) daily unless alternate days.

**Table 2.** Size at stocking and termination, and temperatures during silver perch feeding experiments.

Fish	Experiment no.	Size of fish (g)		Temperature (°C)	
		at stocking <sup>a</sup>	at termination <sup>b</sup>	mean	range
<i>Fingerlings</i>	1	2.0	16.6 - 21.8	23.3	20.2 - 27.5
	2	24.8	26.8 - 35.2	16.8	13.8 - 18.9
	3	14.9	34.2 - 56.5	23.7	20.5 - 28.0
	4	27.7	40.4 - 55.4	27.1	25.1 - 29.4
<i>Large</i>	5	458.6	450.4 - 553.2	15.6	14.1 - 16.9
	6	510.6	492.0 - 598.8	17.3	15.3 - 20.1
	7	162.5	200.4 - 332.0	24.1	20.6 - 27.1
	8	300.7	343.0 - 430.4	27.9	23.9 - 30.6

<sup>a</sup> overall mean; there were no significant differences between means at stocking within each experiment.

<sup>b</sup> range of mean weights across all treatments and replicates.

**Table 3.** Survival of silver perch in the feeding experiments.

Experiment no.	Feeding regime		Survival (%)
	<i>rate (% body weight)</i>	<i>frequency</i>	
1	5.0	2x, 3x	100.0, 98.7, 95.3
	7.5	2x, 3x	92.0, 99.7, 100.0
	10.0	2x, 3x	100.0, 92.7, 95.7
2	1.0	1x, 2x	98.8, 98.3
	2.0	1x, 2x	97.5, 99.8
	3.0	1x, 2x	93.0, 98.0
3	2.5	1x, 2x, 3x	77.3, 81.0, 86.0
	5.0	1x, 2x, 3x	81.3, 85.7, 93.0
	7.5	1x, 2x, 3x	86.7, 82.7, 93.0
4	5.0	1x, 2x, 3x	100, 98.7, 95.3
	7.5	1x, 2x, 3x	92, 99.7, 100
	10.0	1x, 2x, 3x	100, 92.7, 95.7
5	0.5	daily, alt days	99.6, 99.6
	1.0	daily, alt days	100, 98.8
	2.0	daily, alt days	99.2, 98.8
6	0.5	1x	98.8
	1.0	1x	100
	2.0	1x	99.6
7	1.0	1x, 2x	95.0, 99.5
	2.0	1x, 2x	99.5, 100
	3.0	1x, 2x	99.5, 98.5
	4.0	1x, 2x	99.5, 98.0
8	1.0	1x, 2x	100, 98.0
	2.0	1x, 2x	99.6, 100
	3.0	1x, 2x	100, 99.6
	4.0	1x, 2x	100, 100

<sup>a</sup> survival rates are treatment means.

**Table 4.** Water quality variables, dissolved oxygen (DO), pH, total ammonia-nitrogen (TAN), and un-ionised ammonia-nitrogen (NH<sub>3</sub>-N) during silver perch feeding experiments. Data are ranges, except NH<sub>3</sub>-N which is the maximum value.

Experiment	DO (mg/L)	pH	TAN (mg/L)	NH <sub>3</sub> -N (mg/L)
1	7.9 - 10.9	6.9 - 8.3	0.4 - 0.6	0.02
2	7.0 - 12.0	7.1 - 8.9	0.3 - 1.1	0.08
3	5.3 - 9.0	6.5 - 8.3	0.3 - 0.6	0.03
4	6.7 - 11.6	7.0 - 8.4	0.4 - 0.8	0.10
5	6.3 - 12.7	6.7 - 9.1	0.2 - 2.1	0.04
6	6.5 - 11.6	7.0 - 8.4	0.4 - 0.8	0.06
7	3.8 - 13.6	7.5 - 10.1	0.4 - 0.9	0.69
8	6.3 - 12.7	6.9 - 8.8	0.4 - 1.4	0.18

**Table 5.** Recommended feeding rates and frequencies for silver perch at temperatures of 6° to 30°C.

*Feeding rate = % body weight per day; feeding frequency = no. of feeds per day, i.e. once (1x) or twice (2x) e.g. fish to be fed 5.0% body weight, twice daily = 5.0/2x, fish to be fed 0.5% 3 days per week = 0.5 3d/w.*

Fish (g)	Water Temperature (°C)					
	6 - 9	9 - 12 <sup>a</sup>	12 -15	15 - 20	20 -25	25 -30
1 - 15	0.5 3d/w	0.5/1x	1.0/1x	3.0/1x	7.5/2x	7.5/2x
15 - 50	0.5 3d/w	0.5/1x	1.0/1x	2.0/1x	5.0/2x	7.5/2x
50 - 500	0.5 1d/w	0.5/alt.d	0.5/1x	1.0/1x	3.0/2x	2.0/2x
> 500 <sup>a</sup>	0.5 1d/w	0.5/alt.d	0.5/1x	1.0/1x	1.5/1x	1.0/1x
Broodfish	0.5 1d/w	0.5/alt.d	0.5/1x	2.0/2x	2.0/2x	1.0/1x
				spring & early summer; 2.0/1x other seasons	spring & early summer; 2.0/1x other seasons	

<sup>a</sup> recommendations following consultation with industry.

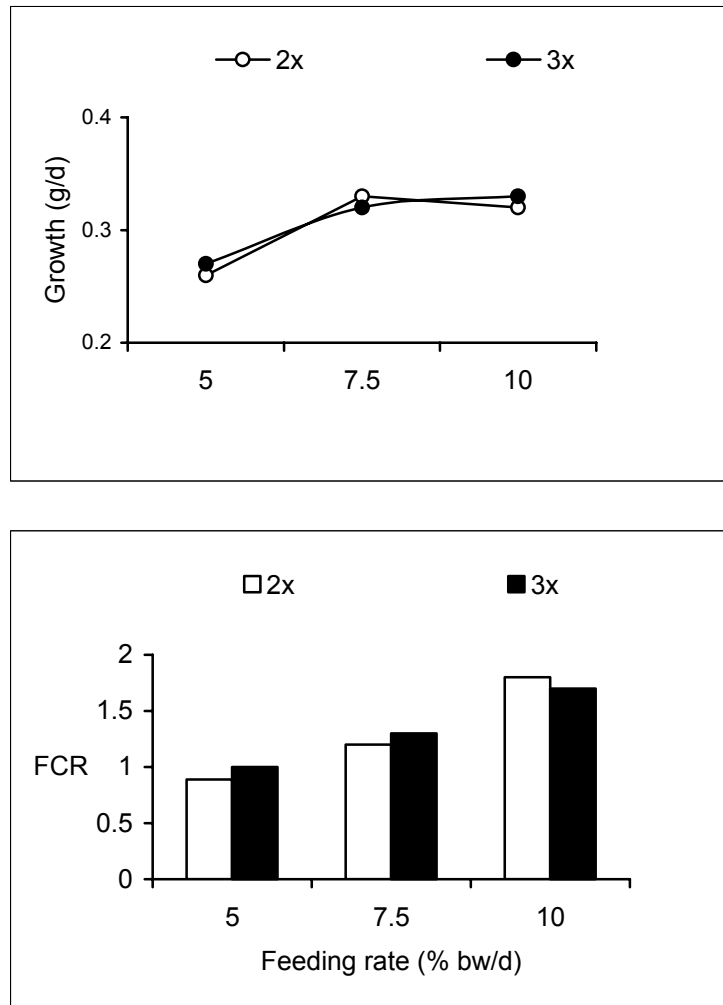
**Table 6.** Recommended feed particle size for silver perch.

*Feed particles generally have similar diameter and length.*

Fish (mm)	Size (g)	Size (mm) and type of feed	
		diameter	crumble (c) or pellet (p)
15-25	0.5	0.6	c
25-35	0.5 - 1.0	1.0	c
35-50	1 - 2	1.5	c
50-75	2 - 5	2.0	c
75-100	5 - 10	1.5	p
100-125	10 - 25	2.0	p
125-175	25 - 100	3.0	p
175-275	100 - 350	4.0	p
> 275	> 350	6.0	p

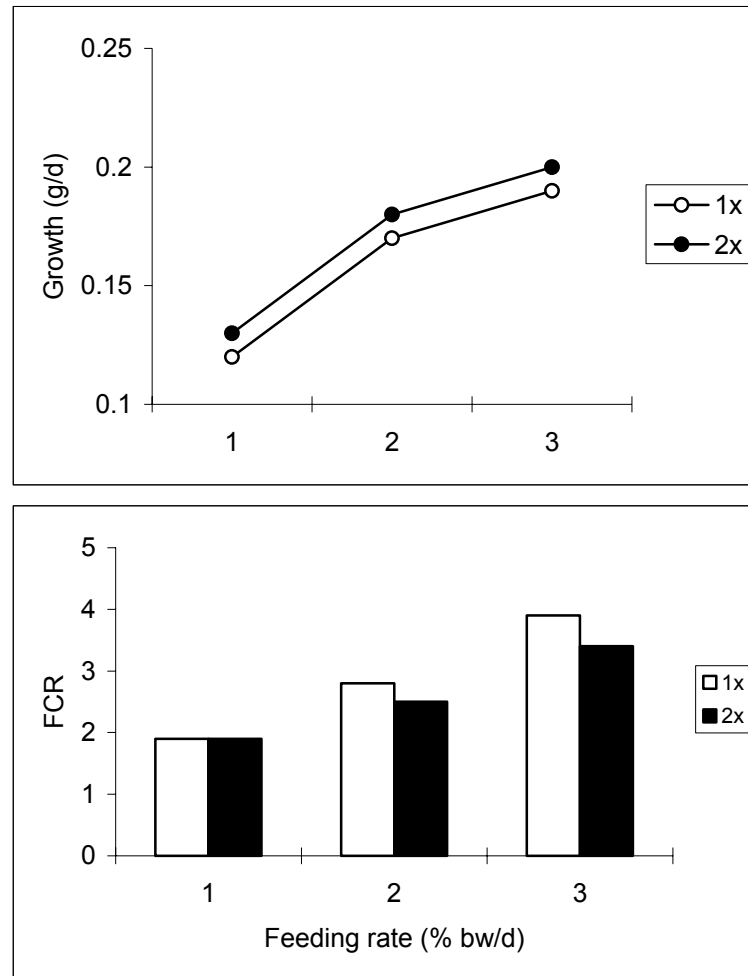
**Table 7.** Growth, FCR and production rates of silver perch under the optimum feeding regimes in the current study. Data for growth rate, FCR and production rate are treatment means.

Fish; weight at stocking (g)	Temperature (°C)	Daily feeding regime	Growth rate (g/d)	FCR	Production rate (kg/m <sup>3</sup> )
<i>Fingerlings</i>					
2.0	20.2 - 27.5	7.5%/2x	0.33	1.3	1.4
24.8	13.8 - 18.9	2.0%/1x	0.18	2.5	3.3
14.9	20.5 - 28.0	5.0%/2x	0.83	1.4	4.9
27.7	25.1 - 29.4	7.5%/2x	0.94	2.3	4.7
<i>Large</i>					
458.6	14.1 - 16.9	0.5%/1x	1.0	2.4	22.8
510.6	15.3 - 20.1	2.0%/1x	1.7	1.7	27.5
162.5	20.6 - 27.1	3.0%/2x	3.5	2.0	14.4
300.7	23.9 - 30.6	2.0%/2x	2.8	2.0	20.7

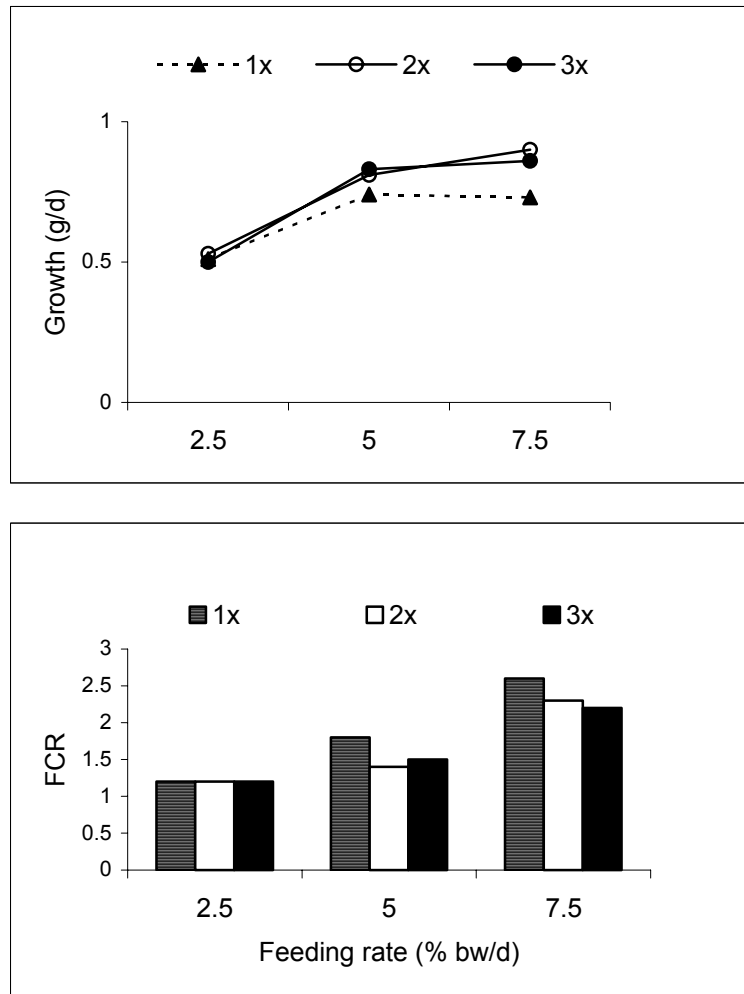


**Figure 1.** Growth and FCR of small silver perch fingerlings (initial mean weight, 2.0 g) fed 5.0%, 7.5% or 10.0% body weight twice (2x) or three times (3x) daily at a mean temperature of 23.3°C.

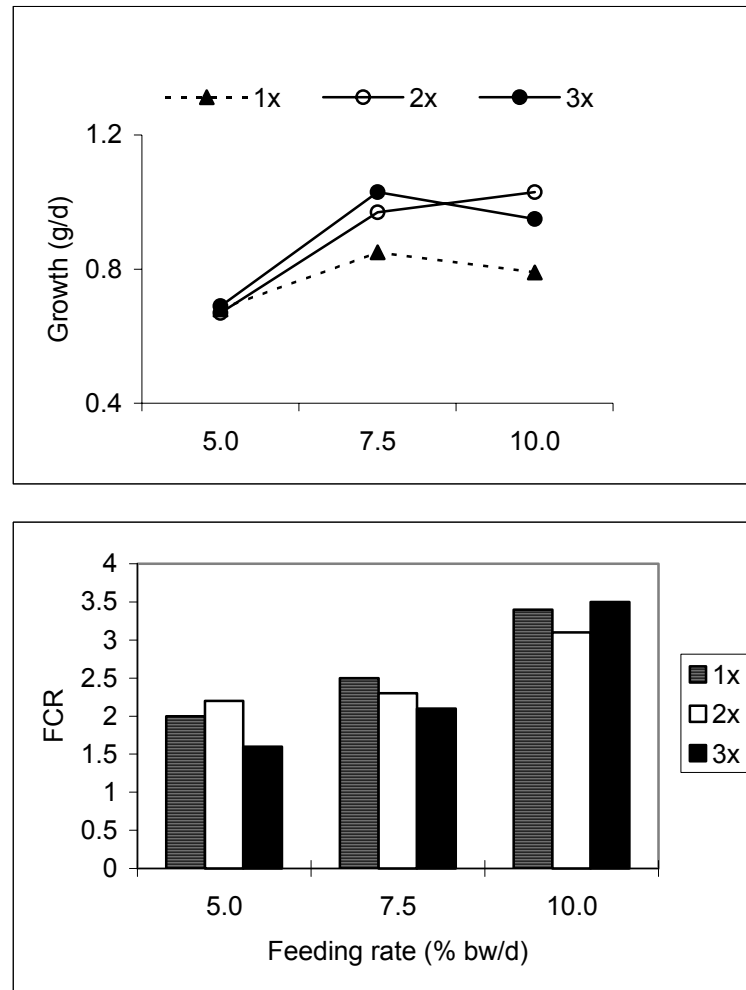




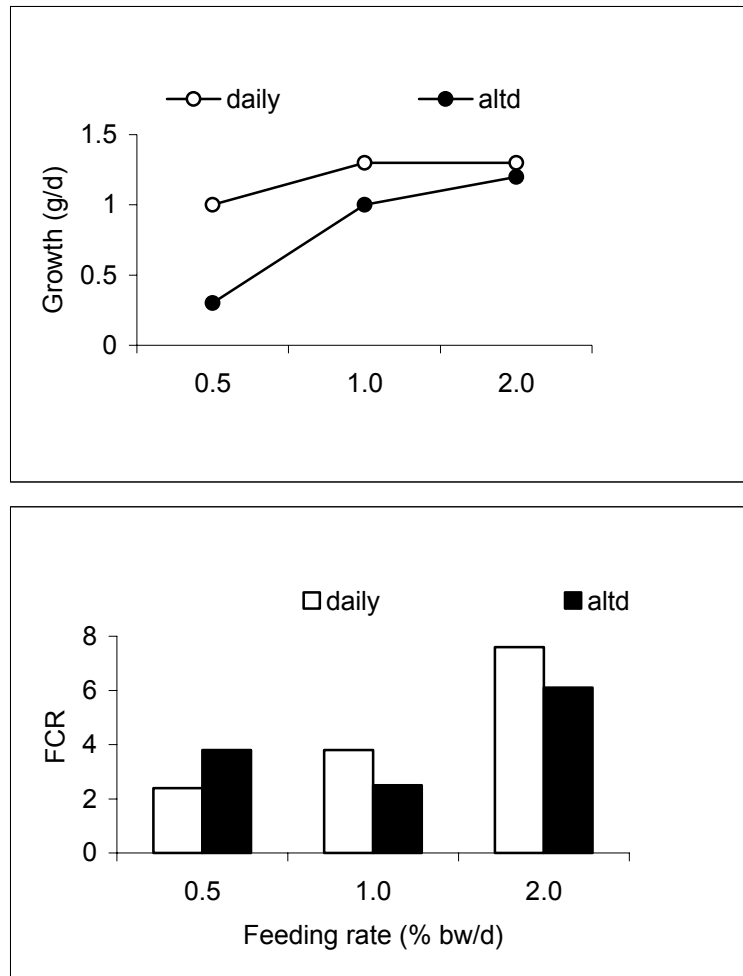
**Figure 2.** Growth and FCR of silver perch fingerlings (initial mean weight, 24.8 g) fed 1.0%, 2.0% or 3.0% body weight once (1x) or twice (2x) daily at a mean temperature of 16.8°C.



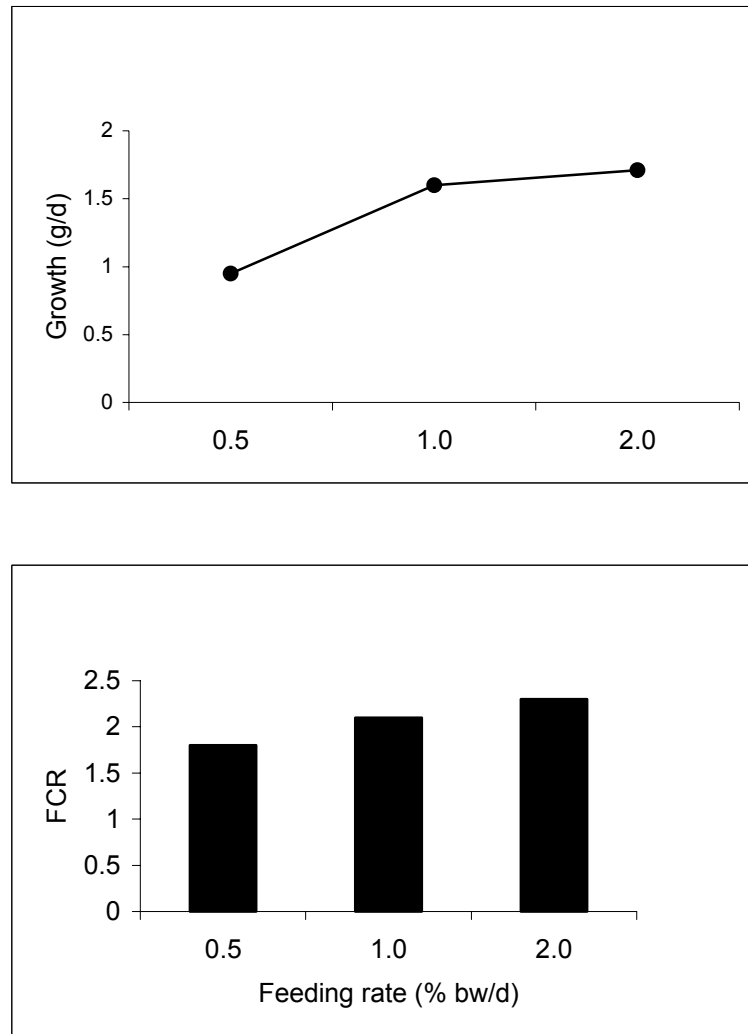
**Figure 3.** Growth and FCR of silver perch fingerlings (initial mean weight, 14.9 g) fed 2.5%, 5.0% or 7.5% body weight once (1x), twice (2x) or three times (3x) daily at a mean temperature of 23.7°C.



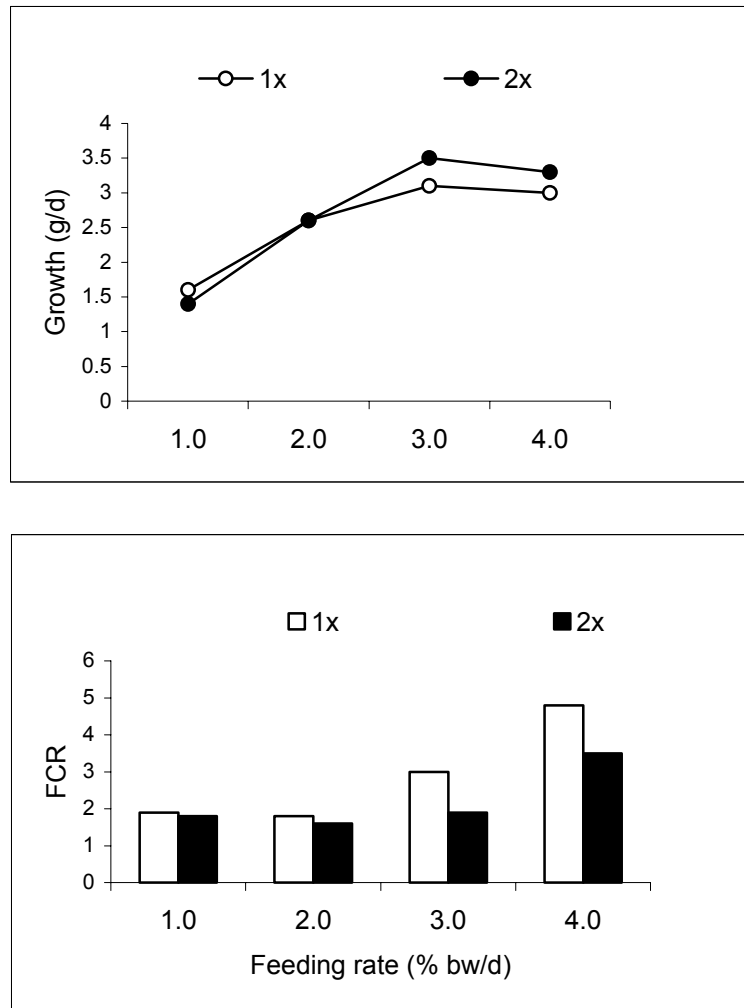
**Figure 4.** Growth and FCR of silver perch fingerlings (initial mean weight, 27.7 g) fed 5.0%, 7.5% or 10.0% body weight once (1x), twice (2x) or three times (3x) daily at a mean temperature of 7.1°C.



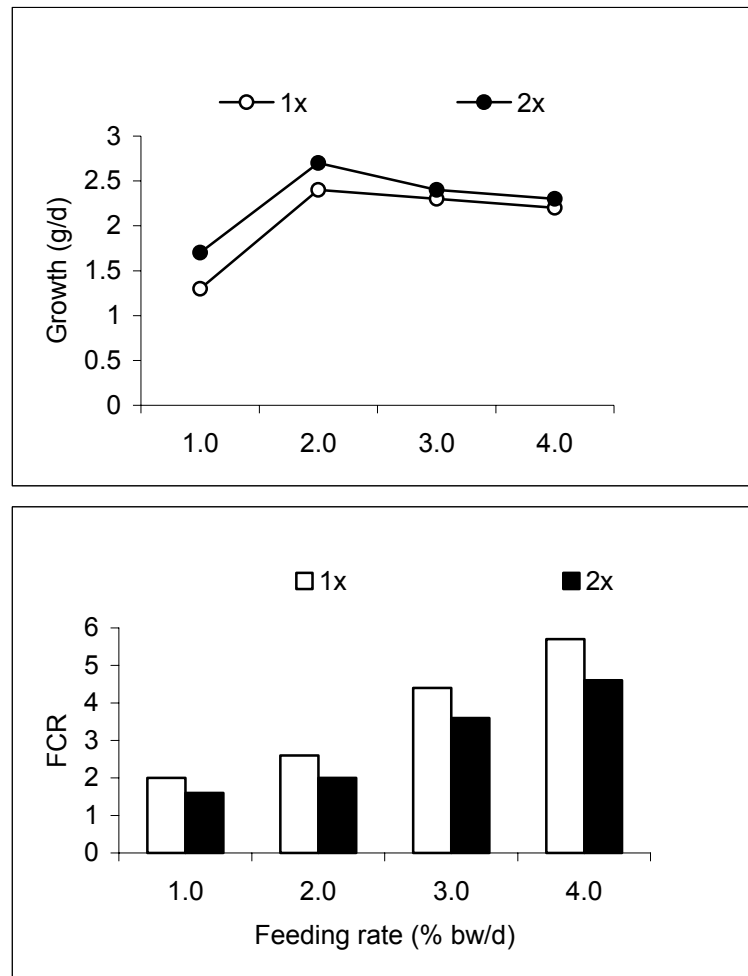
**Figure 5.** Growth and FCR of silver perch (initial mean weight, 458.6 g) fed 0.5%, 1.0% or 2.0% body weight once daily or on alternate days at a mean temperature of 15.6°C.



**Figure 6.** Growth and FCR of silver perch (initial mean weight, 510.6g) fed 0.5%, 1.0% or 2.0% body weight daily at a mean temperature of 17.3°C.



**Figure 7.** Growth and FCR of silver perch (initial mean weight, 162.5 g) fed 1.0%, 2.0%, 3.0% or 4.0% body weight once (1x) or twice (2x) daily at a mean temperature of 24.1°C.



**Figure 8.** Growth and FCR of silver perch (initial mean weight, 300.7 g) fed 1.0%, 2.0%, 3.0% or 4.0% body weight once (1x) or twice (2x) daily at a mean temperature of 27.9°C.

#### 4.9. Performance and sensory evaluation of silver perch (*Bidyanus bidyanus* Mitchell) fed soybean or meat meal-based diets in earthen ponds

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#### Abstract

Two experiments compared the performance of silver perch fed a commercially available diet (95LC2) based on meat meal (38%), grain legumes (18%), oilseeds (10%), wheat millrun (20%), fishmeal (5%) and fish oil (3%), with experimental diets based on alternative protein sources. In Experiment 1 two experimental diets contained similar contents of fishmeal and fish oil as the reference diet, but soybean (25%) and wheat millrun (> 31%) were used to reduce animal protein meals by approximately 50%. The digestible protein and digestible energy of the two experimental diets was either slightly lower (31.5% and 12.8 MJ kg<sup>-1</sup>) or slightly higher (34.9% and 14.3 MJ kg<sup>-1</sup>) than the reference diet (32.1% and 13.2 MJ kg<sup>-1</sup>). In Experiment 2 the two experimental diets contained no fishmeal but included increased contents of rendered animal meals (41-48%). One of the diets had similar digestible protein to the reference diet (32%) while the other had only 25% digestible protein. Fish were stocked at commercially relevant densities and cultured to a market size of greater than 400 g in 0.1 ha earthen ponds at the Grafton Aquaculture Centre. 1500 silver perch (38 g for Experiment 1 and 59 g for Experiment 2), were stocked into each of 9 ponds with fish in three ponds fed each diet for 191 d (Experiment 1) or 187 d (Experiment 2). Survival was > 94% in all ponds and growth rates and food conversion ratios ranged from 2.1-2.4 g fish<sup>-1</sup> day<sup>-1</sup> and 1.7-1.9 respectively for Experiment 1, and 2.3-2.4 g fish<sup>-1</sup> day<sup>-1</sup> and 1.6-1.7 g fish<sup>-1</sup> day<sup>-1</sup> for Experiment 2. There were no significant differences in fish performance indices for any of the three diets in either experiment. A blind consumer sensory evaluation (taste panel) of fish fed the three diets in Experiment 2 rated fish as "highly acceptable". The diet with the lowest digestible protein content produced the best fish in terms of "smell liking", "flavour liking" "muddy flavour strength" and "fresh flavour strength". These results confirm that soybean meal and/or rendered animal protein ingredients including meat meal and poultry offal meal, and wheat can form the basis for high-performance, low-cost diets for intensive pond culture of silver perch.

#### Introduction

Silver perch (*Bidyanus bidyanus*) are being farmed in increasing quantities in Australia. They are omnivorous and readily accept pelleted diets, tolerate crowded conditions and perform well in earthen ponds (Rowland, Allan, Clark, Hollis & Pontifex 1994; 1995). Continued development depends on the development of cost-effective diets. The major constraints in formulating cost-effective diets are a lack of information on nutritional requirements of fish and the suitability of available feed ingredients (Tacon 1998; McGoogan & Reigh 1996).

Allan & Rowland (1992) formulated an experimental diet for silver perch (SP35) that was also used by commercial farmers. This diet produced fast growth (> 2 g fish<sup>-1</sup> day<sup>-1</sup>) and high production (~10 t<sup>-1</sup> ha<sup>-1</sup> yr<sup>-1</sup>) in large-scale farming experiments (Rowland *et al.* 1994; 1995; Rowland 1995), but was based on expensive, imported fishmeal and soybean meal. Additional nutritional research with silver perch has evaluated a large number of Australian agricultural proteins for use in formulated diets. This evaluation has included the determination of digestibility coefficients for dry



matter, energy, nitrogen, amino acids and phosphorus for approximately 60 ingredients, including some processed in different ways (Allan, Parkinson, Frances, Stone, Booth, Rowland & Warner-Smith 2000a). Growth studies to determine maximum inclusion levels for several protein sources considered to have high potential to replace fishmeal have also been completed (Stone, Allan, Parkinson & Rowland 2000). This research culminated in the formulation of new “least-cost” diets based on Australian agricultural ingredients and the success of one of these diets (95LC2) (Allan, Rowland, Mifsud, Glendenning & Ford 2000b) has led to the commercial manufacture and wide availability to farmers. However, 95LC2 still contains 5% fishmeal and relies on lupins and other grain legumes that are difficult for some feed manufacturers to obtain cheaply in eastern Australia where most of the silver perch are cultured.

We ran two experiments to compare the performance of silver perch on diets with alternative protein sources. In Experiment 1, silver perch were fed 95LC2 or one of two diets with 5% fishmeal, but with most of the protein sourced from widely available soybean meal. In Experiment 2, silver perch were fed 95LC2 or one of two diets without fishmeal, containing ingredients selected on a least-cost basis. To ensure that data had direct commercial application, the diets were assessed in large-scale pond trials with fish stocked at commercially-relevant densities and cultured to a market size of greater than 400 g per fish over a 5-6 month grow-out period.

Positive consumer acceptance of culture product is a key goal and replacement of fishmeal with alternative protein sources in silver perch diets will not be successful if the taste of fish is unacceptable to consumers. We conducted a blind sensory evaluation of fish fed the different diets in Experiment 2 to see if consumers could taste a difference in the fish and if so, on what sensory attributes they differed. The evaluation was also designed to identify those attributes which determine consumer acceptance of cooked silver perch.

## **Materials and Methods**

### *Experimental Diets*

In each experiment, fish were fed one of three diets, including 95LC2 as a “control” diet. The ingredient and nutrient composition of experimental diets is given in Tables 1, 2 & 3. The minor differences in ingredient composition for 95LC2 in Experiment 1 and Experiment 2 were due to slight differences in the protein content of the different batches of ingredients used to make the diet. The test diets were formulated using the linear least-cost computer program ‘Feedmania’ (Mania Software, Brisbane, Australia). All diets contained the same vitamin and mineral premixes. With least-cost diet formulation, nutrient concentrations and ingredient contents are specified (minimum and/or maximum levels or unrestricted) and then the cheapest mix of ingredients to supply the specified nutrients are selected.

### *Experiment 1*

For diets E1A and E1B, soybean content was set at approximately 25%. For E1A, similar nutrient specifications to those of 95LC2 were used for digestible energy, digestible crude protein, digestible phosphorus, digestible essential amino acids and linolenic series fatty acids (these nutrients were not allowed to fall more than 5% less than contents in 95LC2). For E1B, nutrient contents were allowed to fall by up to 10% compared with contents in 95LC2.

The diets were manufactured by a commercial feed manufacturer and the diets were ground to  $\leq 800 \mu\text{m}$  particle size and extruded to give 3 or 6 mm diameter sinking pellets.

### Experiment 2

For E2A, similar nutrient specifications to 95LC2 were used for digestible energy, digestible crude protein, digestible phosphorus, digestible essential amino acids and linolenic series fatty acids (these nutrients were not allowed to fall more than 5% less than contents in 95LC2). For E2B, digestible energy and linolenic series fatty acids were also specified to be within 5% of those for 95LC2 but for digestible protein a minimum content of 25% was specified following results from Allan, Johnson, Stone & Booth (2001). Peanut meal and feather meal were restricted to 5% in E2A and E2B. 95LC2 contained 5% fishmeal, 37% meat meal and 18% legumes. E2A and E2B contained approximately 10% poultry offal meal and 37 and 29% high quality meat and bone meal respectively. All diets also contained wheat and/or wheat by-products and other supplements, including fish oil, vitamins and minerals.

E2 diets were manufactured by Select Nutrition Pty Ltd (Windsor, NSW, Australia). The diets were ground to  $\leq 800 \mu\text{m}$  particle size and extruded to give 3 or 6 mm diameter sinking pellets.

### Experimental Fish

Silver perch were artificially bred at the Grafton Aquaculture Centre (GAC) and the fingerlings raised in earthen ponds using techniques described by Rowland (1995) and Thurstan & Rowland (1995). Before the experiment, fingerlings were fed 95LC2 and treated with  $5 \text{ g l}^{-1}$  NaCl for five days to ensure they were free of ectoparasites and to prevent fungal infection (Rowland & Ingram 1991). Immediately prior to stocking, fish were anaesthetised using ethyl  $p$ -aminobenzoate ( $20 \text{ mg L}^{-1}$ ), weighed and distributed among nine ponds by systematic interspersation. A total of 1500 silver perch (Experiment 1 mean weight 38.4 g; range pond means 30.2-42.2 g; Experiment 2 mean weight 57.7 g; range 55.9-60.2 g) were stocked into each 0.1 ha earthen pond with three replicate ponds for each diet.

### Experimental Facilities and Procedures

Experimental earthen ponds were 0.1 ha with a maximum depth of 2 m. The ponds were aerated using a 1-hp paddlewheel aerator for at least 8 h a day, between 0000 and 0800 h. The ponds were static and water was added every four to five weeks to account for evaporative loss and seepage. During Experiment 2, up to 50% of the water in each pond was exchanged during March to alleviate poor water quality (low dissolved oxygen) following the treatment of all ponds with 30 or 40 mg/L formalin to treat infestations of monogenean ectoparasitic *Lepidotrema bidyana*.

The fish were cultured for 191 d (Experiment 1) or 187 d (Experiment 2) from late November (Summer) through to June (Winter). Fish were fed up to 4% body weight  $\text{d}^{-1}$ . Daily rations were divided evenly and fed twice daily by hand at 0800 h and 1500 h, six days a week. Approximately 100 fish  $\text{pond}^{-1}$  were sampled monthly, the mean weight determined, the biomass estimated and the ration adjusted accordingly. Performance was evaluated by survival, mean weight, growth rate and production rate. Food conversion ratio (FCR), production per unit pond area and ingredient cost per unit of fish produced were estimated based on food inputs and survival rates.

Water quality in each pond was monitored twice daily (0800 and 1500 h) at least three days a week using methods described in (Rowland 1995).

### Sensory Evaluation

At the completion of Experiment 2, the silver perch were purged for two weeks in tanks supplied with domestic water to eliminate off-flavours. Fifteen fish from each pond were filleted and transported on ice to Randwick (Sydney) prior to analysis. The fish were then kept in a domestic freezer at  $-20^\circ \text{C}$  until needed. The fish were cooked and evaluated under systematic conditions of

blind sensory evaluation, following Australian best practices, and conducted by the University of NSW, Centre for Chemosensory Research, Randwick, NSW, Australia).

The evaluation panel consisted of 20 female and 25 male consumers of fish, aged 18 to 45 years old. None of the subjects had been in a similar product evaluation for at least three months. At the end of the session, each subject received a cash gratuity and refreshments.

With the evaluation of each sample of fish, a questionnaire was completed by each panellist. The questionnaire items consisted of 21 unstructured line scales with end anchors. The 22nd item on each sensory questionnaire was an open ended "comments" item. The panellist was required to complete the sensory questionnaire in a set sequence, from attributes of visual appearance, through smell, taste, texture, after-taste and overall liking of the product. The magnitude of the rating was obtained by measuring from the left-hand extremity of the scale to the mark made by the subject then calculating the fraction of that distance proportional to the length of the whole line. This is then expressed as a number with a maximum of 100.

The fish were defrosted for 2 hours at room temperature, in batches of three fillets, then placed in a convection oven preheated to 200°C for 7 minutes. The samples were served, skin-side down, one fillet per serve. The cooked fish were presented in a modified "booth" environment, minimising communication between subjects during evaluation of products. The order of presentation was randomised to counterbalance order effects. All samples were presented "blind" and were identified by three digit codes. Each sample was served one at a time (monadic sequentially). Three to five minutes passed between each serving and the end of the previously evaluated sample. This gave the subjects time to sip water and refresh their palates.

#### Statistical Analysis

Performance data were analysed using single-factor ANOVA. Homogeneity of variance was assessed using Cochran's Test. Sensory evaluation data were analysed using ANOVA, Fisher's Least Significant Difference, a matrix of Pearson's correlation coefficients and Biplots of principal components. These procedures determined whether there were significant differences between the attributes for various products and those attributes that had the most influence on the consumers' assessment of the overall acceptability of each product.

## **Results**

### Experiment 1

Growth of fish is presented in Figure 1 and final weight (g/fish), survival (%), absolute growth rate (g fish<sup>-1</sup>day<sup>-1</sup>), production rate (t/ha) and food conversion rate (FCR) in Table 5. There were no significant differences ( $P > 0.05$ ) in any of the performance indices measured. Water quality was good in all ponds throughout the experiment, and no values reached known stressful or lethal levels. Mean monthly water temperatures ranged from 28.1°C in February (maximum 31.8°C) to 18.0°C in May, and pH values from 7.6 to 8.7. The lowest dissolved oxygen concentration (DO) recorded was 3.7 mg L<sup>-1</sup>, and values usually exceeded 4 mg/L each morning. Total ammonia-nitrogen (TAN) ranged from 0.1 to 2.1 mg L<sup>-1</sup>, except in May when values exceeded 3 mg L<sup>-1</sup> in most ponds as blooms of phytoplankton crashed.

### Experiment 2

Results for overall growth over time are presented in Figure 2. Fish production data are presented in Table 6. There were no significant differences ( $P > 0.05$ ) in any of the performance indices measured.

Water quality was high in all ponds throughout the experiment, except for a three day period during March. Mean monthly water temperatures ranged from 28.2°C in January to 18.7°C in May, and pH values from 7.3 to 8.4. DO usually exceeded 5 mg/L. TAN ranged from 0.3 to 1.5 across all ponds between November and April, but in May TAN reached 2.1 to 3.5 mg/L as phytoplankton blooms died off naturally at the end of autumn.

In March, water quality deteriorated significantly following the application of 30 or 40 mg/L formalin to each pond to treat an outbreak of the parasitic monogenean trematode, *Lepidotrema bidyana*. Although the disease was effectively treated, there was a rapid decrease of DO 36-40 h post-treatment, with concentrations in four ponds falling below 2 mg/L (minimum of 1.2 mg/L). The pH decreased in all ponds (to a minimum of 6.3), and TAN rose to 1.5 mg/L or higher in all but one pond.

Increased aeration and water exchange were used to manage the deteriorating water quality, and while silver perch in several ponds showed signs of severe stress, no fish were lost during this episode or over the duration of the experiment.

### Sensory Evaluation

The mean score on the overall liking of the product is taken as the overall acceptance of the product, and is shown in Table 7. The three products did not differ significantly from each other on overall acceptance as shown in the ANOVA. However, the sensory profiles showed five items on which they were differentiated, and examination of these indicates that at least one of the treatments produces a sensory profile that contains several negative attributes. This product (95LC2) also has the lowest acceptance score overall.

The overall acceptability of the products was above 50 (out of 100) for 95LC2 and above 60 for the other two diet treatments etc. While cooked fish products are unlikely to ever score in the 80s or 90s (unlike candy and deserts), these results should be regarded as indicative of a favourable product (Bell 2000; personal communication, Centre for Chemosensory Research, University of NSW, Sydney).

We assume that co-variance between two attributes implies that one "drives" or determines the other (and vice versa), however, this is done in the knowledge that other influences may cause the co-variance.

The correlational analyses identified seven attributes which significantly determine overall acceptance of the products (Table 8). With the exception of Mud Flavour Strength, six of these correlations were positive, i.e. the greater the attribute rating, the greater the overall acceptance can be expected. In the exceptional case, the higher the mud flavour strength, the less the sample of fish was liked overall.

The sensory attributes determining the acceptance of the fish are shown in Table 9.

Figure 3 shows the closeness of each product to the main attributes scores, to each other, and to the other attributes. Fish fed E2A and E2B lie about equidistant from attribute 21 (overall liking) however E2B is more closely related to the determinants of Fresh Flavour Strength, Flesh Colour Liking and Smell Liking (attributes 14, 1 and 5 respectively). E2A has closer association with Aftertaste Liking (attribute 20) while 95LC2 is closely related to the negative driver Muddy Flavour Strength (attribute 9).

Figure 3 confirms the other statistical analysis in this study that the least risk for an acceptable product lies with E2B, the next best is E2A and the product to be avoided is 95LC2.

## Discussion

### Pond trials

Mean daily growth rates of between 2.0 and 2.4 g fish<sup>-1</sup> day<sup>-1</sup> of silver perch fed 95LC2 or one of the four experimental diets during the two experiments was very similar to growth rates (2.0 - 2.3 g fish<sup>-1</sup> day<sup>-1</sup>) achieved using 95LC2 in a previous study by Allan, Rowland, Mifsud, Glendenning & Ford (2000b). In both experiments, there were no sign of differences ( $P > 0.05$ ) in performance of fish between diets, and, when the cost of supplying the ingredients for each diet was calculated (cost of ingredients x FCR), the costs were similar for all diets. The ingredient cost of less than AU\$1 per kg fish (Table 6) is the lowest yet recorded for silver perch. Cost of feeding silver perch on an original diet containing 27% fish oil was around AU\$1 (Rowland *et al.* 1995). Ingredient prices in the current study were obtained from published values or directly from ingredient suppliers or feed manufacturers in 2001/02. They do not include freight charges, although clearly all feed mills will have to pay costs of freight for some ingredients.

During this study, Australian agriculture products, soybean meal and rendered animal meals were successfully used to replace fishmeal in silver perch diets. Results with E1A and E1B show that soybean meal can be used to replace approximately half of the rendered animal meals in the reference diet without compromising performance, although it should be noted that there was a non-significant trend for slower growth on E1A and E1B diets (Figure 1). Soybean meal is probably the most widely used vegetable protein source and has been used successfully in diets for channel catfish (Robinson & Li 2002), rainbow trout (Kaushik, Cravedi, Lalles, Sumpter, Fauconneau & Laroche 1995), Atlantic salmon (Refstie, Storebakken, Baeverfjord & Roem 2001) and seabream (Robiana, Izquierdo, Moyano, Socorro, Vergara, Montero & Fernandez-Palacios 1995). However reductions in performance have also been reported, including for some of the species listed above. Negative effects have included reduced feed intake, digestibility and nutrient absorption (Kissil Lupatsch, Higgs & Hardy 2000; Refstie *et al.* 2001; Ollie & Krogdahl 1995). These reductions have been linked with soybean anti-nutrients such as trypsin inhibitors, agglutinating lectins, isoflavones, antigenic storage protein, phytic acid and unidentified factors causing "distal enteritis" in salmon (Refstie *et al.* 2001).

Current research by the authors has shown that extrusion significantly improved digestibility coefficients of dry matter and energy with silver perch by between 7 - 12 percentage points for soybean products (Allan *et al.*, submitted). Satoh, Higgs, Dosanjh, Hardy, Eales & Deacon (1998) also reported beneficial effects of extrusion for soybean meal and attributed the benefits to improved digestibility and reduced phytic acid content. The current research indicates that the improvements with extrusion recorded in digestibility trials are applicable to larger scale trials with fish cultured to market size.

The excellent results with diets E2A and E2B demonstrate the potential of meat meal, poultry offal meal and wheat to be used as the basis of high performance, low-cost commercial diets for silver perch and confirms the potential shown in earlier digestibility and growth studies (Stone *et al.* 2000).

Other studies have also shown that meat meal, and meat and bone meal can be successfully used to partially replace fishmeal in diets of many fishes, including barramundi, sea bream, tilapia, yellowtail, channel catfish and rainbow trout (Davies, Williamson, Robinson & Bateson 1990; Mohsen & Lovell 1990; Davies, Nengas & Alexis 1991; Aquacop, Orenge, Cuzon & Thouard 1993; Shimeno, Masumoto, Jujita, Mima & Ueno 1993a; Shimeno, Mima, Imanaga & Tomaru 1993b; Watanabe, Pngmaneerat, Sato & Takeuchi 1993; Williams, Allan, Smith & Barlow 1997). In general, meat meal and meat and bone meal have been used to increase diet attractiveness and or

palatability (Mohsen & Lovell 1990; Watanabe *et al.* 1993). Fish fed all diets grew rapidly and there were no observed differences in attractiveness or palatability of the diets.

The value of meat products for use in aquaculture diets will increase if protein content is increased and ash content reduced. Aquaculture diets typically contain much higher protein:energy ratios than diets for pigs or poultry (crude protein contents are usually 35 - 50% for diets compared with 15-22% for pig and poultry diets). For this reason, at least some high protein ingredients are required for aquaculture diets. Fishmeal is a preferred protein source for aquaculture diets as it has a high protein content, excellent amino acid balance, contains essential fatty acids, has no carbohydrate and, provided it is fresh and well processed, contains no anti-nutritional factors (Allan 2000).

Standard meat and bone meal is typically around 50% crude protein, > 30% ash and 10-20% fat (Allan 1994). In general, lower fat contents are desirable as saturated animal fats are undesirable in most aquaculture diets. Low ash, high protein meat meals have shown promise as protein sources to replace fishmeal in diets for rainbow trout (*Oncorhynchus mykiss*) and yellowtail (*Seriola quinqueradiata*) (Shimeno *et al.* 1993a; 1993b; Watanabe *et al.* 1993). Clearly, the value of all meat products in aquaculture diets will depend upon consistency of composition and on the absence of deleterious compounds such as hair or wool (which tend to clog up feed manufacturing equipment). Heat damage in rendering plants can also reduce digestibility of amino acids, e.g. lysine, and reduce the value of meat meal for use in aquaculture diets.

Australia is well placed to utilise agricultural products in aquafeeds. Approximately 450 000 t/y of meat meal is produced while more than 19 million t of wheat is produced.

#### Sensory Evaluation

Although the direct analysis of overall liking scores failed to differentiate the products in terms of overall consumer acceptance, the sensory profiles gave a very strong indication that the three diets are very different in the resulting perceived quality of the cooked fish and clear directions for further development on a preferential basis. E2B deserves the most attention for future commercial development as this diet produced the best product for human appreciation and market position. Silver perch fed on this diet had a sensory profile that was sound in all 21 measured items used in this study. E2A was a good diet and while it is good in terms of aftertaste, it has a couple of weaknesses (weedy and metallic flavours) which indicate it should not be used in preference to E2B. 95LC2 produced a level of acceptance that might be regarded as favourable and produced the most acceptable flesh colour of the three diets. Unfortunately, the number of weak points in its profile rated it overall as the poorest of the three diets.

#### **Conclusion**

The results of this research demonstrates that soybean meal and/or rendered animal protein ingredients including meat meal and poultry offal meal, and wheat can form the basis for high-performance, low-cost diets for intensive pond-culture of silver perch.

#### **Acknowledgments**

We would like to thank the technical staff at the Grafton Aquaculture Centre who committed so much time to the experiments. In particular, Charlie Mifsud, David Glendenning, Mark Nixon and Peter Boyd who carried out many of the daily activities involved with feeding fish. Other temporary employees or work experience students who assisted included Andy Moore, Fiona Copson, Hassarn Jordon, David Kratz and Todd Rowland. Graham Bell and Marilyn Styles at the

University of NSW, Centre for Chemo-sensory Research (Randwick, NSW), arranged the taste panel study. This research was supported by the Fisheries Research and Development Corporation Fishmeal Replacement in Aquaculture Diets Subprogram (93/120) and by the Australian Centre for International Agricultural Research (PN9207). We are grateful to Ridley Agriproducts Pty Ltd for manufacturing the diets and to Australian Native Fish for the supply of silver perch. Mark Booth and Dr Wayne O'Connor kindly commented on the manuscript and Mrs Helena Heasman prepared the manuscript for publication.

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**Table 1.** Ingredient composition of diets for silver perch in Experiment 1.

Ingredient	Diets						Ingredient Purchase Cost (AU\$/t)
	95LC2 REF E1	E1A	E1B	95LC2 REF E2	E2A	E2B	
Fishmeal	5.07	5.03	5.00	5.00	0	0	1500
Fish oil	3.74	3.80	3.75	3.21	3.21	7.62	1400
Blood meal	0	4.90	2.51	0	1.65	0	1000
Meat & bone meal	38.50	15.89	17.82	36.88	37.48	29.39	500
Poultry offal meal	0	7.51	0	0	10.25	10.58	900
Feather meal	0	0	0	0	5.00	5.00	650
Soybean meal	0	24.63	24.50	0	5.00	0	500
Canola Meal	5.23	5.18	5.16	5.00	0	0	380
Peanut meal	4.30	0	0	5.00	5.00	0	325
Lupins (dehulled gung)	9.17	0	4.84	7.36	0	0	517
Field peas (dehulled)	10.59	0	0	10.39	0	0	350
Corn gluten meal	5.39	0	0	5.19	0	0	800
Wheat	0	0	0	0	10.76	25.92	240
Mill run	16.69	31.92	35.29	20.20	20.00	20.00	200
Vitamin premix (NSWF) <sup>1</sup>	0.50	0.50	0.50	0.75	0.75	0.75	14355/9570
Mineral premix (NSWF) <sup>1</sup>	0.50	0.50	0.50	0.75	0.75	0.75	1305/870
DL Methionine	0.32	0.14	0.13	0.27	0.15	0	5120
	100.00	100.00	100.00	100.00	100.00	100.00	
<i>Composition<sup>2</sup></i>							
Crude protein	39.05	40	35.9	38.2	39.4	31.4	
Crude fat	8.5	8.4	7.5	10.3	9.3	13.3	
Ash	16.8	12.9	12.7	15.6	16	12.9	
Carbohydrate	35.7	38.7	43.9	35.9	35.3	42.4	
Gross energy	17.3	18.6	17.1	17.2	17.3	18.2	
Digestible energy	13.2	14.3	12.8	13.3	13.7	14	
Digestible protein	32.1	34.9	31.5	31.1	31.7	25	
Digestible lysine	1.9	2.2	1.9	1.9	1.9	1.5	
Digestible met + cys	1.4	1.4	1.2	1.4	1.6	1.2	
Digestible leucine	2.7	2.7	2.4	2.6	2.5	1.9	
Digestible isoleucine	1.3	1.3	1.2	1.3	1.3	1.1	
Digestible arginine	2.6	2.3	2.2	2.6	2.5	1.8	
Digestible histidine	0.8	1	0.9	0.8	0.8	0.6	
Digestible phenyl.+ tryo.	2.6	2.7	2.4	2.5	2.5	1.9	
Digestible valine	1.6	1.7	1.5	1.5	1.7	1.4	
Digestible threonine	1.3	1.4	1.2	1.3	1.4	1.1	
Digestible phosphorus	0.8	0.6	0.6	0.8	0.7	0.6	

<sup>1</sup> First value is premix for E1 second value is premix for E2. For E1, the premix contained some active ingredients as that for E3 but was more concentrated.

<sup>2</sup> Composition calculated from previous analyses (corrected for measurement of protein of current ingredients) and measured digestibility coefficients (Allan *et al.* 2001).

**Table 2.** Analysed dry matter and protein content of ingredients used in Experiments 1 & 2.

Ingredients	Experiment 1		Experiment 2	
	Dry matter	Protein	Dry matter	Protein
Fishmeal	95.3	67.7	91.3	71.4
Fish oil	100.0	0	100.0	0
Blood meal	95.1	84.3	96.7	88.8
Meat & bone meal	97.2	52.8	95.2	55.0
Poultry offal meal	94.4	56.9	95.4	68.3
Feather meal	87.6	73.9		
Soybean meal	89.1	45.0	87.7	46.2
Canola Meal	95.4	30.4	88.6	38.2
Peanut meal	94.8	39.0	93.2	48.4
Lupins (dehulled gung)	95.0	41.4	91.2	35.7
Field peas (dehulled)	94.4	26.2	90.3	25.7
Corn gluten meal	92.9	57.6	95.1	62.9
Wheat	91.7	11.2		
Mill run	87.6	16.4	86.7	17.4
Vitamin premix (NSWF)	100.0	0	100.0	0
Mineral premix (NSWF)	100.0	0	100.0	0
DL Methionine	100.0	50.0	100.0	50.0

**Table 3.** Composition of vitamin premix<sup>1</sup>.

Raw Material	iu or mg kg <sup>-1</sup> diet	Active ingredient	iu or mg kg <sup>-1</sup> diet
<i>Fat soluble vitamins:</i>			
Vitamin A 500	16 000 iu	Vitamin A	8 000 iu
Vitamin D3 500	2 000 iu	Vitamin D3	1 000 iu
Vitamin E 50 Adsorbate	250.0	Vitamin E	125.0
Hetrazeen FC (22.75 Menadione)	72.53	Vitamin K3	16.5
<i>Water soluble vitamins:</i>			
Thiamine Hcl 89.3% (B1)	11.73	Thiamine (B1)	10.0
Riboflavin S/D 80% (B2)	31.88	Riboflavin (B2)	25.5
Pyridoxine Hcl 81% (B6)	18.51	Pyridoxine (B6)	15.0
Cal D Pantothenate	61.00	D Pantothenate	55.0
Biotin 2%	50.00	Biotin	1.0
Niacin (Nicotinamide)	201.01	Niacin	200.0
Folic acid 80% S/D	5.00	Folic acid	4.0
Vitamin B12 1%	2.00	Vitamin B12	0.02
Vitamin C Stay C 25% (ascorbyl-2-polyphosphate)	1 000.00	Vitamin C (ascorbic acid activity)	250.0
Choline chloride 60%	2 500.00	Choline chloride	1 500.0
Inositol	603.86	Inositol	600.0
Mould inhibitor (Myocurb)	200.00	Mould inhibitor	200.0
Ethoxyquin 66% (antioxidant)	303.03	Ethoxyquin	200.0

<sup>1</sup> Vitamin premix was more concentrated for Experiment 1 than Experiment 2 with inclusion contents of 0.5 and 0.75% respectively. The active ingredients for all vitamins was the same for both experiments.

**Table 4.** Composition of mineral premix<sup>1</sup>.

Raw Material	Mg kg <sup>-1</sup> diet	Active Ingredient	Mg kg <sup>-1</sup> diet
Potassium iodide 68% I	0.684	I	0.465
Copper sulphate 30% Cu	12.00	Cu	3.00
Ferrous sulphate 30% Fe	100.00	Fe	30.00
Magnesium sulphate 10% Mg	5096.84	Mg	500.00
Manganese sulphate 36% Mn	27.78	Mn	10.00
Sodium selenite (diluted to 1%) Se	33.00	Se	0.33
Zinc sulphide 36% Zn	277.78	Zn	100.00
Filler (wheat bran)	<u>1951.96</u>		
	7500.00		

<sup>1</sup> Mineral premix was more concentrated for Experiment 1 than Experiment 2 with inclusion contents of 0.5 and 0.75% respectively. The active ingredients for all minerals was the same for both experiments.

**Table 5.** Experiment 1 Final weight, survival, growth rate, food conversion ratio, production rate and diet ingredient cost for silver perch (stocked at 38.4 g fish<sup>-1</sup>) and fed one of three diets for 191 days<sup>1,2</sup>.

Diet	Final wt. (g/)	Survival (%)	Growth Rate (g fish <sup>-1</sup> day <sup>-1</sup> )	FCR	Production (t ha <sup>-1</sup> )	Ingredient cost (AUS\$ kg fish <sup>-1</sup> )
95LC2	504.2±12.5	95.1±0.7	2.4±0.1	1.8±0.1	7.2±0.2	1.10
E1A	471.6±11.2	96.5±1.01	2.3±0.1	1.7±0.1	6.8±0.1	1.17
E1B	433.9±10.1	94.8±0.4	2.1±0.1	1.9±0.1	6.2±0.1	0.96

<sup>1</sup> Values are means ± SEM for 3 replicate ponds. Means in columns which share the same superscript were not significantly different ( $P > 0.05$ ; ANOVA).

<sup>2</sup> Ingredient cost based per kg fish based on total ingredient cost multiplied by FCR. Ingredient prices were from published values or feed manufacturers (high quality fishmeal).

**Table 6.** Experiment 2. Final weight, growth rate, food conversion ratio, production rate and diet ingredient cost for silver perch (stocked at 57.7 g fish<sup>-1</sup>) fed one of three diets for 187 days<sup>1</sup>.

Diet	Final Weight (g)	Survival (%)	Growth Rate (g fish <sup>-1</sup> day <sup>-1</sup> )	FCR <sup>2</sup>	Production <sup>2</sup> (t ha <sup>-1</sup> )	Ingredient Cost <sup>2</sup> (AUS\$ kg fish <sup>-1</sup> )
95LC2	461.3±9.5 <sup>a</sup>	91.8±0.4	2.1 ± 0.05 <sup>a</sup>	1.6±0.08	6.4 ± 0.15 <sup>a</sup>	0.94
E2A	453.3±6.0 <sup>a</sup>	94.0±0.4	2.1 ± 0.03 <sup>a</sup>	1.7±0	6.3 ± 0.03 <sup>a</sup>	0.96
E2B	432.6±6.8 <sup>a</sup>	93.3±1.1	2.0 ± 0.05 <sup>a</sup>	1.7±0	6.2 ± 0.08 <sup>a</sup>	0.96

<sup>1</sup> Values are means ± SEM for 3 replicate ponds. Means in columns which share the same superscript were not significantly different ( $P > 0.05$ ; ANOVA).

<sup>2</sup> Ingredient cost based per kg fish based on total ingredient cost multiplied by FCR. Ingredient prices were from published values or feed manufacturers (high quality fishmeal) in 2001/02.

**Table 7.** Mean attribute ratings for 3 fish samples.

Attribute (LSD)	95LC2	E2A	E2B
Flesh colour liking*	52.78	51.60	52.18
Colour liking jr	51.71	53.42	52.27
<u>Overall appearance liking</u>	50.20	50.44	50.22
Smell strength (7.8)	50.67	43.89	42.44
Smell liking	52.07	55.24	58.98
Fresh smell liking	54.22	53.53	55.89
Flavour liking	52.31	58.58	59.22
Fish flavour strength	49.36	45.02	45.07
* <u>Muddy flavour strength</u>	42.69	35.44	34.91
Sweet flavour strength	32.64	38.16	37.16
Meaty flavour strength	45.20	48.60	51.89
* Weedy flavour strength (6.6)	35.18	31.31	27.93
* metallic taste strength (7.4)	34.04	28.58	25.67
Fresh flavour strength	52.96	55.93	57.60
Soft-hard	35.11	39.44	38.02
Dry-moist	55.58	58.64	57.91
* stringy	31.20	29.76	30.53
Smooth-rough	38.58	39.67	37.64
Aftertaste strength (8.4)	54.13	42.87	44.40
<u>Aftertaste liking (7.8)</u>	50.84	60.40	59.13
Overall liking	54.91	62.60	62.02

Attributes that are underlined are significant drivers of overall liking.

\* these correlate negatively with overall liking and aftertaste liking.

**Table 8.** Significant Pearson correlations for fish ( $r > 0.292$   $r < -0.292$ ).

	flesh colour liking	colour liking jr	overall appearance liking	smell strength	smell liking	fresh smell liking	flavour liking	fish flavour strength	muddy flavour strength	sweet flavour strength	meaty flavour strength	weedy flavour strength	metallic taste strength	fresh flavour strength	soft-hard	dry-moist	stringy	smooth-rough	aftertaste strength	
colour liking jr	0.319																			
overall appearance	0.646	0.387																		
smell strength																				
smell liking	0.295		0.379																	
fresh smell liking			0.364		0.493															
flavour liking	0.326				0.272															
fish flavour strength				0.289																
muddy flavour strength				0.388			-0.368													
sweet flavour																				
meaty flavour strength				0.245					0.293											
weedy flavour strength									0.541											
metallic taste strength						-0.353			0.398			0.363								
fresh flavour strength						0.443	0.494						-0.308							
soft-hard																				
dry-moist															-0.315					
stringy												0.311								
smooth-rough									0.318						0.358	-0.477	0.458			
aftertaste								0.412	0.417											
aftertaste liking	0.354		0.332		0.455	0.305	0.639		-0.330			-0.306	-0.300	0.486					-0.300	
<b>overall liking</b>	<b>0.400</b>		<b>0.343</b>		<b>0.376</b>		<b>0.715</b>		<b>-0.349</b>					<b>0.486</b>						

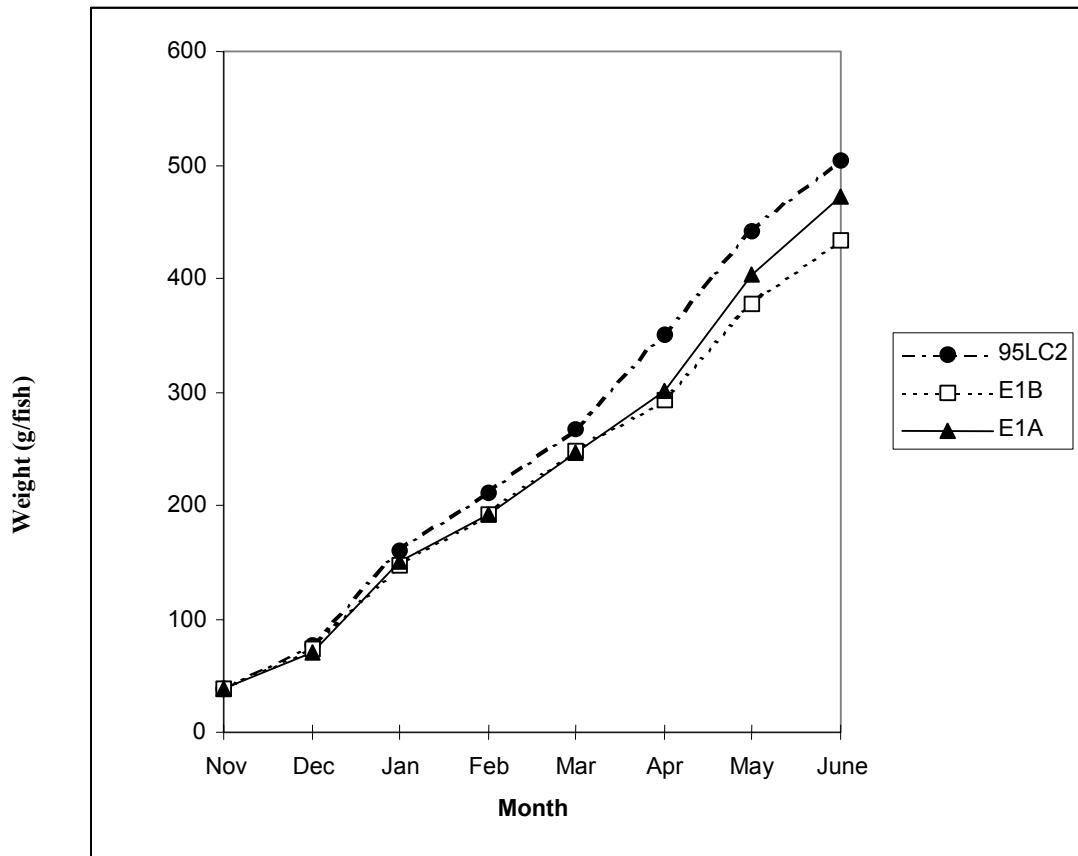
**Table 9.** Main sensory attributes driving the acceptance of the fish.

Attributes	highest scoring fish
Flesh Colour Liking	95LC22
Overall Appearance Liking	E2A
Smell Liking	E2B
Flavour Liking	E2B
Mud Flavour Strength <sup>1,2</sup>	E2B (lowest)
Fresh Flavour Strength	E2B
Aftertaste Liking	E2A

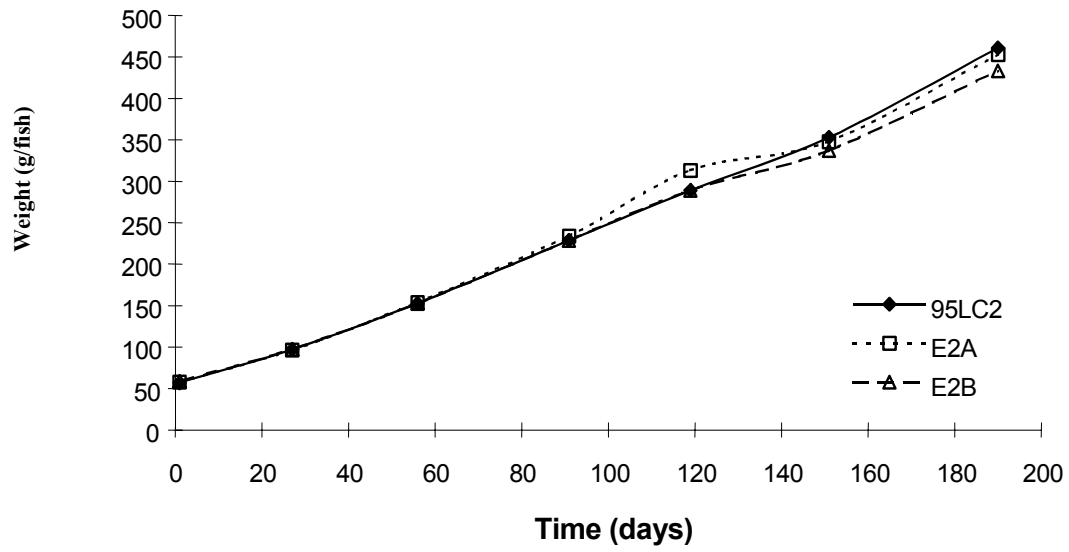
<sup>1</sup>This correlates negatively with acceptance, so E2B is best placed.

<sup>2</sup>These attributes differentiate the three fish and drives Overall Liking.

They are therefore key predictors of whether the fish will gain consumer acceptance.

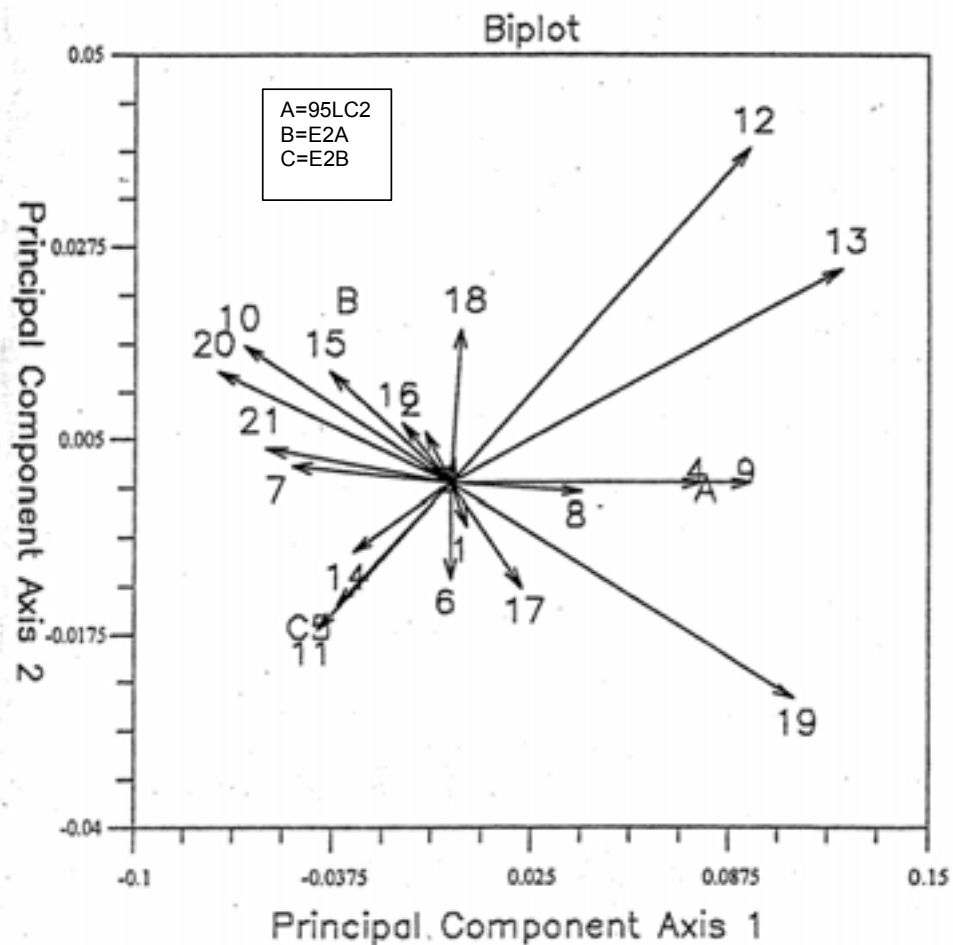


**Figure 1.** Growth of silver perch in 0.1 ha ponds. Values are means (n = 3). There were no significant differences between weights of fish fed different diets at any sampling period ( $P > 0.05$ ; ANOVA).



**Figure 2.** Growth of silver perch in 0.1 ha ponds. Values are means ( $N = 3$ ). There were no significant differences between weights of fish fed different diets at any sampling record ( $P > 0.05$ ).





**Figure 3.** Showing the closeness of each product to the main attributes scores, to each other, and to the other attributes.

#### Main attributes

- |                               |                              |
|-------------------------------|------------------------------|
| 1 – flesh colour liking       | 12 – weedy flavour strength  |
| 2 – colour liking             | 13 – metallic taste strength |
| 3 – overall appearance liking | 14 – fresh flavour strength  |
| 4 – smell strength            | 15 – soft-hard               |
| 5 – smell liking              | 16 – dry-moist               |
| 6 – fresh smell liking        | 17 – stringy                 |
| 7 – flavour liking            | 18 – smooth-rough            |
| 8 – fish flavour strength     | 19 – aftertaste strength     |
| 9 – muddy flavour strength    | 20 – aftertaste liking       |
| 10 – sweet flavour strength   | 21 – overall liking          |
| 11 – meaty flavour strength   |                              |

## 5. BENEFITS

1. Aquaculturists will gain from better, cheaper diets for silver perch, barramundi and prawns which will reduce costs of growing this species and lead to increases in production. Silver perch diets, although still too expensive for the liking of silver perch farmers, are the cheapest pellets and diets for any species of fish or prawn found in Australia. Production of silver perch is based almost entirely on diets developed during this Subprogram (or its precursor the Replacement of Fishmeal in Aquaculture Diets Subprogram) and has increased from less than 50 t in 1995/96 to over 310 t in 2000/01. (Predictions for 2002/03 are over 400 t). (Stuart Rowland, NSW Fisheries, Grafton Aquaculture Centre, September 2002, personal communication). Diets for barramundi, although more expensive are more cost effective. Production of barramundi has increased from 555 t in 1996/97 to 898 t in 2000/01 (ABARE, Fisheries Statistics 2000; 2001). Prawns have increased in production from 1565 t in 1995/96 to 2819 t in 2000/01 (ABARE, Fisheries Statistics, 1997; 2001). It is difficult to attribute production increases to any single factor but improvements in diets and feeding have played a significant part, especially for silver perch and barramundi.
2. The Australian public will benefit from increased production of fish and prawns which will reduce some of the more than 80 000 t of fish and fish products imported annually (including 45 800 t of live, fresh or frozen fish or fillets). Unfortunately, despite increases in aquaculture production, imports of edible fisheries products have increased from 112 706 t in 1995/96 to 144 407 t in 2000/01 (ABARE, Fisheries Statistics 1997; 2001).
3. Agriculture producers will benefit from increased marketing opportunities for Australian agriculture products. This includes the rapidly increasing domestic aquaculture feed market which has nearly doubled in the last two years (1991/92 – 1993/94) and the rapidly increasing Asian aquaculture feed market predicted to reach 2.6 million tonnes in 2000. Statistics for use of agriculture ingredients for aquaculture feeds produced domestically or exported for feeds produced overseas are difficult to obtain. However, thousands of tonnes of Australian ingredients, particularly lupins and meat meals are now being sold offshore, largely as a result of research conducted under this subprogram and its precursor.
4. Feed manufacturers will benefit from having more information to use in silver perch diet formulation including many more ingredients which have been evaluated and new information on nutritional requirements. Demonstrated performance of Australian produced aquaculture feeds in Australia will also lead to marketing opportunities for these diets overseas. Two major manufacturers dominate aquafeed production in Australia – Ridley's Aquafeeds and Skrettings, Australia. Both have expanded their production considerably since the inception of this subprogram. Silver perch diets are also made by Select Nutrition, a small feed manufacturer located at Windsor, NSW. This company sells over 500 t of silver perch feed annually. It did not produce any aquafeed prior to the inception of this subprogram.

## 6. FURTHER DEVELOPMENT

Further development of aquafeeds in Australia has occurred at three levels. Firstly, the commercial feed manufacturers continue to invest in new technology to increase capacity to manufacture aquafeeds for domestic aquaculture. Both Ridley's Aquafeeds and Skrettings Australia (formerly Pivot Aquaculture) invested in the new Aquafin CRC and have on-going collaborative investment in R&D. FRDC have commenced a new Aquaculture Nutrition Subprogram. An expert working group was convened and based on the national significances (benefit, multi-species significance, multiple knowledge inputs required and limiting development and/or viability of aquaculture industries), the following 10 research priorities were identified (the highest priorities are indicated in bold):

### Nutrition Research Tools

- *Capacity to accurately measure feed intake in aquaculture species.*
- *Development of energetic and production models for simulating the responses of aquaculture species to nutritional inputs.*
- *Availability of nutrition research infrastructure and the nutrition research capacity of research providers in and out of Australia.*

### Aquatic Feed Processing

- *Basal larval, broodstock and production diets for existing and emerging species.*
- *Interaction between ingredient source and type and extrusion processing parameters.*

### Feed Evaluation

- *Alternative sources of protein and lipids capable of sustaining aquatic animals exclusive of fresh bait or trash fish, fishmeals and fish oils.*

### Nutrition and Aquatic Animal Health

- *Interaction between nutrition and aquatic animal health.*
- *Nutritional prevention of disease and maintenance of animal health.*

### Nutrition and the Environment

- *Influence of feed form, feeding strategies and feeding regime on the surrounding aquatic environment.*

### Nutrition and Product Quality

- *Influence of manufactured aquatic feeds on aquaculture product quality and food safety hazards.*

## 7. CONCLUSION

The major findings of this study were:

1. Fishmeal can be completely replaced in practical, high-performing diets for silver perch using inexpensive meat and bone meal and wheat.
2. Most of the fishmeal can be replaced in high-performance, nutrient-dense diets for barramundi (using meat and bone meal).
3. Taste panel studies with silver perch and barramundi confirm that provided fish oil is added to diets, completely replacing fishmeal in formulated diets need not negatively affect the taste.
4. A substantial proportion of fishmeal can be replaced in diets for black tiger prawns using meat and bone meal or lupins.
5. Results of long-term growth trials where fish were taken to market size (silver perch and barramundi) or over 6-8 weeks (prawns) in commercially relevant facilities (ponds for silver perch or cages for barramundi and prawns) validated shorter-term laboratory studies in tanks.
6. Cost-effectiveness of alternative diets for all species was demonstrated. For silver perch, diet ingredient costs have been more than halved compared with costs for initial formulations based on fishmeal and soybean meal.
7. Optimal feeding schedules (feeding frequency and feeding rates) have been determined for silver perch for different size fish grown under different temperatures.
8. Optimal feeding frequency has been determined for prawns (3 feeds per day is as good as 6 per day).
9. No evidence for growth compensation (the ability of fish to “catch up”) was recorded when the number of feeds for silver perch was reduced.
10. Research has been commercialised. Commercial silver perch diets are based on research presented in this report (and other reports of the ADD and FMR Subprogram). Although the cheapest and best diets developed so far (in terms of both fish performance and taste) have yet to be manufactured commercially, the formulation for the best diet is available to all current and potential manufacturers of silver perch diets.
11. Data for barramundi obtained during this ADD Subprogram and its precursor (The FMR Subprogram) has been used by researchers and commercial diet manufacturers to make all current commercial barramundi diets.
12. All information for prawns has been made available to the only prawn diet manufacturer in Australia (Ridley Agriproducts Pty Ltd).

## 8. STAFF

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## 9. PUBLICATIONS

These publications flow from the Aquaculture Diet Development Subprogram as well as the earlier Replacement of Fishmeal in Aquaculture Diets Subprogram and appear in all three ADD final reports.

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## **10. APPENDICES**

**10.1. Sensory Profiling of Barramundi**

**10.2. Economic Comparison of Dietary Productivity**

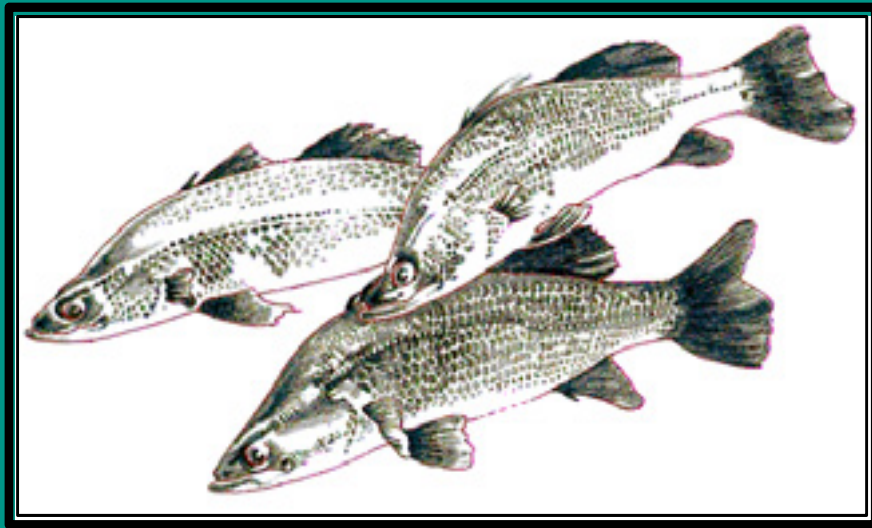
**10.3. Guidelines for Feeding Silver Perch**

## **Appendix 10.1**

### **Sensory Profiling of Barramundi**

# *Sensory Profiling*

## *of Barramundi*



*for*

*Queensland Department of Primary Industries  
Freshwater Fisheries and Aquaculture Centre*

*by*

*Anne Ford and Rob Roberts  
CENTRE FOR FOOD TECHNOLOGY*



*May 1997*

# CONTENTS

	<b>Page</b>
OBJECTIVES .....	1
SAMPLES .....	1
METHODOLOGY .....	2
PREPARATION OF FISH .....	2
COOKING .....	2
SENSORY EVALUATION .....	2
RESULTS AND DISCUSSION .....	3
TRIAL 1 SALT WATER TRIAL (BLUEWATER BARRAMUNDI) .....	4
TRIAL 2 FRESH WATER TRIAL (BARRAMUNDI WATERS) .....	6
CONCLUSIONS .....	8
APPENDIX 1 .....	8
DESCRIPTORS USED FOR PROFILING .....	9
APPENDIX 2 .....	10
COMMENTS ON BARRAMUNDI GROWN IN SALT WATER .....	11
COMMENTS ON BARRAMUNDI GROWN IN FRESH WATER .....	14

## **OBJECTIVES**

To identify the sensory characteristics of Barramundi fed three different diets and cultured under commercial farm conditions in two trials.

### **SAMPLES**

Received 17 March 1997 as frozen whole gutted fish. Fish from five cages of each diet as detailed below (30 cages in all).

Diet 1 15MJ

Diet 2 Walkamin low fat

Diet 3 Walkamin high fat

Trial 1 Salt water Trial (Bluewater Barramundi)

Trial 2 Fresh water Trial (Barramundi Waters)



# METHODOLOGY

Fish were held in a freezer at -18°C until tested in May 1997.

The fish were tasted by replications specified in the original Walkamin experimental design, as listed in Table 1. They were weight ranked within each cage based on the weights of the fish as supplied from Walkamin. Each taster received samples from fish of the same weight ranking from each treatment.

## PREPARATION OF FISH

Five fish from each of three cages (one cage from each diet) were defrosted overnight at 1°C prior to the day of tasting.

Each fish was filleted and one sample (skin off) (20 g $\pm$  1 gram) was cut from the central portion of each fillet. The samples were placed in individual foil dishes and covered with a foil lid.

## COOKING

Samples were placed on trays and cooked in a fan forced electric oven at 200°C for six minutes. They were then transferred to a holding oven at 75°C for up to 20 minutes prior to tasting.

## SENSORY EVALUATION

A total of ten tasters (six male, four female) assessed three samples (one from each dietary treatment) at each of five sessions for each trial using a standard rating test (AS 2542.2.3, 1988). One fillet from each fish was assigned to a taster. 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 and during tasting.

Tasters identified and rated the odour, colour of internal flesh, flavour and texture characteristics on unstructured 19 mm 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. Lists of descriptors used are in Appendix 1.

Data was collected directly into computers using an integrated software package, Compusense five 2.2 (Compusense Inc., Canada 1996).

## RESULTS AND DISCUSSION

**Table 1**

Identity of cages used in each taste session showing total weight (grams) of 5 fish.

<b>Trial 1      Bluewater Barramundi (salt water)</b>			
	15MJ diet	Low fat diet	High fat diet
Session 1	Cage A2 998g	Cage B2 1124.4g	Cage A3 941.9g
Session 2	Cage A6 1175.4g	Cage A4 1141.9g	Cage B5 1031.1g
Session 3	Cage D5 1204g	Cage D6 1244.1g	Cage C6 1180.3g
Session 4	Cage C1 1071.2g	Cage C4 1165.6g	Cage C3 892.2g
Session 5	Cage D2 894.3g	Cage D1 1352.5g	Cage D4 970.7g
<b>Trial 2      Barramundi Waters (fresh water)</b>			
	15MJ diet	Low fat diet	High fat diet
Session 1	Cage 1 1401.6g	Cage 2 1377.9g	Cage 4 639.3g
Session 2	Cage 3 1065.8g	Cage 5 1147.5g	Cage 6 648g
Session 3	Cage 7 1448.6g	Cage 8 1135g	Cage 10 702.5g
Session 4	Cage 9 1284.9g	Cage 11 1140.1g	Cage 12 685.6g
Session 5	Cage 13 989.3g	Cage 14 943.1g	Cage 15 642.1g

Fish were received in good condition but had thawed very slightly in transit.

During preparation for cooking it was observed that the fillets of the fish fed on the low fat diet consistently had a pinker colour than fish from the other diets.

Analysis of variance was performed, and pairwise comparisons were tested at the 5% level of significance and are discussed.

More was a large difference in sizes of fish submitted for tasting. In one case (Trial 1, session 4) both fillets were needed to provide a 20 gram sample. A “dummy” sample was provided for the panellists in this case but the data was not included in the statistical analysis.

The two trials were analysed separately using the panel average for the five sessions and comparing the three diets using analysis of variance. All scores equal to or below 2 were assigned to zero to account for errors in the light pen score for a no detection value.

Means over the five replicates for each trial are shown in tables 2-9.

### **TRIAL 1 SALT WATER TRIAL (BLUEWATER BARRAMUNDI)**

No significant differences ( $p>0.05$ ) were noticed between a fish from the three diets in odour or colour of flesh (Tables 1 and 2).

Scales for all tables following:

0 = None, 100 = Very .....

<sup>1</sup> 0 = Dislike extremely, 100 = Like extremely

<sup>a b c</sup> Different letters signify significant differences between treatments ( $p<.05$ )

**Table 1** Mean Taste Panel Scores - Barramundi Odour profile

	Fishy	Weedy/ herbaceous	Earthy/ muddy	Meaty/ baked	Musty/ mouldy	Milky	Other
MJ15 diet	44.6	9.6	11.2	28.9	3.8	11.8	2.0
Low fat diet	47.0	10.4	8.9	30.1	4.4	11.9	0.8
High fat diet	46.9	10.4	8.4	30.4	6.4	11.1	3.9

**Table 2** Mean Taste Panel Scores - Barramundi Colour Profile and Overall Acceptability

	Grey	Yellow	Brown	Other	Overall acceptability <sup>1</sup>
MJ15 diet	19.8	7.3	15.4	2.0	60.1
Low fat diet	19.3	8.5	13.7	2.1	63.8
High fat diet	18.8	9.3	11.9	0.5	60.2

## *Flavour*

There was a significant difference between all diets for sweetness. The MJ15 diet produced significantly less “fishy” taste.

**Table 3** Mean Taste Panel Scores - Barramundi Flavour Profile

	Metallic	Meaty	Sweet*	Fishy*	Muddy/ earthy	Musty/ mouldy	Stale	Other
MJ15 diet	8.1	36.2	14.8 <sup>a</sup>	43.4 <sup>a</sup>	10.5	3.8	4.7	2.1
Low fat diet	8.6	36.2	22.3 <sup>b</sup>	47.4 <sup>b</sup>	8.7	4.1	5.7	1.5
High fat diet	11.0	38.8	18.1 <sup>c</sup>	50.2 <sup>b</sup>	8.3	4.0	6.8	3.5

## *Texture*

The texture of flesh from all three diets in this trial was noticeably more flaky than in fish from the fresh water trial. Flesh from fish fed the MJ15 diet was much lower in flakiness and more fibrous. It was also perceived to be less moist and more fibrous than the others. Fish from all diets were quality liked.

**Table 4** Mean Taste Panel Scores - Barramundi Texture profile

	Firm	Moist*	Flaky*	Fibrous*	Sticky	Other
MJ15 diet	39.0	46.3 <sup>a</sup>	22.9 <sup>a</sup>	20.7 <sup>a</sup>	17.4	3.1
Low fat diet	39.3	53.1 <sup>b</sup>	27.1 <sup>b</sup>	16.5 <sup>b</sup>	16.0	0.9
High fat diet	37.8	50.5 <sup>ab</sup>	28.2 <sup>b</sup>	18.3 <sup>ab</sup>	16.7	1.4

## TRIAL 2 FRESH WATER TRIAL (BARRAMUNDI WATERS)

Scales for all tables following:

0 = None, 100 = Very .....

<sup>1</sup> 0 = Dislike extremely, 100 = Like extremely

<sup>a b</sup> Different letters signify significant differences between treatments (p<.05)

### *Odour*

The high fat diet canned a significantly (p<0.05) higher “meaty/baked” odour than in the MJ15 diet.

**Table 5** Mean Taste Panel Scores - Barramundi Odour Profile

	Fishy	Weedy/ herbaceous	Earthy/ muddy	Meaty/ baked *	Musty/ mouldy	Milky	Other
MJ15 diet	43.9	8.0	19.5	25.6 <sup>a</sup>	3.7	10.7	1.6
Low fat diet	46.7	6.3	16.6	27.3 <sup>ab</sup>	3.6	10.9	1.8
High fat diet	45.6	8.6	16.0	29.7 <sup>b</sup>	6.4	10.2	2.3

### *Colour*

Flesh from fish fed MJ15 was significantly whiter than from the other two diets, which were rated slightly yellow/brown.

**Table 6** Mean Taste Panel Scores - Barramundi Colour Profile and Overall Acceptability

	Grey	Yellow *	Brown *	Other	Overall acceptability <sup>1</sup>
MJ15 diet	16.4	4.1 <sup>a</sup>	4.7 <sup>a</sup>	0.7	56.1
Low fat diet	17.9	8.1 <sup>b</sup>	8.0 <sup>b</sup>	1.3	58.7
High fat diet	18.7	8.7 <sup>b</sup>	10.2 <sup>b</sup>	1.0	57.3

### *Flavour*

The low fat diet produced sweeter flesh and the high fat diet was rated significantly more “fishy” than the MJ15 diet.

**Table 7** Mean Taste Panel Scores - Barramundi Flavour Profile

	Metallic	Meaty	Sweet *	Fishy*	Muddy /earthy	Musty/ mouldy	Stale	Other
MJ15 diet	9.2	35.2	13.7 <sup>b</sup>	41.9 <sup>a</sup>	9.3	4.2	5.0	2.7
Low fat diet	10.5	34.1	18.5 <sup>a</sup>	45.1 <sup>ab</sup>	8.8	4.0	6.0	2.6
High fat diet	11.8	35.3	12.2 <sup>b</sup>	46.6 <sup>b</sup>	10.1	6.7	7.8	3.6

### *Texture*

Flesh of fish fed MJ15 was significantly softer than from the other diets.

**Table 8** Mean Taste Panel Scores - Barramundi Texture Profile

	Firm*	Moist	Flaky	Fibrous	Sticky	Other
MJ15 diet	22.5 <sup>a</sup>	43.3	9.8	41.0	11.3	1.2
Low fat diet	32.3 <sup>b</sup>	46.9	9.1	43.0	9.8	1.2
High fat diet	31.9 <sup>b</sup>	45.6	10.9	44.0	11.8	0.9

Individual comments made by tasters are listed in Appendix 2.

As in the salt water trial the fish were all liked but scores were generally lower than in the salt water trial.

There were no significant differences between dietary treatments in either trial of overall acceptability of the fish.

## CONCLUSIONS

Fish from all diets from both trials were acceptable and similar in overall acceptability.

The low fat diet produced the sweetest flesh in both trials and the MJ15 diet produced flesh with lower “fishy” flavour scores.

Texture of the fish was affected by diet much more in the salt water trial, where a flaky texture was noted. Flesh from fish fed MJ15 was less moist and flaky and more fibrous. In the fresh water fish this differences was perceived as increased softness.

# APPENDIX 1



## **DESCRIPTORS USED FOR PROFILING**

### ***Odour***

Fishy, weedy/herbaceous, earthy/ muddy, meaty/baked, musty/mouldy, milky, “other”.

### ***Appearance***

Colour of internal flesh - grey, yellow, brown and “other”.

### ***Flavour***

Metallic, meaty, sweet, fishy, muddy/earthy, musty/mouldy, stale, “other”.

### ***Texture***

Firm, moist, flaky, fibrous, sticky, “other”.

All scales were anchored with “None” at the left hand and “very” at the right hand end.

# APPENDIX 2

# Saltwater Barramundi Odour Profile - Mean Sensory Scores

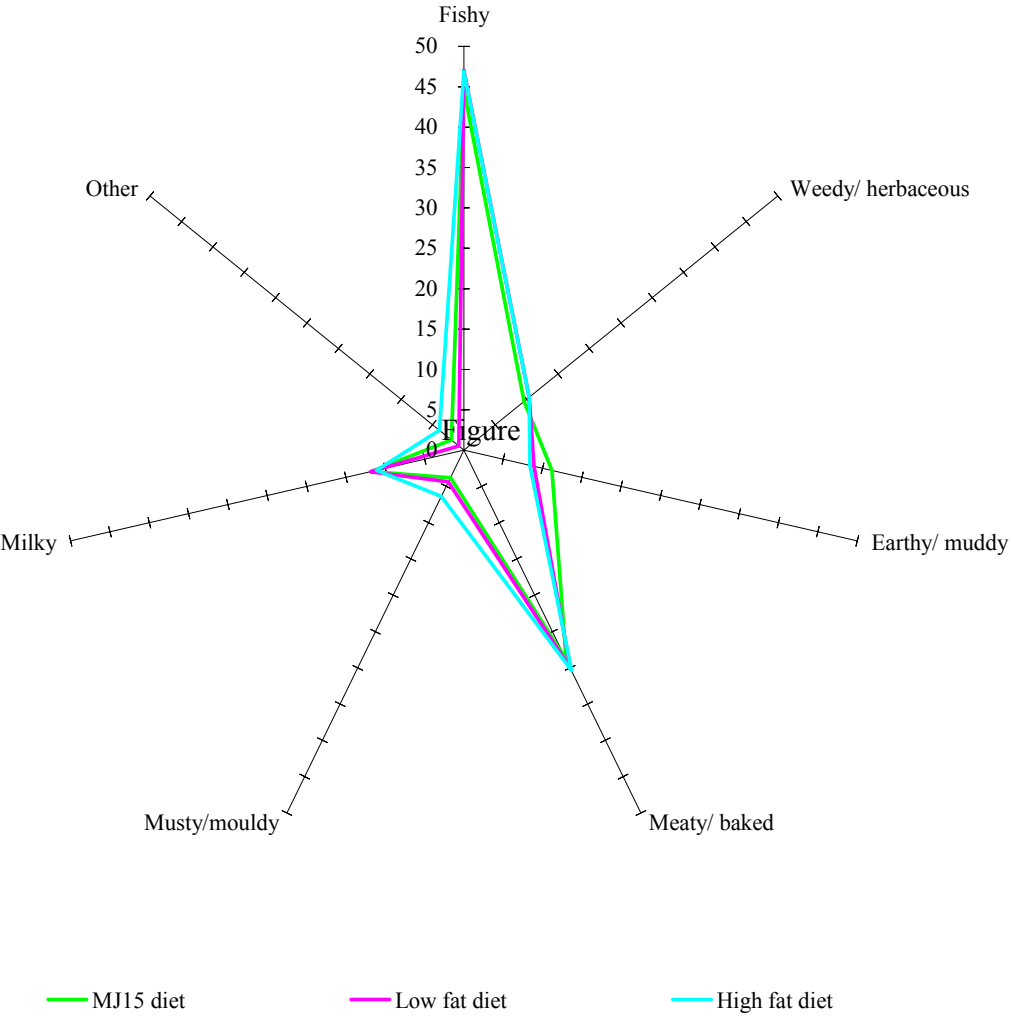


Figure 1

# Saltwater Barramundi Colour Profile - Mean Sensory Scores

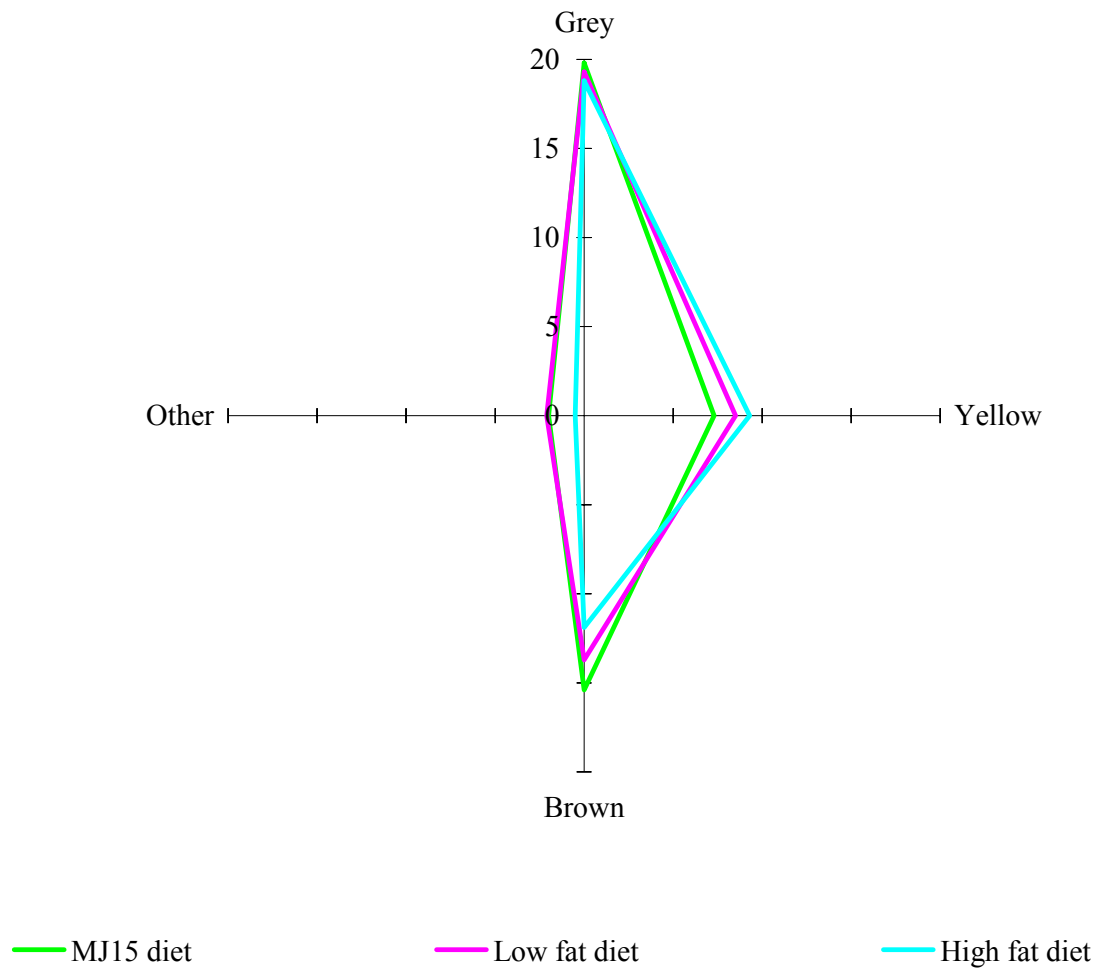


Figure 2

# Saltwater Barramundi Flavour Profile - Mean Sensory Scores

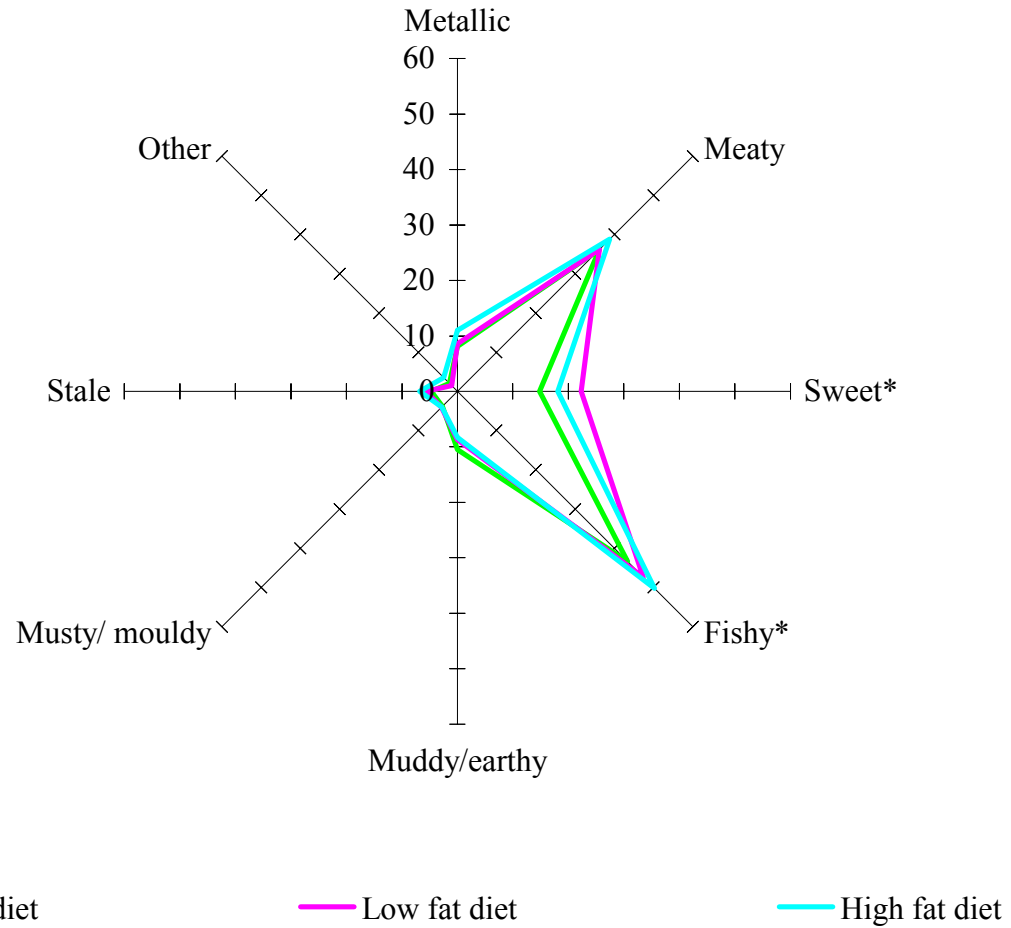


Figure 3

# Saltwater Barramundi Texture Profile - Mean Sensory Scores

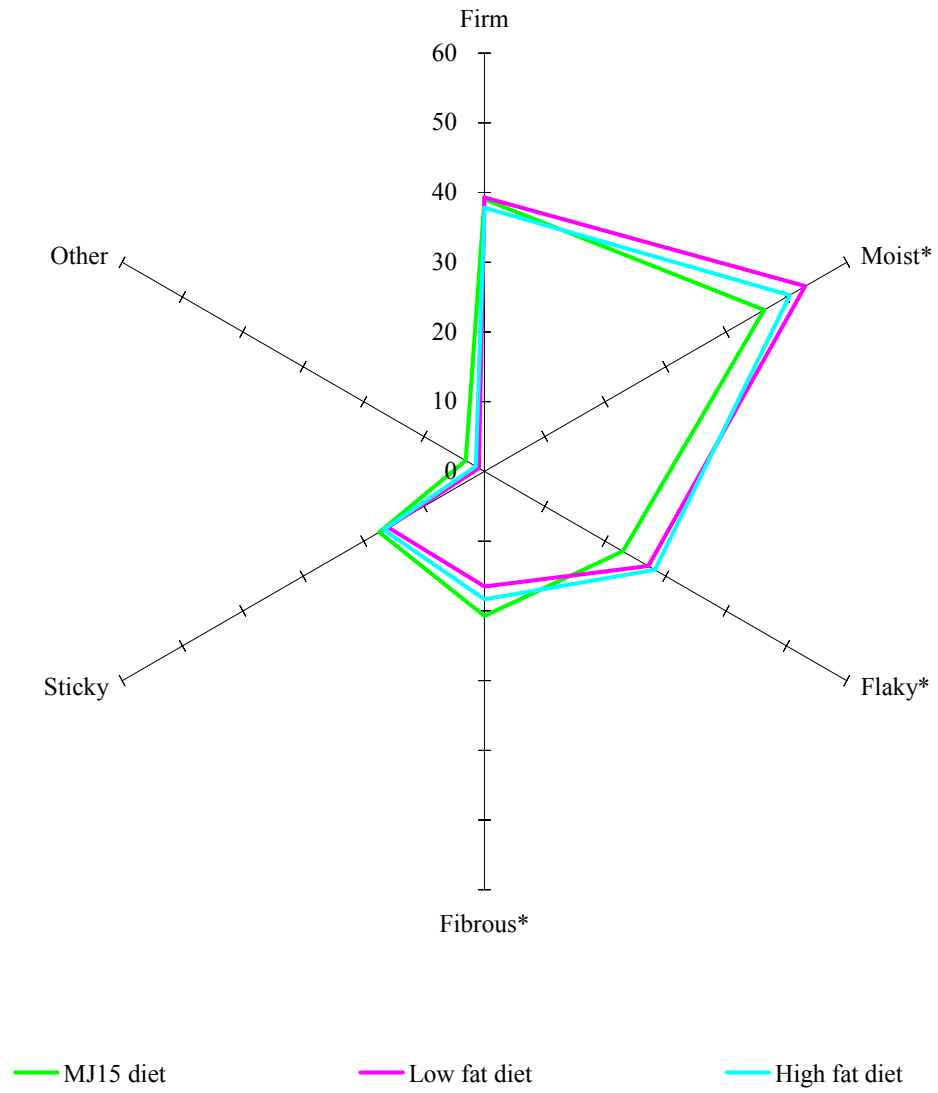


Figure 4

### Freshwater Barramundi Odour Profile - Mean Sensory Scores

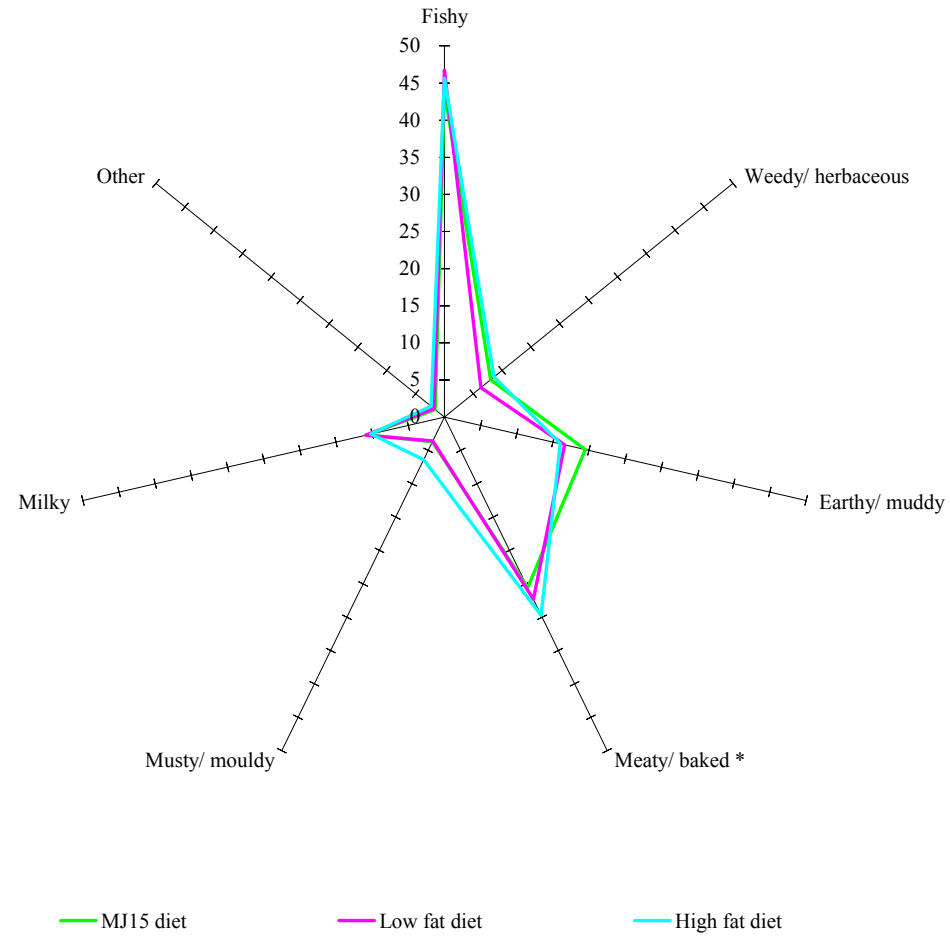


Figure 5

### Freshwater Barramundi Colour Profile and - Mean Sensory Scores

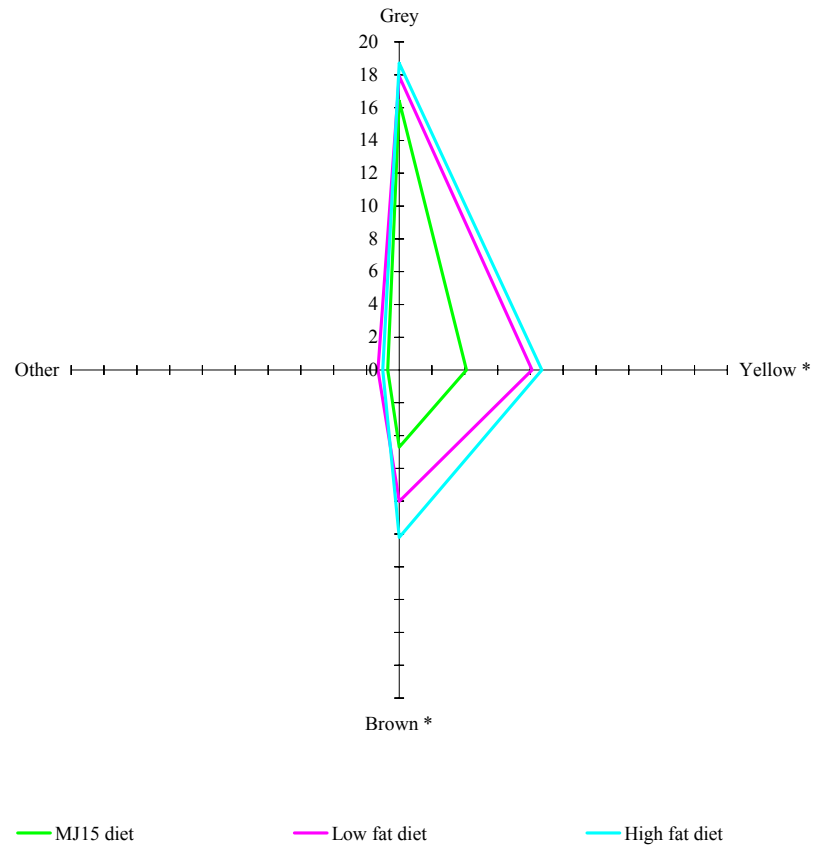


Figure 6



## Freshwater Barramundi Flavour Profile - Mean Sensory Scores

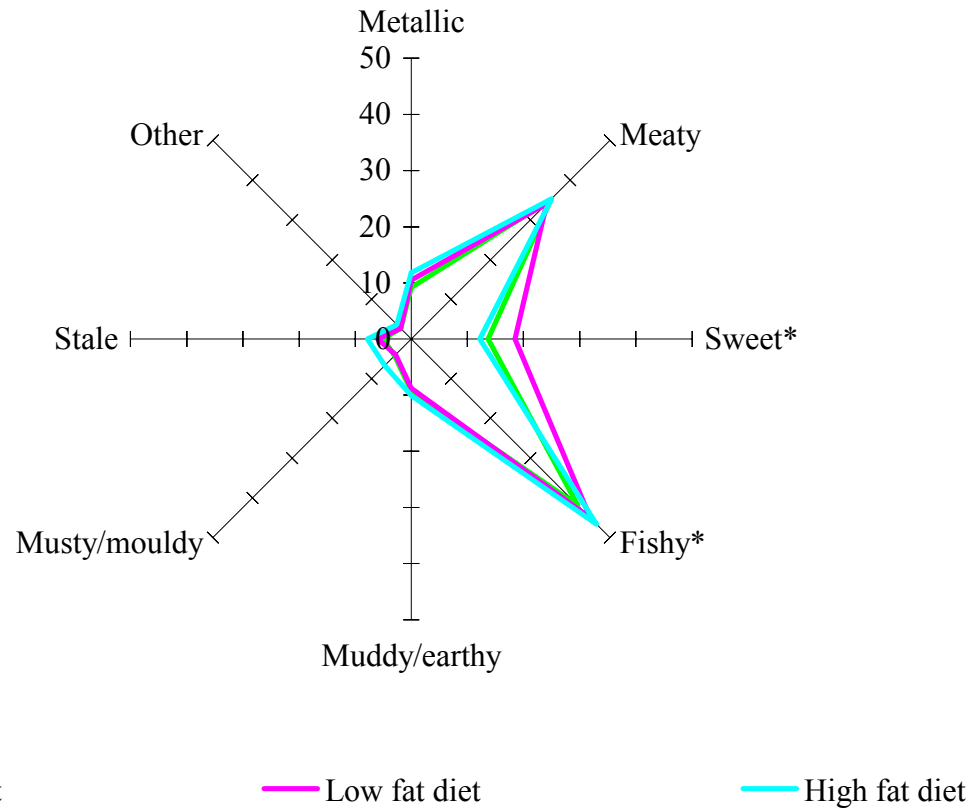


Figure 7

## Freshwater Barramundi Texture Profile - Mean Sensory Scores

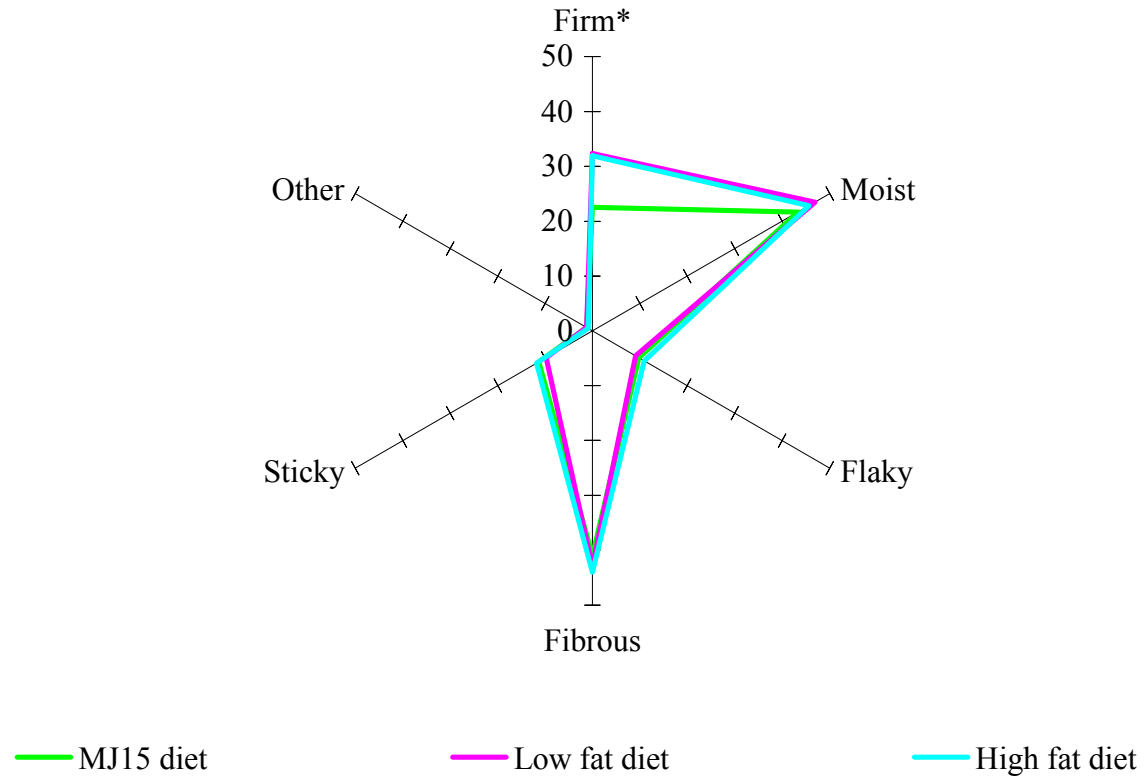


Figure 8

### Adjusted Mean Sensory Scores for Overall Liking of Barramundi

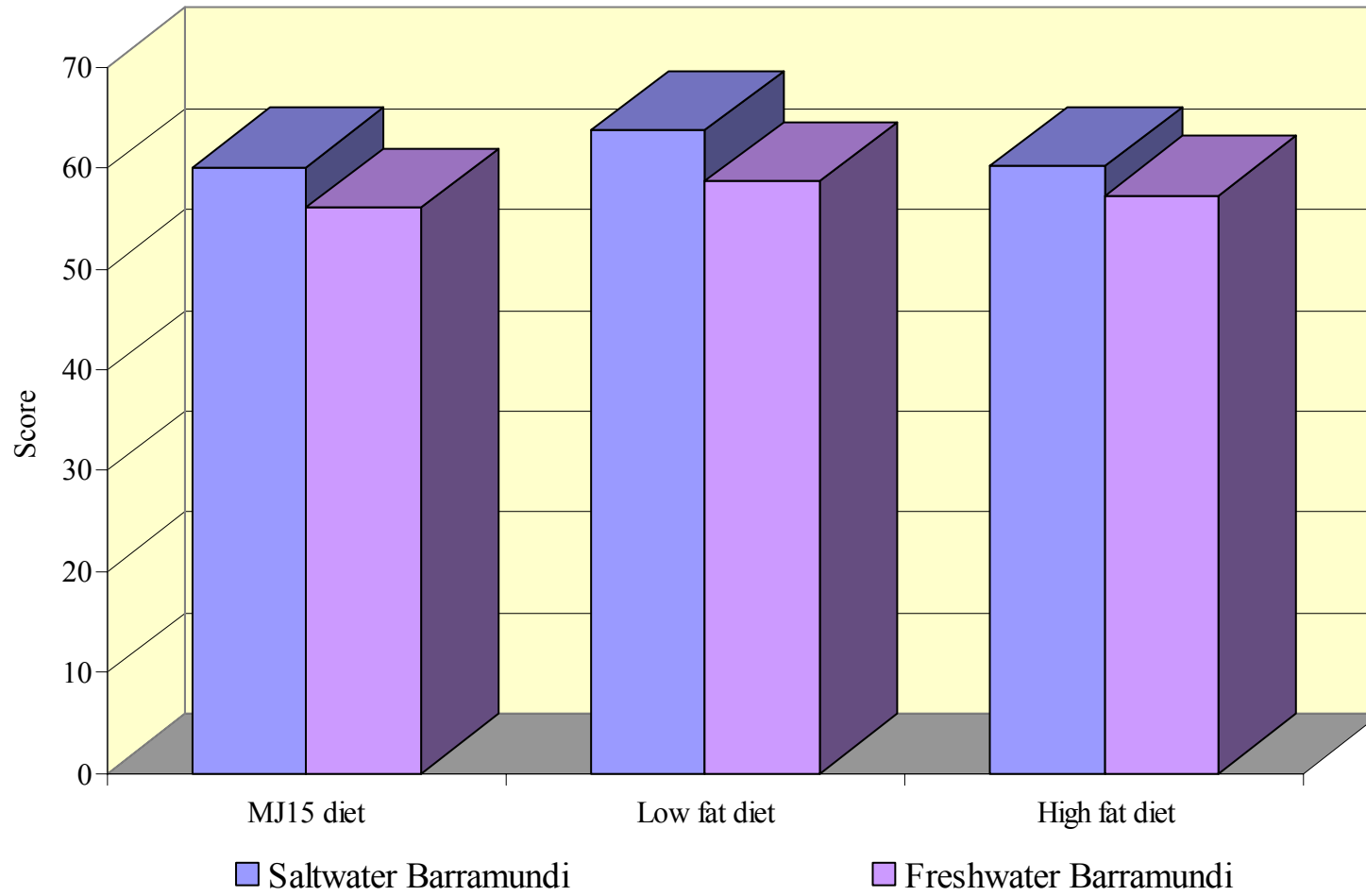


Figure 9



## COMMENTS ON BARRAMUNDI GROWN IN SALT WATER

### 15 MJ Diet

#### *Comments on odour*

- Unpleasant cardboard smell
- Stale rubbery odour
- A bit spicy
- Slight sweaty/sour type smell
- Fresh
- Sharp taste initially
- Old smell

#### *Comments on colour*

- Dark red, black colour
- Pink tinge
- White
- White
- White
- Green tinge

#### *Comments on flavour*

- Bitter off flavour
- Very bland
- Bland in flavour
- Not much fish flavour
- Herbaceous weed
- Bland washed out
- OK but bland

#### *Comments on texture*

- Mushy
- Very sloppy
- Dry tacky
- Slightly mushy

## COMMENTS ON BARRAMUNDI GROWN IN SALT WATER (Continued)

### Low Fat Diet

#### *Comments on odour*

- Low fat greasy/oily
- Not much fish flavour
- Minty pepper smell
- Weedy
- Weedy

#### *Comments on colour*

- Pink colour
- White
- White
- Green tinge
- Greyish skin colour
- Greyish

#### *Comments on flavour*

- Weedy flavour
- Bitter aftertaste
- Not much fish flavour
- Herbaceous taste
- Weedy dry taste on tip of tongue
- Slight old flavour old oil

## COMMENTS ON BARRAMUNDI GROWN IN SALT WATER (Continued)

### High Fat Diet

#### *Comments on odour*

- Strong sweaty/sour type odour
- Sweet uncharacteristic odour, like old perfume
- Old stink
- Weedy
- Weedy/old
- Sweet/floral
- Has a sort of musty/sweaty type odour
- Has a slight sweaty/acidic type odour
- Stale rubbery smell

#### *Comments on colour*

- White
- Green tinge

#### *Comments on flavour*

- Typical baked flavour
- Salty
- Shell fishy than fishy
- Flavour a lot better than expected after off-putting smell
- Quite sour/acid/sweaty type flavour
- Not much fish flavour
- Dry fibrous
- Bland washed out flavour
- Bland

#### *Comments on texture*

- Sludgy
- Old dry i.e. frozen sample

# COMMENTS ON BARRAMUNDI GROWN IN FRESH WATER

## 15 MJ Diet

### *Comments on odour*

- Old rubbery smell
- Cardboard
- Weedy
- Old smell
- Sweet
- OK
- This sample and the sample fed the high fat diet have a slight sweaty odour
- Had an odour - sweet/sweaty
- Meaty

### *Comments on colour*

- Green tinge
- Green
- Greyish (around) edge
- White yellow

### *Comments on flavour*

- Very bitter flavour
- Dry lifeless taste
- Bland as cardboard
- Watery/bland type flavour
- Very bland like boiled in water
- Worked muscle dark flesh flavour
- Bitter throat catch
- Peppery bland

### *Comments on texture*

- Dryish
- Texture OK fine best of all
- Bitter aftertaste
- Quite chewy
- Sticky



## COMMENTS ON BARRAMUNDI GROWN IN FRESH WATER (Continued)

### Low Fat Diet

#### *Comments on odour*

- Slightly weedy
- Slight weedy
- Sweet smell
- Smells a bit stinky/sweaty
- A slight sweaty/sour type odour
- Weedy smell
- Strong kidney - meat smell
- Stale rubbery smell

#### *Comments on colour*

- Colour good
- Green
- Pink colour
- Greyish colour
- OK

#### *Comments on flavour*

- Bitter dry flavour
- Weedy taste tingle on the tongue
- Bland, no salt
- Sour and vegetable (broccoli) like flavour
- Weedy bitter taste
- Sour and brussels sprouts type flavour
- Bitter
- OK not much flavour
- Very bland

#### *Comments on texture*

- This sample is more like fish texture and flavour than any of the other I've tasted
- Dry
- Quite chewy
- OK
- Good
- Leathery
- Good texture

## **COMMENTS ON BARRAMUNDI GROWN IN FRESH WATER (Continued)**

### **High Fat Diet**

#### *Comments on odour*

- Smells a bit stinky/sweaty
- Slightly sour/sweaty type odour
- I've finally worked out what smell is kidney! It's a meaty smell but stronger like kidneys
- Weedy
- Weedy
- Sweet
- OK
- Fresh almost odour

#### *Comments on colour*

- Green tinge
- Pink colour in flesh
- Green
- Greyish slight
- Quite black in patches
- White good

#### *Comments on flavour*

- Bitter flavour
- Dry lifeless
- Bitter
- Bland as cardboard
- Bland lacking salt almost like boiled in water
- Bland and dark flesh flavour - typical of worked muscle
- Sour and brussels sprouts flavour
- Peppery taste

#### *Comments on texture*

- Another 'real fish' it's the combination of flaky and fish flavour - really good
- Dry lifeless
- Little sticky

## **Appendix 10.2**

### **Economic Comparison of Dietary Productivity**

## APPENDIX: Economic Comparison of Dietary Productivity

### Introduction

Assessment of the performance of aquaculture diets needs to be based on food conversion ratios (how much feed is required to produce a standard quantity of fish), growth rates (production time), and price of the feed. In this appendix we bring together the biological parameters of FCR and growth rates, and analyse them in relation to the price of diets. Such analyses provide the basis on which both millers and fish producers can make commercial decisions. The aim of the analyses was to evaluate the economic impact of the diets with respect to fish productivity. Specific questions posed were:

What were the production costs required to produce a kilogram of barramundi for each of the five diets during the 56 day period of the trial?

What were the relative production times when barramundi were grown from approximately 320 to 560 g (ie. a 75 % increase in weight) on each diet?

What is the variation in grow-out time due to the different diets for fish grown approximately 100 to 500 g (using results from the present and other trials in which similar diets were used)?

### Production Cost

Experimental data from the present trial used in these analyses were start weight, finish weight and FCR. These values are given for each diet in (**Error! Reference source not found.**, page **Error! Bookmark not defined.**). These biological parameters were used to calculate the total weight gain (TWG) and total feed intake (TFI) according to the following formulae.

$$TWG = \text{Finish weight} - \text{Start weight}$$

$$TFI = TWG * FCR$$

The costs of the five Gibson's diets at a delivered-to-Townsville price are as follows:

Diet 1	\$1,400 per tonne	(45% protein, 20% oil content)
Diet 2	\$1,375 per tonne	(45% protein, 20% oil content)
Diet 3	\$1,440 per tonne	(45% protein, 20% oil content)
Diet 4	\$1,470 per tonne	Salmon Grower (45% protein, 25% oil content)
Diet 5	\$1,210 per tonne	Barramundi Stock (43% protein, 5% oil content)

(Craig Foster, personal communication, 24<sup>th</sup> June 1998)

The production cost (\$ per kg) is the cost required for each diet to produce one kilogram of barramundi during the 56 day period of the trial. This was calculated according to the following formulae. The cost of feed (\$ per kg) is determined by dividing the tonnage value by 10<sup>3</sup>. The production cost (\$ per kg) can be adjusted to tonnage production cost (\$ per tonne) by multiplying the production cost by 1000.

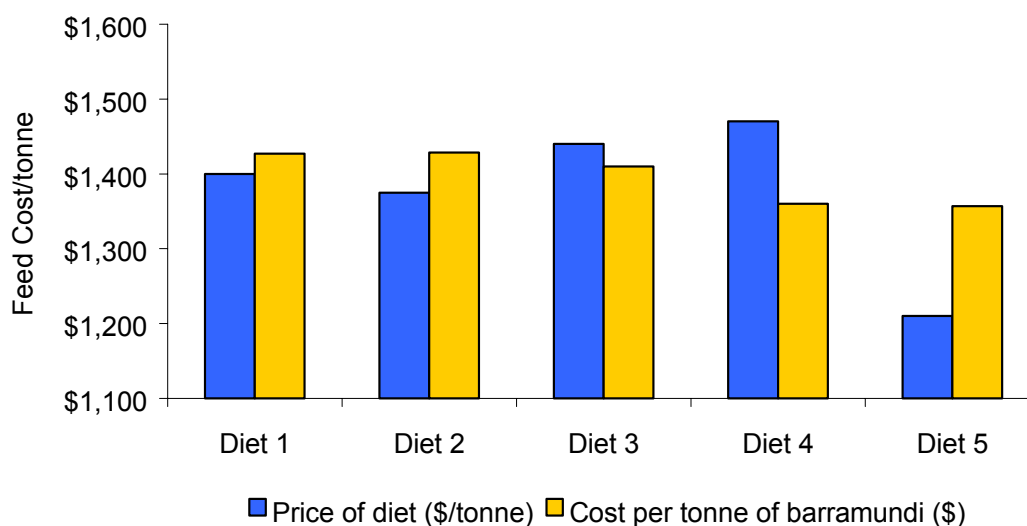
$$\text{Production cost} = \frac{\text{Cost of feed} * TFI}{TWG / 1000}$$

The production costs (\$ per kg) were calculated for each of the five diets in the present trial. The results are presented in Table 1 and Figure 1. The production cost for diets 1 and 2 were highest at \$1.43 per kg. Diets 4 and 5 had the lowest

production cost (\$1.36 per kg). This difference occurred despite diet 1 having a similar feed intake to diets 4 and 5.

**Table 1:** Feed cost, total weight gain, total feed intake and the production cost for each diet following an 8 week growth assay.

Code	Cost of feed (\$/tonne)	Start weight (g)	Finish weight (g)	Total Weight Gain (g)	Total Feed Intake (g)	Production Cost (\$ per kg)
Diet 1	\$1,400	306.6	578.7	272.1	277.4	\$1.43
Diet 2	\$1,375	313.8	611.9	298.1	309.7	\$1.43
Diet 3	\$1,440	311.3	631.1	319.8	313.1	\$1.41
Diet 4	\$1,470	308.6	600.7	292.1	270.3	\$1.36
Diet 5	\$1,210	316.0	564.7	248.7	278.9	\$1.36



**Figure 1:** The price of five Gibson diets and the cost of each diet to produce one tonne of barramundi.

### ***Production Time for Grow-out of Barramundi from 320 to 560 g***

Growth rates from the present trial were best described by the exponential growth equation where  $y = \text{weight (g)}$ ,  $a$  (the intercept on the y-axis when  $x=0$ ) and  $b$  (the slope) are constants,  $e$  is the natural logarithm, and  $x$  is the time in days.

$$y = ae^{bx}$$

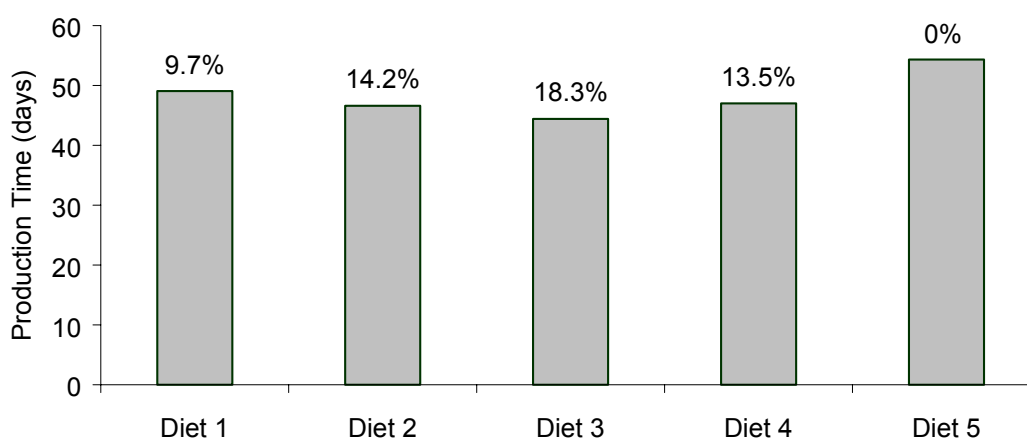
Change in growth at fortnightly intervals during the 8 week trial were used as replicates in fitting the growth equation. The values for the equations are listed in Table 2.

The production time for grow-out of barramundi from 320 to 560 g was determined using the difference in the number of days taken for fish to grow from 320 to 560 grams. This size range was chosen because these values were within the range of the actual start and finish weights (see Table 1), and therefore no extrapolation was required, and because it represented a convenient 75 % increase in fish weight.

Solving the equations for  $y = 320$  and  $y = 560$  enables determination of the production time for fish grown from 320 grams to 560 grams (Table 2 and Figure 2).

**Table 2:** Values for exponential equations describing the growth of barramundi on the five Gibson diets during the 56 days of the trial. Production time in days to grow the fish from 320 grams to 560 grams is also tabulated.

Code	a	b	r <sup>2</sup>	Days 320g	to Days 560g	to Production time (days)
Diet 1	308.78	0.0114	0.9777	3.13	52.22	49
Diet 2	317.86	0.0120	0.9854	0.56	47.19	47
Diet 3	317.04	0.0126	0.9820	0.74	45.15	44
Diet 4	314.05	0.0119	0.9542	1.58	48.60	47
Diet 5	321.49	0.0103	0.9723	-0.45	53.88	54



**Figure 2:** The time in days taken to grow barramundi from 320 grams to 560 grams, using the five Gibson diets and assuming the same culture conditions as in the trial. The figures on each bar are the percentage decrease in growout times compared with the barra stock diet (diet 5).

### ***Production Time for Grow-out of Barramundi from 100 to 500 g***

Calculation of the number of days required for barramundi to grow from 100 to 500g was based on three separate trials for both the improved and standard diets. The standard and improved diets from the present trial were diets 5 and 3 respectively. Similar standard and improved diets from 2 previous trials were used to provide a continuum of fish growth from approximately 100 to 500 g.

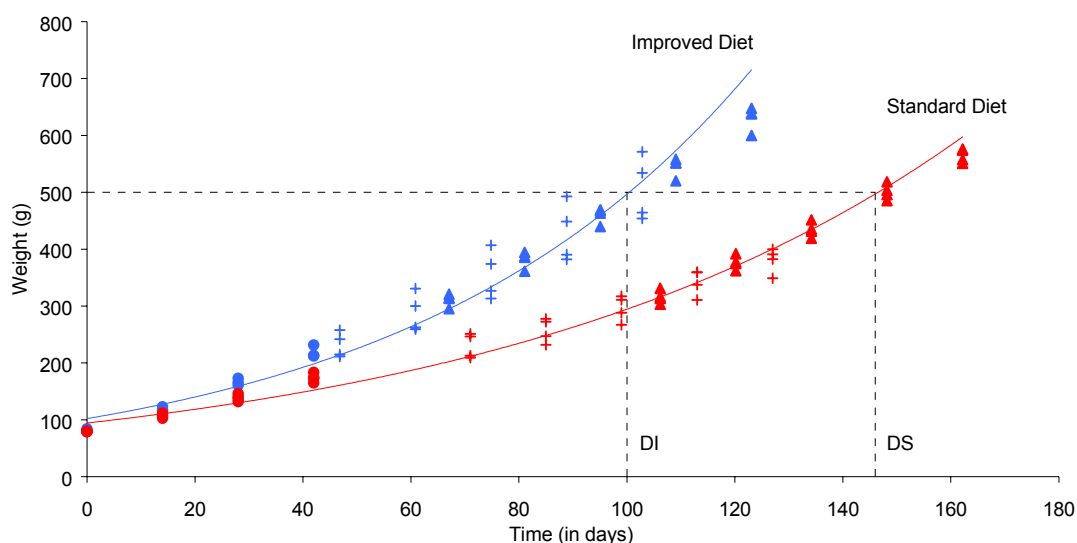
The average weights of barramundi at the beginning and end of each of trial are given in Table 3. There was no overlap in weight at the completion of trial 1 and the commencement of trial 2, although there was a large overlap in weight at the completion of trial 2 and commencement of trial 3.

A common growth curve for the improved and standard diets covering the weight range from the commencement of trial 1 to the completion of trial 3. This required

estimation of the start dates for trials 2 and 3, relative to the end weights of the previous trials. The time taken to complete the previous trial(s) was added to the extrapolated time from the start to the previous trial to the commencement of the next trial. The ongoing date within trial 2 and 3 was then determined by adding the ongoing date within the trial to the estimated trial commencement date. This provided a continuous series of growth increments, based on three separate trials, which could be fitted to the exponential growth curve.

**Table 3:** Average weight (g) of barramundi at the commencement and completion of three separate trials using improved and standard diets.

Diet	Trial	Average weight at commencement	Average weight at completion of trial
IMPROVED DIET	1	82	219
	2	231	506
	3	311	631
STANDARD DIET	1	79	174
	2	230	380
	3	316	565



**Figure 3:** Growth curves for Barramundi grown at 28°C on improved and standard diets. DI and DS indicate the number of days of growth required to reach 500 g on the improved and standard diets respectively.

### Discussion

The three experimental diets (Diets 1 to 3) produced barramundi at a higher food cost than the standard barramundi diet (diet 5) (Table 1 and Figure 1). Diet 3, which gave the fastest growth rate of all diets and second best FCR (after the salmon grower), actually cost 4% more than the standard barramundi diet to produce an equivalent amount of fish. However, the economic benefit derived from the faster growth rates of fish grown on the three experimental diets and the salmon grower

compared with the standard barramundi diet would far outweigh the impact of slightly greater feed costs. As can be seen in Figure 2, fish on diet 3 grew from 320 grams to 560 grams in 44 days compared to 54 days on the standard barramundi diet.

The growth rate curves in Figure 3 demonstrate the effect throughout the grow-out period of the improved diets compared with the standard industry diets. It should be appreciated that the data are compiled from three separate trials, and thus are not an exact account of respective performances of the diets. The overall trends represent the expected growth performance indicating barramundi at 28°C would grow from 100 to 500 g in about 150 days on standard commercial diets, compared with about 100 days on the high specification diets. This variation in growth rate would make an enormous difference to farm profitability.



## **Appendix 10.3**

### **Guidelines for Feeding Silver Perch**

**Table 1 Recommended feeding rates and frequencies for silver perch**

- Feeding rate = % body weight per day; feeding frequency = no. of feeds per day, i.e. once (1x) or twice (2x) e.g. fish to be fed 5.0% body weight, twice daily (i.e. 5% distributed between 2 feeds) = 5.0/2x; fish to be fed 0.5% body weight, 3 days per week = 0.5 3d/w; fish to be fed 0.5% on alternate days = 0.5/alt.d
- Daily ration should be increased or decreased gradually over several days when changing the feeding rate

Fish (g)	Water Temperature (°C)						
	< 9	9 - 12	12 - 15	15 - 18	18 - 21	21 - 25	25 - 30
1 - 15	0.5 3d/w	0.5/1x	1.0/1x	2.0/1x	3.0/1x	7.5/2x	7.5/2x
15 - 50	0.5 3d/w	0.5/1x	1.0/1x	2.0/1x	2.5/1x	5.0/2x	7.5/2x
50 - 250	0.5 3d/w	0.5/1x	1.0/1x	1.5/1x	2.5/1x	4.0/2x	5.0/2x
250 - 500	0.5 1d/w	0.5/alt.d	0.5/1x	1.0/1x	2.0/1x	3.0/2x	2.0/2x
> 500	0.5 1d/w	0.5/alt.d	0.5/1x	1.0/1x	1.0/1x	1.5/1x	1.0/1x
Broodfish	0.5 1d/w	0.5/alt.d	0.5/1x	1.0/1x	2.0/2x spring and early summer; 2.0/1x other seasons	2.0/2x spring and early summer; 2.0/1x other seasons	1.0/1x

**Table 2 Recommended feed particle sizes for silver perch**

- Feed particles generally have similar diameter and length
- Feeds of different sizes should be mixed for several days when changing particle sizes or when there is a large size range of fish

Fish size		Size (mm) and type of feed	
Total length(mm)	Weight(g)	Diameter	Crumble (c) or Pellet (p)
15-25	0.5	0.6	c
25-35	0.5 -1.0	1.0	c
35-50	1 - 2	1.5	c
50-75	2 - 5	2.0	c
75-100	5 - 10	1.5	p
100-125	10 - 25	2.0	p
125-175	25 - 100	3.0	p
175-275	100 - 350	4.0	p
> 275	> 350	6.0	p

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# Guidelines for Feeding Silver Perch



October 2002

## Introduction

Feeding is one of the most important activities in fish farming. The efficient delivery of high quality feeds to fish in ponds, cages and tanks is essential for high survival, fast growth, high production rates, low food conversion ratios, maintenance of good water quality and economic viability.

Feed requirements vary between fish species, with size and age, and with environmental factors, particularly temperature. Omnivorous species such as silver perch require lower levels of protein than carnivorous species, and omnivores can utilise plant proteins and carbohydrates more effectively than carnivores. Small fish require more feed in relation to their size than larger fish. All fish eat less feed at lower temperatures because metabolism is slower and appetite is less than at high temperatures.

High quality feeds for silver perch are now available from feed mills in New South Wales and Queensland. These diets contain around 35% protein, 14 MJ/kg digestible energy and 9% fat, and are made of Australian agricultural products such as meatmeal, wheat, lupins, canola and field peas, with no or low levels of fishmeal.

Different feeding strategies are used in aquaculture and include feeding on demand, feeding to satiation, and feeding a restricted ration based on a proportion of body weight. Currently many silver perch farmers attempt to feed to satiation by observing the feeding activity of the fish at or near the surface of the water. However, satiation can be very difficult to determine in characteristically turbid silver perch ponds, where not all fish feed at the surface. Even where floating feeds are used during warmer periods, many fish do not feed at the surface. Feeding behaviour and activity of silver perch vary with temperature, stocking density and genetic strain, and are also influenced by fish health, water quality, turbidity, availability of natural food, time of day, cloud cover, wind, and bird predation.

Consequently it is easy for silver perch farmers to either over-feed or under-feed their fish. Poor feeding practices increase the cost of production. Over-feeding wastes feed and adversely affects water quality, while under-feeding results in reduced growth and greater size variation because of dominance of available feed by aggressive fish. Both over and under-feeding may increase susceptibility to disease. Feeding to satiation can be time-consuming, and increase labour costs and the overall cost of production.

## Development of a feeding strategy

Research was done by NSW Fisheries to develop a feeding strategy for silver perch. Methods used were: (i) a series of feeding experiments at the Grafton Aquaculture Centre (GAC) using a 35% protein extruded diet to determine the feeding rates (% body weight) and frequencies (no. of feeds/day) which maximised growth, while minimising effort and food conversion ratio (FCR) of fingerling and large fish (up to about 500g) over a range of water temperatures from 13° to 30°C; (ii) a review of the feeding of broodfish in the breeding program at GAC; (iii) consultation with commercial silver perch farmers about feeding practices, particularly for fish over 500g and during winter in regions where water temperatures fall below 12°C; and (iv) a review of feeding strategies used for warmwater species such as channel catfish in other countries.

## Feeding rates, frequencies and times

The resulting feeding strategy for silver perch is presented in Table 1. Recommended feed particle sizes are given in Table 2. The strategy is based on restricted rations where fish are fed a certain percentage of the total body weight or biomass of all the fish in a pond, cage or tank. There is no benefit in feeding silver perch, including small fingerlings, more than twice daily. Silver perch up to 500g perform better if fed twice daily when water temperatures are over 20°C but on large-scale operations, farmers must evaluate the benefits of better performance and water quality against the additional cost and effort of feeding twice daily. At temperatures of 12° to 20°C, feeding once daily is sufficient for fish of all sizes. Feeding activity, growth and food conversion efficiency of silver perch decline at water temperatures over 30°C, and it is recommended that farmers reduce feeding rates and frequencies during such periods. Fish should not be fed during outbreaks of disease, and when ponds and tanks are being treated with formalin and salt. The feeding regimes in Table 1 can also be used as a guideline for farmers wishing to continue feeding to satiation because the recommended daily rations are close to satiation levels.

As with other animals, feeding activities in fishes exhibit distinct daily patterns; however, many studies have shown that regulated feeding in aquaculture overrides (to some degree) the various natural rhythms associated with appetite, feeding and digestion. Consequently, feeding silver perch only during daylight hours will not adversely affect performance. Recommended feeding times when the frequency is twice daily are 08.00h and then 15.00 - 17.00h in the afternoon. Prior to the morning feed, the farmer must ensure there are adequate oxygen concentrations in ponds bearing in mind that minimum levels occur near dawn. The daily ration can be divided evenly between morning and afternoon feeds. Feeding time when the frequency is once daily is 15.00 - 17.00h; the period when the highest temperature and oxygen levels occur.

### Example of calculating daily ration using Table 1:

- a farmer has 4,000 fingerlings in a pond
- a sample of 100 fish weighs 3,000g, therefore the mean weight is 30g
- biomass = 4,000 X 30 = 120,000g = 120kg
- water temperature is 22°C
- daily ration is 5%/2x from Table 1
- 5% of 120kg = 120 X 0.05 = 6kg
- feed particle size is 3mm pellet from Table 2
- feed 3kg at 08.00h, and the other 3kg at 15.00h

## Need for regular adjustment of ration

Where restricted rations are used, the amount of feed that should be fed changes daily because of fish growth, and so the effective use of feeding tables is dependent on the regular estimate of biomass and adjustment of daily rations. Biomass can be estimated by a number of techniques: (i) using known growth rates; (ii) assuming a certain food conversion ratio (FCR) e.g. 2.0; (iii) by sampling fish. At GAC, fish are sampled, weighed and the daily ration adjusted each 2 weeks for small fingerlings and 4 weeks for fish larger than 50g. In ponds, 100 - 200 fish (usually 5 - 10% of the crop) are sampled using a seine net. In experimental cages (1m<sup>3</sup>) all fish are removed and bulk-weighed, but in large cages under commercial conditions a randomly selected sample of around 5% would be sufficient to estimate biomass.

## Maximum daily input of feed to ponds

As fish grow and the pond biomass and daily ration increase, the increasing quantity of feed applied to ponds causes a deterioration of water quality. In particular, dissolved oxygen (DO) concentrations decrease and concentrations of total ammonia-nitrogen (TAN), and usually un-ionised ammonia (NH<sub>3</sub>) increase. To ensure the maintenance of good water quality, it is recommended that total feed inputs into silver perch ponds do not exceed 150 kg/ha/day in small ponds (< 0.3 ha) and 100 kg/ha/day in larger ponds. Farmers must ensure they have adequate aeration in ponds, i.e. around 10 hp/ha, the ability to exchange water and reliable water quality monitoring equipment. Farmers must be cautious when feeding ponds with a large biomass of fish and dense blooms of phytoplankton during summer. Under these circumstances water quality can deteriorate rapidly, necessitating a reduction or cessation of feeding. It may also be necessary to reduce feeding if the weather changes quickly, e.g. if a cold snap occurs, lowering the water temperature by several °C over a few days.

## Feeding during winter

Activity and appetite in warmwater fishes decline markedly as temperatures fall below 20°C, but silver perch will continue to feed at temperatures down to at least 9°C. Feeding in winter can prevent weight loss, achieve small weight gains, and keep fish healthier than those that are not fed.

## Floating and sinking feeds

Floating and sinking feeds are available for silver perch, and both types have advantages and disadvantages. The use of floating feed enables fish that feed at or near the surface to be clearly seen and this aids in management. However, not all silver perch feed at the surface - some feed mid-water, even during warmer months. Uneaten feed may be washed to the edges of ponds and cages and wasted. Sinking feed provides all fish with the opportunity to obtain feed, but some pellets may sink to the bottom where they are generally unavailable to the fish. At low temperatures, silver perch generally do not feed near the surface. We recommend that silver perch farmers use slow-sinking, extruded feed or a combination of sinking and floating feed when water temperatures are over 20°C, and sinking feed at lower temperatures.

## Purchase and storage of feed

Farmers should order feed well before their on-farm supplies are exhausted. On arrival, the feed should be checked for appropriate type and size, and lack of contamination such as mould. All feed should be stored under conditions that are rodent proof, and cool (< 15°C) with low humidity. Feed should be used within 3 months of purchase.

This feeding strategy is recommended as a guideline only for silver perch farmers. Feeding is an important management practice, and there is significant variation in fish behaviour and feeding activity between ponds on any one farm. Farmers need to combine careful observation of feeding activity with other practices, particularly the monitoring and management of water quality and disease.

## Acknowledgments

We thank Charlie Mifsud, Mark Nixon, Peter Boyd, Joel Crispin, Nat Sewell and Todd Rowland for their role in the feeding research at Grafton. We are grateful to Bruce Rhoades, Ian Charles, Bruce Malcolm and Mark Scifleet for information on feeding practices on commercial farms. The research was partly funded by the Fisheries Research and Development Corporation.

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