FISH MEAL REPLACEMENT IN AQUACULTURE FEEDS FOR SILVER PERCH
PROJECT 93/120-03

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FINAL REPORT
TO
FISHERIES RESEARCH AND DEVELOPMENT CORPORATION

March 1998
NSW Fisheries Final Report Series - Report No. 6
ISSN 1440-3544

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13.8 NSW Fisheries Final Report Series

1 PROJECT DETAILS

Project Title:	Fish Meal Replacement in Aquaculture Feeds for Silver Perch
Project No.:	93/120-03
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NB Dr Anderson's work (*in vitro* digestibility) is not included in this document but is detailed as a separate report which collates studies for barramundi, prawns, silver perch and Atlantic salmon, entitled: Anderson, A., 1998. Fish Meal Replacement in Aquaculture Feeds: *In Vitro* Studies on Feed Ingredients for Aquaculture Species. Final Report to Fisheries Research and Development Corporation, Sub-Program 93/120. 58 pp.

2 NON-TECHNICAL SUMMARY

93/120-03 Fish Meal Replacement in Aquaculture Feeds for Silver Perch

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Objectives:

1 To identify potential feed ingredients to replace fish meal in aquaculture diets for silver perch

2 To evaluate promising ingredients in terms of their *in vitro* and *in vivo* digestibility and assimilation

3 To develop and evaluate methods of improving the usefulness of ingredients through processing (eg extrusion or cooking) and the use of enzymes and supplements

4 Identify areas where inadequate knowledge of nutritional requirements may restrict fish meal substitution and determine these requirements for silver perch.

5 To formulate and evaluate diets with reduced contents of fish meal for silver perch.

If aquaculture is to continue to expand in Australia cost-effective diets based on Australian agricultural ingredients urgently need to be developed. The replacement of fish meal as the protein source of choice is a global research priority driven by static or declining supply of fish meal and rapidly expanding aquaculture and aquaculture feed industries. Australia has very poor supplies of fish meal and other aquatic meals but fortunately has abundant supplies of agricultural ingredients with potential for use in aquaculture diets.

In recognition of the need to develop diets for Australian aquaculture species, with reduced contents of fish meal, a number of institutions independantly commenced this type of research in the early 1990's. The Fisheries Research and Development Corporation (FRDC) was approached by a number of institutions to financially support this research. In response, FRDC created their first 'Sub-Program' with the aim of coordinating research to develop Australian aquaculture diets. The two primary objectives were to replace fish meal and obtain an early commitment from commercial aquaculture feed manufacturers to adopt results.

Six separate projects were formed; four on species considered to represent most 'types' of species being farmed in Australia, one on feed processing and one on a technology audit for amino acids. The four 'model' species were prawns *Penaeus monodon*, silver perch *Bidyanus bidyanus*, barramundi *Lates calcarifer* and Atlantic salmon *Salmo salar*. Each project involved a number of collaborating scientists from different institutions and all projects were coordinated through a Sub-Program Steering Committee. Regular meetings with investigators from all projects as well as feed manufacturers, ingredient suppliers and R&D corporations were held twice each year.

This report describes the progress achieved with the silver perch project: Replacement of Fish Meal on Aquaculture Diets for Silver Perch. Silver perch are an omnivorous, freshwater species endemic to eastern Australia. They have shown outstanding potential for culture in static earthen ponds and are one of the few species being cultured, or considered for aquaculture, in Australia which might replace some of the more than 55 000 t/yr of imports of white-fleshed fish. Growth and production potential of silver perch are similar to channel catfish in the USA and carp and tilapia in south-east Asia.

Objective 1: To identify potential feed ingredients to replace fish meal in aquaculture diets for silver perch

Literature and data base searches were conducted and a comprehensive list of ingredients currently available for use in animal feeds in Australia was compiled. Available data on biochemical composition, price and availability of these ingredients was obtained. This was used to select ingredients for further evaluation.

Additional ingredients were identified following discussions with the Grains Research and Development Corporation, the Grain Pool, the Australian Wheat Board and the Meat Research Corporation. The specific features making ingredients worth considering for aquaculture were discussed and the various agencies recommended ingredients their stakeholders produced which might be suitable. Descriptions of ingredients and data on composition can be found in Sections 6.2, 6.3, 6.4, 6.7, 6.8, 6.10 and 6.12.

Objective 2: To evaluate promising ingredients in terms of their *in vitro* and *in vivo* digestibility and assimilation

Initially, a series of three experiments were done to determine the most appropriate techniques for *in vivo* digestibility determination. Results from these experiments demonstrated that collection of faeces by settlement over 18 h was a suitable method for determining digestibility in juvenile silver perch (see Section 6.1 of this report). As the sum of digestibility coefficients calculated separately for individual ingredients was similar to that calculated for a diet comprised of those ingredients, the assumption that digestibility coefficients are additive was validated (see Section 6.1 of this report).

Methods for *in vivo* digestibility were developed or evaluated by Dr Alex Anderson at QUT. Results from these experiments are reported separately (see Fish meal Replacement in Aquaculture Feeds: *in vitro* studies on feed ingredients for aquaculture species. Final Report to FRDC Sub-Program 93/120). In summary, *in vitro* methods were shown to be useful for ranking but not for accurately determining digestibility coefficients for use in diet formulation.

In vivo digestibility determination involves measuring the amount of dry matter, energy or a specific nutrient which is ingested and then subtracting what is excreted. This involves collecting and analysing faeces. It is the first critical stage in evaluating the potential of an ingredient for use in a formulated diet. Following method development and verification (see Section 6.1 of this report), digestibility coefficients for over 60 ingredients, including some processed in different ways, were calculated. Digestibility coefficients for dry matter, energy, protein and, in most cases, individual amino acids (except tryptophan) are presented (see Sections 6.2, 6.3, 6.7, 6.8, 6.10 of this report).

Once digestibility coefficients are available, the next step is to determine the maximum amount of an ingredient which can be used in formulated diets. Many ingredients, especially those derived from plants, have anti-nutrients, some of which affect utilisation of the ingredient but not digestibility. In addition, excessive amounts of some ingredients may reduce the attractiveness of the diet or suppress palatability. Information on how well ingredients are utilised is also crticial for effective diet formulation using new ingredients.

Growth experiments were conducted to provide this information for meat meals, poultry offal meal, feather meal and dehulled lupins. This added to earlier research results to estimate maximum contents of soybean meal, canola meal, peanut meal and lupins. One experiment was also conducted where all fish meal was replaced with specially modified wheat gluten meal. The most promising ingredients evaluated are meat meal, especially low ash meat meal, poultry offal meal and dehulled lupins. The specially-modified wheat gluten meal also deserves further evaluation. These results are reported in Sections 6.4, 6.6, 6.9 and 6.12.

Objective 3: To develop and evaluate methods of improving the usefulness of ingredients through processing (eg extrusion or cooking) and the use of enzymes and supplements

Most ingredients with potential for use in aquaculture diets are inferior to fish meal in terms of their nutritional composition (especially total protein content and amino acid profile),

carbohydrate content or presence of anti-nutrients. Some of these deficiencies can be overcome. Digestibility and utilisation of an ingredient can be improved by processes such as grinding, cooking, and removal of less digestible components such as carbohydrate (eg through dehulling and removal of starch and non-starch polysaccarides) and ash (eg through removal of bone).

We found grinding diets below a particle size of between 710 and 1 000 μm was unnecessary but that steam conditioning or extruding practical diets containing starch improved gelatinisation of starch, digestibility, gustatory characteristics and fish growth (see Sections 6.8 and 6.10). Removal of hulls, by dehulling, improved dry matter and energy digestibility of two species of lupins, field peas, chick peas and vetch but not faba beans (vetch was poorly accepted by silver perch). Further protein concentration, through the removal of starch and/or non-starch polysaccarides further improved energy and dry matter digestibility of lupins, field peas and faba beans (other protein concentrates were not available). Protein digestibility of most pulses was high (see Sections 6.3 and 6.7 of this report).

Removal of part of the ash fraction from meat meals increased total protein content and improved the value of meat meals for use in diets for silver perch (see Section 6.4 of this report).

Some of the most common supplements used to overcome nutritional deficiencies in ingredients and diets are crystalline amino acids. During this study, crystalline lysine, methionine and/or threonine were added during several experiments but there is no conclusive evidence that silver perch responded to these supplements at any time. This may be due to problems with utilisation of crystalline amino acids, and such problems have been widely reported with some species of fish, or indicate that the diets supplemented were not deficient in those amino acids. This area requires further evaluation.

Objective 4: Identify areas where inadequate knowledge of nutritional requirements may restrict fish meal substitution and determine these requirements for silver perch

When we formulated the first reference diets for silver perch we set nutritional specifications using published requirements for other species as a guide. In particular, species such as channel catfish and tilapia, which are omnivorous, were used.

Given the high cost of protein, the major initial task was to estimate requirements of this nutrient for silver perch. As energy might be able to spare requirements for protein, the interaction between energy and protein was also important to quantify.

Preliminary research indicated that growth increased with both protein and energy but that as early diets were formulated before we had accurate information on protein and energy digestibility, results were confusing and difficult to interpret. We were able to conclude that energy could spare requirements for protein but high lipid content diets led to high lipid content of fish tissue, a negative from a marketing perspective.

Recent research with pigs and poultry has introduced the concept of a protein dependant phase and an energy dependant phase for maximum protein deposition. With this concept, at a

certain energy content protein deposition increases during the protein dependant phase and then plateaus out. Further increases in protein deposition require additional energy.

We applied this approach to determine optimum protein and lysine requirements for silver perch. We used a single digestible energy content, one used successfully in practical diets gaining wide commercial acceptance and which we knew did not produce excessive lipid deposition in the fish carcass. During this experiment we determined that for silver perch fed a diet with approximately 14-15 MJ/kg digestible energy the minimum digestible protein content which produced maximum growth was only 25.2% (much lower than most estimates of optimum protein for cultured fish). We also estimated that 14-15 MJ/kg digestible energy diets do not need to contain more than 1.5% digestible lysine for optimum growth (see Section 6.11 of this report).

Objective 5: To formulate and evaluate diets with reduced contents of fish meal for silver perch

Most nutritional research is done using small juvenile fish and small tanks. The applicability of results generated using these methods is often questioned by farmers who wish to grow fish through to a market size in large ponds or tanks. Nutritional research needs to be validated in commercially relevant facilities.

In this study we utilised the results for ingredient digestibility and utilisation efficiency and the effects of diet processing to formulate two 'least-cost' diets for a large-scale farming experiment. Cost of ingredients was estimated as ingredient cost only, not including transport costs as this component is clearly dependant upon where the feed mill is located.

Our least-cost diets contained only 5 or 10% fish meal with the remainder replaced with meat meal, dehulled lupins or dehulled field peas. The least-cost diets out-performed the earlier fish meal/soybean meal reference diet and the cost of producing fish, based on ingredient costs and food conversion ratios, was lower (see Section 6.5).

Keywords: Fish Meal Replacement; Silver Perch; Nutrition; Ingredient Evaluation; Nutrient Requirements; Aquaculture; Meat meals; Lupins; Pulses; Least-cost.

3 BACKGROUND

This project involved collaborative research between NSW Fisheries, NSW Agriculture, Queensland University of Technology, CSIRO Division of Fisheries and CSIRO Division of Food Science and Technology to develop cost-effective diets for silver perch with an emphasis on replacing fish meal in formulated feeds. The project was part of the Fisheries Research and Development Corporation Sub-Program on Fish meal Replacement. The research described here built on very promising results with evaluating the digestibility of a small number of Australian oilseeds and grain legumes in diets for silver perch.

In Australia, 27 312 t of fish meal worth about AUS $17.5 million were imported in 1996/97 (ABARE, 1997). About 30 000 to of aquaculture feeds (almost all for carnivorous fish or prawns) are used each year in Australia Assuming an average fish meal content of 40%, this requires about12 000 t of fish meal. As high quality fish meals are usually used for

aquaculture feeds, with prices of $1 300 for 72% protein fish meal (Danish fish meal) and $1 000/t for 67% protein fish meal (Chilean fish meal), the cost of imported fish meal for aquaculture diets may exceed $8 million each year. Aquaculture in Australia is expanding rapidly, as is the price of fish meal.

Australia currently imported 121 437 t of edible seafood products (worth $601.6 million in 1996/97) each year including 90 289 t of fish and fish products (ABARE, 1997). Much of the fresh, chilled or frozen component (55 042 t worth $184 944 million) could be replaced by cultured fish. Replacement of fish meal in aquaculture diets could prevent a massive escalation in the importation of fish meal into Australia as well as lowering production costs and increasing the commercial viability of fish culture.

One of the major factors limiting the expansion of aquaculture is the development of nutritionally adequate, cost-effective diets. Feeds and feeding can contribute up to 70% of the total operating costs for fish and shrimp farms (Wee, 1992). The most expensive component of pelleted feeds is protein, of which 25-55% is required, depending upon whether the species is herbivorous, omnivorous or carnivorous (NRC, 1993 Lovell, 1989). The major protein source for most aquaculture diets is fish meal (Lovell, 1989) and formulated diets can contain up to 60% fish meal (Wee, 1992; New, 1991).

There are however, some major problems with fish meal. Fish meal and fish oil production is declining (Barlow, 1989). The aquaculture feed industry currently uses more than three million tonees of the global fisheries catch (New and Wijkstrom, 1990) excluding 'trash fish' fed directly to aquaculture species. As aquaculture production increases, demand for fish meal will also increase, inevitably forcing prices to rise. As higher quality fish meal is generally required for aquaculture feeds, species of fish currently used for human consumption will increasingly be targeted by fish meal manufacturers. In Malaysia much of the cheap fish used to produce salted fish for human consumption is now used for aquaculture instead (New, 1991). **While aquaculture remains dependant to this extent upon capture fisheries it will not be a net contributor to human food supplies.**

Apart from a relatively small quantity of fish meal produced in Tasmania during a limited period each year, very little fish meal is produced in Australia (Foster, 1992) and most required for aquaculture feeds is imported (ABARE, 1997). Imported fish meal varies in quality and prices in Australia have risen to about AUS$1 300/tonne for high quality Danish fish meal. Improved growth and food conversion efficiency have been recorded for salmonids when low-temperature fish meals have been used. These special 'aquaculture grade' fish meals are more expensive than ordinary fish meal, some by as much as 35% (Foster, 1992).

The project utilised collaboration between a number of institutions to compare and validate several experimental techniques to evaluate ingredients in diets for silver perch. Compared with warm blooded terrestrial monogastric animals, fish lose relatively little energy due to body heat production, and as measurement of non-faecal losses are much more difficult for fish than terrestrial animals, determination of digestibility is the recommended method for evaluating ingredients (Cho and Kaushik, 1990). Most commonly, faeces are collected by settlement, stripping or dissection and are then analysed (Cho et al., 1982). Nutrients or energy present in the faeces are subtracted from those in the feed to estimate digestibility (Cho and Kaushik, 1990). However, these *in vivo* methods of determining digestibility are

expensive, time consuming and involve large numbers of fish and experimental tanks. When many potential feed ingredients need to be investigated, rapid *in vitro* methods for digestibility determinations may offer many advantages. A large number of potential feed ingredients can be screened in a short time, using very small quantities of material and very few animals, allowing identification of the few best performing ingredients that should be taken on to *in vivo* studies.

Several *in vitro* methods for digestibility determination have been advocated (Akeson and Stahmann, 1964, Hsu et al., 1977; AOAC, 1984; Grabner, 1985, Eid and Matty, 1989; Lan and Pan, 1993) and are being used by scientists at the Queensland University of Technology. Using these methods, ingredients are added to homogenates of fish guts and digestible enzyme activities are measured to assess ingredient digestibility. These methods perform quite satisfactorily, particularly in comparative studies (Neilsen et al., 1988; Lan and Pan, 1993) where a range of ingredients can be compared. They also perform well when the digestive capability of several animal species can be compared. In addition, comparison of *In vitro* digestibility with amino acid content can give specific information on the effects of processing on digestibility (Lan and Pan, 1993). Very good correlation between *in vitro* and *in vivo* methods of digestibility determination has been demonstrated (Grabner, 1985, Eid and Matty, 1989). In addition, good correlation between *in vitro* digestibility of fish meal and fish growth has been reported (Miyazono and Inoue, 1990).

There is also debate over whether digestibility is the best way to evaluate ingredients. An important limitation of digestibility techniques is the lack of discrimination between consumption, leaching and assimilation. In the assessment of dietary ingredients the latter is, of course, a key consideration. An alternative method of tracing the utilisation of formulated feeds by aquaculture species is to use the technique of stable isotope technique. To date, this refined technique has only been tested on penaeid prawns. This Project offers the opportunity to examine the potential of the stable isotope technique in nutritional studies of silver perch and to compare results with those obtained from digestibility determinations and following analyses of whole fish carcasses in synchronised ingredient evaluation trials.

Promising ingredients will be evaluated using the most appropriate techniques and practical, cost-effective techniques to improve the digestibility and availability of lower quality (compared to fish meal) ingredients will be developed. The involvement of the CSIRO Division of Food Science and Technology in this Project (and the entire Sub-Program) will enable fish nutritionists to utilise the latest technology to process ingredients to improve their value in fish diets. In particular, scientists at the CSIRO Division of Food Science and Technology are independently developing technology to fractionate grain legumes to isolate carbohydrate components for use in human foods. The by-products of these are protein enriched, carbohydrate reduced materials which may be cost-effective ingredients for fish feeds. Through contact with commercial food processors, industrial technology to cook or modify ingredients will also be accessed and evaluated. The Division is also purchasing the first pilot-scale, twin-screw extruder in Australia and the Sub-Program will have access to this unit. This extruder will be used to process ingredients and whole diets and be capable of handling small batches of about 50 kg. In the past, the minimum batch size for experimental diets needed for extrusion has been about 1 tonne, greatly restricting the ability of researchers to manipulate and evaluate the variety of factors affecting diet extrusion.

When high quality protein sources like fish meal are replaced with lower quality ingredients, deficiencies in essential nutrients are likely. This Project will identify which nutrients will be the most limiting and the most expensive to supply.

Protein which is supplied in excess of requirements for growth and metabolism is used for energy and might be replaced by other energy sources such as well-digested carbohydrates or fats (El-Sayed and Teshima, 1991; Murai, 1992). Determination of optimum protein contents and protein to energy ratios could lead to significant reduction in protein contents of diets. As alternative protein sources to fish meal are usually lower in protein, lower protein requirements would increase the choice of ingredients which could be considered as protein sources.

As the amino acid balance and fatty acid profile of alternative protein sources to fish meal is often inferior, requirements for limiting amino or fatty acids could also limit potential for fish meal replacement. Requirements for the most important limiting nutrients will be determined for silver perch.

Results from this project will be used to build a comprehensive feed data matrix for use in linear least-cost computer programs to formulate feeds for silver perch. These programs require information on nutritional requirements, and digestibility, restrictions, cost and availability of ingredients. At present these programs are run on assumptions about requirements and ingredients. This project will replace many of these assumptions with rigorous experimental data which will greatly improve the ability to formulate cost-effective diets for silver perch.

Two other projects relate to this one:

1. The Grain Research and Development Corporation funded a preliminary study to evaluate four oilseeds and grain legumes for silver perch. This project overlapped with the FRDC project by approximately six months.

2. The Australian Centre for International Agricultural Research (ACIAR) funded a collaborative project between NSW Fisheries and the Thailand Department of Fisheries entitled Replacing Fish meal in Aquaculture Diets. The ACIAR and FRDC projects had complementary aims but each focused on different aspects. The following table highlights the similarities and differences:

	ACIAR	FRDC
1 Species	Focus on one species in Thailand - hybrid catfish and one in Australia - silver perch (*Bidyanus bidyanus*)	Broad ranging covering penaeid prawns (*Penaeus monodon*), barramundi (*(Lates calcarifer*), silver perch (*Bidyanus bidyanus*) and Atlantic salmon (*Salmo salar*)
2 Feeding habits	Omnivores	One omnivore, rest carnivores
3 Aquaculture industry	Relatively low-value products for high volume production for domestic consumption. Silver perch production will reduce imports of low-value fish in Australia	High-value products, with export market potential
4 Primary beneficiaries	Small scale, low-income fish farmers in Thailand. Producers of relatively low-value fish in Australia. Commercial feed manufacturers and agricultural sector are secondary beneficiaries in Australia	Aquaculture industries generally. Focus on producers of high-value species (except for silver perch farmers). Feed manufacturers and agricultural sector joint primary beneficiaries.
5 Review and analysis of available and potential ingredients	Will be done in collaboration with FRDC Project	Will be done in collaboration with ACIAR Project
6 Evaluation of digestibility of potential ingredients & determination of maximum inclusion levels of these ingredients	Emphasis on currently available ingredients	Emphasis on new or 'improved ingredients' eg protein enriched fractions or specifically developed abattoir by-products
7 Improvements to ingredients	Evaluate commercially available amino acids	Evaluate processing (eg cooking and extrusion) of enzymes, and specifically developed amino acid supplements
8 Determination of nutritional requirements	Protein requirements and protein to energy ratios	Requirements for essential amino acids and, if necessary, essential fatty acids

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4 NEED

The development of cost-effective diets, with reduced contents of fish- and other aquatic-meals is an urgent priority for most fish and crustacean aquaculture industries. The problem is particularly important in Australia which has very poor supplies of aquatic meals. Consequently a number of research institutions and private companies are committed to research of this nature. Although there are a number of specific issues that must be addressed for each species (digestibility of different ingredients for example varies between species as do specific nutritional requirements) much of the research necessary to replace fish meal and develop cost-effective aquaculture diets is generic and will have general application to a number of species. A collaborative project involving a number of species. A collaborative project involving a number of separate research institutions will foster the interchange of ideas and standardisation of methods and will greatly improve the quality of the research and usefulness of the results.

This need was recognised by the Fisheries Research and Development Corporation who created the "Fish Meal Replacement in Aquaculture Diets Sub-Program" in 1993. This Sub-Program was managed by Dr Geoff Allan for NSW Fisheries and involved scientists and technicians from 13 institutions, including State and Commonwealth government research institutions, universities and private companies. Four key species were chosen; prawn, *Penaeus monodon*, barramundi, *Lates calcarifer*, silver perch, *Bidyanus bidyanus*, and Atlantic salmon, *Salmo salar*. Research was coordinated through six projects, one on each species, one on feed processing technology and a project on amino-acid supplementation (this was a technology audit specifically designed to examine the situation with crystalline amino acids and the potential to bioengineer dipeptides or aligopeptides for aquaculture use).

This project is for silver perch which is a freshwater native Australian fish. The omnivorous feeding habits of this species indicate it may be better able to utilise plant protein sources with high carbohydrate contents than other carnivorous aquaculture species.

Silver perch has been chosen as the species within the Sub-Program to use for evaluating the greatest number of potential feed ingredients. The ingredients with the most promise can then be evaluated for other species. The maximum amount of any ingredient which can be used in a diet will be determined by the composition of the ingredient, the digestibility of the ingredient, how well the ingredient is assimilated, the nutritional requirements of the species being fed and what other ingredients are in the diet. Many ingredients have "anti-nutrients" which will affect their use in diets.

Measuring the digestibility of potential ingredients is the first task in evaluation but, unfortunately, digestibility information is available for only a few aquaculture species (eg Channel catfish and rainbow trout). Without reliable data on digestibility, diets for most nutritional studies comparing different ingredients or estimating requirements may have very different digestible energy and digestible dry matter contents which confounds interpretation of results and can invalidate the research.

Following measurement of digestibility, an understanding of the assimilation of ingredients is crucial. This can be determined through growth assays.

Most alternative ingredients of fish meal will be deficient in composition, especially total crude protein and amino acid composition and/or presence of unwanted carbohydrate or ash Processing, through removal or alteration of carbohydrate (in case of plant ingredients) or ash (in case of terrestrial animal meals) can increase the potential use of alternative ingredients In some cases, the addition of lacking nutrients, eg amino or fatty acids, or the use of supplements to enhance attractability or digestibility of diets may also increase use of alternative ingredients to fish meal.

All diets are formulated using specifications of essential nutrients required by the target species These specifications have a major impact on which ingredients can be used However, requirements for aquaculture species have only been reliably designed for a few species Advances in the techniques used to estimate protein and energy requirements for pigs and poultry have led to major changes in the diet formulations for these species The new technology needs to be applied to fish.

5 OBJECTIVES

1 To identify potential feed ingredients to replace fish meal in aquaculture diets for silver perch

2 To evaluate promising ingredients in terms of their *in vitro* and *in vivo* digestibility and assimilation

3 To develop and evaluate methods of improving the usefulness of ingredients through processing (eg extrusion or cooking) and the use of enzymes and supplements

4 Identify areas where inadequate knowledge of nutritional requirements may restrict fish meal substitution and determine these requirements for silver perch.

5 To formulate and evaluate diets with reduced contents of fish meal for silver perch.

6 DETAILED RESULTS

6.1 Nutrient digestibility for juvenile silver perch *Bidyanus bidyanus* (Mitchell): development of methods

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Abstract

Three experiments were undertaken to develop methods for determining digestible energy, dry matter and protein of diets and feed ingredients for juveniles (5-15 g/fish) of the Australian freshwater fish, silver perch (*Bidyanus bidyanus*). The first experiment compared stripping, dissection and settlement as methods of collecting faeces. Stripping was not a suitable method for collecting digesta from juvenile silver perch ≤10 g/fish; insufficient sample was obtained and regurgitation of food contaminated samples. Digestible dry matter calculated using digesta collected by dissection were lower than values using faeces collected following settlement. Values using digesta extracted from the posterior portion of the intestinal tract were higher than those using digesta from the anterior portion. In the second experiment, separate digestibility coefficients were calculated using faeces collected by settlement every two hours (up to 18 h) after tanks were cleaned and these were compared with DCs calculated using faeces collected 6, 12 or 18 h after tanks were cleaned. Digestible energy and digestible dry matter increased with time. Digestible dry matter was similar when calculated using faeces collected over 12 or 18 h and greater than for faeces collected over 2 or 6 h. Digestible energy and digestible dry matter calculated using faeces collected every 2 h and averaged to give a value for 6, 12 or 18 h were similar to values calculated using faeces collected over 6, 12 or 18 h periods. This indicated that leaching did not affect digestibility coefficients after faeces had settled. The third experiment validated the assumption that digestibility coefficients for juvenile silver perch for different feed ingredients are additive. The sum of digestibility coefficients calculated separately for individual ingredients was similar to that calculated for a diet composed of those ingredients. These results demonstrate that collection of faeces by settlement over 18 h is a suitable method for determining digestibility in juvenile silver perch.

Keywords: Silver perch; Fish nutrition; Digestibility; Methods.

1. Introduction

Silver perch (*Bidyanus bidyanus*) are native Australian freshwater finfish with high potential for aquaculture. They readily accept pelleted diets, tolerate crowded conditions and perform well in earthen ponds (Rowland et al., 1994, 1995). Little has been published on their nutritional requirements, but they are omnivorous (Barlow et al., 1987) and there is potential to use agricultural feed ingredients to formulate low-cost, high-performance diets.

The nutritional value of ingredients used in aquaculture diets is dependent upon their composition and bio-availability to the species being fed. Variation in digestibility accounts

for most of the differences affecting the usefulness of ingredients used as energy and nutrient sources, since loss in digesta is the major loss of ingesta (Lovell, 1989; Cho and Kaushik, 1990). Measuring digestibility in fish is more difficult than in terrestrial animals as nutrients and organic matter can leach from the faeces into the water before collection. Various methods have been proposed to overcome this problem, including rapid removal of faeces from the fish tank (Choubert et al., 1982; Cho and Kaushik, 1990), removal of digesta by anal aspiration or following dissection (Windell et al., 1978; Austreng, 1978) and 'stripping', whereby digesta are removed from the rectum by squeezing the abdomen (Windell et al., 1978; Percival and Lee, 1996). When digesta are removed from the fish before they are voided naturally as faeces, incomplete digestion can lead to underestimating digestibility and contamination with various body fluids can cause other inaccuracies (Smith, 1979; NRC, 1993). Where faeces have been in contact with water, loss of dry matter and nutrients due to leaching can lead to over-estimation of digestibility (Smith, 1979; Cho and Kaushik, 1990; NRC, 1993). Leaching will be affected by the physical integrity and water stability of the faecal pellet, the period of time the faeces remain in the water eg the composition of the faeces and the water characteristics eg temperature, flow rate etc. before collection, the degree to which faeces are disturbed. Other factors may affect digestibility, such as ration size, feeding frequency and the time faecal samples are taken (De Silva and Perera, 1983; NRC, 1993).

An assumption behind the use of digestibility coefficients in diet formulation is that they are additive for different ingredients. This has been demonstrated using diets with a range of ingredients and faeces collected either by dissection or settlement (for rainbow trout, tilapia, channel catfish and ayu) (Cho et al., 1982; Wilson and Poe, 1985; Watanabe et al., 1996). No such confirmation has been published for silver perch.

The aims of this study were to compare methods of collecting faeces (or digesta) and the effect of collecting faeces on digestibility coefficients at different times after feeding. The assumption that digestibility coefficients of individual ingredients are additive was tested with a practical diet containing a mixture of plant and animal protein and carbohydrate sources. This research was undertaken to help develop reliable techniques to determine digestibility of different ingredients and diets for silver perch.

2. Materials and Methods

2.1. Experimental Fish

Silver perch were bred at Grafton Research Centre and raised in earthen ponds using similar techniques to those described by Thurstan and Rowland (1995). Before experiments, fish were fed the silver perch reference diet (SP35) of Allan and Rowland (1992) (Table 1) and were treated with 5 g/l^{-1} NaCl to ensure they were free of ectoparasites and to prevent fungal infection (Rowland and Ingram, 1991). Prior to stocking, fish were anaesthesised using a bath of 25 mg/l ethyl-þ-aminobenzoate for 5 min, then caught at random, weighed individually or in groups of 3-5 fish and systematically distributed among 27 tanks. Fish were also weighed at harvest.

2.2. Experimental Facilities

Digestibility tanks were 170 l cylindro-conical tanks (conical base sloped at 35°) fitted with a 65 mm diameter, 250 mm settlement chamber which tapered into a 12 mm diameter,

150 mm length of silicone tubing (Fig. 1). The inside of each tank was black. Continuously-flowing, preheated water was filtered through a sand filter then a diatomaceous earth filter. It was then passed through a UV sterilizer before being supplied to experimental tanks at a flow-rate of 600 ml/min. Effluent water from each tank exited via a 25 mm diameter external stand pipe. 20-25% of this effluent flowed to waste and the rest was collected and recirculated through a 2 000 l biological filter and reused. Each tank was aerated using two air-stone diffusers.

Fish were stocked seven days prior to the start of the faecal collection period to allow for acclimatisation to experimental conditions. During this period, fish were fed the reference diet SP35. Fish were fed to excess using automatic conveyor belt-type feeders for 3 h each day from 0900-1200 h. Within 1 h after feeding ceased, all uneaten food was removed, and the walls of the tank and the settlement chamber were thoroughly cleaned to remove any faeces, uneaten food or bacterial slime. The silicone tubing into which the faeces settled was packed in ice and kept at $\leq 4^{\circ}C$ prior to removal of faeces to reduce bacterial proliferation, which can affect the composition of faeces (Spyridakis et al., 1989). During experiments, dissolved oxygen (always above 6.3 mg/l), pH (between 7.7 and 8.3) nitrite and ammonia (<0.1 mg NO_2-N/l and 0.1 mg total ammonia - N/l respectively) were measured weekly using methods described in Allan et al. (1990).

2.3. Biochemical Analyses

Faecel samples were collected by settlement each morning and dried using silica gel dessicant under vacuum. Samples from each tank were pooled at the end of the experiment and re-dried by the same method. Each sample was freeze dried and ground using a water cooled total recovery grinder prior to analyses.

All chemical analyses of feed and faecal samples were done in duplicate. Dry matter, ash and energy (bomb calorimetry) were measured using the AOAC (1975) procedures, nitrogen using the Kjeldahl or semi-micro Kjeldahl methods (Allan and Frances, 1994) (crude protein = Nx6.25) and chromic oxide was determined by the method described in Scott (1978).

2.4. Digestibility Determinations

The indirect method of Cho and Kaushik (1990) was used to calculate apparent digestibility coefficients, with chromic oxide (1% dry weight basis) as the inert indicator. The apparent digestibility coefficients (ADC's) for energy, protein (Nx6.25) and essential amino acids in experimental diets were calculated as described by Cho and Kaushik (1990):
$ADC = [1-(F/D \times DC_r/FC_r)] \times 100$ where:

F = % nutrient or energy in faeces,
D = % nutrient or energy in diet,
DC_r = % chromic oxide in diet and
FC_r = % chromic oxide in faeces.

2.5. Experiment 1 - Methods of collecting faeces

Digestibility coefficients for dry matter, energy and nitrogen for the reference diet SP35 were calculated and compared using faeces collected by settlement over different periods or using digesta obtained following dissection. Attempts to obtain digesta by stripping were

abandoned as we were unable to collect sufficient quantities from the small fish used in this experiment, and samples were contaminated by regurgitated food. Six treatments were established: faeces collected by settlement 2, 6, 12 or 18 h after tanks were cleaned (Treatments 1 to 4); digesta collected by dissection; the intestinal tract, from the pyloric caeca to the anus, was removed, divided in half and the digesta in the anterior section and the posterior portions were analysed separately (Treatments 5 - anterior and 6 - posterior). Three randomly selected replicate tanks were used for Treatments 1-4, and three in total for Treatments 5 and 6 (15 tanks in total). Ten juvenile silver perch (5.2-6.2 g/fish) were stocked into each tank and after 7 days acclimatisation, faeces were collected daily for 12 days and combined before analyses (Treatments 1-4). Digesta were collected on the last day of faecal collection from fish killed with an overdose of ethyl-þ-aminobenzoate at 1430 h (Treatments 5 and 6). Mean water temperature was 26.3°C (range 25.9-27.2°C).

Differences in digestibility coefficients for dry matter were analysed using single factor ANOVA for Treatments 1-5 ($d.f = 4,10$) and for energy and nitrogen for Treatments 2-4 ($d.f = 3,8$). There were insufficient digesta from Treatments 1 and 5 to analyse for energy or nitrogen. Differences between digestibility coefficients for dry matter for Treatments 5 and 6 were also analysed using single factor ANOVA ($d.f = 1,4$). Variances were homogeneous (Cochrans Test; Winer, 1971) and Student Newman-Keuls (SNK) multiple range test was used to compare more than two means where significant differences ($P<0.05$) were found.

2.6. Experiment 2 - Comparison of settlement periods

Digestibility coeficients of dry matter, energy and nitrogen for the reference diet SP35 were calculated and compared using faeces collected by settlement every 2 h after tanks were cleaned (ie 0-2 h, 2-4 h, 4-6 h, 6-8 h, 8-10 h, 10-12 h, 12-14 h, 14-16 h, 16-18 h; Treatments 1 to 9 respectively) or 6, 12 or 18 h after tanks were cleaned (Treatments 10 to 12 respectively). Five juvenile silver perch (10.4-15.1 g/fish) were stocked into each tank (giving a similar fish biomass to that used in Experiment 1) and, after 7 days acclimatisation, faeces were collected for 17 days. For Treatments 1-9, each of the three replicates consisted of pooled faeces from five randomly selected tanks (15 tanks) while three separate, replicate tanks were used for treatments 10-12 (9 tanks, 24 tanks for the experiment). Mean water temperature was 25.0°C (range 23.8-26.5°C).

Digestibility coefficients for dry matter and nitrogen for these treatments were compared using single factor ANOVA ($d.f = 8,18$) and where significant differences were found ($P<0.05$) means were compared using Student Newman Keuls multiple range test. Variances were homogeneous by Cochrans test (Winer, 1971). Digestibility coefficients for dry matter and nitrogen from Treatments 2, 3 and 4 in Experiment 1 were compared with similar data from Treatments 10, 11 and 12 in Experiment 2 using two factor ANOVA with collection period and experiment number as the two fixed factors ($d.f = 2,1,8$). Digestible energy data from Experiments 1 and 2, calculated using faeces collected by settlement over 18 h after cleaning, were also compared using single-factor ANOVA. For Experiment 2, dry matter and nitrogen digestibility coefficients from Treatments 10, 11 and 12 (6, 12 and 18 h collections) were compared using single factor ANOVA ($d.f = 2,6$) and SNK tests. These data were also compared with the average of digestibility coefficients calculated using 2 hourly collection data for a 0-6 h period (Treatments 1-3; n=3), a 0-12 h period (Treatments 1-6; n=6) and a 0-18 h period (Treatments 1-9; n=9) in two-tailed t-tests.

2.7. Experiment 3 - Additivity of digestibility coefficients

The assumption that digestibility coefficients for different feed ingredients are additive and can be used to predict digestibility coefficients for diets was tested. Digestibility coefficients for each individual ingredient used in the reference diet SP35 was determined and multiplied by the proportion of that ingredient in the reference diet SP35. The sum of these, the calculated digestibility coefficients, should be equal to the digestibility coefficients determined for the entire diet if the assumption of additivity of digestibility coefficients is valid. This assumption was validated by comparing the mean of digestibility coefficients for reference diet SP35 from 12 previous digestibility experiments including experiment 1 and 2 of this study (from treatments where digestibility coefficients were calculated using faeces collected by settlement over 18 h) with calculated digestibility coefficients obtained in Experiment 3. For this analysis, a paired two-tailed t-test was used with the 'expected' values being the mean of the 12 digestibility coefficients from previous experiments and the 'observed' values being those calculated during this experiment.

Experiment 3 consisted of 9 treatments (Table 2) with 3 randomly selected, replicate tanks/treatment. In each treatment, tanks were labelled as replicate 1, replicate 2 or replicate 3 on the basis of their tank number. Three calculated digestibility coefficients were obtained by separately adding all the proportional digestibility coefficients from replicates 1, 2 and 3.

Faeces were collected by settlement over 18 h. The apparent digestibility coefficient (ADC) of each test ingredient = ADC of test diet - (ADC of reference diet SP35 x proportion of reference diet SP35 in test diet)/proportion of test ingredient in test diet (Cho and Kaushik, 1990).

Seven juvenile silver perch (9.8-11.2 g/fish) were stocked into each tank. After 7 days acclimatisation, faeces were collected and pooled for each tank for 12 days. Mean water temperature was 26.0°C (range 25.8-26.2°C).

3. Results

3.1. Experiment 1 - Methods of collecting digesta

Digestibility coefficients for dry matter, calculated using digesta dissected from the anterior half of the intestinal tract, were significantly lower than those calculated using digesta dissected from the posterior portion. Values calculated using digesta dissected from the posterior portion were significantly lower than any values calculated using faeces collected by settlement (Table 3). Digestible dry matter calculated using faeces collected by settlement was similar for faeces collected 2 or 6 h after cleaning, but significantly lower than for faeces collected 12 or 18 h after cleaning. Digestible energy for faeces collected by settlement was significantly higher for faeces collected 18 h after cleaning than for faeces collected 2 h after cleaning. Differences in digestible nitrogen were minor (values ranged from 90.3 - 92.3%) but significantly higher when calculated using faeces collected 12 h after cleaning compared with those collected 2 h after cleaning (Table 3).

3.2. Experiment 2 - Comparison of settlement periods

Digestibility coefficients for dry matter and energy calculated using faeces collected for separate two-hourly periods tended to increase with time (Table 4). Insufficient sample was obtained to analyse each replicate separately for energy so the samples for each time period

after 2-4 h were combined. For dry matter, digestibility coefficients for 0-2, 2-4 and 4-6 h periods were similar and significantly lower than for all other periods. Digestibility coefficients for dry matter for the settlement periods (8 h until 18 h) were similar. Differences for digestible nitrogen calculated using faeces collected every 2 h were not significant (P=0.45) regardless of when these were collected (Table 4).

The results of the two factor ANOVA indicated that digestibility coefficients for dry matter were affected by settlement period (P<0.01) but not by experiment number (Table 5). Conversely, digestibility coefficients for nitrogen were not affected by collection period but were different for each experiment (Table 5). There was no interaction (P>0.05) between experiment number and collection period for either set of data. Digestible energy calculated using faeces collected over 18 h after cleaning was similar for Experiments 1 and 2 (Data in Tables 3 and 4).

Digestibility coefficients calculated using faeces collected over 2 h periods and averaged to give values for 6 h (n=3), 12 h (n=6) or 18 h (n=9) were similar to values calculated using faeces collected over 0-6, 0-12 or 0-18 h for dry matter and nitrogen (Table 4). Values over 18 h for digestible energy were also similar regardless of whether faeces were left in the settlement chamber for the entire 18 h or collected every 2 h (Table 4).

3.3 Experiment 3

Digestibility coefficients for dry matter and energy were significantly higher for fish meal, cod liver oil, corn gluten meal, blood meal and soybean meal than for cereals, wheat, sorghum and millrun (Table 6). Digestible nitrogen was high for all ingredients (86-98%) but differences between ingredients were still significant (Table 6). Calculated digestibility coefficients derived during Experiment 3 were all within 5% and not significantly different (P>0.05) from values measured during 12 previous digestibility experiments, including Experiments 1 and 2 of this study (Table 6).

4. Discussion

Stripping was not an effective method of sampling digesta from juvenile (≤10 g) silver perch and digestibility coefficients calculated using digesta obtained by dissection were considerably lower than those calculated using faeces collected after settlement. The difference between digestibility coefficients calculated using digesta from the posterior or anterior sections of the intestinal tract support the conclusion that digestion occurs throughout the entire digestive tract (Spyridakis et al., 1989; Smith and Lovell, 1973; Austreng, 1978). Other problems noted with obtaining digesta prior to it being naturally voided as faeces (by stripping, anal suction or dissection) is the need to handle fish (sometimes with anaesthesia) which can affect intestinal transit (Spyridakis et al., 1989). Digesta can also be unintentionally taken from different positions in the intestinal tract which may increase variability. Despite these problems, stripping or dissection are the preferred methods of collecting faecal materials for some species, especially where the faeces are loosely bound (Vens-Cappell, 1985; M^{c}Meniman et al., 1996), or when compared with techniques which involved collection from the water and involved some disturbance to the faeces, eg by pipetting, siphoning (Smith and Lovell, 1973) or netting (Windell et al., 1978), or where faeces could lie on the bottom of the fish tank or in the path of flowing water for prolonged periods (Smith, 1971; M^{c}Meniman et al., 1996; Smith et al., 1980; Henken et al., 1985).

Collecting faeces after they have been voided permits leaching of dry matter and nutrients and can lead to overestimation of digestibility. Collection facilities which ensure rapid settlement of faeces (Hajen et al., 1993; Cho and Kaushik, 1990; Satoh et al., 1992) or continuous filtration from the water column (Choubert et al., 1979, 1982; Spyridakis et al., 1989), have been used to reduce this problem. Silver perch faecal pellets are well bound and settled to the terminal end of the collecting chambers used in the present study within 20-30 seconds of being voided by fish. Rapid settlement was facilitated by faeces only having to sink to the settlement chamber, without having to travel along horizontal or nearly horizontal pipe sections. The diameter of the pipe carrying the faeces was constricted immediately prior to connection with the settlement chamber and the diameter of the settlement chamber was increased to rapidly reduce the velocity of the outflowing water, facilitating rapid settlement of particles (see Fig 1). The similarity between the average of digestibility coefficients calculated using faeces collected every 2 h, and those calculated using faeces collected over an 18 h period after tanks were cleaned, indicates that leaching from faeces in the collecting chamber was not a significant pathway for loss of dry matter, nitrogen or energy and that a single collection over 18 h is sufficient. Similarly, Satoh et al. (1992) found minimal differences in lipid or protein digestibility when faeces from rainbow trout were collected using rapid settlement 3, 6, 9, 12 or 15 h after feeding. Conversely, Watanabe et al. (1996) concluded that leaching accounted for an increase in digestible energy coefficients for rainbow trout calculations using faeces collected at increasing periods after the last of three daily feeds. In this study, however, faeces were collected with some disturbance (by siphoning) and were held in a 4 cm wide tube at 17°C without cooling. Faeces left in collecting tubes would have been subject to bacterial decomposition which affects faecal composition (Spyridakis et al., 1989) and this may have increased digestibility coefficients.

Variability in digestibility coefficients has been reported between days (De Silva and Perera, 1983, 1984; Percival and Lee, 1996) and within days (Vens-Cappell, 1985; De Silva et al., 1990; Percival and Lee, 1996). Diets used in digestibility studies often contain unusual ingredients and inert markers and require some time to equilibrate in the digestive tract. Collecting faeces before this time may account for some variation between days, although De Silva and Perera (1983, 1984) and Percival and Lee (1996) all acclimatised fish for 7 days or more, in excess of that recommended (Windell and Norris, 1969; Cho et al., 1982). Lied et al. (1982) found representative faecal sampling could be obtained 72 h after the fish were first fed experimental diets.

Difference within days may be a result of 1) a differential passage of the marker and other dietary components, 2) random differences in digestion and or 3) differences in digestion due to gut retention period. While it is difficult to discount the first alternative, the consistent diurnal differences reported by De Silva and Perera (1983) when using three indigenous components as markers (hydrolysis resistant organic matter, crude fibre and hydrolysis resistant ash), suggests it is unlikely. The consistent pattern whereby digestibility increases with time after feeding, recorded in the present study and those by Lied et al. (1982), De Silva and Perera (1983), Vens-Cappell (1985) and Percival and Lee (1996), support the hypothesis that digestion increases with the time after feeding.

For digestibility estimations to be useful, faecal samples must be representative of those produced from the diet ingested. To account for both within and between day variation in digestibility, faeces should be collected over several days, if not weeks, and include the majority of faeces produced from each meal. If faecal material is sampled by dissection or

stripping, sufficient digesta should be taken at different times to obtain a representative sample.

The use of digestibility information in least-cost diet formulation assumes that digestibility coefficients for separate ingredients are additive. This was confirmed for rainbow trout by separately measuring digestibility of component ingredients of a reference diet and then comparing the sum of these individual components (on a proportional basis) with direct measurement of the complete diet (Cho et al., 1982). We adopted the same approach with our reference diet for silver perch and the similarity in the calculated and measured digestibility of the reference diet supports Cho's research and the assumption of additivity. Similar confirmation was obtained with four freshwater species, carp, rainbow trout, tilapia and ayu (Watanabe et al., 1996) and with channel catfish (Wilson and Poe, 1985). The reliability with which digestibility analysis of component ingredients can be used to predict digestibility of diets confirms the value of determining ingredient digestibility for use in practical diet formulation.

As silver perch faeces are well bound and settle rapidly, collection by settlement is a viable method for digestibility determination. Once faeces had settled into the 12 mm diameter tubing, which was held in ice, negligible leaching of dry matter or protein occurred over 18 h. For silver perch fed to excess once per day (over three hours), a diurnal rhythm in digestibility was observed. Digestibility coefficients for dry matter were lower when derived from faeces voided 2, 4 or 6 h after tanks were cleaned compared with coefficients calculated using faeces voided 8 h and up to 18 h after cleaning. Collection of faeces over the diurnal cycle is recommended. The assumption that digestibility coefficients are additive is valid for silver perch fed a range of vegetable and animal protein and carbohydrate ingredients.

Acknowledgements

We wish to thank Jane Frances, Rebecca Warner-Smith and Jo Pickles at Port Stephens Research Centre and Ken O'Brien and Wendy Peasley at Wollongbar Agricultural Institute for their excellent technical help. John Nell, Wayne O'Connor and Mark Booth made useful comments on the draft manuscript. This research was partially funded by the Australian Fisheries Research and Development Corporation under the Fish meal Replacement Sub-Program. Additional funding was provided to the Sub-Program by the Australian Centre for International Agricultural Research, the Australian Grains Research and Development Corporation and the Australian Meat Research Corporation.

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TABLE 1

Composition of reference diet (SP35)

Ingredient	Amount in SP35 (dry basis) (%)	Composition	
		Energy (MJ/kg)	Protein[1]
Fish meal	26.60	21.27	67.41
Soybean meal	20.19	18.86	48.11
Bloodmeal	2.04	22.56	88.55
Corn gluten meal	3.87	23.16	57.34
Wheat	27.47	17.84	15.89
Sorghum	11.21	16.96	10.87
Millrun	2.01	16.83	21.36
Cod liver oil	0.90	39.03	-
Vitamin premix[2]	0.97	-	
Mineral premix[3]	2.81	-	
Di-calcium phosphate	1.79	-	
DL-methionine	0.13	-	
SP35	-	19.08	39.79

[1] Nx6.25

[2] **Vitamin premix (A)**	**IU**	**mg/kg**
Retinol (A)	8 000	
Cholecalciferol (D3)	1 000	
α-Tocopherol acetate (E)	125	
Menadione sodium bisulphite (K3)		16.5
Thiamine HCl (B6)		10.0
Riboflavin (B2)		25.2
Pyridoxine HCl (B6)		15.0
Folic acid		4
Ascorbic acid (C)		1000
Ca-panothenate		55
Myo-inositol		600
Biotin (2%)		1
Choline chloride		1500
Nicotinamide		200
Cyanocobalamin (B12)		0.02
Ethoxyquin		150
Calcium propionate		25

[3] **Mineral premix**	**g/kg**
Calcium carbonate	7.5
Manganese sulphate	0.3
Zinc sulphate	0.7
Copper sulphate	0.06
Ferrous sulphate	0.5
Sodium chloride	7.5
Potassium iodate	0.002

TABLE 2

Composition of experimental diets used in Experiment 3 (dry basis)

Ingredient (g/100g)	Experimental diets								
	1	2	3	4	5	6	7	8	9
Reference diet (SP35)	99.0	69.3	69.3	69.3	69.3	69.3	69.3	69.3	79.2
Chromic oxide	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Fish meal[1]	-	29.7	-	-	-	-	-	-	-
Soybean meal[1]	-	-	29.7	-	-	-	-	-	-
Bloodmeal[1]	-	-	-	29.7	-	-	-	-	-
Corn gluten meal[1]	-	-	-	-	29.7	-	-	-	-
Wheat[1]	-	-	-	-	-	29.7	-	-	-
Sorghum[1]	-	-	-	-	-	-	29.7	-	-
Millrun[1]	-	-	-	-	-	-	-	29.7	-
Cod liver oil[1]	-	-	-	-	-	-	-	-	19.8

[1] As described in Table 1

TABLE 3

Digestibility coefficients calculated using faeces collected by settlement over different periods, or using digesta dissected from one of two sections of the intestinal tract

Collection method	Digestibility coefficient (%)		
	Dry matter[1]	Energy[1]	Nitrogen[1]
Settlement period (h)[2]			
2	55.7 ± 1.9^{b}	69.7 ± 0.1^{a}	90.3 ± 0.4^{a}
6	58.9 ± 1.4^{b}	70.0 ± 1.4^{a}	91.3 ± 0.6^{ab}
12	63.8 ± 1.0^{c}	74.0 ± 0.9^{ab}	92.3 ± 0.4^{b}
18	64.9 ± 1.0^{c}	76.0 ± 0.5^{b}	90.9 ± 0.2^{ab}
Dissection[3]			
Posterior	50.0 ± 1.7^{a}	-	-
Anterior	42.5 ± 1.2	-	-

1 Values are means ± sem (n=3). In each column, means with the same letter in the superscript were not significantly different (ANOVA; SNK, $P>0.05$).

2 Settlement period = hours after feeding ceased.

3 The intestinal tract (pyloric caeca to anus) was removed and digestibility coefficients calculated using digesta from the anterior half were compared to coefficients calculated using digesta from the posterior half (ANOVA; SNK).

TABLE 4

Digestibility coefficients for dry matter, energy and nitrogen for silver perch where faeces were collected by settlement every 2h, or 6, 12 and 18h after tanks were cleaned. Faeces were pooled over 17 days[1]

Settlement period (h)	Dry matter (%)		Energy (MJ/kg)		Nitrogen (%)	
	2 hourly collection[2]	Extended collection[3]	2 hourly collection[2]	Extended collection[3]	2 hourly collection[2]	Extended collection[3]
2	57.0 ± 1.7[a]		71.2 ± 1.1		86.8 ± 1.0[a]	
4	59.5 ± 0.9[a]		73.9 ± 0.4		88.4 ± 0.2[a]	
6	58.4 ± 0.6[a]	59.8 ± 1.6[a]	79.3	-	88.0 ± 0.2[a]	87.9 ± 0.6[a]
8	63.1 ± 0.5[b]		78.5		88.4 ± 0.1[a]	
10	65.0 ± 0.4[b]		79.8		88.9 ± 0.5[a]	
12	65.3 ± 0.3[b]	61.7± 1.8[a]	80.0	77.4	88.4 ± 0.2[a]	88.1 ± 0.5[a]
14	63.8 ± 1.2[b]		78.3		87.0 ± 0.3[a]	
16	65.7 ± 1.3[b]		78.5		87.6 ± 0.6[a]	
18	65.4 ± 1.3[b]	65.7 ± 1.7[a]	81.5	77.2 ± 0.5[a]	88.4 ± 0.5[a]	86.8 ± 0.7[a]

1 Values are means ± sem for n=3 replicate. In each column, means with the same letter in the superscript were not significantly different (P>0.05) (ANOVA; SNK).

2 Each replicate consisted of faeces pooled from 5 tanks (to allow collection of sufficient faeces). The same 5 tanks were sampled every 2 h for each replicate.

3 Replicates were faeces from separate tanks.

TABLE 5

Results of a two factor ANOVA to compare digestibility coefficients of experiments (Experiments 1 and 2) and settlement periods (6, 12 and 18 h after feeding had ceased)

	Digestibility coefficient[1]	
Experiment	Dry matter (%)	Nitrogen (%)
1	62.6 ± 1.1^{a}	91.5 ± 0.3^{b}
2	63.1 ± 1.2^{a}	87.6 ± 0.4^{a}
Settlement period (h)		
6	59.4 ± 1.0^{a}	89.6 ± 0.9^{a}
12	63.8 ± 0.7^{b}	90.2 ± 1.0^{a}
18	65.3 ± 0.9^{b}	88.9 ± 1.0^{a}

[1] Values are mean ± sem (n = 9 for experiment, and n = 6 for settlement period). The interaction between experiment number and settlement period was not significant ($P>0.05$). In each column, means for experiment (n=9) or settlement period (n=6) with the same letter in the superscript were not significantly different (ANOVA; SNK, $P>0.05$).

TABLE 6

Digestibility coefficients for SP35 diet determined and calculated with silver perch

Ingredient / Diet	Inclusion level (% dry basis)	Dry matter (%)		Energy (MJ/kg)		Nitrogen (%)	
		Dig. Coef.[1]	Proportional[2] Dig. Coef.	Dig. Coef.[1]	Proportional[2] Dig. Coef.	Dig. Coef.[1]	Proportional[2] Dig. Coef.
Ingredients (Exp. 3)							
Fish meal	26.6	91.6 ± 1.3^{d}	25.8 ± 0.4	101.3 ± 1.4^{c}	28.6 ± 0.4	94.2 ± 1.3^{bc}	26.6 ± 0.4
Soybean meal	20.2	80.5 ± 1.7^{c}	17.2 ± 0.4	83.3 ± 1.6^{b}	17.8 ± 0.3	95.4 ± 0.7^{c}	20.4 ± 0.2
Bloodmeal	2.0	98.7 ± 1.7^{de}	2.1 ± 0.1	104.3 ± 8.1^{c}	1.5 ± 0.8	92.4 ± 2.6^{abc}	2.0 ± 0.1
Corn gluten meal	3.9	98.3 ± 2.0^{de}	4.1 ± 0.1	96.4 ± 0.3^{c}	4.0 ± 0.1	97.7 ± 0.2^{c}	4.0 ± 0.1
Wheat	27.5	44.6 ± 2.8^{a}	13.0 ± 0.8	53.0 ± 1.3^{a}	15.5 ± 0.4	87.0 ± 2.4^{ab}	25.4 ± 0.7
Sorghum	11.2	44.1 ± 2.9^{a}	5.2 ± 0.3	52.2 ± 3.8^{a}	6.2 ± 0.5	87.2 ± 1.0^{ab}	10.4 ± 0.1
Millrun	2.0	55.5 ± 1.5^{b}	1.2 ± 0.1	55.8 ± 5.3^{a}	1.2 ± 0.1	86.0 ± 0.9^{a}	1.8 ± 0.1
Cod liver oil	0.9	106.5 ± 5.1^{e}	1.0 ± 0.1	122.2 ± 4.1^{d}	1.2 ± 0.1	-	-
Supplements[3]	5.7	-	-	-	-	-	-
Diet							
Reference (determined)[4]	100	66.6 ± 0.3^{a}-	76.8 ± 0.2^{a}		- 89.1 ± 0.2^{b}	-	
Reference (calculated Exp 3)[5]	100	69.7 ± 0.7^{a}	-	75.9 ± 1.2^{a}	-	90.6 ± 0.3^{a}	-
(%) Difference[6]		-4.7		1.2		-1.7	

1 Values are means ± sem for n=3 replicate tanks. In each column, means for ingredients (df= 7, 16; ANOVA; SNK) or diets (two tailed t-test) with the same letter in the superscript were not significantly different (P>0.05).

2 Digestibility coefficient x inclusion level /(100-Inclusion level of supplements). (Digestibility coefficients for supplements were not determined and were excluded from the calculated digestibility coefficients)

3 Included vitamin and mineral premix (Table 1), 1.79% di-calcium phosphate and 0.13% DL-methionine.

4 The determined digestibility coefficient is based on the average of the reference diets analysed from 12 digestibility experiments.

5 Sum of proportional digestibility coefficients

6 % Difference = (Reference diet [determined] - Reference diet [calculated]) / Reference diet (determined) *100.

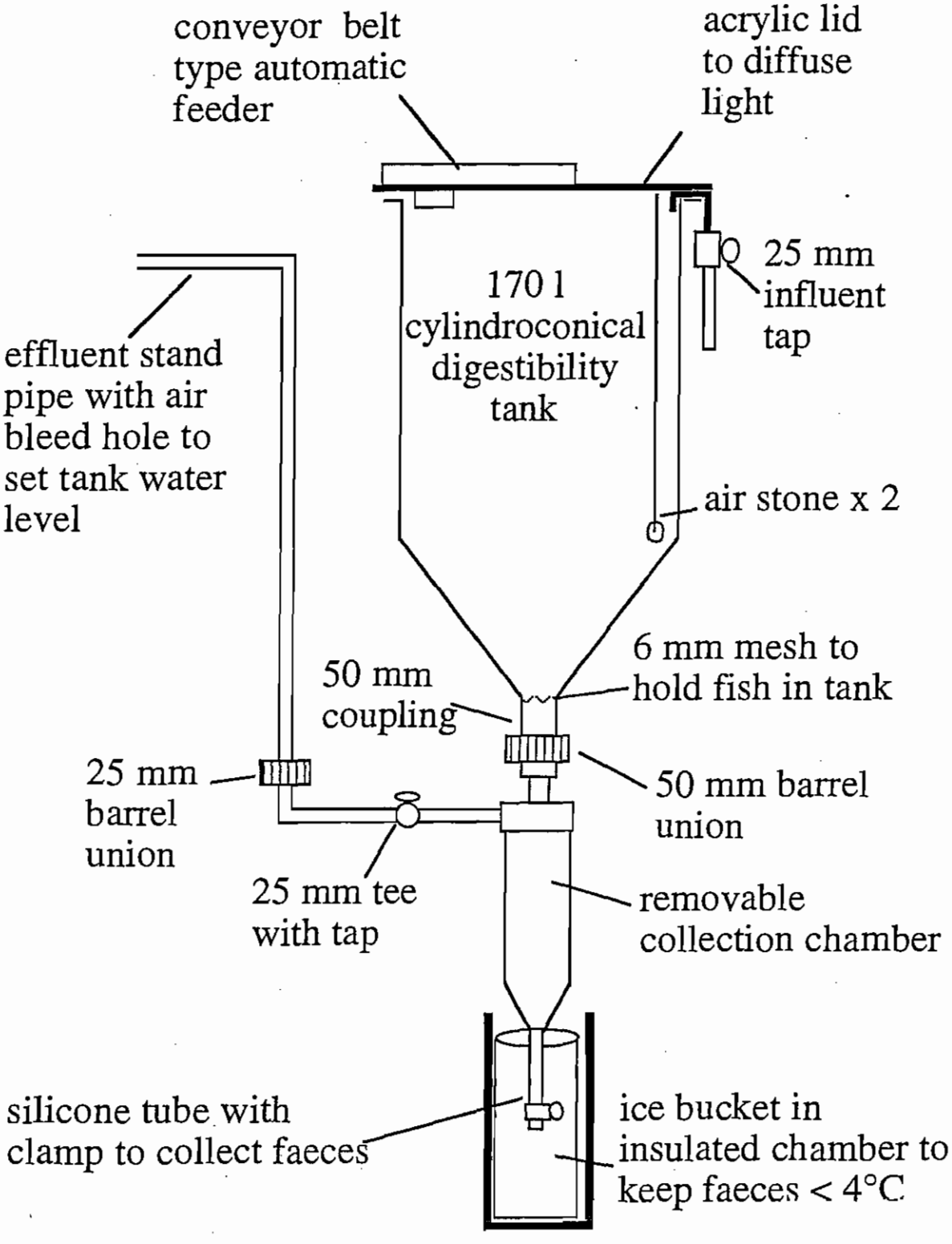

Figure 1 Digestibility tank system (not to scale), a 50 mm diameter stand pipe was used to prevent water loss when the collection chamber was removed for cleaning and faecal collection.

6.2 Replacement of fish meal in diets of silver perch: I. digestibility of alternative ingredients

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Abstract

The measurement of digestibility is critical to the international search for ingredients which, singly or in combination, can be used to reduce or replace fish meal in aquaculture diets. In this study we determined apparent digestibility coefficients (ADCs) for dry matter, nitrogen, energy and individual amino acids for 29 ingredients commercially available for use in formulated diets for fish or terrestrial animals. This research was conducted with silver perch, *Bidyanus bidyanus* (Mitchell), a native Australian freshwater species currently being cultured in Australia, Taiwan and China. ADCs were determined using faeces collected following settlement. Results from 10 experiments are reported. Each experiment included a reference diet and test diets which were composed of 69.3% reference diet, 29.7% test ingredient and 1% chromic oxide (inert indicator). Ingredients tested included Australian, Danish and Peruvian fish meal, bloodmeal, meat and bone meals from beef and lamb, poultry meal, feather meal, soybean and canola meals (both expeller and solvent extracted for each), full fat soybeans, peanut meal, cottonseed meal, linola, two species of lupins, field peas, faba beans, chick peas, vetch, cow peas, wheat gluten, corn gluten meal, two varieties of wheat, millrun and sorghum.

ADCs for dry matter, energy and nitrogen were highest for fish meal, although several other ingredients, including some animal meals and gluten from wheat and corn, had similar ADCs for dry matter and energy. Digestible protein from these ingredients was in the range 51-84% compared with 62-72% for fish meals. Silver perch were capable of digesting protein very effectively in almost all ingredients tested and amino acid digestibility reflected crude protein digestibility except for Peruvian fish meal and the two meat and bone meals, for which digestibility of some amino acids was lower, possibly indicating protein damage during processing. Oilseeds and legumes also tended to have lower ADCs for sulphur amino acids than for other amino acids and for oilseeds, slightly lower ADCs for histidine were recorded compared with ADCs for other amino acids.

Data provided a useful starting point for least-cost formulation of diets for silver perch. Differences in ADCs for nitrogen and individual amino acids indicates the need for individual amino acid digestibility data.

Keywords: Nutrition; *Bidyanus bidyanus*; Digestibility; Meatmeal; Legumes; Oilseeds; Cereals.

1. Introduction

The shift to more intensive culture practices contributed to a global increase in aquaculture production of about 9% per annum from 1984 to 1994 (Tacon, 1996). This shift has only been possible because of the increased availability of formulated diets. In Asia the demand for aquaculture diets increased more than four-fold between 1986 and 1990 (Akiyama, 1991) and New and Csavas (1993) predicted the market in Asia for aquaculture diets would reach 2.6 mt by 2000.

Marine based ingredients, especially fish meals are highly sought after as the protein source of choice for many formulated aquaculture diets. Fish meals provide high contents of essential amino and fatty acids, are low in carbohydrates, well digested and, provided they are fresh, contain few anti-nutritional factors. However, production of fish meal already uses approximately 30% of the total global catch and the proportion used for aquaculture is expected to rise to between 25 and 30% within the next decade (Tacon, 1996). As about 4 kg of wet fish is needed to produce 1 kg fish meal, if diets contain more than 17% fish meal and/or the food conversion ratio exceeds 1.5:1, aquaculture entails a net loss of fish protein.

The search for alternatives to fish meal is an international research priority (Manzi, 1989; Hardy and Kissil, 1997). In Australia, very little fish meal is produced although abundant supplies of lower value agricultural protein sources, such as animal meals, oilseeds, grain legumes and cereals are available. For example, over 460 000 t of meat meal, 178 000 t of canola, 1.2 mt of lupins, 456 000 t field peas and 14.7 mt of wheat, is produced annually (ABARE, 1994; Australasian Agribusiness Services, 1993).

The determination of digestibility is the first step in evaluating the potential of an ingredient for use in the diet for an aquaculture species. In this study digestibility coefficients for 29 commercially available ingredients in Australia was researched. Silver perch (*Bidyanus bidyanus*) is a native Australian freshwater finfish which is being cultured due to its rapid growth, high production in static earthen ponds and excellent eating qualities.

2. Materials and Methods

2.2. Fish

Juvenile silver perch were obtained from NSW Fisheries Grafton Research Centre and held at PSRC in 10 000 l concrete or 10 000 l fibreglass tanks for at least one week prior to each experiment. During this time, fish were fed a reference diet (SP35, Allan and Rowland, 1992) daily to satiation.

Fish were anaesthetised, using a bath of 25 mg/l ethyl-p-aminobenzoate for five minutes, weighed individually or in groups and stocked into digestibility tanks via random interspersion. Fish were fed the reference diet SP35 and allowed to acclimatise to experimental conditions for five to seven days prior to the introduction of chromic oxide marked experimental diets. Faecal collection commenced after fish had been fed experimental diets for at least three days.

2.2. Diets

All experimental diets were composed of 69.3% reference diet (SP35) (Table 1) and 29.7% test ingredient (Tables 2 and 3) on a dry weight basis. Chromic oxide was used as an inert marker and incorporated into the reference and experimental diets at 1% inclusion level. All ingredients and the reference diet were ground to ensure a maximum particle size of 710 μm, then thoroughly mixed in a Hobart mixer (Troy Pty Ltd, Ohio 45374, USA). Approximately 400 ml distilled water/kg dry mix was added to the dry mix prior to being pelleted through a meat mincer (Barnco Australia Pty Ltd Leichhardt 2040 NSW) with a 1, 1.5 or 2 mm die depending on size of experimental fish. Pellets were dried at <35°C in a convection drier for approximately six hours until the moisture content was between 10-15%.

2.3. Laboratory Facility

Twenty-seven 170 l cylindroconical digestibility tanks as described in Allan et al. (unpublished data, section 6.3 in this report) were used in each experiment. Pre-heated (25-26°C) water was passed through a sand filter, a diatomaceous earth filter and a UV steriliser prior to being supplied to digestibility tanks at a flow rate of 600 ml/min. 70% effluent water was recirculated via a 2 000 l biological filter, and 30% of effluent water was replaced with freshwater. Two air stone diffusers were provided to each tank for aeration.

During each experiment, nine treatments, eight experimental diets and the reference diet, were established, with three block-randomised replicates of each treatment.

Faecal samples were allowed to settle overnight (approximately 18 h) and collected each morning (Allan et al., unpublished data, see Section 6.1 in this report). Samples were dried under vacuum using silica gel and kept frozen throughout the experiment. Faecal samples from each tank were pooled, re-dried by the same method, then freeze dried and ground using a water cooled 1 KA total recovery grinder prior to biochemical analysis.

2.4. Water quality

Before each experiment, fish were treated with 5 g/l Na Cl to ensure they were free of ectoparasites and to prevent fungal infection (Rowland and Ingram, 1991). During all experiments, dissolved oxygen (above 6.3 mg/l), pH (between 7.7 and 8.3), nitrite and ammonia (<0.1 mg NO_2-NL and 0.1 mg total ammonia -N/l respectively) were measured weekly using methods described in Allan et al. (1990).

2.5. Biochemical analyses

All chemical analyses of feed and faecal samples were done in duplicate. Dry matter, ash and energy (bomb calorimetry) were measured using the AOAC (1975) procedures, nitrogen using the Kjeldahl or semi-micro Kjeldahl methods (Allan and Frances, 1994) (crude protein = Nx6.25) and chromic oxide was determined by the method described in Scott (1978). Amino

acids were analysed following acid hydrolysis using high pressure liquid chromatography and Waters Pico-Tag (Waters Pty Ltd, Lane Cove, NSW, 2066, Australia). Sulphur amino acids were determined separately following performic acid digestion, and tryptophan, which is lost during acid hydrolysis, was not determined (Cohen et al., 1989).

2.6. Digestibility determinations

The indirect method of Cho and Kaushik (1990) was used to calculate apparent digestibility coefficients, with chromic oxide (1% dry weight basis) as the inert indicator. The apparent digestibility coefficients (ADC's) for energy, protein (Nx6.25) and essential amino acids in the reference and experimental diets were calculated as described by Cho and Kaushik (1990): $ADC = [1-(F/D \times DC_r/FC_r)] \times 100$ where:

F = % nutrient or energy in faeces
D = % nutrient or energy in diet
Dc_r = % chromic oxide in diet and
Fc_r = % chromic oxide in faeces

The apparent digestibility coefficients for ingredients were calculated as described by Cho and Kaushik (1990). ADC of test ingredient = ADC of test diet - (ADC of reference diet SP35 x proportion of reference diet SP35 in test diet)/proportion of test ingredient in test diet.

3. Results

Composition of ingredients are tabulated (Tables 2 and 3). Except for the high protein glutens from wheat and corn, fish meals and other animal meals were generally higher in crude protein than oilseeds, legumes or cereals. Lysine was highest in bloodmeal and fish meals, followed by other terrestrial animal meals, oilseeds, legumes and cereals in that order. Gross energy value for all ingredients ranged from 16.1 for meat and bone meals (beef) to 24.1 MJ/kg for corn gluten meal.

Digestibility coefficients for dry matter, protein and energy and amino acids are tabulated (Tables 4 and 5). For dry matter, digestibility values exceeding 80% were recorded for fish meals (except Peruvian fish meal), bloodmeal, poultry products, expeller soybean meal, and gluten from wheat and corn. Values between 60-80% were recorded for Peruvian fish meal, other soybean meal products, peanut meal, lupins (*L. albus*) and field peas. Values for all other ingredients were above 40% except for linola (28%), high protein wheat (34%) and sorghum (34%). Protein was generally well digested in most ingredients with only meat meal and bone meal (from lamb and beef), linola and vetch yielding digestibility coefficients for protein below 80%.

Dry matter digestibility coefficients were reliable indicators for energy digestibility for all ingredients except for meat meal and bone meals with low dry matter digestibility (due to high ash content) but high energy digestibility and linola and vetch which also had low dry matter digestibility (due to poor protein digestibility) but high energy digestibility.

There were only minor differences in digestibility coefficients for different amino acids for most ingredients, with meat and bone meals (beef and lamb), cottonseed meal, and vetch very notable exceptions (Table 4). Meat and bone meals had much lower digestibility coefficients of glycine, proline, alanine and arginine compared with coefficients for the amino acids. For cottonseed meal, digestibility of leucine was extremely low (32%; the lowest coefficient for any amino acid for any ingredient) and for vetch, cystine, threonine, serine, phenylalanine and tyrosine were all relatively indigestible (Table 5).

The digestible composition of the ingredients tested are presented in Table 6 and 7. The highest digestible protein was for bloodmeal (84%) followed by wheat and corn gluten, fish meals, poultry meal and feather meal, oilseed meals, meat and bone meals, legumes and cereals in that order. The digestible dry matter and energy content of ingredients followed a similar trend.

4. Discussion

The preference for fish meal as the major protein source in formulated feeds for aquaculture is clearly supported by the very high digestibility of dry matter, energy, crude protein and amino acids. Results for silver perch agree well with high digestibility coefficients recorded for other species including rainbow trout (Cho et al., 1982; Smith, 1995; Gomes et al., 1995), chinook salmon (Hajen et al., 1993a), red drum (McGoogan and Reigh, 1996), hybrid striped bass (Sullivan and Reigh, 1995), channel catfish (Robinson, 1989; Wilson, 1991), European eel (Schmitz et al., 1984), tilapia (El-Sayed and Teshima, 1991; Luquet, 1991; Hanley, 1987) and carp (Jauncey, 1982).

Fortunately, digestibility of several other ingredients, including some animal meals, and protein extracts (gluten) for corn and wheat, was similar to fish meals for silver perch and for those ingredients with relatively high total protein content (eg. bloodmeal, poultry offal meal, feather meal and gluten meal), total digestible dry matter, and digestible energy were similar to fish meals and digestible protein was in the range 51-84%, compared with 62-72% for fish meals. The disadvantage of these well digested, high protein ingredients, compared with fish meal, was that the digestible essential amino acid contents and profile of these ingredients were inferior to fish meals.

Silver perch were capable of digesting protein very effectively in almost all ingredients tested. This finding supports results with most species (Ash, 1992; Cho et al., 1992; Sullivan and Reigh, 1995; McGoogan and Reigh, 1996). Protein digestibility coefficients over 95% were recorded for ingredients such as fish meal, soybean meal, peanut meal, lupins and wheat in which protein content ranged from about 15-73%. Ash (1985) also reported that protein digestibility was unaffected by the protein content. However, a positive correlation between protein digestibility and ingredient protein content has been reported for other species including trout and red drum (Smith et al., 1995; Serrano et al., 1992; McGoogan and Reigh, 1996). In the present study, relatively low protein digestibility coefficients were recorded for some plant ingredients with known anti-nutritional components, such as vetch (Petterson and Mackintosh, 1994) and canola (Evans, 1985) and some of the terrestrial animal meals. Excessive heat during the rendering process can damage proteins, especially lysine, and contribute to low protein digestibility of animal meals (Carpenter and Booth, 1973). In addition, digestibility of protein from bone, feathers and connective tissue may not be as well

digested as protein from muscle (NRC, 1993). McGoogan and Reigh (1996) reported that protein digestibility with red drum was highest for ingredients with less than 2% fibre but beyond this level fibre content (as indicative of carbohydrate content) and protein digestibility were not related. Conversely, Hepher (1985) reported protein digestibility was negatively correlated with the dietary carbohydrate content and Falge et al. (1978) proposed that high contents of carbohydrates reduce proteolytic enzyme activity. Wee (1992) concluded that undigested carbohydrate passed rapidly out of the gut taking some undigested protein with it, thus affecting protein digestibility. Protein digestibility coefficients of 92-100% for wheat with only 12-15% protein and over 80% carbohydrates (Novus, 1992) suggests that neither Falge et al.'s (1978) nor Wee's (1992) hypothesis apply to silver perch.

Digestibility coefficients for amino acids tended to reflect digestibility coefficients for crude protein for highly digestible ingredients such as Australian and Danish fish meal, bloodmeal, poultry offal meal and cereal gluten meals. For the lowest quality fish meal tested, Peruvian fish meal, digestibility coefficients for glycine and proline were approximately 10-20% lower than other amino acids or 12% lower than the digestibility coefficient for crude protein. The meat and bone meals have lower digestibility coefficients for lysine than any other fish or animal meals tested. This may indicate heat damage to lysine during the rendering process (Carpenter and Booth, 1973). Low digestibility coefficients for methionine plus cystine, arginine and particularly proline and alanine were also recorded. The same pattern of amino acid and digestibility coefficients was not evident for poultry offal meal or feather meal, possibly suggesting less protein damage during processing for these ingredients.

For blood meal, the imbalance in isoleucine and leucine (very low isoleucine and very high leucine) did not affect digestibility coefficients for these essential amino acids (83% and 89% respectively).

For oilseeds and legumes, digestibility coefficients for the sulphur amino acids tended to be lower than for other amino acids, compounding the problem of low sulphur amino acid content for most oilseed products (Swick, 1995). Slightly lower digestibility coefficients were also recorded for histidine for oilseed meals compared with other amino acids. This was most noticeable for linola for which the digestibility coefficient for histidine was only 30% which was the lowest digestibility coefficient recorded for any amino acid for any ingredient. Data on apparent amino acid availability indicates that this trend does not seem to occur with channel catfish (Robinson, 1989).

In common with most studies, dry matter and energy digestibility coefficients for silver perch were lower for plant ingredients with high carbohydrate contents (Cho et al., 1982; Hajen et al., 1993a; Wilson, 1991; Robinson, 1988; Sullivan and Reigh, 1995; McGoogan and Reigh, 1996; Gomes et al., 1995). Digestibility of carbohydrate in fish is affected by the type of carbohydrate and the digestive system in the fish (Wee, 1992). Digestibility decreases with an increase in carbohydrate structural complexity and some species are also poorly equipped to absorb digested carbohydrates (Wee, 1992). Clearly, removing or reducing the carbohydrate component, as in the wheat or corn gluten meals, results in major increases in dry matter and energy digestibility (Table 4). Allan et al., (unpublished data, section 6.3 in this report) found dehulling lupins removed a significant amount of non-starch polysaccharide and improved dry matter and energy digestibility for this grain legume.

Most fish studied have efficient lipid digestibility (Cho et al., 1982; Sargent et al., 1989; Sullivan and Reigh, 1995) and results reported here for silver perch indicate the energy from lipids in the fish meals, poultry offal and feather meals, and the wheat and corn gluten meals, were well digested. Lower values of digestibility coefficients for the two meat and bone meals may indicate lower digestibility of saturated lipids in these ingredients. McGoogan and Reigh (1995) also recorded low energy digestibility coefficients for meat and bone meal for red drum.

Comparison of digestibility coefficients for any ingredients between species is compromised because of differences in methodology used. Although several authors have reported close agreement in digestibility coefficients derived using different methods of obtaining faeces (eg. Hajen et al., 1993b; Cho et al., 1982), others have found large discrepancies (Smith et al., 1980). Provided the same method is used for different ingredients for a single species the comparison between ingredients is valid and this data is critical if diets are to be formulated from a variety of ingredients with balanced digestible energy and digestible nutrient contents (Cho et al., 1982; Cho and Kaushik, 1990; Gomes et al., 1995). As the assumption that digestibility coefficients calculated separately for different ingredients are additive for silver perch (Allan et al., unpublished data, section 6.5 of this report), the data presented here provide a useful starting point for the least-cost formulation of diets for silver perch. The differences in digestibility coefficients for different amino acids within a single ingredient, indicate the need for amino acid digestibility data when formulating diets which contain a range of ingredients .

Acknowledgements

We wish to thank the following NSW Fisheries staff for assistance with breeding silver perch, running experiments and preparing the manuscript: Charlie Mifsud, David Glendenning, Paul Robertson, Tanya Sushames, Jo Pickles and Helena Heasman. Ken O'Brien and Wendy Peasley (NSW Agriculture) carried out biochemical analysis. The manuscript was critically reviewed by Stewart Fielder and Mike Heasman. The research was part of the Australian Fisheries Research and Development Corporation Sub-Program on fish meal replacement (93/120). Additional support was also provided by the Australian Centre for International Agricultural Research, the Australian Grains Research and Development Corporation and the Australian Meat Research Corporation.

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TABLE 1.

Composition of the practical silver perch diet SP35.

Ingredient	Amount in SP35 (% dry basis)	[1] Vitamin premix (A)	IU	mg/kg	[2] Mineral premix	g/kg
Fish meal	26.20	Retinol (A)	8000		Calcium carbonate	7.5
Soybean meal	20.19	Cholecalciferol (D3)	1000		Manganese ulphate	0.3
Bloodmeal	2.04	α-Tocopheral acatate (E)		125	Zinc sulphate	0.7
Corn gluten meal	3.87	Menadione sodium bisulphate (K3)		16.5	Copper sulphate	0.06
Wheat	27.47	Thiamine HCl (B6)		10.0	Ferrous sulphate	0.5
Sorghum	11.21	Riboflavin (B2)		25.2	Sodium chloride	7.5
Millrun	2.01	Pyridoxine HCl (B6)		15.0	Potassium iodate	0.00
Cod liver oil	0.90	Folic acid		4		
Vitamin premix[1]	0.97	Ascorbic acid (C)		1000		
Mineral premix[2]	2.81	Ca-panothenate		55		
Di-calcium phosphate	1.79	Myo-inositol		600		
DL-methionine	0.13	Biotin (2%)		1		
		Choline chloride		1500		
		Nicotinamide		200		
		Cyanocobalamin (B12)		0.02		
		Ethoxyquin		150		
		Calcium propionate		25		

TABLE 2

Proximate composition and gross energy content (dry matter) of test ingredients.

Test Ingredient	Protein	Fat	Ash	GE	Expt
		(%)		(MJ/kg)	
Fish meals					
1 Australian fish meal	73.2	.NA	.NA	21.3	6
2 Danish fish meal	72.9	.NA	.NA	21.5	6
3 Peruvian fish meal	70.2	11.3	17.6	20.9	7
Animal Meals					
4 Bloodmeal	94.9	.NA	3.1	23.9	9
5 Meat & bonemeal (Beef)	49.2	9.2	36.0	16.1	5
6 Meat & bonemeal (Lamb)	54.3	7.2	34.5	16.2	5
7 Poultry meal	60.3	18.2	15.0	22.7	4
8 Feather meal	84.3	11.2	3.0	24.9	4
Oilseeds					
9 Soybean meal (solvent)	47.8	3.7	8.0	17.0	2
10 Soybean meal (expeller)	47.5	6.4	6.3	20.9	2
11 Soybean meal (full fat)	35.8	19.5	5.5	23.3	2
12 Canola meal (solvent)	36.6	2.6	7.4	19.9	2
13 Canola meal (expeller)	31.8	12.5	6.6	21.8	2
14 Peanut meal	41.2	1.3	5.2	19.7	11
15 Cottonseed meal	48.1	4.6	8.3	19.9	10
16 Linola	29.8	11.3	6.1	21.2	2
Legumes					
17 Lupins-L. *angustifolius*[2.]	34.1	5.7	2.8	17.9	3
18 Lupins-L. *albus*	37.6	6.2	3.7	20.9	3
19 Field pea (Dunn variety)	25.5	1.1	3.4	17.0	1
20 Faba bean	27.7	1.3	3.6	17.3	1
21 Chick pea	20.8	4.7	3.4	19.4	1
22 Vetch	30.9	0.9	3.3	17.9	1
23 Cow peas	25.2	2.3	3.7	18.8	8
Cereals					
24 Wheat gluten	76.9	.NA	.NA	23.1	6
25 Corn gluten meal	62.0	.NA	1.1	24.1	9
26 Wheat 1 (Aust. Std. Wheat)	12.2	.NA	.NA	18.3	6
27 Wheat 2 (High protein)	15.2	.NA	.NA	18.5	6
28 Millrun	22.3	.NA	4.3	19.6	9
29 Sorghum	14.5	.NA	2.3	18.8	9

Key: [2.] Gungarru variety.
.NA Not analysed.

TABLE 3

Amino acid composition (dry matter) of test ingredients

Test ingredient	Amino acid (%)																
	Lys	Meth	Cyst	Threo	Arg	Gly	Ser	Hist	Iso	Leuc	Phenyl	Tyro	Val	Pro	Ala	Glu	Asp
Fish meals																	
1 Australian fish meal	6.9	2.3	1.0	3.9	6.0	5.1	3.6	2.7	3.6	5.9	3.3	2.7	4.0	3.4	5.0	10.2	7.6
2 Danish fish meal	6.2	2.2	0.8	3.6	5.9	5.1	3.6	1.9	3.4	5.6	3.0	2.3	3.8	3.2	4.8	10.2	6.7
3 Peruvian fish meal	5.5	2.0	0.7	3.2	5.1	4.8	3.0	2.3	3.5	5.3	2.9	2.3	3.7	3.5	4.6	9.4	6.2
Animal meals																	
4 Bloodmeal	8.0	1.5	1.4	5.4	3.9	4.1	6.1	5.6	0.9	12.0	6.6	3.0	8.2	3.7	7.9	9.4	10.3
5 Meat & bonemeal (Beef)	2.5	0.7	0.3	1.6	3.9	7.7	2.1	0.8	1.3	2.7	1.5	1.1	2.0	5.0	3.9	5.7	3.3
6 Meat & bonemeal (Lamb)	3.5	1.1	0.7	2.1	4.3	6.7	2.4	1.2	1.8	3.5	1.9	1.5	2.4	4.6	4.0	7.0	3.8
7 Poultry meal	4.0	1.5	1.0	2.8	4.5	5.9	3.1	1.7	2.9	4.7	2.6	2.1	3.3	4.0	4.3	9.1	5.5
8 Feather meal	2.3	0.7	6.8	4.7	6.7	7.1	11.4	1.1	4.9	8.0	4.6	2.7	7.3	9.3	4.6	10.3	6.6
Oilseeds																	
9 Soybean meal (solvent)	3.3	0.7	0.9	2.1	3.7	2.1	2.9	1.3	2.5	3.8	2.6	1.9	2.6	2.6	2.1	9.3	5.8
10 Soybean meal (expeller)	3.0	0.8	1.0	2.1	3.7	2.1	2.9	1.3	2.4	3.8	2.5	1.8	2.5	2.5	2.1	9.2	5.7
11 Soybean meal (full fat)	2.3	0.6	0.8	1.5	2.6	1.5	2.2	0.9	1.7	2.8	1.8	1.3	1.8	1.8	1.5	6.6	4.1
12 Canola meal (solvent)	2.0	0.9	1.1	1.7	2.2	1.9	1.8	1.0	1.7	2.7	1.5	1.1	2.1	2.4	1.6	6.7	2.5
13 Canola meal (expeller)	1.7	0.9	1.1	1.5	2.1	1.7	1.7	0.9	1.5	2.3	1.3	1.0	1.8	2.1	1.5	6.2	2.4
14 Peanut meal	1.7	0.5	0.7	1.5	7.2	3.1	2.9	1.2	2.0	3.5	2.8	2.1	2.3	3.4	2.2	10.9	6.4
15 Cottonseed meal	2.1	1.0	1.1	1.8	6.0	2.0	2.4	1.4	1.7	3.0	2.7	1.5	2.1	2.2	1.9	10.7	4.8
16 Linola	1.2	0.7	0.7	1.2	2.9	1.9	1.7	0.7	1.4	1.9	1.5	0.9	1.7	1.3	1.4	6.3	3.0

TABLE 3 continued

Amino acid composition (dry matter) of test ingredients.

Test ingredient	Amino acid (%)																
	Lys	Meth	Cyst	Threo	Arg	Gly	Ser	Hist	Iso	Leuc	Phenyl	Tyro	Val	Pro	Ala	Glu	Asp
Legumes																	
17 Lupins-*L. angustifolius*[2]	1.4	0.2	0.6	1.3	4.0	1.4	2.1	0.9	1.4	2.4	1.3	1.4	1.3	1.6	1.2	8.2	3.8
18 Lupins-*L. albus*	1.5	0.3	0.9	1.5	4.1	1.5	2.3	0.9	1.7	2.8	1.4	1.8	1.6	1.7	1.3	8.4	4.3
19 Field pea (Dunn variety)	1.7	0.3	0.5	0.8	2.5	1.0	1.3	0.6	1.1	1.7	1.1	0.8	1.2	1.1	1.1	4.3	2.9
20 Faba bean	1.5	0.3	0.5	0.8	2.8	1.0	1.4	0.6	1.1	1.8	1.1	0.8	1.2	1.3	1.1	4.2	2.8
21 Chick pea	1.5	0.4	0.5	0.8	2.0	0.8	1.3	0.5	1.0	1.6	1.2	0.6	1.0	1.0	0.9	3.6	2.5
22 Vetch	1.7	0.3	0.5	0.8	2.4	1.1	1.4	0.6	1.2	1.9	1.1	0.8	1.3	1.2	1.2	5.1	3.4
23 Cow peas	1.8	0.4	0.3	1.0	2.2	1.1	1.4	0.8	1.2	2.0	1.4	0.9	1.3	1.3	1.1	4.7	3.0
Cereals																	
24 Wheat gluten	1.7	1.3	2.1	2.8	3.4	3.3	5.3	2.0	3.7	6.7	5.1	3.5	3.9	12.2	2.5	35.6	3.3
25 Corn gluten meal	1.1	1.6	1.4	2.2	2.0	1.6	3.6	1.1	3.0	11.3	4.1	3.4	3.1	5.8	5.8	14.6	4.2
26 Wheat 1 (Aust. Std. Wheat)	0.3	0.2	0.4	0.3	0.5	0.5	0.6	0.2	0.4	0.8	0.5	0.3	0.5	1.1	0.4	3.3	0.6
27 Wheat 2 (High protein)	0.3	0.3	0.5	0.4	0.6	0.5	0.7	0.3	0.5	1.0	0.6	0.5	0.6	1.4	0.5	4.4	0.7
28 Millrun	1.0	0.5	0.6	0.8	1.5	1.1	1.1	0.4	0.7	1.4	0.8	0.6	1.0	1.3	1.1	4.1	1.6
29 Sorghum	0.3	0.3	0.3	0.5	0.6	0.4	0.7	0.3	0.6	1.8	0.7	0.6	0.7	1.2	1.2	3.1	1.0

TABLE 4

Mean percent (±se) apparent digestibility coefficients for dry matter, crude protein and gross energy of the test ingredients using juvenile silver perch and the settlement method of faeces collection.

Test ingredient	Apparent digestibility (%)		
	Dry matter	Protein	Energy
Fish meals			
1 Australian fish meal	76.5±2.1	95.7±0.6	93.0±2.4
2 Danish fish meal	91.1±1.1	98.6±1.2	102.1±0.6
3 Peruvian fish meal	74.3+0.2	88.8±2.0	89.5±0.6
Animal meals			
4 Bloodmeal	106.4±1.0	88.2±1.2	105.3±1.1
5 Meat & bonemeal (Beef)	48.1±3.3	68.9±2.2	76.4±1.4
6 Meat & bonemeal (Lamb)	53.3±0.5	70.1±0.3	81.4±0.7
7 Poultry meal	83.7±1.7	84.5±2.2	96.4±1.5
8 Feather meal	102.6±1.0	93.3±1.1	105.3±0.6
Oilseeds			
9 Soybean meal (solvent)	73.1±2.0	95.3±0.8	81.5±1.5
10 Soybean meal (expeller)	81.4±0.3	97.2±0.1	85.2±0.3
11 Soybean meal (full fat)	74.9±2.9	92.1±1.7	78.7±2.0
12 Canola meal (solvent)	49.8±3.5	83.1±1.2	56.9±2.9
13 Canola (expeller)	45.3±4.7	81.5±2.7	56.2±2.9
14 Peanut meal	72.0±5.0	100.9±5.7	80.1±3.5
15 Cottonseed meal	48.4±4.3	86.7±1.0	52.4±3.0
16 Linola	28.5±4.0	79.2±1.1	38.0±3.2
Legumes			
17 Lupins-*L. angustifolius*[2]	50.3±3.0	96.6±0.9	59.4±1.0
18 Lupins-*L. albus*	64.7±0.4	96.1±0.9	72.7±1.8
19 Field pea (Dunn variety)	62.0±0.4	83.3±0.3	67.0±0.2
20 Faba bean	55.9±0.3	91.7±1.3	62.3±0.4
21 Chick pea	48.7±0.8	84.8±1.1	53.6±0.8
22 Vetch	41.5±3.2	74.9±2.6	55.5±1.0
23 Cow peas	40.6±8.4	93.0±1.0	45.8±5.9
Cereals			
24 Wheat gluten	97.2±2.9	113.9±1.8	118.8±1.7
25 Corn gluten meal	110.7±0.7	96.7±0.9	111.2±0.4
26 Wheat 1 (Aust. Std. Wheat)	49.9±1.8	94.0±0.7	55.2±1.2
27 Wheat 2 (High protein)	34.1±4.3	99.5±0.5	36.7±3.5
28 Millrun	51.2±3.6	87.9±0.3	55.6±3.1
29 Sorghum	34.6±0.8	86.0±0.3	38.8±0.7

TABLE 5

Mean percent (± sem) apparent digestibility coefficients for amino acids of the test ingredients using juvenile silver perch and the settlement method of faecal collection.

Test ingredient	Apparent Digestibility (%)																
	Lys	Meth	Cys	Threo	Arg	Gly	Ser	Hist	Iso	Leuc	Phenyl	Tyro	Val	Pro	Ala	Glu	Asp
Fish meals																	
1 Australian fish meal	99.9 ±0.1	96.3 ±0.5	97.0 ±2.2	96.3 ±0.6	98.5 ±0.7	90.1 ±2.3	94.6 ±0.4	102.1 ±1.3	100.7 ±0.5	99.6 ±0.6	97.9 ±0.6	96.3 ±0.3	99.4 ±0.5	87.1 ±1.0	94.3 ±1.3	98.0 ±0.4	98.7 ±0.5
2 Danish fish meal	101.8 ±0.3	104.2 ±0.4	103.2 ±1.0	99.2 ±0.3	104.1 ±0.4	107.3 ±0.3	99.0 ±1.5	101.5 ±0.5	102.0 ±0.2	100.9 ±0.4	99.6 ±0.1	97.2 ±0.8	102.8 ±0.2	97.1 ±1.0	102.8 ±0.3	100.4 ±0.3	100.5 ±1.2
3 Peruvian fish meal	96.1 ±0.8	91.0 ±1.4	93.2 ±1.1	92.5 ±0.8	89.6 ±0.7	76.0 ±1.8	89.9 ±1.4	96.2 ±1.0	93.9 ±0.7	94.6 ±0.8	92.1 ±1.0	92.2 ±1.0	93.7 ±0.7	76.9 ±0.5	88.3 ±0.8	93.6 ±0.5	93.9 ±0.8
Animal meals																	
4 Bloodmeal	89.8 ±1.2	91.3 ±1.4	85.4 ±1.7	91.1 ±1.7	90.7 ±1.2	91.4 ±1.7	92.4 ±1.6	92.2 ±1.4	82.7 ±2.4	88.5 ±1.7	91.2 ±1.9	89.4 ±1.7	87.0 ±1.8	89.6 ±1.6	90.7 ±2.0	90.9 ±1.3	91.1 ±1.5
5 Meat & bonemeal (Beef)	77.4 ±1.0	86.4 ±0.9	64.1 ±7.9	76.6 ±0.7	68.8 ±0.9	49.6 ±2.9	71.5 ±0.3	77.5 ±0.3	78.0 ±0.7	79.6 ±0.5	77.5 ±0.9	83.5 ±0.5	75.8 ±0.5	62.4 ±1.7	62.3 ±1.8	77.3 ±0.5	79.1 ±0.9
6 Meat & bonemeal (Lamb)	81.8 ±1.1	85.1 ±1.5	76.3 ±8.7	81.6 ±0.5	69.6 ±1.7	49.1 ±2.9	76.8 ±1.3	87.3 ±0.3	83.4 ±1.0	84.2 ±0.9	82.9 ±0.9	85.7 ±0.4	80.2 ±1.4	65.0 ±2.3	66.4 ±2.2	82.6 ±0.8	87.5 ±0.7
7 Poultry meal	85.7 ±2.4	87.6 ±1.7	83.3 ±2.9	82.1 ±02.7	85.8 ±1.5	78.4 ±2.3	84.5 ±2.7	89.2 ±2.5	82.0 ±2.8	84.9 ±2.4	82.9 ±2.3	83.5 ±2.3	81.9 ±2.5	79.5 ±1.8	82.6 ±1.8	87.1 ±2.2	83.9 ±3.0
8 Feather meal	89.7 ±1.0	95.8 ±0.1	95.2 ±0.4	92.0 ±0.8	97.1 ±0.7	97.7 ±1.2	98.6 ±1.0	90.2 ±1.3	93.9 ±1.1	93.2 ±0.9	94.6 ±0.9	89.4 ±1.1	92.1 ±1.1	93.9 ±1.1	93.5 ±1.1	93.0 ±0.8	89.3 ±0.9
Oilseeds																	
9 Soybean meal (solvent)	98.1 ±0.6	96.4 ±1.4	95.9 ±1.0	96.2 ±0.7	99.9 ±0.8	94.9 ±1.5	97.1 ±0.7	98.3 ±0.9	96.2 ±0.8	95.5 ±0.9	96.9 ±0.7	97.9 ±1.0	95.8 ±1.0	98.3 ±1.3	94.8 ±1.3	98.0 ±0.6	97.7 ±0.2
10Soybean meal (expeller)	97.3 ±0.5	97.6 ±0.3	90.4 ±0.9	96.5 ±0.5	99.6 ±0.3	91.9 ±0.8	96.6 ±0.5	99.1 ±0.4	98.5 ±0.6	96.9 ±0.5	98.7 ±0.3	98.1 ±0.8	97.7 ±0.6	98.1 ±0.9	94.1 ±0.4	97.5 ±0.2	97.3 ±0.6
11 Soybean meal (full fat)	95.3 ±0.8	95.5 ±1.2	95.5 ±1.9	94.1 ±0.6	96.0 ±0.8	88.0 ±2.1	95.3 ±0.7	94.4 ±0.7	91.6 ±0.7	92.4 ±0.7	93.6 ±0.8	94.6 ±0.6	90.9 ±1.1	94.3 ±1.5	91.4 ±1.0	94.8 ±0.4	92.8 ±0.6
12 Canola meal (solvent)	90.8 ±0.5	90.5 ±1.3	76.9 ±5.0	91.2 ±0.6	95.0 ±0.6	90.9 ±1.6	90.5 ±1.1	96.3 ±0.8	88.7 ±0.9	92.6 ±0.9	92.4 ±0.8	93.1 ±0.7	88.5 ±1.0	92.5 ±1.0	91.9 ±1.2	94.3 ±0.8	89.4 ±1.3
13 Canola meal (expeller)	90.4 ±1.2	87.9 ±3.2	74.1 ±5.2	87.3 ±1.4	94.0 ±1.4	87.4 ±2.9	87.1 ±1.7	93.8 ±0.9	88.1 ±2.1	91.4 ±1.5	91.2 ±1.7	90.5 ±1.7	88.1 ±1.8	89.5 ±2.4	89.6 ±2.1	93.2 ±1.0	88.0 ±1.4
14 Peanut meal	93.6 ±1.8	97.4 ±1.5	98.7 ±2.9	91.7 ±2.1	97.9 ±1.3	85.4 ±4.3	93.2 ±2.0	95.3 ±1.9	92.6 ±2.3	92.9 ±2.2	95.6 ±2.1	96.6 ±5.0	93.0 ±2.0	91.7 ±5.6	92.7 ±2.3	95.2 ±1.2	95.1 ±1.1
15 Cottonseed meal	71.4 ±0.4	74.9 ±4.8	68.6 ±4.1	74.7 ±1.3	87.7 ±0.7	75.4 ±1.6	76.1 ±2.3	83.1 ±2.8	74.5 ±1.4	31.7 ±2.1	77.1 ±1.1	78.0 ±1.3	73.7 ±1.7	78.6 ±2.1	73.4 ±1.2	84.3 ±1.0	80.3 ±2.5
16 Linola	88.8 ±0.7	94.7 ±0.6	78.3 ±1.9	83.5 ±1.4	88.7 ±1.2	72.0 ±1.6	82.6 ±1.0	84.4 ±1.1	85.4 ±1.2	86.5 ±0.5	86.2 ±1.3	87.0 ±1.0	84.0 ±1.4	83.3 ±1.0	82.2 ±1.2	87.0 ±0.7	78.9 ±1.0

TABLE 5 continued.

Mean percent (± sem) apparent digestibility coefficients for amino acids of the test ingredients using juvenile silver perch and the settlement method of faecal collection.

Test ingredient	Apparent Digestibility (%)																
	Lys	Meth	Cyst	Threo	Arg	Gly	Ser	Hist	Iso	Leuc	Phenyl	Tyro	Val	Pro	Ala	Glu	Asp
Legumes																	
17 Lupins-*L. angustifolius*[2]	98.1 ±1.5	83.9 ±6.8	89.7 ±2.5	95.8 ±1.9	102.9 ±1.3	100.0 ±2.7	91.4 ±2.7	100.6 ±2.5	95.4 ±2.1	94.9 ±2.0	96.0 ±2.2	95.4 ±2.6	94.6 ±1.9	94.2 ±3.5	96.4 ±1.7	95.5 ±1.7	88.0 ±2.5
18 Lupins-*L. albus*	96.6 ±0.4	92.2 ±1.6	84.4 ±5.8	97.3 ±0.5	102.6 ±0.7	101.8 ±2.2	95.0 ±0.9	98.3 ±2.1	91.8 ±1.2	94.4 ±0.9	94.8 ±0.9	95.6 ±0.5	91.2 ±1.5	96.4 ±1.2	97.1 ±1.3	96.0 ±1.3	93.2 ±1.3
19 Field pea (Dunn variety)	86.3 ±1.1	87.5 ±1.7	63.1 ±1.2	80.5 ±1.2	88.7 ±1.1	76.8 ±0.9	76.9 ±0.6	82.4 ±2.4	81.9 ±1.7	84.5 ±1.5	82.9 ±1.3	81.9 ±0.8	80.8 ±1.2	78.6 ±0.1	83.2 ±1.1	86.9 ±0.6	83.2 ±1.2
20 Faba bean	90.9 ±0.3	93.3 ±0.7	80.9 ±0.6	87.8 ±1.1	94.2 ±1.0	84.9 ±0.2	89.0 ±0.5	89.3 ±1.6	86.5 ±0.8	90.7 ±1.8	89.2 ±0.7	87.9 ±0.5	87.1 ±0.7	86.3 ±0.4	89.8 ±0.8	91.6 ±0.3	87.4 ±0.4
21 Chick pea	80.5 ±0.8	85.3 ±1.2	65.2 ±3.0	67.9 ±1.3	81.2 ±0.7	70.7 ±1.3	69.8 ±0.9	76.8 ±1.3	69.3 ±1.1	75.8 ±0.7	70.8 ±1.0	72.1 ±1.3	70.5 ±0.9	71.0 ±1.0	74.7 ±0.7	78.5 ±0.4	71.6 ±0.7
22 Vetch	72.7 ±1.4	77.8 ±0.9	50.1 ±8.7	56.6 ±0.8	76.6 ±0.5	66.5 ±1.0	60.0 ±1.4	72.1 ±0.9	66.3 ±0.8	71.1 ±0.4	62.8 ±0.8	62.2 ±1.0	65.8 ±0.4	63.4 ±0.8	70.7 ±1.1	73.9 ±0.2	68.4 ±1.4
23 Cow peas	83.3 ±1.2	91.7 ±0.9	93.2 ±2.8	84.6 ±1.0	87.5 ±1.0	74.6 ±2.3	87.6 ±1.2	85.0 ±1.8	78.2 ±1.8	82.0 ±1.3	77.0 ±1.9	77.4 ±2.1	79.7 ±1.3	85.6 ±1.1	83.6 ±1.3	87.3 ±0.8	86.2 ±1.1
Cereals																	
24 Wheat gluten	103.2 ±0.8	109.5 ±1.2	120.4 ±0.4	106.7 ±0.5	108.6 ±0.5	113.6 ±1.2	115.0 ±0.1	111.4 ±0.3	113.5 ±0.7	112.3 ±0.5	114.5 ±0.1	109.4 ±0.1	112.0 ±0.8	125.4 ±1.4	106.3 ±1.0	112.3 ±0.3	103.1 ±1.0
25 Corn gluten meal	93.1 ±0.7	94.6 ±0.8	91.5 ±1.3	95.7 ±0.5	97.2 ±0.5	94.0 ±1.0	98.4 ±0.5	96.6 ±0.5	94.8 ±1.1	101.6 ±0.5	101.3 ±0.7	99.3 ±0.4	95.9 ±0.8	101.4 ±0.5	102.0 ±0.8	99.5 ±0.5	95.5 ±0.6
26 Wheat 1 (Aust. Std. Wheat)	92.4 ±0.4	95.3 ±0.6	84.6 ±3.1	92.7 ±0.9	90.4 ±0.7	79.5 ±0.7	91.8 ±1.4	92.7 ±1.1	90.7 ±0.7	93.9 ±0.9	92.9 ±0.8	91.1 ±0.7	88.8 ±1.2	92.7 ±0.6	85.5 ±0.7	93.7 ±0.6	84.0 ±0.5
27 Wheat 2 (High protein)	97.3 ±0.6	101.6 ±0.6	91.6 ±2.3	89.0 ±0.9	98.0 ±0.7	96.9 ±1.1	97.8 ±0.5	96.6 ±0.8	97.0 ±0.9	95.4 ±0.6	96.6 ±1.1	92.4 ±0.5	93.1 ±0.7	102.4 ±0.8	92.0 ±1.0	97.1 ±0.5	83.5 ±0.5
28 Millrun	91.4 ±1.0	91.9 ±1.7	78.5 ±0.9	85.6 ±1.4	91.0 ±1.2	85.1 ±0.8	86.7 ±1.0	92.8 ±1.8	89.4 ±1.4	88.4 ±1.1	90.2 ±1.0	86.4 ±1.5	87.7 ±1.2	92.5 ±0.6	86.5 ±0.8	92.6 ±0.8	87.2 ±1.2
29 Sorghum	92.5 ±0.8	89.2 ±1.6	73.3 ±4.5	87.9 ±2.3	91.9 ±1.6	86.7 ±2.1	88.2 ±2.2	92.3 ±0.7	90.9 ±1.5	90.7 ±1.2	92.6 ±1.0	88.9 ±1.9	89.9 ±1.6	90.9 ±1.5	88.6 ±1.8	92.8 ±1.2	89.6 ±2.0

TABLE 6

Digestible dry matter, protein and energy values (dry basis) of the test ingredients using juvenile silver perch and the settlement method of faeces collection.

Test ingredient	Digestible dry matter (%)	Digestible protein (%)	Digestible energy (MJ/kg)
Fish meals			
1 Australian fish meal	76.5	70.1	19.8
2 Danish fish meal	91.1	71.9	21.5
3 Peruvian fish meal	74.3	62.3	18.7
Animal meals			
4 Bloodmeal	100.0	83.7	23.9
5 Meat & bonemeal (Beef)	48.1	33.9	12.3
6 Meat & bonemeal (Lamb)	53.3	38.1	13.2
7 Poultry meal	83.7	50.9	21.9
8 Feather meal	100.0	78.7	24.9
Oilseeds			
9 Soybean (solvent)	73.1	45.6	13.9
10 Soybean (expeller)	81.4	46.1	17.8
11 Soybean (full fat)	74.9	33.0	18.3
12 Canola (solvent)	49.8	30.4	11.3
13 Canola (expeller)	45.3	25.9	12.3
14 Peanut meal	72.0	41.2	15.8
15 Cottonseed meal	48.4	41.7	10.4
16 Linola	28.5	23.6	8.0
Legumes			
17 Lupins-*L. angustifolius*[2]	50.3	33.0	10.6
18 Lupins-*L. albus*	64.7	36.1	15.2
19 Field pea (Dunn variety)	62.0	21.2	11.4
20 Faba bean	55.9	25.4	10.8
21 Chick pea	48.7	17.6	10.4
22 Vetch	41.5	23.1	9.9
23 Cow peas	40.6	23.4	8.6
Cereals			
24 Wheat gluten	97.2	76.9	23.1
25 Corn gluten meal	100.0	60.0	24.1
26 Wheat 1 (Aust. Std. Wheat)	49.9	11.5	10.1
27 Wheat 2 (High protein)	34.1	15.1	6.8
28 Millrun	51.2	19.6	10.9
29 Sorghum	34.6	12.5	7.3

Digestible nutrient values are calculated on a 100% basis where digestibility coefficients exceed this value (Table 4).
Digestible nutrient = nutrient composition x ADC of nutrient.

TABLE 7

Digestible amino acid values (dry basis) of the test ingredients using juvenile silver perch and settlement method of faecal collection.

Test ingredient	Digestible Amino Acid (%)																
	Lys	Meth	Cyst	Threo	Arg	Gly	Ser	Hist	Iso	Leuc	Phenyl	Tyro	Val	Pro	Ala	Glu	Asp
Fish meals																	
1 Australian fish meal	6.9	2.2	1.0	3.7	5.9	4.6	3.4	2.7	3.6	5.9	3.2	2.6	4.0	3.0	4.7	10.0	7.5
2 Danish fish meal	6.2	2.2	0.8	3.6	5.9	5.1	3.6	1.9	3.4	5.6	3.0	2.2	3.8	3.2	4.8	10.2	6.7
3 Peruvian fish meal	5.3	1.9	0.7	2.9	4.6	3.6	2.7	2.2	3.2	5.1	2.6	2.1	3.5	2.7	4.0	8.8	5.9
Animal meals																	
4 Bloodmeal	7.2	1.3	1.2	4.9	3.5	3.7	5.6	5.2	0.7	10.6	6.1	2.7	7.1	3.3	7.1	8.5	9.4
5 Meat & bonemeal (Beef)	2.0	0.6	0.2	1.2	2.7	3.8	1.5	0.6	1.1	2.2	1.2	1.0	1.5	3.1	2.4	4.4	2.6
6 Meat & bonemeal (Lamb)	2.9	0.9	0.5	1.8	3.0	3.3	1.8	1.1	1.5	3.0	1.6	1.3	2.0	3.0	2.7	5.8	3.3
7 Poultry meal	3.5	1.3	0.8	2.3	3.9	4.6	2.6	1.5	2.3	4.0	2.1	1.7	2.7	3.2	3.6	7.9	4.6
8 Feather meal	2.1	0.7	6.5	4.3	6.5	6.9	11.2	1.0	4.6	7.4	4.3	2.4	6.7	8.8	4.3	9.6	5.9
Oilseeds																	
9 Soybean meal (solvent)	3.2	0.7	0.8	2.0	3.7	2.0	2.8	1.3	2.4	3.7	2.5	1.8	2.5	2.5	2.0	9.1	5.6
10 Soybean meal (expeller)	2.9	0.8	0.9	2.0	3.7	1.9	2.8	1.3	2.4	3.7	2.5	1.7	2.4	2.5	1.9	8.9	5.5
11 Soybean meal (full fat)	2.2	0.6	0.7	1.4	2.5	1.4	2.1	0.9	1.6	2.6	1.7	1.3	1.6	1.7	1.4	6.3	3.8
12 Canola meal (solvent)	1.8	0.8	0.9	1.5	2.1	1.7	1.7	0.9	1.5	2.5	1.4	1.0	1.8	2.2	1.5	6.4	2.2
13 Canola meal (expeller)	1.5	0.8	0.8	1.3	1.9	1.5	1.4	0.8	1.3	2.1	1.2	0.9	1.6	1.9	1.3	5.8	2.1
14 Peanut meal	1.6	0.5	0.7	1.4	7.0	2.6	2.7	1.2	1.9	3.3	2.6	2.0	2.2	3.1	2.0	10.4	6.1
15 Cottonseed meal	1.5	0.7	0.7	1.3	5.2	1.5	1.8	1.2	1.2	0.9	2.1	1.2	1.6	1.7	1.4	9.1	3.9
16 Linola	1.1	0.7	0.6	1.0	2.5	1.4	1.4	0.6	1.2	1.7	1.3	0.8	1.4	1.1	1.2	5.4	2.4

Digestible amino acid values are calculated on a 100% basis where digestibility coefficients exceed this value (Table 5).

TABLE 7 continued

Digestible amino acid values (dry basis) of the test ingredients using juvenile silver perch and settlement method of faecal collection.

Test ingredient	Apparent Digestibility (%)																
	Lys	Meth	Cyst	Threo	Arg	Gly	Ser	Hist	Iso	Leuc	Phenyl	Tyro	Val	Pro	Ala	Glu	Asp
Legumes																	
17 Lupins-*L. angustifolius*[2]	1.4	0.2	0.5	1.2	4.0	1.4	1.9	0.9	1.3	2.2	1.3	1.3	1.2	1.5	1.2	7.8	3.3
18 Lupins-*L. albus*	1.5	0.2	0.7	1.5	4.1	1.5	2.2	0.8	1.6	2.6	1.4	1.7	1.4	1.6	1.3	8.1	4.0
19 Field pea (Dunn variety)	1.5	0.2	0.3	0.6	2.2	0.8	1.0	0.5	0.9	1.4	0.9	0.6	1.0	0.8	0.9	3.7	2.5
20 Faba bean	1.4	0.3	0.4	0.7	2.6	0.9	1.2	0.5	1.0	1.7	1.0	0.7	1.0	1.1	1.0	3.9	2.5
21 Chick pea	1.2	0.4	0.3	0.5	1.7	0.6	0.9	0.4	0.7	1.2	0.9	0.5	0.7	0.7	0.7	2.8	1.8
22 Vetch	1.2	0.2	0.2	0.4	1.8	0.7	0.8	0.4	0.8	1.4	0.7	0.5	0.9	0.8	0.8	3.8	2.4
23 Cow peas	1.5	0.4	0.3	0.8	1.9	0.9	1.3	0.7	0.9	1.6	1.1	0.7	1.1	1.1	1.0	4.1	2.6
Cereals																	
24 Wheat gluten	1.7	1.3	2.1	2.8	3.4	3.3	5.3	2.0	3.7	6.7	5.1	3.5	3.9	12.2	2.5	35.6	3.3
25 Corn gluten meal	1.0	1.5	1.3	2.1	1.9	1.5	3.6	1.0	2.8	11.3	4.1	3.4	3.0	5.8	5.8	14.5	4.0
26 Wheat 1 (Aust. Std. Wheat)	0.3	0.2	0.3	0.3	0.5	0.4	0.6	0.2	0.4	0.7	0.5	0.3	0.4	1.0	0.4	3.1	0.5
27 Wheat 2 (High protein)	0.3	0.3	0.5	0.4	0.6	0.5	0.7	0.3	0.5	0.9	0.6	0.4	0.6	1.4	0.5	4.2	0.6
28 Millrun	0.9	0.4	0.5	0.6	1.4	0.9	0.9	0.4	0.7	1.2	0.7	0.5	0.9	1.2	1.0	3.8	1.4
29 Sorghum	0.3	0.2	0.2	0.4	0.5	0.4	0.6	0.2	0.6	1.7	0.7	0.5	0.6	1.1	1.1	2.9	0.9

Digestible amino acid values are calculated on a 100% basis where digestibility coefficients exceed this value (Table 5).
Digestible amino acids = composition of amino acids x ADC of amino acids.

6.3 Replacement of fish meal in diets of silver perch: II. digestibility of Australian lupins

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Abstract

This study examines the potential of two species of lupins for use as major ingredients in aquaculture diets for silver perch *Bidyanus bidyanus*, an Australian native freshwater fish. The apparent digestibility of whole and dehulled narrow leaf lupin *Lupinus angustifolius* (Gungurru), and the white lupin *L. albus* by juvenile silver perch were evaluated in order to determine the suitability of lupins as alternative sources of protein to fish meal in aquaculture diets.

Dietary and ingredient apparent digestibility coefficients for dry matter, energy, nitrogen, amino acids, phosphorus, potassium, starch and non-starch polysaccharide (NSP) were calculated for silver perch fed a reference diet containing whole or dehulled *L. angustifolius* or *L. albus* at 30% and 50%. Faeces were collected by settlement and chromic oxide was used as an inert marker at 1%. Results showed apparent digestibility coefficients for diets of 57.9-68.8% for dry matter, 66.4-77.7% for energy, 87.4-93.6% for nitrogen, 36.8-57.4% for phosphorus, 85.0-88.2% for starch, -9.9-11.3% for NSP and 71.7-96.3% for amino acids for both species of lupin included at 30 and 50%, both whole and dehulled. Removing the hulls from both species improved dry matter and energy digestibility coefficients by more than 17% for *L. angustifolius* and between 11-13% for *L. albus*. This is attributed to the removal of 12 and 8% of NSP for *L. angustifolius* and *L. albus* respectively.

Apparent digestibility for protein was not affected by the level of lupin inclusion. Low apparent digestibility for NSP was observed in all diets, indicating that the inclusion of lupins in feeds for juvenile silver perch will increase faecal load which may produce a decline in water quality.

Keywords: Nutrition; *Bidyanus bidyanus*; digestibility; Lupins; Non-starch polysaccharide; Starch; Protein.

1. Introduction

Commercial aquaculture of shrimp and carnivorous and omnivorous finfish relies on high protein diets (Lovell, 1989). Unfortunately, fish meal supply is static or declining while demand is escalating (Barlow, 1989). Very little fish meal is produced in Australia. The search for alternative sources of protein, particularly of plant origin, has been identified as an international research priority (Manzi, 1989). Lupins (*Lupinus* spp.) are extensively cultured in Australia and production has risen from 96,000 tonnes in 1980/81 to 1.4 million tonnes in 1993/94 (ABARE, 1994). They are widely used in terrestrial animal feeds (Batterham, 1992; Farrell, 1992) and have few antinutritional factors (Batterham, 1992; Petterson and Mackintosh, 1994). Two species of sweet lupin are cultured, the narrow-leafed lupin, *Lupinus angustifolius* and Albus or white lupin, *L. albus*. Restrictions are usually placed on lupin use in poultry diets as high contents can give "sticky droppings" (Farrell, 1992). Suggested restrictions for *L. angustifolius* and *L. albus* in pig diets are 40% and 10-15% respectively (Batterham, 1992). Lupin seed meal has been identified as a promising alternative to soybean meal or as a partial substitute for fish meal in diets for rainbow trout, gilthead sea bream and

carp (Hughes, 1988, 1991; Viola et al., 1988; Gomes and Kaushik, 1989; Gomes et al., 1995; Robaina et al., 1995). In those studies, successful diets had lupin contents as high as 45% (Viola et al., 1988). Both species of lupin have been used, although they have not been compared in any study with fish.

Where digestibility of lupins (either as an ingredient or as part of a complete diet) has been determined, relatively low dry matter and energy digestibility values, compared with animal meals and fish meals have been recorded (Gomes et al., 1995; Morales et al., 1994). When included in diets for rainbow trout at the same concentration, dehulled lupins gave better results than whole lupins (Hughes, 1991), and Morales et al., (1994) reported poor digestibility of the carbohydrate fraction of lupins. However, digestibility of dehulled lupins has not been recorded for fish and no digestibility data were found for *L. albus* (hulled or dehulled).

In Australia, aquaculture of silver perch (*Bidyanus bidyanus*), a native freshwater finfish, is expanding rapidly. The species is omnivorous in the wild and should be able to tolerate relatively high contents of plant proteins in formulated diets. Results from preliminary studies show lupins are well digested by silver perch and juvenile growth is not reduced by quite high inclusion levels (>40%). In the current study, apparent digestibility coefficients for dry matter, energy, nitrogen, amino acids, phosphorus, starch and non-starch polysaccharide (NSP) were calculated for juvenile silver perch fed diets containing one of two species of lupins, *Lupinus angustifolius* (Gungurru variety) or *L. albus*, either hulled or dehulled, at one of two inclusion levels (30 or 50%).

2. Materials and methods

2.1. Experimental fish

Silver perch were bred at the Grafton Research Centre and raised in earthen ponds using similar techniques to those described by Thurstan and Rowland (1994). Before experiments, fish were fed the silver perch reference diet (SP35) of Allan and Rowland (1992) (Table 1) and were treated with 5 g/l NaCl to ensure they were free of ectoparasites and to prevent fungal infection (Rowland and Ingram, 1991). Prior to stocking, fish were anaesthetised using a bath of 25 mg/l ethyl-ρ-aminobenzoate for 5 minutes, then caught at random, weighed individually and distributed among 27 tanks by systematic interspersion. Five fish with a mean weight of 11.4 ± 0.2 g were stocked into each tank. Fish were also weighed at harvest.

2.2. Diets

The null hypothesis that lupin species, the presence or absence of hulls or the inclusion content had no effect on digestibility coefficients was tested using three-factor ANOVA. The three fixed factors were: lupin species (*L. angustifolius* [Gungurru variety] or *L. albus*), processing (hulls-on or dehulled) and inclusion content (30 or 50%). Homogeneity of variance was satisfied using Cochran's test (Winer, 1971), and where significant differences ($P<0.05$) were found between means, comparisons were made using Student Newman-Keuls multiple range test.

Nine diets were formulated: the reference diet (Table 1) and eight others including the reference diet at 50 or 70% and one of the lupin products (Table 2). Lupins were obtained from the Grain Pool of Western Australia and milled using a hammer mill fitted with a 800 μm screen. The reference diet and lupin products were mixed with approximately 400 ml distilled water in a Hobart mixer (Troy Pty

Ltd, Ohio, 45374, USA) and then cold pelleted through a meat mincer (Barnco Australia Pty Ltd, Leichardt, 2040, NSW) fitted with a 2 mm die. Pellets were dried at <35°C in a convection drier for approximately 6 h until moisture content was between 10 and 15%. Three replicate digestibility tanks were used for each of the 9 treatments.

2.3. Experimental facilities

Digestibility tanks were 170 l cylindroconical tanks (conical base sloped at 35°) fitted with a 65 mm diameter, 250 mm settlement chamber which tapered into a 12 mm diameter, 150 mm length of silicone tubing (see Allan et al., section 6.1 of this report). The inside of each tank was black. Continuously-flowing, preheated water was filtered through a sand filter and a diatomaceous earth filter, then passed through a UV sterilizer before being supplied to experimental tanks at a flow-rate of 600 ml/min. Effluent water from each tank flowed out the side of the cylindroconical tanks into a 25 mm diameter pipe, and 20-25% of this flowed to waste. The rest was collected and recirculated through a diatomaceous earth filter, a $2m^3$ biological filter and a UV sterilizer prior to being reused. Each tank was aerated using two air-stone diffusers.

Fish were stocked seven days prior to the start of the faecal collection period to allow for acclimation to experimental diets and conditions. Fish were fed to excess using automatic conveyor belt-type feeders for three hours each day from 0830-1130h. Within one hour after feeding ceased, all uneaten food was removed, and the walls of the tank and the settlement chamber were thoroughly cleaned to remove any faeces, uneaten food or bacterial slime. The silicone tubing into which the faeces settled was packed in ice and kept at ≤4°C to reduce bacterial proliferation prior to removal of faeces, which can affect the composition of faeces (Spyridakis et al., 1989). Faeces were collected over 16 days and the total collections for each tank were pooled prior to biochemical analysis. During the experiment, dissolved oxygen (above 7.5 mg/l), pH (8.0), nitrite and ammonia (less than 0.04 mg NO_2-N/l and 0.1 mg total ammonia - N/l respectively) were measured weekly using methods described in Allan et al. (1990).

2.4 Biochemical analyses

Faecal samples were collected by settlement each morning and dried using silica gel dessicant under vacuum. The faecal collection period ran for 17 days. Samples from each tank were pooled at the end of the experiment and re-dried by the same method.

Each sample was freeze-dried and ground using a water-cooled 1KA total recovery grinder prior to analyses. All analyses were carried out in duplicate. Diet and faecal samples were analysed for dry matter, ash, fat and energy (bomb calorimetry) by the AOAC (1975) procedures. Nitrogen was determined by the method of Havilah et al., (1977) and multiplied by 6.25 to calculate crude protein content. Chromic oxide was determined by the method described in Scott (1978). Amino acids were analysed following acid hydrolysis using high pressure liquid chromatography and Waters Pico-Tag (Waters Pty Ltd, Lane Cove, NSW, 2066, Australia). Sulphur amino acids were determined separately following performic acid digestion, and tryptophan, which is lost during acid hydrolysis, was not determined (Cohen et al., 1989).

Moisture was determined by drying samples at 110°C in an oven for 18 h. Phosphorus was measured by the method of Havilah et al., (1977). Neutral detergent fibre was measured on ingredient samples

by the method of Van Soest et al., (1991). Total non-starch polysaccharides (NSP) were determined by colouimetry and by gas liquid chromatography (GLC) according to the methods of Englyst and Hudson (1987) and Englyst and Cummings (1984). Enzymic hydrolysis and determination of starch was carried out using a total starch assay kit (Megazyme amyloglucosidase/α-amylase method AA/AMG 10/94, Sydney, Australia). Uronic acids were determined by the method of Scott (1979). Monosaccharide composition of selected feed and faecal samples were determined as alditol acetate derivatives by GLC (Blakeney et al., 1983).

2.5. Digestibility determinations

Apparent digestibility coefficients (ADC) for dry matter, energy and protein (Nx 6.25) for experimental diets and ingredients were determined following the indirect methods and calculations outlined in Cho and Kaushik (1990).

ADC diet = $[1-(F/D \times DC_r/FC_r)] \times 100$ where:

F = % nutrient or energy in faeces,
D = % nutrient or energy in diet,
DC_r = % chromic oxide in diet and
FC_r = % chromic oxide in faeces.

and ADC for ingredient = 100 (ADC test diet - 0.7 ADC reference diet) / 0.3.

3. Results

Composition of ingredients and diets are tabulated (Tables 3-6). In comparison to the reference diet, all lupin ingredients were higher in gross energy, fat, and NSP. Dehulled lupins were also higher in crude protein. Whole *L. albus* was higher in crude protein and energy than whole *L. angustifolius,* but as more NSP was removed in the dehulling process for *L. angustifolius*, dehulled *L. angustifolius* had a higher crude protein content than dehulled *L. albus*. Energy and NSP contents were similar for both dehulled lupin species (Table 3). There was almost no starch present (<1%) in any of the lupin ingredients, however the reference diet contained 22.9% starch, mostly from wheat. These differences were reflected in the analysed composition of the diets (Table 4).

Similarly, amino acid content of whole *L. albus* was higher than for *L. angustifolius* and, with the exception of methionine where effects were similar, dehulling led to a greater increase in amino acid content for *L. angustifolius* than for *L. albus* (Table 5).

Apparent digestibility coefficients for diets and ingredients are tabulated in Tables 7-10. The significance of the main effects and interaction of digestibility coefficients for ingredients are listed in Table 11.

For dry matter and energy, all fixed factors and the interaction between ingredient and process and ingredient and inclusion level, all significantly affected digestibility coefficients ($P<0.05$). The lowest values were 50% for dry matter and 58% for energy, while the highest values were 78 and 85% for dry matter and energy digestibility coefficients, respectively (Table 8). Digestibility coefficients for crude protein were very high for all products (>95%) and although significant effects

of processing and the interaction between processing and inclusion level and ingredient processing and inclusion level were identified, it is unlikely these small differences were biologically meaningful.

The composition of monosaccharides in NSP (Table 12) indicated a large decrease in glucose as a proportion of NSP in dehulled compared with whole lupins. This demonstrated that removing hulls also reduces cellulose which comprises as much as 50% of the fibre in lupin hulls (Evans et al., 1993). Uronic acids, xylose and mannose expressed as a proportion of NSP were reduced in dehulled lupins indicating a loss of hemipectins due to the removal of hull material.

Digestibility coefficients for NSP for diets and ingredients ranged from -10 to 11% and -37% to 28% respectively. Negative values indicated error in the methodology; this error is greater for ingredients than diets due to the multiplication of error inherent in the method of calculating ingredient digestibility coefficients. Additional analysis of carbohydrate fractions, ingredients, diets and faeces were undertaken to compare the profile of monosaccharides in the diet and faeces. An absence of compositional differences between faeces and diets (Table 13) indicates that digestive changes were minor.

4. Discussion

The determination of digestibility coefficients is necessary before diets which are balanced for energy and essential nutrients can be formulated using different ingredients (Cho et al., 1982). Results in the present study show that protein for lupin is extremely well digested, regardless of the species of lupin or whether the lupin was used whole or dehulled. Protein digestibility coefficients for lupins for silver perch were higher than those reported for rainbow trout (Hughes, 1988; Morales et al., 1994; Gomes et al., 1995). The values for dry matter digestibility and energy digestibility obtained in earlier studies (Hughes, 1988; Gomes et al., 1995) fall within the range of values reported for whole lupins in the present study. No digestibility values for amino acids of lupins have been reported for other species.

The difference in protein digestibility coefficients may be due to differences in methodology or better protein utilization efficiency of silver perch than rainbow trout. All studies collected faeces for digestibility determination following settlement, although more nitrogen may have leached from silver perch faeces than trout faeces. Silver perch consume considerable amounts of vegetable material in the wild and may have adapted mechanisms to more efficiently extract protein from such ingredients than carnivorous trout. Silver perch have well developed proteases in the gut suggesting efficient protein digestion capacity (Anderson, personal communication.)

Removing the hulls from both species of lupins improved dry matter and energy digestibility coefficients by more than 17% for *L. angustifolius* and between 11-13% for *L. albus*. This was not surprising, as dehulling also removed approximately 12 and 8% of NSP in *L. angustigolius* and *L. albus* respectively. NSP is very poorly digested by silver perch. This conclusion is supported by three observations: firstly the direct measurement of apparent digestibility of NSP showed low apparent digestibility of NSP (Table 8). Secondly, no major changes in the monosaccharide profile of NSP extracted from experimental diets and faeces were observed, indicating little or no degradation of fibre (Table 13). The most likely form of fibre degradation in fish is anaerobic bacterial fermentation (Evans et al., 1995), and were this occurring, it would be expected that the proportion of galactose in the faeces would be reduced and the proportion of glucose in the faeces would increase,

as it is unlikely that cellulose would be degraded in these circumstances. The third observation supporting the conclusion of the lack of utilization of NSP by silver perch, is that the apparent dry matter digestibility of the experimental diets range from 57.9-68.8%, indicating that at least 30% of the diet is non-digested (Table 5). Protein and starch, the major components of the diet, are well digested, and together comprise 47.6-59% of the diet. Ash and NSP comprise 27.8-38%, the majority of the remaining dietary components. Poor carbohydrate digestibility of lupins was also reported by Morales et al (1994). In that study, digestibility coefficients for carbohydrate and nitrogen free extract were estimated as 3.72 and 2.80, respectively.

In the only other study where the use of dehulled lupins was compared with whole lupins, significantly better growth results were obtained for rainbow trout with dehulled material (Hughes, 1991). In that comparison, as the lupin content of the diets was the same, the diets with dehulled lupins would have had higher digestible dry matter and digestible energy than those with whole lupins.

In the present study, increasing lupin content from 30-50% reduced digestibility of energy and dry matter for *L. albus* but not *L. angustifolius*. In the present study, there were significant interactions between lupin species and process and lupin species and inclusions level for dry matter and energy digestibility. The greater improvement in dehulled lupins compared with whole lupins for *L. angustifolius* than for *L. albus* is readily explained by the larger proportion of NSP removed by dehulling for *L. angustifolius* compared with *L. albus*. However, increasing the content of lupin in the diet reduced dry matter and energy digestibility for *L. albus* but not for *L. angustifolius*. This cannot be explained by composition of the lupins (for the nutrients measured here) but does indicate presence of some inhibitor to digestion for *L. albus*. By contrast, *L. angustifolius* appear free of anti-nutrient factors. Robaina et al., (1995) found intestinal trypsin activity in gilthead seabream (*Sparus auratus*) increased with increasing *L. angustifolius* content and suggested this might indicate the presence of an antitrypsin factor. However, as Robaina et al., (1995) point out, no antitrypsin factors have been reported for lupins (of either species), and the high protein digestibility tends to refute this suggestion. The very high digestibility of protein for both lupins in the present study suggested an antitrypsin factor is unlikely. In addition, Gouveia et al., (1993) found heating/expanding *L. albus* (145°C, 25 kg/cm^2) did not improve dietary protein utilization with rainbow trout. De la Higuera et al., (1988) reported that autoclaving *L. albus* (120°C for 30 minutes) actually decreased the value of the material for rainbow trout.

Digestibility values reported in the present study will be useful in formulating balanced diets for silver perch containing lupins. Both species of lupins are suitable for use in silver perch feeds, although the reduction in dry matter and energy digestibility for *L. albus* as inclusion levels increased for 30-50% indicated that *L. angustifolius* might be a better ingredient when used in large amounts. Dehulling significantly improves the value of lupins in silver perch diets by removing relatively indigestible non-starch polysaccharide components. Although the adult silver perch is regarded to be an omnivorous feeder, there is as yet no direct evidence of its ability to utilise NSP. As the current study has used only the juvenile form, further research to investigate the herbivorous potential of the adult would be of some interest.

Acknowledgments

The authors would like to thank Aung Htoon, Vartouhi Betchakjian (CSIRO), David Stone, Jane Frances, Scott Parkinson and Rebecca Warner-Smith (NSW Fisheries) and Ken O'Brien and Wendy

Peasley (NSW Agriculture) for their expertise and assistance with methodology and experimental work. Fish were bred at NSW Fisheries, Grafton Research Centre by Stuart Rowland and Charlie Mifsud. Grain was obtained from the Grain Pool of Western Australia with milling and dehulling activities graciously fulfilled by Goodman Fielder Milling. This project was supported by the Australian Fisheries Research and Development Corporation (FRDC), Sub-Program on Fish meal Replacement in Aquaculture Diets (93/120). Additional support was also provided by the Australian Centre for International Agricultural Research and the Australian Grains Research and Development Corporation. The manuscript was critically reviewed Dr John Nell and Wayne O'Connor. Helena Heasman assisted with manuscript preparation.

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Table 1

Formulation and composition of reference diet (SP35) for silver perch.

Ingredients	%
Fish meal	27.0
Soybean meal	20.0
Blood meal	2.0
Corn gluten meal	4.0
Wheat	28.4
Sorghum	11.0
Millrun	2.0
Cod liver oil	1.0
Di-calcium phosphate	2.0
Vitamin / mineral premix	2.5
L-methionine	0.15

Table 2

List of diets tested.

Diet[1]	SP35[2]	Species	Processing	Inclusion (%)
SP35	99	-	-	0
AD30	69.3	*L. albus*	Dehulled	27.7
AD50	49.5	*L. albus*	Dehulled	49.5
GD30	69.3	*L. angustifolius*	Dehulled	27.7
GD50	49.5	*L. angustifolius*	Dehulled	49.5
AW30	69.3	*L. albus*	Hulls on	27.7
AW50	49.5	*L. albus*	Hulls on	49.5
GW30	69.3	*L. angustifolius*	Hulls on	27.7
GW50	49.5	*L. angustifolius*	Hulls on	49.5

[1] All diets contain 1% chromic oxide
[2] Reference diet

Table 3

Composition of ingredients used in test diets for digestibility trials with silver perch. Ingredients were whole (W) and dehulled (D), *L. angustifolius* (Gungurru) (G) and *L. albus* (A).

	Ingredient			
	AD	AW	GD	GW
Dry Matter (%)	95.0	95.4	95.0	94.1
Gross Energy (MJ/kg)	21.5	20.9	20.7	17.9
Ash (%dw)	4.2	3.7	2.9	2.8
Fat (%dw)	8.9	6.2	6.6	5.7
Protein (%dw)	42.8	37.5	43.6	34.1
Phosphorus (%dw)	0.4	0.4	0.3	0.3
Starch (%dw)	0.3	0.8	0.2	0.1
NSP (%dw)	32.5	40.8	35.2	47.6

Table 4

Composition of test diets used in digestibility trials with silver perch. Diets were composed of whole (W) and dehulled (D) *L. angustifolius* (Gungurru) (G) and *L. albus* (A) included with a basal reference diet (R) at 30% or 50%.

	Diet								
	R	AD30	AD50	GD30	GD50	AW30	AW50	GW30	GW50
Dry Matter (%)	96.2	93.9	93.7	95.1	95.3	95.2	94.4	95.6	96.4
Gross Energy (MJ/kg)	16.4	18.5	18.9	18.4	19.0	18.2	18.6	18.3	18.4
Chromic Oxide (%dw)	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Ash (%dw)	16.5	13.7	11.3	12.8	10.3	13.4	11.0	12.9	10.2
Fat (%dw)	9.9	4.3	5.2	4.3	4.6	5.1	6.9	3.4	3.7
Protein (%dw)	38.0	39.0	39.0	38.7	39.3	37.7	37.7	36.8	36.0
Phosphorus (%dw)	1.5	1.1	0.9	1.1	0.9	1.1	0.9	1.1	0.8
Starch (%dw)	21.5	16.0	11.9	16.8	12.4	14.9	11.1	16.0	11.6
NSP (%dw)	11.3	15.0	20.0	17.8	21.8	17.5	25.9	21.1	27.8

Table 5
Amino acid composition of ingredients used in feeds for digestibility trials with silver perch. Ingredients were reference diet material (R), whole (W) and dehulled (D) *L. angustifolius* (Gungurru) (G) and *L. albus* (A).

Amino acid	Ingredient				
	R	AD	AW	GD	GW
Alanine	2.30	1.44	1.30	1.45	1.21
Arginine	2.44	4.49	4.06	5.16	4.01
Aspartine	3.36	4.91	4.26	4.67	3.76
Cystine	0.71	0.94	0.87	0.76	0.57
Glutamic acid	6.50	9.71	8.39	10.30	8.18
Glycine	2.01	1.69	1.50	1.76	1.42
Histidine	1.02	0.96	0.86	1.18	0.87
Isoleucine	1.53	1.93	1.69	1.81	1.36
Leucine	3.19	3.16	2.80	2.94	2.35
Lysine	2.17	1.72	1.53	1.73	1.41
Methionine	1.02	0.32	0.26	0.24	0.20
Phenylalanine	1.68	1.62	1.43	1.64	1.30
Proline	2.26	1.96	1.70	1.98	1.62
Serine	1.97	2.64	2.31	2.57	2.10
Threonine	1.60	1.75	1.51	1.62	1.25
Tyrosine	1.33	2.09	1.75	1.70	1.36
Valine	1.89	1.76	1.56	1.64	1.25

Table 6
Amino acid composition of test diets used in digestibility trials with silver perch. Diets were composed of whole (W) and dehulled (D), *L. angustifolius* (Gungurru) (G) and *L. albus* (A) included in a basal reference diet (R) at 30% and 50%.

Amino acid	Diet								
	R	AD30	AD50	AW30	AW50	GD30	GD50	GW30	GW50
Alanine	2.37	1.88	2.09	2.01	1.77	2.08	1.89	1.97	1.73
Arginine	2.37	3.00	3.53	2.80	3.20	3.29	3.66	2.84	3.13
Aspartine	3.42	3.86	4.31	3.63	3.82	3.84	4.05	3.25	3.54
Cystine	0.61	0.67	0.83	0.65	0.79	0.61	0.55	0.67	0.64
Glutamic acid	6.50	7.47	8.13	6.79	7.24	7.68	8.21	6.69	7.16
Glycine	2.07	1.97	1.87	1.86	1.71	1.97	1.87	1.81	1.69
Histidine	1.04	0.99	0.99	0.93	0.91	1.05	1.06	0.96	0.93
Isoleucine	1.58	1.69	1.75	1.42	1.55	1.62	1.59	1.46	1.48
Leucine	3.33	3.23	3.17	3.02	2.92	3.16	3.09	2.90	2.76
Lysine	2.12	2.02	1.82	1.83	1.82	1.91	1.86	1.92	1.73
Methionine	1.01	0.72	0.65	0.76	0.58	0.67	0.58	0.68	0.63
Phenylalanine	1.73	1.66	1.60	1.55	1.50	1.66	1.64	1.54	1.48
Proline	2.34	2.13	2.08	2.10	1.92	2.21	2.13	2.02	1.89
Serine	2.01	2.18	2.33	2.09	2.11	2.20	2.24	1.91	1.95
Threonine	1.64	1.63	1.66	1.56	1.52	1.60	1.55	1.42	1.38
Tyrosine	1.35	1.57	1.71	1.43	1.53	1.46	1.49	1.30	1.34
Valine	1.97	1.92	1.85	1.63	1.67	1.85	1.69	1.70	1.63

Table 7
Apparent digestibility coefficients (%) of test diets for dry matter (DM), energy, protein, phosphorus (P), starch and non-starch polysaccharide (NSP) for SP35 or SP35 substituted by lupins, *L. albus* (A.) or *L. angustifolius* (G.), whole (W) or dehulled (D) at 30 or 50% inclusion.

	Diet								
	R	AD30	AD50	GD30	GD50	AW30	AW50	GW30	GW50
DM	64.9 ± 0.5	68.8 ± 0.6	66.5 ± 0.2	65.7 ± 1.0	66.9 ± 0.1	64.8 ± 0.1	62.1 ± 0.6	60.5 ± 0.9	57.9 ± 0.7
Energy	74.5 ± 0.3	77.7 ± 0.5	74.6 ± 0.2	74.4 ± 0.7	74.8 ± 0.8	74.0 ± 0.5	70.8 ± 0.2	70.0 ± 0.3	66.4 ± 0.9
Protein	87.4 ± 0.0	91.6 ± 0.1	92.4 ± 0.1	91.3 ± 0.1	93.6 ± 0.5	90.0 ± 0.3	92.2 ± 0.6	90.2 ± 0.3	91.6 ± 0.2
P	36.8 ± 1.0	47.9 ± 1.1	48.9 ± 0.5	49.8 ± 1.4	57.4 ± 1.2	49.0 ± 0.3	51.9 ± 2.3	47.3 ± 1.4	54.4 ± 1.7
Starch	85.0 ± 1.1	85.5 ± 1.0	85.3 ± 0.3	86.3 ± 0.9	86.9 ± 0.4	88.2 ± 0.9	86.8 ± 0.7	86.5 ± 0.9	85.1 ± 1.5
NSP[1]	1.8 ± 3.5	9.7 ± 1.4	11.3 ± 1.7	7.8 ± 3.3	3.4 ± 1.4	-7.7 ± 2.4	-4.1 ± 1.1	-9.9 ± 1.1	-3.5 ± 2.0

Values are mean±sem for three replicates (n=3)
[1] analysis by colourimetry.

Table 8
Apparent digestibility coefficients (%) for ingredients for dry matter (DM), energy, protein, phosphorus (P), and non-starch polysaccharide (NSP) by silver perch, fed diets composed of whole (W) and dehulled (D), *L. angustifolius* (Gungurru) (G) and *L. albus* (A) included in a basal reference diet (R) at 30% and 50%.

	Ingredient							
	AD30	AD50	AW30	AW50	GD30	GD50	GW30	GW50
DM	77.8 ± 2.0	68.2 ± 0.5	64.7 ± 0.4	59.4 ± 1.1	67.6±3.2	68.9±0.3	50.3 ± 3.0	50.8 ± 1.4
Energy	85.2 ± 1.5	74.7 ± 0.4	72.7 ± 1.8	67.1 ± 0.4	74.0±2.3	75.0±1.6	59.4 ± 1.0	58.4 ± 1.7
Protein	101.4 ± 0.3	97.3 ± 0.1	96.1 ± 0.9	97.0 ± 1.3	100.3±0.4	99.9±1.1	96.6 ± 0.9	95.8 ± 0.3
P	73.8 ± 3.7	61.0 ± 1.0	77.5 ± 1.2	67.0 ± 4.6	80.1±4.7	78.0±2.5	71.8 ± 4.7	72.0 ± 3.4
NSP	28.0 ± 4.7	20.8 ± 3.4	-29.9 ± 8.1	-9.9 ± 2.0	21.9±11.1	5.1±2.8	-37.4 ± 3.6	-8.7 ± 3.9

Values are mean±sem for three replicates (n=3)

Table 9

Apparent digestibility coefficients (%) for diets for amino acids by silver perch fed diets composed of whole (W) and dehulled (D), *L. angustifolius* (Gungurru) (G) and *L. albus* (A) included in a basal reference diet (R) at 30% and 50%.

Amino acid	Diet								
	R	AD30	AD50	AW30	AW50	GD30	GD50	GW30	GW50
Alanine	88.8 ± 0.1	92.6 ± 0.1	92.0 ± 0.4	91.3 ± 0.4	91.9 ± 0.3	91.6 ± 0.3	92.8 ± 0.2	91.1 ± 0.5	91.6 ± 0.9
Arginine	90.5 ± 0.4	95.4 ± 0.1	96.3 ± 0.3	94.1 ± 0.2	96.1 ± 0.2	95.2 ± 0.2	96.6 ± 0.1	94.2 ± 0.4	96.0 ± 0.4
Aspartine	93.9 ± 0.3	95.3 ± 0.2	95.0 ± 0.1	93.7 ± 0.4	94.0 ± 0.2	94.7 ± 0.4	95.2 ± 0.1	92.1 ± 0.7	93.1 ± 0.5
Cystine	71.2 ± 5.4	81.5 ± 2.2	86.0 ± 0.5	75.2 ± 1.8	85.4 ± 0.8	73.8 ± 4.2	81.1 ± 2.1	71.7 ± 5.1	73.5 ± 6.0
Glutamic acid	94.1 ± 0.1	96.2 ± 0.2	95.9 ± 0.2	94.7 ± 0.4	95.7 ± 0.2	95.5 ± 0.2	96.0 ± 0.1	94.5 ± 0.5	95.3 ± 0.5
Glycine	81.3 ± 0.3	89.0 ± 0.2	90.0 ± 0.3	87.5 ± 0.7	89.4 ± 0.7	88.3 ± 0.5	90.9 ± 0.2	86.3 ± 0.8	89.2 ± 1.0
Histidine	91.5 ± 0.4	94.4 ± 0.1	94.6 ± 0.5	93.5 ± 0.6	94.5 ± 0.4	94.5 ± 0.5	95.9 ± 0.4	94.2 ± 0.8	95.4 ± 0.6
Isoleucine	90.6 ± 0.4	93.7 ± 0.2	93.6 ± 0.3	91.0 ± 0.4	93.4 ± 0.3	92.7 ± 0.3	94.2 ± 0.3	92.1 ± 0.6	93.4 ± 0.4
Leucine	92.4 ± 0.2	94.5 ± 0.2	94.1 ± 0.2	93.0 ± 0.3	94.1 ± 0.3	93.7 ± 0.3	94.8 ± 0.2	93.1 ± 0.6	93.9 ± 0.5
Lysine	92.0 ± 0.3	95.2 ± 0.2	95.2 ± 0.3	93.4 ± 0.1	95.2 ± 0.2	94.3 ± 0.2	95.8 ± 0.2	93.8 ± 0.5	94.4 ± 0.5
Methionine	88.7 ± 1.1	91.3 ± 0.3	93.0 ± 0.5	89.7 ± 0.5	91.6 ± 1.1	89.6 ± 0.9	92.5 ± 0.4	87.2 ± 2.0	92.1 ± 1.0
Phenylalanine	91.9 ± 0.6	94.4 ± 0.2	93.9 ± 0.2	92.8 ± 0.3	93.8 ± 0.3	93.7 ± 0.4	95.1 ± 0.2	93.2 ± 0.7	94.1 ± 0.4
Proline	86.7 ± 0.4	91.4 ± 0.1	91.1 ± 0.1	89.6 ± 0.4	90.1 ± 0.4	90.5 ± 0.2	92.4 ± 0.1	89.0 ± 1.0	90.6 ± 0.7
Serine	89.4 ± 0.1	93.2 ± 0.1	92.8 ± 0.2	91.1 ± 0.3	91.8 ± 0.6	92.7 ± 0.4	93.8 ± 0.2	90.0 ± 0.8	91.9 ± 0.5
Threonine	91.3 ± 0.3	94.9 ± 0.1	94.6 ± 0.2	93.5 ± 0.1	94.3 ± 0.4	94.8 ± 0.4	95.2 ± 0.2	93.1 ± 0.6	94.6 ± 0.3
Tyrosine	91.8 ± 0.6	94.7 ± 0.2	94.5 ± 0.1	92.9 ± 0.2	94.2 ± 0.2	93.7 ± 0.3	95.4 ± 0.1	92.8 ± 0.8	94.4 ± 0.4
Valine	90.1 ± 0.3	93.2 ± 0.2	92.7 ± 0.4	90.5 ± 0.4	92.3 ± 0.3	92.3 ± 0.3	93.5 ± 0.3	91.5 ± 0.6	92.1 ± 0.6

Values are mean±sem for three replicates (n=3)

Table 10
Apparent digestibility coefficients (%) for ingredients for amino acids by silver perch fed diets composed of whole (W) and dehulled (D), *L. angustifolius* (Gungurru) (G) and *L. albus* (A) included in a basal reference diet (R) at 30% and 50%.

Amino acid	Ingredient[1]							
	AD30	AD50	AW30	AW50	GD30	GD50	GW30	GW50
Alanine	101.6 ± 0.4	95.2 ± 0.8	97.1 ± 1.2	95.1 ± 0.5	98.3 ± 1.2	96.9 ± 0.4	96.4 ± 1.7	94.4 ± 1.8
Arginine	106.8 ± 0.3	102.2 ± 0.5	102.6 ± 0.7	101.8 ± 0.3	106.4 ± 0.7	102.8 ± 0.3	102.9 ± 1.3	101.4 ± 0.7
Aspartine	98.7 ± 0.7	96.2 ± 0.3	93.2 ± 1.3	94.0 ± 0.3	96.6 ± 1.5	96.4 ± 0.2	88.0 ± 2.5	93.5 ± 0.9
Cystine	105.6 ± 7.3	100.7 ± 1.0	84.4 ± 5.8	99.5 ± 1.5	79.9 ± 14.0	91.1 ± 4.1	72.8 ± 16.9	76.8 ± 12.0
Glutamic acid	101.1 ± 0.5	97.8 ± 0.3	96.0 ± 1.3	97.3 ± 0.5	98.6 ± 0.5	97.9 ± 0.1	95.5 ± 1.7	96.5 ± 0.9
Glycine	107.1 ± 0.8	98.7 ± 0.7	101.8 ± 2.2	97.4 ± 1.4	104.7 ± 1.7	100.4 ± 0.4	100.0 ± 2.7	97.1 ± 2.0
Histidine	101.1 ± 0.4	97.6 ± 0.9	98.3 ± 2.1	97.4 ± 0.7	101.6 ± 1.6	100.3 ± 0.8	100.6 ± 2.5	99.3 ± 1.2
Isoleucine	100.8 ± 0.7	96.6 ± 0.6	91.8 ± 1.2	96.2 ± 0.6	97.5 ± 0.9	97.7 ± 0.6	95.4 ± 2.1	96.1 ± 0.9
Leucine	99.5 ± 0.6	95.9 ± 0.5	94.4 ± 0.9	95.8 ± 0.6	96.9 ± 0.9	97.2 ± 0.3	94.9 ± 2.0	95.4 ± 0.9
Lysine	102.5 ± 0.6	98.5 ± 0.5	96.6 ± 0.4	98.4 ± 0.4	99.5 ± 0.7	99.7 ± 0.5	98.1 ± 1.5	96.8 ± 1.0
Methionine	97.3 ± 0.9	97.3 ± 1.1	92.2 ± 1.6	94.5 ± 2.2	91.7 ± 2.9	96.4 ± 0.8	83.9 ± 6.8	95.5 ± 1.9
Phenylalanine	100.0 ± 0.6	95.8 ± 0.4	94.8 ± 0.9	95.6 ± 0.5	98.0 ± 1.2	98.2 ± 0.5	96.0 ± 2.2	96.4 ± 0.8
Proline	102.4 ± 0.5	95.5 ± 0.2	96.4 ± 1.2	93.3 ± 0.9	99.5 ± 0.8	98.2 ± 0.2	94.2 ± 3.5	94.5 ± 1.3
Serine	101.9 ± 0.5	96.1 ± 0.4	95.0 ± 0.9	94.2 ± 1.1	100.4 ± 1.2	98.2 ± 0.4	91.4 ± 2.7	94.3 ± 1.0
Threonine	101.9 ± 0.1	97.3 ± 0.3	97.3 ± 0.4	96.7 ± 0.7	101.4 ± 1.2	98.4 ± 0.5	95.8 ± 1.9	97.2 ± 0.5
Tyrosine	101.6 ± 0.7	97.3 ± 0.3	95.6 ± 0.5	96.6 ± 0.4	98.2 ± 1.1	99.0 ± 0.2	95.4 ± 2.6	97.0 ± 0.8
Valine	100.4 ± 0.7	95.3 ± 0.7	91.2 ± 1.5	94.5 ± 0.6	97.2 ± 0.9	96.8 ± 0.6	94.6 ± 1.9	94.1 ± 1.2

[1] Values are means ± sem for n=3 replicate tanks.

Table 11
The significant effects and interaction of apparent digestibility coefficients for ingredients (%) by silver perch fed diets composed of whole (W) and dehulled (D), *L. angustifolius* (Gungurru) (G) and *L. albus* (A) included in a basal reference diet (R) at 30% and 50%.

Nutrient	Effects			Interactions			
	Ingredient (A)	Process (B)	Inclusion level (C)	A x B	A x C	B x C	A x B x C
Dry Matter	< 0.001	< 0.001	< 0.05	< 0.05	< 0.001	ns	ns
Energy	< 0.001	< 0.001	< 0.01	< 0.05	< 0.01	ns	ns
Nitrogen	ns	< 0.001	ns	ns	ns	< 0.05	< 0.05
Phosphorous	< 0.05	ns	< 0.05	< 0.05	< 0.05	ns	ns
Amino Acids							
Alanine	ns	< 0.05	< 0.01	ns	ns	ns	ns
Arginine	ns	< 0.001	< 0.001	ns	ns	< 0.01	ns
Aspartine	< 0.05	< 0.001	ns	ns	ns	< 0.05	ns
Cystine	< 0.05	ns	ns	ns	ns	ns	ns
Glutamic acid	ns	< 0.001	ns	ns	ns	<0.05	ns
Glycine	ns	< 0.01	< 0.001	ns	ns	ns	ns
Histidine	ns	ns	ns	ns	ns	ns	ns
Isoleucine	ns	< 0.001	ns	ns	ns	< 0.01	< 0.05
Leucine	ns	< 0.01	ns	ns	ns	ns	ns
Lysine	ns	< 0.001	ns	ns	ns	ns	< 0.01
Methionine	ns	ns	< 0.05	ns	ns	ns	ns
Phenylalanine	ns	< 0.01	ns	ns	ns	ns	ns
Proline	ns	< 0.001	< 0.05	ns	ns	ns	ns
Serine	ns	< 0.001	ns	ns	ns	< 0.05	ns
Threonine	ns	< 0.001	< 0.05	ns	ns	< 0.01	ns
Tyrosine	ns	< 0.01	ns	ns	ns	ns	ns
Valine	ns	< 0.001	ns	ns	ns	< 0.05	< 0.05

[1] ns = not significant

Table 12
Monosaccharide composition of NSP extracted from ingredients used in feeds for digestibility trials with silver perch. Ingredients were whole (W) and dehulled (D), *L. angustifolius* (Gungurru) (G) and *L. albus* (A).

Monosaccharide	Ingredient NSP monosaccharide composition (%)			
	AD	AW	GD	GW
Rhamnose	2.0	1.0	0.7	0.6
Fucose	0.0	0.4	0.6	0.5
Arabinose	13.1	11.5	11.8	10.0
Xylose	4.8	10.8	3.0	8.0
Mannose	2.8	2.9	1.2	2.1
Galactose	65.5	38.3	72.0	38.3
Glucose	7.9	31.0	8.7	35.8
Uronic Acid	3.9	4.1	2.0	3.9

Table 13
Monosaccharide composition of NSP extracted from diet and faecal samples (n=1) collected from silver perch during digestibility trials. Diets composed of whole (W) and dehulled (D), *L. angustifolius* (Gungurru) (G) and *L. albus* (A) included in a basal reference diet (R) at 30% were examined.

Monosaccharide	NSP Monosaccharide composition (%)[1]									
	Diet R	Faeces	Diet AD30	Faeces	Diet AW30	Faeces	Diet GD30	Faeces	Diet GW30	Faeces
Rhamnose	0.0	0.4	2.5	1.3	1.4	1.1	2.5	1.4	1.1	0.7
Fucose	0.0	0.7	0.0	0.4	0.0	0.3	0.0	0.4	0.4	0.6
Arabinose	19.5	18.9	16.1	15.2	15.3	14.1	14.0	13.6	12.4	11.4
Xylose	23.2	23.2	13.0	12.4	13.8	14.9	11.7	9.9	11.8	12.4
Mannose	2.5	2.5	2.5	1.5	5.0	2.1	2.0	1.4	2.1	1.8
Galactose	15.6	13.0	42.5	43.8	31.2	30.3	50.8	49.3	33.6	26.1
Glucose	33.4	35.6	18.4	20.2	24.0	30.9	15.1	19.2	33.1	41.5
Uronic Acid	5.9	5.8	5.2	5.2	9.2	6.2	3.8	4.8	5.5	5.6

[1] Monosaccharide composition is expressed as a percentage of the diet or faeces NSP.

6.4 Replacement of fish meal in diets for juvenile silver perch: III. digestibility and growth using Australian meat meal products

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Abstract

Apparent digestibility coefficients for beef meal, lamb meal, a high protein meal from mixed species (mixed meat meal) and from Provine®, a high protein meal based on selected ingredients, were determined for juvenile silver perch (*Bidyanus bidyanus*). Experimental diets comprised a reference diet plus meat meal products at either 15 or 30% inclusion. Silver perch readily accepted diets with up to 30% meat meal. Digestibility coefficients for dry matter, energy, nitrogen and amino acids were determined to assist with the formulation of diets to assess growth of silver perch. Digestibility coefficients for dry matter, energy and nitrogen all increased with increasing protein content in the meat products. Average amino acid digestibility coefficients were highest for the mixed meat meal and Provine®. Digestibility coefficients for alanine, arginine, glycine, methionine, proline and serine were all significantly higher for these products than for either beef or lamb meal. Digestibility of sulphur amino acids was significantly lower in Provine® than in the other products. Compared with fish meal, all meat products contained less lysine, and some meat products were also low in phenylalanine, isoleucine and histidine. An increase in total protein content, through removal of bone, improved the nutritional value of meat meal in silver perch diets.

Juvenile silver perch were grown for 65 days in 10 000-l tanks, using one of five diets with similar digestible nitrogen, energy and dry matter but different contents of fish meal, lamb meal and Provine®. Fish growth was reduced when diets contained less than 13% fish meal and more than 9% Provine® . However, food conversion efficiency and protein retention efficiency were unaffected by diet formulation. These results indicate that meat meal can replace most of the fish meal in silver perch diets without degrading their performance.

Keywords: Bidyanus bidyanus; Digestibility; Nutrient availability; Growth; Meat meal

1. Introduction

Fish meal replacement in aquaculture diets is recognised as a major international research priority (Manzi, 1989; New, 1991; Tacon, 1994), with many dietary development studies using different species and ingredients already having been conducted (Tacon, 1994). The majority have investigated the potential of soybean products to replace fish meal because of the excellent amino acid profile of soybeans (see review by Tacon, 1994). Other studies have investigated a range of different products including rapeseed meal, cottonseed meal (Tacon, 1994), mustard oil cake, linseed and sesame meals (Hosain and Jauncey, 1989a, 1989b) and other less common vegetable proteins (Tacon, 1994).

Few studies with meat meal products have been published (Lovell, 1992; Shimeno et al., 1993a, 1993b; Tacon, 1994; Shimeno et al., 1996) and in general, have been positive with respect to fish meal replacement (Lovell, 1992; Tacon, 1994). In Australia, meat meal and

meat products may have potential to replace significant quantities of fish meal in aquaculture feeds (Allan, 1997). On a cost per unit protein basis, meat meal is an attractive protein source, and for most farmed finfish and aquatic invertebrate species, the absence of significant quantities of carbohydrate, especially fibre, is a significant advantage over vegetable protein sources. In Australia, meat meal is in good supply with 480 Kt of meat meal produced in 1991/92 (Australasian Agribusiness Services, 1993) and 475 Kt produced for 1995/96 (Personal communication, Graeme Banks, Australian Renderers Association)

In terms of digestibility for fish, fish meal is generally superior to terrestrial animal protein sources, which are in turn superior to vegetable protein sources (Lovell, 1989). If ingredient digestibility is not considered when diets to compare different ingredients are formulated, the different diets may vary considerably in the digestible energy levels and in the amounts of specific essential nutrients (eg amino acids) actually available to the fish.

Silver perch (*Bidyanus bidyanus*) is an omnivorous, native Australian freshwater finfish currently being cultured in static ponds in Australia (Rowland and Barlow, 1991; Rowland et al., 1994 and 1995). They readily accept pelleted diets, tolerate crowded conditions and perform well in earthen ponds with low net water exchange (Rowland et al., 1994 and 1995).

The aim of this study was to determine the apparent digestibility coefficients (ADC's) for dry matter, energy, nitrogen and essential amino acids for four different meat meals in diets for silver perch. These results were used to formulate five diets with similar digestible energy, protein and essential amino acids but where fish meal was progressively replaced by a combination of lamb meal and Provine®. Performance and composition of fish fed these five diets for 65 days was measured.

2. Materials and Methods

2.1. Experimental diet preparation

All ingredients were ground or sieved to ensure all particles passed through a 710 μm screen. Dry ingredients were thoroughly mixed in a Hobart mixer (Troy Pty. Ltd., Ohio, USA) then combined with approximately 400 ml distilled water kg^{-1} dry mix before being extruded through a meat mincer (Barnco Australia Pty. Ltd., Leichhardt, NSW, Australia) with a 2 mm die (Experiment 1) or 2 and 3 mm die (Experiment 2). Pellets were dried at 35°C in a convection drier for approximately 6 h until the moisture content was between 10 to 15%, to produce a dry, sinking pellet.

2.2. Experimental fish and water quality

Silver perch (Exp. 1, mean weight ± sem. 6.2 ± 0.3 g and Exp. 2, mean weight 12.1 ± 0.1 g) were bred at the Grafton Research Centre and raised in earthen ponds using similar techniques to those described by Rowland and Thurstan (1994). Before experiments, fish were fed SP35 (Allan and Rowland, 1992) and were treated with 5 g l^{-1} NaCl to ensure they were free of ectoparasites and to prevent fungal infection (Rowland and Ingram, 1991).

Prior to stocking, fish were anaesthetised using a bath of ethyl ρ-aminobenzoate (Experiment 1, 25 mg l^{-1} for 5 min or Experiment 2, 50 mg l^{-1} for 3 min) then caught at random, weighed

(Experiment 1, individually or Experiment 2, in groups of 5 or 10) and distributed among tanks by systematic interspersion. Fish were also weighed at harvest.

During both experiments, water temperature (range 24.3 to 26.7°C), dissolved oxygen (above 5.0 mg l^{-1}), pH (between 7.2 and 8.3) nitrite and ammonia (<0.55 mg l^{-1} NO_2-N l^{-1} and <0.6 mg l^{-1} total ammonia - N l^{-1} respectively) were measured weekly using methods described in Allan et al. (1990).

2.3. Biochemical analyses

All chemical analyses were done in duplicate. Feed, faecal and fish samples were analysed for dry matter, ash, crude fat and energy (bomb calorimetry) by the AOAC (1975) procedures. Nitrogen was determined by the method of Havilah et al. (1977) (crude protein = N x 6.25). Amino acids were determined by the method of Cohen et al. (1989) and analysed following acid hydrolysis using high pressure liquid chromatography and Waters Pico-Tag (Waters Pty Ltd, Lane Cove, NSW, Australia). Sulphur amino acids were determined separately following performic acid digestion, and tryptophan, which is lost during acid hydrolysis (Cohen et al., 1989), was not analysed. Chromic oxide was determined by the method described in Scott (1978).

2.4. Experiment 1, Digestibility

Four meat products were evaluated in this experiment (Table 1a and b). The other components of experimental diets (Table 2) were SP35 (at about 70 or 85% inclusion) and chromic oxide used as an inert marker. SP35 was also the control diet.

Digestibility tanks were 170 l cylindro-conical tanks (conical base sloped at 35°) fitted with a 65 mm diameter, 250 mm settlement chamber which tapered into a 12 mm diameter, 150 mm length of silicone tubing. The inside of each tank was black. Continuously-flowing, preheated water was filtered through a sand filter and a diatomaceous earth filter, then passed through a 2 m^3 biological filter then a UV steriliser before being supplied to experimental tanks at a flow-rate of 600 ml min^{-1} $tank^{-1}$. Effluent water from each tank flowed out the side of the cylindro-conical tanks into a 25 mm diameter pipe. 20-25% of this flowed to waste and the rest was collected and recirculated. Each tank was aerated using two air-stone diffusers.

Fish were stocked (8 fish $tank^{-1}$) seven days before faeces were collected to allow for acclimatisation to experimental conditions. For four of these days, fish were fed SP35 then three days before faeces were collected, fish were fed their respective experimental diets. Fish were fed to excess (10% body weight d^{-1}) using automatic conveyor belt-type feeders for three hours each day from 0830-1130 h. Within one hour after feeding ceased, all uneaten food was removed, and the walls of the tank and the settlement chamber were thoroughly cleaned to remove any faeces, uneaten food or bacterial slime. The silicone tubing into which the faeces settled was packed in ice before faeces were removed (after approximately 18 h) to reduce bacterial proliferation which can affect the composition of faeces (Spyridakis et al., 1989).

Faecal samples were collected by settlement each morning and dried using silica gel desiccant under vacuum. Samples from each tank were pooled at the end of the experiment

and re-dried by the same method. Each sample was freeze dried and ground using a water cooled 1KA total recovery grinder prior to analyses. These methods have been shown to be valid for calculating digestibility coefficients for a range of ingredients fed to silver perch (Allan et al., unpublished data, see Section 6.1 of this report).

The indirect method of Cho and Kaushik (1990) was used to calculate apparent digestibility coefficients, with chromic oxide (1% dry basis) as the inert indicator. The apparent digestibility coefficients (ADC's) for energy, protein and essential amino acids in experimental diets and ingredients were calculated as described by Cho and Kaushik (1990). The proximate body composition of 4 silver perch from each of the 3 tanks for each treatment were analysed.

2.5. Experiment 2, Growth

Using the digestibility data obtained from experiment 1, five diets with approximately the same levels of digestible energy and protein were formulated using a linear least-cost diet formulation computer program 'Feedmania' (Mania Software, Brisbane, Australia) (Table 3). The meat meal products considered for the least cost diet formulation where Provine®, lamb meal and beef meal. Beef meal was not selected in the formulation by the least-cost program. The mixed meat meal product was excluded as it is only currently available in experimental quantities. The experimental diets comprised a reference diet (SP35) with 27% fish meal and three others with 13%, 6% or 0% fish meal with the remainder replaced by a mixture of Provine® and lamb meal. For these three diets L-lysine, DL-methionine and L- threonine were added if necessary to adjust the content of amino acids to that of the reference diet. The fifth diet was formulated without fish meal or added synthetic amino acids.

Experimental tanks were 10 000-l in capacity. Recirculated fresh water was filtered through a rapid rate sand filter before being supplied to experimental tanks at a flow rate of 17 l min^{-1}. Effluent water from each tank flowed out of the bottom of the tank via a 50 mm diameter drain leading to an external stand pipe. Overflow water from the standpipes was collected and ducted into a 7 000-l reservoir and recirculated through a 1 m^3 biological filter, and pumped through a sand filter and then recirculated to the fish tanks. Each tank was aerated using two air-stone diffusers.

Eighty-five silver perch were stocked (November, (spring) 1995) into each of the 15, 10 000 l tanks (three replicate tanks for each diet). Fish were fed by hand to satiation twice daily at 0800 and 1500 h, seven days a week, for a period of 65 days. Fish were then harvested (February, (late summer) 1996) and the survival rate, mean weight increment and food conversion ratio (FCR) = [weight of food, adjusted to 92% dry matter/wet weight fish gain], were calculated from each tank.

Proximate analyses on the whole body composition of five fish randomly selected from each tank were also conducted. From the proximate analyses the following indices were calculated:

• Protein deposition (PD) = [final weight (dry basis) x final protein content (dry basis)/100) - (initial weight (dry basis) x initial protein content (dry basis)/100)]

• Fat deposition (FD) = [final weight (dry basis) x final fat content (dry basis)/100) - (initial weight (dry basis) x initial fat content (dry basis)/100)]
• Protein efficiency ratio (PER) = [individual weight gain(g)/individual protein intake by fish (g dry weight)] (Wilson, 1989)
• Protein retention efficiency (PRE) = [((final dry weight x final % dry weight body protein) - (initial dry weight x initial % dry weight body protein)) / dry weight protein intake x 100]
• Fat retention efficiency (FRE) = [((final dry weight x final % body fat)- (initial dry weight x initial % body fat)) / dry weight fat intake x 100]
• Energy retention efficiency (ERE) = [((final dry weight x final % body energy)- (initial dry weight x initial % body energy)) / dry weight energy intake x 100].

2.6. Statistical analysis

The digestibility experiment (Experiment 1) was designed for analysis using two-factor ANOVA with meat products (B, L, M or P) as the first factor (fixed) and inclusion level (15 or 30%) as the second factor (also fixed). Single factor ANOVA was used to assess the difference between proximate body composition of fish fed experimental diets.

The growth experiment (Experiment 2) was designed for analysis using single-factor ANOVA. Homogeneity of variances was assessed using Cochrans Test (Winer, 1971) and comparison between means were made using Student Newman-Kuels multiple range test. Differences between means were considered significant at $P<0.05$. Unless otherwise stated, all results appear as mean ± standard error of the mean (n=3).

3. Results

3.1. Experiment 1, Digestibility

The analysed proximate composition of meat meals tested in this study were quite variable (Table 1b). When compared to Peruvian fish meal, the beef and lamb meals had less protein, fat and energy, the mixed meat meal had less protein and more fat and energy. Provine® had more protein and energy. Provine® and the mixed meat meal had less ash than the Peruvian fish meal, while the beef meal and lamb meal had more. The amino acid profile of the tested ingredients were also quite variable with lysine being lower in all meat products compared with Peruvian fish meal.

Digestibility coefficients for ingredients were calculated using the values for the reference diet and the proportion of the ingredient used (Table 4). The results of the two-factor ANOVA revealed significant differences between ingredients ($P<0.05$), but there was no significant difference between inclusion level and there was no interaction ($P>0.05$). For dry matter, energy and protein, digestibility coefficients increased significantly with protein content ($P<0.05$); Provine® was the highest, followed by the mixed meal, lamb meal then beef meal. Significant differences are indicated in Table 4. The digestibility coefficients for all amino acids were averaged and the values were 82.9% for the mixed meat meal and Provine®, 73.4% for beef meal and 77.9% for lamb meal. Digestibility coefficients for non-essential amino acids, alanine, glycine, proline and serine were significantly higher for the mixed meat meal and Provine® compared with other products.

The composition of fish fed experimental diets was analysed for protein, energy and fat and there were no significant differences with respect to the body compositions of fish fed different diets ($P>0.05$) (Table 5).

3.2. Experiment 2, Growth

Four fish died in the experiment; two fed the 27% fish meal diet and one fed the 6% fish meal diet and one fed the 0% fish meal diet with added amino acids. Diet had no significant effect on survival, FCR, PRE, FD, ERE or FRE. However, there was a significant diet effect on weight increment, PER and PD, and all of these indices were lower for diets without fish meal (Table 6).

There were no significant differences in the, moisture, crude protein, crude fat and ash compositions of silver perch carcasses fed different experimental diets for 65 days (Table 6). However, there was a difference between the initial and final moisture, crude fat and ash carcass composition for fish from each of the experimental diets. Carcass protein composition remained unchanged.

4. Discussion

In this study, the omnivorous silver perch, readily accepted diets with up to 30% meat meal. This is consistent with other studies, where diets including meat meal 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 (*Oreochromis mossambicus*), gilthead sea bream (*Sparus aurata*), rainbow trout (*Oncorhynchus mykiss*) and yellowtail (*Seriola quinqueradiata*) (Davies et al., 1989; Watanabe & Pongmaneerat, 1991; Davies et al., 1993; Shimeno et al., 1993a and 1993b). Mohsen and 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.

Meat meals were generally well digested by silver perch with higher apparent digestibility coefficients for dry matter, energy and nitrogen for the meat products with less ash (Table 4). Other authors have also found protein digestibility to be negatively correlated with high ash content in meat meals for rainbow trout (Watanabe and Pongmaneerat, 1991) and gilthead sea bream (Nengas et al., 1995).

Nengas et al. (1995) calculated apparent digestibility coefficients for diets containing meat products, fed to gilthead sea bream. For a diet with 43% meat meal (80% protein and 4% ash), protein and energy digestibility coefficients were 92 and 86% respectively, while for a diet with 40% meat and bone meal (52% protein and 27% ash), protein and energy digestibility coefficients were 78 and 75% respectively. The comparable digestibility coefficients for protein and energy for silver perch fed diets with 30% Provine® (81% protein and 3% ash), were 83 and 90% respectively and for diets with 30% lamb meal (54% protein and 35% ash) were 70 and 81% respectively. Given the liklihood of differences in the ingredients used in the different studies, the digesitiblity coefficients are in close agreement.

Very little data for individual amino acid digestibility coefficients has been published. Wilson et al. (1981) determined coefficients for a meat and bone meal (56.1% crude protein) for Channel catfish and the average of amino acid digesitibility values was 74.3%. This is similar to averages for silver perch of 73.4% and 77.9% for beef and lamb meals respectively

calculated from diets with 30% test ingredients; Table 4). Wilson et al. (1981) recorded higher digestibility coefficients for alanine, arginine, glycine and proline compared with those determined for silver perch fed beef or lamb meal, while values for silver perch were higher for aspartic acid, methionine, threonine, serine and tyrosine.

Removal of bone (=less ash) for the mixed species meal resulted in higher average amino acid digestibility (82.9% compared with 73.4% and 74.9% for beef and lamb meal respectively).

For pigs, lower and more variable lysine digestibility 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, personal communication, 1993). This trend was not evident with silver perch for low ash meat meals in this study.

Digestibility coefficients for protein in the present study were lower than those previously recorded with silver perch for fish meals, oilseed meals and cereals (but similar to those recorded for grain legumes (Allan and Rowland, 1994). Protein digestibility coefficients for silver perch fed meat products compared favourably with published values for rainbow trout. Asgard (1988) calculated a protein digestibility coefficient for meat and bone meal with 51.3% protein of 59%, while Alexis et al. (1988) determined protein digestibility values of 60.9 and 59.7% for defatted meat and bone meals (60.2% protein, 2.5% fat, 27.2% ash and 63.1% protein, 3.5% fat, 24.4% ash respectively).

Food conversion efficiency is influenced by dry matter digestibility. Dry matter digestibility coefficients for the mixed meal and Provine® compared favourably with coefficients for lower quality Peruvian fish meal, oilseed meals and grain legumes (Allan and Rowland, 1994). Published dry matter digestibility coefficients for meat products used in fish diets are scarce. McGoogan and Reigh (1996), reported a dry matter digestibility coefficient of 64.7% for meat and bone meal which did not differ significantly form Menhaden fish meal (79.7%) when fed to red drum at the 30% inclusion level. Gaylord and Gatlin (1996) also reported a dry matter digestibility coefficient of 86.2% for meat and bonemeal which were comparable to select Menhaden fish meal (93.9%). For rainbow trout, dry matter digestibility coefficients of 43.2 and 38.8% were determined for fat extracted meat and bone meals (60.2% protein, 2.5% fat, 27.2% ash and 63.1% protein, 3.5% fat and 24.4% ash respectively) (Alexis et al., 1988).

Digestibility coefficients for energy for Provine® were similar to those for Peruvian fish meal, although energy digestibility for other meat products was lower. Digestible energy from all meat products compared favourably with those from oilseed meals and grain legumes (Allan and Rowland, 1994). For silver perch in this study, the digestible energy values for beef meal and lamb meal (30% inclusion) were 12.3 MJ kg^{-1} and 13.2 MJ kg^{-1} respectively. These are comparable with digestible energy values determined for meat and bone meal with 54.1% protein, 10.3% fat, 31.1% ash for channel catfish (12.26 $MJkg^{-1}$) and rainbow trout (13.33 MJ kg^{-1}) (NRC, 1993).

During this study, meat meal products were successfully used to replace half of the fish meal in silver perch diets. Other studies have also shown that meat meal, and meat and bone meal can be successfully used to partially replace fish meal in diets for barramundi (*Lates*

calcarifer) (Aquacop et al., 1993), gilthead sea bream (Davies et al., 1993), (Davies et al., 1989), yellowtail (Shimeno et al., 1993a, 1993b) and rainbow trout (Watanabe et al., 1993). Mohsen and Lovell (1990) found meat meal, or other animal protein meals including fish meal, improved weight gain of channel catfish fed on soybean meal/corn basal diets. Digestibility studies with the prawn, *Penaeus monodon*, also indicate potential for meat meal with this species (Smith, 1995). However, Watanabe and Pongmaneerat (1991) found total substitution of fish meal in rainbow trout diets with either meat meal (80% protein and 4% ash) or meat and bone meal (52% protein and 27% ash) resulted in poor growth compared to when fish were fed diets containing extra white or brown fish meal as the sole protein source.

In Experiment 2, growth and the amount of protein deposited declined when silver perch were fed diets which contained less than 13% fish meal. Possible reasons for this include; 1) a lower concentration of essential nutrients in diets containing less than 13% fish meal, 2) reduced attractiveness or palatability of these diets, causing reduced feed intake and hence reduced growth, or, 3) some growth reducing compound in the diets with less fish meal and more meat products.

As digestible crude protein and digestible energy contents were similar for all diets, differences in protein:energy ratio do not account for the differences in growth. Polyunsaturated fatty acids were also balanced with the addition of fish oil and, therefore, also do not account for these differences. Compared with fish meal, meat products had lower contents of lysine, methionine plus cystine and threonine. Watanabe and Pongmaneerat (1991) attributed poorer performance of rainbow trout fed meat meal based diets, compared to fish meal based diets, to limiting amino acids in meat meal, notably lysine, methionine and tryptophan. However, in our study, with the exception of Diet 5 (0% fish meal, no added amino acids), lysine, methionine and threonine were balanced in all diets using crystalline amino acids. Had amino acids been deficient, a significant difference between Diet 4 and Diet 5 would have been expected and this was not evident. It is possible that the addition of crystalline amino acids was not effective. There is some conjecture about their efficiency in aquaculture diets (Lovell, 1989; Cowey, 1992; Murai, 1992; Davies and Morris, 1997), however, as concentrations of all three amino acids, even in Diet 5, were above published requirements for channel catfish and tilapia (NRC, 1993), deficiencies in amino acids are considered unlikely to have accounted for differences in growth. Davies and Morris (1997) found rainbow trout fed diets where 66% of the protein source was soybean, and containing a multiple amino acid supplementation, performed better than on diets with single or duel supplementation of methionine, methionine and lysine. However, fish fed the multiple supplemented diet did not perform as well as the control diet (fish meal as protein source). In Watanabe and Pongmaneerat's (1991) study, the protein efficiency ratio, net protein utilisation and biological value of fish fed meat meal based diets were all lower than fish fed fish meal based diets. Conversely, in our study, PER and protein retention efficiency were not affected by diet suggesting a different response by silver perch to the meat meals used here compared with the products and fish species used by Watanabe and Pongmaneerat (1991).

Feed intake was lower for fish fed Diets 3, 4 or 5 compared with those fed Diets 1 or 2. This may have been due to reduced attractiveness or palatability of the diets. In general, meat meal and meat and bone meal have been used to increase diet attractiveness and or palatability (Mohsen and Lovell, 1990; Watanabe et al., 1993). Apart from the control diet (Diet 1), the diets used in Experiment 2 contained lamb meal and Provine® as the meat

products used to replace fish meal. The amount of each of these was determined using a least-cost diet formulation program. Nutrients were specified to be similar to Diet 1, the fish meal content was specified at 13, 6 or 0% and then the mix of lamb meal and Provine® to supply these nutrients for each diet at the cheapest price was determined. On this basis, 6.3% and 9.1% of lamb meal and Provine® were used in Diet 2, 7.8% and 14.7% in Diet 3, 8.9% and 18.1% in Diet 4 and 8.9% and 18.9% in Diet 5. Although Provine® was highly digestible, higher contents may have caused the diets to be less attractive or palatable or to have reduced growth. Further investigation on Provine® as a sole ingredient at inclusion levels above 6.3% is warranted if it is hoped that this product is to be used as a major protein source for aquaculture feeds.

The diet cost to produce one kg of silver perch was reduced by feeding diets containing meat meal (Table 6). However, when more than half of the fish meal was replaced a decline in growth was observed. The added cost of the extended grow out period may off-set the benefits of cheaper feed costs if fish are fed diets containing less than 13% fish meal.

Analysed body composition of silver perch fed different experimental diets indicated they had similar protein, fat and energy contents (Table 6). Similarly, Shimeno et al. (1993b), reported the body composition of yellowtail fed diets containing 30% meat meal (65.3 % Protein, 14.8% fat and 11.4% ash) for 30 days, had protein and fat contents similar to fish fed the control diet comprised of fish meal and soybean.

Maximum inclusion levels of ingredients in formulated diets will depend not only upon composition and digestibility but also upon the presence of anti-nutritional factors. Meat can contain high contents of bone fragments which can be deleterious and may restrict the use of meat meal products. High ash might also have a negative effect on pelleting characteristics. Results here show meat products are well digested by silver perch and can be used to replace most of the fish meal in formulated diets without significantly reducing fish performance.

Acknowledgments

We wish to thank, Jane Frances, Rebecca Warner-Smith and Mark Booth at Port Stephens Research Centre and Ken O'Brien and Wendy Peasley at Wollongbar Agricultural Institute for their excellent technical help. Helena Heasman assisted in the manuscript preparation. This research was partially funded by the Australian Fisheries Research and Development Corporation under the Fish Meal Replacement Sub-Program. Additional funding was provided to theSub-Program by the Australian Centre for International Agricultural Research, the Australian Grains Research and Development Corporation and the Australian Meat Research Corporation. Support from Australian Native Fish Ltd is also gratefully acknowledged.

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Table 1b

Analyses of meat meal test ingredients used in experiment 1 (dry basis).

Nutrient	Ingredient				
	Beef meal (B)	Lamb meal (L)	Mixed meat meal (M)	Provine® (P)	Peruvian fishmeal[1]
Protein (%)	49.2	54.3	60.6	81.0	70.2
Fat (%)	9.2	7.2	17.2	10.4	11.3
Energy (MJ kg^{-1})	16.1	16.2	23.5	25.7	20.9
Ash (%)	36.0	34.5	12.1	3.0	17.6
Amino acids (%)					
Alanine	3.9	4.0	4.3	5.3	4.6
Arginine	3.9	4.3	4.5	6.8	5.1
Aspargine	3.3	3.8	4.9	6.9	6.2
Cystine	0.3	0.7	0.8	1.1	0.7
Glutamine	5.7	7.0	7.9	9.9	9.4
Glycine	7.7	6.7	6.6	7.9	4.8
Histidine	0.8	1.2	1.2	1.6	2.3
Isoleucine	1.3	1.8	2.1	3.7	3.5
Leucine	2.7	3.5	4.3	6.2	5.3
Lysine	2.5	3.5	3.7	4.9	5.5
Methionine	0.7	1.1	1.1	1.6	2.0
Phenylalanine	1.5	1.9	2.3	3.4	2.9
Proline	5.0	4.6	4.5	5.2	3.5
Threonine	1.6	2.1	2.6	3.6	3.2
Tyrosine	1.1	1.5	1.9	3.0	2.3
Serine	2.1	2.4	3.0	3.6	3.0
Valine	2.0	2.4	3.0	4.3	3.7

[1] Analysed from previous experiment.

Table 1a.
Suppliers and prices for meat products

Ingredients	Supplier	Address	Tel/Fax	Price $/t	Contact
Lambmeal (L)[1]	Fletcher International	PO Box 764 Dubbo 2830	068 845833 067 842965(F)	453	Peter Breen
Beef meal	Beef City			445	
Mixed meat meal	Midco			500	
Provine® (P)	Aspen Technology	2 Cope Street Preston 3072	03 4806200 03 4804542 (F)	775	Martin Flavin

[1] After lambmeal was used in the growth experiment at PSRC and the formulation finalised for the least-cost experiment at GRC, Fletchers International raised the price of lambmeal to >$700/t as a new export market was identified for this product.

Table 2
Formulation of digestibility diets used in Experiment 1 (% dry basis).

Ingredient	Treatment								
	1	2	3	4	5	6	7	8	9
Reference diet (SP35)[1]	99.0	84.2	69.3	84.2	69.3	84.2	69.3	84.2	69.3
Beef meal (with bones) (M)	-	14.8	29.7	-	-	-	-	-	-
Lamb meal (with bones) (L)	-	-	-	14.8	29.7	-	-	-	-
Mixed meat meal (W)	-	-	-	-	-	14.8	29.7	-	-
Provine® (P)	-	-	-	-	-	-	-	14.8	19.8
Chromic oxide	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0

[1] Formulation and composition of SP35 described in Table 3.

Table 3

Formulation of SP35 (Diet 1) and other experimental diets to evaluate meatmeal as a substitute for fishmeal in rations for silver perch (Expt. 2).

Ingredient	Diet (%)					Ingredient cost (AUS$/t)[1]
	1	2	3	4	5	
Fishmeal (Danish)	27.0	13.0	6.0	0.0	0.0	1300
Soybean meal	20.0	20.0	20.0	20.0	20.0	495
Blood meal	2.0	3.4	3.0	3.9	3.9	900
Lambmeal	-	6.3	7.8	8.9	8.9	453
Provine®	-	9.1	14.7	18.1	18.9	775
Wheat	26.9	22.1	21.9	22.0	22.2	180
Sorghum	11.0	11.0	11.0	11.0	11.0	180
Corn gluten meal	4.0	6.0	6.0	6.0	6.0	700
Millrun	2.0	2.0	2.0	2.0	2.0	190
Fish oil	1.0	1.1	1.1	1.1	1.1	800
DL-methionine	0.2	0.3	0.4	0.5	-	5500
L-lysine	-	0.1	0.2	0.3	-	4250
L-threonine	-	-	0.7	0.1	-	8500
Di-calcium phosphate	2.0	1.7	1.9	2.0	2.0	610
Vitamin premix[2]	1.0	1.0	1.0	1.0	1.0	4000
Mineral premix[3]	3.0	3.0	3.0	3.0	3.0	4000
Diet ingredient cost (AUD$/t)	*756*	*699*	*727*	*652*	*607*	
		Composition (calculated)				
Digestible protein (%)		32.1	34.0	34.1	34.1	34.0
Digestible energy (MJ kg^{-1})		13.0	13.4	13.3	13.2	13.3
Ash (%)		6.9	7.5	7.5	7.5	7.5
ADF (%)		3.1	3.3	3.3	3.3	3.4
Fat (%)		6.4	6.2	6.0	5.7	5.8
Linolenic series fatty acids (%)		0.3	0.3	0.3	0.3	0.3
Digestible arginine (%)		2.0	2.1	2.1	2.1	2.1
" histidine (%)		0.8	0.9	0.8	0.8	0.8
" isoleucine (%)		1.4	1.4	1.3	1.3	1.3
" leucine (%)		2.9	3.1	3.0	3.0	3.0
" lysine (%)		2.1	2.2	2.0	2.0	1.8
" methionine + cys (%)		1.4	1.4	1.4	1.4	0.9
" phenyl + tyro (%)		2.7	2.8	2.7	2.7	2.7
" threonine (%)		1.4	1.4	1.4	1.4	1.3
" valine (%)		1.7	1.7	1.6	1.6	1.6

[1] Diet ingredient cost does not include the cost of processing. Prices from NSW Agriculture, Sydney Retail Feed Ingredient Prices or from commercial feed ingredient manufaturers (does not include freight).

[2] (IU kg^{-1}diet): retinol (A), 8000; cholecalciferol (D3), 1000; α-Tocopherol acetate (E), 125; (mg kg^{-1}diet): ascorbic acid (C), 1000; biotin (2%), 1; calcium panothenate, 55; calcium propionate, 250; choline chloride, 1500; cyanocobalamin (B12), 0.02; ethoxyquin, 150; folic acid, 4; menadione sodium bisulphite (K3), 16.5; myo-inositol, 600; nicotinamide, 200; pyridoxine (B6), 15; riboflavin (B2), 25.2; thiamine HCl (B6), 10.

[3] (mg kg^{-1}diet): calcium carbonate, 7500; manganese sulphate, 300; zink sulphate, 700; copper sulphate, 60; ferrous sulphate, 500; sodium chloride, 7500; potassium iodate, 2.

Table 4

Apparent digestibility coefficients of ingredients for silver perch in Experiment 1 (dry basis)[1,2].

	Ingredient							
	Inclusion level 15%				Inclusion level 30%			
	Beef	Lamb	Mixed	Provine®	Beef	Lamb	Mixed	Provine®
Nutrient								
Dry matter	37.2±2.3^{a}	56.4±1.4^{b}	75.0±5.8^{c}	90.1±3.3^{d}	48.1±3.2^{a}	53.3±0.5^{a}	76.4±1.1^{b}	87.7±1.1^{c}
Energy	69.4±5.8^{a}	81.6±2.0^{ab}	87.5±4.2^{bc}	100.2±4.2^{c}	76.4±1.4^{a}	81.4±0.7^{b}	82.6±0.9^{b}	90.3±1.1^{c}
Protein	64.2±0.7^{a}	68.7±1.8^{a}	83.9±2.3^{b}	83.5±1.3^{b}	68.7±2.1^{a}	70.1±0.3^{a}	81.5±0.9^{b}	83.7±0.3^{b}
Amino acids								
Ala	58.4±1.3^{a}	66.9±1.9^{a}	91.2±2.2^{c}	81.8±4.2^{b}	62.3±1.8^{a}	66.4±2.2^{a}	83.7±1.7^{b}	86.0±1.9^{b}
Arg	62.8±1.2^{a*}	60.1±1.4^{a*}	91.4±1.2^{c}	79.9±4.0^{b}	68.7±0.9^{a*}	70.0±1.7^{a*}	86.0±1.7^{b}	85.4±1.7^{b}
Asp	83.3±2.8^{a}	89.0±3.9^{a}	94.2±3.0^{a}	82.3±3.6^{a}	79.1±0.9^{a}	87.4±0.7^{b}	86.1±0.8^{b}	83.3±1.7^{b}
Cys	72.2±1.9^{b}	72.6±2.1^{b}	79.1±3.6^{b}	53.9±5.8^{a*}	63.3±7.1^{a}	74.7±7.9^{a}	70.5±1.0^{a}	71.8±0.7^{a*}
Glut	78.0±1.5^{a}	81.5±3.3^{a}	90.8±2.1^{a}	84.4±3.7^{a}	77.6±0.5^{a}	82.9±0.8^{b}	85.4±1.0^{b}	85.5±1.4^{b}
Gly	39.2±2.0^{a*}	45.2±3.2^{a}	105.3±2.6^{b*}	98.8±4.3^{b}	49.6±2.9^{a*}	49.1±2.9^{a}	93.3±2.2^{b*}	99.1±2.2^{b}
Hist	63.2±2.1^{a*}	77.6±3.5^{b}	85.7±2.6^{b}	76.6±3.1^{b}	77.5±0.3^{a*}	87.3±0.3^{b}	79.6±1.8^{a}	80.3±2.2^{a}
Isoleu	112.7±0.4^{c*}	81.6±3.2^{b}	84.8±2.7^{b}	67.9±3.8^{a}	78.0±0.7^{a*}	83.4±1.0^{a}	77.8±1.7^{a}	76.6±2.5^{a}
Leue	NA	85.1±2.4^{b}	88.3±2.3^{b*}	74.3±3.8^{a}	79.6±0.5^{a}	84.2±0.9^{a}	80.5±1.6^{a*}	79.3±1.9^{a}
Lys	75.0±0.7^{a}	82.0±2.1^{ab}	86.9±2.7^{b}	80.2±2.4^{ab}	77.4±1.0^{a}	81.8±1.1^{ab}	81.6±1.2^{ab}	84.1±1.4^{b}
Meth	89.8±1.6^{b}	85.0±2.7^{b}	91.0±1.3^{b}	75.1±2.4^{a*}	86.7±0.9^{a}	85.4±1.5^{a}	86.9±1.4^{a}	82.9±0.3^{a*}
Phen	77.5±1.2^{a}	83.1±2.6^{ab}	90.0±2.5^{b*}	75.4±3.9^{a}	77.5±0.9^{a}	82.9±0.9^{a}	80.7±1.9^{a*}	79.5±2.0^{a}
Prol	68.2±1.6^{a}	69.5±2.0^{a}	101.6±2.3^{b*}	93.1±4.7^{b}	62.4±1.7^{a}	65.0±2.3^{a}	90.6±1.7^{b*}	94.5±1.8^{b}
Ser	62.3±2.2^{a*}	73.2±3.0^{ab}	87.0±3.3^{b}	76.7±5.3^{ab}	71.5±0.3^{a*}	76.8±1.3^{b}	81.5±2.1^{b}	80.1±1.4^{b}
Threo	76.4±2.1^{a}	82.9±3.8^{a}	88.2±2.9^{a}	75.8±4.5^{a}	76.6±0.7^{a}	81.6±0.5^{a}	81.4±1.7^{a}	80.3±1.9^{a}
Tyro	84.5±0.8^{ab}	86.6±2.0^{ab}	92.5±2.1^{b}	78.6±3.9^{a}	83.5±0.5^{a}	85.7±0.4^{a}	85.8±1.2^{a}	81.1±1.8^{a}
Val	74.6±0.4^{ab}	78.0±2.5^{ab}	84.7±2.8^{b}	72.0±3.7^{a}	75.8±0.5^{a}	80.2±1.4^{a}	78.5±1.8^{a}	78.7±2.4^{a}
Average	73.6	76.5	90.2	78.0	73.4	77.9	82.9	82.9

[1] Digestibility coefficients for ingredients were calculated using the equation: (digestibility coefficient of experimental diet - digestibility coefficient of SP35 x proportion of SP35 in experimental diet)/proportion of test ingredient in experimental diet.

[2] Values are means ± SEM. Means within the same inclusion level in the same row with the same superscript are not significantly different ($P>0.05$). Means with * appearing in the superscript indicate a significant difference between inclusion levels for that ingredient. Data for protein digestibility coefficients were transformed (arcsine $x^{0.5}$) prior to statistical analysis.

Table 5

Analysed body composition of silver perch fed different experimental diets (Exp. 1)[1].

Diet[2]	Protein (%)	Energy (MJ Kg^{-1})	Fat (%)
Reference (SP35)	50.4±0.3[a]	23.4±0.1[a]	23.3±0.2[a]
Beef meal 15%	48.9±0.4[a]	23.9±0.3[a]	25.7±1.4[a]
Beef meal 30%	49.2±0.3[a]	23.3±0.3[a]	23.3±0.8[a]
Lamb meal 15%	48.8±1.2[a]	23.7±0.1[a]	25.9±0.5[a]
Lamb meal 30%	50.0±0.6[a]	23.5±0.1[a]	25.8±1.0[a]
Mixed 15%	49.0±0.8[a]	23.3±0.1[a]	26.4±0.4[a]
Mixed 30%	49.2±0.6[a]	22.9±0.3[a]	26.6±1.5[a]
Provine® 15%	49.2±1.5[a]	22.9±0.3[a]	25.4±0.7[a]
Provine® 30%	48.6±1.8[a]	23.3±0.1[a]	25.2±1.0[a]

[1] Values are means ± SEM. There were no significant differences ($P>0.05$; ANOVA; SNK) in composition of fish fed different diets.

[2] The percentage indicates inclusion level.

Table 6. Growth performance, survival, feed utilisation and carcass composition of silver perch after a 65 day feeding trial (Exp. 2)[1].

		Diet (% Fishmeal)				
		1 (27)	2 (13)	3 (6)	4 (0)	5 (0)[2]
Initial weight (g)		11.9 ± 0.16^{a}	12.2 ± 0.02^{a}	12.1 ± 0.09^{a}	12.2 ± 0.08^{a}	12.1 ± 0.10^{a}
Weight increment (g)		60.4 ± 2.29^{a}	60.2 ± 1.01^{a}	53.9 ± 2.30^{ab}	52.3 ± 1.75^{b}	50.0 ± 1.47^{b}
Survival (%)		99.2 ± 0.4^{a}	100.0 ± 0.0^{a}	99.6 ± 0.4^{a}	99.6 ± 0.4^{a}	100.0 ± 0.0^{a}
Feed conversion ratio		1.48 ± 0.01^{a}	1.44 ± 0.02^{a}	1.46 ± 0.02^{a}	1.50 ± 0.02^{a}	1.47 ± 0.02^{a}
Protein efficiency Ratio		2.10 ± 0.02^{a}	2.04 ± 0.02^{ab}	2.01 ± 0.03^{ab}	1.96 ± 0.03^{b}	2.01 ± 0.03^{ab}
Protein deposition (g fish^{-1} dry weight)		10.54 ± 0.30^{a}	10.37 ± 0.38^{a}	9.37 ± 0.19^{b}	8.80 ± 0.20^{b}	8.98 ± 0.33^{b}
Protein retention efficiency (%)		36.70 ± 0.26^{a}	35.18 ± 0.71^{a}	34.96 ± 1.18^{a}	32.93 ± 0.72^{a}	36.02 ± 0.63^{a}
Fat deposition (g fish^{-1} dry weight)		11.02 ± 0.76^{a}	11.37 ± 0.38^{a}	10.21 ± 1.20^{a}	10.14 ± 0.86^{a}	9.42 ± 0.23^{a}
Fat retention efficiency (%)		191.99 ± 7.28^{a}	211.80 ± 8.74^{a}	214.96 ± 17.56^{a}	226.41 ± 14.54^{a}	221.62 ± 4.36^{a}
Energy retention efficiency (%)		65.32 ± 0.79^{a}	65.80 ± 1.82^{a}	65.97 ± 2.05^{a}	64.94 ± 2.38^{a}	65.99 ± 0.76^{a}
Feed cost (AUD $/kg fish)[3]		1.12 ± 0.01^{d}	1.01 ± 0.01^{b}	1.06 ± 0.02^{c}	0.98 ± 0.01^{b}	0.89 ± 0.01^{a}
Carcass composition (% dry weight basis of whole fish)						
	Initial					
Moisture	67.37	59.67 ± 0.28^{a}	60.00 ± 0.33^{a}	59.61 ± 0.48^{a}	59.99 ± 0.44^{a}	59.58 ± 0.04^{a}
Crude protein	42.63	41.88 ± 0.52^{a}	41.65 ± 0.91^{a}	41.56 ± 1.34^{a}	40.71 ± 1.42^{a}	42.44 ± 0.40^{a}
Crude fat	31.37	41.91 ± 1.24^{a}	43.54 ± 0.88^{a}	42.71 ± 2.46^{a}	43.95 ± 1.66^{a}	42.44 ± 0.40^{a}
Ash	15.40	10.61 ± 0.39^{a}	10.76 ± 0.03^{a}	11.08 ± 0.52^{a}	10.90 ± 0.39^{a}	10.79 ± 0.13^{a}

1 Values are means ± SEM for 3 replicate tanks. Means in rows which share the same superscript were not significantly different ($P>0.05$; ANOVA; SNK).

2 No added crystalline amino acids.

3 Feed cost = the cost of feed (total for ingredients only) to produce 1 kg fish (FCR x cost of ingredients), and were based on ingredient costs of diets in Table 3.

6.5 Replacement of fish meal in diets for silver perch: IV. least-cost formulation of practical diets.

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Abstract

Silver perch fingerlings (80.7 ± 1.2 g) were stocked at a density of 15 000 fish ha^{-1} earthen ponds (0.1 ha) and cultured for six months to a market size of >350 g $fish^{-1}$. Fish were fed a reference diet (SP35; 27% fish meal) or one of two least-cost diets formulated using digestibility coefficients for a range of Australian agricultural products (meat meal and plant proteins) which replaced all but 5% (95LC2) or 10% (95LC1) of the fish meal content. Survival was high (>95%) in all ponds. There was no significant difference between the performance of silver perch fed the two least-cost diets. The mean weights (431.9 g, 439.8 g), growth rates of 2.5 g $fish^{-1}$ day^{-1} and FCRs (2.0, 1.9) of fish fed 95LC1 and 95LC2 respectively were significantly different ($P<0.05$) to the weight (395.4 g), growth (2.2 g/f/day) and FCR (2.2) of fish fed SP35. A diet ingredient cost of AUD$1.09 to produce 1kg fish for 95LC2 was significantly lower ($P<0.05$) than the costs of the other diets. Diet did not significantly affect body composition (nitrogen, fat, ash or energy) or the sensory quality of the fish, but all silver perch contained a lot of body fat. These results show that least-cost diets containing meat meal and plant proteins (as replacements for all but 5% fish meal) are suitable for silver perch grown to market size in earthen ponds.

Keywords: Bidyanus bidyanus; nutrition; least-cost; fish meal replacement.

1. Introduction

Silver perch (*Bidyanus bidyanus*) is an omnivorous, native Australian, freshwater finfish with high potential for aquaculture (Rowland and Barlow, 1991). They readily accept pelleted diets, tolerate crowded conditions and perform well in earthen ponds (Rowland et al., 1994, 1995). Large-scale commercial culture depends on the development of cost-effective diets. The major constraints in formulating cost-effective diets are a lack of information regarding nutritional requirements of fish and the digestibility of suitable feed ingredients (Tacon, 1994; McGoogan and Reigh, 1996). While a large number of fish species are cultured throughout the world, only the nutritional requirements of rainbow trout, *Oncorhynchus mykiss*, and channel catfish, *Ictalurus punctatus*, have been extensively studied (Lall, 1991). One of the consequences of this lack of information is a heavy reliance on high quality fish meal as a protein base for most intensively-farmed aquaculture species (Lovell, 1989).

Unfortunately, more than 90% of the fish meal used in animal feeds in Australia is imported (ABARE, 1996). However, large quantities of agriculture proteins are produced locally. For

example approximately 480 kt of meat and bone meal (Australasian Agribusiness Services, 1993), up to 1.4 million t of oilseeds and 2.3 million t of pulses are produced annually (ABARE, 1996).

Although there is little information on nutritional requirements of silver perch, data for other species, especially omnivorous species such as channel catfish and tilapia, were used to formulate an experimental diet for silver perch (SP35) (Allan and Rowland, 1992). This diet has produced fast growth (>2 g fish^{-1} day^{-1}) and high production (~10 t^{-1} ha^{-1} yr^{-1}) in large-scale farming experiments (Rowland et al., 1994, 1995; Rowland, 1995a) and forms the basis for several commercially manufactured diets now available to industry. Additional nutritional research with silver perch has evaluated a large number of Australian agricultural proteins for use in formulated diets. This evaluation has included the determination of digestibility coefficients for dry matter, energy, nitrogen, amino acids and phosphorus for approximately 60 ingredients, including some processed in different ways (Allan et al., unpublished data - see Section 6.2). Growth studies to determine maximum inclusion levels for several protein sources considered to have high potential to replace fish meal have also been completed (Allan et al., 1993; Allan et al., unpublished data - see Section 6.6).

Some knowledge of nutritional requirements, information on digestibility coefficients for a wide range of ingredients, and data on the maximum inclusion levels for key ingredients provides the basis for the use of least-cost programming to formulate diets (Cho et al., 1985; NRC, 1993).

In this experiment we evaluated the performance and sensory properties of silver perch fed diets with minimal fish meal (5 or 10%), containing Australian agricultural ingredients selected on a least-cost basis using data on ingredient digestibility and maximum inclusion levels from earlier research (Allan et al., unpublished data - see Section 6.2). The two least-cost diets, which differed in fish meal content and nutrient specifications, were compared with a commercially available diet (SP35) formulated by Allan and Rowland (1992). To ensure that data obtained from this experiment had direct commercial application, the diets were assessed in a large-scale pond trial with fish stocked at commercially-relevant densities and cultured to a market size of greater than 350 g per fish over a six month grow-out period.

2. Materials and Methods

2.1. Experimental diets

The least-cost diets were formulated using the linear least-cost computer program 'Feedmania' (Mania Software, Brisbane, Australia). With least-cost diet formulation, nutrient concentrations and ingredient contents are specified (minimum and/or maximum levels or unrestricted) and then the cheapest mix of ingredients to supply the specified nutrients are selected. For the least-cost diets formulated for this study (95LC1 and 95LC2), nutrients were specified in relation to the nutrient profile in the successful SP35. For both diets, digestible energy, digestible crude protein and digestible phosphorus were restricted to within 5% of the concentrations in SP35. For 95LC1, fish meal content was set at 10% and digestible essential

amino acids and linolenic series fatty acids were restricted to within 5% of the concentrations in SP35. For 95LC2, fish meal was set at 5% and digestible essential amino acids and linolenic series fatty acids were restricted to within 10% of the concentrations in SP35. Peanut meal and canola were excluded from 95LC1 and restricted to 5% in 95LC2. Ingredients and the analysed composition of the experimental diets are shown in Tables 1 and 2.

The least-cost diets, 95LC1 and 95LC2, were manufactured by Ridley Agriproducts (Narangbar, Qld, 4504). The diets were ground to ≤ 500mm particle size, steam conditioned and were manufactured to give 3 or 6 mm diameter sinking pellets (Table 1). The control diet, SP35 (Diet 1, Table 1) was manufactured by Janos Hoey Pty Ltd (Forbes, NSW, 2871) using a pellet press, without steam conditioning, and was also pressed into 3 or 6 mm diameter sinking pellets.

2.2. Experimental fish

Silver perch were artificially bred at the Grafton Research Centre and the fingerlings raised in earthen ponds using techniques described by Rowland (1995a) and Thurstan and Rowland (1995). Before the experiment, fingerlings were fed SP35 and treated with 5 g l^{-1} NaCl for five days to ensure they were free of ectoparasites and to prevent fungal infection (Rowland and Ingram, 1991). Immediately prior to stocking, fish were anaesthetised using ethyl ρ-aminobenzoate (20 mg l^{-1}), weighed and distributed among nine ponds by systematic interspersion. A total of 1 500 silver perch (mean weight 80.7 g) were stocked (density of 15 000 fish ha^{-1} into each 0.1 ha earthen pond (Table 3). Three replicate ponds were used for each diet.

2.3. Experimental facilities and procedures

Experimental earthen ponds were 0.1 ha with a maximum depth of 2 m. The ponds were aerated using a 1-hp paddlewheel aerator for at least 13 hours a day, between 1700 and 0800 h. The ponds were static and water was added every four to five weeks to account for evaporative loss and seepage. Up to 50% of the water in each pond was exchanged during January because of relatively high concentrations of unionised ammonia (>0.3 mg l^{-1}) in most ponds.

The fish were cultured for 143 days from December (Summer, 1995) through to May (Autumn, 1996). Fish were fed a maximum of 3 % bw d^{-1}. Daily rations were divided evenly and fed twice daily by hand at 0800 h and 1500 h, seven days a week. Approximately 100 fish $pond^{-1}$ were sampled monthly, the mean weight determined, the biomass estimated and the ration adjusted accordingly. Feed rates were also readjusted mid-month based on daily growth rates from previous sampling. Fish were harvested by seine net and draining the ponds. All fish were harvested, counted and weighed. Performance was evaluated by measuring survival, growth rate, weight gain, body composition, food conversion ratio (FCR), protein efficiency ratio (PER) = [individual wet weight gain/individual protein intake by fish (dry weight)], production per unit pond area and ingredient cost per unit of fish produced.

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

2.4. Sensory evaluation of silver perch

At the completion of the experiment, the silver perch were purged for three weeks in tanks supplied with domestic water. Eight fish from each of the three ponds for each treatment, were used for sensory evaluation by an experienced seafood profiling panel using a standard rating procedure (Australian Standard 2542.2.3, 1988). Whole silver perch were gutted and frozen at -18°C for shipment and holding prior to analysis. The aim of this component of the experiment was to assess odour, appearance, flavour and texture attributes of cooked silver perch to determine if diet composition affected sensory properties of the flesh.

2.5. Biochemical analyses

All chemical analyses were done in duplicate. Fish samples were analysed for dry matter, ash, crude fat and energy (bomb calorimetry) by the AOAC (1975) procedures. Nitrogen was determined by the method of Havilah et al. (1977) (crude protein = N x 6.25). Amino acids were determined by the method of Cohen et al. (1989) and analysed following acid hydrolysis using high pressure liquid chromatography and Waters Pico-Tag (Waters Pty Ltd, Lane Cove, NSW, 2066, Australia). Sulphur amino acids were determined separately following performic acid digestion, and tryptophan, which is lost during acid hydrolysis, was not analysed (Cohen et al., 1989).

2.6. Statistical analysis

All experiments were designed for analysis using single-factor Analysis of Variance (ANOVA). Homogeneity of variance was assessed using Cochrans' Test, and multiple comparison among means using Student Newman-Keuls procedure. Mean values were considered significant at $P<0.05$.

For each of the sensory variables measured, scores for each diet were compared using a randomised block analysis of variance with sessions and panellists as blocking terms. Where a significant ($P<0.05$) F ratio was found then pairwise comparisons of the mean scores were made using the least significant difference procedure.

3. Results

Survival, growth, FCR and production

Results for survival, growth performance, food conversion ratio (FCR) and fish production rates are presented in Table 4. Survival was above 96.8% in all ponds and there were no significant differences in survival between treatments. Proximate composition of whole fish fed different diets is presented in Table 5. There were no significant differences ($P>0.05$) in

dry matter, nitrogen, ash, energy or fat, in the whole body composition of fish fed different diets. The mean weights at harvest and the mean weight increments were significantly higher ($P<0.05$) for fish fed the 95LC1 and 95LC2 compared with those fed SP35 (Table 4). The growth of fish fed 95LC1 or 95LC2 (2.5 g fish^{-1} day^{-1}) was significantly faster ($P<0.05$) (Table 4) than the growth of fish fed SP35 (2.2 g fish^{-1} day^{-1}).

The growth of silver perch is shown in Fig. 1. Mean production rates of 6.3 and 6.5 t ha^{-1} in ponds fed 95LC1 and 95LC2 respectively were significantly higher than production rates of fish fish fed SP35. FCRs for fish fed LC diets were significantly lower than for those fed SP35 (Table 4).

Water quality

Diet did not have a significant effect (ANOVA; $P>0.05$) on water quality. The ranges of the monthly means for each variable were: water temperature 19.7 to 27.6°C; dissolved oxygen 4.9 to 7.7 mg L^{-1}; pH 6.7 to 9.0; and total ammonia nitrogen ranged from 0.3 to 2.2 mg L^{-1}. Unionised ammonia exceeded 0.1 mg L^{-1} in most ponds during the December-February period, and concentrations as high as 0.4 mg L^{-1} were recorded in some ponds during January. On such occasions feeding rates were reduced and the ponds were flushed.

Sensory evaluation

The results for sensory evaluation of silver perch undertaken at the Centre for Food Technology, QDPI, are presented in Figures 2, 3 and 4. They showed that fish from all dietary treatments were highly acceptable. Fish fed diet 95LC2 had whiter flesh than those on the other diets. There were no significant differences ($P>0.05$) in odour or flavour characteristics of the flesh (Figures 2 and 4 respectively), although a slightly stronger weedy/herbaceous flavour was detected in fish fed diet 95LC1. This diet also produced flesh which was rated significantly ($P<0.05$) more flaky in texture (Figure 3). None of these differences significantly altered overall liking scores.

4. Discussion

The high survival (95%) of silver perch achieved in the current study is characteristic of this species when good fish husbandry and pond management techniques are used (Rowland, 1995b). The mean daily growth rate of 2.2 g fish^{-1} day^{-1} of silver perch fed SP35 was very similar to growth rates (2.0-2.3 g fish^{-1} day^{-1}) achieved using this diet in previous studies by Rowland (1995a) and Rowland et al. (1995). The significantly faster growth (2.5 g fish^{-1} day^{-1}) of fish fed 95LC1 and 95LC2 (Table 4) suggests that the least-cost diets enhanced growth. This growth rate is the fastest yet reported for silver perch, confirming the suitability of the 95LC1 and 95LC2 diets. The slower growth over the last 28 days (Figure 1) may have been due to decreasing water temperatures during autumn and/or a discrepancy between the last monthly sample, in which only ~10% of the fish were collected using a seine net to estimate mean weight, and the final mean weight which was calculated using the actual biomass and total number of fish harvested from each pond.

During this study, Australian agriculture products were successfully used to replace most (all but 5 or 10%) of the fish meal in silver perch diets. The experiment was conducted in 0.1 ha earthen ponds (which are similar to many used by industry) with fish stocked at commercial densities and cultured to a market size of >400 g. The excellent results with diets 95LC1 and 95LC2 ; which contained 10 and 5% fish meal respectively, demonstrate the potential of meat meal, lupins and field peas to be used as the major protein source in commercial diets for silver perch. This confirms the potential indicated by the earlier digestibility and growth studies. (Allan et al., unpublished data - see Sections 6.2, 6.3 and 6.6 of this report; Stone et al., unpublished data, see Section 6.4 of this report).

Other studies have also shown that meat meal, and meat and bone meal can be successfully used to partially replace fish meal in diets for barramundi (*Lates calcarifer*) (Aquacop et al., 1993; Williams et al., 1997), sea bream (*Sparus aurata*) (Davies et al., 1991), tilapia (*Oreochromis mossambicus*) (Davies et al., 1989), yellowtail (*Seriola quinqueradiata*) (Shimeno et al., 1993a, 1993b) and rainbow trout (*Oncorhynchus mykiss*) (Watanabe et al., 1993). Mohsen and Lovell (1990) found meat meal, or other animal protein meals including fish meal, improved weight gain of channel catfish fed on soybean meal/corn basal diets. Digestibility studies with the prawn, *Penaeus monodon*, also indicate potential for meat meal with this species (Smith, 1995; Williams et al., 1997).

In general, meat meal and meat and bone meal have been used to increase diet attractiveness and or palatability (Mohsen and Lovell, 1990; Watanabe et al., 1993). Fish fed 95LC2, a diet containing only 5% fish meal, with most protein supplied from meat meal, grew more rapidly than fish fed the other diets. These diets were clearly as palatable and, as observed during feeding, just as attractive as the control diet, SP35.

FCR of 95LC1and 95LC2 are significantly lower than SP35, and were comparable to those achieved in previous production trials with silver perch fed with SP35 which ranged from 1.6 to 2.0:1 (Rowland et al., 1994), and commercial catfish ponds which are near 2.0:1 1992). The lower FCR's and increased weight gains for 95LC1 and 95LC2 should in turn lead to lower production costs.

Differences in performance of fish fed SP35, 95LC1 or 95LC2 were significant ($P<0.05$), and, when the cost of supplying the ingredients for each diet was calculated (cost of ingredients x FCR), there were large differences between diets ($1.09/kg fish from 95LC1 and $1.69/kg fish from SP35). Ingredient prices were obtained from NSW Agriculture, Sydney Retail Feed Ingredient Prices, which are compiled mainly for pig and poultry farmers, or directly from ingredient suppliers or feed manufacturers. They do not include any freight charges, although clearly all feed mills will have to pay costs of freight for some ingredients.

Lupins and field peas have been used as feed ingredients in the pig and poultry industries in Australia with promising results (Petterson and Mackintosh, 1994). When compared to other ingredients such as soybean, lupins and field peas have the additional advantage of being relatively free of anti-nutritional factors, therefore, thermo-processing to neutralise anti-

nutritional factors is not required, and a saving is made in ingredient processing costs (Petterson and Mackintosh, 1994).

Australia is well placed to utilise agricultural products in aquafeeds. Lupins and field pea production were 1.5 and 0.6 million t respectively, for the 1993/94 period, and accounted for 88% of the Australian total annual pulse production (ABARE, 1996). Projected production estimates for 1995/96 are expected to at least equal 1993/94 production (ABARE, 1996). While Australian soybean production of 0.8 million t for 1993/94 is expected to remain static in 1995/96 (ABARE, 1996).

Some of the differences between the performance of fish fed SP35 and the other least-cost diets could be attributed to differences in processing and manufacture. SP35 was manufactured by a different company than the other diets as it was important that the control diet was a commercially available diet and one which had been used in large scale experiments at GRC previously and, therefore, had 'performance credentials'. The least-cost diets were formulated at a plant equipped with commercial scale grinding facilities, steam conditioning and with experience in large scale manufacture of aquaculture diets.

Research conducted at PSRC in 10 000 L tanks, concurrently with this trial at GRC demonstrated that silver perch grew significantly faster on SP35 which was steam conditioned, compared with SP35 which was not ($P<0.05$) (Booth et al., unpublished data, see Section 6.10 of this report).

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

Standard meat and bone meal is typically around 50% crude protein, >30% ash and 10-20% fat (Allan, 1994). In general, lower fat contents are desirable as saturated animal fats are undesirable in most aquaculture diets. Low ash, high protein meat meals have shown promise as protein sources to replace fish meal in diets for rainbow trout (*Oncorhynchus mykiss*) and yellowtail (*Seriola quinqueradiata*) (Shimeno et al., 1993a; 1993b; Watanabe et al., 1993).

Carcass composition of silver perch at the conclusion of the feeding trial, indicated that fish fed all three diets were high in total fat. High levels of fat were also observed during preparation of fish for the sensory tests. Deposits of fat were noticed on the flesh surface below the dorsal fin, around the belly flaps and inside the gut cavity and tasters frequently commented on "oily" or "buttery" flavours. However, neither carcass composition (nitrogen, ash, fat or energy) or overall sensory evaluation of fish fed any of the three diets differed

significantly. During the sensory evaluation, all fish were rated acceptable by all 'tasters', and as "some of the best silver perch eaten" by one 'taster'.

The results of this research demonstrates meat meal, lupins and field peas have the potential to become a major protein source for aquaculture diets for silver perch. Clearly, the value of all meat products in aquaculture diets will depend upon consistency of composition and on the absence of deleterious compounds such as hair or wool (which tend to clog up feed manufacturing equipment). Heat damage in rendering plants can also reduce digestibility of amino acids, eg lysine, and reduce the value of meat meal for use in aquaculture diets.

Acknowledgments

We would like to thank David Stone, Jane Frances, Mark Booth, Scott Parkinson and Rebecca Warner-Smith at PSRC for their assistance with experiments. The experiment at GRC was run with help from Justin Shipman and Paul Robertson. Staff of the Centre for Food Technology seafood panel tasted the fish and results were statistically analysed by Stephen Nottingham, biometrician for the Centre.

We are grateful to Dr David Overend, Mr Kevin Smyth and Mr Gary Hoey from Ridley Agriproducts for their assistance with manufacturing diets used at GRC and to Mr Tony Hoey and Ms Kerry Ruge at Janos Hoey for manufacturing SP35 and supplying some ingredients and the vitamin/mineral premixes. Biochemical analyses were carried out at NSW Agriculture, Wollongbar Agricultural Institute by Ms Elspeth Berger and Ms Wendy Peasley. Australian Native Fish Pty Ltd, through a contract with NSW Fisheries, provided funds for constructing the ponds at GRC used in this study and for wages for two of the technicians. Their support for research and development towards establishing a commercial silver perch industry is gratefully acknowledged.

The research described in the report was specifically commissioned by the Meat Research Corporation but financial support for fish meal replacement research has also been provided by the Fisheries Research and Development Corporation, the Australian Centre for International Agricultural Research and the Grains Research and Development Corporation. All agencies are gratefully acknowledged for providing the support necessary for the coordinated national and international (Thailand) research on fish meal replacement and aquaculture diet development currently under way in Australia.

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Table 1
Formulation for SP35, 95LC1 and 95LC2

Ingredient	Quantity in diet (g/100g dry matter)			Dry matter (%)	Protein content (%)	Assumed cost[1] (AUD$/t)
	SP35	95LC1	95LC2			
Fish meal (Danish)	27.00	10.00	5.00	94.70	65.86	1300
Meat meal (lamb meal)	-	21.71	36.88	97.15	52.75	400
Blood meal	2.00	2.09	-	88.65	85.32	900
Corn gluten meal	4.00	3.77	5.19	92.90	57.68	700
Soybean meal	20.00	-	-	89.11	45.55	495
Canola	-	-	5.00	91.74	40.00	325
Peanut meal	-	-	5.00	94.75	39.00	335
Field peas (*Pisum sativum)*	-	14.92	10.39	88.55	24.44	235
Lupins (Gungaroo) dehulled	-	25.50	7.36	95.02	43.61	350
Wheat	26.85	-	-	90.78	13.70	180
Sorghum	11.00	4.70	-	89.59	14.62	180
Millrun	2.00	10.00	17.70	89.67	19.62	190
Fish oil	1.00	2.91	3.21	100.00	-	800
DL-methionine	0.15	0.40	0.27	100.00	78.60	5500
Vit[2]/min premix[3]	4.00	4.00	4.00	100.00	-	4000
Di-Calcium phosphate	2.00	-	-	100.00	-	610

1 Based on prices published by NSW Agriculture, Sydney Retail Feed Ingredient Prices, NSW Agriculture, Orange, NSW, 2800, Australia), or from commercial feed manufacturers.

2 (IU/kg diet): retinol (A), 8000; cholecalciferol (D3), 1000; a-Tocopheral acetate (E), 125; (mg/kg diet): ascorbic acid (C), 1000; biotin (2%), 1; calcium panothenate, 55; calcium propionate, 250; choline chloride, 1500; cyanocobalamin (B12), 0.02; ethoxyquin, 150; folic acid, 4; menadione sodium bisulphite (K3), 16.5; myo-inositol, 600; nicotinamide, 200; pyridoxine (B6), 15; riboflavin (B2), 25.2; thiamine HCl (B6), 10.

3 (mg/kg diet): calcium carbonate, 7500; manganese sulphate, 300; zink sulphate, 700; copper sulphate, 60; ferrous sulphate, 500; sodium chloride, 7500; potassium iodate, 2.

Table 2

Composition of SP35, 95LC1 and 95LC2[1].

Nutrient	Quantity in diet (dry matter basis)		
	SP35	95LC1	95LC2
Digestible protein (%)	36.08	36.41	34.01
Digestible energy (MJ/kg)	13.71	14.53	14.00
Fat (%)	6.97	9.18	9.01
Linolenic series fatty acids (%)	1.25	1.19	1.11
Digestible lysine (%)	2.24	2.10	1.97
" methionine + cys (%)	1.65	1.53	1.44
" isoleucine (%)	1.58	1.47	1.39
" leucine (%)	3.26	3.04	2.86
" arginine (%)	2.20	3.16	2.74
" histidine (%)	0.91	0.99	0.86
" phenyl + tyro (%)	2.98	2.90	2.72
" valine (%)	1.87	1.75	1.64
" threonine (%)	1.57	1.53	1.39
" phosphorous (%)	0.68	0.62	0.83

1 Based on previously determined composition and digestibility coefficients for each ingredient in all diets.

Table 3

Mean weight of fish at commencement of feeding of experimental diets[1].

Experimental diet	Mean individual weight[2] (g)
SP35	80.9 ± 1.5[a]
95LC1	80.4 ± 1.5[a]
95LC2	80.8 ± 1.0[a]
Mean stocking weight at commencement of experiment[3]	80.7 ± 1.2

1 70 - 100 fish sampled from each pond prior to commencement of experiment.

2 Values are means ± SEM (n = 3). Means with the same letter in the superscript were not significantly different ($P>0.05$; ANOVA; SNK).

3 Value of the overall mean is based on mean of 9 ponds.

Table 4

Survival, growth , food conversion ratio, protein efficiency ratio, production rate and diet ingredient cost for silver perch fed three diets for 143 days

Diet	Survival (%)	Weight increment (g)	Growth rate (g fish^{-1} day^{-1})	FCR	PER	Production (kg ha^{-1})	Ingredient Cost AUS$ kg fish^{-1}
SP35	97.5 ± 1.1[a]	314.5 ± 11.9[a]	2.23 ± 0.07[a]	2.23 ± 0.03[b]	1.33 ± 0.02[a]	5779 ± 127[a]	1.69[a]
95LC1	97.0 ± 0.4[a]	353.4 ± 10.8[b]	2.53 ± 0.09[b]	1.97 ± 0.09[a]	1.37 ± 0.06[a]	6283 ± 117[b]	1.22[b]
95LC2	96.8 ± 0.5[a]	360.9 ± 7.2[b]	2.53 ± 0.03[b]	1.93 ± 0.03[a]	1.41 ± 0.02[a]	6450 ± 83[b]	1.09[c]

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

Table 5

Proximate body composition of whole silver perch at completion of nutrition experiment.

Fish Sample	Dry Matter (%)	Nitrogen (% dry basis)	Ash (% dry basis)	Energy (MJ/kg dry basis)	Fat (% dry basis)
SP35	41.23 ± 0.34[a]	6.18 ± 0.11[a]	7.88 ± 0.05[a]	29.72 ± 0.17[a]	50.92 ± 0.45[a]
95LC1	43.14 ± 0.05[a]	5.39 ± 0.08[a]	6.87 ± 0.20[a]	30.55 ± 0.13[a]	55.04 ± 0.51[a]
95LC2	42.92 ± 1.78[a]	5.68 ± 0.05[a]	7.65 ± 0.67[a]	30.12 ± 0.34[a]	54.40 ± 2.05[a]

[1] Values are means ± SEM for fish sampled from 3 replicate ponds (fish sampled from 2 ponds for SP35 diet).
Means in columns which share the same superscript were not significantly different ($P>0.05$; ANOVA).

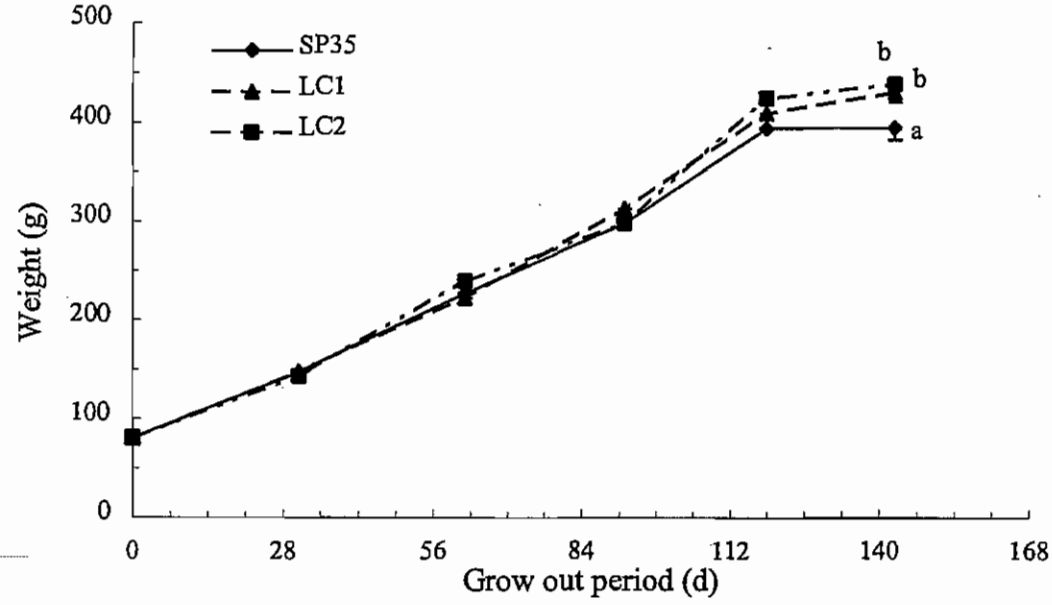

Figure 1.
Growth of silver perch in 0.1 ha ponds at GRC. Values are means ± SEM (n = 3). For weight at harvest, means with the same letter were not significantly different ($P>0.05$; ANOVA; SNK).

Silver Perch diet comparison - Mean taste panel scores

Odour Profile

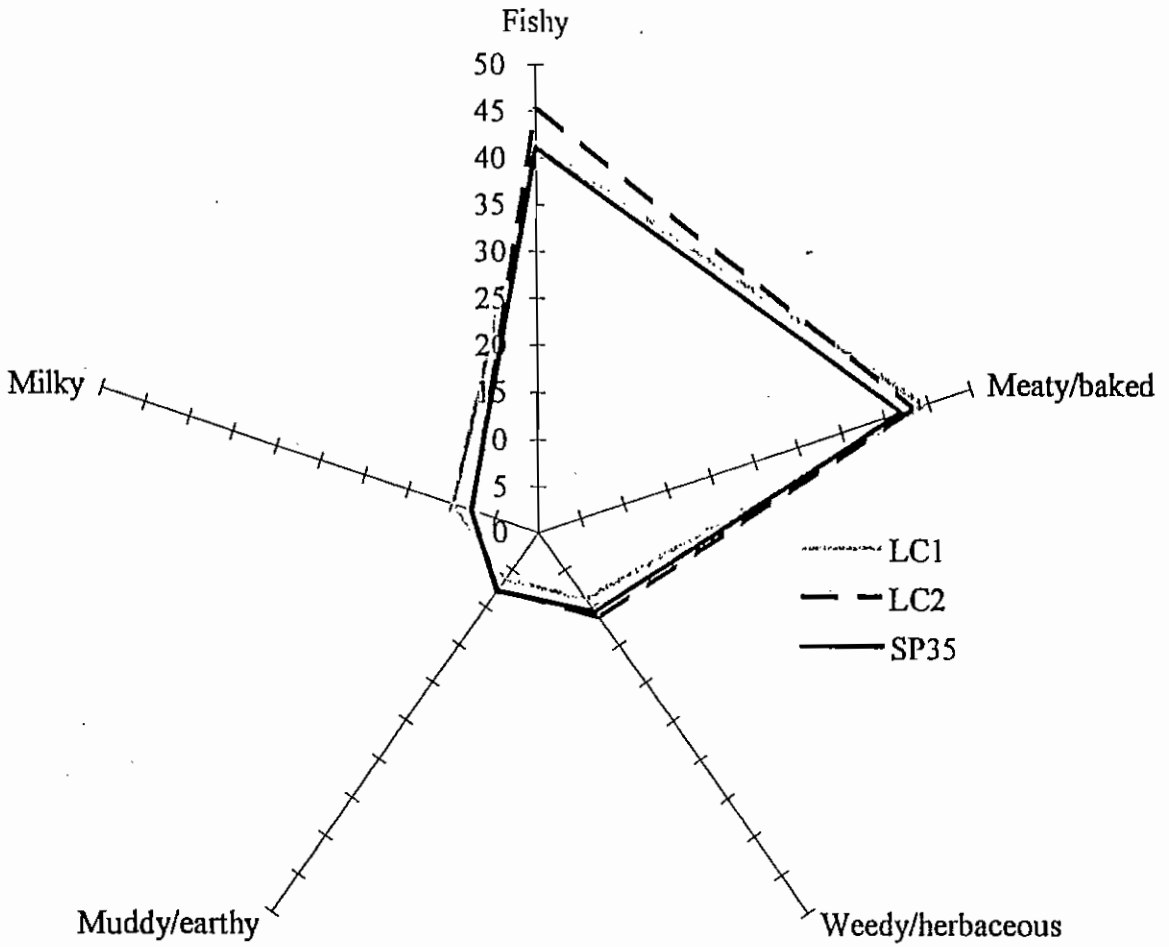

Figure 2

Silver Perch diet comparison - Mean taste panel scores

Texture Profile

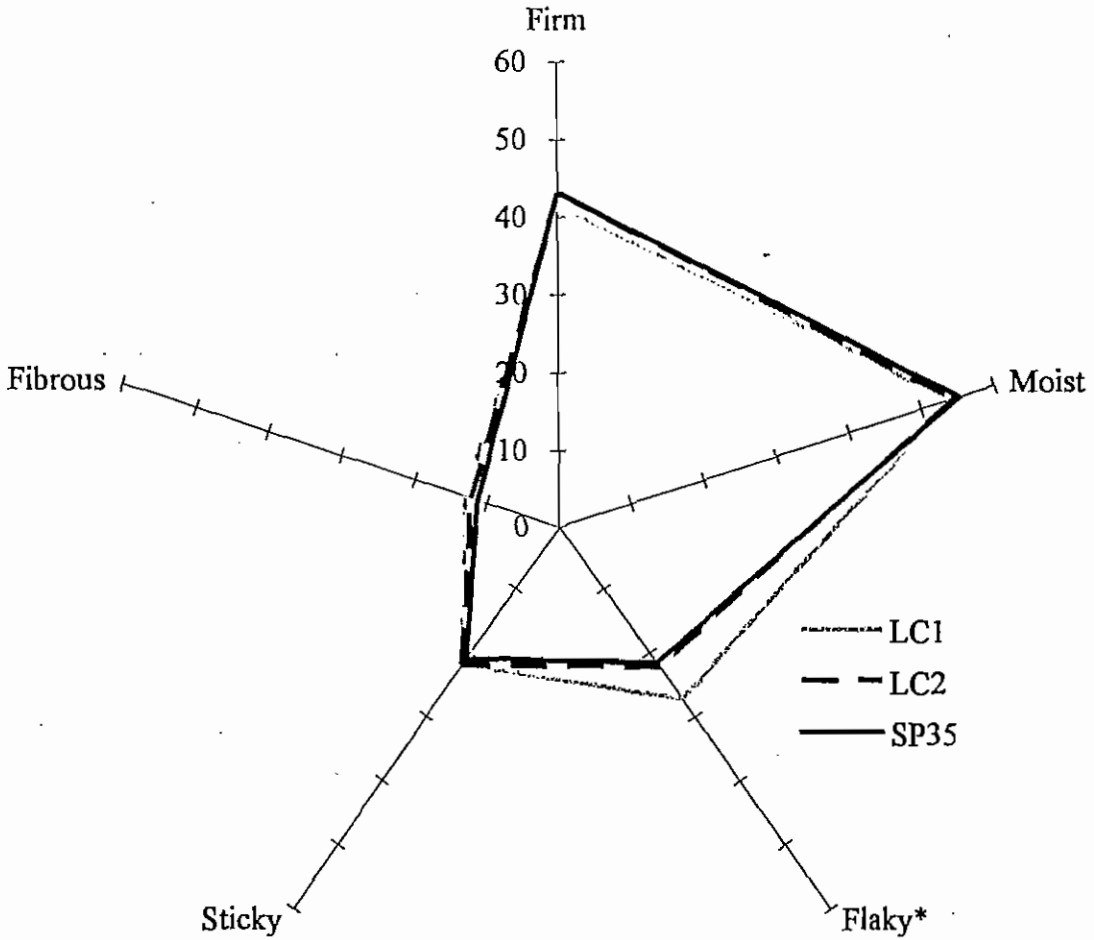

Figure 3

Silver Perch diet comparison - Mean taste panel scores

Flavour Profile

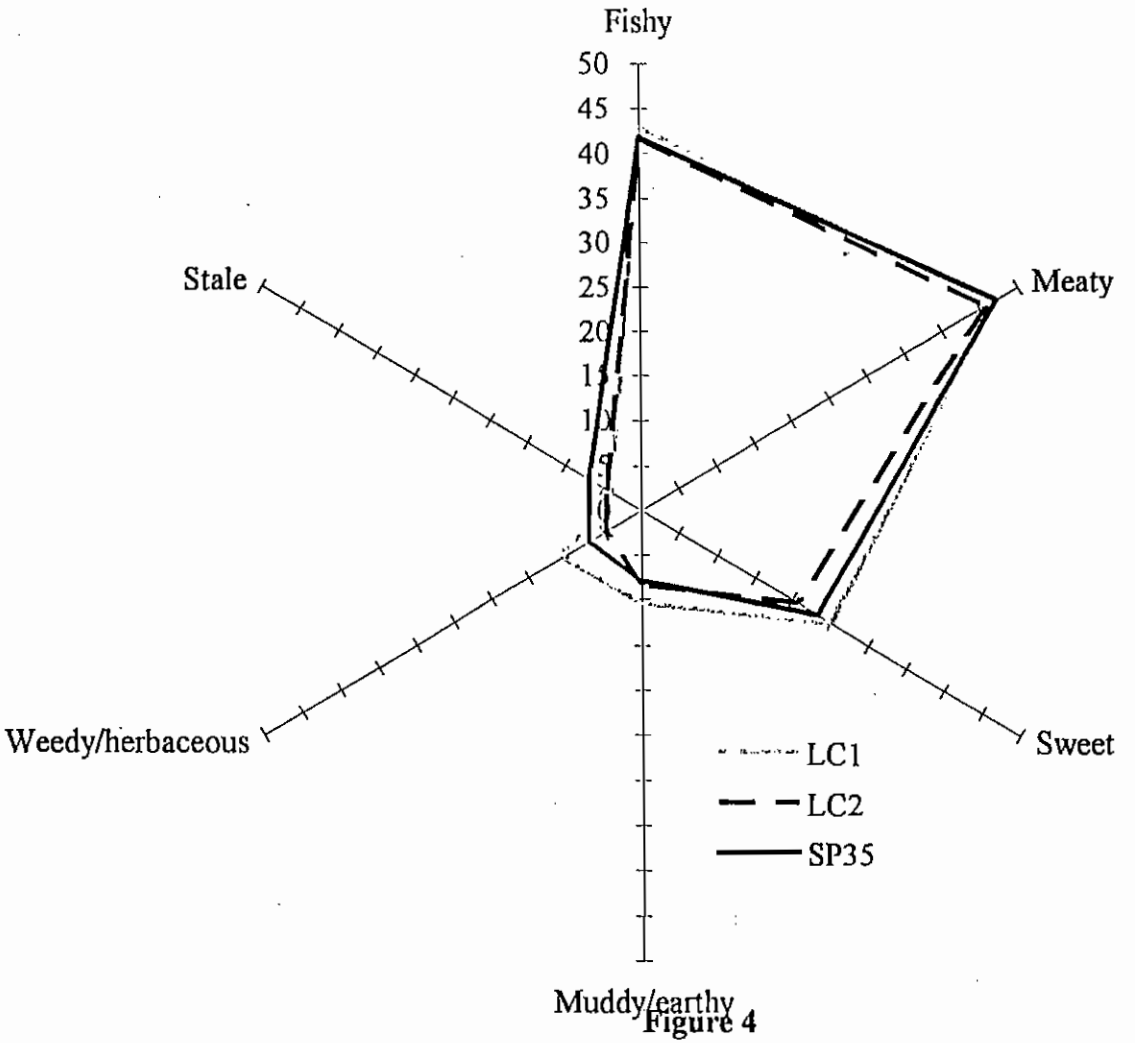

Figure 4

6.6 Replacement of fish meal in diets for silver perch, *Bidyanus bidyanus*: V. effects of increasing poultry offal meal and feather meal content on growth and body composition

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Abstract

Poultry offal meal and feather meal are both well digested by silver perch. In this study the contribution of both ingredients to growth and body composition of silver perch was measured using the summit/dilution method. During this experiment, a 35% protein reference diet (SP35) was used as the summit diet and then three series of diets where this diet was progressively replaced with poultry offal meal, feather meal or an inert diluent, diatomaceous earth, were formulated (17 diets in total). Fish were fed on a restricted feeding regime at approximately 90% of satiation, adjusted according to biomass. The difference in growth response and carcass composition for fish fed the three series of diets was compared and the series of diets with the inert diluent allowed measurement of the exact contribution from the test ingredient (poultry offal meal or feather meal).

Six fish (5-7 g/fish) were stocked into each 70 l aquarium, four replicate aquaria were used for each diet, and the experiment ran for 71 days. Weight gain, protein and lipid deposition and food conversion ratio (FCR) all increased with increasing poultry offal meal content in the diets and protein efficiency ratio and protein retention efficiency were unaffected by inclusion content. By contrast, increasing the content of feather meal did not affect weight gain, protein or lipid deposition but FCR and protein efficiency ratio and protein retention efficiency declined. Weight gain, protein and lipid deposition were inversely related to inclusion content. Diet significantly affected carcass protein and lipid content for each diet series. Carcass protein was similar for fish fed either the poultry offal meal diet series or the feather meal diet series and was stable or tended to decline as inclusion content of either ingredient increased. Carcass lipid content increased with increasing inclusion content for fish fed either diet series. By contrast, carcass lipid decreased and carcass protein increased with increasing diatomaceous earth content.

The results indicate that both poultry offal meal and feather meal are effectively utilised for body weight gain and protein deposition. The similarity in protein retention efficiency for fish fed diets containing poultry offal meal, irrespective of content, do not indicate a need to set an upper limit for this ingredient for use as a protein source, provided energy and nutrients are balanced. The protein retention efficiency of feather meal was 7% less than for poultry offal meal at 13% inclusion content and this deteriorated to 14% less at 78% inclusion content. Differences in digestibility did not account for the difference between ingredients and it is unlikely absolute amino acid contents were limiting. Excessive protein:energy ratio for feather meal diets may have contributed to reduced utilisation and thus warrants further investigation.

Keywords: Nutrition; *Bidyanus bidyanus*; Growth; Poultry offal meal; Feather meal; Summit/dilution; Nutrient utilisation.

1. Introduction

Fish meal is the traditional protein source of choice in aquaculture diets (Lovell, 1989; Rumsey, 1993), but as world fish meal production is predicted to decline (FAO, 1991; New, 1991), the continued expansion of aquaculture is dependent on securing alternate protein sources. Very little fish meal is produced in Australia, but abundant supplies of agricultural proteins are available (ABARE, 1997; Allan, 1997). In Australia, aquaculture production of a number of species is increasing (ABARE, 1997), including silver perch, an omnivorous native freshwater fish which performs very well in static pond culture (Rowland et al., 1995). If this trend is to continue, agricultural protein sources will need to be used as the basis of Australian aquaculture diets.

Poultry offal meal and feather meal are two terrestrial animal-based protein sources which may have potential for use in aquaculture diets. They are both widely available for use in manufactured animal feeds, have high crude protein content, do not contain poorly digested carbohydrates, and provided they are processed correctly, contain few if any anti-nutritional factors.

Digestibility coefficients for poultry by-products have been calculated for a number of species, including silver perch, rainbow trout, tilapia and carp. Although, in general, these studies have reported lower protein and energy digestibility of poultry by-product than fish meal, most concluded they had potential as at least partial substitutes (Degani, et al., 1997; Alexis et al., 1985; Hanley, 1987; Allan et al., unpublished data, Section 6.2 of this report).

Digestibility information is critical to allow formulation of diets with known digestible energy and digestible nutrients. Following this, diet formulators need to know how well the ingredient is utilised by the animal being fed and the maximum amount of an ingredient that can be included in a diet before growth or other indicators of fish performance or fish composition decline.

Different methods have been used to provide this information for poultry by-products. Most commonly, a series of diets are formulated where different amounts of poultry by-products are used to replace fish meal. Some studies found poultry by-products had potential as complete replacements (eg for rainbow trout [Alexis et al., 1985]), but most reported that performance was not affected provided replacement was only partial. For example, 15 or 20% replacement of fish meal with feather meal or poultry by-product meal did not reduce growth of Chinook salmon (Fowler, 1990, 1991), up to 66% replacement of fish meal with feather meal did not reduce growth of Coho salmon fingerlings (Higgs et al., 1979) and 56% replacement of fish meal with poultry/feather meal did not affect weight gain of rainbow trout (although carcass fat was increased) (Steffens, 1994).

These type of studies are useful but they do not directly estimate the contribution to growth from the poultry by-products. If energy and nutrients are oversupplied in test diets, even a poorly utilised ingredient will not reduce growth until its inclusion content is quite high.

An alternative approach to measure how well a particular ingredient is utilised is the summit/dilution method first used as a measure of assessing methionine requirements of poultry (Fisher and Morris, 1970). In this approach, a summit diet is chosen and two series of other diets are made by progressively replacing the summit diet with a test ingredient or inert diluent. The differences in growth response and composition of tissue deposited for fish fed the two series of diets allow for a direct measure of the contribution made by the test ingredient at each inclusion level.

The aim of this experiment was to measure the contribution of poultry offal meal and feather meal to growth and body composition of juvenile silver perch fed a series of diets in which a summit diet was systematically replaced with graded levels of each ingredient.

2. Materials and Methods

2.1 Experimental diets and feeding strategy

The summit diet chosen for this experiment was the reference diet of Allan and Rowland (1992; Table 1) which has been used in large-scale grow-out trials with silver perch (Rowland et al., 1995). The other experimental diets were made by progressively substituting part of this diet with poultry offal meal, feather meal or the inert filler diatomaceous earth (Table 2). All ingredients were ground or sieved to ensure all particles passed through a 710 μm screen. Dry ingredients were thoroughly mixed in a Hobart mixer (Troy Pty. Ltd., Ohio 45374, USA) then combined with approximately 400 ml distilled water kg^{-1} dry mix before being pelleted through a meat mincer (Barnco Australia Pty. Ltd., Leichhardt, 2040, NSW) with a 1.5 mm die. Pellets were dried at <35°C in a convection drier for about 6 h until the moisture content was between 10-15%, to produce a dry, sinking pellet.

The fish were allowed to acclimatise to experimental conditions for 10 days, during which time all fish were fed the summit diet twice daily (40% am, 60% pm) until satiation levels were established for each tank. Once satiation levels were determined, fish were switched to experimental diets (Table 2). A restricted feeding regime was used, with experimental diets offered at 90% satiation. Uneaten feed was collected, dried and weighed during both phases of the experiment and total feed inputs for each tank were adjusted accordingly at the end of the trial. In order to maintain the restricted feeding regime, total biomass for each aquarium was determined by anaesthetising and weighing all fish every two weeks. Feed rates were then adjusted on the basis of the new biomass, according to the equation:

$$\text{Food Intake} = a\text{Weight}^{b} \quad \text{(Jobling, 1988);}$$

where a is a constant. The scaling or weight exponent b relates feeding ration to body weight and is less than 1 so that ration increases allometrically with increasing body weight In the present study, the weight exponent chosen was 0.8, which represents the average weight exponent used in other studies with cultured fish (Paloheimo and Dicki, 1966; Jobling, 1983).

2.2 *Experimental fish*

Juvenile silver perch were obtained from NSW Fisheries' Grafton Research Centre and held in 10 000 L fibreglass tanks prior to use in this trial. During this time they were fed on the reference diet of Allan and Rowland (1992) (SP35) *ad libitum* daily.
Prior to the experiment, fish were graded, anaesthetised and individually weighed. Each aquarium was stocked via random interspersion with six fish between 5 and 7 g (5.90±0.03; mean ± sem; n=432). During the course of the experiment, any mortalities were weighed and then replaced with individually weighed, fin-clipped fish. At the conclusion of the trial (71 days) all fish were individually weighed, and five fish from each tank were randomly selected for whole body proximate composition.

Fin-clipped fish were later excluded from all calculations involving average individual weight gain, protein and lipid deposition and protein and lipid retention efficiency. The net weight gain $tank^{-1}$ was used for estimating food conversion, protein and energy efficiency ratios. The following formula apply:

Weight gain (g/fish) = (final individual weight -initial individual weight).

Protein deposition (g) = (final ind. wt x DM final carcass x protein composition final carcass) - (initial ind. wt x DM initial carcass x protein composition initial carcass).

Lipid deposition (g) = (final ind. wt x DM final carcass x lipid composition final carcass) - (initial ind. wt. x DM initial carcass x lipid composition initial carcass).

Food conversion ratio = dry weight of food consumed / wet weight gain of fish.

Protein efficiency ratio = wet weight gain of fish / amount protein consumed (dry basis).

Energy efficiency ratio = wet weight gain fish / amount energy consumed (dry basis).

Protein retention efficiency (%) = individual protein deposition (dry basis)/individual protein consumption (dry basis) x 100.

Lipid retention efficiency = individual lipid deposition (dry basis)/individual lipid consumption (dry basis) x 100.

2.3 *Laboratory facilities and water quality*

Sixty eight, 70 l aquaria were supplied with pre-heated (25-26°) particle filtered (<10 μm) water at a rate of 400 ml min^{-1}. Seventy-five percent of water was recirculated through a 2 m^3 biological filter; 25% of water was replaced daily. Four replicate aquaria were provided for each of the seventeen treatments (68 aquaria). Replacement fish were held in a 200 l, polyethylene tank in the same laboratory and supplied with water (single pass) from the same

sytem. Flourescent lighting was automatically controlled in order to provide a 12 hour light/12 hour dark cycle.

Water temperature, pH, dissolved oxygen, nitrite and ammonia were monitored weekly from 20 representative aquaria (at least one per treatment) selected on a rotating basis, following methods described in Allan et al. (1990). Values ranged from 23.6-27.1^{0}C for temperature, 7.2-8.5 for pH, 4.6-8.3 ppm for dissolved oxygen and nitrite (NO_2-Nl^{-1}) was always less than 100ugl^{-1} and ammonia (total-ammonia-Nl^{-1}) was always less than 300ugl^{-1}.

2.4 Biochemical analysis

All chemical analyses were done in duplicate. Final fish carcass, experimental diets (1-17) and ingredients (poultry/feathermeal) were analysed for dry matter and crude fat (lipid) by AOAC (1975) procedures. Nitrogen was determined for these samples by the method of Havilah et al. (1977) (crude protein = N x 6.25). Gross energy (bomb calorimetry) was determined by AOAC (1975) procedures for poultry and feather meal ingredients and the summit diet. Energy values for diets 2-17 were then estimated as the sum of the proportion of summit, times the summit energy and the proportion of poultry offal meal or feather meal times the energy for that ingredient. Diatomaceous earth was assumed to contribute no energy. Digestible energy and digestible protein contents were determined in a similar way using experimentally determined digestibility coefficients for energy and protein from poultry offal meal and feather meal obtained from the same source (and batch) (Allan et al., unpublished data, see Section 6.2 of this report).

2.5 Statistical analysis

Homogeneity of variance for all data was confirmed using Cochran's Test (Winer et al., 1971). The effect of inclusion level (0, 13, 26, 39, 52, 65 or 78%) and ingredient type (poultry offal meal or feather meal) on performance indices were examined by two-factor ANOVA (both factors were considered fixed). Significant interactions ($P<0.05$) were found for all indices. Consequently, single-factor ANOVA was used to examine the effect of inclusion level for each ingredient for each index, and the effect of ingredient type for each inclusion level for each index (Table 3). Single-factor ANOVA was also used to examine statistical differences between the inclusion level of ingredient (poultry offal meal, feather meal or diatomaceous earth) and carcass protein and lipid composition, as well as differences between the carcass composition of fish fed different ingredients but at similar inclusion levels (Table 4). Where significant differences were determined by ANOVA, a Student-Newman-Keuls test was used to compare means. The relationships between test ingredient inclusion level and performance indices were modelled using regression analysis, and best fit curves were chosen on the basis of their estimated reliability with respect to R-squared values (Microsoft Excel, Version 5, 1993; Microsoft Corporation, USA).

3. Results

Weight gain of fish fed diets substituted with graded levels of poultry offal meal increased ($P<0.05$) as inclusion level increased (Table 3, Figure 1a). This trend was also evident for

protein and lipid deposition (Figures 1b and c). Food conversion ratio decreased (improved) with increasing levels of poultry offal meal ($P<0.05$) (Figure 2a) although protein efficiency ratio, energy efficiency ratio (Figures 2b and c), and protein retention efficiency (Figure 3a) were unaffected by poultry offal meal inclusion ($P>0.05$). Lipid retention efficiency decreased with increasing poultry offal meal inclusion ($P<0.05$) (Figure 3b).

Conversely, increasing inclusion of feather meal did not affect weight gain, protein deposition, lipid deposition (Figures 1a, b and c) or food conversion ratio ($P>0.05$) (Figure 2a). Protein efficiency ratio, energy efficiency ratio (Figures 2b and c), protein retention efficiency and lipid retention efficiency (Figures 3a and b) all declined ($P<0.05$) with increasing feather meal inclusion.

Relationships between inclusion level for each ingredient for each index are presented in Table 3 and Figures 1, 2 and 3. There were significant differences between ingredients for each index at the two highest inclusion levels (65 and 78%) and for all indices except lipid retention efficiency for 52% inclusion. Differences between ingredients were also significant for some indices at all inclusion levels (Table 3).

The diluent effect of diatomaceous earth on the summit diet was clearly demonstrated by the inverse linear relationships between inclusion level and weight gain and protein deposition (Figures 1a and b). Food conversion ratio, protein efficiency ratio and energy efficiency ratios were similar for the fish fed diets containing diatomaceous earth up to 26% and then deteriorated rapidly at higher inclusion levels (Figures 2a, b and c). The response of protein retention efficiency was similar and there was a rapid decrease in lipid retention efficiency after 13% inclusion of diatomaceous earth (Figures 3a and b).

Diet significantly affected carcass protein and lipid content for each diet series. The carcass protein content was higher for fish fed the summit diet than for fish fed the poultry offal meal or feather meal diets (Table 4). By contrast, as the summit diet was progressively replaced with diatomaceous earth, carcass protein content increased (Table 4). The lipid content of fish fed the poultry offal meal diets or the feather meal diets increased with increasing inclusion content while the lipid contents of fish fed the diatomaceous earth series declined with increasing inclusion content. Differences between composition of fish fed poultry offal meal diets and feather meal diets were significant only for protein at one inclusion content (65%). The relationship between carcass protein and carcass lipid to the digestible protein:digestible energy ratio of diets was examined (Figures 4a and b). Differences in carcass content for fish fed the different diet series were clearly greater than any effects of changing protein:energy ratio.

4. Discussion

Data from the present study does not indicate a basis for recommending an upper limit of poultry offal meal inclusion in silver perch diets where adequate digestible protein and energy are provided. This is supported by increasing growth, and protein gain and improving food conversion ratio with increasing poultry offal meal inclusion and no effect of inclusion on protein efficiency ratio or protein retention efficiency. This supports research by Steffens

(1994) who found poultry offal meal could replace 50% of the fish meal in diets for rainbow trout, and Higgs et al. (1979) who found defatted poultry offal meal could replace up to 75% of the fish meal in diets for Coho salmon, without reducing growth. Belal et al. (1995) tested poultry offal silage at up to 20% inclusion in tilapia diets (as replacement for fish meal) and found no effects on growth or body composition under the experimental conditions. However, Steffens (1987) recommended total replacement of fish meal with poultry offal meal in trout diets required lysine and methionine supplementation. Later, Sadiku and Jauncey (1995) also warned that phenylalanine and tyrosine deficiencies would result from excessive inclusion of poultry offal meal in diets for tilapia and catfish.

Amino acids were not supplemented in the present study and the similarity in growth of fish fed all poultry offal meal diets clearly shows amino acids were not deficient and that imbalances relative to fish meal did not reduce growth. It must be noted, however, that total protein increased with increasing poultry offal meal content so absolute amino acid deficiencies would not be expected in the present study.

The protein retention efficiency of fish fed the poultry offal meal diets were in the range 29-31%, similar to the protein retention efficiency of fish fed the summit (30%) and close to the range of 30-40% proposed by Cowey (1994) as the range of net retention of dietary N for fish. El-Sayed (1994) reported lower net protein retention efficiencies of 24.3, 17.8 and 14.7% for silver seabream (*Rhobdosargus sarba*) fed 40% protein and 11-18% lipid diets where fish meal (43, 30 or 15%) was replaced with poultry offal meal (15, 30 or 45%).

In another similar study where silver perch were fed diets where dehulled lupins were used to replace the same summit diet (SP35), net protein retention efficiencies were similar or slightly lower (lupins -28.7-24.1% for diets containing from 10-60% dehulled lupins (Allan et al., unpublished data, see Section 6.9 of this report). In another study where fish meal was progressively replaced with meat meal (in this case digestible energy and digestible protein were balanced) net protein retention efficiencies were slightly higher (33-37%) (Stone et al., unpublished data, see Section 6.4 of this report).

By contrast with the poultry offal meal series of diets, increasing concentration of feather meal had no affect on weight gain, protein deposition or food conversion efficiency (1/FCR). However, protein efficiency ratio and protein retention efficiency declined with increased dietary content of feather meal. The positive results for weight gain and food conversion efficiency are supported by results from other studies. Fowler (1990) reported feather meal could be used at up to 15% in Chinook salmon diets without affecting growth or food conversion efficiency and that this allowed replacement of approximately 50% of the fish meal in the basal diet. Kikuchi et al. (1994) examined performance of Japanese flounder fed diets containing up to 50% feather meal and found fish accepted all diets well and growth was unaffected by inclusion levels of up to 25% feather meal. Higher inclusion content reduced performance.

Feather meal has also successfully been used in combination with poultry offal meal with several species, including rainbow trout (Tiews et al., 1979), Chinook salmon (Higgs et al., 1979) and tilapia (Rodriguez-Serna et al., 1996).

However, in the present study the results for feather meal indicate that this ingredient was less efficiently utilised than poultry offal meal. This is evident from the significantly worse protein efficiency ratios and protein retention efficiencies for feather meal compared with poultry offal meal at most inclusion contents.

The difference between ingredients cannot be attributed to differences in poorer digestibility of feather meal as has been suggested by some authors (Brannon et al., 1976; Roley et al., 1977). Determination of digestibility with silver perch gave digestibility coefficients for dry matter, energy and nitrogen of 102.6%, 105.3% and 93.3% respectively for feather meal compared with 83.7%, 96.4% and 84.5% respectively for poultry offal meal (Allan et al., unpublished data, see Section 6.2 of this report). These digestibility coefficients were calculated using diets with 30% feather meal or poultry offal meal and 70% reference diet (SP35).

Deficiencies in essential amino acids may also be responsible for reduction in performance with feather meal based diets (Fowler, 1990). Kikuchi et al. (1994) found the nutritive value of feather meal was slightly improved with the addition of crystalline amino acids to diets for Japanese flounder As feather meal is much higher in protein content than the reference diet, direct substitution of the reference diet with feather meal in the present study increased total protein content and the amount of essential amino acids also increased. Although the amino acid profile was different to fish meal, imbalances in amino acids should not have caused growth reduction provided requirements for all essential amino acids were met. Although an imbalance in hystine/methionine in feather meal has been reported to affect terrestrial animals, they have not been reported to affect fish fed synthetic amino acids (Tacon and Jackson, 1985). Provided adequate or surplus digestible protein (amino acids) was available, poor utilisation of feather meal compared with poultry offal meal might be attributed to poor protein:energy ratio for the feather meal diets or to the presence of some anti-nutrients in the feather meal.

The protein:energy ratio for the poultry offal meal series of diets ranged from 2.2-2.4:1while for the feather meal series it increased from 2.5 to 3.0:1 (Table 2). As the ratio increased, protein efficiency ratio and protein retention efficiency also decreased indicating that at higher inclusion levels (higher total protein), proportionately more of the protein in the feather meal series of diets was utilised for energy than in the poultry offal meal series of diet, leaving less protein available for tissue deposition.

Utilisation of protein for energy is inefficient compared with use of lipid (NRC, 1993). The digestible protein content of feather meal diets ranged from 51.4% to 69.6% for diets with 13% to 78% feather meal compared with 37% to 53.8% for the poultry offal meal diets over the same range of inclusion contents. The calculated digestible energy contents for the two series of diets were more similar (14.4 to 20.0 MJ kg^{-1} and 14.8 to 22.3 MJ kg^{-1} for the poultry offal meal and feather meal series respectively). The major difference was that more of the digestible energy was from protein in the feather meal series compared with the poultry offal meal series where lipid increased with inclusion content. For this to account for differences in protein efficiency ratio and protein retention efficiency of fish fed the different series of diets,

the lipid from poultry offal meal would need to be effectively utilised as an energy source for silver perch. Higgs et al. (1979) reported that Coho salmon were able to utilise the lipid in poultry by-product meal as well as fish oil as a source of energy for growth and this was supported by Yu et al. (1977) with trout. Gallagher and Degani (1988) reported poultry oil was inferior to fish oil although eels fed diets with 10% poultry oil grew as well as fish fed diets with 10% fish oil.

The possibility of anti-nutrients in feather meal cannot be discounted. At lower total inclusion contents, antagonism between essential amino acids might have reduced protein retention efficiency or damage to proteins during the processing of feather meal and may have rendered some amino acids digestible but less available. Further research is needed to investigate these suggestions.

The decline in carcass protein and increase in carcass fat with increasing inclusion of poultry meal or feather meal indicates the diets in both these series contain surplus energy. Carcass composition of other fish fed diets with poultry meal or feather meal reveal that if test diets had similar protein and lipid contents, the carcass composition was also similar (Kikuchi et al., 1994 for Japanese flounder; Sadiku and Jauncey, 1995 for tilapia). Where diet protein was stable but diet lipid increased with increasing poultry meal or feather meal content, carcass protein was relatively stable or decreased slightly while carcass lipid increased (Steffens, 1994 for rainbow trout; Fowler, 1990, 1991 for Chinook salmon). Too much dietary lipid can result in an imbalance in the protein:energy ratio and excessive carcass lipid deposit (NRC, 1993). In the present study, however, protein: energy ratio was similar for the poultry meal series of diets and increased for the feather meal series of diets. There was no clear relationship between protein:energy ratio of diets and carcass protein or lipid content. This may reflect that in both series both protein and energy were in excess but that there was an extra energetic cost of processing diets with high protein: energy content (feather meal series) contributing to a reduction in protein efficiency ratio and protein retention efficiency. Excessive carcass lipid, especially if it is deposited in the visceral cavity and tissues (much of the lipid is in these regions in silver perch), is undesirable as it reduces effective meat yield, product quality, storage (NRC, 1993) and perhaps market acceptance.

By restrictively feeding fish a series of diets with increasing amounts of an inert filler (diatomaceous earth), the effective ration available for fish was progressively reduced even though the protein:energy ratio was maintained. The large increase in carcass protein and decrease in carcass lipid for fish fed this series of diets indicates that lipid reserves were used preferentially to protein reserves.

These results indicate both poultry meal and feather meal are effectively utilised for body weight gain and protein deposition by silver perch. The similarity in protein retention efficiency for fish fed diets containing poultry meal, irrespective of content, indicates that there is no need to set an upper restriction on use of this ingredient in diets for silver perch (provided diets meet or exceed specifications for essential nutrients and that they do not contain excessive energy which will result in excess carcass lipid). Feather meal was less effectively utilised by silver perch. Protein retention efficiency for feather meal was 6.7% less than for poultry meal at 13% inclusion content and this difference increased to 13.8% at 78%

inclusion. Differences in digestibility did not account for differences between the ingredients, and in absolute terms, it is unlikely that amino acids were limiting. Excessive protein:energy ratio for feather meal may have reduced utilisation and this warrants further investigation.

Acknowledgements

We would like to thank Dr Stuart Rowland and other staff at the NSW Fisheries Grafton Research Centre for providing the juvenile silver perch used in this experiment. Mr Scott Parkinson, David Stone and Helena Heasman provided excellent technical or clerical assistance. Biochemical analysis of diets and fish were done at NSW Agriculture, Wollongbar by Ken O'Brien and Wendy Peasley. We also thank Dr Kevin Williams (CSIRO, Cleveland, Queensland, Australia) for suggesting this method for evaluating dietary ingredients. We are grateful to John Nell and Wayne O'Connor for critically reviewing earlier drafts of the manuscript. This research was partially funded by the Australian Fisheries Research and Development Corporation under the Fish Meal Replacement Sub-Program. Additional funding was provided to the Sub-Program by the Australian Centre for International Agricultural Research, the Australian Grains Research and Development Corporation and the Australian Meat Research Corporation. Support from Australian Native Fish also is also gratefully acknowledged.

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Table 1
Formulation of SP35 (Dry basis).

Ingredient	Diet (g/100g)
Fish meal (Danish)	26.62
Soybean meal	20.19
Blood meal	2.04
Wheat	27.47
Sorghum	11.21
Corn gluten meal	3.87
Millrun	2.01
Cod liver oil	0.90
DL-methionine	0.13
Di-calcium phosphate	1.79
Vitamin premix[1]	0.97
Mineral premix[2]	2.81

[1] (IU/kg diet): retinol (A), 8000; cholecalciferol (D3), 1000; α-tocopherol acetate (E), 125; (mg/kg diet): ascorbic acid (C), 1000; biotin (2%), 1; calcium panothenate, 55; calcium propionate, 250; choline chloride, 1500; cyanocobalamin (B12), 0.02; ethoxyquin, 150; folic acid, 4; menadione sodium bisulphite (K3), 16.5; myo-inositol, 600; nicotinamide, 200; pyridoxine (B6), 15; riboflavin (B2), 25.2; thiamine HCl (B6), 10.

[2] (mg/kg diet): calcium carbonate, 7500; manganese sulphate, 300; zinc sulphate, 700; copper sulphate, 60; ferrous sulphate, 500; sodium chloride, 7500; potassium iodate, 2.

Table 2. Ingredient and nutrient composition of experimental diets

Diet[1,2]	Ingredients (%)				Composition					
	Reference[3]	Poultry meal	Feather meal	Diatomac. earth	Protein[4] %	Gross Energy $Mjkg^{-1}$ (4)	Lipid[4]	Digestible Energy MJ kg^{-1} (4)	Digestible Protein[5] %	Digestible protein: Digestible energy[5]
1	100	-	-	-	36.4	17.9	1.9	14.1	32.9	2.3
2	87	13			37.0	18.7	6.9	15.3	35.1	2.3
3	74	26			39.1	19.4	9.4	16.4	37.4	2.3
4	61	39			42.5	20.2	11.6	16.8	39.6	2.4
5	48	52			45.6	20.9	14.3	18.6	41.9	2.2
6	35	65			51.5	21.6	14.5	19.8	44.1	2.2
7	22	78			53.8	22.4	17.1	20.9	46.3	2.2
8	87		13		51.4	18.8	4.6	15.5	38.6	2.5
9	74		26		45.2	19.7	5.8	16.9	44.3	2.6
10	61		39		50.7	20.6	6.3	18.3	50.0	2.7
11	48		52		57.5	21.5	8.6	19.7	55.7	2.8
12	35		65		64.6	22.4	9.5	21.1	61.4	2.9
13	22		78		69.6	23.3	9.8	22.4	67.1	3.0
14	87			13	28.5	15.6	4.2	12.3	28.6	2.3
15	74			26	24.1	13.3	3.4	10.5	24.3	2.3
16	61			39	19.2	10.9	0.7	8.6	20.1	2.3
17	48			52	15.5	8.6	1.4	6.8	15.8	2.3

1 To all diets was added 1% vitamin premix and 3% mineral premix

2 2% carboxymethyl cellulose was added to all diets as a binder

3 Table 1

4 Analyses (% dry basis)

5 Based on experimentally determined digestibility coefficients for reference diet, poultry meal or feather meal used at 30% in the diet (Allan et al., unpublished data; see section 6.2 of this report).

ype and ingredient content on performance indicators for juvenile silver perch

	Experimental Diet (ingredient inclusion level - %)						
	0	13	26	39	52	65	78
oultry meal	8.4±0.2a	9.2±0.6ab	9.8±0.5ab*	11.0±1.2abc	11.6±0.2bc*	13.6±1.1c*	13.3±0.3c*
feather meal	8.4±0.2a	9.6±0.8a	7.9±0.4a*	9.0±1.1a	7.7±1.0a*	7.5±1.2a*	6.6±0.6a*
Protein depos..ion (g/fish)							
poultry meal	1.6±0.1a	1.7±0.1a	1.8±0.1a*	2.0±0.2ab	2.1±0.0ab*	2.4±0.2b*	2.4±0.1b*
feather meal	1.6±0.1a	1.8±0.2a	1.4±0.1a*	1.6±0.2a	1.4±0.1a*	1.3±0.2a*	1.2±0.1a*
Lipid deposition (g/fish)							
poultry meal	0.6±0.1a	1.0±0.1b	1.2±0.2bc	1.5±0.2cd	1.6±0.1cd	2.1±0.1de*	1.8±0.1e*
feather meal	0.6±0.1a	1.1±0.2a	1.1±0.1a	1.0±0.2a	1.1±0.3a	1.1±0.2a*	1.0±0.1a*
Food conversion ratio							
poultry meal	1.7±0.1a	1.6±0.1a	1.5±0.1a	1.6±0.1a	1.3±0.0b*	1.3±0.0b*	1.3±0.0b*
feather meal	1.7±0.1a	1.5±0.1a	1.7±0.1a	1.7±0.1a	1.8±0.1a*	1.9±0.2a*	1.8±0.1a*
Protein efficiency ratio							
poultry meal	1.5±0.0a	1.7±0.1a*	1.7±0.1a*	1.6±0.1a	1.7±0.0a*	1.6±0.1a*	1.5±0.1a*
feather meal	1.5±0.0a	1.3±0.1ab*	1.3±0.1ab*	1.2±0.1bc	1.0±0.1cd*	0.9±0.1d*	0.9±0.1d*
Energy efficieney ratio							
poultry meal	3.2±0.1a	3.4±0.1a	3.4±0.1a	3.3±0.3a	3.8±0.1a*	3.7±0.1a*	3.6±0.1a*
feather meal	3.2±0.1ab	3.5±0.2b	3.0±0.1ab	3.0±0.2ab	2.7±0.2a*	2.5±0.2a*	2.5±0.1a*
Protein retention effieiency (%)							
poultry meal	29.9±0.8a	31.3±0.9a*	30.6±0.8a*	31.3±2.5a*	30.7±0.8a*	28.8±1.1a*	29.5±1.9 a *
feather meal	29.9±0.8a	24.6±1.4b*	23.1±1.0b*	23.2±1.4b*	18.9±1.0c*	16.1±1.5c*	15.7±0.5c*
Lipid retention efficiency (%)							
poultry meal	209.6±20.4a	102.4±10.0b*	94.4±10.6b*	92.1±9.4b	81.2±6.8b	97.8±4.7b*	73.75.3b*
feather meal	209.6±20.4a	174.7±20.1ab*	153.5±9.4abc*	121.9±23.2bc	106.4±21.5c	97.0±11.4c*	94.72.0c*

Values are mean ± sem for four replicates. * indicates a significant difference ($P<0.05$) between feather and poultry meals for each index at each inclusion level. Different letters in superscript indicate a signifieant difference ($P<0.05$) between inclusion levels for either ingredient for each performance index.

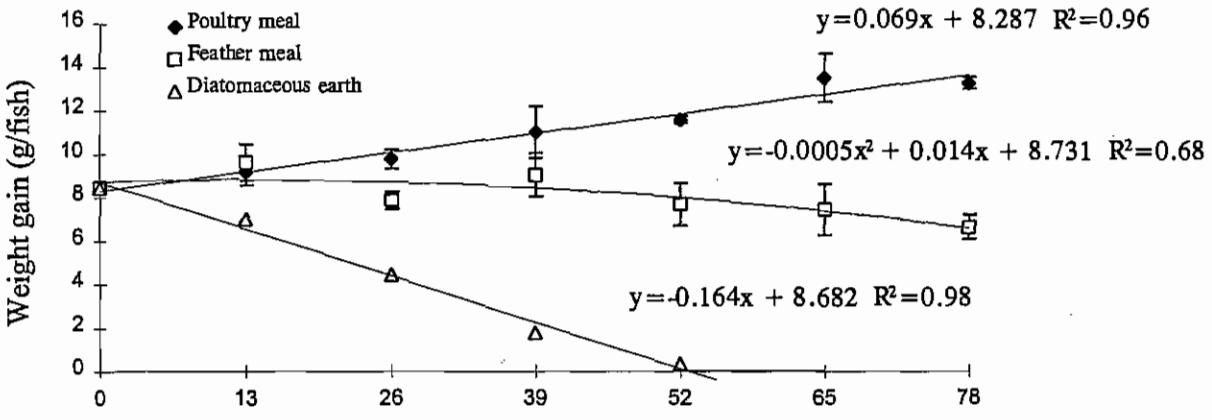
Table 4

Carcass protein and lipid composition (dry basis) of silver perch fed graded diets of either poultry meal, feather meal or diatomaceous earth for a period of 71 days.

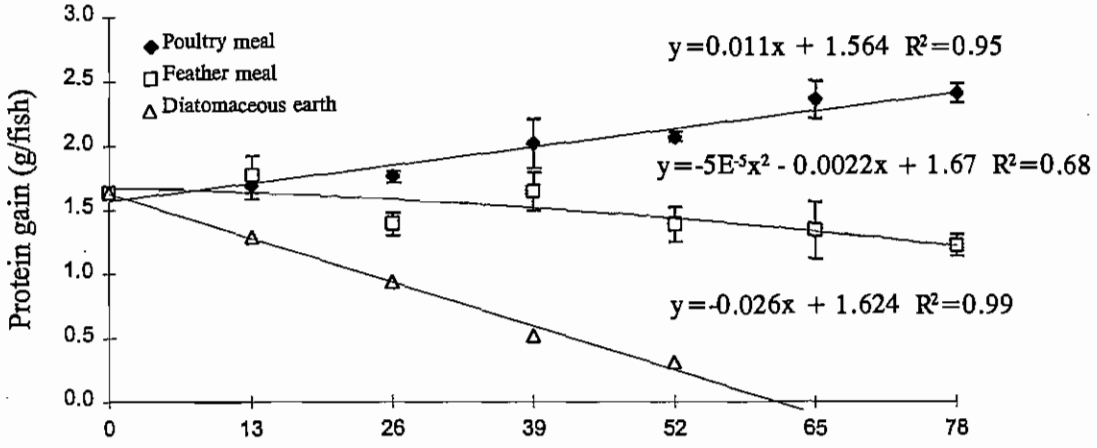
	Experimental Diet (ingredient inclusion level -%)						
	0 (Ref)	13	26	39	52	65	78
Carcass Protein							
Poultry	58.2±1.3^{a}	53.0±0.9^{b}	52.2±1.3^{b**}	51.3±0.9^{b}	49.5±1.0^{b}	46.2±1.1^{c}	51.0±0.9^{b}
Feather	58.2±1.3^{a}	53.3±0.5^{b}	49.5±1.3^{b*}	53.1±1.7^{b}	50.1±2.7^{b}	50.8±0.8^{b*}	51.6±0.4^{b}
D. Earth	58.2±1.3^{ab}	51.1±1.1^{c}	55.4±0.6^{a*}	56.9±1.9^{ab}	61.5±1.3^{b*}		
Carcass Lipid							
Poultry	23.4±1.0^{a}	28.6±1.5^{b}	32.2±2.2^{bc}	34.0±1.1^{c}	34.1±1.6^{c}	36.4±1.1^{c}	34.6±0.7^{c}
Feather	23.4±1.0^{a}	30.4±1.3^{b}	33.7±1.2^{b}	30.0±2.6^{b}	33.3±2.6^{b}	35.6±1.2^{b}	34.7±0.4^{b}
D. Earth	23.4±1.0^{ab}	30.2±3.1^{b}	25.6±1.6^{ab*}	17.7±1.7^{a*}	18.6±3.2^{a*}		

Values are means ± sem of four replicates n=4. Different letters in superscript indicate significant differences ($P<0.05$) between inclusion levels for each ingredient. Significant differences between carcass protein and or lipid composition at each inclusion level are indicated by asterix *.

Figure 1. Performance of silver perch fed experimental diets for 71 days.



a) Individual weight gain.



b) Individual protein deposition.

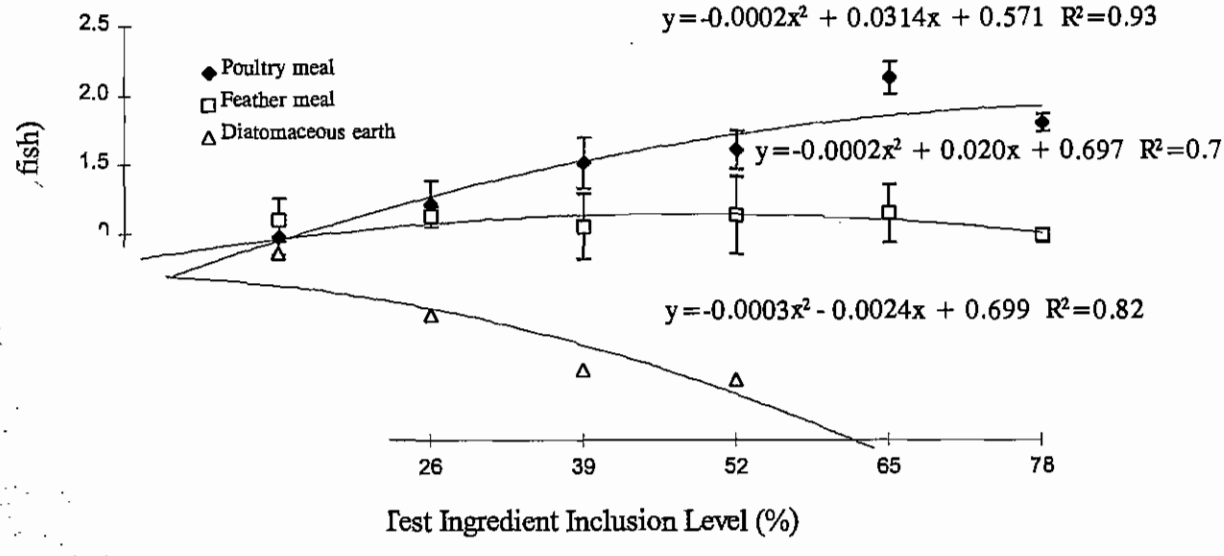


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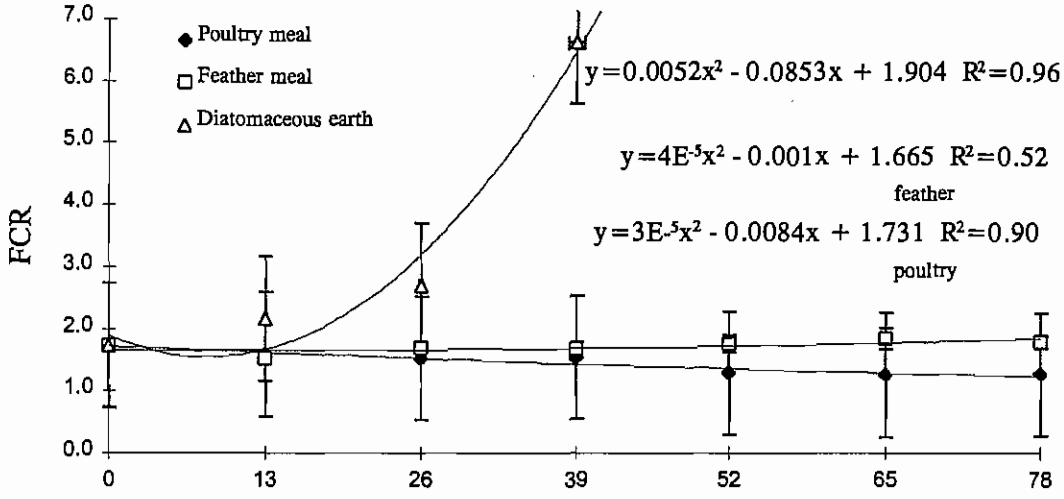
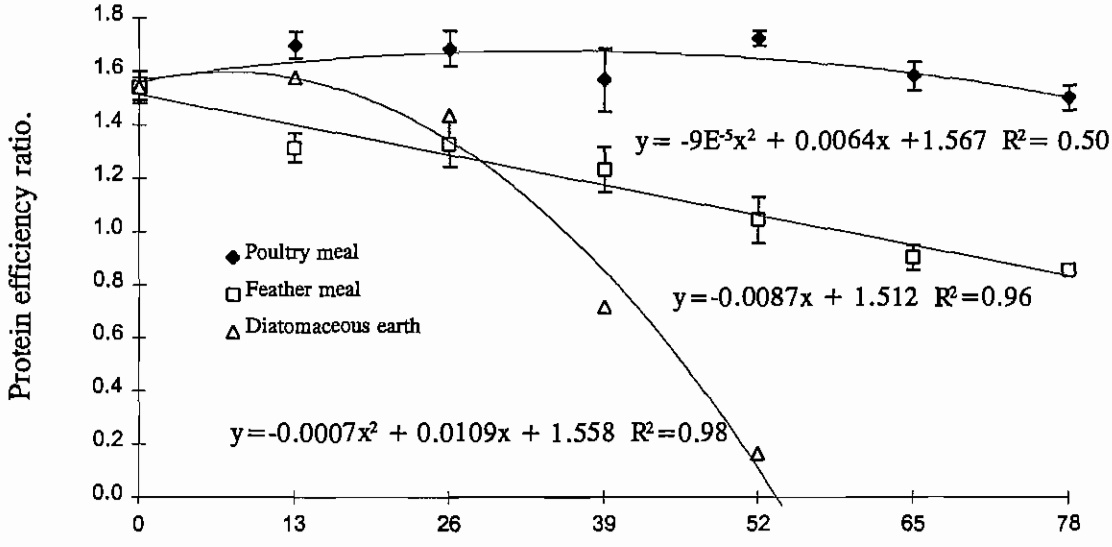
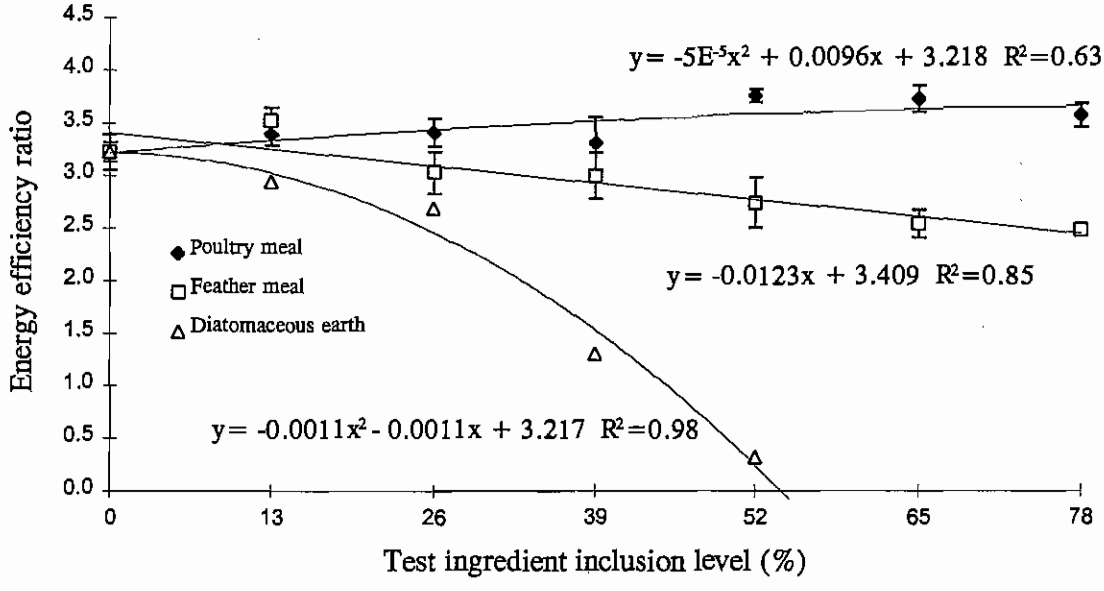
Figure 2. Conversion ratios of silver perch fed experimental diets for 71 days.



a) Food conversion ratio.

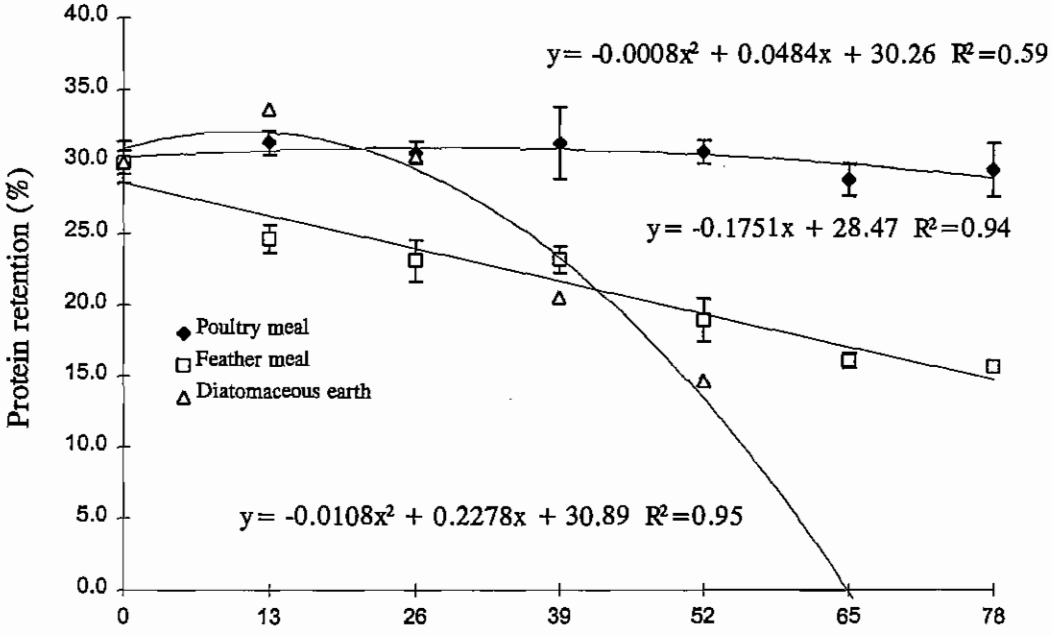


b) Protein efficiency ratio.

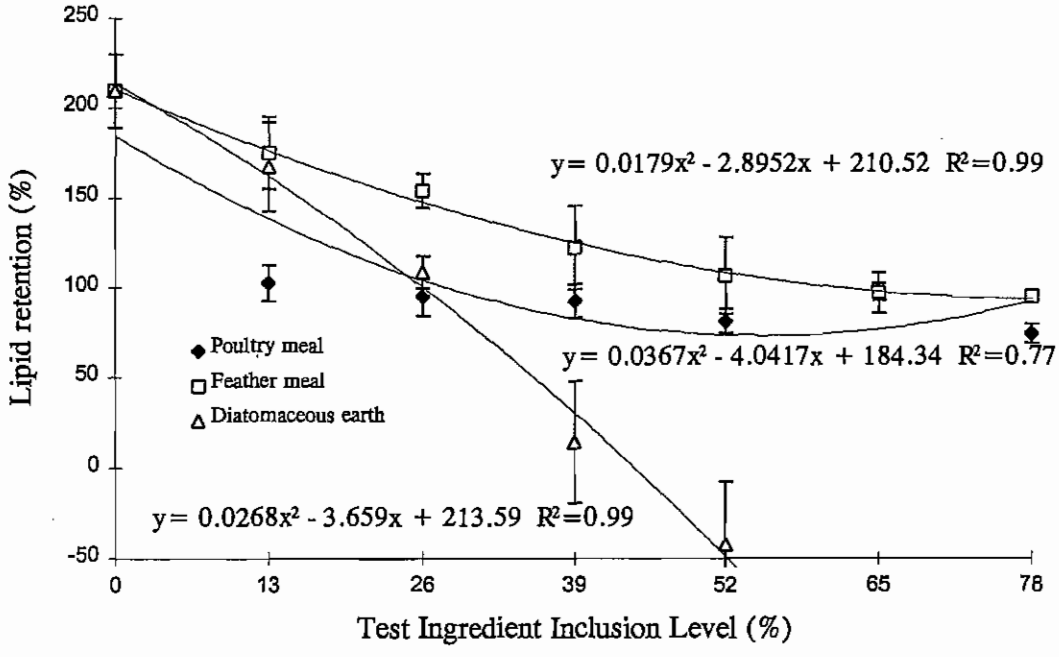


c) Energy efficiency ratio.

Figure 3. Retention efficiencies for silver perch fed on experimental diets for 71 days.

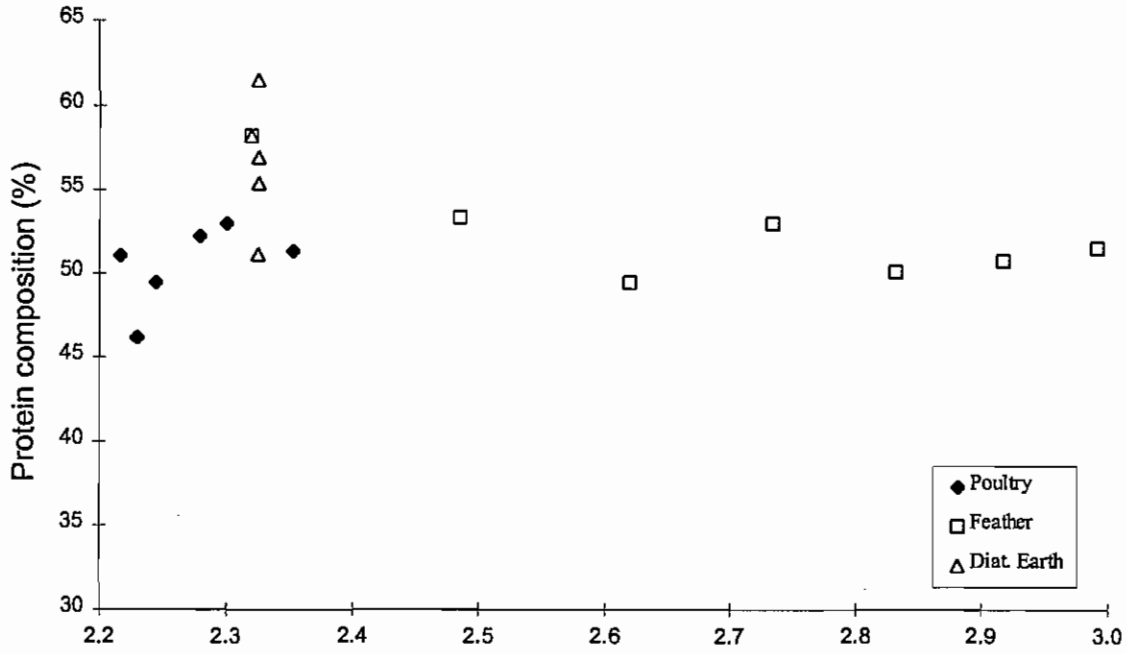


a) Individual protein retention efficiency.

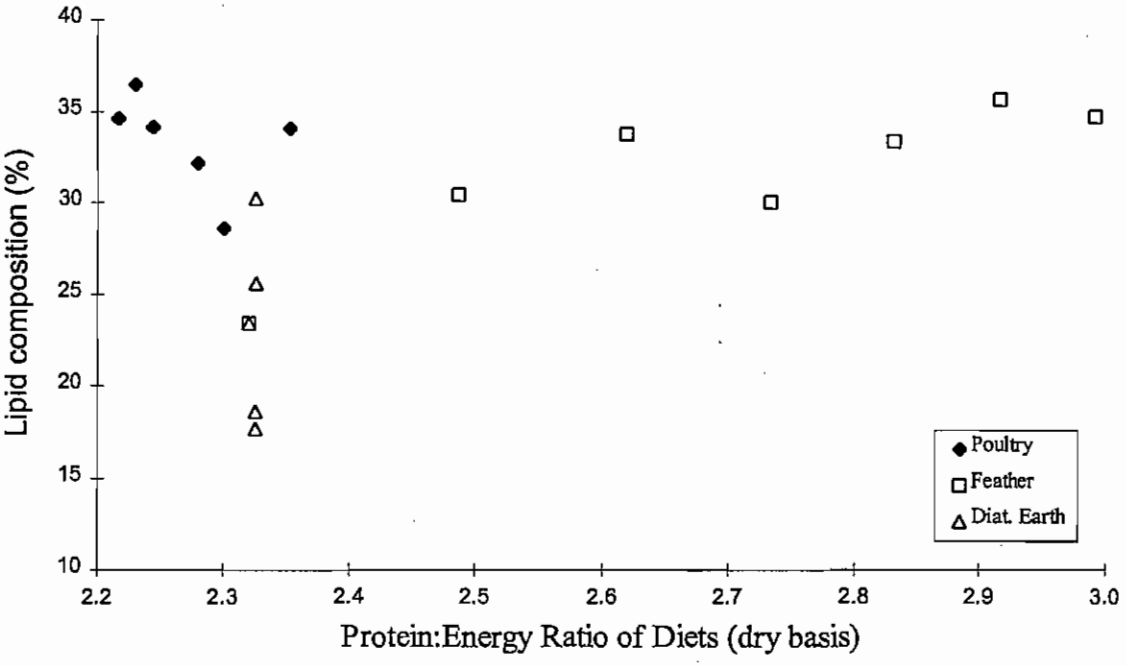


b) Individual lipid retention efficiency.

Figure 4. Carcass protein and lipid composition (dry basis) of silver perch fed experimental diets for 71 days.



a) Individual carcass protein composition (dry basis).



b) Individual carcass lipid composition (dry basis).

6.7 Replacement of fish meal in diets of silver perch: VI. effects of dehulling and protein concentration on the digestibility of four Australian grain legumes in diets for silver perch (*Bidyanus bidyanus*).

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Abstract

Grain legumes have the potential to partially replace fish meal in aquaculture diets. Recent studies with the legumes *Lupinus angustifolius* and *Lupinus albus* showed that removal of complex carbohydrates by dehulling or protein concentration greatly improved digestibility for both species when fed to juvenile silver perch (*Bidyanus bidyanus*). In the present study, four additional grain legumes were evaluated using the same fish species.

Two experiments were conducted to determine apparent digestibility coefficients (ADC's) for dry matter, energy, protein and amino acids. Chromic oxide (1%) was used as an inert indicator and faeces were collected by settlement. In the first experiment, eight diets were evaluated in a factorial design. Two hull-types (whole or dehulled) and four ingredients, field pea (*Pisum sativum*), faba bean (*Vicia faba*), chick pea (*Cicer arietinum*), and vetch (*Vicia sativa*) were tested. In the second experiment, protein concentrates (air classification : density separation) of field peas and faba beans were tested.

For the first experiment, both factors and their interaction were significant for all ADC's ($P<0.05$). Dry matter ADC's for whole grains ranged from 41-56% with faba beans>field pea>chick peas>and vetch. Energy ADC's for whole grains ranged from 53-62% with faba bean>vetch>field pea>and chick peas. Dry matter and energy digestibility was significantly improved for all dehulled grains with the exception of faba beans. Dehulled vetch proved to have the highest overall dry matter (78%) and energy (82%) digestibility of all grain legumes (excluding the protein concentrates), but test diets containing 30% of the vetch product were poorly accepted by silver perch.

Protein digestibility of the legumes studied was high, ranging from 74-91% for whole grains and 81-97% for dehulled grains. There was significant improvement in the protein digestibility of dehulled field peas, faba beans and vetch, but not chick peas. Protein digestibility was highest for the field pea concentrate (99%) and lowest for whole vetch (74%). Dry matter and energy ADC's for protein concentrates of field peas and faba beans were much higher than for respective whole or dehulled grains. Digestibility of amino acids were generally improved by dehulling and protein concentration, with all amino acid ADC's significantly improved for dehulled vetch. Cystine had the lowest content and was the most poorly digested of the amino acids in whole grains.

In this study, silver perch have generally demonstrated high protein digestibilities for the legumes evaluated. Removal of carbohydrates (ie: non starch polysaccharides) by dehulling improved the digestibility of field peas, chick peas and vetch, but had less effect on faba beans. The use of protein concentrates in the test diets dramatically improved dry matter and energy digestibilities for field peas and faba beans, but had little effect on improving the

already high protein digestibility of these legumes. The cost effectiveness of using grains processed in this way is yet to be determined.

Keywords: Silver perch; Fish nutrition; Digestibility; Legumes

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1. Introduction

One of the major constraints limiting the development of aquaculture production is the high cost of feeds. However, these costs may be reduced if locally available low-cost protein sources can be utilised. The use of plant proteins to replace more traditional protein sources in aquaculture diets has become more prevalent in recent years. Among the most promising are species of grain legumes (de la Higuera et al., 1988; Hughes, 1988; Gouveia et al., 1993; Hughes, 1991; Robaina et al., 1995; Allan et al., 1997; unpublished data - section 6.3). Soybean has traditionally been the focus for much of this research but it to has become increasingly expensive and may also become prohibitive in comparison to less expensive protein sources (Hughes, 1991; Anon, 1997).

Grain legumes are cultivated on a large scale in many countries. In Australia, production of total grain legumes was projected to reach 2334.5 kt in 1995-96 (ABARE, 1996). Lupin species predominate, accounting for approximately 60% of production. The remainder consists mainly of field pea, chick pea, faba bean and vetch with 19%, 11%, 6% and 1.6% of production respectively. Legumes are already valued as ingredients in compound stock feeds due to their relatively high protein and energy values (Petterson and Mackintosh, 1994; Khorasani and Kennelly, 1997). While the protein content of Australian grain legumes is adequate for supplementing livestock diets, fish require less energy and their diets contain significantly more crude protein. Estimates for dietary protein requirements for fish range between 30-55% depending on the species and its state of maturity (NRC, 1993).

Apart from lower protein content, grain legumes can also contain anti-nutritional factors (Krogdahl, 1989; Petterson and Mackintosh, 1994), and have been implicated in poor protein utilisation and growth in several studies on fish (Viola et al., 1983; Olvera et al., 1988; Webster et al., 1995). In addition, like many plant feed ingredients, grain legumes have lower contents of one or more of the limiting amino acids (Wee, 1991), usually lysine and methionine compared with marine based ingredients like fish meal.

Legumes are also high in soluble and insoluble complex carbohydrates. Fish have no immediate dietary requirement for carbohydrate, although some species can utilise carbohydrate as a non-protein energy source (Shimeno et al., 1979; Anderson et al., 1984; Brauge et al.,1994; Wilson, 1994). Carbohydrate can also be important as a binding agent for the production of stable pellets (Hui-Meng, 1989). However, excessive amounts of carbohydrate in fish diets has been associated with problems such as glycaemia (Brauge et al., 1994), reductions in digestibility (Wilson, 1994; McGoogan and Reigh, 1996), increased consumption and faster gastric evacuation times (Hilton et al., 1983). Reduced digestibility and increased rates of gastric evacuation may pre-dispose intensive culture systems to water quality problems given the high energy / nutrient loadings of faecal outputs (Cho, 1991; Jirsa et al., 1997).

In their raw form, all Australian grain legumes are well below the protein content of fish meals (70%) and soybean meals (44%) ranging between about 20-30%. This implies that proportionally more of these ingredients will be required to balance dietary protein in diets for fish than would be required when using the more traditional sources. Therefore, unless protein content can be elevated in some way, the use of grain legumes (excluding oilseeds) as a practical alternative to other protein sources will be limited. Dehulling and protein concentration are processing techniques which may overcome some of these inherent deficiencies.

Recent nutritional studies undertaken on the dominant Australian grain legumes *Lupinus angustifolius* and *Lupinus albus*, have shown that removal of non-digestible carbohydrate by dehulling or protein concentration greatly improved digestibility coefficients for each species when fed to silver perch (Allan et al., unpublished data, see Section 6.3 of this report). This study aimed to extend this research by evaluating the potential use of four additional Australian grain legumes as major ingredients in aquaculture diets for silver perch (*Bidyanus bidyanus*). Those evaluated include the field pea - dunn (*Pisum sativum*), faba bean - fijord (*Vicia faba*), chick pea - desi (*Cicer arietinum*) and vetch - blanch flur (*Vicia sativa*). All legumes were evaluated as whole or dehulled seeds. Field pea and faba bean were further processed following fine grinding, air classification and density separation to remove additional carbohydrate.

2. Materials and Methods

21 Experimental fish

Silver perch were initially bred and reared at the Grafton Research Centre following techniques described by Thurstan and Rowland (1995). They were transferred to the Port Stephens Research Centre where they were held in 10 000 l tanks and fed the silver perch reference diet of Allan and Rowland (1992) until used in experiments. Fish were treated with 5mg/l NaCl before stocking to remove ectoparasites and reduce the risk of fungal infection (Rowland and Ingram, 1991).

During all stocking procedures, fish were anaesthetised (25mg/l ethyl-p-aminobenzoate) and weighed individually, or in small groups, before being systematically dispersed to experimental tanks. Spare fish to replace any mortalities were stocked into separate holding tanks (100 l) and fed the appropriate test diets.

2.2 Diets

Two digestibility experiments were performed. In the first study, 8 diets were evaluated under a 2 X 4 factorial design. Two hull types (whole or dehulled) and four ingredients (field pea, faba bean, chick pea, vetch) were tested.

In the second study, digestibility coefficients for test diets containing protein concentrates of field peas and faba beans were determined. These legumes are typically lower in oil content than soybean and cotton seed meals, and as a consequence, are much more suited to dry processing techniques. Protein was isolated by first grinding field peas and faba beans to an extremely fine flour (<30um) which produced two physically distinct fractions, each with a different particle size and density (Cheftel et al., 1985; Greg Pointing, Goodman-Fielder, pers.

comm.). Fractions were predominently protein bodies or starch granules, and were then separated using an air classification system similar to that described in Tan (1991). For these concentrates, the starch fraction was discarded, leaving a protein rich fraction for incorporation into test diets.

In both experiments, diets were formulated on a dry matter basis to contain 70% of the reference diet SP35 (Allan and Rowland, 1992) and 30% of the test ingredient. 1% chromic oxide was included in experimental diets as an inert indicator. All components of each test diet were then thoroughly dry mixed before the addition of about 500ml/kg distilled water (Hobart Mixer: Troy Pty Ltd, Ohio, 45374, USA). The mixture was then pelleted through a meat mincer fitted with a 1.5mm die (Barnco Australia Pty Ltd, Leichhardt, 2040, NSW). After pelleting, diets were dried in a convection drier at $< 35^{O}$ C for approximately 6 hours until all diets had moisture contents of less than 10%.

2.3 Experimental facilities

Digestibility tanks were housed in a light / temperature controlled environment. These tanks were 170 l cylindro-conical tanks fitted with a 65mm diameter settlement chamber which tapered into a 150mm length of silicone tubing which collected the faecal pellets. Fresh pre-filtered water was initially drawn from a 50 000 l reservoir and passed through a $2m^3$ biofilter where incoming water was heated. Water then flowed directly from the biofilter through an ultra violet conditioning unit into the experimental tanks at a flow rate of 600 ml/min. Effluent water exited each tank via a 25mm standpipe and returned to a common sump where 25% of the effluent was directed to waste. The remaining water passed through a twin cartridge membrane filter before being returned to the biofilter for recirculation. Each tank was aerated with two air stone diffusers and fitted with an automatic belt feeder attached to a clear perspex diffuser.

Fish were fed in excess of their daily requirements once a day for a period of 3 hours (between 0830 and 1130h). Approximately 1 h after all feed had been delivered to the digestibility tanks both the upper tanks and lower collection chambers were thoroughly cleaned. The silicone collection tubes were then packed in ice and maintained at temperatures of approximately 4^{0}C to reduce bacterial activity during the collection period. Faeces was collected by settlement over a period of 18 h. Faecal samples were collected each morning prior to feeding and dried over silica gel in vacuum desiccators. Individual tank samples from daily collections were pooled to provide sufficient sample for bio-chemical analyses.

After stocking, each test and reference diet was randomly assigned to three replicate digestibility tanks (n=3). Tanks were stocked with five silver perch (mean 16.3g) in the first digestibility trial and six (mean 18.1g) fish in the second trial. Fish were acclimated to experimental conditions and diets for a minimum of 10 days before collection of faeces. Water quality was monitored weekly in both experiments following procedures described in Allan et al. (1990). In each case temperature was always 26.0 ± 2.0^{0}C, dissolved oxygen 7.3 $\pm$ 1.1 mg/l, and pH 8.1 $\pm$ 0.4 units.

2.4 Bio-chemical analyses

All analyses were carried out in duplicate on samples of feed and faecal material at the Wollongbar Agricultural Institute, Bruxner Highway, NSW, 2477. Samples were freeze dried

and ground using a water cooled total recovery grinder prior to analyses. Dry matter and energy (bomb calorimetry) were determined following procedures described in AOAC (1975). Nitrogen was determined following methods outlined by Havilah et al. (1977) and multiplied by 6.25 to establish crude protein contents. Determination of chromic oxide was by the method described in Kimura and Miller (1957). Amino acids were analysed using HPLC and Water Pico-Tag (Waters Pty Ltd, Lane Cove, NSW, 2066, Australia) after being subject to acid hydrolysis. Tryptophan was not determined. Sulphur amino acids were determined separately following performic acid digestion. Acid detergent fibre (ADF) was determined by methods described in AOAC (1975).

2.5 Calculation of digestibility coefficients

Apparent digestibility coefficients (ADC) for whole diets and ingredients were calculated following the indirect procedures described in Cho and Kaushik (1990):

ADC for a diet = $[1-(F/D \times DC_r/FC_r)] \times 100$ where:

F = % nutrient or energy in faeces,
D = % nutrient or energy in diet,
DC_r = % chromic oxide in diet and
FC_r = % chromic oxide in faeces.

and ADC% for ingredient = 100(ADC test diet - 0.7xADC reference diet)/0.3.

2.6 Statistical analyses

In the first trial, a 2 X 4 factorial experiment with n=3 replicates per treatment was used. Both factors (hull-type and ingredient) were fixed and individual digestibility coefficients for dry matter, energy, protein and amino acids were analysed using two factor ANOVA. Both factors and their interaction were significant for all coefficients ($P<0.05$). As interpretation of main effects following interaction are inappropriate (Huck et al., 1974; Underwood, 1997), a comparison of simple main effects was undertaken using oneway ANOVA. All data was tested for homogeniety of variance (Cochran's test), and differences between means, where significant, were determined by Student Newman-Keuls test. Data that failed tests for variance were subject to arc-sin transformation before oneway ANOVA, and except for data in two tests, arc-sin transformation failed to improve variances. All data are presented in their original form.

3. Results

3.1 Composition

Nutrient composition for the four grain legumes evaluated in this study shows dehulling has had little effect on the gross energy composition of most ingredients, with only a slight increase recorded for vetch (Table 1). Protein composition of all ingredients was elevated by removal of hulls. Vetch, field pea, chick pea and faba bean had an approximately 1.4, 2.2, 3.4 and 3.6% improvement in crude protein content respectively. Dehulling significantly reduced the levels of Acid Detergent Fibre (ADF) in all legumes, with greatest reductions for faba bean and chick pea (Table 1)

Protein concentration improved both the energy and protein content of field peas and faba beans (Table 1). Elevations of approximately 15% in energy for each of these grains is directly linked to a concomitant increase in the fat content of each concentrated legume. The crude protein content of concentrated field peas was 16.9% higher and faba beans 20.6% higher than respective whole grains.

Levels of the sulphur amino acids cystine and methionine were low for all grains tested. The amino acid levels of all dehulled legumes were equivalent to, or slightly higher than whole grains (Table 2), with the exception of proline in dehulled faba beans. Protein concentration dramatically improved the amino acid composition of field peas and faba beans, with the majority of amino acids showing an almost two-fold increase in content when compared to the whole ingredients. Despite this fact, values for cystine and methionine remained relatively low.

3.2 Apparent Digestibility Coefficients

In the first experiment, both factors and their interaction were significant for all proximates and amino acids ($P<0.05$). All subsequent tests on ADC's were reduced to oneway analyses of variance. Differences were found between the dry matter digestibilities of whole grains (Table 3). From highest to lowest, dry matter ADC's for whole grains were: faba beans>field peas>chick peas>and vetch, and for energy ADC's: faba bean>vetch>field pea>and chick peas. Protein digestibility was greater than 75% for all whole grain legumes.

Dehulling significantly improved dry matter, and energy ADC's for all grains with the exception of faba beans. Dry matter digestibility for faba beans showed a minor numerical increase, but energy digestibility was significantly reduced. For dehulled material, vetch returned the highest digestibility coefficients for dry matter and energy, however diets incorporating vetch (whole and dehulled) were poorly accepted by silver perch. Dehulling significantly increased protein digestibility for field peas, faba beans and vetch, but no difference was detected between whole and dehulled chick peas. Dry matter and energy ADC's for protein concentrates of field peas and faba beans were much higher than for whole or dehulled grains. Protein ADC's for concentrates were highest in field peas, and protein ADC's for concentrates of faba bean were equivalent to protein ADC's for dehulled material. Statistical comparisons between energy ADC's for dehulled grains and between energy ADC's for whole and dehulled field peas failed tests for homogeneity of variance (Cochran's $P<0.02$ in each case). Comparisons between dry matter ADC's for dehulled grains also failed tests for homogeneity (Cochrans $P=3.8\times10^{-3}$) (Table 3)

Dehulling significantly improved the digestibility of all amino acids for vetch, and improved a limited number of amino acids in field and chick peas (Table 4). Only the levels of lysine and cystine were significantly improved by dehulling faba beans. Similar to the effects on proximate digestibility, protein concentration elevated the digestibility of all amino acids for field pea and faba bean, with the exception of cystine and methionine for faba beans.

In addition to data from the present study, we also present complimentary data for two species of lupin. Compositional data and apparent digestibility coefficients for whole and dehulled *Lupinus angustifolius* (gungarru variety) and *Lupinus albus* were previously determined under experimental conditions similar to those described above (Allan et al., 1997; unpublished data

data - section 6.3). As well as the above, formerly unpublished digestibility coefficients for a protein concentrate of *L. angustifolius* (gungarru variety), prepared and evaluated in the same way as those of field pea and faba bean concentrates is also presented (Table 5). For this experiment six fish were stocked into digestibility tanks with a mean initial weight of 10.9g and faeces was collected over a three week period.

Whole *L.albus* was more digestible than whole *L. angustifolius* for dry matter and energy, however both species had similar protein digestibility for whole and dehulled grains. Dehulling markedly improved the proximate digestibility of both species and notable gains were achieved after protein concentration of *L. angustifolius* in dry matter and energy digestibility. Protein concentration did not result in increased protein digestibility for *L. angustifolius*. Similarly, protein concentration did not increase the digestibility of amino acids for *L. angustifolius* above levels of dehulled material, however this ingredient was evaluated in a separate experiment and care should be exercised when drawing comparisons between these ingredients. Dehulling generally increased the digestibility of all amino acids for both species of lupin.

4. Discussion

Results of the present study indicate that dehulling and/or protein concentration appear to be effective methods of removing certain complex carbohydrates (fibre and starch) from whole grain legumes, while at the same time maintaining or improving energy, protein and amino acid profiles. In general, both processes improved the dry matter, energy and protein digestibility of all grains with the exception of faba beans, which showed a significant reduction in energy digestibility. ADC's for amino acids were also generally improved for all grains after processing. In addition, while dry matter digestibilities for either whole or dehulled grains were sometimes very low (<50%), protein digestibilities remained relatively high (with the exception of whole vetch), indicating the exceptional ability of silver perch to utilise proteins, even from poorly digested plant sources.

Other information on the effects of dehulling or concentration of grain legumes to improve their digestibility for fish species is minimal. Specific reference to dehulling is made by Eusebio (1991) in his evaluation of dehulled legumes fed to tiger prawns (*Penaeus monodon*), and other authors (eg: Hughes, 1991) have speculated on the benefits this procedure would confer on grains such as lupins when fed to rainbow trout (*Oncorhynchus mykiss*). Data on the use of protein concentrates in diets for fish is also rare, however one study (Kaushik et al., 1995) was found which evaluated the use of a soybean protein concentrate (SPC) fed to rainbow trout (*Oncorhynchus mykiss*). Apparent dry matter, energy and protein digestibility coefficients for SPC fed to rainbow trout were 74, 83 and 96% respectively, with digestible protein and energy values of 69% and 15MJ/kg of dry matter. The ADC's are similar to values we report for concentrates of *L. angustifolius* and dehulled *L. albus*, however the soybean concentrate had > 70% crude protein.

Whole lupins have been used successfully in several performance and metabolism trials with fish (De la Higuera et al., 1988; Hughes, 1988; Gomes and Kaushik, 1989; Hughes, 1991; Moyano, 1992; Morales et al., 1994; Robaina et al., 1995; Smith et al., 1995), as have several other less well known legumes (De Silva and Gunasekera, 1989; Keembiyehetty and De Silva, 1993). However, metabolism trials following processing techniques such as dehulling are scarce. In contrast, the effects of processing techniques such as extrusion (Gouveia et al,

1993; Gomes et al., 1993), autoclaving (De La Pena et al., 1987; Martinez-Palacios et al., 1988; Allan and Rowland, 1994) and soaking (Olvera et al., 1988), which aim to remove or reduce anti-nutrients from legume seeds to improve either their digestibility or acceptability are more common.

Results from this study reflect similar trends to those reported for whole and dehulled *Lupinus angustifolius* and *Lupinus albus* in the previous trial (Table 5). In that study, improvements in the digestibility of dehulled lupins for silver perch was attributed to the reduction of poorly digested non-starch polysaccharides (NSP) (Allan et al., 1997 unpublished data, see Section 6.3 of this report). Similar conclusions about the improvement in digestibility for dehulled field peas, chick peas and vetch may also be drawn, however NSP was not measured for any of the grains reported in this study.

Starch content and digestibility were not determined for any of the protein concentrates evaluated in the present study, or for the protein concentrates of *L. angustifolius*. However, given the specificity of the air classification:density separation process, it is highly probable the improvements in the digestibility of these concentrates is related to dramatic reductions in not only the starch fraction, but also the further reduction in NSP from the kernals. As a result, the protein concentrates of faba beans and *L. angustifolius* showed about a 10% improvement and those for field peas a 20% improvement in dry matter and energy digestibility above that of the dehulled material.

Gains for dry matter, energy and protein ADC's for protein concentrates of field pea were much greater than those for similarly treated faba beans (Table 3). This difference cannot be related to the starch content of each legume, as field peas and faba beans both contain similar amounts of starch (30-50%) (Petterson and Mackintosh, 1994; Evans and Htoon, 1997; unpublished data). Given that the concentration step should have removed a similar proportion of the starch from each grain, the differences in their digestibility must be related to other factors.

Of particular interest in this study was the high protein digestibility of all the legumes (excluding whole vetch), regardless of whether or not they were processed. Similar trends were apparent for both lupin species. This would suggest either the presence of well developed intestinal proteases in silver perch (Allan et al., unpublished data, see Section 6.3 of this report) or the lack of significant amounts of proteinase type inhibitors in these grains. Both field and chick peas are reported to contain fairly low levels of common anti-nutrients such as tannins, oligosaccharides and lectins (Petterson and Mackintosh, 1994) which might otherwise limit protein digestibility for silver perch.

In real terms, dehulling has only marginally improved the protein digestibility of field peas, faba beans and lupins. In fact protein digestibility for chick peas showed a slight reduction. This suggests, that for these legumes, dehulling has a greater effect on improving dry matter and energy digestibility than it does in improving protein digestibility. This was not the case for vetch, which had a 13% increase in protein digestibility after dehulling, rising from 74-88% (Table 3). Vetch also showed more dramatic increases in dry matter (nearly 40%), energy and amino acid digestibility after being dehulled than the other three legumes.

However, these gains were confounded by the fact that silver perch were reluctant to accept the vetch diets.

This suggests the fibre from the hulls of this grain reduce protein digestibility in silver perch in a different way to the fibre of the other legumes, or that the hull contains certain anti-nutrients which reduce protein digestibility. The similarity in protein digestibility for dehulled vetch compared with that of dehulled field peas indicates that if anti-nutrients were present in the kernals (after dehulling), they were present at fairly low levels. Vetch is reported to contain cyanoalanine compounds (toxic non-protein amino acids = neuro toxins) within the seed that may limit its use (Petterson and Mackintosh, 1994), and others have suggested tannins may be a problem with this grain (R. Barneveld , pers. comm.). Our results suggest these anti-nutrients were ineffective in reducing the digestibility of dehulled material for silver perch. However, they may have been responsible for reducing the acceptability of these diets.

Another point of interest in this study was that although both faba beans and chick peas had proportionally more carbohydrate removed during the dehulling process than field peas or vetch (Table 1), their gains in dry matter and energy digestibility were much lower. Again, this would suggest that for silver perch, removal of a particular type of fibre is more important in improving digestibility than the actual amount removed.

For faba beans, the reduction in energy digestibility, although significant is probably a reflection of experimental variation more than a real reduction in digestibility, especially considering that dry matter digestibility was not significantly improved after removal of hulls. In general, reduced dry matter and energy digestibility have been linked to high levels of dietary fibre in the diets of fish (Hilton et al., 1983, Gomes et al., 1993), and thus for most fish species removal of this component would be expected to increase digestibility. Faba beans are known to contain tannins, which are concentrated in the hulls, and which can effect protein digestibility, but this would not appear to be the case in this study, given the exceptionally high protein digestibility of this grain under all processing conditions.

Despite high protein digestibility, the effectiveness of feeding poorly digested ingredients such as whole vetch, chick peas, faba beans and *L. angustifolius* to silver perch in a production environment would appear questionable. Poorly digested feeds, especially those high in fibre (Leary and Lovell, 1975) are the primary source of pollution in aquaculture (Cho, 1991) and tend to decrease water quality. With this in mind, the trend towards using highly digestible, low pollution, nutrient dense type feeds or ingredients which minimise faecal outputs is now a major priority (Cho, 1991; Cho et al., 1994; Sadiku and Jauncey, 1995; Jirsa et al., 1997).

Fish such as rainbow trout appear to utilise some of the raw legumes tested in these trials more effectively than silver perch. Gomes et al., (1995) report dry matter digestibility coefficients using *Oncorhynchus mykiss* (rainbow trout) for *Lupinus angustifolius*, *Pisum sativum* and *Vicia faba* of 63.3, 66.1 and 66.1% respectively. Energy digestibility was almost identical to that reported in our trial, but silver perch exhibited superior protein digestibility for each ingredient.

Digestibility coefficients for autoclaved (121^{0}C, 5 min) legumes have been determined using juvenile silver perch (Allan and Rowland, 1994). Protein digestibility was high for all grains with 86.5, 82.9 and 83.2% for field pea, chick pea and cow peas (*Vigna unguiculata*)

respectively, but gross energy ADC's were extremely low at 52.0, 48.7 and 45.8%. In comparison to our results for unprocessed legumes, it would appear that autoclaving at 121^{0}C for 5 minutes did not effect digestibility. The thermal treatment was probably insufficient to affect gelatinisation of starch within the legumes, as gelatinisation of starch has been shown to affect digestibility of starch for silver perch and other species (Bergot and Breque, 1983; Kaushik et al., 1989; Stone et al., unpublished data, see Section 6.8 of this report). For legumes with low starch content such as lupins, heating is unlikely to improve digestibility. This is supported by results reported by de la Higuera et al. (1988) for *L. angustifolius* fed to rainbow trout.

In conclusion, all whole grain legumes evaluated in this study with the exception of vetch, had high apparent protein digestibility, despite having low dry matter and energy digestibility. In general, dehulling significantly improved dry matter and energy digestibility for most grains (excluding faba beans), however practical gains in protein digestibility were only small for field peas, faba beans and chick peas. Although dehulled vetch showed the greatest gains in digestibility for all proximates and amino acids, silver perch were reluctant to accept diets containing this material.

Protein concentrates of field pea, faba beans and *L. angustifolius* all had much higher dry matter, energy and amino acid digestibilities than respective dehulled material, but protein digestibility was not necessarily greater for silver perch fed these ingredients. The cost effectiveness of this procedure needs to be determined using commercial scale protein concentration before such products can be incorporated into practical diets.

Of the four legumes evaluated in this study, dehulled field peas appears to be the most promising, despite its high starch content. There would appear to be little justification for dehulling either faba beans or chick peas, as only marginal gains were made from this process, and poor overall digestibilities indicate these grains are less suitable for production type diets than other legumes tested here.

Acknowledgments

We would like to thank the following for their technical input to experiments: Dr Stuart Rowland, Charlie Mifsud and other staff at NSW Fisheries Grafton Research Centre for producing juvenile silver perch and support for nutrition research; Rebecca Warner-Smith and David Stone at Port Stephens Research Centre; Mr Ken O'Brien and Wendy Peasley (NSW Agriculture) for their assistance with biochemical analysis; Dr Tony Evans, (CSIRO Division of Food Science and Technology, North Ryde, NSW) for supplying the diet ingredients; Mr Wayne O'Connor and Mr Stewart Fielder for cirtically reviewing the manuscript and Mrs Helena Heasman for assisting with the preparation of the manuscript. The research developed was part of the Fisheries Research and Development Corporation Sub-Program on Replacing Fish Meal in Aquaculture Diets (93/120). Financial support from the Australian Centre for International Agricultural Research, the Australian Grain Research and Development Corporation and the Meat Research Corporation is also gratefully acknowledged.

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Table 1

Proximate composition of selected pulses (dry basis) subject to different processing techniques.

		Ingredient			
Proximate	Process	Field pea	Faba bean	Chick pea	Vetch
Energy MJ/kg	Whole	17.0	17.3	19.4	17.9
	Dehulled	17.3	17.6	19.3	18.6
	Concentrate	*19.8*	*19.9*		
Protein %	Whole	25.5	27.7	20.8	30.9
	Dehulled	27.7	31.3	24.2	32.3
	Concentrate	*42.4*	*48.3*		
Fibre (ADF) %	Whole	8.7	12.0	13.4	7.2
	Dehulled	2.8	3.3	2.5	4.1
	Concentrate	*NA*	*NA*		
Fat %	Whole	1.1	1.3	4.7	0.9
	Dehulled	1.0	1.3	5.0	0.9
	Concentrate	*2.8*	*2.6*		

Table 2

Amino acid composition (dry basis) of selected pulses subject to different processing techniques.

		Ingredient			
Amino acid %	Process	Field pea	Faba bean	Chick pea	Vetch
Aspartic acid	Whole	2.9	2.8	2.5	3.4
	Dehulled	3.2	3.1	2.8	3.9
	Concentrate	*5.7*	*5.0*		
Glutamic acid	Whole	4.3	4.2	3.6	5.1
	Dehulled	4.7	4.7	3.9	5.9
	Concentrate	*8.7*	*8.0*		
Serine	Whole	1.3	1.4	1.3	1.4
	Dehulled	1.4	1.6	1.3	1.6
	Concentrate	*2.6*	*2.5*		
Glycine	Whole	1.0	1.0	0.8	1.1
	Dehulled	1.1	1.1	0.9	1.2
	Concentrate	*2.0*	*1.9*		
Histidine	Whole	0.6	0.6	0.5	0.6
	Dehulled	0.7	0.7	0.6	0.8
	Concentrate	*1.1*	*1.1*		
Arginine	Whole	2.5	2.8	2.0	2.4
	Dehulled	2.8	3.1	2.1	2.7
	Concentrate	*5.3*	*5.1*		
Threonine	Whole	0.8	0.8	0.7	0.8
	Dehulled	0.9	1.0	0.8	1.1
	Concentrate	*1.7*	*1.6*		
Alanine	Whole	1.1	1.1	0.9	1.2
	Dehulled	1.2	1.2	1.0	1.2
	Concentrate	*2.2*	*2.0*		
Proline	Whole	1.1	1.3	1.0	1.2
	Dehulled	1.1	1.2	1.0	1.4
	Concentrate	*2.2*	*2.1*		
Tyrosine	Whole	0.8	0.8	0.6	0.8
	Dehulled	0.9	0.9	0.7	1.0
	Concentrate	*1.6*	*1.6*		
Valine	Whole	1.2	1.2	1.0	1.3
	Dehulled	1.3	1.3	1.1	1.5
	Concentrate	*2.5*	*2.4*		
Isoleucine	Whole	1.1	1.1	1.0	1.2
	Dehulled	1.2	1.2	1.1	1.4
	Concentrate	*2.3*	*2.2*		
Leucine	Whole	1.7	1.8	1.6	1.9
	Dehulled	1.8	2.0	1.7	2.2
	Concentrate	*3.5*	*3.5*		
Phenylalanine	Whole	1.1	1.1	1.2	1.1
	Dehulled	1.2	1.2	1.3	1.2
	Concentrate	*2.3*	*2.0*		
Lysine	Whole	1.7	1.5	1.5	1.7
	Dehulled	1.8	1.9	1.5	1.7
	Concentrate	*3.4*	*3.0*		
Cystine	Whole	0.5	0.5	0.5	0.5
	Dehulled	0.6	0.5	0.6	0.5
	Concentrate	*0.8*	*0.7*		
Methionine	Whole	0.3	0.3	0.4	0.3
	Dehulled	0.3	0.3	0.5	0.3
	Concentrate	*0.4*	*0.4*		

Table 3

Apparent digestibility coefficients (dry basis) for dry matter, energy and protein of selected pulses subject to different processing techniques.

		Apparent Digestibility Coefficients %			
Proximate	Process	Field pea	Faba bean	Chick pea	Vetch
Dry Matter	Whole	$48.9 \pm 2.2^{ab*}$	55.9 ± 0.3^{b}	$48.7 \pm 0.8^{ab*}$	$41.5 \pm 3.2^{a*}$
	Dehulled	$62.0 \pm 0.4^{a*}$	58.2 ± 1.3^{a}	$58.4 \pm 0.7^{b*}$	$78.3 \pm 3.9^{c*}$
	Concentrate	*85.9 ± 4.0*	*66.3 ± 1.8*		
Energy	Whole	$54.5 \pm 2.2^{a*}$	$62.2 \pm 0.4^{b*}$	$53.6 \pm 0.8^{a*}$	$55.5 \pm 1.0^{a*}$
	Dehulled	$67.0 \pm 0.2^{a*}$	$58.8 \pm 0.7^{b*}$	$60.2 \pm 0.7^{b*}$	$81.8 \pm 2.3^{c*}$
	Concentrate	*91.1 ± 2.8*	*73.4 ± 2.0*		
Protein	Whole	$83.3 \pm 0.3^{a*}$	$91.6 \pm 1.3^{b*}$	84.8 ± 1.0^{a}	$74.9 \pm 2.6^{c*}$
	Dehulled	$88.1 \pm 1.0^{a*}$	$96.6 \pm 0.8^{b*}$	81.2 ± 3.5^{a}	$87.7 \pm 0.8^{a*}$
	Concentrate	*98.6 ± 2.0*	*95.0 ± 1.4*		

Values are means ± sem for three replicates. Row means with different letters in superscript indicate a significant difference between the ADC's of ingredients for each particular method of processing ($P<0.05$, SNK). Significant differences ($P<0.05$) between ADC's of whole and dehulled ingredients are indicated by *. Data in italics represent ADC's for protein concentrates which were not statistically compared.

Table 4

Apparent digestibility coefficients for amino acids (dry basis) of selected pulses subject to different processing techniques.

Amino acid	Process	Apparent Digestibility Coefficient %			
		Field pea[a]	Faba bean	Chick pea	Vetch
Aspartic acid	Whole	83.2 ± 1.2^{a}	87.4 ± 0.4^{b}	$71.6 \pm 0.7^{c*}$	$68.4 \pm 1.4^{c*}$
	Dehulled	85.3 ± 0.6^{a}	86.9 ± 0.9^{a}	$76.6 \pm 1.0^{b*}$	$82.0 \pm 1.2^{c*}$
	Concentrate	*96.4 ±2.2*	*93.5 ±1.0*		
Glutamic acid	Whole	$86.9 \pm 0.6^{a*}$	91.6 ± 0.3^{b}	$78.5 \pm 0.4c$	$73.9 \pm 0.2^{d*}$
	Dehulled	$90.6 \pm 0.7^{a*}$	92.5 ± 0.8^{a}	90.6 ± 9.2^{a}	$85.0 \pm 1.4^{a*}$
	Concentrate	*99.3 ±1.2*	*98.2 ±0.5*		
Serine	Whole	$76.9 \pm 0.6^{a*}$	89.0 ± 0.5^{b}	$69.8 \pm 0.9^{c*}$	$60.1 \pm 1.4^{d*}$
	Dehulled	$83.1 \pm 1.1^{a*}$	88.4 ± 0.8^{b}	$75.0 \pm 1.2^{c*}$	$78.1 \pm 2.0^{c*}$
	Concentrate	*95.0 ±2.2*	*96.4 ±0.6*		
Glycine	Whole	$76.8 \pm 0.9^{a*}$	84.9 ± 0.2^{b}	$70.7 \pm 1.3^{c*}$	$66.5 \pm 1.0^{d*}$
	Dehulled	$83.2 \pm 1.0^{a*}$	87.4 ± 1.5^{a}	$81.0 \pm 2.8^{a*}$	$82.7 \pm 1.0^{a*}$
	Concentrate	*94.9 ±2.4*	*91.8 ±1.0*		
Histidine	Whole	$82.4 \pm 2.4^{a*}$	89.3 ± 1.6^{b}	76.8 ± 1.3^{c}	$72.1 \pm 0.9^{c*}$
	Dehulled	$90.2 \pm 0.4^{a*}$	89.9 ± 2.9^{a}	81.7 ± 2.8^{a}	$84.5 \pm 0.6^{a*}$
	Concentrate	*98.8 ±1.8*	*96.5 ±0.3*		
Arginine	Whole	$88.7 \pm 1.1^{a*}$	94.2 ± 1.0^{b}	81.2 ± 0.7^{c}	$76.5 \pm 0.4^{d*}$
	Dehulled	$94.0 \pm 0.6^{a*}$	95.4 ± 0.9^{a}	84.3 ± 2.1^{b}	$87.0 \pm 1.4^{b*}$
	Concentrate	*100.8 ±0.9*	*99.2 ±0.7*		
Threonine	Whole	80.5 ± 1.2^{a}	87.8 ± 1.1^{b}	67.9 ± 1.3^{c}	$56.6 \pm 0.8^{d*}$
	Dehulled	83.2 ± 1.3^{a}	86.3 ± 1.3^{a}	75.2 ± 5.5^{a}	$73.3 \pm 1.4^{a*}$
	Concentrate	*93.7 ±1.9*	*95.2 ±0.5*		
Alanine	Whole	83.2 ± 1.1^{a}	89.8 ± 0.8^{b}	$74.7 \pm 0.7^{c*}$	$70.7 \pm 1.1^{d*}$
	Dehulled	85.5 ± 0.9^{a}	89.8 ± 0.9^{b}	$77.1 \pm 0.2^{c*}$	$82.4 \pm 1.8^{a*}$
	Concentrate	*95.3 ±1.9*	*93.0 ±1.0*		
Proline	Whole	$78.6 \pm 0.1^{a*}$	86.3 ± 0.4^{b}	$71.0 \pm 1.0^{c*}$	$63.4 \pm 0.8^{d*}$
	Dehulled	$84.9 \pm 0.8^{a*}$	88.1 ± 1.1^{a}	$78.7 \pm 1.3^{b*}$	$80.4 \pm 1.8^{b*}$
	Concentrate	*94.6 ±1.8*	*93.3 ±0.7*		
Tyrosine	Whole	81.9 ± 0.8^{a}	87.9 ± 0.5^{b}	72.1 ± 1.3^{c}	$62.2 \pm 1.0^{d*}$
	Dehulled	84.6 ± 0.7^{a}	87.6 ± 1.3^{a}	74.3 ± 0.8^{b}	$76.4 \pm 1.8^{b*}$
	Concentrate	*95.4 ±1.6*	*94.5 ±0.6*		
Valine	Whole	80.8 ± 1.2^{a}	87.1 ± 0.7^{b}	70.5 ± 0.9^{c}	$65.8 \pm 0.4^{d*}$
	Dehulled	82.1 ± 0.8^{a}	87.1 ± 1.4^{b}	71.5 ± 0.7^{c}	$78.1 \pm 2.4^{a*}$
	Concentrate	*94.6 ±1.8*	*93.5 ±0.7*		
Isoleucine	Whole	81.9 ± 1.7^{a}	86.5 ± 0.8^{b}	69.3 ± 1.1^{c}	$66.3 \pm 0.7^{c*}$
	Dehulled	82.6 ± 0.7^{a}	87.5 ± 1.3^{b}	71.5 ± 0.9^{c}	$78.3 \pm 1.6^{d*}$
	Concentrate	*95.3 ±1.7*	*94.0 ±0.6*		
Leucine	Whole	84.5 ± 1.5^{a}	90.7 ± 1.8^{b}	75.8 ± 0.7^{c}	$71.1 \pm 0.4^{d*}$
	Dehulled	87.9 ± 0.7^{a}	90.1 ± 1.0^{a}	75.0 ± 1.9^{b}	$82.2 \pm 2.0^{c*}$
	Concentrate	*96.1 ±1.3*	*95.5 ±0.5*		
Phenylalanine	Whole	82.9 ± 1.3^{a}	89.2 ± 0.7^{b}	70.8 ± 1.0^{c}	$62.8 \pm 0.7^{d*}$
	Dehulled	85.1 ± 0.9^{a}	89.3 ± 1.0^{a}	72.5 ± 0.8^{b}	$76.7 \pm 2.0^{b*}$
	Concentrate	*96.1 ±1.3*	*94.6 ±0.5*		
Lysine	Whole	86.3 ± 1.1^{a}	$90.9 \pm 0.3^{b*}$	80.5 ± 0.7^{c}	$72.7 \pm 1.4^{d*}$
	Dehulled	88.6 ± 0.7^{a}	$94.2 \pm 0.7^{b*}$	83.3 ± 0.6^{c}	$86.7 \pm 1.6^{a*}$
	Concentrate	*98.2 ±1.5*	*98.2 ±0.7*		
Cystine	Whole	63.1 ± 1.2^{a}	$80.9 \pm 0.6^{b*}$	65.2 ± 3.0^{a}	$50.1 \pm 8.7^{a*}$
	Dehulled	66.3 ± 1.1^{a}	$76.5 \pm 1.6^{a*}$	48.8 ± 19.7^{a}	$77.4 \pm 3.2^{a*}$
	Concentrate	*85.2 ±4.0*	*80.3 ±1.7*		
Methionine	Whole	87.5 ± 1.7^{a}	93.3 ± 0.7^{b}	85.3 ± 1.2^{a}	$77.8 \pm 0.9^{c*}$
	Dehulled	91.2 ± 0.4^{a}	94.2 ± 0.7^{b}	83.2 ± 1.2^{c}	$88.1 \pm 0.4^{d*}$
	Concentrate	*94.4 ±1.4*	*91.1 ±1.6*		

Values are mean ± sem for 3 replicate tanks. Row means with different letters in superscript indicate a significant difference between the ADC's of ingredients for each particular method of processing ($P<0.05$, SNK). Significant differences ($P<0.05$) between ADC's of whole and dehulled ingredients for individual amino acids are indicated by *. Data in italics represent ADC's for protein concentrates which were not statistically compared.

Table 5

Composition and apparent digestibility coefficients (dry basis) for *L. angustifolius* and *L. albus* subject to different processing techniques.

		Composition		Apparent digestibility coefficient %	
	Process	*L. angustifolius*	*L.albus*	*L.angustifolius*	*L.albus*
Proximate					
Dry Matter %	Whole	94.1	95.4	50.3 ± 3.0	64.7 ± 0.4
	Dehulled	95.0	95.0	67.7 ± 3.2	77.8 ± 2.0
	Concentrate	*96.3*		*78.4 ± 3.2*	
Energy MJ/Kg	Whole	17.9	20.9	59.4 ± 1.0	72.7 ± 1.8
	Dehulled	20.7	21.4	74.0 ± 2.3	85.2 ± 1.5
	Concentrate	*22.7*		*82.0 ± 2.5*	
Protein %	Whole	34.1	37.5	96.6 ± 0.9	96.1 ± 0.9
	Dehulled	43.6	42.8	100.3 ± 0.4	101.4 ± 0.3
	Concentrate	*61.4*		*97.4 ± 1.0*	
Amino acid %					
Aspartic acid	Whole	3.8	4.3	87.9 ± 2.4	93.2 ± 1.3
	Dehulled	4.7	4.9	96.6 ± 1.5	98.6 ± 0.7
	Concentrate	*6.8*		*97.4 ± 0.5*	
Glutamie acid	Whole	8.2	4.3	95.5 ± 1.7	96.0 ± 1.3
	Dehulled	10.3	4.9	98.6 ± 0.5	101.1 ± 0.5
	Concentrate	*14.8*		*98.3 ± 0.7*	
Serine	Whole	2.1	2.3	91.4 ± 2.7	95.0 ± 0.9
	Dehulled	2.6	2.6	100.4 ± 1.2	101.9 ± 0.5
	Concentrate	*3.6*		*96.7 ± 0.7*	
Glycine	Whole	1.4	1.5	100.0 ± 2.7	101.8 ± 2.2
	Dehulled	1.8	1.7	104.7 ± 1.7	107.1 ± 0.7
	Concentrate	*2.6*		*94.6 ± 1.4*	
Histidine	Whole	0.9	0.9	100.6 ± 2.5	98.3 ± 2.1
	Dehulled	1.2	1.0	101.6 ± 1.6	101.1 ± 0.3
	Concentrate	*1.5*		*94.2 ± 1.6*	
Arginine	Whole	4.0	4.1	102.9 ± 1.3	102.6 ± 0.7
	Dehulled	5.2	4.5	106.3 ± 0.7	106.8 ± 0.3
	Concentrate	*7.4*		*101.5 ± 1.0*	
Threonine	Whole	1.3	1.5	95.8 ± 1.9	97.3 ± 0.5
	Dehulled	1.6	1.7	101.3 ± 1.2	101.8 ± 0.1
	Concentrate	*2.4*		*96.5 ± 1.2*	
Alanine	Whole	1.2	1.3	96.4 ± 1.7	97.1 ± 1.2
	Dehulled	1.5	1.4	98.3 ± 1.1	101.6 ± 0.4
	Concentrate	*2.1*		*92.0 ± 1.5*	
Proline	Whole	1.6	1.7	94.2 ± 3.5	96.4 ± 1.2
	Dehulled	2.0	2.0	99.5 ± 0.8	102.4 ± 0.4
	Concentrate	*2.7*		*96.2 ± 2.0*	
Tyrosine	Whole	1.4	1.8	95.4 ± 2.6	95.6 ± 0.5
	Dehulled	1.7	2.1	98.2 ± 1.1	101.6 ± 0.7
	Concentrate	*2.5*		*97.3 ± 2.3*	
Valine	Whole	1.3	1.6	94.6 ± 1.9	91.2 ± 1.5
	Dehulled	1.6	1.8	97.2 ±0.9	100.4 ± 0.7
	Concentrate	*2.6*		*97.3 ± 1.2*	
Isoleucine	Whole	1.4	1.7	95.4 ± 2.1	91.8 ± 1.2
	Dehulled	1.8	1.9	97.5 ± 0.9	100.8 ± 0.7
	Concentrate	3.0		97.3 ± 1.2	
Leucine	Whole	2.4	2.8	94.9 ± 2.0	94.4 ± 0.9
	Dehulled	2.9	3.2	96.8 ± 0.9	99.5 ± 0.6
	Concentrate	*4.5*		*95.8 ± 0.8*	
Phenylalanine	Whole	1.3	1.4	96.0 ± 2.2	94.8 ± 0.9
	Dehulled	1.6	1.6	98.0 ± 1.2	100.0 ± 0.6
	Concentrate	*2.6*		*95.6 ± 0.8*	
Lysine	Whole	1.4	1.5	98.1 ± 1.5	96.6 ± 0.4
	Dehulled	1.7	1.7	99.5 ± 0.7	102.5 ± 0.6
	Concentrate	*3.1*		*95.5 ± 1.0*	
Cystine	Whole	0.6	0.9	89.6 ± 2.5	84.4 ± 5.8
	Dehulled	0.8	0.9	79.9 ± 14.0	105.6 ± 7.3
	Concentrate	*0.9*		*82.8 ± 4.5*	
Methionine	Whole	0.2	0.3	83.9 ± 6.8	92.2 ± 1.6
	Dehulled	0.2	0.3	91.7 ± 2.9	97.3 ± 0.9
	Concentrate	*0.3*		*91.0 ± 0.4*	

Values for apparent digestibility coefficients are mean ± sem for 3 replicates.
Table adapted from Allan et al., 1997; unpublished data , see Section section 6.3 of this report.

6.8 Replacement of fish meal in diets for juvenile silver perch: VII. effects of cooking on digestibility of a practical diet containing different starch products

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Abstract

The interactive effects of cooking and starch type on the dry matter, gross energy and protein digestibility of diets fed to fingerling silver perch were examined. Nine experimental diets were made: a reference diet either uncooked or cooked, and six other diets comprising uncooked reference diet supplemented with 30% uncooked or cooked wheat (WS), maize (MS) or potato starch (PS) and one diet comprising uncooked reference diet supplemented with 15% pregelatinised maize starch (PGMS). Cooking involved autoclaving at 121°C for 15 minutes. The indirect method was used to calculate apparent digestibility coefficients, and chromic oxide (1%) was used as the inert indicator. Both ingredient and cooking influenced dry matter and gross energy digestibility of diets. All diets had significantly different digestibility coefficients in the following order (from most to least digestible): Reference > wheat > maize > potato, for both dry matter, and gross energy. Cooking significantly increased the apparent digestibility of diets, for both dry matter and gross energy. There was no significant effect of ingredient or cooking, or their interaction on protein digestibility. Amino acid digestibility of the diets for serine, arginine, threonine, proline, isoleucine and cystine were affected by ingredient type, however, the differences were minor and may not have been biologically significant. The apparent digestibility for diets, of all other analysed amino acids were unaffected by ingredient. With the exception of cystine, there was no significant effect of cooking on the apparent digestibility of amino acid for the diets, and there were no significant interactions. Dry matter and energy digestibility of the diet containing pregelatinised maize starch were significantly higher than for diets containing cooked or uncooked starch products. Ingredient digestibility followed the same trend as diet digestibility. The results from this study indicate that the successful use of starch in practical diets for silver perch is dependent on the origin and processing of the starch. The significant benefits of cooking on SP35 suggest pelleted diets should be cooked (eg steam conditioned or extruded).

Keywords: *Bidyanus bidyanus*; Digestibility; Processing; Nutrient availability; Carbohydrate; Starch; Foodstuffs

1. Introduction

The use of plant protein ingredients as alternative protein sources to replace fish meal in aquaculture diets is becoming more common (Tacon, 1994; Wilson, 1994). This substitution produces an increase in carbohydrates, particularly starch, and enhances the binding characteristics of pellets (Shimeno, 1991). Fish do not require dietary carbohydrate, although

carbohydrate may provide a cheap energy source and, therefore, a protein and lipid sparing effect (Hidalgo et al., 1993; Wilson, 1994; Catacutan and Coloso, 1997).

The ability to digest and utilise dietary carbohydrates varies among fish species (Wilson, 1994), where carnivorous species such as yellowtail (Shimeno, 1991) and Atlantic salmon (Anderson and Lipovsek, unpublished data) lack the required digestive enzymes to efficiently digest or metabolise high levels of carbohydrates. When digestible dietary carbohydrate exceeds 20% carnivorous fish exhibit symptoms similar to diabetic animals, in that they can not regulate blood glucose (Shimeno, 1991). In contrast, omnivorous and herbivorous species have higher activities of digestive and metabolic enzymes and are better able to digest and metabolise dietary carbohydrates (Wilson, 1994). As a consequence, researchers have recommended that dietary carbohydrate contents should not exceed 20% in diet formulations for many carnivorous species (Wilson, 1994; Shimeno, 1991; Catacutan and Coloso, 1997), while higher inclusion levels of carbohydrates (25-56%) have been recommended in diets for omnivorous species (Ding, 1991; Lim, 1991; Satoh; 1991; Wilson, 1994).

Starch is the most commonly used carbohydrate in practical and experimental diet formulations. Starch digestibility in fish may be variable, ranging from 25 to 95% (Wilson, 1994). Digestibility of starch may depend upon the origin and complexity of the starch, inclusion level, feeding strategy, processing methods such as cooking or extrusion, and species (Cowey and Walton, 1989; Wilson, 1994).

Cooking of diets containing starch increases the gelatinisation of dietary starch (Rooney and Pflugfelder, 1986; Thomas et al., 1997). This has been reported to enhance dry matter and energy digestibility for rainbow trout (Bergot and Breque, 1983; Kaushik et al., 1989) and common carp (Hernandez et al., 1994), but to reduce digestibility for Atlantic salmon (Arnesen and Krogdahl, 1993).

A program is currently in progress to replace fish meal and develop least-cost diets for the omnivorous species, silver perch, *Bidyanus bidyanus*. A major component of this program is to compile a digestibility data base of practical feed ingredients for inclusion into least-cost diet formulations. The aim of the present study was to investigate the effect of cooking on the dry matter, gross energy and nitrogen digestibility of practical diets containing wheat starch, maize starch or potato starch, and on the starch-rich reference diet (SP35) of Allan and Rowland (1992) fed to juvenile silver perch.

2. Materials and Methods

2.1. Experimental diets and preparation

Four plant starches (analysed compositions Table 1), and the reference diet (SP35), were assessed during this study. All starch ingredients were either uncooked or cooked (autoclaved for 15 minutes at 121°C) and test diets contained 30% of one of the ingredients plus 70% SP35. An additional diet contained 15% pregelatinised maize starch and 85% SP35. (Diets with higher contents of the pregelatinised starch could not be extruded through the meat mincer because of the agglutinating properties of the starch). Diet formulations (n=9 diets)

and analysed compositions are presented in Tables 2, 3 and 4. All ingredients were ground or sieved to ensure all particles passed through a 710 μm screen. Dry ingredients were thoroughly mixed in a Hobart mixer (Troy Pty. Ltd, Ohio, USA) then combined with approximately 400 ml distilled water kg^{-1} dry mix before being pelleted through a meat mincer (Barnco Australia Pty. Ltd., Leichhardt, NSW, Australia) with a 3 mm die. Pellets were dried at < 35°C in a convection drier for approximately 6 h until the moisture content was between 10 - 15%, to produce a dry, sinking pellet.

2.2. Experimental Fish

Silver perch (mean weight ± sem, 12.8 ± 0.1 g) were bred at the Grafton Research Centre and raised in earthen ponds using similar techniques to those described by Thurston and Rowland (1994). Before the experiment, fish were fed SP35 and were treated with 5 g l^{-1} NaCl to ensure they were free of ectoparasites and to prevent fungal infection (Rowland and Ingram, 1991). Prior to stocking, fish were anaesthetised using a bath of ethyl p-aminobenzoate (50 mg l^{-1} for 3 min) then caught at random, individually weighed, and distributed among 27 tanks by systematic interspersion. Eight fish were stocked into each tank, in triplicate, for each treatment. Fish were also weighed at harvest.

2.3. Experimental Facilities

Digestibility tanks were 170 l cylindro-conical tanks (conical base sloped at 35°) fitted with a 65 mm diameter, 250 mm settlement chamber which tapered into a 12 mm diameter, 150 mm length of silicone tubing. The inside of each tank was black. Continuously-flowing, preheated water was filtered through a sand filter and a diatomaceous earth filter, then passed through a 2 m^3 biological filter then a UV steriliser before being supplied to experimental tanks at a flow-rate of 600 ml min^{-1}. Effluent water from each tank flowed out the side of the cylindro-conical tanks into a 25 mm diameter pipe. 20-25% of this flowed to waste and the rest was collected and recirculated. Each tank was aerated using two air-stone diffusers.

Fish were stocked ten days prior to the start of the faecal collection period to allow for acclimatisation to experimental conditions. During this period, fish were fed the reference diet, SP35, for 3 days and then the experimental diets for 7 days prior to faecal collection. Fish were fed to excess (8% body weight day^{-1}) using automatic conveyor belt-type feeders for three hours each day from 0830-1130. Within one hour after feeding ceased, all uneaten food was removed, and the walls of the tank and the settlement chamber were thoroughly cleaned to remove any faeces, uneaten food or bacterial slime. The silicone tubing into which the faeces settled was packed in ice and kept at ≤4°C prior to collection of faeces to reduce bacterial proliferation, which can affect the composition of faeces (Spyridakis et al., 1989).

Faecal samples were collected by settlement each morning and dried using silica gel desiccant under vacuum. Daily faecal samples were collected for 14 days from each tank and then pooled. Each sample was freeze dried and ground using a water cooled 1KA total recovery grinder prior to analyses. These methods have been shown to be valid for calculating digestibility coefficients for a range of ingredients fed to silver perch (Allan et al., unpublished data, see Section 6.1 of this report).

During this experiment, water temperature ranged from 25.9-26 °C and dissolved oxygen (always above 5.1 mg l^{-1}), pH (between 7.9 and 8.0), and nitrite and ammonia (< 0.02 mg NO_2-N l^{-1} and 0.3 mg total ammonia - N l^{-1} respectively) were measured weekly using methods described in Allan et al. (1990).

2.4. Digestibility Determinations

The indirect method of Cho and Kaushik (1990) was used to calculate apparent digestibility coefficients (ADC's), with chromic oxide (1% dry basis) as the inert indicator. The ADC;s for gross energy, crude protein (N x 6.25) and essential amino acids in experimental diets were calculated as described by Cho and Kaushik (1990);
$ADC = [1-(F/D \times DC_r/FC_r)] \times 100$ where:
F = % nutrient or energy in faeces,
D = % nutrient or energy in diet,
DC_r = % chromic oxide in diet and
FC_r = % chromic oxide in faeces.

The ADC of each test ingredient was calculated as follows:
ADC of test ingredient = ADC of test diet - (ADC of reference diet SP35 x proportion of reference diet SP35 in test diet)/proportion of test ingredient in test diet (Cho and Kaushik, 1990).

2.5. Biochemical Analyses

All chemical analyses were done in duplicate. Feed, ingredient and faecal samples were analysed for dry matter, ash, crude fat, ADF and energy (bomb calorimetry) by the AOAC (1975) procedures. Nitrogen was determined by the method described by Havilah et al. (1977) (crude protein = N x 6.25). Amino acids were determined by the method described by Cohen et al. (1989) and analysed following acid hydrolysis using high pressure liquid chromatography and Waters Pico-Tag (Waters Pty Ltd, Lane Cove, NSW, Australia). Sulphur amino acids were determined separately following performic acid digestion. Tryptophan, which is lost during acid hydrolysis, was not analysed (Cohen et al., 1989). Chromic oxide was determined by the method described in Scott (1978).

2.6. Statistical analysis

Data for digestibility coefficients for dry matter, energy and nitrogen for diets (n=9) and ingredients (n=7) were analysed separately using single-factor ANOVA. To examine interactive effects of ingredient type and cooking on digestibility, data for diets (excluding the pregelatinised maize starch diet; n=8) or ingredients (excluding SP35 and pregelatinised maize starch; n=6) were analysed using two-factor ANOVA with ingredient and process (cooked or uncooked) as the two fixed factors. Homogeneity of variance was assessed using Cochrans Test (Winer, 1971), and where significant, comparison between means were made using Student Newman-Keuls multiple range test. Means were considered significant at $P<0.05$. Unless otherwise stated, all results appear as mean ± standard error of the mean (n=3).

3. Results

Diet digestibility

Silver perch readily accepted the diets containing 30% starch in this study.

Single factor ANOVA indicated dry matter and energy digestibility of the cooked SP35 was significantly higher than either the uncooked SP35 or diets containing cooked or uncooked starch products ($P<0.001$; SNK) (Table 5). Protein digestibility was not affected by diet ($P>0.05$).

The results of the two-factor ANOVA indicated that dry matter and gross energy digestibility of the diets were affected by both ingredient ($P<0.001$) and process ($P<0.01$), and there was no significant interaction between the two ($P>0.05$). All diets had significantly different digestibility coefficients for dry matter and gross energy ($P<0.05$; SNK) in the following order (from most to least digestible): SP35 > wheat > maize > potato. Cooking significantly increased the apparent dry matter and energy digestibility of diets. There was no significant affect of ingredient or process, and no interaction on protein digestibility coefficients ($P>0.05$).

Results of the two-factor ANOVA of dietary digestibility coefficients indicated that digestibility of serine, arginine, threonine, proline, isoleucine and cystine were affected by ingredient ($P<0.05$). The apparent digestibility of all other analysed amino acids were unaffected by diet ($P>0.05$). Except for cystine, cooking did not affect amino acid digestibility ($P>0.05$), and there were no significant interactions between diet and cooking ($P>0.05$).

The digestibility of cystine was significantly affected by ingredient ($P<0.001$) and process ($P<0.01$), and there was a significant interaction ($P<0.001$). It should be noted however, that cystine analysis has been variable and somewhat unreliable.

Ingredient digestibility

Ingredient digestibility followed similar trends to diet digestibility in this study (Table 6), but as the reference diet already contained ~25% starch, it is not possible to differentiate between test ingredient (30% starch) and the starch component of the SP35.

The dry matter and gross energy digestibility coefficients for all ingredients including pregelatinised maize starch, were analysed using single-factor ANOVA. Dry matter and energy digestibility of the pregelatinised maize starch were significantly higher than both cooked and uncooked starch products ($P<0.001$; SNK) (Table 6).

The results of the two-factor ANOVA indicated that dry matter and energy digestibility for starch ingredients were affected by both ingredient ($P<0.001$) and process ($P< 0.05$), and there were no significant interactions ($P>0.05$). All starch ingredients had significantly different dry matter and energy digestibility coefficients ($P<0.05$, SNK) in the following order (from

most to least digestible): wheat > maize > potato. Cooking significantly increased the digestibility of both dry matter and gross energy. There was no significant affect of ingredient or process, and there was no interaction (P>0.05), on protein digestibility.

4. Discussion

Dry matter and energy digestibility of diets for silver perch were influenced by both the origin of the starch (with SP35 > wheat > maize > potato), and cooking (cooked > uncooked). Silver perch digested dry matter and energy more efficiently from diets which were cooked. Ingredient digestibility followed the same trends. The reference diet, SP35 is starch rich, (~25% starch), which originates mainly from wheat and sorghum (Booth et al., unpublished data, see Section 6.10 of this report). Thus, the total starch content of the test diets in this study were ~ 47.5% for diets containing 30% starch inclusion levels. Starch inclusion level has been reported to be inversely related to digestibility and has been shown to have a negative affect on both dry matter and energy digestibility in a number of fish species (Table 7). This may account for the differences in dry matter and energy digestibility for silver perch fed SP35 or the diets substituted with 30% pure starch.

Dry matter and energy digestibility results suggest silver perch were better able to digest diets containing 30% wheat or maize starch than diets containing 30% potato starch. In general, cereal starches have been reported to be more digestible than tuberous starches (Shetty et al., 1974; Banks and Greenwood, 1975; Moran, 1982; Rooney and Pflugfelder, 1986). Banks and Greenwood (1975) reported *in vitro* digestibility (the relative susceptibility to bacterial α-amylase attack) of isolated native maize, wheat and potato starches to be 100, 98 and 37% respectively. Moran (1982) confirmed these findings and showed *in vitro* digestibility (the relative susceptibility to pancreatic α-amylase attack of maize starch) of isolated native maize, wheat and potato starches to be 100, 100 and 7% respectively.

Rooney and Pflugfelder (1986) concluded that the differences in digestibility between native starches may be attributed to the physical structure and amylose content of the native starch granules. As all starches used in this study have been reported to have similar amylose contents (24-26%) (Manners, 1974) (Table 1), there is the suggestion that physical characteristics may be the limiting factor for digestibility. Starch degrading enzymes act on the surface area of the granule (Manners, 1974; Shetty et al, 1974), and potato starch granules are relatively large (20- 100μm) compared to maize starch (10-20μm) (French, 1973), or wheat starch (<20μm) (Manners, 1974). The smaller surface area to volume ratio of potato starch granules may have directly influenced their susceptibility to enzymatic attack and resulted in lower digestibilities for silver perch. Another possible contributing factor to the inefficient digestibility of diets containing potato starch may have been adsorption of the digestive enzyme, α-amylase by raw potato starch. This phenomenon has been demonstrated in rainbow trout fed raw potato starch (Spannhof and Plantikow, 1983).

Cooking significantly improved the dry matter and energy digestibility of all diets for silver perch, when compared to the uncooked diets. The improvements in dry matter and energy digestibility for silver perch may be attributed to the increased gelatinisation of starch during cooking. Gelatinisation (swelling and melting of the starch granule) effectively breaks down

the starch granule exposing the bound amylose fraction, and increases the surface area, which renders the starch more susceptible to enzymatic attack (French, 1973; Rooney and Pflugfelder, 1986). This has been demonstrated using *in vitro* starch degradability tests (using amyloglucosidase), where the degradability of raw wheat starch and maize starch was increased by gelatinisation from 25-82% and 29-79% respectively (Pfeffer et al., 1991; Pfeffer, 1995).

Dry matter and energy digestibility of SP35 by silver perch in this study were consistent with previous work where dry matter and energy digestibilities were progressively increased when SP35 was unprocessed, steam conditioned or extruded (Booth et al., unpublished data, see Section 6.10 of this report) (Table 7). These findings are also consistent with dry matter and energy digestibility reported for common carp and rainbow trout (Table 7). However, they conflict with the response of the carnivorous Atlantic salmon. Arnesen and Krogdahl (1993) reported that dry mater and energy digestibility actually decreased with pre-extrusion (Table 7). A possible explanation for the difference observed for dry matter and energy digestibility may be the higher levels of intestinal amylase activity in silver perch compared with Atlantic salmon. Intestinal amylase activity is probably the key factor in the digestibility coefficients of diets containing starch fed to fish (Shimeno, 1991). The omnivorous silver perch has been reported to have moderate levels of intestinal amylase activity which is half those of the herbivorous species such as the tilapia, *Oreochromis mossambicus*, but 7 and 16 times higher than those of the carnivorous barramundi, *Lates calcarifer,* and Atlantic salmon (Anderson and Lipovsek, unpublished data, Section 6.4 of Final Report to FRDC, Fish Meal Replacement in Aquaculture Feeds: *In Vitro* Studies on Feed Ingredients for Aquaculture Species).

An interesting aspect of this study was the increased dry matter and energy digestibility of the diet containing 15% pre-gelatinisation maize starch compared with diets containing 30% cooked or raw maize starch. This may have been due to a lower inclusion level, or a higher degree of gelatinisation as a result of a more efficient processing method. The diet containing 15% of pregelatinised maize starch would have had a total starch content of ~36%. Several researchers have demonstrated that starch digestibility is negatively correlated with inclusion level (Singh and Nose, 1967; Pfeffer, 1995). Research currently being carried out on wheat starch digestibility for silver perch indicates that the dry matter digestibility for raw wheat starch is ~62% when included at 30%, and 31% when included at 60% of the diet (Stone et al., unpublished data). The dry matter digestibility coefficients from this study correlate well with the unpublished findings, and indicate that dry matter digestibility of starch is negatively correlated to starch inclusion level in diets fed to silver perch. However, the increased digestibility of maize starch by silver perch is consistent with the findings of Pfeffer (1995) who reported an increase in starch digestibility in rainbow trout when fed diets containing maize starch with increasing degrees of gelatinisation (Table 7). Similarly, extrusion improved dry matter and energy digestibility of raw maize starch compared to extruded maize starch when included at 38% in diets for rainbow trout (Kaushik et al., 1989) (Table 7).

Gelatinisation of starch is dependent on the combination of free water and heat (French, 1973; Rooney and Pflugfelder, 1986). The pregelatinised maize starch is prepared by cooking starch in a slurry of water at a temperature of >80°C until it forms a paste, where it approaches 100%

gelatinisation (Rooney and Pflugfelder, 1986). In the present study, the cooked maize starch was exposed to steam and heat during the cooking (autoclave) process, which may have resulted in a lower degree of gelatinisation (Rooney and Pflugfelder, 1986) and possibly increased levels of indigestible retrograded starch (French, 1973) compared to the pregelatinised starch.

Digestibility of dietary protein in test diets fed to silver perch ranged from 88-90 %, with no significant effect of starch type, processing method or interaction between the two. In general the digestibility of most individual amino acids were also unaffected by starch inclusion in the reference diet. However, digestibility of serine, arginine, threonine, proline, isoleucine and cystine was significantly affected by starch type, but the differences were small and may not be biologically significant. These findings are in agreement with digestibility studies for a range of species (Table 7). The fact that there was no change in protein digestibility for diets containing high levels of starch, which were either uncooked or cooked, suggest that there was no evidence of an interaction between starch and lysine (Maillard reaction) during processing (Carpenter and Booth, 1973).

Silver perch are more efficient at digesting wheat starch than either maize or potato starch. Given that wheat is one of Australia's major export commodities (AUD$ 4.3 billion, 1996/97; Anon, 1997), this product appears to be the logical choice for further investigation for incorporation into aquaculture diets for silver perch. Cooking (autoclaving at 121°C for 15 min) increased dry matter and energy digestibility of the reference diet and all starch ingredients tested in this study, while protein digestibility was not affected. Consequently, aquaculture diets for silver perch should be cooked, through steam pelleting or extrusion, (rather than be pelleted without cooking), to enhance the digestibility of diets containing high levels of native plant starches. The reduction of feed waste due to increased dry matter digestibility may also contribute to better pond water quality, an area of aquaculture that is coming under closer scrutiny.

Acknowledgments

We wish to thank Rebecca Warner-Smith, Mark Booth and Helena Heasman at Port Stephens Research Centre, and Ken O'Brien and Wendy Peasley at Wollongbar Agricultural Institute for their excellent technical and clerical help. The research was part of the Australian Fisheries Research and Development Corporation's Fish Meal Replacement Sub-Program (93/120). Additional support was also provided by the Australian Centre for International Agricultural Research, the Australian Grains Research and Development Corporation and the Australian Meat Research Corporation.

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Table 1
Analysed composition of starch test ingredients (dry basis)[1,2].

Nutrient	Ingredient			
	Wheat starch (WS)	Maize starch (MS)	Pregelatinised Maize starch (PGMS)	Potato starch (PS)
Dry matter (%)	93.0	91.2	93.7	86.7
Gross energy (MJ/kg)	17.5	17.1	16.6	16.9
Protein (%)	0.9	0.9	0.4	0.4
ADF (%)	0.0	0.0	0.0	0.0
Ash (%)	0.1	0.1	0.5	0.3
Amylose content (%)	25	26	26	24

[1] Wheat starch (Wheaten cornflour), maize starch (3401C) and pregelatinised maize starch (Instant FTD 176) were produced by Goodman Fielder Mills, Australia, while the potato starch was produced by Avebe Pty. Ltd. Holland.

[2] Amylose content of starch products were not analysed in this study. Data supplied by Goodman Fielder Australia.

Table 2
Formulation of diets used in digestibility experiment (% dry basis)[1].

Ingredient	Diet								
	1 SP35	2 CSP35	3 MS	4 CMS	5 WS	6 CWS	7 PGMS	8 PS	9 CPS
Reference diet (SP35)[2]	99.0	-	69.3	69.3	69.3	69.3	84.2	69.3	69.3
Cooked reference diet[2]	-	99.0	-	-	-	-	-	-	-
Maize starch	-	-	29.7	-	-	-	-	-	-
Cooked maize starch	-	-	-	29.7	-	-	-	-	-
Wheat starch	-	-	-	-	29.7	-	-	-	-
Cooked wheat starch	-	-	-	-	-	29.7	-	-	-
Pregelatinised maize starch	-	-	-	-	-	-	14.8	-	-
Potato starch	-	-	-	-	-	-	-	29.7	-
Cooked potato starch	-	-	-	-	-	-	-	-	29.7
Chromic oxide	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0

[1] For diets 3-6 and 8-9 the ingredient inclusion level was 30%; diets 1 and 2 were 100% of test ingredient and an ingredient inclusion level of 15% was used in diet 7.

[2] The formulation and composition of SP35 is presented in Table 3.

Table 3
Formulation of SP35 (As is basis).

Ingredient	Diet (g/100g)
Fish meal (Danish)	26.62
Soybean meal	20.19
Blood meal	2.04
Wheat	27.47
Sorghum	11.21
Corn gluten meal	3.87
Millrun	2.01
Cod liver oil	0.90
DL-methionine	0.13
Di-calcium phosphate	1.79
Vitamin premix[1]	0.97
Mineral premix[2]	2.81

[1] (IU/kg diet): retinol (A), 8000; cholecalciferol (D3), 1000; α-tocopherol acetate (E), 125; (mg/kg diet): ascorbic acid (C), 1000; biotin (2%), 1; calcium panothenate, 55; calcium propionate, 250; choline chloride, 1500; cyanocobalamin (B12), 0.02; ethoxyquin, 150; folic acid, 4; menadione sodium bisulphite (K3), 16.5; myo-inositol, 600; nicotinamide, 200; pyridoxine (B6), 15; riboflavin (B2), 25.2; thiamine HCl (B6), 10.

[2] (mg/kg diet): calcium carbonate, 7500; manganese sulphate, 300; zink sulphate, 700; copper sulphate, 60; ferrous sulphate, 500; sodium chloride, 7500; potassium iodate, 2.

Table 4. Analysed composition of experimental diets for silver perch (dry basis).

Nutrient	Diet 1 (REF)	Diet 2 (CREF)	Diet 3 (MS)	Diet 4 (CMS)	Diet 5 (WS)	Diet 6 (CWS)	Diet 7 (PGMS)	Diet 8 (PS)	Diet 9 (CPS)
Dry matter (%)	95.90	95.63	95.19	96.05	95.65	96.09	92.13	95.47	95.08
Energy MJ/kg	18.87	19.06	18.30	18.22	18.16	18.34	18.64	18.27	18.32
Protein (%)	38.94	38.19	28.56	28.69	27.50	27.56	32.81	27.44	27.50
Fat (%)	4.44	4.60	2.96	2.94	3.02	3.00	2.26	3.28	2.97
Ash (%)	11.35	11.43	8.16	8.08	8.05	8.01	10.30	8.11	8.29
ADF (%)	4.65	4.52	na	na	na	na	na	na	na
Starch (%)[1]	25.0	25.0	47.5	47.5	47.5	47.5	36.3	47.5	47.5
Amino acids (%)									
Alanine	2.29	2.37	1.62	1.61	1.61	1.63	1.91	1.57	1.62
Arginine	2.26	2.27	1.58	1.57	1.58	1.62	1.86	1.50	1.56
Aspartic acid	3.45	2.87	2.43	2.40	2.44	2.46	2.89	2.41	2.44
Cystine	0.63	0.52	0.48	0.44	0.44	0.47	0.48	0.48	0.44
Glutamic acid	6.27	6.24	4.37	4.37	4.41	4.43	5.23	4.32	4.38
Glycine	1.95	1.97	1.33	1.33	1.33	1.38	1.61	1.35	1.35
Histidine	0.77	0.79	0.51	0.50	0.51	0.60	0.63	0.52	0.53
Isoleucine	1.60	1.63	1.10	1.07	1.06	1.03	1.31	1.13	1.16
Leucine	3.11	3.16	2.16	2.13	2.14	2.19	2.56	2.14	2.16
Lysine	2.53	2.49	1.69	1.63	1.63	1.77	2.04	1.71	1.70
Methionine	1.14	1.06	0.84	0.77	0.76	0.89	0.93	0.87	0.81
Phenylalanine	1.69	1.70	1.15	1.12	1.13	1.17	1.39	1.15	1.18
Proline	1.93	1.97	1.36	1.35	1.36	1.40	1.60	1.32	1.35
Serine	1.87	1.81	1.28	1.29	1.32	1.39	1.55	1.25	1.24
Threonine	1.45	1.33	0.96	0.93	0.99	1.07	1.19	0.90	0.85
Tyrosine	1.16	1.17	0.81	0.79	0.81	0.82	0.99	0.82	0.81
Valine	1.88	1.92	1.31	1.28	1.25	1.22	1.57	1.38	1.38

[1] Starch composition for SP35 measured in previous experiments, while the starch composition of diets 3-9 are based on the assumption that 70% of Diet 1 contains 17.5% starch or 85% of Diet 1 contains 21.25% starch + the inclusion of either 30 or 15% starch in test diets.
na = not analysed

Table 5. Apparent dry matter, gross energy and amino acid digestibility coefficients (%) of diets for silver perch (dry basis)[1].

Nutrient	Reference diet		Maize starch			Wheat starch		Potato starch	
	Cooked	Uncooked	Cooked	Uncooked	Pregelatinised	Cooked	Uncooked	Cooked	Uncooked
Dry matter	72.2±2.2^{e}	64.8±1.1^{d}	56.4±1.13^{bc}	53.5±1.8^{ab}	65.2±0.6^{d}	60.2±0.8^{c}	57.7±0.9^{bc}	51.5±0.5^{a}	50.0±1.4^{a}
Gross energy	81.8±1.8^{e}	74.4±0.8^{d}	64.1±1.2^{bc}	61.5±1.7^{ab}	73.3±0.5^{d}	67.7±0.6^{c}	65.7±0.7^{c}	61.2±0.5^{ab}	58.8±1.0^{a}
Protein	88.8±0.9^{a}	89.0±0.7^{a}	88.7±0.5^{a}	88.9±0.3^{a}	90.0±0.6^{a}	89.0±0.4^{a}	89.3±0.4^{a}	88.6±0.4^{a}	89.7±0.3^{a}
Amino acids									
Alanine	90.9±1.0^{a}	90.9±0.4^{a}	90.5±0.3^{a}	90.2±0.2^{a}	91.1±0.7^{a}	90.5±0.5^{a}	90.8±0.4^{a}	89.8±0.2^{a}	90.1±0.2^{a}
Arginine	93.9±0.6^{a}	92.7±0.5^{a}	92.4±0.2^{a}	92.4±0.2^{a}	92.8±0.6^{a}	92.4±0.4^{a}	92.7±0.3^{a}	91.9±0.2^{a}	92.4±0.3^{a}
Aspartic acid	87.9±1.2^{a}	90.2±0.5^{a}	89.3±0.5^{a}	89.4±0.7^{a}	90.1±1.1^{a}	89.0±0.9^{a}	89.4±0.6^{a}	87.5±0.5^{a}	87.6±0.6^{a}
Cystine	88.9±0.4^{e}	87.1±0.8^{cde}	84.1±0.1^{b}	86.0±0.4^{bcd}	87.9±1.1^{de}	84.2±0.1^{b}	84.9±0.9^{bc}	81.1±0.6^{a}	84.8±0.4^{bc}
Glutamic acid	94.2±0.6^{a}	93.8±0.2^{a}	93.7±0.3^{a}	93.4±0.1^{a}	93.8±0.8^{a}	93.6±0.5^{a}	93.7±0.3^{a}	93.0±0.3^{a}	93.1±0.4^{a}
Glycine	89.8±1.0^{a}	89.4±0.3	88.6±0.5^{a}	88.2±0.5^{a}	89.3±0.8^{a}	88.5±1.0^{a}	89.3±0.4^{a}	87.7±0.2^{a}	88.3±0.4^{a}
Histidine	89.2±1.2^{a}	90.7±0.5^{a}	90.8±0.3^{a}	90.0±0.7^{a}	90.6±0.7^{a}	92.3±0.6^{a}	91.0±0.4^{a}	90.9±0.2^{a}	90.5±0.6^{a}
Isoleucine	92.4±0.8^{b}	90.1±0.6^{ab}	89.5±0.4^{a}	89.5±0.5^{a}	90.5±0.9^{ab}	89.3±0.6^{a}	90.3±0.4^{ab}	90.4±0.2^{ab}	90.9±0.3^{ab}
Leucine	91.5±0.9^{a}	91.8±0.4^{a}	91.6±0.3^{a}	91.4±0.3^{a}	91.9±0.8^{a}	92.0±0.4^{a}	91.9±0.4^{a}	91.6±0.2^{a}	91.7±0.4^{a}
Lysine	92.8±0.8^{a}	93.1±0.5^{a}	92.6±0.5^{a}	93.1±0.3^{a}	93.9±0.6^{a}	93.7±0.5^{a}	93.5±0.2^{a}	93.4±0.2^{a}	94.0±0.3^{a}
Methionine	94.3±0.3^{a}	93.6±0.5^{a}	93.5±0.2^{a}	93.9±0.4^{a}	94.3±0.8^{a}	93.3±0.6^{a}	93.9±0.6^{a}	92.7±0.3^{a}	93.7±0.4^{a}
Phenylalanine	91.0±1.0^{a}	91.0±0.5^{a}	90.8±0.4^{a}	90.6±0.4^{a}	91.2±0.8^{a}	91.2±0.5^{a}	91.1±0.4^{a}	91.0±0.2^{a}	91.3±0.4^{a}
Proline	92.4±0.7^{a}	91.9±0.4^{a}	91.8±0.3^{a}	91.3±0.4^{a}	91.5±0.8^{a}	91.7±0.8^{a}	91.9±0.3^{a}	90.6±0.2^{a}	90.4±0.4^{a}
Serine	90.2±0.9^{a}	90.3±0.5^{a}	90.7±0.3^{a}	90.0±0.3^{a}	90.4±0.7^{a}	91.0±0.6^{a}	90.3±0.3^{a}	88.5±0.1^{a}	88.4±0.7^{a}
Threonine	88.8±1.2^{a}	89.4±0.5^{a}	88.3±0.3^{a}	88.2±0.7^{a}	89.8±0.8^{a}	90.1±0.5^{a}	89.4±0.6^{a}	86.8±0.2^{a}	88.3±0.5^{a}
Tyrosine	92.4±0.7^{a}	91.1±0.5^{a}	90.6±0.3^{a}	90.7±0.4^{a}	91.5±0.8^{a}	91.5±0.8^{a}	91.6±0.3^{a}	91.2±0.1^{a}	91.7±0.6^{a}
Valine	89.9±1.2^{a}	89.9±0.5^{a}	89.1±0.5^{a}	89.2±0.5^{a}	90.1±0.9^{a}	88.8±0.7^{a}	89.8±0.3^{a}	90.0±0.3^{a}	90.6±0.3^{a}

[1] Values are means ± SEM (n=3 pooled replicate tanks). Means in the same row with the same superscript are not significantly different (One factor ANOVA, $P>0.05$; SNK).

Table 6
Apparent digestibility coefficients of starch ingredients for silver perch (dry basis)[1,2].

Nutrient	Maize starch			Wheat starch		Potato starch	
	Cooked	Uncooked	Pregelatinised	Cooked	Uncooked	Cooked	Uncooked
Dry matter	36.7±3.8^{bc}	27.1±6.1^{ab}	67.4±3.4^{d}	49.4±2.5^{c}	41.0±3.1^{bc}	20.3±1.6^{a}	15.5±4.7^{a}
Energy	40.0±4.0^{bc}	31.4±5.6^{ab}	67.4±3.6^{d}	52.2±2.1^{c}	45.6±2.3^{c}	30.3±1.7^{ab}	22.2±3.3^{a}

1 Digestibility coefficients for ingredients were calculated using the equation (digestibility coefficient of experimental diet - digestibility coefficient of SP35 x proportion of SP35 in experimental diet)/proportion of test ingredient in experimental diet

2 Values are means ± SEM (n=3 pooled replicate tanks). Means in the same row with the same superscript are not significantly different (One factor ANOVA, P>0.05; SNK).

Table 7
Comparison of dry matter, protein and energy apparent digestibility coefficients (ADC %) of diets containing starch products for silver perch and other fish species.

Species	Carbohydrate origin	Processing method	Inclusion (%)	ADC(%) DM	Protein	Energy	Author
Silver perch	SP35	raw	100	64.8	89.0	74.4	this study
" "	"	cooked	100	72.2	88.8	81.8	" "
" "	Wheat	raw	30	57.7	89.3	65.7	" "
" "	"	cooked	30	60.2	89.0	67.7	" "
" "	Maize	raw	30	53.5	88.9	61.5	" "
" "	"	cooked	30	56.4	88.7	64.1	" "
" "	"	pregelatinised	15	65.2	90.0	73.3	" "
" "	Potato	raw	30	50.0	89.7	58.8	" "
" "	"	cooked	30	51.5	88.6	61.2	" "
" "	SP35	raw	100	64.8	89.0	76.5	Booth et al., unpublished data
" "	"	steam	100	67.0	90.2	78.0	" "
" "	"	extruded	100	71.4	89.3	83.2	" "
Rainbow trout	Maize starch	raw	30	63	88	-	Bergot and Breque, 1983
" "	Maize starch	pregelatinised	30	79	88	-	" " "
" "	Maize starch	raw	38	55	-	64.0	Kaushik et al., 1989
" "	Maize starch	pregelatinised	38	80	-	86.0	" " "
" "	Potato starch	raw	20	73	90	82	Podiskina et al., 1997
" "	Potato starch	gelatinised (129°C)	20	81	91	89	" " "
Atlantic salmon	Whole wheat	raw	30	73	89	-	Arnesen and Krogdahl, 1993
" "	Whole wheat	pre-extruded	30	55	78	-	" " "
" "	Whole wheat	raw	45	57	86	-	" " "
" "	Whole wheat	pre-extruded	45	49	84	-	" " "
" "	Wheat flour	extruded	9	90	94	-	Hemre et al., 1995
" "	Wheat flour	extruded	31	83	93	-	" " "
Common carp	Maize meal	extruded (28%gel)	40	-	93.3	77.6	Hernandez et al., 1994
" "	Maize meal	extruded (72%gel)	40	-	95.9	84.6	" " "
" "	Maize meal	extruded (100%gel)	40	-	96.7	87.3	" " "
Eel	Maize starch	cooked	25	-	60.2	-	Hidalgo et al., 1993
"	Maize starch	cooked	40	-	64.3	-	" " "
"	Maize starch	pregelatinised	40	-	68.3	56.0	Gallego et al,1994
Sturgeon	Wheat starch	pregelatinised	20	61.9	70.9	73.4	Médale eta l., 1991

6.9 Replacement of fish meal in diets for silver perch: VIII. effects of increasing dehulled lupins *Lupinus angustifolius* (var. Gungarru) on growth and body composition

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Abstract

Dehulled lupins contain about 44% crude protein and as they are well digested by silver perch and have few anti-nutrients, they have considerable potential to at least partially replace fish meal as a protein source. In this study the contribution of dehulled lupins (*Lupinus angustifolius* var. Gungarru) to growth and body composition of juvenile silver perch was measured using the summit/dilution method. A 35% protein, fish meal/ soybean meal based diet was used as the summit diet and then three series of diets were made where the summit was progressively diluted with dehulled lupins, cellulose or diatomaceous earth. Fish were fed on a restricted feeding regime at approximately 90% of satiation, adjusted according to biomass. The difference in growth response and carcass composition for fish fed the dehulled lupin series of diets was compared with that of fish fed the cellulose and diatomaceous earth series of diets.

Eight fish (1.0-2.5 g/fish) were stocked into each 70 l aquarium, four replicate tanks were used for each diet, and the experiment ran for 75 days. Weight gain, protein deposition, protein efficiency ratio and protein retention efficiency all decreased with increasing dehulled lupin content in the diets while FCR also deteriorated. However, compared with the control diet, differences were not significant until diets contained more than 60% dehulled lupins (50% for protein efficiency ratio). Reduced performance of fish fed diets containing more than 60% lupins may have been due to insufficient energy for maximum protein utilisation. The similarity in response to diluting the summit diet with cellulose or diatomaceous earth indicates that fibre (cellulose) acts as a diluent not as an anti-nutrient. The excellent results for diets with contents of 50% or less confirm the potential of dehulled lupins for use in diets of silver perch.

Keywords: Nutrition; *Bidyanus bidyanus*; Growth; Lupins; summit/dilution.

1. Introduction

Development of aquaculture in Australia will be restrained unless suitable ingredients for use in cost-effective diets are found. Very little fish meal is produced in Australia, although fortunately there are alternative sources of agricultural protein including vegetable protein sources (Allan, in press) and rendered animal meals (Williams et al., 1997; Allan et al., unpublished data, Section 6.6 of this report). Many of these have shown potential for use in aquaculture diets including those for silver perch (Williams et al., 1997; Allan et al., unpublished data, Section 6.6 of this report).

Among the vegetable protein sources, pulses have been successfully included in diets for a number of species including rainbow trout, gilthead seabream and carp (Viola et al., 1988; Hughes, 1991; Moyano et al., 1992; Morales et al., 1994; Robaina et al., 1995).

Lupins (*Lupinus* spp) have been the most commonly used pulse and have been successfully used as a complete replacement for soybean meal or full fat soy in diets for rainbow trout and carp (Hughes, 1991; Viola et al., 1988). Digestibility data indicates that protein digestibility is good but dry matter and energy digestibility are inferior to fish meals or animal protein meals (Gomes et al., 1995; Morales et al., 1994). At the same concentration in the diet, dehulled lupins were better than whole lupins for rainbow trout (Hughes, 1991). Earlier research with silver perch has shown that dehulling (*L.angustifolius*) significantly improved energy and dry matter digestibility by more than 17% and this was attributed to the removal of about 12% of the non-starch polysaccharide fraction (Allan et al., unpublished data, see Section 6.3 of this report). Once digestibility information is available, diet formulators need to know how well an ingredient is utilised by the animal being fed and to know the maximum amount of an ingredient that can be included in a diet before growth or other indicators of fish performance or carcass composition decline.

One method of providing this information is the use of a summit/dilution technique first used to measure methionine requirements of poultry (Fisher and Morris, 1969). For this method, a reference or summit diet is chosen and then two other series of diets are made where this summit diet is progressively replaced with either the ingredient under evaluation or an inert filler (such as diatomaceous earth or cellulose). The difference in growth response and carcass composition for fish fed the two series of diets allows a direct measurement of the contribution made by the test ingredient at each inclusion content.

The main aim of this experiment was to measure the contribution made by dehulled lupins (*L. angustifolius* var. Gungarru) to growth and carcass composition of juvenile silver perch. A second aim was to determine if fibre (cellulose) acts as an anti-nutrient or simply as a diluent in diets for silver perch.

2. Materials and Methods

2.1 Experimental diets and feeding strategy

The summit diet used was the reference diet (SP35) of Allan and Rowland (1992; Table 1) which has been used in large-scale grow-out trials with silver perch (Rowland et al., 1995). The other experimental diets were made by replacing part of this diet with dehulled lupins, *Lupinus angustifolis* (var. Gungarru), cellulose or diatomaceous earth (Table 2). All ingredients were ground or sieved to ensure all particles passed through a 710 μm screen. Dry ingredients were thoroughly mixed in a Hobart mixer (Troy Pty. Ltd., Ohio 45374, USA) then combined with approximately 400 ml distilled water kg^{-1} dry mix before being pelleted through a meat mincer (Barnco Australia Pty. Ltd., Leichhardt, 2040, NSW) with a 1.5 mm die. Pellets were dried at <35°C in a convection drier for about 6 h until the moisture content was between 10-15%, to produce a dry, sinking pellet.

The fish were allowed to acclimatise to experimental conditions for 14 days, during which time all fish were fed the summit diet twice daily (40% am, 60% pm), initially at 4% biomass for the first three days, and then to satiation twice daily. Any uneaten feed was siphoned from each aquarium approximately 30 minutes after feeding, dried and weighed.

After 14 days, the amount of diet required to feed fish in each aquarium to satiation was established. After this period, feeding of experimental diets started. A restricted feeding regime was established whereby experimental diets were offered at a rate of 90% satiation. (Again, uneaten feed was collected after feeding had ceased, dried and weighed for adjustment of total feed consumption at the conclusion of the experiment). During the course of the experiment, total biomass in two replicate aquaria for each treatment was determined by anaesthetising and weighing all fish every two weeks. Feed rates were then adjusted on the basis of the new biomass, according to the equation:

Food Intake = $a\text{Weight}^b$ (Jobling, 1988);

where a is a constant. The scaling or weight exponent b relates feeding ration to body weight and is less than 1 so that ration increases allometrically with increasing body weight In the present study, the weight exponent chosen was 0.8, which represents the average weight exponent used in other studies with cultured fish (Paloheimo and Dicki, 1966; Jobling, 1983).

2.2 Experimental fish

Juvenile silver perch were obtained from NSW Fisheries' Grafton Research Centre and held in 10 000 l fibreglass tanks for at least one week, until required. Fish were fed the reference diet of Allan and Rowland (1992) (SP35) *ad libitum* daily.

Prior to the experiment, fish were graded, anaesthetised and individually weighed. Initial fish were selected for whole body proximate composition. Each aquarium was stocked via random interspersion with eight fish between 1.0 and 2.5 g (1.82±0.01; mean ± se; n=576). During the course of the experiment, any mortalities were weighed and then replaced with individually weighed, fin-clipped fish. The experiment was terminated after 75 days. At the conclusion of the trial all fish were individually weighed, and five fish from each tank were randomly selected for whole body proximate composition.

Fin-clipped fish were later excluded from all calculations involving individual weight gain, protein and lipid deposition and protein and lipid retention efficiency. The net weight gain per tank was used for estimating food conversion, protein and energy efficiency ratios. The following performance indices were then calculated:

* Weight gain (g/fish) = final individual weight -initial individual weight.
* Protein deposition (g) = final ind. wt (dry basis) x protein composition final carcass - (initial ind. wt [dry basis] x protein composition initial carcass).
* Lipid deposition (g) = (final ind. wt [dry basis] x lipid composition final carcass) - (initial ind. wt. [dry basis] initial carcass x lipid composition initial carcass).
* Food conversion ratio = dry weight of food consumed / wet weight gain of fish.
* Protein efficiency ratio = wet weight gain of fish / amount protein consumed (dry basis).
* Energy efficiency ratio = wet weight gain fish / amount energy consumed (dry basis)

* Protein retention efficiency (%) = individual protein deposition (dry basis)/individual protein consumption (dry basis) x 100.
* Lipid retention efficiency = individual lipid deposition (dry basis)/individual lipid consumption (dry basis) x 100.

2.3 Laboratory facilities and water quality

Experimental aquaria were 70 l acrylic tanks with four randomly allocated tanks for each treatment. Continuously-flowing, preheated water was filtered through a sand filter and a cartridge filter (nominal pore size 10μm), then passed through a 2 m^3 biological filter then a UV steriliser (Vf-9 Big Blue, Australian Ultra-Violet Products Pty. Ltd., Seven Hills, 2147, NSW) before being supplied to experimental aquaria at a flow-rate of 400 ml min^{-1}. Effluent water from each aquarium flowed out the side. Twenty five percent of this flowed to waste and the rest was collected and recirculated. Each aquarium was aerated using two air-stone diffusers and fluorescent lighting provided a 12 h light:12 h dark cycle. Spare fish were held in a 200 l polyethylene tank in the same laboratory until required.

During the experiment, water temperature (range 25.0 to 26 5°C), dissolved oxygen (always >6.0 mg l^{-1}), pH (between 6.9 and 8.2) nitrite and ammonia (<0.2 mg l^{-1} NO_2-N l^{-1} and <0.6 mg l^{-1} total ammonia - N l^{-1} respectively) were measured weekly using methods described in Allan et al. (1990).

2.4 Biochemical analysis

All chemical analyses were done in duplicate. Feed, ingredient and fish samples were analysed for dry matter, crude fat (lipid), and energy (bomb calorimetry) by the AOAC (1975) procedures. Nitrogen was determined by the method of Havilah et al. (1977) (crude protein = N x 6.25).

2.5 Statistical analysis

Prior to analysis, all data was checked for homogeneity of variance using Cochrans Test (Winer et al., 1971). The effect of including graded levels of dehulled lupins at either 10, 20, 30, 40, 50, 60, 70 and 80% was then examined for specific performance indices using one-way ANOVA. Where significant differences ($P<0.05$) were determined by ANOVA, a Student-Newman-Keuls test (SNK) was used to establish differences between the means.

The relationships between inclusion level and performance indices were modelled using regression analysis, and best-fit curves were chosen on the basis of their estimated reliability with respect to R-squared values (Microsoft Excel, Version 5, 1993, Microsoft Corporation, USA). Two-factor ANOVA was used to investigate differences between the weight gain of fish fed diets diluted with either diatomaceous earth or cellulose at four inclusion levels (10, 20, 30 and 40% of diet). Both ingredients (factor 1) and inclusion level (factor 2) were considered to be fixed.

One-way ANOVA (SNK) was also used to examine differences between moisture, protein, lipid and energy composition (all on a dry basis) of fish carcass after being fed the experimental diets for the duration of the trial (Table 3).

3. Results

As more dehulled lupin meal was substituted for the summit diet, weight gain of fish declined ($P<0.05$; one-way ANOVA), although compared with the summit diet, the difference was not significant ($P<0.05$, SNK) until the diet contained approximately 70% dehulled lupins (Table 4, Fig. 1a). This trend was similar for protein deposition and lipid deposition was unaffected by diet (Figs. 1b and 1c).

Food conversion ratio increased (deteriorated) with increasing dehulled lupin content and again, compared with the summit, the difference was significant only when the diet contained ≥70% dehulled lupins ($P<0.05$, SNK) (Table 4, Fig. 2a). Protein efficiency ratio and energy efficiency ratio also both decreased (deteriorated) with significant differences compared with the summit diet when diets contained ≥60% or ≥70% dehulled lupins respectively ($P<0.05$, SNK) (Table 4, Figs. 2b and 2c).

Protein retention efficiency increased and lipid retention efficiency decreased with increasing dehulled lupin content and, compared with the summit diet, differences were significant when the diet contained ≥70% dehulled lupins ($P<005$, SNK) (Table 4, Figs. 3a and 3b).

The diluent effect of diatomaceous earth and cellulose was similar, indicating that fibre had no anti-nutrient effects (Fig. 1a). (Type of filler was not significant; $P>0.05$; and there was no interaction between type of filler and inclusion content; $P>0.05$; two-factor ANOVA). Weight gain of fish decreased as content of filler increases. Results for each content were different for all others ($P<0.05$; SNK).

The protein and lipid composition of fish was significantly affected by diet ($P<0.05$) and there was a trend for protein content to decrease and lipid content to increase as dehulled lupin content increased. The reverse was evident as the proportion of inert filler increased (Table 3).

4. Discussion

Data from the present study indicate that on the basis of fish growth, protein deposition and protein retention efficiency, diets should contain ≤60% dehulled lupins. Protein efficiency ratio declined when fish were fed diets with more than 50% dehulled lupins. Results with diets of up to 50% dehulled lupins supports research with other species where diets with over 43% lupins performed as well or better than a fish meal control diet for rainbow trout (Morales et al., 1994) or a fish meal/soybean meal control diet for carp (Viola et al., 1988).

These results further demonstrate an excellent potential for dehulled lupins and confirms the effective absence of significant anti-nutrients in the material used for this study. Robaina et al. (1995) found increased intestinal trypsin activity in gilthead seabream (*Sparus auratus*)

when dietary content of *L. angustifolius* was increased and suggested this might indicate the presence of an anti-trypsin factor in lupins. However, the very high protein digestibility of lupins, even where diets contained 50% lupins (Allan et al., unpublished data, Section 6.3 of this report), tends to refute this suggestion. In addition, Gouveia et al. (1993) found heating/expanding *L. albus* (145°C, 25 kg/cm^2) did not improve dietary protein utilisation with rainbow trout.

Protein retention efficiencies for dehulled lupins, when included at up to 60% in the diet, were 28.7-24.1% and were similar to those reported when similar methods were used to evaluate poultry offal meal (protein retention efficiencies were 31.3 to 29.5% when dietary content of poultry offal meal was increased from 13 to 78%) and superior to feather meal (protein retention efficiencies were 24.6 to 15.7% when dietary content of feather meal was increased from 13 to 78%).

The decline in protein retention efficiency for diets with 70 and 80% dehulled lupins cannot be attributed to a decline in digestible protein (which increased) or digestible energy (which stayed relatively constant; Table 2). Other possible explanations include a deficiency in essential amino acids, presence of anti-nutrient(s) which inhibit protein utilisation or an imbalance in the protein:energy ration.

Compared with fish meal, lupins are typically low in lysine and sulphur amino acids and this has been listed as a possible restriction to their use in diets for pigs and poultry (Petterson et al., 1997). Using analysed amino acid composition and digestibility coefficients for the reference diet and *L. angustifolius* (Allan et al., unpublished data, see Section 6.3 of this report), digestible lysine for the reference diet, the diets containing 30% reference and 70% lupins and the diet containing 20% reference and 80% lupins were calculated to be 1.95, 1.86 and 1.72% respectively. Recent studies indicate requirements for digestible lysine for juvenile silver perch are not more than 1.5% (Allan et al., unpublished data, see Section 6.11 of this report). The contents are also above published requirements for channel catfish, tilapia, trout and gilthead sea bream, and within 0.28% of requirements for all other species listed in NRC (1993) except carp. It is unlikely that deficiencies in lysine restricted protein retention in very high lupin content diets. Digestible sulphur amino acid content (methionine plus cystine) were 1.33, 1.18 and 0.94% in the reference, 30% reference 70% dehulled lupins and the 20% reference 80% dehulled lupins diets. These values are similar or above published requirements for trout, channel catfish and tilapia but are less than those reported for salmon, carp and Japanese eels (NRC, 1993).

Anti-nutrients in lupins are typically very low (Petterson et al., 1997) and although the non-starch polysaccharide content of lupins is indigestible (Allan et al., unpublished data, see Section 6.3 of this report), there is no evidence that it can act as an anti-nutrient. As we have seen in this experiment, increasing inert materials such as cellulose or diatomaceous earth have a simple diluent effect.

The digestible protein:digestible energy ratios of the three highest lupin content diets were higher than for the rest of the series (2.5:1 compared with 2.2-2.4:1). In a previous study, where similar methods to those described here were used to evaluate feather meal as a protein

ingredient for silver perch diets, protein retention efficiency declined as the protein:energy ratio increased from 2.5-3.0:1 (Allan et al., unpublished data, see Section 6.6 of this report). Reduced protein retention efficiency was associated with a trend toward a decrease in carcass protein and an increase in carcass lipid with increasing dehulled lupin content. For this series of diets, digestible energy content remained fairly constant (14.5-15.1 MJ kg^{-1}). The energy from protein in the lupin diets increased at the expense of energy from carbohydrate. The change in carcass composition and decrease in protein retention efficiency may indicate that for the higher lupin diets, protein was in excess of requirements and this excess was converted to carcass lipid more efficiently than digestible energy from carbohydrate. Protein utilisation may have been improved if digestible energy had increased with increasing digestible protein. A similar pattern of decreasing carcass protein and increasing carcass lipid was recorded when increasing amounts of poultry offal meal were used to substitute a reference diet for silver perch. However, in that case digestible energy also increased with increasing dietary digestible energy (Allan et al., unpublished data, see Section 6.6 of this report). Further research on the effects of changing protein and energy content on nutrient retention efficiency is warranted.

The results of this study confirm the potential of dehulled lupins in diets for silver perch. Maximum inclusion contents should probably not exceed about 50%, but up to this content, weight gain, protein efficiency ratios and protein retention efficiency were all unaffected by dehulled lupin content.

Acknowledgments

We would like to thanks Dr Stuart Rowland and other staff at the NSW Fisheries Grafton Research Centre for providing the juvenile silver perch used in this experiment. David Stone, Scott Parkinson and Helena Heasman provided excellent technical or clerical assistance. Biochemical analysis of diets and fish were done at NSW Agriculture, Wollongbar by Ken O'Brien and Wendy Peasley. We also thank Dr Tony Evans (CSIRO, North Ryde, NSW, Australia) for supplying the dehulled lupins and Dr Kevin Williams (CSIRO, Cleveland, Queensland, Australia) for suggesting this method of evaluating dietary ingredients. This research was partially funded by the Australian Fisheries Research and Development Corporation under the Fish Meal Replacement Sub-Program. Additional funding was provided to the Sub-Program by the Australian Centre for International Agricultural Research and the Australian Grains Research and Development Corporation.

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Table 1. Formulation of SP35 (Dry basis).

Ingredient	Diet (g/100g)
Fishmeal (Danish)	26.62
Soybean meal	20.19
Blood meal	2.04
Wheat	27.47
Sorghum	11.21
Corn gluten meal	3.87
Millrun	2.01
Cod liver oil	0.90
DL-methionine	0.13
Di-calcium phosphate	1.79
Vitamin premix[1]	0.97
Mineral premix[2]	2.81

[1] (IU/kg diet): retinol (A), 8000; cholecalciferol (D3), 1000; α-tocopherol acetate (E), 125; (mg/kg diet): ascorbic acid (C), 1000; biotin (2%), 1; calcium panothenate, 55; calcium propionate, 250; choline chloride, 1500; cyanocobalamin (B12), 0.02; ethoxyquin, 150; folic acid, 4; menadione sodium bisulphite (K3), 16.5; myo-inositol, 600; nicotinamide, 200; pyridoxine (B6), 15; riboflavin (B2), 25.2; thiamine HCl (B6), 10.

[2] (mg/kg diet): calcium carbonate, 7500; manganese sulphate, 300; zinc sulphate, 700; copper sulphate, 60; ferrous sulphate, 500; sodium chloride, 7500; potassium iodate, 2.

Table 2. Ingredient and nutrient composition of experimental diets

Diet[1,2]	Ingredients (%)				Composition					
	Reference[3]	Lupin	Diatomac. earth	Cellulose	Protein[4] %	Lipid %[4]	Energy Mj/Kg [4]	Digestible Energy MJ kg^{-1} [(4,5)]	Digestible Protein[4,5] %	Digestibl protein: Digestibl energy[4,5]
1	100	-	-	-	36.9	5.0	19.5	14.5	32.3	2.2
2	90	10	-	-	34.1	4.9	18.9	14.6	33.1	2.3
3	80	20	-	-	36.0	5.1	19.1	14.7	34.0	2.3
4	70	30	-	-	35.1	5.5	19.3	14.7	34.8	2.4
5	60	40	-	-	36.4	5.7	19.3	14.8	35.6	2.4
6	50	50	-	-	37.6	5.9	19.2	14.9	36.5	2.5
7	40	60	-	-	38.6	5.7	19.7	15.0	37.3	2.5
8	30	70	-	-	38.5	4.7	19.5	15.0	38.1	2.5
9	20	80	-	-	42.7	5.0	19.8	15.1	39.0	2.6
10	90	-	10	-	33.1	4.0	16.1	13.1	29.0	2.2
11	80	-	20	-	NA	NA	NA	11.6	25.8	2.2
12	70	-	30	-	NA	NA	NA	10.2	22.6	2.2
13	60	-	40	-	21.5	2.4	11.3	8.7	19.4	2.2
14	90	-	-	10	30.5	3.7	18.2	13.1	29.0	2.2
15	80	-	-	20	NA	NA	NA	11.6	25.8	2.2
16	70	-	-	30	NA	NA	NA	10.2	22.6	2.2
17	60	-	-	40	22.0	2.3	17.8	8.7	19.4	2.2

1 To all diets 1% vitamin premix and 3% mineral premix was added

2 2% carboxymethyl cellulose was added to all diets as a binder

3 Table 1

4 Analyses (% dry basis)

5 Calculated from experimentally determined digestibility coefficients for reference diet and dehulled lupins (Gungarru variety) (see Allan et al., unpublished data; section 6.3 of this report).

Table 3.

Carcass composition of silver perch fed experimental diets containing graded levels of the summit diet (SP35) and either dehulled lupins, diatomaceous earth or cellulose.

	Composition (dry basis)[1]			
Diet	Moisture %	Protein %	Lipid %	Energy MJ/kg
Summit (SP35)	68.2±0.7^{abc}	45.7±2.1^{abc}	29.8±1.2^{def}	24.0±0.4^{bc}
90/10 lupins	68.1±0.5^{abc}	46.8±1.2^{abc}	29.9±0.6^{def}	23.8±0.3^{bc}
80/20 lupins	66.9±1.2^{abc}	46.8±1.5^{abc}	31.1±0.5^{d}	23.9±0.5^{bc}
70/30 lupins	66.2±0.7^{abc}	42.1±1.0^{bc}	32.1±1.0^{d}	24.0±0.5^{bc}
60/40 lupins	65.1±1.0^{c}	40.8±1.3^{bc}	32.9±1.2^{d}	24.2±0.6^{bc}
50/50 lupins	64.8±1.0^{c}	40.7±2.2^{c}	35.8±0.9^{c}	24.5±0.4^{bc}
40/60 lupins	65.8±0.8b^{c}	44.5±1.3^{abc}	37.5±0.9^{bc}	24.9±0.2^{b}
30/70 lupins	65.6±0.3b^{c}	41.5±0.4^{bc}	39.2±0.7^{b}	26.1±0.2^{a}
20/80 lupins	64.6±0.4^{c}	41.9±1.1^{bc}	41.8±0.9^{a}	26.3±0.2^{a}
90/10 diat earth	66.3±0.9^{abc}	45.5±1.5^{abc}	30.4±0.7^{de}	23.4±0.6^{bcd}
60/40 diat earth	69.2±0.1^{ab}	47.2±0.7^{ab}	27.5±0.4^{efg}	22.9±0.1^{cde}
90/10 cellulose	65.8±1.4^{bc}	43.6±0.7^{abc}	27.1±0.5^{fg}	22.2±0.4^{de}
60/40 cellulose	69.6±0.5^{a}	48.5±0.9^{a}	24.9±0.2^{g}	21.9±0.1^{e}

[1.] Values are means ± sem of four replicates (n=4). Means within columns which share a common superscript are not significantly different (P>0.05).

Table 4.

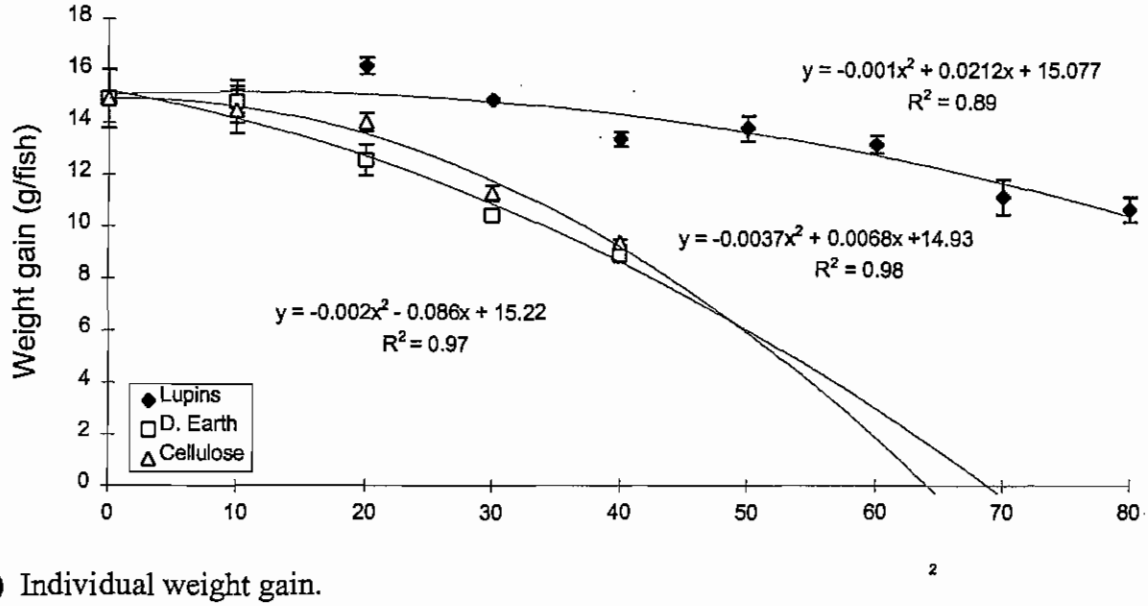
Effect of feeding diets with different amounts of dehulled lupins on performance, conversion ratio and retention efficiency of juvenile silver perch over 75 days.

Diet	Inclusion level lupins (%)	Weight[1] gain g/fish	Protein[2] deposition g/fish	Lipid[3] deposition g/fish	Food[4] conversion ratio	Protein[5] efficiency ratio	Energy[6] efficiency ratio	Protein[7] retention (%)	Lipid[8] retention (%)
1	0	14.9 ± 1.1^{ab}	2.2 ± 0.1^{bc}	1.4 ± 0.2^{a}	1.5 ± 0.1^{a}	1.8 ± 0.1^{ab}	3.4 ± 0.1^{ab}	26.2 ± 0.6^{cd}	127.3 ± 12.8^{a}
2	10	14.8 ± 0.5^{ab}	2.2 ± 0.1^{b}	1.4 ± 0.0^{a}	1.6 ± 0.1^{ab}	1.8 ± 0.1^{ab}	3.3 ± 0.1^{abc}	28.7 ± 0.7^{ab}	127.4 ± 2.5^{a}
3	20	16.2 ± 0.3^{a}	2.5 ± 0.0^{a}	1.7 ± 0.0^{a}	1.5 ± 0.0^{a}	1.9 ± 0.0^{a}	3.6 ± 0.1^{a}	29.7 ± 0.3^{a}	140.8 ± 2.7^{a}
4	30	14.9 ± 0.1^{ab}	2.1 ± 0.0^{bc}	1.6 ± 0.0^{a}	1.6 ± 0.1^{ab}	1.8 ± 0.1^{ab}	3.3 ± 0.1^{ab}	27.2 ± 0.1^{bc}	135.1 ± 0.9^{a}
5	40	13.4 ± 0.3^{b}	1.9 ± 0.0^{c}	1.6 ± 0.0^{a}	1.6 ± 0.0^{ab}	1.7 ± 0.0^{abc}	3.2 ± 0.1^{abc}	24.4 ± 0.5^{d}	130.4 ± 3.2^{a}
6	50	13.8 ± 0.5^{b}	2.0 ± 0.0^{bc}	1.8 ± 0.1^{a}	1.7 ± 0.1^{ab}	1.6 ± 0.1^{bcd}	3.2 ± 0.1^{abc}	24.7 ± 0.9^{d}	144.1 ± 10.5^{a}
7	60	13.2 ± 0.3^{b}	2.0 ± 0.1^{bc}	1.8 ± 0.1^{a}	1.7 ± 0.0^{ab}	1.5 ± 0.0^{cd}	3.0 ± 0.1^{bcd}	24.1 ± 0.6^{d}	142.3 ± 5.8^{a}
8	70	11.2 ± 0.7^{c}	1.6 ± 0.1^{d}	1.6 ± 0.1^{a}	1.8 ± 0.1^{bc}	1.5 ± 0.1^{d}	2.9 ± 0.1^{cd}	22.1 ± 1.0^{e}	179.0 ± 9.7^{b}
9	80	10.7 ± 0.5^{c}	1.6 ± 0.1^{d}	1.7 ± 0.1^{a}	1.9 ± 0.1^{c}	1.3 ± 0.0^{e}	2.7 ± 0.1^{d}	19.2 ± 0.7^{f}	170.9 ± 9.0^{b}

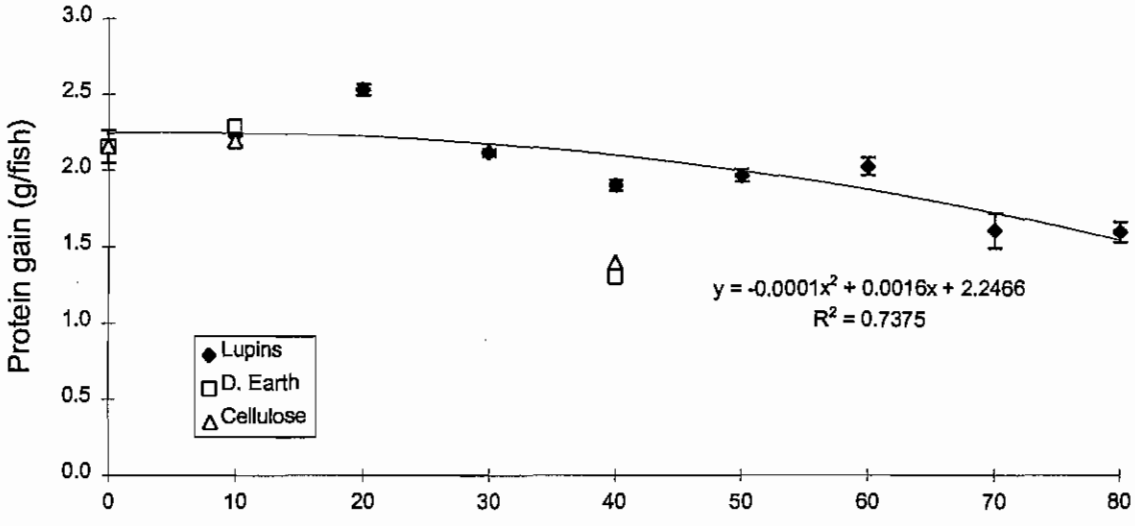
Values are mean ± sem of four replicates n=4. Different letters in superscript indicate a significant difference ($P<0.05$) between column means.

1. Weight gain (g) = (final individual weight - initial individual weight)
2. Protein deposition (g) = (final ind. wt [dry basis] x protein composition final carcass) - (initial ind. wt [dry basis] x protein composition initial carcass)
3. Lipid deposition (g) = (final ind. wt [dry basis] x fat composition final carcass) - (initial ind. wt [dry basis] x fat composition initial carcass)
4. Food conversion ratio = dry weight of food consumed / wet weight gain of fish
5. Protein efficiency ratio = wet weight gain of fish / amount protein consumed (dry basis)
6. Energy efficieney ratio = wet weight gain of fish / amount energy consumed (dry basis)
7. Protein retention efficiency (%) = individual protein deposition (dry basis) / individual protein consumption (dry basis) x 100
8. Lipid retention efficiency (%) = individual lipid deposition (dry basis) / individual lipid consumption (dry basis) x 100

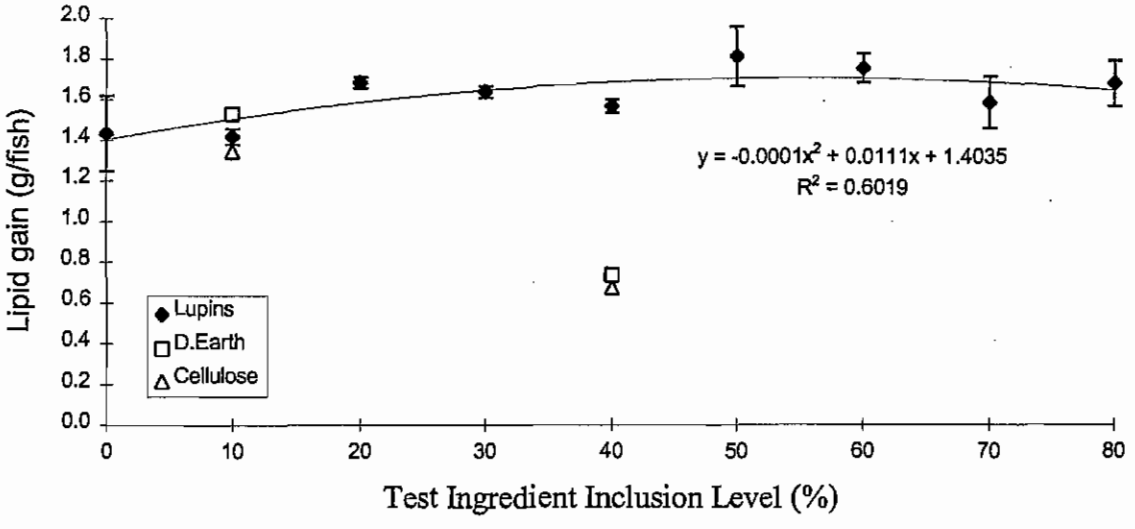
Figure 1. Performance of silver perch fed experimental diets for 75 days.



a) Individual weight gain.

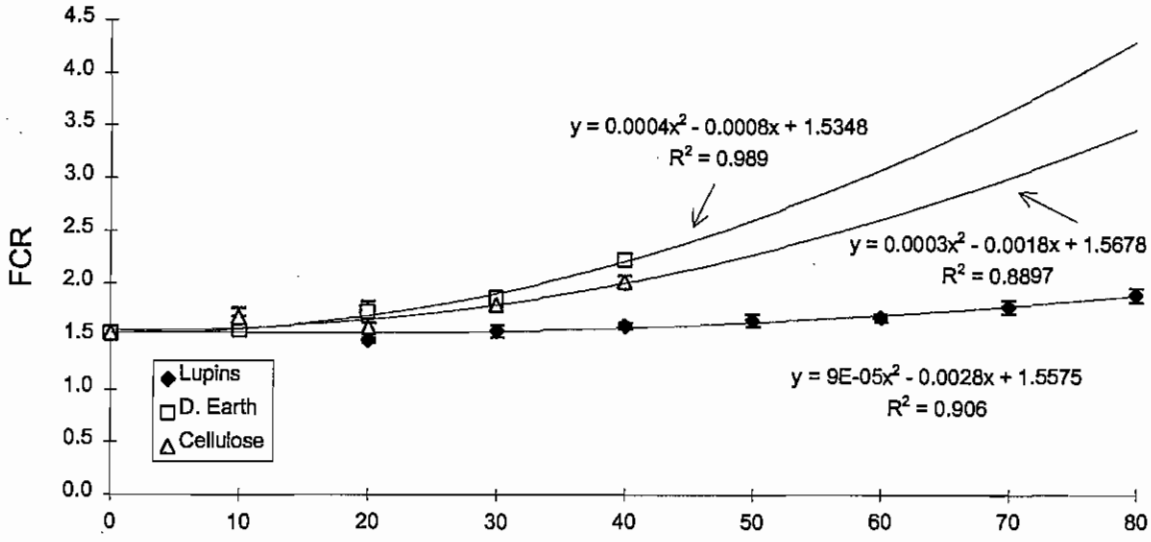


b) Individual protein deposition

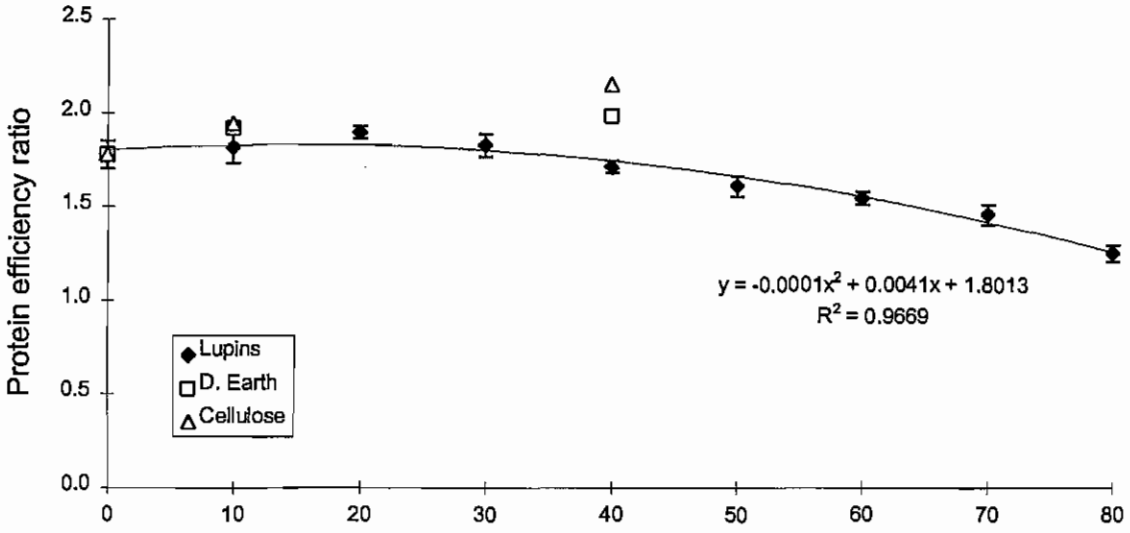


c) Individual lipid deposition.

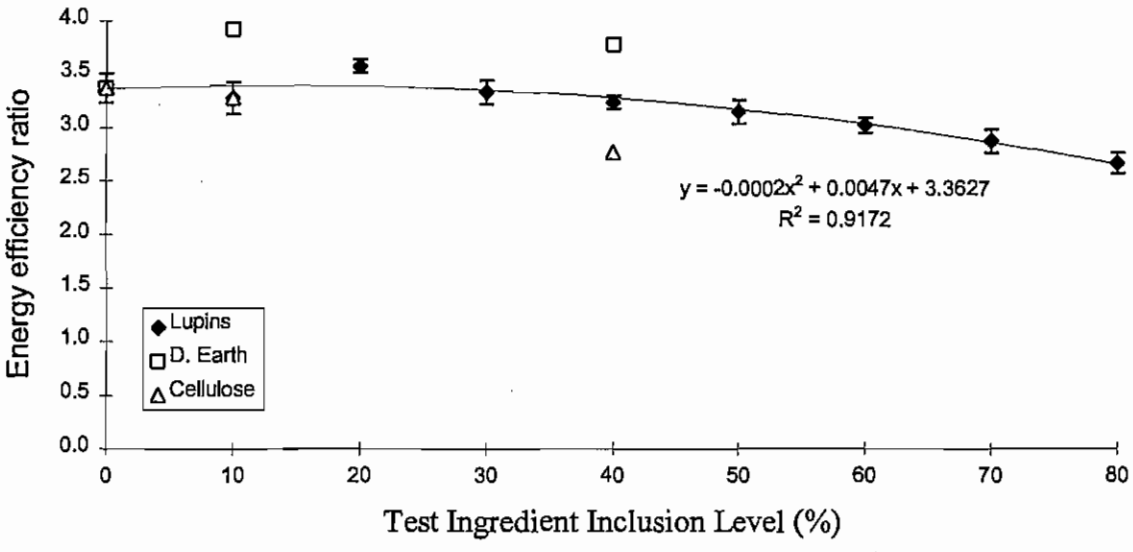
Figure 2. Conversion ratios of silver perch fed on experimental diets for 75 days.



a) Food conversion ratio.

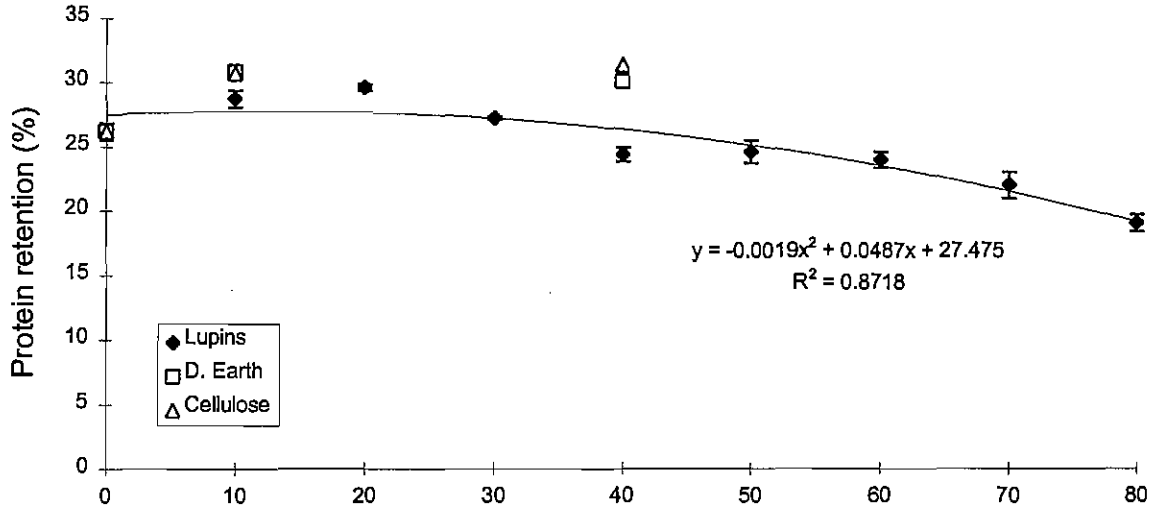


b) Protein efficiency ratio.

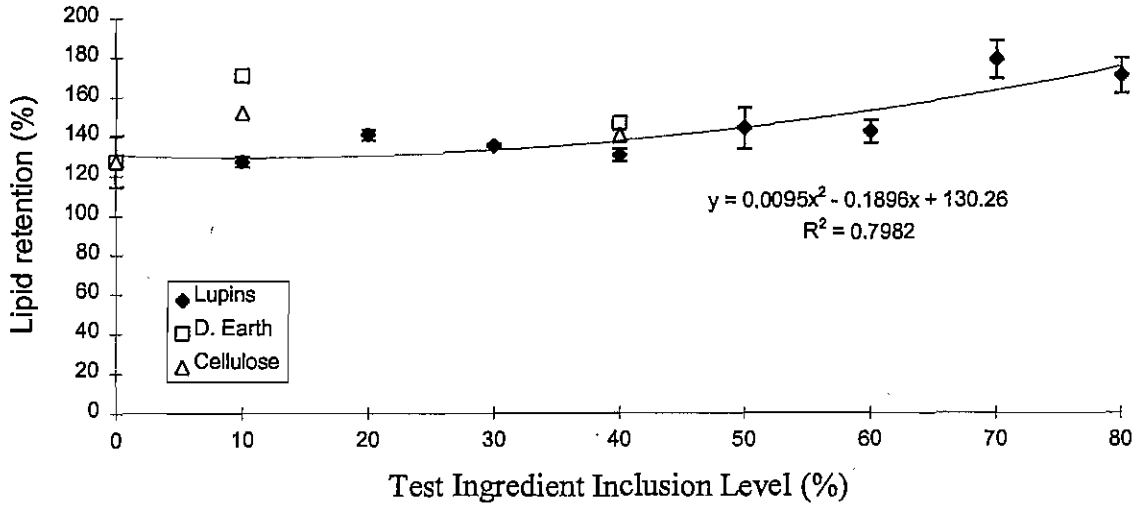


c) Energy efficiency ratio.

Figure 3. Retention efficiencies of silver perch fed experimental diets for 75 days.



a) Protein retention efficiency.



b) Lipid retention efficiency.

6.10 Effects of grinding, steam conditioning and extrusion of a practical diet on digestibility and growth of silver perch, *Bidyanus bidyanus*

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Abstract

In addition to ingredient composition, processing can affect the digestibility and utilisation of diets and performance of fish. In this study, we examined the effects of grinding and steam conditioning and extrusion of a single commercially available diet (SP35) on growth and performance of the native Australian freshwater silver perch *Bidyanus bidyanus* (Mitchell).

SP35 (35% protein, 18 MJ/kg gross energy) with approximately 70% of particles between 710 and 1000 µm was either left (unground) or finely ground to 500 µm (ground). Both unground and ground fractions were pelleted in a commercial steam pelleting mill with or without the addition of steam (90°C) to process four of five diets (Diets 1-4) and a fifth diet was processed by pelleting finely ground material in a single-screw extruder (after the addition of approximately 5% fish oil) at a temperature of 120°C. The extruded diet floated. Each diet was fed to 50 juvenile silver perch (mean initial weight 17.8 g) in each of three replicate 10 000 l tanks for 112 days. Fish gained between 55-71 g/fish during the experiment, and food conversion ratio ranged from 1.5-2.01:1. For Diets 1-4, steam conditioning significantly improved weight gain and food conversion efficiency while neither grinding nor the interaction between grinding and steam conditioning had any effect. Fish were reluctant to feed on the floating extruded diet and grew less on this diet than on the steam conditioned diets, although food conversion efficiency was better than for all other diets.

Ground diets, uncooked and steamed, and the extruded diet were subsequently reground, 1% chromic oxide was added as an inert indicator and each of these three diets was fed to juvenile silver perch (mean initial weight 2.5 g) in 170 l cylindroconical tanks from which faeces were collected by settlement to determine digestibility coefficients for dry matter, energy and nitrogen. Digestibility coefficients for dry matter and energy were higher for the extruded diet but similar for the unsteamed and steamed diets. Nitrogen digestibility was unaffected by processing.

These results indicate grinding of diets below approximately 710-1000 µm was unnecessary for silver perch. Steam conditioning improved fish performance and this was related to the effect on physical stability of the pellet and possible effects of steam conditioning on improving gustatory characteristics of the diets. Extrusion improved digestibility and food conversion efficiency but consumption of the floating extruded pellets was reduced in our tanks. Sinking, extruded diets deserve evaluation.

Keywords: Nutrition; *Bidyanus bidyanus*; Digestibility; Growth; Diet processing.

1. Introduction

In recent years, the focus on processing technologies used to produce commercial diets for use in aquaculture has increased. This focus has been primarily driven by the rapid expansion in global aquaculture which has resulted in an increased demand by producers for high quality diets designed to meet their specific requirements (Lovell, 1992). This demand for nutritionally adequate, cost effective diets has also driven the search for high quality ingredients to either wholly or partially replace the expensive fish meal component in aquaculture diets. In many instances, these alternative ingredients and diets often require some form of processing to improve their nutritional or physical qualities.

Three processing techniques currently dominate the production of pelleted diets in aquaculture and their use often, but not always, facilitates improvement in the raw product; grinding, steam conditioning and extrusion. These techniques inevitably affect both the physical and chemical characteristics of a feed (Hilton et al., 1981). They include water stability and durability, pellet hardness, nutrient availability (Tan, 1991) and digestibility (Walker, 1980; Hui-Meng, 1989; Hardy, 1989). Other factors influenced by processing such as the palatability and organoleptic properties of a diet may affect the amount of feed consumed by a target species.

While processing techniques such as those described can improve the nutritional and physical qualities of diet ingredients, heating can also have detrimental effects. Heat labile vitamins and nutrients can be lost at elevated temperatures (Slinger et al.,1979; Kiang, 1989; Springate, 1991). For example ascorbic acid has been shown to be unstable during heat treatments such as steam conditioning and extrusion (Slinger et al., 1979), and overheating can reduce the available levels of essential amino acids such as lysine and cystine (Evans and Butts, 1951; Viola et al., 1983; Carpenter and Booth, 1973).

In Australia, aquaculture of silver perch (*Bidyanus bidyanus)*, a native freshwater finfish, is expanding rapidly. One of the major reasons for this expansion was the development of an experimental diet known as SP35 (Allan and Rowland, 1992). After extensive evaluation, the diet is now commercially available and is capable of supporting rapid growth and production outputs approaching 10 t/ha/year (Rowland, et al., 1995).

Processing technologies such as fine grinding, steam conditioning and extrusion have the potential to improve the physio-chemical nature of aquaculture diets. To investigate the effects these processing techniques have on SP35, fish growth and performance were evaluated and, in a separate experiment, digestibility coefficients were determined.

2. Materials and Methods

2.1. Experimental fish

Silver perch were initially bred and reared at the Grafton Research Centre following techniques described by Thurstan and Rowland (1994). Afterwards they were held in 10 000 l indoor tanks and fed SP35 until transferred to experimental facilities and diets.

During all stocking procedures, fish were anaesthetised (25 mg/l ethyl-p-aminobenzoate) and weighed individually, or in small groups, before being systematically dispersed to experimental tanks. In the digestibility trial, spare fish to replace any mortalities were stocked into separate holding tanks (100 l) and fed the appropriate test diets.

2.2. Diets

SP35 (Table 1) was subject to three commercial processing techniques for the purpose of the growth trial; grinding, steam conditioning and extrusion. All processing of SP35 was carried out by Ridley Aquafeeds, Narangbar, Queensland. The complete diet was initially supplied as a 3mm pellet (Janos Hoey Pty Ltd.) and was subsequently broken down through a commercial hammermill fitted with a 3mm screen. The bulk of this coarse or 'unground' diet (≈80%) consisted of particles ranging between 710-1000um. Half this material was then subject to fine grinding using a pulverizer/air classification system similar to that described in Tan (1991), which ensured an accurate grind size of 500um with low variance. Both the coarsely and finely ground diets were then separately re-pelleted in a commercial steam pellet mill with or without the addition of steam (90^0C). This produced four diets for evaluation.

A fifth diet was produced to evaluate the effects of extrusion by pelleting the finely ground material in a commercial, single screw extruder at a temperature of 120^0C. Extrusion of this diet after grinding proved difficult and in order to produce a practical pellet the addition of fish oil was required which resulted in a 5% increase in fat content. All diets apart from the extruded diet were isonitrogenous and isoenergetic after processing (Table 2).

Physical pellet characteristics of the five diets used in the growth study are reported in Table 3. Characteristics for each of the five diets was determined following the methods outlined by Gleeson and Evans (unpublished; CSIRO Division of Food Science and Technology). Wet stability tests (0.5h) indicate the ground extruded diet exhibited superior water stability in comparison to all other diets, followed by those subject to steam conditioning. The ground-unsteamed treatment proved to be the most unstable pellet in water with greater than 50% of material collected as particulate matter. Values for other characteristics such as water absorption and dry pellet durability were similar, however the extruded diet showed the greatest increase in gelatinisation of starch with approximately 82% of raw starch modified. All diets with the exception of the extruded diet sank immediately they were administered.

Results obtained from the growth trial indicated that steam conditioning improved performance and extruding the diet improved food conversion ratio. To evaluate these improvements in relation to digestibility, diets were reground through a 1.5 mm hammermill (Raymond Laboratory Mill, Transfield Technologies Pty Ltd, Rydalmere, 2116, Australia) and 1% chromic oxide was added. This reprocessing ensured pellets from the extruded diets sank at a similar rate to other diets and major physical differences were eliminated. After grinding, greater than 92% of each diet was less than 710 μm. Diet and marker were then thoroughly dry mixed before the addition of ≈ 600ml/l distilled water (Hobart Mixer: Troy Pty Ltd, Ohio, 45374, USA). The mixture was then pelleted through a meat mincer fitted with a 1.5 mm pellet die (Barnco Australia Pty Ltd, Leichhardt, 2040, NSW). After pelleting, diets were dried in a convection drier at $<35^0$C for approximately 6 hours until all diets had moisture contents of less than 10%.

2.3. *Growth trial*

The growth experiment was undertaken in a large hot-house facility which housed 15 x 10 000 l tanks. The trial ran between December 1995 and April 1996. Water was circulated through each tank at approximately 17 l/min then returned to a large sump containing a submerged biofilter. Water was drawn from the sump via two rapid sand filters before returning to experimental tanks. Each tank was provided with two air stone diffusers and covered with black shade cloth to reduce the growth of algae. Tanks were siphoned once a week to remove accumulated faeces.

Each of the five diets was allocated to 3 randomly selected tanks (n=3). Tanks were stocked with 50 fish (mean 17.8 g, range 17.2-18.3 g) which were hand fed twice daily (0830 and 1500 h) until satiated for a period of 112 days. Feed intake was recorded daily. Each tank was weighed monthly over the course of the experiment with fish starved one day prior to weighing. At the end of the growth trial individual weight gain and food conversion ratio was determined for each tank to allow comparisons among different processing techniques.

2.4. *Digestibility trial*

The digestibility experiment was performed in a light / temperature controlled environment. Experimental units were 170 l cylindroconical tanks fitted with a 65 mm diameter settlement chamber which tapered into a 150 mm length of silicone tubing. Fresh pre-filtered water was pumped from a 50 000 l reservoir into a 3000 l header tank where it was heated. Water then flowed directly from the header tank, via an ultra violet light conditioning unit, into the experimental tanks at a flow rate of 600 ml/min. Effluent water exited each tank via a 25mm standpipe and returned to a common sump where 25% of the effluent was directed to waste. The remaining water passed through a twin cartridge membrane filter before being filtered through a 2 m^3 biofilter. Water was then returned to the header tank for recirculation. Each tank was aerated with two air stone diffusers and fitted with an automatic belt feeder attached to a clear perspex diffuser.

Fish were fed in excess of their daily requirements once a day for a period of 3 h (0830 and 1130 h). Approximately 1 h after all feed had been delivered to the digestibility tanks both the upper tanks and lower collection chambers were thoroughly cleaned. Silicone collection tubes were then packed in ice and maintained at temperatures of approximately 4^0C to reduce bacterial activity during the collection period. Faeces was collected by settlement over a period of 18 h. Faecal samples were collected each morning prior to feeding and dried over silica gel in vacuum desiccators. Individual tank samples from daily collections were pooled to provide sufficient sample for biochemical analyses.

Each of the test diets was randomly assigned to 3 replicate digestibility tanks (n=3) after completion of the stocking procedure. Digestibility tanks were stocked with 12 fish (mean 2.5 g, range 2.3-2.8 g) and they were acclimated on experimental diets for 7 days prior to collection of faeces. The experiment was run for 24 days.

2.5. *Water quality analysis*

Water quality variables were monitored weekly in both experiments following analyses described in Allan et al., (1990). Over the course of the growth trial they ranged between 23.8-28.3^{0}C for temperature, 6.2-7.7 mg/l dissolved oxygen, 6.1-8.5 pH, 20-60ug/l $N0^2$-N and 20-100ug/l total ammonia-N. As ambient temperatures dropped approaching April (1996) heaters were installed in the sump to maintain temperatures above 23^{0}C.

Values for the digestibility experiment ranged between 25-26.5^{0}C for temperature, 6.9-8.8 mg/l dissolved oxygen, pH 7.75-8.32, $N0_2$-N <57 ug/l and total ammonia-N between 181-233 ug/l.

2.6. *Biochemical analyses*

All analyses were carried out in duplicate on samples of feed and faecal material by NSW Agriculture, Wollongbar Agricultural Institute, Bruxner Highway, 2477. Values for dry matter, fat and energy (bomb calorimetry) were determined following procedures described in AOAC (1975). Nitrogen was determined following methods outlined by Havilah et al., (1977) and multiplied by 6.25 to establish the content of crude protein. Determination of chromic oxide was by the method described in Kimura and Miller (1957). Amino acids were analysed using HPLC and Water Pico-Tag (Waters Pty Ltd, Lane Cove, NSW, 2066, Australia) after being subject to acid hydrolysis. Tryptophan was not determined. Sulphur amino acids were determined separately following performic acid digestion.

2.7. *Calculation of digestibility coefficients*

Apparent digestibility coefficients for dry matter, energy and nitrogen for experimental diets were calculated following indirect procedures similar to those outlined in Cho and Kaushik (1990). All values are presented on a dry basis.

$ADC = [1-(F/D \times DC_r/FC_r)] \times 100$ where:

F = % nutrient or energy in faeces,
D = % nutrient or energy in diet,
DC_r = % chromic oxide in diet and
FC_r = % chromic oxide in faeces.

2.8. *Statistical analyses*

Data from four diets used in the growth trial were subject to a 2-factor ANOVA (steam conditioned / not steam conditioned; ground to 500 μm / not ground) in order to investigate interaction between level of grind and presence or absence of steam conditioning. Data from the extruded treatment were excluded from statistical examination of results for the growth trial as extruded pellets floated, and this clearly reduced food consumption. Digestibility coefficients for dry matter, energy and nitrogen determined from the digestibility trial were compared with one way ANOVA after tests for homogeneity of variance were satisfied (Cochran's). Differences between means was determined by Student Newman-Keul's

multiple range test. Retrospective tests for power, where reported were calculated from equations presented in Searcy-Bernal (1994).

3. Results

3.1. Effect of processing on growth

Figure 1 is a graphical representation of mean individual monthly growth increment for each of the five diets fed in the growth trial. Differences in individual growth were apparent after the second growth check (60 days) and become more pronounced toward the end of the study (112 days). Individual weight gain, food conversion ratio (FCR) and individual food consumption for juvenile silver perch grown on different diets are presented in Table 4. Mean values for individual weight gain ranged between 54.5 g and 71.3 g for the unground, unsteamed treatment and unground steam conditioned treatment respectively. FCR's were poorest in both the unsteamed diets and markedly improved for both the steamed and extruded diets. Improvements in FCR for fish fed the extruded diet was probably related to their feeding behaviour. The extruded diet tended to float, and fish were reluctant to feed at the surface during the trial. As a consequence less food was delivered in satiation feeding. Although less feed was delivered to this particular treatment, individual gains in weight matched those of the unground, unsteamed treatment despite the fact they consumed considerably more feed (Table 4).

Results for the 2 Factor analyses of variance on weight gain and FCR indicate steam conditioning significantly improved the growth parameters investigated, while grinding to 500 μm had no effect. There was no interaction present in any test (Table 5).

3.2. Effect of processing on digestibility

Mean digestibility coefficients for dry matter, energy, nitrogen and amino acids of the process modified diets are presented in Table 6. One way analysis of variance (Factor: diet treatment) on dry matter and energy proved to be significant ($P<0.05$) with differences detected between the two alternative diets and the extruded treatment. The ground extruded treatment returned the highest digestibility values for both dry matter and energy coefficients. No difference was found between digestibility coefficients for nitrogen (ANOVA, P=0.055, power 0.59) and digestibility coefficients for amino acids were not statistically tested.

4. Discussion

Steam conditioning of SP35 improved the growth and performance of silver perch reared under experimental conditions. This is supported by significant increases in individual growth and FCR above those of unsteamed diets regardless of the level of grind (Table 5) and despite no differences in analysed nutrient composition (Table 2). The improvement might have been due to an improvement in gelatinisation, reduction in anti-nutrients or an improvement in the physical characteristics of the pellet.

Thermal treatments such as steam conditioning have the potential to gelatinise a percentage of the raw starch present in a diet (Hastings and Higgs, 1980; Hui-Meng, 1989; Hardy, 1989)

which is generally considered to improve its digestibility (Bergot, 1991; Wilson, 1994). However, in this study, the steam conditioning had only a small effect on starch gelatinisation and did not account for the difference in fish performance. For instance, despite a 5% difference in percent gelatinisation between the two steam conditioned diets (ground:unground), growth performance and food consumption on these two diets was almost identical (Table 4). Further, the ground unsteamed diet had 21% of its starch gelatinised but performance of silver perch on this diet was only marginally better than that of the control diet with 17% gelatinisation. Similar differences in percentage gelatinisation occurred between both ground diets (steamed:unsteamed) studied in the digestibility trial (Table 3), yet neither diet varied in digestibility coefficients for dry matter, energy or nitrogen ($P>0.05$) and digestibility coefficients for the amino acids remained unchanged (Table 6).

Mild heat treatments are well known to reduce the anti-nutritional factors present in plant proteins that are associated with reductions in performance and digestibility (Del Valle, 1981; Chin, 1989; Robaina et al., 1995). SP35 contains significant levels of soybean meal (20.2%) which is known to contain certain inhibitors of trypsin and chymotrypsin (Krogdahl, 1989; Mitchell et al.,1991; Wee, 1991). Wheat (27.5%) contains α-amylase inhibitor which has been linked to reduced digestibility in trout (Hofer and Sturmbauer, 1985), carp and tilapia (Natarajan et al., 1992). However a reduction in anti-nutrients in the steam conditioned diet, which might have improved performance, is not supported by the digestibility results. Digestibility coefficients for the uncooked diet were statistically similar to those of the cooked diet, indicating levels of anti-nutrients in the uncooked diets were not responsible for limiting their digestibility.

The greatest physical difference between the steamed and unsteamed pellets is in wet pellet stability. Steamed pellets exhibited superior stability in water with a subsequent reduction of free particulate matter, while both the unsteamed diets had less than 50% of pellets remaining after 30 minutes immersion. The physical integrity of steam conditioned diets would give fish the option of returning to them after the initial satiation response has passed. Satiation feeding is an arbitrary response, and in this trial small amounts of food (20-30 pellets) would sometimes remain on the bottom after fish had ceased active feeding ("boiling"). Therefore, uneaten pellets may have been consumed after the initial feeling of fullness had passed. Similarly, due to the hierarchal structure often established in fish groups, less dominant fish would have an opportunity to forage on these pellets immediately after the dominant fish were satiated. Ingesting a greater proportion of all the feed offered would ensure a greater feeding efficiency and this may explain the observed increases in weight gain and FCR's. These scenarios are less likely to have occurred in tanks where the unsteamed diets were fed, as these pellets were very unstable in water and tended to disintegrate when approached by swimming fish.

Alternatively, gains in performance may be directly related to the increased consumption of the steam conditioned diets. Reasons why the steam conditioned diet might be more acceptable to silver perch are difficult to establish, however these diets may have "incitant or stimulatory" effects related to either chemical or gustatory cues (Mackie and Mitchell, 1985; Ishida and Kobayashi, 1992) that are more pronounced than in uncooked diets. Of the pellet characteristics examined, hardness index is the only one related to a gustatory effect. Steam conditioned diets were softer than unsteamed diets even though they exhibited greater water stability, and silver perch may prefer to consume a softer pellet. The increased hardness of the

unsteamed pellets may have had a slightly "suppressant" effect (Mackie and Mitchell, 1985) on silver perch which may have caused an early cessation of feeding.

Extrusion of SP35 returned a highly acceptable FCR, however the feeding behaviour of fish in this treatment was dramatically different and this precludes them from any fair comparisons against the remaining treatments. Extruded pellets had a low bulk density (579.48 g/l) and slow sinking rate (0.022 m/s) which resulted in a floating pellet that silver perch in our tanks were reluctant to feed on. As a consequence, feeding to satiation was difficult, and as a result estimates of consumption for this treatment are unreliable. Better FCR for the extruded diet may be accounted for with a restricted feeding regime which was dissimilar to that for other diets. Secondly, the 5% addition of fish oil required to manufacture an acceptable pellet may have affected the attractiveness and/or palatability of the diet and the increased energy content may have increased food conversion efficiency. The extruded diet also had a much higher degree of starch gelatinisation (82%) than all other diets. This major increase in gelatinisation may have accounted for the significant improvement in digestibility coefficients for dry matter and energy in comparison to the steamed and unsteamed diets (Table 6).

Extrusion has clearly improved the digestibility of SP35, and improved the degree of gelatinised starch within the diet. Use of this technology to produce a sinking pellet which can be ingested at or near the bottom may lead to greater improvements in fish performance given the gains reported for digestibility. Gains such as these could help to offset higher plant costs associated with producing an extruded pellet (Springate, 1991) and also lead to significant reductions in the pollution associated with intensive aquaculture (Cho, 1991; Jirsa et al., 1997).

Grinding SP35 pellets to 500 μm has had no effect on the performance of silver perch in this study. This finding is contrary to the generally accepted benefits of such a process. For example, fine grinding is generally thought to improve the overall physical characteristics of a pellet (Hui Meng, 1989; Botting ,1991), improve digestibility (Walker, 1980; Hardy, 1989; Hui-Meng, 1989) and ensure the non-selective ingestion of ingredients (Tan, 1991).

Grinding SP35 to 500 μm did not improve wet pellet stability. In fact grinding SP35 below 500um in the absence of steam resulted in a highly unstable pellet, potentially prone to nutrient losses and likely to reduce water quality. The most stable pellet was produced from coarsely ground SP35 (3 mm screen) subject to steam conditioning. These results indicate that for this study, the additional expense associated with fine grinding is unwarranted with respect to gains in either fish performance or improvements in pellet stability.

Acknowledgments

We would like to thank the following for their technical input to experiments: Dr Stuart Rowland, Charlie Mifsud and other staff at NSW Fisheries Grafton Research Centre for producing juvenile silver perch and support for nutrition research; Scott Parkinson and David Stone at Port Stephens Research Centre; Mr Ken O'Brien and Wendy Peasley (NSW Agriculture) for their assistance with biochemical analysis; Mr David Overend, Mr Kevin Smyth and Mr Gary Hoey of Ridley Agriproducts Pty Ltd for their assistance with processing the diets; Mr Tony Hoey and Ms Kerry Page at Janos Hoey Pty Ltd for supplying the diet SP35; Mr Wayne O'Connor and Mr Stewart Fielder for critically reviewing the manuscript

and Mrs Helena Heasman for assisting with the preparation of the manuscript. The research developed was part of the Australian Fisheries Research and Development Corporation Sub-Program on Replacing Fish Meal in Aquaculture Diets (93/120). Financial support from the Australian Centre for International Agricultural Research, the Australian Grain Research and Development Corporation and the Meat Research Corporation is also gratefully acknowledged.

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TABLE 1

Composition of the practical silver perch diet SP35.

Ingredient	Amount in SP35 (% dry basis)
Fish meal	26.20
Soybean meal	20.19
Bloodmeal	2.04
Corn gluten meal	3.87
Wheat	27.47
Sorghum	11.21
Millrun	2.01
Cod liver oil	0.90
Vitamin premix[1]	0.97
Mineral premix[2]	2.81
Di-calcium phosphate	1.79
DL-methionine	0.13

[1] Vitamin premix (A)	IU	mg/kg
Retinol (A)	8000	
Cholecalciferol (D3)	1000	
α-Tocopherol acatate (E)		125
Menadione sodium bisulphate (K3)		16.5
Thiamine HCl (B6)		10.0
Riboflavin (B2)		25.2
Pyridoxine HCl (B6)		15.0
Folic acid		4
Ascorbic acid (C)		1000
Ca-panothenate		55
Myo-inositol		600
Biotin (2%)		1
Choline chloride		1500
Nicotinamide		200
Cyanocobalamin (B12)		0.02
Ethoxyquin		150
Calcium propionate		25

[2] Mineral premix	g/kg
Calcium carbonate	7.5
Manganese sulphate	0.3
Zinc sulphate	0.7
Copper sulphate	0.06
Ferrous sulphate	0.5
Sodium chloride	7.5
Potassium iodate	0.002

TABLE 2

Proximate analysis (dry basis) of process modified SP35.

Processing method	Protein %	Energy MJ/Kg	Fat %
Unground, no steam conditioning	33.67	18.94	5.38
Unground, steam conditioned	37.11	18.66	4.29
Ground, steam conditioned	35.57	18.70	4.36
Ground, no steam conditioning	35.31	18.32	3.03
Ground, extruded	33.66	20.02	9.56

TABLE 3

Physical characteristics of the practical silver perch diet SP35 after processing.

Physical Characteristic		Processing Method				
		Unground, no steam	Unground, steam	Ground, steam	Ground, no steam	Ground, extruded
Pellets retained after	0.5h (%)	48.60 ± 3.04	73.13 ± 0.39	56.48 ± 12.03	38.38 ± 0.74	94.45 ± 0.53
Particulates after	0.5h (%)	39.16 ± 2.34	17.20 ± 0.07	33.44 ± 10.41	51.01 ± 1.06	1.35 ± 0.08
Soluble material after	0.5h (%)	12.24 ± 0.70	9.68 ± 0.32	10.07 ± 1.62	10.62 ± 0.32	4.18 ± 0.45
Water absorption after	0.5h (%)	50.71 ± 5.33	46.37 ± 5.64	48.87 ± 4.26	47.48 ± 3.34	51.96 ± 0.33
Breaking shear force	(N)	12.96 ± 0.54	9.55 ± 0.48	12.22 ± 1.01	15.44 ± 0.89	20.37 ± 0.58
Breaking shear force	(N/mm^2)	1.53 ± 0.07	1.15 ± 0.06	1.49 ± 0.13	1.84 ± 0.11	1.48 ± 0.08
Hardness index		1.24 ± 0.05	0.91 ± 0.04	1.18 ± 0.10	1.56 ± 0.09	1.37 ± 0.05
Bulk density	(g/l)	810.54 ± 0.00	784.93 ± 8.26	789.88 ± 0.04	841.18 ± 0.08	579.48 ± 6.14
Durability	(%)	97.69 ± 0.11	98.26 ± 0.01	97.94 ± 0.05	97.24 ± 0.16	98.46 ± 0.18
Sinking rate	(m/s)	0.108 ± 0.007	0.114 ± 0.009	0.112 ± 0.003	0.107 ± 0.008	0.022 ± 0.006
Degree of gelatinisation	(%)	16.65	20.05	14.94	20.65	82.07
Total starch	(%dw)	30.180 ± 0.52	24.15 ± 0.15	25.13 ± 0.48	23.71 ± 0.71	21.77 ± 0.86

Values arc means ± sem for n=2 replicates.

TABLE 4

Results for weight gain, food conversion ratio (FCR) and food consumption for individual juvenile silver perch grown on the practical diet SP35 after processing.

SP35 Processing Method	Individual Weight Gain g/fish	FCR[1]	Individual food consumption (dry wt) g/fish
Unground, no steam conditioning	54.5 ± 5.5	2.01 ± 0.04	106.9 ± 9.1
Unground, steam conditioned	71.3 ± 3.2	1.75 ± 0.04	123.3 ± 2.3
Ground, steam conditioned	70.7 ± 1.5	1.76 ± 0.02	122.7 ± 5.3
Ground, no steam conditioning	61.3 ± 2.1	1.91 ± 0.09	116.0 ± 3.7
Ground, extruded	58.2 ± 4.9	1.50 ± 0.01	86.3 ± 7.0

Values are means ± sem for n=3 replicates. Experimental period t = 112 days.

1. FCR = dry weight feed/wet weight fish.

TABLE 5

Results of a 2 FACTOR ANOVA on individual weight gain and food conversion ratio for juvenile silver perch grown on the practical diet SP35 after processing (Factor 1-steam conditioned, not steam conditioned; Factor 2-ground or unground).

Growth Parameter	Steam conditioning	Grinding	SteamxGrind	Error
Individual weight gain				
Mean square	512.48	29.45	40.85	35.69
F	14.36*	0.83	1.15	
Food conversion ratio				
Mean square	0.13	0.004	0.009	0.008
F	14.99*	0.524	1.08	

* denotes $P < 0.05$. df = 1 for steam conditioning, grinding and steam x grind. df = 8 for error.

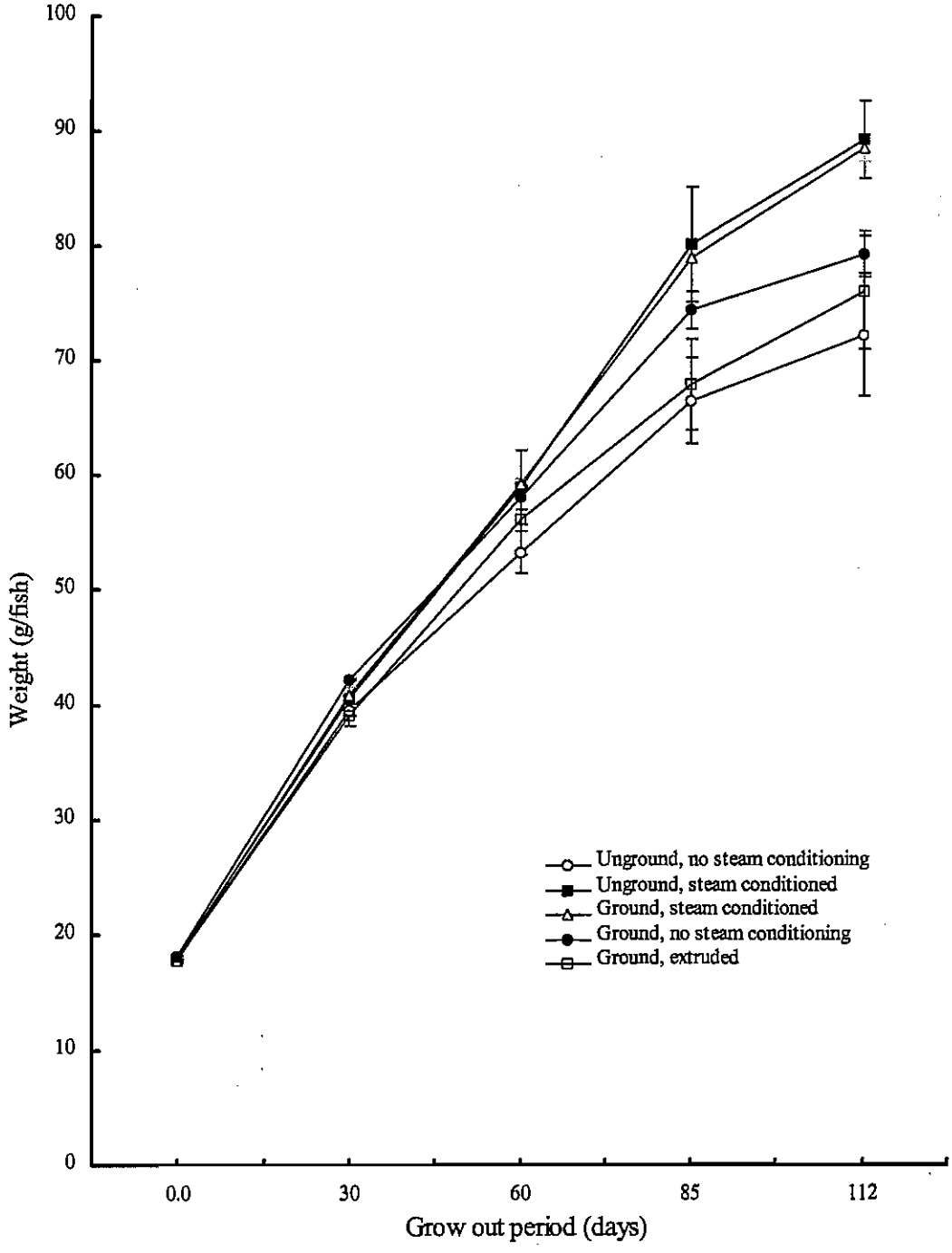
TABLE 6

Apparent digestiblity coefficients for proximates and amino acids of ground diets (500 μm) fed to silver perch.

	Processing Method		
Digestibility coefficient %	Steam conditioned	No steam conditioning	Extruded
Proximate			
Dry Matter	67.00 ± 1.39[a]	64.76 ± 0.97[a]	71.40 ± 0.32[b]
Energy	77.95 ± 1.10[a]	76.47 ± 0.73[a]	83.22 ± 0.22[b]
Nitrogen	90.16 ± 0.39[a]	88.97 ± 0.49[a]	89.28 ± 1.62[a]
Amino acids			
Aspartic acid	94.1 ± 0.5	93.9 ± 0.3	94.9 ± 0.1
Glutamic acid	95.2 ± 0.1	94.7 ± 0.2	95.8 ± 0.1
Serine	92.0 ± 0.4	91.4 ± 0.3	91.5 ± 0.5
Glycine	86.3 ± 0.4	83.9 ± 0.5	87.4 ± 0.4
Histidine	95.3 ± 0.4	93.8 ± 0.3	91.6 ± 0.4
Arginine	94.0 ± 0.2	92.8 ± 0.2	94.1 ± 0.2
Threonine	94.8 ± 0.4	94.2 ± 0.4	94.0 ± 0.4
Alanine	91.5 ± 0.3	90.5 ± 0.2	91.3 ± 0.3
Proline	86.8 ± 0.7	84.6 ± 0.3	87.7 ± 0.4
Tyrosine	94.6 ± 0.4	93.7 ± 0.3	94.6 ± 0.3
Valine	92.4 ± 0.3	90.9 ± 0.3	91.4 ± 0.2
Isoleucine	93.4 ± 0.3	92.1 ± 0.3	94.5 ± 0.2
Leucine	93.7 ± 0.4	92.8 ± 0.3	92.6 ± 0.2
Phenylalanine	94.2 ± 0.6	92.7 ± 0.3	92.5 ± 0.1
Lysine	94.5 ± 0.4	93.8 ± 0.2	93.7 ± 0.2
Cystine	94.4 ± 2.8	90.4 ± 0.8	89.4 ± 1.4
Methionine	96.8 ± 1.6	94.4 ± 0.1	93.9 ± 1.2

Values are means ± sem for n=3 replicates. Row means with similar letters in superscript are not significantly different (P>0.05, ANOVA, SNK, Cochran's).

Figure 1. Individual monthly growth increment (mean ± sem) of silver perch fed experimental diets for a period of 112 days.



6.11 Estimating digestible protein and lysine requirements of silver perch

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Abstract

Lysine is usually the first limiting amino acid in fish diets and may limit fish meal substitution. In this study, we attempted to estimate requirements for lysine using intact protein sources. Using digestibility data for silver perch for a large number of ingredients, we formulated a 'summit' diet to contain 1.8 times the expected requirements for essential amino acids except lysine and 1.4 times the expected requirements of lysine (based on requirements for channel catfish). A 'diluent' diet was formulated to contain 0.4-0.5 times the expected requirements of all essential amino acids. Both 'summit' and 'diluent' diets contained similar digestible energy (15.1 MJ kg^{-1} for the summit and 13.8 MJ kg^{-1} for the diluent). Six diets with the following amounts of summit:diluent diets were prepared: 100:0, 80:20, 60:40, 40:60, 20:80, 0:100. Three additional controls were used: a practical control diet and the 40:60 summit:diluent diet with either additional crystalline lysine or a mix of all essential amino acids, except lysine in crystalline form. Ten fish (2.1-2.6 g) were stocked into each 70 l aerated aquaria (5/diet). Fish were fed twice daily to satiation for 54 days. Final individual fish weight ranged from 4 g to 15.5 g.

Results were modelled using intersecting linear regression analysis. The optimum digestible dietary protein and lysine contents for diets with 13.8-15.1 MJ kg^{-1} digestible energy, after which protein and lysine deposition did not increase significantly, were 25.2 and 1.5% respectively. The minimum lysine requirement was not conclusively determined as there was no additional response to the 40:60 summit:diluent diet with added crystalline lysine. This was either due to a failure of the crystalline lysine to elicit a growth response or a limitation in lysine plus one or more of the other essential amino acids. This needs to be further investigated.

Keywords: Nutrition; silver perch; *Bidyanus bidyanus;* protein requirements; lysine requirements; summit/diluent

1. Introduction

Requirements for crude protein for monitoring growth have been estimated for at least 25 species of fish to be between 31-55% of the diet on an as fed basis (NRC, 1993). Lysine is usually the first limiting amino acid in feed ingredients used in diets for warmwater fish especially plant proteins (Robaina et al., 1980). Published requirements range from 1.3-2.9% of dry diet (or 3.7-6.1% of dietary protein) (NRC, 1993). This range encompasses published requirements for chinook and chum salmon, common carp, Nile and Mossambique tilapia, channel catfish, Japanese eel, gilthead sea bream and rainbow trout, but the minimum and maximum estimates are both for rainbow trout derived during different studies (NRC, 1993). The issue of whether published differences in requirements reflect genuine differences between species or differences in experimental conditions or methodology is discussed by Cowey (1994).

Several methods of estimating requirements for specific amino acids have been used. The most common are dose-response experiments with weight gain as the response. The protein in experimental diets is usually made up of some intact protein, eg casein and gelatin, plus a mixture of crystalline amino acids. Diets with graded levels of the amino acid being studied are provided. Total protein in the crystalline amino acid mix are balanced using non-essential crystalline amino acids. Growth rates of fish fed on these diets are often inferior to those fed protein diets with similar amino acid composition, possibly at least partly due to relatively poor utilisation of crystalline amino acids and purified protein sources (NRC, 1993; Cowey, 1994; Nose and Murai, 1990). Other possible causes of discrepancies include differences in digestible or metabolisable energy density for different ingredients or fish species and consequent differences in the protein to energy ratios of experimental diets within a study or in different studies.

There is also considerable evidence that while some species, such as rainbow trout, appear able to effectively utilise crystalline acids, even in quite high concentrations, other species such as carp are either unable to utilise crystalline amino acids or grow very slowly and only after the crystalline amino acid component is carefully neutralised with NaOH (Cowey, 1994).

In studies with pigs and poultry, optimum requirements for protein have been determined using two diets with similar digestible or metabolisable energy contents. One, the summit diet, has protein or amino acid contents well in excess of those required, while the other (diluent diet) has contents approximately half of those required. A series of diets are manufactured by combining different amounts of the summit and diluent mix (Fisher and Morris, 1970; Bikker, 1994). This method facilitates the use of practical protein sources, avoids the need to use large amounts of crystalline amino acids, and provided digestibility of the summit and diluent diets is determined, ensures that differences in digestible energy do not confuse interpretation of results.

Silver perch (*Bidyanus bidyanus*) are native Australian freshwater fish with considerable potential for aquaculture (Rowland et al., 1994, 1995). Very little is known of their nutritional requirements although preliminary studies indicate diets with 32-36% crude protein and 13-15 MJ kg^{-1} digestible energy will be sufficient (Allan and Rowland, 1991) and effective practical diets with approximately 35% protein and 13-15 MJ kg^{-1} digestible energy have been successfully used in large-scale farming trials and are now available commercially (Allan and Rowland, 1992; Allan, 1995). For silver perch aquaculture to expand in Australia, cost-effective diets will need to be developed. Very little fish meal is produced in Australia, however, considerable success with diets where protein is derived mainly from Australian agricultural proteins such as meat meal and legumes have been reported (Allan, 1997; Allan et al., unpublished data, see Section 6.4 of this report; Williams et al., 1997). Australian agricultural ingredients usually contain less crude protein and lysine than fish meal and this may reduce their use.

In research to date, addition of crystalline lysine and other crystalline amino acids has not been effective in increasing growth rates of silver perch, raising suspicion that this species may not be able to effectively utilise crystalline amino acids. The aim of the study was to determine the requirement of juvenile silver perch for crude protein and lysine when fed diets based on intact protein sources with a digestibile energy content of about 15 MJ kg^{-1}.

2. Materials and Methods

2.1. Experimental diets

Two diets were formulated using the least-cost diet program Feedmania (Mania Software, Brisbane,Queensland, 4000). All ingredients were ground or sieved to ensure all particles passed through a 710 μm screen. Dry ingredients were thoroughly mixed in a Hobart mixer (Troy Pty. Ltd, Ohio 45374, USA) then combined with approximately 400 ml distilled water kg^{-1} dry mix before being pelleted through a meat mincer (Barnco Australia Pty. Ltd., Leichhardt, 2040, NSW) with a 1.5 mm die. Pellets were dried at < 35°C in a convection drier for about 6 h until the moisture content was between 10 - 15%, to produce a dry, sinking pellet.

For the first diet, the summit diet, the nutrient profile specified 15 MJ kg^{-1} digestible energy and that all amino acids were to be supplied (on a digestible basis) at approximately 1.8 times "expected requirements" except lysine which was to be supplied at 1.4 times the 'expected requirement'. For the second diet, the diluent diet, the nutrient profile specified the same digestible energy as the summit diet, but digestible amino acid specifications were 0.4-0.5 times the 'expected requirements'. The published requirements for channel catfish were used as "expected requirements" (NRC, 1993). Fish were fed the summit or diluent diets or different mixtures of the two (Table 1, 2 and 3), to give a range of diets with similar digestible energy but different digestible amino acid contents, with lysine expected to be the first limiting amino acid in all diets. Three other control diets were included, SP35 (reference diet; Table 1), 40:60 mix (summit:diluent) with an additional 1.15% L-lysine, and a 40:60 mix (summit:diluent) with all nine other essential amino acids (except lysine) added in crystalline form to bring the total digestible level of these amino acids to that of the summit diet (all crystalline amino acids were assumed to be 100% digestible). The formulation of diets with similar digestible energy and the required digestible protein and digestible amino acid contenets were critical to the design of this experiment. Apparent digestibility coefficients (ADC's) of all ingredients were calculated before diets were formulated (Allan et al., unpublished data, see Sections 6.1 and 6.2 of this report; Stone et al., unpublished data, see Section 6.8 of this report) and Allan et al., (unpublished data, see Section 6.1 of this report), showed that digestibility coefficients for silver perch were additive. This was confirmed during a separate digestibility experiment where digestibility coefficients for energy, crude protein and amino acids were determined for the summit and diluent diets, and a 50:50 mix of the two diets. This experiment was conducted before the growth experiment commenced.

2.2 Feeding strategy

The fish were fed their respective experimental diets twice daily (40% at 8.30am and 60% at 3.00pm), initially at 4% biomass/ day for the first four days, and then to satiation twice daily for the remainder of the experiment. Any uneaten feed was siphoned from each aquarium approximately 30 minutes after feeding, dried and weighed. Fish were fed for a period of 54 days.

2.3. Experimental Fish

Silver perch (range 2.1-2.6 g; mean weight 2.32 ± 0.01 g) were bred at the Grafton Research Centre and raised in earthen ponds using similar techniques to those described by Thurston and Rowland (1994). Before experiments, fish were fed SP35 to satiation twice daily and were treated with 5 g l^{-1} NaCl to ensure they were free of ectoparasites and to prevent fungal infection (Rowland and Ingram, 1991).

Prior to stocking, fish were anaesthetised using a bath of ethyl ρ-aminobenzoate (50 mg l^{-1} for 3 min.) then caught at random, weighed individually and distributed among tanks by systematic interspersion until a total of ten fish were stocked in each tank. Five replicate aquaria were provided for each of the nine treatments and fish in nine additional aquaria were fed one of each of the nine diets throughout the experiment and were used to replace mortalities in the experimental tanks. During the experiment any mortalities were replaced with individually weighed, fin-clipped fish which were excluded from estimates of weight gain and composition of final fish.

Weight checks were carried out every 4 weeks, and also at harvest. At the first weight check, two fish were randomly removed from each tank, thus leaving 8 fish for the remainder of the experiment to reduce stocking density to minimise the chance of density reducing growth.

Fish were then harvested (February, (late summer) 1996) and the survival rate, mean weight increment, food consumption (% fish biomass/day) (=average daily food intake x 100/average fish biomass (g)) and food conversion ratio (FCR)[= dry weight of food/wet weight fish gain], were calculated from each tank. Average fish biomass was estimated as (initial weight + final weight)/2. Digestible protein and digestible lysine intake were calculated as: Digestible protein intake = analysed dietary protein x ADC for protein/100 x food intake, and digestible lysine intake = analysed dietary lysine x ADC for lysine/ 100 x food intake. ADC's for each diet were calculated as: (proportion of the summit diet in that experimental diet x summit ADC/100) + (proportion of the diluent diet in that experimental diet x diluent ADC/100). ADC's for the summit and diluent were determined from a previous experiment.
Proximate analysis of the whole body composition for 5 randomly selected fish from each tank were also determined. From the proximate analyses the following indices were calculated:

Protein deposition (PD) = final weight (dry basis) x final protein content (dry basis) - initial weight (dry basis) x initial protein content (dry basis);
Fat deposition (FD) = final weight (dry basis) x final fat content (dry basis) - initial weight (dry basis) x initial fat content (dry basis).

2.4 Laboratory facilities and water quality

Experimental aquaria were 70 l acrylic tanks. Continuously-flowing, preheated water was filtered through a sand filter and a cartridge filter (nominal pore size 10μm), then passed through a 2 m^3 biological filter then a UV steriliser (Vf-9 Big Blue, Australian Ultra-Violet Products Pty. Ltd., Seven Hills, 2147, NSW) before being supplied to experimental tanks at a flow-rate of 400 ml min^{-1}. Effluent water from each aquaria flowed out the side of the tank. Twenty five percent of this flowed to waste and the rest was collected and recirculated. Each tank was aerated using two air-stone diffusers and fluorescent lighting was provided on a 12 h light:12 h dark basis.

During the experiment, water temperature (range 25.6 to 27.5°C), dissolved oxygen (range 5.7 to 7.7 mg l^{-1}), pH (between 6.7 and 8.1) nitrite and ammonia (<0.04 mg l^{-1} NO_2-N l^{-1} and <0.2 mg l^{-1} total ammonia - N l^{-1} respectively) were measured weekly using methods described in Allan et al. (1990).

2.6. Biochemical Analyses

All chemical analyses were done in duplicate. Diet and fish samples were analysed for dry matter, ash, crude fat and energy (bomb calorimetry) by the AOAC (1975) procedures. Nitrogen was determined by the method of Havilah et al. (1977) (crude protein = N x 6.25). Amino acids were determined by the method of Cohen et al. (1989) and analysed following acid hydrolysis using high pressure liquid chromatography and Waters Pico-Tag (Waters Pty Ltd, Lane Cove, NSW, 2066, Australia). Sulphur amino acids were determined separately following performic acid digestion, and tryptophan, which is lost during acid hydrolysis, was not analysed (Cohen et al., 1989).

2.7. Statistical analysis

Homogeneity of variances was assessed using Cochrans Test (Winer et al., 1971). Single-factor ANOVA was used to examine the effect of diet for each index and comparison between means were made using Student Newman-Kuels multiple range test. Means were considered significant at $P<0.05$. The relationships between protein and lysine and growth performance indices were modelled for the summit/diluent series of diets using intersecting linear regression analysis (Sedgewick, 1979; Allan et al., 1990). The first regression included means for the summit diet and all treatments which did not differ significantly from the summit. The second regression included the lowest mean from the first group of data plus all other means in the series. Unless otherwise stated, all results appear as mean ± standard error of the mean (n=3).

3. Results

3.1 Growth Performance

Results for fish growth are presented in Table 4 and Figure 1. Weight gain increased with increasing amount of dietary digestible protein or dietary digestible lysine in a curvilinear fashion, reaching a plateau between the 60S:40D and 40S:60D diets. Weight gain for fish fed the summit diet, 80S:20D and 60S:40D was similar ($P>0.05$) and higher than for all other diets. Weight gain of fish fed the 40S:60D, the 20S:80D and the reference diet (SP35) were similar and greater than of fish fed the Diluent diet. The minimum dietary digestible protein and lysine contents for maximum weight gain were estimated by intersecting linear regression analysis to be 25.2% and 1.5% respectively (Figures 1a and b). Weight gain of fish fed the 40S:60D diet supplemented with L-lysine or a mix of all essential amino acids except L-lysine were not significantly different from the unsupplemented 40S:60D diet.

Similar trends were apparent when protein deposition was plotted against digestible protein intake and when digestible lysine deposition was plotted against lysine intake (Figures 2a and b). Intersecting regression analysis was used to predict a minimum digestible protein and digestible lysine intake of 3.83 g/fish and 0.22 g/fish respectively (Figures 2a and b). For fish of an average biomass of 6.9 g (initial weight plus final weight/2), this is equivalent to an

intake of approximately 10.2 g digestible protein/kg fish/day. For lysine, this intake is 0.57 g digestible lysine/kg fish/day.

3.2 Food Consumption and Efficiency

Food consumption was affected by diet ($P<0.05$) although differences were minor (range for summit/diluent series 3.4-4.0% fish biomass/day) (Figure 3a). Food conversion ratio (FCR) tended to increase (deteriorate) exponentially with increasing porportion of the diluent diet and compared with the summit diet, the FCR's of fish fed the 20S:80D and the diluent diets were significantly different (Figure 3b).

4. Discussion

The results show that for silver perch fed diets with a digestible energy content of between 13.8-15.1 MJ kg^{-1}, increasing dietary digestible protein or dietary digestible lysine above 25.2 or 1.5% respectively does not significantly increase weight gain or improve food conversion ratios. This protein requirement is below most estimates of protein requirements published for fish (NRC, 1993; Wilson,1989) although maximum growth rates with channel catfish have been reported for fish fed diets with protein contents ranging from 22-40% (Garling and Wilson, 1976). One of the reasons for the relatively low estimation of requirements for silver perch reported here is that in the present study values are all for digestible protein (and digestible lysine) not total crude protein as is commonly used. In addition the present experiment was conducted with relatively low energy diets. As energy increases, requirements for protein and amino acid also increase (Wilson, 1989; Cowey, 1994). Most studies on protein requirements attempt to formulate diets on an isocaloric basis but as pointed out by Wilson (1989), digestible or metabolisable energy values for the diets and ingredients have not usually been determined for the species being studied. Instead, most researchers have used estimated physiological fuel values which range from 14.6-23.8 MJ kg^{-1} for protein, 33.5-39.7 MJ kg^{-1} for lipid and 4.2-16.7 MJ kg^{-1} for carbohydrate for different species (Wilson, 1989).

With the enormous range in these estimated energy values for each of the major nutrients, as well as the large differences that have been reported for different types of protein, fat and carbohydrate, formulating genuinely isocaloric diets covering a range of protein contents is difficult.

In an earlier experiment with silver perch we attempted to formulate a series of diets with one of three digestible energy contents for each of five digestible protein contents. We used published digestible energy values for channel catfish to formulate these diets. Unfortunately, subsequent direct measurement of digestible energy for silver perch for the ingredients used indicated that our estimation of digestible energy for the protein source (fish meal) was too low while that for corn starch (the major carbohydrate source) was far too high. We were left with a series of diets where energy increased with protein content.

For many earlier studies, diets were formulated using relatively purified ingredients such as casein, gelatin, whole egg protein or mixtures of crystalline amino acids (NRC, 1993; Wilson, 1989). Growth on diets based on these protein sources can be inferior to that on diets based on intact protein sources (Cowey, 1994; Wilson, 1989) and this might lead to an overestimation of requirements. In the present study, fish performance on experimental diets

with adequate protein was similar or superior to growth on a practical reference diet which is widely used in industry.

Other differences between studies, even with the same species, have included fish size, water temperature and other methodological differences (Cowey, 1994 and Wilson, 1989).

Research with both pigs and poultry has led to the development of a concept of two phases in protein deposition, a protein dependant phase and an energy dependant phase. At a constant energy intake, protein deposition increases with protein intake until a plateau has been reached after which there is no response to increasing protein intake. At a higher energy intake, the protein deposition will respond to a higher protein intake (Bikker, 1994).

Provided this model applies to silver perch, the results presented here suggest that for diets with between 13.8 and 15.1 MJ kg^{-1} digestible energy, the protein dependant phase was ≤25.2% digestible protein (Figure 1a) or up to an intake of 3.83 g protein/fish (Figure 2a).

Despite being a major objective, quantatitive requirements of silver perch for lysine were not determined during the present study. For this to have occurred, it was necessary to demonstrate that lysine was the first limiting amino acid in the summit/diluent series.

As there was not significant improvement in weight gain, protein or lysine deposition for fish fed the 40S:60D diet supplemented with L-lysine compared to unsupplemented 40S:60D diet, this premise was not demonstrated. However, there was also no response to the 40S:60D diet with supplemental amino acids (except lysine). Possible explanations for this include: 1) crystalline lysine, and possibly other crystalline amino acids, were poorly utilised or 2) lysine plus at least one other amino acid were limiting.

There is considerable evidence for poor response to crystalline amino acids (Wilson, 1989; Cowey, 1994; Nose and Murai, 1990) and there is no evidence that silver perch responded to supplementation of crystalline lysine, methionine and/or threonine in diets containing high contents of meat products, pulses, poultry meal, feather meal or wheat gluten, despite all ingredients being considered to be deficient in essential amino acids (Allan et al., unpublished data, see Section 6.4, 6.6, 6.9 and 6.12). This explanation could be verified by the inclusion of a treatment containing all 10 essential amino acids (including lysine) and would be a valuable exercise.

Despite having failed to determine absolute requirements for lysine, the data presented here indicates that no more than 1.5% dietary digestible lysine is needed in silver perch diets containing between 13.8-15.1 MJ kg^{-1}. This amount is towards the low end of published requirements for other species (NRC, 1993; Wilson, 1989) but it is similar to requirements published for channel catfish (1.2 and 1.5% of diets with 24 and 30% total crude protein respectively) (Wilson, 1989), and Nile tilapia (1.43% of a diet with 28% total crude protein) (NRC, 1993).

Fish, like most animals, are considered to eat primarily to satisfy energy needs (Smith, 1989). In the curent experiment, all diets (except the practical reference diet) had equal digestible energy contents but very different protein, carbohydrate and lipid contents. Although there were significant differences between food consumption (as a % fish biomass/day basis), the similarity in food consumption (3.4 - 4.0 g) for the summit/diluent series of diets supports the hypothesis that energy needs drive consumption.

The significant deterioration in food conversion ratio for the 20S:80D diet and the diluent diet, indicated that the proportion of protein in these diets used for maintenance was much higher than for the other diets. The fact that some growth was recorded for fish fed these diets indicated that at about 14 MJ kg^{-1} digestible energy content, maintenance requirements were above 9.5% digestible protein and 0.58% digestible lysine. For fish fed the diluent diet, the digestible protein intake was approximately 3.6 g protein/kg fish/day which is above the maintenance requirements of between 0.95 - 1.6 g protein/kg body weight/day reported by Wilson (1989).

Data presented here indicates silver perch have a relatively low requirement for digestible protein (25.2%) when fed diets with 13.8-15.1 MJ kg^{-1} digestible energy. For diets with this digestible energy content, 1.5% dietary lysine is sufficient. These results indicate ingredients considered relatively low in lysine may still have potential for use in silver perch diets.

Acknowledgments

The authors would like to thank Scott Parkinson, Rebecca Warner-Smith and Mark Booth for technical assistance with running the experiment described here. Dr Stuart Rowland, Charlie Mifsud and David Glendenning from the NSW Fisheries, Grafton Research Centre bred the silver perch used in this study.

Mr Carl Rayner (State Chemistry Laboratories, Victoria) analysed diets and fish for proximate and amino acid composition. The earlier digestibility experiment using the summit and diluent diets was analysed by Mr Ken O'Brien and Ms Wendy Peasley who were employed at the time by NSW Agriculture at Wollongbar. Mr Roger Campbell (Bunge Meats, Victoria, Australia) provided a very useful account of the use of this method in pig nutrition. Helena Heasman assisted with manuscript preparation.

Financial support was provided by the Australian Fisheries Research and Development Corporation under the Fish Meal Replacement Sub-Program, the Australian Centre for International Agricultural Research and the Australian Grains Research and Development Corporation. Australian Native Fish Ltd is also gratefully acknowledged.

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Table 1

Formulation of SP35, summit and diluent diets used to evaluate lysine requirements for silver perch[1].

Ingredient	Diet (g/100g as is)		
	SP35	Summit	Diluent
Fish meal (Australian)	27.0	34.2	8.2
Wheat	26.9	43.0	10.0
Sorghum	11.0	-	-
Corn gluten meal	4.0	4.5	-
Cooked wheat starch	-	-	33.5
Pregeled corn starch	-	-	10.0
Wheat gluten	-	3.0	-
Soybean meal	20.0	-	-
Blood meal	2.0	-	-
Millrun	2.0	-	-
Peanut meal	-	9.3	4.1
Carboxymethylcellulose	-	-	8.8
Fish oil	1.0	0.0	17.0
DL-methionine	0.2	-	-
Di-calcium phosphate	2.0	2.0	4.4
Vitamin premix[2]	1.0	1.0	1.0
Mineral premix[3]	3.0	3.0	3.0
	Digestible composition (dry basis)[4]		
Digestible protein (%)	37.9	40.4	9.5
Digestible energy (MJ kg-1)	15.4	15.1	13.8
Ash (%)	11.06	10.9	18.3
Lipid (%)	-	4.4	17.2
Digestible arginine (%)	2.1	2.8	0.6
" histidine (%)	0.8	1.0	0.2
" isoleucine (%)	1.4	1.6	0.4
" leucine (%)	2.9	2.9	0.7
" lysine (%)	2.1	2.2	0.5
" methionine (%)	1.4	0.9	0.2
" cystine (%)	1.4	0.5	0.1
" phenylaline (%)	1.4	1.7	0.4
" tyrosine (%)	2.7	1.3	0.3
" threonine (%)	1.4	1.4	0.4
" valine (%)	1.7	1.9	0.5

1 Analysed composition of individual dietary ingredients are displayed in table 2.

2 (IU/kg diet): retinol (A), 8000; cholecalciferol (D3), 1000; α-Tocopherol acetate (E), 125; (mg/kg diet): ascorbic acid (C), 1000; biotin (2%), 1; calcium panothenate, 55; calcium propionate, 250; choline chloride, 1500; cyanocobalamin (B12), 0.02; ethoxyquin, 150; folic acid, 4; menadione sodium bisulphite (K3), 16.5; myo-inositol, 600; nicotinamide, 200; pyridoxine (B6), 15; riboflavin (B2), 25.2; thiamine HCl (B6), 10.

3 (mg/kg diet): calcium carbonate, 7500; manganese sulphate, 300; zink sulphate, 700; copper sulphate, 60; ferrous sulphate, 500; sodium chloride, 7500; potassium iodate, 2.

4 Analysed composition of experiment diet multiplied by digestible coefficients (as a proportion) determined in a previous experiment.

Table 2

Analysed proximate composition of diet ingredients (dry basis).

Ingredient	Protein (%)	Ash (%)	Gross energy MJ kg^{-1}
Fish meal (Australian)	73.2	.NA	21.3
Wheat	15.2	.NA	18.5
Sorghum	14.5	2.3	18.8
Corn gluten meal	62.0	1.1	24.1
Cooked wheat starch	0.4	0.1	17.0
Pregeled corn starch	0.4	0.5	16.6
Wheat gluten	76.9	.NA	23.1
Soybean meal	47.8	8.0	17.0
Blood meal	94.9	3.1	23.9
Millrun	22.3	4.3	19.6
Peanut meal	41.2	5.2	19.7
Fish oil	.NA	.NA	39.0

.NA = not analysed

Table 3

Formulation of experimental diets (% dry basis).

Treatment	Diet ingredient				
Diet	Summit	Diluent	Reference	L-lysine	L-Eaa (not lysine)
1					
2	-	-	100	-	-
3	100	-	-	-	-
4	80	20	-	-	-
5	60	40	-	-	-
6	40	60	-	-	-
7	20	80	-	-	-
8	0	100	-	-	-
9	40	60	-	1.15	-
10	40	60	-	-	mix[1]

[1] mix = essential amino acids (L-form) except lysine to meet specifications of summit diet

Table 4. Nutrient composition of experimental diets and effect of diets on performance indicators for juvenile silver perch

	Diet				Performance indices[1]						
Diet	Protein (db%)	Digestible protein (db%)	Lysine (db%)	Digestible lysine (db%)	Weight gain (g)	Food consumed (% biomass/day)	FCR (db)	Digestible protein intake (db g/fish)	Digestible lysine intake (db g/fish)	Protein deposition. (db g/ fish)	Lysine deposition (db g/ fish)
Reference	41.90	37.87	2.43	2.28	6.81^{b}	3.27^{a}	1.53^{ab}	3.85^{d}	0.23^{d}	1.23^{c}	0.09^{dc}
Summit	45.10	40.37	2.32	2.15	9.51^{c}	3.44^{ab}	1.41^{a}	5.34^{f}	0.28^{e}	1.61^{d}	0.11^{f}
80S:20D	38.00	33.96	2	1.86	9.68^{c}	3.65^{abc}	1.46^{ab}	4.73^{e}	0.26^{de}	1.52^{d}	0.11^{f}
60S:40D	31.20	27.84	1.64	1.52	9.21^{c}	3.83^{bc}	1.60^{ab}	4.01^{d}	0.22^{d}	1.48^{d}	0.10^{ef}
40S:60D	24.90	22.18	1.31	1.22	7.40^{b}	3.85^{bc}	1.72^{b}	2.75^{c}	0.15^{c}	1.12^{c}	0.08^{cd}
20S:80D	17.20	15.30	0.94	0.87	5.88^{b}	4.00^{c}	2.00^{c}	1.75^{b}	0.10^{b}	0.79^{b}	0.05^{b}
Diluent	10.70	9.50	0.58	0.54	2.63^{a}	3.45^{ab}	2.81^{d}	0.66^{a}	0.04^{a}	0.33^{a}	0.02^{a}
40S:60D+L	25.00	22.27	2.26	2.10	7.24^{b}	3.54^{ab}	1.60^{ab}	2.53^{c}	0.24^{d}	1.11^{c}	0.08^{cd}
40S:60D+M	29.30	26.10	1.09	1.01	6.64^{b}	3.62^{abc}	1.68^{b}	2.86^{c}	0.11^{b}	1.02^{c}	0.07^{bc}

[1] Values are means ± sem (n=5 pooled replicate tanks). Means in the same column which share the same letter in the superscript are not significantly different (One-factor ANOVA, P>0.05; SNK). db= dry basis

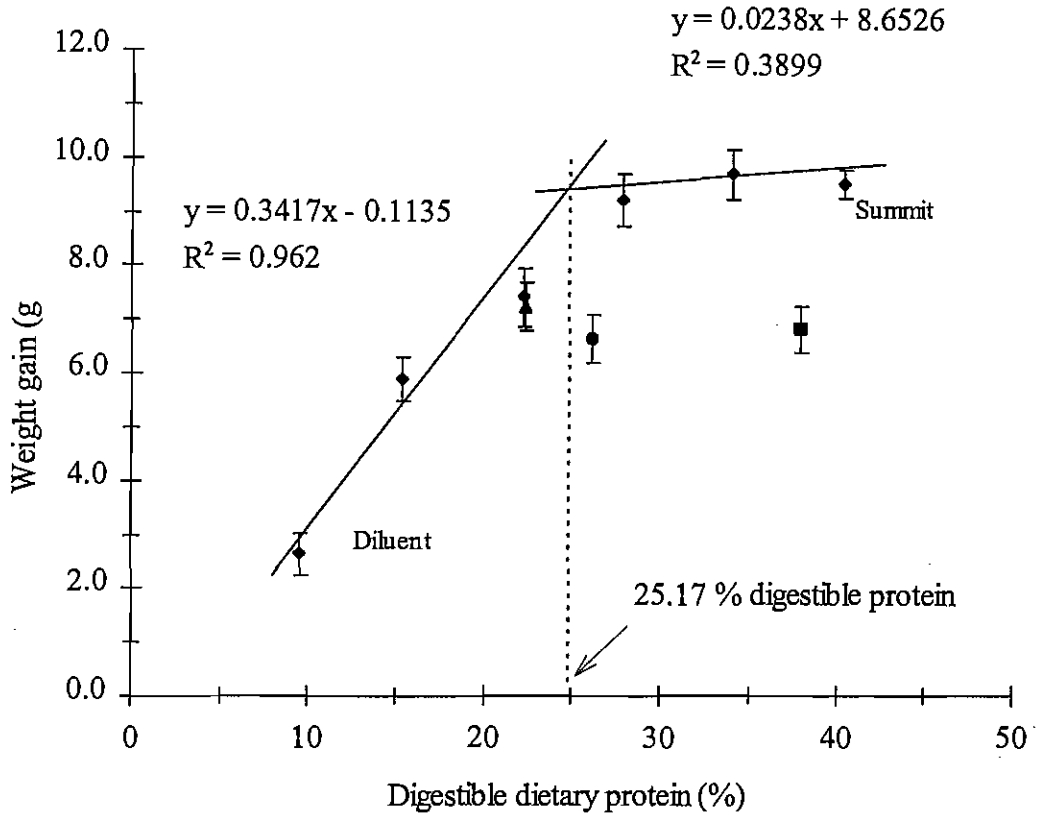

Figure 1a. Weight gain versus dietary digestible protein (%). ◆ = Summit:Diluent series; ▲ = 40S:60D + lysine; ● = 40S:60D + eaa mix (excluding lysine); ■ = Reference diet (SP35).

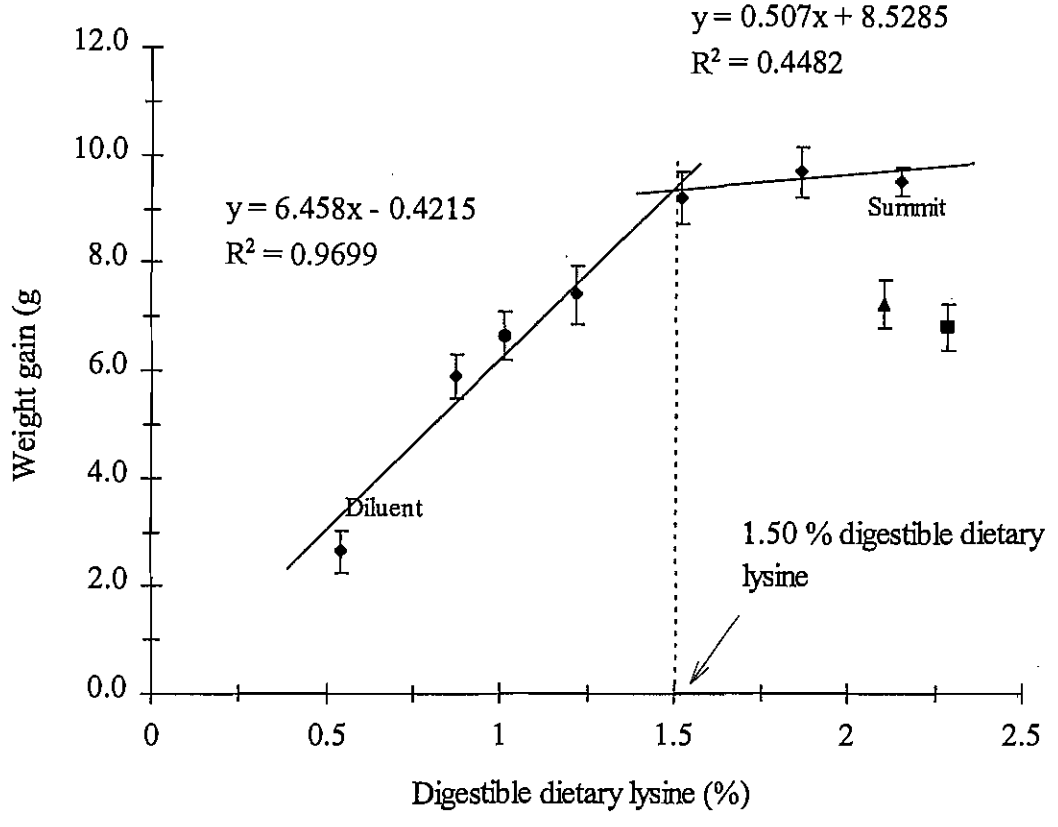

Figure 1b. Weight gain versus dietary digestible lysine (%). ◆ = Summit:Diluent series; ▲ = 40S:60D + lysine; ● = 40S:60D + eaa mix (excluding lysine); ■ = Reference diet (SP35).

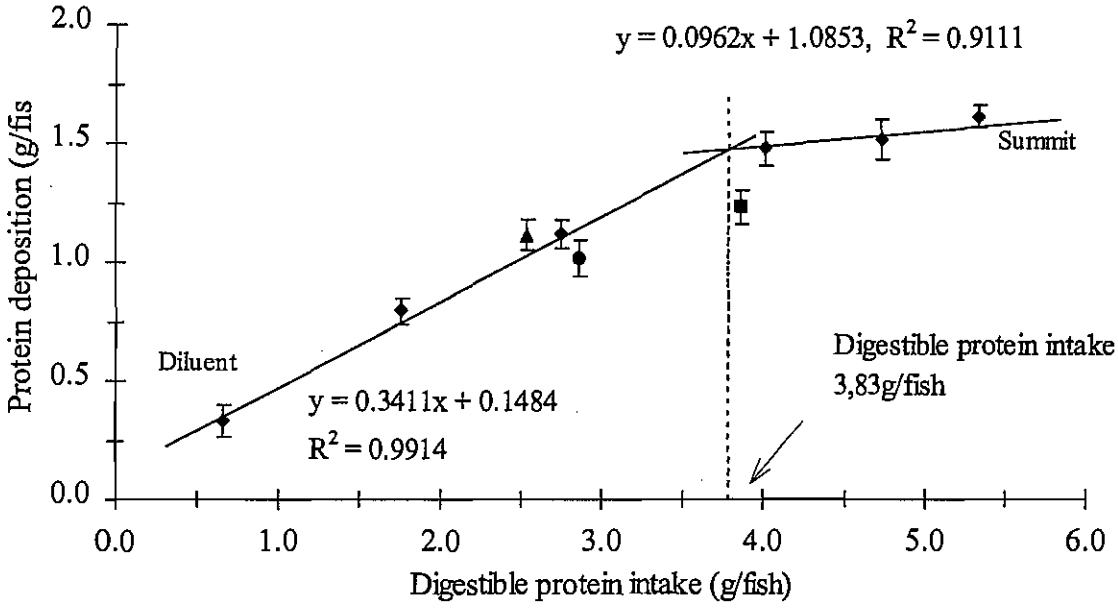

Figure 2a. Protein deposition versus protein intake. ◆ = Summit:Diluent series; ▲ = 40S:60D + lysine; ● = 40S:60D + eaa mix (excluding lysine); ■ = Reference diet (SP35).

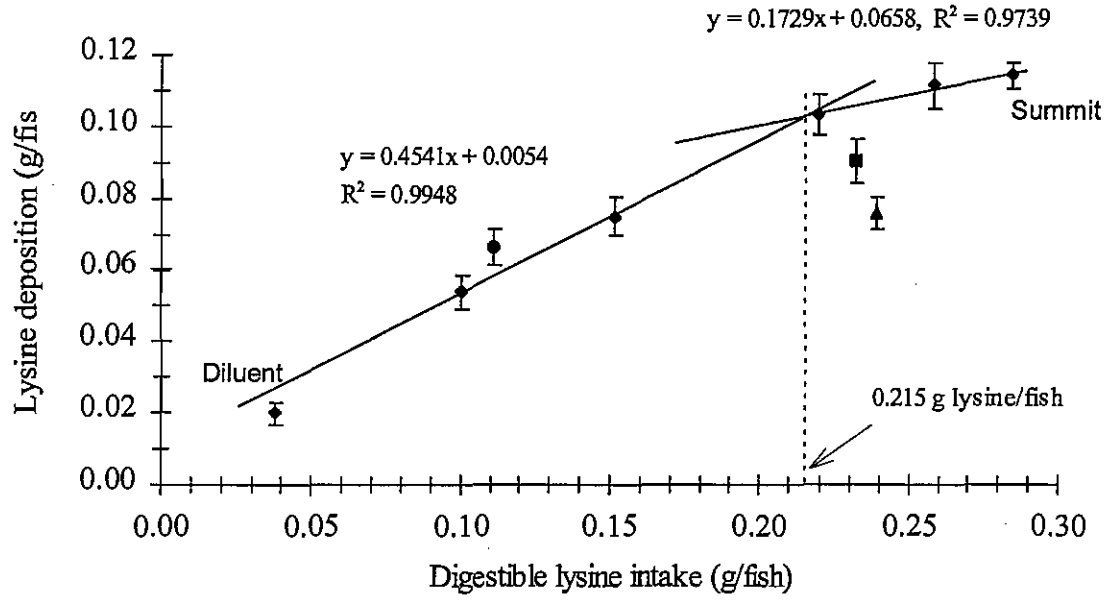

Figure 2b. Lysine deposition versus lysine intake. ◆ = Summit:Diluent series; ▲ = 40S:60D + lysine; ● = 40S:60D + eaa mix (excluding lysine); ■ = Reference diet (SP35).

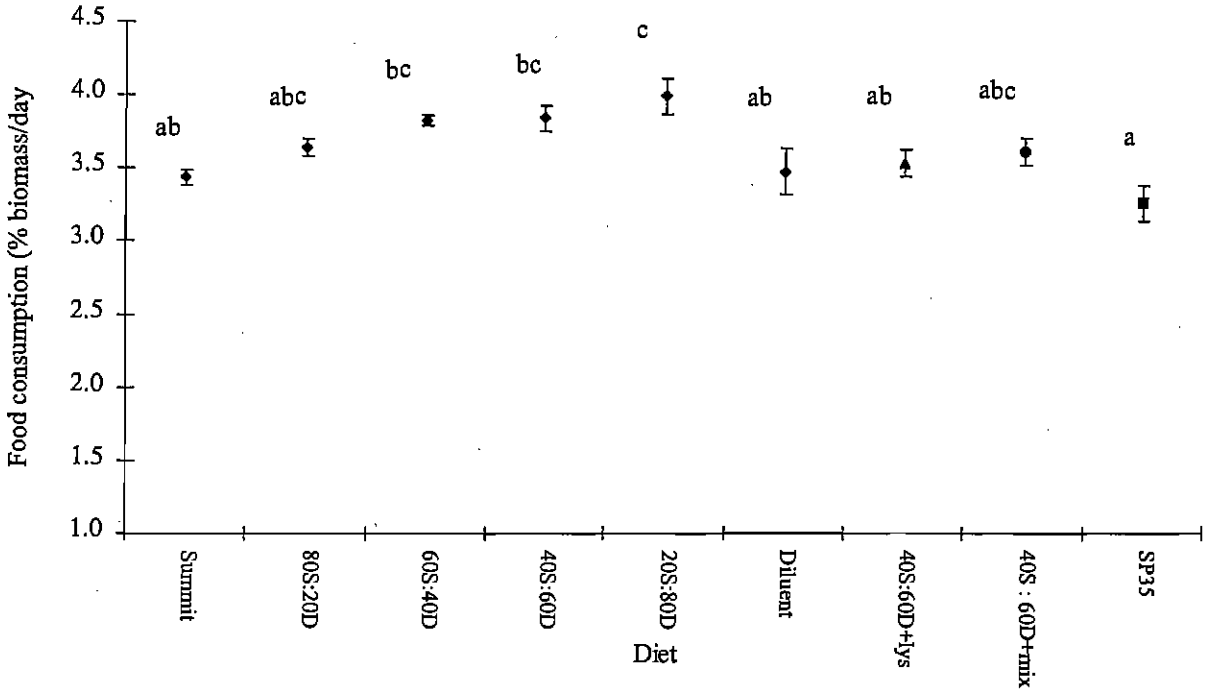

Figure 3a. Average daily food consumption of silver perch. ◆ = Summit:Diluent series; ▲ = 40S:60D + lysine; ● = 40S:60D + eaa mix (excluding lysine); ■ = Reference diet (SP35).

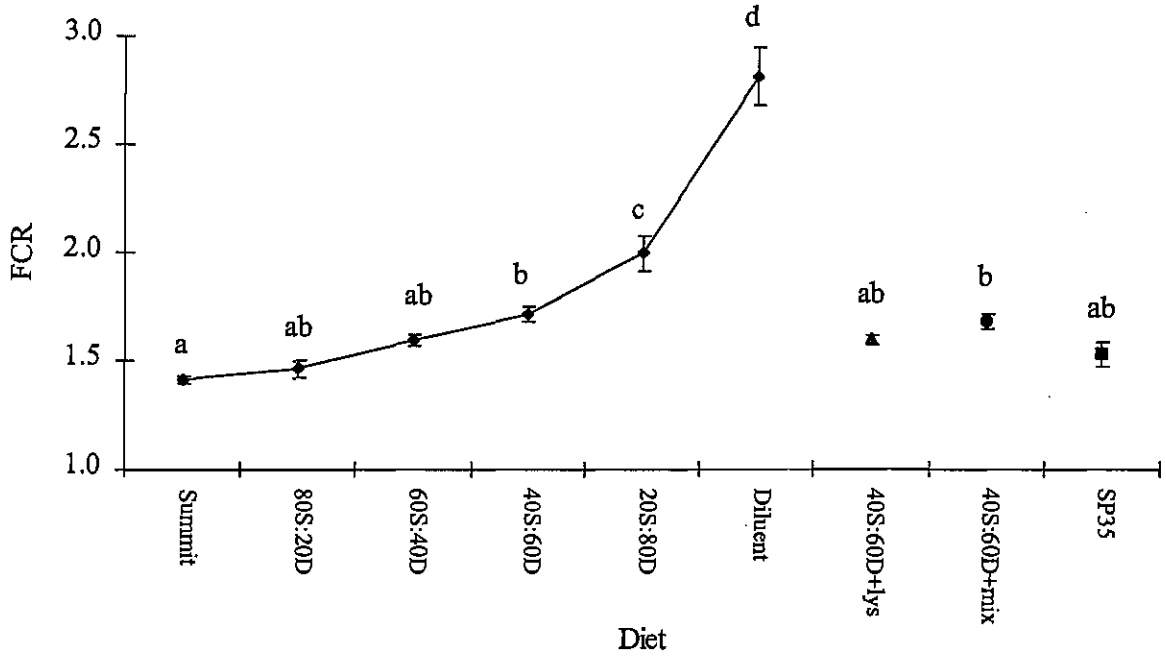

Figure 3b. Food conversion ratio (FCR). ◆ = Summit:Diluent series; ▲ = 40S:60D + lysine; ● = 40S:60D + eaa mix (excluding lysine); ■ = Reference diet (SP35).

6.12 Growth of juvenile silver perch (*(Bidyanus bidyanus*) on modified wheat gluten*

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1. Summary

The replacement of fish meal in aquaculture diets is recognised as a major international research priority. Most aquaculture diets are based primarily on fish meal, although this protein source is expensive, decreasing in availability and mostly imported into Australia.

Wheat gluten meal is a highly digestible source of protein to silver perch (*Bidyanus bidyanus*) and has been used as a binder in aquaculture diets for a number of species. Its use as a protein source (at levels above about 5%) has been limited by strong agglutinating properties and a price of about $3000/t. Modified wheat gluten products with reduced agglutinating properties have been produced by the Academy of Grain Technology with an estimated price of $350/t. Such products could be produced in abundance, and if the agglutinating properties could be sufficiently reduced to permit inclusion levels of 30-50% without reducing nutritive value, modified wheat gluten meal would have enormous potential for use in the Australian and international feed industry. The market for aquaculture feeds in Asia is estimated to be approximately 2.6 million tonnes per year.

The aim of the present study was to measure growth of silver perch, a native Australian freshwater fish with great potential for aquaculture, fed diets based on modified wheat gluten meals. The first task in evaluating protein sources is to assess their digestibility for target species. Digestibility of traditional wheat gluten meal fed to silver perch was evaluated and for the purpose of this study, digestibility of the modified wheat gluten meals was assumed to be similar. Three diets were formulated. The first was a reference diet used in previous research with silver perch, and the other two had similar nutritional composition (on a digestibility basis) but all the fish meal and soybean meal in the reference diet was replaced with either modified wheat gluten meal type 1 (WG1) or type 2 (WG2). The two types of modified wheat gluten meals differed in the processing used during their manufacture to reduce agglutination.

A second aspect of the study was to compare the effects of evaluating fish performance on diets using replicate aquaria containing single fish with replicate aquaria containing groups of eight fish.

Silver perch performed well on diets where all the fish meal and soybean meal were replaced with modified wheat gluten meal type 1. The difference between growth of fish fed the reference diet and the diet with modified wheat gluten meal Type 1 was 7%, and this difference was not significant (results from two-factor ANOVA). Similar results were apparent for specific growth rates. Growth of fish fed the diet with modified wheat gluten meal type 2 was 30% less than growth of fish for the reference diet, and 24% less than growth of fish fed the diet with modified wheat gluten meal type 1. These differences were significant ($P<0.05$). Food conversion ratio was best (lowest) for fish fed the reference diet ($P<0.05$), and

* This report formed part of the Final Report (ISBN 0 7310 6400 3) to the Academy of Grain Technology on research that commenced October 1994 and was completed by December 1994. The project was funded by NSW Fisheries, Fisheries Research and Development Corporation and the Academy of Grain Technology.

although it was higher for the fish fed the diet with modified wheat gluten type 2 compared with the diet with modified wheat gluten type 1, the difference between these two diets was not significant ($P>0.05$).

The experimental power of comparison between diets made with fish stocked with 8 fish/aquarium was much greater (>0.99) than with single fish in each aquarium (about 0.24). On this basis, comparison between diets using similar facilities to those described should contain groups of fish rather than single fish.

Further research to develop and evaluate modified wheat gluten meal is recommended. The focus should be on further reducing agglutinating properties while retaining nutritive value. Protein damage due to processing effects, especially through heating, should be minimised.

2. Background

Wheat gluten meal is a high protein ingredient often used as a binder in aquaculture diets (Lovell, 1989). It has not been used as a protein source for two reasons; firstly it is usually too expensive (typically about AUD$3 000/t) and secondly, its agglutinating properties prohibit inclusion above about 5%. Recently, the Academy of Grain Technology has been developing modified wheat gluten products which are significantly cheaper (approximately AUD$350/t) and which have had much of their agglutinating properties destroyed. If, as hoped, these products have a similar protein content to traditional wheat gluten (around 77%), a price of AUD$350/t, and their inclusion level is unrestricted by agglutination, modified wheat gluten products could be very attractive protein sources for aquaculture diets.

One of the major factors limiting the expansion of aquaculture is the development of nutritionally adequate, cost-effective diets. Feeds and feeding can contribute up to 70% of the total operating costs for fish and shrimp farms (Wee, 1992). The most expensive component of pelleted feeds is protein, of which 25-55% is required, depending upon whether the species is herbivorous, omnivorous or carnivorous (Lovell, 1989; NRC, 1993). The major protein source for most aquaculture diets is fish meal (Lovell, 1989) and formulated diets can contain up to 60% fish meal (New, 1991; Wee, 1992).

There are, however, some major problems with fish meal. Fish meal and fish oil production is declining (Barlow, 1989). The aquaculture feed industry currently uses more than 3 million tonnes of the global fisheries catch (New and Wijkstrom, 1990) excluding 'trash fish' fed directly to aquaculture species. As aquaculture production increases, demand for fish meal will also increase, inevitably forcing prices to rise. As higher quality fish meal is generally required for aquaculture feeds, species of fish currently used for human consumption will increasingly be targeted by fish meal manufacturers. In Malaysia, much of the cheap fish which was used to produce salted fish for human consumption is now used as aquaculture feed instead (New, 1991).

Apart from a relatively small quantity of fish meal produced in Tasmania during a limited period each year, very little fish meal is produced in Australia (Foster, 1992) and most required for aquaculture feeds is imported (ABARE, 1991). Imported fish meal varies in quality and prices in Australia have risen to about AUS $1100/tonne for high quality Danish fish meal. Improved growth and food conversion efficiency have been recorded for salmonids when low-temperature fish meals have been used. These special 'aquaculture grade' fish meals are more expensive than ordinary fish meal, some by as much as 35% (Foster, 1992).

If Australian aquaculture is to develop, suitable alternatives to fish meal must be found.

The need to replace fish meal in aquaculture diets is recognised as a major international research priority (Manzi, 1989; New, 1991) and was recognised as one of the major challenges facing aquaculture nutrition researchers at the Aquaculture Nutrition Workshop held in 1991 (Allan and Dall, 1992).

Australian agriculture has much to gain from developing new products for use in aquaculture feeds and from selling existing products in this market. Forecasts of the world's aquaculture feed production for the year 2000 range from a projected 3.5 million tonnes (New, 1991) to 6.6 million tonnes (Akiyama, 1991). By far the largest consumer is the Asian region, with a market estimated at 2.6 million tonnes in 1990 (Akiyama, 1991). New and Csavas' (1993) estimate is more conservative, predicting an Asian market of 2.6 million tonnes by 2000. This market is expanding, and will continue to expand rapidly.

The push throughout Asia to increase aquaculture production is leading to a much greater demand for formulated feeds. This is evident in the much greater increase in the production of aquaculture diets than in production of fish and crustaceans from aquaculture. Between 1986 and 1990, production of aquaculture feeds increased more than four fold (Akiyama, 1991). The aquaculture feed market could offer an outlet for tens or even hundreds of thousands of tonnes of Australian products if these are shown to be well utilised by fish and crustaceans and are competitively priced.

Australian feed manufacturers also have the opportunity to enter the rapidly expanding aquaculture feeds market. Although Asian fish and crustacean feed manufacturing technology is currently at the forefront of international feed development, Australian companies could access this market if low cost ingredients could be produced from Australian agricultural products. This would require appropriate formulations and rigorous evaluation of diets. The development of new technology to improve the digestibility of Australian agricultural products to fish and the manufacture of new protein or amino acid supplements could give Australian feed manufacturing companies significant advantages over rival overseas companies. Value adding to our agricultural products by combining them into high value exportable aquaculture diets could greatly increase export earnings.

A large number of studies using different species and ingredients have already been conducted. The majority have investigated the potential of soybean meals or soybean products to replace fish meal (eg Smith et al., 1988; Dabrowski et al., 1989; Shiau et al., 1989; Mohsen and Lovell, 1990; Balogun and Ologhobo, 1989; Lim and Dominy, 1990) because of the excellent amino acid profile of soybeans. Other studies have investigated a range of different products including rapeseed meal (Smith et al., 1988; Davies et al., 1990), cottonseed meal (Robinson and Brent, 1989; El-Sayed, 1990), mustard oil cake, linseed and sesame meals (Hossain and Jauncey, 1989a, 1989b) and other less common vegetable proteins (Martinez-Palacios et al., 1988; Olvera-Novoa et al., 1990). Unfortunately, many of these studies have been conducted on an *ad-hoc* basis and, with the exception of channel catfish, very little systematic research has been conducted for warmwater species. Although the first task in evaluating the potential use of a feed ingredient is to assess its digestibility (Cho et al., 1982), digestibility of alternative protein sources to fish meal has not been determined for many warmwater species apart from catfish (Halver, 1989; NRC, 1993). The measurement of digestibility involves measuring the amount of energy, or a specific nutrient such as protein or fat, which is ingested, and subtracting the amount remaining in the faeces. For highly digestible ingredients like fish meal, very little energy or specific nutrients remain in the faeces. In terms of digestibility to fish, fish meal is generally superior to terrestrial protein sources, which are in turn superior to vegetable protein sources (Lovell, 1989). Fish do not have well developed mechanisms to digest the large amounts of carbohydrate or fibre often present in vegetable protein ingredients (New, 1987), although omnivorous or herbivorous species are more capable of utilising carbohydrates than carnivorous species.

If digestibility of ingredients is not considered when diets are formulated to compare different ingredients, the different diets may vary considerably in the digestible energy levels and in the amounts of specific nutrients (eg protein) actually available to the fish.

Digestibility of traditional wheat gluten meal was evaluated for silver perch (*Bidyanus bidyanus*)(Allan et al., unpublished data, see Section 6.2 of this report). Dry matter, energy, protein and essential amino acid digestibility coefficients were all similar or superior to fish meals (including high quality low temperature fish meal), indicating that wheat gluten meal is well digested by fish. Compared with fish meal, however, wheat gluten meal is deficient in lysine, arginine, threonine and methionine. No published information was found which evaluated the effects of using wheat gluten as a major protein source in fish diets. In this study we used previously obtained digestibility information for wheat gluten meal to design two wheat gluten meal diets for silver perch which we compared with our successful reference diet (Allan et al., unpublished data, see Section 6.1 of this report). The two wheat gluten diets contain different modified wheat gluten products.

In the past, growth of fish in small (70 l) aquaria was variable within aquaria and within replicates for a single treatment. We suspected behavioural interactions among the fish (commonly 6-10 per aquarium) may have increased this variation. In larger tanks (eg.10 000 l) with more fish (>50), schooling behaviour was more evident and fish were noticeably less aggressive. A second aim of this study was to compare growth of single fish with the means of groups of eight fish and to investigate if there was an interaction between the type of diet (reference, wheat gluten type 1 or wheat gluten type 2) and number of fish (1 or 8/aquarium).

3. Objectives

3.1 To evaluate growth and food conversion efficiency of silver perch in 70 l aquaria fed diets based on:

a fish meal and soybean meal (silver perch reference diet)
b modified wheat gluten type 1, or
c modified wheat gluten type 2

3.2 To recommend a strategy for further research to evaluate the potential use of modified wheat gluten products in aquaculture diets.

3.3 To compare growth of fish in aquaria containing one fish with that in aquaria containing eight fish and to investigate interactions between type of diet and number of fish/aquarium.

4. Methods

4.1 Modified wheat gluten products and experimental diets

Two modified wheat gluten meal products were provided by Dr Michael Wootton, Academy of Grain Technology. They differed in the processing used to destroy the agglutinating properties. (For more information on the products supplied please contact Dr Michael Wootton, C/- Academy of Grain Technology.)

Previous research had been carried out to determine the digestibility of traditional wheat gluten meal to juvenile silver perch in laboratories at NSW Fisheries, Port Stephens Research Centre (Table 1). This information was used to formulate both modified wheat gluten diets. Ingredients and calculated composition of experimental diets are presented in Table 2.

All ingredients were ground using a laboratory hammer mill (Jones and Rickard Pty Ltd, Waterloo, NSW, 2017) and sieved to exclude particles >710 μm. Dry ingredients were then thoroughly mixed in a Hobart mixer (Troy Pty Ltd, Ohio, 45374, USA), combined with fish oil and approximately 400 ml water/kg dry mix and then pelleted through a meat mincer (Barnco, Australia Pty Ltd, Leichhardt, NSW, 2040) with a 2 mm diameter die. Pellets were dried at <35°C in a convection dryer for about 6 hours until the moisture content was between 20 and 30%.

4.2 Experimental fish

Juvenile silver perch (mean initial stocking weight 6.0g; range 5.0-7.0g) were stocked into aquaria. Mean initial weights were similar for all treatments (P=0.3). The fish were bred by Dr Stuart Rowland at the NSW Fisheries, Grafton Research Centre. Fish were anaesthetised using a bath of 25 mg/L ethyl p-aminobenzoate for 5 minutes and then weighed individually and distributed to aquaria (1 or 8 fish/aquarium) by systematic interspersion.

4.3 Experimental facilities and procedures

Experimental aquaria were 70 L acrylic aquaria fitted with a 12 mm stand pipe. Continuously-flowing, preheated water (mean 26.0°C; range 23.4-27.0°C) was filtered through a diatomaceous earth filter then passed through a UV steriliser before being supplied to aquaria at a flow-rate of 240 mL/min. Effluent water from all aquaria was collected, combined and approximately 75% was recirculated through a 3 m^3 biological filter, a diatomaceous earth filter, a UV steriliser and was then re-used.

Each aquarium was aerated using two air-stone diffusers. Fish were stocked and fed the reference diet for five days prior to the start of the experiment. This acclimation period was provided to allow replacement of fish which died or were damaged following stocking. During the experiment, fish were fed to apparent satiation twice daily at about 0830 and 1600 h. Feed rates were adjusted on the basis of consumption. Exactly 5 minutes after each aquarium was fed in the morning, the number of uneaten pellets was counted. If no pellets were left, feed rates for that aquarium for that day were increased by 0.05 g for aquaria with single fish and 0.4 g for aquaria with 8 fish. If pellets remained, these were removed and feed rates were decreased by that amount.

Water quality was monitored weekly. Temperature, dissolved oxygen, pH, nitrite and ammonia were measured as described by Allan et al. (1990). Dissolved oxygen remained above 6.5 mg/L (mean se 7.4±0.08; range 6.5-8.6 mg/L) pH was 8.3±0.03 (mean ± se; range 8.1-8.6), nitrite remained below 0.02 mg NO_2-N/L and ammonia below 0.02 mg NH_3-N/L.

The experiment was run for 42 days, after which time fish were removed from each aquarium, weighed individually and results used to calculate weight gain, weight gain per day and specific growth rate ((In final weight - In initial weight)/time [day] x 100; Hopkins, 1992). The total amount of feed used in each

aquarium was determined and used to calculate food conversion ratio (amount of dry feed/ amount of wet weight gain of fish).

4.4 Statistical procedures

For each diet (3) x number of fish (2) treatment combination (6 in total) 12 replicate aquaria were provided (72 aquaria in total). Data for initial fish weights, weight gain, specific growth rate and food conversion ratio were analysed using two-factor ANOVA with diet as Factor A (3 levels) and number of fish/aquarium as Factor B (2 levels). Both factors were assumed to be fixed.

Single-factor ANOVA's were run with fish stocked at 8 fish/aquarium to investigate effects of diet on weight gain, specific growth rate and food conversion ratio.

The statistical power of separate single-factor ANOVA's to investigate effects of diets on weight gain, was calculated (Searcy-Bernal, 1994) for single fish/aquarium and for 8 fish/aquarium.

For all analyses, homogeneity of variance was assessed using Cochran's Test (Winer, 1971) and multiple comparison among means, where significant differences were identified using ANOVA, were made using Student-Newman-Keuls test (SNK) (Sokal and Rohlf, 1982).

5. Results

For all indices - weight gain, specific growth rate and food conversion ratio - fish fed the reference diet performed better than fish fed either of the modified wheat gluten meal diets. There was also a clear deterioration in performance of fish fed the modified wheat gluten meal type 2 diet compared with the type 1 diet. Results from the two-factor ANOVA's are presented in Table 3. For these analyses, weight gain of fish fed the reference diet and the diet containing the modified wheat gluten product type 1 was not significantly different ($P>0.05$). The values for specific growth rate follow the same pattern, although for this analysis it should be noted that variances were heterogeneous so results should be interpreted cautiously.

Food conversion ratio data for the two-factor ANOVA were also variable and again variances were heterogeneous. It was apparent, however, that food conversion ratio was poorer for fish fed wheat gluten diets than those fed the reference diet. For this index the number of fish in each tank significantly affected food conversion ratio with single fish more efficiently converting food ($P<0.001$). Single factor ANOVA's run with data from 8 fish/aquarium indicated that significant ($P<0.05$) differences existed between all diets for each indice.

Results of the single factor ANOVA's conducted on weight gain of single fish indicated that effects of diet were not significant (Table 4), although the power of this analysis was only about 0.24. This is considered low (Searcy-Bernal, 1994). Conversely, in the analysis of aquaria which contained eight fish, weight gain of fish fed all diets were significantly different from each other ($P<0.05$) (Table 5). The power of this test, where the average weight gain for eight fish was used to generate an 'aquarium mean', was much higher (>0.99).

6. Discussion

The results for this experiment indicate that wheat gluten protein is suitable for silver perch diets and that even with all fish meal and soybean meal replaced with wheat gluten meal, fish performance was still acceptable. At $350/t, wheat gluten meal type 1 as evaluated here is a very attractive protein source. The cost per unit digestible protein to silver perch ($0.46/kg digestible protein) is much less than any other commonly available protein source (Table 6). The relatively low content of lysine in wheat gluten meal is reflected in a cost of $20.30/kg digestible lysine which is higher than for some oilseed meals, grain legumes and meatmeal but lower than for fish meal ($22.90-$24.20/kg digestible lysine) (Table 6).

The digestible amino acid profile for traditional wheat gluten meal is presented in Table 6.2. Although amino acid requirements for silver perch have not been described compared with fish meal, wheat gluten meal is deficient in lysine, arginine, threonine, methionine and valine. Deficiencies in lysine, methionine and threonine might be overcome with the addition of synthetic amino acids, although the efficacy of these products in fish diets is the subject of some debate (Lovell, 1989; Cowey, 1992; Murai, 1992); they are also expensive.

The two types of modified wheat gluten meal used in this study differed in the degree to which agglutination was reduced. In type 1, agglutination was reduced compared with traditional wheat gluten meal, although it was still significant enough to prevent large-scale processing with inclusion levels above about 15-20%. This product, however, gave significantly better fish performance than the other product, type 2, where agglutination was further reduced allowing somewhere between 20-25% to be included in large-scale processing using facilities such as those used in this experiment. The inclusion level of 32% for modified wheat gluten used in the diets here was only achieved by pelleting a number of very small batches. When more than about 200 g for wheat gluten meal type 1 or 300 g for wheat gluten meal type 2 was mixed and pelleted at one time, the agglutination caused by the meals prevented the dough from passing through the meat mincer. Clearly, further reduction of agglutination is necessary before inclusion levels above about 10-15% could be recommended for commercial diet manufacture. In addition, the process used to reduce agglutination in wheat gluten meal type 2 compared with wheat gluten meal type 1 also significantly reduced its nutritive value to silver perch.

The comparison of single fish per aquarium with eight fish per aquarium clearly demonstrated the importance of using groups of fish rather than single fish. The power of the comparison with groups of eight fish was much higher (>0.99) than with single fish (about 0.24). This resulted from minimising variation within each aquarium by calculating an average weight for a group of fish rather than using the weight for a single fish.

Further research into modified wheat gluten products is warranted. Future product development and evaluation should concentrate on keeping cost of production as low as possible (eg $350/t) and on reducing agglutination without affecting nutritive value. (Minimising heat damage during processing is important.) Any new products should be evaluated specifically for aquaculture species. The first step should be an assessment of digestibility. For the products evaluated in this study, a digestibility study may have identified processing damage to amino acids resulting in reduced digestibility and helped explain the reduction in the nutritive value of the modified wheat gluten type 2 compared with type 1. Following digestibility determination, a series of long experiments with fish fed balanced diets with different contents of the best wheat gluten meal products would assist with evaluating commercial potential for these products. Feed manufacturers should be involved as early as possible to ensure modified wheat gluten meals can be processed using commercial feed manufacturers' equipment.

7. Conclusions and Recommendations

a Modified wheat gluten products are suitable for use in silver perch diets, although the degree of processing affects their nutritive value.

b The diet based on the modified wheat gluten product type 1 was significantly better at promoting fish growth than the diet based on the modified wheat gluten product type 2.

i When all fish meal and soybean meal was replaced with modified wheat gluten type 1, weight gain was reduced by 7% ($P>0.05$, two-factor ANOVA; Table 5.1).

ii When all fish meal and soybean meal was replaced with modified wheat gluten meal type 2, weight gain was reduced by 24% ($P<0.05$; two-factor ANOVA; Table 5.1).

c Experiments of this kind should be conducted with groups of fish rather than single fish.

d Food conversion efficiency was reduced when fish were fed diets with all the fish meal and soybean meal replaced by modified wheat gluten products. Food conversion ratio was poorer for the fish fed the diet containing the modified wheat gluten meal type 2.

e Modified wheat gluten products may be very cost-effective sources of protein. Future research should focus on developing products at similar cost ($350/t) to that estimated for the products tested here, but with reduced agglutinating properties and intact nutritive value. Heat damage during processing should be minimised.

f Evaluation of modified wheat gluten products should continue for use in aquaculture diets. The first step should be evaluation of digestibility to target species, followed by growth studies using balanced diets with different contents of modified wheat gluten products.

8. Acknowledgments

I would like to thank Dr Michael Wootton, Academy of Grain Technology, for his efforts in producing the product used here and in supporting this study. This research could not have been completed without expert technical assistance from Mr Scott Parkinson, Ms Rebecca Warner-Smith, Mr David Stone and Ms Jane Frances from the Port Stephens Research Centre. I also thank Ms Jo Pickles for preparing the manuscript.

Partial funding for this study was provided from the Academy of Grain Technology through the Fisheries Research and Development Corporation Sub-Program on Fish meal Replacement in Aquaculture Diets. I would like to thank Mr Peter Dundas-Smith and Mr Simon Prattley (Fisheries Research and Development Corporation) for their support for this Sub-Program. Funding for complimentary research was also provided by the Australian Centre for International Agricultural Research; Mr Barney Smith's assistance as Fisheries Program Coordinator is gratefully acknowledged.

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Table 1

Digestible dry matter, digestible energy, and digestible nutrients of wheat gluten meal (traditional) fed to juvenile silver perch[1]

Digestible dry matter (%)	97.4
Digestible energy (MJ/kg)	23.1
Digestible crude protein (Nx6.25) (%)	76.9
Digestible arginine (%)	3.43
Digestible histidine (%)	1.99
Digestible isoleucine (%)	3.67
Digestible leucine (%)	6.59
Digestible lysine (%)	1.72
Digestible methionine[2] (%)	3.19
Digestible phenylalanine[3] (%)	8.32
Digestible threonine (%)	2.44
Digestible valine (%)	3.76

1 Composition of wheat gluten multiplied by experimentally determined digestibility coefficient

2 Methionine plus cystine

3 Phenylalanine plus tyrosine

Table 2

Ingredient and composition of experimental diets

	Experimental diets (% as is)			Assumed ingred.cost
	1 (reference)	2[1]	3[1]	(AUD $/t)
Ingredient				
Fish meal	27.00	-	-	1 300
Soybean meal	20.00	-	-	495
Modified wheat gluten[1]	-	32.00	32.00	350
Bloodmeal	2.00	2.00	2.00	987
Corn gluten meal	4.00	4.00	4.00	700
Wheat (ASW)	26.85	34.70	34.70	147
Sorghum	11.00	11.00	11.00	180
Millrun	2.00	2.00	2.00	165
Fish oil	1.00	4.50	4.50	2 000
Di-calc phosphate	2.00	3.70	3.70	610
Vitamin/mineral premix	4.00	4.00	4.00	6 250
DL-methionine	0.15	0.30	0.30	5 500
L-lysine	-	1.80	1.80	4 250
Total ingredient cost (AUD$/t)	851	689	689	
Composition (dry basis)				
Ash (%)	9.8	5.9	6.0	
Digestible crude protein (%)	39.6	27.0	31.2	
Digestible energy (MJ/kg)	19.0	19.7	19.7	
Fibre (ADF) (%)	3.2	4.5	3.9	
Crude fat (%)	4.1	6.6	5.9	
Digestible arginine	2.6	1.3	1.5	
Digestible histidine	0.8	0.4	0.5	
Digestible isoleucine	1.6	0.8	1.0	
Digestible leucine	3.2	2.0	2.3	
Digestible lysine	2.6	1.7	1.9	
Digestible methionine[2]	1.7	1.4	1.7	
Digestible phenylaline[3]	2.9	1.9	2.2	
Digestible threonine	1.4	0.7	0.9	
Digestible valine	2.0	1.1	1.3	

[1] The two wheat gluten diets contained either modified wheat gluten type 1 or modified wheat gluten type 2

[2] Methionine plus cystine

[3] Phenylalanine plus tyrosine

Table 3

Weight gain, specific growth rate and food conversion ratio for fish fed one of three diets (reference, a diet based on modified wheat gluten type 1 or a diet based on modified wheat gluten type 2) and stocked with either 1 fish/aquarium or 8 fish/aquarium for 42 days.

Level	Count	Weight gain[1] g/fish	SGR[1,2] %/day	FRC[1,3]
Grand mean	72	7.4±0.3	1.84±0.06	2.14±0.05
Diet				
Reference	24	8.4±0.6[a]	2.04±0.11[ab]	1.76±0.09[a]
Wheat Gluten 1	24	7.9±0.6[a]	1.94±0.11[ab]	2.21±0.09[a]
Wheat Gluten 2	24	5.9±0.6[b]	1.55±0.11[b]	2.46±0.08[a]
Fish number				
1/aquarium	36	7.2±0.5	1.75±0.09	1.89±0.08[a]
8/aquarium	36	7.6±0.5	1.93±0.09	2.39±0.08[a]
Diet x fish number				
Reference 1 fish/aquarium	12	7.9±0.8	1.92±0.15	1.59±0.12
Reference 8 fish/aquarium	12	8.9±0.8	2.15±0.15	1.92±0.12
Wheat Gluten 1 1 fish/aquarium	12	7.8±0.8	1.90±0.15	1.92±0.12
Wheat Gluten 1 8 fish/aquarium	12	7.7±0.8	1.98±0.15	2.49±0.12
Wheat Gluten 2 1 fish/aquarium	12	5.8±0.8	1.44±0.15	2.16±0.12
Wheat Gluten 2 8 fish/aquarium	12	6.0±0.8	1.66±0.15	2.76±0.12

1 Values are means ± pooled standard errors. Interactions between diet and fish number were non-significant (P>0.05) for each index (two-factor ANOVA). Where differences between diet (all indices) or fish number (FCR only) were significant, means were allocated a different letter in the superscript. Variances for SGR and FCR were heterogeneous and to reduce chances of type 1 errors differences were only considered significant if P 0.01.

2 SGR = Specific growth rate (In final weight - In initial weight/days x 100)

3 FCR = Food conversion ratio (weight food used dry matter/wet weight gain of fish)

Table 4

Summary of weight gain data and analysis of variance table for aquaria containing single fish fed the reference diet, a diet based on modified wheat gluten meal type 1 or a diet based on modified wheat gluten meal type 2 (single-factor ANOVA)

a Data summary

Diet	Mean	Standard deviation	Standard error
Ref	7.91	3.90	1.13
WG1	7.83	3.65	1.05
WG2	5.81	3.95	1.14

b Analyses of variance table

Source of variation	Sum of squares	df	mean sq	F. ratio	P
Between groups	33.94	2	16.97	1.16	0.33
Within groups	484.82	33	14.69		
Total	518.75	35			

Table 5

Summary of weight gain data and analysis of variance table for aquaria containing eight fish fed the reference diet, a diet based on modified wheat gluten meal type 1 or a diet based on modified wheat gluten meal type 2 (Single-factor ANOVA)

a Data summary

Diet	Mean	Standard deviation	Standard error
Ref	8.90	1.23	0.36
WG1	7.74	1.21	0.35
WG2	6.01	0.75	0.22

b Analyses of variance table

Source of variation	Sum of squares	df	mean sq	F. ratio	P
Between groups	50.88	2	25.44	21.54	<0.001
Within groups	38.98	33	1.18		
Total	89.86	35			

Table 6
Cost of digestible protein for wheat gluten meal, fish meal and selected vegetable protein sources

Ingredient	Digestible protein	Digestible lysine	Price	Price	
	(%)	(%)	($/t)	$/kg digestible protein	$/kg digestible lysine
Wheat gluten meal[1]	76.9	1.72	350	0.46	20.3
Danish fish meal	68.1	5.23	1200	1.76	22.9
Danish fish meal (low temp)	72.2	6.19	1500	2.08	24.2
Peruvian fish meal	62.0	5.23	750	1.21	14.3
Meatmeal (lamb)	38.1	2.74	453	1.19	16.5
Soybean meal	47.6	2.82	440	0.92	15.6
Canola meal	40.3	2.25	330	0.82	14.7
Peanut meal	39.4	1.56	380	0.96	24.4
Cottonseed meal	41.6	1.49	370	0.89	24.8
Lupins (*Lupinus angustifolius*) hulls on	30.8	1.58	298	0.97	18.9

[1] Digestibility data calculated using traditional wheat gluten (normal retail price approx $3 000/t). Price of $350/t is for modified wheat gluten product (M. Wootton, Academy Grain Technology, pers. comm., 1994). Digestibility experiments need to be conducted with modified products to confirm digestibility is not reduced.

Table 7

Digestible energy and digestible nutrients for low temperature Danish fish meal and meat products compared with requirements for channel catfish (NRC, 1993)

Nutrient	Ingredient		
	Wheat gluten meal	Danish fish meal	Requirements
Digestible dry matter (%)	97.4	91.4	
Digestible energy (MJ/kg)	23.1	21.5	12.6
Digestible protein (%)	76.9	72.2	28.0
Amino acids (g/16 g nitrogen)			
Digestible arginine	4.5	8.1	4.3
Digestible histidine	2.6	2.6	1.5
Digestible isoleucine	4.8	4.6	2.6
Digestible leucine	8.6	7.7	3.5
Digestible lysine	2.2	8.5	5.1
Digestible methionine[1]	4.2	3.0	2.3
Digestible phenylalanine[2]	10.8	7.2	5.0
Digestible threonine	3.2	5.0	2.0
Digestible valine	4.9	5.2	3.0

[1] Including cystine

[2] Including tyrosine

7 BENEFITS

Australia will benefit in four major ways from this project. Firstly, the research will lead to much better, cheaper diets for aquaculture. This will improve the economic viability of aquaculture, and hopefully lead to reduced prices for aquaculture products. This will improve the chance of replacing some of the 90 289 t of fish and fish products imported annually. Secondly, marketing opportunities for Australian agriculture products will be substantially increased, both from an increase in production of aquaculture feeds for the growing Australian industry and as ingredients for aquaculture feeds produced in Asia. The global market for aquaculture feeds is enormous. In Asia, the region where aquaculture is growing most rapidly, the feeds market was estimated at around 26 million tonnes in 1990 and this market grew more than four fold between 1986 and 1990 (Akiyama, 1991). There is a great potential to market Australian agricultural products, including oilseed, grain legumes, other cereal crops and animal protein sources like bloodmeal and meatmeal as ingredients in aquaculture diets Research into cost-effective methods of increasing the value of agricultural products in aquaculture diets, through processing or the addition of enzymes or amino acids will further improve the marketing potential of these products. Thirdly, Australian feed manufacturers will benefit from this research as they will be able to use the results to manufacture better diets The possibility of selling diets in Asia offers major marketing opportunities for dynamic Australian feed manufacturers. Finally, Australian aquaculture research workers will benefit from close interaction with scientists from other disciplines who have much to contribute to this research topic.

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

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8 INTELLECTUAL PROPERTY

The focus is to conduct public domain research so all stakeholders can benefit. Data on digestibility is presented here and will be published as soon as possible and disseminated widely.

Results of comparisons between raw ingredients or those improved through processing or addition of supplements are presented here and will be published although the actual methods of improving ingredients may be subject to intellectual property agreements developed

outside this project. Data on nutrient requirements are presented here and will be published and disseminated widely.

It is not anticipated that any patents or commercial intellectual property will arise from this project.

9 FURTHER DEVELOPMENTS

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

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

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

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

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

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

In Australia, aquaculture will not develop beyond a small scale unless aquaculturists can purchase cheap, efficient feeds. We will not have the luxury of using cheap fish meal to produce these feeds and so must develop viable alternatives. Fortunately, Australia has abundant sources of cheap agricultural proteins and results from the Replacement of Fish meal in Aquaculture Diets Sub-Program have been excellent. Scientists involved with the Sub-Program have developed and validated techniques to determine diet and ingredient digestibility for silver perch, prawns, barramundi and salmon and diets replacing all but 5 or

10% of fish meal with Australian agricultural proteins have been used on an experimental scale without compromising growth. Fish meal alternatives have also been identified for prawns, *Penaeus monodon*. Large scale, commercially relevant trials are underway or planned to validate these results for all these species.

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

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

Results from this project are critically important for two related applications on Aquaculture Diet Development; Nutrient Requirements and Diet Validation and Feeding Strategies. Armed with comprehensive data on ingredient digestibility and growth effects, it is possible to determine the cost of providing different nutrient specifications in formulated rations made from a range of ingredients. This analysis has clearly shown that digestible lysine and methionine plus cystine are the first limiting amino acids and that meeting published requirements for fatty acids is also expensive. Defining these requirements precisely is critical to ensure maximum use can be made of cheaper ingredients which are often deficient in one or more of the essential amino acids. These requirements need to be proven in commercially relevant situations before feed manufacturers or farmers will adopt the results. Diets are a major component of feed costs but feeding practices need to be optimised to lower operating costs. Optimum feeding frequency is also affected by physical characteristics of the diet and, to some extent, by composition.

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

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10 CONCLUSION

Results generated during this project have shown that Australian agricultural ingredients can be successfully used to replace all but 5% of the fishmeal in diets for silver perch.

The formulation of these successful diets have been made available to commercial feed manufacturers and at least one company has produced and marketed diets based on the most successful formulation.

The most promising alternative ingredients to fishmeal include meatmeal, poultry meal, dehulled lupins and dehulled field peas. The nutritional value of plant ingredients is improved by the removal of carbohydrates (and elevation of protein content) through dehulling and removal of starch and non-starch polysaccarides. Meat meal products are improved through the removal of ash (bone). For all ingredients the benefits of improving their nutritional value need to be weighed against the cost of such processes. Poor commercial availability of some ingredients, such as dehulled lupins and field peas and low ash meat meals, will affect the volume of the products used in Australian aquaculture diets.

Information on ingredient digestibility and utilisation has been provided to all interested feed companies and ingredient suppliers. The Grain Pool and the Meat Research Corporation have both used results from this project, and other projects within the Sub-Program, to market ingredients produced by their stakeholders for use in aquaculture diets in south-east Asia.

Grinding particles for silver perch diets to below 710 - 1 000 μm is unnecessary but diets should be cooked using steam conditioning or extrusion.

Requirements for protein and lysine for silver perch are low relative to other species of cultured fish. For diets with approximately 14-15 MJ/kg digestible energy a digestible dietary protein of 25.2% produced equivalent growth to diets with digestible protein contents of up to 40.4%. For 14-15 MJ/kg digestible energy diet, a digestible lysine content of 1.5% was sufficient for optimum growth.

11 ACKNOWLEDGMENTS

Assistance for technical, administrative and financial support is acknowledged separately at the end of each of the sections detailing technical progress. However, the authors would like to acknowledge assistance from a number of people for the entire project. The Fish Meal Replacement Project was initially conceived by Dr Bob Kearney (then Director of Research, NSW Fisheries) and since his resignation has been supported by Dr Rick Fletcher, Director of Research (NSW Fisheries) The continuing support from the Fisheries Research and Development Corporation, especially from the Executive Director, Mr Peter Dundas-Smith,

Programs Manager, Mr Patrick Hone and Projects Administrator, Ms Annette Lyons, is gratefully acknowledged. Financial support from the Australian Centre for International Agricultural Research (especially from Fisheries Program Coordinator Mr Barney Smith), The Grains Research and Development Corporation (especially Mr Russell Hilton) and the Meat Research Corporation (especially Mr Jon Marlow, Mr Dennis Roberts and Mr Michael Perry) has been crucial to the success of this project.

Ms Helena Heasman has done an outstanding job helping with the coordination of the Fish Meal Replacement Sub-Program Final Reports (silver perch, prawns, barramundi, feed processing and *in vitro* digestibility) and with helping coordinate the new Aquaculture Diet Development Sub-Program. We would also like to thank all the staff at the NSW Fisheries, Port Stephens Research Centre and the NSW Fisheries, Grafton Research Centre who have assisted with research on the project. We would also like to acknowledge the outstanding contribution to research made by Australian Native Fish Pty Ltd through their contract with NSW Fisheries. Without their support for this research the large scale feeding experiments described here could not have been done.

Feed manufacturing companies, Ridley Agriproducts Pty Ltd and Janos Hoey Pty Ltd assisted with manufacturing of the experimental diets and with supplying some ingredients.

12 STAFF

The following staff were employed at NSW Fisheries to work on the FRDC and/or the ACIAR project:

Name	Position	Highest Qualification	% Time
Geoff Allan[1]	Principal Aquaculture Scientist	PhD	40
Stuart Rowland[2]	Scientist	PhD	10
Jane Frances[1]	Fisheries Technician	BSc	50
David Stone[1]	Fisheries Technician	BSc (Hons)	100
Paul Robertson[2,3]	Fisheries Technician	HSC	100
Justin Shipman[2,3]	Fisheries Technician	HSC	100
Scott Parkinson[1,4]	Fisheries Technician	Dip.App.Sci.	100
Mark Booth[1,4]	Fisheries Technician	BSc (Hons)	100
Rebecca Warner-Smith[1]	Fisheries Technician	Biol.Tech. Cert.	50

1 Employed at NSW Fisheries, Port Stephens Research Centre
2 Employed at NSW Fisheries, Grafton Research Centre
3 Justin Shipman replaced Paul Robertson when Paul resigned
4 Mark Booth replaced Scott Parkinson when Scott resigned

APPENDIX 13.1

Alternative feed ingredients for intensive aquaculture

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Summary

The rapid expansion in aquaculture has accelerated the search for alternatives to feed ingredients of marine origin. Some Australian agricultural ingredients are already widely used in aquafeeds and considerable potential exists to increase their use both domestically and overseas. Plant breeding programs to reduce concentrations of anti–nutritional factors and increase essential nutrients, and processing to remove less digestible components such as fibre and carbohydrates will improve the usefulness of vegetable ingredients. For animal by–products, reducing ash and saturated fat levels and improving and standardising processing conditions will increase the scope for use of these products. For all ingredients the use of attractants, synthetic amino acids and enzymes offers the potential for formulation of successful aquaculture diets with reduced reliance on marine ingredients.

Introduction

Demand for seafood is escalating as global population rises and the popularity of seafood increases (Anon., 1994, Liao, 1996). Production from capture fisheries is declining and aquaculture offers the only chance to meet this demand. Aquaculture production has risen from 15% of total fisheries production in 1989 to 22% in 1993 (Liao, 1996).

A shift to more intensive culture practices, made possible by the availability of better, formulated diets, has been partly responsible for the increase in aquaculture production. From 1986 to 1990, Akiyama (1991) estimated that demand for aquafeeds in Asia increased more than four–fold and New and Csavas (1993) predicted the Asian aquafeed market would reach 2.6 million tonnes by the year 2000.

Marine based ingredients, especially fishmeal and fish oil, are preferred protein and energy sources as they provide high levels of essential amino and fatty acids, are low in carbohydrates and are well digested and utilised. However, production of fishmeal already uses approximately 33% of the total global fish catch and the proportion of fishmeal used for aquaculture is predicted to rise to between 25–30% within the next decade (Tacon, 1996). In the same period, the proportion of fish oil used for aquaculture is expected to reach 30–50% of total production (Tacon, 1996). As about 4 kg wet fish is needed to produce 1 kg fishmeal, if diets contain more than 17% fishmeal and/or the food conversion ratio exceeds 1.5:1 or both, the aquaculture operation entails a net loss of fish protein.

In this paper, Australian ingredients with the potential to replace fishmeal and other marine ingredients will be reviewed and constraints to their use discussed. Methods for evaluating these ingredients and increasing their use will be examined.

Identifying alternative feed ingredients to those of marine origin

To evaluate feed ingredients, information is needed on availability and price, the biochemical composition (proximates, amino acids, fatty acids, carbohydrates and anti–nutritional factors) and digestibility and availability to the target species. Although very little fishmeal is produced in Australia, abundant supplies of terrestrial agricultural ingredients are available and large volumes are exported. Table 1 lists production and export volume and average export price of major commodities.

A large number of grains and grain by–products are used in aquafeeds, ranging from high quality soybean meal to cereals like wheat and rice. The most commonly available oilseed meals in Australia include soybean meal, canola meal, peanut meal, cottonseed meal and sunflower meal. Globally, soybean meal is probably the most widely used plant protein source for

aquaculture diets (New *et al.* 1993). Grain legumes, excluding soybeans and peanuts which are typically considered as oilseeds, usually have a lower crude protein content but are also used widely as animal feed ingredients. Lupins, mungbeans, chick peas, cow peas and field peas are examples. Cereals generally have the lowest protein content but can be important sources of energy and useful for binding diets. Cereal by–products, from which most of the starch has been removed, can be much higher in crude protein (>60%). Corn gluten meal and wheat gluten meal are examples. In Australia wheat, barley, oats, sorghum and triticale are produced in large quantities (Table 1).

Animal by–products can be very useful ingredients with a relatively high crude protein content and a valuable source of essential amino acids. Bloodmeal, meat and bone meals, ungraded slaughterhouse wastes and other by–products of beef, sheep, pigs and poultry are widely available. Brewing residues, such as distillers grains and solubles and brewers draff have been successfully used as ingredients in aquafeeds for a number of species (Kohler and Pagan–Font, 1978;

Table 1 Production and export volumes of Australian Agricultural Feed Ingredients 1992/93[1].

Ingredient	Production (kt)	Export volume (kt)	Export price ($/t)
Wheat	14738	10310	210
Barley	5397	2909	172.5
Oats	1937	207	153.7
Triticale	278	–	–
Sorghum	548	62	161.2
Maize	199	26	230
Millet/panicum	43	23	348.7
Cereal rye	28	–	–
Lupins	1195	786	206.2
Field peas	456	350	265
Chick peas	172	183	318.7
Faba beans	99	–	315
Mung beans	15	4.8	747
Navy beans	4	–	–
Soybeans	49	2.8	587
Soymeal	99.8	2.8	–
Soyoil	18	0.2	–
Canola	178	52	391
Canola meal	70	–	–
Canola oil	29	–	–
Sunflower	50	1.3	2953
Sunflower meal	26.8	–	–
Sunflower oil	11.2	0.6	–
Safflower	24	15.6	431
Safflower meal	3	–	–
Safflower oil	1	0.2	–
Linseed	4	0.2	639
Linseed meal	2.1	–	–
Linseed oil	0.8	0.1	–
Peanuts	32	1.5	1394
Peanut meal	18	–	–
Peanut oil	6.7	0.1	–
Meatmeal	460	145	383.7
Tallow (edible)	100	68	655
Meat offal (edible)	124	–	1000

[1]ABARE (1994) and Australasian Agribusiness Services (1993)

Hughes, 1987; Webster *et al.* 1992). General discussions of benefits and limitations of different types of ingredients can be found in Evans (1985), Hardy (1989), Hardy and Dong (1995) and Swick (1995).

Price is a major regulator of which ingredients will be considered for inclusion in aquaculture diets, especially where there are many to choose from. Fishmeal is the protein source of choice for most formulated aquafeeds and ranged in price in 1992 from $650–$1,300/t depending upon source, protein content and quality and country of purchase (New *et al.* 1993, NSW Agriculture, Sydney Retail Feed Ingredient Prices, 1992) [NSW Agriculture, Kite Street, Orange NSW 2800]. Fish oil is also traded on the international commodity market and prices range from approximately $1,000–$2,000/t. Alternative protein feed ingredients will usually need to compete economically on a price per unit protein (or limiting amino acids) with fishmeal.

Consistency of composition and availability is critical; feed manufacturers must be able to access ingredients when they need them and have confidence that the nutrient composition will be similar for different batches of the same ingredient.

Biochemical composition of ingredients will determine their consideration as replacements for marine ingredients. For vegetable ingredients, composition will vary depending upon which cultivars are grown, soil and weather conditions, and processing and storage methods. For animal by–products, composition will depend upon the species composition, rendering equipment and methods and storage conditions. Even so, sufficient information is available for an initial assessment (for example Evans, 1985; AEC, 1987; Novus, 1992; NRC, 1993; New, 1987; Petterson and Mackintosh, 1994; New *et al.* 1993). Analysed dry matter, energy and protein for a range of ingredients used, or considered for use, in aquafeeds in Australia is presented in Table 2, and essential amino acid composition of a subset of these ingredients is presented in Table 3.

Table 2 Analysed composition for dry matter, energy and protein for various feeds available in Australia (Allan *et al.* unpublished data, 1993–1996).

Ingredient	Dry matter (%)	Energy (MJ/kg dry basis)	Protein (% dry basis)
Danish fishmeal		22.1	74.4
Danish fishmeal (LT[1])	94.7	21.5	73.1
Aust fishmeal	94.6	21.3	73.1
Peruvian fishmeal	20.7	70	
Soybean meal (defatted, hexane)	89.1	19.7	50.6
Canola meal	91.7	20.0	43.8
Peanut meal	94.8	19.7	41.3
Cottonseed meal	90.7	19.9	48.1
Lupins (L. *angustifolius*)	94.1	17.9	34.4
Field peas	88.6	18.6	27.5
Chick peas	86.9	18.9	23.1
Cow peas	86.8	18.8	25
Wheat (low Protein)	91.7	18.3	12.5
Wheat (high Protein)	90.8	18.5	15
Peanut meal	94.8	19.7	41.3
Wheat	90.8	18.5	15
Wheat offal	89.7	19.6	22.5
Sorghum	89.6	18.8	14.4
Millrun		18.9	15
Wheat gluten meal	94.0	23.1	76.9
Corn gluten meal		24.4	43.4
Poultry meal	94.4	22.7	60
Feather meal	87.6	24.9	84.4
Bloodmeal	88.7	23.9	95
Meat and bone meal	97.0	16.1	49.4

[1]LT = Low temperature

TABLE 3
Essential amino composition of fishmeal and some agricultural proteins (g/16 g N)[a]

Ingredient		Amino acid								
	Crude Protein (%)	Arginine	Histidine	Isoleucine	Leucine	Lysine	Methionine + cystine	Phenylalanine + tyrosine	Threonine	Valine
Fishmeal[b]	72.9	8.06	2.55	4.62	7.68	8.52	4.07	5.85	4.96	5.17
Soybean	48.5	6.87	2.16	4.69	7.58	5.84	3.43	8.10	3.76	4.59
Canola	43.6	6.61	2.57	4.17	7.00	5.55	5.07	6.83	4.47	4.79
Cottonseed meal	48.0	12.45	2.89	3.43	6.16	4.25	4.27	8.70	3.73	4.41
Lupins meal	30.8	10.89	2.43	4.02	6.58	5.22	2.50	7.30	3.47	3.50
Field peas	27.6	12.54	2.28	4.38	7.32	7.03	2.46	8.04	3.80	4.82
Chick peas	22.8	12.29	2.41	4.12	7.28	6.32	2.90	8.38	3.42	3.99
Poultry meal	60.25	7.52	2.76	4.73	7.73	6.71	4.02	7.68	4.65	5.43
Feather meal	84.31	5.56	1.33	5.8	9.43	2.72	8.93	8.58	5.56	8.63
Peanut meal	41.16	17.4	2.94	4.96	8.5	4.06	3.01	11.76	3.64	5.69
Wheat	15.22	4.14	1.97	3.29	6.37	2.23	5.19	7.16	2.69	3.88
Wheat offal	22.3	6.91	1.93	3.23	6.14	4.3	5.02	6.28	3.36	4.48
Sorghum	14.51	4.07	1.79	4.14	12.61	2.07	2.46	8.75	3.1	4.89
Bloodmeal	94.9	4.11	5.88	0.92	12.61	8.44	3.01	10.12	5.72	8.64
Meat and bone meal	49.17	7.87	1.61	2.73	5.57	5.13	2.03	5.41	3.25	4.01
Lamb meal	54.3	7.9	2.23	3.35	6.5	6.43	3.17	6.28	3.94	4.49

[a] Analysed dry matter composition, tryptophan was not measured (Allan et al., unpublished)
[b] Danish fishmeal (LT)

Evaluating feed ingredients

Armed with information on availability, price and composition, the next step is to determine digestibility for the target species. Measuring digestibility of an ingredient does not take into account losses which can occur in the production of urine and heat but normally accounts for the majority of the differences between ingredients for fish (Lovell, 1989). (Cho *et al.* 1982 or Cho and Kaushik 1990.) In addition, digestibility coefficients are additive (Cho *et. al.* 1982; Allan *et al.* unpublished data), so digestibility of diets can be calculated based on digestibility values for individual ingredients. Digestibility coefficients for a number of ingredients available in Australia fed to silver perch (*Bidyanus bidyanus*) are presented in Table 4. Aquaculture species have varying capacities to digest different ingredients, depending largely upon their digestive system and the presence and activity of various endogenous enzymes (Wee, 1992). Digestibility coefficients for energy and protein for a number of aquaculture species for some common feed ingredient, are presented in Table 5. Although the various methodologies used to calculate these values will have influenced the results, the comparison is useful to indicate differences between species with different digestive systems.

Digestibility information allows ingredients to be compared on the basis of cost of digestible protein or digestible limiting amino acids (*e.g.* lysine). After digestibility information is available, the maximum amount of an ingredient which can be used without suppressing growth or causing other adverse effects, should be determined. With this information, diets containing alternative ingredients to those of marine origin can be formulated on a least–cost basis. Ultimately diets containing new ingredients need to be validated under commercial farming conditions.

Table 4 Digestibility coefficients for dry matter, energy and protein for various feeds (values are means, n=3) fed to silver perch (*Bidyanus bidyanus*).

	Digestibility Coefficient (%)		
Ingredient	**Dry matter**	**Energy**	**Protein**
Danish fishmeal	91.1	102.1	98.6
Danish fishmeal (LT)[1]	100.0	100.0	100.0
Aust fishmeal	76.5	93.1	95.7
Peruvian fishmeal	74.3	89.5	88.8
Poultry meal	83.7	96.4	84.5
Feather meal	100.0	100.0	93.3
Bloodmeal	100.0	100.0	88.2
Meat and bone meal	48.1	76.4	68.9
Soybean meal (defatted, hexane)	73.1	81.6	95.3
Canola meal	49.8	56.8	83.1
Peanut meal	72.0	80.1	100.0
Cottonseed meal	48.4	52.4	86.7
Lupins (L. *angustifolius*)	50.3	59.4	96.6
Field peas	62.0	67.0	83.3
Chick peas	48.7	53.6	84.8
Cow peas	40.6	45.8	93.0
Wheat (low protein)	49.9	55.23	99.0
Wheat (high protein)	34.1	36.6	99.5
Millrun	51.2	55.6	87.9
Wheat gluten meal	97.2	118.7	113.9
Corn gluten meal	100.0	97.4	96.6
Sorghum	34.6	38.8	86.0

[1] LT = Low temperature

Table 5 Average percent digestibility coefficients for common feedstuffs used in aquafeeds

Ingredients	Digestibility coefficients for energy (%)					Digestibility coefficients for protein				
	Trout[1]	Salmon[2]	Tilapia[3]	Catfish[4]	Silver[5] perch	Trout[1]	Salmon[2]	Tilapia[3]	Catfish[4]	Silver[5] perch
Fishmeal	91	84	80	92	100	92	83	86	85	99
Poultry offal meal	71	65	59	-	96	68	74	74	-	85
Blood meal	89	-	-	-	100	99	-	-	-	88
Meat meal	85	-	-	76	76	85	-	-	61	69
Soybean meal	75	70	57	72	82	96	77	91	97	95
Canola meal	45	65	-	-	57	77	85	-	-	83
Lupin seed meal	61[6]	-	-	-	59	86[6]	-	-	-	97
Corn	39	-	76	57	-	95	-	83	97	-
Wheat middlings	46	45	58	-	55	92	86	76	-	88

1 Cho and Kaushik, 1990.
2 Hajen, W. E., Higgs, D. A., Beames, R. M. and Dosanjh, B. S., 1993.
3 Hanley, F., 1987.
4 Wilson, R. P., 1991.
5 Allan *et al.*, unpublished data
6 Gomes, E. F., Rema, P. and Kaushik, S. J., 1995

Table 6 Anti–nutritional factors present in oilseeds and grain legumes currently used in aquaculture diets[1].

Grain	Anti–nutrition factors	Comments
Soybean meal	Protease inhibitors	Deactivated by heat but heating can also reduce availability of some amino acids especially lysine.
	Haemagglutenating agents	Deactivated by pepsin in stomach
	Other anti–nutritional factors have been implicated	
Cottonseed meal	Gossypol (highly reactive polyphenolic compounds)	Low gossypol varieties are available but generally not widely grown. Supplementation with iron sulphate may reduce problems with gossypol
Peanut meal	Tannins	May affect protein availability
	Frequently contaminated by aflatoxins	
Rapeseed meal	Glucosinolates Erucic acid	Selective breeding has produced varieties of rapeseed low in glucosinolates and erucic acid These are called canola
Linseed meal	Linatin (anti–pyridoxine factor)	For poultry, use of linseed meal requires supplementation with pyridoxine. Effects on fish not reported
	Linamarin (cyanogenic glucoside)	Mostly a problem in immature seeds Toxicity to fish unknown
Sunflower meal	Relatively free of anti–nutritional factors	
Safflower meal	Phenolic glucosides	Reduces palatability in poultry diets
Lupins	Alkaloids Tannins	Low levels of alkaloids and tannins in lupins should not affect inclusion levels
Field peas	Tannins	Relatively free of anti–nutritional factors
Faba bean	Tannins	Low tannin cultivars are available and dehulling overcomes most problems.
	Protease inhibitors	Protease inhibitors inactivated by heating
Chick peas	Tannins	Relatively free of anti–nutritional factors

[1]Ravindran and Blair, 1992; NRC 1993; Petterson and Mackintosh, 1994.

Major constraints to replacing marine ingredients

Compared with fishmeal, grains contain large amounts of carbohydrates, including fibre, and some species contain anti–nutrients or may be contaminated by mycotoxins. Grains and ingredients of animal origin are often deficient in essential amino acids and essential fatty acids, compared with marine ingredients, and this can suppress palatability and attractiveness of diets.

Carbohydrate includes starches, non–starch polysaccharides, oligosaccharides and some free sugars. No requirement for carbohydrates has been demonstrated for fish (NRC, 1993), although they can provide an energy source and reduce the need for using expensive protein for energy. Carbohydrates, especially starch, also have an important role in binding extruded and pelleted feeds. The ability of different species of fish to utilise carbohydrate will limit the inclusion level of many unprocessed grains.

Although enzymes necessary for carbohydrate digestion have been detected in fish, some species are clearly better able to digest carbohydrates than others (NRC, 1993). The digestibility of carbohydrates is influenced by the digestive system of the fish, with carnivorous species least equipped to digest carbohydrates. Processing, *e.g.* cooking or steam treatment also influences digestibility, as does the structural complexity of the carbohydrate (NRC, 1993; Robinson, 1989).

Grains can also contain a number of anti–nutritional factors, including trypsin inhibitors, gossypol, glucosinolates, erucic acid, haemagglutinating agents, cyclopropenoic fatty acids and alkaloids (Table 6).

Mycotoxins produced by fungi can also contaminate feed ingredients and formulated feeds. Peanut meal is particularly susceptible to contamination, but other grains are also affected. Contamination can occur during growth of the crop or storage and distribution of grains or feeds (Williams and Blaney, 1992).

Another limitation with the use of grains is the presence of phytates. Phytates are found in all plant materials and are the major storage form of phosphorus in seeds (Reddy *et al.* 1982). The amount of phosphorus present as phytate in grains ranges from about 40–90% of the total phosphorus (Ravindran and Blair, 1992) and is considered to be almost unavailable to fish (NRC, 1993). In addition, phytates may reduce the bio–availability of protein and several essential minerals (NRC, 1993).

Compared with marine ingredients, other ingredients are usually deficient in essential amino acids, particularly lysine and methionine. The essential amino acid content (as a percentage of protein) of a number of feed ingredients, including grains, is listed in Table 3.

Maximum inclusion levels of ingredients in formulated diets will depend not only upon composition and digestibility, but also on the presence of anti–nutritional factors. Although meatmeal has fewer anti–nutritional factors than plant protein sources, it can contain high contents of bone fragments which can be deleterious. Excessive heat during the rendering process can damage proteins, especially lysine, and may contribute to lower protein digestibility. Consistent temperature throughout rendering facilities is important (Carpenter and Booth, 1973).

Excessive amounts of hair or wool also make processing difficult, as can high contents of fat. In general, provided essential fatty acid requirements are met, saturated animal fats have no adverse effects on fish (Reinitz, 1980) and they are a good, cheap source of energy. However, fish fed diets with high concentrations of saturated fat tend to have a body composition lower in unsaturated fatty acids which may become a marketing disadvantage. Reduction of fat content, through mechanical or chemical extraction, will result in meals with a higher protein content which is an advantage for aquaculture diet formulation.

Contamination of meatmeal products with pesticides or bacteria, particularly salmonella, is a genuine concern and industry specifications on these contaminants are needed (Australasian Agribusiness Services, 1993). Concern with exotic diseases like bovine spongiform encephalopathy (or Mad Cow Disease) has reduced use of meat products in animal feeds in some countries (Australasian Agribusiness Services, 1993).

One of the major factors which has prevented the use of meatmeals in animal feeds has been inconsistent composition. This was recognised in Australia in the review commissioned by the Meat Research Corporation into the meatmeal and tallow industry and markets (Australasian Agribusiness Services, 1993). The inconsistency in the composition of meat meals is especially notable when compared with vegetable protein sources such as soybean meal. The variability is a result of a number of factors, including the differing nature of raw materials, especially where mixed species are rendered. The practice of rendering processors to 'take what's left' contributes to this variability.

Improving the nutritional value of alternative ingredients

Plant breeding programs have been very successful in improving the nutritive value of some grains. Varieties of maize which are high in lysine and tryptophan (Opaque –2) or lysine and methionine (Floury –2) are examples (Farrell, 1992). Low glucosinolate, low erucic acid varieties of rapeseed (called canola), low alkaloid varieties of lupins (to improve palatability) and tannin–free cultivars of faba beans have all been produced for livestock feeding (Farrell, 1992).

Changes in the nutritive value of feeding ingredients can also be achieved by processing, including grinding, classification, sieving, mixing, heating, drying, and extrusion. Grinding to reduce particle size improves digestibility and is especially important for crustacean diets. Many forms of processing involve heat treatment, including pelleting and extrusion. Heat is important to deactivate some of the anti–nutritional factors present in grains, such as trypsin inhibitors (NRC, 1993), and can also be used to gelatinise starch compounds which usually improves digestibility (Hardy, 1989; Table 7). Heating can also have detrimental effects including reducing the digestibility of some essential amino acids (Hardy, 1989). Lysine and cystine are the most likely amino acids to be adversely affected, but digestibility of arginine, threonine, leucine and tryptophan may also be affected (Ravindran and Blair, 1992). Heat sensitive vitamins, e.g. ascorbic acid, may also be damaged by some processing treatments which involve heating (Halver, 1989).

Increasing the protein content of grains by removing some of the carbohydrate material should enable use of higher contents of grains in aquafeeds. Dehulling and protein fractionation are examples. Effects of dehulling and removing some of the carbohydrate fraction of some Australian grain legumes on dry matter, energy and protein digestibility to silver perch are presented in Table 8. Wheat or corn gluten meals, produced by removing starch, are generally highly digestible to fish (Table 4; Allan, 1995; unpublished data) although they are often expensive, and maximum inclusion of wheat gluten meal is limited by the agglutinating properties of this product. The Academy of Grain Technology in Australia is currently investigating ways to produce much cheaper wheat gluten, with reduced agglutinating properties, for use in animal feeds.

There has been increasing interest in using exogenous enzymes to improve utilisation of nutrients in animal feeds. These include proteases, cellulases, pectinases, β–glucanases, lipases and phytases (Batterham, 1992). Use of these products offers the potential to increase the use of non–marine ingredients, especially grains, although efficacy for some products with fish has not yet been clearly demonstrated. For rainbow trout, supplementation of plant protein sources with phytase significantly increased phosphorus availability (Riche and Brown, 1996).

Meat and bone meals and poultry waste products can be improved through the reduction of bone and fat. In studies with silver perch, digestible dry matter, energy and protein all increased for meat meals with more protein (through reduction in ash–bone) (Allan, 1994).

Supplements of synthetic amino acids such as L–lysine, DL–methionine and DL–threonine are used extensively and successfully in pig and poultry diets (Batterham, 1992). Unfortunately, synthetic amino acids leach rapidly in water and they are absorbed much more rapidly than protein–bound amino acids. Fish can utilise free amino acids, although their efficiency in overcoming deficiencies is the subject of some debate (Lovell, 1989; Cowey, 1992; Murai, 1992).

Ingredients of marine origin contain various attractants and are usually highly palatable. Replacement of these ingredients can lead to problems with reduced feed intake and deterioration in performance. Not only may feeding stimulants be diluted or removed, but some ingredients actually contain

Table 7 Digestibility coefficients for cooked and uncooked reference diet (Allan and Rowland 1992), starch products and pregelled corn starch fed to silver perch (*Bidyanus bidyanus*)[1].

	Digestibility coefficient (%)[1]					
	Dry matter		Energy		Protein	
Ingredient	Cooked[2]	Uncooked	Cooked[2]	Uncooked	Cooked[2]	Uncooked
Reference diet	72.2±2.2	64.8±1.1	81.8±1.8	74.4±0.8	88.8±0.9	89±0.7
Corn starch	36.7±3.8	27.1±6.1	40.0±4.0	31.4±5.6		
Wheat starch	49.4±2.5	41.0±3.1	52.2±2.1	45.6±2.3		
Potato starch	20.3±1.6	15.5±4.7	30.3±1.7	22.3±3.3		
Pregelled corn starch		66.1±2.0			70.9±1.8	

[1]Values are means ± SE for 3 replicate aquaria (Allan *et al.* unpublished data)
[2]Autoclaved for 15 minutes at 121°C

Table 8 Composition and digestibility of processed and unprocessed legumes fed to silver perch (*Bidyanus bidyanus*) diets.

	Proximate composition (dry basis)		Digestibility coefficient (%)[1]		
	GE[2] MJ/kg	Protein %	DM[3]	GE[2]	Protein
Faba beans					
Hulls on	17.34	27.69	55.86±0.32	62.25±0.43	91.74±1.26
Dehulled	17.58	31.31	58.17±1.26	58.83±0.67	96.2 ±0.84
Protein conc	19.93	48.31	66.34±1.77	73.45±2.01	95.05±1.45
Field peas[4]					
Hulls on	17.02	25.44	62.03±0.44	66.97±0.2	84.05±0.31
Dehulled	17.31	27.75	48.93±2.21	54.46±2.2	88.68±1.0
Protein conc	19.81	42.44	85.93±3.99	91.05±2.85	98.56±2.03
Lupins *Lupinus albus*					
Hulls on	20.87	37.55	64.65±0.42	72.71±1.79	96.07±0.91
Dehulled	21.45	42.82	77.79±1.99	85.19±1.52	101.44±0.26
***Lupinus angustifolisus*[5]**					
Hulls on	17.87	34.14	50.33±2.97	59.37±1.0	96.62±0.86
Dehulled	20.74	43.61	67.62±3.2	73.97±2.3	100.29±0.36
Protein conc	22.66	61.39	78.42±3.2	82.02±2.5	97.4 ±0.96

[1]Values are means ± SE for data from groups of fish in each of n=3 replicate tanks (Allan *et al.* unpublished data)
[2]GE = gross energy
[3]DM = dry matter
[4]Dunn variety
[5]Gungarru variety

deterrents (Mackie and Mitchell, 1985). It may be possible to address problems with reduced diet attractiveness and palatability by the addition of stimulants and 'palatability enhancers' (Mackie and Mitchell, 1985; Viana *et al.* 1994). Examples of substances which have been shown to improve feeding behaviour include amino acids (especially in dipeptide linkages), betaine, inosine and organic acids (NRC, 1993). Carnivores tend to show the most positive response to alkaline and neutral additives such as glycine, proline, taurine, valine and betaine, while herbivores respond more positively to more acidic additives like aspartic acid and glutamic acid (NRC, 1993).

Fishmeal replacement research in Australia

In Australia, with little fishmeal production but abundant agricultural production, aquaculture will not develop unless aquafeeds based on agricultural proteins are developed. The Australian Fisheries Research and Development Corporation recognised the importance of fishmeal replacement research and created a separate Sub–Program to coordinate national research in 1993. Additional funding for this research has been provided by the Australian Centre for International Agricultural Research (for collaborative research with the Thailand Department of Fisheries), the Australian Grains Research and Development Corporation, the Australian Meat Research Corporation and the Australian Academy of Grain Technology. The overall aim of the Sub–Program is to produce cost–effective aquafeeds, specifically by replacing imported fishmeal (trash fish in Thailand) with cost–effective locally produced alternative protein sources. In summary, the methods used to achieve this objective include:

- Identifying and then evaluating alternative protein sources (digestibility, net energy utilisation, tracking stable isotopes, growth responses)
- Developing and evaluating processing methods to improve utilisation of ingredients
- Evaluating methods of increasing inclusion by using supplements, *e.g.* synthetic amino acids and enzymes

- Examining the role of attractants
- Defining requirements for nutrients, *e.g.* amino acids and energy, where previously reported requirements are limiting fishmeal replacement.

The research was perceived to be of value to most fish and crustacean species, but for practical purposes it was decided to concentrate efforts on four 'representative' species in Australia; sea bass (*Lates calcarifer*), shrimp (*Penaeus monodon*), silver perch (*Bidyanus bidyanus*) and salmon (*Salmo salar*), and on hybrid walking catfish (*Clarias macrocephalus* X *C. gariepinus*) in Thailand.

Acknowledgments

Much of this information was extracted from a paper (Allan, 1996) presented at Victam International, Feed and Food Industries Show, November 14–15, 1996, Bangkok, Thailand. Feed Production on the Threshold of the Next Age. Victam International, Leidein, The Netherlands, pp. 125–152). Technical assistance for research with silver perch was provided by Scott Parkinson, David Stone, Jane Frances, Rebecca Warner–Smith, Ken O'Brien and Wendy Peasley. Jo Pickles and Jane Frances assisted with the preparation of the manuscript. Support from the Australian Fisheries Research and Development Corporation (Fishmeal Replacement Sub–Program), Australian Centre for International Agricultural Research, Australian Meat Research Corporation and the Australian Academy of Grains Technology for fishmeal replacement research is gratefully acknowledged.

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APPENDIX 13.2
Potential for Pulses in Aquaculture Systems

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Short Title: Pulses in aquaculture

Abstract

Aquaculture is expanding rapidly as demand for seafood increases and aquaculture productivity intensifies through use of improved, cost-effective formulated diets. Demand for fish meal for use in these diets is escalating, while supply is static or declining, and this has driven a global search for alternative ingredients. Pulses have potential to replace at least some of the fish meal in aquaculture diets. This potential is influenced by biochemical composition of pulses; especially protein content, amino acid profile and carbohydrate content and profile, digestibility and biological availability of pulses to the target species and price and supply. Research indicates that pulses such as lupins, field peas and faba beans are generally well utilised by fish. The potential to use pulses is aquaculture diets can be improved through plant breeding programs to reduce anti-nutrients or increase content of essential amino acids, processing to reduce the content of poorly digested carbohydrate fractions or increase starch gelatinization, or addition of exogenous enzymes to assist digestion. Coordinated aquaculture nutrition research within Australia has identified pulses (i.e. lupins, field peas and faba beans) as having potential to partially replace fish meal in diets for silver perch, barramundi (sea bass), salmon and shrimp.

Introduction

The demand for seafood is escalating rapidly as global population increases and there is an increase the preference for seafood. As production of seafood from capture fisheries is static or declining (Tacon, 1996), the increase in demand can only be met from aquaculture.

Aquaculture is one of the fastest growing food production industries. It has expanded by approximately 9%/year over the past decade (Tacon, 1996) to the stage where in 1993, 22% of total fishery products were from aquaculture (Liao, 1996).

One of the main reasons this increase has been possible is the shift to more intensive production made possible by the availability of cost-effective formulated diets. The market for aquaculture diets in Asia increased more than four-fold from 1986 to 1996 (Akiyama, 1991) and New and Csavas (1993) predicted 2.6 million tonnes will be required in Asia by the year 2000.

Marine based ingredients, especially fish meal and fish oil, are preferred protein and energy sources as they are rich in essential amino acids and fatty acids, are well digested and low in anti-nutritional factors. Unfortunately, production of fish meal already uses 33% of the total global fish catch and within the next decade, Tacon

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[1] Paper presented at International Food Legume Research Conference III, Adelaide, Australia, September 22-26, 1997.

(1996) predicts the proportion of total fish meal and fish oil used for aquaculture will rise to 25-30% and 30-50% of total production respectively.

Pulses are grain legumes which are widely used in stock feeding industries and may have potential to replace at least some of the fish meal in aquaculture diets.

Availability and composition of pulses

The major pulses produced in Australia which are used in stock feeds include lupins (*L. angustifolius* and *L. albus*), field peas (*Pisum sativum*), chick peas (*Cicer arietinum*) and faba beans (*Vicia faba*) while smaller quantities of mung beans (*Vigoria radiata*), navy beans (*Phaseolus vulgaris*) and vetch (*Vicia sativa*) are also grown (ABARE, 1996). (For a full list of grain legumes produced in Australia, see Pettersen and MacIntosh, 1994). In total, more than 2.3 million tonnes of legumes are produced, approximately half of which are exported (ABARE, 1996; Table 1).

The biochemical composition of pulses (and any other ingredients) will have a major influence on their potential for use in aquaculture diets. Total protein and energy content and the amino acid profile are of primary importance. Fish do not have a requirement for carbohydrates although carbohydrate can be a relatively inexpensive source of energy and spare requirements for protein and lipid (NRC, 1993). Carbohydrate, especially starch, is also important in binding formulated diets.

The digestion and utilization of carbohydrate depends upon its structure. Complex carbohydrates, including plant cell wall material such as cellulose, hemicellulose, lignan and pentosan offer little nutritional benefit, are poorly digested and ultimately pollute the culture environment. In contrast, glucose, maltose and sucrose contributed to the best growth rates when different carbohydrates were fed to chinook salmon at 10% of the diet, followed by dextrin, fructose and galactose (Buhler and Halver, 1961; cited in NRC, 1993). Starch, in particular gelatinised starch, is relatively well digested and digestibility coefficients for dry matter and energy for silver perch were 58 and 66% for raw wheat starch, 60 and 68% for cooked (autoclaved at 121°C for 15 minutes) wheat starch, and 65 and 73% for pregelatinised maize starch respectively, (Stone and Allan, in press). Carbohydrates in five pulses is presented in Table 2. Anti-nutrients can also restrict inclusion contents of some pulses (Table 3).

Evaluating pulses for aquaculture diets

Ultimately, the choice of which ingredients are used in aquaculture rations will depend mainly on availability, price and composition and the ability of the aquaculture species to digest and utilise the ingredients. In addition, factors such as storage capacity for ingredients at the feed mill and proven performance of fish under commercial conditions when fed diets containing the ingredients of interest, will influence which ingredients are used.

Measurement of digestibility involves subtracting the amount of energy or nutrients which are voided in the faeces from the amount of energy or nutrients provided in the diet. Measuring digestibility does not take into account losses which occur in the production of heat or urine but these are much less for fish than terrestrial animals because fish are cold-blooded and expend far less energy in locomotion and excretion.

(See Cho et al., 1982 and Cho and Kaushik, 1990 for reviews of fish energetics.) For fish, digestibility coefficients for different ingredients are additive (Cho et al., 1982; Allan et al., unpublished data) and account for most of the differences between ingredients (Lovell, 1989). Aquaculture species have varying capacities to digest different ingredients, especially ingredients like pulses which can have considerable amounts of carbohydrates. This capacity depends upon the digestive system of the fish species and the presence and acitivity of various endogenous enzymes (Wee, 1992; Alex Anderson, unpublished data). Apparent digestibility coefficients (ADC's) for a number of pulses fed to silver perch (*Bidyanus bidyanus*), a native Australian freshwater omnivorous fish which is being cultured in NSW, Queensland and Victoria, and rainbow trout, are presented in Table 4. ADC's for juvenile silver perch (<10 g) were determined after faeces were collected by settlement using similar methods to those described by Cho and Kaushik (1990). Although different methods were used for different studies, when compared with trout, silver perch had higher ADC's for protein but lower ADC's for dry matter. The overall high protein digestibility augers well for use of protein sources from pulses. For both species, ADC's for dry matter and energy for pulses are much lower than for fish meal, reflecting the poorer digestibility of carbohydrates in pulses.

Positive results have been reported where pulses, most often lupins, have been used to replace fish meal or other protein sources, for rainbow trout, gilthead sea bream and carp (Viola et al., 1988; Hughes, 1991; Moyano et al., 1992; Morales et al., 1994; Robaina et al., 1995). Hughes (1991) for rainbow trout and Viola et al. (1988) for carp reported that lupin was successful as a complete replacement for soybean meal or full fat soy and diets with over 43% lupins performed as well or better than a fish meal control diet for rainbow trout (Morales et al., 1994) or a fish meal/soybean meal control diet for carp (Viola et al., 1988).

Morales et al. (1994) reported a protein retention efficiency of 41.2% for rainbow trout fed a diet containing 43% lupins, which was significantly higher than that recorded for a fish meal-based control diet. Similarly, Gomes and Kaushik (1989) reported protein retention efficiencies of 40-43% for rainbow trout for diets containing 10-30% lupins.

Gouveia et al. (1991) compared performance of rainbow trout fed diets where lupins (*L. albus*), field peas or faba beans were used to replace 20% of the dietary protein from fish meal. Best results were obtained with lupins although all the diets yielded superior performance to the fish meal control diet. (Gouveia et al., 1991).

Improving the value of pulses for use in aquaculture diets

Plant breeding programs to increase essential nutrients and reduce anti-nutrients have been very successful for a number of grains, including maize, rapeseed, lupins and faba beans (Farrell, 1992). Lupins and faba beans cultured with low alkaline or tannin contents have increased the value of these pulses for use in stock feeds (Farrell, 1992).

Improving the nutritive value of pulses can also be achieved by processing techniques such as grinding, dehulling, classification cooking and expansion. Grinding to reduce particle size improves digestibility of grains, especially for crustaceans. Protein concentration, through dehulling and removal of starch or non-starch polysaccharides

can increase digestibility of pulses for silver perch (Tables 5 and 6). Silver perch have also grown well in laboratory studies on diets with very high contents of dehulled lupins. In one experiment, eight juvenile silver perch (1.8 g/fish) were stocked into 60 litre aquaria (four were used for each diet) and grown for 75 days on experiment diets comprising a reference diet or that diet diluted with different amounts of dehulled lupins or an inert filler (either diatamaceous earth or cellulose). Growth of silver perch was not significantly different for fish fed the reference diet or that diet diluted with up to 60% dehulled lupins (Figure 1). Compared with the reference diet, protein retention efficiency in that experiment was not reduced until diets contained >60% dehulled lupins (Figure 2).

Cooking/expansion improved the nutritional value to rainbow trout of field peas and faba beans but not lupins (Gouveia et al., 1991). This is not surprising as lupins contain almost no starch and the improvements, following cooking, to field peas and faba beans are most likely due to partial or complete gelatinisation of starch in those products. A co-extruded product containing rapeseed and field peas, "Colzapro", has been evaluated as a fish meal replacement in diets for rainbow trout and levels of 45% in the diet did not affect protein efficiency (although lower protein and energy reduced growth) (Gomes and Kaushik, 1989).

There is also potential to increase the utilisation of pulses in animal feeds through the addition of exogenous enzymes. Such enzymes include proteases, cellulases, pectinases, β-glucanases, lipases and phytases (Batterham, 1992). Riche and Brown (1996) significantly increased phosphorus availability by supplementing plant protein sources with phytase.

Fish meal replacement research in Australia

In Australia, with little fish meal production but abundant agricultural production, aquaculture will not develop unless aquafeeds based on agricultural proteins are developed. The Australian Fisheries Research and Development Corporation recognised the importance of fish meal replacement research and aquaculture diet development and created a separate Sub-Program to coordinate national research in 1993. Additional funding for this research has been provided by the Australian Centre for International Agricultural Research (for collaborative research with the Thailand Department of Fisheries), the Australian Grains Research and Development Corporation, the Australian Meat Research Corporation and the Australian Academy of Grain Technology. The overall aim of the coordinated research is to produce cost-effective aquafeeds, specifically by replacing imported fish meal with cost-effective, locally-produced, alternative protein sources, defining critical nutritional requirements, evaluating and developing attractants and additives to improve diet palatability, and determining optimum feeding strategies. The research was perceived to be of value to most fish and crustacean species, but for practical purposes it was decided to concentrate efforts on four 'representative' species in Australia; barramundi or sea bass (*Lates calcarifer*), shrimp (*Penaeus monodon*), silver perch (*Bidyanus bidyanus*) and salmon (*Salmo salar*).

A strong focus is on developing highly digestible, "low waste-producing" diets. Results have been very promising: methods for determining digestibility in shrimp (*Penaeus monodon*) and fish have been developed or improved and large data bases

of digestibility coefficients for a range of ingredients generated for silver perch (*Bidyanus bidyanus*), barramundi (*Lates calcarifer*) and shrimp (*P. monodon*).

Growth studies have been conducted to measure the contribution for different ingredients, including pulses, oilseeds and animal protein meals and protein and protein/energy requirements investigated. Recently, we formulated, on a least-cost basis, diets for silver perch based on meatmeal, lupins and field peas with only 5 or 10% fish meal. Fish performance on these diets was similar or superior to that on fish meal/soybean meal control diets in commercially relevant facilities where fish were grown to market size (Allan, 1996) (Tables 7 and 10). Composition of the diets did not affect sensory qualities of the fish flesh. Diets for barramundi with no fish meal have also been developed and for both fish species, results are now being used as the basis of commercial formulations being sold by feed manufacturers.

Acknowledgments

Research with silver perch in ponds was conducted under Dr Stuart Rowland at the NSW Fisheries, Grafton Research Centre and assistance from the following colleagues from NSW Fisheries, CSIRO Fisheries, CSIRO Food Science, Queensland Department of Primary Industries and Ridley Agriproducts Pty. Ltd. is gratefully acknowledged: Dave Stone, Jane Frances, Mark Booth, Scott Parkinson, Rebecca Warner-Smith, Helena Heasman, Charlie Mifsud, David Glendenning, Kevin Williams, David Smith, Tony Evans, Vince Gleeson, Zafer Sarac, Chris Barlow and David Overend.

Financial support for Australian aquaculture diet development research has been provided by the Australian Fisheries Research and Development Corporation, Australian Centre for International Aquaculture Research, Australian Grain Research and Development Corporation and the Australian Meat Research Corporation and Australian Native Fish Pty. Ltd.

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TABLE 1

Production and value of Australian pulses[1]

	Lupins		Field Peas		Chick Peas		Total[2]	
	1994/ 1995	1995/ 1996	1994/ 1995	1995/ 1996	1994/ 1995	1995/ 1996	1994/ 1995	1995/ 1996
Area planted (x1000 ha)	1258	1308	407	350	169	207	1982	2033
Production (kt)	865	1429	214	465	69	258	1231	2335
Exports (kt)	291	674	134	217	36	112	547	1133
Average unit value (AUD$/t)	185	199	300	266	559	368	240	239
Gross value ($ million)	160	284	64	124	39	95	295	558

[1] Data from ABARE, 1996

[2] Includes lupins, field peas, chick peas, faba beans, mung beans and vetch.

TABLE 2

Carbohydrates in grain legumes (whole seed %)[1].

Type	Lupins	Field peas	Chick peas	Faba beans	Vetch
Starch	0.30	34.8-54.1	36.0-47.7	31.4-41.8	32.0
Cellulose	11.4-17.7	2.4-7.9	6.0	8.5-10.0	4.7
Sugar	4.8-5.4	1.0-5.7	4.4	1.5-4.1	-
Crude fibre	5.0-12.9	4.9-6.3	5.2-13.0	6.2-11.4	3.2-5.9
ADF	15.4-15.8	6.0-8.7	10.0-16.7	7.2-10.6	6.9-8.0
NDF	18.7-18.8	10.0-12.0	25.4-30.2	9.6-16.8	11.8-21.9

[1] Data from Novus, 1992 and Petterson & Macintosh, 1994.

TABLE 3

Some antinutrients in selected Australian pulses[1]

	Lupins		Field Peas	Chick Peas	Faba Beans	Vetch
	L.angustifolius	*L. albus*				
Alkaloids (%)	0.02	<0.01				
Oligosaccharides %)	5.16	6.69	3.69		2.93	
Phytate (%)	0.58	0.79	0.48	0.63		0.66
Tannins (total) (%)	0.32	0.37	0.25	0.49	0.75	0.64
Trypsin inhibitors activity	0.14	0.13	1.01	4.79	0.39	2.40
Chymotrypsin inhibitor activity		0.08	1.60	7.72	0.40	2.25

[1] Data from Pettersen and MacIntosh, 1994.

TABLE 4

Comparison of digestibility coefficients (%) for dry matter, energy and protein for rainbow trout (*Oncorhynchus mykiss*) and silver perch (*Bidyanus bidyanus*)

	Lupins *(L.angustifolius)*	**Field Peas** *(Pissum sativum)*	**Faba Beans** *(Vicia faba)*	**Fishmeal**
Dry Matter				
silver perch[1]	50.3±3.0	67.0±0.2	56.9±0.3	91.1±1.1
rainbow trout[2]	63.3±0.7	66.1±0.6	66.1±0.9	89.4±0.02
Energy				
silver perch[1]	59.4±1.0	62.0±0.4	62.3±0.4	102.1±0.6
rainbow trout[2]	61.2±0.6	59.2±0.5	60.2±1.0	93.8±0.02
rainbow trout[3]	66.1±2.7			
rainbow trout[4]	64.0±1.9			
Protein				
silver perch[1]	96.6±0.9	84.1±0.3	91.7±1.3	98.6±1.2
rainbow trout[2]	85.5±0.7	80.4±0.5	80.2±0.9	92.3±0.02
rainbow trout[3]	85.2±1.3			
rainbow trout[4]	85.3±1.1			

[1] Data from Allan et al., unpublished data, fishmeal was Danish, 72% protein
[2] Data from Gomes et al., 1995, fishmeal was from Portuguese sardines.
[3] Data from Hughes, 1988.
[4] Data from Smith et al., 1995.

TABLE 5

Proximate composition and apparent digestibility coefficients for processed (HO = hulls on; DH = dehulled; PC = protein concentrate) and unprocessed pulses fed to silver perch (*Bidyanus bidyanus*)

	Ingredients														
	L. angustifolius[1]			*L. albus*		*Field pea*[2]			*Faba bean*			*Chick pea*		*Vetch*	
Composition[3]	*HO*[4]	*DH*[4]	*PC*[5]	*HO*[4]	*DH*[4]	*HO*[6]	*DH*[6]	*PC*[7]	*HO*[6]	*DH*[6]	*PC*[1]	*HO*[6]	*DH*[6]	*HO*[6]	*DH*[6]
Dry matter %	94.1	95.0	96.3	95.4	95.0	93.5	94.4	90.9	93.9	94.6	91.2	92.5	95.2	94.7	94.9
Energy MJ-kg	17.9	20.7	22.7	20.9	21.4	17.0	17.3	19.8	17.3	17.6	20.6	19.4	19.3	17.9	18.6
Protein %	34.1	43.6	61.4	37.5	42.8	25.5	27.7	42.4	27.7	31.3	48.3	20.8	24.2	30.9	32.3
Apparent Digestibility Coefficients %[8]															
Dry matter %	50.3 3.0	67.6 3.2	78.4 3.2	64.7 0.4	77.8 2.0	48.9 2.2	62.0 0.4	85.9 4.0	55.9 0.3	58.2 1.3	66.3 1.8	48.7 0.8	58.4 0.7	41.5 3.2	78.3 3.9
Energy %	59.4 1.0	74.0 2.3	82.0 2.5	72.7 1.8	85.2 1.5	54.5 2.2	67.0 0.2	91.1 2.8	62.2 0.4	58.8 0.7	73.4 2.0	53.6 0.8	60.2 0.7	55.5 1.0	81.8 2.3
Protein %	96.6 0.9	100.3 0.4	97.4 1.0	96.1 0.9	101.4 0.3	83.3 0.3	88.1 1.0	98.6 2.0	91.6 1.3	96.6 0.8	95.0 1.4	84.8 1.0	81.2 3.5	74.9 2.6	87.7 0.8

Values for digestibility coefficients are mean (± sem) for 3 replicates

[1] Gungarru variety; [2] Dunn variety; [3] Analysed composition (see Allan and Frances, 1994 for methods of analysis)

[4] Allan et al, unpublished data August 1994; [5] Allan et al, unpublished data October 1994; [6] Allan et al, unpublished data December 1994

[7] Allan et al, unpublished data August 1995; [8] Values are means with sem below (n=3 replicates)

TABLE 6

Amino acid composition and apparent digestibility coefficients for processed (HO=hulls on; DH=dehulled; PC=protein concentrate) and unprocessed pulses fed to silver perch (*Bidyanus bidyanus*)

	Ingredients														
	L. angustifolius[1]			*L. albus*		*Field pea*[2]			*Faba bean*			*Chick pea*		*Vetch*	
% Composition[3]	*HO*[4]	*DH*[4]	*PC*[5]	*HO*[4]	*DH*[4]	*HO*[6]	*DH*[6]	*PC*[7]	*HO*[6]	*DH*[6]	*PC*[7]	*HO*[6]	*DH*[6]	*HO*[6]	*DH*[6]
Arg	4.0	5.2	7.4	4.1	4.5	2.5	2.8	5.3	2.8	3.1	5.1	2.0	2.1	2.4	2.7
Hist	0.9	1.2	1.5	0.9	1.0	0.6	0.7	1.1	0.6	0.7	1.1	0.5	0.6	0.6	0.8
Iso	1.4	1.8	3.0	1.7	1.9	1.1	1.2	2.3	1.1	1.2	2.2	1.0	1.1	1.2	1.4
Leuc	2.4	2.9	4.5	2.8	3.2	1.7	1.8	3.5	1.8	2.0	3.5	1.6	1.7	1.9	2.2
Lys	1.4	1.7	3.1	1.5	1.7	1.7	1.8	3.4	1.5	1.9	3.0	1.5	1.5	1.7	1.7
Meth	0.2	0.2	0.3	0.3	0.3	0.3	0.3	0.4	0.3	0.3	0.4	0.4	0.5	0.3	0.3
Phen	1.3	1.6	2.6	1.4	1.6	1.1	1.2	2.3	1.1	1.2	2.0	1.2	1.3	1.1	1.2
Threo	1.3	1.6	2.4	1.5	1.7	0.8	0.9	1.7	0.8	1.0	1.6	0.7	0.8	0.8	1.1
Val	1.3	1.6	2.6	1.6	1.8	1.2	1.3	2.5	1.2	1.3	2.4	1.0	1.1	1.3	1.5
Apparent Digestibility Coefficients (%)[8]															
Arg	102.9	106.3	101.5	102.6	106.8	88.7	94.0	100.8	94.2	95.4	99.2	81.2	84.3	76.5	87.0
Hist	100.6	101.6	94.2	98.3	101.1	82.4	90.2	98.8	89.3	89.9	96.5	76.8	81.7	72.1	84.5
Iso	95.4	97.5	97.3	91.8	100.8	81.9	82.6	95.3	86.5	87.5	94.0	69.3	71.5	66.3	78.3
Leuc	94.9	96.8	95.8	94.4	99.5	84.5	87.9	96.1	90.7	90.1	95.5	75.8	75.0	71.1	82.2
Lys	98.1	99.5	95.5	96.6	102.5	86.3	88.6	98.2	90.9	94.2	98.2	80.5	83.3	72.7	86.7
Meth	83.9	91.7	91.0	92.2	97.3	87.5	91.2	94.4	93.3	94.2	91.1	85.3	83.2	77.8	88.1
Phen	96.0	98.0	95.6	94.8	100.0	82.9	85.1	96.1	89.2	89.3	94.6	70.8	72.5	62.8	76.7
Threo	95.8	101.3	96.5	97.3	101.8	80.5	83.2	93.7	87.8	86.3	95.2	67.9	75.2	56.6	73.3
Val	94.6	97.2	94.0	91.2	100.4	80.8	82.1	94.6	87.1	87.1	93.5	70.5	71.5	65.8	78.1

[1] Gungarru variety; [2] Dunn variety; [3] Analysed composition (see Allan and Frances, 1994 for methods of analysis); [4] Allan et al., unpublished data, August 1994; [5] Allan et al., unpublished data, October 1994; [6] Allan et al., unpublished data, December 1994; [7] Allan et al., unpublished data, August 1995; [8] Values are means (n=3 replicates)

TABLE 7

Formulation of control diet (SP35) and two least-cost diets based on meatmeal and pulses (95LC1 and 95LC2).

Ingredient	Quantity in diet (g/100g)			Dry matter (%)	Protein content (%)	Ingredient cost[1] (AD$/t)
	SP35	95LC1	95LC2			
Fishmeal (Danish)	27.00	10.00	5.00	94.70	65.86	1300
Lambmeal	-	21.71	36.88	97.15	52.75	400
Bloodmeal	2.00	2.09	-	88.65	85.32	900
Corn gluten meal	4.00	3.77	5.19	92.90	57.68	700
Soybean meal	20.00	-	-	89.11	45.55	495
Canola	-	-	5.00	91.74	40.00	325
Peanut meal	-	-	5.00	94.75	39.00	335
Field peas	-	14.92	10.39	88.55	24.44	235
Lupins (Gungaroo) dehulled	-	25.50	7.36	95.02	43.61	350
Wheat	26.85	-	-	90.78	13.70	180
Sorghum	11.00	4.70	-	89.59	14.62	180
Millrun	2.00	10.00	17.70	89.67	19.62	190
Fish oil	1.00	2.91	3.21	100.00	-	800
DL-methionine	0.15	0.40	0.27	100.00	78.6	5500
Vit/min premix	4.00	4.00	4.00	100.00	-	4000
C-Calcium phosphate	2.00	-	-	100.00	-	610

[1] Prices from NSW Agriculture Sydney Retail Feed Ingredient Prices or from commercial feed manufacturers (does not include freight).

TABLE 8

Fish performance on control and least-cost diets[1,2]

Diet	Survival (%)	Weight (g)	Growth rate (g/fish/day)	Production (kg/ha)	FCR	Diet ingredient cost ($/kg fish)[3]
SP35	97.5±1.1[a]	395.4±11.6[a]	2.23±0.07[a]	5779±127[a]	2.23±0.03[b]	1.69±0.03[c]
95LC1	97.0±0.4[a]	431.9±9.9[b]	2.53±0.09[b]	6283±117[b]	1.97±0.09[a]	1.22±0.06[b]
95LC2	96.8±0.5[a]	439.8±5.6[b]	2.53±0.03[b]	6450±83[b]	1.93±0.03[a]	1.09±0.02[a]

[1] Fish were grown from an average initial weight of 81 g from December 1995 to May 1996 in aerated, static 0.1 ha earthen ponds.

[2] Values are means ± sem for 3 replicate ponds, means in columns which share the same superscript were not significantly different (P>0.05; ANOVA; SNK).

[3] Diet ingredient costs = the cost of feed (total for ingredients only) to produce 1 kg fish (FCR x cost of ingredients) and were based on ingredient costs of SP35 ($756.38/t), 95LC1 ($619.2/t), and 95LC2 ($566.3/t).

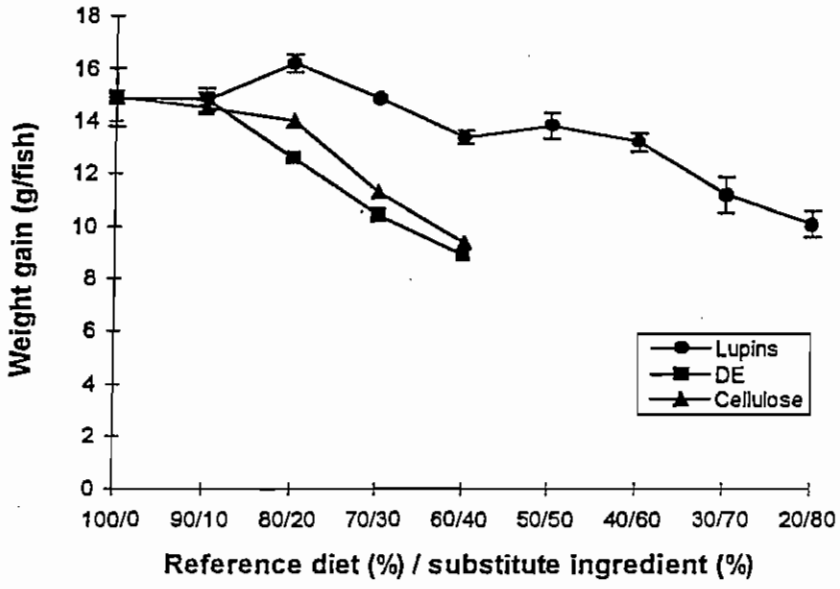

Figure 1. Growth of silver perch (*Bidyanus bidyanus*) fed experimental diets composed of a reference diet and either dehulled lupins, diatomaceous earth or cellulose.

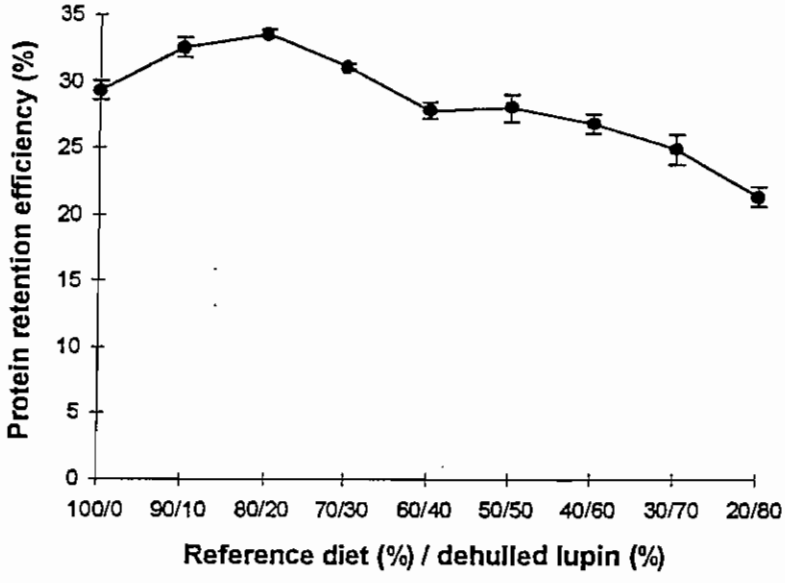

Figure 2. Protein retention efficiency of silver perch (*Bidyanus bidyanus*) fed experimental diets composed of different ratios of a reference diet and dehulled lupins.

Fishmeal Replacement in Aquaculture Diets using Rendered Protein Meals

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Abstract

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

Introduction

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

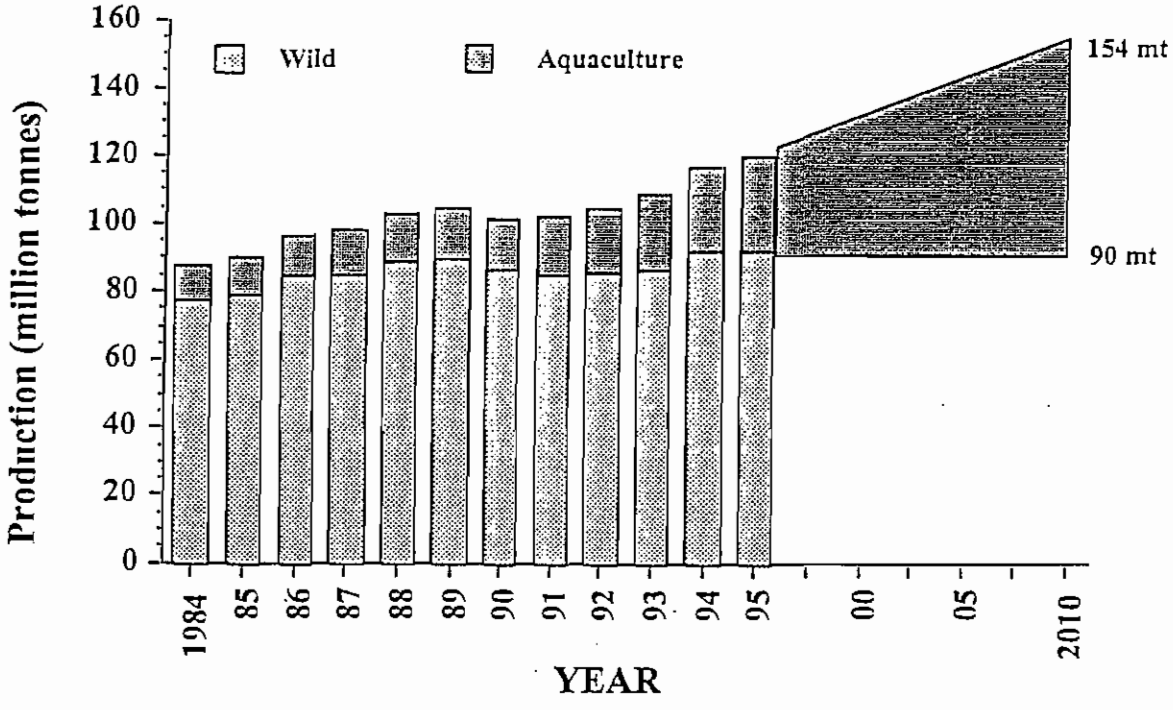
Responding to the need for sustainable non-marine protein sources, the Australian Fisheries Research and Development Corporation (FRDC) funded a nationally coordinated research program in 1993 to find suitable alternatives to fishery product for four Australian 'template' species – a marine, predominantly carnivorous prawn (giant tiger prawn, *Penaeus monodon*), a warm-water carnivorous euryhaline fish (barramundi, *Lates calcarifer*), a freshwater omnivorus fish (silver perch, *Bidyanus*

bidyanus) and a cold-water diadromous carnivorous fish (Atlantic salmon, *Salmo salar*). This research was extended through funding from the Meat Research Corporation to examine rendered protein meals as replacements of fish meal in diets for the giant tiger prawn, barramundi and silver perch. This paper reviews the expanding role of aquaculture globally and the Australian research that has been done to determine the suitability of locally available meat meals as cheaper alternatives to fish meal in manufactured aquafeed.

Aquaculture expansion and aquafeed requirements

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

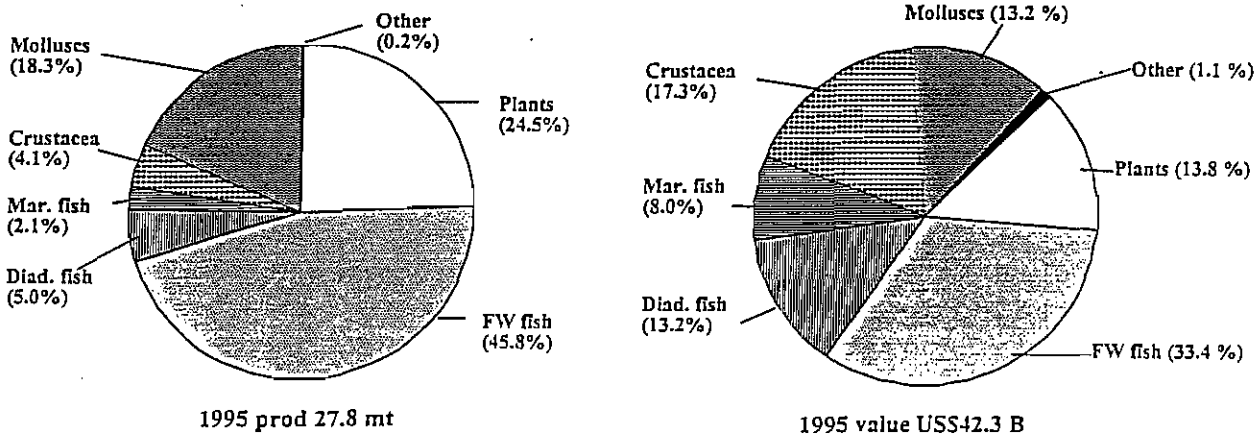
Figure 1. World fisheries and aquaculture production and forecast aquaculture requirement to year 2010



Although more than 250 aquatic species are currently being cultured world wide (Rana 1997), 50% of the 1995 production by weight was derived from just seven species (kelp, four carp species, yesso scallop and pacific oyster) with all of the animal species being either inland freshwater herbivorous fish or marine filter feeders. However, crustaceans (predominantly the giant tiger prawn, *Penaeus monodon*), diadromous fish (predominantly the salmonids rainbow trout, *Oncorhynchus mykiss* and Atlantic salmon, *S. salar*) and marine fish (predominantly Japanese or red seabream, *Pagrus major*) comprise almost 40% (US$16.3 B) of the total

(Figure 2). Species in this group are all strict or essentially carnivorous in feeding habit and all rely on compounded aquafeeds. Within developed countries, it is this group of cultured animals that has shown the greatest increases in production (Tacon 1996) and in turn, stimulated a massive expansion in manufactured aquafeed.

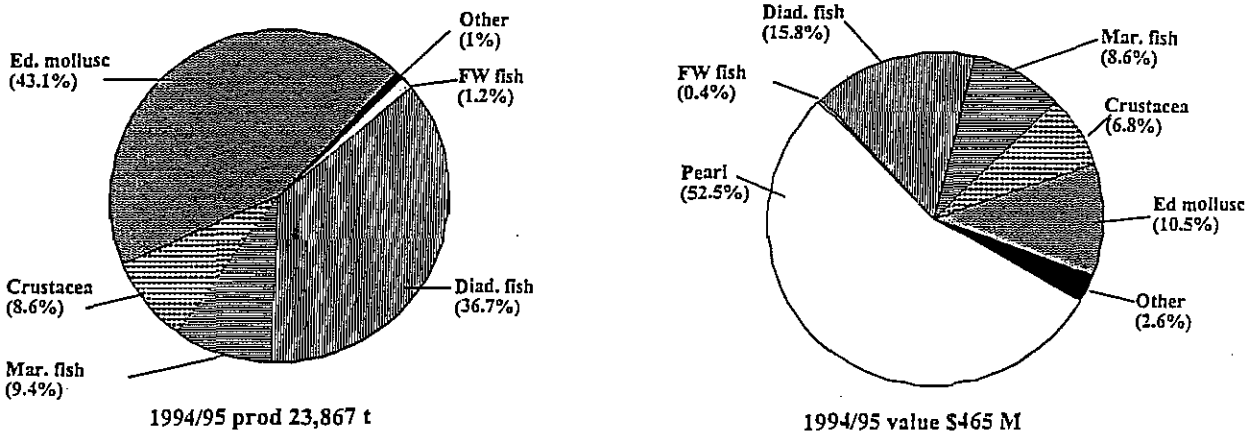
Figure 2. World production and value of aquaculture for major culture groups in 1995 (after Rana 1997)



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

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

Figure 3. Australian production and value of aquaculture for major culture groups in 1994/95 (after O'Sullivan and Kiley 1996)



Need for fish meal alternatives

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

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

Nationally-coordinated research on fish meal replacement

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

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

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

Nutritive value of rendered protein (meat) meals

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

Table 1. **Analysed dry matter (DM), ash, protein, lipid and energy composition for various protein concentrate feeds available in Australia**

Ingredient	DM (%)	Ash (% DM)	Protein (% DM)	Lipid (% DM)	Energy (kJ/g DM)	Cost ($/kg CP)
Meat meal						
AMH (mixed)	94.2	32.6	55.9	10.4	16.8	0.84
Aspen (Provine®)	94.3	9.4	80.9	13.0	25.0	1.02
Beef city (beef)	97.0	38.6	46.7	7.4	13.9	0.98
Fletcher (lamb)	97.2	34.5	53.4	7.4	15.7	0.87
Midco (mixed)	97.7	12.1	60.6	14.5	23.5	0.85

Fishmeal						
Tasmanian	93.5	14.2	75.8	9.9	21.4	1.41
Danish	93.1	13.0	73.2	11.4	21.9	1.76
Peruvian	90.1	15.0	70.5	12.9	21.8	1.42
Soybean (solv ext)	89.1	7.3	52.9	1.6	19.9	1.05
Canola	91.7	6.8	40.9	3.1	19.7	0.87
Lupin (de-hulled)	90.5	3.5	44.8	7.1	20.6	0.86
Wheat gluten	94.0	1.5	76.9	0.5	23.1	1.04

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

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

Table 2 Apparent dry matter, protein and energy digestibility coefficients of various protein concentrates determined with prawns (P), silver perch (SP) and barramundi (B)[1]

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

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

The apparent digestibility of the meat meals was generally lower than for the fish meals with all three aquatic species and particularly so for energy. Interestingly, gluten was highly digestible in all species while that of soybean meal was as well digested as the meat meals. Apparent digestibility values with barramundi were generally lower than with either prawns or silver perch for all feed ingredients other

than Danish fish meal and gluten, both of which were highly digestible in all species. The more carnivorous barramundi may be less capable of digesting terrestrial feeds. The digestibility values for meat meals compare favourably with estimates derived with rainbow trout (Asgard 1988; Alexis et al. 1988).

The apparent essential amino acid digestibility of fish meal and various meat meals as determined with prawns is shown in Table 3. Each of the essential amino acids was digested equally as well but tended to be slightly lower than that for the overall protein; digestibility of the fish meal was high and similar to that of the overall protein. With silver perch, the apparent digestibilities of the individual essential amino acids were similar both to one another and to that for the whole protein except for arginine which was from 10 to 20% lower than the other amino acids.

Table 3. The essential amino acid content (Con; g/100g DM) of fish meal and various meat meals (MM) and their apparent digestibility (AD; %) for prawns

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

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

Table 4. The apparent digestibility (AD) and cost of protein (P) and lysine (Lys) of alternative protein feed ingredients as determined for silver perch

Feed ingredient	ADP (%)	ADLys (%)	Cost $/t	$/kg ADP	$/kg ADLys)
Meat meal					
Beef city (beef)	33.8	1.91	445	1.32	23.3
Fletcher (lamb)	38.1	2.74	453	1.19	16.5
Midco (mixed)	49.4	2.85	500	1.01	17.5
Aspen (Provine)	67.8	3.53	775	1.14	22.0

Fishmeal					
Danish	68.1	5.23	1200	1.76	22.9
Peruvian	62.0	5.23	850	1.37	16.2
Soybean meal	47.6	2.82	440	0.92	15.6
Peanut meal	39.4	1.56	380	0.96	24.4
Canola meal	40.3	2.25	330	0.82	14.7
Cottonseed	41.6	1.49	370	0.89	24.8
Lupin (hull-on)	30.8	1.58	298	0.97	18.9

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

Nutrient requirements

It is beyond the scope of this paper to review in any detail the nutrient requirements of the three aquatic species - giant tiger prawn, silver perch and barramundi - that were used to examine the comparative value of rendered protein meals. For many of the micro-nutrients including vitamins, essential fatty acids and essential amino acids, requirements have not been well defined and even optimum protein (amino acid) to energy relationships are at best working guesstimates rather than precise quantitative requirements. However, a broad awareness of the gross requirements of the different species is necessary to evaluate the extent to which alternative protein meals might be able to substitute for fish meal. Table 5 provides information on 'typical' high fish meal-based formulations, ingredient cost of the diets and key dietary specifications for each of the three target species. For more specific information on nutrient requirements, the reader is referred to Allan and Rowland (1992) for silver perch, D'Abramo et al. (1997) for prawns and Williams and Barlow (1996) for barramundi.

Table 5 **Formulation, ingredient cost and key nutrient specifications of typical high fish meal diets for giant tiger prawn, silver perch and barramundi**

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

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

Nutrient assimilation of rendered meat meals

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

barramundi and silver perch to determine nutrient retention following the feeding of diets containing serial increments of meat meal. An aquarium study with prawns was also carried out to examine the effect of meat meal substitution of fish meal.

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

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

Fig. 4A

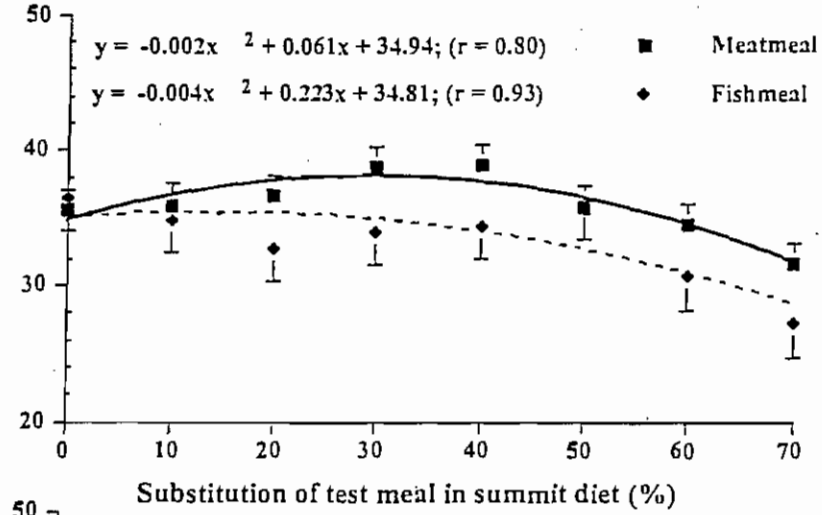
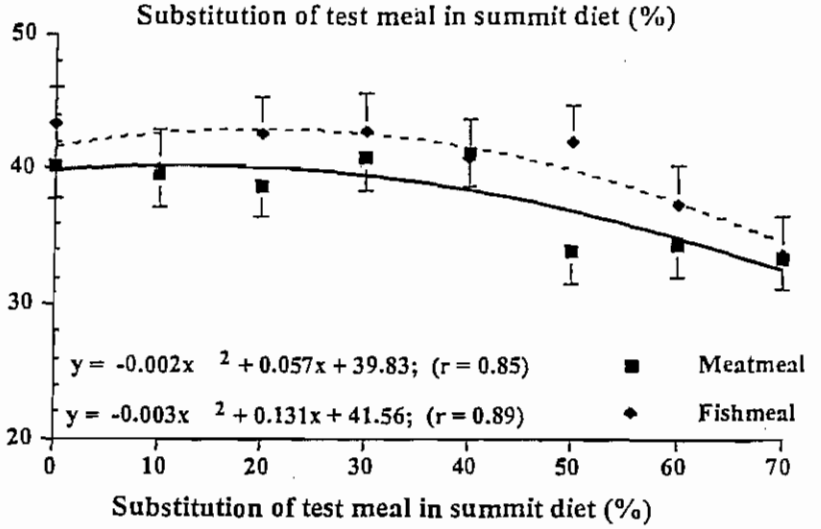


Fig. 4B



As is evident from Figure 4, net protein retention of the meat meal series diets was similar to, if not better than that for fish meal whereas the reverse was the case for net energy retention. However, the overall retention of protein was low (viz. <38% as compared to >50% for pigs; Williams et al. 1993) but similar to that of European bass (*Dicentrarchus labrax*) where retention of dietary protein has been reported to vary

from as low as 16 to 18% (Hidalgo and Alliot 1988; Tibaldi et al. 1994) to more typical values of 40 to 46% (Ballestrazzi et al. 1994). Energy retention has similarly been found to vary from 20% to >60% (Cho et al. 1982; Knights 1985; Steffens 1989) and to be affected particularly by the protein to energy (lipid) ratio of the diet. Given their carnivorous feeding habit and dependence on dietary protein for metabolic energy, it is not surprising that retention of dietary protein by barramundi is low. This is the most likely explanation why the relatively poorer essential amino acid composition of meat meal protein did not adversely affect protein deposition (somatic growth) in the barramundi. As a comparatively large proportion of the absorbed amino acids would have been used for energy rather than protein synthesis metabolism, the least abundant essential amino acids would presumably therefore be preferentially conserved for protein synthesis by the animal. Thus, the essential amino acid composition of the dietary protein appears to be much less important in barramundi (and almost certainly in other carnivorous aquatic animals where metabolic energy is derived mainly from dietary protein) than terrestrial monogastrics (pigs and poultry) where dietary protein is used predominantly as a source of amino acids for protein synthesis with carbohydrate being the primary source of metabolic energy.

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

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

Table 6. Main constituents and critical nutrient content of experimental diets evaluating meat meal for silver perch at PSRC

Attribute	Diet				
	1	2	3	4	5
	Formulation (g/100g)				
Fishmeal (Danish)	27.0	13.0	6.0	0	0
Meat meal (Fletcher)	0	6.3	7.8	8.9	8.9
Meat meal (Provine)	0	9.1	14.7	18.1	18.9
Blood meal	2.0	3.4	3.0	3.9	3.9
Soybean meal	20.0	20.0	20.0	20.0	20.0
Gluten (corn)	4.0	6.0	6.0	6.0	6.0
Grain product	39.9	35.1	34.9	35.0	35.2
Other[1]	7.1	7.1	7.6	8.1	7.1
	Composition (air-dry)				
Dig. protein (%)	32.1	34.0	34.1	34.1	34.0
Dig. energy (kJ/g)	13.0	13.4	13.3	13.2	13.3
Lipid (%)	6.4	6.2	6.0	5.7	5.8
Ash (%)	10.0	11.2	11.2	11.2	11.2
Dig. lysine (%)	2.1	2.2	2.0	2.0	1.8

Dig. meth + cystine (%)	1.4	1.4	1.4	1.4	0.9
Dig. threonine (%)	1.4	1.4	1.4	1.4	1.3

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

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

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

Table 7. Performance of silver perch fed diets reducing in fish meal content in the tank experiment at PSRC

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

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

Possible reasons for the reduced growth of the silver perch on the low fish meal diets are: a), insufficient essential nutrients; b), reduced attractiveness of the high meat meal diets; and/or c), some growth-reducing effect of the meat meal or conversely, some growth-enhancing effect of the fish meal. As digestible protein and digestible energy contents were similar for all diets, differences in protein to energy ratio do not explain the differences in growth rate. Similarly, essential fatty acid contents were equalised through the addition of fish oil and therefore would seem not to be the reason. Although the essential amino acid composition of meat meal was lower than for fish meal, this also was compensated for by crystalline amino acid supplementation. Had amino acids limited growth, a more marked difference between the amino acid supplemented (diet 4) and the non-supplemented (diet 5) diets would have been expected. While there is some conjecture about the efficacy of crystalline amino acids in aquatic animals (Lovell 1989; Cowey 1992), essential amino acid concentrations in all diets (including the non-supplemented diet 5) were above published requirements for channel catfish (NRC 1993) and moreover, protein retention was unaffected by diet. Food consumption of the fish on the high meat meal diets was lower than for those on the high fish meal diets which suggests a palatability effect. This is surprising as meat meal has been found to be highly attractive to other fish, including barramundi (Moshen and Lovell 1990; Watanabe et

al. 1993; Williams and Barlow 1996). The increased concentration of saturated fatty acids in the high meat meal diets may have contributed to the apparent reduced palatability of these diets.

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

Table 8. Ingredient composition of diets[1] with increasing replacement of fish meal using two meat meal sources trialed with prawns in aquaria

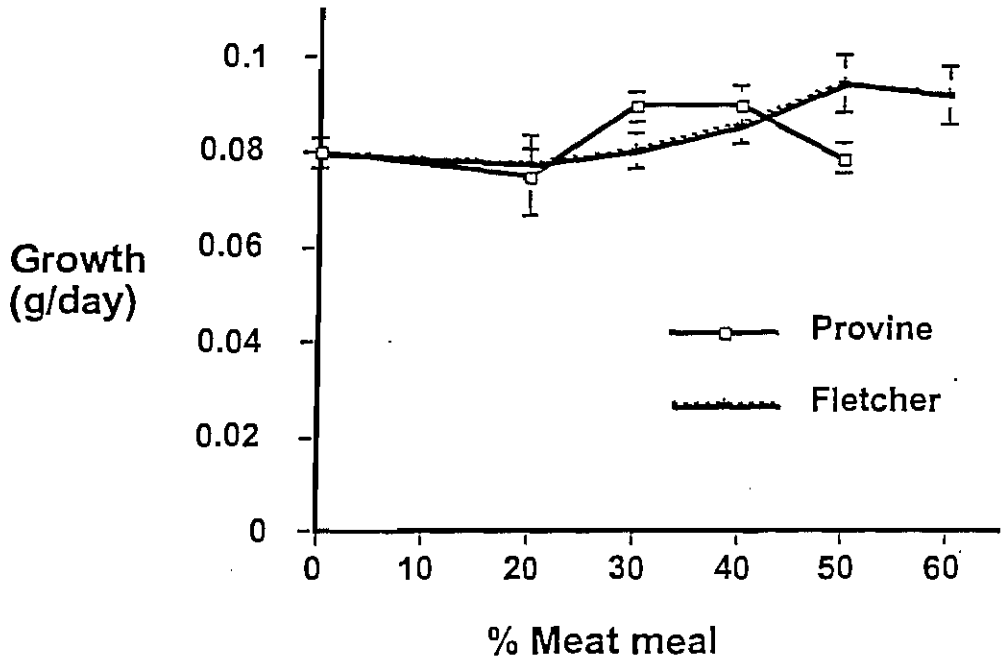
Ingredient	Diet designation									
	Base	Provine				Fletcher				
		20%	30%	40%	50%	20%	30%	40%	50%	60%
Fishmeal (Tas)	38.9	23.3	15.5	7.7	0	27.1	21.2	15.4	9.5	3.6
Meat meal (Provine)	0	20.0	30.0	40.0	50.0	0	0	0	0	0
Meat meal (Fletcher)	0	0	0	0	0	20.0	30.0	40.0	50.0	60.0
Squid mince (dried)	5.0	5.0	5.0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

1 Formulated to constant digestible protein content of 35.0%.

2 Comprised vitamin, squid oil and essential lipid supplements.

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

Figure 5. **Average daily growth of juvenile prawns fed diets with increasing replacement of fish meal using two meat meal sources trialed with prawns in aquaria at CSIRO**



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

Suitability and economics of meat meal under farm conditions

The partial or complete replacement of fish meal in diets for silver perch and barramundi diets has been examined under farm conditions. In the silver perch work, two least-cost diets (LC1 and LC2) were formulated to be of equivalent nutrient specification to the reference diet (SP35) when the fish meal was constrained to inclusions of either 10 or 5 % (Table 9). The LC1 and LC2 diets were 18 and 25% less expensive than the reference diet, respectively and contained up to Å37% meat meal. All three diets were manufactured commercially and fed at 3% bodyweight/d to fish stocked in 0.1 ha ponds (1,500 fish/pond; 28 g initial weight) at the Grafton Research Centre (GRC). Three replicate ponds were assigned to each diet and the fish were cultured to a marketable weight of ³400 g.

Table 9. Main constituents and critical nutrient content of the Reference and least-cost diets trialed with silver perch in pond experiment at GRC

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

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

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

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

Attribute	Diet designation			
	SP35	LC1	LC2	Pooled sem
Growth rate (g/d)	2.23^{B}	2.53^{A}	2.53^{A}	0.06
Food conversion (g:g)	2.23^{B}	1.97^{A}	1.93^{A}	0.05
Survival (%)	97.5^{A}	97.0^{A}	96.8^{A}	0.7
Production (t/ha pond)	5.78^{B}	6.28^{A}	6.45^{A}	0.11
Productivity cost ($/kg gain)	1.69^{C}	1.22^{B}	1.09^{A}	0.04

Fish composition				
Dry matter (DM; %)	41.2^{A}	43.1^{A}	42.9^{A}	0.7
Nitrogen (% DM)	6.18^{A}	5.39^{A}	5.68^{A}	0.08
Ash (% DM)	7.9^{A}	6.9^{A}	7.7^{A}	0.3
Fat (% DM)	50.9^{A}	55.0^{A}	54.4^{A}	0.7
Energy (kJ/g DM)	29.72^{A}	30.55^{A}	30.12^{A}	0.21
Sensory evaluation[1]				LSD
Yellow appearance	7.3^{A}	6.8^{A}	3.6^{B}	2.5
Fishy flavour	41.7	42.8	41.6	ns
Muddy flavour	7.9	10.4	8.4	ns
Flaky texture	21.6^{B}	27.3^{A}	22.3^{B}	4.6
Overall liking	66.7	68.5	67.8	ns

A,B,C Within row comparisons, means without a common superscript letter differ (P<0.05); LSD, (P<0.05).

1 All scores were scaled from zero (none) to 100 (very).

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

In the barramundi work, a high fish meal control (Ctl) diet was evaluated against a commercial grow-out barramundi diet (Rid1 or Rid2 in Experiments B1 or B2, respectively) and two diets containing high inclusions of meat meal (Table 11). In Experiment B1, two sources of meat meal were compared, one being a conventional high-ash product from the Casino abattoir with a crude protein content of 52% while the other was a low-ash product from the Midco abattoir with a crude protein content of 60%. These two meat meals were compared when each contributed 55% of the total dietary crude protein content and diets (M1 and M2 respectively) were formulated to be isonitrogenous and isoenergetic with the Ctl diet. Diets M1 and M2 both contained 10% fish meal (contributing 15% of the total dietary protein content). In Experiment B2, the Casino meat meal was used as the major source of dietary protein in two diets (M3 and M4) at the total exclusion of fish meal. Diet M3 was formulated to be isonitrogenous and isoenergetic with the Ctl diet while diet M4 was formulated to have a higher digestible energy content (15 vs 16.2 kJ/g, respectively) but with the same protein to energy ratio of 29 mg/kJ. All diets were commercially manufactured.

Table 11. Formulation and critical nutrient composition of the low-fish meal diets fed to barramundi in two (B1 and B2) on-farm cage experiments

Attribute	Diet description and experiment						
	Ctl	M1	M2	M3	M4	Rid1[1]	Rid2[1]
Expt	B1, B2	B1	B1	B2	B2	B1	B2
	Formulation (%) and cost						
Wheat	30.4	18.1	29.9	16.1	10.4		
Fish meal (Chile)	35.0	10.0	10.0	0	0		
Meat meal (Casino)	0	45.0	0	50.0	50.0		
Meat meal (Midco)	10.0	0	40.0	0	0		
Blood meal (ring)	0	0	0	7.0	9.0		
Soybean (fullfat)	16.0	16.0	5.0	15.0	10.0		
Soybean (solvent)	0	0	5.0	0	0		
Gluten	5.0	5.0	5.0	5.0	10.0		
Fish oil	2.5	4.0	3.3	5.0	6.0		
Vit & min premix	1.1	1.9	1.8	1.9	4.6		
Ingredient cost ($/t)	884	650	1129	621	678	---	---
	Composition (air dry basis)						
Dig. energy [2] (kJ/g)	15.0	15.0	15.2	15.0	16.2	15.0	15.0
Protein (%)	43.8	43.0	43.0	42.5	47.8	54.3	50.1
Ash (%)	9.5	14.9	9.5	14.6	14.1	9.3	7.6
Lysine (%)	2.83	2.67	2.74	2.77	3.16	4.61	4.11
Meth + Cyst (%)	1.07	0.89	0.92	1.01	1.17	1.08	1.35
Threonine (%)	1.70	1.47	1.56	1.45	1.65	2.39	2.15

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

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

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

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

There were no significant ($P>0.05$) differences between diets for fish productivity in Experiment B1 while in Experiment B2, fish fed the M4 diet performed best overall, growing faster ($P<0.05$) than those fed either Ctl or Rid2 diets (Table 12). Fish fed the

Ctl diet in Experiment B2 had the lowest food issued and the best food conversion which was better than either M3 or Rid2 diets (P<0.05).

Table 12. Production responses and dressing-out percentage of barramundi fed low-fish meal diets in two (B1 and B2) on-farm cage experiments

Attribute[1]	Diet						
	Ctl	M1	M2	M3	M4	Rid1/Rid2	± sem
	Experiment B1						
Growth (g/week)	18.1^{A}	17.6^{A}	17.9^{A}			21.2^{A}	1.75
Food conversion (g:g)	1.44^{A}	1.43^{A}	1.47^{A}			1.25^{A}	0.070
Fish recovered (%)	97.6^{A}	96.6^{A}	96.8^{A}			98.3^{A}	0.85
Dressing-out (%)	88.8^{A}	89.5^{A}	89.6^{A}			88.6^{A}	0.30
Food cost ($/kg gain)[2]	1.27^{B}	0.93^{A}	1.65^{C}			^{AB}	0.060
	Experiment B2						
Growth (g/week)	20.8^{B}			21.4^{AB}	23.2^{A}	20.3^{B}	1.75
Food conversion (g:g)	1.22^{A}			1.44^{B}	1.31^{AB}	1.37^{B}	0.070
Fish recovered (%)	94.6^{A}			97.8^{A}	97.9^{A}	99.2^{A}	2.96
Dressing-out (%)	89.9^{A}			88.7^{A}	88.6^{A}	89.4^{A}	0.30
Food cost ($/kg gain)[2]	1.08^{B}			0.89^{A}	0.88^{A}	^{B}	0.038

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

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

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

Replacing fish meal with Casino meat meal reduced the ingredient cost of the diet by 30% and also the food productivity cost ($ food cost/kg fish gain) by 18 to 23%. In the prevailing economic climate and in the absence of an environmental incentive, a low-ash meat meal could be expected to attract a price premium of about 15% above that of a high-ash product. This premium is primarily a function of the relative difference in protein content between the alternative products. Increasing the digestible energy content of the diet in Experiment B2 from 15 to 16.2 kJ/g caused a 10% increase in the ingredient cost of the diet (from $621 to $678/t) but this was offset by increased fish productivity such that the food productivity cost was essentially the same (0.89 vs 0.88 $/kg gain).

Differences between diets in sensory scores (Table 13) were evident only in the flesh of fish from Expt B1 where stronger (P<0.05) fishy and sweet flavours were found for the M1 and M2 diets as compared to the Rid1 diet; fish fed the Ctl diet were softer in

texture than those fed other diets. Irrespective of diet, the overall liking of the flesh of the fish was very high (scores of >58) and with scores for all undesirable taints such as "muddy", "weedy" or "metallic" being very low (² 18).

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

Attribute[1]	Diet						
	Ctl	M1	M2	M3	M4	Rid1/ Rid2	±sem
	Experiment B1						
Appearance							
Greyish	23.8	24.6	25.5			24.3	0.98
Yellow	8.6	9.4	8.7			6.9	0.75
Flavour							
Fishy	35.2^{AB}	37.3^{A}	36.8^{A}			33.0^{B}	1.30
Sweet	19.2^{B}	21.7^{A}	22.4^{A}			18.1^{B}	1.28
Muddy	16.1	18.2	14.8			17.7	1.57
Texture (firm)	31.5^{B}	36.1^{A}	35.9^{A}			37.1^{A}	1.71
Overall liking	58.4	62.1	62.8			59.3	1.23
	Experiment B2						
Appearance							
Greyish	10.5			9.7	10.5	9.5	1.18
Yellow	6.9			9.1	8.8	7.6	1.26
Flavour							
Fishy	49.0			45.5	47.3	46.8	1.29
Sweet	29.9			27.5	29.9	28.9	1.50
Muddy	14.8			15.9	13.9	16.6	1.55
Texture (firm)	46.5			44.3	46.9	47.3	1.72
Overall liking	60.0			61.2	64.3	63.5	1.65

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

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

Meat meal, either alone or in combination with other terrestrial protein concentrates such as soybean meal and gluten meal, has been successfully used for the partial replacement of fish meal in diets for cultured carnivorous fish including channel catfish *Ictalurus punctatus* (Mohsen and Lovell, 1990; Lovell, 1992) yellowtail *Seriola quinqueradiata* (Shimeno et al., 1993a,b), rainbow trout *O. mykiss* (Watanabe et al., 1993; Yamamoto et al., 1995). sea bream *S. aurata* (Davies et al., 1991) and European

sea bass *D. labrax* (Langar and Metailler, 1989). In these cited studies, meat meal was used to replace from 30 to 91% of the protein contributed by fish meal without any adverse effect on fish growth. Higher levels of fish meal replacement were not examined possibly for fear of the diets not being palatable to the fish. The only known report where meat meal has been used as a replacement of fish meal in diets for barramundi is the French study of Aquacop et al. (1991). They found including greaves meal (a rendered high fat meat meal product) at 22% of the diet as a partial substitute of fish meal to have no adverse effect on its digestibility nor did it cause a decrease in the performance of the fish. In more herbivorous/omnivorous species, meat meal has similarly been found to be suitable for the partial replacement of fish meal in tilapia *Oreochromis mossambicus* (Davies et al. 1989) and prawns (Tacon 1993).

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

Conclusions

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

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

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

premium of from 15 to 20% (on a per unit of digestible protein basis) but only if the fat content is kept below about 7 to 8%.

Acknowledgements

The work reported in this paper was carried out under the auspice of the "Fishmeal Replacement Sub-program" of the Australian Fisheries Research and Development Corporation with additional funding provided principally by the Meat Research Corporation. We acknowledge the support received from Messrs Peter Dundas-Smith and Denis Roberts of the two aforementioned Corporations respectively in facilitating the funding of these studies. Reference has been made to digestibility values determined with barramundi by Dr Neil McMeniman, University of Queensland, and this contribution is gratefully acknowledged. Our thanks are extended to the teams of dedicated technical and support staff at Bribie Island Aquaculture and Research Centre, Cleveland Marine Laboratory, Port Stephens Research Centre and Walkamin Freshwater and Fisheries Centre without which this work would not have been possible.

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Development of an experimental diet for silver perch (*Bidyanus bidyanus*)

Stuart Rowland

NSW Fisheries is conducting a research project aimed at developing technology for growing silver perch in earthen ponds. This article details a diet that has been formulated for the initial experiments and which has produced encouraging results.

By Geoff Allan[1] & Stuart Rowland[2]

Freshwater finfish is the major component of world aquaculture production. In 1987, approximately 6.8 million t of finfish were produced, of which 88.4% was farmed in freshwater (Nash and Kensler, 1990). Although there are many indigenous freshwater fish in Australia that are highly regarded for their edible qualities, many of these species are no longer abundant. Hatchery techniques have been developed for some species (Rowland, 1989); however, with the exception of barramundi (*Lates calcarifer*) there has been no research into the grow-out of native finfish.

Currently there is only a small industry (1613 t in 1989-90) based on the freshwater production of the exotic rainbow trout, *Oncorhynchus mykiss* (O'Sullivan, 1992).

Rowland and Barlow (1991) suggested that the native freshwater fish silver perch (*Bidyanus bidyanus*) has high potential for aquaculture because hatchery techniques are established and the species is hardy, grows rapidly in farm dams, is omnivorous and readily accepts pellets.

A major research project to determine the feasibility and develop techniques for the intensive culture of silver perch commenced at NSW Fisheries', Eastern Freshwater Fish Research Hatchery (EFFRH), Grafton, in 1990. A component of the project, the evaluation of feeds, is being partly funded by the Fisheries Research and Development Corporation. Formulated feed represents one of the major costs in finfish aquaculture, accounting for up to 60% of total operating costs (Manzi, 1989). The development of nutritionally adequate, cost-effective diets is therefore one of the major factors limiting the establishment of an economically successfully aquaculture industry. One of the research priorities for the silver perch project at EFFRH is to determine protein requirements. Requirements for other omnivorous freshwater species, such as channel catfish (*Ictalurus punctatus*) are in the range 25-36% protein (Robinson, 1989) while requirements for carnivorous freshwater species such as rainbow trout (*Oncorhynchus mykiss*) are higher (40-45%; Halver, 1989).

The initial nutrition experiment was conducted with fry (0.6 g) stocked in 1,000 litre aerated tanks and fed isoenergetic diets with protein contents of 21, 36 and 49%. The fastest growth was recorded with the 36% protein diet; however, differences in growth between fish on this diet and the 49% protein diet were not significant (Allan and Rowland, 1991). The results indicated that the dietary protein requirement for juvenile silver perch would exceed 21% and would probably be closer to those required by other omnivorous freshwater species than to those required by carnivorous freshwater species such as rainbow trout.

These results provided the basis for the formulation of a diet to be used in pond trials. The diet, SP35 (Table 1) was also formulated to satisfy or exceed the published requirements for channel catfish of essential amino acids, digestible energy to protein ratio and available phosphorus (NRC, 1983; Lovell, 1989; Robinson, 1989). Published results for nutrient digestibility and phosphorus availability for catfish (NRC, 1983; Robinson, 1989) were also used. Even though requirements for essential fatty acids are likely to be lower for silver perch than for carnivorous marine species (Anderson and Arthington, 1992), fish oil was added to the diet to ensure that essential fatty acid deficiencies did not depress growth in in silver perch.

The diet was manufactured in the form of crumbles (2 mm; 3 mm) for fry and fingerlings, and pellets (3 x 12 mm; 6 x 12 mm) for larger fish. All experimental diets were manufactured by Janos Hoey Pty Ltd, Forbes, NSW, and stored at 15°C until used.

SP35 was first used in a fingerling production experiment. Silver perch fry (0.6 g) were stocked into six, aerated 0.1 ha earthen ponds and fed 2 mm and 3 mm crumbles at rates up to 3% body weight per day. Within two weeks, fry were readily feeding on the crumbles. Fingerlings (16 g) were harvested after 12 weeks; survival rates ranged from 97 to 100% and the food conversion ratios ranged from 1.0 to 1.3 (S. Rowland,

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unpublished data, 1992).

A grow-out phase experiment is currently underway in the earthen ponds. Fingerlings were stocked in May and fed SP35 at rates up to 3% body weight daily. Fish fed throughout winter, and growth has been rapid since late spring. Mean weights of silver perch in six ponds at the end of February, 1992, ranged from 436 to 581 g and assuming high survival, estimated standing crops in some ponds may exceed 8 t/ha (S.Rowland, unpublished data, 1992).

Stuart Rowland

Harvesting fingerlings from EFFRH ponds at Grafton, NSW

The results of the nutrition and production research to date, suggest that the experimental diet, SP35, is suitable for the pond production of fingerling and market size (400-500 g) silver perch.

The formulation of this diet will probably be modified after further nutrition experiments. Experiments to define the optimum protein requirements and to determine the digestibility of a number of protein sources are underway at EFFRH and Brackish Water Fish Culture Research Station. Subsequent research will concentrate on ways to reduce the cost of silver perch diets by defining optimum protein to energy ratios, and formulating practical diets with reduced fishmeal and increased soybean (or other plant protein) content.

TABLE 1: Formulation and biochemical composition of the experimental diet, SP35, for silver perch.

Ingredients	%
Fish meal	27.0
Soybean meal	20.0
Blood meal	2.0
Corn gluten meal	4.0
Wheat	28.4
Sorghum	11.0
Millrun	2.0
Cod liver oil	1.0
Di-calcium phosphate	2.0
Vitamin/mineral premix[1]	2.5
L-methionine	0.15
Proximate composition (as fed basis)	%
Crude protein (N x 6.25)[2]	35.6
Crude fat (ether extract)[2]	5.5
Linolenic series (n-3) fatty acids[3]	1.1
Fibre (acid detergent)[2]	4.4
Carbohydrate (difference)[2]	52.1
	(g/kg)
total methionine[4]	7.4
Total lysine[4]	22.6

[1] Included the following (per kg diet) Retinol 2.4 mg: Cholecalciferol 25 µg: ∞-Tocopherol acetate 125 mg: Menadione sodium bisulfite 16.5 kg: Thiamin. HCl 10 mg: Riboflavin 25.5 mg: Nicotinamide 200 mg: Ca-pantothenate 54.5 mg: Pyridoxine. HCl 15 mg: Cyanobalamin 20 µg: folic acid 4 mg: Biotin 1 mg: Ascorbic acid 450 mg: Myo-inositol 600 mg: Choline chloride 1500 mg: $CaCO_3$ 7.5 g: $MnSO_4$ 0.3 g: $ZnSO_4$ $7H_2O$ 0.7 g: $FeSO_4$ $7H_2O$ 0.5 g: $CuSO_4$ 60 mg: NaCl 7.5 g: KIO_3 2 mg.

[2] Methods described by Faichney and White (1983).

[3] Neutral and polar lipid fractions were separated by chromatoghraphy and lipid classes were separated by thin layer chromatography.

[4] Amino acid profiles analysed using high pressure liquid chromatography and Waters (Lane Cove, NSW) Pico-Tag.

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Research underway on replacement of fishmeal in aquaculture diets

Around the world a number of vegetable proteins are being tested for their use as a replacement for fishmeal in aquaculture feeds. Dos O'Sullivan reports on a strategic research and development (R&D) program which, if successful, will dramatically reduce the Australian aquaculture industry's reliance on fishmeal as an ingredient in the diets of prawns, salmon, barramundi and other cultured species.

By Dos O'Sullivan

In Australian aquaculture, as else where in the world, a large proportion of the annual operating costs are for foods and feeding. In many sectors of the finfish and crustacean culture industries specially formulated pelleted diets are used for convenience in storage and distribution. An economic analysis of the industry undertaken by the Australia Bureau of Agricultural and Resource Economics and the University of Tasmania (Treadwell et al. 1991) showed that for freshwater trout, prawns, crocodiles, Atlantic salmon, ocean trout, and barramundi the pelleted feed costs were over 40% of the total operating costs (Table 1). Adding labour costs for unloading, storing and distributing the feeds, as well as the costs of the feeding equipment (hoppers, blow feeders, demand feeders, etc.), makes the total expenditure of supplying the nutritional requirements of the culture species very high indeed.

A large percentage of the cost of pelleted feeds is due to the utilisation of fishmeal and fish oils. At present Australia imports some 30,000 tonnes of fishmeal (worth $17 million) mostly for fertilisers and for ingredients in the diets of pigs and poultry. However the highest grade (and hence most expensive) fishmeal is imported for aquaculture. In addition, up to 9,000 tonnes per annum has been produced by a Tasmanian fishmeal plant from jack mackerel, although the catches of this fish vary greatly from year to year.

Thus whilst demand for the fishmeal is high, supply is limited, fuelling a price spiral. Over the past few years a number of R&D projects have chipped away at the goal of reducing our reliance on fishmeal in aquaculture. Now, however, there is a national approach, with a collaborative sub-program funded by the Fisheries Research and Development Corporation (FRDC) – a joint government and industry funded body. The budget for the first year is $0.5 million. Entitled "Replacement of Fishmeal in Aquaculture Diets," the sub-program is being co-ordinated by Dr. Geoff Allan, a scientist at the Brackish Water Fish Culture Research Station, Salamander Bay, New South Wales.

The sub-program is expected to run for 3 years and aims to produce cost effective aquaculture feeds. "Our primary objective is to replace fishmeal in aquaculture feeds with cost effective alternatives," explains Dr. Allan. "This may be partial or total replacement."

Collaborative groups include CSIRO Fisheries (Cleveland, Qld), Queensland Department of Primary Industry (Bribie Island Research Labs and International Food Institute of Queensland), Queensland University of Technology (Brisbane), Curtin University (Perth), CSIRO Food Science and Technology (Ryde), N.S.W. Fisheries (Port Stephens and Cronulla), N.S.W. Agriculture (Wollongbar), University of Tasmania (Launceston), SALTAS (Dover) and University of Queensland (Brisbane). The involvement of the feed manufacturing industry in all phases, including research direction, manufacture of experimental diets and commercialisation of results, is an integral part of the sub-program.

Ethics of fishmeal use questioned

Addressing an International Symposium on Fish Nutrition, held in late 1993 in Hobart, Tasmania, Allan commented that the ethics of using fishmeal to produce fish for human consumption was being questioned, especially when many of the fish species targeted for fishmeal production support large artesinal fisheries.

"It takes five tonnes of edible fish to produce one tonne of fishmeal," says Allan. "In excess of 35% of the total world fish catch, some 6.5 million tonnes, was used for fishmeal production in 1989. Approximately 12% of that was used for aquaculture, but it is suggested that the figure will be 20-25% by the year 2,000. However, it is also estimated the total fishmeal production will drop by 5 % by the year 2,000. Thus while the supply of fishmeal is expected to fall, demand and therefore price, will be increased; hence the need for R&D into fishmeal replacement."

The sub-program is divided into six separate but co-ordinated projects, each of which involve several collaborating resource groups. Four will look at fishmeal replacement in aquaculture feeds for specific target species, one will examine the feed processing technology while the last will undertake a technology audit on amino acid supplementation of aquaculture diets.

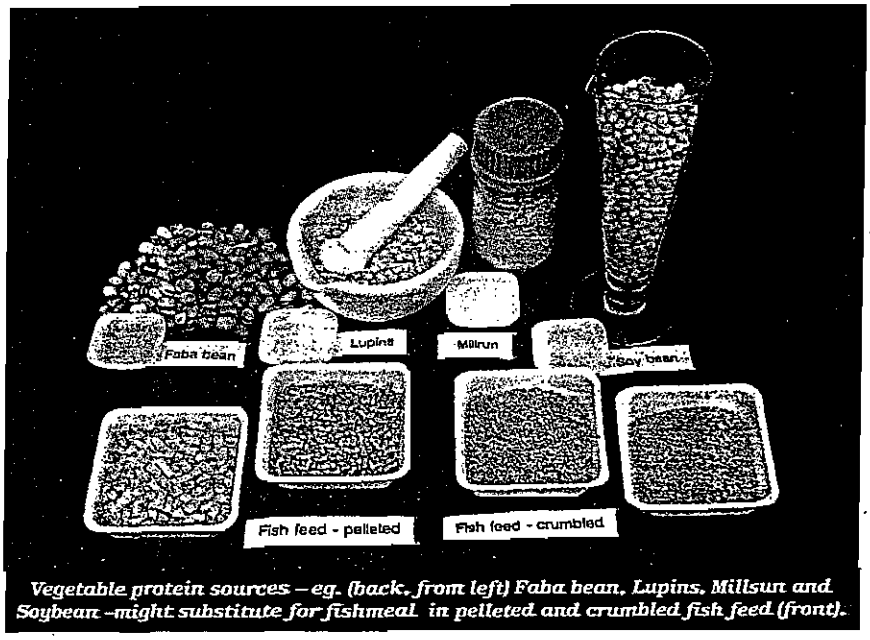
Throughout the sub-program there will be co-ordinated experimental design and planning workshops to standardise methodology and discuss results; co-ordinate the purchase of ingredients, feeds and equipment; analyse and evaluate potential feed ingredients; maintain the R&D direction; and facilitate the commercialisation of the results into cost effective aquaculture diets.

Key species chosen

Australia's aquaculture industry consists of more than 60 commercial and semi-commercial species. In order that the R&D program be wide ranging in its outcomes, but specific enough in its experimentation, a number of key representative species have been chosen for the feeding trials. A number of criteria were used to select these species:

1. They were the basis of a large industry, or showed outstanding potential.
2. The cost of feed use was a major limiting factor to industry development.
3. Fishmeal was currently the primary protein source.
4. There was a perceived potential to replace fishmeal.
5. There was an ability to do all the necessary R&D.
6. The feed manufacturing companies currently making diets for the species are interested in replacing feed meal in these diets.

The species chosen were the leader or black tiger prawn (*Penaeus monodon*),



Vegetable protein sources – eg. (back, from left) Faba bean, Lupins, Millsun and Soybean –might substitute for fishmeal in pelleted and crumbled fish feed (front).

silver perch (*Bidyanus bidyanus*), Atlantic salmon (*Salmo salar*) and barramundi (*Lates calcarifer*).

"There are many agricultural products produced in Australia (including vegetable and animal protein meals) which may have the potential to substitute for fishmeal in aquacultural diets," explains Allan. "Many of these are produced in abundance (Table 2) and some have been successfully used in diets for aquaculture species overseas and in pig and poultry diets in Australia.

"The first step in the sub-program is to analyse these ingredients with the most potential, evaluate them with the target aquaculture species and then determine the maximum amount which can be included in diets for each species.

"The second step is to investigate methods to improve the digestibility and availability of these ingredients. These methods will include extrusion, microwave, enzyme treatment and protein concentration. Using the pig and poultry industry as models, the effectiveness of different additives such as limiting amino acids, enzymes and attractants will also be investigated to overcome intrinsic deficiencies of some of the new ingredients compared with fishmeal.

"The third step is to define the requirements for the nutrients which are most expensive and supply in diets for the target species."

Allan says that one of the key factors to the potential success of the work is the establishment of an early commitment from the feed manufacturers to incorporate its results. "We are involving the feed manufacturers in all stages," he explains. "Information on ingredient analysis and evaluation is being disseminated quickly. We are identifying and developing promising ingredients in conjunction with agricultural industry groups - such as the Grain Research and Development Council, Wheat Board, and Meat Research Corporation. Processing technology developments will be distributed, and we are identifying and developing new markets for the feeds."

Table 1: Feeds as an percentage of annual operating costs for aquaculture farms (Treadwell et al, 1991).

Species	Costs of Food ($,000)	Total Costs ($,000)	% Total Costs.
Freshwater trout	90.0	187.3	48.1
Prawns	270.0	600.0	45.0
Crocodiles	169.4	401.0	42.2
Atlantic salmon	238.0	583.0	40.8
Ocean trout	181.0	512.0	35.4
Barramundi	115.3	425.7	27.1
Marron	26.0	341.4	0.8
Redclaw	10.0	216.2	0.5
Yabbies	13.5	249.0	0.5

Early success

The N.S.W. Fisheries Department believe that silver perch shows outstanding potential for culture. Attributes include white flesh and small head – hence a large fillet – as well as high production rates in ponds, equivalent to 10 t/ha/yr. Research into fishmeal replacement for silver perch diets has been underway in New South Wales for nearly three years with funding from the Grains Research and Development Corporation. This research has already led to the development of a successful reference diet which contains some Australian oilseeds (see *Austasia Aquaculture* May/June 1992. Vol 6 No. 3, pages 39-40) and has provided the impetus for the national sub-program.

Allan says that the reference diet has formed the basis of three commercial silver perch diets produced by Janos, Kinta and Cropp King (Barastoc). "There are some 72 licences issued for silver perch in New South Wales," he says. "Previously many of these were using inappropriate diets which were prepared for chickens, barramundi or salmon. Now they can use specifically formulated pellet diets."

Table 2: Annual production and market prices of grain legumes and oilseeds in Australia compared with the price for imported fishmeal. (Australian production figures from ABARE Commodity Statistics Bulletin 1992 and prices from NSW Agriculture, Sydney Retail Feed Ingredient Prices, December, 1993)

Type	Aust. Production (,000 t/yr)	Price/ tonne $A
Lupins	1,038	202
Soybean meal	51	495
Canola meal	161	325
Peanut	40	335
Cottonseed meal	749	400
Chick peas	221	225
Field peas	463	232
Cow peas	4	600
Imported fishmeal	-	800-1,200

To date the N.S.W. experiments have concentrated on the use of Australian oilseed meals (soybean, canola,

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Continued from page 45

cottonseed and peanut) and grain legumes (lupins, chick pea, field pea and cow pea). The digestibility of these ingredients has been examined, and maximum inclusion levels for the four most promising ingredients determined. Silver perch were found to be good at digesting vegetable protein. The next phase is to formulate and evaluate least-cost diets.

The peanut meal gave the best growth, followed by soybean meal, lupins and canola meal. Although fish growth slowed and FCR deteriorated as the content of vegetable protein was increased, the weight gain from using soybean meal, peanut meal or lupins was not significantly different to the fishmeal controls (42 % of diet) when 38 or 64 % of the fishmeal was replaced by any one of these ingredients. Allan says that the FCR was worst in diets with high fibre contents: however dehulling could result in major improvements.

For more information contact Dr. Geoff Allan, Brackish Water Fish Culture Research Station, Post Office, Salamander Bay, N.S.W. 2301, Australia. Telephone: (049) 821-232 Fax: (049) 821-107.

Reference : Treadwell, R., McKelvie, L. and Maguire, G., 1991. Profitability of selected aquaculture species. ABARE Discussion Paper 91.11, 85 pp.

meatmeals, grain legumes, particularly dehulled lupins and field peas, and modified wheat gluten meals.

5. Data for *in vitro* digestibility of protein supported *in vivo* results and showed that protein from a range of sources was well digested by silver perch. Unfortunately, cheaper *in vitro* techniques could not be used in place of *in vivo* methods. Research with *in vitro* techniques to determine carbohydrate digestibility and effects of processing has highlighted important differences between species and supported the contention that silver perch are better equipped to cope with diets containing higher carbohydrate contents than carnivorous fish.
6. Experiments on selected ingredients have investigated the effects of steam cooking, grinding and dehulling. Most forms of processing have improved digestibility, although to different extents for different ingredients. Further research will be completed during the term of the current FRDC/ACIAR projects to evaluate effects of extrusion on digestibility of oilseeds and grain legumes. Liquid enzyme preparations have been obtained for future evaluation.
7. Preliminary trials have determined the approximate crude protein requirements for silver perch as being around 35% (Allan and Rowland, 1991) and this has been confirmed in subsequent experiments. Protein in excess of requirements is used for energy and for silver perch we have shown that this portion of protein can be replaced with cheaper energy sources such as lipid and carbohydrates (Allan et al., 1994). However, deposition of body fat (a noted problem with silver perch) increased when high energy diets were used (Hunter et al., 1994).
8. A series of trials have been conducted in 70L aquaria, 10,000L tanks and 0.1 ha earthen ponds. The first trial in 0.1 ha ponds compared two diets: our reference diet (SP35) and a new diet based on vegetable protein (primarily peanut meal, canola and lupins). Unfortunately performance on the vegetable protein diet was inferior. Anti-nutritional factors in peanut meal and canola may have been responsible and inclusion of these ingredients has been limited in subsequent diets until potential problems can be fully investigated. Additional experiments with lupins have shown this ingredient is unlikely to have reduced growth.
9. Diets where meatmeal products, have been used to replace over half the fishmeal have produced equal growth and food conversion efficiency to SP35 in trials in 10,000 L tanks.
10. Two other low-cost diets with only 5 or 10% fishmeal are being compared with SP35 in 0.1 ha ponds at Grafton Research Centre. These diets are based on meatmeal, lupins and dehulled field peas. The new diets contain similar digestible energy and nutrients to SP35 but were made from relatively cheap Australian ingredients, mainly meatmeal, dehulled lupins and dehulled field peas. At the time this article was written the experiment was still being harvested but it is clear the fish fed the two low-cost diets have grown faster than fish fed SP35. Discussions to produce a commercial version of these diets is underway with a large feed manufacturer.
11. An experiment to investigate the effects of different processing conditions, used to produce SP35 pellets, on growth of juvenile silver perch was run in 10,000L tanks at Port Stephens Research Centre. Grinding diets to < 500mm has no significant effect on growth of juvenile fingerlings but fish fed the diets which were steam conditioned grew significantly (23%) faster. An additional treatment where fish were fed the same diet processed using a single-screw extruder was also included. These pellets floated and feed intake was reduced. Food conversion efficiency for these diets was however excellent.
12. An experiment to determine requirements for lysine (an essential and often first limiting amino acid) indicated that the lysine content of SP35 is higher than that required by juvenile silver perch. This has important implications as diets containing less lysine than SP35 will be less expensive and can be made from a large number of low-cost, readily available ingredients.

Nutrition Research Priorities

Effective, low-cost diets will meet but not over-supply requirements for essential nutrients and be formulated predominantly using locally available Australian ingredients. We now have much of the information necessary to assist in the ingredient choice for these diets but we still need to know the maximum contents of high-potential Australian ingredients (singly and in combination) which can be included. Wherever pos-

sible, this research should be done in large tanks or ponds to provide commercially relevant information. Research into utilization of carbohydrates is needed to ensure that maximum use can be made of Australian grains.

Armed with comprehensive data on ingredient digestibility, it is possible to determine the cost of providing different nutrient specifications in formulated rations. This analysis has clearly shown that digestible lysine, methionine plus cystine, isoleucine and threonine are the first limiting amino acids, and that supplying essential fatty acids is also expensive. An experiment has been conducted to determine optimum lysine requirements at one energy content, but requirements for other amino acids, and the interactive effects of amino acid:energy ratios, and the requirements for essential fatty acids need to be determined.

Diets are a major component of feed and feeding costs, but feeding practices also need to be optimised to lower operating costs. Labour is the major feeding cost and feeding once per day, rather than more often, and not feeding on one or both weekend days, could significantly reduce costs, provided fish performance was not affected. Compensatory growth (growth following periods of starvation allowing fish to 'catch up' to continuously fed fish) has been demonstrated for several species of fish (Quinton and Blake, 1990; Sullivan and Smith, 1982). As silver perch have evolved in a 'flood and drought' regime where periods of low food availability are common, it is worth investigating if compensatory growth occurs in this species.

The FRDC have approved a new three year Sub-Program on Aquaculture Diet Developments which will include silver perch. Three new projects will be initiated: Ingredient Evaluation, Nutritional Requirements and Diet Validation and Feeding Strategies.

Conclusion

As silver perch can effectively use feeds based on plant proteins and meatmeal, and grow rapidly under crowded conditions, costs of production are relatively low. Because of this, silver perch is the only species currently identified as having potential for culture in Australia, which might be able to replace some of the more than 40,000 t/year of cheap imported fresh or frozen fish.

To capitalise on this potential, research to develop cheap, effective diets which meet nutritional requirements, but are based on Australian protein sources will continue as a major industry priority.

Acknowledgments

Research summarised here was only possible with the excellent technical assistance of Scott Parkinson, David Stone, Jane Frances and Rebecca Warner-Smith at Port Stephens Research Centre, Charlie Mifsud, Phil Read, Paul Robertson, Justin Shipman and David Glendenning at Grafton Research Centre and Ken O'Brien and Wendy Peasley at Wollongbar Agricultural Institute. Financial support from FRDC and ACIAR is gratefully acknowledged. Ms Jo Pickles expertly typed the manuscript.

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Encouraging results for meatmeal as fishmeal replacement

Aquaculture's heavy reliance on expensive, and at best static, supplies of fishmeal as the basis of feeds is a major barrier to sustainable development. Recent research has shown that not only can most fishmeal be replaced with terrestrial proteins without affecting growth rates, but that the seafood produced looks good, smells good, and tastes good.

In any animal farming system a dependable supply of nutritious and reasonably priced feed is critical if the industry is to survive, and aquaculture is no different. Hailed as the solution to static or declining supplies of wild-caught seafood, aquaculture's success relies on its sustainability, which means finding feed sources that are renewable as well as nutritious and cost-effective.

Many of the fish and crustaceans reared on farms are either omnivorous or carnivorous feeders. Fishery product, either as low-value "trash" fish and fishery waste or rendered as fishmeal, has been the traditional source of protein for these animals and may constitute as much as 70 per cent of the feed by weight.

But fishmeal production, being static, can no longer keep pace with the increasing demand of the aquaculture industry, which is expected to increase twofold or threefold by the year 2010. Predictions are that aquaculture will use 30 per cent of global fishmeal production by the year 2000 unless alternative non-marine sources of feed are found.

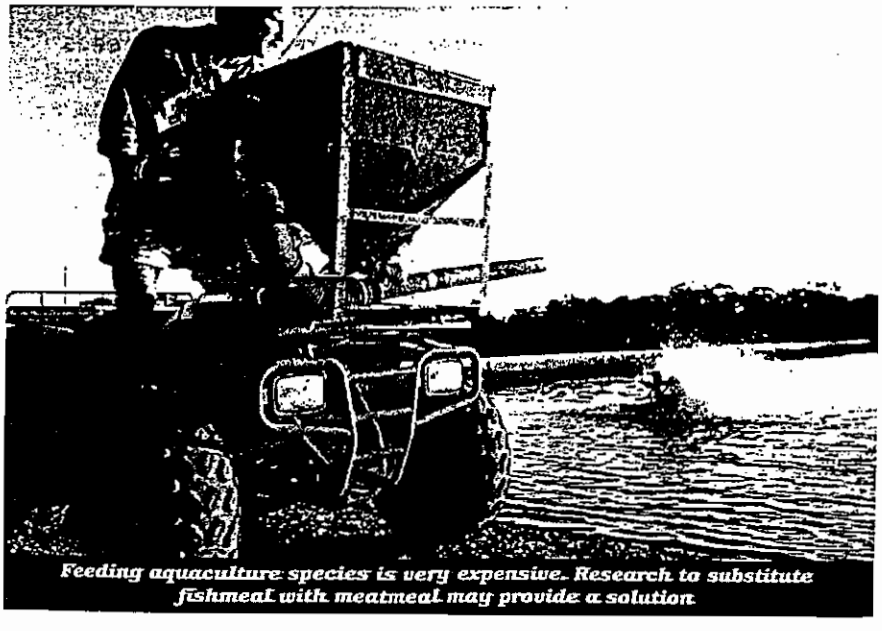
In response to this international call, the Australian Fisheries Research and Development Corporation (FRDC) funded a research program in 1993 to find alternatives to fish-based feeds for four "template" species: a marine and mainly carnivorous prawn (giant tiger prawn, *Penaeus monodon*); a warm-water carnivorous euryhaline (can live in fresh and salt water) fish (barramundi, *Lates calcarifer*); a freshwater omnivorous fish (silver perch, *Bidyanus bidyanus*); and a cold-water carnivorous fish (atlantic salmon, *Salmo salar*). These species represent a variety of habitat types from marine to freshwater and a range of feed preferences, carnivorous and omnivorous. The research has been supported by funding from the Meat Research Corporation, the Grains Research and Development Corporation, and the Australian Centre for International Agricultural Research. Several industry partners (both feed-millers and farmers), universities, and state and federal government research agencies are involved in the study, which is coordinated by Dr Geoff Allan from NSW Fisheries.

The Meat Research Corporation specifically funded an examination of the suitability of rendered meatmeals as replacements for fishmeal in the feeds of three of these species: giant tiger prawns, barramundi, and silver perch.

Three years later, the research has proved to be very successful. Experimental feeds have been developed based on meatmeal – produced as a byproduct of livestock processing – in combination with vegetable protein meals. These feeds are far less expensive and should result in growth rates equal to or better than those based on fishmeal. The studies have shown that meatmeal has enormous potential for replacing fishmeal in aquafeeds. And the good news for Australia is that we have a distinct advantage when it comes to supplies of this protein resource. Given Australia's extensive livestock industry, meatmeal is abundant in supply (470,000 tonnes a year), of high quality, and price-competitive with other land-based sources of protein.

The first challenge for the research team from CSIRO and the Queensland and New South Wales Departments of Primary Industry was to determine the digestibility of meatmeals for the three species. This enabled feeds to be formulated that provided digestible nutrients in the correct amounts required by the particular animal. A key consideration was the extent to which the animals liked the new feeds and the effect of these feeds on growth rate.

In both laboratory and field trials,



Feeding aquaculture species is very expensive. Research to substitute fishmeal with meatmeal may provide a solution

Meatmeal was put through exhaustive laboratory tests

meatmeal was shown to be able to replace at least two-thirds of the fishmeal in the feeds of prawns and silver perch, and all fishmeal in the feed of barramundi, without any adverse effect on production.

Dr Kevin Williams presented the key findings of this research to the 4th International Symposium of the Australian Renderers Association in Melbourne, September 1997.

Barramundi

The barramundi work was carried out at QDPI's Walkamin laboratory and on three barramundi farms in north Queensland. The research was co-led by CSIRO's Kevin Williams and QDPI's Chris Barlow and showed that meatmeal-based feeds were equal to or better than a reference feed containing a high proportion of fishmeal.

Particular care was taken in formulating the feeds to include sufficient fish oil to satisfy the fish's requirement for omega-3 fatty acids. "This also ensured that the desirable 'fishy flavour' was maintained and that high amounts of omega-3 fatty acids were present in the harvested fish to satisfy a growing awareness of their human health benefits", Dr Williams told Austasia Aquaculture.

Feeding trials on the barramundi farms were run for about ten weeks with the fish confined in floating cages either in aerated freshwater ponds or in an estuary. The experimental fish were managed exactly the same as the other fish on the farm and were usually fed only once daily. They were reared from a starting weight of about 250-300g to a harvest weight greater than 400g. At the end of the experiments, representative samples of fish were taken to QDPI's Centre for Food Technology for taste-testing by a panel of professional tasters.

The research has shown that all fishmeal could be replaced by meatmeal without any adverse effect on barramundi growth performance. "Using meatmeal instead of fishmeal reduced the ingredient cost of the feed by as much as 30 per cent and reduced the overall production cost by 18-23 per cent", Kevin Williams said. The colour, odour, flavour, and texture of the flesh and its overall appeal were unaffected by replacing fishmeal with meatmeal.

QDPI predicts Australia will produce 1000 tonnes of farmed barramundi by the year 2000. Replacement of fishmeal by meatmeal will help ensure the profitability and sustainability of the growing industry.

According to Kevin Williams, the next challenge is to improve the shelf life of the new feeds to better withstand the sometimes unfavourable feed storage conditions on farms during the hot and humid north Queensland summer.

Silver perch

Results for meatmeal replacement in the feeds of silver perch were also very promising, showing that meatmeal can replace almost all the fishmeal in silver perch feeds.

In large-scale pond experiments, two silver perch feeds containing meatmeal (lamb meal) were compared to a reference feed based on a high inclusion of fishmeal. The ponds were stocked with silver perch at commercially relevant densities and cultured to a market weight of about 400g.

In the pond experiment, the best feed in terms of growth rate contained only five per cent fishmeal; the predominant sources of protein were meatmeal and grain legumes. The fish fed this feed grew faster than those fed the reference feed and resulted in a heavier harvest weight of 440g as against 395g for the reference feed. Fish survival was excellent (>96%) for all ponds.

The productivity cost for every kilogram of fish weight gain of the

meatmeal-based feed was about $1.09 compared to $1.69 for the fishmeal-based reference feed.

These results show unequivocally the cost-effectiveness of using meatmeal in combination with vegetable protein meals to replace most of the fishmeal in feeds for silver perch.

According to research leader Dr Geoff Allen from the New South Wales Department of Fisheries, feed conversion of the meatmeal-based feeds was also better than for the reference feed. "In the case of meatmeal-based feed, 1.9kg of feed produced 1kg of fish", he said. This was significantly better than for the fishmeal reference feed where 2.2kg of feed was needed to produce 1kg of fish.

Prawns

CSIRO's David Smith, the principal researcher for the prawn-feed study, says his research has shown that meatmeal can also successfully replace a significant amount of the fishmeal currently being used in prawn feeds.

"In laboratory trials, meatmeal could replace two-thirds of the fishmeal without any adverse effect on the growth rate of prawns", he said. "But the prawns still appear to need some high-quality marine protein in their feeds."

And the taste test? An expert panel of tasters found that prawns fed on feeds that consisted mostly of meatmeal tasted no different to those fed fishmeal-based feeds.

Meatmeal also represented a cheaper source of protein than fishmeal in prawn feeds. At high levels of meatmeal in the feed, there was a saving of more than ten per cent in the ingredient cost of the feeds.

David Smith explained that the next stage of the research will be to extend the findings in the laboratory to the field by trialing meatmeal-based feeds in prawn ponds.

Australia's rapidly growing prawn farming industry relies heavily on imported feeds which make up 60 per cent of on-farm production costs. The development of low-cost, high-performance prawn feeds based on renewable resources such as meatmeal will be a welcome outcome of the research.

Conclusions

Based on these findings, meatmeal has the potential to become a major protein source in aquaculture feeds.

The take-home message of the meatmeal research is that feeds containing even a moderate inclusion of meatmeal as a substitute for fishmeal will result in lower feed costs and an improvement in farming profitability, according to Kevin Williams. "It's also in the industry's long-term interest to base aquaculture on sustainable and renewable resources, given that in less than 30 years the global demand for aquafeed is expected to double if not treble", he said.

There are also very significant import replacement opportunities. Australia produces 7000 tonnes of fishmeal each year but imports 63,000 tonnes for use in animal feeds. Instead, we should be exporting meatmeal or better still, exporting aquafeeds containing meatmeal, in the opinion of Kevin Williams. "Even if meatmeal replaced only 20 per cent of the fishmeal in aquafeeds," he said, "the Asian market alone would use 500,000 tonnes of meatmeal. That's equivalent to Australia's total annual production of meatmeal."

The research has also shown that if we are to get the most out of this opportunity, Australian renderers will need to produce meatmeal low in ash (<20%) and fat (<7%), but high in protein (>60%).

The researchers have prepared final reports of their meatmeal studies to the MRC and all of the fishmeal replacement research is being compiled as final reports to FRDC.

Katherine Johnson
CSIRO Division of
Marine Research

THE NEED FOR FISHMEAL ALTERNATIVES

- Fishmeal is the basis (50-70%) of aquafeeds for carnivorous fish and prawns
- Global fishmeal production is static at 6.2 million tonnes each year, about half of which is available for export
- Aquafeeds used 1.1 million tonnes of fishmeal globally in 1995
- Aquafeed will use two million tonnes of fishmeal, which is 32% of global total fishmeal production, by the year 2000

AQUAFEED

- Global production is 4.3 million tonnes (US $3 B, 1994)
- Main users:
  - carnivorous fish (40%)
  - marine prawns (25%)
- Main producers:
  - Asia (60%)
  - Europe (21%)
- Demand predicted to be 4.5-7.5 million tonnes by year 2000
- Australia uses 22,000 t (AUD $32 M, 1995)

CONCLUSIONS OF RESEARCH

- Meatmeals are highly palatable and digestible by prawns, silver perch and barramundi
- More than 75% of fishmeal protein can be replaced with meatmeal with no adverse effect on growth, taste, or appearance
- Meatmeals are up to 40% cheaper sources of protein than the Australian and Peruvian fishmeals
- Production of meatmeals with reduced fat and ash and higher protein contents would improve their suitability and usage in aquafeeds

Source: Williams, Allan, Smith and Barlow, "Fishmeal Replacement in Aquaculture Diets using Rendered Protein Meals", 4th International Symp. on Animal Nutrition. Protein, Fats and the Environment, 24-26 September 1997, Melbourne.

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ISSN 1440-3544

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