Final Report

AQUAFIN CRC PROJECT 1B.3: INCREASING THE PROFITABILITY OF SNAPPER FARMING BY IMPROVING HATCHERY PRACTICES AND DIETS
VOLUME 1: DIET DEVELOPMENT

Mark A. Booth, Geoff L. Allan, D. Stewart Fielder and Alex Anderson

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NSW DEPARTMENT OF PRIMARY INDUSTRIES

QUT

Australian Government
Fisheries Research and Development Corporation

Aquafin CRC is established and supported under the Australian Government's Cooperative Research Centres Program

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LIST OF ACRONYMS

$AUD	Australian dollar
ABARE	Australian Bureau of Agricultural & Resource Economics
ADC	Apparent digestibility coefficient
ANCOVA	Analysis of covariance
ANOVA	Analysis of variance
AOAC	Association of Official Analytical Chemists
BV	Biological value
BW	Body weight
CHO	Carbohydrates
CP	Crude protein
CRC	Cooperative Research Centre
CSIRO	Commonwealth Scientific & Industrial Research Organisation
DE	Digestible energy
df	Degrees of freedom
DO	Dissolved oxygen
DP	Digestible protein
DPI	Department of Primary Industries
exp	Exponent
FAO	Food & Agriculture Organisation
FBW	Final body weight
FCR	Feed conversion ratio
FRDC	Fisheries Research & Development Corporation
GE	Gross energy
GMBW	Geometric mean body weight
GTT	Glucose Tolerance Test
HSI	Hepatosomatic index
IBW	Initial body weight
ICP-MS	Inductively coupled plasma – mass spectrometer
MJ	Mega joule
NFE	Nitrogen free extract
NPU	Net protein utilisation
NRC	National Research Council
OM	Organic matter
PER	Protein efficiency ratio
PPV	Productive protein value
PRE	Protein retention efficiency
PSFC	Port Stephens Fisheries Centre
RAS	Recirculating aquaculture system
SARDI	South Australian Research and Development Institute
SD	Standard deviation
SEM	Standard error of mean
SGR	Specific growth rate
SKM	Saturation kinetics model
SNK	Student Newmans Kuels test
TGC	Thermal growth coefficient

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ACKNOWLEDGEMENTS

This work formed part of a project of Aquafin CRC, and received funds from the Australian Government's CRCs Program, the Fisheries R&D Corporation and CRC Participants.

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EXECUTIVE SUMMARY

Australian snapper (*Pagrus auratus)*, known as red sea bream in Japan, is a premium table fish that fetches high market prices in eastern Australia. Commercial culture of this species has been constrained by the high costs of feeds and feeding and fingerlings. The work described in this report (in three volumes) details results of research to address these constraints. This research has increased our knowledge of the nutritional requirements of Australian snapper *Pagrus auratus* and provided information on the potential of Australian feed ingredients to reduce the level of fishmeal in diets for this species. To meet the general and specific aims of this study, a research strategy based on determination of apparent digestibility coefficients to evaluate the potential for a range of potential feed ingredients, establishment of requirements for digestible energy and protein, and an assessment of how well different nutrients from key ingredients was adopted. Nutritional requirements are presented here as are details of the value of different feed ingredients. Trials were conducted that showed that fishmeal could be reduced to approximately 16% provided digestible energy and protein contents were maintained.

Farmed snapper are generally darker and less red in appearance than those obtained from the wild harvest, leading to lower market prices. This issue was raised as a priority among pioneer snapper farmers in Australia. The research described here has increased our knowledge of the factors that affect skin colour and developed practical methods to improve the appearance of farmed snapper. The combination of culturing snapper in light coloured tanks and feeding diets high in the natural pigment, astaxanthin, produced 'pink' coloured snapper that met consumer preferences.

Before this project started, snapper larvae were cultured in hatcheries using intensive techniques and while several hatcheries had successfully produced fingerlings, production costs were high. During this project, new protocols for intensive production were developed for managing environmental variables such as light intensity, photoperiod and temperature and live and inert feeds and feeding regimes. These new protocols allowed greatly increased hatchery production and lower costs. In addition, prior to this project there was no information on the potential or procedures to culture larvae extensively in outdoor, fertilised ponds. Results presented here have provided an understanding of zooplankton production in ponds and allowed strategic stocking of snapper larvae. High quality juvenile snapper were successfully produced in large numbers in fertilised ponds, demonstrating that extensive larval rearing of snapper is a viable alternative to traditional intensive culture.

Snapper, in common with other marine fish, occasionally suffer from infestation of the ectoparasite, *Amyloodinium ocellatum.* During this project, this parasite was genetically characterised and an understanding was made of the methods in which infestation can take place within hatcheries. A variety of control methods including chemotherapeutics were evaluated and recommendations for hatchery biosecurity were made.

This research has been transferred to industry through publications and workshops. Three PhD projects were completed during the project, two have been awarded and the last one will be submitted soon.

Although snapper was the focus of early marine finfish farming operations in New South Wales, Western Australia, South Australia and Queensland, more recently, emphasis has shifted to other marine species, mulloway (*Argyrosomus japonicus*) and yellowtail kingfish (*Seriola lalandi*). Much of the research described in this series of reports is relevant to all temperate species of marine fish and research methods used are generally applicable for a range of aquaculture species facing similar constraints.

NON-TECHNICAL SUMMARY

2001/208	Aquafin CRC – Increasing the profitability of snapper farming by improving hatchery practices and diets

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OBJECTIVES:

(1) Improve production of snapper fingerlings by developing extensive, fertilised-pond rearing techniques for the advanced production of snapper juveniles.

(2) Improve production of snapper fingerlings by developing larval feeding strategies to reduce the use of live feeds, in particular *Artemia*, by weaning larvae at an early age onto commercial and/or experimental artificial diets.

(3) Improve production of snapper fingerlings by developing methods to reduce and/or treat the incidence of parasite infestation.

(4) Improve the skin colour of farmed snapper by reducing melanisation and improving skin pigmentation.

(5) Determine digestibility for, and ability of fish to utilize, new ingredients with potential for use in low-polluting snapper diets.

(6) Evaluate ability of snapper to utilize carbohydrate and lipid sources for energy.

(7) Determine optimum protein:energy ratio for fish grown at one favourable temperature.

(8) Provide recommendations for feeding strategies to minimise overfeeding and maximise fish production.

This final report is published in three volumes. **Objectives 5, 6 & 7** pertain to Volume 1: Diet Development; Objective 4 pertains to Volume 2: Skin Colour; Objectives 1, 2, 3 and 8 pertain to Volume 3: Fingerling Production and Health.

The Executive Summary, Background, Need, Objectives, Benefits and Adoption, Further Development and Planned Outcomes are common to all three volumes.

NON TECHNICAL SUMMARY:

OUTCOMES ACHIEVED

This research has increased our knowledge of the nutritional requirements of Australian snapper *Pagrus auratus* and provided information on the potential of Australian feed ingredients to reduce the level of fishmeal in diets for this species. To meet the general and specific aims of this study, a research strategy based on determination of apparent digestibility coefficients (ADCs), establishment of DP:DE requirements, efficacy of high or low protein diets and an understanding of how well different nutrients from key ingredients was adopted.

This part of the project contributed to the Aquafin CRC contract outcome: Support for new and emerging sectors of finfish aquaculture; More cost-effective and environmentally-sustainable feeding strategies and grow-out feeds.

Digestibility coefficients

This research has determined apparent digestibility coefficients (ADCs) for a range of potential feed ingredients at different dietary inclusion levels. This was achieved by applying an indirect method of determination (marker method; chromic oxide) and collecting faecal material from snapper by passive settlement. The digestibility methods we used for the preparation of diets and the collection of faeces was standardised across all experiments in this study, and our results provide reliable information on the digestibility of different feed ingredients by snapper. Similar methods were used by Allan, *et al.* (1999), to investigate the digestibility of an extensive range of feed ingredients for silver perch *Bidyanus bidyanus.* Individual feed ingredients were selected on the basis of their potential for use in snapper diets, either because of an elevated protein or energy content, the perceived ability to provide reasonable levels of essential amino or fatty acids or their ability to provide functional qualities to experimental or commercial diets. Ingredients investigated included those typically used in many carnivorous finfish feeds such as fishmeal and fish oil, but also protein rich rendered by-product meals such as poultry offal meal, meat meal and blood meals. In addition, the ADCs of two forms of soybean meal (expeller vs solvent extracted) were determined, as was the digestibility of extruded wheat and fully gelatinised wheat starch (see Sections 4.1 & 4.3).

The protein component of all individual feedstuffs we tested, with the exception of high-ash meat and bone meal, was extremely well digested. The protein component of low-ash high-protein meat meals was extremely well digested. The organic matter, protein, fat and gross energy from fishmeal or fish oil were highly digestible and these ingredients will remain the benchmark by which other ingredients are judged (see Section 4.1). Excluding ADCs for low ash, meat and bone meal, protein ADC's for all other ingredients ranged from 84.9 to 105.4%. In addition, the protein, fat or gross energy digestibility of rendered animal meals were not affected by the inclusion levels we tested. The protein digestibility of the meat meal was inferior to other protein sources, possibly due to processing damage, and a protein ADC of between 62.2-65.3% was recorded. The gross energy ADC's were also lower for this product. However, when low ash, high protein meat meal products were tested, ADC's were extremely high and similar to fishmeal of similar composition. The protein, fat and gross energy digestibility of all types of meat meal was not affected by the inclusion levels tested in this study.

Carbohydrates offer a cheap source of energy in the diets of finfish and may be of some benefit to carnivorous species such as snapper. For this reason, the digestibility of different levels of extruded wheat (see Section4.1) or fully gelatinised wheat starch was determined (see Section 4.3). Extruded or gelatinised products were selected for evaluation because most modern feed mills employ extruder technology to manufacture aquafeeds. This process invariably gelatinises the majority of raw starch in these feeds, and therefore pre-extruded products serve as useful substitutes for evaluation in experimental cold-pressed feeds. Extruded wheat contains a low level of crude protein (172 g kg^{-1}), but pregelatinised wheat starch is a pure starch product, so serves only as a potential source of energy. Our results demonstrated that protein from extruded wheat was well digested and independent of inclusion level, however, the digestibility of organic matter and gross energy from both ingredients varied inversely with inclusion level.

We proposed that the reduction in digestibility of starch as inclusion levels increased was related to the saturation of the carbohydrate digestive mechanism, as reported for other species. This outcome confirms that gross energy and organic matter ADCs for snapper fed ingredients that contain high levels of starch based CHO are not additive (see Sections 4.1 & 4.5). Consequently, it is imperative to determine gross energy or nutrient ADCs for these types of ingredients over a practical range of inclusion levels before formulating experimental or commercial aquafeeds.

Rapid elevation in circulating levels of plasma glucose coupled with prolonged hyperglycaemia following a glucose tolerance test (GTT) also indicated snapper were intolerant of highly available forms of CHO (see Section 4.3) compared to omnivorous species such as silver perch. However, the uptake of more complex forms of CHO (i.e. gelatinised wheat starch) from the digestive system of snapper appeared to be more regulated and did not cause significant elevations in plasma glucose concentration after 3 h (see Section 4.3). This data suggests that utilisation of CHO by snapper, apart from being affected by the route of assimilation is affected by the complexity of the CHO source, as described for other fish species.

Additivity of apparent digestibility coefficients

Once determined, ingredient ADCs for protein and energy were used throughout this study to formulate experimental diets on a digestible protein (DP) and digestible energy (DE) basis for snapper using a limited range of energy and nutrient sources. This strategy required that the assumption of additivity hold true for the ingredients supplying DP and DE in the experimental feeds we formulated. This assumption was confirmed by the close approximation of formulated versus measured DP and DE values of test diets fed to snapper in Section 4.2, despite the fact that these diets were composed of variable levels of fishmeal, extruded wheat and fish oil (see Section 4.2). Although there were minor differences between the formulated and measured DE values, differences between DP values were greater. Regression of formulated versus measured DP values indicated that the relationship was linear (i.e. measured DP value = 1.005(±0.0285) x formulated DP value – 20.37(±11.34); R^2=0.99), but that measured DP values were consistently lower than formulated values by about 20 units. This is best explained by the fact that a different batch of fishmeal was used in this study to that used to determine the ADC of fishmeal in Section 4.1. Thus, the ADC of protein for the fishmeal used in Section 4.2 is likely to be lower than that used in Section 4.1. However, the linear nature of the relationship between formulated and measured DP values confirms the additivity of protein ADCs for the ingredients used to formulate these diets.

Application of digestibility coefficients

The ingredient composition data and digestibility coefficients presented in this report will improve the accuracy of feed formulation and provide feed manufacturers with practical alternatives to fishmeal. Besides the digestibility of ingredients determined in this study (see Sections 4.1 & 4.3), these alternatives include wheat gluten and lupin kernal meals (*Lupinus angustifolius*), as well as solvent extracted and expeller canola meals, canola protein concentrates and high protein soybean meal (determined by Glencross *et al.*, 2003a, b, c & 2004). Low ash, high protein meat meals (e.g 3-10% ash, 75-88% crude protein) were extremely well digested with ADC's being similar to those for fishmeal of similar composition. Cereal and oilseed based protein concentrates (e.g. wheat, soy, canola) should also be investigated. However, for all ingredients, inclusion contents will be determined by cost, particularly relevant to fishmeal.

Estimating protein and energy requirements

Historically, the quantification of nutrient or energy requirements for different fish species has been undertaken using dose-response studies, where graded levels of nutrients (protein, amino acids, fatty acids, minerals etc) or energy are fed and changes in response variables such as weight gain, protein or energy deposition and feed conversion ratio etc. are recorded for a suitable period. These relationships are then studied by applying *ANOVA* or regression models (linear or quadratic functions; bent-stick models; logistic functions; 4-SKM etc) to experimental data in order to determine nutrient or energy requirements for maintenance and growth. More recently, the application of factorial models or D-optimal design strategies has become prevalent in fish nutrition. Irrespective of the approach selected, estimates of the requirement are highly dependant on the chosen response criteria. The more specific this response (e.g. protein deposition rather than weight gain), the better the estimate of the true-requirement.

Each of these approaches has particular strengths and weaknesses. For example, the classical dose-response approach relies on the premise that the nutrient of interest is the only variable limiting the expression of the response variable. This means that if other nutrients or energy unknowingly become limiting, or interactions exist between ingredients supplying the nutrient of interest, then the true-requirement can be underestimated. Factorial models are based on the assumption that requirements equal the sum of nutrients needed for maintenance, growth and reproductive outputs and excretions. This approach attempts to model the overall response to nutrient intake. However, factorial models also estimate nutrient requirements using a series of smaller dose-response studies and are therefore prone to the same problems encountered in classical studies. In addition, the influence of such factors as genetic make-up, stage of development, activity level and the nutrient density of diets on model parameters is poorly understood. Notwithstanding these problems, provided data are accurate and similar specific response variables are selected for investigation (e.g. protein deposition), differences between requirements obtained using a dose response approach and those determined using factorial models should be minor.

This research has confirmed that snapper, like the majority of marine carnivores, has a high protein requirement. It has also demonstrated that weight gain and protein deposition in juvenile snapper (30-90 g $fish^{-1}$) is highly dependent on the ratio of DP:DE. Application of a four parameter mathematical model for physiological responses (4-SKM) developed by Mercer (1980 & 1982) to the data presented in Section 4.2 indicated juvenile snapper require approximately 28 g DP MJ DE^{-1} to optimise protein deposition and feed conversion ratio (see Section 4.2). This estimate is almost identical to that reported for similar sized gilthead seabream *Sparus aurata* and Australian snapper determined using factorial models. The close agreement between our estimate (e.g. determined using a dose- response approach) and that of the previous authors suggest that factorial models can be used as a basis for formulating diets for fish outside the range we studied (> 90 g) (see Section 4.5). The agreement in DP:DE requirement values and the possibility that snapper might have been protein limited on high-energy, high-lipid diets formed the basis of the experimental design in Section 4.5.

Utilization of carbohydrates and lipid for energy

The major outcomes of experiments described in Sections 4.2 and 4.5 indicate that for snapper fed to apparent satiation, feed intake is primarily governed by the DE content of the diet, as reported for other species (NRC 1993). In this study we have also shown that this is true regardless of whether DE is supplied in the form of protein, lipid or CHO as supported by the similarity in relative feed intake for dietary treatments with DE derived from different sources. Consequently, weight gain in snapper is governed by the DP content of diets and the energy-regulated intake of DP (see Sectiopns 4.2 and 4.5). Secondly, our data indicate that snapper perform better on high-energy, high-protein diets provided a significant proportion of DE is in the form of highly digestible protein. The appropriate level appears to be between 60% (Koshio, 2002) and 68% of total dietary DE (see Section 4.5). The fact that feed conversion ratio (FCR) in snapper from these experiments consistently improved as the level of DP in diets was increased also suggests that protein may be the preferred energy source for this species. Data from Chapter 4 did not provide unequivocal evidence that lipid or CHO energy sources were able to spare protein for growth. However, wide variations in the ratio of lipid and CHO did not significantly affect weight gain and performance in snapper fed the majority of test diets containing similar levels of DP, indicating the utilisation of both energy sources is similar for snapper when based on their DE values. This result demonstrated that these two energy sources can be reliably exchanged within the diet matrix provided inclusion levels and ratios are similar to those tested in the present study.

In terms of weight gain and FCR, productivity of snapper could be increased by using nutrient dense feeds (high protein), although these benefits must be more fully assessed in terms of carcass composition before these specifications can be unequivocally recommended.

An additional series of experiments was undertaken to determine whether addition of potassium to the diet of snapper grown in potassium deficient saline groundwater could negate or reduce the need to adjust potassium by dissolving potassium directly in cuture water. Unfortunately, dietary supplementation of KCl did not improve survival or growth when fish were reared in potassium deficient water. Culture of snapper and probably other marine or estuarine species, requires modified rearing water.

Conclusion

Work presented in this report culminated in two experiments designed to evaluate the potential of poultry offal meal, meat meal, blood meal and soybean meal to partially replace fishmeal in diets for this species. All diets were formulated to contain similar DP and DE levels according to the composition and ADC's of individual feed ingredients. In the semi-commercial pond experiment described in Section 4.4, diet formulations were a compromise between nutritional requirements and the practicalities of manufacturing an extruded aquafeed.

Our results have shown that snapper can tolerate high dietary levels of poultry meal (360 g kg^{-1}), meat meal (345 g kg^{-1}) and soybean meal (420 g kg^{-1}) before performance or feed intake is unduly affected. In addition, the combinations of these three ingredients (and blood meal) were able to effectively replace all but 160 g fishmeal kg^{-1} in commercially manufactured diets for snapper (see Section 4.4), reducing the ingredient cost of production for 1 kg of fish from $AUD2.51 to $AUD2.25 in diets containing 600 or 160 g fishmeal kg^{-1}, respectively. With the ever-increasing price of fishmeal due to escalating demand and static world supply, the relative ingredient cost savings reported by the current research will increase over time.

KEYWORDS:

Diet development; Nutrition; *Pagrus auratus*; Snapper

1. BACKGROUND

This project formed part of the Research Program of the CRC for Sustainable Aquaculture of Finfish ("Aquafin CRC"), and employed funds invested out of the CRC's Commonwealth grant and by FRDC and other participants of the CRC.

When this project was conceived, aquaculture of snapper, *Pagrus auratus*, in Australia was developing and commercial farms were operating in NSW and SA (Fielder, unpublished data). The potential for snapper aquaculture was indicated by the huge (approximately 50 000 t/yr) industry for this species in Japan. Although technology used in Japan formed the basis for early investigation of snapper culture in Australia, development of this infant industry, especially in NSW, had only been possible following research conducted by NSW Fisheries, FRDC ("Potential of snapper *Chrysophrys auratus* for aquaculture" (1989-1992)) and the CRC for Aquaculture (Project No. C4.2) to develop technology for broodstock management, intensive larval rearing, and evaluation of seacages and inland saline ponds for growout, and diet development.

Some key differences between the industry in Australia and Japan have influenced technology transfer and the need for research described in this study. Most importantly, the market price for fish in Japan is approximately three times that received in Australia. This fact lessens the need to reduce production costs which is so important if snapper aquaculture is to reach its potential in Australia. Secondly, the hatchery sector in Japan is based on many generations of domestication. We found that just one generation of domestication with Australian snapper conferred major advantages in inducing spawning by manipulating phototherm regimes and expect that as subsequent generations are reared, advantages will compound. In general, hatchery production in Japan is based on greenwater systems. This is economically viable in Japan and even though extensive pond larval culture may be more cost-effective, many hatcheries in Japan have limited access to sufficient land for extensive ponds (land in general is very expensive in many areas in Japan where red sea bream hatcheries are located).

The red sea bream industry in Japan was built on feeding trash fish. It wasn't until the bait-fish industry in Japanese waters collapsed in the late 1980's and early 1990's that farmers were forced to replace trash fish. As late as 1995, commercial practices were still blending frozen whole fish with a formulated premix. Diet development research in Japan since then has been conducted primarily by private feed companies and is generally not available in the technical or scientific literature.

In Australia, it is now possible to maintain captive broodstock snapper and to spawn high quality eggs on-demand, year-round. Also, intensive rearing of snapper larvae has been improved by identifying the optimum physical parameters such as photoperiod, salinity and temperature (Fielder & Allan, unpublished data). As a consequence, the time to rear snapper larvae to fully-weaned, metamorphosed fish has been reduced by approximately 2 weeks compared with the time taken using previous best-practice techniques. However, intensive larval fish rearing requires high capital and labour inputs as well as operation of facilities to culture live feeds such as rotifers and *Artemia*. These ancillary culture systems can be unreliable and expensive to operate. There is also a world shortage of *Artemia* cysts and purchase price has tripled in the last 12 months. Current industry estimates the cost of production of snapper at $1.00 /fingerling. In comparison, industry costs for producing other marine fingerlings are 34c for barramundi (Lobegeiger, 2001) and about 46c for bass. To improve profitability, there is an obvious need to develop techniques to reduce the cost and improve the vigour of fingerlings. Alternative hatchery methods or live foods and their replacement with artificial diets therefore needed to be identified.

Scientists at Port Stephens Fisheries Centre (PSFC) linked with counterparts at SARDI who regularly breed large numbers of snapper for industry. Advances in larval rearing techniques have

been validated at SARDI facilities. This has been an important conduit for technology transfer for South Australian hatcheries.

Extensive larval rearing, where ponds are fertilised to promote zooplankton populations (McCarty, Geiger, Sturmer, Gregg & Rutledge, 1986; Fielder, Bardsley & Allan, 1999), has been used to rear large numbers of several marine fish species such as red drum *Sciaenops ocellatus* (McCarty, *et al.* 1986), barramundi *Lates calcarifer* (Rutledge & Rimmer, 1991), Australian bass *Macquaria novemaculeata* (Battaglene, Talbot & Allan, 1992) and mulloway *Argyrosomus japonicus* (Fielder, *et al.* 1999). Growth and quality of fish can be high from fertilised ponds and cost of fingerling production can be low due to the need for relatively unsophisticated facilities and low operating costs. However, survival of larvae can be variable due to sub-optimal environmental conditions. In the successful FRDC Project No. 95/148, survival of mulloway larvae in fertilised ponds increased as the age of larvae that were stocked increased (Fielder, *et al.* 1999). These results indicated that a combination of initial intensive rearing from hatch to consumption of *Artemia* (~14 d), when larvae are vulnerable to fluctuations in environmental conditions, followed by on-growing in fertilised ponds can optimise fingerling production. Preliminary trials during the CRC for Aquaculture project C4.2 demonstrated that snapper juveniles could be reared in commercial larval fish ponds; however, survival was low and the power of experiments was very low due to low or no replication. These techniques may be suitable for large-scale production of cheap, high quality, healthy juvenile snapper but this must be verified in rigorous, replicated experiments.

Development of methods to reduce the reliance on *Artemia* as a live feed in intensive culture of juvenile snapper may also decrease the cost of production. Preliminary larval snapper rearing trials have demonstrated that *Artemia* can be replaced completely by extending the period of rotifer feeding and early addition of high quality imported weaning diets (Fielder, unpublished data). Indeed replacement of *Artemia* in aquaculture in Australia is viewed as a priority (McKinnon, Rimmer & Kolkovski, 2000) and FRDC have funded a project (2001-220) "Aquaculture Nutrition Subprogram: development of marine fish larval diets to replace *Artemia*" (PI Dr Sagiv Kolkovski), to investigate production of artificial microdiets for marine fish larvae. This technique has been evaluated in terms of fish quality, survival, reliability and cost of production. Also, replacement of *Artemia* with cultured copepods for marine fish larval rearing has recently attracted significant attention by larval fish culturists and was highlighted as a priority research area at the recent FRDC sponsored Live Feeds Workshop. As an alternative to intensive production of copepods (Rippingale, 1994), it may be possible to produce sustainable cultures of copepods in outdoor ponds, which could then be harvested and fed to snapper larvae in intensive tanks. Hundreds of thousands of juvenile Australian bass have been produced at the PSFC using this method, resulting in complete replacement of both rotifers and *Artemia*.

The research proposed in this application to decrease the cost of fingerling snapper has relevance to many other species of marine finfish. Feeding marine fish larvae is expensive and reducing mortality remains an international priority. Technologies developed and refined here will assist in efforts to reduce fingerling production costs for other species. Facilities at PSFC are unique as they include both large-scale production facilities and replicated smaller-scale facilities for larval rearing (intensive, semi-intensive and extensive). These types of facilities will be especially critical in the pursuit of successful tuna propagation and fingerling production.

New health management strategies are required to minimise the impact of disease in hatcheries. Disease outbreaks reduce vigour of fish and periodically cause excessive mortality resulting in increased cost of fingerling production. Infestations of the ectoparasite *Amyloodinium* sp. in particular have caused significant mortality in Australian snapper hatcheries (Fielder & Allan, unpublished data) and is a major problem in overseas marine fish hatcheries (Paperna, 1983). Strategies to minimise losses include management to exclude or reduce the prevalence of disease, enhancement of fish resistance, and application of new methods of control.

A post-graduate Certificate student at the University of Queensland, Mr James Stopford, recently developed an excellent method to produce large number of *Amyloodinium* parasites in the laboratory. He operated his cultures for at least 10 generations and used the product to conduct preliminary investigations on the parasite. His work provided an excellent foundation for this project.

The cost of purchasing and delivering feeds is the single highest operating cost for most types of fish culture. To farm fish profitably, there is an obvious economic imperative to develop high-performance diets and feeding systems which are cost-effective. The diets also need to produce fish with desirable marketing traits. These traits include skin and flesh colour and flesh composition. To fetch premium prices, snapper need to have pink skin and white flesh. Large deposits of intestinal fat are perceived to be a marketing disadvantage. Fish are marketed as a "healthy" product, largely because fish fat has relatively high contents of the omega-3 highly unsaturated fatty acids. However, while replacing fish meal and fish oil in fish diets may reduce diet cost, and may also reduce P content in the effluent from farms, it will also reduce these health benefits.

There is also an important environmental imperative to minimise the amounts of nutrients and organic matter that are discharged from fish farms. To achieve these goals, diets need to satisfy but not oversupply essential nutrients and be made from high quality, highly digestible, readily obtainable ingredients. Diets also need to stimulate maximum consumption and deliver optimal feed conversion efficiency.

Excretion of nitrogen (N), phosphorus (P) and organic matter (mainly carbon, C) are the major pollutants from fish farms and their release is regulated by environmental protection agencies in many states in Australia. Feed is the sole source of these potential pollutants. To minimise N loss, N (protein) digestibility and retention must be increased. The recommended approach is to optimise protein quality and minimise diet protein content. This might be achieved by optimising the protein:energy ratio and using as much non-protein energy as possible. Strategies to minimise P loss include: selecting ingredients with high P bioavailability and selecting P supplements with high P absorbtion and low water solubility. To reduce organic matter pollution, diets must be highly digestible, promote maximum feed consumption and feed conversion efficiency. Minimising feed wastage through ensuring optimal pellet stability and determining the best feeding frequencies and feeding rates are critical factors in reducing pollution from fish farms.

FRDC currently fund snapper diet development research through project 99/323 "Aquaculture Diet Development (ADD) Subprogram: rapid development of diets for Australian snapper." Research under this project has determined:

1. Effects of shading and dietary astaxanthin source on skin colour. Results have demonstrated that although shading will reduce "skin blackness", shaded, farmed fish are still darker than wild fish and although shading is a viable option for some smaller, inshore fish farms, it is very difficult for large, offshore farms. To harvest genuinely "light" fish, farmers will need to find better ways to prevent melanisation. Addition of astaxanthin in either the free or esterified form increased the intensity of the "red" colour in the skin but this colour was not the highly desired "pink" of wild fish. More needs to be done to evaluate combinations of commercially available pigments to make fish pink and to reduce skin melanisation.

2. Identification of the best available commercial diet for snapper. Previous research under the ADD Subprogram for barramundi demonstrated the cost-effectiveness of nutrient-dense diets. It was expected that these would also benefit snapper but this has not been apparent in results from this commercial diet evaluation. Clearly, snapper have different nutrient requirements to barramundi, indicating that determination of their protein:energy requirements and ability to utilize carbohydrates for energy warrant independent investigation.

3. Protein:energy requirements have been determined at one temperature. This research will commence as soon as digestibility of experimental ingredients is completed.

4. Digestibility and utilization of key ingredients with potential to replace fishmeal and be incorporated into lower cost, high performance diets for snapper. This research is underway and will allow full investigation of digestibility of up to 16 ingredients and utilization of up to 8 ingredients.

5. Preliminary experiment to determine whether ionic deficiencies in inland saline water can be overcome through nutrition supplements. This research is planned to commence within six months.

The nutrition component of this research was fully integrated with FRDC 99/323. Already, the current FRDC project 99/323 addressed important questions and allowed formulation of better diets. However, diet development is an ongoing process. Increased funding for poultry and pig diet development is being allocated each year in recognition of the ongoing importance of feed and feeding to animal husbandry. This additional research on snapper diet development has proven to be a good investment for industry. The research on skin colour has identified the best mix of pigments and reduction of melanisation, beyond reduction possible using shading. Farmers have claimed they could receive up to an extra $3/kg for light and pink snapper. Research on alternative ingredients and on protein:energy requirements has been completed, in light of the apparent dissimilarity of snapper nutritional requirements to those of barramundi.

Research has also focussed on eco-friendly feeds that minimise concentration of N, P and C from uneaten or poorly utilized diets. One of the only ways to reduce N is to provide as much non-protein dietary energy as fish can utilize. The two non-protein dietary energy sources are lipid and carbohydrate. Utilization of these ingredients have been investigated and effects on body composition and taste determined.

Finally, recommendations have been made to optimize feeding strategies to minimize over-feeding.

REFERENCES:

Battaglene, S.C., Talbot, R.B. & Allan, G.L. (1992). Supplementary feeding with brine shrimp, *Artemia*, in the extensive brackish water culture of Australian bass, *Macquaria novemaculeata* (Steindachner). In: Allan, G.L. & Dall, W. (eds.) Proceedings of Aquaculture Nutrition Workshop, 15 April 1991, Salamander Bay, NSW. NSW Fisheries. Pp. 197-198.

Fielder, D.S., Bardsley, W.J. & Allan, G.L. (1999). Enhancement of mulloway (*Argyrosomus japonicus*) in intermittently opening lagoons. NSW Fisheries Final Report Series 14. NSW Fisheries, Sydney. 98 pp.

Glencross, B., Curnow, J., Hawkins, W., Kissil, G.W.M. & Peterson, D. (2003a). Evaluation of the feed value of a transgenic strain of the narrow leaf lupin (*Lupinus angustifolius*) in the diet of the marine fish, *Pagrus auratus*. Aquaculture Nutrition **9**:197-206.

Glencross, B., Hawkins, W. & Curnow, J. (2003b). Evaluation of canola oils as alternative lipid resources in diets for juvenile red sea bream *Pagrus auratus*. Aquaculture Nutrition **9**:305-315.

Glencross, B., Hawkins, W. & Curnow, J. (2004). Nutritional assessment of Australian canola meals. 1. Evaluation of oil extraction method and meal processing conditions on the digestible value of canola meals fed to the red sea bream (*Pagrus auratus*, Paulin). Aquaculture Research **35**:15-24.

Glencross, B., Kolkovski, S., Jones, B., Felsing, M. & Saxby, S. (2003c). Temperate marine fin-fish R&D in Western Australia. In: Allan, G.L. (ed.) Proceedings of the Aquafin CRC Snapper Workshop held on 26 September 2002 at the Airport Motel Convention Centre,

Melbourne (Aquafin CRC 2001/208). NSW Fisheries, Cronulla, NSW, Australia, pp 38-44.

Lobegeiger, R. (2001). Report to Farmers. Queensland Aquaculture Production Survey 1999-2000. Department of Primary Industries, Queensland. Information Series Q101019. 24 pp.

McCarty, C.E., Geiger, L.N., Sturmer, L.N., Gregg, B.A. & Rutledge, W.P. (1986). Marine finfish culture in Texas: a model for the future. In: Stroud, R.H. (ed) Fish culture in fisheries management. American Fisheries Society, Fish Culture Section & Fisheries Management Section, Bethesda, Maryland pp. 249-262.

McKinnon, D., Rimmer, M. & Kolkovski, S. (2000). Hatchery Feeds Research and Development Plan 2000-2005. http/www.aims.gov.au/pages/research/hatchery-feeds/r&d-plan.doc.

Paperna, I. (1983). Reproduction cycle and tolerance to temperature and salinity of *Amyloodinium ocellatum* (Brosn, 1931) (Dinoflagellida). Annales de Parasitologie Humaine et Comparee **50**(1):7-30.

Rimmer, M. & Rutledge, B. (1991). Extensive rearing of barramundi larvae. Information Series Queensland Department of Primary Industries no. Q191012. QDPI, Brisbane. 6 pp.

Rippingale, R.J. (1994). A calanoid copepod *Gladioferens imparipes*, holding to surfaces. Hydrobiologica **292-293**:351-360.

2. NEED

This project extended previous work, which demonstrated the feasibility of snapper farming in both marine and inland saline waters. It sought to reduce production costs by improving fingerling survival and growth and reducing input (feed) costs.

A reliable supply of cheap, high quality, healthy fingerlings is essential for development of viable snapper farming. When this project was conceived industry estimated the cost of production of snapper at $1.00 per fingerling. This compares with less than 35¢ for barramundi fingerlings and about 46¢ for bass. To improve profitability, there is a need to reduce the cost and improve the vigour of fingerlings and to develop cost-effective high-performance diets and feeding systems for both hatchery and grow-out. This need has been recognised through the FRDC sponsored Hatchery Feeds R&D Plan (McKinnon *et al.*, 2000: http://www.aims.gov.au/hatchery-feeds), This project improved hatchery methods and replaced live feeds, such as brine shrimp (*Artemia*) whose supply and quality are unreliable, with alternative live feeds or artificial feeds. The project also developed better strategies for combining intensive and extensive rearing methods so as to optimise fingerling survival and quality. Research will have application for other species, including tuna.

Grow-out diets need to produce fish with desirable marketing traits, including colour. Fish are marketed as a "healthy" product, largely because fish fat has relatively high contents of the omega-3 highly unsaturated fatty acids. However, while replacing fishmeal and fish oil in fish diets may reduce diet costs, it will also reduce these health benefits. Minimising feed wastage through ensuring optimal pellet stability and determining the best feeding frequencies and feeding rates are critical factors in reducing pollution from fish farms. To achieve these goals, diets have been developed that satisfy but not oversupply essential nutrients and that are made from high quality, highly digestible, readily obtainable ingredients. Diets have been designed to stimulate maximum consumption and deliver optimal feed conversion efficiency. This research has built on successful results with snapper diet development under the previous FRDC Aquaculture Diet Development Subprogram snapper diet development project. The nutrition component of this project was fully integrated with the FRDC snapper diet development project.

Finally, the project sought to reduce disease-induced mortality by developing treatment methods for common parasites and establishing a foundation for immunological approaches to fish skin diseases.

3. OBJECTIVES

1. Improve production of snapper fingerlings by developing extensive, fertilised-pond rearing techniques for the advanced production of snapper juveniles.

2. Improve production of snapper fingerlings by developing larval feeding strategies to reduce the use of live feeds, in particular *Artemia*, by weaning larvae at an early age onto commercial and/or experimental artificial diets.

3. Improve production of snapper fingerlings by developing methods to reduce and/or treat the incidence of parasite infestation.

4. Improve the skin colour of farmed snapper by reducing melanisation and improving skin pigmentation.

5. **Determine digestibility for, and ability of fish to utilize, new ingredients with potential for use in low-polluting snapper diets.**

6. **Evaluate ability of snapper to utilize carbohydrate and lipid sources for energy.**

7.
8. **Determine optimum protein:energy ratio for fish grown at one favourable temperature.**

9. Provide recommendations for feeding strategies to minimise overfeeding and maximise fish production.

This final report is published in three volumes. **Objectives 5, 6 & 7** pertain to this **Volume 1: Diet Development;** Objective 4 pertains to Volume 2: Skin Colour; Objectives 1, 2, 3 and 8 pertain to Volume 3: Fingerling Production and Health.

4. RESULTS AND DISCUSSION

4.1 Investigation of the nutritional requirements of Australian snapper *Pagrus auratus* (Bloch & Schneider, 1801): apparent digestibility of protein and energy sources

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ABSTRACT

Two experiments were done to determine the apparent crude protein (CP), organic matter (OM), fat and gross energy (GE) digestibility coefficients (ADCs) of several protein and energy sources (ingredients) for Australian snapper using the indirect method of determination and collection of faeces by passive settlement. The first experiment determined ADCs for one level of fishmeal (500g kg^{-1} diet), three levels of extruded wheat (200, 300 or 400g kg^{-1} diet) and two levels of fish oil (150 or 250g kg^{-1} diet). The second experiment determined ADCs for two levels each of meat meal or poultry meal (300 or 500g kg^{-1} diet), one level each of haemoglobin powder or blood meal (150g kg^{-1} diet) and one level each of solvent extracted soybean meal or a low-allergenic, cold pressed soybean meal (300g kg^{-1} diet). Similar ingredients and where appropriate, different inclusion levels were compared using one or two-way ANOVA. Fishmeal was almost completely digested and ADC values ranged between 94.3 and 99.2%. Fish oil was also well digested with ADC values ranging between 97.6 and 106.0% and was not significantly affected by inclusion level. Linear regression analysis indicated that there was no relationship between the inclusion level of extruded wheat and either CP (ADCs ranged from 100.1 to 105.4%) or fat digestibility (ADCs ranged from 89.1 to 104.4%). However, there was a significant negative linear relationship between the inclusion level of extruded wheat and GE digestibility (GE_{ADC} = 86.51 – 0.031 x inclusion level; R^2=0.49). Two-way ANOVA indicated that CP, OM and GE ADCs of poultry meal (i.e. 85.9, 89.7 and 91.3% respectively) were significantly higher than those determined for meat meal (i.e. 63.8, 63.4 and 71.3% respectively), but ADCs were not affected by inclusion level or the interaction between inclusion level and ingredient type. The fat digestibility coefficients of meat and poultry meal were not significantly different (ADCs ranged from 92.3 to 95.0%). The CP digestibility of haemoglobin powder (95.1%) was significantly higher than that of ring dried blood meal (81.6%), but there was no difference between the digestibility of OM (77.0%) or GE (80.4%) from these products. There was no difference between the CP (88.9%), OM (56.9%) and GE (65.6%) digestibility of the solvent extracted soybean meal and the low allergenic, cold pressed soybean meal. These coefficients will be useful in formulating both practical and research based diets for this species.

1. INTRODUCTION

Fishmeal and fish oil remain the most important protein and energy sources in formulated diets for carnivorous fin fish (Tacon & Forster, 2001; Coutteau, Ceulemans, Van Halteren & Robles, 2002; Tacon, 2003). In 2000, the production of compound aquafeeds was estimated to have consumed about 2.41 million tonnes of fishmeal and 0.55 million tonnes of fish oil, or approximately 35 and 41% of the total global production of fishmeal and fish oil respectively (Tacon, 2003). Of the total consumption in 2000, production of carnivorous fin fish species such as salmon, trout, marine fish and eels accounted for 58% of the fishmeal and 88% of the fish oil. The next largest consumers

were marine shrimp and feeding carp which together accounted for about 33% of the remaining fishmeal and 7% of the remaining fish oil (Tacon, 2003). Although there is no evidence that the increasing use of these resources in aquaculture diets is affecting fishmeal or fish oil production (Pike & Barlow, 2003), the finite supply of fishmeal and fish oil underscores the urgency to find alternative protein and energy sources for use in aquatic diets (Tidwell & Allan, 2001).

Practical and informed reductions in the content of fishmeal in aquaculture diets can only be made after the digestibility coefficients of alternative ingredients are determined (Cho, Slinger & Bayley, 1982; Bureau, Kaushik & Cho, 2002). Ideally, these coefficients should be determined over a range of inclusion levels. For carnivorous species, initial focus will be on terrestrial-based ingredients that offer high protein and energy contents. These may include rendered products such as meat and bone meals, blood meal, poultry offal and feather meals, industrial tallows, feed grade fats (Tacon & Forster, 2001) and soybean meal. In some cases, the investigation of protein concentrates may be warranted (Refstie & Storebakken, 2002). The need to reduce the overall cost of feeds by limiting the use of high cost proteins will also mean that the digestibility of cheaper carbohydrate sources such as wheat and other cereal grains or by-products should be investigated. This is particularly important for the carnivorous species, which generally have a lower tolerance for these types of energy sources (Wilson 1994; Sargent, Tocher & Bell, 2002).

Pagrus auratus (Australian snapper), a marine carnivore that is closely related to the Japanese red sea bream *P. major* (Paulin, 1990; Tabata & Taniguchi, 2000), has been grown commercially in Australian waters for nearly a decade. Initial interest in the intensive production of this species was fostered by its high market price and the successful overseas culture of the red and gilthead sea breams *Sparus auratus* (Bell, Quartararo & Henry, 1991). Between them, these two sparids account for about 17% of global marine finfish production (combined production equalled 137 kt in 1998; Tacon & Forster, 2001). By comparison, annual production of Australian snapper, which is presently limited to small sea-cage operations on the east coast of New South Wales (NSW) and South Australia (SA) fluctuates between about 20 to 40 metric tonnes. Earlier studies with snapper recognised that, unless low cost, locally available alternatives to fishmeal could be identified, the development of a large-scale Australian industry based on this species was unlikely (Quartararo, Bell & Allan, 1998a). This need is still paramount, primarily because large volumes of fishmeal will not be produced in Australia. Consequently, the cost of incorporating imported fishmeal into locally produced aqua-feeds links Australian feed manufacturers to the global supply of fishmeal and the volatility of foreign exchange rates (Akiyama & Hunter, 2001). Independence from these economic constraints will only be possible when suitable feed alternatives are identified.

Despite the promise of the Australian snapper industry, little has been done in regard to diet development (Quartararo, Allan & Bell, 1998b) and farmers still rely on feeds formulated specifically for Atlantic salmon *Salmo salar* or barramundi *Lates calcarifer*. More recently, specific digestibility coefficients for snapper fed Australian lupin and canola meals have been published (Glencross, Crunow, Hawkins, Kissil & Peterson, 2003; Glencross, Hawkins & Curnow, 2004). In contrast, digestibility coefficients for a diverse range of ingredients have been published for the red sea bream (Yamamoto, Akimoto, Kishi, Unuma & Akiyama, 1998) and the gilthead sea bream (Nengas, Alexis, Davies & Petichakis, 1995; Lupatsch, Kissil, Sklan & Pfeffer, 1997). These data serve as useful comparisons, however it is imperative that specific digestibility coefficients for snapper are determined within the context of the local industry, especially with regard to the type, quality and supply of ingredients. In this study, we determined the apparent digestibility coefficients of several protein and energy sources for snapper. The apparent digestibility of ingredients of greater interest was investigated at different inclusion levels.

2. MATERIALS AND METHODS

Two experiments were done to determine the apparent digestibility coefficients (ADCs) of several protein and energy sources for Australian snapper using the indirect method of determination and collection of voided faeces by passive settlement. The first experiment (Exp.1) determined ADCs for one level of fishmeal (500g kg^{-1} diet), three levels of extruded wheat (200, 300 or 400g kg^{-1} diet) and two levels of fish oil (150 or 250g kg^{-1} diet). The second experiment (Exp.2) determined ADCs for two levels each of meat meal or poultry meal (300 or 500g kg^{-1} diet), one level each of haemoglobin powder or blood meal (150g kg^{-1} diet) and one level each of solvent extracted soybean meal or a low-allergenic, cold pressed soybean meal (300g kg^{-1} diet). Different levels of extruded wheat were combined with a reference diet composed mainly of fishmeal in order to reduce possible interactions between carbohydrate test ingredients and carbohydrates in the reference diet. All other test ingredients were combined with a reference diet based on equal contents of fishmeal and extruded wheat.

2.1 Diets

Ingredients used in this study were obtained from local and interstate livestock feed providers or feed ingredient specialists (Table 1). In each experiment, dry ingredients, vitamin and mineral premixes and the inert marker were combined on a dry matter basis according to the formulations presented in Tables 2 and 3. Each dietary mash was then thoroughly mixed (Hobart Mixer: Troy Pty Ltd, Ohio, USA) before being finely ground in a laboratory scale hammer mill fitted with a 1.5 mm screen (Raymond Laboratory Mill, Transfield Technologies, Rydalmere, NSW, Australia). The ground mash was thoroughly re-mixed and fortified with 1.0g vitamin C kg^{-1} (Rovimix® Stay-C® 35; F. Hoffman-La Roche, Basel, Switzerland) before being combined with wet ingredients (distilled water and fish oil) and formed into sinking 3.0mm pellets using a meat mincer (Barnco Australia Pty Ltd, Leichhardt, NSW, Australia). Moist pellets were then dried for about six hours (≈ 35º C) in a convection drier until moisture contents were < 100g kg^{-1} diet. Following preparation, all diets were stored frozen at < -15º C until required.

2.2 Facilities

Twenty-seven purpose built 170L cylindro-conical digestibility tanks were used to house fish and collect faeces. A detailed description of this system is given in Allan, Rowland, Parkinson, Stone & Jantrarotai, (1999). Digestibility tanks were housed inside an insulated laboratory where temperature (22-26ºC) and photo-period (12L:12D; incandescent lighting) were controlled. Briefly, digestibility tanks consisted of an upper tank with a sloping base (35º) that was connected to a removable lower settlement chamber separated by a 6mm mesh screen and a 50mm ball valve. The lower chamber terminated in a 250mm length of silicone tubing closed off with a plastic clamp which collected uneaten feed or faecal material. Each digestibility tank was supplied continuously with pre-heated, particle-filtered water at a flow rate of approximately 1L min^{-1}. The effluent water from each tank then flowed to a common collection point where about 25% was directed to waste. The remainder was collected in a ground level sump and pumped through a sand and cartridge filter (10-15 µm) to a raised biological filter before being recirculated to the laboratory via gravity flow. Pre-filtered and disinfected replacement water was stored in a 47 kL reservoir tank and pumped into the biological filter on demand. Each digestibility tank was fitted with a prismatic polycarbonate lid, a 12 h mechanically operated belt-feeder (AGK, Wallersdorf, Germany) and two fine bubble air-stone diffusers.

Water quality was monitored daily in both experiments using one of two hand held water quality analysers; either a Model 611 (Yeo-Kal Electronics, Brookvale, NSW, Australia) or a Horiba U-10 (Horiba, Japan). Total ammonia [NH_3 + NH_4^+] was monitored daily in one randomly selected experiment tank and from the laboratory influent using a rapid test kit procedure (Model

1.08024.0001, E. Merck, Darmstadt, Germany). During the faecal collection phase of Exp.1, temperature, dissolved oxygen (DO_2), salinity and pH ranged from 21.8-25.9º C, 4.3-6.9mg L^{-1}, 3.4-3.6% and 7.4-8.1 units respectively with always < 0.2mg L^{-1}. Recorded values for Exp.2 in the same order were, 23.7-25.3º C, 4.0-6.5mg L^{-1}, 3.4-3.6% and 7.7-8.1 units respectively with [NH_3 + NH_4^+] always < 0.4mg L^{-1}.

2.3 Fish

Snapper used in both experiments were progeny of first generation brood-stock held at the NSW Fisheries Port Stephens Fisheries Centre (PSFC). Prior to use in these experiments, snapper were grown at low densities in large 10kL tanks and fed twice daily on a commercial barramundi Lates calcarifer feed (Ridley Aqua-Feeds, Narangba, Qld, Australia; reported nutrient composition: 50% crude protein; 12% crude fat; 18.0MJ kg^{-1} gross energy). A prophylactic formalin bath (200mg formaldehyde L^{-1}) was given to all fish to reduce parasitic loads before they were transferred to the digestibility laboratory. Snapper were anaesthetised prior to handling (20-30mg L^{-1} ethyl-ρ-aminobenzoate), then weighed individually or in small groups and placed into experiment tanks. Each tank was stocked with seven fish (Exp.1, range 59.7 – 73.8g $fish^{-1}$; Exp.2, range 85.3 – 106.4g $fish^{-1}$) that were re-weighed at the conclusion of each experiment.

2.4 Feeding and collection of faeces

In each experiment, individual dietary treatments were randomly assigned to three digestibility tanks (n=3). Snapper were then acclimatised to their test diets and experimental conditions for 16 (Exp.1) and 7 days (Exp.2) respectively before collection of faeces. All diets were offered once a day in excess of normal feed requirements for a period of approximately 3h between 0830 and 1130h. The unrestricted delivery of test diets was maintained by ensuring that a quantity of uneaten pellets was always present in the collection chambers at the end of each feeding period. After feeding had ceased, all belt feeders were checked, air stones were removed and the walls and floors of the upper tanks were carefully cleaned to dislodge uneaten feed or faeces. Upper tanks were then left undisturbed for about 45 minutes to allow all suspended material to settle out of the water column. Subsequently, the lower collection chambers were isolated from the upper tanks by closing the ball valves, removed and thoroughly brushed and washed clean. A significant volume of water was then flushed from upper tanks to dislodge any settled material before the faecal collection chambers were refitted and packed in ice. Air stones were replaced. Faeces were allowed to settle overnight (≈ 18 h) and were removed the following morning prior to feeding. Daily tank samples were centrifuged at 3000 rpm (1000 g) for 25 min (Multex; MSE Limited, Beckenham, Kent, UK) after which the supernatant liquid was discarded and the faecal matter frozen (< -15º C). Daily faecal collections from individual tanks were pooled until a sufficient quantity was obtained for chemical analyses. Afterwards, faecal samples were dried in vacuum dessicators (-70 mm Hg) at room temperature using silica gel as a dessicant. The dried samples were then finely ground (Waring model 32 BL 80, New Hartford, Connecticut, USA) and dispatched to individual service providers for chemical analyses. Exp.1 and Exp.2 were run for a total of 30 and 23 days, respectively.

2.5 Chemical analyses

Analyses of crude protein (NX6.25; Kjeldahl nitrogen), moisture (105º C for 16 h), ash (550º C for 2 h) and chromium oxide (ICP-MS analysis) in ingredient, diet and faecal samples was done by the CSIRO Analytical Services Facility (CSIRO-Livestock Industries, Indooroopilly, QLD, Australia). Analyses of moisture and total fat (modified Bligh-Dyer method) was done by the State Chemistry Laboratory (SCL) (Victoria Agriculture, Werribee, VIC, Australia) and gross energy (bomb calorimetry) was determined by the Pig and Poultry Production Institute (SARDI) (Henley Beach, SA, Australia) on sub-samples drawn from those prepared by SCL.

2.6 *Apparent digestibility calculations*

Apparent digestibility coefficients (ADC) for reference and test diets were calculated according to equation:

$$ADC\ (\%) = 100\ X\ [1 - (\ F / D\ x\ D_{Cr} / F_{Cr})] \qquad \text{Equation 1.}$$

where F = % nutrient or gross energy in faeces; D = % nutrient or gross energy in diet; D_{Cr} = % chromic oxide in diet; F_{Cr} = % chromic oxide in faeces (Cho, *et al.* 1982).

To account for differences between the nutrient or gross energy content of the reference diet and individual test ingredients as well as variations in inclusion contents, the ADCs of ingredients were calculated according to the following equation:

$$ADC_{ING}\ (\%) = [(Nutr_{TD}\ X\ AD_{TD}) - (P_{RD}\ X\ Nutr_{RD}\ X\ AD_{RD})] / [(P_{ING}\ X\ Nutr_{ING})] \quad \text{Equation 2.}$$

where ADC_{ING} = apparent digestibility of nutrient or gross energy in the test ingredient; $Nutr_{TD}$ = the nutrient or gross energy concentration in test diet; AD_{TD} = the apparent digestibility of the nutrient or gross energy in the test diet; P_{RD} = proportional amount of reference diet; $Nutr_{RD}$ = the nutrient or gross energy concentration in the reference diet; AD_{RD} is the apparent digestibility of nutrient or gross energy in the reference diet; P_{ING} = proportional amount of test ingredient; $Nutr_{ING}$ is the nutrient or gross energy concentration in the test ingredient (Sugiura, *et al.* 1998).

2.7 Statistical analyses

One-way analysis of variance (ANOVA) was used to compare the effect of diet on the SGR of fish within each experiment. Due to similarities in experimental design, one-way ANOVA was also used to compare the digestibility coefficients of diet Ref. 2 between experiments. Linear regression analysis was used to examine the effect of inclusion level on the ingredient digestibility of extruded wheat, whereas the effect of inclusion level on the digestibility of fish oil (Exp.1), or products derived from blood meal or soybean meal were compared with one-way ANOVA (Exp.2). Two-way ANOVA was used to examine the effects of ingredient type and inclusion level on the digestibility of meat meal and poultry meal (Exp. 2). Prior to conducting ANOVA, data were tested to ensure that treatment variances were homogenous (Cochran's test). The significance level for all ANOVA and multiple comparisons tests (Student Newman Keul's) was set at 0.05 and data were statistically analysed using Statgraphics Plus, version 4.1 (Manugistics Inc., Rockville, MD, USA, 1998).

3. RESULTS

Snapper remained healthy and increased in weight in both Exp.1 and Exp. 2 (Tables 4 & 5). SGR was lowest for snapper fed the diets containing 50% poultry meal (Exp. 2) and 25% fish oil (Exp. 1), however treatment variances were large and one-way ANOVA could not detect significant differences between diets within each experiment. No fish died in Exp.1, but one replicate from the reference treatment in Exp. 2 was lost mid-way through the faecal collection phase. Enough faeces were collected from this replicate to determine the concentrations of chromic oxide, crude protein and ash, but gross energy and fat could not be determined. Consequently, where indicated in Table 5, data values were calculated from two replicate tanks.

The crude protein and gross energy digestibility of diet Ref. 2 was significantly higher in Exp. 1 than in Exp. 2 ($P<0.05$) while the organic matter and fat digestibility of diet Ref. 2 was similar in both experiments ($P>0.05$) (Tables 4 & 5).

The protein digestibility of fishmeal (batch 1) was high and by way of comparison, was very similar to the protein ADC of diet Ref 1 which was composed of 90% fishmeal. In addition, the organic matter, fat and gross energy from this batch of fishmeal was almost completely digested. Fish oil was completely digested and not affected by the inclusion contents tested in this study (P>0.05) (Table 4).

Linear regression analysis indicated there was a non-significant relationship between the inclusion level of extruded wheat and either crude protein (df=1,7; F=0.01, P=0.934), or fat digestibility (df=1,7; F=2.60, P=0.151). However, there was a significant negative linear relationship between the inclusion level of extruded wheat and gross energy digestibility (df=1,7; F=6.69, P=0.036; gross energy$_{ADC}$ = 86.506 – 0.0308 x inclusion level; R^2=0.49). A similar relationship occurred for organic matter digestibility, but the relationship was only significant at the 90% confidence interval (df=1,7; F=4.55, P=0.070; organic matter$_{ADC}$ = 82.26 – 0.0312 x inclusion level; R^2=0.39); both coefficients gradually decreased as the dietary content of extruded wheat was increased from 200 to 400g kg^{-1}.

The crude protein digestibility of haemoglobin powder was significantly higher than that of blood meal (P<0.05), but there was no difference between the digestibility of organic matter and gross energy from these products (Table 5). The crude protein digestibility of haemoglobin powder was about 5% (absolute units) higher than any other protein source in Exp.2 and was comparable to that of fishmeal in Exp.1 (≈ 95%). There was no difference between the digestibility of the solvent extracted soybean meal and the low allergenic, cold pressed soybean meal (P>0.05; Table 5).

Two-way ANOVA indicated that the crude protein, organic matter and gross energy ADCs of poultry meal were significantly higher than those determined for meat meal (P<0.001 for each ADC) by approximately 22, 26 and 19% (absolute units; n=6) respectively. However, the crude protein, organic matter, fat and gross energy ADCs for meat and poultry meals were not significantly affected (P>0.05) by inclusion level (300 or 500g kg^{-1}) or the interaction between inclusion level and ingredient type (P>0.05). The fat digestibility coefficients of meat and poultry meal were not significantly different (P>0.05).

We note that all mean protein and one fat ADC for extruded wheat and all organic matter and one gross energy ADC for fish oil were greater than 100% (Table 4). Digestibility coefficients greater than 100% or lower than 0% may indicate that interactions between the reference diet and these test ingredients was occurring (Sugiura, *et al.* 1998). However, given the numerical sensitivity of Equation 2, the digestibility coefficients we present which are slightly greater than 100% more likely reflect the propagation of analytical and experimental errors (Table 4).

4. DISCUSSION

4.1 Digestibility of energy sources

4.1.1 Fish oil

Fish oil was all but completely digested by snapper (Table 4). It is generally acknowledged that fish oils are well digested by most fish species and lipid ADCs are usually equal to or above 80% (NRC, 1993). Rainbow trout *Oncorhynchus mykiss* digest fish oil efficiently (Cho & Kaushik, 1990) as does the omnivorous silver perch *Bidyanus bidyanus*, for which dry matter and lipid ADCs of cod liver oil were 106 and 122% respectively when included at 200g kg^{-1} diet (Allan *et al.*, 1999). The high digestibility of fish oil coupled with its excellent n-3HUFA content (20-25% of total as lcHUFA; Lovell, 1989) makes it an invaluable source of dietary energy for marine fin fish, capable of supplying up to 38MJ digestible energy kg^{-1}.

4.1.2 Extruded wheat

Extruded wheat appears to be a particularly good energy source for snapper according to proximate digestibility, however its DE value is still less than half that of fish oil (Table 6) which will reduce its use in energy dense diets. Most notable in our evaluation of extruded wheat was the significant reductions in gross energy ($P<0.05$) and organic matter ($P<0.10$) digestibility as the inclusion content of this product was increased from 20% to 40%, reductions that could be reliably described by linear regression. These reductions in digestibility may be related to increased levels of dietary fibre, a response which is documented by others (Hilton, Atkinson & Slinger, 1983), or simply indicate that the carbohydrate digestive mechanism in snapper was progressively overwhelmed as inclusion levels increased.

Other sparids seem relatively capable of digesting CHO from cereal grains. In a factorial study to evaluate the effects of starch form (native or gelatinised) and starch inclusion content in gilthead sea bream, Georgopoulos and Conindes (1999) found that dietary CHO and energy digestibility increased significantly when similarly formulated diets substituted equivalent amounts of native starch for gelatinised starch (i.e. 10, 20 & 30% of diet). These authors found energy digestibility increased significantly as either native or gelatinised starch levels were increased from 10 to 20% of the diet but either plateaued or fell when inclusion levels reached 30%. Another experiment with gilthead sea bream investigated the digestibility of diets containing 40% raw as opposed to extruded wheat (Venou, Alexis & Fountoulaki, 2003). Of particular interest in that study was that gross energy and CHO digestibility of diets containing raw wheat were high and almost identical to those of their test diet containing extruded wheat (90-92%). This result agrees with the findings of Lupatsch, *et al.* (1997), who determined the ingredient ADCs of raw wheat flour (231g kg^{-1} of diet) fed to gilthead sea bream (crude protein, CHO and gross energy ADCs were 82, 77 & 88% for respectively), indicating that sparids (including snapper) may have an inherent capacity to digest moderate levels of dietary carbohydrate. This result contrasts with that of red drum *Sciaenops ocellatus*, which could efficiently digest the protein (96.8%) and lipid (97.9) from raw wheat but not the organic matter (46.9%) or gross energy (61.6%) (Gaylord & Gatlin III, 1996).

In the current study, neither the protein nor fat digestibility of extruded wheat was affected by inclusion content. However, Hajen, Higgs, Beames and Dosanjh, (1993) showed that increasing the level of extruded wheat from 15 to 30% in diets for chinook salmon *Oncorhynchus tshawytcha* reduced protein and organic matter digestibility by about 3% (absolute units), a consequence they attribute to an increase in faecal nitrogen production. Cheng and Hardy (2003) also recorded significant reductions in protein and fat digestibility of extruded wheat and in protein digestibility of extruded barley compared to the raw products for rainbow trout *Oncorhynchus mykiss*. While not significant at the 95% confidence interval, there was a downward trend in fat digestibility for snapper as levels of extruded wheat were increased (Table 4). A 1.8-2.6% (absolute units) improvement in protein digestibility of extruded versus malt protein flour was recorded for red sea bream (Yamamoto, *et al.* 1998).

4.2 Digestibility of protein sources

4.2.1 Fishmeal

In agreement with results for most fin fish species, the high digestibility of fishmeal was confirmed for snapper. Its capacity to supply diets with high levels of digestible protein (719g DP kg^{-1}; Table 6) containing a well balanced suite of essential amino acids (Hardy & Barrows, 2002) will mean it will remain an important source of protein for this species.

The protein digestibility recorded in our study was similar to the apparent and true protein digestibility coefficients (92.9-96.4%) cited for fingerling red sea bream fed a high quality white

fishmeal (Yamamoto, *et al.*, 1998). Digestibility of the fishmeal used in our study is also similar to other closely related species which determined digestibility coefficients using settlement techniques. For example, Fernandez, Miquel, Cumplido, Guinea and Ros, (1996) reported that carbon and nitrogen digestibility of a diet composed predominantly of fishmeal (910g kg^{-1} diet) by gilthead sea bream was 85.6% and 92.3% respectively. Glencross, *et al.* (2003), using indirect settlement techniques, found that 100g snapper digested slightly less of the crude protein (87.5%) and the gross energy (87.8%) from a commercial source of fishmeal incorporated at similar levels to that used in our study (e.g. 500g kg $diet^{-1}$). This difference is best explained by differences between the fishmeals used and the experimental conditions applied (Bureau, *et al.* 2002). Lupatsch *et al.* (1997) recorded lower ADCs for a high protein fishmeal (70%) fed to gilthead sea bream than that of snapper in our study, but they determined ADCs by stripping faeces (ADCs for crude protein, crude lipid and gross energy were 83, 95 and 80% respectively). Fernandez, *et al.* (1996) also determined the digestibility of a fishmeal diet using stripping techniques and found that ADCs for stripped faeces were as much as 15-30% lower than those collected via settlement. This has certain implications for the present study, because the degree of leaching occurring in snapper faeces collected under our protocols is unknown. However, the debate about different faecal collection techniques continues and it is generally acknowledged that all methods introduce particular types of errors (Bureau, *et al.* 2002). Of major importance is that the ingredient digestibility coefficients used by feed formulators for the same species are determined under similar experimental conditions (Allan, Parkinson, Booth, Stone, Rowland, Frances & Warner-Smith, 2000; Bureau, *et al.* 2002).

4.2.2 Meat and poultry meals

The protein and energy digestibility of poultry meal was approximately 20% (absolute units) higher than similar inclusion levels of meat meal (Table 5) and reflects similar differences between the digestibility of meat and poultry meal products tested in one study with gilthead sea bream (Nengas, *et al.* 1995). In contrast, organic matter, protein, lipid and energy digestibility were all lower for a poultry by-product meal than a meat and bone meal fed to red drum *Sciaenops ocellatus* (Gaylord & Gatlin III, 1996), while others have found that the protein (80%) and gross energy (78%) digestibility of meat and poultry meals were almost similar (Lupatsch, *et al.* 1997). The higher gross energy digestibility of poultry meal in our study is likely related to the fact that the fat content of poultry meal was almost twice that of meat meal and it contained slightly more crude protein. Poultry meat meals were well digested by salmonids (Sugiura, *et al.* 1998; Cheng & Hardy, 2002) and if processed correctly, appear to offer a real alternative to lower grade fishmeals due to a favourable amino acid profile (Cheng & Hardy, 2002).

The low protein and organic matter digestibility of the meat meal tested in our study may indicate that the rendering process had over-heated or damaged the protein in some way. Nengas, *et al.* (1995) found that the protein digestibility of several different meat meal products that had been subjected to different processing conditions ranged between 35-72.2% and gross energy digestibility ranged between 14-69.2%, indicating that the composition and processing employed in the manufacture of these products can have significant impacts on digestibility.

The inclusions levels of meat and poultry meal we tested did not affect gross nutrient or energy digestibility in snapper and reflect the results of Stone, Allan, Parkinson & Rowland (2000), who found no difference between the digestibility of various contents of meat meal in diets for the omnivorous silver perch *Bidyanus bidyanus*. However, Pfeffer, Kinzinger and Rodehutscord, (1995) found small but significant differences between the protein and lipid digestibility of poultry meal included at 250 and 500g kg^{-1}. Poultry meal also appears to be well utilised by snapper and other sparids, with several growth studies indicating that high levels of fishmeal replacement are possible (Quartararo, *et al.* 1998b; Nengas, Alexis & Davies, 1999; Takagi, Hosokawa, Shimeno & Ukawa, 2000).

4.2.3 Blood meals

Digestibility of blood meals was high, particularly the haemoglobin powder meal which had a similar protein digestibility coefficient to that of fishmeal (95%). In fact, protein digestibility of the haemoglobin powder closely reflected the pepsin digestibility coefficient cited for this product (% pepsin dig. = 97.4). Protein, organic matter and energy digestibility of the ring dried blood meal was lower than that of poultry meal which may indicate this product had been heat damaged during the processing stage. Reported coefficients for blood meal products vary considerably. For example, protein and energy digestibility of a steam dried blood meal (ruminant origin) was as low as 46% and 58% respectively for gilthead sea bream in one study (Nengas, *et al.*, 1995), but as high as 90% and 83% for a spray dried product tested in another (Lupatsc,h *et al.*, 1997). Other processes appear to affect the protein digestibility of blood meal including the application of organic acid preservatives (Laining, Rachmansyah, Ahmad & Williams, 2003). A recent investigation of blood meal inclusion contents on the digestibility and performance of the Murray cod *Maccullochella peelii peelii* found that both digestibility and performance decreased as the levels of blood meal were increased from 8 to 32% of the diet (Abery, Gunasekera & de Silva, 2002).

4.2.4 Soybean meals

The digestibility of protein, energy and organic matter from the solvent extracted soybean meal tested in our study was approximately 7-8% higher than for a similarly treated product fed to snapper by Glencross, *et al.* (2004), a difference most probably related to the different feeding and collection methods they used. Protein digestibility was similar to that cited for red sea bream (Yamamoto, *et al.*, 1998), gilthead seabream (Lupatsch, *et al.* 1997) and rainbow trout (Pfeffer, *et al.* 1995; Sugiura, Dong, Rathbone & Hardy, 1998). However, the organic matter and gross energy digestibility of soybean meals tended to be lower and was similar to that cited for a dehulled soybean meal fed to red drum (Gaylord & Gatlin III, 1996). One possibility for these low values is the presence of indigestible hull material (non-starch polysaccharides; NSP). For example, gilthead sea bream digested about the same amount of protein (90%) from an undecorticated, hexane extracted soybean meal as snapper, but digested only 45% of the gross energy (Nengas, *et al.* 1995).

The major difference between the soybean meal products we tested was the method of lipid extraction. Anecdotally, hexane residues have been implicated in negative effects with solvent extracted soybean meal. However, although there were minor differences in digestibility of the products in our study, they were not significantly different. Likewise, a study on the inhibition of digestive proteases in gilthead sea bream found that there was no difference between in-vitro assays testing either a raw or solvent extracted soybean meal (Alarcon, Moyano & Diaz, 1999). Both products inhibited approximately 41% of digestive enzyme activity. In complete contrast, a recent digestibility study with snapper fed either an expeller versus solvent extracted canola meal found that protein (93.6% vs 83.2%), energy (61.6% vs 43.9%) and organic matter (52.7% vs 19.6%) digestibility coefficients varied considerably (Glencross, *et al.* 2004). These authors also implicated NSP as a possible cause for the low coefficients observed in canola meals and is obviously an area that needs further investigation.

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REFERENCES

Abery, N.W., Gunasekera, R.M. & De Silva, S.S. (2002). Growth and nutrient utilisation of Murray cod *Maccullochella peelii peelii* (Mitchell) fingerlings fed diets with varying levels of soybean meal and blood meal. Aquaculture Research **33**:279-289.

Akiyama, D. & Hunter, B. (2001) .A review of the Asian aquafeed industry. International Aquafeed Directory & Buyers Guide **2001**:36-38.

Allan, G.L., Rowland, S.J., Parkinson, S., Stone, D.A.J. & Jantrarotai, W. (1999). Nutrient digestibility for juvenile silver perch *Bidyanus bidyanus*: development of methods. Aquaculture **170**:131-145.

Allan, G.L., Parkinson, S., Booth, M.A., Stone, D.A.J., Rowland, S.J., Frances, J. & Warner-Smith, R. (2000). Replacement of fishmeal in diets for Australian silver perch, *Bidyanus bidyanus*: I. Digestibility of alternative ingredients. Aquaculture **186**:293-310.

Alarcon, F.J., Moyano, F.J. & Diaz, M. (1999). Effect of inhibitors present in protein sources on digestive proteases of juvenile sea bream (*Sparus aurata*). Aquatic Living Resources **12**(4):233-238.

Bell, J.D., Quartararo, N. & Henry, G.W. (1991). Growth of snapper, *Pagrus auratus*, from south-eastern Australia. New Zealand Journal of Marine and Freshwater Research **25**:117-121.

Bureau, D.P., Kaushik, S.J. & Cho, C.Y. (2002). Bioenergetics. In: Fish Nutrition 3rd Edition (ed. by J.E Halver & R.W. Hardy), pp. 2-59. Academic Press, London UK.

Cheng, Z.J. & Hardy, R.W. (2002). Apparent digestibility coefficients of nutrients and nutritional value of poultry by-product meals for rainbow trout *Oncorhynchus mykiss* measured in-vivo using settlement. Journal or the World Aquaculture Society **33**(4):458-465.

Cheng, Z.J. & Hardy, R.W. (2003) Effects of extrusion processing of feed ingredients on rainbow trout (*Oncorhynchus mykiss*). Aquaculture Nutrition **9**:77-83.

Cho, C.Y., Slinger, S.J. & Bayley, H.S. (1982). Bioenergetics of salmonid fishes: energy intake, expenditure and productivity. Comparative Biochemistry & Physiology **73B**(1):25-42.

Cho, C.Y. & Kaushik, S.J. (1990). Nutritional energetics in fish: energy and protein utilisation in rainbow trout *Oncorhychus mykiss*. World Review of Nutrition and Dietetics **61**:132-172.

Coutteau, P., Ceulemans, S., Van Halteren, A. & Robles, R. (2002). Fishmeal and fish oil in aquafeeds; how narrow is the bottleneck for marine fish? International Aquafeed Directory & Buyers Guide **2002**:20-24.

Fernandez, F., Miquel, A.G., Cumplido, L.R., Guinea, J. & Ros, E. (1996). Comparisons of faecal collection methods for digestibility determinations in gilthead sea bream. Journal of Fish Biology **49**:735-738.

Gaylord, T.G. & Gatlin III, D.M. (1996). Determination of digestibility coefficients of various feedstuffs for red drum (*Sciaenops ocellatus*). Aquaculture **139**:303-314.

Georgopoulos, G.B. & Conindes, A.J. (1999) Study on the effects of dietary starch on growth, food conversion and digestibility of adult gilthead seabream (*Sparus aurata* L. 1758). Journal of Aquaculture in the Tropics **14**(2):143-152.

Glencross, B., Curnow, J., Hawkins, W., Kissil, G.W.M. & Peterson, D. (2003). Evaluation of the feed value of a transgenic strain of the narrow leaf lupin (*Lupinus angustifolius*) in the diet of the marine fish, *Pagrus auratus*. Aquaculture Nutrition **9**:197-206.

Glencross, B., Hawkins W. & Curnow J. (2004) .Nutritional assessment of Australian canola meals. 1. Evaluation of oil extraction method and meal processing conditions on the digestible value of canola meals fed to the red sea bream (*Pagrus auratus*, Paulin). Aquaculture Research **35**:15-24.

Hajen, W.E., Higgs, D.A., Beames, R.M. & Dosanjh, B.S. (1993). Digestibility of various feedstuffs by post-juvenile Chinook salmon (*Oncorhynchus tshawytcha*) in sea water. 2. Measurement of digestibility. Aquaculture **112**:333-348.

Hardy, R.W. & Barrows, F.T. (2002). Diet formulation and manufacture. In: Fish Nutrition 3rd Edition (ed. by J.E Halver & R.W. Hardy), pp. 506-600. Academic Press, London UK.

Hilton, J.W., Atkinson, J.L. & Slinger, S.J. (1983). Effect of increased dietary fibre on the growth of rainbow trout (*Oncorhynchus mykiss*). Canadian Journal of Fisheries & Aquatic Science **40**:81-85.

Laining, A., Rachmansyah, Ahmad, T. & Williams, K. (2003). Apparent digestibility of selected ingredients for humpback grouper, *Cromileptes altevis*. Aquaculture **218**:529-538.

Lovell, T. (1989). Nutrition and feeding of fish. Chapter 5; Feed formulation and Processing, pp. 107-127. Van Nostrand Reinhold, New York.

Lupatsch, I., Kissil, G.Wm., Sklan, D. & Pfeffer, E. (1997). Apparent digestibility coefficients of feed ingredients and their predictability in compound diets for gilthead seabream, *Sparus aurata* L. Aquaculture Nutrition **3**:81-89.

Nengas, I., Alexis, M.N., Davies, S.J. & Petichakis, G. (1995). Investigation to determine digestibility coefficients of various raw materials in diets for gilthead seabream, *Sparus auratas* L. Aquaculture Research **26**:185-194

Nengas, I., Alexis, M.N. & Davies, S.J. (1999). High inclusion levels of poultry meals and related by-products in diets for gilthead seabream *Sparus aurata* L. Aquaculture **179**:13-23.

National Research Council (NRC) (1993). Nutrient requirements of fish. National Academy Press Washington, D.C., USA.

Paulin, C.D. (1990). *Pagrus auratus*, a new combination for the species known as 'snapper' in Australian waters (Pisces: Sparidae). New Zealand Journal of Marine and Freshwater Research **24**:259-265.

Pfeffer, E. Kinzinger, S. & Rodehutscord, M. (1995). Influence of the proportion of poultry slaughter by-products and of untreated or hydrothermically treated legume seeds in diets for rainbow trout, *Oncorhynchus mykiss* (Walbaum), on apparent digestibilities of their energy and organic compounds. Aquaculture Nutrition **1**:111-117.

Pike, I.H & Barlow, S.M. (2003). Impact of fish farming on fish stocks. International Aquafeed Directory & Buyers Guide **2003**:24-29.

Quartararo, N., Bell, J.D. & Allan, G.L. (1998a). Substitution of fishmeal in a diet for the carnivorous marine fish *Pagrus auratus* (Bloch and Schneider) from southeastern Australia. Asian Fisheries Science **10**:269-279.

Quartararo, N., Allan, G.L. & Bell, J.D. (1998b). Replacement of fishmeal in diets for Australian snapper, *Pagrus auratus*. Aquaculture **166**:279-295.

Refstie, S. & Storebakken, T. (2002). Antinutritional factors in plant seeds; how they limit the utilisation of plant proteins by salmonid fish and measures to overcome their effects. International Aquafeed Directory & Buyers Guide **2002**:14-19.

Sargent, J.R., Tocher, D.R. & Bell, J.G. (2002). The lipids. In: Fish Nutrition 3rd Edition (ed. by J.E Halver & R.W. Hardy), pp. 181-257. Academic Press, London UK.

Stone, D.A.J., Allan, G.L., Parkinson, S. & Rowland, S.J. (2000). Replacement of fishmeal in diets for Australian silver perch, *Bidyanus bidyanus*. III. Digestibility and growth using meat meal products. Aquaculture **186**:311-326.

Sugiura, S.H., Dong, F.M., Rathbone, C.K. & Hardy, R.W. (1998). Apparent protein digestibility and mineral availabilities in various feed ingredients for salmonid feeds. Aquaculture **159**:177-202.

Tabata, K. & Taniguchi, N. (2000). Differences between *Pagrus major* and *Pagrus auratus* through mainly mtDNA control region analysis. Fisheries Science **66**:9-18.

Tacon, G.J. & Forster, I.P. (2001) Global trends and challenges to aquaculture and aquafeed development in the new millennium. International Aquafeed Directory & Buyers Guide **2001**:4-25.

Tacon, G.J. (2003). Global trends in aquaculture and compound aquafeed production – A Review. International Aquafeed Directory & Buyers Guide 2003:8-23.

Takagi, S., Hosokawa, H., Shimeno, S. & Ukawa, M. (2000); Utilization of poultry by-product meal in diet for red sea bream *Pagrus major*. Nippon Suisan Gakkaishi **66**(3):428-438.

Tidwell, J.H. & Allan, G.L. (2001). Fish as food: aquaculture's contribution. European Molecular Biology Organisation (EMBO) **2**(11):958-963.

Venou, B., Alexis, M.N., Fountoulaki, E., Nengas, I., Apostolopoulou, M. & Castritsi-Cathariou, I. (2003). Effect of extrusion of wheat and corn on gilthead sea bream (*Sparus aurata*) growth, nutrient utilization efficiency, rates of gastric evacuation and digestive enzyme activities. Aquaculture **225**:207-223.

Wilson, R.P. (1994). Utilisation of dietary carbohydrate by fish. Aquaculture **124**:67-80.

Yamamoto, T., Akimoto, A., Kishi, S., Unuma, T. & Akiyama, T. (1998). Apparent and true availabilities of amino acids from several protein sources for fingerling rainbow trout, common carp and red sea bream. Fisheries Science **64(**3):448-458.

TABLE 1
Measured composition of individual feed ingredients (g kg^{-1} or MJ kg^{-1} dry matter).

Ingredient	Crude Protein	Ash	Organic matter[1]	Fat	Gross energy (MJ)
Wheat standards[2,a]	129.0	23.0	977.0	31.5	18.0
Gelatinised starch[2,b]	4.0	1.0	999.0	8.7	16.6
Fishmeal[2,c (batch 1)]	762.5	140.0	860.0	126.1	21.3
Extruded wheat[2,d]	171.9	23.0	977.0	55.1	18.9
Fish oil[2,e]	-	<10.0	990.0	990.0	38.0
Fishmeal[3,c (batch 2)]	700.0	166.0	834.0	102.6	20.2
Extruded wheat[3,d]	183.8	21.0	979.0	49.7	19.0
Meat meal[3,f]	571.3	296.0	704.0	105.8	16.9
Poultry meal[3,g]	687.5	75.0	925.0	200.6	23.8
Haemoglobin powder[3,h]	981.3	18.0	982.0	14.7	24.1
Blood meal[3,i]	999.0	13.0	987.0	9.6	24.0
Soybean meal[3,j]	523.1	63.0	937.0	28.7	21.9
LA soybean meal[3,k]	521.9	51.0	949.0	74.8	19.6

[1] organic matter calculated by difference = (1000 – measured ash content)
[2] ingredients used in experiment 1
[3] ingredients used in experiment 2
[a] Koombi Feeds, Kootingal, NSW, Australia
[b] Goodman Fielder, Summer Hill, NSW, Australia (100% pre-gelatinised wheat starch)
[c] Pivot Aquaculture, Rosny Park, Tasmania, Australia
[d] Ridley Agriproducts, Murray Bridge, SA, Australia
[e] Janos Hoey Pty. Ltd., Forbes, NSW, Australia (cod liver oil)
[f] Ridley Aqua-Feed, Narangba, Qld, Australia
[g] Barter Enterprises Pty. Ltd. (Steggles), Beresfield, NSW, Australia
[h] Australian Meat Holdings (AMH) Pty. Ltd., Dinmore, Qld, Australia
[i] Lachley Meats Pty. Ltd., Forbes, NSW, Australia (ring dried)
[j] Gibsons Ltd., Cambridge, Tasmania, Australia (commercially available solvent extracted meal)
[k] Hyfeed, Scientific Feeds, Toowoomba, Qld, Australia (cooked @ 130ºC, cold pressed without use of solvents, low-allergenic (LA)

TABLE 2
Calculated ingredient and measured nutrient composition of diets used in experiment 1 (g kg^{-1} or MJ kg^{-1} of dry matter).

	Diet[1]							
	Ref 1	EW20	EW30	EW40	Ref 2	FM50	FO15	FO25
Ingredient								
Fishmeal	900.0	716.3	624.5	532.7	490.0	740.0	415.0	365.0
Wheat standards	40.0	31.8	27.8	23.7	-	-	-	-
Fish oil	-	-	-	-	-	-	150.0	250.0
Gelatinised starch	40.0	31.8	27.8	23.7	-	-	-	-
Extruded wheat	-	200.0	300.0	400.0	490.0	240.0	415.0	365.0
Vitamin premix[2]	7.5	7.5	7.5	7.5	7.5	7.5	7.5	7.5
Mineral premix[3]	7.5	7.5	7.5	7.5	7.5	7.5	7.5	7.5
Chromic oxide	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0
Nutrient								
Crude protein	700.0	594.0	542.0	489.0	462.0	612.0	393.0	346.0
Ash	141.0	117.0	106.0	94.0	92.0	116.0	78.0	73.0
Organic matter[4]	859.0	883.0	894.0	906.0	908.0	884.0	922.0	927.0
Fat	111.0	100.0	94.0	89.0	86.0	106.0	223.0	305.0
NFE[5]	48.0	189.0	258.0	328.0	360.0	166.0	306.0	276.0
Gross energy (MJ)	20.9	20.5	20.3	20.1	20.1	20.7	22.8	24.6

[1] Ref diet 1 was replaced with 200, 300 or 400g kg^{-1} of extruded wheat (EW). Ref diet 2 was replaced with either 500g kg^{-1} of fishmeal (FM) or 150 or 250g kg^{-1} fish oil (FO)
[2] (IU kg^{-1} diet): retinol A, 8000; cholecalciferol D3, 1000; DL-α-tocopherol acetate E, 125. (mg kg^{-1}): menadione sodium bisulphite K3, 16.5; thiamine hydrochloride B1, 10.0; riboflavin B2, 25.2; pyridoxine hydrochloride B6, 15.0; folic acid, 4.0; ascorbic acid C, 1000; calcium-D-pantothenate, 55.0; myo-inositol, 600; D-biotin H (2%), 1.0; choline chloride, 1500; nicotinamide, 200; cyanocobalamin B12, 0.02; ethoxyquin (anti-oxidant) 150; calcium propionate (mould inhibitor) 25.0
[3] (mg kg^{-1} diet): calcium carbonate, 7500; manganese sulphate monohydrate, 300; zinc sulphate monohydrate, 700; copper sulphate pentahydrate, 60, ferrous sulphate heptahydrate, 500, sodium chloride, 7500; potassium iodate, 2.0
[4] Organic matter calculated by difference = (1000-measured ash content)
[5] Nitrogen free extractives calculated by difference (NFE) = 1000 – (crude protein + ash + fat)

TABLE 3
Calculated ingredient and measured nutrient composition of diets used in experiment 2 (g kg^{-1} or MJ kg^{-1} of dry matter).

	Diet[1]								
	Ref 2	MM30	MM50	PM30	PM50	HP15	BM15	SB30	LA30
Ingredient									
Fishmeal	490.0	340.0	240.0	340.0	240.0	415.0	415.0	340.0	340.0
Extruded wheat	490.0	340.0	240.0	340.0	240.0	415.0	415.0	340.0	340.0
Meat meal	-	300.0	500.0	-	-	-	-	-	-
Poultry meal	-	-	-	300.0	500.0	-	-	-	-
Haemoglobin meal	-	-	-	-	-	150.0	-	-	-
Blood meal	-	-	-	-	-	-	150.0	-	-
Soybean meal	-	-	-	-	-	-	-	300.0	-
LA soy bean meal	-	-	-	-	-	-	-	-	300.0
Vitamin premix[2]	7.5	7.5	7.5	7.5	7.5	7.5	7.5	7.5	7.5
Mineral premix[2]	7.5	7.5	7.5	7.5	7.5	7.5	7.5	7.5	7.5
Chromic oxide	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0
Nutrient									
Crude protein	412.0	459.7	491.6	494.6	549.7	497.3	502.0	445.3	444.9
Ash	102.0	160.2	199.0	93.9	88.5	89.4	88.7	90.3	86.7
Organic matter[3]	898.0	839.8	801.0	906.1	911.5	910.6	911.4	909.7	913.3
Fat	74.8	84.1	90.3	112.5	137.7	65.8	65.0	61.0	74.8
NFE[4]	411.2	296.0	219.1	299.0	224.1	347.5	344.3	403.4	393.6
Gross energy (MJ)	19.6	18.8	18.2	20.9	21.7	20.3	20.3	20.2	19.6

[1] Ref diet 2 was replaced with 300 or 500g kg^{-1} of meat meal (MM); 300 or 500g kg^{-1} of poultry meal (PM); 150g kg^{-1} each of haemoglobin meal (HP) and blood meal (BM) and 300g kg^{-1} each of soybean meal (SB) and low allergenic soybean meal (LA)
[2] Vitamin and mineral premix as per Table 2
[3] Organic matter calculated by difference = (1000-measured ash content)
[4] Nitrogen free extractives calculated by difference (NFE) = 1000 – (crude protein + ash + fat)

TABLE 4
Mean apparent digestibility coefficients (ADCs) for diets and ingredients and specific growth rate (SGR) of snapper used in experiment 1.

	Apparent digestibility coefficient (ADC)[1]							
	Ref 1	EW20	EW30	EW40	Ref 2	FM50	FO15	FO25
Dietary ADC %								
Crude protein	93.1	93.6	94.3	94.1	94.1	94.2	94.0	93.5
	(0.4)	(0.3)	(0.4)	(0.4)	(0.4)	(0.3)	(0.3)	(0.4)
Organic matter	89.6	86.5	84.4	80.9	78.2	88.3	82.7	84.1
	(0.5)	(0.5)	(0.8)	(0.9)	(0.6)	(0.5)	(0.7)	(0.7)
Fat	90.7	92.2	91.6	90.3	88.5	93.9	94.8	95.7
	(2.5)	(1.0)	(0.9)	(1.7)	(3.7)	(0.7)	(0.8)	(1.0)
Gross energy	91.6	89.6	87.5	85.1	82.3	91.0	86.8	88.5
	(0.5)	(0.3)	(0.6)	(0.5)	(0.6)	(0.6)	(0.9)	(0.8)
Ingredient ADC %								
Crude protein	-	100.6	105.4	100.1	-	94.3	-	-
	-	(4.6)	(3.9)	(2.7)	-	(0.5)	-	-
Organic matter	-	75.6	73.7	69.4	-	98.9	106.0	100.2
	-	(2.1)	(2.4)	(2.1)	-	(1.0)	(4.4)	(2.7)
Fat	-	104.4	96.0	89.1	-	97.7	97.9	97.6
	-	(8.9)	(5.4)	(7.0)	-	(1.1)	(1.1)	(1.2)
Gross energy	-	80.5	76.9	74.4	-	99.2	100.5	98.3
	-	(1.9)	(2.2)	(1.3)	-	(1.2)	(3.6)	(2.2)
Weight gain[2]	23.3	23.2	23.9	23.3	22.4	27.9	22.2	15.0
	(3.0)	(2.0)	(4.0)	(2.8)	(3.2)	(0.7)	(2.8)	(0.51)
SGR[3]	1.0	1.1	1.0	1.0	1.0	1.2	1.0	0.7
	(0.1)	(0.1)	(0.1)	(0.1)	(0.1)	(0.0)	(0.1)	(0.0)

Values represent mean (± SEM) of three replicate tanks.
[1] Diet abbreviations as per Table 2
[2] Individual weight gain (g $fish^{-1}$) = (biomass at harvest – biomass at stocking) / 7
[3] Specific growth rate (SGR) = 100 [ln(individual final weight) – ln(individual initial weight)] / 30days

TABLE 5
Mean apparent digestibility coefficients (ADCs) for diets and ingredients and specific growth rate (SGR) of snapper used in experiment 2.

	Apparent digestibility coefficient (ADC)[1]								
	Ref 2	MM30	MM50	PM30	PM50	HP15	BM15	SB30	LA30
Dietary ADC %									
Crude protein	89.1	79.1	75.3	87.4	87.7	90.9	86.8	88.5	89.7
	(0.4)	(1.8)	(1.5)	(0.4)	(0.2)	(0.4)	(0.6)	(1.8)	(0.7)
Organic matter	76.5	73.2	70.6	80.2	83.7	76.0	77.1	70.5	70.3
	(0.5)	(1.5)	(2.0)	(0.4)	(0.1)	(0.7)	(0.5)	(1.4)	(0.6)
Fat	86.5ǂ	88.8	89.9	90.8	92.7	85.5	86.1	81.5	86.4
	(1.1)	(1.2)	(1.9)	(0.8)	(0.7)	(3.0)	(1.3)	(3.3)	(4.6)
Gross energy	77.9ǂ	76.3	74.5	82.4	85.3	78.2	78.5	74.3	73.8
	(0.8)	(1.5)	(1.2)	(0.3)	(0.2)	(0.5)	(1.0)	(1.5)	(1.1)
Ingredient ADC %									
Crude protein	-	62.2	65.3	84.9	86.9	95.1	81.6	87.2	90.7
	-	(5.0)	(2.6)	(1.0)	(0.4)	(1.3)	(2.0)	(5.1)	(1.9)
Organic matter	-	63.5	63.2	88.5	90.6	73.8	80.4	57.1	56.6
	-	(6.0)	(4.4)	(1.4)	(0.2)	(4.1)	(3.3)	(4.7)	(1.8)
Fat	-	92.5	92.3	94.6	95.0	-	-	-	-
	-	(3.1)	(3.2)	(1.5)	(0.9)	-	-	-	-
Gross energy	-	72.0	70.5	91.1	91.4	79.5	81.3	66.8	64.3
	-	(5.5)	(2.7)	(0.9)	(0.4)	(3.1)	(5.7)	(4.6)	(3.5)
Weight gain[2]	23.2ǂ	23.8	17.5	24.5	13.8	18.4	21.5	17.8	20.8
	(0.4)	(3.4)	(3.4)	(2.0)	(4.9)	(3.8)	(2.1)	(3.9)	(2.9)
SGR[3]	0.99ǂ	0.97	0.75	0.96	0.56	0.75	0.91	0.77	0.87
	(0.0)	(0.16)	(0.14)	(0.07)	(0.17)	(0.13)	(0.05)	(0.15)	(0.13)

Values represent mean (± SEM) of three replicate tanks. ǂ Indicates mean (± SEM) of two replicate tanks.
[1] Diet abbreviations as per Table 3
[2] Individual weight gain (g fish^{-1}) = (biomass at harvest – biomass at stocking) / 7
[3] Specific growth rate (SGR) = 100 [ln(individual final weight) – ln(individual initial weight)] / 23days

TABLE 6
Digestible protein and energy values of test ingredients fed to snapper.

Ingredient[1] (inclusion level)	Digestible nutrient	
	Digestible protein (g kg^{-1})	Digestible energy (MJ kg^{-1})
Fishmeal[2,c] (50)	719	21.1
Fish oil[2,e] (15)	-	38.0
Fish oil[2,e] (25)	-	37.3
Extruded wheat[2,d] (20)	172	15.2
Extruded wheat[2,d] (30)	172	14.5
Extruded wheat[2,d] (40)	172	14.0
Meat meal[3,f] (30)	355	12.2
Meat meal[3,f] (50)	372	11.9
Poultry meal[3,g] (30)	583	21.7
Poultry meal[3,g] (50)	597	21.7
Haemoglobin powder[3,h] (15)	933	19.1
Blood meal[3,i] (15)	815	19.5
Soybean meal[3,j] (30)	456	14.6
LA soybean meal[3,k] (30)	473	12.6

Digestible nutrient or energy values calculated by multiplying specific ingredient ADC x ingredient protein or energy content. ADCs were limited to 100 if calculated values from Table 4 exceeded 100%.
[1] Ingredient superscripts as per Table 1.

4.2 Investigation of the nutritional requirements of Australian snapper *Pagrus auratus* (Bloch & Schneider, 1801): effects of digestible energy content on utilisation of digestible protein.

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ABSTRACT

This study used a four-parameter mathematical model for physiological responses (4-SKM) to investigate the effects of different digestible energy (DE) levels on the digestible protein (DP) requirements of juvenile snapper *Pagrus auratus*. For each energy level (15, 18 or 21 MJ kg^{-1}), DP content was increased from about 210 to 560 g kg^{-1} in seven evenly spaced increments by formulating a summit (highest DP content) and diluent (lowest DP content) diet pair. Each diet pair was then combined in various ratios to achieve the desired DP content, ensuring DE level remained relatively stable. The apparent digestibility of test diets was confirmed at the completion of the dose-response feeding trial. Each of the 21 test diets was fed to four replicate groups of snapper (mean stock weight = 31 g; 8 fish per 200 L floating cage) twice daily to apparent satiation for 57 days. A commercial barramundi feed was included in the trial to indicate relative performance of fish fed the experimental diets (66 cages in total). Cages were secured in circular 10 kL tanks connected to a saltwater recirculating aquaculture system inside a large plastic covered hot-house. At the completion of the trial, fish were weighed and killed for chemical analysis. According to the models fitted to our data, diets formulated for snapper weighing between 30 – 90 g and reared at temperatures ranging from 20-25ºC should contain a minimum of 28 g DP $MJDE^{-1}$ to promote optimal weight gain and protein deposition. Based on this study, these levels could be achieved with practical diets containing approximately 519 g DP and 18 MJ DE or 480 g DP and 17 MJ DE. Diets that incorporate higher levels of DE (mostly as lipid) without a concomitant increase in DP to balance this ratio lead to major shifts in the efficiency of protein utilisation and the amount of protein expended on maintenance.

1. INTRODUCTION

Protein is the basic component of all animal tissues and constitutes about 65-75% of the dry matter in fish tissues (Wilson, 2002). For this reason, protein is considered an essential nutrient, providing a suite of indispensable and dispensable amino acids for both maintenance and growth. However, because fish have a limited capacity for the de novo synthesis of proteins, they must be provided in the diet (Hepher, 1988). For most cultured fish species, this requirement has been found to be between 30-55% of the diet (Hepher, 1988; NRC, 1993). Reasons for higher protein requirements in fish include a lower energy requirement due to the efficient excretion of metabolic waste products to hypotheses about the obligate or facultative use of dietary protein for energy (Hepher, 1988).

Feed proteins are expensive and their inclusion in aquaculture diets has a significant impact on overall feed costs. This is one of the fundamental reasons for attempting to optimise the amount of dietary protein in aquaculture feeds. Secondary benefits associated with optimised dietary protein levels include rapid and cost efficient growth, reduced environmental impacts and improvements in water quality (Azevedo, Cho, Leeson & Bureau, 1998; Kaushik & Bureau, 1998). It is therefore critical that protein is used efficiently and that most of the growth is associated with protein rather than fat deposition (Bikker, 1994). Many factors affect the efficiency by which dietary protein is utilised for maintenance and growth. These include the quantity and quality of the dietary protein

(i.e. amino acid profile), the digestibility of the protein, body weight and age of the fish, feed intake and numerous environmental conditions (Hepher, 1988; De Silva & Anderson, 1995; Wilson, 2002; Halver & Hardy, 2002). One of the most important factors affecting the dietary protein requirement of fish for rapid growth (i.e. excluding genetic potential; Halver & Hardy, 2002) is its relationship to dietary energy content (Lupatsch, Kissil &Sklan, 2001a; Wilson, 2002).

Modern aquaculture feeds aim to optimise dietary protein levels by incorporating high levels of non-protein energy into the diet in the form of lipids or carbohydrates (CHO). These energy sources are incorporated to reduce the catabolism of proteins for energy and improve protein retention efficiency (Halver & Hardy, 2002). However, the ability of these energy sources to "spare" protein is highly dependent on the capacity of a given species to utilise them for metabolic energy demands (Hepher, 1988; Wilson, 1994). Not all fish species are tolerant of diets high in non-protein energy (Halver & Hardy, 2002). Incorrect dietary energy levels, regardless of the source (i.e. protein, lipid or CHO) can have significant effects on voluntary feed intake that can ultimately reduce potential weight gains (Lupatsch, Kissil, Sklan & Pfeffers, 2001b) and affect carcass composition (Hepher, 1988; Shearer, 1994).

In studies with poultry and pigs, optimum requirements for protein or amino acids have been determined using two diets with similar digestible or metabolisable energy contents. One, the summit diet, has a protein or amino acid content which aims to be in excess of that thought necessary for maximum growth whereas the other, the diluent diet, has a protein or amino acid content well below that thought necessary for maximum growth. A series of diets is then manufactured by blending different amounts of the summit and diluent mixtures (Fisher & Morris, 1970; Bikker, 1994). This approach has been applied successfully to elucidate the digestible protein (DP) requirements of juvenile silver perch *Bidyanus bidyanus* using DP and digestible energy (DE) values derived from predetermined ingredient digestibility coefficients rather than less appropriate physiological fuel equivalents (Allan, Johnson, Booth & Stone, 2001; Allan & Booth, 2004).

Here, we have applied the same approach to determine the DP requirements of juvenile Australian snapper *Pagrus auratus*, with the exception that we have used the four-parameter mathematical model for physiological responses to interpret our results (Mercer, 1982). Thus, the objectives of this study were to determine the optimal balance of DP and DE content in diets for juvenile snapper by evaluating their combined effects on weight gain, protein deposition and carcass composition.

2. MATERIALS AND METHODS

A growth experiment was done to evaluate the effects of dietary DE content on the DP requirement of juvenile snapper. Three series of diets were formulated to have a fixed level of DE (i.e. 15, 18 or 21 MJ kg^{-1}). Within each of these energy levels, DP content was incrementally increased from about 210 to 560 g kg^{-1} by formulating a summit (highest DP content) and diluent (lowest DP content) diet pair for each level of DE. Paired diets were then combined at various ratios to achieve the desired DP content, thus ensuring calculated DE levels remained relatively constant. In total, 21 test diets were prepared. A commercial barramundi diet (Ridley Aqua-Feeds, Narangba, Qld, Australia), commonly used by the snapper industry, was included in the experiment as an internal control.

Immediately after the growth experiment was terminated, a digestibility experiment was done using the same fish to confirm the digestible nutrient content of the summit and diluent diets as well as the intermediate DP diet in each series. The digestibility of the commercial diet was also determined.

2.1 Diet formulation

Summit and diluent test diets were formulated on a digestible nutrient basis using ingredient digestibility coefficients determined in earlier experiments with Australian snapper (Booth, Allan

& Anderson, 2005). In addition, diet composition was kept relatively simple by using only fish meal, fish oil and extruded wheat as the sources of dietary protein and energy. Consequently, where necessary, inert fillers and binders (i.e. diatomaceous earth or carboxy-methyl cellulose) were incorporated into diets to balance dry matter. Diets were also fortified with a vitamin / mineral premix (15 g kg^{-1} diet; NSW Fisheries formulation) and extra L-Ascorbic acid phosphate (1 g kg^{-1} diet; Rovimix® Stay-C® 35; F. Hoffman-La Roche, Basel, Switzerland) (Table 1).

Before incorporation into test diets, the fishmeal and extruded wheat products were finely ground. Subsequently, all dry ingredients were thoroughly mixed (Hobart Mixer: Troy Pty Ltd, Ohio, USA) according to individual dietary formulations before the addition of the correct amount of fish oil and various quantities of distilled water. The wet mash was cold pressed into 3 mm sinking pellets using a bench top meat mincer (Barnco Australia Pty Ltd, Leichhardt, NSW, Australia) after which pellets were dried for about six hours (≈ 35º C) in a convection drier or until moisture content was < 100 g kg^{-1} diet. Following preparation, all diets were stored frozen at < -15ºC until required. The commercial barramundi diet was finely ground, fortified with Stay-C® 35 and re-made into 3 mm pellets using the same techniques.

Ten diets were selected at the conclusion of the growth trial to confirm the digestible nutrient content of dietary formulations. These diets were reground (1.5 mm screen; Raymond Laboratory Mill, Transfield Technologies, Rydalmere, Australia) and thoroughly dry mixed with chromic oxide marker (5 g kg^{-1} diet; dry matter basis) before the addition of a suitable quantity of distilled water. Pellets were formed, dried and stored according to the description above.

2.2 Fish

The snapper used in this study were progeny of first generation brood-stock held at the NSW DPI Fisheries Port Stephens Fisheries Centre (PSFC). Before being used in experiments, snapper were grown at low densities in large 10 kL tanks and fed twice daily on a commercial barramundi *Lates calcarifer* feed (Ridley Aqua-Feeds, Narangba, Qld, Australia; reported nutrient composition: 50% crude protein; 12% crude fat; 18.0 MJ kg^{-1} gross energy). Fish were anaesthetised during all handling procedures (20-30 mg ethyl-ρ-aminobenzoate L^{-1}) and starved for 24 h prior to weighing. Experiment cages used in the growth experiment were stocked with 8 juvenile snapper weighing between 25 to 36 g $fish^{-1}$ (mean weight = 31.5 g $fish^{-1}$). At the conclusion of the growth experiment, selected groups of snapper were anaethetised, reweighed and transferred to the digestibility laboratory.

Two snapper, each from a different treatment group (D9 & D18) were removed during the growth experiment because of eye damage. These fish were replaced with tagged fish (fin clipped) of a similar size. The tagged fish were later identified during the harvest procedure and excluded from chemical analyses, however, they were used to investigate data on feed intake. Seven fish from one replicate assigned to D18 died during an intermediate weight check after failing to recover from anaesthesia. Data for this replicate was eliminated from the results.

2.3 Growth experiment

The experiment was carried out in a saltwater recirculating system that consisted of 9 x 10 kL circular fibreglass tanks (3.4 m diameter x 1.2 m depth) housed within a "greenhouse" at PSFC. Each of the 10 kL tanks contained 8 cylindrical floating cages (dimensions approximately 0.2 m^3; 0.6 m diameter x 0.7 m submerged depth; constructed of 10 mm perforated plastic oyster mesh) fitted with a lid and a fine mesh feeding screen secured at the base (1.6 mm perforated oyster mesh). Cages were firmly secured to the outer perimeter of 10 kL tanks and remained in the same position during the entire experiment. Each 10 kL tank was provided with approximately 40 L min-1 of pre-filtered water pumped from a submerged bio-filter. Effluent water from each tank was drained through a 1 mm filter screen to remove coarse solids (NikaFilt; Taylor Made Fish Farm, Bobs Farm, NSW, Australia), collected in a common concrete sump (2.5 kL) and pumped back to

the bio-filter through a pair of twin cartridge pool filters (10-15 µm); cartridges were cleaned and replaced daily. All tanks were provided with 4 x 100 mm air stone diffusers and fitted with black shade cloth covers to reduce the proliferation of algae.

Fish were acclimatised for 10 days to the experimental conditions before test diets were introduced. During this period fish were restrictively fed the commercial barramundi feed, ensuring all feed was consumed, with 50% of the total ration offered at 0830 h and 1430 h respectively.

Before the acclimation period ended, each of the 22 dietary treatments was randomly allocated to 3 replicate cages (66 cages in total). Snapper were then switched to the experimental diets and fed to apparent satiation at the pre-established times six days a week (Monday – Saturday). Fish were fed to apparent satiation only once on Sundays (0830 h). Feeding was conducted in two stages to ensure complete satiation. During the first stage, experimental feeds were offered generously, after which there was usually a small amount of feed left on the screen (5-10 pellets). Approximately five minutes later, feed was re-offered in small quantities provided no pellets remained from the initial feed and fish continued to feed actively. A subjective measure of apparent satiation was judged after 4-8 pellets settled on the feeding screen. A final check of feeding screens was made after all cages had been fed to ensure that all pellets had been eaten.

Water quality was maintained by vacuuming tanks once or twice weekly to remove the build up of accumulated solids. Fresh, disinfected water was pumped into tanks from a 47 kL storage reservoir to replace water lost through cleaning processes and water exchange. Water temperature was kept above ambient winter temperature by installing a single 2 kW immersion heater in each 10 kL tank. Water quality parameters were recorded daily from each tank using one of two hand held water quality analysers; either a Model 611 (Yeo-Kal Electronics, Brookvale, NSW, Australia) or a Horiba U-10 (Horiba, Japan). Total ammonia [NH3 + $NH4^+$] was monitored from tanks using a rapid test kit procedure (Model 1.08024.0001, E. Merck, Darmstadt, Germany). During the experiment, temperature, dissolved oxygen (DO^2), salinity and pH ranged from 19.26-24.24ºC, 6.01-8.61 mg L^{-1}, 3.27-3.41% and 7.86-8.68 units respectively with [NH3 + $NH4^+$] always ≤ 0.4 mg L^{-1}.

Fish were harvested and individually weighed on day 57. To determine changes in the body composition of snapper, a representative sample of 10 fish was selected during stocking procedures, killed with an overdose of anaesthetic and frozen (<-15ºC). This procedure was repeated at the end of the growth experiment, at which time 2 fish from each replicate cage were randomly selected, killed and frozen (<-15ºC).

In addition to the controlled feeding experiment, two groups of snapper were held in a separate tank in the same type of floating cages, subjected to the same ambient conditions and starved for a period of 28 days. Each group was replicated 4 times, with 15 small (initial mean weight ± SEM; 14.3 ± 0.2 g) or 5 large snapper (initial mean weight ± SEM ; 99.5 ± 3.2 g) per replicate cage. Fish weight was determined after 28 days and used to calculate the relative weight and protein loss of starved, free swimming snapper.

Performance based calculations:

Data on weight or nutrient gains and data on feed or nutrient intakes were expressed as a proportion of geometric mean body weight (GMBW) before investigations of fish performance;

GMBW = (average initial weight fish * average harvest weight of fish)$^{0.5}$

In addition, further investigation of weight gain, feed intake and digestible protein retention data were referred to the metabolic weight exponent of 0.70; an exponent that has previously been determined for Australian snapper (Glencross *et al.* unpublished data) and the closely related gilthead sea bream *Sparus aurata* (Lupatsch, Kissil, Sklan & Pfeffer 1998);

Weight gain (g $kgBW^{-0.7}d^{-1}$)= weight gain $fish^{-1}$/(($GMBW/1000)^{0.7}$)/57 days
Feed intake (g $kgBW^{-0.7}d^{-1}$) = feed intake $fish^{-1}$/(($GMBW/ 1000)^{0.7}$)/57 days
Protein intake (g protein $kgBW^{-0.7}d^{-1}$) = protein intake $fish^{-1}$/(($GMBW/1000)^{0.7}$)/57 days
Protein gain (g protein $kgBW^{-0.7}d^{-1}$) = protein gain $fish^{-1}$/(($GMBW / 1000)^{0.7}$)/57 days

2.4 Digestibility experiment

The remaining 6 fish from each replicate cage were kept in their established groups and immediately transferred to the digestibility laboratory where they were stocked into one of 27 randomly selected digestibility tanks. According to the design of the digestibility experiment, only the remaining fish from the 3 replicates assigned to each of the summit or diluent diets (D1, D7, D8, D14, D15 & D21), 2 randomly chosen replicates assigned to the intermediate dietary treatment in each series (D4, D11 & D18), and the 3 replicates assigned to the commercial barramundi diet were transferred (D22).

Twenty-seven purpose built, 170 L cylindro-conical digestibility tanks were used to house fish and collect faeces by passive settlement. A detailed description of this system is given in Allan, Rowland, Parkinson, Stone & Jantrarotai (1999) while the specific procedures used to collect and store faeces from snapper are described in Booth *et al.* (2005). Snapper were fed approximately 24h after being stocked into digestibility tanks with same diets as in the growth experiment but diets were marked with 0.5% chromic oxide. Fish were fed for 5 days before the collection of faeces commenced. All diets were fed to excess (confirmed by the presence of uneaten feed in faecal collection tubes) for a period of approximately 3 h between 0830 and 1130 h each day. Faeces were allowed to settle overnight (≈ 18 h) and were removed the following morning prior to feeding. The digestibility experiment was terminated after 34 days.

Water quality parameters and total ammonia [NH3 + $NH4^{+}$] were monitored daily as previously described. During the faecal collection phase of the experiment, temperature, dissolved oxygen (DO2), salinity and pH ranged from 19.6-25.0ºC, 5.2-8.22 mg L^{-1}, 3.07-3.21% and 7.66-8.09 units respectively with [NH3 + $NH4^{+}$] always ≤ 0.2 mg L^{-1}.

2.5 Digestible nutrient calculations

The apparent digestibility coefficients of the test diets was calculated according to the following equation:

ADCDiet (%) = 100 X [1 - (F / D x DCr / FCr)]

where ADC = % apparent digestibility coefficient of diet; F = % nutrient or gross energy in faeces; D = % nutrient or gross energy in diet; DCr = % chromic oxide in diet; FCr = % chromic oxide in faeces (Cho, Slinger & Bayley 1982). Subsequently, the digestible nutrient or energy content of individual test diets was calculated by multiplying the measured nutrient or energy content of the diet (Table 1) by its respective ADCDiet. The DP and DE content of diets not tested in the digestibility experiment were then estimated by regressing the measured DP and DE of diets within each energy series against their formulated values. Each regression was then used to obtain a set of corrected values that were used in performance-based calculations involving DP and DE intake (Table 1).

2.6 Chemical analyses

Analyses of crude protein (NX6.25; Kjeldahl nitrogen), fat (hexane solvent; Dionex ASE®), moisture (105ºC for 16 h), ash (550ºC for 2 h) and chromium oxide (ICP-MS analysis) in ingredient, diet or faecal samples was done by the CSIRO Analytical Services Facility (CSIRO-Livestock Industries, Indooroopilly, QLD, Australia). Analyses of gross energy (bomb calorimetry) in diets and faecal samples was done by the Pig and Poultry Production Institute (SARDI), SA,

Australia). Analyses of crude protein (NX6.25; Kjeldahl nitrogen), fat (ether extraction), moisture (105ºC for 16 h) and ash (550ºC for 2 h) in whole fish samples was done by the State Chemistry Laboratory (SCL) (Victoria Agriculture, Werribee, VIC, Australia). The gross energy (bomb calorimetry) content of whole fish was determined by SARDI on sub-samples prepared by SCL.

2.7 Statistical analyses and curve fitting

Prior to conducting ANOVA, data were tested to ensure that treatment variances were homogenous (Cochran's test). The significance level for all ANOVA and multiple comparisons tests (Student Newman Keuls; SNK) was set at 0.05 and data were statistically analysed using Statgraphics Plus, version 4.1 (Manugistics Inc., Rockville, MD, USA; 1998). To assess the interactive effects of dietary DE and DP content on different response criteria, dietary DE and DP contents were considered as two fixed factors with three and seven levels, respectively. For DP content, level means were assigned values of 1 through 7 for diets containing the lowest to highest DP contents, respectively.

The effect of DP content, relative protein intake or dietary DP:DE ratio on relative protein deposition in snapper from each DE series was investigated using the four-parameter mathematical model for physiological responses (4-SKM; Mercer, Flodin & Morgan 1978; Mercer 1980; Mercer 1982). Models were fitted using GraphPad Prism V4 (GraphPad Software Inc., San Diego CA, USA), which uses the Marquardt-Levenberg algorithm to minimize the sum-of-squares. The nutrient-response relationship was described by the function;

$$R = [b*(K_{0.5})^n + R_{MAX}*(I)^n] / [(K_{0.5})^n + (I)^n]$$

where R = physiological response, I = nutrient intake or concentration in the diet, b = intercept on the response axis, R_{MAX} = maximum theoretical response, n = apparent kinetic order and $K_{0.5}$ = intake for ½ R_{MAX}-b. Several other points were derived from the fitted functions according to equalities presented by Mercer *et al.* (1978);

Intake at maximum efficiency;	$Ime = K_{0.5*}(n-1)^{1/n}$
Intake at maximum slope;	$Ims = K_{0.5*}(n-1/n+1)^{1/n}$
Intake at zero response;	$Iro = K_{0.5*}(-b/R_{max})^{1/n}$
Maximum slope at Ims;	$dr/dI = ((R_{MAX}-b)*(n*K_{0.5*}I^{n-1}))/(K_{0.5}+I^n)^2$

The response value at each of these intakes was calculated by substituting derived values into the original nutrient response function. The efficiency of nutrient utilisation is highest at Ime and is calculated from;

Maximum efficiency; Max eff. = (rIme – b)/ Ime

Each of the models fitted to our data approaches an asymptote (R_{MAX}), which, by mathematical definition, is the limiting value. This asymptotic value is approached but never reached; therefore, there is no maximum point on the response curve by which to judge the definitive requirements in terms of DP concentration or DP intake. This means from a practical standpoint, we must choose an arbitrary point on the response curve to define a useful or "optimal" requirement for the dependent variables explored in our study. In this study, we have chosen conservative concentration and intake values that represent an 85% reduction in maximum slope (Mercer, May & Dodds, 1989) for each model ($I_{85\%SR}$ and $R_{85\%SR}$), using software to calculate the respective derivatives of each curve (GraphPad Prism V4: GraphPad Software Inc., San Diego CA, USA).

3. RESULTS

3.1 Effects of DP and DE on feed intake and growth

Relative feed intake (g $kgBW^{-0.7}d^{-1}$) was significantly affected by the DE level of the test diets (df=2/42, F=15.09, *P*<0.0001), but not by DP level (df=6/42, F=1.35, *P*=0.256) or the interaction between each factor (df=12/42, F=1.67, *P*=0.109). Multiple comparison procedures indicated that relative feed intake was significantly higher in snapper fed the 21 MJ kg^{-1} diets, but similar in snapper fed either of the lower energy diets (21 MJ kg^{-1} > 15 MJ kg^{-1} = 18 MJ kg^{-1}; *n*=21, *SNK*). Average relative DE intake was calculated to be 375.8, 314.1 and 277.2 kJ $kgBW^{-0.84}d^{-1}$ for the HE, ME and LE series, respectively (*n*=21).

Feed conversion ratio (FCR) was significantly affected by DP level (df=6/42, F=80.40, *P*<0.0001), DE level (df=2/42, F=48.89, *P*<0.0001) and the interaction term (df=12/42, F=8.32, *P*<0.0001). The strong interaction between DP and DE level was mainly due to the very poor FCR exhibited by snapper fed the 3 lowest DP contents within the HE series compared with snapper fed similar amounts of DP at the lower DE levels (Table 2). Generally, FCRs decreased rapidly as DP content increased and converged at level 4 where most FCRs had declined to approximately 2:1. After this point, FCRs stabilised and decreased slowly towards a minimum FCR of about 1.6:1 for the remaining diets.

Two-way ANOVA indicated relative weight gain (g $kgBW^{-0.7}d^{-1}$) and relative protein deposition (g $kgBW^{-0.7}d^{-1}$) were both significantly affected by the DP (*P*<0.0001) and DE (*P*<0.0001) level of diets, but only relative weight gain was affected by the interaction between these two factors (df=12/42, F=3.27, *P*=0.0022). This interaction was driven by the greater weight gain of snapper fed D13 from the mid-energy series (Table 2). Relative protein deposition was similar and significantly higher in snapper fed diets with lower DE than for snapper fed the 21 MJ kg^{-1} diets (21 MJ kg^{-1} < 15 MJ kg^{-1} = 18 MJ kg^{-1}; *n*=21, *SNK*). Protein deposition increased in response to increasing DP level and significant differences were identified between all groups with the exception of levels 6-7, indicating protein deposition had begun to plateau (7 = 6 > 5 > 4 > 3 > 2 > 1; *n*=9, *SNK*). DP content of the 3 diets at level 6 ranged between 489.4 and 547.5 g kg^{-1} diet.

3.2 Effects of DP and DE on carcass composition

Fish size affects carcass composition (Shearer 1994). Therefore, before evaluating the interactive effects of DP and DE level on the carcass composition of snapper, a statistical adjustment of the data was made by including individual harvest weight as a single covariate and assuming homogeneity of slopes. Results of two-way ANCOVA indicated that carcass protein was not affected by the DP (df=6/41, F=1.19, P=0.33) or DE level (df=2/41, F=0.47, P=0.62) of test diets or by the interaction of these terms (df=12/41, F=0.62, P=0.81). Similarly, the ash content of snapper was not affected by the DP (df=6/41, F=1.04, P=0.41) or the DE level (df=2/41, F=0.82, P=0.45) of test diets or the interaction between these terms (df=12/41, F=0.67, P=0.77). Juvenile snapper maintained their carcass protein and ash composition within a narrow range, with mean ± STDEV for each variable of 17.42 ± 0.83% and 4.96 ± 0.27%, respectively (*n*=63; Table 2). However, two-way ANCOVA found that carcass energy, carcass fat and carcass moisture content were all significantly affected (*P*<0.05) by the interaction between the DP and DE level of test diets. Despite the interaction, carcass fat tended to decrease as dietary DP levels increased. However, decreases in fat content were mostly balanced by increases in carcass moisture (Table 2).

3.3 Curve fitting

The relationship between relative protein deposition and DP concentration or DP intake was similar, so only figures depicting protein retention are presented (Figure 1). Parameter estimates for each model are presented in Tables 3 and 4. According to the results of two-way ANOVA, relative protein deposition had plateaud in snapper fed diets containing the highest levels of DP, therefore,

a global modelling approach was employed (Motulusky & Christopoulos, 2003) in which R_{MAX} was constrained to an unknown, but shared value in each model. All parameters were estimated using values determined for fed and starved fish.

Relative weight and protein loss in 90 g snapper starved for 28 days averaged -2.03 g kgGMBW$^{-0.7}$d^{-1} and -0.35 g kgGMBW$^{-0.7}$d^{-1}, respectively. Although the starved fish in our study were not incorporated to determine metabolic weight exponents for protein, a power function fitted to our limited data set indicated that absolute protein losses in snapper could be described by; protein loss (g fish^{-1}day^{-1}) = 0.392* kgGMBW$^{-0.754}$ (R^2=0.99). The constant and exponent proved to be similar to that reported for the closely related gilthead sea bream and the European seabass *Dicentrarchus labrax* reared at similar water temperatures (Lupatsch, *et al.* 1998 & 2001a).

Higher dietary levels of DE shifted the parameter estimates (i.e. $K_{0.5,}$ Ime, Ims & Iro) for each model to the right of the determinant axis (Table 3; Figure 1), indicating that dietary energy level had a significant impact on the growth potential of snapper. To elucidate this observation, relative protein deposition was modelled as a function of the DP:DE of each diet series. Estimates for each of the 3 parameters were extremely close after fitting the models separately. Consequently, a post-hoc comparison of the parameters in each of the three models indicated one set of parameters was appropriate to describe all the data (df=8/12, F=0.66, *P*=0.71; GraphPad Prism 4; F-test). The associated response curve is presented in Figure 2.

4. DISCUSSION

4.1 Requirements for maintenance and growth

In line with many other carnivorous marine fin fish, juvenile Australian snapper were found to require diets containing high levels of DP in order to maximize weight gain and protein deposition (NRC, 1993; Wilson, 2002; Koshio, 2002: Lupatsch, Kissil & Sklan, 2003). With regard to DP content, optimal protein deposition ranged between 1.27 and 1.30 g kgGMBW$^{-0.7}$d^{-1}. Accordingly, diets formulated to contain either 21, 18 or 15 MJkg^{-1} would need to supply approximately 631, 519 or 481 g DP kg^{-1} to promote optimal protein deposition (Table 3). The highest of these estimated levels is clearly outside the range of DP contents we were able to provide to snapper reared on the HE diet series, but suggests fish on these diets may have been "protein limited" to some extent and that we may have inadvertently restricted optimal protein deposition. Similar results were presented by Catacutan & Coloso (1995), who demonstrated that weight gain and feed efficiency in Asian seabass was inferior on high-energy diets (i.e. higher lipid levels) unless the increases in energy was accompanied by a concomitant increase in protein to maintain optimal crude protein:energy ratio.

With respect to DP intake, requirements for optimal protein deposition were very similar, with optimal deposition occurring in snapper fed between 5.0 and 6.54 g DP kg GMBW-0.7d-1 (Table 3). Protein deposition was then limited by either the genetic potential of snapper for protein synthesis or the nutritional limitations of the diet. Maintenance protein requirements were estimated as 1.47, 1.87 and 2.36 g DP kg GMBW-0.7d-1 for snapper fed the LE, ME and HE series respectively. Our values for maintenance are somewhat elevated compared to those presented for Australian snapper (0.82 g kgGMBW$^{-0.7}$d^{-1}; Glencross *et al.* unpublished data) and gilthead seabream when protein utilisation was studied using linear regression analysis (0.86-0.96 g kgGMBW$^{-0.7}$d^{-1}; Lupatsch, Kissil, Sklan & Pfeffer, 1998), but lower than those cited for yellowtail kingfish *Seriola quinqueradiata* (Watanabe, Hara, Ura, Yada, Kiron & Satoh, 2000a; Watanabe, Ura, Yada, Kiron, Satoh & Watanabe, 2000b). When maintenance requirements for protein in gilthead seabream were estimated using a non-linear approach, values of between 0.62 and 1.30 g kg GMBW$^{-0.7}$d^{-1} have been recorded (Lupatsch, *et al.* 2001; Lupatsch, *et al.* 2003). The particular model used to determine requirements obviously has a major impact on the estimate. In our case, the use of a logistic curve would tend to overestimate maintenance requirements compared to estimates determined using linear models or rectangular hyperbola.

The partial efficiency of protein utilisation for growth varied between 0.28, 0.36 and 0.39 for the HE, ME and LE series, respectively (Table 3; Figure 1). These intake levels (Ime), represent the point at which protein deposition in snapper was at its most efficient in terms of production. The intake at maximum slope (Ims) represents the point where snapper were most sensitive to changes in the dependant variable and necessarily occurs at the inflection point. Here, the rate of change is approaching a maximum, and small changes in protein intake produce large changes in protein deposition (Mercer, Watson & Ramlet, 1981). As the response and intake variables are in similar units, we can derive the maximum instantaneous protein utilisation for the HE, ME and LE series to be approximately 0.51, 0.66 and 0.61 respectively (i.e. max slope; Table 3).

The partial efficiency of protein utilisation for growth for the snapper in this study (0.39 for diets with 15 $MJkg^{-1}$ DE) is slightly lower than values cited for red seabream (i.e. 0.46; Glencross, *et al.* unpublished data) and gilthead seabream (i.e. 0.34-0.47; Lupatsch, *et al.* 1998 & 2001) fed a single diet under increasing intake levels and using linear regression to estimate protein efficiency. In a more detailed study, Lupatsch and her colleagues (2001b) varied both the DP and DE contents of test diets fed to gilthead seabream. They found that protein retention efficiency was better described by a non-linear function (i.e. $y = a*[1-\exp(-b(x-c))]$) that predicted protein deposition would reach a plateau at levels close to 1.25 g $DPkgGMBW^{-0.7}d^{-1}$ with a corresponding maintenance requirement of 1.30 g $DPkgGMBW^{-0.7}d^{-1}$. Partial efficiencies of protein utilisation for growth varied between highly efficient (0.6) to moderate levels (0.33), reflecting the values described in the present study.

Protein deposition in juvenile snapper proved to be highly dependent on the ratio of dietary DP:DE (Table 3; Figure 2). According to estimates from the combined model, maximum efficiency occurred at an intake of 16.65 g DP $MJDE^{-1}$, however, to optimise protein deposition diets for snapper weighing between 30 to 90 g should be formulated to contain at least 28 g DP $MJDE^{-1}$. These recommendations are almost identical to the ratios determined by Glencross *et al.* (unpublished data) for snapper using a factorial approach (i.e. 27.6-25.8 g DP $MJDE^{-1}$) and are similar to those of red drum (McGoogan & Gatlin, 1997). Ratios for optimal weight gain and protein retention in juvenile rainbow trout and Atlantic salmon are somewhat lower at between 19-24 and 20 g DP $MJDE^{-1}$, respectively (Azevedo, *et al.* 2004). In addition, recommendations have been made that diets for juvenile gilthead seabream contain between 21 and 28.5 g DP $MJDE^{-1}$ (Lupatsch, *et al.* 1998; 2003) while diets for European seabass contain about 25.2 g DP $MJDE^{-1}$ (Lupatsch, *et al.* 2003).

4.2 Effects of DP and DE on feed intake and feed conversion

Relative feed intake in snapper proved to be governed within narrow limits by the DE content of the test diets and was not affected by DP content, a result that has also been reported by others for the closely related gilthead seabream (Lupatsch, *et al.* 2001b; Fournier, Gouillou-Coustans, Metailler, Vachot, Guedes, Tulli, Oliva-Teles, Tibaldi & Kaushik, 2002). Contrary to our results, Sabaut & Luquet (1973) and Santinha, Gomes & Coimbra, (1996) found that voluntary feed intake increased in gilthead seabream fed diets with decreasing DP content and Azevedo, *et al.* (2004) presented data that showed rainbow trout and Chinook salmon fed isoenergetic diets with varying amounts of DP displayed similar weight gains but increased the absolute intake of diets with lower DP; higher feed intake corresponded with lower DP:DE ratio of the diets. Similar results were presented for rainbow trout, turbot and European seabass (Fournier, *et al.* 2002). Azevedo, Leeson, Cho & Bureau, (2004) suggested this feed regulation might be driven by a "growth target" and its associated "nutrient demands" rather than overall energy requirements.

Data from our study showed that relative feed intake was significantly higher in snapper fed the 21 $MJkg^{-1}$ diets compared to snapper fed lower amounts of DE (Table 3). This was considered counter-intuitive as we presumed that relative feed intake would be lower in snapper fed diets from the high-energy series. For example, a similar study in which DP and DE ratios were manipulated using practical feed ingredients found that relative feed intake in gilthead seabream fitted a

quadratic response and increased in fish fed diets containing up to 18 MJDEkg^{-1} after which it decreased (Lupatsch, *et al.* 2001b; range of DE from 10 to 22 MJkg^{-1} diet). In practical terms, the difference between the relative feed intake of each of the DE series in our study was minor and probably reflects the fact that there was a certain amount of "drift" in the formulated as opposed to the actual DE content of test diets. The likelihood that snapper fed under our daily feeding regime may have eaten to their physical capacity rather than an "energy requirement" is also possible. The average amount of energy consumed by snapper within each energy group was clearly different (375.8, 314.1 and 277.2 kJ kgGMBW$^{-0.8}$4d^{-1} for the HE, ME and LE series respectively; n=21), but approximated the maximum voluntary DE intake cited for rapidly growing gilthead seabream (264 kJ kg$^{-0.8}$4d^{-1}; Lupatsch, *et al.* 2001b).

Feed conversion ratio steadily improved as the DP content of each diet series increased and plateaud in diets containing between 374 and 379 g DPkg-1 (Table 2). While FCRs improved slightly beyond this level, they were not statistically different within each energy series. Improvements in FCR under increasing dietary protein regimes are common in the literature for both omnivorous and carnivorous fish species (Yone, 1976; Jauncey, 1982; Takeuchi, Shiina & Watanabe, 1991; Santinha, *et al.* 1996; Allan, *et al.* 2001; Fournier, *et al.* 2002; Espinos, Tomas, Perez, Balasch & Jover 2003). In gilthead seabream, FCRs of 1.6:1 and 1.23:1 were obtained in fish fed diets with 414 g DPkg^{-1} and 13 MJDE (ratio of DP:DE = 32.9) or 549 g DPkg^{-1} and 19.5 MJDE (ratio of DP:DE = 28.2) respectively (Lupatsch *et al.* 2001b). The strong interactions between DP and DE in this study suggest that feeding efficiency (FCR^{-1}*100) was also governed by the DP:DE ratio of diets. This relationship was appropriately described by the exponential function: Feed efficiency (%) = 70.35(±4.96)*[1-exp(-0.133(±0.032)*(DP:DE–8.77(±0.75))], (R^2=0.93). The curve of the fitted data can be used to estimate the DP:DE intake requirements at the point where the feed efficiency was numerically highest in each series. These values were 60.9, 65.8 and 64.5% for the HE, ME and LE series respectively corresponding to DP:DE intakes of 23.9, 29.4 and 27.5 g DP MJ DE^{-1}. The best feed conversion ratio's from each series also corresponded with the highest weight gains, and were achieved in fish fed diets with more than 27 g DP MJDE^{-1}.

4.3 Effects of DP and DE on carcass composition

Protein and ash composition of snapper was not affected by the changes in dietary DP and DE content of test diets. The relative stability of these tissue constituents in whole fish of various sizes is also well documented in other species (Jauncey, 1982; Shearer, 1994; Lupatsch,*et al.* 1998; Allan & Booth, 2004). Similar to other studies investigating protein requirements, we found a negative correlation between fat and moisture composition (Allan & Booth, 2004). Whole body fat content also tended to decrease as the amount of dietary DP or DP:DE ratio increased, however these effects were more pronounced in the carcass of fish sampled from the high-energy diet series. The pattern of increasing lipid deposition with increasing dietary fat levels under low dietary protein intake has been reported in many studies including those on gilthead seabream (Santinha, *et al.* 1996; Lupatsch 2001b), silver perch (Allan & Booth, 2004) and several species of salmonid (Azevedo, *et al.* 2004). Whole body fat depots in snapper were as high as 30% (dry matter basis) in some treatments, and as much as 50% of this fat could be associated with the viscera (Oku & Ogata, 2000).

5. CONCLUSION

This study has indicated that weight gain and protein deposition of fast growing snapper is particularly sensitive to the dietary balance of digestible protein and energy. As such, diets formulated for snapper weighing between 30 – 90 g and reared at temperatures ranging from 20-25ºC should contain a minimum of 28 g DP MJDE^{-1} to promote optimal protein deposition. Based on the outcomes of this study, these levels could be achieved with practical diets containing approximately 519 g DP and 18MJ DE or 480 g DP and 17MJ DE. Diets that incorporate higher

levels of DE (as lipid) without a concomitant increase in DP to balance this ratio lead to major shifts in the efficiency of protein utilisation and the amount of protein expended on maintenance.

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REFERENCES

Allan, G.L., Rowland, S.J., Parkinson, S., Stone, D.A.J. & Jantrarotai, W. (1999). Nutrient digestibility for juvenile silver perch *Bidyanus bidyanus*: development of methods. Aquaculture **170**:131-145.

Allan, G.L., Johnson, R.J., Booth, M.A. & Stone, D.A.J. (2001). Estimating the digestible protein requirements of silver perch, *Bidyanus bidyanus*. Aquaculture Research **32**:337-347.

Allan, G.L.& Booth, M.A. (2004). The effects of dietary digestible protein and digestible energy content on protein retention efficiency of juvenile silver perch *Bidyanus bidyanus* (Mitchell). Aquaculture Research **35**:970-980.

Azevedo, P.A., Cho, C.Y., Leeson, S. & Bureau, D.P. (1998). Effects of feeding level and water temperature on growth, nutrient utilisation and waste outputs of rainbow trout (*Onchorhynchus mykiss*). Aquatic Living Resources **11**(4):227-238.

Azevedo, P.A., Leeson, S., Cho, C.Y. & Bureau, D.P. (2004). Growth, nitrogen and energy utilisation of juveniles from four salmonid species: diet, species and size effects. Aquaculture **234**:393-414.

Bikker, P. (1994). Protein and lipid accretion in body components of growing pigs: effect of body weight and nutrient intake. PhD Thesis, Agricultural University, Wageningen.

Booth, M.A., Allan, G.L. & Anderson, A.J. (2005). Investigation of the nutritional requirements of Australian snapper *Pagrus auratus* (Bloch & Schneider, 1801): apparent digestibility of protein and energy sources. Aquaculture Research **36**:378-390.

Catacutan, M.R. & Coloso, R.M. (1995) Effect of dietary protein to energy ratios on growth, survival and body composition of juvenile Asian seabass, *Lates calcarifer***.** Aquaculture **131**:125-133.

Cho, C.Y. & Bureau, D.P. (2001). A review of diet formulation strategies and feeding systems to reduce excretory and feed wastes in aquaculture. Aquaculture Research **32**:349-360.

Cho, C.Y., Slinger, S.J. & Bayley, H.S. (1982). Bioenergetics of salmonid fishes: energy intake, expenditure and productivity. Comparative Biochemistry & Physiology **73B**(1):25-42.

Cowey, C.B. (1992). Nutrition: estimating requirements of rainbow trout. Aquaculture **100**:177-189.

De Silva, S.S. & Anderson, T.A. (1995). Fish Nutrition in Aquaculture. Chapman & Hall Aquaculture Series, London.

Espinos, F.J., Tomas, A., Perez, L.M., Balasch, S. & Jover, M. (2003). Growth of dentex fingerlings (*Dentex dentex*) fed diets containing different levels of protein and lipid. Aquaculture **218**:479-490.

Fisher, C. & Morris, T.R. (1970). The determination of the methionine requirement of laying pullets by a diet dilution technique. British Poultry Science **11**:67-82.

Fournier, V., Gouillou-Coustans, M.F., Metailler, R., Vachot, C., Guedes, M.J., Tulli, F., Oliva_Teles, A., Tibaldi, E. & Kaushik, S.J. (2002). Protein and arginine requirements for maintenance and nitrogen gain in four teleosts. British Journal of Nutrition **87**:459-469.

Halver, J.E. & Hardy, R.W. (2002). Nutrient flow and retention. In: Fish Nutrition, Third Edition (Eds. John E. Halver & Ronald W. Hardy). Chapter 14:755-770. Academic Press, California, US.

Hepher, B. (1988). Nutrition of pond fishes. Cambridge University Press, Sydney.

Jauncey, K. (1982). The effects of varying dietary protein level on the growth, food conversion, protein utilisation and body composition of juvenile Tilapias (*Sarotherodon mossambicus*). Aquaculture **27**:43-54.

Koshio, S. (2002). Red sea bream, *Pagrus major*. In: Nutrient requirements and feeding of finfish for aquaculture, Chapter 4 (eds. C.D. Webster & C. Lim). CAB International.

Lupatsch, I., Kissil, G.WM., Sklan, D. & Pfeffer, E. (1998). Energy and protein requirements for maintenance and growth in gilthead seabream (*Sparus aurata* L.). Aquaculture Nutrition **4**:165-173.

Lupatsch, I., Kissil, G.WM. & Sklan, D. (2001a). Optimization of feeding regimes for European seabass *Dicentrarchus labrax*: a factorial approach. Aquaculture **202**:289-302.

Lupatsch, I., Kissil, G.WM., Sklan, D. & Pfeffer, E. (2001b). Effects of varying dietary protein and energy supply on growth, body composition and protein utilisation in gilthead seabream (*Sparus aurata* L.). Aquaculture Nutrition **7**:71-80.

Lupatsch, I., Kissil, G.WM. & Sklan, D. (2003). Comparison of energy and protein efficiency among three species: gilthead seabream (*Sparus aurata*), European seabass (*Dicentrarchus labrax*) and white grouper (*Epinephelus aenus*): energy expenditure for protein and lipid deposition. Aquaculture **225**:175-189.

McGoogan , B.B. & Gatlin III, D.M. (1998). Metabolic requirements of red drum, *Sciaenops ocellatus*, for protein and energy based on weight gain and body composition. Nutrient Requirements and Interactions. American Society for Nutritional Sciences, 123-129.

Mercer, L.P., Flodin, N.W. & Morgan, P.H. (1978). New methods for comparing biological efficiency of alternate nutrient sources. Journal of Nutrition **108**:1244-1249.

Mercer, L.P. (1980). Mathematical models in nutrition. Nutrition Reports International **21**:189-198.

Mercer, L.P., Watson, D.F. & Ramlet, J.S. (1981). Control of food intake in the rat by dietary protein concentration. Journal of Nutrition **111**:1117-1123.

Mercer, L.P. (1982). The quantitative nutrient-response relationship. Journal of Nutrition **112**:560-566.

Mercer, L.P, Gustafson, J.M., Higbee, P.T., Geno, C.E., Schweisthal, M.R. & Cole, T.B. (1984). Control of physiological response in the rat by dietary nutrient concentration. Journal of Nutrition **114**:144-152.

Mercer, L.P., May, H.E. & Dodds, S.J. (1989). The determination of nutritional requirements in rats: mathematical modelling of sigmoidal, inhibited nutrient-response curves. Journal of Nutrition **119**:1465-1471.

Motulusky, H.J. & Christopoulos, A. (2003). Fitting models to biological data using linear and nonlinear regression. A practical guide to curve fitting. GaphhPad Software Inc. San Diego, CA.

National Research Council (NRC) (1993). Nutrient requirements of fish. National Academy Press Washington, D.C., USA.

Oku, H. & Ogata, Y. (2000). Body lipid deposition in juveniles of red sea bream *Pagrus major*, yellowtail *Seriola quinqueradiata*, and Japanese flounder *Paralichthys olivaceus*. Fisheries Science **66**:25-31.

Sabaut, J.J. & Luquet, P. (1973). Nutritional requirements of the gilthead bream *Sparus aurata*. Quantitative protein requirements. Marine Biology **18**:50-54.

Santinha, P.J.M., Gomes, E.F.S. & Coimbra, J.O. (1996). Effects of protein level of the diet on digestibility and growth of gilthead seabream, *Sparus auratus* L. Aquaculture Nutrition **2**:81-87.

Shearer, K.D. (1994). Factors affecting the proximate composition of cultured fishes with emphasis on salmonids. Aquaculture **119**:63-88.

Shearer, K.D. (1995). The use of factorial modelling to determine the dietary requirements for essential elements in fishes. Aquaculture **133**:57-72.

Shearer, K.D. (2000). Experimental design, statistical analysis and modelling of dietary nutrient requirement studies for fish: a critical review. Aquaculture Nutrition **6**:91-102.

Takeuchi, T., Shiina, Y. & Watanabe, T. (1991). Suitable protein and lipid levels in diets for red sea bream *Pagrus major*. Nippon Suisan Gakkaishi **57**(2):293-299.

Yone, Y. (1976). Nutritional studies of red sea bream. In: Proceedings of the First International Conference on Aquaculture Nutrition, 39-64. (Eds. Kent S. Price, William N. Shaw & Karin S. Danberg), October 14-15th 1975, Lewes/Rehoboth, Delaware.

Watanabe, K., Hara, Y., Ura, K., Yada, T., Kiron, V., Satoh, S. & Watanabe, T. (2000a). Energy and protein requirements for maximum growth and maintenance of bodyweight of yellowtail. Fisheries Science **66**:884-893.

Watanabe, K., Ura, K., Yada, T., Kiron, V., Satoh, S. & Watanabe, T. (2000b). Energy and protein requirements of yellowtail for maximum growth and maintenance of body weight. Fisheries Science **66**:1053-1061.

Wilson, R.P. (2002). Amino acids and proteins. In: Fish Nutrition, Third Edition (eds. John E. Halver & Ronald W. Hardy). Chapter 3, 144-179. Academic Press, California, US.

TABLE 1
Composition of test diets (g kg^{-1} or MJ kg^{-1} of dry matter).

	High energy diet series							*Mid energy diet series*							*Low energy diet series*							
	Summit						*Diluent*	*Summit*						*Diluent*	*Summit*						*Diluent*	*Com.*
	D1	D2	D3	D4	D5	D6	D7	D8	D9	D10	D11	D12	D13	D14	D15	D16	D17	D18	D19	D20	D21	D22
Ingredient formulation																						
Fish meal[1]	770.0	683.3	601.7	515.0	428.3	346.7	260.0	780.0	686.5	598.5	505.0	411.5	323.5	230.0	650.0	575.2	504.8	430.0	355.2	284.8	210.0	-
Ext. wheat[2]	34.0	90.3	142.3	197.5	252.8	304.8	360.0	100.0	156.9	210.6	267.5	324.5	378.1	435.0	100.0	145.9	189.1	235.0	280.9	324.1	370.0	-
Fish oil[3]	180.0	200.4	219.6	240.0	260.4	279.6	300.0	80.0	103.8	126.2	150.0	173.8	196.2	220.0	60.0	79.6	97.9	117.5	137.1	155.5	175.0	-
Diat. earth[4]	-	6.0	12.5	19.0	25.8	32.1	40.0	-	13.6	26.4	39.0	52.5	65.3	79.0	139.0	145.7	152.3	159.0	165.7	172.1	179.0	-
CMC[5]	-	4.0	7.9	12.5	16.7	20.8	24.0	24.0	23.2	22.3	22.5	21.7	20.9	20.0	35.0	37.6	39.9	42.5	45.1	47.5	50.0	-
Premix[6]	15.0	15.0	15.0	15.0	15.0	15.0	15.0	15.0	15.0	15.0	15.0	15.0	15.0	15.0	15.0	15.0	15.0	15.0	15.0	15.0	15.0	-
Stay-C® 35	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	-
Nutrient or energy(measured)																						
Protein	567.5	536.3	482.5	426.9	370.6	325.0	266.9	588.1	547.5	485.6	416.3	361.9	326.3	245.6	498.8	489.4	396.3	361.3	304.4	268.1	218.8	524.4
Ash	134.8	125.2	119.3	120.8	107.7	102.5	102.7	140.4	135.7	134.8	143.2	135.0	143.0	139.8	255.3	227.8	230.2	230.0	238.8	210.9	245.3	112.3
Fat	230.0	243.0	263.0	267.0	295.0	305.0	234.0	141.0	156.0	175.0	185.0	211.0	160.0	134.0	64.0	106.0	121.0	86.0	99.0	93.0	83.0	124.0
NFE[7]	67.7	95.5	135.2	185.3	226.7	267.5	396.4	130.5	160.8	204.5	255.6	292.1	370.8	480.5	181.9	176.8	252.5	322.7	357.8	428.0	453.0	239.3
Gross energy	23.6	24.0	24.2	24.0	24.4	24.5	24.0	21.8	21.9	21.9	21.8	22.2	20.8	21.7	18.5	18.9	18.8	18.8	18.5	19.1	18.3	21.7
Digestible nutrient or energy[8]																						
Protein	519.3	470.1	422.9	373.7	324.5	277.2	228.0	546.6	489.2	435.9	378.5	321.1	267.8	210.4	472.6	424.1	378.9	331.5	283.0	237.7	190.3	na
Energy	21.2	21.2	21.9	21.5	21.5	21.5	20.0	20.2	19.5	19.5	18.9	18.3	18.3	17.7	16.9	16.9	16.9	16.2	15.6	14.9	14.3	na
DP:DE	24.5	22.2	19.3	17.5	15.1	12.9	11.4	27.1	25.1	22.4	20.0	17.6	14.6	11.9	28.0	25.1	22.4	20.5	18.1	16.0	13.3	22.7

[1] Austral Group VLT (very low temperature) steam dried fish meal (Prime quality) via Ridley Aquafeeds Pty. Ltd., Narangba, QLD, Australia.
[2] Extruded wheat purchased from Ridley Agriproducts Pty. Ltd., Murray Bridge
3 Cod liver oil purchased from Janos Hoey Pty. Ltd.
[4] Amorphous diatomaceous earth purchased from Recreational Water Products, East Melbourne, Australia.
[5] CMC = carboxy-methyl cellulose
[6] NSW Fisheries premix contains vitamins (IU kg^{-1} diet): retinol A, 8000; cholecalciferol D3, 1000; DL-α-tocopherol acetate E, 125. (mg kg^{-1}): menadione sodium bisulphite K3, 16.5; thiamine hydrochloride B1, 10.0; riboflavin B2, 25.2; pyridoxine hydrochloride B6, 15.0; folic acid, 4.0; ascorbic acid C, 1000; calcium-D-pantothenate, 55.0; myo-inositol, 600; D-biotin H (2%), 1.0; choline chloride, 1500; nicotinamide, 200; cyanocobalamin B12, 0.02; ethoxyquin (anti-oxidant) 150; calcium propionate (mould inhibitor) 25.0 and minerals (mg kg^{-1} diet): calcium carbonate, 7500; manganese sulphate monohydrate, 300; zinc sulphate monohydrate, 700; copper sulphate pentahydrate, 60, ferrous sulphate heptahydrate, 500, sodium chloride, 7500; potassium iodate, 2.0.
[7] Nitrogen free extractives calculated by difference NFE = 1000 – (protein + ash + fat)
[8] Predicted digestible nutrient values based on relationships (regression) between formulated and measured digestible nutrient values from this study.

TABLE 2
Performance of snapper in the growth experiment.

	High energy diet series							*Mid energy diet series*							*Low energy diet series*							
	Summit						*Diluent*	*Summit*						*Diluent*	*Summit*						*Diluent*	*Com.*
	D1	D2	D3	D4	D5	D6	D7	D8	D9	D10	D11	D12	D13	D14	D15	D16	D17	D18*	D19	D20	D21	D22
Performance index																						
Initial weight	31.0	30.5	30.5	30.1	30.2	30.8	30.6	31.4	31.7	30.6	30.6	30.6	30.2	30.4	31.3	30.0	29.9	31.3	29.3	30.7	30.8	30.7
Harvest weight	77.5	77.8	69.8	66.2	55.2	47.9	43.5	85.4	79.8	74.3	72.6	64.4	64.5	47.3	81.0	76.9	73.8	69.2	63.6	59.6	53.3	77.3
Weight gain	46.5	47.3	39.3	36.1	25.0	17.1	12.9	54.0	48.1	43.7	42.0	33.8	34.2	16.9	49.6	46.9	43.9	38.0	34.3	28.9	22.6	46.6
Feed intake	83.3	77.3	76.6	73.2	73.1	67.7	65.5	81.6	73.5	71.7	73.5	68.0	71.5	65.2	76.9	75.6	74.9	77.3	72.1	67.8	66.9	82.0
Relative intake	12.1	11.2	11.6	11.3	12.0	11.7	11.7	11.4	10.5	10.6	10.9	10.6	11.2	11.3	10.9	11.1	11.2	11.6	11.4	10.8	11.1	11.9
FCR	1.79	1.64	1.97	2.03	2.97	4.12	5.08	1.52	1.53	1.66	1.76	2.02	2.09	3.86	1.55	1.63	1.72	2.04	2.12	2.36	2.96	1.77
DP intake	42.1	35.7	32.1	27.5	24.3	19.8	26.4	43.2	35.0	30.8	27.9	22.5	20.2	15.2	35.6	31.8	28.5	22.6	21.3	17.5	14.5	32.0
DE intake	1729	1604	1635	1537	1534	1418	1285	1621	1412	1378	1374	235	1299	1149	1305	1280	1270	1090	1139	1031	984	1413
Protein gain	8.01	7.82	6.62	6.11	4.46	2.53	1.76	9.82	8.93	7.78	6.72	6.00	5.86	2.35	9.07	8.14	7.50	6.70	5.89	4.82	3.28	8.27
Energy gain	427.2	492.8	386.4	383.8	260.2	178.1	151.2	502.4	451.4	433.6	433.4	371.4	369.8	189.0	461.2	421.4	406.1	341.8	337.4	300.1	262.5	456.8
Fat gain	3.9	7.0	4.6	5.6	3.6	3.0	2.9	7.2	5.7	6.6	6.7	6.2	5.7	2.7	6.6	5.8	6.4	5.5	5.3	5.0	4.5	6.9
Harvest carcass composition (% as rec'd basis or MJ kg^{-1})																						
Moisture	65.8	63.6	64.8	63.4	64.4	65.0	64.9	65.4	65.2	64.5	64.2	63.5	63.2	64.5	65.2	65.5	65.2	65.0	64.9	64.5	64.0	64.9
Protein	17.6	17.2	17.3	17.4	17.8	16.7	16.5	18.2	18.3	17.8	16.9	17.8	17.5	16.4	18.2	17.8	17.5	17.8	17.5	17.3	16.4	17.9
Ash	4.9	4.8	4.6	4.7	5.1	5.0	5.1	5.0	5.0	4.8	4.8	5.0	4.8	5.4	5.0	5.1	5.1	4.9	4.9	4.9	5.0	4.9
Fat	7.1	11.1	8.7	10.8	9.2	9.3	10.2	10.4	9.1	11.0	11.4	12.1	11.2	9.0	10.2	9.6	10.7	10.3	10.7	11.0	11.3	10.9
Gross energy	8.7	9.5	8.9	9.3	9.0	8.7	8.9	8.8	8.8	9.1	9.3	9.5	9.4	9.0	8.7	8.6	8.7	8.4	8.9	9.0	9.4	9.0

* data based on 2 replicate cages
Weight gain (g $fish^{-1}$) = average harvest weight – average initial weight
Feed intake (g $fish^{-1}$; dry basis) = average total feed intake per cage / 8 fish
Relative feed intake (g $kgBW^{-0.7}d^{-1}$) = average total feed intake per fish / $((GMBW/1000)^{0.7})$/57 days
Feed conversion ratio (FCR) = average dry basis feed intake per fish / average wet weight gain per fish
Digestible protein intake DP (g $fish^{-1}$) = average dry basis feed intake per fish x mean digestible protein content of diet
Digestible energy intake DE (kJ $fish^{-1}$) = average dry basis feed intake per fish x mean digestible energy content of diet
Protein, energy or fat gain (g or kJ $fish^{-1}$) = average nutrient or energy carcass content of fish at harvest – carcass nutrient or energy content of initial fish sample (dry matter basis)

TABLE 3
Parameter estimates ± standard error derived from fitting relative protein deposition in snapper as a function of DP content or DP intake.

	Digestible protein content			Digestible protein intake		
	HE	ME	LE	HE	ME	LE
Best-fit values						
B	-0.35	-0.35	-0.35	-0.35	-0.35	-0.35
$K_{0.5}$	290.90	227.8	196.4	3.44	2.70	2.37
N	2.78	2.47	2.15	3.70	3.74	2.91
$R_{MAX\ (shared)}$	1.50	1.50	1.50	1.40	1.40	1.40
Std. error of best fit values						
B	0.04	0.04	0.02	0.04	0.04	0.02
$K_{0.5}$	11.10	10.53	4.84	0.08	0.07	0.04
N	0.25	0.36	0.14	0.31	0.05	0.18
R_{MAX}	0.12	0.12	0.12	0.08	0.08	0.08
R^2	0.96	0.95	0.99	0.96	0.95	0.98
Slope calculations						
Concentration (Ims)	222.05	160.89	123.15	2.96	2.34	1.85
Response at Ims	0.24	0.20	0.15	0.29	0.29	0.23
Slope at Ims (max slope)	0.005	0.006	0.006	0.51	0.66	0.61
Efficiency calculations						
Concentration (Ime)	358.13	266.25	209.90	4.50	3.54	2.95
Response at Ime	0.84	0.75	0.64	0.93	0.93	0.80
Maximum efficiency	0.003	0.004	0.005	0.28	0.36	0.39
Maintenance (Iro)	172.15	126.94	99.88	2.36	1.87	1.47
Concentration at $I_{85\%SR}$	631.8	519.1	481.5	6.54	5.13	5.00
Response at $R_{85\%SR}$	1.30	1.29	1.27	1.26	1.26	1.23

TABLE 4
Parameter estimates ± standard error derived from fitting relative protein deposition in snapper as a function of DP:DE ratio of test diets. Data presented as different curves for each data set and one curve for all data sets

	Relative protein deposition			Relative protein deposition
	HE	ME	LE	(combined parameters)
Best-fit values				
B	-0.35	-0.35	-0.35	-0.35
$K_{0.5}$	12.9	13.1	13.7	13.3
N	3.61	2.52	2.69	2.93
R_{MAX}	1.33	1.58	1.52	1.47
Std. error of best fit values				
B	0.04	0.11	0.01	0.03
$K_{0.5}$	0.54	2.57	0.30	0.62
N	0.60	1.44	0.22	0.47
R_{MAX}	0.10	0.47	0.06	0.12
R^2	0.99	0.98	1.00	0.98
Slope calculations				
Intake (Ims)	11.02	9.39	10.25	10.43
Response at Ims	0.26	0.23	0.24	0.25
Slope at Ims (max slope)	0.13	0.11	0.11	0.11
Efficiency calculations				
Intake (Ime)	16.83	15.47	16.65	16.65
Response at Ime	0.86	0.81	0.82	0.85
Maximum efficiency	0.07	0.08	0.07	0.07
Maintenance (Iro)	8.93	7.23	7.94	8.17
Intake at $I_{85\%SR}$	24.8	29.7	29.7	28.3
Response at $R_{85\%SR}$	1.19	1.36	1.31	1.29

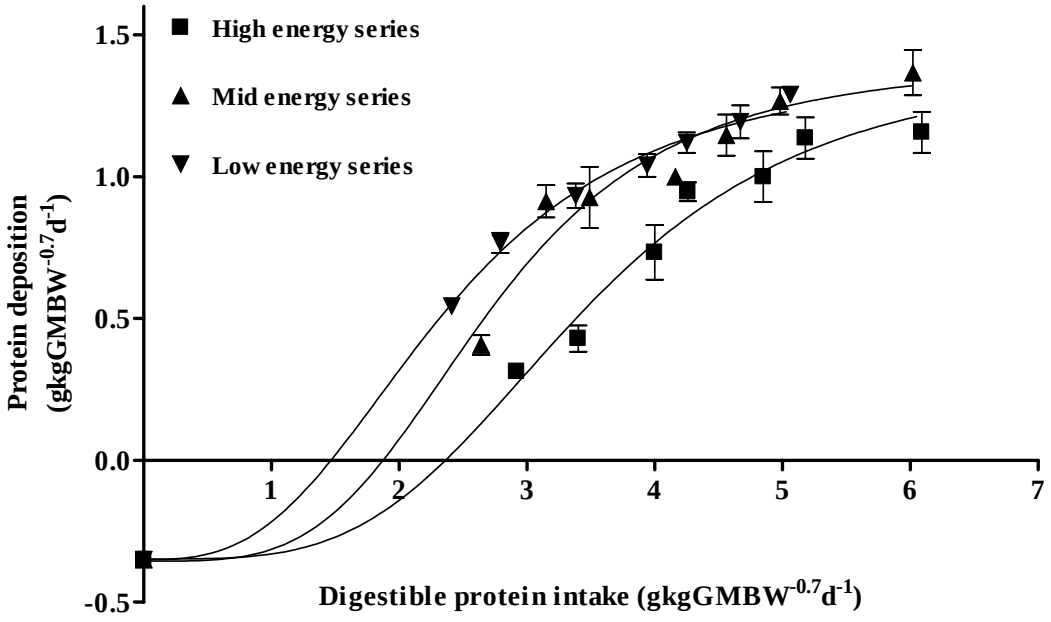

FIGURE 1

Effect of digestible protein intake on relative protein deposition in Australian snapper after 57 days on test diets. Parameter R_{MAX} shared.

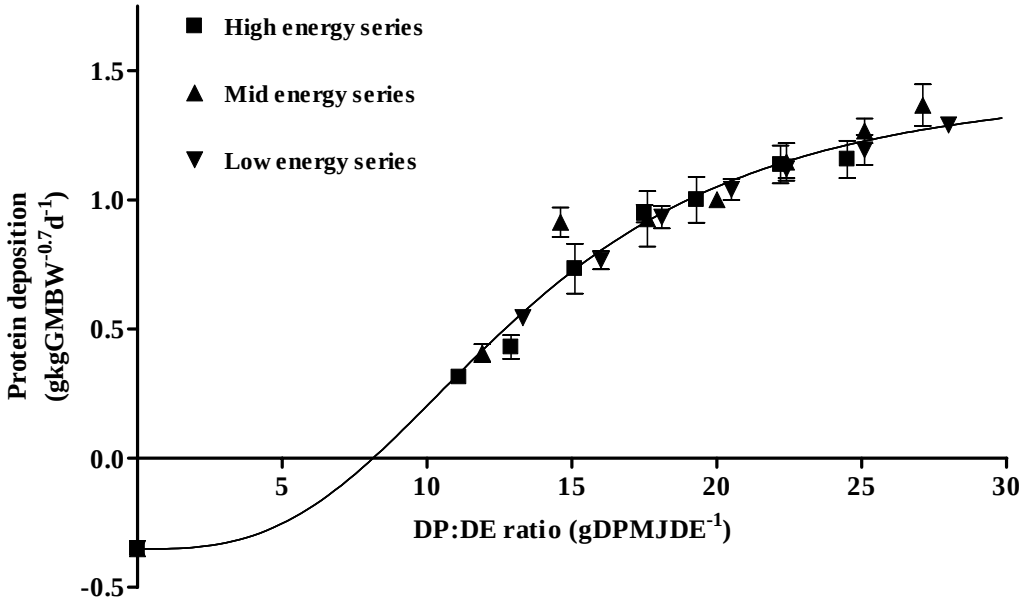

FIGURE 2

Effect of digestible protein (DP) and digestible energy (DE) ratio on relative protein deposition of Australian snapper after 57 days on test diets. All parameters shared

4.3 Investigation of the nutritional requirements of Australian snapper *Pagrus auratus* (Bloch & Schneider, 1801): digestibility of gelatinised wheat starch and clearance of an intra-peritoneal injection of D-glucose.

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ABSTRACT

Two experiments were done to investigate the digestibility and utilisation of carbohydrate sources by Australian snapper *Pagrus auratus*. In the first experiment, snapper of two different size classes (110 g and 375 g) were fed a reference diet containing no starch (REF) or diets containing 150 (PN15), 250 (PN25), 350 (PN35) or 450 g kg^{-1} (PN45) of 100% gelatinised wheat starch to investigate the interactive effects of fish size and starch inclusion level on apparent organic matter (OM) or gross energy (GE) digestibility (ADC), post-prandial plasma glucose concentration, hepatosomatic index (HSI) and liver or tissue glycogen content. A second experiment employed a 72 h time course study to investigate the ability of larger snapper (300-481 g) to clear an intra-peritoneal injection of 1 g D-glucose kg^{-1} body weight (BW).

OM and GE ADCs declined significantly in both fish sizes as the level of starch increased (PN45<PN35<<PN25<PN15). Only GE ADC was significantly affected by fish size (ADCenergy small fish < ADCenergy large fish). There was no interaction between fish size and inclusion level with respect to GE or OM ADC. GE ADC for both sized fish could be described by a linear function; GE ADC = 104.97 (±3.39) – 0.109 (±0.010) * inclusion level (R2 = 0.86). HSI, liver and muscle glycogen concentrations were significantly elevated in both small and large snapper fed diets containing gelatinised starch compared to snapper fed the REF diet. Three-hour post-prandial plasma glucose concentrations were not significantly affected by fish size, inclusion level, or the interaction of these factors (REF=PN15=PN25=PN35=PN45) and ranged between 1.60 and 2.5 mm.

Resting levels of plasma glucose (0 h) ranged from 0.4 to 4.6 mM. Circulating levels of plasma glucose in snapper peaked at 18.9 mM approximately 3 h after intra-peritoneal injection and fish exhibited hyperglycaemia for at least 12-18 h. There were no significant differences between the plasma glucose concentrations of snapper sampled 0, 18, 24, 48 or 72 h after injection (0 h=18 h=24 h=48 h=72 h<12 h<1 h<3 h=6 h), indicating snapper required almost 18 h to regulate their circulating levels of glucose to near basal concentrations.

Australian snapper are capable of digesting moderate levels of gelatinised wheat starch, however, increasing the dietary content of starch has an immediate negative impact on OM and GE digestibility. Smaller snapper appear to be less capable of digesting gelatinised starch than larger fish, and levels above 250 and 350 g kg^{-1} of diet are not recommended for small and large fish respectively. Snapper subjected to an intra-peritoneal injection of D-glucose suffer from prolonged hyperglycaemia, however the post-prandial response to the uptake of glucose from normally digested gelatinised starch appears to be more regulated.

1. INTRODUCTION

Australian snapper *Pagrus auratus*, also known as red sea bream (*P. auratus* = *P. major*; Paulin, 1990; Tabata & Taniguchi, 2000), is a marine carnivore being farmed in small numbers in sea-cage operations around Australia. A recent investigation of the ability of snapper to digest increasing contents of extruded wheat indicated they were highly efficient at digesting the protein from this carbohydrate (CHO) source, but gross energy digestibility decreased linearly as the inclusion level of wheat increased (Booth, Allan & Anderson, 2005). These results indicated that energy apparent digestibility coefficients (ADCs) for CHOs are not entirely additive, a result that has also been reported for CHO ADCs in gilthead seabream *Sparus auratus* (Lupatsch, Kissil, Sklan & Pfeffer, 1997). This variability in CHO digestibility underscores the need to determine the ADCs of CHO sources over a wide range of inclusion levels in order to accurately formulate research and commercial feeds. Other studies have confirmed that sparids are reasonably efficient at digesting the energy from wheat, provided inclusion levels are not excessive (Georgopoulos & Conindes, 1999; Venou, Alexis, Fountoulaki, Nengas & Apostologpoulou, 2003). Clearly, CHO sources (excluding non-starch polysaccharides; NSP) have potential for use in sparid diets, but this potential will ultimately be governed by the specific protein and lipid requirements of the species in question which in turn dictates the "formulation space" available for carbohydrate inclusion.

Initial and rapid investigations of CHO utilisation in fish and other species is often elucidated using glucose tolerance tests (Moon, 2001; Stone, Allan & Anderson, 2003a) due to the fact that CHO energy sources that are digested (i.e. starch) are generally reduced to monosaccharides such as glucose during the digestive process (De Silva & Anderson, 1995; Davies & Gouveia, 2004). Glucose serves as the primary energy source in mammalian metabolism, however its role in fish metabolism is not as well understood (Moon, 2001; Hemre, Mommsen & Krogdahl 2002). Most fish species exhibit a prolonged state of hyperglycaemia when subjected to acute loads of glucose and, in a clinical sense, are considered to exhibit impaired glucose tolerance (Wilson, 1994; Moon, 2001). It is also common for fish to deposit excessive loads of CHO as glycogen, which itself becomes a relatively unavailable source of endogenous energy due to inefficiencies in the glycogenolysis pathway (De Silva & Anderson, 1995).

In the present study, we have extended our investigation of CHO utilisation in snapper by evaluating the effect of gelatinised starch inclusion level on gross energy digestibility, post-prandial plasma glucose concentration and liver and tissue glycogen content. The fact that improvements in the digestibility of carbohydrates for fish can be made by reducing the structural complexity of starch granules through thorough gelatinisation is well documented in nearly all fish species (Stone, 2003). For this reason, we have investigated a 100% gelatinised product, which should approximate the degree of gelatinisation obtained during the cooking/expansion process applied in most modern feed mills. In a separate experiment, snapper were subjected to a glucose tolerance test (GTT) to evaluate their ability to regulate a rapid influx of highly available CHO in the form of D-glucose.

2. MATERIALS AND METHODS

The snapper used in both experiments were progeny of first generation broodstock held at the NSW DPI Fisheries Port Stephens Fisheries Centre (PSFC). Prior to use in these experiments, snapper were grown at low densities in large 10 kL tanks and fed twice daily on a commercial barramundi Lates calcarifer feed (Ridley AquaFeed Pty. Ltd., Narangba, Qld, Australia; reported nutrient composition: 50% crude protein; 12% crude fat; 18 MJ kg^{-1} gross energy).

2.1 Digestibility of pregelatinised wheat starch

The chemical composition of ingredients used in the digestibility experiment is presented in Table 1. The apparent digestibility of 100% gelatinised wheat starch (Pregel-N; Penford Australia Ltd., Lane Cove, NSW, Australia) was evaluated by substituting a reference diet composed predominantly of fish meal with either 150, 250, 350 or 450 g kg^{-1} of gelatinised wheat starch. All dry ingredients, vitamin and mineral premixes and the inert marker (5 g chromic oxide kg^{-1} diet) were combined on a dry matter basis according to the formulations presented in Table 2. Each dietary mash was then thoroughly mixed (Hobart Mixer; Troy Pty Ltd, OH, USA) before being ground in a laboratory scale hammer mill fitted with a 1.5 mm screen (Raymond Laboratory Mill, Transfield Technologies, Rydalmere, NSW, Australia). The ground mash was thoroughly re-mixed, combined with wet ingredients (distilled water and fish oil) and formed into 4.0 mm pellets using a meat mincer (Barnco Australia Pty Ltd, Leichhardt, NSW, Australia). Moist pellets were then dried for 5 to 6 h (≈ 35º C) until moisture content was < 100 g kg^{-1} diet. Following preparation, all diets were stored frozen at < -15ºC until required.

Groups of snapper were anaesthetised (10-25 mg L-1 ethyl-ρ-aminobenzoate) and transferred from their holding facilities to a laboratory that contained 27 digestibility tanks. A detailed description of this system is given in Allan, Rowland, Parkinson, Stone & Jantrarotai (1999), while the specific procedures used to collect and store faeces from snapper are described in Booth *et al.* (2005). Fifteen tanks were each stocked with 12 small snapper (mean individual weight ± SD = 110 ± 3.5 g; n=180), while the remaining 12 tanks were each stocked with 5 large snapper (mean individual weight ± SD = 375 ± 50 g; n=60). Following stocking, each of the five dietary treatments was randomly assigned to three replicate tanks containing small snapper. All dietary treatments, with the exception of the diet containing 250 g kg^{-1} gelatinised wheat starch, were randomly assigned to three replicate tanks containing large snapper. All snapper were acclimated to the experimental conditions (24L:0D photoperiod) and test diets for 12 days prior to the collection of faeces. Test diets were fed to excess (confirmed by the presence of uneaten feed in faecal collection tubes), over a period of approximately 3 h between 0830 and 1130 h each day. Tanks and collection tubes were cleaned and rinsed before faecal matter was allowed to settle overnight (≈ 18 h). Faeces were removed the following morning prior to feeding. Daily faecal collections from individual tanks were pooled and kept frozen (< -15ºC) until a sufficient quantity was obtained for chemical analyses. Afterwards, faecal samples were oven dried (60º C), finely ground (Waring, model 32 BL 80, New Hartford, CT, USA) and then re-dried under absorbent silica gel in vacuum desiccators (70 mm Hg).

Apparent digestibility coefficients (ADC) for reference and test diets were calculated according to the following equation:

$$ADC\ (\%) = 100*[1 - (F/D*DCr/FCr)], \qquad \text{Equation 1.}$$

where F = % nutrient or gross energy in faeces; D = % nutrient or gross energy in diet; DCr = % chromic oxide in diet; FCr = % chromic oxide in faeces (Cho, Slinger & Bayley 1982). Apparent digestibility coefficients for ingredients were calculated according to the following equation:

$$ADCING\ (\%) = [(NutrTD*ADTD) – (PRD*NutrRD*ADRD)]/[(PING*NutrING)], \qquad \text{Equation 2.}$$

where ADCING = apparent digestibility of nutrient or gross energy in the test ingredient; NutrTD = the nutrient or gross energy concentration in test diet; ADTD = the apparent digestibility of the nutrient or gross energy in the test diet; PRD = proportional amount of reference diet; NutrRD = the nutrient or gross energy concentration in the reference diet; ADRD is the apparent digestibility of nutrient or gross energy in the reference diet; PING = proportional amount of test ingredient; NutrING is the nutrient or gross energy concentration in the test ingredient (Sugiura, Dong, Rathbone & Hardy 1998).

2.2 Post-prandial plasma glucose, HSI and glycogen evaluations

Snapper were maintained on their respective test diets using similar feeding protocols for a further 4 days (overall total of 34 days). All fish were then starved for approximately 40 h before each tank was re-offered an unrestricted amount of their respective diet in one sitting lasting 5 min. Feeding of individual digestibility tanks was systematically staggered over a period of 2.75 h (tank 1 through tank 27) in preparation for blood sampling procedures. Exactly 3 h after feeding had ceased, individual tanks of snapper were rapidly anaesthetised (30-40 mg L-1 ethyl-ρ-aminobenzoate) and 3 fish were randomly selected and removed for immediate blood sampling. Approximately 2 mL of blood was withdrawn from the caudal vein of each fish using a 23 gauge x 1.25 mm hypodermic needle and 3 mL syringe (Becton-Dickinson B-D, Singapore). Blood samples were collected within 2 min of capture to prevent stress mediated glucose responses (Stone *et al.* 2003a). To prevent haemolysis, needle tips were removed before whole blood samples were transferred into specialised 2 mL collection tubes prepared to prevent coagulation and halt glycolysis (VACUETTE Greiner Bio-one FE; Sodium Fluoride / EDTA K3). Labelled samples were refrigerated and immediately transferred to a NATA accredited pathology laboratory for analysis of plasma glucose.

The snapper used to obtain blood samples were euthanased (overdose of anaesthetic), weighed and then placed into labelled bags and frozen (< -15ºC). The remaining fish were weighed and returned to the holding facilities. Frozen fish were later thawed to determine hepatosomatic index (HSI) and collect liver and muscle tissue samples for glycogen analysis. Because fish stomachs contained significant volumes of feed, they were excised and weighed (wet weight) in order to accurately calculate HSI. A single tissue sample (muscle) was taken from an area of deep muscle, located on the lateral line approximately 20 mm from the operculum. The stomach and intestinal contents were also inspected to confirm whether all fish had consumed test diets prior to blood sampling. Individual response data obtained for fish in each tank were averaged to provide a single replicate value for statistical comparisons.

2.3 Chemical analyses

Analyses of ingredient, diet or faecal samples were performed by the Food & Agriculture Laboratory of Australia (FALA; Coopers Plains, Qld, Australia). Crude protein (N x 6.25) was analysed by the Leco Method. Total fat was determined after extraction using chloroform and methanol (Folch method) while moisture (oven drying), ash (muffle furnace) and gross energy (bomb calorimeter) were determined using standard procedures (AOAC 1995). Faecal collections from large fish randomised to the reference diet were pooled in order to perform gross energy analysis due to inadequate collections from individual tanks.

Plasma glucose was determined using an enzymatic reference method that used hexokinase to convert D-glucose to NADH. The concentration of NADH was determined by measuring its absorbance at 340 nm (COBAS INTEGRA 700; Hunter Area Pathology Service, Newcastle, NSW, Australia).

The glycogen content of liver or muscle tissue samples was measured (Newcastle University, NSW, Australia) as total hydrolysed CHO (ug/mg) following the phenol-sulfuric acid reaction method described in Dubois, Gilles, Hamilton, Rebers & Smith (1956)

2.4 Acute glucose tolerance test

A time course study was used to investigate the ability of snapper to deal with a rapid influx of dietary carbohydrate by subjecting fish to an intra-peritoneal injection of 1 g D-glucose kg^{-1} body weight (BW). A stock glucose solution was prepared by dissolving 50 g anhydrous analytical grade D-glucose (Ajax Finechem, Seven Hills, NSW, Australia) in 100 mL of sterilised distilled water (autoclaved). In addition, two control treatments were employed to confirm that neither handling nor injection procedures unduly influenced plasma glucose concentrations (Stone, *et al.* 2003a).

All fish were injected or handled and sampled only once. Afterwards they were returned to holding tanks to recover. Fish subjected to the sham control were injected with a sterile saline solution (0.9% sodium chloride; AstraZeneca), while fish subjected to the handling control were exposed to exactly the same experimental procedures but were not injected. The volume of glucose or saline solution injected ranged between 0.60 – 0.96 mL, depending on fish weight.

Groups of snapper, ranging in weight from 300 to 481 g, were held in 1200 L tanks lined with black plastic and fasted for 72 h prior to beginning each trial. On each occasion, fish were lightly sedated (20 mg L-1 ethyl-ρ-aminobenzoate) before 3 randomly selected fish were sampled to establish resting plasma glucose levels (0 hour), weighed and removed from the experiment. The remaining fish were removed individually and weighed before being randomly allocated to one of the experimental treatments (glucose, sham or handling). The time was recorded and each fish was placed into a separate 200 L floating cage secured inside a circular 10 kL tank. The inside of each 200 L cage was lined with a black plastic insert to prevent the inadvertent disturbance of fish during the experiment. Fluorescent lighting provided a 24L:0D photoperiod. Blood samples were withdrawn from un-anaesthetised fish within 90 seconds of capture 3, 6, 12, 18, 24, 48 and 72 h after being injected or handled. The experimental procedure was repeated over a number of days to provide multiple replicates for each collection time (individual fish were treated as replicates; minimum of n=4 per time). Procedures for collection of blood and analysis of plasma glucose were as previously described.

2.5 Water quality

Water quality was monitored daily in both experiments using one of two hand held water quality analysers; either a Model 611 (Yeo-Kal Electronics, Brookvale, NSW, Australia) or a Horiba U-10 (Horiba, Japan). Total ammonia [NH3 + NH4+] was monitored regularly using a rapid test kit procedure (Model 1.08024.0001, E. Merck, Darmstadt, Germany). During the faecal collection phase of Exp.1, temperature, dissolved oxygen (DO2), salinity and pH ranged from 20.6-25.7º C, 4.3-6.0 mg L-1, 27-30‰ and 7.2-7.9 units respectively with total ammonia always < 0.4 mg L-1. Recorded values for Exp.2 in the same order were, 20.2-21.0º C, 6.0-7.0 mg L-1, 34-36‰ and 7.9-8.2 units respectively with total ammonia always < 0.2 mg L^{-1}.

2.6 Statistical analyses

Where appropriate, the results were subjected to one-way or multifactor ANOVA after data was tested to ensure that treatment variances were homogenous (Cochran's test). With respect to digestibility of gelatinised starch, only the data for ingredient ADCs were examined. The significance level for all ANOVA and multiple comparisons tests (Student Newman Keul's) was set at 0.05 and data were statistically analysed using Statgraphics Plus, version 4.1 (Manugistics Inc., Rockville, MD, USA, 1998). Linear regression analysis was used to investigate the relationship between the inclusion content of gelatinised starch and apparent gross energy digestibility.

3. RESULTS

Snapper were capable of digesting approximately 90% of the organic matter and gross energy from pre-gelatinised wheat starch providing inclusion levels did not exceed 150 g kg^{-1} diet (Table 3). The interactive effects of fish size and the inclusion level of gelatinised starch on organic matter or gross energy ADCs was investigated by eliminating the 250 g kg-1 treatment assigned to smaller snapper from a two-way ANOVA. In both cases, the interaction term was non-significant (P<0.05). Organic matter digestibility was significantly affected by inclusion level (P<0.0001; PN45<PN35<PN15), but not fish size (P<0.521). Gross energy digestibility was affected by inclusion level (P<0.0001; PN45<PN35<PN15) and fish size (P=0.0147; small fish < large fish), however, the size effect explained only a small proportion of the total variance (3.5%) compared to the amount explained by inclusion level (88.1%). Subsequently, data for energy ADCs for different size fish were pooled to model the response of gross energy digestibility to increasing dietary level of gelatinised starch. The data were adequately described by the linear function; gross energy ADC = 104.97 (±3.39) – 0.109 (±0.010) x inclusion level (R2 = 0.86; *Figure* 1).

Examination of fish stomachs during dissection revealed that all fish had consumed their respective feeds prior to withdrawal of blood samples. In many cases, the stomach was tightly packed with feed and there was partially digested material in the anterior intestine. Intact pellets were observed in the stomachs of some fish, especially those fed diets containing 450 g kg-1 gelatinised starch.

The effect of fish size and the inclusion level of gelatinised starch on HSI or 3 h post-prandial plasma glucose concentration was examined with two-way ANOVA by including data values for the reference diet treatments (zero CHO) but again excluding data from the 250g kg^{-1} treatment. The HSI of snapper was significantly affected by fish size (P<0.0001) and inclusion level (diet; P<0.0001), but not the interaction between these terms. Smaller snapper exhibited significantly higher HSI than larger snapper and the HSI of snapper fed the reference diet was significantly lower than the HSI of fish fed the other dietary treatments (REF<PN15=PN35=PN45; Table 4). A statistical comparison (one-way ANOVA) of HSI in smaller snapper that included the data recorded for the 250 g kg-1 treatment indicated there was no difference between the HSI of smaller snapper fed diets containing gelatinised starch (REF<PN15=PN25=PN35=PN45; Table 4). The livers of snapper fed CHO diets were white and pale compared to those fed the reference diet.

Three-hour post-prandial plasma glucose concentrations were not significantly affected by fish size, inclusion level, or the interaction of these factors (Table 4).

Two-way ANOVA indicated both fish size and the presence or absence of dietary starch (REF vs PN45), but not the interaction of these terms, significantly affected muscle or liver glycogen concentration in snapper. Snapper reared on diets containing 450 g kg^{-1} gelatinised starch had significantly elevated levels of muscle and liver glycogen compared to snapper fed a reference diet devoid of starch, and glycogen concentrations were consistently higher in the organs of smaller snapper regardless of diet (Table 4).

Circulating levels of plasma glucose in snapper peaked at 18.9 mM approximately 3 h after injection of 1 g $kgBW^{-1}$ D-glucose and fish exhibited prolonged hyperglycaemia for at least 12 h (Figure 2). One-way ANOVA revealed there were no significant differences between the plasma glucose concentrations of snapper sampled 0, 18, 24, 48 or 72 h after injection with glucose, indicating snapper of this size required almost 18 h to regulate their circulating levels of glucose to near basal concentrations (P<0.0001; 0 h=18 h=24 h=48 h=72 h<12 h<1 h<3 h=6 h). Resting levels of plasma glucose (0 h) ranged from 0.4 to 4.6 mM. Minor fluctuations were recorded in the plasma

concentrations of snapper subjected to the sham injection or handling stress, most notably within the first hour, but these fluctuations were small compared to the effects of glucose loading.

4. DISCUSSION

4.1 Digestibility of gelatinised starch

Organic matter and gross energy ADCs for snapper fed pre-gelled wheat starch closely approximated the gross energy ADC reported for similar quantities of wheat flour fed to gilthead seabream (Lupatsch, *et al.* 1997) and for gelatinised starch fed to European seabass *Dicentrarchus labrax* (Peres & Oliva-Teles, 2002). There was some evidence that larger snapper appeared to be slightly more capable of digesting the energy from pre-gelled wheat starch at higher inclusion levels, but there is little information on this subject in the literature. Explanation for this difference is most probably related to feed intake. Although this was not recorded, larger snapper are likely to have consumed less feed per unit body weight than smaller snapper. This may have increased digestibility, as demonstrated in rainbow trout *Oncorhynchus mykiss* and gilthead seabream (Bergot & Breque, 1983; Fernandez, Miquel, Guinea & Martinez, 1998).

Limited data are available on CHO digestibility or utilisation in red sea bream (i.e. snapper). Earlier work described decreases in the growth of juveniles (70 g) fed increasing contents of glucose (Furuichi, *et al*. 1971, cited in Koshio 2002). Feeding high levels of dextrin (>300 g kg-1) caused poor growth and feed efficiency, high hepatic glycogen concentration and reductions in protein absorption (Furuichi & Yone, 1980, cited in Koshio, 2002). A later study indicated that α-starch was better utilised than the less complex CHOs dextrin and glucose when measured in terms of growth and feed efficiency (Furuichi & Yone, 1982 cited in Koshio, 2002). In contrast, CHO digestibility was lower for native potato starch and dextrin (0.67) than glucose when included at 250 g kg-1 (Furuichi, 1983, cited in Stone, 2003), demonstrating that digestibility coefficients for CHOs can be particularly misleading when used as a guide to overall utilisation (Stone, 2003).

The size of fish used in this study had little impact on either organic matter or gross energy digestibility of gelatinised wheat starch. In contrast, the inclusion level of gelatinised wheat starch had a significant negative impact on the digestibility of these components, reflecting the results of a previous experiment with snapper that showed the organic matter and gross energy digestibility of extruded wheat declined as inclusion levels were increased from 200 to 400 g kg-1 diet (Booth, *et al.* 2005). Linear reductions in the digestibility of dextrin or potato α-starch have also been demonstrated in yellowtail (Shimeno, Hosokawa & Tadeka, 1979), juvenile rainbow trout as ingredient inclusion levels were increased from 200 to 600 g kg-1 diet (Singh & Nose, 1967) and in cod fed dextrinised potato starch, as levels increased from as little as 33 to 124 g kg-1 diet (Hemre, Lie, Lied & Lambertsen, 1989). Stone, Allan & Anderson (2003b) reported significant reductions in dry matter, energy and starch ADCs in the omnivorous silver perch *Bidyanus bidyanus* when dietary levels of gelatinised starch were increased from 300 to 600 g kg^{-1}. In contrast, a positive relationship between CHO digestibility and inclusion level of raw starch (corn flour) was reported in common carp (Appleford & Anderson, 1996).

This result confirms that for snapper, gross energy digestibility coefficients for CHOs are not additive, and individual coefficients must be determined for a range of inclusion levels in order to accurately formulate research or commercial aquaculture feeds. Experiments with gilthead seabream have demonstrated that although digestible protein and lipid levels in compound feeds can be accurately predicted using ADCs determined for individual ingredients, predictions for digestible CHO and gross energy were far less accurate (Lupatsch, *et al.* 1997).

4.2 HSI and glycogen concentration

In the present study, diets containing as little as 150 g kg^{-1} gelatinised starch significantly increased the relative liver weight (HSI) and the liver and tissue glycogen concentrations of both small and large snapper compared to fish fed the reference diet. The snapper fed on starch diets also had discoloured livers which may be indicative of lipid accumulation (De Silva & Anderson, 1995), although this was not measured. The ratio of liver to muscle glycogen content also appears to be well conserved despite the differences in dietary treatments. These results are typical of many studies investigating CHO metabolism in fish (Brauge, Medale & Corraze, 1994; Hemre, *et al.* 2002; Krogdahl, Sundby & Olli, 2004), and may indicate that excessive energy from CHO has been deposited in the liver as glycogen (Yone, 1976; Shimeno, *et al.* 1979; Meton, *et al.* 2003) and possibly encouraged lipogenesis (Brauge, *et al.* 1994; Brauge, *et al*. 1995; Hung & Storebakken, 1994; Hemre, *et al.* 2002; Peres & Oliva-Teles, 2002; Stone, 2003; Venou, *et al.* 2003).

Elevated HSI and liver glycogen content can be indicative of liver dysfunction in fish resulting in suppression of immune functions (Hemre, *et al.* 2002). For example, excessive deposits of glycogen (140 g kg^{-1} wet weight) caused liver dysfunction in rainbow trout fed on high starch diets (Baeverfjord, 1992; cited in Hemre, *et al.* 2002). These levels are almost double the highest concentration recorded in the liver of snapper fed diet PN45 (Table 4). However, liver glycogen levels of Atlantic salmon were unaffected by carbohydrate inclusion level and remained close to 6.4 g kg^{-1} after being fed diets containing up to 210 g kg^{-1} gelatinised starch (Arnesen & Krogdahl, 1996).

With regard to differences in fish size, Austreng, Risa, Edwards & Hvidsten (1977) suggested that larger rainbow trout might be more efficient at metabolising CHO than smaller fish, based on reductions in the CHO content (measured as glucose) of fish livers examined after 24 weeks feeding on diets containing up to 38% metabolisable energy from CHO. This observation is supported by the lower HSI and glycogen concentrations of the larger snapper in the present study. In addition, much higher levels of liver glycogen were also recorded in small gilthead seabream (18.5 g) fed a diet containing 32% CHO (approximately 180 g kg^{-1} wet weight; Meton, Fernandez & Baanante, 2003), which supports other evidence of the greater capacity of larger fish to deal with higher CHO levels (Hemre, Mangor-Jensen, Rosenlund, Waagbo & Lie, 1995; Hemre, Shiau, Deng, Storebakken & Hung, 2000). In our study, the similarity in HSI of snapper fed all CHO diets may be indicative of the livers capacity to store glycogen, and that even moderate levels of highly available CHO (e.g. 150 g kg^{-1}) exceed the capacity of this organ, as suggested by Hemre, Lie & Sundby (1993) for cod. Whether or not this has any long term nutritional or health implications for snapper as demonstrated in rainbow trout remains to be determined.

4.3 Glucose tolerance

The ability to absorb and rapidly regulate plasma glucose to basal circulating levels after either an injected or fed dose of highly available CHO such as glucose, is used as a relative measure of CHO tolerance (Moon, 2001). Accordingly, the slightly slower assimilation and prolonged hyperglycaemia exhibited by snapper indicates impaired glucose homeostasis when glucose is administered via the intra-peritoneal cavity. Handling or sham controls only had a minor effect on circulating plasma glucose concentrations, as was found in Atlantic salmon (Hemre & Krogdahl, 1996), white sturgeon *Acipenser transmontanus* (Deng, Reftsie & Hung, 2001) and silver perch (Stone, *et al.* 2003a).

The glucose response in snapper is similar to that reported for other carnivorous fish such as gilthead seabream, European seabass (Peres, Goncalves & Oliva-Teles, 1999) and turbot *Scophthalmus maximus* (Garcia-Riera & Hemre, 1996), challenged with an intra-peritoneal injection of glucose, but was not as rapid as the uptake or clearance of glucose in the plasma of the more omnivorous silver

perch (Stone, *et al.* 2003a). Peak response and length of hyperglycaemia in snapper in the present study tended to be higher than that recorded for red sea bream (Yone, 1976) challenged with an oral dose of 1.67 g glucose kgBW-1 (peak of 13.8 mM after 2 h). This may indicate the route of assimilation of glucose into the bloodstream differs between the peritoneal cavity and digestive tract, as suggested by Stone, *et al.* (2003a). The majority of evidence for poor glucose removal from the plasma compartment in fish now points to mechanisms involving either a lack of peripheral white muscle glucose transporters sensitive to insulin or other rate limiting steps in glucose metabolism (Wright, O'Hali, Yang, Han & Bonen, 1998; Moon, 2001; Hemre, *et al.* 2002; Gisbert, Sainz & Hung, 2003; Stone, 2003). The fate of glucose assimilated by snapper in the present study was not tested, however several pathways for clearance of excess glucose exist in fish including glycosuria (Deng, *et al.* 2001) and excretion across the gill (Stone, *et al.* 2003a).

Unlike the rapid response to an intra-peritoneal injection of glucose, plasma glucose concentrations measured 3 h after feeding were not significantly affected by fish size or starch inclusion level. On closer inspection, a minor increase in plasma glucose concentrations in fed snapper is apparent, although not significant, as is the possibility that larger snapper may be more capable of controlling the uptake of CHO from the digestive system. In red sea bream, peaks in plasma glucose occurred approximately 2 h after oral administration of 1.67 to 1.7 g glucose $kgBW^{-1}$ (Yone, 1976; Furuichi & Yone, 1981). The lack of a significant response in snapper fed gelatinised wheat starch is likely related to the well-bound nature of the CHO based diets and the complexity of the pre-gelatinised wheat starch, effectively slowing the digestive process through a need to hydrolyse starch which ultimately delayed the assimilation of glucose. Evidence for this hypothesis is supported by the study of Deng, *et al.* (2001), who demonstrated CHOs of higher complexity, such as potato starch, potato dextrin and corn starch had less effect on elevating plasma glucose concentration over time than orally administered glucose or maltose fed to white sturgeon. Peak plasma glucose concentrations also occurred later in white sturgeon fed complex starches. Similar effects were seen in rainbow trout fed diets containing either gelatinised wheat or raw wheat starch (Brauge, *et al.* 1994). Deng, *et al.* (2001) also developed a plasma glycaemic index which correlated well with digestibility coefficients as well as peak plasma concentration for sturgeon, and ranked ingredients glucose>maltose>corn dextrin>potato dextrin>corn starch>potato starch. In the case of snapper fed different levels of gelatinised wheat starch, plasma glucose levels may have increased after the 3 h period as indicated by the slight upward trend in plasma glucose concentration (Table 4). Although the post-prandial sampling time is not given, Hemre, Waagbo, Hjeltnes & Aksnes (1996) demonstrated a positive correlation between CHO inclusion level and plasma glucose concentration in Atlantic salmon fed between 24 to 230 g pre-gelatinised starch (maize:wheat, 1:1). However, plasma glucose concentrations were remarkably stable and only varied from 3.13 to 6.89 mM.

5. CONCLUSIONS

Australian snapper are capable of digesting moderate levels of gelatinised wheat starch. However, increasing the dietary content of gelatinised starch results in a predictable reduction in digestibility. In terms of the capacity to digest gelatinised starch, smaller snapper appear to be less capable than larger fish, and levels above 250 and 350 g kg^{-1} of diet are not recommended for small and large fish respectively. Snapper subjected to an intra-peritoneal injection of highly available glucose suffer from prolonged hyperglycaemia, however the post-prandial response to the uptake of glucose from normally digested gelatinised starch appears to be more regulated, either because of the physical complexity of the starch or the functional qualities it provides to the pellet.

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REFERENCES

Allan, G.L., Rowland, S.J., Parkinson, S., Stone, D.A.J. & Jantrarotai, W. (1999). Nutrient digestibility for juvenile silver perch *Bidyanus bidyanus*: development of methods. Aquaculture **170**:131-145.

AOAC (1995). *Official methods of analysis of the association of official chemists.* (ed. K. Helrich), 15th ed., Published by the Association of the Official Analytical Chemists, Inc., Arlington, Virginia, USA.

Appleford, P. & Anderson, T.A. (1996). The effect of inclusion level and time on digestibility of starch for common carp (*Cyprinus carpio*, Cyprinidae). Asian Fisheries Science **9**:121-126.

Arnesen, P. & Krogdahl, A. (1996). Gelatinized wheat in diets for Atlantic salmon, *Salmo salar*, L. A full-scale production experiment. Aquaculture Nutrition **2**:49-54.

Austreng, E., Risa, S., Edwards, D.J. & Hvidsten, H. (1977). Carbohydrate in rainbow trout diets. II. Influence of carbohydrate levels on chemical composition and feed utilisation of fish from different families. Aquaculture **11**:39-50.

Bergot, F. & Breque, J. (1983). Digestibility of starch by rainbow trout: effects of the physical state of starch and of intake level. Aquaculture **34**:203-212.

Booth, M.A., Allan, G.L. & Anderson, A.J. (2005). Investigation of the nutritional requirements of Australian snapper *Pagrus auratus* (Bloch & Schneider, 1801): apparent digestibility of protein and energy sources. Aquaculture Research **36**:378-390.

Brauge, C., Medale, F. & Corraze, G. (1994). Effect of dietary carbohydrate levels on growth, body composition and glycaemia in rainbow trout, *Oncorhynchus mykiss*, reared in seawater. Aquaculture **123**:109-120.

Brauge, C., Corraze, G. & Medale, F. (1995). Effects of dietary levels of carbohydrate and lipid on glucose oxidation and lipogenesis from glucose in rainbow trout, *Oncorhynchus mykiss*, reared in freshwater or seawater. Comparative Biochemistry and Physiology **111A**(1):117-124.

Cho, C.Y., Slinger, S.J. & Bayley, H.S. (1982). Bioenergetics of salmonid fishes: energy intake, expenditure and productivity. Comparative Biochemistry & Physiology **73B**(1):25-42.

Davies, S. & Gouveia, A. (2004). Cereal processing and improved carbohydrate digestibility in over wintering diets for juvenile gilthead sea bream. International Aquafeed **7**(4):18-23.

Deng, D-F., Reftsie, S. & Hung, S.S. (2001). Glycemic and glycosuric responses in white sturgeon (*Acipenser transmontanus*) after oral administration of simple and complex carbohydrates. Aquaculture **199**:107-117.

De Silva S.S. & Anderson T.A. (1995). Fish Nutrition in Aquaculture. Chapter 3 Metabolism, pp 41-102. Chapman & Hall, South Melbourne, Victoria, Australia.

Dubois, M., Gilles, K.A., Hamilton, J.K., Rebers, P.A. & Smith, F. (1956). Colorimetric method for determination of sugars and related substances. Analytical Chemistry **28**:350-356.

Fernandez, F., Miquel, A.G., Guinea, J. & Martinez, R. (1998).Digestion and digestibility in gilthead sea bream (*Sparus aurata*): the effect of diet composition and ration size. Aquaculture **166**:67-84.

Furuichi, M. & Yone, Y. (1981). Change of blood sugar and plasma insulin levels of fishes in glucose tolerance test. Bulletin of the Japanese Society of Scientific Fisheries **47**:761-764.

Garcia-Riera, M.P. & Hemre, G-I. (1996). Glucose tolerance in turbot, *Scophthalmus maximus* (L.). Aquaculture Nutrition **2:**117-120

Georgopoulos, G.B. & Conindes, A.J. (1999) Study on the effects of dietary starch on growth, food conversion and digestibility of adult gilthead seabream (*Sparus aurata* L. 1758). Journal of Aquaculture in the Tropics **14**(2):143-152.

Gisbert, E., Sainz, R.D. & Hung, S.S. (2003). Glycemic responses in white sturgeon after oral administration of graded doses of D-glucose. Aquaculture **224**:301-312.

Hemre, G-I., Lie, O., Lied, E. & Lambertsen, G. (1989). Starch as an energy source in feed for cod (*Gadus morhua*): digestibility and retention. Aquaculture **80**:261-270.

Hemre, G-I., Lie, O. & Sundby, A. (1993). Dietary carbohydrate utilization in cod (*Gadus morhua*): metabolic responses to feeding and fasting. Fish Physiology and Biochemistry **10**(6):455-463.

Hemre, G-I., Mangor-Jensen, A., Rosenlund, G., Waagbo, R. & Lie, O. (1995). Effect of dietary carbohydrate on gonadal development in broodstock cod, *Gadus morhua* L. Aquaculture Research **36**:399-408.

Hemre, G-I. & Krogdahl, A. (1996). Effect of handling and fish size on secondary changes in carbohydrate metabolism in Atlantic salmon, *Salmo salar* L. Aquaculture Nutrition **2**:249-252.

Hemre, G-I., Waagbo, R., Hjeltnes, B. & Aksnes, A. (1996)..Effect of gelatinized wheat and maize in diets for large Atlantic salmon (*Salmo salar* L.) on glycogen retention, plasma glucose and fish health. Aquaculture Nutrition **2**:33-39.

Hemre, G-I., Shiau, S.Y., Deng, D.F., Storebakken, T. & Hung, S.S. (2000). Utilisation of hydrolysed potato starch by juvenile Atlantic salmon *Salmo salar* L., when using a restricted feeding regime. Aquaculture Research **31**:207-212.

Hemre, G-I., Mommsen, T.P. & Krogdahl, A. (2002). Carbohydrates in fish nutrition: effects on growth, glucose metabolism and hepatic enzymes. Aquaculture Nutrition **8**:175-194.

Hung, S.S. & Storebakken, T. (1994). Carbohydrate utilization by rainbow trout is affected by feeding strategy. Journal of Nutrition **124**:233-230.

Koshio, S. (2002). Red sea bream, *Pagrus major*. In: Nutrient Requirements and Feeding of Finfish for Aquaculture (eds. C.D. Webster & C. Lim). Chapter 4, 51-63. CAB International.

Krogdahl, A., Sundby, A. & Olli, J.J. (2004). Atlantic salmon (*Salmo salar*) and rainbow trout (*Oncorhynchus mykiss*) digest and metabolize nutrients differently. Effects of water salinity and dietary starch level. Aquaculture **229**:335-360.

Lupatsch, I., Kissil, G.Wm., Sklan, D. & Pfeffer ,E. (1997). Apparent digestibility coefficients of feed ingredients and their predictability in compound diets for gilthead seabream, *Sparus aurata* L. Aquaculture Nutrition **3**:81-89.

Meton, I., Fernandez, F. & Baanante, I.V. (2003). Short- and long-term effects of refeeding on key enzyme activities in glycolysis-gluconeogenesis in the liver of gilthead seabream (*Sparus aurata*). Aquaculture **225**:99-107.

Moon, T.W. (2001). Glucose tolerance in teleost fish: fact or fiction? Comparative Biochemistry and Physiology Part B **129**:243-249.

Paulin, C.D. (1990). *Pagrus auratus*, a new combination for the species known as 'snapper' in Australian waters (Pisces: Sparidae). New Zealand Journal of Marine and Freshwater Research **24**:259-265.

Peres, H., Goncalves, P. & Oliva-Teles, A. (1999). Glucose tolerance in gilthead seabream (*Sparus aurata)* and European seabass (*Dicentrarchus labrax*). Aquaculture **179**:415-423.

Peres, H. & Oliva-Teles, A. (2002). Utilization of raw and gelatinized starch by European sea bass (*Dicentrarchus labrax*) juveniles. Aquaculture **205:**287-299.

Shimeno, S., Hosokawa, H. & Takeda, M. (1979). The importance of carbohydrate in the diet of a carnivorous fish. Proceedings of the World Symposium on Finfish Nutrition and Feed Technology, Hamburg 20-23 June 1978. Berlin. Vol 1:127-143.

Singh, R.P. & Nose T. (1967). Digestibility of carbohydrates in young rainbow trout. Bulletin of the Freshwater Fisheries Research Laboratory **17**:21-25.

Stone, A.J. (2003). Dietary carbohydrate utilization by fish. Reviews in Fisheries Science **11**(4):337-369.

Stone, D.A.J., Allan, G.L. & Anderson, A.J. (2003a). Carbohydrate utilization by juvenile silver perch, *Bidyanus bidyanus* (Mitchell). I. Uptake and clearance of monosaccharides following intra-peritoneal injection. Aquaculture Research **34**:1-11.

Stone, D.A.J., Allan, G.L. & Anderson, A.J. (2003b). Carbohydrate utilization by juvenile silver perch, *Bidyanus bidyanus* (Mitchell). II. Digestibility and utilisation of starch and its breakdown products. Aquaculture Research **34**:109-122.

Sugiura S.H., Dong F.M., Rathbone C.K. & Hardy R.W. (1998). Apparent protein digestibility and mineral availabilities in various feed ingredients for salmonid feeds. Aquaculture 159:177-202.

Tabata, K. & Taniguchi, N. (2000). Differences between *Pagrus major* and *Pagrus auratus* through mainly mtDNA control region analysis. Fisheries Science **66**:9-18.

Venou, B., Alexis, M.N., Fountoulaki, E., Nengas, I., Apostolopoulou, M. & Castritsi-Cathariou, I. (2003). Effect of extrusion of wheat and corn on gilthead sea bream (*Sparus aurata*) growth, nutrient utilization efficiency, rates of gastric evacuation and digestive enzyme activities. Aquaculture **225**:207-223.

Wilson, R.P. (1994). Utilisation of dietary carbohydrate by fish. Aquaculture **124**:67-80.

Wright ,J.R., O'Hali, W., Yang, H., Han, X. & Bonen, A. (1998). Glut-4 deficiency and severe peripheral resistance to insulin in the teleost fish tilapia. General and Comparative Endocrinology **111**:20-27.

Yone, Y. (1976). Nutritional studies of red sea bream. Proceedings of the First International Conference on Aquaculture Nutrition. October 14-15 (1975):39-64. Lewes/Rehoboth, Delaware.

TABLE 1
Measured chemical composition of feed ingredients used in experiment 1 (g kg^{-1} or MJ kg^{-1} dry matter basis).

Ingredient	Crude Protein	Ash	Organic matter[1]	Fat	Gross energy (MJ)
Fish meal[2]	757.9	153.3	846.7	94.8	21.5
Fish oil[3]	-	10.0	990.0	990.0	38.0
Maize gluten[4]	630.9	35.6	964.4	69.6	22.2
Pregelatinised starch[5]	8.0	3.6	996.4	10.5	15.7

[1] Organic matter calculated by difference = (1000 – measured ash content)
[2] Austral Group VLT (very low temperature) steam dried (Prime quality) via Ridley Aquafeeds Pty. Ltd., Narangba, QLD, Australia.
[3] Supplied by Skretting (Nutreco), Tasmania, Australia.
[4] Penford Australia Ltd., Lane Cove, NSW, Australia. Maize gluten 60% Plus,.
[5] Penford Australia Ltd., Lane Cove, NSW, Australia. 100% pregelatinised wheat starch – Pregel N.

TABLE 2
Calculated ingredient and measured nutrient composition of test diets used in experiment 1 (g kg^{-1} or MJ kg^{-1} of dry matter).

	Diet[1]				
	REF	PN15	PN25	PN35	PN45
Ingredient					
Fishmeal	800.0	677.5	595.9	514.3	432.7
Fish oil	110.0	93.2	81.9	70.7	59.5
Maize gluten	70.0	59.3	52.1	45.0	37.9
Gelatinised starch	0.0	150.0	250.0	350.0	450.0
Vit/min premix[2]	15.0	15.0	15.0	15.0	15.0
Chromic oxide	5.0	5.0	5.0	5.0	5.0
Nutrient or gross energy					
Crude protein	680.7	619.5	531.6	431.8	374.5
Ash	129.6	116.7	102.4	90.9	78.6
Organic matter[3]	870.4	883.3	897.6	909.1	921.4
Fat	174.5	149.7	132.1	117.6	105.0
NFE[4]	15.2	114.1	234.0	359.7	441.9
Gross energy (MJ)	23.2	22.5	21.8	21.0	20.6

[1] The reference diet was replaced with 150, 250, 350 or 400g kg^{-1} of 100% pregelatinised wheat starch (Pregel-N).
[2] NSW DPI Fisheries premix contains vitamins (IU or mg active ingredient): retinol A 8000iu; cholecalciferol D3 1000iu; DL-α-tocopherol acetate E 125; menadione sodium bisulphite K3 16.5; thiamine hydrochloride B1 10.0; riboflavin B2 25.5; pyridoxine hydrochloride B6, 15.0; folic acid, 4.0; ascorbic acid C, 250; calcium-D-pantothenate, 55.0; myo-inositol, 600; D-biotin H (2%), 1.0; choline chloride 1500; nicotinamide 200; cyanocobalamin B12 0.02; ethoxyquin (anti-oxidant) 200; calcium propionate (mould inhibitor) 200 and minerals (mg active ingredient): manganese sulphate (36%) 500; magnesium sulphate (10%) 500; zinc sulphide (36%) 100; copper sulphate (30%) 30; ferrous sulphate (30%) 30; sodium selinite 0.33; potassium iodate, 2.0.
[3] Organic matter calculated by difference = (1000-measured ash content)
[4] Nitrogen free extractives calculated by difference (NFE) = 1000 – (crude protein + ash + fat)

TABLE 3
Apparent organic matter and gross energy digestibility coefficients for snapper fed diets containing increasing levels of gelatinised wheat starch.

	Small snapper (110 g)					Large snapper (375 g)			
	REF	PN15	PN25	PN35	PN45	REF[1]	PN15	PN35	PN45
ADC of Diet									
Organic matter	90.33	90.77	85.22	79.65	77.08	87.03	88.06	80.57	70.67
	(0.58)	(0.11)	(1.90)	(1.31)	(1.33)	-	(0.54)	(1.39)	(2.24)
Gross energy	93.65	92.61	89.57	82.36	81.89	91.88	90.86	84.60	83.50
	(0.28)	(0.10)	(0.58)	(1.29)	(0.39)	-	(0.49)	(0.49)	(0.41)
ADC of ingredient									
Organic matter	-	89.30	70.34	67.24	54.39	-	89.61	72.66	43.03
	-	(0.65)	(6.83)	(2.69)	(3.47)	-	(3.19)	(2.85)	(5.83)
Gross energy	-	90.10	73.89	63.86	50.25	-	88.34	72.62	60.14
	-	(0.84)	(2.92)	(3.38)	(1.33)	-	(4.20)	(1.30)	(1.40)
Weight gain (g/fish)	34.75	46.69	43.76	40.97	41.71	16.45	35.06	55.69	35.18
	(0.36)	(0.18)	(1.68)	(1.80)	(3.29)	(1.59)	(2.64)	(14.51)	(5.20)

[1] Due to inadequate sample volume, faecal material from 3 replicate tanks was combined (n=1). All other values represent mean ± (SEM) of three replicate tanks.

TABLE 4
Hepatosomatic index (HSI), 3 h post-prandial plasma glucose concentration and liver or tissue glycogen concentration of snapper fed test diets with different levels of gelatinised wheat starch.

	Small snapper (110 g)					Large snapper (375 g)			
	REF	PN15	PN25	PN35	PN45	REF	PN15	PN35	PN45
Peformance index									
HSI (%)	0.83	1.88	1.90	1.78	1.68	0.67	1.21	1.31	1.36
	(0.04)	(0.14)	(0.09)	(0.14)	(0.11)	(0.04)	(0.12)	(0.04)	(0.05)
Post-prandial plasma glucose (mM)	1.88	2.33	2.42	2.53	2.09	1.59	1.77	2.00	2.31
	(0.04)	(0.29)	(0.39)	(0.20)	(0.35)	(0.16)	(0.13)	(0.19)	(0.53)
Glycogen (ug mg^{-1})									
Muscle	11.95	-	-	-	28.88	3.35	-	-	24.71
	(2.86)	-	-	-	(0.65)	(1.37)	-	-	(3.74)
Liver	30.20	-	-	-	67.03	13.53	-	-	54.80
	(0.80)	-	-	-	(6.54)	(4.77)	-		(3.90)

Values represent mean ± (SEM) of three replicate tanks (n.b. average tank value based on 3 fish per tank).
Hepatosomatic index (HSI) = 100 * (wet weight liver / (wet weight whole fish – wet weight stomach).

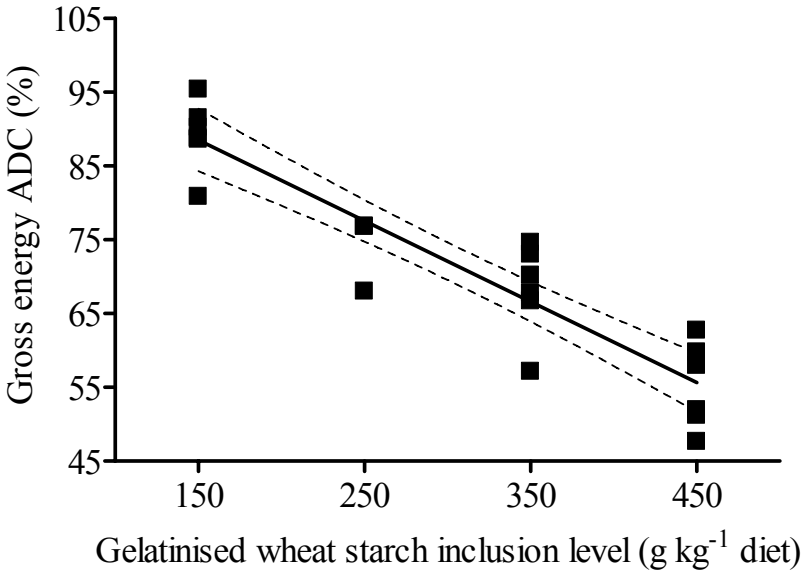

FIGURE 1
Effect of gelatinised wheat starch inclusion level on gross energy ADC. Outer curves represent 95% confidence limits. Gross energy ADC = 104.97 (±3.39) – 0.109 (±0.010) x inclusion level (R^2 = 0.86)

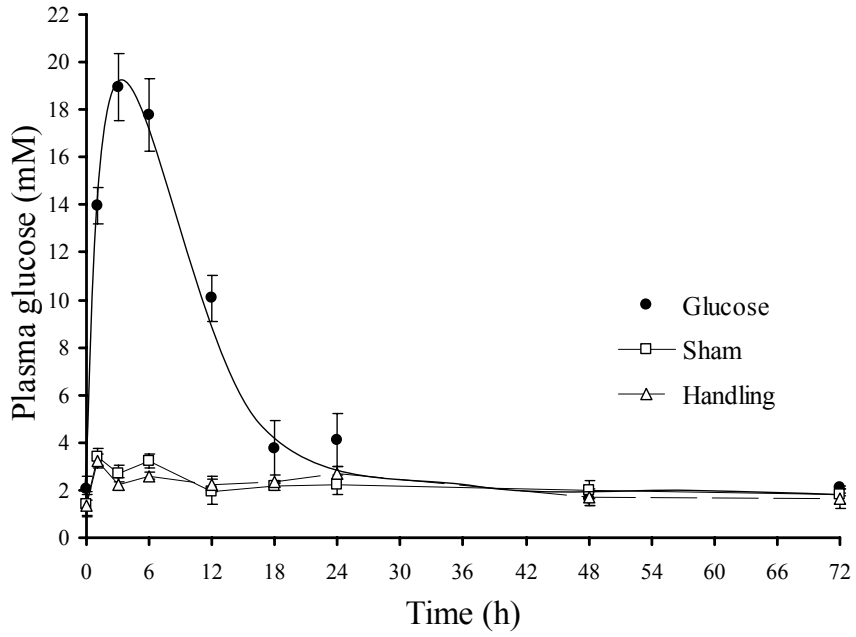

FIGURE 2

Effect of intra-peritoneal injection of 1 g D-glucose kgBW-1, a sham injection of saline or a handling stress on the 72 h plasma glucose response of snapper

4.4 Investigation of the nutritional requirements of Australian snapper *Pagrus auratus* (Bloch & Schneider, 1801): influence of poultry offal, meat or soybean meal inclusion level on weight gain and performance

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ABSTRACT

Two experiments were done to evaluate the effects of fishmeal replacement on the performance of Australian snapper *Pagrus auratus*. In each experiment, test diets were formulated with similar contents of digestible protein (DP) and digestible energy (DE) and fish performance was evaluated against snapper fed a commercially available barramundi *Lates calcarifer* feed (COM). In experiment 1, snapper (14 g) were fed four diets containing 360, 480, 610 or 730 g kg^{-1} poultry offal meal (PM); three diets containing 345, 320 or 500 g kg^{-1} meat meal (MM) and three diets containing 420, 600 or 780 g kg^{-1} solvent extracted soybean meal (SM). MM diets also contained 120 g kg^{-1} blood meal (BM). In experiment 2, snapper (87 g) were fed for 104 days on three commercially extruded test diets in which combinations of PM, MM, SM or BM replaced all but 600 (MF60), 250 (MF25) or 160 g kg^{-1} fishmeal (MF16).

Experiment 1 indicated weight gain was highest in snapper fed diets containing 360, 345 and 420 g kg^{-1} of PM, MM or SM respectively and was similar ($P>0.05$) to snapper fed the COM diet. In all cases, weight gain, protein gain and protein retention efficiency tended to decrease as the amount of each test ingredient was increased. Relative feed intake was not affected by the inclusion levels of PM, MM or BM we tested but declined significantly in snapper fed diets containing 600 or 780 g kg^{-1} SM. Feeding behaviour indicated fish found diets containing 600 or 780 g kg^{-1} SM unpalatable. The composition of test diets had little impact on carcass composition.

In experiment 2, the final weight of snapper fed the three test feeds was similar ($P>0.05$), but lower than snapper fed the commercial diet (i.e. 234 vs 256 g $fish^{-1}$). Feed conversion ratio was lowest in snapper fed the COM diet (1.53) compared to snapper fed MF16 (1.66) or MF25 (1.70), but similar ($P>0.05$) to fish fed the MF60 diet (1.60). Changes in thermal growth coefficients (TGC) over time suggest that snapper required at least 40-70 days to adjust to the physical and ingredient composition of the test feeds.

Australian snapper will readily accept feeds containing high levels of PM, MM or SM and diets containing these feed ingredients will support rapid weight and protein gains with little affect on whole body composition. In combination, these feed ingredients were able to replace all but 160g kg^{-1} of fishmeal in a commercially extruded diet. As such, they serve as valuable alternatives to fishmeal and extend the manufacturing options available to aqua-feed producers.

1. INTRODUCTION

Aquaculture of high value species like Australian snapper (*Pagrus auratus* = red sea bream, *P. major*; Paulin, 1990; Tabata & Taniguchi, 2000) is generally dependant on feeds that contain high levels of fishmeal and fish oil (Coutteau, Ceulemans, Van Halteren & Robles, 2002). It is now acknowledged however, that the global demand for these resources will eventually outstrip supply and alternative ingredients sourced from more sustainable sectors will have to be used (Tacon, 2003; Williams, Barlow, Rodgers & Ruscoe, 2003; Allan, 2004).

Fishmeal replacement studies are common in the literature, and studies involving sparids are no exception (Robaina, Moyano, Izquierdo, Socorro, Vegara & Montero, 1997; Nengas, Alexis & Davies, 1999; Kissil, Lupatsch, Higgs & Hardy, 2000; Gomez-Requeni,Mingarro, Calduch-Giner, Medale, Martin & Houlihan, 2004). Much of the recent literature has focused on plant proteins, but rendered animal meals still appear to be a more practical alternative for carnivorous fish in terms of protein content and cost (Williams, *et al.* 2003). This is especially true in Australia, where stocks of rendered animal meals are readily available and generally of high quality. However, there are contradictory reports about the ability of sparids to utilise animal by-product meals for growth. For example, two separate studies showed that weight gain of Australian snapper was inversely related to diets that replaced fishmeal with increasing contents of poultry offal meal and soybean meal (Quartararo, Allan & Bell, 1998a; Quartararo, Bell & Allan, 1998b). In contrast, a study by Takagi, Hosokawa, Shimeno & Ukawa (2000), indicated that weight gain in red sea bream fed diets with up to 590 g poultry offal meal kg^{-1} as the only protein source was equivalent to, if not superior to those fed a fishmeal control diet. Results for juvenile fish (54 g) fed similar diets were less clear and weight gains, while statistically similar, appeared to decrease substantially after diets contained more than 410 g poultry meal kg^{-1}.

Australian aquafeed producers also regularly use soybean meals in their formulations when it becomes cost effective. Again, there is contradictory evidence within the literature with regard to appropriate inclusion levels in sparid diets. Some studies have indicated that feed intake and weight gain of fish is not affected (Robaina, Izquierdo, Moyano, Socorro, Vergara, Montero & Fernandez-Palacios, 1995), whereas others have found clear differences (Kissil, *et al.* 2000). Many of these differences may be associated with the level of inclusion tested, degree of processing and whether or not amino acid deficiencies have been accounted for by the addition of crystalline amino acids or mixing with complimentary protein sources.

One of the limitations in many replacement studies is that test diets designed to examine increasing inclusion levels of alternative ingredients are formulated on an isocaloric and isonitrogenous basis without any regard to the apparent digestibility of the test ingredients. Ideally, it would be preferable to predetermine apparent digestibility coefficients before planning substitution experiments. In this way, experimental diets can be formulated applying similar constraints to those used to design practical feeds for industry. Consequently, experimental results and conclusions would be more robust.

Aquafeed producers require not only a comprehensive database of digestibility coefficients with which to formulate diets, they also require information on practical levels of inclusion for ingredient stocks. Therefore, this paper describes two experiments designed to evaluate the effects of increasing inclusion levels of poultry offal meal, meat meal and solvent extracted soybean meal on the performance, weight gain and protein retention of Australian snapper.

2. MATERIALS AND METHODS

2.1 Diets Experiment 1

Test diets were formulated to a single DP (450 g kg^{-1} diet dry basis) and DE content (17 $MJkg^{-1}$ diet dry basis) using previously determined apparent digestibility coefficients for Australian snapper (Booth, Allan & Anderson, 2005). The level of individual test ingredients was increased at the expense of fishmeal, extruded wheat or fish oil, with the remaining dry matter balanced by the addition of diatomaceous earth.

Four diets (P36, P48, P61 & P73) were formulated that contained 360, 480, 610 and 730 g kg^{-1} poultry offal meal. Three diets (M35, M32 & M50) were formulated which contained 345, 320 and 500 g kg^{-1} meat meal. Diets M32 and M50 also contained blood meal. The final three diets (S42, S60 & S78) contained 420, 600 and 780 g kg^{-1} solvent extracted soybean meal. All ingredients used in Exp.1 were obtained from local and interstate livestock feed providers or feed ingredient specialists (Table 1). In each experiment, dry ingredients, vitamin and mineral premixes were combined on a dry matter basis according to the formulations presented in Table 2. Each dietary mash was then thoroughly mixed (Hobart Mixer: Troy Pty Ltd, Ohio, USA) before being finely ground in a laboratory scale hammer mill fitted with a 1.5 mm screen (Raymond Laboratory Mill, Transfield Technologies, Rydalmere, NSW, Australia). The ground mash was thoroughly re-mixed and fortified with 1.0 g vitamin C kg^{-1} (Rovimix® Stay C®-35; F. Hoffman-La Roche, Switzerland) before being combined with distilled water and fish oil and formed into sinking 2.0 mm pellets using a meat mincer (Barnco Australia Pty Ltd, Leichhardt, NSW, Australia). Moist pellets were then dried for about six hours (≈ 35º C) in a convection drier until moisture contents were <100 g kg^{-1}. Following preparation, all diets were stored frozen at < -15ºC until required. A commercial barramundi *Lates calcarifer* feed manufactured by Ridley Aqua-Feeds Pty. Ltd. (Narangba, Qld, Australia) was included in this experiment to assess comparative growth and performance. This diet was ground and repelleted using the same procedures.

2.2 Diets Experiment 2

All ingredients used in Exp.2 were obtained by Ridley Aquafeeds Pty Ltd. Three diets were formulated using a linear least-cost feed program (FeedMania;A.B.R.I. University of New England, Armidale, NSW, Australia) to replace fishmeal with a practical combination of the protein sources investigated in Exp.1. Diets were designated MF16, MF25 and MF60, which contained 160, 250 and 600 g kg^{-1} fishmeal respectively (Table 3). These formulations were a compromise between the desire to test relatively high inclusion levels of particular ingredients and the manufacturing constraints dictated by the operator. Under these constraints, the final DP and DE content of diets was calculated to be approximately 410 g kg^{-1} and 18 MJ kg^{-1} respectively on an as received basis. All diets were produced on a single screw extruder (Wenger X185; Ridley Aquafeeds Pty Ltd) and were manufactured using similar system configurations. The aim was to extrude the test diets to produce a slowly sinking 6.0 mm pellet. Extruder settings are presented in Table 3. A commercial barramundi feed (Ridley Aquafeeds Pty Ltd) was included in this experiment for comparative growth purposes. The commercial diet was more buoyant than the experimental diets with > 90% of pellets floating. Extrusion details for this feed are confidential.

2.3 Fish

Snapper used in both experiments were progeny of first generation brood-stock held at the NSW Fisheries Port Stephens Fisheries Centre (PSFC). Prior to use in experiments, snapper were grown at low densities in large 10 kL tanks and fed twice daily on a commercial barramundi feed (Ridley Aqua-

Feeds Pty. Ltd., reported nutrient composition: 50% crude protein; 12% crude fat; 18.0 MJ kg^{-1} gross energy). A prophylactic formalin bath (200 mg formaldehyde L^{-1}) was given to all fish before they were transferred to experiment cages. Snapper were then starved for 24 h, anaesthetised (20-30 mg L^{-1} ethyl-ρ-aminobenzoate), weighed (individually or in small groups) and systematically distributed to experiment cages. Fifteen snapper were stocked into each cage in Exp.1 (initial weight ± STDEV = 14.2 ± 0.3 g $fish^{-1}$) and 47 snapper were stocked into outdoor cages in Exp.2 (initial weight ± STDEV = 87.7 ± 10.0 g $fish^{-1}$). A random sample of the fish used to stock Exp.1 was killed for proximate analysis with an overdose of anaesthetic.

2.4 Facilities

2.4.1 Experiment 1

Experiment 1 was carried out in a saltwater re-circulation system that consisted of 7 x 10 kL circular fibreglass tanks (3.4 m diameter x 1.2 m depth) housed within a plastic covered shade house at PSFC. Each of these tanks contained 8 cylindrical floating cages (dimensions approximately 0.2 m^3; 0.6 m diameter x 0.7 m submerged depth) constructed of 10 mm perforated plastic mesh. Each cage was fitted with a lid to prevent the escape of fish and a feeding screen secured at the base to prevent the loss of feed (1.6 mm plastic mesh). Cages were firmly secured to the outer perimeter of 10 kL tanks and remained in the same position during the entire experiment. Each 10 kL tank was provided with approximately 36 L min^{-1} of pre-filtered water pumped from a submerged bio-filter. Effluent water from each tank was drained through a 1 mm filter screen to remove coarse solids (NikaFilt; Taylor Made Fish Farm, Bobs Farm, NSW, Australia), collected in a common concrete sump (2500 L) and pumped back to the bio-filter through a pair of twin cartridge pool filters (10-15 μm); cartridges were exchanged daily. All 10 kL tanks were provided with 4 x 100 mm air stone diffusers and fitted with black shade cloth covers to reduce the proliferation of algae. The floor of each 10 kL was vacuumed at least once a week to ensure removal of accumulated faecal material and facilitate water exchange.

Prior to feeding, 5 experiment cages were randomly assigned to each of the 11 dietary treatments. Fish were switched to experimental feeds the day after stocking and fed to apparent satiation twice daily (0830 and 1500 h), Monday through Saturday, but only once on Sundays (0830 h). At the completion of the experiment (50 days), all fish were individually weighed after which 3 fish from each cage were randomly selected and killed for proximate analysis (overdose of ethyl-p-amino benzoate). Fish were starved in the 24 h prior to harvest.

During Exp.1, snapper became infested with the dinoflagellate *Amyloodinium ocellatum*. In order to control this outbreak, snapper were kept in their individual cages but consolidated within 10 kL tanks. Fish were subsequently treated once daily with 200 mg formalin L^{-1} over a period of 7 days. They were not fed during the entire treatment period. After fish had recovered, they were bulk weighed and returned to their original positions within each tank and feeding was recommenced.

2.4.2 Experiment 2

Experiment 2 was conducted in a large outdoor, plastic lined pond at PSFC (approximately 0.1 ha; 0.5 ML). A 20 m articulated, pontoon bridge designed to support 16 x 1 m^3 experiment cages was constructed and floated longitudinally in the pond. The experiment cages were fabricated from 8 mm knot-less mesh and held in shape by rigid PVC pipe fixed at the top and bottom (weighted) of each cage. Eight experiment cages were secured on either side of the bridge with a minimum distance of 0.4 m provided between each cage. Each cage was fitted with a plastic lid (50 x 50 mm oyster mesh) to prevent the escape of fish and an internal feeding collar to prevent pellets floating between cages. In

addition, a feeding mat (3 mm oyster mesh) was attached at the base of each cage to prevent the loss of feed. Unfiltered or filtered estuarine water was continuously passed through the pond at approximately 0.34 ML d^{-1}. Localised levels of dissolved oxygen were elevated around the experiment cages by placement of 8 x 100mm air stone diffusers. Four air-lift pumps were also employed to impart a unidirectional flow within the pond to prevent stratification.

Prior to feeding, 4 experiment cages were randomly assigned to each of the test diets. Snapper were then fed their respective test diets to apparent satiation twice daily, Monday through Saturday at approximately 0930 and 1500 h and once on Sunday (0930 h), but starved in the 24 h prior to weighing procedures. Dietary performance was assessed by measuring weight and survival of snapper 15, 40, 70 and 104 days (harvest) after stocking. The general health of fish was also assessed at these times and the experiment cages were exchanged. Fish density was reduced on day 70 by removing 7 snapper from each cage during weight check procedures.

2.5 Water quality

Water quality parameters were recorded in both experiments using one of two hand held water quality analysers; either a Model 611 (Yeo-Kal Electronics, Brookvale, NSW, Australia) or a Horiba U-10 (Horiba, Japan). Total ammonia [$NH_3 + NH_4^+$] was monitored using a rapid test kit procedure (Model 1.08024.0001, E. Merck, Darmstadt, Germany). During Exp.1, temperature, dissolved oxygen (DO_2), salinity and pH ranged from 21-30ºC, 4.3-8.8 mg L^{-1}, 2.8-3.6% and 7.4-8.5 units respectively with [$NH_3 + NH_4^+$] always $\leq$ 0.6 mg L^{-1}. In Exp.2, ambient temperature, dissolved oxygen (DO_2), salinity and pH ranged from 18.6-26.7ºC, 5.0-9.3 mg L^{-1}, 2.9-3.6% and 6.8-8.7 units respectively.

2.6 Chemical analyses

Ingredient, diet and whole fish samples generated in Exp.1 were analysed by the CSIRO Analytical Services Facility (CSIRO - Livestock Industries, Indooroopilly QLD, Australia). Samples for nitrogen were dried, weighed and digested in sulphuric acid/catalyst mixture for determination by Kjeldahl Nitrogen. Continuous flow analysis (auto analyser) procedures were used to determine nitrogen content. Crude protein was calculated as N x 6.25. Moisture in diets was determined gravimetrically after samples were oven dried at 105º C for 16 h and ash by muffle furnace. Moisture in whole fish was determined by weighing, freeze drying and reweighing sub samples. Fat content was determined gravimetrically after hexane extraction using a Dionex ASE system. Gross energy was determined by bomb calorimetry.

Duplicate diet samples from Exp.2 were analysed by the Food & Agriculture Laboratory of Australia (FALA – Coopers Plains, Qld, Australia). Crude protein (N x 6.25) was analysed by the Leco Method. Total fat was determined after extraction using chloroform and methanol (Folch method) while moisture (oven drying), ash (muffle furnace) and gross energy (bomb calorimeter) were determined using standard procedures (AOAC 1995).

Statistical analyses

The effect of diet type on the performance of snapper in Exp.1 and Exp.2 was analysed using one-way analysis of variance (ANOVA). The effect of diet type on carcass composition in Exp.1 was analysed using one-way analysis of covariance (ANCOVA), with harvest weight as the covariate (Shearer 1994). When the covariate was significant, adjusted treatment means are presented (Table 4). When the covariate was not significant, the analysis was reduced to one-way ANOVA and observed treatment means are presented (Table 4). Before ANOVA, all data was assessed to ensure variances were homogeneous (Cochran's test). Data for feed conversion ratio (FCR) was log transformed in order to

satisfy this assumption, however the untransformed treatment means are presented in Table 4. The significance level for all ANOVA, ANCOVA and multiple comparisons tests (Student Newman Keul's) was set at 0.05. Data was statistically analysed using Statgraphics Plus, version 4.1 (Manugistics Inc., Rockville, MD, USA, 1998).

3. RESULTS

3.1 Experiment 1

No significant differences were found in the survival of snapper reared on the experimental or commercial feeds in Exp.1. Average final weights and individual weight gains were highest for snapper fed test diets containing 360, 350 or 420 g kg^{-1} poultry offal meal, meat meal or soybean meal (i.e. diets P36, M35, S42) and were similar to snapper fed the commercial diet. Despite similarities in the DP and DE content of each test diet, weight gain tended to decrease gradually as the amount of each test ingredient was increased (Table 4). There were significant differences between the individual feed intakes of different treatments, however, differences between the feed intakes of poultry offal meal, meat meal or meat and blood meal diets were minor. More obvious were the reductions in feed intake for snapper reared on diets containing high levels of soybean meal (S78), which may be indicative of reduced palatability. This is further highlighted when data is considered on a relative feed intake basis (Table 4). The feeding behaviour of snapper fed diets containing the two highest levels of soybean meal was clearly different to other treatments, with fish quickly losing interest in consuming these feeds. Feed conversion ratio (FCR) was less than 2.0:1 for all snapper except those fed the basal diet and was superior in snapper fed P36, M35, S42 or the commercial diet. Average individual protein deposition was highest and statistically similar in fish fed P36, P48, M35, M32, S42 or the commercial feed (COM), while protein retention was superior in snapper fed diets M35, S42 and COM. As was the case with the aforementioned indices, protein deposition, protein retention efficiency and lipid deposition also declined as the levels of respective test ingredients increased. There were significant but minor differences between the protein, ash and fat composition of whole fish, however, there was no difference in gross energy content (Table 4).

3.2 Experiment 2

Survival of snapper was extremely high, with only 8 mortalities occurring during the entire experimental period. Mortality was recorded in MF16 (3 fish), MF25 (1 fish), MF60 (3 fish) and the commercial feed treatment (1 fish). Unfortunately, attempts to produce a slow sinking pellet from each of our test formulations failed, and there were clear differences between the buoyancy of each feed. MF16 and MF25 sank rapidly while approximately 20% of the MF60 pellets floated. The commercial feed was more buoyant (> 90% of pellets floated). Despite these physical differences, snapper readily accepted all diets, however it became somewhat difficult to feed sinking diets to apparent satiation in turbid conditions as these fish became accustomed to feeding below the surface of the water. Average final weight, weight gain and daily weight gain were statistically similar for snapper fed the 3 test diets but significantly lower than snapper fed the commercial diet (Table 5). Daily weight gains proved to be rapid in Exp.2, particularly during periods of elevated water temperature (i.e. stocking to day 40, midsummer), and gains of 2.35 g $fish^{-1}$ d^{-1} were recorded. Total and individual feed intake was similar and significantly higher in snapper reared on MF25 or the commercial feed compared to fish reared on MF16 or MF60 (Table 5). Feed conversion ratio (FCR) was significantly lower in snapper fed the commercial diet compared to snapper fed MF16 or MF25, but similar to fish fed the MF60 diet. Protein efficiency ratio (PER) proved to be significantly higher only in fish fed the commercial diet (Table 5).

Thermal growth coefficients (TGC) were calculated using the modal temperature and weight gains recorded between each weight check procedure. These coefficients were elevated at the beginning of the trial and gradually decreased over time as water temperatures decreased and weight gains slowed. Significant differences between coefficients were recorded during the first 40 days, however, coefficients for all dietary treatments became statistically similar ($P>0.05$) after this time (Table 5).

4. DISCUSSION

The approach taken in Exp.1 was aimed at assessing increasing levels of the test ingredients in diets formulated to have a similar ratio of digestible protein to digestible energy (i.e. 26 g DP $MJDE^{-1}$). These specifications were based on previously determined apparent digestibility coefficients for the tested ingredients and the digestible protein requirements of 50-90 g snapper (Booth, *et al.* 2005; Booth *et al.* unpublished data). This type of experimental approach is different to the majority of replacement studies that generally use only crude protein and gross energy values or "average" physiological fuel equivalents to formulate diets designed to test the utilisation of alternative ingredients. Our approach removes the effect of ingredient differences attributable to digestibility and allows direct comparison of the effects different ingredient compositions have on tissue growth and feed intake. Formulation to similar DP and DE contents relies on the assumption that digestibility coefficients for ingredients are additive, and remain constant as inclusion levels are increased. This assumption has been confirmed for snapper fed diets containing 300 or 500 g kg^{-1} poultry offal meal or meat meal (Booth, *et al.* 2005) and for the closely related gilthead seabream fed a range of ingredients similar to the ones tested here (Lupatsch, Kissil, Sklan & Pfeffer, 1997).

Once the effects of digestibility have been accounted for, changing dietary ingredient composition can affect fish performance because of three factors. Firstly, the utilisation of different ingredients can affect tissue growth. This is usually apparent if growth and feed conversion efficiency change (i.e. deteriorate as inclusion contents increase). Secondly, different ingredients can affect attractability or palatability of diets. This is usually apparent if feed intake changes (e.g. declines), but feed conversion efficiency remains similar. Some ingredients affect both utilisation and palatability, and for these, changes (e.g. reductions) in feed intake, growth and feed conversion efficiency would be expected. Thirdly, different ingredients can affect the diet manufacturing process, resulting in diets with different physical characteristics such as buoyancy, stability or hardness. These effects can be more difficult to separate from other factors, because the physical characteristics of a diet can affect feed intake (e.g. some fish will not eat floating pellets) and apparent feed conversion efficiency (e.g. poorly bound or fragile feeds resulting in the loss of dry matter). In the present study, results for different diets indicate that different ingredients have affected each of these factors.

4.1 Effects of ingredients on utilisation

The weight gain of snapper fed diets containing 420 g kg^{-1} soybean meal (S42), 360 g kg^{-1} poultry offal meal (P36) or 345 g kg^{-1} meat meal (M35) was statistically similar to that recorded for fish fed the commercial feed (COM) in Exp.1. However, for each of these key ingredients, as inclusion increased above those contents, growth, feed conversion efficiency and protein retention efficiency (PRE) declined, indicating that utilisation was reduced, possibly because the balance of amino acids was inferior.

In terms of growth utilisation, there has been considerable success in using soybean meal in controlled replacement studies with sparids (Robaina, *et al.* 1995, Kissil, *et al.* 2000) and many other fish species (Abery, Gunasekera & de Silva, 2002; Saitoh, Koshio, Harada, Watanable, Yoshida, Teshima &

Ishikawa, 2003; Catacutan & Pagador, 2004). Our results support these findings, but indicate that for snapper, practical soybean meal inclusion levels should not exceed about 420 g kg^{-1} diet.

Within the poultry meal series, weight gain, protein gain, FCR and PRE were similar in snapper fed diets containing up to 480 g kg^{-1} poultry offal after which performance clearly deteriorated (Table 4). Similarly, the performance indices of juvenile red sea bream fed diets containing poultry by-product meal compared to a fishmeal control deteriorated after diets incorporated more than 410 g kg^{-1} poultry meal, but interestingly the same product was capable of totally replacing fishmeal in the diets of older fish (i.e. 280 g; Takagi, *et al.* 2000). In general, poultry offal meals appear to well utilised by many species in terms of weight gain, however performance indices such as FCR and protein retention tend to decline as inclusion levels are increased. These reductions appear to be mostly associated with inconsistency in product quality, with some being deficient in particular amino acids and others having poor digestibility. Despite these anomalies, most researchers have found these products capable of replacing significant quantities of dietary fishmeal (El-Sayed, 1998; Kureshy, Davis & Arnold, 2000).

Snapper grown on the diet containing 350 g kg^{-1} meat meal (M35) exhibited an excellent FCR and a PRE that was statistically similar to the proprietary feed. However, a minor reduction in meat meal content (from 350 to 320 g kg^{-1} diet) coupled with an increase in blood meal (from 0 to 120 g kg^{-1} diet) had a negative impact on weight gain, FCR and PRE (M35 to M32). A subsequent increase in meat meal content (320 to 500 g kg^{-1} diet) while holding the blood meal levels constant at 120 g kg^{-1} resulted in significantly worse FCR and protein deposition (Table 4). There was no reduction in feed intake with increasing meat meal content, indicating this ingredient did not negatively affect attractiveness or palatability of the diet. Although the protein from blood meal was almost totally digested by snapper (Booth, *et al.* 2005), as a protein source this particular batch appears to have been poorly utilised. Moderate to high levels (47 to 188 g kg^{-1} diet) of spray-dried blood meal were also unsuccessful in the test diets of Murray cod *Maccullochella peelii peelii*. In this case, the authors implicated factors such as poor digestibility and excessive dietary concentrations of iron and zinc on the poor performance of fish (Abery, *et al.* 2002).

Gilthead seabream readily accepted diets containing between 140 to 280 g kg^{-1} meat meal and recorded similar weight gains and PRE values to a fishmeal control diet (Robaina, *et al.* 1997). These inclusion levels are somewhat lower than those we have tested, but suggest that only moderate inclusion levels of conventionally rendered meat meals are appropriate for sparids. Similar recommendations were made for juvenile red drum (Kureshy, *et al.* 2000). Japanese flounder fed increasing levels of meat and bone meal at the expense of fishmeal also exhibited generalised reductions in performance, with the authors of this study recommending diets should include no more than about 200 g kg^{-1} (Kikuchi, Sato, Furuta, Sakaguchi & Deguchi, 1997). In contrast, studies with barramundi have found that diets composed predominantly of conventionally rendered meat meal (500 g kg^{-1} diet; cattle sheep and goat meat) in combination with small amounts of soybean meal (104-161 g kg^{-1} diet) and wheat flour returned equivalent if not better growth than barramundi reared on fishmeal control diets. In this case, diets were supplemented with crystalline lysine and methionine (Williams, *et al.* 2003).

4.2 Effects of ingredients on diet palatability

Given our diets provided equivalent amounts of DP and DE, we expected relative feed intake to be similar among treatments (i.e. that fish would eat to satisfy energy requirements; NRC, 1993). This proved to be the case for snapper fed diets containing poultry offal meal and diets containing meat or a combination of meat and blood meal. In contrast, relative feed intake in snapper fed the soybean meal diets steadily declined as inclusion levels increased, suggesting that snapper found diets containing more than 420 g kg^{-1} soybean meal unpalatable. The feeding behaviour of snapper fed diets S60 and

S78 was clearly different to that of other treatments, with fish quickly loosing interest in the feed. On many occasions, fish would mouth the pellets for a short time but then reject them. This result supports evidence presented by Kissil, *et al.* (2000), who found an inverse relationship between soybean meal inclusion level and feed intake in gilthead seabream when fish were subjected to a similar range of inclusion contents to those tested in our study. This relationship was not evident in gilthead seabream when levels of soybean meal were kept below 300 g kg^{-1} diet (Robaina, *et al.* 1995). Apart from being less palatable, many studies have also indicated that even low levels of soybean meal can induce chronic physiological or pathological changes in fish (Robaina, *et al.* 1995; Krogdahl, Bakke-McKellop & Baeverfjord, 2003; Catucutan & Pagador, 2004; Rondan, Hernandez, Egea, Garcia, Jover, Rueda & Martinez, 2004).

4.3 Effects of ingredients on physical characteristics of diets

At the beginning of Exp.2, we were concerned about the differences in the buoyancy of feeds possibly confounding the outcomes of the study. This was especially concerning for the commercial feed that was more buoyant than the experimental diets. It was also the diet that the fish were reared on prior to the commencement of the experiment. The longer-term acclimation to this diet may have given the snapper randomised to this treatment a slight advantage during the early phases of the experiment. Evidence for this is can be seen in the calculated thermal growth coefficients for each phase of the study. During the first 40 days of the trial, TGC's for the commercial feed remained equivalent to if not superior to the experimental feeds, which tended to fluctuate. However, by day 70, all TGC's had stabilised and in fact remained statistically similar ($P>0.05$) until the conclusion of the trial. This statistical equivalence in growth rates suggests that snapper fed the experimental diets required between 41 to 70 days to become completely accustomed to the physical and / or ingredient composition of the test diets.

4.4 Combinations of ingredients

Data from Exp. 2 demonstrated that diets formulated from relatively simple blends of rendered animal by-product meals and plant proteins, similar to those evaluated in Exp.1, can support rapid growth and acceptable feed conversion in snapper grown in outdoor cages. With respect to the experimental diets, fishmeal content could be reduced from 600 to as little as 160 g kg^{-1} without significant reductions in survival, weight gain or PER after 104 days.

A previous growth experiment with snapper also looked at a blend of different protein sources with which to replace increasing levels of fishmeal, however these diets were not formulated on a DP or DE basis or subjected to a commercial pelleting procedure (Quartararo, *et al.* 1998a). In that study lupins, poultry meal, sorghum, soybean meal and wheat were blended to achieve reductions in dietary fishmeal content from 640 to 100 g kg^{-1}. In all cases the weight gain and FCR of snapper decreased as the amount of fishmeal in the diets decreased, even though diets were fortified with additional crystalline amino acids to balance possible deficiencies. In that experiment digestibility of ingredients was not measured and although digestibility of the two highest fishmeal diets was similar (DP and DE), the digestibility of the other diets was not determined. In addition, feed intake could not be measured in the system used, making it difficult to determine the factors responsible for reduced performance with increasing fishmeal replacement.

Based on current commodity prices (Table 3) and the FCRs obtained in this study, the ingredient cost of producing 1 kg of snapper is approximately $1.03, $1.14 and $1.26 for the MF16, MF25 and MF60 diets respectively (Table 5). These reductions clearly demonstrate the type of cost savings that can be made by replacing fishmeal with alternative feed ingredients such as those tested in the present study.

However, these cost reductions must also be considered in terms of the total number of production days required to produce a marketable fish. Extrapolating from the TGC's calculated for each diet over 104 days (Table 5) indicates that for a 1kg fish, production time increases by approximately 15 days if snapper are grown on FM25 or FM60 compared to the COM diet, but by almost 30 days if grown on FM16.

5. CONCLUSION

This study has shown that Australian snapper will readily accept feeds containing moderate to high levels of poultry offal meal, meat meal or soybean meal and that diets containing these feed ingredients will support rapid weight and protein gains with little affect on whole body composition. In combination, these feed ingredients were able to replace all but 160 g kg^{-1} of fishmeal in a commercially produced extruded diet. As such, they serve as valuable alternatives to fishmeal and extend the manufacturing options available to aqua-feed producers. Further research is needed to elucidate the effects of blood meal inclusion in the diets of snapper. In addition, the utilisation of the ingredients in this study was affected by nutritional quality, palatability and the physical characteristics of the test diets.

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REFERENCES

Abery, N.W., Gunasekera, R.M. & De Silva, S.S. (2002). Growth and nutrient utilisation of Murray cod *Maccullochella peelii peelii* (Mitchell) fingerlings fed diets with varying levels of soybean meal and blood meal. Aquaculture Research **33**:279-289.

Allan, G.L. (2004). Fish for feed vs fish for food. In: Fish, Aquaculture and Food Security; sustaining fish as a food supply (ed. A.G. Brown). Record of a conference conducted by the ATSE Crawford Fund, Parliament House, Canberra, ACT, Australia, 11 August 2004.

AOAC (1995). Official methods of analysis of the association of official chemists (K. Helrich, ed.), 15th ed. Published by the Association of the Official Analytical Chemists, Inc., Arlington, Virginia, USA.

Booth, M.A., Allan, G.L. & Anderson, A.J. (2005). Investigation of the nutritional requirements of Australian snapper *Pagrus auratus* (Bloch & Schneider, 1801): apparent digestibility of protein and energy sources. Aquaculture Research **36**:378-390.

Catacutan, M.R. & Pagador, G.E. (2004). Partial replacement of fishmeal by defatted soybean meal in formulated diets for the mangrove red snapper *Lutjanus argentimaculatus* (Forsskal 1775). Aquaculture Research **35**: 299-306.

Coutteau, P., Ceulemans, S., Van Halteren, A. & Robles, R. (2002). Fishmeal and fish oil in aquafeeds; how narrow is the bottleneck for marine fish? International Aquafeed Directory & Buyers Guide **2002**:20-24.

El-Sayed, A-F. M. (1998). Total replacement of fishmeal with animal protein sources in Nile Tilapia, *Oreochromis niloticus* (L.), feeds. Aquaculture Research **29**:275-280.

Gomez-Requeni, P., Mingarro, M., Calduch-Giner, J.A., Medale, F., Martin, S.A.M., Houlihan, D.F., Kaushik, S. & Perez-Sanchez, J. (2004). Protein growth performance, amino acid utilisation and somatotropic axis responsiveness to fishmeal replacement by plant protein sources in gilthead seabream (*Sparus aurata*). Aquaculture **232**:493-510.

Kissil, G.Wm., Lupatsch, I., Higgs, D.A. & Hardy, R.W. (2000). Dietary substitution of soy and rapeseed protein concentrates for fishmeal, and their effects on growth and nutrient utilization in gilthead seabream *Sparus aurata* L. Aquaculture Research **31**:595-601.

Kikuchi, K., Sato, T., Furuta, T., Sakaguchi, I. & Deguchi, Y. (1997). Use of meat and bone meal as a protein source in the diet of juvenile Japanese flounder. Fisheries Science **63**(1):29-32.

Krogdahl, A., Bakke-McKellop, A.M. & Baeverfjord, G. (2003). Effects of graded levels of standard soybean meal on intestinal structure, mucosal enzyme activities, and pancreatic response in Atlantic salmon (*Salmo salar* L.). Aquaculture Nutrition **9**:361-371.

Kureshy, N., Davis, D.A. & Arnold, C.R. (2002) Partial replacement of fishmeal with meat and bone meal, flash dried poultry by-product meal, and enzyme-digested poultry by-product meal in practical diets for juvenile red drum. North American Journal of Aquaculture **62**:266-272.

Lupatsch, I., Kissil, G.Wm., Sklan, D. & Pfeffer, E. (1997). Apparent digestibility coefficients of feed ingredients and their predictability in compound feeds for gilthead seabream, *Pagrus aurata* L. Aquaculture Nutrition **3**:81-89.

National Research Council (NRC) (1993). Nutrient requirements of fish. National Academy Press, Washington, D.C., USA.

Nengas, I., Alexis, M.N. & Davies, S.J. (1999). High inclusion levels of poultry meals and related by-products in diets for gilthead seabream *Sparus aurata* L. Aquaculture **179**:13-23.

Paulin, C.D. (1990). *Pagrus auratus*, a new combination for the species known as 'snapper' in Australian waters (Pisces: Sparidae). New Zealand Journal of Marine and Freshwater Research **24**:259-265.

Quartararo, N., Allan, G.L. & Bell, J.D. (1998a). Replacement of fishmeal in diets for Australian snapper, *Pagrus auratus*. Aquaculture **166**:279-295.

Quartararo, N., Bell, J.D. & Allan, G.L. (1998b). Substitution of fishmeal in a diet for the carnivorous marine fish *Pagrus auratus* (Bloch and Schneider) from southeastern Australia. Asian Fisheries Science **10**:269-279.

Robaina, L., Izquierdo, M.S., Moyano, F.J., Socorro, J., Vergara, J.M., Montero, D. & Fernandez-Palacios, H. (1995). Soybean and lupin seed meals as protein sources in diets for gilthead seabream (*Sparus aurata*): nutritional and histological implications. Aquaculture **130**:219-233.

Robaina, L., Moyano, F.J., Izquierdo, M.S., Socorro, J., Vegara J.M. & Montero, D. (1997). Corn gluten and meat and bone meals as protein sources in diets for gilthead seabream (*Sparus aurata*): Nutritional and histological implications. Aquaculture **157**:347-359.

Rondan, M., Hernandez, M.D., Egea, M.A., Garcia, B., Jover, M., Rueda, F.M. & Martinez, F.J. (2004). Effects of fishmeal replacement with soybean meal as protein source, and protein replacement with carbohydrates as an alternative energy source on sharpsnout sea bream, *Diplodus puntazzo*, fatty acid profile. Aquaculture Research **35**:1220-1227.

Saitoh, S, Koshio, S., Harada, H., Watanabe, K., Yoshida, T., Teshima, S-I. & Ishikawa, M. (2003). Utilisation of extruded soybean meal for Japanese flounder Paralichthys olivaceus juveniles. Fisheries Science **69**:1075-1077.

Shearer, K.D. (1994). Factors affecting the proximate composition of cultured fishes with emphasis on salmonids. Aquaculture **119**:63-88.

Tabata, K. & Taniguchi, N. (2000). Differences between *Pagrus major* and *Pagrus auratus* through mainly mtDNA control region analysis. Fisheries Science **66**:9-18.

Tacon, G.J. (2003). Global trends in aquaculture and compound aquafeed production – A Review. International Aquafeed Directory & Buyers Guide **2003**:8-23.

Takagi, S., Hosokawa, H., Shimeno, S. & Ukawa, M. (2000). Utilization of poultry by-product meal in diet for red sea bream *Pagrus major*. Nippon Suisan Gakkaishi **66**(3):428-438.

Williams, K.C., Barlow, C.G., Rodgers, L.J., and Ruscoe, I. (2003) Potential of meat meal to replace fishmeal in extruded dry diets for barramundi, *Lates calcarifer* (Bloch). I. Growth performance. Aquaculture Research **34**:23-32.

TABLE 1
Measured composition of individual feed ingredients in Exp.1 (g kg^{-1} or MJ kg^{-1} dry matter).

Ingredient	Crude Protein	Ash	Organic matter[1]	Fat	Gross energy (MJ)
Fishmeal[2]	743.8	163.0	837.0	90.0	17.6
Extruded wheat[3]	168.8	29.0	977.0	40.0	22.3
Fish oil[4]	-	<10.0	990.0	990.0	38.0
Meat meal[5]	571.3	296.0	704.0	105.8	16.9
Poultry meal[6]	687.5	84.0	916.0	208.0	23.9
Blood meal[7]	999.0	13.0	987.0	9.6	24.0
Soybean meal[8]	523.1	63.0	937.0	28.7	21.9

[1] Organic matter calculated by difference = (1000 – measured ash content)
[2] Austral Group; VLT steam dried prime quality (via Ridley Aquafeeds Pty. Ltd., Narangba, Qld, Australia)
[3] Ridley Agriproducts Pty Ltd., Murray Bridge, SA, Australia
[4] Janos Hoey Pty. Ltd., Forbes, NSW, Australia (cod liver oil)
[5] Ridley Aqua-Feeds, Narangba, Qld, Australia
[6] Barter Enterprises Pty. Ltd. (Steggles), Beresfield, NSW, Australia (includes feathers, meat and some blood, high temp, oil extracted by pressing)
[7] Lachley Meats Pty. Ltd., Forbes, NSW, Australia (ring dried)
[8] Gibsons Ltd., Cambridge, Tasmania, Australia (commercially available solvent extracted meal)

TABLE 2
Ingredient and nutrient composition of test diets used in Exp.1 (g kg^{-1} or MJ kg^{-1} dry matter).

	Poultry meal diets				Meat meal diets			Soybean meal diets			
	P36	P48	P61	P73	M35	M32	M50	S42	S60	S78	COM
Ingredient[1]											
Fishmeal	300.0	200.0	100.0	-	430.0	330.0	195.0	300.0	200.0	100.0	-
Fish oil	50.0	30.0	20.0	20.0	105.0	115.0	120.0	60.0	60.0	60.0	-
Extruded wheat	144.0	144.0	104.0	54.0	104.0	54.0	44.0	204.0	124.0	44.0	-
Vitamins[2]	7.5	7.5	7.5	7.5	7.5	7.5	7.5	7.5	7.5	7.5	-
Minerals[3]	7.5	7.5	7.5	7.5	7.5	7.5	7.5	7.5	7.5	7.5	-
Stay-C 3[4]	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	-
Blood meal	-	-	-	-	-	**120.0**	**125.0**	-	-	-	-
Poultry meal	**360.0**	**480.0**	**610.0**	**730.0**	-	-	-	-	-	-	-
Meat meal	-	-	-	-	**345.0**	**320.0**	**500.0**	-	-	-	-
Soybean meal	-	-	-	-	-	-	-	**420.0**	**600.0**	**780.0**	-
Diatomaceous earth[5]	130.0	130.0	150.0	180.0	-	45.0	-	-	-	-	-
Measured nutrient											
Crude protein	508.2	517.5	528.8	525.0	536.3	557.5	561.3	482.5	475.0	482.5	519.4
Ash	215.0	216.0	229.0	249.0	189.0	204.0	209.0	91.0	89.0	79.0	105.0
Fat	141.0	137.0	141.0	148.0	174.0	160.0	181.0	91.2	85.0	70.2	135.0
Gross energy (MJ)	22.1	21.2	22.1	21.0	22.8	23.1	23.2	24.0	23.8	24.2	25.0
Digestible nutrient[6]											
Digestible protein	449.8	451.3	452.1	445.2	441.63	451.9	423.6	436.4	434.9	433.3	390.4
Digestible energy	17.6	17.7	17.7	17.7	17.2	17.0	16.7	17.3	16.8	16.2	17.2
DP:DE ratio	25.6	25.5	25.6	25.2	25.7	26.6	25.3	25.2	25.9	26.8	22.7

1 Ingredients as described in Table 1
2 (IU kg-1 diet): retinol A, 8000; cholecalciferol D3, 1000; DL-α-tocopherol acetate E, 125. (mg kg-1): menadione sodium bisulphite K3, 16.5; thiamine hydrochloride B1, 10.0; riboflavin B2, 25.2; pyridoxine hydrochloride B6, 15.0; folic acid, 4.0; ascorbic acid C, 1000; calcium-D-pantothenate, 55.0; myo-inositol, 600; D-biotin H (2%), 1.0; choline chloride, 1500; nicotinamide, 200; cyanocobalamin B12, 0.02; ethoxyquin (anti-oxidant) 150; calcium propionate (mould inhibitor) 25.0
3 (mg kg-1 diet): calcium carbonate, 7500; manganese sulphate monohydrate, 300; zinc sulphate monohydrate, 700; copper sulphate pentahydrate, 60, ferrous sulphate heptahydrate, 500, sodium chloride, 7500; potassium iodate, 2.0
4 Roche Vitamins Australia Pty. Ltd. ROVIMIX® STAY-C® 35. Vitamin C phosphate
5 Diatomaceous earth as inert filler
3 DP and DE content of test diets and commercial diet based on data from a previous study (Booth, Allan & Anderson; in press)

TABLE 3
Ingredient, nutrient and energy composition of extruded diets used in Exp.2 (g kg^{-1} or MJ kg^{-1} of dry matter).

	Diet type					
	MF16	MF25	MF60	COM	Ingredient cost[4] ($AUD/tonne) 2005	Ingredient cost[5] ($AUD/tonne) 2008
Ingredient[1]						
Blood meal	150.0	128.0	40.0	-	451.00	980.00
Wheat	100.0	100.0	215.0	-	250.00	490.00
Fishmeal	160.0	250.0	600.0	-	750.00	1500.00
Fish oil	150.0	150.0	130.0	-	876.00	2300.00
Meat meal	50.0	50.0	-	-	478.00	630.00
Poultry meal	182.7	84.4	-	-	318.00	1000.00
Soybean meal	192.3	222.6	-	-	221.00	700.00
Vit/Min. premix[2]	15.0	15.0	15.0	-	10440.00	15000.00
Ingredient cost of diet $/t 2005	625.15	658.17	792.27			
Ingredient cost of diet $/t 2008	1354.81	1391.16	1568.55			
Measured nutrient						
Crude protein	504.0	462.0	459.0	508.5		
Ash	89.2	84.2	94.5	126.0		
Fat	188.5	203.5	194.5	128.5		
Gross energy (MJ)	28.4	27.2	27.5	25.8		
Formulated digestible nutrient (as rec'd basis)[3]						
Digestible protein	408.8	404.6	419.9	390.4		
Digestible energy	18.1	17.9	18.6	17.2		
DP:DE ratio	22.6	22.6	22.6	22.7		

Extruder system	
Pre Conditioner:	54DDC (Differential Diameter Conditioning)
Extruder Type:	Wenger X185 Single Screw
Drier: Wenger	Double Pass Drier
Fat Coating:	UAS Vacuum Infusion Mixer
Running conditions	
Pre Conditioning Temp:	80ºC
Extruder Feed Rate:	4000kg/Hr
Oil added in Extruder:	2%
Extruder Discharge Temp:	Not Measured
Extruder Die Pressure:	1850 kPa
Mechanical energy:	9.9 kWh/tonne
Specific Thermal Energy:	51 kcal/kg
Drier Temps:	118ºC
Vacuum Coating Setpoint:	400 mBar

[1] All ingredients sourced by Ridley Aquafeeds Pty. Ltd. (Narangba, Qld, Australia).
[2] Ridley Aquafeeds Pty. Ltd. (Narangba, Qld, Australia) formulation.
[3] DP and DE content of commercially manufactured test diet based on data from a previous study (Booth, Allan & Anderson 2005). Dry matter composition of formulated diets approximately 93%.
[4] Ingredient costs based on Hammersmith Marketing (15 January 2005 & AUD$=0.7528$USD).
[5] Ingredient costs based on prices supplied by Ridley Aquafeeds Pty Ltd (16 May 2008 & AUD$=0.937997).

TABLE 4
Performance of juvenile snapper fed diets with increasing levels of poultry meal, meat meal or soybean meal after 50 days (Exp.1).

	Poultry meal diets				Meat meal diets			Soybean meal diets				
	P36	P48	P61	P73	M35	M32	M50	S42	S60	S78	COM	Pooled SEM
Performance index												
Initial weight	14.2	14.1	14.2	14.0	14.3	14.1	14.2	14.1	14.1	14.3	14.2	(0.1)
Final weight	33.2^{def}	31.5^{cde}	30.3^{c}	27.0^{b}	34.4^{f}	31.2^{cd}	29.2^{c}	32.9^{def}	30.1^{c}	25.2^{a}	33.9^{ef}	(0.7)
Weight gain	19.0^{de}	17.4^{cd}	16.1^{c}	13.0^{b}	20.1^{e}	17.1^{cd}	14.9^{c}	18.8^{de}	16.1^{c}	10.8^{a}	19.8^{e}	(0.6)
Survival (%)	97.3	98.7	96.0	98.7	100.0	96.0	98.7	98.7	98.7	100.0	100.0	(1.6)
Feed intake	28.9^{e}	28.0^{cde}	26.5^{cd}	25.9^{c}	28.0^{cde}	28.5^{de}	29.0^{e}	26.4^{cd}	24.2^{b}	19.5^{a}	28.9^{e}	(0.6)
Relative feed intake	8.4^{cde}	8.2^{cde}	7.9^{c}	7.8^{c}	8.8^{ef}	8.2^{cde}	8.7^{de}	8.1^{cd}	7.1^{b}	5.9^{a}	9.3^{f}	(0.2)
Feed conversion ratio‡	1.5^{ab}	1.6^{bc}	1.7^{bc}	2.0^{d}	1.4^{a}	1.7^{bc}	1.9^{d}	1.4^{a}	1.5^{ab}	1.8^{cd}	1.5^{ab}	(0.1)
Feed efficiency (%)	65.6^{cd}	62.1^{bc}	60.6^{bc}	50.2^{a}	71.7^{d}	60.1^{bc}	51.5^{a}	71.5^{d}	66.4^{cd}	55.3^{ab}	68.0^{cd}	(1.9)
Protein gain	3.23^{e}	3.08^{de}	2.65^{cd}	2.15^{ab}	3.28^{e}	2.87^{de}	2.36^{bc}	3.39^{e}	2.64^{cd}	1.86^{a}	3.28^{e}	(0.1)
Energy gain	200.7^{b}	179.1^{b}	146.3^{ab}	130.4^{ab}	190.8^{b}	164.8^{b}	135.6^{ab}	184.6^{b}	136.4^{ab}	90.3^{a}	197.9^{b}	(16.0)
Fat gain	2.6^{cd}	2.1^{bc}	1.9^{bc}	1.7^{b}	2.9^{d}	2.2^{bc}	2.1^{bc}	2.2^{bc}	1.7^{b}	1.0^{a}	3.2^{d}	(0.2)
Protein retention (%)	24.8^{cd}	24.3^{cd}	22.1^{bc}	18.6^{a}	26.5^{de}	22.4^{bc}	19.2^{ab}	29.0^{e}	25.2^{cd}	22.0^{bc}	29.0^{e}	(0.9)
Harvest carcass composition (% as rec'd basis or MJ kg^{-1})												
Crude protein	17.2^{ab}	17.6^{b}	16.9^{ab}	17.0^{ab}	16.8^{ab}	17.1^{ab}	16.6^{a}	17.6^{b}	16.9^{ab}	17.4^{ab}	17.0^{ab}	(0.2)
Ash†	5.3^{cd}	5.4^{cd}	5.3^{cd}	5.3^{cd}	5.5^{cd}	5.5^{cd}	5.7^{d}	5.3^{cd}	4.7^{b}	4.3^{a}	5.2^{c}	(0.1)
Fat†	10.9^{ab}	10.6^{ab}	10.8^{ab}	12.1^{b}	11.1^{ab}	10.9^{ab}	12.1^{b}	9.9^{a}	10.2^{a}	10.9^{ab}	12.2^{b}	(0.5)
Gross energy† (MJ)	9.2	9.0	8.4	8.7	8.7	8.7	8.3	8.8	8.1	7.9	8.9	(0.4)

Data are mean of 5 replicate cages: ‡ indicates data were log transformed for ANOVA. † indicates covariate (harvest weight) was significant; data presented as adjusted means; Row means with different superscript letters are significantly different ($P<0.05$, SNK).
Weight gain (g fish^{-1}) = average harvest weight – average initial weight
Feed intake (g fish^{-1}; dry basis) = average total feed intake per cage / 15 fish
Relative feed intake (g kgBW$^{-0.7}$d^{-1}) = average total feed intake per fish / ((GMBW/1000)$^{0.7}$)/50 days
Feed conversion ratio (FCR) = average dry basis feed intake per fish / average wet weight gain per fish
Feed efficiency % = (FCR^{-1}*100)
Protein, energy or fat gain (g or kJ fish^{-1}) = average nutrient or energy carcass content of fish at harvest – carcass nutrient or energy content of initial fish sample (dry matter basis)
Protein retention efficiency % = dry basis protein gain / dry basis digestible protein intake * 10

TABLE 5
Performance of snapper grown in 1m^3 cages in an outdoor pond at PSFC for 104 days (Exp 2).

	Diet type			
	MF16	MF25	MF60	COM
Performance index				
Initial weight (g)	87.6±0.3	87.5±0.8	86.2±0.7	89.4±0.7
Final weight (g)	232.7±3.4^a	241.7±5.3^a	233.0±3.3^a	255.8±2.9^b
Weight gain (g)	145.1±3.19^a	154.1±5.0^a	146.8±3.5^a	166.3±3.3^b
Daily weight gain (g)	1.40±0.03^a	1.48±0.05^a	1.41±0.03^a	1.60±0.03^b
Total feed intake (kg)	11.29±0.18^a	12.26±0.23^b	11.03±0.13^a	11.89±0.10^b
Individual feed intake (g)	240.30±3.73^a	260.92±5.01^b	234.63±2.78^a	252.95±1.98^b
Relative feed intake (g)	1.62±0.02^a	1.73±0.04^b	1.60±0.01^a	1.61±0.02^a
Feed conversion (FCR)	1.66±0.03^b	1.70±0.04^b	1.60±0.03^{ab}	1.53±0.03^a
Feed efficiency %	60.38±1.18^a	59.05±1.56^a	62.55±1.01^{ab}	65.75±1.25^b
PER %	1.48±0.03^a	1.46±0.05^a	1.49±0.03^a	1.68±0.03^b
TGC $^{(day\ 1\text{-}15;\ 26.15ºC)}$	0.0891^a	0.1162^b	0.1254^b	0.1071^b
TGC $^{(day\ 16\text{-}40;\ 26.15ºC)}$	0.1050^b	0.0882^a	0.0855^a	0.1088^b
TGC $^{(day\ 41\text{-}70;\ 23.35ºC)}$	0.0522^a	0.0618^a	0.0623^a	0.0589^a
TGC $^{(day\ 71\text{-}104;\ 23.14ºC)}$	0.0365^a	0.0412^a	0.0316^a	0.0422^a
TGC $^{(day\ 1\text{-}104;\ 24.70ºC)}$	0.0666^a	0.0676^a	0.0697^{ab}	0.0730^b
Ingredient cost per kg fish produced				
($AUSD)	1.03	1.14	1.26	-

Data are mean and SEM of 4 replicate outdoor cages. Row means with different superscript letters are significantly different (P<0.05, SNK).
Weight gain (g fish^{-1}) = average harvest weight – average initial weight.
Daily weight gain (g fish^{-1} day^{-1}) = average weight gain / 104 days.
Total feed intake (kg) = average total feed per treatment over 104 days.
Feed intake (g fish^{-1}; dry basis) = average total feed intake per cage / 47 fish.
Relative feed intake (%BW$\cdot$d^{-1}) = average total feed intake per fish / (GMBW/104 days) *100
Feed conversion ratio (FCR) = average dry basis feed intake per fish / average wet weight gain per fish .
Feed efficiency % = (FCR^{-1}*100).
Protein efficiency ratio (PER) = average wet weight gain per fish / average digestible protein intake per fish.
Thermal growth coefficient (TGC) = [(FBW$^{1/3}$ –IBW$^{1/3}$) / (23ºC * 104 days)] * 100; (Bureau, Kaushik & Cho 2002).
Ingredient cost per kg fish produced = (ingredient cost for 1 tonne of diet / 1000) * FCR

4.5 Investigation of the nutritional requirements of Australian snapper *Pagrus auratus* (Bloch & Schneider, 1801): Weight gain and performance on diets providing an optimal ratio of digestible protein:digestible energy, but different digestible protein and energy contents.

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ABSTRACT

Australian snapper *Pagrus auratus* (170-290 g) were fed for 51 days on test diets formulated with optimal or sub-optimal ratios of digestible protein (DP) to digestible energy (DE), but in which the absolute content of DP and DE was varied. All diets were formulated using fishmeal as the protein source, fish oil as the lipid source, gelatinised wheat starch as the carbohydrate (CHO) source and diatomaceous earth (filler). Diets were also fortified with vitamins and minerals. Three diet groups contained DP and DE in optimised ratios of 320:15, 390:18 or 490:23 (i.e. 21 g DP MJ DE^{-1}). The sub-optimal diet group contained DP and DE in the ratio 390:21 (i.e. 18 g DP MJ DE^{-1}). The DE content of the sub-optimal group was elevated by increasing dietary lipid content. Each diet group consisted of 4 diets (i.e. 16 experimental feeds) and each of these diets was fed to n=4 replicate cages containing 7 fish. Within each diet, the ratio of lipid to CHO was varied according to their DE values in order to determine if different ratios of these ingredients affected fish performance. Lipid inclusion levels ranged from 20-220 g kg^{-1} and gelatinised starch levels ranged from 40-370 g kg^{-1} diet.

Results demonstrated that voluntary feed intake in snapper was governed by the DE content of test diets, irrespective of whether DE was supplied predominantly as lipid, CHO or protein, and decreased in a linear fashion as the DE content of diets increased; relative feed intake (g kg $BW^{-0.7}$ d^{-1}) = $14.0^{(\pm 0.79)} - 0.27^{(\pm 0.04)}$ x DE content; (R^2=0.77). Consequently, feed intake regulated DP intake, which affected weight gain in a linear fashion; weight gain = $29.0^{(\pm 4.4)}$ x DP intake – $5.3^{(\pm 15.0)}$; (R^2=0.77).

Weight gain and feed conversion ratio (FCR) was significantly better in snapper fed optimised test diets containing the highest levels of DP and DE (i.e. 490DP:23DE). These diets also provided more DE in the form of protein. In diets with lower, but similar levels of DP (i.e 390 g DP kg^{-1}), increasing DE content from 18 to 21 MJ DE kg^{-1} (i.e. from an optimised to sub-optimal DP:DE ratio) by increasing dietary lipid level reduced feed intake and weight gain, presumably because energy needs were satisfied before there was sufficient intake of DP for maximum weight gain. Results from these diets indicated lipid was of little use in sparing dietary protein. Maximum weight was also restricted in snapper fed diets with an optimised DP:DE ratio, but low protein (i.e. 320DP:15DE), because, while fish attempted to increase intake to satisfy DE requirements, they were unable to consume enough and therefore DP intake was restricted.

Within the digestible nutrient range of diets formulated for this study, varying the source of DE by changing the ratio of lipid (fish oil) to carbohydrate (gelatinised wheat starch) had little effect on performance provided diets contained 390-490 g DP kg^{-1}. In nutrient sparse diets providing 320DP:15DE and where a greater proportion of DE was derived from gelatinised starch, reductions in weight gain and performance were noted. This may be due either to an inappropriately low level of total dietary lipid or because lipid may have a greater protein sparing effect than carbohydrate in low protein diets. Snapper perform best on high protein, high energy diets where increases in DE are driven by increasing DP content. Low protein, low energy diets restrict the intake of sufficient DP to

allow maximum weight gains to be reached. For this reason, high performance diets for Australian snapper should be formulated with high levels of DP.

1. INTRODUCTION

Optimising protein retention in farmed fish improves the overall efficiency of feeds and reduces the environmental impacts of excreted nitrogen (Halver & Hardy, 2002). Improved retention of proteins can be achieved by improving the quality of feed proteins and limiting the unnecessary catabolism of protein for energy yielding processes by ensuring that the correct ratio of digestible protein to digestible energy (DP:DE) is maintained (Cho, 1992; Einen & Roem, 1997; Santinha, Medale, Corraze & Gomes, 1999). Feeding nutritionally adequate diets which are balanced with the correct ratio of DP:DE with respect to fish size or growth stage should ensure that optimal protein deposition is achieved, providing feed intake and hence protein intake is not limited in some way (Bikker, 1994; Bureau, Azevedo, Tapia-Salazar & Cuzon, 2000). This has been demonstrated in snapper (Booth, *et al*. in press) and several studies with the gilthead seabream *Sparus aurata* (Lupatsch, Kissil & Sklan, 2001a; Lupatsch, Kissil & Sklan, 2003b). Nevertheless, within these constraints there is tremendous capacity to vary the absolute amounts of DP and DE and the dietary sources of DP and DE. Even where diets are formulated to appropriate DP:DE requirements, manipulating sources of protein and energy, particularly energy sources such as lipids and carbohydrates (CHO), can impact upon pellet quality, feed cost, feed intake and utilisation or carcass composition (Hepher, 1988; Bureau, Kaushik & Cho, 2002; Wilson, 2002).

Diets for carnivorous species have generally been formulated with high levels of protein (e.g. 400 – 500 g kg^{-1}; NRC, 1993), which has made them relatively expensive. However, recent formulation trends for Atlantic salmon *Salmo salar* have seen the development of "high energy" aquafeeds based on significant increases in dietary lipid (e.g. 300 –400 g kg^{-1} diet) coupled with reductions in the dietary levels of protein and CHO (Einen & Roem, 1997). These "nutrient dense" feeds have resulted in significant improvements in FCR and nutrient outputs, due to the ability of salmon to utilise lipid-energy to spare protein. However, these lipid rich diets may not be suitable for other carnivorous species. For example, Booth, *et al.* (in press), observed that maximum weight gain and protein deposition in juvenile snapper reared on high-energy, high lipid diets were suppressed compared to snapper reared on lower energy diets providing similar levels of digestible protein. Williams, Barlow, Rodgers, Hockings, Agcopra & Ruscoe (2003) found that increasing dietary lipid-energy content in the diet of barramundi, *Lates calcarifer,* was of limited benefit in terms of demonstrating a protein sparing effect, and that dietary protein levels must be kept high in order to maintain maximum growth potential (i.e. barramundi have a limited ability to use lipid as a primary energy source unless diets are adequately supplied with protein). Similar effects were proposed in red drum, *Sciaenops ocellatus* (Ellis & Reigh, 1991). In addition, increasing the levels of dietary lipid did not improve protein retention in gilthead seabream (Company, Caldich-Giner, Perez-Sanchez & Kaushik, 1999). De Silva, Gunasekera, Collins & Ingram (2002) also noted that carcass lipid content in Murray cod, *Maccullochella peelii peelii,* increased significantly in response to increasing dietary lipid, with much of this fat deposited in the peritoneum, suggesting that this species is also incapable of efficiently utilising lipid to spare protein.

Due to the cheaper cost of CHOs, the ability of CHOs to spare either dietary protein or lipid energy sources has also received attention (Wilson, 1994; Stone, 2003), but results are often ambiguous. In fact, little work has been presented on the differential utilisation or interchangeable nature of these energy sources in complete diets. We note, however, that Ellis & Reigh (1991) fed juvenile red drum isonitrogenous diets providing high and low lipid levels that also incorporated various levels of carbohydrate. Weight gain and net protein retention was superior on high lipid, low carbohydrate diets, which the authors postulate was due to the improved utilisation of lipid compared to

carbohydrate. But, because these diets were formulated on crude protein and gross energy values, without regard to the digestibility of the individual ingredients, the differential utilisation of lipid or carbohydrate is difficult to interpret. Catacutan & Coloso (1997) presented weight gain and performance data for juvenile barramundi based on diets that had similar protein contents (fishmeal) but that crossed two CHO levels with three lipid levels. These authors suggested that lipid could be spared by CHO. However, as in the previous case, diets were formulated based on the proximate composition of dietary ingredients. Nevertheless, other carnivorous species such as the European seabass, *Dicentrarchus labrax,* appear to be capable of utilising reasonable levels of starch (120 – 250 g kg^{-1}) and protein-sparing effects have been demonstrated (Peres & Oliva-Teles, 2002).

Australian snapper *Pagrus auratus* (*P. auratus* = *P. major*; Paulin, 1990; Tabata & Taniguchi, 2000), is a marine sparid cultured in small numbers in sea cage operations based in the Australian states of New South Wales and South Australia. We recently used a dose-response approach to determine the DP:DE requirements of small snapper (range 30-80 g body weight) reared at 26ºC to be 28 g DP MJ DE^{-1} (Booth, *et al.* in press). This requirement is similar to that cited for similar sized snapper (Glencross & Lupatsch; unpublished data), gilthead seabream, *Sparus auratus* (Lupatsch, *et al.* 2001a; Lupatsch, Kissil & Sklan, 2003a) and European sea bass (Lupatsch, Kissil & Sklan, 2001b) determined using bio-energetic models. The agreement between the estimates from our study and those determined by the aforementioned authors for similar sized fish indicates that both methods are suitable for determining the DP:DE ratio of feeds for these species. Bio-energetic or factorial models have been used to predict these ratios for different sized fish and have indicated that the optimum DP:DE ratio declines as fish size increases, generally falling from about 31 g DP MJ DE^{-1} to 20 g DP MJ DE^{-1} as fish size increases from 10 g to 300 g respectively (Lupatsch, *et al.* 2001b & 2003b).

This study aimed to elucidate the effects of varying the absolute levels of DP and DE in diets for Australian snapper while maintaining the correct ratio of DP:DE. In addition, the non-protein sources of DE were varied by adjusting the ratio of fish oil to gelatinised wheat starch in order to determine if different ratios of these ingredients affected fish performance. The snapper that were available for this experiment were outside the size range investigated in a previous study (Booth, *et al.*, in press), therefore we used the recommended DP:DE requirements reported for 100-300 g snapper (Glencross & Lupatsch, unpublished) and gilthead seabream (Lupatsch, *et al.* 2001a & 2003b) to formulate test feeds for this experiment that contained an optimal ratio of 20-22 g DP MJ DE^{-1}.

2. MATERIALS AND METHODS

2.1 Experimental diets

Sixteen experimental diets were formulated on a digestible nutrient basis using ingredient digestibility coefficients determined in earlier experiments with Australian snapper (Booth, Allan & Anderson, 2005). Ingredient composition is presented in Table 1. Diets were primarily composed of fishmeal, fish oil and 100% gelatinised wheat starch. Where necessary, amorphous diatomaceous earth was used to balance dry matter (Recreational Water Products, East Melbourne, Victoria, Australia) and all formulations were fortified with a vitamin / mineral premix (15 g kg^{-1} diet; NSW DPI Fisheries formulation) and L-Ascorbic acid phosphate (1 g kg^{-1} diet; Rovimix® Stay-C® 35; F. Hoffman-La Roche, Basel, Switzerland).

Four diet groups were established and each group contained 4 diets (i.e. 16 experimental diets). Three of these groups were formulated to have an optimal balance of digestible protein (DP) and digestible energy (DE) appropriate for the size of fish used in this study (i.e. 21-22 g DP MJ DE^{-1}). These three groups differed in absolute amounts of DP and DE, with a “low protein” group containing approximately 310 g DP kg^{-1} and 15 MJ DE kg^{-1}, an “intermediate protein” group containing

approximately 390 g DP kg^{-1} and 18 MJ DE kg^{-1}, and a "high protein" group (nutrient dense) containing approximately 490 g DP kg^{-1} and 23 MJ DE kg^{-1}. The fourth group was formulated to have a sub-optimal ratio of DP:DE (18 g DP MJ DE^{-1}) and contained approximately 390 g DP kg^{-1} and 22 MJ DE kg^{-1}. The sub-optimal group of diets was formulated to have the same DP content as the intermediate series, but these diets were provided with more DE by increasing lipid content.

Within each of the four diet groups, the level of fishmeal was kept constant, but the ratio of lipid (fish oil) to CHO (gelatinised starch) was varied (Table 2). This was done in order to evaluate the utilisation of the different energy sources. In effect, this ratio established a negative linear relationship between lipid and CHO inclusion level within each diet group. Diets were prepared and manufactured into 4.0 mm sinking pellets using similar techniques and equipment to that described by Booth *et al.* (2005).

Comparative weight gain and performance of snapper in this experiment was assessed using a commercial barramundi feed (Ridley Aquafeeds Pty Ltd, Narangba, Qld, Australia; reported nutrient composition 50% crude protein, 12% crude fat). This diet was also finely ground, fortified with L-Ascorbic acid phosphate (Stay-C® 35) and re-made into 4.0 mm pellets using the same techniques.

2.2 Fish

The snapper used in this study were progeny of first generation brood-stock held at the NSW DPI (Fisheries) Port Stephens Fisheries Centre (PSFC). Before the experiment, they were grown at low densities in circular 10 kL tanks and fed twice daily on a floating barramundi feed (Ridley Aqua-Feeds Pty Ltd). Fish were always anaesthetised before handling (20-30 mg L^{-1} ethyl-ρ-aminobenzoate) and starved for 24 h prior to any weighing procedures.

Sixty-eight experiment cages were each stocked with 7 individually weighed snapper (mean weight ± STDEV = 171.6 ± 12.5 g; n=476). Interim and harvest weight check procedures were undertaken 28 and 51 days after stocking, respectively. Any snapper that died or became moribund during the experiment were removed and replaced with tagged fish (i.e. fin clipped) of a similar size that were subsequently identified during the harvest procedure. Because only 9 fish became moribund or died during the experiment, the growth and feed intake attributable to the tagged fish was incorporated in investigations of feed intake and feed conversion. All snapper were returned to larger holding tanks at the conclusion of the experiment.

2.3 Experimental Facilities

The experiment was carried out in a saltwater recirculating system that consisted of 9 x 10 kL circular fibreglass tanks (3.4 m diameter, 1.2 m depth) housed within a plastic covered "hot-house" at PSFC. Each of the 10 kL tanks contained 8 cylindrical floating cages (dimensions approximately 0.2 m^3; 0.6 m diameter x 0.7 m submerged depth; constructed of 10 mm perforated plastic mesh). Each cage was fitted with a lid to prevent the escape of fish and a plastic mesh screen secured at the base of the cage to prevent the loss of pellets (1.6 mm perforated plastic mesh). Cages were firmly secured to the outer perimeter of 10 kL tanks and remained in the same position during the entire experiment. Each 10 kL tank was provided with approximately 40 L min^{-1} of pre-filtered water (15-20 µm) pumped from a submerged bio-filter. Effluent water from each tank was drained through a 1 mm filter screen to remove coarse solids (NikaFilt; Taylor Made Fish Farm, Bobs Farm, NSW, Australia), collected in a common concrete sump (2.5 kL) and pumped back to the bio-filter through a pair of twin cartridge pool filters (10-15 µm); cartridges were cleaned and replaced daily. All tanks were aerated with 4 x 100 mm air stone diffusers and an autumn (fall) photo-period was maintained using fluorescent lighting (12 h light:12 h dark). Each of the 17 dietary treatments was randomly allocated to 4 replicate

cages within the "hot-house" system. Four additional cages holding spare snapper of a similar size were also established and these fish were fed the commercial barramundi feed

2.4 Feeding

Snapper were switched to their respective feeds 24 h after stocking and fed to apparent satiation twice daily at approximately 0830 and 1500 h each day, six days a week (Monday – Saturday). Fish were fed to apparent satiation only once on Sundays (0830 h). Feeding was conducted in two stages to ensure complete satiation. During the first stage, experimental feeds were offered generously, after which there was usually a small amount of feed left on the screen (5-10 pellets). Approximately five minutes later, feed was re-offered in small quantities provided no pellets remained from the initial feed and fish continued to feed actively. A subjective measure of apparent satiation was judged after 4-8 pellets settled on the feeding screen. A final check of feeding screens was made after all cages had been fed to ensure that all pellets had been eaten.

2.5 Water quality

Water quality was maintained by vacuuming tanks once or twice weekly to remove the build up of accumulated solids. Fresh, disinfected (chlorinated / dechlorinated) water was pumped into tanks from a 47 kL storage reservoir to replace water lost through cleaning processes and water exchange. Water temperature was kept above ambient winter temperature by installing a single 2 kW immersion heater in each 10 kL tank. Water quality parameters were recorded daily from each tank using one of two hand held water quality analysers; either a Model 611 (Yeo-Kal Electronics, Brookvale, NSW, Australia) or a Horiba U-10 (Horiba, Japan). Total ammonia [$NH_3 + NH_4^+$] was monitored from tanks using a rapid test kit procedure (Model 1.08024.0001, E. Merck, Darmstadt, Germany). During the experiment, temperature, dissolved oxygen (DO_2), salinity and pH ranged from 20.2-25.2ºC, 5.8-7.5 mg L^{-1}, 3.1-3.5% and 7.4-8.2 units respectively with [$NH_3 + NH_4^+$] always $\leq$ 0.4 mg L^{-1}.

2.6 Chemical analyses

Diet and ingredient samples were analysed by the Food & Agriculture Laboratory of Australia (FALA – Coopers Plains, Qld, Australia). Crude protein (N x 6.25) was analysed by the Leco Method. Total fat was determined after extraction using chloroform and methanol (Folch method) while moisture (oven drying), ash (muffle furnace) and gross energy (bomb calorimeter) were determined using standard procedures (AOAC 1995).

2.7 Statistical analyses

Data were assessed using one-way analysis of variance *ANOVA* procedures. Prior to conducting *ANOVA*, data were tested to ensure that treatment variances were homogenous (Cochran's test) and data was normally distributed (standardised skewness test). The significance level for all *ANOVA* and multiple comparisons tests (Student Newman Keuls; SNK) was set at 0.05 and all data were statistically analysed using Statgraphics Plus, version 4.1 (Manugistics Inc., Rockville, MD, USA; 1998). Linear regressions were fitted to data using GraphPad Prism V4 (GraphPad Software Inc., San Diego CA, USA).

3. RESULTS

With the exception of snapper fed on diet 13 (D13), snapper reared on the experimental feeds grew rapidly and generally performed as well or better than snapper reared on the commercial feed (Table 3). Diet 13 contained the highest level of diatomaceous earth (360 g kg^{-1} diet) which did not affect the

relative feed intake of snapper fed this diet, but, feed conversion (FCR) and protein efficiency ratio (PER) for this treatment were extremely poor. High levels of diatomaceous earth did not negatively affect relative feed intake, FCR or PER in snapper fed other experimental feeds. Clearly, the level of diatomaceous earth used to balance the dry matter in D13 affected the utilisation of this diet. As this response was anomalous within the nutrient sparse group (Group 4) and compared to the remaining experimental diets, it was deleted from any statistical analyses (i.e. nutrient sparse n=12; Table 3).

Preliminary statistical evaluation of the performance indices of snapper within each diet group (one-way *ANOVA*) indicated there were no significant differences (P>0.05) between the dietary treatments allocated to snapper in Group 2 (D5-D8); the group of diets with the sub-optimal DP:DE ratio (high lipid), or Group 3 (D9-D12); the group of diets with an optimal DP:DE ratio with the highest protein content (Table 3). Harvest weight, weight gain and daily weight gain in snapper allocated to the group of diets with an optimal DP:DE ratio and an intermediate protein level (Group 1; D1-D4) were not affected (P>0.05) by dietary treatment, but, feed intake, DP intake, FCR and PER of fish in this group were affected (P<0.05; Table 3). Only harvest weight, weight gain, daily weight gain and PER were significantly affected (P<0.05) by dietary treatment in snapper allocated to the group of diets with an optimal ratio of DP:DE but a low level of dietary protein (Group 4; D14-D16) (Table 3).

With respect to dietary group, snapper reared on nutrient dense diets containing an optimal ratio of DP:DE and 490 g DP kg^{-1} performed significantly better than snapper reared on optimally balanced diets containing either 390 or 320 g DP kg^{-1} (i.e. Group 1 and Group 4), or snapper reared on sub-optimal diets containing 390 g DP kg^{-1} (Group 2; Table 4).
Feed intake among diet groups was influenced by the DE content of the test diets, with relative feed intake suppressed on diets containing high levels of DE (Group 2 and Group 3) compared to diets containing lower levels of DE (Group 1 and Group 4; Table 4). The relationship between relative feed intake and DE content was linear (Figure 1) and could be described by the function:

$$\text{Relative feed intake (g kg BW}^{-0.7}\text{ d}^{-1}\text{)} = 14.0^{(\pm 0.79)} - 0.27^{(\pm 0.04)} \text{ x DE content; } (R^2=0.77, n=16) \quad (1)$$

Because DE content influenced feed intake it also regulated DP intake, which ultimately affected weight gain. The relationship between relative DP intake and weight gain was also linear (Figure 2) over the range of DP intake experienced by snapper in this study and could be described by the function:

$$\text{Weight gain (g fish}^{-1}\text{)} = 29.0^{(\pm 4.4)} \text{ x DP intake} - 5.3^{(\pm 15.0)}; (R^2=0.77, n=15; \text{excludes data for D13}) \quad (2)$$

The *SNK* unplanned multiple comparisons test clearly separated groups means with respect to FCR (Table 4), however, for PER, the *SNK* test could only discriminate between the lowest (1.49; Group 4) and highest (1.64; Group 2) PER values, respectively, indicating little practical difference between this performance index among dietary groups.

4. DISCUSSION

4.1 Weight gain and performance

This study has shown, that under our feeding protocols, maximum weight gain in Australian snapper is influenced by energy mediated DP intake and the DP and DE content of diets. High-energy diets, regardless of whether DE was supplied predominantly in the form of protein (e.g. Group 3; 23 MJ DE kg^{-1}) or lipid (e.g. Group 2; sub-optimal formulation; 22 MJ DE kg^{-1}) reduced relative feed intake (Table 4). This effect has been documented in other sparids (Santinha, *et al.* 1999; Lupatsch, *et al.* 2001a; Koven, 2002). However, despite reductions in the feed intake of high-energy diets, provided

high-energy diets supplied a significant proportion of DE in the form of highly digestible protein (i.e. approximately 67% of DE from DP; Table 3), maximum weight gain was approached (Group 3; Table 4). Diets formulated with lower amounts of DE (e.g. Group 4; 15 MJ DE kg^{-1}) and lower amounts of DP (320 g DP kg^{-1}), resulted in snapper attempting to increase feed intake in order to meet DE, as is reported for fish generally (NRC, 1993) and possibly DP requirements as reported for salmonid species (Azevedo, Leeson, Cho & Bureau, 2004).

Digestible protein intake by snapper on these diets was inadequate, and while satisfying maintenance requirements, did not satisfy requirements for maximum growth. Diets that provided an "optimised" ratio of DP:DE, but in which DE and DP levels were increased to 18 MJ DE and 390 g DP kg^{-1} respectively (Group 1), allowed snapper to increase relative feed intake (and thus DP intake), resulting in increased weight gain. These gains were intermediate between those recorded for snapper fed optimised low and high protein diets respectively (Table 4).

According to factorial models presented for snapper (Glencross & Lupatsch, unpublished data) and gilthead seabream (Lupatsch, *et al.* 2003b), maximum daily weight gain for fish that weigh approximately 230 g (average for range in this study) is about 1.9 to 2.1 g $fish^{-1}$ day^{-1}. The similarity in these predicted values and that of the snapper consuming the high protein diets in this study indicates that maximum weight gain (2.1 g $fish^{-1}$ day^{-1}) was approached. Maximum weight gain for the 230 g snapper in this study corresponds to a DP intake of approximately 3.9 g DP kg $BW^{-0.7}$ d^{-1} (Table 4), which is slightly higher than the values of 3.0 (Lupatsch, *et al.* 2001a) and 3.1 g DP kg $BW^{-0.7}$ d^{-1} (Glencross & Lupatsch, unpublished data) predicted for gilthead seabream and snapper respectively. Relative DE intake was also significantly higher in snapper fed the high protein, nutrient dense feeds (227 kJ kg $BW^{-0.84}$ d^{-1}) compared to those fed dietary groups with lower levels of DE. This outcome probably reflects the fact that the energy requirements for growth in this group of snapper were slightly elevated due to their substantial improvement in weight gain (Lupatsch, *et al.* 2003a). In any case, DE intake was within the range described for gilthead seabream (200-220 kJ kg $BW^{-0.83}$ d^{-1}) fed twice daily to apparent satiation on feeds containing 17 to 22 MJ DE kg^{-1} (Lupatsch, *et al.* 2001a).

To date, all factorial models presented for snapper (Glencross & Lupatsch, unpublished data), gilthead seabream (Lupatsch, Kissil, Sklan & Pfeffer, 1998; Lupatsch, *et al.* 2001a; 2003b), European seabass and white grouper *Epinephelus aenus* (Lupatsch, *et al.* 2003b), predict that the DP:DE ratio of diets will decline as fish size increases. This is apparently due to the changing ratio of protein to energy of the weight gain and the increasing proportion of energy used to satisfy maintenance requirements (absolute) of larger fish (Lupatsch, *et al.* 2001a). Importantly, our study has demonstrated that the performance of snapper fed diets that are "optimised" according to factorial or bioenergetic models derived for gilthead seabream (Lupatsch, *et al.* 2001a) or snapper (Glencross & Lupatsch, unpublished data), also appear to be highly dependant on the DP content of this theoretical ratio and the contribution DP makes to the dietary pool of DE.

Considerable changes in the feed formulation strategies for farmed fish have occurred over the last decade. This is exemplified by the dramatic reductions in the protein and carbohydrate contents of farmed salmonid diets and the switch to high-energy feeds containing up to 400 g lipid kg^{-1} (Cho, Hynes, Wood & Yoshida, 1994; Azevedo, *et al*. 2004). However, this type of strategy will be inappropriate for production of snapper because firstly, snapper fed diets containing 390 g DP kg^{-1} and high levels of DE due to elevated lipid levels (i.e. sub-optimal ratio) performed poorly compared to snapper fed diets with the same DP content but lower levels of DE (Tables 4.3 & 4.4). Secondly, the apparent need to provide a high proportion of DE in the form of DP (to ensure maximum weight gain) will limit the "dietary space" available for other energy sources. These results are significant for the snapper industry because they indicate that maximum weight gains in snapper of this size farmed under similar temperatures and feeding protocols will not be achieved unless diets are formulated to

have reasonably high DP contents that provide approximately 68% of DE in the form of DP. Koshio (2002) also noted that juvenile red sea bream required almost 60% of dietary energy in the form of protein to obtain optimal growth. This means that high-energy production diets for snapper will need to contain higher rather than lower levels of DP.

These results reflect the outcomes of our earlier study with snapper that appeared to indicate high-energy diets (increased lipid) that failed to provide high levels of DP in the correct DP:DE ratio also limited weight gain (Booth, *et al.*, in press). In this sense, snapper appear to be similar to barramundi, which also have a limited ability to use lipid as a primary energy source unless their diets are also adequately supplied with protein. Under this scenario, it is possible that more nutrient dense feeds formulated to the correct ratio of DP:DE and providing approximately 60% of DE in the form of DP may produce even greater weight gains (Williams, *et al.* 2003). These diets will ultimately be more expensive than lower protein diets, however significant reductions in FCR should alleviate some of these concerns. Replacing fishmeal with less expensive blends of different protein sources may also reduce these costs for snapper and improved feed conversion efficiencies have obvious benefits for the environment (Cho & Bureau, 2001). However, the potential for rapid weight gains in snapper fed high-energy diets (high protein) may be tempered by the partition of this increased energy between fat and lean tissue gain. For barramundi, increases in the nutrient density of diets increased weight gain, however, increases in the energy content of diets also increased lipid deposition (Williams, *et al.* 2003). These outcomes affect the marketability and profitability of fish depending on whether they are sold whole or gilled and gutted. These issues remain to be resolved for snapper and should be investigated before the use of high-energy diets (high protein) can be unequivocally recommended to the snapper industry.

As expected, there was little variation in PER within or between dietary groups containing 390-490 g DP kg^{-1} (Tables 2 & 3). This was not surprising given fishmeal was the sole source of protein in all the experimental diets. PER is generally used to evaluate only the source of protein in a diet, not the diet itself or the level of protein in the diet. Nevertheless, where experimental systems, diets and conditions are quite similar, it serves as a useful comparison of protein utilisation (Hepher, 1988). In contrast, FCR improved significantly as the DP content (intake) of optimised diets increased and reflects similar improvements in FCR of snapper (Booth *et al.*, in press) and red sea bream (Takeuchi, *et al*. (1991) fed increasing levels of intact dietary protein (fishmeal). Our data also suggests that feeds for 170-290 g snapper should be formulated to contain between 18 - 23 MJ DE kg^{-1}. This will prevent energy mediated feed intake limiting DP intake when diets are constrained to a DP:DE ratio of approximately 22 g DP MJ DE^{-1}.

4.2 Effect of lipid and carbohydrate ratio on performance

It is widely reported that while digestibility of protein and lipid sources are relatively independent of inclusion content, digestibility of CHO sources decrease with increasing inclusion content (Wilson,1994; Stone, 2003; Booth, *et al.* 2005; Booth, *et al.* in press). This infers that variations in lipid and particularly CHO content and source may also impact on the utilisation of diets. In this study, we have formulated diets on a digestible nutrient basis according to predetermined digestibility coefficients for the particular ingredients used. These ingredients were investigated over a range of inclusion levels (Booth, *et al.* 2005; Booth, *et al.* in press). This approach should substantially improve the capacity to study the utilisation of different ingredients by comparing different measures of performance. In the present trial, we incorporated gelatinised wheat starch and fish oil into test diets based on their DE values and found that varying the ratio of lipid to CHO (i.e. varying the composition of DE) within the high and intermediate DP groups we tested had little impact on weight gain or feed intake. This indicates that, within the constraints of the formulations we have presented for these diet groups (Table 3), gelatinised wheat starch could effectively be interchanged with fish oil

without affecting fish performance. Thus, for diets containing 390 g DP kg^{-1} with an optimised DP:DE ratio (Group 2), levels of gelatinised starch between 50 –290 g kg^{-1} could be used. For diets optimised with 490 g DP kg^{-1} (Group 3), gelatinised starch incorporation levels can range from 40 – 129 g kg^{-1} (Table 2). In contrast to these results, Morris & Davies (1995; cited in Koven, 2002) reported that based on metabolic energy values, lipid and carbohydrate could not be interchanged in the diets of gilthead seabream. Presumably, these metabolic energy values were based on physiological fuel equivalents, demonstrating the importance of determining the digestibility of different energy sources before they can be reliably interchanged.

The effect of varying the lipid and CHO ratio on fish performance in optimised diets with a low DP and DE content (Group 4) was more difficult to interpret. Clearly lower, but similar DP intakes had reduced the potential weight gains of snapper from this group (Table 3). However, significant reductions in harvest weight, weight gain and PER were noted in snapper fed diets D15 and D16 (Group 4). In this case, weight gain and PER declined as the level of fish oil was reduced from 84 to 51 and 20 g kg^{-1} at the same time the level of gelatinised starch was increased (Table 3). Recommended levels of crude lipid in the diets of juvenile red seabream vary between about 100 to 150 g kg^{-1} (Yone, 1976; Takeuchi, Shiina & Watanabe, 1991; Koshio, 2002), while similar levels have been recommended for gilthead seabream (Marias & Kissil, 1979). Lipid levels of 200 g kg^{-1} actually reduced weight gain, feed efficiency and PER in juvenile red seabream fed diets containing 420 or 520 g kg^{-1} crude protein (Takeuchi, *et al.* 1991). These recommendations suggest that we may have inadvertently formulated diets D15 and D16 to be deficient in total dietary lipid. Alternatively, reductions in weight gain and the apparent decline in FCR and PER for snapper fed optimised but low protein diets may suggest that pregelled wheat starch is less capable of meeting the DE requirements of snapper when used in low DP diet formulations. Thus, under this type of formulation scenario, lipid (i.e. fish oil) has a greater protein sparing effect than carbohydrate.

5. CONCLUSIONS

This experiment has shown for diets "optimised" to a previously determined DP:DE ratio for 100-300 g sparids (i.e. 21-22 g DP $kgBW^{-1}$), increasing total DP and DE content improved the weight gain and performance of snapper. Importantly, diets must supply a reasonable proportion of DE in the form of highly digestible protein. For diets with 390 g DP kg^{-1}, increasing DE from 18 to 21 MJ DE kg^{-1} reduced feed intake and weight gain, presumably because energy needs were satisfied before there was sufficient intake of DP for maximum weight gain. Diets with an optimised ratio of DP:DE and containing 320 g DP and 15 MJ DE kg^{-1} also prevented snapper consuming enough DP in order to satisfy requirements for maximum weight gain. Within the digestible nutrient range of diets formulated for this study, varying the source of DE by changing the ratio of lipid (fish oil) to carbohydrate (gelatinised wheat starch) had little effect on performance provided diets contained more than 390-490 g DP kg^{-1}.

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REFERENCES

AOAC (1995). *Official methods of analysis of the association of official chemists*. (ed. K. Helrich), 15th ed., Published by the Association of the Official Analytical Chemists, Inc., Arlington, Virginia, USA.

Azevedo, P.A., Leeson, S., Cho, C.Y. & Bureau, D.P. (2004). Growth, nitrogen and energy utilization of juveniles from four salmonid species: diet, species and size effects. Aquaculture **234**:393-414.

Bikker, P. (1994). Protein and lipid accretion in body components of growing pigs: the effects of body weight and nutrient intake. PhD Thesis, Department of Animal Nutrition, Wageningen Agricultural University, Haasteeg 4, Wagengingen, The Netherlands.

Booth, M.A., Allan, G.L. & Anderson, A.J. (2005). Investigation of the nutritional requirements of Australian snapper *Pagrus auratus* (Bloch & Schneider, 1801): apparent digestibility of protein and energy sources. Aquaculture Research **36**:378-390.

Bureau, D.P., Azevedo, P.A., Tapia-Salazar, M. & Cuzon, G. (2000). Pattern and cost of growth and nutrient deposition in fish and shrimp: potential implications an applications. In: Advances en Nutricion Acuicola V. Memorias del V Simposium Internacional de Nutricion Acuicola (ed. by L.E. Cruz-Suarez, D. Ricque-Marie, M. Tapia-Salazar, M.A. Olivera-Novoa & R. Civera-Cerecedo). 19-22 Noviembre, 2000. Merida, Yucutan, Mexico.

Bureau, D.P., Kaushik, S.J. & Cho, C.Y. (2002). Bioenergetics. In: Fish Nutrition, 3rd Edition, Chapter 1 (ed. by J.E. Halver & R.W. Hardy). Academic Press, California, US.

Catacutan, M.R. & Coloso, R.M. (1997). Growth of juvenile Asian seabass, *Lates calcarifer*, fed varying carbohydrate and lipid levels. Aquaculture **149**:137-144.

Cho, C.Y. (1992). Feeding systems for rainbow trout and other salmonids with reference to current estimates of energy and protein requirements. Aquaculture **100**:107-123.

Cho, C.Y., Hynes, J.D., Wood, K.R. & Yoshida, H.K. (1994). Development of high-nutrient dense, low pollution diets and prediction of aquaculture wastes using biological approaches. Aquaculture **124**:293-305.

Cho, C.Y. & Bureau, D.P. (2001). A review of diet formulation strategies and feeding systems to reduce excretory and feed wastes in aquaculture. Aquaculture Research **32**:349-360.

Company, R., Caldich-Giner, J.A., Perez-Sanchez, J. & Kaushik, S.J. (1999). Protein sparing effect of dietary lipids in common dentex (*Dentex dentex*): a comparative study with sea bream (*Sparus aurata*) and sea bass (*Dicentrarchus labrax*). Aquatic Living Resources **12**(1):23-30.

De Silva, S.S., Gunasekera, R.M., Collins, R.A. & Ingram, B.A. (2002). Performance of juvenile Murray cod, *Maccullochella peelii peelii* (Mitchell) fed with diets of different protein to energy ratio. Aquaculture Nutrition **8**:79-85.

Einen, O. & Roem, A.J. (1997). Dietary protein/energy ratios for Atlantic salmon in relation to fish size: growth, feed utilization and slaughter quality. Aquaculture Nutrition **3**:115-126.

Ellis, S.C. & Reigh, R.C. (1991). Effects of dietary lipid and carbohydrate levels on growth and body composition of juvenile red drum, *Sciaenops ocellatus*. Aquaculture **97**:383-394.

Halver, J.E. & Hardy, R.W. (2002). Nutrient flow and retention. In: Fish Nutrition, 3rd Edition, Chapter 14 (ed. by J.E. Halver & R.W. Hardy). Academic Press, California, US.

Hepher, B. (1988). Nutrition of pond fishes. Cambridge University Press, Sydney.

Koshio, S. (2002), Red sea bream, *Pagrus major*. In: Nutrient Requirements and Feeding of Finfish for Aquaculture, Chapter 4 (ed. by C.D. Webster & C. Lim). CAB International.

Koven, W. (2002). Gilthead sea bream, Sparus aurata. In: Nutrient Requirements and Feeding of Finfish for Aquaculture, Chapter 5 (ed. by C.D. Webster & C. Lim). CAB International.

Lupatsch, I., Kissil, G.WM., Sklan, D. & Pfeffer, E. (1998). Energy and protein requirements for maintenance and growth in gilthead seabream (*Sparus aurata* L.). Aquaculture Nutrition **4**:165-173.

Lupatsch, I., Kissil, G.WM. & Sklan, D. (2001b). Optimization of feeding regimes for European seabass *Dicentrarchus labrax*: a factorial approach. Aquaculture **202**:289-302.

Lupatsch, I., Kissil, G.WM., Sklan, D. & Pfeffer, E. (2001a). Effects of varying dietary protein and energy supply on growth, body composition and protein utilisation in gilthead seabream (*Sparus aurata* L.). Aquaculture Nutrition **7**:71-80.

Lupatsch, I., Kissil, G.WM. & Sklan, D. (2003a). Comparison of energy and protein efficiency among three species: gilthead seabream (*Sparus aurata*), European seabass (*Dicentrarchus labrax*) and white grouper (*Epinephelus aenus*): energy expenditure for protein and lipid deposition. Aquaculture **225**:175-189.

Lupatsch, I., Kissil, G.WM. & Sklan, D. (2003b). Defining energy and protein requirements of gilthead seabream (*Sparus aurata*) to optimise feeds and feeding regimes. The Israeli Journal of Aquaculture – Bamidgeh **55**(4):243.247.

Marias, J.F.K. & Kissil, G.W.M. (1979). The influence of energy level on the feed intake, growth, food conversion and body composition of *Sparus aurata***.** Aquaculture **17**:203-219.

National Research Council (NRC) (1993); Nutrient requirements of fish. National Academy Press Washington, D.C., USA.

Paulin, C.D. (1990); *Pagrus auratus*, a new combination for the species known as 'snapper' in Australian waters (Pisces: Sparidae). New Zealand Journal of Marine and Freshwater Research **24**:259-265.

Peres, H. & Oliva-Teles, A. (2002); Utilisation of raw and gelatinized starch by European sea bass (*Dicentrarchus labrax*) juveniles. Aquaculture **205**:287-299.

Santinha, P.J.M., Medale, F., Corraze, G. & Gomes, E.F.S. (1999) Effects of the dietary protein:lipid ratio on growth and nutrient utilization in gilthead seabream (*Sparus aurata* L.). Aquaculture Nutrition **5**:147-156.

Stone, D.A.J. (2003). Dietary carbohydrate utilization by fish**.** Reviews in Fisheries Science **11**(4):337-369.

Takeuchi, T., Shiina, Y. & Watanabe, T. (1991). Suitable protein and lipid levels in diets for red sea bream *Pagrus major*. Nippon Suisan Gakkaishi **57**(2):293-299.

Tabata, K. & Taniguchi, N. (2000). Differences between *Pagrus major* and *Pagrus auratus* through mainly mtDNA control region analysis. Fisheries Science **66**:9-18.

Williams, K.C., Barlow, C.G., Rodgers, L.J., Hockings, I., Agcopra, C. & Ruscoe, I. (2003). Dietary protein to energy relationships in barramundi. In: Aquaculture Diet Development Subprogram: Nutrient Requirements of Aquaculture Species, 101-117 (ed. by D.M. Smith, *et al.*). FRDC Final Report Series No. 59. DPI NSW Fisheries, Port Stephens Fisheries Centre, Taylors Beach, Australia.

Wilson, R.P. (1994). Utilisation of dietary carbohydrate by fish**.** *Aquaculture 124*:67-80.

Yone, Y. (1976). Nutritional studies of red sea bream. In: Proceedings of the First International Conference on Aquaculture Nutrition, 39-64. (ed. by K.S. Price, *et al*.), October 14-15, 1975. Lewes/Rehoboth, Delaware.

TABLE 1
Measured chemical composition of major feed ingredients (g kg^{-1} or MJ kg^{-1} dry matter basis).

Ingredient	Crude Protein	Ash	Organic matter[1]	Fat	Gross energy (MJ)
Fish meal[2]	734.1	150.0	850.0	132.8	22.23
Fish oil[3]	-	10.0	990.0	990.0	38.00
Pre-gelatinised wheat starch[4]	8.0	3.6	996.4	10.5	15.65

[1] Organic matter calculated by difference = (1000 – measured ash content)
[2] Pesquera S.A. Product of Chile. Talcahuano, South Chile. Steam dried prime quality.
[3] Supplied by Skretting (Nutreco), Tasmania, Australia.
[4] Penford Australia Ltd., Lane Cove, NSW, Australia. 100% pregelatinised wheat starch – Pregel N.

TABLE 2
Ingredient composition and calculated digestible protein or energy content of test diets fed to snapper (g kg^{-1} or MJ kg^{-1} dry matter).

	Optimal ratio 390/18 Intermediate protein Group 1				Sub-optimal ratio 390/21 High energy Group 2				Optimal ratio 490/23 High protein (nutrient dense) Group 3				Optimal ratio 320/15 Low protein (nutrient sparse) Group 4				COM
Ingredient	D1	D2	D3	D4	D5	D6	D7	D8	D9	D10	D11	D12	D13	D14	D15	D16	D17
Fish meal	570.0	567.0	563.0	560.0	570.0	563.0	557.0	550.0	710.0	705.0	700.0	695.0	460.0	457.0	453.0	450.0	-
Fish oil	150.0	125.0	100.0	75.0	220.0	207.0	193.0	180.0	190.0	180.0	170.0	160.0	115.0	84.0	51.0	20.0	-
Pregelled starch	50.0	129.0	211.0	290.0	70.0	113.0	157.0	200.0	40.0	69.0	100.0	129.0	50.0	156.0	264.0	370.0	-
Premix[1]	15.0	15.0	15.0	15.0	15.0	15.0	15.0	15.0	15.0	15.0	15.0	15.0	15.0	15.0	15.0	15.0	-
Stay-C® 35[2]	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	-
Diat. earth[3]	214.0	163.0	110.0	59.0	124.0	101.0	77.0	54.0	44.0	29.0	15.0	-	359.0	288.0	215.0	144.0	-
Nutrient or energy (measured)																	
Crude protein	426.0	429.0	423.0	419.0	435.0	423.0	410.0	417.0	523.0	527.0	526.0	520.0	359.0	346.0	341.0	337.0	540.0
Fat	208.0	197.0	171.0	164.0	281.0	296.0	249.0	242.0	256.0	246.0	229.0	226.0	183.0	152.0	121.0	78.0	138.0
Ash	307.0	257.0	212.0	159.0	223.0	196.0	180.0	156.0	170.0	156.0	142.0	129.0	223.0	426.0	370.0	222.0	137.0
NFE[4]	59.0	117.0	195.0	258.0	58.0	84.0	160.0	185.0	51.0	71.0	102.0	125.0	235.0	75.0	168.0	364.0	184.0
Gross energy	19.0	20.0	20.0	21.0	22.0	23.0	22.0	23.0	23.0	23.0	23.0	23.0	20.0	16.0	16.0	17.0	21.0
Digestible nutrient or energy (calculated[5])																	
DP	395.0	393.0	391.0	389.0	395.0	391.0	386.0	382.0	492.0	488.0	485.0	482.0	319.0	317.0	315.0	314.0	-
DE	19.0	19.0	18.0	18.0	22.0	22.0	21.0	21.0	23.0	23.0	23.0	23.0	15.0	15.0	15.0	14.0	-
DP:DE ratio	20.8	20.7	21.7	21.6	17.9	17.8	18.4	18.2	21.4	21.2	21.1	20.9	21.3	21.1	21.0	22.4	-
% DE from DP	67.0	66.0	67.0	67.0	58.0	58.0	57.0	58.0	68.0	68.0	67.0	67.0	68.0	66.0	67.0	69.0	
% DE from oil	29.0	24.0	19.0	15.0	37.0	35.0	32.0	31.0	30.0	28.0	27.0	25.0	28.0	20.0	12.0	5.0	
% DE from CHO	4.0	10.0	13.0	18.0	5.0	7.0	10.0	11.0	2.0	4.0	6.0	8.0	5.0	14.0	21.0	26.0	

[1] NSW DPI (Fisheries) Premix; formulation described in Booth, Allan & Anderson (2005)
[2] Stay-C® 35, vitamin C, Hoffman La-Roche, Basel, Switzerland.
[3] Amorphous diatomaceous earth purchased from Recreational Water Products, East Melbourne, Australia.
[4] Nitrogen free extractives calculated by difference NFE = 1000 – (protein + ash + fat)
[5] Predicted digestible nutrient values based on measured nutrient or energy content of ingredients (Table 1) and apparent digestibility coefficients determined by Booth, Allan & Anderson (2005) and Booth *et al.* (in press)

TABLE 3
Performance of snapper after 51 days on test diets.

	Optimal ratio 390/18 Intermediate protein				Sub-optimal ratio 390/21 High energy				Optimal ratio 490/23 High protein (nutrient dense)				Optimal ratio 320/15 Low protein (nutrient sparse)				
	Group 1				Group 2				Group 3				Group 4				COM
	D1	D2	D3	D4	D5	D6	D7	D8	D9	D10	D11	D12	D13	D14	D15	D16	D17
Initial wt (g)	173.0 (2.1)	172.5 (2.4)	171.0 (1.9)	167.8 (1.6)	171.8 (2.0)	168.2 (1.8)	173.1 (1.0)	169.4 (2.8)	169.8 (3.8)	172.0 (1.8)	172.7 (1.5)	173.4 (1.7)	172.2 (1.3)	173.7 (1.1)	173.1 (3.6)	171.6 (0.9)	171.0 (0.9)
Harvest wt. (g)	263.3 (1.3)	263.8 (6.3)	266.2 (6.5)	260.8 (6.4)	260.7 (5.5)	258.8 (3.8)	248.3 (3.7)	243.9 (6.7)	271.5 (7.6)	291.0 (3.7)	271.8 (7.1)	286.5 (4.2)	188.3 (1.4)	261.6^{b} (2.5)	251.4^{ab} (4.6)	244.9^{a} (3.5)	274.8 (4.8)
Weight gain (g)	90.3	91.3	95.1	93.1	88.8	90.6	75.2	74.5	101.7	119.0	99.1	113.1	16.1	87.9^{b}	78.3^{a}	73.3^{a}	103.8
Daily gain (gd^{-1})	1.8	1.8	1.9	1.8	1.8	1.8	1.5	1.5	2.0	2.3	2.0	2.2	0.4	1.7^{b}	1.5^{a}	1.4^{a}	2.0
Feed intake (g)	167.0^{b}	144.9^{a}	153.9^{ab}	155.6^{ab}	134.4	133.0	121.3	124.9	133.1	152.2	132.2	144.7	145.3	172.4	168.2	169.9	174.3
Relative intake	9.7^{b}	8.4^{a}	8.9^{ab}	9.1^{ab}	7.8	7.8	7.2	7.5	7.7	8.5	7.6	8.1	9.5	10.0	9.9	10.1	10.0
DP intake	3.8^{b}	3.3^{a}	3.5^{a}	3.5^{a}	3.1	3.1	2.8	2.9	3.8	4.2	3.7	3.9	3.0	3.2	3.1	3.2	na
DE intake	224.1^{b}	195.6^{a}	204.3^{a}	208.8^{a}	208.8	209.2	190.3	195.1	219.5	241.7	215.3	230.3	180.3	189.4	183.6	180.3	na
FCR	1.9^{b}	1.6^{a}	1.6^{a}	1.7^{a}	1.5	1.5	1.6	1.7	1.3	1.3	1.3	1.3	9.9	2.0	2.1	2.3	1.7
PER	1.4^{a}	1.6^{b}	1.6^{b}	1.5^{b}	1.7	1.8	1.6	1.6	1.6	1.6	1.5	1.6	0.3	1.6^{b}	1.5^{a}	1.4^{a}	na

Values represent mean ± (SEM) of 4 replicate cages. Diet 13 excluded from statistical comparisons
Within each diet group, different superscript letters indicate significant differences between means ($P<0.05$, ANOVA-SNK)
Weight gain (g $fish^{-1}$) = average harvest weight – average initial weight
Feed intake (g $fish^{-1}$; dry basis) = average total feed intake per cage / 7 fish
Relative feed intake (g kg $BW^{-0.7}$ d^{-1}) = average total feed intake per fish / (GMBW/1000)/51 days
Relative digestible protein intake (g DP kg $BW^{-0.7}$ d^{-1}) = g DP intake per fish /$((GMBW/1000)^{0.7}$/51 days
Relative digestible energy intake (kJ DE kg $BW^{-0.84}$ d^{-1}) = kJ DE intake per fish / $(GMBW/1000)^{0.84}$/51 days
Feed conversion ratio (FCR) = average dry basis feed intake per fish / average wet weight gain per fish
Protein efficiency ratio (PER) = wet weight gain per fish / dry basis DP intake per fish

TABLE 4
Group performance of snapper reared on optimal or sub-optimal diets for 51 days.

	Optimal ratio Intermediate protein	Sub-optimal ratio High energy	Optimal ratio High protein (nutrient dense)	Optimal ratio Low protein (nutrient sparse)
	21 g DP MJDE^{-1}	18 g DP MJDE^{-1}	21 g DP MJDE^{-1}	21 g DP MJDE^{-1}
Performance index[1]	Group 1	Group 2	Group 3	Group 4[2]
Harvest wt. (g)	263.5^{b}	252.9^{a}	280.2^{c}	252.6^{a}
Weight gain (g)	92.5^{b}	82.3^{a}	108.2^{c}	79.8^{a}
Daily gain (gd-1)	1.8^{b}	1.6^{a}	2.1^{c}	1.6^{a}
Feed intake (g)	155.4^{c}	128.4^{a}	140.6^{b}	170.1^{d}
Relative intake	9.0^{b}	7.6^{a}	7.9^{a}	10.0^{c}
Relative DP intake	3.5^{c}	2.9^{a}	3.9^{d}	3.1^{b}
Relative DE intake	208.2^{b}	201.0^{b}	226.7^{c}	183.1^{a}
FCR[3]	1.7^{c}	1.6^{b}	1.3^{a}	2.1^{d}
PER	1.5^{a}	1.6^{b}	1.6^{ab}	1.5^{a}

[1] Indices described in Table 4.3.

[2] Values represent mean of 16 replicate tanks except for Group 4 where n=12. Within each row, different superscript letters indicate significant differences between group means (P<0.05, ANOVA, SNK).

[3] Data for FCR were heterogeneous and were log transformed prior to ANOVA. Actual data means are presented in the table.

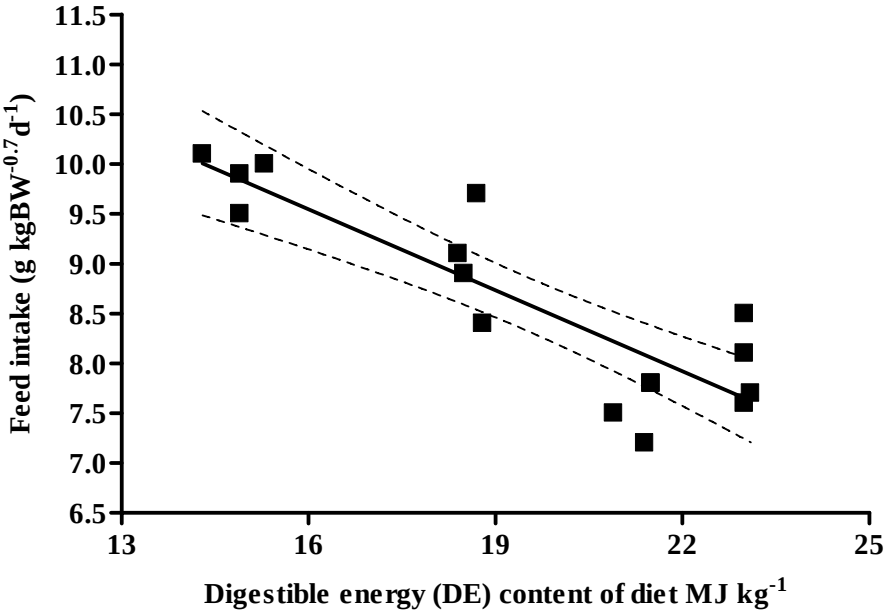

FIGURE 1
Effect of digestible energy (DE) content on relative feed intake in juvenile snapper. Points represent mean of 4 replicate cages. Outer curves represent 95% confidence intervals.

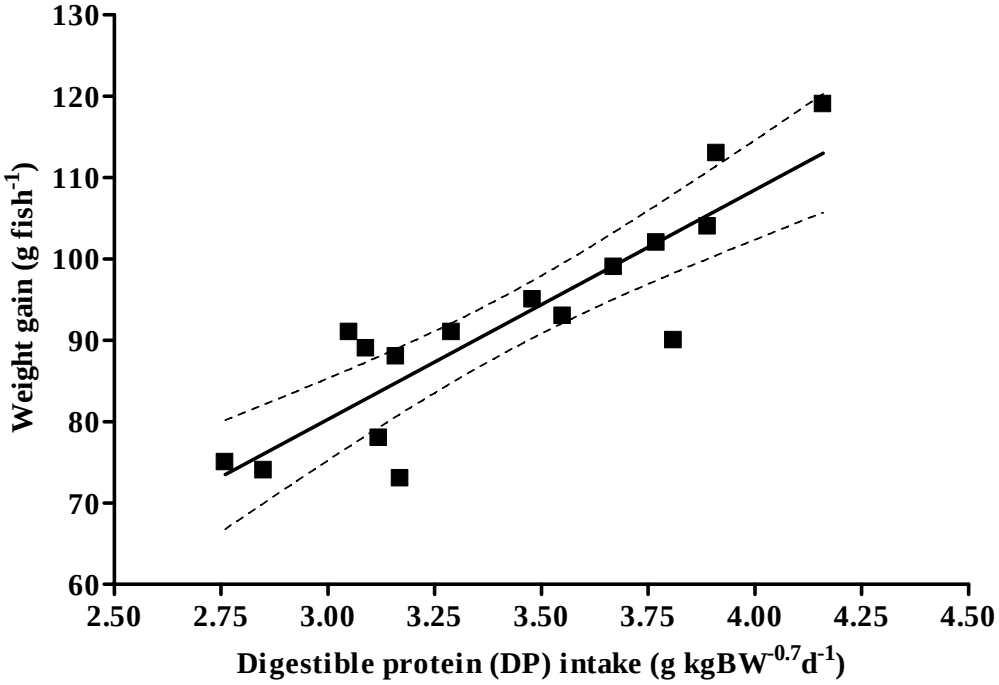

FIGURE 2
Effect of relative digestible protein (DP) intake on weight gain of juvenile snapper. Points represent mean of 4 replicate cages. Outer curves represent 95% confidence intervals.

4.6 Apparent digestibility of high-protein ovine and bovine meals by Australian snapper *Pagrus auratus* (Bloch & Schneider, 1801).

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ABSTRACT

An experiment was done with 180 g Australian snapper *Pagrus auratus* to investigate the effect of inclusion level (30 or 60%) on the apparent digestibility of two low ash, high protein ovine meals (16.5 and 9.8% ash and 74.3 and 83.1% crude protein [dry basis] respectively) and a low ash, high protein bovine meal (3.0% ash, 86.8% crude protein [dry basis]). Apparent digestibility coefficients (ADC's) for organic matter, crude protein, fat and gross energy were determined using indirect methodology (5000 mg Cr_2O_3 kg^{-1} diet) and faecal material was collected using a modified Guelph settlement system. Snapper were acclimated to experimental conditions and diets for 8 days before collection of faeces. Inclusion content did not affect any of the ingredient ADC's and there was no interaction between inclusion content and ingredient type for any of the ADC's. ADC's for the lower protein ovine meal, higher protein ovine meal and the bovine meal for organic matter were 86%, 99% and 92% respectively, for crude protein were 83%, 91%, 95% respectively, for fat were 85%, 94%, and 95% respectively and for gross energy were 85%, 95% and 89% respectively. Results indicate that for low ash, high protein meat meals, ADC's for snapper were high and similar to reported values for fishmeal of similar nutrient composition.

1. INTRODUCTION

Aquaculture was the fastest growing food producing primary industry in the world from 1970 to 2005 (at annual compounded growth rate of 8.8%) and in 2005 contributed 47.8 million tonnes or 43% of global supplies of food fish (FAO, 2007). The expansion in the use of formulated feeds has been a key factor in the growth of aquaculture as reflected in the growth in the production of aquaculture feeds from approximately 4 million tonnes in the early to mid 1990's (Tacon, 1998) to around 23 million tonnes in 2006 (FAO, 2007). Over 50% of global fishmeal supplies are now used in aquafeed production and finding suitable alternative protein sources remains a key global challenge (FAO, 2007).

Marine finfish are generally strictly carnivorous and most are fed on fishmeal-based, high protein diets (>45% protein) or even directly fed low-value fish (De Silva – pers. comm. NACA Kochi Trash Fish Workshop). Increasing amounts of vegetable proteins are being used but, for marine carnivores, a requirement for high dietary protein, driven mainly by an inability to tolerate high dietary carbohydrate or the ability to utilise carbohydrate for energy (for example see Glencross, 2006), has restricted fishmeal replacement to date.

However, rendered animal protein sources, such as poultry offal meal, meat and bone meal and blood meal products are excellent protein sources with no carbohydrates and few anti-nutrients. Globally, approximately 50 million tones of raw animal material is rendered each year (Swisher, 2006), producing around 12 million tonnes of rendered animal meal. This is roughly twice as much fishmeal as is normally available (Shepherd IFFO, 2007, pers. comm.). There is a substantial body of literature demonstrating the generally positive benefits of using rendered animal products in aquafeeds for freshwater finfish species (Lovell, 1992; Tacon, 1994; Allan, Parkinson, Booth, Stone, Rowland, Frances & Warner-Smith, 2000; Stone, Allan, Parkinson & Rowland, 2000; Allan & Rowland, 2005; Bureau, 2006). Williams, Allan, Smith & Barlow (1998) and Allan *et al.* (2000) reported on positive results with the catadromous barramundi (*Lates calcarifer*) following digestibility studies, laboratory

based studies with graded replacements on the basis of digestibility followed by confirmatory studies with fish on a commercially relevant scale.

There is, however, less data available for carnivorous marine species. Nengas, Alexis, Davies & Petichakis (1995) measured digestibility coefficients for a range of rendered animal products for gilthead sea bream (*Sparus auratus*) and Shimeno, Masumoto & Hujita. (1993a), Shimeno, Mima & Imanaga (1993b) and Shimeno, Hosokawa & Masumoto (1996) reported meat meal was well accepted by the marine carnivore yellowtail kingfish (*Seriola quinqueradiata*). Booth, Allan & Anderson (2005) measured apparent digestibility coefficients with snapper for meat and bone meal (57% crude protein, dry basis) and poultry meal (69% crude protein, dry basis) (each at two inclusion contents; 30 & 50 %) and reported that coefficients for the meat and bone meal were lower than for the higher protein poultry meal. Shimeno *et al.* (1993a,b 1996) reported that meat meal with other ingredients could replace half of the fishmeal, they did not measure digestibility of meat meal products. For carnivorous species, the difficulty with finding suitable high protein ingredients has limited the substitution of fishmeal in aquafeeds. Williams *et al.* (1998) concluded that use of high protein, low ash and low fat rendered products had the most potential. The aim of this study was to determine digestibility coefficients for high protein, low ash rendered animal products.

2. MATERIALS AND METHODS

2.1 Diets

The imported ovine and bovine meat meals tested in this study were produced by Bakels Edible Oils (N.Z.) Ltd., (Mt Maunganui, New Zealand) and were obtained from Camilleri Stockfeeds Pty. Ltd., (Maroota, NSW, Australia). Two "premium" high protein ovine meal products were evaluated; one reported to contain a minimum of 60% (Ovine meal–60) and the other reported to contain a minimum of 70% crude protein (Ovine–70), respectively. A "premium" high protein bovine meal reported to contain a minimum of 70% crude protein was also tested (Bovine–70). The chemical composition of these ingredients is presented in Table 1. Test diets were made by substituting 300 or 600 g kg^{-1} of a simple reference diet with each test ingredient. The reference diet was composed of fish meal and extruded wheat mixed in a 1:1 ratio (Booth *et al.* 2005). Dry ingredients, supplements and the inert marker Cr_2O_3 were combined on a dry matter basis according to the formulations presented in Table 2. Each dietary mash was then thoroughly mixed (Hobart Mixer: Troy Pty Ltd, Ohio, USA) before being finely ground in a laboratory scale hammer mill fitted with a 1.5 mm screen (Raymond Laboratory Mill, Transfield Technologies, Rydalmere, NSW, Australia). The ground mash was then re-mixed and combined with distilled water and formed into sinking pellets using a meat mincer fitted with a 3.0 mm die plate (Barnco Australia Pty Ltd, Leichhardt, NSW, Australia). Moist pellets were then dried for about six hours (≈35ºC) until moisture contents were < 100 g kg^{-1} diet. Following preparation, all diets were stored frozen at < -15º C until required.

2.2 Facilities

Twenty-seven purpose built 170 L cylindro-conical digestibility tanks were used to house fish and collect faeces. A detailed description of this system is given in Allan, Rowland, Parkinson, Stone & Jantrarotai (1999). Digestibility tanks were housed inside a temperature-controlled laboratory. A skylight provided fish with a natural summer photoperiod (14 L: 10 D). Each digestibility tank consisted of an upper tank with a sloping base (35º) that was connected to a removable lower settlement chamber separated by a 6mm mesh screen and a 50mm ball valve. The lower chamber terminated in a 250 mm length of silicone tubing closed off with a plastic clamp which collected uneaten feed or faecal material. Each digestibility tank was supplied continuously with pre-heated, particle-filtered water at a flow rate of approximately 2 L min^{-1}. The effluent water from each tank then flowed to a ground level sump where 4 L effluent water min^{-1} was discharged. The remainder was pumped through a sand and cartridge filter (10-15 μm) to a raised biological trickle filter before being re-circulated to the laboratory via gravity flow. Pre-filtered and disinfected replacement water was stored in a 47 kL reservoir tank and pumped into the biological filter on demand. Each digestibility

tank was fitted with a prismatic polycarbonate lid, a mechanically operated belt-feeder (AGK, Wallersdorf, Germany) and two fine bubble air-stone diffusers.

Water quality was monitored daily using one of two hand held water quality analysers; either a Model 611 (Yeo-Kal Electronics, Brookvale, NSW, Australia) or a Horiba U-10 (Horiba, Japan). Total ammonia [$NH_3 + NH_4^+$] was monitored daily from selected experiment tanks and from the laboratory influent using a rapid test kit procedure (Model 1.08024.0001, E. Merck, Darmstadt, Germany). During the experiment, temperature, dissolved oxygen (DO_2), salinity and pH ranged from 23.1-28.3ºC, 4.5-7.0 mg L^{-1}, 32.8-34.8 ‰ and 7.3-7.9 units respectively with total ammonia always < 1.0 mg L^{-1}.

2.3 Fish

Snapper were progeny of brood-stock held at NSW DPI (Fisheries) Port Stephens Fisheries Centre (PSFC). Prior to use, juvenile snapper were grown at low densities in large 10 kL tanks and fed twice daily on a commercial barramundi *Lates calcarifer* feed (Ridley Aqua-Feeds, Narangba, Qld, Australia; reported nutrient composition: 50% crude protein; 12% crude fat; 18 MJ kg^{-1} gross energy). A prophylactic formalin bath (200 mg formaldehyde L^{-1} saltwater) was given to the fish before they were transferred to the digestibility laboratory.

Snapper were anaesthetised prior to handling (20-30 mg L^{-1} ethyl-ρ-aminobenzoate), then weighed in small groups and placed into experiment tanks. Each digestibility tank was stocked with 8 fish (average individual weight ± SD = 181 ± 5 g). Snapper were re-weighed at the conclusion of the experiment. One fish died during the acclimation period and was replaced with a fish of similar size. All remaining fish survived.

2.4 Feeding and collection of faeces

Each dietary treatment was randomly assigned to three digestibility tanks (n=3). Snapper were then acclimatised to their test diets and experimental conditions for 8 days before collection of faeces. Diets were offered to slight excess once daily between 0830 and 1130 h using belt-feeders. The unrestricted delivery of test diets was maintained by ensuring that a quantity of uneaten pellets was always present in the collection chambers at the end of each feeding period. Nonetheless, feeds were weighed accurately each day and were offered initially at 1% body weight d^{-1} according to the stocked biomass of each tank, increasing to approximately 3% body weight d^{-1} by the conclusion of the experiment. After feeding had ceased, all belt feeders were checked, air stones were removed and the walls and floors of the upper tanks were carefully cleaned to dislodge uneaten feed or faeces. Upper tanks were then left undisturbed for about 45 minutes to allow all suspended material to settle out of the water column. Subsequently, the lower collection chambers were isolated from the upper tanks by closing the ball valves, removed and thoroughly brushed and washed clean. A significant volume of water was then flushed from upper tanks (≈ ⅓ tank volume) to dislodge any settled material before the faecal collection chambers were refitted and packed in ice. Air stones were replaced.

Faeces were allowed to settle overnight (≈ 18 h) and were removed the following morning prior to feeding. Wet faecal samples were dried for 24 h at room temperature under absorbent silica gel in vacuum-sealed desiccators (70 mm Hg). Daily faecal collections from respective tanks were pooled, re-dried and finely ground (Waring blender, model 32-BL-80, New Hartford, Connecticut, USA) before they were submitted for chemical analyses. The experiment was run for 29 days.

2.5 Chemical analyses

All chemical analyses on ingredient, diet and faecal samples was performed by the NATA certified Food & Agricultural Laboratories of Australia Pty. Ltd. (FALA; Archerfield, Qld, Australia). Crude protein (N X 6.25) was determined by the Leco method, moisture by oven drying at 105º C for 16 h, ash by muffle furnace at 550ºC for 2 h and chromium by ICP-MS analysis. Total fat of samples was

determined by a modified Bligh-Dyer method and gross energy was determined by standard adiabatic bomb calorimetry.

2.6 Apparent digestibility calculations

Apparent digestibility coefficients (ADC) for reference and test diets were calculated according to equation:

$$ADC\ (\%) = 100\ X\ [1 - (\ F / D\ x\ D_{Cr} / F_{Cr})] \qquad \text{Equation 1.}$$

where F = % nutrient or gross energy in faeces; D = % nutrient or gross energy in diet; D_{Cr} = % chromic oxide in diet; F_{Cr} = % chromic oxide in faeces (Cho et al. 1982).
To account for differences between the nutrient or gross energy content of the reference diet and individual test ingredients as well as variations in inclusion contents, the ADCs of ingredients were calculated according to the following equation:

$$ADC_{ING}\ (\%) = [(Nutr_{TD}\ X\ AD_{TD}) - (P_{RD}\ X\ Nutr_{RD}\ X\ AD_{RD})] / [(P_{ING}\ X\ Nutr_{ING})] \qquad \text{Equation 2.}$$

where ADC_{ING} = apparent digestibility of nutrient or gross energy in the test ingredient; $Nutr_{TD}$ = the nutrient or gross energy concentration in test diet; AD_{TD} = the apparent digestibility of the nutrient or gross energy in the test diet; P_{RD} = proportional amount of reference diet; $Nutr_{RD}$ = the nutrient or gross energy concentration in the reference diet; AD_{RD} is the apparent digestibility of nutrient or gross energy in the reference diet; P_{ING} = proportional amount of test ingredient; $Nutr_{ING}$ is the nutrient or gross energy concentration in the test ingredient (Sugiura, Dong, Rathbone & Hardy, 1998).

2.7 Statistical analyses

Two-way analysis of variance (*ANOVA*) was used to study the interaction between ingredient type and ingredient inclusion level on the organic matter, protein, fat or gross energy digestibility of ingredients. Both factors were considered fixed. Prior to conducting multi-factor *ANOVA*, data was tested to determine if treatment variances were homogeneous (Cochran's C test). The significance level for all *ANOVA* and multiple comparisons tests (Student Newman Keul's - *SNK*) was set at 0.05 and data were statistically analysed using Statgraphics Plus for Windows 4.1 (Manugistics Inc. Rockville, Maryland, U.S.A.).

3. RESULTS

Snapper remained healthy and grew rapidly during this experiment despite the variable nutritional composition of the test diets and the daily disturbance from tank cleaning. Average daily growth rate reached 2.03 ± 0.33 g fish d^{-1} (mean ± SD), indicating fish were relatively unstressed and had quickly become accustomed to the experimental protocols (Table 3).

Mean ADC's for each test ingredient at each of the respective inclusion levels is presented in Table 4. All ingredients were well digested, and ADC's for all proximate categories were above 82%. The inclusion levels tested in this study had little effect on the proximate digestibility of the Ovine-60, Ovine-70 and Bovine-70 meals. However, we note there was a minor reduction in the gross energy digestibility of the Ovine-60 meal when included at 600 g kg^{-1} compared to the 300 g kg^{-1} diet. The organic matter digestibility of this product also decreased slightly at the highest inclusion level, but neither protein nor fat digestibility was affected (Table 4).

Increasing the protein content of ovine meal from 60 to 70% (as received basis; Table 1) by reducing ash content elevated the organic matter, protein, fat and gross energy digestibility coefficients of the Ovine-70 meal, regardless of inclusion level.

Neither the effect of inclusion level nor the interaction between ingredient type and inclusion level significantly affected organic matter, protein, fat or gross energy ADC's of ingredients. However two-way *ANOVA* revealed a significant main effect of ingredient type on each of the proximate categories. Results of this analysis are presented in Table 5.

4. DISCUSSION

In this study, the carnivorous snapper readily accepted diets with up to 60% of the high protein meat meals and while assessment of fish performance was not a major objective of this study, the growth of around 2 gd^{-1} indicated the ingredients were well utilised. This is consistent with other studies where diets including meat meal at contents ranging from 30% to 70%, as a substitute for fish meal, have been readily accepted by both omnivorous and carnivorous species such as tilapia, channel catfish, rainbow trout, and yellowtail kingfish (Davies, Williamson, Robinson & Bateson, 1989; Davies, Nengas & Alexis, 1993; Watanabe & Pongmaneerat, 1991; Watanabe, Pongmaneerat & Sato, 1993; Shimeno *et al.* 1993a & b). Mohsen & Lovell (1990) found that meat and bone meal at an inclusion level of 11% increased the palatability of soybean meal / corn based diets for channel catfish.

The high protein meat meals were very well digested by snapper with higher ADCs for organic matter, energy and nitrogen for the low ash products than have been recorded previously. Booth *et al.* (2005) recorded ingredient ADC's for protein of 62.2 and 65.3% for high ash meat and bone meal (29% ash; 57.1% crude protein) included at 30 and 50% in test diets, respectively. Higher coefficients of 84.9 and 86.9% were reported for lower ash poultry meal (7.5% ash, 68.8% crude protein) included at 30 and 50% in test diets, respectively. Other authors have also found protein digestibility to be negatively correlated with high ash content in meat meals for rainbow trout (Watanabe & Pongmaneerat 1991) and gilthead sea bream (Nengas *et al.* 1995). Nengas *et al.* (1995) recorded diet ADC's for protein of 78% and 92% for a diet with low ash meat meal (4% ash, 80% crude protein) and high ash (27% ash, 52% crude protein) respectively. For the freshwater silver perch, Stone *et al.* (2000), also reported higher ADC's for protein for low ash meat meal products (85.5% for a 3% ash, 81% crude protein) than for higher ash meat meal products (e.g. 69.7% for a 36% ash, 49.2% crude protein meat meal).

Results for energy ADC's for different levels of the same ingredient were fairly similar. Booth *et al.* (2005) recorded ingredient ADC's for energy of 72.0 and 70.5% for the high ash meat and bone meal at 30 and 50% inclusion content, respectively and 91.1 and 91.4% for the lower ash poultry meal at 30 and 50% inclusion, respectively. Nengas *et al.*'s (1995) energy ADC's were 86% and 75% for their low and high ash meat meal diets, respectively. Stone *et al.*'s (2000) energy ADC's were 89.9% and 71.4% for his low and high ash products respectively.

Lower protein ADC's for some meat and bone meal products for fish have been attributed to processing damage (Booth *et al.* 2005). Nengas *et al.* (1995) reported protein ADC's for several different meat meal products that had been subjected to different processing conditions of between 35-72.2% and gross energy ADC's of between 14-69.2%, indicating that the composition and processing employed in the manufacture of these products can have significant impacts on digestibility. For pigs, lower and more variable lysine availability coefficients were reported for low ash meat meal (50–60% protein) compared with high ash meat meal (43–44% protein) and this was attributed to a higher chance of processing damage to lysine for meals rendered without bone (Ted Batterham, pers. comm. 1993). Batterham felt that presence of bones in the digestion chamber could assist with the even spread of heat and reduce surface associated temperature fluctuations that might have reduced lysine availability for pigs. However, results from Nengas *et al.* (1995), Stone *et al.* (2000), Booth *et al.* (2005) and the current study indicate that this has not been a problem with fish, possibly reflecting the type of digestion process used for rendering the low ash meat meal used in the various studies.

Booth *et al.* (2005) reported ADC's for protein and energy of 94.3% and 99.2% respectively for fishmeal (14% ash, 72.3% crude protein) when tested at 50% inclusion. The best ADC's for protein and energy for the lowest ash meat meal (3% ash, 86.7% crude protein [dry matter basis]) tested in the

current study at 60% inclusion were 95.1% and 90.9%, indicating that low ash meat meals are excellent sources of both protein and energy for snapper.

In conclusion, feed formulators can have confidence in using low ash meat meal products for snapper diets. In addition, if high protein rendered products are processed to ensure minimal damage, feed formulators can expect digestibility coefficients to be similar to good quality fishmeal.

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REFERENCES

Allan, G.L., Rowland, S.J., Parkinson, S., Stone, D.A.J. & Jantrarotai, W. (1999) Nutrient digestibility for juvenile silver perch *Bidyanus bidyanus*: development of methods. Aquaculture **170**:131-145.

Allan, G.L., Parkinson, S., Booth, M.A., Stone, D.A.J., Rowland, S.J., Frances, J. & Warner-Smith, R. (2000) Replacement of fishmeal in diets for Australian silver perch, *Bidyanus bidyanus*: I. Digestibility of alternative ingredients. Aquaculture **186**:293-310.

Allan, G.L. & Rowland, S.J. (2005) Performance and sensory evaluation of silver perch (*Bidyanus bidyanus* Mitchell) and water quality in earthen ponds. Aquaculture Research **36**:1322-1332.

Booth, M.A., Allan, G.L. & Anderson, A.J. (2005) Investigation of the nutritional requirements of Australian snapper *Pagrus auratus* (Bloch & Schneider, 1801): apparent digestibility of protein and energy sources. Aquaculture Research **36**:378-390.

Bureau, D.P. (2006) Rendered products in fish aquaculture feeds. In: D.L. Meeker (ed.) Essential Rendering. National Renderers Association, pp. 179-194.

Davies, S.J., Williamson, J., Robinson, M. & Bateson, R.I. (1989) Practical inclusion levels of common animal by-products in complete diets for tilapia (*Oreochromis mmossambicus*, Peters). In: M. Takeda & T. Watanabe (eds.) The Current Status of Fish Nutrition in Aquaculture. Tokyo Univ. Fisheries, Tokyo, Japan, pp. 325-332.

Davies, S.J., Nengas, I. & Alexis, M. (1993) Partial substitution of fish meal with different meat meal products in diets for sea bream (*Sparus aurata*). In: S.J. Kaushi & P. Luquet (eds.) Fish Nutrition in Practice. Coll. Les Colloques No. 61 INRA, Paris, pp. 908-911.

FAO (2007) The State of World Fisheries and Aquaculture 2006. FAO Fisheries and Aquaculture Department, Rome, Italy. 180 pp.

Glencross, B.J. (2006) The nutritional management of barramundi (*Lates calcarifer*) – a review. Aquaculture Nutrition **12**(4):291-309.

Lovell, R.T. (1992) Replacing fish meal in channel catfish diets In: G.L. Allan and W. Dall (eds.) Proceedings of the Aquaculture Nutrition Workshop, NSW Fisheries, Salamander Bay, Australia, pp. 15-17.

Mohsen, A.A. & Lovell, R.T. (1990) Partial substitution of soybean meal with animal protein sources in diets for channel catfish. Aquaculture **90**:303-311.

Nengas, I., Alexis, M.N., Davies, S.J. & Petichakis, G. (1995) Investigation to determine digestibility coefficients of various raw materials in diets for gilthead seabream, *Sparus auratas* L. Aquaculture Research **26**:185-194

Quinn & Keough (2002) Experimental Design and Data Analysis for Biologists. Cambridge University Press, Port Melbourne, Victoria 3207 Australia. 537pp.

Shimeno, S., Masumoto, T. & Hujita, T. (1993a) Alternative protein sources for fish meal in diets of young yellowtail. Nippon Suisan Gakkaishi **59**:137-143.

Shimeno, S., Mima, T. & Imanaga, T. (1993b) Inclusion of combination of defatted soybean meal, meat meal, and corn gluten meal to yellowtail diets. Nippon Suisan Gakkaishi **59**:1889-1895.

Shimeno, S. Hosokawa, H. & Masumoto, T. (1996) Addition of combined defatted soybean meal, malt protein flour, and meat meal to yellowtail diet. Nippon Suisan Gakkaishi **62**:243-247.

Stone, D.A.J., Allan, G.L., Parkinson, S. & Rowland, S.J. (2000) Replacement of fishmeal in diets for Australian silver perch, *Bidyanus bidyanus*. III. Digestibility and growth using meat meal products. Aquaculture **186**:311-326.

Sugiura, S.H., Dong, F.M., Rathbone, C.K. & Hardy, R.W. (1998) Apparent protein digestibility and mineral availabilities in various feed ingredients for salmonid feeds. Aquaculture **159**:177-202.

Swisher, K.J. (2006) The global market for rendered products. In: D.L. Meeker (ed.) Essential Rendering. National Renderers Association, pp. 213-228.

Tacon, G.J. (2003) Global trends in aquaculture and compound aquafeed production – A review. International Aquafeed Director 2003, pp. 9-23.

Tacon, A.G.J. (1994) Feed ingredients for carnivorous fish species: alternatives to fish meal and other fishery resources. FAO Fisheries Circular No. 881, Rome, Italy.

Watanabe, T. & Pongmaneerat, J. (1991) Quality evaluation of some animal protein sources for rainbow trout *Oncorhynchus mykiss*. Nippon Suisan Gakkaishi **57**:495-501.

Watanabe, T.J., Pongmaneerat, J. & Sato, S. (1993) Replacement of fish meal by alternative protein sources in rainbow trout diets. Nippon Suisan Gakkaishi **57**:495-501.

Williams, K.C., Allan, G.L., Smith, D.M. & Barlow, C.G. (1998) Fishmeal replacement in aquaculture diets using rendered protein meals. In: Proceedings of the Fourth International Symposium on Animal Nutrition, Protein, Fats and the environment, held at the Novotel Bayside Hotel, St Kilda, Melbourne, Australia, 24-26 September 1997. Australian Renderers Association Inc., 13-26.

TABLE 1
Measured composition of individual feed ingredients (g kg^{-1} or MJ kg^{-1}dry matter).

Ingredient	Crude Protein	Ash	Organic matter[1]	Fat	Gross energy (MJ)
Fish meal[2]	734.1	150.1	849.9	132.8	22.6
Extruded wheat[3]	173.3	27.3	972.7	59.7	19.2
Ovine meal 60[4]	743.4	164.6	835.4	115.3	22.1
Ovine meal 70[4]	830.7	98.1	901.9	120.6	24.4
Bovine meal 70[4]	867.6	30.3	969.7	169.1	27.2
Proprietary blend[5]	619.9	227.0	773.0	159.6	20.9

[1] Organic matter calculated by difference = (1000 – measured ash content)
[2] Prime quality fish meal sourced from Ridley Aquafeed, Narangba, Qld, Australia
[3] Extruded wheat sourced from Ridley Agriproducts Pty. Ltd., Murray Bridge, S.A., Australia
[4] Manufactured by Bakels Edible Oils (N.Z.) Ltd., Mt Maunganui, New Zealand; obtained from Camilleri Stockfeeds Pty. Ltd., Maroota, NSW, Australia
[5] Proprietary blend sourced from Camilleri Stockfeeds Pty. Ltd., Maroota, NSW, Australia

TABLE 2
Calculated ingredient and measured nutrient composition of diets (g kg^{-1} or MJ kg^{-1} of dry matter).

	Diet[1]						
	Reference	Ovine meal (60-30)	Ovine meal (60-60)	Ovine meal (70-30)	Ovine meal (70-60)	Bovine meal (70-30)	Bovine meal (70-60)
Ingredient (calculated)							
Fish meal	490.0	340.0	190.0	340.0	190.0	340.0	190.0
Ovine meal-60	-	300.0	600.0	-	-	-	-
Ovine meal-70	-	-	-	300.0	600.0	-	-
Bovine meal-70	-	-	-	-	-	300.0	600.0
Proprietary blend-65	-	-	-	-	-	-	-
Extruded wheat	490.0	340.0	190.0	340.0	190.0	340.0	190.0
Vitamin premix[1]	7.5	7.5	7.5	7.5	7.5	7.5	7.5
Mineral premix[2]	7.5	7.5	7.5	7.5	7.5	7.5	7.5
Chromic oxide	5.0	5.0	5.0	5.0	5.0	5.0	5.0
Nutrient (measured)							
Organic matter[3]	897.7	871.7	855.3	896.9	899.1	914.3	931.6
Crude protein	445.8	538.4	625.7	559.3	676.4	570.5	689.6
Fat	91.8	112.0	106.0	107.8	108.6	114.3	142.0
Ash	102.3	128.3	144.7	103.1	100.9	85.7	68.4
NFE[4]	360.1	221.2	123.6	229.8	114.2	229.6	100.0
Gross energy (MJ kg^{-1})	20.3	20.9	21.1	21.4	22.2	21.9	23.6

[1] (IU kg^{-1} diet): retinol A, 8000; cholecalciferol D3, 1000; DL-α-tocopherol acetate E, 125. (mg kg^{-1}): menadione sodium bisulphite K3, 16.5; thiamine hydrochloride B1, 10.0; riboflavin B2, 25.2; pyridoxine hydrochloride B6, 15.0; folic acid, 4.0; ascorbic acid C, 1000; calcium-D-pantothenate, 55.0; myo-inositol, 600; D-biotin H (2%), 1.0; choline chloride, 1500; nicotinamide, 200; cyanocobalamin B12, 0.02; ethoxyquin (anti-oxidant) 150; calcium propionate (mould inhibitor) 25.0
[2] (mg kg^{-1} diet): calcium carbonate, 7500; manganese sulphate monohydrate, 300; zinc sulphate monohydrate, 700; copper sulphate pentahydrate, 60, ferrous sulphate heptahydrate, 500, sodium chloride, 7500; potassium iodate, 2.0
[3] Organic matter calculated by difference = (1000-measured ash content)
[4] Nitrogen free extract (NFE) calculated by difference = 1000 – (crude protein + ash + fat)

TABLE 3
Apparent digestibility coefficients (ADCs) for reference and test diets.

	Apparent digestibility coefficient (ADC)						
	Reference	Ovine meal (60-30)	Ovine meal (60-60)	Ovine meal (70-30)	Ovine meal (70-60)	Bovine meal (70-30)	Bovine meal (70-60)
Dietary ADC %							
Organic matter	60.7±2.9	69.0±1.0	74.6±1.5	73.1±1.7	84.2±0.4	70.1±1.0	82.7±0.4
Crude protein	89.9±0.7	86.7±0.8	85.1±0.7	90.3±0.7	91.9±0.1	92.3±0.2	95.2±0.1
Fat	85.1±2.1	85.0±0.6	85.2±1.4	86.9±1.0	91.2±0.7	90.6±0.5	89.8±0.5
Gross energy	67.3±2.5	74.2±0.9	77.8±1.3	77.6±1.4	86.6±0.4	75.8±0.4	86.0±0.3
Weight gain g $fish^{-1}$	58.9±5.7	60.3±1.8	59.3±3.8	65.2±2.5	68.8±5.7	51.0±6.3	60.6±8.1

Table values represent mean (± SEM) of 3 replicate tanks.
Average individual weight gain (g $fish^{-1}$) = (average weight fish at harvest - average weight fish at stocking); experiment run for 29 days and initial weight of fish = 181 ± 5 g.

TABLE 4
Apparent digestibility coefficients (ADCs) of test ingredients.

		Apparent digestibility coefficient (ADC)		
	Inclusion (g kg^{-1})	Ovine meal - 60	Ovine meal – 70	Bovine meal - 70
Nutrient or gross energy				
Organic matter	300	88.1±4.0	100.1±5.5	89.1±3.1
	600	83.9±2.5	99.3±0.8	95.0±0.7
Crude protein	300	83.3±1.8	89.8±1.6	94.3±0.4
	600	83.5±0.9	92.6±0.1	95.1±0.2
Fat	300	84.5±1.6	95.9±2.8	96.2±1.2
	600	85.1±2.2	93.7±1.1	94.8±0.8
Gross energy	300	89.5±2.9	96.1±4.2	86.9±1.2
	600	82.4±2.1	94.1±0.6	90.9±0.5

Table values represent mean ± SEM of 3 replicate tanks.

4.7 Fortification of diets with potassium chloride (KCl) does not improve the survival or performance of Australian snapper *Pagrus auratus* (Bloch & Schneider, 1801) reared in potassium deficient saline ground water.

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ABSTRACT

Potassium deficiency is a common problem in saline groundwater and while fortification of culture water is effective it is expensive. This study was conducted to investigate whether a potassium deficiency could be overcome through dietary supplementation for Australian snapper *Pagrus auratus* (Bloch & Schneider, 1801). Experiment 1 investigated whether pre-feeding juvenile snapper on diets fortified with 0, 25 or 50g KCl kg^{-1} affected performance and survival of fish transferred directly from estuarine water to raw groundwater (20‰). All fish survived and fed vigorously during the 6-day pre-feeding phase but following transfer to saline groundwater treatments, fish exhibited symptoms of potassium deficiency – they were distressed, had tetany, were hemorrhaging around the mouth, failed to feed, became moribund and started to die. Diet had no impact on symptoms. No mortality or abnormal behaviour was recorded in fish reared in estuarine water, although a reduction in voluntary intake of diets fortified with 25 or 50g KCl kg^{-1} was noted.

Experiment 2 investigated whether a diet fortified with either 0, 10 or 25g KCl kg^{-1} affected performance of snapper reared for 13 days in estuarine water or improved the performance and survival of snapper reared in estuarine water or saline groundwater fortified to 40% or 100% the $[K^+]$ of equivalent salinity estuarine water. Diet did not affect performance in either estuarine or groundwater and there was no interaction between diet and water type. Weight gain and FCR were affected by water-type with better results for fish reared in estuarine water or groundwater fortified to 100% the $[K^+]$ of equivalent salinity estuarine water. Feed intake was not affected by water type. Results clearly demonstrate that snapper were unable to utilize the additional dietary source of K^+ (as KCl) and were reliant on sequestering K^+ ions from the water column in order to maintain functions involving intra-cellular homeostasis.

1. INTRODUCTION

In Australia, as is the case in many countries, rising water tables have carried large amounts of salt to the surface, reducing the fertility and productivity of agricultural land (Smith, 1999). In some areas, notably the Murray-Darling Basin in Australia, this problem is ameliorated by large interception schemes, which pump saline ground water from subterranean aquifers into evaporation basins. These schemes offer huge potential for production of euryhaline or marine aquaculture species (Allan & Fielder, 1999; Fielder, Bardsley & Allan, 2001; Sowers, Gatlin, Young, Islely, Browdy & Tomasso, 2005) but this potential may be limited by the ionic composition of these waters. Fielder, *et al.* (2001), demonstrated that juvenile snapper transferred from coastal seawater to saline groundwater from Australia's largest interception scheme (Wakool-Tullakool Subsurface Drainage Scheme (WTSDS), NSW), failed to feed, lost equilibrium and became moribund within 3 days due to an inadequate concentration of potassium in the raw groundwater. The concentration of potassium in saline groundwater and coastal seawater diluted to 20‰ in that study was 9.2 mg L^{-1} and 203 mg L^{-1} respectively. Fish survival and normal behaviour was restored when the saline groundwater was fortified with potassium chloride (KCl), to provide 60% or more of the concentration of potassium found in coastal seawater. Snapper survived in saline groundwater fortified to 40% of the concentration of coastal seawater, but feed intake and growth were reduced (Fielder, *et al.* 2001). This result clearly demonstrated that snapper were sequestering potassium from the water column and, for snapper and other estuarine species, grow-out ponds drawing water from the WTSDS have been

"dosed" with KCl (as potash) to provide potassium concentrations equivalent to that of coastal seawater (Fielder, *et al*. 2001; 2007; Doroudi, Fielder, Allan & Webster, 2006). Apart from the additional cost associated with the purchase of potash, the management and dispersal of this chemical on-farm increases labour costs. Provision of potassium via the diet may overcome these issues and improve the efficiency of snapper production.

Little data exists with respect to the dietary potassium requirement of fish (Lall, 1989). Early studies focused on the requirements of freshwater fish such as Chinook salmon (Shearer, 1988) and channel catfish (Wilson & El Naggar, 1992), because the potassium requirements of marine species were generally thought to be more than satisfied by the potassium concentration of seawater. In addition, dietary potassium supplementation for fish has received little attention because the residual potassium concentration of most complete feeds is thought to be above the level required by most animals (c.a. 0.26% for pig, rat and chick; Wilson & El Naggar,1992). In the only known study on marine finfish, Sakamoto & Yone (1978). reared juvenile red sea bream (*Pagrus major = P. auratus*; Paulin, 1990) in seawater and fed groups of fish diets with (2.2 g kg^{-1}) and without (0.4 g kg^{-1}) potassium for 63 days, but found no significant changes in growth rate, feed efficiency, condition factor, hepatosomatic index, histopathological or hematological characteristics. They concluded dietary potassium was not essential.

Sodium, potassium and chloride are the most abundant electrolytes in the body of living organisms. Sodium and chloride are the major cation and anion, respectively, of extra-cellular fluids of the body, whereas potassium and magnesium are the major intra-cellular cations. They serve a vital role in controlling osmotic pressures and acid base equilibrium (Wilson & El Naggar, 1992; Lall, 2002). Potassium deficiency causes overall muscle weakness, resulting in intestinal distension, weakness of cardiac and respiratory muscles and ultimately the failure of these organs. Signs of potassium deficiency in Chinook salmon included anorexia, convulsions, tetany and death (Shearer, 1988).

Clearly, the symptoms of potassium deficiency for marine fish will be most evident in water bodies deficient in this cation, such as the saline groundwater studied by Fielder, *et al.* 2001. While this water source is more abundant in calcium (Ca^{2+}) and deficient in potassium, compared to coastal seawater, the remaining ionic constituents are almost equivalent (Fielder, *et al.* 2001). As such, the unique water chemistry of the WTSDS presents an opportunity to study the efficacy of fortifying diets for Australian snapper with potassium.

The aim of this trial was to determine if snapper fingerlings were capable of surviving and growing in potassium deficient groundwater obtained from the WTSDS when fed diets fortified with different concentrations of KCl.

2. MATERIALS AND METHODS

2.1 Background

Two experiments were done to evaluate the efficacy of fortifying a commercial barramundi feed with analytical reagent grade potassium chloride (99.5%; Chem-Supply Pty. Ltd., Gillman, SA, Australia). The first experiment investigated whether pre-feeding juvenile snapper on diets fortified with 0 (residual), 25 or 50 g KCl kg^{-1} improved performance and survival of fish transferred directly from estuarine water to raw groundwater of equivalent salinity. The second experiment investigated whether a commercial diet fortified with either 0 (residual), 10 or 25 g KCl kg^{-1} affected performance of snapper reared in estuarine water or improved the performance and survival of snapper reared in saline groundwater fortified to 40 or 100% the potassium concentration of equivalent salinity estuarine water. In both experiments, the performance and survival of snapper was compared to snapper fed on the aforementioned diets but reared in saltwater drawn from the estuary adjacent to the NSW DPI Port Stephens Fisheries Centre (PSFC).

2.2 Experimental diets

The same batch of a commercial barramundi *Lates calcarifer* feed (Ridley Agriproducts Pty. Ltd., Narangba, Qld, Australia; reported nutrient composition: 50% crude protein; 12% crude fat; 18.0 MJ kg^{-1} gross energy) was used in both experiments. This diet was finely ground through a laboratory scale hammer mill fitted with a 1.5 mm screen (Raymond Laboratory Mill, Transfield Technologies, Rydalmere, NSW, Australia) before incorporation of KCl. Four diets were prepared by adding 0, 10, 25 or 50 g KCl kg^{-1} of mash and dry mixing for a minimum of 15 minutes before addition of a suitable quantity of distilled water (Hobart Mixer: Troy Pty Ltd, Ohio, USA). Sinking pellets were formed by passing the damp mash through a meat mincer fitted with a 2.0 mm die plate (Barnco Australia Pty Ltd, Leichhardt, NSW, Australia). Moist pellets were then dried (≈ 35º C) until moisture contents were <100 g kg^{-1}. Following preparation, all diets were stored frozen at < -15ºC until required. Proximate and mineral composition of experimental diets as well as several common feed ingredients are presented in Table 1. Chemical analysis of diets and ingredients were performed exclusively by NATA accredited Food & Agricultural Laboratories of Australia Pty. Ltd. (FALA; Coopers Plains, Qld, Australia, http://www.fala.com.au/).

2.3 Water sources

The saline groundwater used in this study was trucked from NSW DPI Inland Saline Aquaculture Centre at Wakool and stored on-site at PSFC in a covered fibreglass holding tank until required (75-80‰ at time of transfer). Indicative water chemistry of diluted saline groundwater (20‰) from the WTSDS is tabulated in Fielder *et al.* (2001). To allow comparisons with previous research (i.e. Fielder *et al.* 2001), experimental water treatments in this study were adjusted to a salinity of 20‰ using rainwater obtained from a single source at PSFC. Prior to adjusting salinity, the potassium concentration of stored volumes of raw saline groundwater, local estuarine water and rainwater were determined in order to accurately fortify experimental volumes (200 L) of diluted saline groundwater with KCl. Before experiments commenced, samples of undiluted and diluted water were collected and analysed for potassium concentration and salinity (Table 2). Analysis of water samples was performed by Hunter Water Laboratories (Warabrook, NSW, Australia; http://www.hwa.com.au/).

Water quality parameters were regularly recorded from each tank using one of two hand held water quality analysers; either a Model 611 (Yeo-Kal Electronics, Brookvale, NSW, Australia) or a Horiba U-10 (Horiba, Japan). Total ammonia [NH3 + NH4+] was monitored from tanks using a rapid test kit procedure (Model 1.08024.0001, E. Merck, Darmstadt, Germany). During experiment 1, temperature, dissolved oxygen (DO_2), salinity and pH ranged from 20.3-26.0ºC, 4.9-8.2 mg L^{-1}, 20.1-28.9% and 7.0-8.3 units respectively with [$NH_3 + NH_4^+$] always ≤ 1.0 mg L^{-1}.

2.4 Fish handling procedures

The snapper used in this study were progeny of first generation brood-stock held at the NSW DPI Fisheries Port Stephens Fisheries Centre (PSFC). These snapper were grown at low densities in large 10 kL tanks and fed twice daily on the aforementioned barramundi feed. Before either experiment, juvenile snapper were lightly sedated (10 mg ethyl-ρ-benzoate L^{-1}) and transferred from 10 kL holding tanks to the laboratory in 200 L plastic tubs aerated with oxygen. Groups of fish were then anaethetised (20 mg ethyl-ρ-benzoate L^{-1}) before individual fish were selected according to a predetermined weight range (experiment 1, 20-25 g $fish^{-1}$; experiment 2, 35-40 g $fish^{-1}$). These fish were lightly dried with a damp absorbent cloth, weighed and systematically placed into experimental tanks in small groups until each tank contained 8 (experiment 1) and 6 fish (experiment 2) respectively. Fish were individually weighed during and at the completion of each experiment.

2.5 Experimental facility

Experimental units were housed in a temperature / photoperiod (12 h light:12 h dark) controlled laboratory at PSFC. Each unit consisted of a 60 L clear acrylic aquaria connected to a 9 L plastic sump by 20 mm clear plastic tubing. Each sump contained approximately 2 L of bio-media ("B-Cell"; Water Management Technologies Inc., Baton Rouge, USA). A small fountain pump (Watermaster, White International, Alexandria, NSW, Australia) was housed within the plastic sump to re-circulate the water in each system. Water was pumped from the sump (≈ 1.6 L min^{-1}) into the aquaria before returning to the sump via gravity-assisted flow through a simple particle trap constructed from a perforated plastic container (200 mL) filled with filter wool. Each aquarium and associated sump was aerated using fine-bubble air-stone diffusers and the filter wool from each particle trap was removed each day, washed in freshwater (< 0.3 ‰) and replaced.

In order to maintain the water quality of individual systems and control the build up of organic matter, 3-5 L of water was siphoned daily from each tank and replaced with an equivalent volume of unsullied water associated with the respective experimental treatment.

2.6 Experiment 1. Effect of pre-feeding diets fortified with KCl on direct transfer to 20‰ saline groundwater.

Snapper were transferred to experimental diets (i.e. 0, 25 or 50 g kg^{-1} KCl fortification) 24 h after being placed into experiment tanks containing undiluted estuarine water. Initially, each dietary treatment was randomly allocated to 6 experimental units (e.g. 18 units in total) and fish were fed to apparent satiation twice daily at 0830 and 1500 h. Snapper were fed their respective experimental feeds for 6 days prior to being transferred to 20‰ raw inland saline groundwater or 20‰ estuarine saltwater (procedural control). During this period, the salinity of the undiluted estuarine rearing water in each system was gradually reduced to 20‰ by using pre-diluted estuarine water (20‰) to replace the water siphoned for cleaning purposes. Transfer procedures were similar for all units and were undertaken immediately after the morning feed (i.e. 1000 h). The procedure involved rapidly draining all but 5 L of the estuarine water from each tank (n.b. sumps were completely drained), before refilling respective tanks with raw saline groundwater or estuarine saltwater that had been pre-diluted to a salinity of 20‰ and adjusted to a similar temperature. After water transfer, flows were restored in each of the experimental units and feed was offered according to the aforementioned protocol. Feed intake, survival and performance of snapper were recorded over the next 48 h.

2.7 Experiment 2. Effects of KCl fortification on feed intake and performance of snapper reared in estuarine water or KCl fortified saline groundwater.

This experiment was conducted in 2 halves and involved 27 experimental units as described above. In the first half, snapper were reared for 13 days in undiluted estuarine saltwater and fed diets that had been fortified with 0, 10 or 25 g KCl kg^{-1} to determine if dietary concentration of KCl had impacted on the short term feed intake or performance of fish (n=9). In the second half of the experiment, 3 replicates from each diet group were randomly assigned to one of 3 water treatments; saline groundwater fortified to 40% the potassium concentration of estuarine water, saline groundwater fortified to 100% the potassium concentration of estuarine water, or an estuarine water control. The nominal salinity of all water treatments was 20‰. Ostensibly, this was done to determine if weight gain and performance of snapper reared in saline groundwater fortified with KCl to either a minimum (40%) or maximum (100%) standard (Fielder, *et al.* 2001) would improve or match the weight gain and performance of snapper reared in estuarine saltwater. Transfer to water treatments was incorporated with the weight check performed on day 13. At this time, snapper were systematically removed from experiment tanks and bulk weighed. During this procedure, experiment tanks and sumps were thoroughly washed, rinsed clean and filled with the appropriate water source before fish were returned to their respective tanks. The experiment was run for a further 21 days before it was terminated.

2.8 Statistical analyses

Data from experiment 1 was statistically analysed using one-way *ANOVA*. Data from the first phase of experiment 2 was analysed using one-way *ANOVA* while data from the second part of this experiment was analysed using two-way *ANOVA*. Prior to conducting ANOVA, data were tested to ensure that treatment variances were homogenous (Cochran's test). Where treatment variances were heterogeneous, data were log transformed. The significance level for all *ANOVA* and multiple comparisons tests (Student Newman Keuls; SNK) was set at 0.05 and data were statistically analysed using Statgraphics Plus, version 4.1 (Manugistics Inc., Rockville, MD, USA; 1998).

3. RESULTS

3.1 Experiment 1. Effect of pre-feeding diets fortified with KCl on direct transfer to 20‰ saline groundwater.

3.1.1 Behaviour and mortality

No snapper died and all fish ate vigorously during the 6-day pre-feeding phase of experiment 1. There were no obvious signs of distress in snapper subjected to the saline groundwater or estuarine water treatments immediately after transfer, and most fish consumed some pellets during the afternoon feed, despite being disturbed during the water exchange procedures. No mortality was recorded prior to departure from the laboratory on this day (1700 h) and the behaviour of all fish appeared normal.

By the following morning (0815 h), 6 snapper subjected to the saline groundwater treatment had died. These mortalities were not restricted to any one specific dietary treatment. The behaviour of snapper in saline groundwater was also different to the behaviour displayed by snapper reared in estuarine water. Snapper in saline groundwater were clearly distressed, exhibited a fixed mouth gape (tetany) and appeared red around the mouth. At this stage, they were still somewhat responsive to external stimuli (e.g. tapping lightly on tank wall), but were not as responsive to this stimulus as snapper in estuarine water. Fish in saline groundwater remained stressed throughout the morning, failed to feed and individuals continued to die. By 1300 h, a further 10 fish from the saline groundwater treatment had died. At this time, saline groundwater treatments were fortified with KCl to prevent further loss of fish. Recovery of surviving snapper in the saline groundwater treatment was reasonably rapid and only one more fish (which was moribund at 1200 h), died. By 1500 h, signs of tetany had declined and surviving fish feigned at, or ate a small amount of feed that was offered that afternoon. Normal vigour and feeding behaviour was evident in these fish the following morning. One-way *ANOVA* indicated there were no significant differences between survival of snapper pre-fed on diets containing different levels of KCl (Table 3). No mortality, loss of appetite or abnormal behaviour was recorded in fish reared in estuarine saltwater (i.e. controls).

3.1.2 Feed intake, weight gain & FCR

Voluntary feed intake was clearly affected in snapper transferred to saline groundwater due to the fact these fish became extremely distressed or moribund. For this reason, the affect of dietary treatment on voluntary feed intake, weight gain and FCR was assessed using only harvest data collected from the estuarine water treatments. One-way *ANOVA* indicated voluntary feed intake was significantly affected by diet type, with snapper consuming significantly more of the unfortified treatment than snapper fed diets fortified with KCl (25 mg kg^{-1} = 50 mg kg^{-1} < 0 mg kg^{-1}; n=3). Weight gain (g $fish^{-1}$) and feed conversion ratio (FCR) were also significantly affected by diet type, with weight gain tending to decline in response to increasing dietary levels of KCl while FCR tended to increase (Table 3).

3.1.3 Experiment 2. Effects of KCl fortification on feed intake and performance of snapper reared in estuarine water or KCl fortified saline groundwater.

All snapper survived in the first phase of experiment 2. In addition, there were no significant differences between the individual weights, feed intake or FCR of snapper fed diets fortified with 0, 10 or 25 g KCl kg^{-1} and reared in undiluted estuarine water (one-way *ANOVA*, Table 4).

Five snapper, each from different replicate tanks, died during the second phase of experiment 2. Three of these fish were fed on the unfortified commercial feed (0 g KCl kg^{-1}) and were each reared in one of the different water treatments. The remaining 2 individuals were fed on diets fortified with 10 g KCl kg^{-1}, but one was reared in saline groundwater fortified to 40% and the other in saline groundwater fortified to 100% the potassium concentration of estuarine water respectively.

There were no significant interactions detected between water-type or diet treatment (P>0.05) for harvest weight, individual weight gain, feed intake (as % BW d^{-1}), or feed conversion ratio (FCR) during the second part of experiment 2. In addition, none of these response variables was significantly affected by dietary treatment (P>0.05). In contrast, harvest weight (P<0.006), individual weight gain (P<0.0003) and FCR (P<0.0176) were significantly affected by water-type, but feed intake was not affected by this factor (P>0.2380). Factor means (n=9) are presented in Table 5.

4. DISCUSSION

Fish in K deficient water lost appetite, exhibited tetany, became unresponsive to feed and external stimuli and, in a relatively short period of less than 20 hours, were haemorrhaging around the mouth and starting to die. These deficiency signs in snapper are consistent with those reported for juvenile chinook salmon (Shearer, 1988) and mirror earlier results for snapper using water from a similar source (Fielder *et al*., 2001). Results of the present study also confirm previous findings (Fielder *et al*. 2001; Fielder, Allan & Pankhurst, 2007; Doroudi *et al.* 2006) that K^+ deficiency in seawater can be ameliorated by addition of KCl to the water. Fielder *et al.* (2001) speculated that a deficiency in the water might be overcome through dietary supplementation of K^+, thereby sparing the considerable expense of having to dose culture systems with KCl. This hypothesis was supported by a positive response to additions of dietary KCl for Chinook salmon (Shearer, 1988) and tilapia (Shiau and Hsieh, 2001). For both the former studies, water concentrations of potassium (<1 mg/l and approximately 1-3.6 mg/l respectively) were at the lower end for typical freshwater (usually around 3 mg/l and <10 mg/l; Shearer, 1988) and are in contrast to the results from the study by Wilson & Naggar (1992) who reported that that channel catfsh did not respond to dietary KCl. Wilson & Naggar (1992) used water with a potassium concentration of 4 mg/l. Studies with estuarine or marine species did not show a response to additions of dietary KCL (redlip mullet - El-Zibdeh, Yoshimatsu, Matsui & Furuichi, 1996; red snapper - Sakamoto & Yone, 1978) but those studies were done in seawater, presumably with typical K^+ concentrations of (380 mg/l K^+, Sverdrup *et al.*, 1942; Spotte, 1979), and the authors both opined that fish were able to sequester their requirements of K^+ from the water.

However, the hypothesis that dietary KCl can be used to overcome a deficiency of K in rearing water was not supported by results from the present study. The response of fish to transfer from coastal seawater of a salinity of 20‰ with approximately 200 mg/l K^+ to water of the same salinity but with only approximately 20 mg/l K^+ was similar regardless of whether fish were fed diets containing up to 50 g /kg KCl (= 26.2 g K/kg) for 6 days prior to transfer. Possible reasons for this include that the dietary supplementation of K^+ was inadequate and that fish were too stressed by the very low K^+ concentration in the saline groundwater to continue to feed and benefit from dietary K^+. It is unlikely that the maximum dietary concentration tested was inadequate. The maximum dietary requirement, estimated for chinnock salmon in K^+ deficient seawater, was 8.0 g K/kg (Shearer, 1988) and this is believed to be an overestimation with more typical requirements being 2-3g K/kg (Wilson & Naggar, 1992; Shiau & Hsein, 2001). Fish consumed the feed prior to transfer although feed consumption and FCR were reduced for fish fed the diet with 50 g KCL/kg. Fish were obviously stressed following the

transfer to saline groundwater treatments and response to feeding; irrespective of dietary K^+ concentration, was reduced, and fish started dying within 20 hours of exposure.

To address this, the second experiment was conducted with fish exposed to saline groundwater with K^+ concentrations fortified to 40% or 100% of that in equivalent salinity coastal seawater (coastal seawater was used as the control). Previous research had shown that snapper survived and grew at a K^+ concentration of 40% of that in seawater but that growth, feed consumption and FCR were all significantly reduced compared with results for fish in coastal seawater or saline groundwater with K^+ concentrations fortified to 100% using KCl (Fielder *et al.,* 2001). For this experiment, fish were acclimated for 13 days to experimental diets with 0, 10 or 25 mg KCL/kg and then exposed to one of the three water treatments. Diet had no effect nor was there any interaction between diet and water treatments. Attempting to ameliorate K^+ deficiency in rearing water through dietary supplementation has not been attempted but dietary supplementation of NaCl (20 g/kg) was effective in improving FCR and weight gain of the euryhaline red drum when fish were exposed to fresh or brackish water but not when fish were exposed to seawater (Gatlin, MacKenzie, Craig & Neil, 1992). Conversely Scarpa & Gatlin (1993) found that feeding channel catfish diets supplemented with calcium did not improve growth or reduce spinal deformities when fish were grown in calcium deficient waters. Sowers *et al.,* (2005) found that dietary salt did not offer an advantage to shrimp (*Litopenaeus vannamei*) when shrimp were cultured in low salinity water.

Gills are the most important extrarenal organ responsible for osmoregulation in fish (Shiau & Hsieh, 2001) and results from the present study indicate that snapper are unable to adequately compensate for suboptimal Na^+-K^+ exchange across the gills in a K^+ deficient medium through Na^+-K^+ exchange across the stomach and intestinal membranes.

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REFERENCES

Allan, G.L. & Fielder, D.S. (1999). Inland saline aquaculture activities in NSW. In: Inland Saline Aquaculture Workshop. Proceedings of a Workshop held on 6 & 7 August 1997 in Perth (b. Smith & Barlow, C. eds.). ACIAR Proceedings No. 83, Australian Centre for International Agricultural Research, Canberra, ACT, pp. 14-15.

Doroudi, M.S., Fielder, D.S., Allan, G.L., Webster, G.K. (2006). Combined effects of salinity and potassium concentration on juvenile mulloway (*Argyrosomus japonicus*, Temminick and Schlegel) in inland saline groundwater. Aquaculture Research **37:**1034-1039.

El-Zibdeh, M., Yoshimatsu, T., Matsui, S. & Furuichi, M. (1996). Effects of the deletion of K, Mg or Fe from purified diets on growth and efficiency of feed utilization of redlip mullet. Journal of the Faculty of Agriculture, Kyushu University **40**:383-390.

Fielder, D.S., Bardsley, W.J., Allan, G.L. (2001). Survival and growth of Australian snapper, *Pagrus auratus*, in saline groundwater from inland New South Wales, Australia. Aquaculture **201**:73-90.

Fielder, D.S., G.L. Allan and P.M. Pankhurst, (submitted 2007). The effects of potassium concentration in saline groundwater on osmoregulation and chloride cell morphology of juvenile Australian snapper, *Pagrus auratus*. Aquaculture Research.

Gatlin, D.M. III, MacKenzie, D.S., Craig, S.R. & Neil, W.H. (1992). Effects of dietary sodium chloride on red drum juveniles in waters of various salinities. Progressive Fish Culturist **54**: 220-227.

Lall, S.P. (1989). The minerals. In: Fish Nutrition 2nd Edition (J.E. Halver ed.) Academci Press, New York, pp. 219-257.

Paulin, C.D. (1990). *Pagrus auratus*, a new combination for the species known as "snapper" in Australasian waters (Pisces:Sparidae). New Zealand Journal of Marine and Freshwater Research **24**: 259-265.

Sakamoto, S. & Yone, Y. (1978). Requirement of red sea bream for dietary Na and K. Journal of the Faculty of Agriculture, Kyushu University **23**: 79-84.

Scarpa, J. & Gatlin, D.M. III (1993). Responses of channel catfish (*Ictalurus punctatusi)* swim-up fry to dietary calcium in solf and hard water. Comparative Biochemistry and Physiology **106A**: 803-808.

Shearer, K.D. (1988). Dietary potassium requirement of juvenile Chinook salmon. Aquaculture **73**: 119-129.

Shiau, S.Y. & Hsieh, J.R. (2001). Dietary potassium requirement of juvenile grass shrimp *Penaeus monodon*. Fisheries Science 67: 592-595.

Sowers, A.D., Gatlin, D.M., Young, S.P., Islely, J.J., Browdy, C.L. & Tomasso, J.R. (2005). Responses of *Litopenaeus vannamei* (Boone) in water containing low concentrations of total dissolved solids. Aquaculture Research **36**: 819-823.

Smith, B. (1999). Introduction. In: Smith, B. and Barlow, C. (Eds.) Inland Saline Aquaculture. Proceedings of a workshop held in Perth, Western Australia, 6-7 August 1997. ACIAR, p. 5.

Spotte, S. (1979). Fish and Invertebrate Culture: Water Management in Closed Systems. 2nd Edition. Wiley, New York, NY, 179 pp.

Sverdrup, H.U., Johnson, M.W. & Fleming, R.H. (1942). The oceans their physics, chemistry, and general biology. Prentice-Hall, Inc., Englewood Cliffs, N.J. 1087 pp.

Wilson, R.P. & El Naggar, G. (1992). Potassium requirement of fingerling channel catfish, *Ictalurus puntatus*. Aquaculture **10**8: 169-175.

TABLE 1
As received composition of unfortified and fortified barramundi feed used in both experiments and selected feed ingredients.

	Com. feed unfortified	Com. feed 1.0% KCl	Com. feed 2.5% KCl	Com. feed 5.0% KCl	Fish meal	Meat meal	Poultry meal	Blood meal	Soybean wheat	Extruded meal	Lupin meal
Ash	9.63	10.30	12.00	13.70	15.90	31.30	7.54	1.77	6.81	2.60	3.20
Chloride %	0.64	1.07	1.72	2.08	2.46	0.83	0.53	0.44	0.23	0.25	0.27
Calcium %	2.39	2.65	2.65	2.49	3.18	10.90	6.20	0.02	0.24	0.09	0.17
Magnesium %	0.15	0.15	0.15	0.14	0.29	0.23	0.14	0.01	0.30	0.17	0.18
Sodium %	0.41	0.41	0.42	0.40	1.53	0.62	0.64	0.41	0.006	0.02	0.02
Potassium %	**0.49**	**1.02**	**1.63**	**2.75**	**1.01**	**0.28**	**0.40**	**0.06**	**2.28**	**0.59**	**2.28**
Phosphorous %	3.19	1.60	1.60	1.51	2.43	5.73	3.23	0.05	0.65	0.40	0.40
Iron (mg kg^{-1})	537.00	467.00	473.00	437.00	166.00	324.00	317.00	2094.00	119.00	127.00	58.10
Copper (mg kg^{-1})	11.00	10.50	10.50	11.90	8.47	6.66	9.29	4.33	16.5	14.5	4.92
Zinc (mg kg^{-1})	169.00	171.00	173.00	169.00	6903.00	73.10	85.60	15.80	66.70	58.70	32.60
Gross energy (MJ kg^{-1})	23.60	16.00	21.40	12.10	23.30	17.00	19.80	27.7	21.4	18.7	19.40
Fat %	13.10	13.60	15.90	10.30	10.10	9.06	18.90	0.93	1.91	4.20	5.44
Iodine (mg kg^{-1})	3.26	<7.50	<7.50	<7.5	3.78	3.77	4.67	3.87	3.37	2.00	9.10
Moisture %	6.66	5.34	3.64	5.62	7.67	4.13	7.14	6.26	10.30	11.30	9.58
Crude protein %	51.70	52.00	52.70	50.20	68.20	50.50	63.40	92.10	48.10	15.50	34.90

TABLE 2
Composition of raw and diluted volumes of saline groundwater, estuarine water and rainwater used in experiments.

	Experiment	Salinity (‰)	$[K^+]$ (mg L^{-1})	pH
Undiluted saline ground water	1.	78.4	21.9	7.86
	2.	79.0	21.8	7.56
Undiluted estuarine water	1.	24.1	272.0	8.40
	1.	22.8	218.0	
	2.	29.7	338.0	8.04
	2.	31.7	360.0	7.81
Undiluted rainwater	1.	3.3	1.5	6.52
	2.	<0.1	0.3	8.72
Dilute saline ground water	1.	19.7	5.1	7.70
	2.	19.7	4.7	8.22
Diluted estuarine water	1.	18.8	193.0	7.30
	1.	18.0	195.0	7.43
	1.	19.3	239.0	7.44
	2.	18.4	200.0	7.53
	2.	19.7	235.0	7.87
Dilute saline groundwater 40%	2.	19.1	92.0	7.19
	2.	19.3	88.0	
Dilute saline groundwater 100%	2.	18.4	210.0	7.08
	2.	18.0	210.0	

TABLE 3
Average individual weight, performance and survival of snapper from experiment 1.

Performance index	Dietary potassium concentration (mg kg^{-1}) 0	25	50	Pooled SEM
Estuarine treatment				
survival (%)	100.0	100.0	100.0	na
stock weight (g fish^{-1})	22.49	22.77	22.16	0.51
final weight (g fish^{-1})	24.81	24.09	22.79	0.67
weight gain (g fish^{-1})	2.31^{b}	1.32^{ab}	0.63^{a}	0.30
feed intake (% BW day^{-1})	2.17^{b}	1.64^{a}	1.72^{a}	0.10
feed conversion ratio‡	1.86^{a}	2.50^{a}	5.76^{b}	1.05
Saline groundwater treatment				
survival (%)	70.83	79.17	83.33	12.50
stock weight (g fish^{-1})	22.26	22.66	22.74	0.26
final weight (g fish^{-1})†	23.05	21.68	22.15	0.41

† Final weight of surviving fish from each treatment at harvest.
‡ FCR data were log transformed prior to *ANOVA* to transform heterogeneous variances. Untransformed data are presented in the table.

TABLE 4
Average individual weight, performance and survival of snapper after 13 days on diets containing different levels of KCl and reared in undiluted estuarine water (experiment 2; n=9).

Performance index	Dietary potassium concentration (mg kg^{-1}) 0	10	25	Pooled SEM
Survival (%)	100.00	100.00	100.00	na
Stock weight (g fish^{-1})	37.31	37.46	37.83	0.21
Interim weight (g fish^{-1})	44.64	44.45	45.16	0.50
Weight gain (g fish^{-1})	7.32	6.99	7.34	0.42
Feed intake (% BW d^{-1})	1.87	1.92	1.85	0.06
Feed conversion ratio	1.32	1.39	1.38	0.05

TABLE 5
Performance of juvenile snapper reared in different water-types and fed test diets containing different levels of KCl for 21 days (experiment 2; *n*=9).

Factor mean	Interim weight (g fish^{-1})	Harvest weight (g fish^{-1})	Weight gain (g fish^{-1})	Feed intake (% BW d^{-1})	FCR
Water-type					
estuary	44.53	55.69^{b}	11.16^{b}	1.74	1.65^{a}
40%	45.15	52.66^{a}	7.51^{a}	1.70	2.85^{b}
100%	44.50	57.71^{b}	13.20^{b}	1.84	1.52^{a}
Diet-type					
0	44.64	54.86	10.22	1.73	2.11
15	44.46	56.50	10.25	1.73	2.21
25	45.09	54.70	11.41	1.82	1.69
pooled SEM	*0.48*	*0.96*	*0.78*	*0.06*	*0.32*

Significant differences (P<0.05) between the level means of each factor are indicated by different superscript letters in each column.

4.8 Benchmarking commercial feeds for juvenile Australian snapper *Pagrus auratus*; growth performance and apparent digestibility

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1. INTRODUCTION

Several commercial diets are available for farmers of temperate marine finfish in Australia. These diets are commonly fed to barramundi, Atlantic salmon, snapper, mulloway and more recently yellowtail kingfish. Some of the available diets are specifically formulated for one species (e.g. A. salmon or barramundi), and are therefore probably not ideal for other temperate fish in terms of nutrient content, nutrient balance or ingredient composition. This dilemma is common in aquaculture, where many new fish are being evaluated and dietary requirements are poorly understood. Therefore, farmers which diversify into new species are basically constrained to feeding these animals what diets are available. The goal then becomes to feed the diet, that as far as possible, matches the perceived or best guess nutritional requirements of the species until more information is available. The first step in such an investigation is to evaluate or benchmark the weight gain and performance of the new species on a range of readily available commercial diets. This research gives invaluable insights into the gross nutritional requirements of a species by indicating which of the available diets promote better growth and feed conversion. More detailed evaluation may also involve determination of the apparent digestibility of feeds and how the physical qualities of feeds affect things such as feed intake.

The aim of this experiment was to compare the growth of juvenile Australian snapper on six commercial feeds. Two of the selected feeds were of international origin and four were from Australian feed manufacturers. Two laboratory made reference feeds were also included in this study for comparison. One of the reference feeds has been used in previous published trials with snapper (Quatararo, Bell & Allan, 1998), and the other in ingredient evaluation trials with barramundi (McMeniman, 2002). The apparent digestibility of all diets was determined at the conclusion of the growth trial.

2. METHODS

2.1 Diets and pellet manufacture

Eight diets were evaluated in the growth study (Table 1). All commercial diets and the ingredients used in the 2 reference feeds were ground through a laboratory scale hammer mill fitted with a 1.5mm screen. Each mash was dry mixed (Hobart mixer) and fortified with 1g vitamin C kg^{-1} (Rovomix® Stay-C® 35, Roche Pty Ltd). before the addition of an adequate amount of distilled water. The wet mash was then cold pressed into sinking pellets using an electric meat-mincer fitted with a 3 mm die (Barnco Pty Ltd).

Diets for use in the digestibility study were made by combining the left-over amount of each feed from the growth study with 0.5 g Cr_2O_3 kg^{-1} dry matter. Pellets were mixed and formed as previously described.

2.2 Growth trial

Each of the 8 diets was randomized to 6 experiment tanks in a temperature / photoperiod controlled laboratory. Individual experiment tanks (60 L rectangular clear acrylic) were supplied with continuously flowing (≈ 2 L min^{-1}) saltwater pumped from an estuary adjacent to PSFC. This water was initially pre-filtered by large sand-filters, disinfected with liquid chlorine, de-chlorinated with

sodium-thiosulphate solution and stored in a large 50 kL reservoir. Recirculated water from the laboratory was pumped through a twin-cartridge pool filter (nominal pore size 20 μm), and over a 2 m^3 trickling bio-filter before reaching the experiment tanks. Approximately 25% of the effluent stream water was exchanged daily and replaced with fresh saltwater from the reservoir. Each tank was covered with a clear perspex lid and aerated with two air-stone diffusers. Individual fluorescent lighting was provided over each tank and was automatically controlled by an electric time-clock to provide a 12L:12D (0600 h to 1800 h) photoperiod. Water temperature was maintained at 24 ± 2°C and other water quality parameters were maintained within a suitable range for temperate marine finfish.

TABLE 1
List and origin of commercial and experimental feeds trialled with juvenile snapper in growth and digestibility trial.

Diet	Description	Origin
1	GAS*	Quatararo *et al.* (1998)
2	Basal NM**	McMeniman (2002)
3	Pivot barramundi grower 43/15	Pivot Aquaculture, Rosney Park, Tasmania
4	Pivot barramundi grower 45/20	Pivot Aquaculture, Rosney Park, Tasmania
5	Ridley barramundi grower 50/12	Ridley Aqua-Feed, Narangba, QLD
6	Ridley Supreme 54/18	Ridley Aqua-Feed, Narangba, QLD
7	Biomar sparid feed	Sweden-France
8	Chindou red sea bream	Taiwan

*GAS formula dry weight basis: fishmeal 64%, lupins 22%, fish oil 3.1%, poultry meal 4.0%, sorghum 10.1%, wheat 10.8% vitamin / mineral premix 1.0%
** Basal formula dry weight basis: fish meal 80.2%, corn gluten 11.3%, fish oil 7%, vitamin / mineral premix 1.5%

Each of the 48 experiment tanks was systematically stocked with 4 juvenile snapper having a mean ± sd weight of 32.7 ± 4.5 g (n=192). All tanks were fed to apparent satiation twice daily, with 60% of the total daily ration offered at 1500h (PM) and 40% of the ration offered the following morning at 0830h (AM). All tanks were initially allocated a starting ration of 1% of their biomass day^{-1}. After this, the total daily ration for each tank was adjusted using a 4 point scoring system that increased or decreased the following days ration based on the average number of uneaten pellets counted in a tank during the current PM:AM cycle. As such, if no pellets were counted, a score of 0 was recorded and the daily ration was increased by 0.3g. If between 1 and 5 pellets were counted a score of 1 was recorded and the feed ration remained unchanged. If a between 5-10 pellets was counted a score of 2 was recorded and the ration was decreased by 0.15g. If more than 10 pellets were counted a score of 3 was recorded and the ration was decreased by 0.3g. In this way the following day's feed could be accurately pre-weighed. In addition, uneaten feed was carefully collected and later subtracted from the total amount of feed placed into each tank.

Fish were weighed individually at stocking, bulk weighed after 28 and 42 days to monitor progress and weighed individually at harvest (56 days). Fish were not fed in the 24 h prior to any weighing procedures. Specific performance criterion were calculated including weight gain, feed intake and feed conversion ratio (FCR).

2.3 Digestibility trial

Twenty-four purpose built 170 L cylindro-conical digestibility tanks housed inside a temperature / photoperiod controlled laboratory were used to collect faeces from snapper. A detailed description of this system is given in Allan, Rowland, Parkinson, Stone & Jantrarotai (1999). Each digestibility tank was supplied continuously with pre-heated, particle-filtered water at a flow rate of approximately 2 L min^{-1}. The effluent water from each tank then flowed to a ground level sump where a proportion of the

effluent water was discharged. The remainder was pumped through a sand and cartridge filter (10-15 µm) to a raised biological trickle filter before being re-circulated to the laboratory via gravity flow. Pre-filtered and disinfected replacement water was stored in a 47 kL reservoir tank and pumped into the biological filter on demand. Each digestibility tank was fitted with a prismatic polycarbonate lid, a mechanically operated belt-feeder (AGK, Wallersdorf, Germany) and two fine bubble air-stone diffusers. Water temperature was maintained at 23 ± 2°C and other water quality parameters were maintained within a suitable range for temperate marine finfish.

Snapper from the growth trial were kept in their dietary treatment groups and moved to the digestibility laboratory for collection of faeces. This was achieved by randomly selecting 3 fish from each of 2 replicate tanks in the growth laboratory and stocking them into one of 3 digestibility tanks. In this way each digestibility tank contained 6 fish already acclimated to their respective feeds. After stocking, fish were acclimated to one feed per day at 0830 h and pellets were delivered to tanks using the belt-feeders. Snapper were switched to the diets containing Cr_2O_3 three days after stocking and faecal collections commenced 5 days later. After feeding ceased (1200h), the top and bottom sections of each tank were thoroughly cleaned and flushed to remove uneaten feed and faecal material before the collection chambers were packed in ice (1400h). Faeces were collected each morning (0800h) and immediately frozen (-15˚C). Daily tank samples were pooled, oven dried (105˚C for 24h) then finely ground before chemical analysis.

The apparent dry matter, organic matter and protein digestibility of diets was calculated using the following equation;

$$ADC\ (\%) = 100\ X\ [1 - (\ F / D\ x\ D_{Cr} / F_{Cr})]$$

where F = % nutrient in faeces; D = % nutrient in diet; D_{Cr} = % chromic oxide in diet; F_{Cr} = % chromic oxide in faeces (Cho, Slinger & Bayley, 1982).

2.4 Chemical analyses

Diets used in the growth trial were analysed in duplicate for dry matter, crude protein (N x 6.25), crude fat (ether extract), ash, gross energy (bomb calorimeter), neutral detergent fibre and phosphorous (ICP) by the State Chemistry Laboratory of Victoria, Werribee, Victoria, Australia (Table 2). Diets and faecal material from the digestibility experiment were analysed for dry matter, crude protein (N x 6.25), ash and chromium by CSIRO Livestock Industries Analytical Services Facility, Indooroopilly, Queensland, Australia. All analyses were conducted using in-house or AOAC (1995) methodology.

Table 2
Nutrient composition of commercial and experimental feeds trialled with juvenile snapper in growth and digestibility trial (g $100g^{-1}$ or MJ kg^{-1} dry basis).

Diet	Description	Protein	Fat	Ash	Gross energy (NDF)	Fibre	Phosphorous
1	GAS	55.9	9.9	11.6	21.1	6.9	1.8
2	Basal NM	68.2	14.3	13.0	22.7	3.8	2.0
3	Pivot 43/15	50.5	16.9	12.5	22.2	6.3	2.1
4	Pivot 45/20	49.3	22.1	12.3	23.6	5.3	2.0
5	Ridley 50/12	53.7	12.8	10.7	21.9	8.7	1.7
6	Ridley 54/18	57.9	18.7	11.2	23.3	3.5	1.7
7	Biomar	51.3	14.2	8.2	22.4	4.4	1.1
8	Chindou	45.5	7.3	15.8	18.8	3.4	2.3

Statistical analyses

Treatment means for different criterion were compared using one-way *ANOVA* after homogeneity of treatment variances were confirmed using Cochran's T test. Where *ANOVA* revealed a significant effect of treatment, the Student-Newman-Keul's multiple comparison test was used to separate means. Tests were performed at the 95% confidence interval using StatGraphics Plus for Windows, Version 4.1 (Statistical Graphics Corporation).

3. RESULTS

3.1 Growth trial

Weight gain and performance criterion of snapper fed the 6 commercial and 2 reference feeds are presented in Table 3. Preliminary data investigation identified outliers in snapper fed the GAS diet (i.e. harvest weight = 41.2 g; individual feed intake = 92.2 & 78.7 g) and snapper fed the Biomar diet (i.e. individual feed intake = 55.8 g). These outliers were removed prior to each *ANOVA*. There was a significant difference between the harvest weights of snapper ($F_{7/39}$=4.29, P=0.0013), with fish fed the GAS, Basal, Chindou or Ridley Supreme diets being heavier than fish fed the Pivot 45/20 diet. There was a significant difference between the individual feed intake of snapper ($F_{7/37}$=14.20, P<0.0001). Two discrete groups were identified, those that consumed a total of less than 70 g and those that consumed a total of greater than 80 g over the 56 day trial period (Pivot 45/20 = Ridley 54/18 = Ridley 50/12 = Pivot 43/15 < Basal = Chindou = GAS = Biomar; Table 3). Data for FCR was problematic and values were higher than anticipated for all diets. Several tanks recorded extremely high FCRs of greater than 10:1, which was considered highly unusual (i.e. GAS = 11.1; Pivot 45/20 = 10.3 & 11.6; Biomar = 10.6 & 12.1), and these tanks were dropped from the one-way *ANOVA.* The subsequent *ANOVA* identified significant differences between treatment means ($F_{7/35}$=3.05, P=0.013), with fish fed the Ridley 54/18 recording the lowest FCR and fish fed the Pivot 45/20 the highest (Table 3).

TABLE 3

Average performance of individual snapper fed six commercial and two experimental diets to apparent satiation for a period of 56 days.

Diet	Description	Stock wt (g)	Harvest wt (g)	Feed intake (g/fish)	FCR
1	GAS[1]	32.2	55.1[b]	84.0[b]	3.9[ab]
2	Basal NM	32.7	51.4[b]	81.3[b]	4.6[ab]
3	Pivot 43/15	32.6	46.6[ab]	67.7[a]	5.4[b]
4	Pivot 45/20[2]	33.1	40.8[a]	57.0[a]	6.0[b]
5	Ridley 50/12	32.3	50.1[ab]	66.2[a]	4.2[ab]
6	Ridley 54/18	32.6	56.6[b]	65.6[a]	2.9[a]
7	Biomar[3]	32.8	45.8[ab]	88.2[b]	5.5[b]
8	Chindou	32.9	52.0[b]	83.2[b]	4.5[ab]
	pooled sem	*0.6*	*2.7*	*3.2*	*0.7*

Similar superscript letters in columns indicate homogenous treatment groups (SNK)
[1] For the GAS diet, one outlier was removed from harvest weight (n=5), two outliers from individual feed intake (n=4) and one extraneous value from FCR (n=5) prior to one-way ANOVA.
[2] For the Pivot 45/20 diet, two extraneous values were removed from FCR (n=4) prior to one-way ANOVA.
[3] For the BIOMAR diet, one outlier was removed from individual feed intake (n=5) and two extraneous values from FCR (n=4) prior to one-way ANOVA.

3.2 Digestibility trial

The amount of faeces collected from small fish using settlement methods in this trial was adequate for determination of dry matter, chromium, nitrogen and ash. This allowed calculation of dry matter, organic matter and protein digestibility coefficients for the 8 feeds. One-way ANOVA indicated that diet type significantly affected the dry matter ($F_{7,16}$=42.10, P<0.0001), organic matter ($F_{7,16}$=37.14, P<0.0001) and nitrogen digestibility ($F_{7,16}$=54.94, P<0.0001) of feeds. Mean apparent digestibility coefficients and the results of post-hoc comparisons (SNK) are presented in Table 4. Dry matter digestibility ranged from 60 to 78%, organic matter digestibility ranged from 65 to 85% and protein ADCs ranged from 75 to 93%.

TABLE 4
Apparent digestibility coefficients of test feeds used in growth trial

Diet	Description	Dry matter ADC (%)	Organic matter (%)	Protein ADC (%)
1	GAS[1]	60.8[a]	65.2[a]	88.1[c]
2	Basal NM	77.2[c]	83.4[cd]	87.6[c]
3	Pivot 43/15	67.5[b]	79.1[c]	85.4[bc]
4	Pivot 45/20[2]	69.2[b]	80.1[c]	85.0[bc]
5	Ridley 50/12	62.3[a]	69.0[b]	67.3[a]
6	Ridley 54/18	78.2[c]	85.3[d]	87.7[c]
7	Biomar[3]	77.6[c]	80.2[c]	92.8[d]
8	Chindou	60.5[a]	70.0[b]	83.2[b]

4. DISCUSSION

Snapper fed a range of commercial and reference feeds exhibited variable feed intake and growth response. The apparent digestibility of these feeds also varied. In terms of performance, small snapper fed the high protein, high lipid Ridley diet (i.e. 54/18) were slightly heavier at harvest than the other treatments. Fish in this group also had the lowest recorded feed conversion ratio of 2.9:1. However, feed conversion efficiency was poor across all the diets tested in this study and may indicate that other factors such as low stocking density or interactions between individual fish in tanks could have been affecting feeding behaviour. The fact that all uneaten feed was recovered from tanks excludes over feeding as a cause of poor feed conversion. Underfeeding is also unlikely because feed input was systematically governed by the aforementioned feed scoring protocols. Nonetheless, FCRs as high as these would be catastrophic in a farm situation, regardless of the species.

Dry basis crude protein content of diets ranged from as low as 45% for the Chindou feed to a high of 68.2% for the reference diet composed mainly of fishmeal (i.e. Basal NM). Dry matter (DM) digestibility of all diets was above 60% and as high as 77% for the Basal NM, Biomar and Ridley Supreme feeds. Organic matter (OM) digestibility reflected DM values (DM ADC% = 0.88 x OM ADC% + 15.87; R_2=0.81). Protein digestibility was above 85% for most feeds, however, the protein ADC of the Ridley standard feed was only 67.3% which is unusually low and may indicate that the protein/s were heat damaged in this batch of feed.

There was some indication that increasing dietary levels of gross energy ($y = -1.6491x^2 + 65.66x – 568.9$; R_2=0.42) and crude fat ($y = -0.1173x^2 + 1.629x + 77.55$; R_2=0.63) were suppressing feed intake in juvenile snapper, however, the relationships were fairly weak. Of greater interest was the relationship between dietary protein content and harvest weight, which was typical of a dose-response model (Figure 1). However, for reasons explained later, harvest data for snapper fed the Chindou diet did not fit this relationship. The tangent to the fitted quadratic function suggests that diets containing about 57% crude protein are adequate for juvenile snapper. Based on the average protein ADC for the test diets used in this study (i.e. 87.1%) would mean that snapper weighing between 30-60g require feeds that contain approximately 49.6% digestible protein to approach maximum weight gain. Obviously, this amount is subject to energy balance and potential feed intake. This recommendation is similar to that made by others who used a 4 parameter mathematical model to determine the protein requirements of juvenile snapper fed diets with known digestible protein and energy contents (Booth, Allan & Anderson, 2005). The amount of faecal material collected in the digestibility study did not allow determination of gross energy and by default the digestible energy content of these diets. However, if harvest data are modelled against the crude protein to gross energy ratio of test diets, an underlying pattern becomes clear (Figure 2).

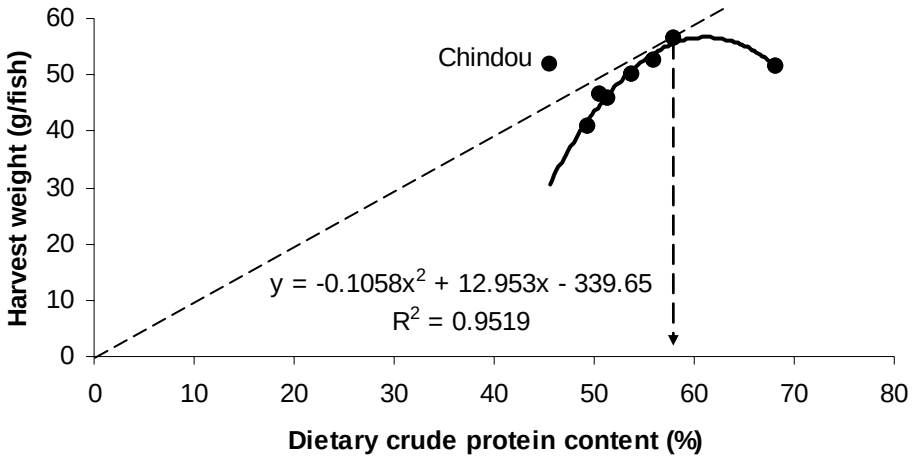

FIGURE 1
Relationship between dietary crude protein content and harvest weight of juvenile snapper. Data point for Chindou diet is excluded from model.

Harvest weight steadily increased in response to protein: energy ratio up until the point where the ratio reached 27 g CP MJ GE^{-1} (quadratic inflection point at y=0). This response demonstrates that high levels of dietary protein are critical in terms of growth performance in juvenile snapper under 60g, and diets which provide protein and energy at lower ratios than this will result in poorer weight gain. It also underscores the fact that protein is probably the preferred energy source of juvenile snapper during this growth stage, as fish fed diets with a higher fat content but similar protein and gross energy contents (e.g. Pivot 43/15, Pivot 45/20, Biomar), experienced slightly lower weight gains than those fed diets with less fat (limited protein sparing). Only when elevations in fat content are matched with increased levels of protein at or near the correct CP:GE ratio does growth respond (i.e. Ridley 54/18). As a result of its high CP:GE ratio (i.e. 24.2; Table 2), the Chindou diet, although having the lowest crude protein, crude fat and gross energy content, sustained weight gains statistically similar to the best of the other diets. The CP:GE ratio that promoted the highest weight gains in this study is not dissimilar to the cited values of 23g DP MJ DE^{-1} given for similar sized snapper in a recent study by Booth, Allan & Anderson (2007).

This study was one of the first in a series of many experiments designed to evaluate feeds and elucidate the basic nutritional requirements of juvenile snapper. The feeds selected in this study were of commercial origin and obtained from local manufacturers (i.e. Ridley or Pivot) or from overseas (Biomar or Chindou). Two laboratory made reference feeds were included in the study as benchmarks. One was formulated for snapper and composed of several common feed ingredients (Quatararo, *et al*. 1998) while the other was composed predominantly of fish meal (McMeniman, 2002). Both these diets performed as well as the best commercial feeds, suggesting that the manipulation of feed ingredients in diets for snapper should pose no great problems for feed formulators, providing these manipulations are done using sound digestible nutrient or energy data for specific ingredients and that protein and energy requirements for different life stages are satisfied. The commercial diets tested in this trial were representative of feeds that were available to snapper farmers in 1999-2000. These feeds are no longer available, however, the information gained from this initial investigation has underpinned and directed further nutritional research on this animal.

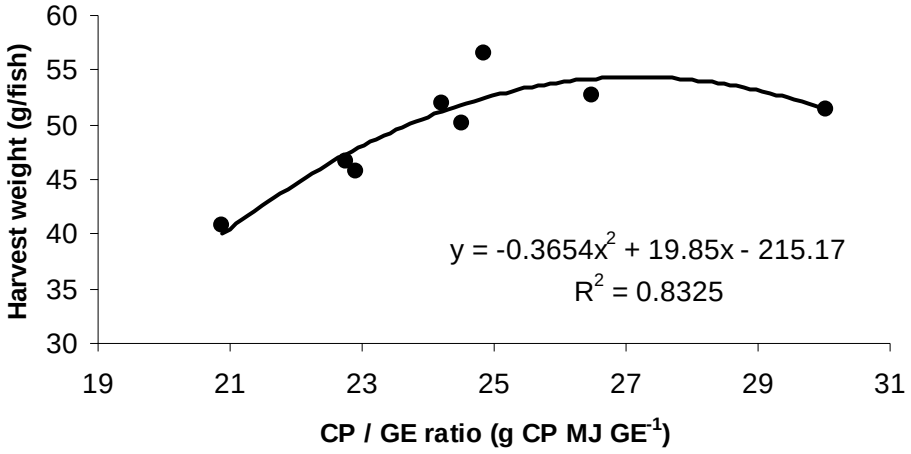

FIGURE 2
Relationship between dietary crude protein / gross energy ratio and harvest weight of juvenile snapper.

ACKNOWLEDGEMENTS

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REFERENCES

Allan, G.L., Rowland, S.J., Parkinson, S., Stone, D.A.J. & Jantrarotai, W. (1999). Nutrient digestibility for juvenile silver perch *Bidyanus bidyanus*: development of methods. Aquaculture **170**:131-145.

AOAC (1995). Official methods of analysis of the association of official analytical chemists. (ed. K. Helrich), 15th ed., Published by the Association of the Official Analytical Chemists, Inc., Arlington, Virginia, USA.

Booth M.A, Allan G.L. & Anderson A.J. (2005). Effects if digestible energy content on utilisation of digestible protein. In: Investigation of the nutritional requirements of Australian snapper *Pagrus auratus* (Bloch & Schneider 1801). Doctoral Thesis, Chapter 3:49-80. School of Life Sciences, Queensland University of Technology (QUT), Brisbane, Australia, 198pp.

Booth M.A, Allan G.L. & Anderson A.J. (2007). Investigation of the nutritional requirements of Australian snapper *Pagrus auratus* (Bloch & Schneider 1801): effects of digestible energy content on utilisation of digestible protein. Aquaculture Research **38**:429-440.

Cho, C.Y., Slinger, S.J. & Bayley, H.S. (1982). Bioenergetics of salmonid fishes: energy intake, expenditure and productivity. Comparative Biochemistry & Physiology **73B**(1):25-42.

McMeniman N.P. (2002). Digestibility and utilisation of starch by barramundi. In: Allan G.L., *et al*. (eds.). Aquaculture Diet Development Subprogram. Final report to Fisheries Research & Development Corporation Project 1996/391, NSW DPI Fisheries Final Report Series No. 58, 142-148.

Quartararo, N., Bell, J.D. & Allan, G.L. (1998). Substitution of fishmeal in a diet for the carnivorous marine fish *Pagrus auratus* (Bloch and Schneider) from southeastern Australia. Asian Fisheries Science **10**:269-279.

5. BENEFITS AND ADOPTION

- The research strategy of Aquafin CRC has been developed explicitly to deliver the essential technologies needed by the Australian finfish farming industry. The industry partners agreed to invest in a CRC, defined the major goals which they believed a CRC could best achieve, and clearly indicated the weight of effort which should be applied to each of these goals. These goals and weightings were first defined at a workshop of potential CRC participants in December 1999.

- The industry participants have continued to refine their priorities during the subsequent development and industry participants and researchers have met regularly (several times per year) to discuss results, implications of results and how they might be used, and refined plans for subsequent experiments.

- While there is currently no commercial snapper farming in Australia (fish farmers have moved to other faster growing marine fish like mulloway and yellowtail kingfish) the research has contributed in several major ways to Australian marine fish aquaculture:

    - Diets developed for snapper have helped form the basis for formulations for other marine carnivorous species.

    - One of the most significant benefits is the confidence among feed manufacturers to use alternative ingredients to fishmeal in response to reductions in availability and/or increases in price. The progression towards least-cost formulation (as is industry practice for terrestrial monogastric animal feed formulation) relies on rigorous ingredient evaluation, accurate estimation of nutritional requirements and systematic validation of different formulations.

    - Hatchery practices and nursery technology has been refined providing a much clearer understanding of the importance of abiotic factors, larval feeding strategies and diets on the cost-effective production of snapper fingerlings. Low-cost technology for extensive production of snapper larvae in fertilised ponds has also been developed. The new technology has been applied to other marine fish species providing an excellent starting point from which to refine larval rearing techniques for those species.

    - A clear understanding of how to improve skin colour of farmed snapper through manipulation of the culture environment and optimisation of dietary pigments.

    - Although a "solution" has not been found, the project has led to a much greater understanding of "velvet disease" caused by the parasitic dinoflagellate *Amyloodinium occelatum* and best management practices to help avoid major problems.

    - The development of successful research methods cannot be underestimated as a benefit of this project. Research methods for diet development, larval rearing and nursery production and health management have all assisted in the design of new research to address similar problems for other species.

    - Research capacity at an institutional (NSW DPI and Ridley Aquafeeds) and personal level has been expanded.

- Adoption has been made possible because of the involvement of Ridley Aquafeeds throughout the project. The commercial fish farmers who were partners in the project are currently

represented by Anthony O'Donohue (Clear Water Mulloway) who is presently using the Ridley Marine Fish Diet for production of mulloway and yellowtail kingfish.

6. FURTHER DEVELOPMENT

Despite the commercial investment in snapper farming at the commencement of this project, snapper farming has not developed in Australia. This is mainly because the economics of snapper culture, when the species was farmed as the only species in sea cage operations, were sub-optimal. It was therefore not surprising that, without exception, operators moved to faster growing species such as mulloway and yellowtail kingfish. However, even though the aquaculture production of snapper has not developed, marine fish farming in Australia has expanded considerably. The research conducted during this project has assisted with the development of culture techniques for mulloway and yellowtail kingfish. In particular, results have been adapted during the Aquafin CRC project "Feed technology for temperate fish species" for both the hatchery/nursery and the diet development components, and information gained is being used by feed manufacturers and hatchery operators.

Assuming commercial production costs are equivalent for different diets, use of ingredients similar to those tested in this study can reduce the levels of fishmeal and thus the cost of snapper (and marine fish) feeds. Due to the high protein requirements of marine fish, the fact that productivity improvements can be achieved by feeding high-protein feeds and the increasing demand on existing fishmeal supplies, replacement of fishmeal in diets will be increasingly important in the future. During the course of this project, the cost of fishmeal has increased by at least 100% and at times has been virtually unavailable in Australia because of reductions in production and massive increases in demand, particularly from China. This rapidly changing supply/demand relationship for fishmeal has major implications for marine fish farming, particularly in Australia, where low production reduces the ability to negotiate large volume purchases of fishmeal at globally attractive prices. This reinforces the priority for continued investment in fishmeal replacement research.

In addition, because the "formulation space" for other energy sources will be reduced in nutrient dense feeds, the challenge is to identify and test high protein ingredients that in combination have a similar nutritional quality to fishmeal but at a lower cost. The paradox for Australian marine fish farmers is that rather than lowering the cost of feeds, high-protein nutrient dense feeds will inevitably cost more per kilogram than those formulated with a lower nutrient specification. However, the increased growth and improved FCR of this feeding strategy should make the use of more expensive, nutrient dense diets economically sound. The imperative for future research is to ensure whole-farm economics are considered when modelling different nutritional and feeding strategies. A key aspect is to ensure research is conducted with large fish most relevant to farming operations.

This project has led to considerable improvements in hatchery technology for snapper. Hatcheries dedicated to producing snapper are now able to produce nearly twice as many batches of snapper juveniles as they could using the "best practice" technology available at the start of the project. As the emphasis has shifted to other temperate marine fish species, e.g. mulloway, yellowtail and even southern bluefin tuna, it will be very important that hatchery procedures for each species are optimised. The research capacity developed during the project will be invaluable. In the past, meetings of marine fish hatchery managers and technicians have been very effective as a means of sharing new technology and identifying bottlenecks in production. Further hatchery development for marine finfish in Australia would benefit greatly from an expansion of this concept.

As production intensifies, health management will become increasingly important. The research during this project has progressed knowledge of velvet disease, caused by the parasitic dinoflagellate *Amyloodinium occelatum.* However, other diseases will also emerge to limit aquaculture production. This is a critical area of research for future marine fish aquaculture development.

7. PLANNED OUTCOMES

1. Profitable, expanding industry for snapper aquaculture in Australia. **Not met**. However, development of other marine fish farming industries has occurred and research methods developed during this project, as well as research results, have assisted with the development of the marine fish culture industry in Australia.

2. Viable hatcheries, breeding vigorous low-cost snapper fingerlings (for aquaculture or stock enhancement). Our goal is to reduce fingerling costs to around 25 cents/fish. (Specific outcome for Fingerling Production and Health Component). **Achieved.** Several marine fish hatcheries in Australia are now able to successfully produce snapper fingerlings and "commercial-scale" validation of new improved hatchery technology has occurred in South Australia, Western Australia and NSW.

3. Development of techniques for management exclusion of ectoparasites in marine fish hatcheries. *Amyloodinium ocellatum* infests many fish species in hatcheries throughout the world. All Australian marine fish hatcheries will potentially benefit from this technology. (Specific outcome for Fingerling Production and Health Component). **Achieved.** While a successful treatment for *Amyloodinium ocellatum* was not developed, methods for excluding the problem from hatcheries and nurseries were developed and successfully applied.

4. Commercially-available, cost-effective, high performance and low polluting diets for Australian snapper that help produce highly marketable fish of the desired colour. (Specific outcome for Diet Development and Skin Colour Component). **Achieved.** While there are no snapper being farmed at present, research results from the project have assisted the commercial feed manufacturer, Ridley Aquafeeds, with formulation of their Marine Fish Diet that is very effective for snapper. The Ridley Marine Fish Diet is recommended for grow out and the same diet with the addition of astaxanthin is recommended as a finisher diet to impart the desirable red pigment. A tank-based finishing treatment is recommended in addition to the pigmented finisher diet to ensure the best skin colour for farmed snapper.

5. Increased availability of snapper for domestic (and export) markets. **Not achieved**. There has, however, been considerable expansion in production of other temperate marine finfish species and research and commercial experience with snapper has helped lay the foundation for this expansion.

8. CONCLUSIONS

Data presented in this report demonstrates that by applying a research strategy based on determination of digestibility coefficients, an understanding of basic nutrient requirements and ingredient utilisation, diets can be formulated to optimise growth and minimise feed conversion ratio. Importantly, this approach also allows feed manufacturers to choose between a range of alternative feed ingredients that in combination can replace significant levels of fishmeal in the diets of Australian snapper before weight gain and performance is negatively affected.

The major conclusions and findings of this research are:

- Australian snapper are efficient at digesting the crude protein from a range of ingredients including fishmeal, poultry offal meal, blood and haemoglobin meals, solvent and expeller extracted soybean meals and extruded wheat. They are less efficient at digesting the protein from a rendered meat meal by-product, possibly because this particular meat meal was over processed.

- The crude protein and gross energy ADCs of poultry offal meal, meat meal and extruded wheat were not affected by dietary inclusion level within the range examined.

- The gross energy and organic matter ADCs of extruded wheat and pregelatinised wheat starch are inversely related to inclusion level.

- Gross energy ADCs for snapper fed CHO based ingredients are not additive, and ADCs for these ingredients or those like them should be determined over a wide range of inclusion levels before formulating experimental or commercial diets.

- Australian snapper are incapable of rapidly regulating their blood glucose after an intra-peritoneal injection of glucose and remain hyperglycaemic for 18 h.

- The optimum digestible protein (DP):digestible energy (DE) ratio of diets for juvenile snapper weighing 30-90 g was determined to be 28 g DP MJ DE^{-1}.

- Dietary levels of extruded wheat and fish oil can be exchanged according to their DE values in diets for snapper that provide 390-490 g DP kg^{-1} without unduly compromising weight gain and performance.

- Semi-commercial production diets for Australian snapper can be formulated from a combination of alternative Australian based feed ingredients to replace all but 160 g fish meal kg^{-1}

- Fortification of diets with potassium does not improve survival or growth of snapper cultured in potassium deficient saline groundwater. For successful culture of snapper in potassium deficient saline groundwater, potassium must be added directly to the water.

9. APPENDICES

9.1 Intellectual Property

All information brought into this project or developed during the project is public domain.

9.2 Staff

- Dr Geoff Allan, Principal Investigator/Research Leader, Aquaculture and Port Stephens Fisheries Centre Director.
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- Mr Ian Russell, Fisheries Technician, Port Stephens Fisheries Centre.
- Mr Luke Cheviot, Fish Hatchery Manager, Port Stephens Fisheries Centre.
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