Aquaculture Diet Development Subprogram: Ingredient Evaluation

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NON-TECHNICAL SUMMARY

1996/391	Aquaculture Diet Development Sub-Program: Ingredient Evaluation

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OBJECTIVES:

1. Determine nutrient digestibility of major terrestrial protein and energy feed ingredients for which information is not currently available.
2. Determine contribution to fish and prawn growth of the most promising new ingredients and identify the maximum amounts which can be included in practical diets.
3. Evaluate carbohydrate utilisation and determine effects of carbohydrate type, content and processing on digestibility.
4. Use results to determine first limiting nutrients for fish diets based on Australian ingredients, and to formulate practical diets for evaluation in commercially relevant facilities.
5. Communicate results to producers of feed ingredients, feed manufacturers, aquaculturists and the scientific community.

OUTCOMES ACHIEVED

- Digestibility coefficients for oilseeds, legumes and carbohydrate sources were determined for silver perch and for carbohydrate sources for barramundi. Effects of processing on digestibility of a number of ingredients were also determined for silver perch. These data provide feed formulators with accurate information to allow ingredients not previously used to be incorporated into diets. This is critical for replacement of expensive, difficult to obtain, imported fishmeal.
- Utilisation of protein and energy sources for silver perch were detected using summit/diluent diet formulation and carcass slaughter techniques. Maximum contents of meat meal, peanut meal, canola meal, field peas, wheat, wheat starch (raw and gelatinised) and dextrin (a breakdown product of starch) were estimated.
- Carbohydrate utilisation was studied in detail for silver perch and barramundi. Silver perch are able to efficiently digest and utilise starch (but not non-starch polysaccharides). In contrast, barramundi are very inefficient at digesting starch, regardless of whether it is raw or gelatinised. For both species carbohydrate tolerance tests were carried out to measure uptake and clearance rates of glucose, galactose and xylose. Results from these experiments demonstrated major differences between the species.
- For silver perch the protein sparing effects of wheat starch based carbohydrates were measured. Silver perch utilised processed starch, gelatinised starch or dextrin, better than raw starch or wheat meal.

- Data on digestibility and utilisation clearly demonstrate that no ingredients are as 'good' as fishmeal. However, alternatives can be used to completely replace fishmeal provided information on digestibility and utilisation is available and provided requirements for major nutrients are understood. Protein and energy are the key requirements to understand for cost-effective formulation of the diets with either zero or minimal fishmeal.
- Information from this project has been extensively disseminated. Over 60 scientific manuscripts, journal articles and conference presentations have been prepared (or delivered) and commercial feed manufacturers personally briefed on results. The majority of commercial silver perch diets now sold are based on research generated during this sub-program or its predecessor, the Fishmeal Replacement in Aquaculture Diets Sub-Program.

The overall aim of this project was to identify and improve Australian ingredients for use in aquafeeds. The focus was on replacement of expensive, imported fishmeal. Since this project commenced the global situation with fishmeal has worsened considerably. In 1998, more than 42% of the total fishmeal available worldwide was used in aquaculture feeds (an increase from around 11% in 1993). Global production of fishmeal is static. Australian production of fishmeal, miniscule by world standards at best, has declined. Almost all ingredients that might be used to replace fishmeal are inferior in terms of total protein content, amino acid profile, fatty acid profile, carbohydrate contents and content of anti-nutrients. One of the key goals of this project was to investigate methods of processing that could increase the potential use for some of these alternative ingredients.

Effects of processing (no cooking, steam conditioning or extrusion) on three diets for silver perch that differed in ingredient composition, demonstrated that cooking, through steam conditioning or extrusion, improved performance. Extrusion improved digestibility and both methods of cooking improved utilisation. However, food intake was reduced for extruded compared with steam-conditioned diets.

Extrusion processing, in addition to dehulling (to remove less digestible carbohydrates), was studied for its effect on digestibility of individual ingredients. Dehulling greatly improved lupins but was of little benefit for peas. Conversely, extrusion was very effective in improving digestibility of starch-rich peas but conferred no benefits to lupins as lupins contain insignificant amounts of starch (their carbohydrates are non-starch polysaccharides, chiefly galactose and xylose). Soybean was a better ingredient than canola and was further improved when extruded. Extrusion actually reduced the digestibility of canola. These results demonstrate the mistake of commonly expressed simplifications such as "extrusion improves ingredients" or "dehulling increases utilization".

The utilisation of nitrogen and energy from key agricultural ingredients was measured. Summit/diluent methods, where a high performance reference diet was progressively replaced by either a test ingredient or an inert filler, were used. Using carcass slaughter, and a comparison between the inert filler series of diets and those containing test ingredients, the utilisation of each ingredient was measured and the maximum practical inclusion content estimated. Ingredients tested for silver perch were peanut meal, canola meal, meat meal or dehulled field peas.

Although actual requirements for amino acids for protein deposition are relatively similar for most species of fish (and for that matter, terrestrial animals), diets for herbivorous, warm water omnivorous, and carnivorous fish differ significantly in their protein content. Herbivores and omnivores do very well on diets with less than about 30-40% protein while carnivores seem to require more than about 45% protein. This has little to do with actual protein requirements but instead reflects the ability of herbivores and omnivores to effectively utilise non-protein sources for

energy. For many warm water species, lipids are either poorly utilised or lead to excess carcass lipid that can negatively impact on market acceptance. The understanding of carbohydrate metabolism and investigation of ways to improving it therefore is essential.

In this project, carbohydrate utilisation was investigated using *in vivo* digestibility studies, carbohydrate tolerance tests and summit/dilution growth studies. The first step in carbohydrate utilisation is digestibility. For silver perch, research with wheat and peas showed wheat starch was more digestible and that gelatinisation improved digestibility of wheat starch. Digestibility of various starch breakdown products, dextrin, maltose and glucose were also measured and the least complex carbohydrate was most digestible. Increasing inclusion contents reduced digestibility.

In contrast to silver perch, barramundi were very inefficient at digesting carbohydrates and gelatinising wheat starch actually reduced digestibility, possibly because of the inability of that species to utilise glucose, the initial breakdown product of starch.

Carbohydrate tolerance tests involve injecting fish with a carbohydrate (most commonly glucose, the breakdown product of starch) and then measuring uptake of the carbohydrate in the blood and then clearance over time (usually over 24-48 h). Rapid uptake and clearance are indicators that the carbohydrate is metabolised efficiently. Studies were conducted with barramundi and silver perch with glucose, galactose and xylose (galactose and xylose are non-starch polysaccharides, and are the primary carbohydrate products in lupins). Tilapia were also used in glucose tolerance tests for comparison and, in terms of ability to clear glucose following uptake, were the most efficient species in metabolising this ingredient. Silver perch were much better at metabolising glucose than barramundi. Barramundi was also intolerant of galactose and silver perch could only metabolise galactose to a limited extent. Neither silver perch nor barramundi could metabolise xylose.

Results of growth studies (summit/diluent) confirmed that silver perch are efficient at utilising carbohydrate for energy to spare protein. Up to 30% wheat meal, raw wheat starch, gelatinised wheat starch or dextrin elicited no negative response on performance on carcass composition.

With poor digestibility and an inability to metabolise carbohydrates, further research on carbohydrates with barramundi was suspended.

The final stage in this research was to determine if addition of enzymes could improve carbohydrate digestibility. Enzyme preparations tested with silver perch contained ∝-amalyse (designed to increase starch digestibility) or a blend of β-glucanase and β-xylanase (designed to improve digestibility of non-starch polysaccharides). Although a minor improvement in digestibility of raw wheat starch followed the addition of ∝-amylase, the improvement was small compared with the improvement following gelatinisation of raw wheat starch. ∝-Amylase had no effect on the already highly digestible gelatinised wheat starch. The blend of β-glucanase and β-xylanase had no effect on digestibility of non-starch polysaccharides and did not improve digestibility of lupins.

Finally, effects of a preparation containing phytase to improve ingredient driveability was tested with silver perch. Phytase has been shown to improve phosphorus digestibility and also the utilisation of protein for some ingredients (e.g. soybean). No clear benefits were found with the enzyme for silver perch.

During this project research on ingredient digestibility and utilisation were conducted. The information generated allows feed formulators to include alternate ingredients to fishmeal and to better understand the ability of carbohydrate-rich ingredients (i.e. grains) to be used to spare protein. Silver perch are efficient at utilising starch for energy and so perform well on low-protein diets (26-28% digestible protein for diets with 13-15 MJ/kg DE and 30% for diets with 17 MJ/kg

DE; see project 96/392 ADP: nutrient requirements). Barramundi are inefficient at utilising carbohydrates and consequently require diets with high >40% digestible protein as much as their energy needs must come from protein.

Keywords: Aquaculture nutrition, ingredient evaluation, digestibility, utilisation, silver perch, *Bidyanus bidyanus*, barramundi, *Lates calcarifer*, extrusion.

1.   BACKGROUND

This project successfully identified and improved Australian ingredients for use in aquaculture diets. The major focus was on protein ingredients to replace expensive, imported fishmeal and it built on successful research results from a previous FRDC Subprogram on Replacement of Fishmeal in Aquaculture Diets. During the earlier project high priority Australian ingredients were identified and many of these have been evaluated for silver perch, barramundi, prawns and salmon.

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

1.   Global fishmeal production currently requires more than 30 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 fishmeal is based on the Jack Mackerel fishery in Tasmania. However, quotas for this fishery have been reduced, catch effort slashed to less than half previous levels and production will be far less than the previous 7 000 t/yr maximum.

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

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

In Australia, aquaculture will not develop beyond a small scale unless aquaculturists can purchase cheap, efficient feeds. We will not have the luxury of using cheap fishmeal to produce these feeds and so must develop viable alternatives. Fortunately, Australia has abundant sources of cheap agricultural proteins and results from the FRDC Replacement of Fishmeal in Aquaculture Diets Subprogram and the soon to be completed Aquaculture Diet Development Subprogram have been excellent. Scientists involved with the Subprograms have developed and validated techniques to determine diet and ingredient digestibility for silver perch, prawns, barramundi and salmon. Silver perch and barramundi diets with all but 5 or 10% of the fishmeal has been replaced with Australian agricultural proteins, have been used on an experimental scale without compromising growth. Fishmeal alternatives have also been identified for prawns, *Penaeus monodon.* Large scale, commercially relevant trials have also been undertaken 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 coefficients 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. 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, e.g. 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 projects 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 nutrients. For all species, demonstrating the performance of new diets, (with newly defined nutrient specifications and comprised of different ingredients), needs to be accomplished 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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2. NEED

Effective, low-cost diets will meet but not over-supply requirements for essential nutrients and be formulated predominately using locally available Australian ingredients. We now have much of the information necessary to assist with ingredient selection for these diets but we still need to evaluate some ingredients and determine the maximum contents of high potential Australian ingredients (singly and in combination) which can be included. Effects of successful processing techniques including cooking and protein concentration require further evaluation especially for barramundi.

Using lower-priced, carbohydrate-containing feeds (grains and grain legumes) to spare protein in diets may be an effective way of reducing feed costs. Use of such feedstuffs, however, will result in diets with higher contents of starch and non-starch polysaccharides. The digestibility of starch is known to vary among aquatic animals and to vary with the amount present in the diet. Fish have different capacities to digest starch (Anderson, 1994) and their capacity to utilise the glucose produced is not known. Carnivorous fish generally have a low tolerance to glucose, leading to health problems such as hepatomegaly and fatty liver, and to an altered carcass composition (Wilson, 1994). Fish are highly adapted to derive their energy needs through oxidation of fatty acids, rather than glucose which occurs as an end product of starch digestion. Thus, the balance between starch and fat in the diet may be as important a determinant of nutritive value as protein. Aspects requiring investigation are the digestibility of starch at varying inclusion levels; the optimal dietary starch-to-fat balance and the capability of fish to utilise the digested starch for energy production and storage.

3.   OBJECTIVES

1. Determine nutrient digestibility of major terrestrial protein and energy feed ingredients for which information is not currently available.

2. Determine contribution to fish and prawn growth of the most promising new ingredients and identify the maximum amounts which can be included in practical diets.

3. Evaluate carbohydrate utilisation and determine effects of carbohydrate type, content and processing on digestibility.

4. Use results to determine first limiting nutrients for fish diets based on Australian ingredients, and to formulate practical diets for evaluation in commercially relevant facilities.

5. Communicate results to producers of feed ingredients, feed manufacturers, aquaculturists and the scientific community.

4. RESULTS / DISCUSSION

4.1. Effects of steam pelleting or extrusion on digestibility and performance of silver perch *Bidyanus bidyanus*

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Abstract

The effects of different processing techniques on apparent digestibility coefficients (ADC's) and performance of silver perch *Bidyanus bidyanus* were evaluated. Results of a factorial digestibility trial in which three diets (SP35, 95LC1, 95LC2) and three processing methods (cold-pelleted, steam-pelleted, extruded) were evaluated, indicated that extrusion, but not steam conditioning, significantly improved ADC's of dry matter (DM) and energy. ADC's of DM and energy of cold-pelleted diets were statistically similar to steam-pelleted diets and ADC's of nitrogen were unaffected by processing method. No interaction was found between diet type and processing method for either DM, energy or nitrogen ADC's. A performance trial indicated that feed intake, weight gain and specific growth rate of fish fed steam-pelleted diets was greater than fish fed extruded diets. Feed conversion and digestible protein efficiency was better in fish fed extruded diets. Results indicated that extruded diets were better utilised than steam-pelleted diets, however, voluntary intake of extruded diets may have been limited. Fish fed cold-pelleted SP35 exhibited inferior performance compared to fish fed steam-pelleted or extruded SP35. Reduced performance of fish fed this diet may relate to poor utilisation of digestible protein or reduced palatability. Diets for silver perch with similar formulations to SP35 and 95LC2 should be steam-pelleted.

Introduction

The availability of data concerning the digestibility of alternative protein and energy sources for juvenile silver perch continues to grow (e.g. Allan, Parkinson, Booth, Stone, Rowland, Frances & Warner-Smith 2000a; Allan, Rowland, Mifsud, Glendenning, Stone & Ford 2000b; Stone, Allan, Parkinson & Rowland 2000; Booth, Allan, Frances & Parkinson 2001), as does research investigating the nutrient requirements of this species (e.g. Ngamsnae, De Silva & Gunasekera 1999; Harpaz, Sklan, Karplus, Barki & Noy 1999; Harpaz, Jiang & Sklan 2001; Booth, Allan & Stone 2000b; Allan, Johnson, Booth & Stone 2001). There is however, only limited information currently available on the utilisation of specific ingredients for silver perch (Booth, Allan & Stone 1999) and few reports dealing with the effects that current and novel processing techniques can have on ingredients and diets which are fed to this species (Gleeson, O'Sullivan & Evans 1999; Booth, Allan & Warner-Smith 2000a). These investigations are important because manufacturing processes can influence the utilisation of ingredients and feed intake (Booth *et al.* 2000a). The effects of processing can differ for different species and it is important to apply the appropriate processing technique to maximise production efficiency at the lowest possible cost (Tacon 1990). In many cases the additional cost of some processing can be unwarranted. For example, we found no improvements in either weight gain or feed conversion ratio (FCR) of juvenile silver perch when test diets were subjected to fine grinding (< 500 μm) compared to course grinding (710-1000

µm) (Booth *et al*. 2000a). The same formulation, when steam conditioned, increased feed intake and led to better weight gain and FCR than when extruded.

As a result of the recommendations in Booth *et al*. (2000a) to evaluate a sinking extruded diet for silver perch, this study aims to compare the effects of steam-pelleting and extrusion processing on the digestibility and performance of three commercial diets for silver perch.

Materials and Methods

Diet selection and experimental design

Three diets were selected to investigate the effects of steam conditioning or extrusion on digestibility and fish performance (Table 1). The first diet, SP35 (Allan and Rowland 1992), was commercially available and was used as the control. The second (95LC1) and third (95LC2) diets were formulated using linear least cost programming (LLCP) (Mania Software, Brisbane, Qld, Australia) and were previously evaluated using silver perch in pond trials by Allan *et al*. (2000b). Digestibility coefficients for 95LC1 and 95LC2 have not previously been reported. Ingredient and digestibility data for use in LLCP were drawn from experiments conducted exclusively on silver perch *Bidyanus bidyanus* (Allan, Rowland, Parkinson, Stone & Jantrarotai 1999; Allan *et al*. 2000a; Stone *et al*. 2000). Briefly, 95CL1 was formulated to match the digestible nutrient profile of SP35 but fish meal content was constrained to 100 g fish meal kg^{-1} and minimum digestible protein, energy, essential amino acids, phosphorous and linoleic series fatty acids were restricted to within 5 % of the concentrations in SP35. For 95LC2, fish meal content was constrained to 50 g fish meal kg^{-1} and minimum digestible contents for the aforementioned categories were allowed to vary by approximately 15 % of the concentrations in SP35 (Allan *et al*. 2000b). In addition, peanut and canola meals were excluded from 95LC1 and restricted to 50 g kg^{-1} in 95LC2 (Table 1).

Using these formulations in their uncooked form and then applying either steam conditioning or extrusion to each of them produced nine diets for evaluation. The digestibility of each of these dietary treatments was evaluated in an orthogonal two factor ANOVA. Fixed factors were Diet type (SP35, 95LC1 and 95LC2) and Process (cold-pelleted, steam-pelleted and extruded). Each dietary treatment was replicated three times (*n* = 3).

Due to the constraints of our grow-out facility, only five diets were evaluated in the performance trial. Diets tested were cold-pelleted, steam-pelleted and extruded SP35, and steam-pelleted and extruded 95LC2. Each dietary treatment was replicated three times (*n* = 3). SP35 was included on the basis that it was the only diet available commercially for silver perch at that time and had been used extensively in previous research. In addition, comparative performance and digestibility data for silver perch that had been fed steam-pelleted and extruded SP35 were also available (Booth *et al*. 2000a) which provided a benchmark for this study. Steam-pelleted or extruded 95LC2 were included at the expense of the remaining diets based on its significantly lower ingredient cost (Allan *et al*. 2000b) and that the prospects of 95LC1 or 95LC2 being manufactured as a cold pressed pellet was unlikely given the move by many large scale feed producers towards extrusion technology (Springate 1991).

Extruded diets

The control diet SP35 was obtained from Janos Hoey Proprietary (Pty.), Limited (Ltd.), Forbes, NSW, Australia. Ingredients for 95LC1 and 95LC2 were mostly obtained from commercial feed manufacturers (details available in Gleeson *et al*. 1999), however, dehulled field peas (*Pisum sativum*) were provided by the Grain Pool of Western Australia. Prior to manufacture, all ingredients were passed through a hammer mill (model RD-8-K32; Rietz Manufacturing Company, Santa Rosa, CA., USA) fitted with a 1.6 mm screen to achieve a particle size of < 1.0 mm.

Ingredients were sieved (2 mm screen), then mixed in a cement mixer and 100 kg ribbon blender (Gleeson *et al.* 1999). Formulations were mixed on a dry weight basis and sufficient quantities of 95LC1 and 95LC2 were prepared to provide adequate material for use in the preparation of steam conditioned and cold-pelleted dietary treatments in the digestibility and performance trials.

Extruded diets were produced in a pilot scale, twin screw extruder (model APV MFP40; APV-Baker, Peterborough, England), at CSIRO, Division of Food Science and Technology, North Ryde, NSW, Australia. Diets were produced after extrusion processing conditions with respect to the moisture content of the extruder barrel and specific mechanical energy of the process had been optimised for physicochemical characteristics (Gleeson *et al.* 1999). To provide extruded diets for the digestibility trial, a quantity of mash was withdrawn from each batch and mixed with chromic oxide (5 g kg^{-1} dry basis) prior to being passed through the extruder. Pellets were dried (70 °C) until moisture contents reached about 10 % using a fluid bed dryer (Drytec, Tonbridge, Kent, England). Pellets had a nominal diameter of 2 mm and a length of 3-4 mm (Gleeson *et al.* 1999).

Steam-pelleted diets

SP35 for use in steam-pelleted and cold-pelleted diets was obtained from Janos Hoey Pty., Ltd., Forbes, NSW, Australia, but was from a different batch to that used to produce extruded diets. Prior to manufacture of steam conditioned SP35, the raw mash was ground through a hammer mill (Raymond Laboratory Mill, Transfield Technologies, Rydalmere, NSW, Australia) fitted with a 1.5 mm screen. An adequate quantity was ground to provide enough material for use in the cold-pelleted treatment. Experimental diets were then produced in a steam pellet press (W-500B Junior Ace pellet mill, Sprout Waldron, PA, USA) by CSIRO, Division of Animal Production, Prospect, NSW, Australia. Steam settings were set to 75 °C and the temperature of pellets exiting the die ranged from 77-82 °C. Pellets were air-dried. To provide steam-pelleted diets for the digestibility trial, a quantity of mash was withdrawn from each batch and mixed (at PSFC) with chromic oxide (5 g kg^{-1} dry basis) prior to being passed through the steam pellet press at the end of a run. Pellet diameter was approximately 2.2 mm.

Cold-pelleted diets

All cold-pelleted diets were processed at Port Stephens Fisheries Centre (PSFC). Mash was dry mixed before the addition of water then cold pelleted using a small-scale meat mincer (Barnco Australia Pty Ltd, Leichhardt, NSW, Australia) fitted with a 2 mm die. Diets for use in the digestibility trial had 5 g chromic oxide kg^{-1} diet included as the inert indicator. Pellets were then dried at < 40 °C in convection dryers for about 5-6 h until moisture contents were below 10 %.

Experimental facilities and procedures

Silver perch used in both experiments were bred and reared at NSW Fisheries, Grafton Research Centre, after which they were held at PSFC in 10 000-L tanks and fed SP35 exclusively. However, due to problems with the supply of fingerlings, separate stocks of fish were used in the digestibility and performance trials. Prior to use in experiments, fish were given a prophylactic treatment (10 g L^{-1} NaCl) to reduce the presence of protozoan ectoparasites (Callinan & Rowland 1995). During stocking procedures all fish were anaesthetised in a bath of 30 mg ethyl-ρ-amino benzoate L^{-1}, then caught at random, weighed in small groups and distributed among tanks by systematic interspersion.

Digestibility trial

The digestibility trial was performed in a light / temperature controlled environment. Digestibility tanks (27 x 190-L cylindro-conical tanks) consisted of an upper tank and lower settlement chamber separated by a mesh screen which prevented movement of fish to the settlement chamber. The settlement chamber terminated in a 250 mm length of silicone tubing which collected faecal materials (Allan *et al*. 1999). 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 1 L min^{-1}. Effluent water exited each tank via a 25 mm 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 stocked with 13 juvenile silver perch of 5.6 g average weight and fish were then acclimated to their respective diets and laboratory conditions for 6 days prior to collection of faeces. Silver perch were fed in excess of their requirements (approximately 10 % of total biomass day^{-1}) for a period of 3 h (0830 and 1130 h) using clockwork type, belt feeders (Fischtechnik Fredelsloh, Moringen, Germany). Approximately 1 h after all feed had been delivered to the digestibility tanks both the upper tanks and lower collection chambers were thoroughly cleaned. Faecal collection tubes were then packed in ice and faecal materials collected by passive settlement over a period of 18 h (Allan *et al*. 1999). Faecal samples were removed each morning prior to feeding and dried under vacuum at room temperature ($\approx$ 20 °C, silica gel desiccant) and then frozen ($\leq$ -15 °C). Individual daily tank samples were pooled over the course of the experiment until enough material was obtained for chemical analyses. Following chemical analyses of feed and faecal material, apparent digestibility coefficients (ADC) were calculated using the following formula:

ADC = [1- (F/D X DCr/ FCr)] where F is the percent of nutrient or energy in faeces, D is the percent of nutrient or energy in diet, DCr is the percent of chromic oxide in diet and FCr is the percent of chromic oxide in faeces (Cho & Kaushik 1990)

Performance trial

The performance trial was undertaken in a hot-house facility which housed 15 circular 10 000-L fibreglass tanks (diameter 3.4 m; height 1.2 m). Fresh water was circulated through each tank at approximately 17 L min^{-1} then returned to a common sump (5000-L) containing a submerged biofilter. Water was pumped from the sump via two rapid sand filters before returning to experimental tanks. Each tank was provided with two large air stone diffusers and covered with black shade cloth to reduce the proliferation of algae. Tanks were siphoned once a week to remove accumulated faeces. Each of the five dietary treatments was randomly allocated to three tanks (*n* = 3). Tanks were stocked with 70 fish (average individual weight 7.38 g) which were hand fed to apparent satiation twice daily (0830 h and 1500 h) for 92 days.

Observation of the feeding response in individual tanks was aided by the clarity of water in our experimental tanks which ensured delivery of excess pellets was minimised. However, in the first week after feeding commenced, it became apparent fish fed on extruded SP35 were being restricted due to the unexpected buoyancy of this diet. Following the recommendations of Booth *et al*. (2000a), all diets for use in the performance trial were designed to sink slowly. However, approximately 24 % of extruded SP35 fed to fish in grow-out tanks remained buoyant. In order to overcome this problem, a different strategy was adopted for delivering feed to the fish in this treatment. Pellets were carefully delivered in excess in order that fish might feed on them close or near to the bottom (Booth *et al*. 2000a) Uneaten floating pellets were then collected from the surface after fish were judged to be satiated, dried to a constant weight (105 °C, 24 h) and subtracted from the total feed delivered at the end of the experiment. The range in sinking rates (Evans, Gleeson & McCann 1998) for each of the five diets tested in the performance trial were as

follows; cold-pelleted SP35 (5-7.7 cm s^{-1}), steam-pelleted SP35 (10-11.1 cm s^{-1}), extruded SP35 (floating-3.7 cm s^1), steam-pelleted 95LC2 (8.3-10.2 cm s^{-1}) and extruded 95LC2 (2.8-4.5 cm s^{-1}). A preliminary weight check was undertaken one month after stocking, after which the trial was run to completion. Fish were harvested and the following performance indices calculated for each tank; survival, weight gain, feed intake and feed conversion ratio. In addition, digestible energy and protein intake and digestible protein efficiency ratio were investigated.

Water quality

Temperature, pH, dissolved oxygen and salinity were monitored using a Model 611 electronic water quality analyser (Yeo-Kal Electronics, Brookvale, NSW, Australia). Colourimetric methods were adopted to measure total ammonia nitrogen (Dal Pont, Hogan & Newell 1973) and nitrite (Major, Dal Pont, Kyle & Newell 1972). During the digestibility trial, temperature ranged between 25.5-28.0 °C, pH 7.8, dissolved oxygen 6.0-7.5 mg L^{-1} and salinity 3.3-7.5 g L^{-1}. Background salinity was raised in this trial as a cautionary measure to prevent the outbreak of protozoan diseases that had been prevalent at that time. Total ammonia-N ranged between 20-40 ug L^{-1} and NO_2-N remained below 20 ug L^{-1}.

During the performance trial, water temperature ranged between 22.1-28.0 °C, pH 7.6-8.5, salinity 1.3-5.2 mg L^{-1}, dissolved oxygen 6.7-7.4 mg L^{-1}. Total ammonia-N and NO_2-N remained below 100 ug L^{-1} and 200 ug L^{-1} respectively. Prior to stocking (October), all tanks were fitted with 1 kW heaters to maintain water temperatures above a minimum of 20 °C. They remained in use until early summer temperatures were adequate to sustain temperatures closer to 26 °C. Over the course of this trial several prophylactic treatments were administered to prevent the outbreak of protozoan diseases. These included regular elevation of salinity by the addition of either pool salt or near inshore ocean water (Port Stephens) and two treatments (November and December) with 20 mg formalin L^{-1} (Rowland & Ingram 1991; Callinan & Rowland 1995).

Chemical analyses

Chemical analyses (excluding determination of gross energy and chromic oxide) of diets and faeces were performed by the State Chemistry Laboratory (SCL) (Victoria Agriculture, Werribee, Vic, Australia). Crude protein was determined from an adaptation of the standard Kjeldahl method (AOAC 1995) using automated Tecator distillation apparatus. Fat was extracted from samples with diethyl ether in a continuous extraction procedure using an automated Soxtherm apparatus (Gerhardt) after which oven dried residue was weighed to calculate "crude fat" (AOAC 1995). Sample moisture was determined after oven drying at 105 °C for 16 h and ash after oxidation (muffle furnace at 550 °C for 5-6 h).

Gross energy analysis (bomb calorimetry) was performed by the South Australian Research and Development Institute (SARDI) on sub-samples drawn from those prepared by SCL. Analysis of diets and faeces for chromic oxide indicator was performed by CSIRO, Tropical Agriculture Analytical Services, St Lucia, Qld, Australia, using inductively coupled plasma - mass spectrometry (ICP-MS) techniques.

Statistical analyses

All data was subjected to tests for homogeneity of variance using Cochran's Test (Winer, Brown & Michels 1991) before proceeding with analysis of variance (ANOVA). Nine dietary treatments from the digestibility trial were subjected to a two-factor ANOVA to investigate the interaction between diet type (SP35, 95LC1 and 95LC2) and processing method (cold-pelleted, steam-pelleted and extruded). The five dietary treatments evaluated in the performance trial were initially compared with one-way ANOVA. Following this, data for cold-pelleted SP35 was excluded from analyses, and the remaining data subjected to a two-factor ANOVA. Where ANOVA was

significant ($P < 0.05$), differences among treatment means were distinguished using the Student Newman-Keuls test. Data were statistically analysed using Statgraphics Plus, version 4.1 (Manugistics Inc., Rockville, MD, USA; 1998).

Results

Digestibility trial

Two-factor ANOVA indicated that there was no interaction between diet type and processing method for dry matter (DM) ($P = 0.250$), energy ($P = 0.112$) or nitrogen ($P = 0.337$) digestibility coefficients. Both factors significantly affected DM ($P = 0.0001$ for diet; $P = 0.0012$ for process) and energy ($P < 0.0001$ for diet; $P < 0.0001$ for process) digestibility, but only diet type affected nitrogen digestibility ($P < 0.0001$). Analysis of main effects ($n = 9$) indicated that SP35 was significantly ($P < 0.05$) more digestible than either 95LC1 or 95LC2 for all ADCs, while only extrusion of diets significantly improved the digestibility of DM and energy. Nitrogen digestibility was not significantly affected by processing (Table 2).

Performance trial

100% survival was recorded for all treatments. One-way ANOVA indicated that individual weight gain ($P < 0.0001$), feed consumption ($P < 0.0001$) and FCR ($P = 0.0001$) were all significantly affected by dietary treatment (Table 3). Weight gain was the highest in fish fed steam-pelleted and extruded 95LC2, however, FCRs were better for fish fed SP35 diets (Table 3). Fish consumed significantly more of the steam-pelleted 95LC2 on a percent biomass basis than any other dietary treatment.

After eliminating the control diet (cold-pelleted SP35) from statistical analysis, results of a two-factor ANOVA indicated that for weight gain, interactions between diet type and processing method were not significant ($P > 0.05$). Weight gain of fish fed 95LC2 was significantly better than fish SP35, regardless of processing technique ($P < 0.0001$). Steam processing produced significantly higher weight gain than extrusion processing applied to the same diets ($P = 0.0013$). Two-way ANOVA revealed a significant interaction between main effects for FCR ($P = 0.009$) and feed consumption ($P = 0.023$).

Discussion

The results of our factorial digestibility trial indicate that steam conditioning had little effect on digestibility, but extrusion significantly improved DM and energy digestibility. Nitrogen digestibility was unaffected by the processing techniques employed in this study (Table 2). SP35, was significantly more digestible than either 95LC1 or 95LC2 for each ADC while 95LC1 and 95LC2 were similar for DM and energy digestibility. 95LC2 had the lowest nitrogen digestibility.

The improvements in DM and energy digestibility of SP35 after extrusion relate directly to the higher gelatinised starch content of this diet (Booth *et al*. 2000a). Contributions to total starch in SP35 come only from the cereal grains of wheat and sorghum and a small contribution from millrun. In contrast, energy digestibility was lower for 95LC1 and 95LC2, probably because greater dietary contributions were made by ingredients which contained little or no starch such as lupins, peanut meal and canola meal. Although content of millrun was higher in these diets, the starch content of millrun is typically low and its composition can be inconsistent (Gleeson *et al*. 1999). In addition, most of the protein in SP35 is derived from fish and soybean meals, for which silver perch exhibit relatively high DM ADCs of > 75 % (Allan *et al*. 2000a). By comparison, 95LC1 and 95LC2 diets carry about 22 and 37 % standard quality meat meal respectively, for

which silver perch exhibit DM ADCs of between only 50-55 % (Allan *et al*. 2000a). Incorporation of high-quality low-ash meat meals in diets for silver perch have resulted in dramatic improvements in DM and energy digestibility > 80 % (Stone *et al*. 2000). Recently, Allan *et al*. (2000c) showed that extrusion significantly improved DM and energy digestibility of soybeans and field peas, but had no effect on lupins and actually reduced DM and energy digestibility of canola meal fed to juvenile silver perch. Extrusion had no major effects on the nitrogen digestibility of canola or soybean meals, but did improve field peas.

Cooking proteins in the presence of carbohydrates and water can lead to Maillard type reactions (Whistler & Daniel 1985) resulting in a nutritional loss of L-lysine and other susceptible amino acids, possibly reducing protein utilisation (Hilton, Cho & Slinger 1981; Vens-Cappell 1984). The absence of a reduction in nitrogen digestibility tends to discount the influence of these reactions. We found no evidence of a reduction in apparent availability of amino acids in previous studies with silver perch fed cold-pelleted, steam-pelleted or extruded SP35 (Booth *et al*. 2000a). Given that weight gain of silver perch fed the cold-pelleted SP35 was lower than for fish fed either the steam-pelleted or extruded SP35, it is unlikely that protein degradation had been a factor and the prospect that these types of reactions had reduced the bio-availability of dietary protein were discounted. Cooking may not have affected apparent protein digestibility, but this does not preclude the possibility that anti-nutrients present in cold-pelleted SP35 which affect palatability were reduced or eliminated by cooking, consequently leading to the increased intake of cooked and extruded SP35.

DM digestibility of SP35 diets was unexpectedly low, and compares poorly to digestibility coefficients determined for this diet in earlier studies. For instance Booth *et al*. (2000a) previously determined DM digestibility for raw, steam-pelleted and extruded SP35 as 65, 67 and 71 % respectively. Nitrogen ADC's for the same diets were 89, 90 and 89 %, while gross energy values were 76, 78 and 83 MJ kg^{-1} respectively. Lower values for these digestibility determinations may be related to batch differences between the previous and the new ingredients, the fact that different fish stocks were used, or the fact that this trial was run at slightly elevated salinity (e.g. average 5.9 g L^{-1} NaCl) to guard against the outbreak of protozoan diseases. Silver perch have been shown to be highly tolerant of elevated salinity (Gou, Mather & Capra 1995), however, MacLeod (1977) showed that feed adsorption efficiency of DM, energy and protein was negatively correlated with salinity in trout *Oncohynchus mykiss*. More recently, Storebakken, Shearer, Refstie, Lagocki & McCool (1998) reported ADCs of organic matter, protein, fat and carbohydrate for trout were significantly lower in salt water than in fresh water. Fortunately, it is likely that any such effect in our study applied across all treatments.

On the basis of our digestibility results (Table 2), fish fed similar formulations of steam-pelleted and extruded diets should have performed comparably, with a tendency towards improved performance on extruded diets. Although fish fed on extruded diets did not gain as much weight as fish fed on steam-pelleted diets, they performed better than fish fed similar cold-pelleted diets and weighed on average, only 10g less than those fed steam-pelleted diets. In addition, fish fed on extruded diets had better FCR and better digestible protein efficiency ratio (DPER) than fish fed similar steam-pelleted diets (Table 3), indicating extruded diets were probably better utilised than steam-pelleted diets. While improvements in FCR and DPER are probably related to the increased energy digestibility of extruded diets, the fact remains that consumption of extruded diets was significantly lower ($\approx$ 20 %) than similar steam-pelleted diets and suggests that some other factor/s may be restricting the consumption of these diets. In previous work with processed diets, we found that buoyancy of extruded SP35 in 10 000-L clear water tanks affected the feeding behaviour of silver perch resulting in reduced voluntary intake (Booth *et al*. 2000a). For this reason, extruded diets evaluated in the present study were designed to sink, allowing fish unrestricted access to the diet as well as providing a more accurate monitoring of the satiation response. Despite these improvements in the presentation of the extruded pellets, voluntary intake remained well below that of the steam-pelleted diets (Table 3). There is some evidence to suggest that the gastrointestinal

evacuation rates of extruded diets affects both feed consumption and FCRs in fish. In a 13-week study with rainbow trout *Onchorhynchus mykiss*, Hilton *et al.* (1981) found that weight gain of fish reared on steam-pelleted diets was significantly higher than those reared on extruded diets of the same composition, although, feed efficiency was higher for extruded diets. In addition, they found that stomach emptying rates were faster in trout fed on steam-pelleted diets than for those fed on extruded diets (e.g. on average it took fish 305 min as opposed to 534 min to half empty their stomachs of the steam-pelleted and extruded dietary treatments respectively). These differences were loosely linked to the increased water stability of their extruded pellets. It was also noted that fish from both treatments consumed essentially the same amount of feed at the beginning of the day, but fish fed the extruded diets consumed considerably less at subsequent feedings. We recorded only daily intake data, but in many respects, our results approximate those described by Hilton *et al.* (1981) and the possibility that the lower weight gain exhibited by silver perch fed extruded diets is linked to a restricted intake during afternoon feeding due to delayed gastric emptying cannot be discounted.

The relationships between dietary energy and nutrient balance are beyond the scope of this study, but there were minor differences between the digestible energy contents of respective pairs of steam-pelleted and extruded diets (Table 2) which may have affected consumption (Smith 1989; Tacon 1990; NRC 1993). In fact, there is a strong linear relationship between the digestible energy content of steam-pelleted and extruded diets and voluntary feed intake of fish per kg of average body weight (ABW), (i.e. dry basis intake g $kgABW^{-1}$ d^{-1} = 51.93 – 1.71 x digestible energy content ; R^2 = 0.92). Vens-Cappell (1984) also reported linear relationships between intake and energy digestibility of pelleted and extruded diets fed to trout (species not given). Given that silver perch may have been regulating intake of cooked diets due to digestible energy content, the fact that steam-pelleted diets contained slightly more digestible protein per unit of digestible energy compared to extruded diets (Table 2) may explain why fish fed steam-pelleted diets were, on average, about 10 g heavier than those fed similar extruded diets at harvest. There is, however, no evidence to suggest that the digestible protein content of our test diets which contained about 13 MJ digestible energy kg^{-1} was limiting growth (Allan *et al.* 2001).

Voluntary intake of cold-pelleted SP35 was not consistent with the previously described regression. Fish fed this diet also exhibited the poorest weight gain coupled with a low DPER (Table 3), indicating protein utilisation (not digestibility) may be a problem with this diet. The overall lower performance of fish on SP35 diets compared to 95LC2 diets also suggests that this formulation was inferior. One of the major differences in the formulation was the inclusion of 20 % soybean meal. Plant protein was supplied by lupins and field peas in the 95LC2 diets. Given the increasing evidence of the antinutritional effects of solvent extracted soybean meal (van den Ingh, Krogdahl, Olli, Hendriks & Koninkx, 1991; Bureau, Harris, & Cho 1998; Kroghdahl & Bakke-Mckellep 2001), it is possible that this ingredient reduced palatability or depressed growth of silver perch fed on cold-pelleted SP35.

Although the mechanisms influencing feed consumption and growth in the feeding study remain unclear, the results emphasize the importance of investigating the nutritional profile (e.g. composition, digestibility, measures of utilisation) of diets as well as investigating the effects of feeding frequency when comparing diets of a different physical composition. A measure of gastric evacuation rates for silver perch fed diets processed under different conditions warrants investigation and may prove a useful tool in determining the appropriate feeding frequencies for maximum gain or efficiency under a variety of conditions. In this way, the performance of fish reared on extruded diets may be better understood. The influence of digestible energy on voluntary and restricted feed intake also deserves evaluation, as does investigation of protein requirements at digestible energy contents below 13 MJ kg^{-1} diet.

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Table 1.   Formulation and composition of experimental diets.

	SP35			95LC1			95LC2		
	Cold pelleted	Steam pelleted	extruded	Cold pelleted	Steam pelleted	extruded	Cold pelleted	Steam pelleted	extruded
Formulation g kg⁻¹									
Fish meal (Peruvian)	270.0	270	270	100.0	100.0	100.0	50.0	50.0	50.0
Meat meal (lamb meal)	-	-	-	217.0	217.0	217.0	369.0	369.0	369.0
Blood meal	20.0	20.0	20.0	21.0	21.0	21.0	-	-	-
Corn gluten meal	40.0	40.0	40.0	38.0	38.0	38.0	52.0	52.0	52.0
Soybean meal	200.0	200.0	200.0	-	-	-	-	-	-
Canola meal	-	-	-	-	-	-	50.0	50.0	50.0
Peanut meal	-	-	-	-	-	-	50.0	50.0	50.0
Field peas	-	-	-	149.0	149.0	149.0	104.0	104.0	104.0
Lupins	-	-	-	255.0	255.0	255.0	74.0	74.0	74.0
Wheat	284.0	284.0	284.0	-	-	-	-	-	-
Sorghum	110.0	110.0	110.0	47.0	47.0	47.0	-	-	-
Millrun	20.0	20.0	20.0	125.0	125.0	125.0	202.0	202.0	202.0
Fish oil	-	-	-	29.0	29.0	29.0	32.0	32.0	32.0
Cod liver oil	10.0	10.0	10.0	-	-	-	-	-	-
DL-methionine	2.0	2.0	2.0	4.0	4.0	4.0	3.0	3.0	3.0
Vitamin premix[1]	13.0	13.0	13.0	8.0	8.0	8.0	8.0	8.0	8.0
Mineral premix[2]	13.0	13.0	13.0	8.0	8.0	8.0	8.0	8.0	8.0
Di-calcium phosphate	20.0	20.0	20.0	-	-	-	-	-	-
Measured composition g kg⁻¹									
Crude protein	420.0	399.0	390.0	422.0	428.0	441.0	438.0	437.0	429.0
Fat	21.0	43.0	20.0	85.0	93.0	87.0	102.0	97.0	88.0
Ash	172.0	155.0	166.0	117.0	118.0	116.0	162.0	163.0	159.0
Gross energy MJ kg⁻¹	17.9	17.9	17.8	19.7	19.6	19.7	19.6	18.4	19.0
Starch[3]	-	-	240.0	-	-	161.0	-	-	125.0
Gelatinisation (%starch)[3]	-	-	81.8	-	-	70.7	-	-	82.1

[1] (IU kg⁻¹ diet): retinol A, 8000; cholecalciferol D3, 1000; DL-α-tocopherol acetate E, 125. (mg kg⁻¹): menadione sodium bisulphite K3, 16.5; thiamine hydrochloride B1, 10.0; riboflavin B2, 25.2; pyridoxine hydrochloride B6, 15.0; folic acid, 4.0; ascorbic acid C, 1000; calcium-D-pantothenate, 55.0; myo-inositol, 600; D-biotin H (2%), 1.0; choline chloride, 1500; nicotinamide, 200; cyanocobalamin B12, 0.02; ethoxyquin (anti-oxidant) 150; calcium propionate (mould inhibitor) 25.0.
[2] (mg kg⁻¹ diet): calcium carbonate, 7500; manganese sulphate monohydrate, 300; zinc sulphate monohydrate, 700; copper sulphate pentahydrate, 60, ferrous sulphate heptahydrate, 500, sodium chloride, 7500; potassium iodate, 2.0.
[3] Determined by Gleeson *et al.* (1999).

Table 2.   Apparent digestibility coefficients and digestible nutrients for experimental diets fed to silver perch.

	SP35			95LC1			95LC2		
	Cold pelleted	Steam pelleted	extruded	Cold pelleted	Steam pelleted	extruded	Cold pelleted	Steam pelleted	extruded
Apparent digestibility coefficients (n = 3)									
Dry matter %	57.2	59.9	59.7	46.8	51.9	57.4	48.0	49.6	55.3
Gross energy %	72.6	73.6	76.8	58.6	63.5	70.0	63.1	63.8	69.5
Nitrogen %	87.3	86.4	84.5	78.9	83.7	80.7	75.2	73.0	73.8
Digestible nutrient content (n = 3)									
Digestible protein %	36.6	34.5	33.0	33.3	35.8	35.6	33.0	31.9	31.7
Digestible energy MJ kg^{-1}	13.0	13.1	13.6	11.6	12.4	13.8	12.4	11.7	13.2

Apparent digestibility coefficients (n = 9)

	Main effect diet			Main effect process			
	SP35	95LC1	95LC2	Cold pelleted	Steam pelleted	extruded	pooled sem
Dry matter %	58.93[a]	52.04[b]	50.96[b]	50.68[x]	53.81[x]	57.46[y]	1.07
Gross energy %	74.34[a]	64.04[b]	65.48[b]	64.79[x]	66.97[x]	72.11[y]	0.76
Nitrogen %	86.05[a]	81.11[b]	74.01[c]	80.49[x]	81.03[x]	79.66[x]	1.02

Values are means of *n* = 9 replicate tanks. There was no interaction between diet type and processing method ($P > 0.05$). For each factor, means in the same row with the same superscript are not significantly different ($P > 0.05$).

Table 3.   Performance characteristics of silver perch fed experimental diets for 92 days.

	SP35			95LC2		
Performance index	cold-pelleted	steam-pelleted	extruded	steam-pelleted	extruded	pooled sem
Initial weight (g fish^{-1})	7.4[a]	7.3[a]	7.4[a]	7.4[a]	7.4[a]	0.045
Final weight (g fish^{-1})	50.3[a]	78.5[b]	66.5[c]	100.2[d]	90.9[e]	2.000
Weight gain (g fish^{-1})	42.7[a]	71.1[b]	58.8[c]	93.7[d]	83.1[e]	1.990
Total feed (kg tank^{-1})	5.16[a]	8.27[b]	6.75[c]	11.14[d]	9.24[e]	0.214
FCR[1]	1.47[a]	1.51[b]	1.46[a]	1.57[c]	1.46[a]	0.011
Intake (g kgABW^{-1} d^{-1})[2]	27.9[a]	29.9[b]	28.5[a]	31.8[c]	29.3[b]	0.212
DPER[3]	1.59[a]	1.75[b]	1.85[c]	1.85[c]	1.99[d]	0.013

Means (*n* = 3) in the same row with similar superscript are not significantly different ($P > 0.05$).

[1] Feed conversion ratio (FCR) = dry weight feed tank^{-1} / wet weight gain tank^{-1}.

[2] Intake (g kgABW^{-1} d^{-1}) = [dry basis intake per fish (g) / (ABW per fish / 1000) / 92 days]. Average body weight (ABW).

[3] Digestible protein efficiency ratio (DPER) = wet weight gain fish^{-1} / digestible protein intake fish^{-1}

4.2.   **Effects of extrusion processing and dehulling on digestibility of extruded peas, lupins, soybean and canola in silver perch (*Bidyanus bidyanus*) diets**

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Abstract

Two experiments were conducted to investigate effects of processing on digestibility of legumes and oilseeds for silver perch, *Bidyanus bidyanus*. Beneficial effects of processing, including removal of hulls and extrusion cooking have been reported but such practices can be expensive. The first experiment was a three fixed factor ANOVA design to evaluate interactive effects of ingredients (lupins or field peas), processing (whole seed; hulls on or hulls off) and extrusion cooking (raw or extruded) on digestibility of juvenile silver perch (~4 g/fish). The second experiment was a three fixed factor ANOVA design to evaluate interactive effects of ingredients (soybean meal or canola meal), extrusion cooking (raw or extruded) and inclusion content (30 or 50% of the diet) on digestibility of juvenile silver perch (~4g/fish). Apparent digestibility coefficients (ADCs) were calculated after collecting faeces by settlement. The protein from lupins was more digestible than for peas (ADC for protein 91% vs 85% for peas) but the organic matter was less digestible (ADC for organic matter 50% vs 67% for peas). For lupins, dehulling significantly improved ADCs for all indices (dry matter, organic matter, energy and protein) while extrusion had either no effect (ADCs for dry matter, organic matter and energy or slightly reduced ADCs for protein). Extrusion was not beneficial because lupins do not contain starch or heat-labile anti-nutrients. Conversely, for starch-rich peas, both dehulling and extrusion significantly improved ADCs. Peas also contain trypsin inhibitors which are heat labile. Digestibility of soybean meal was much higher than of canola meal. For soybean meal, neither processing, content nor their interaction affected digestibility but extrusion improved ADCs for dry matter, organic matter and energy but there was an interaction with content. Although higher overall, digestibility for these indices declined with increasing content for extruded product while there were only minor effects of inclusion for raw product. Benefits of extrusion were attributed to reductions in anti-nutrients, including phytic acid. For canola, there were no interactions between extrusion and content for any ADC. Increasing content reduced ADCs for protein, dry matter and organic matter but did not effect energy. Surprisingly, extrusion of canola also reduced digestibility for all ADCs. Dehulling improved both lupins and peas. Protein for all ingredients was well digested with lupins > soybean meal > peas > canola meal. Energy digestibility was best for soybean meal and worst for lupins. Extrusion greatly improved digestibility of peas and to a lesser extent soybean meal, gave no benefits to lupins and was detrimental for canola.

Introduction

Static or declining supplies of fishmeal and increasing production of carnivorous and omnivorous aquaculture species have increased the proportion of fishmeal going to aquaculture from about 12% in 1984 to over 37% in 1997 (Tacon 2000). This has driven the need to find alternatives and there is now an increasing amount of information available on the potential to use plant ingredients. Fishmeal has been at least partially replaced by plant proteins for many species (Tacon 1994; Kaushik, Cravedi, Lalles, Sumpter, Fauconneau & Laroche 1995; Satoh, Higgs, Dosanjh, Hardy, Eales & Deacon 1998; Burel, Boujard, Kaushik, Boeuf, van der Geyten, Mol, Kuhn, Quinsac,

Krouti & Ribaillier 2000; Carter & Hauler 2000; Allan, Parkinson, Booth, Stone, Rowland, Frances & Warner-Smith 2000a; Allan, Rowland, Mifsud, Glendenning, Stone & Ford 2000b; Refstie, Storebakken, Baeverfjord & Roem 2001). The majority of studies are on soybean meal as this is the most commonly used plant protein in aquaculture feeds (e.g. Kaushik *et al*. 1995; Robaina, Izquierdo, Moyano, Socorro, Vergara, Montero & Fernandez-Palacios 1995; Kissil, Lupatsch, Higgs & Hardy 2000; Refstie *et al*. 2001). However, other ingredients that have been used successfully include canola meal (Higgs, McBride, Markert, Dosanjh, Plotnikoff & Clarke 1982; Higgs, Dosanjh, Prendergast, Beames, Hardy, Riley & Deacon 1995; Lim, Klesius & Higgs 1998; Satoh *et al*. 1998), lupins; (Higuera, Garcia-Gallego, Sanz, Cardenete, Suarez & Moyano 1988; Hughes 1988; Hughes 1991; Gouveia, Oliva-Teles, Gomes & Rema 1993; Robaina *et al*. 1995; Booth, Allan & Warner-Smith 2000; Burel *et al*. 2000a) and peas; (Pfeffer, Kinzinger & Rodenhutscord 1995; Gouveia & Davies 1998; Carter & Hauler 2000; Gouveia & Davies 2000; Burel, Boujard, Tulli & Kaushik 2000b). In Australia, aquaculture of the freshwater native silver perch (*Bidyanus bidyanus*) is increasing, especially in NSW and Queensland and to a lesser extend Western Australia. One of the attributes of this species is that it is omnivorous and can be cultured using relatively low protein diets which contain no fishmeal (Allan & Rowland 2001). Because of these attributes, silver perch diets are now the cheapest formulated feeds for any intensively cultured species in Australia.

Our research has demonstrated that silver perch are extremely efficient at digesting protein from plant ingredients (Allan *et al*. 2000a) and that dry matter and energy digestibility for legumes is usually improved when hulls and other carbohydrate components are removed (Booth, Allan, Frances & Parkinson 2001). This research was done using experimental diets processed without heat and digestibility values now being used by commercial feed manufacturers were generated using cold processed ingredients and diets. However, in other research, the beneficial effects of extrusion on practical diets has been demonstrated. Pfeffer *et al*. (1995) reported that pressure cooking or autoclaving significantly increased the digestibility of energy, crude protein and total carbohydrates of soybean, field peas (*Pisum sativum*) and field beans (*Vicia faba*) fed to rainbow trout while (Satoh *et al*. 1998) reported that extrusion cooking of canola meal improved its nutritive value for Chinook salmon. (Carter & Hauler 2000) suggested that the higher energy digestibilities recorded in their study with lupin (*Lupinus angustifolius*) and pea (*Pisum sativum*) protein concentrates fed to Atlantic salmon, compared with earlier studies, were due to improvements following extrusion.

Beneficial effects of heat processing may include deactivation of anti-nutritional factors (Pfeffer *et al*. 1995), increased starch gelatinisation (Watanabe, Pongmaneerat, Sato & Takeuchi 1993; Carter & Hauler 2000; Stone, Allan & Anderson, in press) and increased utilization of nitrogen-free extracts or other components (Bangoula, Parent & Vellas 1993; Burel *et al*. 2000a). Extrusion may also confer important benefits to physical characteristics of pellets such as binding, water stability and buoyancy (Hardy 1989). However, the effects of heat processing on any ingredient will depend on the carbohydrate (especially starch) composition and the concentration of heat labile anti-nutritional factors. Because anti-nutrients and carbohydrates are not distributed equally within whole grains, other types of processing, such as dehulling, can also have large effects on digestibility and nutritive value of grains (Booth *et al*. 2001). Finally, although research has demonstrated that digestibility coefficients are largely additive, where ingredients contain anti-nutrients or high carbohydrate contents, inclusion contents can affect digestibility (Bergot & Breque 1983; Pfeffer *et al*. 1995). Other factors such as inclusion content will affect the impact of any anti-nutrients on digestibility and nutritive value of grains to fish.

The aim of this study was to investigate the effects of extrusion cooking on digestibility of plant ingredients included in diets for silver perch. Results from two experiments are reported; the first was designed to assess interactive effects of extrusion and dehulling on digestibility of lupins and

field peas and the second to assess the interactive effects of extrusion and inclusion content on digestibility of soybean and canola.

Materials and Methods

Experimental design

Both digestibility experiments were designed for fully orthogonal, three factor ANOVA. Each factor had two levels and each level was replicated three times. The first experiment was designed to evaluate the effects of different processing techniques on the digestibility of two legumes; field peas (*Pisum sativum* –cv. Dunn) and lupins (*Lupinus angustifolius*-cv. Gungurru). Fixed factors for this experiment were ingredient (field peas or lupins), processing method (whole seed or dehulled seed) and cooking method (raw or extruded). The second digestibility experiment was designed to evaluate the effects of different inclusion levels and different cooking methods on the digestibility of two oilseed meals; soybean meal (*Glycine max*) and canola meal (*Brassica* sp.). Fixed factors for this experiment were ingredient (soybean or canola), cooking method (raw or extruded) and inclusion content (30 or 50 % of diet).

Test ingredients and experimental diets

All test ingredients were provided by the Commonwealth Scientific and Industry Research Organisation (CSIRO), Division of Food Science and Technology, North Ryde, Australia. This facility was also responsible for the extrusion of the test legumes used in both digestibility experiments. Soybean and canola meals were solvent extracted (commercial supplier) prior to extrusion. Ingredients were extruded in a pilot scale, twin screw extruder (model APV MFP40, APV- Baker, Peterborough, England) and extrusion conditions were managed by varying screw speed, barrel melt temperature and barrel moisture content (Gleeson, O'Sullivan & Evans 1999).

In both experiments, diets were formulated on a dry matter basis. In addition, the vitamin / mineral content of all test diets was adjusted to account for the dilution effect of mixing test ingredients with the reference diet. For the experiment in which legumes were evaluated, diets were prepared by mixing a 50:50 ratio of the reference diet (Allan & Rowland, 1992; Table 1; supplied by Janos Hoey Pty. Ltd., Forbes, NSW, Australia) and the individual test ingredient of interest. For the trial in which oilseeds were evaluated, our experimental design required two different inclusion contents. As such, diets were prepared by mixing either a 70:30 or 50:50 ratio of reference diet to individual test ingredients. 500 mg ytterbium (III) acetate, tetrahydrate $[(CH_3CO_2)_3Yb4H_2O]$ kg^{-1} dry basis was included in each diet as the inert indicator. The required amount of indicator was first dissolved in about 100 ml of warm distilled H_2O. This suspension was then sprayed onto the diet with the aid of a plastic atomiser after the diet had been thoroughly dry mixed and spread in a thin layer on a large table. Each diet was then dry mixed (Hobart Mixer: Troy Pty Ltd, City OH, USA) before the addition of a suitable quantity of distilled water. The wet mash was then cold pressed into 3mm pellets using a meat mincer (Barnco Australia Pty Ltd, Leichhardt, NSW, Australia) and dried in a convection drier (< 40 ° C) for approximately 6 hours. Afterwards, to facilitate a homogeneous dispersion of the $(CH_3CO_2)_3Yb4H_2O$ each diet was then reground through a hammer mill fitted with a 1.5 mm screen (Raymond Laboratory Mill, Transfield Technologies, Rydalmere, Australia), thoroughly remixed (Hobart Mixer) then cold pressed using the same meat mixer described above into 2 mm pellets with the addition of a suitable quantity of distilled water. All diets were dried in a convection drier (<40°C) until moisture contents reached approximately 10 %.

Experimental facilities

The treatment of fish, stocking procedures and experimental facilities used in all experiments are similar to those described by (Allan, Rowland, Parkinson, Stone & Jantrarotai 1999). Briefly, digestibility tanks consisted of an upper tank and lower settlement chamber separated by a mesh screen which prevented movement of fish to the settlement chamber. The settlement chamber terminated in a 250 mm length of silicone tubing which collected faecal pellets. Digestibility tanks were stocked with twenty silver perch *Bidyanus bidyanus* (mean weight = 3.8 g) in the first digestibility experiment and seventeen fish in the second digestibility experiment (mean weight = 4.4 g). Each experimental treatment was randomly assigned to three replicate digestibility tanks. Experimental diets were introduced to fish over three to four days by feeding decreasing amounts of the reference diet mixed with increasing amounts of the test diet. Fish were acclimated to experimental conditions and diets for a minimum of 10 days before collection of faeces. Silver perch were fed in excess of their daily requirements ($\approx$10 % of tank biomass) between 0830-1130 h using clockwork feeders (Fischtechnik Fredesloh, Moringen, West Germany). Approximately 1 h after all feed had been delivered, both upper and lower sections of the digestibility tanks were thoroughly cleaned. Faecal collection tubes were then packed in ice and faecal pellets collected by passive settlement over a period of 18 h (Allan *et al*. 1999). Faecal samples were removed each morning prior to feeding and dried at room temperature under vacuum using silica gel as the desiccant. Individual tank samples from daily collections were pooled to provide sufficient sample for chemical analyses and stored at < - 15 °C until analysed. Experimental tanks were continuously supplied with pre-heated, particle filtered fresh water at a flow rate of approximately 1 l min^{-1}. Effluent from each tank then flowed to a common collection point where about 25 % was directed to waste and the rest recirculated through a biological filter before returning to the laboratory. Experiments ran for 25 (legumes) and 29 (oilseeds) days respectively.

Water quality was monitored regularly in both experiments. Salinity was increased ($\leq$ 10 g l^{-1}) at the beginning of each experiment by the addition of filtered estuarine water (PSFC) to reduce the presence of protozoan ectoparisites (Callinan & Rowland 1995). During the faecal collection period, water temperature ranged between 23 –28.5 °C, dissolved oxygen between 5.4 – 7.3 mg l^{-1}, pH between 8.3 - 8.4 and salinity between 1.0 – 6.0 g l^{-1} NaCl (Model 611-Intelligent Water Quality Analyser; Yeo-Kal Electronics Pty Ltd, Brookvale, NSW, Australia). Colourimetric methods were adopted to measure total-ammonia nitrogen (Dal Pont, Hogan & Newell 1973) and nitrite (Major, Dal Pont, Kyle & Newell 1972). In both experiments total ammonia nitrogen ranged between 20 - 100 ug l^{-1} and NO_2-nitrogen was always less than 20 ug l^{-1}.

Chemical analyses

At the conclusion of each experiment, individual faecal samples from each tank were redried under vacuum using silica gel desiccant. Following drying, individual diet and faecal samples were homogenised in a high speed blender (Waring, model 32 BL 80) before being divided for shipping to analytical service providers. Chemical analyses (excluding determination of gross energy and ytterbium (III) acetate tetrahydrate) of diets and faeces were performed by the State Chemistry Laboratory (SCL) (Victoria Agriculture, Werribee, Australia). Crude protein was determined from an adaptation of the standard Kjeldahl method (AOAC 1995) using automated Tecator distillation apparatus. Fat was extracted from samples with diethyl ether in a continuous extraction procedure using an automated Soxtherm apparatus (Gerhardt) after which oven dried residue was weighed to calculate "crude fat" (AOAC 1995). Sample moisture was determined after oven drying at 105 °C for 16h and ash after oxidation (muffle furnace at 550 °C for 2h). Total phosphorous was measured after microwave digestion using inductively coupled plasma - mass spectrometry (ICP-MS) techniques

Gross energy analysis (bomb calorimetry) was performed by the South Australian Research and Development Institute (SARDI) on sub-samples drawn from those prepared by SCL. Analysis of

diets and faeces for ytterbium (III) acetate tetrahydrate indicator was performed by CSIRO, Tropical Agriculture, Analytical Services, St Lucia, QLD, Australia, using (ICP-MS) techniques.

Calculation of digestibility coefficients

Apparent digestibility coefficients (ADC) for reference and test diets were calculated from the formula ADC (%) = 100 X [1 - (F / D x DCr / FCr)] where F = % nutrient or energy in faeces, D = % nutrient or energy in diet, DCr = % chromic oxide in diet and FCr = % chromic oxide in faeces (Cho & Kaushik 1990). Apparent digestibility coefficients for ingredients were then determined after considering the disparity between the nutrient or energy content of the reference diet and test ingredients within each experiment. The following formula was applied: AD_{ING} (%) = ($Nutr_{TD}$ X AD_{TD} − 0.699 X $Nutr_{RD}$ X AD_{RD}) / (0.299 X $Nutr_{ING}$), where AD_{ING} is apparent digestibility of nutrient or energy in the test ingredient; $Nutr_{TD}$ is the nutrient or energy concentration in test diet; AD_{TD} is the apparent digestibility of the nutrient or energy in the test diet; $Nutr_{RD}$ is the nutrient or energy concentration in the reference diet; AD_{RD} is the apparent digestibility of nutrient or energy in the reference diet and $Nutr_{ING}$ is the nutrient or energy concentration in the test ingredient (Sugiura, Dong, Rathbone & Hardy 1998).

Statistical analyses

Data on dry matter, organic matter, protein and energy digestibility coefficients for ingredients were statistically analysed using *Statgraphics Plus* (Version 4.1; 1998, Manugistics Inc., Rockville, Maryland, USA). All data was tested for heterogeneity of variances (Cochran's test) before conducting ANOVA. For legumes, variances were heterogenous in ADC's of organic matter. Transformation of data failed to remove heterogeneity. One outlier was identified in the lupin-whole-extruded group and removed from the data set (Table 4). For oilseeds, variances were heterogeneous in ADC's of protein. Transformation of this data failed to remove heterogeneity. Two outliers were identified, one from the canola-raw-50 group and one from the soy-raw-30 group. Both outliers were removed from the data set (Table 5).

Results

Composition of reference diets, legume and oilseed ingredients

There was a small difference between the measured protein content of the reference diet used in each experiment (36 versus 39 % protein; Tables 2 & 3), however, gross energy, fat and ash contents were similar. Differences in protein content were probably related to batch differences. Lupins had higher protein and fat contents than field peas. In addition, dehulling had a greater effect on increasing the protein and fat content of lupins than for field peas (Table 2). These increases relate to the fact that the hull of lupins accounts for approximately 24 % of the seed weight as opposed to about 10-15 % for field peas (Gleeson *et al*. 1999). Extrusion of legumes had little effect on the composition of either whole or dehulled legumes apart from reducing the fat content of field peas (Table 2). Solvent extracted soy bean meal had a considerably higher content of protein, but lower content of fat than solvent extracted canola meal. Ash and gross energy contents were similar for each oilseed. Extrusion had no apparent effect on the protein and energy contents of either oilseed, however minor reductions in fat content were noted for extruded versus raw oilseeds (Table 3).

Apparent digestibility coefficients for legume seeds

Three factor ANOVA indicated no significant third order interactions ($P < 0.05$). The protein from lupins was more digestible than from peas (overall mean 93% compared with 88%) but the non-protein component was less digestible (overall mean for organic matter digestibility 48% for lupins compared with 68% for peas).

As there were a number of second order interactions involving ingredients, data for each ingredient were examined separately using two-factor ANOVA to examine effects of processing (whole or dehulled) and extrusion cooking.

For lupins, there were no interactions between extrusion and dehulling for any of the digestibility coefficients (dry matter, organic matter, energy or crude protein) and for all coefficients except crude protein, dehulling significantly increased digestibility ($P < 0.05$) (Table 4). Effects of extrusion were not significant ($P > 0.05$) for dry matter, organic matter, energy or crude protein (Table 4). For peas, there was no interaction between extrusion and dehulling for dry matter, organic matter or energy but there was an interaction ($P = 0.02$) for crude protein. In that case, the interaction resulted from the greater improvement in digestibility following dehulling for extruded peas than raw peas. For all other digestibility coefficients, effects of both extrusion and dehulling were highly beneficial (and significant $P < 0.001$) (Table 4).

Apparent digestibility coefficients for oilseeds

Three-factor ANOVA indicated no significant third order interactions ($P > 0.05$). Digestibility of soybean meal was higher for all indices than for canola (Table 5). Overall differences were between 7 and 16 percentage points for crude protein digestibility and between 12 and 35% points for energy digestibility. As there were a number of second order interactions involving ingredients, data for each ingredient were analysed separately using two-factor ANOVA to examine effects of extrusion and inclusion content. For soybean, there were no significant effects of inclusion or the interaction between inclusion and extrusion for any of the digestibility coefficients. However, extrusion significantly improved the digestibility of all coefficients for soybean meal ($P < 0.05$) (Table 5). Digestibility coefficients for dry matter, organic matter and energy were all higher for extruded soybean meal compared with raw product but for extruded soybean meal, increasing content from 30 to 50% slightly reduced digestibility. At 30% inclusion content, extrusion improved digestibility coefficients of dry matter, organic matter and energy by between 7-12 percentage points compared with between 2-5 percentage points where inclusion content was 50% (Table 5).

For canola, there were no significant interactions between inclusion content and extrusion ($P > 0.05$). For dry matter, organic matter and energy, increasing inclusion content from 30 to 50% did not affect digestibility ($P < 0.05$), however, protein digestibility of canola was significantly reduced at higher inclusion contents ($P < 0.05$). The dry matter, organic matter and energy digestibility coefficients of raw canola were significantly higher ($P < 0.05$) than extruded canola, but the effect was not significant for protein (Table 5).

Discussion

Results from this study support earlier research with lupins and peas that showed protein in these ingredients is generally well digested and that, in general, dry matter and energy digestibility are reduced by high carbohydrate contents (Allan *et al*. 2000a; Booth *et al*. 2000; Robaina *et al*. 1995; Pfeffer *et al*. 1995; (Gouveia & Davies 2000; Burel *et al*. 2000a; Higuera *et al*. 1988; Hughes 1988; Hughes 1991); Gomes & Kaushik, 1989).

Our values for dry matter and energy digestibility for whole peas were 63 and 64% respectively, compared with 66 and 69% for rainbow trout and 72 and 78% for turbot (Burel *et al*. 2000a). Differences were greater for lupins. Our values for dry matter and energy digestibility for extruded dehulled lupins were 49 and 62% respectively, compared with 70 and 77% for trout and 81 and 85% for turbot (Burel *et al*. 2000a). Gross composition of the ingredients used in study and that of (Burel *et al*. 2000a) appears almost identical but differences in measuring digestibility, particularly in the composition of the reference diet, as well as differences between the species may account for the discrepancy.

The most interesting finding in the present study is the difference in the effect of extrusion on peas compared with lupins. Both peas and lupins were improved with dehulling. Digestibility coefficients for dry matter and energy increased between 8-13% for both ingredients, with results confirming earlier research with silver perch described in detail in Booth *et al*. (2001).

However, while extrusion processing led to improvements in digestibility of peas of between about 10-15% for dry matter and energy and between 3-7% for protein, extrusion had little affect or actually reduced the dry matter, organic matter and energy digestibility of lupins.

The predominant carbohydrate in field peas is starch while lupins contain non-starch polysaccharides, chiefly galactose and xylose. Extrusion gelatinises the starch granules in field peas which effectively increases the surface area of the starch granules available for enzymatic attachment (Watanabe *et al*. 1993). As silver perch are efficient at digesting starch (Stone *et al*. in press), this greatly improves dry matter and energy digestibility. (Gouveia & Davies 2000) found European sea bass were able to digest carbohydrate from diets with up to 30% pea seed meal very efficiently, and attributed this partly to the beneficial effect of extrusion on starch gelatinisation. Carter & Hauler (2000) also commented that the high energy digestibility of pea starch in diets for Atlantic salmon was due to extrusion.

Field peas also contain trypsin inhibitors (Petterson & Mackintosh 1994; Saini 1995) which are deactivated by extrusion (Watanabe *et al*. 1993; Krogdahl, Berg & Olli 1994) and this may help explain the modest improvement in protein digestibility following extrusion. The improvement following dehulling for both cooked and raw peas suggests that the hulls may contain significant amounts of trypsin inhibitors.

By contrast, lupins contain negligible amounts of trypsin inhibitors (Petterson & Mackintosh 1994; Saini 1995) and carbohydrates are predominantly non-starch polysaccharides with little or no starch. Improvements resulting from increasing starch gelatinisation are not expected for fish. Higuera *et al*. (1988) specifically advised against "thermal treatment" of lupin seed meal, reporting a reduction in protein digestibility when lupin seed meal was heated. Those authors also reported a decline in food intake of trout fed diets containing pre-heated lupins compared with those fed diets containing crude lupin. Contrasting results were reported by Bangoula *et al*. (1993) who found that extrusion increased the utilization of components of white lupin (*Lupinus albus*) fed to rainbow trout. Cooking lupins has been reported to improve feeding efficiency in broiler chickens (Molina, Sanz, Boza & Aquilera 1983).

In the present study, digestibility coefficients for dry matter and energy were reduced by between 0-6% for extruded lupins compared with raw lupins while protein digestibility was relatively stable. These minor decreases may just indicate that heating had little effect and may not reflect biologically meaningful trends. Given the design of the feeding regime, we used in the present study, any results cannot be attributed to reduction in feed intake caused by cooking lupins. Higuera *et al*. (1988) speculated that heating lupins might have increased digestibility of galactans leading to an accumulation in the liver, hypoglycaemia and reduced fish health. It is unlikely that increased digestibility of galactans occurred in the present study; if it had, dry matter digestibility

might have been expected to have increased following extrusion rather than decline slightly. However, it is possible that alterations in carbohydrate chemistry following extrusion has a negative impact on lupin digestibility. These effects are worth exploring in longer-term studies.

Results from the second experiment also demonstrated marked differences between ingredients. In that experiment, digestibility values were much lower for canola than soybean meal for all indices regardless of cooking or inclusion content. Digestibility values for dry matter, energy and protein were all similar to those reported for rainbow trout by Hilton & Slinger (1986). Extruding canola reduced digestibility by around 12% points for dry matter and energy regardless of inclusion content. In contrast, extrusion increased the digestibility of dry matter and energy of soybean meal (by between 3-14% points).

Soybean meal and other soybean products can have negative effects on feed intake, digestibility and nutrient absorption (Kissil *et al*. 2000; Refstie *et al*. 2001; Olli & Krogdahl 1995). For some species effects are either not noticeable or far less of a problem (e.g. Channel catfish – Lim *et al*. 1998; Satoh, Porn-Ngam, Akimoto, Takeuchi & Watanabe 1997).

The factors which may cause negative effects with soybean include trypsin inhibitors, agglutinating lectins, soybean isoflavones, antigenic storage proteins, phytic acid and as yet unidentified factors that cause "distal enteritis" in salmonids (Refstie *et al*. 2001). Clearly, the effects of these are species dependent and some species such as channel catfish are able to digest and utilise soybean meal based diets without any problems (Lim *et al*. 1998). (Kissil *et al*. 2000) found reduced performance of gilthead sea bream when soybean meal protein concentrates replaced 30% of fishmeal, and attributed this to reduced feed intake, although (Robaina *et al*. 1995) found no impacts on feed intake or growth when soybean meal replaced 30% of the fishmeal in diets for the same species. Similar differences have been recorded for Atlantic salmon when soybean meal was included in diets (see Refstie *et al*. 2001).

Previous studies where impacts of extrusion on oilseeds were investigated concluded that extrusion had overall beneficial impacts (Satoh *et al*. 1998; Burel *et al*. 2000a; Burel *et al*. 2000b). Our results for soybean meal support these conclusions. Extrusion significantly improved digestibility of dry matter, organic matter and energy. Satoh *et al*. (1998) suggested improvements were due, at least in part, to increased digestibility (particularly of energy) and reduction in the phytic acid content.

The results with canola in our study were therefore surprising. Satoh *et al*. (1998) found that when commercial solvent extracted canola meal was used to replace 15 or 30% of the herring meal in a control diet for Chinook salmon, growth and feed intake were markedly reduced. In contrast, when canola meal was extruded either at low temperature (100°C) or higher temperature (150°C), canola meal substitution elicited no negative effects. Satoh *et al*. (1998) suggested that extrusion improved energy digestibility and also demonstrated reduced phytic acid content (by 20%). Higgs *et al*. (1995) described sequential processing techniques used to improve the quality of canola meal on protein concentration in diets for salmonids. Subjecting dehulled seeds to boiling water deactivates myrosinase, the enzyme responsible for glucosinolate hydrolysis. As it is likely that extrusion would have a similar beneficial effect, glucosinolate toxicity would not appear to be the cause of poor digestibility of canola meal in our study and does not account for reduction in digestibility following extrusion. Burel *et al*. (2000b) also recommended heat treatment to improve digestibility of rapeseed meal, however, inspection of their results indicates that extrusion tended to improve digestibility of dry matter, energy and protein for turbot but did slightly reduce digestibility for trout. The power of this experiment was low (partly because of low replication) but trends were similar to those reported in the present study with silver perch. Possible reasons for a reduction in digestibility following extrusion include a reduction in the protein digestibility because of heat induced denaturation and cross-linkage (Carpenter & Booth 1973). We found a significant

reduction in the protein digestibility of canola due to increased inclusion content (about 4%), but, this does not necessarily explain the larger 10% reduction in dry matter or energy digestibility. Other heat mediated impacts on carbohydrates may have rendered them less digestible and this warrants further investigation, as does the possibility of antagonistic interactions between canola and other ingredients.

In conclusion, dehulling was very beneficial for both lupins and field peas, confirming results of earlier studies. Protein from all ingredients was well digested although protein from lupins and soybean meal was better digested than protein from field peas or canola. Energy digestibility values were best for soybean meal followed by extruded dehulled peas, raw canola, extruded whole peas, raw dehulled peas and dehulled lupins. Extrusion delivered major benefits to digestibility of field peas, less but still significant benefits for soybean meal, no benefits for lupins and negative effects for canola. Where diets are formulated according to digestibility values, impacts of processing should be considered.

Acknowledgments

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Table 1.   Indicative formulation and premix composition of reference diet.

Ingredient	Amount in SP35 (% dry basis)	* Vitamin premix	IU	mg kg-1	** Mineral premix	g kg-1
Fish meal	262.0	Retinol (A)	8000		Calcium carbonate	7.5
Soybean meal	202.0	Cholecalciferol (D3)	1000		Manganese sulphate monohydrate	0.3
Blood meal	20.0	dl-α-tocopherol acetate (E)	125		Zinc sulphate monohydrate	0.7
Corn gluten meal	39.0	Menadione sodium bisulphite (K3)		16.5	Copper sulphate pentahydrate	0.06
Wheat	287.0	Thiamine hydrochloride (B1)		10.0	Ferrous sulphate heptahydrate	0.5
Sorghum	112.0	Riboflavin (B2)		25.2	Sodium chloride	7.5
Millrun	34.0	Pyridoxine hydrochloride (B6)		15.0	Potassium iodate	0.002
Cod liver oil	9.0	Folic acid		4		
Vitamin premix*	8.0	Ascorbic acid (C)		1000		
Mineral premix **	8.0	Calcium D-Pantothenate		55		
Di-calcium phosphate	18.0	Myo-inositol		600		
DL-methionine	10.0	d-Biotin (H) (2%)		1		
		Choline chloride		1500		
		Nicotinamide		200		
		Cyanocobalamin (B12)		0.02		
		Ethoxyquin (anti-oxidant)		150		
		Calcium propionate (mould inhibitor)		25		

Table 2.   Analytically measured composition of legume ingredients and experimental diets containing test ingredients at the 50% inclusion level (dry basis).

	Reference diet	Peas raw whole	Peas raw dehull	Peas ext whole	Peas ext dehull	Lupin Raw whole	Lupin raw ehull	Lupin ext whole	Lupin ext dehull
Test ingredient composition									
Protein %	35.8	25.8	26.2	25.7	27.4	31.0	44.4	34.4	41.7
Fat %	5.1	1.2	1.6	0.5	0.7	6.3	7.8	5.8	6.4
Ash %	15.8	3.3	2.8	3.4	2.8	2.6	2.8	2.7	2.8
Gross Energy MJ Kg^{-1}	18.1	18.1	18.5	18.4	18.5	19.8	20.7	20.1	20.6
Phosphorus %	1.4	0.5	0.4	0.5	0.4	0.3	0.3	0.3	0.3
Test diet composition									
Protein %		29.9	31.8	31.9	30.3	34.3	38.2	33.5	38.1
Fat %		2.3	1.7	1.6	1.7	2.9	4.3	5.1	3.6
Ash %		9.5	9.3	9.6	9.2	9.2	9.3	9.5	9.3
Gross Energy MJ kg^{-1}		18.1	18.4	18.2	17.5	18.9	19.2	18.7	19.3
Phosphorous %		0.9	1.0	1.0	1.0	0.9	0.9	0.9	0.9

Table 3.   Analytically measured composition of oilseed ingredients and experimental diets containing test oilseeds at the 30 and 50% inclusion level (dry basis).

	Reference diet	Canola raw	Canola raw	Canola ext	Canola ext	Soy raw	Soy raw	Soy ext	Soy ext
Inclusion content %	100	30	50	30	50	30	50	30	50
Test ingredient composition									
Protein %	39.4	39.3	-	38.7	-	54.3	-	53.9	-
Fat %	3.8	4.3	-	3.8	-	1.6	-	1.0	-
Ash %	15.5	6.8	-	6.5	-	6.7	-	6.8	-
Gross Energy MJ kg^{-1}	18.0	19.9	-	19.9	-	19.5	-	19.6	-
Test diet composition									
Protein %		39.8	39.3	39.3	39.3	43.9	46.3	44.7	48.9
Fat %		3.2	3.3	4.3	4.6	3.2	2.4	2.7	1.4
Ash %		12.4	10.8	12.5	11.1	13.0	11.2	12.7	11.2
Gross Energy MJ kg^{-1}		18.6	18.9	18.5	18.9	18.5	18.9	18.5	18.8

Table 4. Mean ± (sem) apparent digestibility coefficients (ADC) of test diets and individual ingredients fed to silver perch at the 50% inclusion level (dry basis).

	Reference	Peas raw whole	Peas raw dehull	Peas ext whole	Peas ext dehull	Lupin Raw whole	Lupin raw ehull	Lupin ext whole	Lupin ext dehull
Dietary ADC %									
Dry matter	59.36	57.31	63.69	63.81	67.84	51.20	57.87	50.41	53.72
	(0.99)	(0.50)	(0.48)	(1.53)	(0.48)	(0.82)	(0.08)	(2.06)	(0.61)
Organic matter	68.15	61.07	67.89	68.53	72.54	54.73	61.55	54.54	57.81
	(0.82)	(0.57)	(0.44)	(1.21)	(0.56)	(0.76)	(0.11)	(1.99)	(0.51)
Protein	87.52	84.29	87.82	85.28	90.44	89.03	90.54	88.93	89.76
	(0.18)	(0.44)	(0.16)	(0.24)	(0.30)	(0.56)	(0.18)	(0.52)	(0.24)
Gross energy	72.68	63.71	70.14	71.03	74.96	61.84	67.45	61.27	66.26
	(1.93)	(0.57)	(0.37)	(1.00)	(0.60)	(1.59)	(0.11)	(1.88)	(0.53)
Ingredient ADC %									
Dry matter	-	56.43	69.31	69.56	77.69	44.07	57.56	42.47	49.17
		(1.01)	(0.97)	(3.10)	(0.98)	(1.65)	(0.17)	(4.17)	(1.23)
Organic matter	-	56.06	68.93	70.15	77.70	44.16	56.98	40.06[†]	49.93
		(1.08)	(0.82)	(2.28)	(1.05)	(1.43)	(0.21)	(0.76)	(0.97)
Protein	-	81.85	90.33	84.23	96.36	92.71	94.64	92.23	93.38
		(1.06)	(0.38)	(0.59)	(0.66)	(1.21)	(0.32)	(1.06)	(0.45)
Gross energy	-	56.12	69.05	70.85	78.67	53.15	64.17	52.17	61.90
		(1.12)	(0.75)	(1.99)	(1.16)	(3.07)	(0.21)	(3.62)	(0.99)

[†] Denotes mean ± (sem) values for two replicates after removal of extreme outlier.

Table 5. Mean ± (sem) apparent digestibility coefficients (ADC) of test diets and oilseed ingredients fed to silver perch at the 30 and 50% inclusion level (dry basis).

	Reference	Canola raw	Canola raw	Canola ext	Canola ext	Soy raw	Soy raw	Soy ext	Soy ext
Inclusion content %	100	30	50	30	50	30	50	30	50
Dietary ADC									
Dry matter %	64.34	62.97	62.05	59.49	55.82	67.89	71.13	71.62	73.73
	(1.02)	(0.16)	(1.99)	(0.91)	(0.91)	(0.67)	(0.27)	(0.71)	(0.47)
Organic matter %	72.52	70.20	70.07	66.10	63.36	75.02	78.28	77.34	79.66
	(1.06)	(0.22)	(1.61)	(0.19)	(0.66)	(0.51)	(0.13)	(0.67)	(0.30)
Protein %	89.21	88.49	87.26	87.77	85.50	91.49	93.00	91.04	94.40
	(0.59)	(0.13)	(1.20)	(0.19)	(0.68)	(0.07)	(0.25)	(1.57)	(0.10)
Gross Energy %	76.29	73.75	73.78	69.62	67.68	78.34	81.35	80.98	82.77
	(0.80)	(0.32)	(1.37)	(0.27)	(0.56)	(0.47)	(0.10)	(0.66)	(0.22)
Ingredient ADC									
Dry matter %	-	61.90	61.01	50.19	48.42	78.47	79.36	91.01	84.61
		(0.55)	(4.02)	(0.65)	(1.85)	(2.26)	(0.54)	(2.40)	(0.96)
Organic matter %	-	67.48	69.20	54.56	56.28	82.69	85.03	89.98	87.68
		(0.70)	(3.11)	(0.60)	(1.28)	(1.62)	(0.26)	(2.09)	(0.59)
Protein %	-	89.79	84.78[†]	87.33	83.47	97.84	97.51	98.63[†]	99.85
		(0.45)	(1.40)	(0.67)	(1.40)	(0.19)	(0.43)	(1.37)	(0.17)
Gross Energy %	-	70.68	72.93	57.70	61.18	85.24	87.60	93.57	90.32
		(1.02)	(2.64)	(0.85)	(1.07)	(1.51)	(0.19)	(2.11)	(0.44)

[†] Denotes mean ± (sem) values for two replicates after removal of extreme outlier.

4.3.   Utilisation of digestible nitrogen and energy from four agricultural ingredients by juvenile silver perch *Bidyanus bidyanus* 1

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Abstract

A comparative slaughter, growth assay was conducted using juvenile silver perch to evaluate different inclusion contents of peanut meal, canola meal, meat meal and dehulled field peas. Each ingredient was combined with a nutritionally balanced basal diet composed mainly of fishmeal (27%), soybean meal (21%), wheat (28%) and sorghum (11%) such that between 15 and 75% of the basal diet was wholly replaced by the test ingredient. In addition, the basal diet was replaced with 15, 30 or 45% of an inert filler (diatomaceous earth) in order to compare diets containing test ingredients and the inert filler. Fish were fed respective test diets under a slightly restricted feeding regime (90% apparent satiation), twice a day for 56 days. Weight gain of silver perch decreased steadily as the basal diet was systematically replaced with diatomaceous earth, confirming the limiting contribution to weight gain from the basal diet under a restricted feeding regime. Silver perch fed diets containing a mixture of the basal diet and either peanut meal, meat meal, canola meal or up to 60% field peas gained more weight than fish fed diets containing similar contents of the inert filler, indicating silver perch were able to utilise these ingredients to support growth. Regression analysis was applied to investigate protein and energy retention and models were fitted with 95% confidence and prediction intervals. Inspection of these relationships indicated various outliers which greatly affected the fitted models. We postulate that these outliers represent test diets which contain ingredients that are poorly utilised, or poorly utilised at particular inclusion contents. Removal of these outliers greatly improved the fit of each model. Using this approach, the partial efficiency of digestible protein for growth above maintenance was constant at 0.45 after diets containing more than 45% of peanut meal and 75% of field peas were removed from the fitted model. The partial efficiency of digestible energy for growth above maintenance was constant at 0.68 after diets containing 75% of field peas and 75% of canola were removed from the fitted model. Adherence of other diets containing test ingredients to the slope of each regression suggests that silver perch are capable of utilising any of the protein sources tested at all but the inclusion contents described above. Confirmation of this approach under different feeding regimes is required.

Introduction

Silver perch *Bidyanus bidyanus,* is an omnivorous Australian freshwater species that is capable of efficiently digesting a wide range of intact protein and energy sources (Allan *et al.* 1999; Allan *et al.* 2000a; Booth *et al.* 2001). Digestibility for many of these ingredients is to be additive, which has allowed the least-cost formulation of commercial diets based on the protein and energy composition of ingredients and their respective digestibility coefficients (Allan *et al.* 2000b). The equitable performance of silver perch reared on diets containing meat meal, cereal grains, legumes or oilseeds, compared to fish fed on diets containing significant quantities of fishmeal,

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1 This section was also included in Final Report 1996/392 "Aquaculture Diet Development Subprogram: Nutrient Requirements of Aquaculture Species" as the work is relevant to objectives of both reports.

demonstrates this species is also able to utilise the protein and energy from these ingredients (Allan *et al.* 2000c; Booth *et al.* 2000). This apparent ability to readily utilise ingredients indicates that high levels of replacement may be possible, but, to recommend upper inclusion contents for particular ingredients, ingredient utilisation under increasing inclusion contents must be measured.

The use of regression analysis to explore the relationships between nutrient intake and nutrient retention in fish has become more prevalent in aquaculture research (e.g. Lupatsch *et al.* 1998; Lupatsch *et al.* 2001a & b; Rodehutschord & Pfeffer 1999; Shearer 2000). Regression analysis is typically applied to data generated from dose-response experiments. The resulting relationships or models are usually used to estimate optimal nutrient requirements based on a maximum response in the variable of interest (Mercer 1982; Shearer 2000). Regression can also be used to estimate nutrient retention efficiency by evaluating the slope produced from the regression of nutrient intake data against nutrient accretion data (Rodehutschord & Pfeffer 1999; Lupatsch *et al.* 1998, 2001a & b). Data points may fit linear or non-linear models (Hepher 1988; Shearer 2000) and points which depart or deviate from the resultant slope may indicate that the nutrient of interest is being poorly utilised. If this departure can be linked to the source of the nutrient (i.e. the ingredient), then it may provide some biological measure of the suitability of the ingredient in meeting the nutritional needs of the animal at both low and high levels of inclusion.

In this paper, we relate the retention of dietary protein and energy by juvenile silver perch to increasing contents of peanut meal, canola meal, dehulled field peas, meat meal or diatomaceous earth (inert filler). This experiment was designed to investigate the effects of increasing the content of each of the aforementioned ingredients at the expense of a balanced reference diet (Allan & Rowland 1992). As such, the practical measure of utilisation was expected to be shown by the difference in weight gain between fish fed a mix of the reference diet and a specific ingredient and the reference diet and the same content of the inert filler. Direct substitution of the reference diet with the test proteins inevitably resulted in a wide variation in the digestible protein (DP) and digestible energy (DE) contents of respective diets, effectively subjecting silver perch to a dose-response situation. Therefore, by evaluating the growth of fish in this experiment with respect to variations in ingredient content and the estimated digestible protein and energy content of test diets, we aimed to gain a greater insight into the overall response of silver perch to ingredient substitution and nutrient utilisation.

Materials and Method

Diets

A 56 day growth experiment was performed in order to evaluate different inclusion contents of commercial grades of peanut meal, canola meal, a premium grade meat meal (Ridley Aquafeeds Pty., Ltd., Australia) and dehulled field peas *Pisum sativum*. Each ingredient was systematically combined with a nutritionally balanced basal diet (SP35; Table 1; Allan & Rowland 1992; Allan *et al*. 1999) composed mainly of fishmeal (27%), soybean meal (21%), wheat (28%) and sorghum (11%) such that between 15 and 75% of the basal diet was wholly replaced by the test ingredient. In addition, the basal diet was systematically replaced with 15, 30 and 45% of diatomaceous earth (Melcann Ltd., West Footscray, VIC, Australia) (Tables 1 & 2). In total, 24 diets were manufactured for this experiment. The basal diet mixture was sourced as a mash (Janos Hoey Pty., Ltd., Forbes, NSW, Australia), and did not contain added vitamins or minerals. All test diets and the basal diet mixture were later fortified with 7.5 g kg^{-1} (dry basis) of a formulated vitamin/mineral pre-mix (Table 1). Diets were prepared by dry mixing the basal diet mixture, vitamins and minerals and test ingredients in their selected ratios before passing them through a laboratory scale hammer mill (Raymond Laboratory Mill, Transfield Technologies, Rydalmere, Australia) fitted with a 1.5 mm screen. Diets were then thoroughly re-mixed (Hobart Mixer: Troy Pty Ltd, OH, USA) and combined with distilled water before being cold pelleted through a meat

mincer fitted with a 2.0 mm pellet die (Barnco Australia Pty Ltd, Leichhardt, NSW, Australia). Wet diets were dried in a convection drier (< 35^0C) for about six hours or until moisture contents were < 100 g kg^{-1}. Following preparation, all diets were stored at < $^-$15ºC until required.

Fish and feeding strategy

Silver perch *Bidyanus bidyanus* were bred at the NSW Fisheries Grafton Research Centre and raised in earthen ponds using similar techniques to those described by Thurstan & Rowland (1994) before being transported to Port Stephens Fisheries Centre (PSFC). Before experiments, silver perch were fed exclusively on SP35 and periodically treated with 5-10 g l^{-1} NaCl to ensure they were free of ectoparasites and to prevent fungal infection (Rowland & Ingram 1991). Fish were anaesthetised using a bath of ethyl ρ-aminobenzoate (20-30 mg l^{-1}), then caught at random, weighed individually then systematically distributed to 70 l experiment tanks. Eight fish (3.01 g ± 0.01; mean ± sem) were stocked into each aquaria with three aquaria randomly assigned to each dietary treatment. A representative selection of fish from the same pool were killed and frozen for initial chemical analysis. After stocking, fish were acclimatised to their respective test diets for 12 days during which time they were fed twice daily (0830 and 1500 h) to establish apparent satiation levels. After the acclimatisation phase, fish from individual aquaria were sedated and bulk weighed and then fish were switched to a slightly restricted feeding regime based on 90% of their current biomass (Jobling 1983). Fish were fed a slightly restricted ration to overcome perceived palatability problems and prevent fish increasing feed intake in response to any nutritional deficiencies. Fish were subsequently sedated and weighed every 14 days thereafter to adjust their feeding rations. When fish failed to consume their total ration on consecutive days, feed inputs were reduced accordingly. Any uneaten feed was collected from each tank after 20 – 30 min, dried to a constant weight and subtracted from the total feed input for that tank at the conclusion of the experiment. Fish which died during the experiment were weighed and replaced with weighed, fin clipped fish to maintain the stocking density of each aquaria. Fin clipped fish were identified at the end of the experiment and excluded from calculations involving weight gain and nutrient deposition. At the completion of the experiment, a representative selection of fish was removed from each aquaria, killed with an overdose of ethyl ρ-aminobenzoate and frozen (< $^-$15° C) for chemical analysis.

Laboratory facilities

Individual aquaria were supplied with continuously-flowing (400 – 500 ml min^{-1}), temperature controlled water. Before reaching experiment tanks, re-circulated water was filtered through a rapid sand filter and a cartridge filter (nominal pore size 10 μm), then passed through a 2 m^3 trickling bio-filter and an ultra-violet steriliser (Vf-9 Big Blue, Australian Ultra-Violet Products Proprietary. Limited., Seven Hills, NSW, Australia). Approximately 25% of water was exchanged each day. Each tank was covered with a clear perspex lid and aerated with two air-stone diffusers. Fluorescent lighting was automatically controlled to provide a 12 h light (0600 h to 1800 h) and 12 h dark photo-period.

Water temperature (range 23.4 to 27.1°C), dissolved oxygen (range 4.6 to 8.0 mg l^{-1}) and pH (between 6.9 and 8.5) were monitored regularly with a Model 611-Intelligent Water Quality Analyser (Yeo-Kal Electronics Proprietary Ltd, Brookvale, NSW, Australia). Colourimetric methods were adopted to measure total ammonia nitrogen (Dal Pont, Hogan & Newell 1973) and nitrite (Major, Dal Pont, Kyle & Newell 1972) once per week from 18-20 experiment tanks. Total ammonia-N remained below 600 μg l^{-1} and N0$_2$-N remained below 200 μg l^{-1} during the course of the trial.

Chemical analyses

Chemical analyses (excluding gross energy) of feeds and whole fish were performed by the State Chemistry Laboratory (SCL) (Victoria Agriculture, Werribee, Australia). Nitrogen was determined from an adaptation of the standard Kjeldahl method (AOAC 1995) using an automated Tecator distillation apparatus. Fat was extracted from samples with diethyl ether in a continuous extraction procedure using an automated Soxtherm apparatus (Gerhardt) after which oven dried residue was weighed to calculate crude fat (AOAC 1995). Moisture and ash for feed samples were determined according to AOAC (1995), however, ash values for carcass samples were determined by difference. Gross energy analysis (bomb calorimetry) on feeds and whole fish were performed by the South Australian Research and Development Institute (SARDI) on sub-samples drawn from those prepared by SCL (Tables 2 & 3).

Calculations

Silver perch fed the reference diet exhibited exponential growth with respect to time (weight (g) = $2.92^{0.0265*day}$; $R^2 = 0.99$). As such, body weight (BW) was calculated as the geometric mean according to the formula presented in Lupatsch *et al.* (2001b) where BW = (initial weight x final weight)$^{0.5}$. To our knowledge, the metabolic weights of protein and energy have not been determined for silver perch. Therefore, an iterative approach using linear regression analysis, similar to that presented in Rodehutscord and Pfeffer (1999), was used to estimate a weight exponent to which BW could be referred. Using this approach, a linear model based on BW raised to the power of 0.6 explained more of the variability in the data for each nutrient than models where BW was standardised by raising data to a power of 0.8 or 1.0, respectively (Table 4). Subsequently, intake and nutrient accretion data were standardised by referring original data values to the metabolic body weight of 0.6 (e.g. g kgBW$^{-0.6}$ d^{-1} or kJ kgBW$^{-0.6}$ d^{-1}). Unless otherwise stated, all data displayed in figures represents the average value of three replicate tanks. Digestible nutrient values were calculated using the analytically measured nutrient contents of test ingredients and the reference diet and the apparent digestibility coefficients (ADC's) for these components which were previously determined for silver perch; ADC's are based on data for 30% dietary substitutions (Allan *et al*. 1999 & 2000; Booth *et al*. 2001).

Curve fitting

After preliminary evaluation of the data sets, the relationship between dietary DP content and protein deposition was fitted with a 2nd order polynomial regression. The relationship between DP intake and protein deposition was adequately described by a simple linear regression. Both models were fitted with 95% confidence and prediction intervals. Inspection of these figures indicated various outliers which greatly affected the fitted models. Our premise is, that these outliers represent test diets which contain ingredients that are either poorly utilised or are poorly utilised at particular inclusion contents. Thus, exclusion of these outliers improves (reduces) the error term associated with each model, subsequently improving the R^2 statistic. Only data points which fell outside the 95% prediction intervals were excluded from respective models. Similar procedures were applied to investigate the relationship between DE intake and energy deposition in silver perch. In this case, the relationship was best described by a simple linear regression. All regressions and statistical analysis were performed using *Statgraphics Plus V4.1* (Manugistics® Incorporated, Rockville, MD, USA).

Results

There was an inverse relationship between the whole body moisture and whole body fat content of fish. In addition, the fat content of whole fish carcass appeared to be affected by the lipid content of the respective dietary treatments, and increased as dietary lipid content approached 6% (Table 3). Comparative performance indices for silver perch reared on test diets are presented in Table 5.

Two data point outliers which corresponded to diets which contained 75% each of field peas or canola meal were removed from the model which described the relationship between DP content of test diets and protein deposition (Figure 1). Removal of these points increased the adjusted R^2 statistic for this particular relationship from 0.69 to 0.84 with ANOVA indicating a highly significant relationship between the variables ($F = 55.4$; $P < 0.0001$; Equation 1). Equation 1 was used to predict the dietary DP content that gave maximum protein deposition. This value was 41.1%.

Protein deposition (g kgBW$^{-0.6}$ d^{-1}) = -0.00126x^2 + 0.1037x – 1.587   **(Equation 1)**

The relationship between DP intake and protein deposition was linear and indicated that protein deposition was not limited by DP intake in this study (Figure 2). In this case, four outliers were excluded from the full data set corresponding to diets which contained 45, 60 or 75% peanut meal and those diets containing 75% field peas. Exclusion of these points improved the R^2 statistic from 0.71 to 0.89 with ANOVA indicating a highly significant relationship between the variables ($F = 106.5$; $P < 0.0001$; Equation 2).

Protein deposition (g kgBW$^{-0.6}$ d^{-1}) = 0.452x – 0.276   **(Equation 2)**

An estimate of the protein requirement for maintenance (zero protein deposition) was made by extrapolation of Equation 2 to the x axis. This requirement was found to be 0.61g DP kgBW$^{-0.6}$ d^{-1}. Based on this equality, the partial efficiency of DP for growth above maintenance was constant and determined as 0.45.

The relationship between DE intake and energy deposition was linear and was improved by removal of two outliers corresponding to diets which contained 75% field peas and 75% peanut meal (Figure 3). This improved the R^2 statistic from 0.76 to 0.92 with ANOVA indicating a highly significant relationship between the variables ($F = 224.0$; $P < 0.0001$; Equation 3). Energy deposition was not limited by DE intake within the range investigated in this study. Energy requirements for maintenance in this study were estimated to be approximately 37.76 kJ kgBW$^{-0.6}$ d^{-1} while the partial efficiency of DE for growth above maintenance was found to be 0.68 (Equation 3).

Energy deposition (kJ kgBW$^{-0.6}$ d^{-1}) = 0.682x – 25.096   **(Equation 3)**

Discussion

In this study, weight gain of silver perch decreased steadily as the basal diet was systematically replaced with higher contents of diatomaceous earth, confirming the limiting contribution to weight gain from the basal diet under a restricted feeding regime. Silver perch fed diets containing a mixture of the basal diet and either peanut meal, meat meal, field peas (excluding the 75% inclusion level) or canola meal gained more weight under this protocol than fish fed diets containing similar contents of diatomaceous earth, indicating that silver perch were able to utilise these particular agricultural ingredients to support growth (protein deposition).

Appraisal under a nutrient-response relationship identified that the increase in weight gain of silver perch in this study may have been strongly related to the intake of dietary protein or energy. Generally, the relationships between intake and physical accretion of ingested nutrients are thought to be graded and systematic in nature (Mercer 1982; Shearer 2000). The approach taken in this paper depends on the premise that, like other fish species, silver perch probably exhibit a predetermined, biologically driven pattern of growth. This pattern will, however, be affected by environmental and nutritional factors acting on the fish. Such a concept is discussed in a review by Bureau *et al.* (2000). The pattern of growth is therefore a "plastic one", able to respond to the

positive and negative factors impacting on it (Brett 1979; Bureau *et al*. 2000). In our study, we rely on the premise that although growth may respond to these factors, there is an underlying pattern of growth that is identifiable and relatively stable. This pattern can then be used to identify and exclude diets (i.e. in our case = ingredients) which result in departures from the "normal" modelled response. Secondly, after removal of outlying data points which belong to diets that we suggest are poorly utilised, the modelled response should be comparable to those presented for silver perch or other, similar species.

This assumption was first checked by comparing the response of silver perch to increasing levels of dietary DP in our trial to those of an earlier experiment with fish of similar size (Allan *et al.* 2001). Optimum DP requirements for silver perch were previously determined using intersecting linear regression analysis after fish were fed diets in which DP varied between 9.5 and 40.4% and DE was held constant (13.4 – 15 MJ kg^{-1} diet; Allan *et al*. 2001). The optimum dietary DP (that content after which further increases in weight gain with increasing DP were not significant) was estimated as 28%. By re-analysing their original data set with a 2nd order polynomial regression, the dietary DP content which was predicted to give maximum protein deposition was 38.6%. This value is similar but slightly lower to 41.1% determined in this study. The possibility that we may have overestimated the DP and DE content of diets with high inclusion contents of the test ingredients in the present study cannot be discounted, as we used values based on apparent digestibility coefficients determined for 30% inclusion contents.

Comparisons with published values for the utilisation efficiency of DP and DE can also be made. We determined that values for the coefficients of utilisation for DP and DE are constant (linear) at 0.45 and 0.68, respectively. An indication of the coefficient of utilisation for DP from the linear portion of the data presented in Allan *et al*. (2001) was found to be 0.34. For species such as channel catfish, the utilisation coefficient for crude protein derived from a series of purified diets averaged about 0.26 (Gatlin III *et al*. 1986). After interpretation of the linear portion of data presented in Jauncey (1982), the coefficient of utilisation for crude protein (white fish meal) in juvenile tilapia *Sarotherodon mossambicus* of similar size to silver perch used in our study was found to be 0.40, while the utlisation coefficient of metabolisable energy for growth above maintenance in tilapia *Oreochromis niloticus* was found to be about 0.67 (Meyer-Burgdorff *et al*. 1989). In separate studies, Lupatsch *et al*. (1988 and 2001b) found that utilisation of DE for growth above maintenance in gilthead seabream *Sparus aurata* was linear and ranged between 0.46 and 0.50 regardless of energy intake. In the same studies, utilisation of DP for growth above maintenance ranged between 0.28 (linear response) and an optimum of 0.47 (exponential response) (Lupatsch *et al*. 1988 and 2001b). Azevedo *et al*. (1998) and Rodehutscord & Pfeffer (1999) determined that the utilisation of DE for growth above maintenance in rainbow trout *Oncorhynchus mykiss* was 0.61 and 0.68, respectively. Similarly utilisation of DE and DP for growth above maintenance in European seabass *Dicentrarchus labrax* were linear at 0.68 and 0.52, respectively (Lupatsch *et al*. 2001a). The close agreement of our values to those presented by the aforementioned authors would appear to indicate that the responses seen in this study to nutrient density and nutrient intake are both systematic and realistic.

Protein utilisation of the diets tested in this study was constant at 0.45 after all diets which contained more than 45% of peanut meal and 75% of field peas were excluded from the data set (Figure 2). Energy utilisation was constant at 0.68 after diets containing 75% of field peas and canola meal were removed from the data set. Adherence of other diets to the slope produced by each of these regressions suggests that silver perch are capable of utilising any of the protein sources tested in this study at all but the inclusion contents just described. Silver perch also exhibit a high apparent protein digestibility for the ingredients tested in this trial, with values for peanut meal, meat meal, field peas and canola of 98, 71, 88 and 83% respectively (Allan *et al*. 2000a; Booth *et al*. 2001). A high coefficient of digestibility may therefore be indicative of the ability of silver perch to utilise protein from these ingredients. Grow-out trials have further demonstrated the ability of silver perch to readily utilise diets which contain a variety of agricultural protein sources at various inclusion contents. Ingredients used include meat meal, corn gluten, soybean meal,

canola meal, peanut meal, lupins, wheat, sorghum and millrun (Stone *et al.* 2000; Allan *et al.* 2000b & c). These diets were generally formulated to DP and DE densities of 35% and 14MJ kg^{-1}, respectively, with dietary contents of close to 40% meat meal and 30% wheat promoting the same weight gains as that of a commercial silver perch control diet (Allan *et al.* 2000c). While a practical formulation for an aquaculture diet would be unlikely to contain more than 40 or 50% of any ingredient, other than fish meal, the fact that silver perch are capable of utilising locally available ingredients at high dietary inclusion contents gives feed manufacturers greater flexibility with their formulations.

Feeding fish either a satiation or *ad-libitum* ration may have changed the outcome of the approach used in this study, possibly reducing the efficiency of utilisation; assuming that maintenance costs remained similar. Feeding below satiation may also mask palatability or intake effects related to particular ingredients, especially as inclusion contents increase (Lupatsch *et al*. 2001b). These issues should be investigated as the value of an ingredient is determined by its influence on both nutrient utilisation and intake. Formulating diets for silver perch with balanced DP and DE densities but with varying amounts of the ingredients tested in the present study (excluding peanut meal above 30%, canola and field peas above 60%) should produce similar weight gains, at least for juvenile fish, provided no other nutrients are deficient.

Acknowledgments

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Table 1. Indicative composition of commercial silver perch diet (SP35).

Ingredient	Amount in SP35 (g kg-1 dry basis)	* Vitamin premix	IU	mg/kg	** Mineral premix	g/kg
Fish meal	26.2	Retinol (A)	8000		Calcium carbonate	7.5
Soybean meal	20.2	Cholecalciferol (D3)	1000		Manganese sulphate monohydrate	0.3
Blood meal	2.0	dl-α-tocopherol acetate (E)	125		Zinc sulphate monohydrate	0.7
Corn gluten meal	3.9	Menadione sodium bisulphite (K3)		16.5	Copper sulphate pentahydrate	0.06
Wheat	27.5	Thiamine hydrochloride (B1)		10.0	Ferrous sulphate heptahydrate	0.5
Sorghum	11.2	Riboflavin (B2)		25.2	Sodium chloride	7.5
Millrun	4.6	Pyridoxine hydrochloride (B6)		15.0	Potassium iodate	0.002
Cod liver oil	1.0	Folic acid		4		
Vitamin premix*	0.8	Ascorbic acid (C)		1000		
Mineral premix **	0.8	Calcium D-Pantothenate		55		
Di-calcium phosphate	1.8	Myo-inositol		600		
DL-methionine	0.1	d-Biotin (H) (2%)		1		
		Choline chloride		1500		
		Nicotinamide		200		
		Cyanocobalamin (B12)		0.02		
		Ethoxyquin (anti-oxidant)		150		
		Calcium propionate (mould inhibitor)		25		

Table 2. Measured composition (dry basis) of ingredients and estimated composition (dry basis) of experimental diets fed to juvenile silver perch. [56 days; *n* = 3 aquaria; 8 fish/aquaria].

		Protein[1] (%)	Energy (MJ kg^{-1})	Crude fat (%)	Ash (%)	DP[2] (%)	DE[3] (MJ kg^{-1})	DP/DE[4]
Ingredient								
Basal (SP35)		39.6	18.5	4.6	13.3	35.3	14.1	2.5
Peanut meal (solvent extracted)		54.7	21.0	7.2	5.0	53.7	16.2	3.3
Meat meal		54.3	17.2	10.2	31.9	40.1	13.9	2.9
Field pea (dehulled)		26.4	18.3	1.4	2.7	23.3	11.6	2.0
Canola meal (solvent extracted)		38.7	19.7	2.2	6.7	32.9	11.4	2.9
Diet[5]	*basal/ingredient ratio*							
Basal (SP35)	100/0	39.6	18.5	4.6	13.3	35.3	14.1	2.5
Peanut meal	85/15	41.9	18.8	5.0	12.1	38.0	14.4	
Peanut meal	70/30	44.1	19.2	5.4	10.8	40.8	14.7	
Peanut meal	55/45	46.4	19.6	5.8	9.6	43.6	15.0	
Peanut meal	40/60	48.7	20.0	6.2	8.3	46.3	15.3	
Peanut meal	25/75	50.9	20.4	6.6	7.1	49.1	15.7	
Meat meal	85/15	41.8	18.3	5.4	16.1	36.0	14.1	2.6
Meat meal	70/30	44.0	18.1	6.3	18.9	36.7	14.0	2.6
Meat meal	55/45	46.2	17.9	7.1	21.7	37.4	14.0	2.7
Meat meal	40/60	48.4	17.7	8.0	24.5	38.2	14.0	2.7
Meat meal	25/75	50.6	17.5	8.8	27.3	38.9	13.9	2.8
Field pea	85/15	37.6	18.4	4.1	11.7	33.5	13.7	2.5
Field pea	70/30	35.6	18.4	3.6	10.1	31.7	13.3	2.4
Field pea	55/45	33.7	18.4	3.2	8.5	29.9	13.0	2.3
Field pea	40/60	31.7	18.4	2.7	6.9	28.1	12.6	2.2
Field pea	25/75	29.7	18.4	2.2	5.4	26.3	12.2	2.2
Canola meal	85/15	39.5	18.6	4.2	12.3	34.9	13.7	2.6
Canola meal	70/30	39.3	18.8	3.9	11.3	34.5	13.3	2.6
Canola meal	55/45	39.2	19.0	3.5	10.3	34.2	12.9	2.7
Canola meal	40/60	39.1	19.2	3.2	9.3	33.8	12.5	2.7
Canola meal	25/75	38.9	19.4	2.8	8.4	33.5	12.1	2.8
Diatomaceous earth	85/15	33.7	15.7	3.9	26.3	30.0	12.0	2.5
Diatomaceous earth	70/30	27.7	12.9	3.2	39.3	24.7	9.9	2.5
Diatomaceous earth	55/45	21.8	10.1	2.5	52.3	19.4	7.7	2.5

[1] Crude protein = Nx6.25.

[2] Digestible protein (DP) = apparent nutrient digestion coefficient (Allan *et al*. 2000a; Booth *et al*. 2001) x tabulated nutrient content.

[3] Digestible energy (DE) = apparent energy digestion coefficient (Allan *et al*. 2000a; Booth *et al*. 2001 in press) x tabulated nutrient content.

[4] Digestible protein to digestible energy ratio.

[5] Calculated values based on the analytically measured nutrient contents for the basal and individual test ingredients.

Table 3. Final weight (FW), Moisture, crude protein (CP), gross energy (GE), fat and ash content (as received basis) of whole juvenile silver perch taken at the beginning and at completion of the feeding experiment [56 days; $n = 3$ aquaria; 8 fish/aquaria].

	basal/ingredient	FW (g)	Moisture (%)	CP (%)	GE (MJ kg⁻¹)	Crude fat (%)	Ash[1] (%)
Initial fish sample	-	(3.01)*	72.1	16.8	6.8	7.3	3.8
Basal (SP35)	100/0	12.2	71	16.6	7.0	7.9	4.5
Peanut meal	85/15	12.9	70.7	17.1	7.1	8.5	3.7
Peanut meal	70/30	13.2	71.2	16.3	7.1	8.7	3.8
Peanut meal	55/45	12.1	70.1	16.5	7.6	9.7	3.7
Peanut meal	40/60	9.7	71.1	17.1	7.2	8.7	3.1
Peanut meal	25/75	9.1	70.6	16.0	7.5	9.5	3.9
Meat meal	85/15	11.9	70.9	16.5	7.2	8.7	3.9
Meat meal	70/30	12.1	70.2	17.2	7.3	8.5	4.1
Meat meal	55/45	11.0	70.4	16.9	7.2	8.2	4.5
Meat meal	40/60	9.7	71.4	17.2	6.8	6.8	4.6
Meat meal	25/75	8.1	72.0	17.1	6.6	6.7	4.2
Field pea	85/15	11.1	72.6	16.4	6.6	6.9	4.1
Field pea	70/30	10.2	71.9	16.7	6.6	7.0	4.4
Field pea	55/45	9.5	70.9	16.6	7.3	8.7	3.8
Field pea	40/60	8.2	71.6	17.1	6.9	7.7	3.6
Field pea	25/75	4.5	72.5	16.4	5.9	6.1	5.0
Canola meal	85/15	11.3	71.0	17.0	6.9	7.4	4.6
Canola meal	70/30	10.6	71.6	17.8	6.7	6.7	3.9
Canola meal	55/45	10.0	73.3	17.3	6.3	6.1	3.3
Canola meal	40/60	9.2	73.5	17.9	6.2	5.4	3.2
Canola meal	25/75	8.7	74.2	16.6	6.1	5.0	4.2
Diatomaceous earth	85/15	10.2	72.0	17.1	6.6	7.1	3.8
Diatomaceous earth	70/30	6.7	71.2	17.7	6.7	6.5	4.6
Diatomaceous earth	55/45	4.3	71.8	17.7	6.1	5.2	5.3
Pooled sem	-	0.65	0.43	0.27	0.17	0.35	0.26

Each value represents the average composition of three replicate aquaria ($n = 3$). Values for each replicate aquaria. were determined from a homologous mix of randomly selected whole fish.

[1] Ash calculated by difference.

* Value for average initial weight (g) of all fish stocked in experiment.

Table 4. R^2 and $S_{y,x}$ for linear regressions describing protein deposition as a function of DP intake and energy deposition as a function of DE intake ($n = 72$).

Protein models	R^2	$S_{y,x}$
g kgBW$^{-1.0}$ d^{-1}	0.43	0.75
g kgBW$^{-0.8}$ d^{-1}	0.56	0.25
g kgBW$^{-0.6}$ d^{-1}	0.65	0.08
Energy models		
kJ kgBW$^{-1.0}$ d^{-1}	0.38	40.30
kJ kgBW$^{-0.8}$ d^{-1}	0.56	12.48
kJ kgBW$^{-0.6}$ d^{-1}	0.69	3.88

Table 5. Selected performance characteristics for silver perch fed on experimental diets [56 days; *n* = 3 aquaria; 8 fish/aquaria].

	S1 (%)	WG2 (g fish-1)	PD3 (g fish-1)	ED4 (kJ fish-1)	LD5 (g fish-1)	I6 (g fish-1)	DPI7 (g fish-1)	DEI8 (g fish-)
Diet basal/ingredient								
Summit	100/0	100	9.26	1.54	65.19	0.75	14.1	4.94	197.52
Peanut meal	85/15	96	9.90	1.71	71.69	0.87	14.4	5.13	194.41
Peanut meal	70/30	96	10.11	1.63	73.04	0.93	14.7	5.62	202.63
Peanut meal	55/45	92	9.10	1.49	71.63	0.97	15.0	5.55	191.05
Peanut meal	40/60	88	6.67	1.15	49.56	0.62	15.3	5.03	166.24
Peanut meal	25/75	79	6.02	0.94	47.83	0.65	15.7	4.25	135.81
Meat meal	85/15	100	8.90	1.46	65.65	0.84	14.1	4.82	188.81
Meat meal	70/30	100	9.04	1.56	67.28	0.79	14.0	4.75	181.37
Meat meal	55/45	100	7.98	1.35	58.57	0.68	14.0	4.77	178.51
Meat meal	40/60	96	6.70	1.17	45.28	0.44	14.0	4.32	158.27
Meat meal	25/75	100	5.12	0.88	33.70	0.32	13.9	3.78	135.07
Field pea	85/15	96	8.01	1.30	52.40	0.54	13.7	4.47	182.88
Field pea	70/30	100	7.18	1.20	47.13	0.49	13.3	4.11	172.64
Field pea	55/45	100	6.44	1.07	48.75	0.61	13.0	3.72	161.68
Field pea	40/60	100	5.28	0.91	37.44	0.41	12.6	3.23	144.74
Field pea	25/75	100	1.60	0.25	6.91	0.06	12.2	2.45	113.80
Canola meal	85/15	92	8.36	1.43	58.65	0.62	13.7	4.55	178.80
Canola meal	70/30	92	7.50	1.37	50.38	0.48	13.3	4.23	163.12
Canola meal	55/45	88	6.92	1.21	42.30	0.38	12.9	4.16	157.02
Canola meal	40/60	79	6.25	1.15	37.21	0.27	12.5	3.88	143.47
Canola meal	25/75	83	5.69	0.95	32.64	0.21	12.1	3.80	137.34
Diatom. earth	85/15	96	7.20	1.24	47.74	0.51	12.0	3.86	154.29
Diatom. earth	70/30	100	3.62	0.67	23.88	0.21	9.9	2.73	109.46
Diatom. earth	55/45	100	1.17	0.24	4.99	0.00	7.7	1.99	78.86

Values in table represent the average of three replicate aquaria.
[1] Survival (S) = 100 x (initial fish remaining at end of experiment / 8).
[2] Weight gain (WG) = individual harvest weight – individual stocking weight.
[3] Protein deposition (PD) = dry basis carcass protein content at harvest – dry basis carcass protein content at stocking.
[4] Energy deposition (EA) = dry basis carcass energy content at harvest – dry basis carcass energy content at stocking.
[5] Fat deposition (FD) = dry basis carcass fat content at harvest– dry basis carcass fat content at stocking.
[6] Feed intake (I) = dry basis feed intake g fish[-1].
[7] Digestible protein intake (DPI) = g DP fish[-1].
[8] Digestible energy intake (DEI) = kJ DE fish[-1].

Figure 1. Effect of digestible protein density on protein deposition in silver perch. Outer curves represent 95% prediction and confidence intervals. Inner curve represents the quadratic model. Points (diets) excluded from the data set are indicated by a cross. All points represent mean of three replicate tanks.
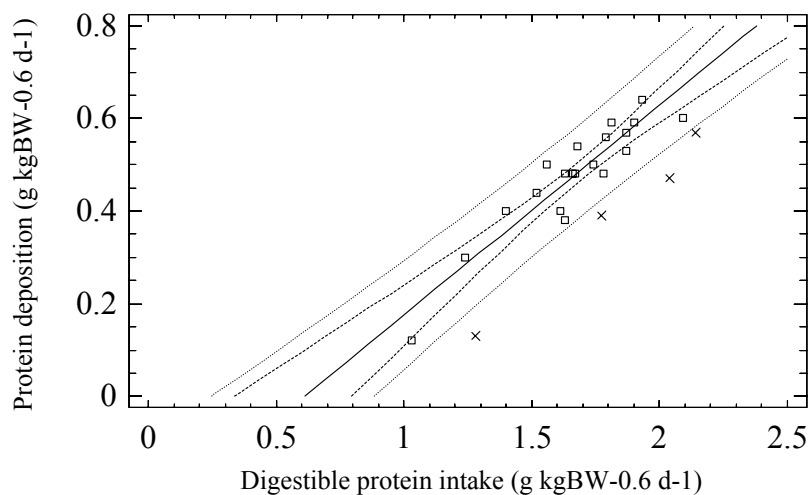
Figure 2. Effect of digestible protein intake on protein deposition in silver perch. Outer curves represent 95% prediction and confidence intervals. Inner curve represents the quadratic model. Points (diets) excluded from the data set are indicated by a cross. All points represent mean of three replicate tanks.

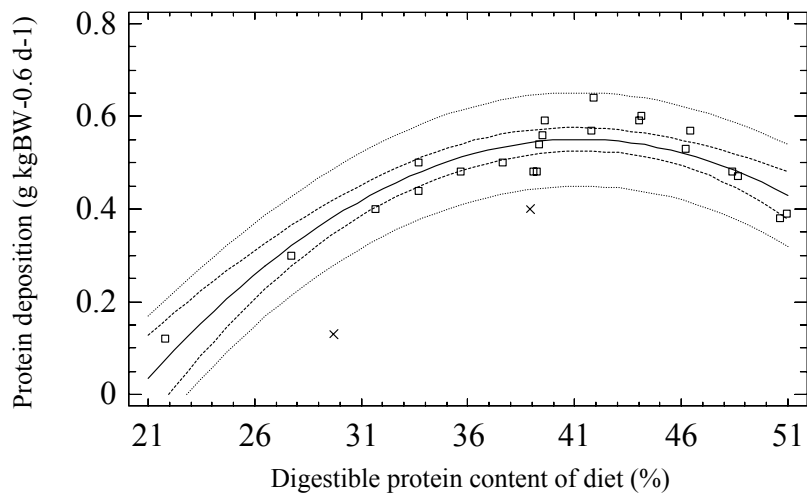**Figure 3.** Effect of digestible energy intake on energy deposition in silver perch. Outer curves represent 95% prediction and confidence intervals (linear regression). Inner curve represents the quadratic model. Points (diets) excluded from the data set are indicated by filled circles. All points represent mean of three replicate tanks.
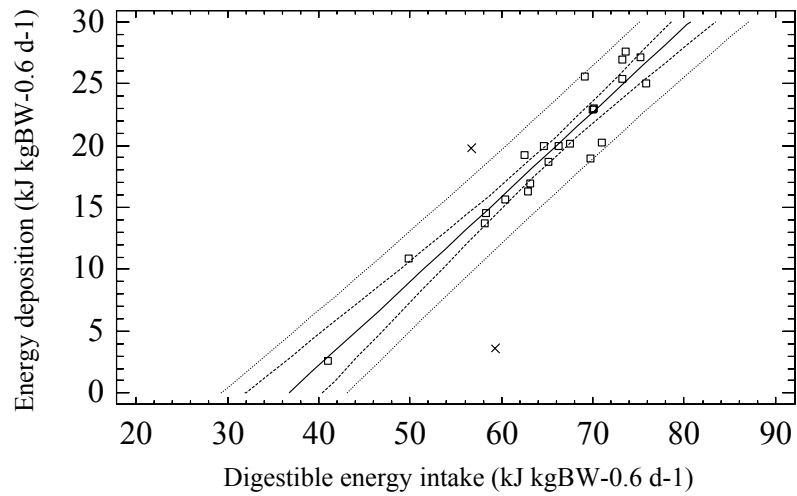
4.4. Carbohydrate utilisation by juvenile silver perch *Bidyanus bidyanus* (Mitchell): I. Uptake and clearance of monosaccharides following intra-peritoneal injection

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Abstract

Intra-peritoneal carbohydrate tolerance tests were done to assess the ability of silver perch, *Bidyanus bidyanus*, to utilise the predominant monosaccharides in plant ingredients currently being used in the formulation of aquaculture feeds for this species. Preliminary experiments carried out to assess baseline plasma glucose concentrations indicated that blood glucose levels were elevated within 2 min of handling and silver perch required a period of 48h without feeding before plasma glucose levels remained constant. In the first carbohydrate test, either glucose, galactose or xylose was administered by injection into the intra-peritoneal cavity at a dose rate of 1 g carbohydrate kg body weight (BW)$^{-1}$. In the second carbohydrate test, glucose was administered at a dose rate of either 2 or 4 g glucose kg BW^{-1}. Following injection, uptake and clearance rate of the carbohydrates from the blood stream was monitored over a 24 h period. Silver perch were significantly more efficient at the uptake and clearance of glucose from the blood stream than xylose or galactose. Maximum plasma glucose concentrations (22.2 mM) were recorded at 1 h following injection and basal levels (3.44 mM) were attained between 6-12 h following injection. For both galactose and xylose, maximum concentrations were recorded at 1 and 3 h, respectively, and concentrations of both monosaccharides remained significantly elevated 24 h after the administration. Plasma glucose concentrations of silver perch administered with either 2 or 4 g glucose kg BW^{-1} were significantly elevated and peaked at similar levels (30.2 mM and 30.7mM, respectively) 3 h after injection. Basal plasma glucose concentrations were attained in silver perch injected with 2 g glucose kg BW^{-1} at 24 h following administration. Plasma glucose concentrations remained significantly elevated in fish injected with 4 g glucose kg BW^{-1} after 24 h. These findings indicate that silver perch are more efficient at utilising glucose than either xylose or galactose, and that there are also differing maximum threshold for the inclusion of ingredients rich in glucose, galactose and xylose into the diets of silver perch.

Introduction

Increasing effort is being made to use more plant ingredients, such as wheat, lupins and field peas as protein sources, or to spare energy in aquaculture diets (Tacon 1994; Wilson 1994; Allan, Parkinson, Booth, Stone, Rowland, Frances & Warner-Smith 2000). One of the key limitations to the increased use of plant ingredients is the ability of fish to utilise the inherent carbohydrates (Allan *et al.* 2000).

While digestibility and growth trials are the principal methods to measure utilisation of nutrients in fish, both methods are relatively expensive and time consuming. A rapid and relatively cheap screening method for assessing the ability of fish to utilise carbohydrates is to introduce carbohydrate, either orally or into the intra-peritoneum of fish, and then measure uptake and clearance of the carbohydrate from the blood stream. When glucose is used this method is commonly referred to as the glucose tolerance test. Glucose tolerance tests have been conducted on

a range of fish species (Palmer & Ryman 1972; Furuichi & Yone 1982; Shimeno 1982; Furuichi Taira & Yone 1986; Wilson 1994; Lin, Ho & Shiau 1995; Shiau & Chuang 1995; Deng, Refstie & Hung 2001).

In general, fish of a low trophic level (herbivorous or omnivorous) tend to be more efficient at both the uptake and clearance of glucose compared to carnivorous species (Furuichi & Yone 1981; Garcia-Riera & Hemre 1996; Peres, Goncalves & Oliva-Teles 1999).

Enzyme pre-treatment of dietary plant material with carbohydrases (α-Amylase, β-glucanases and β-xylanases) may enhance energy digestibility in fish by releasing previously unavailable glucose, galactose and xylose. Relatively few tolerance studies have investigated the ability of fish to utilise galactose or xylose (Hung 1991; Shikata, Iwanaga & Shimeno 1994; Anderson, unpublished data). Results from these studies tend to suggest that herbivorous species are more galactose and xylose tolerant than carnivorous species.

Silver perch (*Bidyanus bidyanus)* is an omnivorous freshwater species, currently being cultured in Australia on relatively low protein (<35%) diets containing carbohydrates, including starch rich ingredients such as wheat and field peas, as well as lupins that contain a high proportion of galactose and xylose (Allan & Rowland 1998).

The aim of this study was to investigate the uptake and short-term clearance rates of glucose, galactose and xylose from the blood stream of silver perch.

Materials and Methods

Experimental fish and holding facilities

Silver perch (Experiment 1, mean weight ± sem: 42.5 ± 4.5 g [*n* = 36], Experiment 2, 25.8 ± 7.0 g [*n* = 63], Experiment 3, 58.1 ± 1.1 g [*n* = 180] and Experiment 4, 45 ± 1.2 g [*n* = 72]) were bred at the NSW Fisheries Grafton Research Centre (GRC) (29° 40'S, 153° 00'E) and raised in earthen ponds using similar techniques to those described by Thurstan & Rowland (1994). Except for Experiment 1 which was conducted at GRC, fish used in experiments were transported by road to the NSW Fisheries Port Stephens Fisheries Centre (PSFC) (32° 44'S, 152° 08'E) and held in a 7000-L tank supplied with re-circulating freshwater (bore water). Fish were fed twice a day with a low carbohydrate basal diet (digestible protein, 60.0; lipid, 13.4; total carbohydrate, 7.9; ash 15.6% and digestible energy 20.3 MJ kg^{-1}). Water temperature was held at 24 ± 2°C by the use of two, 2 kw immersion heaters. The fish were held at PSFC for at least 3 weeks prior to the commencement of each experiment.

Two preliminary experiments were performed to validate blood sampling and fasting protocols for glucose tolerance in silver perch.

Experiment 1: Baseline plasma glucose levels and acute glucose stress response of pond reared silver perch

Plasma glucose levels in fish has been shown to be elevated by handling stress (Mazeaud, Mazeaud & Donaldson 1977; Pickering 1981, 1993; Schreck 1982; Carragher & Rees 1994), and stress can, therefore, be a major confounding factor and influence results obtained from glucose tolerance tests. This experiment was designed to investigate the plasma glucose response of silver perch following capture and handling stress. The aim was to determine base line plasma glucose concentrations of silver perch and also to determine how quickly blood samples had to be collected from silver perch following an initial disturbance before plasma glucose concentrations were influenced by handling stress.

Silver perch used in this experiment were maintained in a 0.1 ha pond at GRC. In order to monitor the glucose kinetics of silver perch following capture and confinement, a time course study was adopted. Sampling times were 0.5, 0.75, 1, 2, 5 and 10 min following initial disturbance. Fish were fasted for a period of 48 h, prior to the experiment. Silver perch were captured from the pond by angling and blood samples were withdrawn either immediately or following confinement. Fish were confined in a 20-L plastic bucket containing fresh pond water supplied with oxygen. Six fish were used for each sampling time. Blood samples were obtained from the caudal vessel of fish using a 1 mL syringe and a 27 gauge hypodermic needle. Following collection, blood was transferred immediately into a 1.5 mL microfuge tube containing anti-coagulant, EDTA**,** (5 mg mL blood^{-1}) and glycolysis inhibitor, NaF, (2 mg mL blood^{-1}). Blood samples were immediately centrifuged at 1250 G and the plasma was separated and stored at -20°C prior to analysis. Plasma glucose concentrations were measured.

Results from experiment 1 indicated that blood glucose levels of silver perch are elevated rapidly following a handling disturbance (after 1 min; Figure 1). Therefore, to eliminate the confounding effects of stress on plasma glucose concentrations, all blood samples taken from silver perch in the following experiments were obtained within 1 min of initial disturbance at each sampling time.

Experiment 2. Post-prandial plasma glucose levels of silver perch

This experiment was designed to investigate the post-prandial glycaemic response of silver perch following normal feeding. The aim was to determine how long fish needed to be starved before glucose tolerance tests to ensure that blood glucose concentrations were due to the test diet and not previous feeding.

Three silver perch were stocked into each of 21, 70-L acrylic aquaria (3 aquaria per sampling time) in a photo therm controlled room (17 h light:7 h dark, water temperature range 24 -26°C). Fish were acclimated to the facility for a period of two weeks, during which time they were fed the basal diet twice daily (50% am/50% pm) at a rate of 4% biomass day^{-1}. To monitor the post-prandial glucose kinetics of silver perch, a time-course study was adopted. Sampling times were 0 (before feeding) 3, 6, 12, 24, 48 and 72 h following feeding. To minimise disturbance of fish during sampling, each 70-L aquaria was isolated by black plastic screens on front, back and sides.

On the morning of the commencement of the experiment, six fish were sampled (2 from each of three aquaria), and the remaining fish were fed a single meal (2% biomass). At each sampling time fish were anaesthetised with ethyl ρ-aminobenzoate (Sigma Chemical Co., Castle Hill, NSW, Australia) at a concentration of 300 mg L^{-1} then captured using a dip net. Blood samples were withdrawn as described in Experiment 1. Two fish from each of three aquaria were sampled. Fish were then weighed and plasma glucose was analysed.

Experiment 3. Carbohydrate tolerance test for glucose, galactose and xylose at a dose rate of 1 g carbohydrate kg BW^{-1}

Design, facilities and fish

This experiment was designed to investigate the ability of silver perch to regulate blood carbohydrate levels following an intra-peritoneal injection of either D-glucose, or D-galactose, or D-xylose (Sigma Chemical Co.), at a dose rate of 1 g carbohydrate kg BW^{-1}, over a period of 24 h.

The experiment was carried out in a recirculating system consisting of sixteen 10 000-L tanks held within a 40 x 15 m plastic greenhouse. Each 10 000-L tank contained six experimental floating cages (n = 96). Experimental cages were 200-L in capacity (0.6 m diameter and 0.7 m submerged depth, walls were constructed of 9 mm plastic mesh and the top and bottom were constructed of 1.6 mm plastic (Kinnears Pty Ltd, Smithfield, NSW, Australia). To minimise disturbance to the fish during acclimation and sampling, each 10 000-L tank was divided into six segments using black plastic sheeting. One cage was held within each segment. The 1.6 mm mesh lid on each cage also helped shield the fish from disturbances.

Freshwater was filtered through a sand filter before being supplied to experimental tanks at a flow-rate of 17-L min^{-1}. Effluent water from each tank flowed by gravity from the bottom of the tanks, into a 2 m^3 biological filter within a common 7000-L reservoir and was then pumped back to the sand filter. Aeration was provided to each tank by two air-stone diffusers and each tank was heated using a 2 kw immersion heater. Fluorescent lighting was used to control photo-period at 17 h light:7 h dark.

Prior to stocking, fish were captured by dip net from the 7000-L holding tank and anaesthetised in a 200-L container using a bath of 50 mg L^{-1} ethyl ρ-aminobenzoate for 3 min. The fish were then caught at random, individually weighed, and distributed among the 200-L cages by systematic interspersion. During the acclimation phase of both experiments, 20 fish were held in each 200-L cage. Twelve cages of fish (n = 240 fish) were used. The fish were fed the basal diet at 4% BW day^{-1} and acclimated to the experimental facilities for a period of 35 days prior to the experiment. At the completion of the acclimation phase the fish were not fed for a further 48 h prior to the administration of carbohydrate (following results from Experiment 2).

To confirm handling stress did not influence plasma glucose levels of fish, two groups of control fish were included. The first control group were exposed to the same handling procedures as the fish injected with carbohydrate but received no injection. The second group were also exposed to the same handling procedures as the fish injected with carbohydrate, but also received a "sham" injection of sterile isotonic saline solution (0.9% NaCl).

Stock solutions and injections

For ease of injection, the carbohydrates were mixed with distilled water and sterilised to create a stock solution containing 200 mg carbohydrate mL water^{-1}. To achieve the desired dose rate, each fish was weighed and the desired volume of stock solution was injected. This resulted in a constant ratio of stock solution volume to fish weight. Injections were administered using a 27 gauge needle and 0.5 mL syringe.

Sampling procedures

Fish were removed from a 200-L holding cage and a blood sample was withdrawn immediately from one fish (as for Experiment 1). The sample from this fish was randomly assigned to each treatment and used as the initial sample (basal level). The remaining fish were transferred simultaneously into a 100-L bin containing 50 mg L^{-1} ethyl ρ-aminobenzoate and anaesthetised, weighed and randomly assigned to each treatment series. The fish were then processed, either no injection (handling control group) or injected with either glucose, galactose, xylose or saline solution ("sham" control group) at a dose rate of 1 g carbohydrate kg BW^{-1}. Immediately following handling and injection, single fish were returned to individual 200-L cages to minimise disturbance at subsequent sampling times. Blood samples were obtained (as described in Experiment 1) from fish at 1, 3, 6, 12 and 24 h following injection. This procedure was repeated for each holding cage of fish until six fish were sampled for each sampling time for each treatment. After blood sampling, each fish was removed from the experiment and placed back into a separate tank for recovery.

Experiment 4. Glucose tolerance test at a dose rate of 2 or 4 g carbohydrate kg BW^{-1}

Silver perch appeared to be efficient at the uptake and clearance of glucose from their blood when administered at 1 g glucose kg BW^{-1}. Therefore, this experiment was done to assess the uptake and clearance capacity of silver perch with increasing levels of glucose over a period of 24 h following an intra-peritoneal injection with either 2 or 4 g glucose kg BW^{-1}.

This experiment was conducted using the same facilities and techniques described for Experiment 3, except the fish were acclimated to the facilities for 14 days and there were 6 cages of fish ($n = 120$ fish) used. In addition, as the results from Experiment 3 indicated that the injection procedures had no significant confounding effects on the glucose response of silver perch, the controls used for this experiment were the fish sampled initially without anaesthetic or injection. In an attempt to keep the injection volume constant between experiments, the concentration of the stock solution used in this experiment was 400 mg glucose mL distilled water^{-1}. Injection procedures, sampling times and experimental facilities for this experiment were the same as those described for Experiment 3. Six fish were used for each treatment for each sampling time.

Water Quality

During experiments, water temperature (range 23 -26°C), dissolved oxygen (above 5.0 mg l^{-1}), and pH (between 7.4 and 8.2) were measured daily using a Yeo-Kal 611 water quality analyser (Yeo-Kal Electronics, Brookvale, Sydney, NSW Australia). Nitrite and ammonia (<0.2 mg L^{-1} NO_2-N and <0.4 mg L^{-1} total ammonia-N, respectively) were measured using colourmetric methods described by Major, Dal Pont, Kyle & Newell (1972) and Dal Pont, Hogan & Newell (1973).

Biochemical analysis

Plasma glucose was determined for all blood samples. The glucose oxidase – peroxidase method (Fleming & Pegler 1963) (Kit 510A, Sigma Chemical Co.) was used. Plasma galactose was determined for blood samples of fish injected with galactose by difference using the Somogyi-Nelson method for total reducing sugars (Dische 1962a). Plasma xylose was measured in blood samples from fish injected with xylose using the method of Dische (1962b).

Statistical analysis

Statistical evaluation of the data was carried out using the *Statgraphics Plus* for Windows 4.1 software package (Manugistics Inc., Rockville, Maryland, USA; 1998). Homogeneity of variance was assessed using Cochran's Test (Winer 1991). Experiments 1 and 2 were designed for analysis of plasma glucose concentrations of silver perch using one-factor ANOVA with sampling time as the fixed factor. Experiment 3 was designed for analysis of plasma glucose concentrations of silver perch using two-factor ANOVA with treatment type (no injection [handling only], sham injection, glucose injection, galactose injection or xylose injection) as the first fixed factor and sampling time as the second fixed factor. Concentrations of plasma galactose and xylose in fish injected with either of these carbohydrates were analysed using single factor ANOVA with sampling time as the fixed factor. Experiment 4 was designed for analysis of plasma glucose concentrations of silver perch using two-factor ANOVA with glucose injection concentration (2 or 4 g glucose kg BW^{-1}) as the first fixed factor and sampling time as the second fixed factor. Single factor ANOVA was used to assess the differences of plasma glucose concentrations within treatments over time. Where significant differences were found, comparisons among means were made using Student-Newman-Kuel's multiple range test. Differences between means were considered significant at $P < 0.05$. Unless otherwise stated, all results appear as mean ± standard error of mean ($n = 6$).

Results

Experiment 1: Baseline plasma glucose levels and acute glucose stress response of pond reared silver perch

Resting, or baseline plasma glucose concentrations of silver perch were 4.41 ± 0.30 mM, and were significantly elevated within 2 min of initial disturbance ($P < 0.05$) (Figure 1).

Experiment 2. Post-prandial plasma glucose levels of silver perch

Post-prandial plasma glucose concentrations of silver perch changed significantly during the experiment ($P < 0.05$) (Figure 2). Glucose concentrations approached their lowest levels following 48 h of starvation, and were not significantly different after 72 h ($P > 0.05$) (Figure 2). As there was no significant reduction of plasma glucose concentrations between 48 and 72 h, a starvation period of 48 h was adopted before the carbohydrate administration for the subsequent glucose tolerance tests.

Experiment 3. Carbohydrate tolerance test for glucose, galactose and xylose at a dose rate of 1 g carbohydrate kg BW^{-1}

There was a significant effect of treatment on the plasma glucose concentrations of silver perch, in order of magnitude: glucose injection > control = saline injection = galactose injection = xylose injection ($P <0 .05$, Figure 3). There was also a significant effect of time on the glucose concentrations of silver perch with plasma glucose concentrations elevated between 1 and 12 h following treatment ($P < 0.05$). There was also a significant interaction between treatment and time ($P < 0.05$). The interaction may be explained by the large elevation of plasma glucose concentrations of silver perch following the injection of glucose, when compared to the minimal elevation of plasma glucose concentrations of fish, which received either no injection or an injection of, galactose, xylose or saline solution. This was confirmed by excluding plasma glucose concentrations of fish injected with glucose from the two-factor ANOVA with treatment type (no injection [handling only], sham injection, galactose injection or xylose injection) as the first fixed factors and sampling time as the second fixed factor. Results of this two-factor analysis indicated that there was a significant effect of time on plasma glucose concentrations of silver perch ($P < 0.05$), and that there was no significant effect of treatment type and there was no interaction ($P > 0.05$).

Initial basal concentrations of glucose in plasma (3.43 mM) were similar for all groups. Plasma glucose levels of silver perch injected with glucose were significantly elevated ($P < 0.05$) and peaked (~22 mM) 1 h after injection. From this point onwards glucose levels declined until basal levels were attained at 12 h after injection ($P > 0.05$). In contrast, silver perch, which received no injection (control), exhibited a slight increase in plasma glucose concentrations, which peaked at 3 h and returned to basal levels between 6 and 12 h of injection. Plasma glucose concentrations of fish, administered with galactose, xylose or the sham injection, followed the same trend as the control (no injection) fish ($P > 0.05$).

There were no measurable concentrations of galactose found in the blood of silver perch prior to galactose administration. Silver perch injected with 1g galactose kg BW^{-1} exhibited a significant elevation of plasma galactose that peaked between 1 and 3 h after injection ($P < 0.05$) (Figure 4). Plasma galactose concentrations tended towards basal levels over the following 24 h, but remained significantly elevated ($P < 0.05$).

There were no measurable concentrations of xylose observed in the blood of silver perch prior to xylose administration. Fish injected with 1 g xylose kg BW^{-1} exhibited a significant elevation of plasma xylose between 1 and 3 h after the injection ($P < 0.05$) (Figure 4). From this point plasma xylose concentrations slowly declined but were still significantly elevated 24 h following injection ($P < 0.05$).

Experiment 4. Silver perch glucose tolerance test

There was a significant effect of glucose dose rate on the plasma glucose concentrations of silver perch (4g glucose kg BW^{-1} > 2 g glucose kg BW^{-1}) ($P < 0.05$, Figure 5). There was also a significant effect of time on the glucose concentrations of silver perch ($P < 0.05$). There was also a significant interaction between glucose dose rate and time ($P < 0.05$). The interaction may be explained by the failure of fish injected with 4 g glucose kg BW^{-1} to attain basal levels, whereas fish injected with 2 g glucose kg BW^{-1} attained basal levels 24 h following injection.

Initial basal concentrations of plasma glucose (4.78 mM) of silver perch were similar for both groups. Plasma glucose levels of silver perch injected with either 2 or 4 g glucose kg BW^{-1} were significantly elevated ($P < 0.05$) and peaked at 30.2 mM and 30.7 mM, 3 h after injection respectively. Basal plasma glucose concentrations for fish injected with 2 g glucose kg BW^{-1} were reached at 24 h following injection ($P > 0.05$). In contrast, plasma glucose concentrations of silver perch injected with 4 g glucose kg BW^{-1} remained significantly elevated ($P < 0.05$) throughout the experiment.

Discussion

Experimental procedures, such as crowding, repeated sampling from the same tank or handling and blood sampling, have been reported to induce a primary stress response in fish (Mazeaud *et al*. 1977; Pickering 1981, 1993; Schreck 1982). In fact, plasma glucose concentrations have been used as a measure of secondary stress in fish (Mazeaud *et al*. 1977; Carragher & Rees 1994). The failure to adequately address this aspect of fish physiology during glucose tolerance tests can easily confound results. This was evident in a number of studies (Shikata *et al*. 1994; Garcia-Riera & Hemre 1996). The basal glucose concentrations observed in the plasma of silver perch during Experiments 2, 3 and 4 of the present study were within the range reported for silver perch sampled from the pond at GRC (Figure 1), and are also within the range reported for other species (Furuichi & Yone 1981; Wilson & Poe 1987; Hung 1991; Peres *et al*. 1999; Deng *et al*. 2001). This indicates the silver perch had settled into the experimental facilities and stress was minimised prior to the experiment. The injection procedure had a minimal effect on plasma glucose concentrations in

silver perch. There was a slight increase in plasma glucose of silver perch in both handling or "sham" injected control groups when compared to the initial basal concentrations, however, these were small compared to increased plasma glucose concentrations from silver perch receiving an injection of glucose (Figure 3).

The ability of an organism to assimilate and clear glucose from the blood stream is predominantly dependent upon active transport mechanisms (Champe & Harvey 1994). The route of assimilation of glucose into the bloodstream following intra-peritoneal injection may differ to that following oral administration. In the case of intra-peritoneal injection, the assimilation of glucose through the digestive system maybe by-passed, and as glucose does not passively diffuse into cells, active transport must occur predominantly through the epithelial cells of the peritoneum cavity or surrounding organs (Champe & Harvey 1994).

Silver perch challenged with an intra-peritoneal injection of glucose at a dose rate of 1 g kg BW^{-1} were extremely efficient at the assimilation of glucose from the peritoneum cavity into the blood stream. The assimilation of glucose by silver perch was rapid, with maximum glucose concentrations observed within 1 h of administration (Figure 3). When compared to the assimilation rate reported for the omnivorous common carp challenged with the same dose of glucose (Peres *et al*. 1999), it appears that silver perch have a more efficient transport mechanism. As expected of an omnivorous species, silver perch were more efficient at assimilating glucose from the bloodstream than a wide range of carnivorous species (Hemre, Sandnes, Lie & Waagbø 1995; Peres *et al.* 1999; Deng *et al.* 2001).

The utilisation of assimilated glucose as an energy source relies upon facilitated transport of extra-cellular glucose from the blood into cells and involves specific, cell-bound glucose transporters (Champe & Harvey 1994). Glucose may also be stored as glycogen, or incorporated into fat or protein (Champe & Harvey 1994). Other clearance routes do operate, for example, when the renal threshold is exceeded glucose may also be passed in the urine (glycosuria) (Champe & Harvey 1994; Deng *et al.* 2001). Glycosuria has been reported for the carnivorous yellowtail and white sturgeon (Furuichi *et al*. 1986; Deng *et al. 2001*). Glucose excretion, although minimal, has also been detected via the gills of Atlantic cod (*Gadus morhua*) (Hemre & Kahrs 1997).

Silver perch injected with 1 g glucose kg BW^{-1} were efficient at the clearance of glucose from the blood stream, with basal glucose concentrations achieved between 6 to 12 h following administration (Figure 3). This is only slightly longer than the clearance rate of glucose observed for the herbivorous carp or tilapia which required 4 to 8 h to achieve basal concentrations following carbohydrate administration (Hertz, Madar, Hepher & Gertler 1989; Lin *et al.* 1995). When compared to the clearance rate of glucose by carnivorous species such as Atlantic salmon, turbot or sturgeon (Hemre *et al.* 1995; Garcia-Riera & Hemre 1996; Deng *et al.* 2001), silver perch appear to be more efficient at utilising assimilated glucose. However, from the experiments reported in this study it is not possible to say what proportion of circulating blood glucose was utilised or simply excreted as waste via the urine or gills.

As with all other species of fish, silver perch exhibited a rapid and prolonged elevation of plasma glucose, which peaked within 1 h of administration at ~22 mM, and returned to basal levels within 12 h.

A comparison of the magnitude of the effects of increased glucose loading on silver perch from Experiments 2 and 3, indicates silver perch maintained the ability for rapid assimilation of glucose from the intra-peritoneal cavity into the blood stream (Figure 5). However, when the dose rate of glucose was increased from 1 to 2 to 4 g kg BW^{-1}, there appeared to be a progressive overload of the metabolic pathways responsible for the assimilation of glucose and prolonged hyperglycemia

was observed. This may have major implications on feed formulations and feeding strategies adopted for silver perch production.

Dietary carbohydrate level, complexity and feeding frequency have been reported to influence carbohydrate utilisation in fish (Brauge, Medale & Corraze, 1994, Shiau, 1997; Deng *et al.* 2001). This indicates that fish must be able to clear glucose from a previous meal before the next meal is given, otherwise hyperglycaemia may occur, and a progressive decline in fish growth performance may result. Hyperglycemia may also be reduced by feeding fish more complex carbohydrates. Slower digestibility of the more complex carbohydrates such as starch or dextrin by fish has been reported to result in a lower glycaemic response compared to fish fed the more easily digested glucose (Wilson & Poe 1987; Deng *et al.* 2001).

The administration of glucose at the dose rate of 1 g kg BW^{-1} equates to approximately the same amount of glucose that would be derived from one meal (1% BW^{-1}) of a commercial diet containing 30% digestible starch for silver perch. The uptake and clearance capacity of glucose following the intra-peritoneal injection at this dose rate complements digestibility and utilisation data for gelatinised starch or dextrin when included at 30% dietary inclusion content from previous studies for silver perch (Stone *et al.*, a & b, in press). In a culture situation, it appears that increasing the feeding frequency when feeding diets containing moderate levels of digestible carbohydrate may improve carbohydrate utilisation by silver perch.

Silver perch appear to have similar initial uptake rates for xylose and galactose into the blood stream (Figure 4). However, silver perch are very inefficient at the assimilation and utilisation of xylose and galactose compared with glucose. Even though the uptake of both xylose and galactose into the blood stream was only approximately half that of glucose, plasma concentrations remained elevated at 24 h following the injection. This suggests silver perch are both xylose and galactose intolerant at the dose rates tested in this study. Using similar methods to those reported in the present study, Anderson, (unpublished data, 2000) concluded barramundi were also both xylose and galactose intolerant (they also failed to clear elevated blood sugar levels within 48 h following an intra-peritoneal injection of 1 g carbohydrate kg BW^{-1}). Tilapia cleared elevated blood galactose concentrations within 4 h (Anderson, unpublished data) but blood xylose levels of tilapia peaked rapidly within 1 h of administration and remained significantly elevated after 24 h.

In humans, galactose shares many of the same uptake mechanisms as glucose and may even be converted to glucose during the absorption process (Champe & Harvey 1994). It appears that silver perch lack the required conversion mechanisms as there was no evidence of the conversion of galactose into glucose observed in the plasma of silver perch injected with galactose (Figure 3). The conversion mechanism appears to be species dependent as Shikata *et al.* (1994) also reported that carp fed galactose lacked the ability to convert the galactose to glucose in the absorption process while the opposite has been reported for white sturgeon (Hung 1991). Hung (1991) reported elevated blood glucose in sturgeon following oral administration of galactose.

An interesting point regarding galactose utilisation is that most fish, including silver perch, fail to efficiently clear elevated concentrations of galactose from their blood stream and appear to be galactose intolerant. There may be a reduction in the growth and health of silver perch if fed diets high in galactose. Galactosemia has been reported to have negative effects on fish performance. Shikata *et al.* (1994) reported a remarkable reduction in growth, reduced feeding activity, low body and serum fat levels and reduced hepatopancreatic enzyme activity in carp fed diets containing 30% galactose. Inefficient utilisation of galactose has also been reported for Chinook salmon (*Oncorhynchus tshawytscha*), brook trout (*Salvelinus fontinalis*) and chum salmon fry (*Oncorhynchus keta*) (Buhler & Halver 1961; McCartney 1971).

The inefficient utilisation of galactose and xylose observed here for silver perch lends further support to previous findings from digestibility studies which have indicated that this species is inefficient at digesting galactose or xylose from plant ingredients such as lupins (Allan & Rowland 1998). It also brings into question the potential of pre-treatment of dietary NSPs with enzymes (β-glucanases and β-xylanases) to enhance dry matter and energy digestibility for silver perch.

As the digestibility of NSPs by silver perch is insignificant (Allan & Rowland 1998), the potential for the availability of excessively high levels of dietary galactose or xylose in commercial diets is low. However, caution will have to be exercised if an attempt is made to enhance the digestibility of NSPs by the use of endogenous carbohydrase enzymes, as an increase in the availability of dietary galactose and xylose may have negative impacts on fish health and growth performance.

The results from this study indicate that there are marked differences in carbohydrate utilisation between the silver perch and other species. And even though hyperglycemia was evident, silver perch appear to be extremely glucose tolerant at the dose rate of 1 g glucose kg BW^{-1}. They maintained the ability to clear excessive blood glucose the before delivery of the next meal. As silver perch appear to be galactose and xylose intolerant, dietary levels of galactose and xylose should be restricted. In a culture situation, the restriction of high contents of glucose, galactose and xylose from diets may also contribute to an improvement in pond water quality due to a reduction in waste output from faeces.

Acknowledgements

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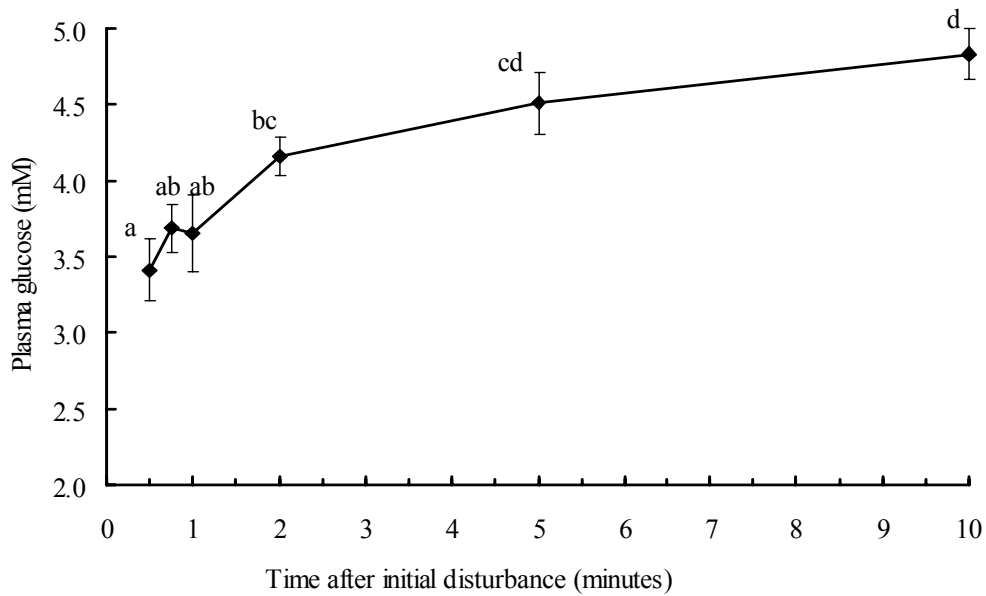
Figure 1.   Plasma glucose concentrations of silver perch following capture and confinement. Values are means ± sem (*n* = 6 fish per sampling time). Means which share the same superscript are not significantly different (*P* > 0.05, ANOVA, SNK).
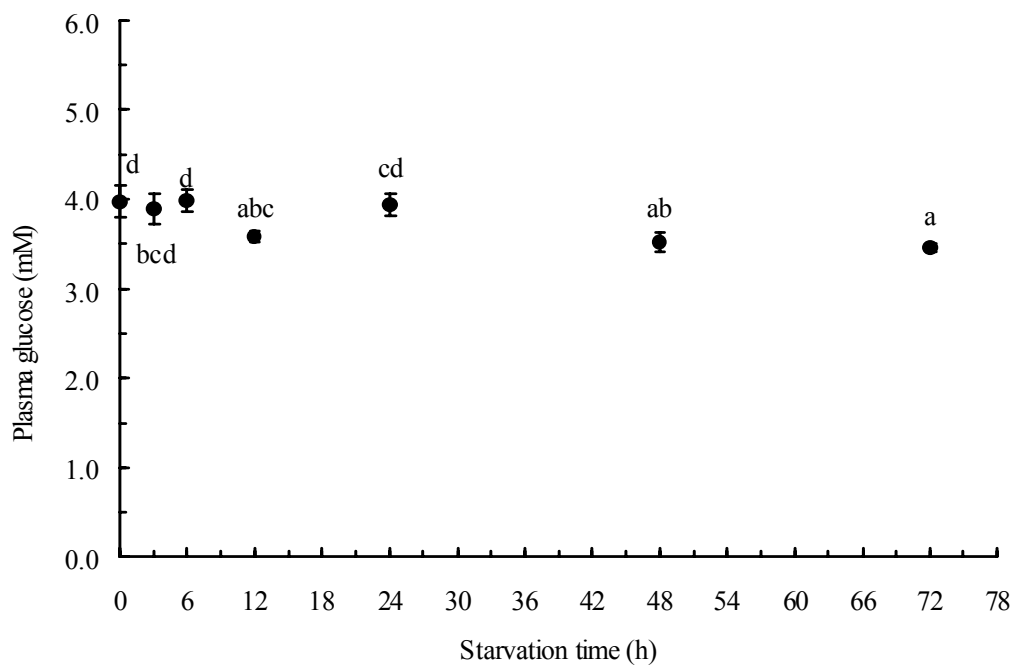
Figure 2.   Post-prandial plasma glucose levels of starved silver perch. Values are means ± sem (*n* = 3 replicates consisting of 2 fish per tank) Means which share the same superscript are not significantly different (*P* > 0.05,ANOVA, SNK).
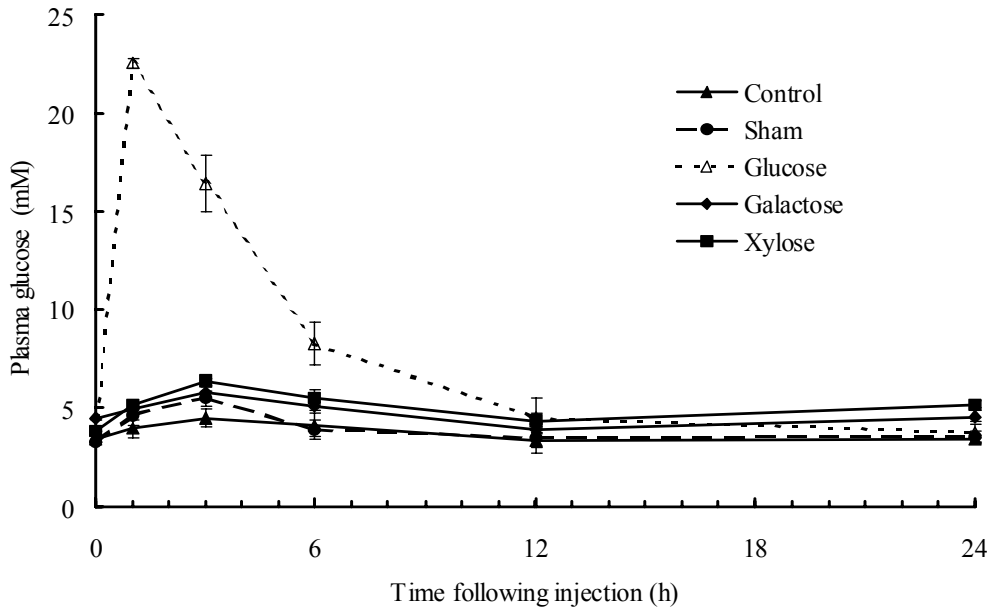
Figure 3. Plasma glucose concentrations of silver perch which were either anaesthetised (control) or anaesthetised and administered with an intra-peritoneal injection of carbohydrate (1 g carbohydrate kg BW^{-1}) or isotonic saline solution (mean ± sem; n = 6 fish for each sampling time within each series).
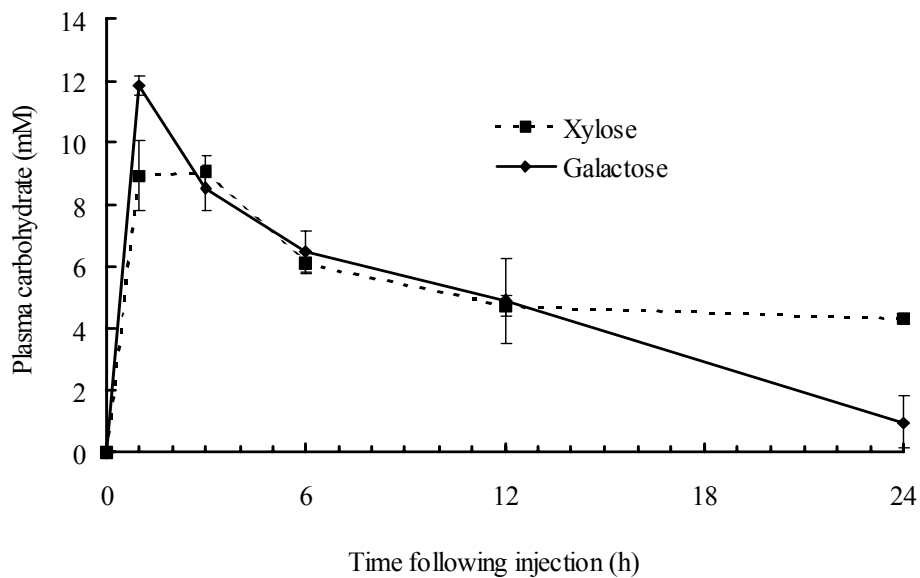
Figure 4. Plasma galactose and xylose concentrations of silver perch following an intra-peritoneal injection of either 1 g galactose or 1 g xylose kg BW^{-1} (mean ± sem; n = 6 fish for each sampling time within each series).
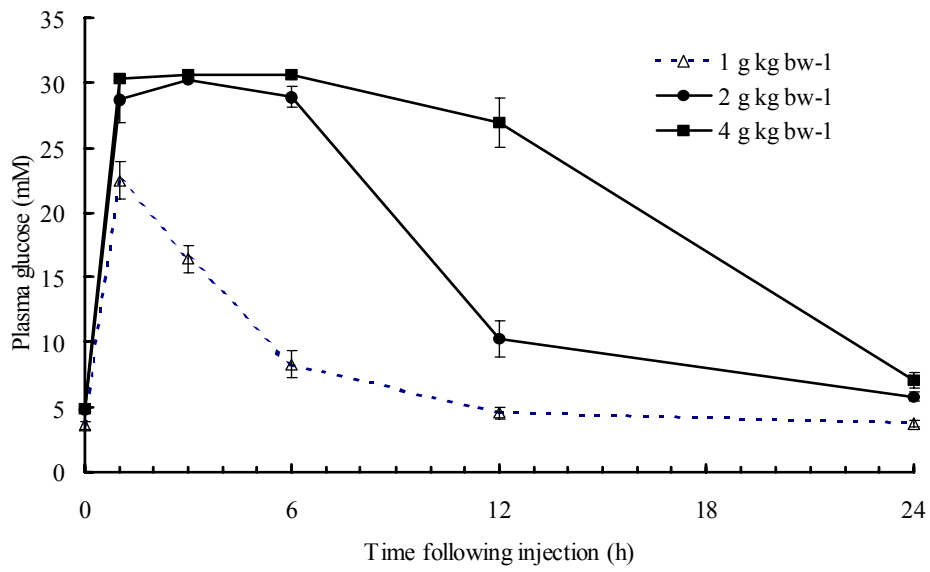
Figure 5.   Glucose response of silver perch following an intra-peritoneal injection of glucose at either 1 or 2 or 4 g kg BW^{-1} (mean ± sem; n = 6 fish for each sampling time within each series

4.5.   Carbohydrate utilisation by juvenile silver perch *Bidyanus bidyanus* (Mitchell): II. digestibility and utilisation of starch and its breakdown products

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Abstract

The ability of silver perch, (*Bidyanus bidyanus*) to digest and utilise dietary starch or starch breakdown products was investigated. For Experiment 1 the ability of silver perch (2.7±0.01g) to digest wheat starch at two dietary inclusion levels (30 or 60%) each at four levels of gelatinisation (0, 25, 50 or 80%) was investigated over a 31 day period. For Experiment 2, the ability of silver perch (15.9±0.25g) to digest wheat starch, dextrin (at 3 levels of dextrinisation), maltose, glucose and pea starch all at the 30% inclusion level was investigated over a 41 day period. Water temperature for both experiments was 25±1°C. Apparent digestibility coefficients (ADC) for starch, dry matter (DM) and energy were affected by both degree of gelatinisation (80 > 50 > 25 = 0%) and inclusion level (30 > 60%). Specific growth rate was unaffected by the inclusion of 30% starch; however, it was reduced at the 60% starch content. Degree of gelatinisation had no effect on SGR. For Exp. 2, there were significant differences between carbohydrate and DM ADC's for the test ingredients. The carbohydrate, DM and energy ADC's were ranked as follows: Dextrin (Fieldose 9) = Dextrin (Fieldose 17) = Dextrin (Fieldose 30) = gelatinised wheat starch = maltose = glucose > raw wheat starch > raw pea starch. Protein ADC of diets, post-prandial plasma glucose concentration and SGR were all unaffected by ingredient type for both experiments, HSI tended to increase with carbohydrate inclusion. Liver glycogen concentrations were also elevated But muscle glycogen and liver and muscle TAG concentrations were unaffected. Digestibility of starch by silver perch is clearly affected by inclusion content and processing.

Introduction

In the search for alternative protein sources to fishmeal, plant ingredients, such as wheat, corn, and field peas, have been incorporated into diets for fish (Allan, Rowland, Parkinson, Stone & Jantrarotai 1999; Allan, Parkinson, Booth, Stone, Rowland, Frances & Warner-Smith 2000a; Allan, Booth, Stone, Williams & Smith 2000b). When compared to many animal meals most plant ingredients contain relatively less protein, and much higher carbohydrate contents. Starch is the predominant carbohydrate in most grains and legumes, and may comprise up to 60% of the total grain (NRC 1993). Fish may be able to utilise dietary carbohydrates for energy, which in turn may spare the more expensive protein component of the diet for growth (Buhler & Halver 1961; Wilson & Poe 1987; Shiau 1997). However, efficient utilisation is linked to efficient digestibility and the capacity of fish to digest carbohydrate has been reported to be variable between species (Shimeno 1982; Furuichi 1983; Wilson 1994; Shiau 1997).

Three major factors affecting starch digestibility in fish are physical state (e.g. degree of gelatinisation), complexity (e.g. molecular weight), and dietary inclusion levels (Singh & Nose, 1967; Wilson 1994; Shiau 1997). It is common practice to employ steam pelleting or heat and pressure extrusion to produce aquaculture diets. Both methods involve heat and moisture, and as a

result, the starch component of diets may be altered by either gelatinisation or a reduction in complexity (Hilton, Cho & Slinger 1981; Takeuchi, Jeong & Watanabe 1990).

Raw starch is a complex carbohydrate and is relatively insoluble in cold water (Knight 1969). Processing of starch may effect its physical characteristics, or lead to a reduction in molecular complexity, which increases water solubility (Knight 1969). Although, the ability of fish to digest processed starch is species dependent, in general, processing has a positive effect on starch digestibility. For example, for channel catfish (*Ictalurus punctatus*) cooked corn starch was 17% more digestible than raw starch when fed at a 25% dietary level (Lovell 1989a). Singh & Nose (1967) reported that rainbow trout (*Oncorhynchus mykiss*) were more efficient at digesting glucose than either dextrin or potato starch. Glucose digestibility appears to be high (ADC >90%) for most species (Wilson 1994). This may be explained by the fact that glucose does not require pre-hydrolysis by digestive enzymes prior to absorption, as it is already in the required form for uptake by intestinal glucose transporters (Buddington 1987).

As dietary levels increase, fish appear to exhibit a reduced capacity to digest starch, or its less complex breakdown products (with the exception of glucose). The reduced capacity for digestion of increasing contents of dietary carbohydrate has been linked to inadequate digestive carbohydrase activities associated with the trophic level of the species (Singh & Nose 1967; Wilson 1994).

Silver perch (*Bidyanus bidyanus*) is an omnivorous freshwater fish cultured in Australia. Digestibility values for dry matter, protein, lipid and energy have been determined for a range of animal and plant ingredients for silver perch and these have formed the basis for a useful database for least-cost diet formulation (Allan *et al*. 1999, 2000a, 2000b; Allan, Rowland, Mifsud, Glendenning, Stone & Ford 2000c; Stone, Allan, Parkinson & Rowland 2000;). Silver perch are very efficient at digesting protein from animal and plant ingredients; but, energy digestibility was variable for the ingredients tested with values ranging from 31% for wheat to 98% for fish meal, with animal ingredients generally providing more energy than plant ingredients (Allan *et al*. 2000a).

Previous research has indicated that this species is relatively tolerant to blood glucose loading and is more efficient at digesting energy from wheat starch than maize or potato starch at 30% inclusion content. Cooking also enhanced energy digestible of starch (Stone, Allan & Anderson, in press) The aim of this study was to examine the effect of dietary content and processing on the digestibility and utilisation of starch by silver perch.

Materials and Methods

Test ingredients

Eight carbohydrate products were evaluated during this study; raw wheat starch (Wheaten cornflour), gelatinised wheat starch (100% pregelatinised starch - Pregel N), dextrin with one of three increasing glucose equivalents (Fieldose 9, Fieldose 17, Fieldose 30), maltose (maltose grade II crystalline monohydrate), glucose (dextrose monohydrate B 60) and raw pea starch (Table 1).

Experiment 1. Inclusion content and degree of gelatinisation of wheat starch, digestibility and utilisation

We investigated the ability of silver perch to digest and utilise wheat starch at two inclusion contents each with four different levels of gelatinisation. Silver perch were fed a basal diet containing no starch (Ingredient composition g kg^{-1}: Chilean fish meal, 801; corn gluten 113.5; fish oil 70.0; vitamins 7.5; minerals 7.5). The basal diet for the test diets was substituted with 30% or 60% of wheat starch with 0, 25, 50 or 80% gelatinisation to give 9 diets in total (Tables 2 & 3),

each fed to three replicate tanks for 31 d. Two starch products, raw wheat starch and 100% gelatinised wheat starch were evaluated in this experiment. Gelatinisation treatments were produced by mixing raw and 100% gelatinised wheat starch, at one of four ratios, to give similar gelatinisation values commonly measured in raw, steam pelleted and extruded diets. At harvest, weight gain was recorded and the livers and fillets were immediately removed from three randomly selected fish from each tank and stored at –20°C prior to glycogen and triacylglycerol (TAG) analysis.

Experiment 2. Digestibility and utilisation of starch or its constituent breakdown products

We examined the ability of silver perch to digest and utilise diets containing raw wheat starch or its constituent breakdown products, and raw pea starch. Silver perch were fed a basal diet (containing no carbohydrates of starch origin), or the basal diet substituted with 30% of either raw wheat starch, gelatinised wheat starch, dextrin with one of three increasing glucose equivalents (Fieldose 9, 17 or 30), maltose, glucose or raw pea starch (to give nine diets in total, Tables 4 & 5), each fed to three replicate tanks for 41 days. At harvest, fish weight gain was measured and, the livers and fillets were immediately removed from three randomly selected fish from each tank and stored at –20°C prior to glycogen and triacylglycerol (TAG) analysis.

At the end of the experiment the fish were left in their 200-L tanks and fed their respective diets for 3 days and then, on the morning of sampling, fish were fed to excess over a 3 h period. Fish were then anaesthetised using ethyl ρ-aminobenzoate (Sigma Chemical Co., Castle Hill, NSW, Australia) (300 mg L^{-1} for 30 seconds), captured with a dip net and blood samples were collected from three fish from each tank within 1 minute of initial disturbance to eliminate the confounding effects of handling stress on blood glucose levels (Stone *et al*. in press). Blood samples were obtained from the caudal vessel of fish using a 1mL syringe and a 27-gauge hypodermic needle. Following collection, blood was transferred immediately into a 1.5 mL micro-tube containing anti-coagulant (EDTA, 5 mg mL blood^{-1}) and glycolysis inhibitor (NaF, 2mg mL blood^{-1}). Blood samples were immediately centrifuged at 1250 *g* and the plasma was separated and stored at -20°C prior to glucose analysis.

Diet preparation

All ingredients used in the basal diet and test ingredients were ground using a hammer mill (C-E Raymond Inc., IL, USA) or sieved to ensure all particles passed through a 710 μm screen. Dry ingredients were then thoroughly mixed in a Hobart mixer (Troy Pty. Ltd., OH, USA). Ytterbium chloride, an inert indicator, was then sprayed onto each batch of diet mash at 0.1g kg^{-1} dry basis. The mash was then combined with approximately 400 mL distilled water kg^{-1} dry mix before being cold pelleted through a meat mincer (Barnco Australia Pty. Ltd., Leichhardt, NSW, Australia) with a 4 mm die. The diet was then dried in a convection drier at 35°C until the moisture content was approximately 20%. The diets were then reground in a hammer mill to ensure homogenous mixing of the ytterbium chloride throughout the mash. The appropriate amount of fish oil was added to each batch of mash and then each diet was re-pelleted through a 2 mm die. 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.

Fish and holding facilities

Silver perch (Experiment 1, mean weight ± sem: 2.7 ± 0.01 g; Experiment 2, 15.9 ± 0.25 g) were bred at the NSW Fisheries Grafton Research Centre. Fish were transported to the NSW Fisheries Port Stephens Fisheries Centre. Prior to the experiments, fish were held in a 10 000-L tank supplied with heated (24 ± 2°C) recirculating freshwater (bore water; salinity, 0.05 g L^{-1}) and fed to apparent satiation twice a day with a commercial silver perch diet (95LC2; digestible protein, 34.0%;

digestible lipid, 9.0%; carbohydrate, 31.8%; ash 17.8% and digestible energy 14.0 MJ kg^{-1}; Allan *et al*. 2000c).

Experimental facilities and stocking

Digestibility tanks were 170-L cylindro-conical tanks (conical base sloped at 35°). The design and operation of this system is described by Allan *et al*. (1999). Photoperiod was held at 17 h light:7 h dark. Prior to stocking, fish were anaesthetised using a bath of ethyl ρ-aminobenzoate (30 mg L^{-1} for 5 min) then caught at random, weighed (Experiment 1, in groups of 5 or 10; Experiment 2, individually) and distributed among tanks by systematic interspersion. Fish were stocked (Experiment 1, 25 fish tank^{-1}; Experiment 2, 6 fish tank^{-1}) and allowed to acclimatise to their experimental diets and facilities for a period of 11 days prior to the collection of faeces. Fish were fed their respective experimental diets to excess (9% body weight d^{-1}) using automatic conveyor belt-type feeders for 3 h each day from 0830-1130 h. Faecal samples were collected by settlement as described by Allan *et al*. (1999). During both experiments, water temperature (range 24.5 to 26.0°C), dissolved oxygen (above 5.0 mg L^{-1}) and pH (between 7.2 and 8.5) were measured weekly using a Yeo-Kal 611 electronic water quality analyser (Yeo-Kal Electronics, Brookvale, Sydney, NSW Australia). Nitrite and ammonia (< 0.02 mg L^{-1} NO$_2$-N and < 0.06 mg L^{-1} total ammonia - N respectively) were measured weekly using colourmetric methods described by Major, Dal Pont, Kyle & Newell (1972) and Dal Pont, Hogan & Newell (1973).

Digestibility determinations

The apparent digestibility coefficients (ADC's) for dry matter, starch, carbohydrate, energy, protein (Experiments 1 and 2) and lipid (Experiments 1) in experimental diets were calculated using the indirect procedures described by Cho & Kaushik (1990). Ytterbium chloride (0.1% dry basis) was used as the inert indicator. Then apparent digestibility coefficients (%) for energy for test ingredients for both experiments were calculated using the methods described by Sugiura, Dong, Rathbone & Hardy (1998).

Performance indices

For each experiment, three silver perch from each of the three replicate tanks for each treatment were randomly selected and analysed for proximate body composition. At the completion of each experiment liver and muscle samples were obtained from three fish from each tank for each treatment for each experiment, and analysed for liver and muscle glycogen, and triacylglycerol (TAG). Specific growth rate (SGR % day^{-1}) was determined using the following equation: [SGR % day^{-1} = (ln final wt g - ln initial wt g) / length of experiment in days x 100]. The hepatosomatic index of fish was determined using the following equation: [HSI% = wet weight liver / whole wet body weight x 100].

Biochemical analyses

Feed, faecal and fish samples were analysed for dry matter, ash, crude fat and energy (bomb calorimetry) by the AOAC (1990) procedures. Nitrogen was determined using Kjeldahl or semi-micro Kjeldahl methods (AOAC 1990) and crude protein content was estimated by multiplying nitrogen by 6.25. Total starch, dextrin, maltose and glucose content of the basal diet, ingredients and faeces were analysed enzymatically (Megazyme total starch assay AA/AMG 9/97, Megazyme International Ltd, Ireland). Dextrin and maltose concentrations of diets and faeces were analysed enzymatically using amyloglucosidase with modified Megazyme methods. Plasma glucose from blood samples was determined using the glucose oxidase – peroxidase method (Fleming & Pegler 1963). Analysis of diets and faeces for ytterbium chloride was performed by CSIRO, Tropical Agriculture Analytical Services, St Lucia, Qld Australia, using inductively coupled plasma-mass spectroscopy (ICP-MS) techniques. Liver and muscle glycogen concentrations were analysed by the amyloglucosidase digestion method of Murat & Serfaty (1974). Liver and muscle

triacylglycerol concentrations were analysed using the Hantzsch reaction following extraction and saponification (Teitz 1987).

Statistical analysis

Homogeneity of variances was assessed using Cochran's Test (Winer 1991). Experiment 1 was designed for analysis using two-way ANOVA with starch content (30 or 60%) and the degree of gelatinisation (0, 25, 50 or 80%) as the two fixed factors. Dry matter (DM), starch, gross energy and protein digestibility, HSI, SGR and proximate body composition for diets 2 - 9 were compared using two-way ANOVA. Where significant interactions were found one-way ANOVA was used to compare treatments 1 - 9. One-way ANOVA was used to compare the specific growth rate of fish fed the basal diet and diets containing starch. The second digestibility experiment was designed for analysis using one-way ANOVA with carbohydrate type as the fixed factor. One-way ANOVA was used to assess the difference between apparent digestibility coefficients and other utilisation indices. Where significant differences were found, means were separated using Student Newman-Kuel's multiple range test. Means were considered significant at $P < 0.05$. Unless otherwise stated, all results appear as mean ± standard error of the mean ($n = 3$). Statistical evaluation of the data was carried out using the *Statgraphics Plus* for Windows 4.1 software package (Manugistics Inc., Rockville, MD USA; 1998).

Results

Experiment 1. Inclusion content and degree of gelatinisation of wheat starch, digestibility and utilisation

For starch digestibility (starch ADC), dry matter digestibility (DM ADC) and energy digestibility (energy ADC) of the diets the two-way ANOVA indicated that inclusion content (30>60%) and degree of gelatinisation (0 = 25 < 50 < 80%) had a significant effect, and there were significant interactions ($P < 0.05$) (Table 6). Although increasing degree of gelatinisation led to increased ADC's, a significant interaction between inclusion content and degree of gelatinisation was caused by the much greater effect of gelatinisation at the 60% inclusion (here starch, DM and energy ADC's increased from 46 to 70%, 53 to 73% and 63.7 to 80.1%, respectively) than at 30% inclusion (starch, DM and energy ADC's increased from 76-88%, 79 to 84% and 88.5 to 92.5% respectively) (Table 7). A similar pattern was evident for energy ADCs for ingredients.

For lipid ADC's of the diets the two-way ANOVA indicated there was a significant effect of both starch content (30 > 60%) and degree of gelatinisation and there was no interaction between the two ($P < 0.05$) (Table 6). Lipid digestibility of all diets were relatively high, suggesting that 30 or 60% inclusion of starch has a relatively minor affect on lipid digestibility for silver perch. For protein digestibility the two-way ANOVA indicated that there was no significant effect of starch content or degree of gelatinisation, and there was no interaction ($P > 0.05$), again suggesting that there are no antagonistic effects of carbohydrate on protein digestibility for these diets when fed to silver perch (Table 6).

For specific growth rate, the two-way ANOVA indicated that starch inclusion content had a significant effect ($P < 0.05$). Silver perch fed diets containing 30% starch exhibited better growth than fish fed diets containing 60% starch (Table 6). There was no significant effect of degree of gelatinisation on specific growth rate, and there was no significant interaction ($P > 0.05$). SGR of silver perch fed diets containing wheat starch at 30% inclusion ranged from 2.74 to 3.06 and were not significantly different to the SGR of the basal diet (2.92) (one-way ANOVA, SNK, $P > 0.05$). However, silver perch fed diets containing 60% starch had significantly lower SGRs (2.09 - 2.38) than fish fed the basal diet (one-way ANOVA, SNK, $P < 0.05$).

For carcass composition, results from the two-way ANOVA's indicated there was no significant effects of starch inclusion content or degree of gelatinisation on moisture (range 69.3 - 72.1%), protein (13.6 - 14.6%), lipid (9.7 - 11.7%), gross energy (7.1 - 8.1MJ kg^{-1}) and ash (10.3 - 12.0%) of silver perch ($P > 0.05$). There were also no significant interactions ($P > 0.05$).

For HSI, the two-way ANOVA indicated that both starch content and degree of gelatinisation had a significant effect ($P < 0.05$) (Table 6). There was also a significant interaction ($P < 0.05$). The results from the one-way ANOVA indicate that the HSI of silver perch increased as the starch inclusion content was increased from 30 to 60% when gelatinisation was $\geq 25\%$ (Table 7).

For liver glycogen concentrations, the two-way ANOVA indicated that there was no significant effect of starch content, degree of gelatinisation or interaction between the two ($P > 0.05$). However, a comparison of the liver glycogen concentrations of silver perch fed the basal diet (no starch) (54 mg glycogen g^{-1} wet tissue) to those fed either series of starch diets (one-way ANOVA), revealed levels were significantly elevated ($P < 0.05$) in fish receiving any one of the starch diets (range, 103 - 113 mg glycogen g^{-1} wet tissue).

For muscle glycogen concentrations the two-way ANOVA indicated that there were no significant effects of starch content, degree of gelatinisation (range, 0.73 – 1.35 mg glycogen g^{-1} wet tissue) or their interaction ($P > 0.05$). For liver TAG concentrations the two-way ANOVA indicated that there were no significant effects of starch content, degree of gelatinisation (range, 44.4 - 53.0 mg TAG g^{-1} wet tissue) or their interaction ($P > 0.05$). For muscle TAG concentrations the two-way ANOVA indicated that there were no significant effects of starch content, degree of gelatinisation (range, 23.3 - 26.1 mg TAG g^{-1} wet tissue), or their interaction ($P > 0.05$).

Experiment 2. Digestibility and utilisation of starch or its constituent breakdown products

Carbohydrate, dry matter and energy ADCs of the test diets were significantly affected by carbohydrate type (one-way ANOVA, $P < 0.05$) (Table 8). Carbohydrate, dry matter and energy ADCs of diets containing processed starch or less complex carbohydrates were significantly higher than for diets containing raw wheat starch or raw pea starch. Diets containing raw pea starch had significantly lower carbohydrate, dry matter and energy ADCs than diets containing raw wheat starch (one-way ANOVA, $P < 0.05$). Protein ADCs of the test diets were significantly effected by carbohydrate type (one-way ANOVA, $P < 0.05$) (Table 8). However, all protein ADCs were high (94 - 96%) suggesting carbohydrate in the diet did not interfere with protein digestion for silver perch.

Energy ADC's of the test ingredients were significantly effected by carbohydrate type (one-way ANOVA, $P < 0.05$) (Table 8). The energy digestibility of ingredients in the order of highest to lowest was Fieldose 9 > Fieldose 17 = Fieldose 30 = gelatinised wheat starch = glucose = maltose > raw wheat starch > raw pea starch.

Specific growth rate of silver perch was not significantly effected by test ingredients (one-way ANOVA, $P > 0.05$). Specific growth rate ranged from 1.40% day^{-1} for silver perch fed diets containing glucose to 1.77% day^{-1} for fish fed diets containing dextrin in the form of Fieldose 9 (Table 8).

Silver perch fed diets containing carbohydrate tended to have higher HSI compared to fish fed the basal diet, however, the differences were not significant (one-way ANOVA, $P > 0.05$) (Table 8). There was a significant effect of diet on liver glycogen concentrations of silver perch (one-way ANOVA, $P < 0.05$) with fish fed diets containing carbohydrate tending to have increased levels of liver glycogen compared to fish fed the basal diet (Table 8). Silver perch fed diets containing carbohydrate tended to have elevated concentrations of muscle glycogen compared to fish fed the basal diet, however, the differences were not significant ($P > 0.05$).

Liver and muscle TAG concentrations of silver perch were not significantly affected by the incorporation of carbohydrate into their diet (one-way ANOVA, $P > 0.05$). Liver and muscle TAG concentrations ranged from 31.9 - 57.2 and 13.0 - 17.0 mg TAG g^{-1} wet tissue respectively.

Post-prandial plasma glucose concentrations of silver perch were not significantly affected by ingredient type within 3 h after feeding (one-way ANOVA, $P > 0.05$). Plasma glucose concentrations ranged from 4.28mM for silver perch fed diets containing dextrin in the form of Fieldose 9 to 5.25mM for fish fed diets containing glucose.

Discussion

Silver perch digest gelatinised starch better than the omnivorous channel catfish and common carp and as well as tilapia (*Oreochromis niloticus* x *O. aureus*), when fed at a similar dietary inclusion content (30%) (Lovell 1989a; Hernandez, Takeuchi & Watanabe 1994; Shiau & Liang 1995).

The positive effect of gelatinisation on digestibility of starch for silver perch is consistent with results for channel catfish, rainbow trout and carp (Singh & Nose 1967; Lovell 1989a; Hernandez *et al*. 1994;). For example gelatinisation of starch by extrusion was reported to improved digestibility of wheat starch, corn starch and potato starch at the ~30% dietary inclusion content for rainbow trout (Takeuchi *et al*. 1990). In contrast, increasing gelatinisation reduced digestibility of wheat starch for barramundi (McMeniman pers. comm., 2000) and Atlantic salmon (Arnesen & Krogdahl 1993).

In the current study the digestibility of wheat starch was reduced as inclusion content increased. This response is consistent with starch digestibility data reported for rainbow trout, cod (*Gadus morhua*), channel catfish and Atlantic salmon (Singh & Nose 1967; Hemre, Lie, Lied & Lambertsen 1989; Lovell, 1989(a); Arnesen & Krogdahl, 1993). The reduction of starch digestibility with increasing dietary inclusion may be explained by an overload on the digestive carbohydrase enzymes present in silver perch. Given that silver perch have moderate levels of α-amylase (Anderson & Lipovsek 1998), it is likely that there is a threshold where the enzyme system is saturated by substrate, and no further digestion can occur. This appears to have occurred at the 60% inclusion content.

The slightly lower digestibility reported for raw starches compared to gelatinised wheat starch may be attributed to their reduced solubility, or to the impediment of α-amylase activity. In order for ingested starch to be digested it must be soluble in aqueous solution. The solubility of raw starch is reduced in cold water and requires relatively strong alkaline conditions to enhance solubility (Knight 1969). Starch digestibility occurs predominantly in the anterior section of the digestive tract of fish (Fänge & Grove 1979; Lovell 1989b). The pH optimum for α-amylase activity of the anterior section of the digestive tract of silver perch is reported to be neutral to slightly alkaline (Anderson & Lipovsek 1998). As the temperature of the fluids in the digestive tract of fish is related to the surrounding water temperature (26°C), the solubility of raw starch would be lower than gelatinised starch and digestibility would be impeded. As the inclusion content increased, the solubility and digestibility of raw starch would be reduced even further.

Raw wheat starch was more digestible than raw pea starch reflecting the different characteristics of these ingredients. Raw starch granules are classified into three different groups depending on the crystalline structure of the amylose and amylopectin within the granule. Wheat starch has been classified into group A, while high amylose corn starch, barley starch and pea starch have been classified into the B and C groups (Gallant, Bouchet, Buleon & Perez. 1992). *In vitro* digestibility studies using bacterial or pancreatic α-amylase have demonstrated that the different crystalline

characteristics of the starch granules has a significant effect on their susceptibility to enzymatic degradation, with A group starches being more susceptible to enzymatic degradation than B or C group starches (Gallant *et al.* 1992). *In vivo* digestibility studies with fish appear to confirm this. Energy from wheat starch has been reported to be more digestible by fish than starches classified into the B and C groups. The energy from the carbohydrate component of wheat (Energy ADC 68%) was digested more efficiently than energy from either corn (50%) or barley (36%) by common carp (Degani, Yehuda, Viola & Degani 1997a). Tilapia, (*Oreochromis aureus x O. niloticus*) were more efficient at digesting the energy from the carbohydrate component of wheat flour (Energy ADC 96%) than from either corn (Energy ADC 82%) or barley (Energy ADC 77%) (Degani , Viola & Yehuda 1997b).

Silver perch were more efficient at digesting carbohydrates of lower molecular weight (increased water solubility), such as dextrin, maltose and glucose, when compared to raw wheat starch or raw pea starch. Dextrin was more efficiently digested than raw starch by silver perch. This result is in agreement with results reported for rainbow trout, carp, yellowtail and white sturgeon (Singh & Nose, 1967; Shimeno 1982; Furuichi 1983; Herold, Hung & Fynn-Aikins 1995). The digestion of glucose by silver perch was also high and consistent with glucose ADC's reported for both omnivorous and carnivorous fish (Wilson 1994).

Given that lower molecular weight compounds are water soluble, it is possible that the effects of leaching may have led to overestimations of digestibility for these ingredients. Silver perch produce a relatively intact faecal pellet and when faeces were collected after settlement in a 12 mm diameter, 100 mm long silicone tube, the faeces and all of the water surrounding the faeces in the tube were retained and dried. Any soluble carbohydrate in the water in the faecal collection tube would have been retained. Previous research with a range of ingredients indicates leaching was not a significant pathway of loss of dry matter or protein given the method used (Allan *et al*. 1999).

α-Amylase hydrolyses the α-1,4 or α-1,6- linkages between the glucose units in the starch molecule while α-glucosidase hydrolyses the glucose units in oligosaccharides and maltose to yield single monomers of glucose available for digestion (Gallant *et al*. 1992). The inclusion of water-soluble carbohydrates in the pre-hydrolysed form of dextrin, maltose or glucose into the diet for silver perch effectively minimises the amount of work required from the endogenous α-amylase and α-glucosidase present in the digestive tract. This may explain the enhanced digestibility of these products compared to raw starches.

Due to restrictions on the availability of fish, different sized fish were used for each experiment. Fish of different sizes digested the protein and lipid component of the basal and tests diets in a similar manner (> 90%) indicating that silver perch are efficient at protein and lipid digestion regardless of their size difference. Also carbohydrate at the 30 or 60% dietary inclusion content had little effect on the digestibility of protein or lipid for silver perch. However, the apparent digestibility of raw wheat starch at the 30% inclusion content in Experiment 1 (76.23%) was lower than observed in Experiment 2 (90.20%). This raises the possibility that digestion of wheat starch in silver perch is affected by fish size. Kuz'mina (1996) reported age had an effect on the enzyme activity of several freshwater fish. Shiau (1997) reported larger tilapia (4.55 g) utilised glucose more efficiently for growth than smaller fish (0.46 g), although size did not effect starch utilisation.

Although the inclusion of dietary starch did not influence the whole body composition of silver perch, and liver and muscle TAG and muscle glycogen concentrations remained relatively unaffected, liver glycogen concentrations were elevated and there was some evidence of enlarged livers. The HSI of silver perch was elevated at 60% dietary starch inclusion when gelatinisation was ≥ 25%. It appears the enlarged livers were caused by the deposition of glycogen, as liver triacylglyceride concentrations were not elevated. The effect of increasing solubilisation and inclusion content of carbohydrate on HSI of silver perch is similar to results reported for Chinook

salmon (*Oncorhynchus tshawytscha*) and Siberian sturgeon (*Acipenser baeri*) (Buhler & Halver 1961; Kaushik, Luquet, Blanc & Paba 1989). It does appear, however, that silver perch tolerate higher levels of dietary carbohydrate before an enlargement of the liver is evident. This may be an indication of the relatively high tolerance to dietary carbohydrates by silver perch.

Silver perch were capable of maintaining relatively constant levels of plasma glucose when fed diets containing 30% carbohydrate. However, plasma glucose concentrations of silver perch fed the 30% glucose diet did appear to be slightly elevated. This may have been a result of the rapid uptake of dietary glucose into the blood stream due to it being available in the pre-digested form (Buddington 1987). In contrast Hemre, Waagbo, Hjeltnes & Aksnes (1996) recorded a progressive elevation of plasma glucose concentration in Atlantic salmon fed diets containing dietary starch (ranging from 2.4 – 23%) over a prolonged period. Hemre *et al.* 1989) and Bergot (1979) reported similar responses in cod and rainbow trout fed diets with increasing carbohydrate contents. The differences in post-prandial plasma glucose concentrations of silver perch and the cold water and marine species support the theory that warm water fish are more tolerant to carbohydrate and are more efficient at utilising higher levels of dietary carbohydrate.

The specific growth rate of silver perch was depressed in fish fed diets containing 60% wheat starch regardless of degree of gelatinisation. The incorporation of 60% starch in diets for silver perch may have resulted in excessive levels of non-protein energy that may have overloaded the metabolic enzymes responsible for carbohydrate assimilation. The inclusion of extruded wheat starch at levels of >20% into diets fed to Atlantic salmon also led to a reduction in growth (Arnesen & Krogdahl 1993).

The trend for reduced growth of fish fed glucose as an energy source has been observed in warm water species such as carp, channel catfish and tilapia (Wilson & Poe 1987; Wilson 1994; Shiau 1997). In contrast, cold water and marine species such as, rainbow trout, cod and white sturgeon appear to utilise glucose more efficiently for growth than more complex carbohydrates, such as dextrin or starch (Hung, Fynn-Aikins, Lutes & Xu 1989; Hung & Storebakken 1994; Wilson 1994). Channel catfish had depressed growth when fed diets containing 30% glucose when compared to diets containing dextrin or raw starch (Wilson & Poe 1987). Growth data reported in this study, although inconclusive, points toward the possibility that silver perch utilise either dextrin or gelatinised starch more efficiently than glucose for growth.

In conclusion, silver perch are efficient at digesting carbohydrates of starch origin at 30% dietary content. However, as the dietary inclusion content of starch was increased from 30 to 60% digestibility and growth was reduced. Gelatinisation improved digestibility of wheat starch, especially when included at 60% content. A reduction in the complexity of the starch also enhanced digestibility of carbohydrates. However, the enhanced digestibility of the less complex carbohydrate did not necessarily lead to an improvement in growth. Bearing these factors in mind the production of diets using heat extrusion methods would be beneficial in enhancing the availability of wheat starch as an energy source for silver perch. The reduced digestibility of raw starch and starch at high inclusion contents presents several problems for feed manufacturers. If diets are formulated using gross energy data of ingredients, or assuming inclusion content of starch does not effect digestibility, the digestible energy content of the diet may be under estimated. Effectively, this means a large proportion of raw or gelatinised starch may act simply as a diluent. Diets deficient in digestible energy may retard growth and also contribute to an increase in the amount of waste produced as faeces. Bearing this in mind, energy digestibility data for carbohydrates are essential in diet formulation. However, even when digestibility data is used to formulate diets, the ability of silver perch to utilise and assimilate digestible energy from carbohydrates needs to be considered.

Acknowledgements

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Table 1. Carbohydrate profile, energy and ash composition of test ingredient (dry basis).

Ingredient	Dextrose[1] equivalent (%)	Saccharide type (%)[1]				Energy[2] (MJ kg^{-1})	Ash[2] (%)
		Mono-	Di-	Tri-	Tetra and >		
Raw wheat starch[3]	na	<1	<1	<1	99.0	16.87	<0.1
Pre-Gel wheat starch[3]	na	<1	<1	<1	99.0	16.87	<0.1
Dextrin - Fieldose 9[3]	7-10	0.4	1.2	1.9	96.5	17.15	0.8
Dextrin - Fieldose 17[3]	17-20	1.2	5.4	9.8	83.6	16.56	0.4
Dextrin - Fieldose 30[3]	27-30	3.4	11.5	16.2	68.9	16.41	0.8
Maltose[4]	50	3.0	90	7	0	15.61	<0.1
Glucose[3]	99.5	99.5	<1	<1	0	13.13	<0.1
Raw pea starch[5]	na	<1	<1	<1	99.0	16.50	0.1

[1] Technical data supplied by Manufacturer.
[2] Analysed composition.
[3] Product of Starch Australasia, Summer Hill, Sydney, NSW, Australia.
[4] Product of Sigma Chemicals, Australia.
[5] Product of Nastar, Cosucra S.A., Fontaroy, Belgium.
na = not available.

Table 2. Diets for Experiment 1 (dry basis)[1].

Diet[2]	Amount of Basal diet (g kg^{-1})	Raw wheat starch (g kg^{-1})	Gelatinised wheat starch (g kg^{-1})
1. Basal	1000	-	-
2. 30S 0G	700.0	300.0	-
3. 30S 25G	700.0	225.0	75.0
4. 30S 50G	700.0	150.0	150.0
5. 30S 80G	700.0	60.0	240.0
6. 60S 0G	400.0	600.0	-
7. 60S 25G	400.0	450.0	150.0
8. 60S 50G	400.0	300.0	300.0
9. 60S 80G	400.0	120.0	480.0

[1] Ytterbium chloride was used at 0.1g kg^{-1} as the inert indicator.
[2] S = total starch content in experimental diet (%) and G = degree of gelatinisation (%).

Table 3.   Analysed composition of diets for Experiment 1 (dry basis).

Diet	Starch[1] (g kg^{-1})	Energy MJ kg^{-1}	Protein (g kg^{-1})	Lipid (g kg^{-1})	Ash (g kg^{-1})	CHO[2] (g kg^{-1})
1. Basal	0	22.9	702	148	115	25
2. 30S 0G	300	21.2	505	109	88	298
3. 30S 25G	300	21.0	501	117	86	296
4. 30S 50G	300	21.2	503	109	86	302
5. 30S 80G	300	21.1	505	103	84	308
6. 60S 0G	600	19.3	309	62	44	585
7. 60S 25G	600	20.0	278	57	47	618
8. 60S 50G	600	19.5	275	49	48	628
9. 60S 80G	600	19.8	271	51	42	636

[1] Starch content not analysed.
[2] Carbohydrate calculated by difference; CHO including fibre (%) = 1000-(protein + fat + ash).

Table 4.   Diets for Experiment 2 (dry basis)[1].

Diet	Basal mash (g kg^{-1})	Test ingredient (g kg^{-1})
1. Basal diet	1000	-
2. Raw wheat starch	700	300
3. Gelatinised wheat starch	700	300
4. Dextrin, Fieldose 9	700	300
5. Dextrin, Fieldose 17	700	300
6. Dextrin, Fieldose 30	700	300
7. Maltose	700	300
8. Glucose	700	300
9. Raw pea starch	700	300

[1] Ytterbium chloride was used at 0.1g kg^{-1} as the inert indicator.

Table 5.   Analysed composition of diets for Experiment 2 (dry basis).

Diet	Energy MJ kg^{-1}	Protein (g kg^{-1})	Lipid (g kg^{-1})	Ash (g kg^{-1})	CHO1 (g kg^{-1})
1. Basal[2]	22.4	690	159	123	27
2. Raw wheat starch	20.7	481	106	88	325
3. Gelatinised wheat starch	20.7	474	84	81	361
4. Dextrin, Fieldose 9	20.9	483	92	76	348
5. Dextrin, Fieldose 17	20.2	492	77	88	343
6. Dextrin, Fieldose 30	20.8	492	78	87	343
7. Maltose	20.3	495	109	74	322
8. Glucose	20.7	510	108	81	301
9. Raw pea starch	20.6	484	106	84	326

[1] Carbohydrate calculated by difference; CHO (g kg^{-1}) = 1000-(protein + lipid + ash).
[2] The basal diet contained < 5 g kg^{-1} of glucose derived from either starch or glucose from ingredients.

Table 6. Apparent digestibility coefficients (%) of diets and ingredients, specific growth rate and hepatosomatic index for Experiment 1[1].

Indice	Starch content (%)		Pooled SE (±)	Degree of gelatinisation (%)				Pooled SE (±)	Interaction
	30	60		0	25	50	80		
Diet ADC (%)									
Dry Matter	81.4	62.1*	0.43	66.2[a]	67.9[a]	73.9[b]	79.1[c]	0.61	<0.05
Starch	79.3	54.2*	1.34	58.6[a]	60.3[a]	68.5[b]	79.5[c]	1.90	<0.05
Energy	90.0	70.7*	0.30	76.2[a]	77.4[a]	81.6[b]	86.3[c]	0.42	<0.05
Lipid	98.3	95.1*	0.72	96.0[ab]	94.3[a]	97.5[ab]	99.0[b]	1.01	ns
Protein	94.4	93.9	0.18	93.8	93.7	94.4	94.6	0.25	ns
Ingredient ADC (%)									
Energy	73.2	48.5*	1.38	51.1[a]	53.01[a]	64.4[b]	74.8[c]	1.91	<0.05
Growth performance(%)									
SGR (%)[2]	2.90	2.30*	0.05	2.72	2.63	2.62	2.41	0.07	ns
HIS (%)[3]	2.67	3.48*	0.11	2.67[a]	2.88[ab]	3.23[bc]	3.52[c]	0.16	<0.05

[1] Values are means±se. For starch content * denotes significant difference (*n* = 12 pooled replicate tanks for starch content). For gelatinisation means in the same row with the same letter in the superscript are not significantly different (*n* = 6 pooled replicate tanks) (two-way ANOVA, SNK, *P* > 0.05).

[2] SGR% = 1n (final wt) – 1n (initial wt) / days x 100.

[3] HIS% = wet weight liver/whole wet body weight x 100.

Table 7. Results for hepatosomatic index (HSI %), and starch, dry matter and energy dietary and ingredient digestibility coefficients analysed using one-way ANOVA for Two-way interactions observed in Experiment 1[1].

Diet	HIS (%)	DM ADC Diet (%)	Energy ADC Diet (%)	Energy ADC Ingredient (%)	Starch ADC (%)
1. Basal	2.70±0.50[a]	83.6±0.46[f]	95.3± 0.30[h]	-	-
2. 30% S 0% G	2.58± 0.70[a]	79.1±0.67[e]	88.6± 0.19[ef]	67.3±0.77[d]	76.2±1.02[de]
3. 30% S 25%G	2.62± 0.30[a]	79.5±0.27[e]	86.6± 1.63[e]	65.8±1.56[d]	73.0±1.85[d]
4. 30% S 50%G	2.76± 0.60[a]	82.6±0.60[f]	90.7± 0.34[fg]	76.2±1.42[e]	79.1±0.73[e]
5. 30% S 80%G	2.72± 0.70[a]	84.6±0.68[f]	92.5± 0.29[g]	83.6±1.22[f]	88.6±1.36[f]
6. 60% S 0% G	2.74± 1.30[a]	53.3±1.30[a]	63.7± 0.92[a]	34.9±1.74[a]	40.9±2.26[a]
7. 60% S 25%G	3.14± 1.30[a]	56.3±1.33[b]	66.5± 1.01[b]	40.2±1.92[b]	47.5±3.35[b]
8. 60% S 50%G	3.71± 0.60[ab]	65.1±0.58[c]	73.1± 0.30[c]	52.6±0.56[c]	57.9±0.32[c]
9. 60% S 80%G	4.32± 0.90[b]	73.5±0.87[d]	80.1±0.71[d]	66.0±1.34[d]	70.4±1.30[d]

[1]Means±se in the same column with the same superscript are not significantly different ($n = 3$ pooled replicate tanks) (one-way ANOVA, SNK, $P > 0.05$).

Table 8. Diet and ingredient apparent digestibility coefficients (ADC%), specific weight gain (SGR %), hepatosomatic index (HSI %), and liver and muscle glycogen concentrations (mg glycogen g^{-1} wet tissue) of silver perch fed the basal diet and diets containing different carbohydrates at 30% inclusion content for Experiment 2[1].

Treatment	ADC of diets (%)				Energy ADC (%) ingredient	SGR (% day)	HIS (%)	Liver glycogen	Muscle glycogen
	Dry Matter	CHO[2]	Energy	Protein					
Basal	84.5±0.3[b]	-	95.4±0.2[c]	95.1±0.1[ab]	-	1.70±0.21	1.78±0.3	54.6±3.0[a]	0.48±0.2
Raw wheat Starch	85.0±0.2[b]	90.0±0.2[b]	92.5±0.2[b]	93.9±0.1[a]	83.4±0.8[b]	1.47±0.03	2.24±0.2	61.5±0.5[b]	1.40±0.1
PG wheat starch	88.8±0.4[c]	98.1±0.3[c]	95.3±0.4[c]	94.7±0.2[ab]	93.4±1.4[cd]	1.73±0.20	2.49±0.4	59.5±1.2[ab]	1.40±0.3
Fieldose 9	90.0±0.1[c]	99.7±0.1[c]	96.1±0.1[c]	94.8±0.1[ab]	101.3±0.3[e]	1.77±0.13	1.97±0.1	61.9±1.3[b]	1.86±0.4
Fieldose 17	88.3±1.1[c]	99.6±0.1[c]	95.4±0.6[c]	94.8±0.7[ab]	95.7±2.5[d]	1.60±0.21	2.05±0.2	60.2±1.1[ab]	1.93±0.5
Fieldose 30	89.2±0.1[c]	99.7±0.1[c]	96.1±0.1[c]	95.2±0.1[ab]	94.5±0.3[cd]	1.60±0.10	1.87±0.1	59.0±0.7[ab]	1.09±0.2
Maltose	90.3±0.2[c]	99.7±0.1[c]	96.3±0.1[c]	95.6±0.1[b]	91.2±0.6[c]	1.70±0.06	1.85±0.1	63.0±1.1[b]	0.80±0.3
Glucose	90.2±0.2[c]	99.8±0.1[c]	96.6±0.1[c]	96.0±0.1[b]	92.5±0.2[cd]	1.40±0.12	1.83±0.2	59.6±0.9[ab]	1.12±0.3
Raw pea starch	82.6±0.9[a]	75.8±2.5[a]	89.3±0.6[a]	94.7±0.2[a b]	69.9±2.6[a]	1.50±0.17	2.31±0.2	62.6±0.3[b]	0.88±0.1

[1] Means±se (n = 3 replicates) in the same column which share the same superscript are not significantly different (one-way ANOVA, SNK, $P > 0.05$).
[2] CHO = carbohydrate of starch origin.

Table 9.   Gross and digestible energy contents of test ingredient for silver perch (dry basis).

Ingredient	Dietary inclusion (%)	Gross energy (MJ kg^{-1})	Energy ADC (%)	Digestible energy [1] (MJ kg^{-1})
Experiment 1				
Wheat starch 80% gelatinised	30	16.87	83.61	14.11
Wheat starch 50% gelatinised	30	16.87	76.24	12.86
Wheat starch 25% gelatinised	30	16.87	65.79	11.10
Raw wheat starch	30	16.87	67.28	11.35
Wheat starch 80% gelatinised	60	16.87	66.01	11.14
Wheat starch 50% gelatinised	60	16.87	52.60	8.87
Wheat starch 25% gelatinised	60	16.87	40.24	6.79
Raw wheat starch	60	16.87	34.94	5.89
Experiment 2				
Dextrin - Fieldose 9	30	17.15	100.00	17.15
Dextrin - Fieldose 17	30	16.56	95.70	15.85
Dextrin - Fieldose 30	30	16.41	94.50	15.51
Gelatinised wheat starch	30	16.87	93.40	15.76
Glucose	30	13.13	92.50	12.15
Maltose	30	15.61	91.20	14.24
Raw wheat starch	30	16.87	83.40	14.07
Raw pea starch	30	16.50	69.60	11.48

[1] Digestible energy = gross energy x energy ADC.

4.6.   Carbohydrate utilisation by juvenile silver perch *Bidyanus bidyanus* (Mitchell): III. The protein sparing effect of wheat starch based carbohydrates

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Abstract

The ability of juvenile silver perch (*Bidyanus bidyanus*) to utilise dietary raw wheat meal, raw wheat starch, gelatinised wheat starch or dextrin as energy sources to spare protein for growth was quantified. Energy utilisation and protein sparing were assessed by comparing the weight gain, energy retention efficiency, protein retention and body composition of silver perch that had been fed a series of diets in which the basal diet (low carbohydrate) was systematically replaced with graded levels of each carbohydrate ingredient or an inert diluent, diatomaceous earth. The protein content decreased as the carbohydrate content increased, giving four different protein to energy ratios for each of the four carbohydrate sources (except for the 60% inclusion level where only 3 carbohydrate sources were tested).

Silver perch were efficient at utilising carbohydrate for energy to spare protein. Silver perch fed diets containing up to 30% wheat meal, raw wheat starch, gelatinised wheat starch or dextrin exhibited similar growth, protein retention and energy retention efficiency to the fish fed the basal diet. Weight gain of silver perch fed diets containing wheat meal or carbohydrates at 45% inclusion content had significantly reduced weight gain when compared to fish fed the basal diet. However, protein retention and energy retention efficiency were similar or better. Whole body protein levels of silver perch remained constant regardless of carbohydrate sources and there was no evidence of increasing whole body lipid concentrations for fish fed diets with up to 60% dietary carbohydrate. Silver perch were more efficient at utilising processed starch (either gelatinised starch or dextrin), than wheat meal or raw wheat starch.

Introduction

Protein is the most expensive macro-nutrient in fish diets and, if over supplied, amino acids excess to the fishes immediate requirements may be catabolised for energy rather than growth (Phillips 1972). A great deal of research has been conducted on the protein sparing potential of non-protein ingredients, such as lipids and carbohydrates (Phillips 1972; Watanabe, Takeuchi & Ogino 1979; Cho & Kaushik 1985; Beamish & Medland 1986; Wilson & Poe 1987; Ellis & Reigh 1991; Shiau & Peng 1993; Brauge, Medale & Corraze 1994; Wilson 1994). Lipids, such as fish oil, have been shown to be an excellent alternative energy source to protein and, at the correct ratio to protein, have been demonstrated to exhibit a significant protein sparing effect (Phillips 1972; Watanabe *et al*. 1979; Cho & Kaushik 1985). While lipids are an excellent energy source, they are also expensive relative to plant carbohydrates.

Compared with warm-blooded animals, fish have poor control over their blood glucose levels (Furuichi & Yone 1981, 1982; Wilson 1994). Following carbohydrate digestion, blood glucose concentrations in fish rapidly increase and remain elevated for many hours (Wilson, 1994). Absorbed carbohydrate may be used immediately as energy, stored as glycogen in the liver and

muscle, synthesised into compounds such as triglycerides and non-essential amino acids or excreted (Furuichi 1983; Lovell 1988). As amino acids are not stored as such, excess amino acids may be deaminated and used for immediate energy requirements, or converted to lipid, carbohydrate or other compounds. Because fish oxidise deaminated amino acids for energy more efficiently and preferentially than glucose, only sufficient quantities of protein to meet anabolic requirements should be supplied in the diet to gain a protein sparing effect using carbohydrate (Lovell 1988).

Starch and non-starch polysaccharides (NSP) are the predominant groups of carbohydrates present in plant ingredients used for the production of aquaculture feeds. NSPs have a structural role in plants and seeds and are relatively indigestible. Therefore, they are unavailable to most warm and cold water fish as an energy source. Starch is comprised predominately of glucose and is potentially a source of energy. Hence, much effort has been directed at investigating the ability of fish to utilise energy from starch either in its raw or processed form (Buhler & Halver 1961; Singh & Nose 1967; Wilson & Poe 1987; Wilson 1994; Shiau 1997). Molecular complexity, physical state and inclusion content of starch are factors that have been reported to have an influence on both digestibility and efficient utilisation (Buhler & Halver 1961; Singh & Nose 1967; Anderson, Jackson, Matty & Capper 1984; Wilson & Poe 1987; Wilson 1994; Shiau 1997). While digestibility data is essential in formulating well balanced diets, digestibility is not the same as utilisation and ingredients can be well digested but poorly utilised. For example, Furuichi, Taira & Yone (1986) reported that yellowtail (*Seriola quinqueradiata*) digested glucose more efficiently (ADC 94%) than gelatinised potato starch (ADC 52%), at the 30% dietary inclusion level, but fish fed the glucose diet grew poorly compared with those fed the potato starch diet.

Silver perch (*Bidyanus bidyanus)* is an omnivorous freshwater species, currently being cultured in Australia on relatively low protein (≤ 35%) diets containing moderate levels of plant carbohydrates, including starch rich ingredients such as wheat and field peas (Allan, Rowland, Mifsud, Glendenning, Stone & Ford 2000a)). Previous research investigating carbohydrate tolerance, digestibility and utilisation by silver perch has indicated that this species has excellent potential to use carbohydrate to spare protein (Stone, Allan & Anderson, in press a), b); Allan, Booth, Stone, Williams & Smith 2000b); Allan, Parkinson, Booth, Stone, Rowland, Frances & Warner-Smith 2000c)).

Carbohydrate tolerance tests have indicated that the ability of silver perch to uptake and clear glucose from the bloodstream is comparable to that of carp and tilapia; both of which efficiently utilise carbohydrate (Furuichi & Yone 1981, 1982; Shikata, Iwanaga & Shimeno 1994; Lin, Ho & Shiau 1995; Stone *et al.* in press (a)). Carbohydrate digestibility is influenced by inclusion level and carbohydrate complexity for silver perch. Energy digestibility values ranged from 83 to 91 to 100% for raw wheat starch, gelatinised wheat starch, and dextrin respectively when included in the diet at 30% (Stone *et al.* in press b). Energy digestibility values for wheat starch for silver perch decreased from 71 to 48% when inclusion level increased from 30 to 60% (Stone *et al.* in press b). Compared to warm water omnivorous species currently being cultured such as channel catfish, carp or tilapia, silver perch appear to be relatively efficient at digesting starch and its less complex breakdown products (Wilson & Poe 1987; Lovell 1989; Hernandez, Takeuchi & Watanabe 1994; Shiau & Liang 1995). However, little quantifiable data is available on how well silver perch utilise digested carbohydrates. In this study we measured the utilisation of wheat meal, raw wheat starch, gelatinised wheat starch and dextrin as protein-sparing energy sources for silver perch.

Materials and Methods

Experimental fish and holding facilities

Silver perch (mean weight 26.5 g, range 23.3 - 30.1 g) were obtained from the NSW Fisheries Grafton Research Centre, and transported to the NSW Fisheries Port Stephens Fisheries Centre (PSFC). Prior to the experiment fish were held in 10 000-L tanks supplied with re-circulating freshwater (bore water; salinity 0.05 g L^{-1}) and fed twice a day with a commercial silver perch diet (95LC2, Allan *et al*. 2000a) (digestible protein, 34%, lipid, 9% and digestible energy 14 MJ kg^{-1}) until transferred to the experimental cages. Water temperature was held at 24 ± 2°C by the use of two, 2 kw immersion heaters.

Experimental diets

Four carbohydrates of wheat origin were evaluated in this study: raw wheat meal; raw wheat starch; gelatinised (100%) wheat starch; and dextrin (Table 1). A high protein, low carbohydrate basal diet was used. The basal diet contained the following (g kg^{-1}): Chilean fish meal, 801.5; corn gluten, 113.5; cod liver oil, 70; vitamin premix, 7.5; mineral premix, 7.5: The vitamin and mineral premixes were as described by Allan & Rowland (2002) except 1.0 mg biotin (2%) and 0.33 mg sodium selenite (44%) were added. For all other test diets, part of the basal diet was substituted with one of the carbohydrate test ingredients at 15, 30, 45 or 60%, or diatomaceous earth (as an inert filler) at 15, 30 or 45%. Diets containing 60% dextrin or 60% diatomaceous earth were not included in the experiment as it was not possible to manufacture pellets with this high level of either ingredient. A total of 19 diets were manufactured and evaluated (Tables 2 & 3).

A single batch of basal diet was prepared by mixing and grinding through a 1.5 mm screen in a hammer mill (Raymond Laboratory Mill, Transfield Technologies Pty Ltd, Rydalmere, NSW, Australia) and then, when required, substituting with individual test ingredients. All diets contained the same amount of the vitamin and mineral premixes. After dry mixing, approximately 600 mL of distilled water was added per kg of mash, and each batch was mixed using a Hobart mixer (Troy Pty Ltd, OH, USA). The wet mash was then cold pelleted through a meat mincer fitted with a 3 mm pellet die (Barnco Australia Pty Ltd, Leichhardt, NSW, Australia) to produce a 3 mm sinking pellet. After pelleting, diets were dried in a convection drier at < 35°C for approximately 6 h until all diets had moisture contents of < 10%.

Experimental facilities

The experiment was done in a re-circulating system comprised of ten 10 000-L fibreglass tanks in a 40 x 15 m plastic greenhouse. Each 10 000-L tank contained six cylindrical floating cages spaced evenly around the perimeter. Floating cages were 200 L in capacity (diameter = 0.6 m; submerged depth = 0.7 m) with walls constructed of 9 mm plastic mesh and the top and bottom were constructed of 1.6 mm plastic mesh (Kinnears Pty Ltd, Footscray, Vic. Australia). A plastic tray (diameter 0.5 m) was placed on the bottom of each cage to collect uneaten food. Each 10 000-L tank was covered with a black shade cloth lid to reduce algae growth. Freshwater (0.05 g L^{-1}), filtered through a rapid sand filter, was supplied to each experimental tank at a flow-rate of 17 L min^{-1}. Effluent water from each tank flowed out through the bottom of the tank into a 2 m^3 biological filter within a common 7000-L reservoir. This water was then returned to the sand filter. Aeration was provided to each tank by two air-stone diffusers and each tank was heated using a 2 kw immersion heater. Fluorescent lighting was used to control photoperiod at 16 h light:8 h dark. Tanks were siphoned every two weeks to remove accumulated wastes.

Experimental procedure

At stocking, fish were captured by dip net from each 10 000-L holding tank and anaesthetised in a 200-L container using a bath of clove bud oil (17 mg L^{-1}) (Branson and Jacobs, Sydney, NSW, Australia). The fish were then caught at random, individually weighed, and distributed among the 200-L cages by systematic interspersion.

Each of the 19 diets was allocated to 3 randomly selected cages (n = 3 cages diet^{-1}). Cages were stocked with 10 fish which were hand fed twice daily (0830 and 1500 h) on their respective restricted ration (85% satiation) for a period of 61 days. Ten min following feeding, uneaten food was collected from the tray at the bottom of each cage and the dry weight recorded. The biomass of each cage was weighed fortnightly over the course of the experiment with feed withheld for 24 h prior to weighing. At the end of the growth trial, fish were harvested and weighed individually.

Water quality analysis

During the experiment, water temperature (range 24-28°C), dissolved oxygen (above 6.0 mg L^{-1}), and pH (between 7.4 and 8.5) were measured daily using a Yeo-Kal 611 water quality analyser (Yeo-Kal Electronics, Brookvale, Sydney, NSW Australia). Nitrite and ammonia (<60ug L^{-1} NO_2-N and <100 ug L^{-1} total ammonia-N respectively) were measured weekly using colourmetric methods described by Major, Dal Pont, Kyle & Newell (1972) and Dal Pont, Hogan & Newell (1973).

Plasma glucose and blood sampling

At the completion of the experiment blood samples were obtained from three fish from each cage to measure plasma glucose. To minimise the disturbance to the fish during the collection of blood samples for glucose analysis at the final weight check, each 10 000-L tank was divided into six segments using black plastic sheeting two weeks before the completion of the growth trial. One cage was held within each segment. Feed was withheld from fish for 24 h prior to blood sampling. Blood samples were obtained from fish within 1 min of initial disturbance of each cage to eliminate the confounding effects of stress on blood glucose levels (Stone *et al.* in press a).

Blood samples were obtained from the caudal vessel of each fish using a 1mL syringe and a 27 gauge hypodermic needle. Following collection, blood was transferred immediately into a 1.5 mL micro-tube containing the anti-coagulant, EDTA (5 mg mL blood^{-1}) and the glycolysis inhibitor NaF (2 mg mL blood^{-1}). Blood samples were immediately centrifuged at 1250 g and the plasma was separated. Samples were stored at -20°C prior to glucose analysis.

Biochemical analyses

Whole body proximate composition of four fish (freeze dried, ground and homogenised) randomly selected from each cage were determined at the end of the feeding trial. All feed analyses were carried out in duplicate. Values for dry matter, lipid, energy (bomb calorimetry) and ash were determined for fish and diets following procedures described in AOAC (1990). Nitrogen was determined using Kjeldahl or semi-micro Kjeldahl methods (AOAC 1990) and crude protein content was estimated by multiplying nitrogen by 6.25. Plasma glucose was determined from all blood samples using the glucose oxidase – peroxidase method (Fleming & Pegler 1963) (Sigma 510A method).

Performance indices

Individual weight gain % [= (final weight – initial weight) / (initial weight x 100)], feed consumption and feed conversion ratio [FCR = dry weight feed consumed/ wet weight gain of fish] were determined for each cage to allow comparisons of fish performance among different diets.

Indices for protein retention [PR % = (final body protein – initial body protein) / protein intake x 100] and energy retention efficiency [ERE % = (final body energy – initial body energy) / energy intake x 100] were calculated.

Statistical analyses

Statistical evaluation of the data was carried out using the *Statgraphics Plus* for Windows 4.1 (1998) software package (Manugistics Inc., Rockville, MD, USA). Homogeneity of variances was assessed using Cochran's C Test (Winer 1991). Initially the experiment was designed for analysis using two-factor ANOVA with ingredient inclusion content (15, 30, 45 and 60%) and ingredient type (wheat, raw wheat starch, gelatinised wheat starch, dextrin and diatomaceous earth) as the two fixed factors. However, due to the problems associated with the manufacture of the diets containing 60% dextrin and 60% diatomaceous earth, which resulted in an unbalanced two-factor ANOVA, two sets of data that were statistically analysed (all of the diet series containing 15 - 45% or 15 - 60%). This analysis allowed investigation of the main effects of both carbohydrate inclusion content and ingredient type as well as any interactions that occurred between the two factors. There were significant interactions found for all indices except plasma glucose and whole body protein levels, so one-factor ANOVA was used to compare the indices for all treatments (including the basal diet), both at each inclusion level within each diet series and also between ingredient types at each separate inclusion level. Where significant differences were found, comparison between means were made using Student Newman-Kuel's multiple range test. Means were considered significant at $P < 0.05$. Unless otherwise stated, all results appear as mean ± standard error of the mean ($n = 3$).

Results

Performance indices

Weight gain of silver perch was similar for fish fed the basal diet or diets containing wheat meal, raw wheat starch, gelatinised wheat starch or dextrin at inclusion contents of up to 30% (Table 4). For each ingredient, fish weight gain declined at inclusion levels above 30%. Silver perch fed diatomaceous earth diets which contained greater than 15% of the filler exhibited a significant reduction in weight gain when compared to fish fed the basal diet or fish fed any of the carbohydrate test ingredients.

FCR was similar for fish fed the basal diet or diets with up to 45% carbohydrate, but deteriorated when carbohydrate content increased to 60% (Table 4). FCR for diets containing diatomaceous earth decreased with increasing inclusion content (Table 4).

Energy retention efficiency was similar for fish fed the basal diet or diets with any of the carbohydrates at up to 45% inclusion content (Table 4). Beyond this inclusion content, energy retention efficiency declined with the biggest reduction being for raw wheat starch, wheat meal and gelatinised wheat starch (Table 4). Energy retention efficiency decreased with increasing content of diatomaceous earth (Table 4).

Protein retention was similar for the basal diet and for diets containing up to 60% wheat meal, 60% gelatinised wheat starch and 45% dextrin (Table 4). There was a significant reduction in protein retention for diets containing 60% raw wheat starch or 45% diatomaceous earth (Table 4).

Proximate body composition

Whole body protein concentrations ranged from 16.8 to 18.6 g 100 g^{-1} and there were no significant effects of diet, inclusion content or their interactions for any of the diets.

Fish fed the gelatinised starch or dextrin had similar concentrations of body dry matter (DM) to fish fed the basal diet regardless of the inclusion level. There was a significant reduction in body DM of silver perch fed diets containing wheat meal (> 45% inclusion) or raw wheat starch (> 45% inclusion) compared to fish fed the basal diet (Table 5). There was a significant reduction in body DM content of fish fed the diatomaceous earth at > 30% inclusion contents compared to fish fed the basal diet.

Fish fed the gelatinised starch or dextrin series of diets had similar concentrations of whole body lipid as fish fed the basal diet regardless of the inclusion level. There was a significant reduction in body lipid concentrations of silver perch that were fed diets containing wheat meal (> 45% inclusion), raw wheat starch (> 30% inclusion), or diatomaceous earth (> 15% inclusion level) compared to fish fed the basal diet (Table 5).

Fish fed the gelatinised starch or dextrin series of diets had similar concentrations of whole body gross energy (GE) as fish fed the basal diet regardless of the inclusion level. There was a significant reduction in body GE concentrations of silver perch which were fed diets containing wheat meal (> 45% inclusion) or raw wheat starch (> 30% inclusion) compared to fish fed the basal diet (Table 5). There was a significant reduction of body GE content in fish fed the diatomaceous earth diets at > 15% inclusion contents compared to fish fed the basal diet or any of the carbohydrate test ingredients at > 15% inclusion content.

Silver perch fed the wheat meal or dextrin series of diets had similar concentrations of whole body ash as fish fed the basal diet regardless of the inclusion level. There was a significant increase in body ash concentrations of silver perch that were fed diets containing raw wheat starch or gelatinised wheat starch at > 45% inclusion content when compared to fish fed the basal diet (Table 5). There was a significant increase of body ash content in fish fed the diatomaceous earth diets at > 30% inclusion contents compared to fish fed the basal diet or any of the diets containing carbohydrate at > 15% inclusion content.

Plasma glucose

Plasma glucose concentrations ranged from 3.99 mM to 4.37 mM. There was no significant effect of diet, inclusion content, or their interaction, on the plasma glucose concentrations of silver perch.

Discussion

With each progressive increase in diatomaceous earth content (reduction in basal diet), there was a progressive reduction in growth performance as indicated by a decrease in weight gain, energy and protein retention and an increase in FCR. There was also a reduction in whole body lipid and gross energy content with a concomitant increased in whole body ash content. As the digestible protein:energy ratio of the diatomaceous earth series of diets remained constant (3:1) the observed reduction in growth performance was attributed to a progressive reduction in digestible dietary protein and energy intake. A significant reduction in growth performance was not evident in any of the diet series containing carbohydrate until the dietary inclusion content of the test carbohydrate exceeded 30%. The difference between weight gain (and other indices) of fish for the series of diets containing test carbohydrates and diatomaceous earth is a quantification of carbohydrate utilisation in silver perch. The positive relationship between PR and increasing dietary carbohydrate content

(up to the 45% inclusion content) for each series of diets demonstrates the protein sparing effect of the carbohydrate source. Gelatinised starch and dextrin were utilised most efficiently as energy sources to spare protein although differences between ingredients were not significant except at the 60% inclusion level where raw wheat starch was significantly poorer than other ingredients.

The interpretation above relies on the assumption that diatomaceous earth is a nutrient diluent and does not interfere with feed intake, digestibility or growth of silver perch. This assumption is supported by the lack of evidence of reduced palatability of diets due to the incorporation of diatomaceous earth. Silver perch readily accepted diets containing diatomaceous earth, with no apparent difference in the time taken to consume their daily ration compared to the basal diet (< 2 min). The linear reduction in weight gain of silver perch fed increasing amounts of diatomaceous earth was consistent with previous research where silver perch (Allan, Frances & Booth 1998) and European sea bass (Dicentrarchus labrax) (Dias, Huelvan, Dinis & Métailler 1998) were fed diets containing inert fillers, indicating that the diatomaceous earth used in this study had minimal negative nutritional effects. Allan *et al*. (1998) reported that juvenile silver perch, fed a series of diets in which α-cellulose or diatomaceous earth replaced similar contents of a control diet, exhibited similar weight gain, FCR, and protein retention at each inclusion level. Dias *et al*. (1998) investigated the effects of incorporating of silica (similar to diatomaceous earth), cellulose or zeolite at either 10 or 20% inclusion levels as bulking agents (inert fillers) in diets on growth performance of juvenile European sea bass. When feed rates were adjusted in proportion to the percent dilution of the control diet without the bulking agent, there were no significant effects of filler on protein digestibility, protein retention or weight gain indicating that the fillers did not effect assimilation of dietary nutrients.

The inclusion of up to 60% carbohydrate in the diets had no effect on plasma glucose concentrations of silver perch 24 h following a meal. Plasma glucose concentrations were within the range of normal concentrations found for similar sized silver perch (~ 3.44 mM) (Stone *et al.* in press a). Stone *et al.* (in press a) found that silver perch are able to clear glucose from the blood stream and attain basal plasma glucose levels within 12 h of an intra-peritoneal injection of glucose at 0.1% BW. In contrast, Hemre, Waagbø, Hjeeltnes & Aksnes (1996) recorded a progressive elevation of plasma glucose concentration in Atlantic salmon (*Salmo salar* L.) fed diets containing from 2.4 to 23% dietary starch over a prolonged period. Hemre, Lie, Lied & Lambertsen (1989) and Bergot (1979) reported similar responses in cod and rainbow trout fed diets with increasing carbohydrate contents. The differences in post-prandial plasma glucose concentrations of silver perch and the cold water and marine species support the theory that warm water fish are more tolerant to glucose and are more efficient at utilising higher levels of dietary carbohydrate (Wilson, 1994).

The growth of fish is affected by both the inclusion content of dietary starch and level of complexity (Wilson & Poe 1987; Hung, Fynn-Aikins, Lutes & Xu 1989; Shiau & Peng 1993; Hung & Storebaken, 1994; Shikata *et al*. 1994). Based on weight gain and protein and energy retention, it appears that silver perch were more efficient at utilising carbohydrates that had undergone some form of processing, i.e., gelatinisation or dextrinisation. These findings support previous studies where the energy from dextrin and gelatinised wheat starch were more efficiently digested than wheat meal or raw wheat starch by silver perch (Stone *et al* in press b).

The protein retention observed for silver perch in this study was comparable to calculated PR values reported for silver perch fed commercial diets at different feed rates (Harpaz, Jiang & Sklan 2001). Weight gain and protein retention of silver perch fed carbohydrates in the current study also exhibited similar trends to tilapia fed diets containing graded levels of raw maize starch or dextrin at up to 40% of the diet (Anderson *et al*. 1984). As for silver perch, tilapia, a warm water species, exhibited better growth performance and protein sparing when fed diets containing dextrin as opposed to raw starch at each inclusion content.

Previous research has indicated that although silver perch digest simple carbohydrates such as glucose extremely efficiently (~100%) when included in the diet at 30% inclusion content, weight gain was increased by 46% for fish fed gelatinised wheat starch at the same dietary inclusion content (Stone *et al.* in press b). This is in contrast to cold water or marine species, which utilise less complex carbohydrates such as glucose or maltose more efficiently to spare protein (Hung, Fynn-Aikins, Lutes & Xu 1989; Hung & Storebaken 1994,).

In the case of raw wheat starch and wheat meal, the comparative reduction in growth performance of silver perch would have resulted from the lower digestibility of these ingredients at greater than 30 and 45% inclusion contents, respectively. Previous digestibility experiments using silver perch have indicated that the digestibility of wheat starch is negatively correlated with increasing inclusion content and energy digestibility of wheat is relatively low when included at 30% (Energy ADC ~53%) (Allan *et al.* (2000c); Stone *et al.* in press b). The slightly improved performance of fish fed the wheat meal compared to raw wheat starch may be attributed to the slightly higher protein concentrations in the wheat meal diets compared to the raw wheat starch diets (Table 2).

It has been reported that fish do not have a carbohydrate requirement (NRC 1993). However, the recommended dietary inclusion of digestible carbohydrate is up to 20% for diets fed to salmonids and marine fish and up to 40% for warm water species (see review, Wilson 1994). Recent research with rainbow trout suggests that carbohydrates play a vital role in fish nutrition and growth. Peragón, Barroso, Garcia-Salguero, de la Higuera & Lupiáñez (1999) fed rainbow trout two diets, one containing no digestible carbohydrate the other 23% digestible carbohydrate in the form of pre-cooked starch, and reported suppressed daily weight gain for fish fed the diet with no digestible carbohydrate. Feed conversion efficiency, protein retention, white muscle weight gain and the RNA:DNA ratio were also reduced in rainbow trout that were fed the diet with no digestible carbohydrate. Peragón *et al.* (1999) concluded that the growth suppression resulted from white muscle cell hypotrophy (rather than a reduction in cell numbers) from increased protein degradation and decreased protein synthesis. Effectively, the absence of dietary carbohydrate led to a significant proportion of the amino acids from digested dietary protein and muscle protein breakdown being utilised for gluconeogenic purposes and not for protein synthesis and growth (Peragón *et al.* 1999). An interesting finding in the current study was that there was a trend for slightly better all round growth performance in silver perch which were fed diets containing gelatinised starch or dextrin at 15% inclusion content than fish fed the basal diet. Although effects were not significant, the trend suggests that the inclusion of digestible carbohydrate in diets for silver perch may be beneficial. This is worthy of further investigation.

High levels of dietary energy originating from carbohydrate have been reported to produce fatty fish (Anderson *et al.* 1984; Shiau & Peng 1993; Hemre, Sandes, Lie & Waagbø 1995; Yamamoto & Akiyama 1995; Catacutan & Coloso 1997; Shiau 1997; Nankervis, Matthews & Appleford 2000). However, whole body lipid levels of silver perch in this study were comparable to those of similar sized silver perch (13% lipid) fed a commercial diet containing 32% digestible protein and 13 MJ kg digestible energy^{-1} (protein to energy ratio of 2.5:1) (Stone, Allan, Parkinson & Rowland 2000) and there was a slight reduction of body lipid in silver perch fed diets containing greater than 30% raw wheat starch or greater than 45% wheat meal. This reduction in body lipid level may have been due to the restricted energy availability resulting from inefficient digestibility, which led to the mobilisation of body lipid to meet energy requirements for growth and maintenance. Similar inverse relationships between dietary carbohydrate level and whole body lipid and energy content for turbot (*Scophthalmus maximus*) and walking catfish (*Clarius batrachus*) fed increasing levels of non-protein energy (starch) have been reported (Nijhof & Bult 1994; Erfanullah & Jafri 1998).

In conclusion, the inclusion of wheat starch or its constituent breakdown products into diets for silver perch has a protein sparing effect that may increase growth and, therefore, reduce feed costs.

Silver perch are able to utilise the dietary carbohydrates tested in his study at levels of up to 30% inclusion in diets containing ~42% digestible protein and digestible energy levels of ~19 MJ kg^{-1}. The efficiency of utilisation of each product was positively related to digestibility. Silver perch were more efficient at utilising dextrin and gelatinised wheat starch as energy sources for growth than wheat meal or raw wheat starch. As it is likely that raw wheat starch or its constituent breakdown products will be supplied from wheat meal in commercial diets for silver perch, it is essential that diets undergo some form of heat processing to increase gelatinisation.

Acknowledgments

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Table 1. Analysed composition of diet ingredients (dry basis).

Diet	Composition (g 100 g^{-1} or MJ kg^{-1})					
	Dry matter	Crude protein	Fat	Gross energy	Ash	CHO[1]
Fish meal	90.4	72.6	7.4	19.8	18.0	2.0
Corn gluten	90.2	38.4	2.3	21.1	1.2	58.1
Raw wheat meal	88.8	13.5	1.5	17.9	2.1	82.9
Raw wheat starch[2]	88.2	0.5	0.0	16.9	0.2	99.3
Gelatinised wheat starch[2]	94.2	0.3	0.0	16.6	0.2	99.5
Dextrin[2]	94.7	0.1	0.0	16.6	0.5	99.4

[1] CHO = total carbohydrate (including fibre), calculated by difference (Total CHO g 100 g^{-1} = (100 – protein + fat + ash).
[2] Supplied by Starch Australasia, Summer Hill, Sydney NSW, Australia.

Table 2. Analysed composition of experimental diets (dry basis).

Diet	Composition (g 100 g^{-1} or MJ kg^{-1})					
(Test ingredient inclusion content into basal diet)	Dry matter	Crude protein	Lipid	Gross energy	Ash	Total CHO[1,2]
Basal diet (100)	92.3	63.1	13.4	21.2	15.6	7.9
Wheat meal (15)	93.1	55.6	11.4	20.6	13.8	19.2
Wheat meal (30)	94.8	47.7	10.0	19.9	11.7	30.6
Wheat meal (45)	89.8	40.1	7.9	19.6	9.8	42.2
Wheat meal (60)	91.3	33.5	5.9	18.7	7.9	47.3
Raw wheat starch (15)	94.5	54.2	10.6	20.2	13.3	21.9
Raw wheat starch (30)	92.4	44.7	9.0	19.5	10.8	35.5
Raw wheat starch (45)	90.5	35.1	7.1	18.7	8.8	49.0
Raw wheat starch (60)	92.0	25.4	5.4	18.4	6.6	62.6
Gelatinised wheat starch (15)	94.7	54.0	10.4	20.3	13.2	22.4
Gelatinised wheat starch (30)	92.2	43.9	8.4	20.9	11.0	36.7
Gelatinised wheat starch (45)	91.2	35.2	7.0	18.9	8.9	48.9
Gelatinised wheat starch (60)	91.7	24.8	4.9	18.1	6.5	63.8
Dextrin (15)	94.7	54.8	10.9	20.3	13.3	21.0
Dextrin (30)	90.3	44.7	8.3	19.3	11.3	35.7
Dextrin (45)	89.3	35.2	7.1	19.4	8.9	48.8
Diatomaceous earth (15)	95.5	53.6	11.4	17.7	27.5	7.5
Diatomaceous earth (30)	96.2	44.0	9.0	14.6	40.2	6.8
Diatomaceous earth (45)	95.6	34.5	7.0	11.3	52.5	6.0

Total CHO = total carbohydrate (including fibre) = (100 – protein + lipid + ash).
Basal diet contained >0.5% of total CHO from starch origin.

Table 3.   Digestible nutrient composition of experimental diets (dry basis).

Diet (Test ingredient inclusion content %)	Digestible dry matter (g 100 g^{-1})	Digestible protein (g 100 g^{-1})	Digestible energy (MJ kg^{-1})	DP : DE
Basal diet (100) [1]	78.0	60.0	20.3	3.0 : 1
Wheat meal (15) [2]	76.5	52.0	18.7	2.8 : 1
Wheat meal (30) [2]	76.1	44.0	17.0	2.6 : 1
Wheat meal (45) [2]	70.6	36.5	15.4	2.4 : 1
Wheat meal (60) [2]	70.2	30.1	13.8	2.2 : 1
Raw wheat starch (15) [3]	80.1	50.9	18.3	2.8 : 1
Raw wheat starch (30) [1]	78.5	42.0	17.3	2.4 : 1
Raw wheat starch (45) [3]	60.1	33.0	13.9	2.4 : 1
Raw wheat starch (60) [1]	49.0	23.9	11.7	2.0 : 1
Gelatinised wheat starch (15) [3]	82.0	50.7	19.1	2.7 : 1
Gelatinised wheat starch (30) [1]	81.9	41.6	20.0	2.1 : 1
Gelatinised wheat starch (45) [3]	72.6	33.1	16.4	2.0 : 1
Gelatinised wheat starch (60) [1]	67.3	23.2	14.5	1.6 : 1
Dextrin (15) [3]	82.5	51.5	19.4	2.7 : 1
Dextrin (30) [1]	81.3	42.4	18.5	2.3 : 1
Dextrin (45) [3]	82.7	33.0	18.7	1.8 : 1
Diatomaceous earth (15) [3]	80.7	51.3	16.9	3.0 : 1
Diatomaceous earth (30) [3]	81.3	41.8	13.9	3.0 : 1
Diatomaceous earth (45) [3]	80.7	32.8	10.8	3.0 : 1

[1] Digestible dry matter, protein and energy calculated using apparent digestibility coefficients of diets from Stone *et al.* (in press b).
[2] Apparent digestibility coefficients used to calculate digestible energy of wheat meal diets were from Allan *et al*. (2000c).
[3] Apparent digestibility coefficients used to calculate digestible dry matter, protein and energy of diets were estimated using linear regression of apparent digestibility coefficients and ingredient inclusion levels from Stone *et al.*(in press b).

Table 4. Performance indices of silver perch fed a basal diet or the basal diet containing graded levels of wheat meal, raw wheat starch, gelatinised wheat starch, dextrin or diatomaceous earth[1,2].

Performance indices and diet series	Test ingredient inclusion level into basal diet (%)				
	0 (*Basal*)	15	30	45	60
Individual weight gain (%)[3]					
Basal		107.9±10.9 [w]	107.9±10.9 [x]	107.9±10.9 [y]	107.9±10.9 [y]
Wheat meal	107.9±10.9[b]	104.1±3.9[b w]	97.9±5.0[b x]	78.3±1.2[a x]	65.6±3.0[a x]
Raw wheat starch	107.9±10.9[c]	103.9±8.5[c w]	89.3±1.1[c x]	58.8±1.6[b x]	17.6±1.1[a w]
Gelatinised wheat starch	107.9±10.9[cd]	116.4±5.5[d w]	93.2±3.6[bc x]	78.3±2.7[b x]	51.9±1.9[a x]
Dextrin (Fieldose 9)	107.9±10.9[ab]	119.3±8.1[b w]	104.6±9.4[ab x]	76.1±3.5[a x]	-
Diatomaceous earth	107.9±10.9[c]	86.1±5.6[c w]	52.2±6.1[b w]	12.2±1.4[a w]	-
FCR[4]					
Basal		1.7±0.1 [w]	1.7±0.1 [x]	1.7±0.1 [x]	1.7±0.1 [y]
Wheat meal	1.7±0.1[a]	1.7±0.1[a w]	1.8±0.1[a x]	2.1±0.1[a x]	2.7±0.3[b x]
Raw wheat starch	1.7±0.1[a]	1.7±0.1[a w]	1.9±0.1[a x]	2.6±0.2[a x]	7.8±0.4[b w]
Gelatinised wheat starch	1.7±0.1[bc]	1.6±0.1[c w]	1.8±0.1[bc x]	2.0±0.1[b x]	2.9±0.1[a x]
Dextrin (Fieldose 9)	1.7±0.1[ab]	1.6±0.1[b w]	1.8±0.2[ab x]	2.1±0.1[a x]	-
Diatomaceous earth	1.7±0.1[b]	2.0±0.1[b w]	3.0±0.3[b w]	11.1±1.1[a w]	-
Energy retention efficiency (%)[5]					
Basal		28.53±3.7 [w]	28.53±3.7 [x]	28.53±3.7 [x]	28.53±0.37 [y]
Wheat meal	28.5±3.7[b]	26.56±0.9[b w]	27.08±0.7[b wx]	21.69±1.4[ab x]	16.96±2.4[a x]
Raw wheat starch	28.5±3.7[b]	28.49±3.5[b w]	27.06±1.4[b wx]	18.72±2.6[b x]	4.32±1.4[a w]
Gelatinised wheat starch	28.5±3.7[ab]	32.86±2.8[b w]	25.49±0.8[ab wx]	26.22±1.3[ab x]	21.21±0.7[a xy]
Dextrin (Fieldose 9)	28.5±3.7[a]	31.74±2.4[a w]	30.04±3.5[a x]	28.03±3.0[a x]	-
Diatomaceous earth	28.5±3.7[b]	26.92±2.7[b w]	17.28±2.7[b w]	-3.48±3.8[a w]	-
Protein retention (%)[6]					
Basal		17.0±2.8 [w]	17.0±2.8 [w]	17.0±2.8 [x]	17.0±2.8 [x]
Wheat meal	17.0±2.8[a]	17.7±0.5[a w]	18.6±1.3[a w]	22.9±1.9[a x]	18.8±2.3[a x]
Raw wheat starch	17.0±2.8[b]	17.4±2.0[b w]	18.7±0.7[b w]	18.7±2.7[b x]	6.1±1.9[a w]
Gelatinised wheat starch	17.0±2.8[a]	19.2±0.6[a w]	20.5±0.4[a w]	21.1±2.0[a x]	21.3±0.5[a x]
Dextrin (Fieldose 9)	17.0±2.8[a]	19.5±0.3[a w]	21.4±3.1[a w]	24.7±1.9[a x]	-
Diatomaceous earth	17.0±2.8[b]	16.9±1.5[b w]	13.8±1.3[b w]	5.8±2.4[a w]	-

[1] Values are means ± se from three groups of fish. Different superscripts [abcd] indicate significant differences between inclusion levels within each diets series; [wxyz] indicate differences between diets within each inclusion level ($P < 0.05$; One factor ANOVA; SNK).
[2] Data for the basal diet was included in all ANOVA's.
[3] Individual weight gain % (= [final weight – initial weight] / initial weight x 100).
[4] Feed conversion ratio (FCR = dry weight feed consumed / wet weight increment).
[5] Protein retention (PR (%) = [final body protein – initial body protein] / protein intake x 100).
[6] Energy retention efficiency (ERE (%) = [final body energy – initial body energy] / energy intake x 100).

Table 5. Proximate body composition of silver perch fed a basal diet or the basal diet containing graded levels of wheat meal, raw wheat starch, gelatinised wheat starch, dextrin or diatomaceous earth[1, 2].

Proximate composition indice and diet series	Test ingredient inclusion level into basal diet (%)				
	0 (Basal)	15	30	45	60
Dry matter (g 100g^{-1})					
Basal		34.5±0.6 ^{w}	34.5±0.6 ^{x}	34.5±0.6 ^{x}	34.5±0.6 ^{x}
Wheat meal	34.5±0.6^{b}	33.8±0.5$^{b\,w}$	33.4±0.4$^{b\,x}$	33.3±0.8$^{ab\,x}$	31.0±0.7$^{a\,w}$
Raw wheat starch	34.5±0.6^{b}	33.7±0.8$^{b\,w}$	34.4±0.6$^{b\,x}$	31.9±0.6$^{ab\,x}$	30.8±0.4$^{a\,w}$
Gelatinised wheat starch	34.5±0.6^{a}	34.1±0.6$^{a\,w}$	33.6±0.5$^{a\,x}$	34.3±0.8$^{a\,x}$	34.3±0.1$^{a\,x}$
Dextrin (Fieldose 9)	34.5±0.6^{a}	34.7±0.4$^{a\,w}$	33.8±0.4$^{a\,x}$	33.8±0.1$^{a\,x}$	-
Diatomaceous earth	34.5±0.6^{b}	33.3±0.4$^{b\,w}$	31.5±0.3$^{ab\,w}$	29.1±1.3$^{a\,w}$	-
Crude Protein (g 100g^{-1})					
Basal		17.5±0.5 ^{w}	17.5±0.5 ^{w}	17.5±0.5 ^{w}	17.5±0.5 ^{w}
Wheat meal	17.5±0.5^{a}	17.6±0.3$^{a\,w}$	16.8±0.1$^{a\,w}$	18.6±0.6$^{a\,w}$	16.8±0.4$^{a\,w}$
Raw wheat starch	17.5±0.5^{a}	17.0±0.4$^{a\,w}$	17.2±0.3$^{a\,w}$	17.1±0.4$^{a\,w}$	17.1±0.3$^{a\,w}$
Gelatinised wheat starch	17.5±0.5^{a}	17.1±0.1$^{a\,w}$	17.1±0.1$^{a\,w}$	17.0±0.6$^{a\,w}$	17.2±0.2$^{a\,w}$
Dextrin (Fieldose 9)	17.5±0.5^{a}	17.4±0.4$^{a\,w}$	16.9±0.5$^{a\,w}$	17.5±0.1$^{a\,w}$	-
Diatomaceous earth	17.5±0.5^{a}	17.7±0.2$^{a\,w}$	18.0±0.1 $^{a\,w}$	18.5±0.8$^{a\,w}$	-
Lipid (g 100g^{-1})					
Basal		11.8±0.5 ^{w}	11.8±0.5 ^{x}	11.8±0.5 ^{yz}	11.8±0.5 ^{x}
Wheat meal	11.8±0.5^{b}	12.0±0.4$^{b\,w}$	11.6±0.4$^{b\,x}$	10.3±0.3$^{ab\,xy}$	8.7±0.9$^{a\,w}$
Raw wheat starch	11.8±0.5^{b}	11.3±0.5$^{b\,w}$	12.2±0.4$^{b\,x}$	9.3±0.4$^{a\,x}$	8.2±0.4$^{a\,w}$
Gelatinised wheat starch	11.8±0.5^{a}	12.2±0.6$^{a\,w}$	12.0±0.3$^{a\,x}$	12.5±0.7$^{a\,z}$	11.8±0.3$^{a\,x}$
Dextrin (Fieldose 9)	11.8±0.5^{a}	12.2±0.5$^{a\,w}$	12.5±0.2$^{a\,x}$	11.3±0.4$^{a\,yz}$	-
Diatomaceous earth	11.8±0.5^{c}	10.6±0.6$^{c\,w}$	8.1±0.4$^{b\,w}$	4.6±0.6$^{a\,w}$	-
Gross energy (MJ kg^{-1})					
Basal		8.5±0.2 ^{w}	8.5±0.2 ^{x}	8.5±0.2 ^{x}	8.5±0.2 ^{x}
Wheat meal	8.5±0.2^{b}	8.4±0.2$^{b\,w}$	8.4±0.1$^{b\,x}$	8.0±0.2$^{b\,x}$	7.3±0.3$^{a\,w}$
Raw wheat starch	8.5±0.2^{b}	8.5±0.4$^{b\,w}$	8.6±0.2$^{b\,x}$	7.6±0.2$^{a\,x}$	7.1±0.2$^{a\,w}$
Gelatinised wheat starch	8.5±0.2^{a}	8.9±0.3$^{a\,w}$	8.4±0.2$^{a\,x}$	8.6±0.1$^{a\,x}$	8.6±0.1$^{a\,x}$
Dextrin (Fieldose 9)	8.5±0.2^{a}	8.8±0.2$^{a\,w}$	8.4±0.2$^{a\,x}$	8.7±0.2$^{a\,x}$	-
Diatomaceous earth	8.5±0.^{c}	8.1±0.2$^{bc\,w}$	7.3±0.2$^{b\,w}$	6.1±0.4$^{a\,w}$	-
Ash (g 100g^{-1})					
Basal		4.4±0.2 ^{w}	4.4±0.2 ^{w}	4.4±0.2 ^{w}	4.4±0.2 ^{w}
Wheat meal	4.4±0.2^{a}	4.0±0.3$^{a\,w}$	4.3±0.1$^{a\,w}$	4.0±0.3$^{a\,w}$	4.5±0.2$^{a\,w}$
Raw wheat starch	4.4±0.2^{a}	4.2±0.1$^{a\,w}$	4.2±0.1$^{a\,w}$	4.6±1.3$^{a\,w}$	5.2±0.1$^{b\,x}$
Gelatinised wheat starch	4.4±0.2^{ab}	3.9±0.1$^{a\,w}$	4.2±0.1$^{ab\,w}$	4.3±0.2$^{ab\,w}$	4.7±0.1$^{b\,w}$
Dextrin (Fieldose 9)	4.4±0.2^{a}	4.2±0.1$^{a\,w}$	3.9±0.1$^{a\,w}$	4.3±0.1$^{a\,w}$	-
Diatomaceous earth	4.4±0.2^{a}	4.5±0.1$^{a\,w}$	4.8±0.1$^{a\,x}$	5.7±0.1$^{b\,x}$	-

[1] Values are means ± se from three groups of fish. Different superscripts ^{abcd} indicate significant differences between inclusion levels within each diets series; ^{wxyz} indicate differences between diets within each inclusion level ($P < 0.05$; One factor ANOVA; SNK).
[2] Data for the basal diet was included in all ANOVA.

4.7.   Carbohydrate utilisation by juvenile silver perch *Bidyanus bidyanus* (Mitchell): IV. Can dietary enzymes increase digestible energy from wheat starch, wheat and dehulled lupin?

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Abstract

The effects of exogenous digestive enzyme supplements on the digestibility of wheat starch or diets containing either wheat or de-hulled lupin (*Lupinus angustifolius* var. Gungurra) by silver perch (*Bidyanus bidyanus*) were investigated. In the first experiment, Natustarch® (α-amylase supplement specific to starch) was added at three nominal concentrations (0, 50, 100 or 150 mg kg⁻¹diet) to diets containing either, raw or 100% gelatinised wheat starch (30% dietary inclusion content) and fed to silver perch. The apparent digestibility coefficients (ADCs) for dry matter, starch and energy were calculated. The action of Natustarch® on the diet and in the digestive tract was also investigated. The addition of Natustarch® to diets containing raw and gelatinised wheat starch led to an average increase in reducing sugar content of diets of 67 and 340%, respectively, indicating that the α-amylase was more efficient at hydrolysing wheat starch in the gelatinised form. Gelatinised wheat starch was digested more efficiently than raw wheat starch. However, although the addition of Natustarch® at ≥ 50 mg kg⁻¹ led to a significant increase in digestibility of raw wheat starch, the digestibility of gelatinised wheat starch which was already high was not further improved. Leaching due to immersion in water caused a minor loss of α-amylase activity from diet pellets treated with Natustarch® (~ 13% after 5 min). The α-amylase activity in the anterior section of the intestinal tract of silver perch fed diets containing Natustarch® was not affected, indicating that the α-amylase had been denatured by the acidic conditions in the stomach of silver perch. In the second experiment, diets containing wheat or lupin (at the 30% inclusion content) were treated with Natugrain-blend® (an enzyme supplement containing β-glucanase and β-xylanase, specific to non-starch polysaccharides [NSPs]) at three nominal concentrations (0, 75, 150 or 300 μL kg⁻¹) and fed to silver perch. ADCs for energy and protein were calculated. The addition of Natugrain-blend® had no effect on dry matter, energy or protein digestibility of the diets or ingredients.

Introduction

Wheat and lupin are common plant ingredients either already being used or considered for use in aquaculture diets in Australia (Allan & Rowland 1998; Allan, Booth, Stone, Williams & Smith 2000a; Allan, Parkinson, Booth, Stone, Rowland, Frances & Warner-Smith 2000b, Allan, Rowland, Mifsud, Glendenning, Stone & Ford 2000c; Carter 2000). When compared to animal meals such as fish meals, meat and bone meals and poultry meals, the protein content of wheat (~ 15%) and lupin (~ 36%) is low, and both contain high levels of carbohydrate (Novus 1992; Stone, Allan, Parkinson & Rowland 2000; Allan *et al.* 2000a; 2000b). Although the carbohydrate component of grains and legumes may provide a cheap source of dietary energy for fish, it is poorly utilised by most fish compared with protein and lipid (Buhler & Halver 1961; Singh & Nose 1967; Shimeno 1982; Furuichi 1983; Wilson & Poe 1987; Lovell 1989; Wilson 1994; Allan *et al.* 2000a, 2000b).

Starch is the predominant energy storage carbohydrate in wheat and may comprise approximately 60% of the total grain. In contrast, lupins contain insignificant levels of starch (< 3%), with the non-starch polysaccharide (NSP), β-(1,4)-galactan acting as the primary energy storage carbohydrate (Novus 1992; van Barneveld 1999). In general, NSPs are a complex group, composed predominantly of linked monomers of hexoses and pentoses, e.g., galactose, glucose, aribinose, xylose, and mannose (Stone 1996; Dudley-Cash 1997; van Barneveld 1999). The NSP content in wheat and lupin may account for 25 and 50 % of the total grain and seed, respectively (Novus 1992).

Starch not only differs from NSPs in the type of monosaccharides present, but also in the type of chemical bonds linking the respective polysaccharides. Starch is composed entirely of glucose monomers linked exclusively by α-glycosidic bonds, whereas the monomers of NSPs are linked predominantly by β-glycosidic bonds. This has a profound effect on digestibility, as different enzymes are required to hydrolyse α and β-glycosidic bonds (Smith 1989). The capacity of fish to digest dietary carbohydrate has been reported to be variable between species (Shimeno 1982; Furuichi 1983; Wilson 1994; Shiau 1997). The variability may be explained by the differing endogenous digestive carbohydrase activity in different fish (Smith 1989; Kuz'mina 1996; Hidalgo, Urea & Sanz 1999). Carbohydrase activity in fish has been reported to be highest in the anterior section of the intestinal tract, where the majority of carbohydrate absorption takes place (Fänge & Grove 1979; Kuz'mina 1996; Krogdahl, Nordrum, Sorensen, Brudeseth & Rosjo 1999). The predominant starch digestive enzymes are α-amylase (1,4-α -D-glucan glucanohydrolase) and α-glucosidase (1,4-α-glucosidase). In combination, these enzymes specifically hydrolyse the α-glycoside bonds of starch to yield glucose. Low, medium and high activities of these enzymes have been measured in the intestinal tract of carnivorous, omnivorous and herbivorous fish, respectively (Smith 1989; Kuz'mina 1996).

In fish, digestive enzymes for NSPs such as β-glucanases or β-xylanases are scarce or non-existent (Stickney & Shumway 1974; Lindsay & Harris 1980; Smith 1989; Chakrabarti, Gani, Chaki, Sur & Misra 1995; Kuz'mina 1996). Low levels of cellulase (1,4-β-D-glucan glucanohydrolase) have been reported for herbivorous species such as grass carp (*Ctenopharyngodon idella*) and milkfish (*Chanos chanos*) (Lindsay & Harris 1980; Smith 1989; Das & Tripathi 1991; Chakrabarti *et al.* 1995; Kuz'mina 1996) and insignificant activities have been reported in omnivorous and carnivorous species (Stickney & Shumway 1974; Lindsay & Harris 1980; Smith 1989; Chakrabarti *et al.* 1995; Kuz'mina 1996).

Consequently, dietary NSPs remain indigestible and, therefore, unavailable as an energy source for carnivorous and most omnivorous species.

Exogenous dietary enzyme supplements, isolated from plants and bacteria, have been used successfully in the pig and poultry industries to overcome the negative effects of the soluble fraction of dietary NSP (Batterham 1992; Farrell 1992; Campbell & Bedford 1992; Chesson 1993; Bedford 1996; Dudley-Cash 1997). The anti-nutritional effects of soluble dietary fibre were alleviated by a reduction in solubility due to a reduction in chain length of the NSP (Chesson 1993; Dudley-Cash 1997). The addition of exogenous carbohydrase enzymes to aquaculture diets has also been reported to enhance the utilisation of otherwise unavailable dietary carbohydrates by Atlantic salmon (*Salmo salar*), larval gilthead seabream (*Sparus aurata*) and tiger prawns (*Penaeus monodon*) (Kolkovski, Tandler, Kissil & Gertler 1993; Carter, Houlihan, Buchanan & Mitchell 1994; Buchanan, Sarac, Poppi & Cowan 1997). NSPase's may also hydrolyse the NSP component of cell walls and release otherwise unavailable nutrients, such as protein and starch (Chesson 1993; Dudley-Cash 1997).

Silver perch (*Bidyanus bidyanus*), an omnivorous freshwater fish, are currently being cultured in Australia using diets containing moderate to high levels of carbohydrate (20 – 40%) and fibre

(~15%) (Rowland, Allan, Clark, Hollis & Pontifex 1994; Rowland, Allan, Hollis & Pontifex 1995; Allan *et al*. 2000c). Carbohydrate digestibility in silver perch has been demonstrated to be influenced by its type, origin, physical state, complexity and inclusion content (Stone, Allan, Parkinson & Frances, in press a; Stone, Allan & Anderson, in press b). Digestibility of energy from raw wheat starch by silver perch was increased from 83 to 93% by gelatinisation, but, to date, the ability of silver perch to digest energy from the NSP component of lupin has been found to be negligible (Allan & Rowland 1998). There is a possibility that the use of supplemental digestive enzymes may enhance energy digestibility of diets containing starch and NSP by silver perch.

The aim of this study was to investigate if the addition of supplemental carbohydrate enzymes to diets containing either, raw or gelatinised wheat starch, wheat or dehulled lupin, would enhance dietary energy digestibility when fed to silver perch.

Materials and Methods

Supplemental enzymes and test ingredients

Two enzyme products and four carbohydrate sources were evaluated during this study (Tables 1 & 2). The two enzyme products were: Natustarch®, a commercial α-amylase (α-1, 4-glucan hydrolase EC 3.2.1.1) supplement in powder form; and Natugrain-blend®, a commercial enzyme supplement in liquid form containing a mixture of endo-1, 3(4)-β-glucanase (EC 3.2.1.6) and endo-1,4-β-xylanase (EC 3.2.1.8) (Table 1). Both enzymes where supplied by BASF, Sydney, NSW, Australia. The four carbohydrate products were: raw wheat starch (wheaten cornflour); 100% gelatinised wheat starch (Pregel N); wheat; and dehulled lupin (*Lupinus angustifolius* var. Gungurru) (Table 2).

Experiment 1. The effect of Natustarch® on the digestibility of raw or gelatinised wheat starch

In this experiment we investigated the effect of Natustarch® added at one of three different concentrations on the digestibility of either raw or gelatinised wheat starch by silver perch. Silver perch were fed a basal diet (Table 3) or test diets that contained 70% basal diet and 30% of either raw wheat starch or gelatinised wheat starch. The test diets were treated with Natustarch® to give concentrations of 0, 50, 100 or 150 mg Natustarch® kg^{-1} diet (nine diets in total) (Table 4). The experiment ran for 36 days**.**

At the completion of this experiment, a series of three separate experiments was conducted to: a) determine if Natustarch® predigested the wheat starch in the diets; b) determine if feeding any of the test diets to silver perch led to increased α-amylase activity in the anterior portion of their intestinal tract; and c) assess the loss of α-amylase activity from the diet due to leaching in the water.

Experiment 1a. Total reducing sugar level of diets containing raw or gelatinised wheat starch due to pre-treatment with Natustarch®

To determine if Natustarch® predigested the wheat starch in the diets prior to feeding, the total reducing sugar content (measure of dextrinisation) of each diet was measured (*n* = 2 for each diet). Following the application of Natustarch® to the diets (described in enzyme preparation and application section), the pellets were allowed to dry for a period of 3 h at 25°C. The diets were then stored at -15°C until analysed. To assess the relative differences in the pre-digestion of the raw wheat starch and the gelatinised wheat starch component of diets due to Natustarch®, the percent increases of total reducing sugar content for each diet series were determined using the following equation; % increase in total reducing sugar = (reducing sugar in test diet - reducing sugar in control diet) / reducing sugar in control diet) x 100.

Experiment 1b. α-Amylase activity in the anterior portion of the intestinal tract following the feeding of silver perch

At the completion of the digestibility experiment fish were held in their respective tanks and fed at the same rate for a further two days. Thereafter, the intestinal tract was dissected from silver perch which were fed the basal diet, the raw wheat starch diets containing either 0, 50 or 100 mg Natustarch® kg[-1] diet, and the diet containing gelatinised wheat starch and 100 mg Natustarch® mg[-1] diet. On the day of sampling, fish were fed their respective diet for 3 h and then the fish were killed using ethyl ρ-aminobenzoate (Sigma Chemical Co., Castle Hill, NSW, Australia) (400 mg l^{-1} for 2 min). The fish were then dissected and the anterior portion of the intestinal tract was removed from three randomly selected fish from each tank from each treatment. Samples were immediately frozen and stored at -20°C. The anterior section of the intestinal tract of silver perch was analysed for α-amylase activity.

Experiment 1c. Loss of α-amylase activity from the diet due to leaching in water

Two grams of the diet containing 30% gelatinised wheat starch and 100 mg Natustarch® kg[-1] were placed into a 15 mL conical bottom tube and 3 mL of distilled water were then added to the tube with an auto pipette. Separate batches of diet pellets (*n* = 2 for each time) were then left immersed in water for either 5 - 10 s, 15 - 20 s, 30 - 35 s, 1 min or 5 min. These immersion times were selected to represent the range of times that pellets were likely to be in the water before being eaten by silver perch. After the desired time had elapsed, the water was then removed from the bottom of the tube using a hypodermic needle and syringe. The total α-amylase activity of the diet (315.2 mg reducing sugar[-1] min^{-1} g^{-1} of diet) was determined by mixing 2 g of diet with 3 mL water and shaking for 20 min before collecting the supernatant by centrifugation (5 minutes at 1250 *g*). All samples were held on ice prior to analysis. The supernatant was analysed for α-amylase activity.

Experiment 2. The effect of Natugrain-blend® on the digestibility of energy from wheat or dehulled lupin by silver perch

In this experiment we investigated the effect of the Natugrain-blend® added at three different concentrations on the digestibility of either the wheat or dehulled lupins by silver perch. Silver perch were fed a basal diet (Table 3), or test diets that contained 70% basal diet and 30% of either wheat or dehulled lupin. The test diets were treated to give concentrations of 0, 75, 150 or 300 μL of the Natugrain-blend® kg[-1] diet (nine diets in total) (Table 5). The experiment ran for 26 days.

Diet preparation

All ingredients used in the basal diet and test ingredients were ground using a hammer mill (C-E Raymond Inc., IL, USA) or sieved to ensure all particles passed through a 710 μm screen before being thoroughly dry mixed in a Hobart mixer (Troy Pty. Ltd., OH, USA). Ytterbium chloride, an inert indicator, was then sprayed onto each dry batch of diet mash at 0.1g kg^{-1} (dry basis). The mash was then combined with approximately 400 mL distilled water kg^{-1} dry mix before being cold pelleted through a meat mincer (Barnco Australia Pty. Ltd., Leichhardt, NSW, Australia) with a 4 mm die. The diet was then dried in a convection oven at 35°C until the moisture content was approximately 20%. The diets were then reground in a hammer mill to ensure homogenous mixing of the ytterbium chloride throughout the mash. The appropriate amount of fish oil was then added to each batch of mash and then each diet was re-pelleted through a 2 mm die. 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.

Enzyme preparation and application

To achieve the desired concentration of enzyme on each diet the required amount of either Natustarch® (powder form) or Natugrain-blend® (liquid) was added to 50 mL of distilled water kg^{-1} of diet to give a stock solution. To account for possible losses of enzymes during spraying (in the aerosol form) an extra 5% of enzyme stock solution was added to the volume required for each batch of diet. The enzyme stock solution was mixed thoroughly and sprayed onto the diet pellets. For those diets without the enzyme, distilled water was sprayed onto the diet pellets. All pellets were allowed to dry for a period of 3 h at 25°C and then stored at -15°C until fed to the fish.

Fish and holding facilities

Silver perch (Experiment 1, mean weight ± sem: 15.0 ± 0.4 g, Experiment 2, 41.7 ± 0.7 g) were bred at the NSW Fisheries Grafton Research Centre (E 153° 00, S 29° 40') and raised in earthen ponds using similar techniques to those described by Thurstan & Rowland (1994). Fish were transported by road to the NSW Fisheries, Port Stephens Fisheries Centre (PSFC) (E 152° 20, S 32° 40'). Prior to the experiments, fish were held in a 10 000-L tank supplied with heated (24 ± 2°C) re-circulating freshwater (bore water, salinity 0.05 g L^{-1}) and fed *ad libitum* twice a day with a commercial silver perch diet (95LC2, digestible protein, 34.0%; digestible lipid, 9.0%; carbohydrate, 31.8%; ash 17.8% and digestible energy 14.0 MJ kg^{-1}; Allan *et al*. 2000c).

Experimental facilities and stocking

Digestibility tanks were 170-L cylindro-conical tanks (conical base sloped at 35°). The design and operation of this system is described by Allan, Rowland, Parkinson, Stone & Jantrarotai (1999). Photo-period was held at 17 h light/7 h dark. Prior to stocking, fish were anaesthetised using a bath of ethyl ρ-aminobenzoate (40 mg L^{-1} for 5 min) then caught at random, weighed individually and distributed among tanks by systematic interspersion. Fish were stocked (Experiment 1, 10 fish tank^{-1}; Experiment 2, 5 fish tank^{-1}) and allowed to acclimatise to their experimental diets and facilities for a period of 11 days prior to the collection of faeces. Fish were fed their respective experimental diets to excess (9% body weight d^{-1}) using automatic conveyor belt-type feeders for 3 h each day from 0830 - 1130 h. Faecal samples were collected by settlement as described by Allan *et al.* (1999). During both experiments, water temperature (range 26 ± 1°C), dissolved oxygen (above 5.0 mg L^{-1}), and pH (between 7.0 and 8.2) were measured weekly using a Yeo-Kal 611 electronic water quality analyser (Yeo-Kal Electronics, Brookvale, Sydney, NSW Australia). Nitrite and ammonia concentrations (< 0.03 mg L^{-1} NO$_2$-N and < 0.03 mg L^{-1} total ammonia - N respectively) were measured weekly using colormetric methods described by Major, Dal Pont, Kyle & Newell (1972) and Dal Pont, Hogan & Newell (1973).

Digestibility determinations

The indirect method of Cho & Kaushik (1985) was used to calculate apparent digestibility coefficients for dry matter, energy, starch and protein in experimental diets, with ytterbium chloride (0.1g kg^{-1} dry basis) as the inert indicator. Then, apparent digestibility coefficients for energy of test ingredients were calculated using the methods described by Sugiura, Dong, Rathbone & Hardy (1998).

Biochemical analyses

Feed and faecal samples were analysed for dry matter, ash, nitrogen and energy (bomb calorimetry) by the AOAC (1990) procedures. Nitrogen was determined using Kjeldahl or semi-micro Kjeldahl methods (AOAC 1990) and crude protein content was estimated by multiplying percent nitrogen by 6.25. Analysis of diets and faeces for ytterbium chloride was performed by CSIRO, Tropical Agriculture Analytical Services, St Lucia, QLD Australia, using inductively coupled plasma-mass spectroscopy (ICP-MS) techniques. The diets were analysed for total reducing sugar content using the methods of Teitz (1982). α-Amylase activity determinations of diets and the anterior portion of the intestinal tract were conducted using the methods of Teitz (1982). The total starch content of the basal diet, ingredients and faeces was analysed enzymatically (Megazyme total starch assay AA/AMG 9/97, Megazyme International Ltd, Bray, Ireland).

Statistical analysis

Homogeneity of variances was assessed using Cochran's Test (Winer 1991). The experiments were designed for analysis using two-factor ANOVA with ingredient type (Experiment 1, raw wheat starch or gelatinised wheat starch; Experiment 2, wheat or dehulled lupin) and the concentration of enzyme supplement (Experiment 1, Natustarch® at either 0, 50, 100 or 150 mg kg^{-1}; Experiment 2, Natugrain-blend® at either 0, 75, 150 or 300 μL kg^{-1}) as the two fixed factors. Dry matter (DM), starch (Experiment 1 only), energy and protein digestibility for diets containing test ingredients for each experiment was compared using two-factor ANOVA. The total reducing sugar content of diets from each series were statistically analysed using two-factor ANOVA. Where significant interactions were found between main effects, one-factor ANOVA was used to compare simple effects. The loss of α-amylase activity from the diet due to leaching and α-amylase activity in the anterior portion of the intestinal tract were statistically analysed using one-factor ANOVA. Starch digestibility for diets containing wheat in Experiment 2 were analysed using one-factor ANOVA. When results were significant, comparison between means were made using Student Newman-Kuel's 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). Statistical evaluation of the data was carried out using the *Statgraphics Plus* for Windows 4.1 software package (Manugistics Inc., Rockville, Maryland, USA; 1998).

Results

Experiment 1. The effect of Natustarch® on the digestibility of raw or gelatinised wheat starch by silver perch.

Apparent dry matter and energy digestibility coefficients of the diets were significantly affected by ingredient type (gelatinised starch > raw starch) (two-factor ANOVA; *P* < 0.05) (Table 6). For dry matter digestibility, the addition of Natustarch® led to a minor, but significant increase (*P* < 0.05). For energy digestibility there was no significant effect of Natustarch®. There were no significant interactions between the two factors for either dry matter or energy digestibility (*P* > 0.05).

Starch digestibility was significantly affected by ingredient type, with gelatinised starch being digested more efficiently than raw starch (*P* < 0.05) and a minor but significant effect of Natustarch® level on starch digestibility (*P* < 0.05) (Table 6). The significant interaction between ingredient type and Natustarch® level (*P* > 0.05), may be explained by the greater effect of Natustarch® on the diets containing raw wheat starch (here starch ADCs increased from 84 to 92%) than on the diets containing gelatinised wheat starch (starch ADCs remained unaffected at 99%).

The protein digestibility of diets was not significantly affected by carbohydrate type (*P* > 0.05) but the addition of Natustarch® led to a slight increase in protein digestibility (*P* < 0.05). There was no

interaction ($P > 0.05$) between ingredient type and Natustarch[®] level. Protein ADCs of diets were high (> 90%) suggesting carbohydrate or the addition of Natustarch[®] did not interfere with protein digestion for silver perch.

Energy digestibility of ingredients followed similar trends to dietary energy digestibility.

Experiment 1a. Total reducing sugar level of diets containing raw or gelatinised wheat starch due to pre-treatment with Natustarch[®].

There was a significant effect of ingredient (gelatinised starch > raw starch) on total reducing sugar levels (two-factor ANOVA; SNK; $P < 0.05$). There was also a significant effect of Natustarch[®] level on total reducing sugar levels of the diets ($0 < 50 = 100 < 150$ mg kg^{-1} diet) ($P < 0.05$). There was a significant interaction between the two factors ($P < 0.05$) due to the greater effect of Natustarch[®] on gelatinised wheat starch than raw wheat starch (Table 7). The total reducing sugar levels increased by 289 – 363% for the diets containing gelatinised starch and Natustarch[®] compared to an increase of only 35- 99% for the diets containing raw wheat starch and Natustarch[®].

Experiment 1b. α-Amylase activity in the anterior portion of the intestinal tract following feeding.

α-Amylase activity in the anterior section of the intestinal tract ranged from 3.59 to 4.52 mg reducing sugar min^{-1} g^{-1} of tissue and was not affected by the addition of Natustarch[®] (up to 100 mg kg^{-1}) to diets containing 30% wheat starch (one factor ANOVA; $P > 0.05$).

Experiment 1c. Loss of α-amylase activity from the diet due to leaching in water.

When immersed in water for a period of 10 s to 5 min, there was a slight loss of α-amylase activity (range 7.6 to 13.3%) from the diet containing 30% gelatinised wheat starch and Natustarch[®] added at 100 mg kg^{-1} diet. The loss was not affected ($P > 0.05$) by increasing time, indicating that a high proportion (>85%) of the enzyme was ingested by the fish via the diet.

Experiment 2. The effect of Natugrain-blend[®] on the digestibility of energy from wheat or dehulled lupin by silver perch

Apparent dry matter and energy digestibility coefficients of diets were significantly affected by ingredient type with wheat being digested more efficiently than lupins (Table 8; two-factor ANOVA; SNK; $P < 0.05$). There was no significant effect of the Natugrain-blend[®] on the digestibility of dietary dry matter or energy ($P > 0.05$) and no significant interactions between the two factors ($P > 0.05$).

Dietary protein digestibility was not significantly affected by ingredient type, concentration of the Natugrain-blend[®] or their interaction (Table 8; two-factor ANOVA; $P > 0.05$). All protein ADCs were high (> 87%) suggesting carbohydrate in the diet did not interfere with protein digestion for silver perch.

For diets containing wheat, starch digestibility was high ranging from 93.5 to 96.3%, and was not significantly affected by the addition of the Natugrain-blend[®] (one-factor ANOVA, $P > 0.05$).

Discussion

The enzymes used in this study were selected to increase the digestibility of dietary carbohydrate, specifically starch and non-starch polysaccharides present in wheat and dehulled lupin. There was evidence of a positive effect of Natustarch® on the digestibility of dietary wheat starch; however, the addition of the Natugrain-blend® did not enhance the digestibility of diets containing wheat or lupin.

The improved digestibility of wheat starch due to gelatinisation is consistent with previous findings for silver perch (Stone *et al*. in press b), and the high ADCs further demonstrate that silver perch are extremely efficient at digesting starch when compared to other omnivorous fish species currently cultured (Wilson 1994).

Natustarch® degrades the wheat starch in diets prior to feeding and does not enhance enzyme activity in the intestinal tract of silver perch. This was apparent by the increase of the reducing sugar content of the diets containing wheat starch following the application of Natustarch, ingestion of significant quantities of enzyme, and the lack of any evidence of increased intestinal amylase activity due to consumption of diets containing the enzyme supplement.

The active constituent of Natustarch®, α-amylase, acts on the α-glycosidic bonds of starch. The addition of Natustarch® to diets containing 30% wheat starch resulted in an increased reducing sugar content. This is an indication that α-amylase had acted on the starch molecule and hydrolysed the α-glycosidic bonds of the polysaccharide, and increased dextrinisation of the starch. Natustarch® degraded gelatinised wheat starch more effectively than raw wheat starch. This may be explained by the increased susceptibility of the gelatinised starch molecule to enzymatic hydrolysis due to its increased surface area and is clearly demonstrated by the much greater level of total reducing sugar in enzyme-treated gelatinised wheat starch diets than enzyme-treated raw wheat starch diets. However, paradoxically, although the digestibility of raw starch was improved by the addition of Natustarch®, digestibility of gelatinised starch was not affected. This is a reflection of the efficiency by which silver perch digest gelatinised starch. Natustarch® effectively converted gelatinised starch to dextrin but as silver perch digest dextrin and gelatinised wheat starch equally well (Stone *et al.* in press b) the enzyme had no effect on digestibility. Addition of Natustarch® to diets for silver perch is useful where diets contain raw uncooked starch, but unnecessary where gelatinisation of starch is achieved through heat processing. However, other species, less efficient at starch digestibility, may respond more positively to Natustarch® addition. This could easily be determined by comparing the digestibility of gelatinised and dextrinised starch. The greater the difference, the more likely it is that Natustarch® would increase digestibility.

The application of Natustarch® to diets containing 30% raw wheat starch resulted in an 8% increase in raw starch digestibility. When the application rate of Natustarch® was reduced to 50 mg kg^{-1}, digestibility of raw starch was comparable to that achieved at the recommended application rate of 100 mg kg^{-1} diet (BASF, Australia). This indicates that the application rate of Natustarch® may be halved while maintaining similar performance. However, even though improved, the digestibility of Natustarch® treated raw wheat starch was still 5% less than that achieved for diets containing gelatinised wheat starch without enzyme treatment.

At present commercial diets for silver perch are manufactured by either steam pelleting or cooking-extrusion. Both methods result in the production of a durable pellet, and have also been shown to increase the degree of gelatinisation and improved digestibility and utilisation of dietary starch by fish (Podoskina, Podoskin & Bekina 1997; Booth, Allan & Warner-Smith 2000; Obaldo, Dominy & Ryu 2000).

The application of the Natugrain-blend® to diets containing 30% wheat or dehulled lupin did not result in an improvement of dry matter, energy or protein digestibility, even when included at twice the recommended level (Table 8). Dry matter and energy digestibility of wheat was higher than dehulled lupin. The efficient protein digestibility of diets is consistent with previous digestibility studies where silver perch have been reported to be very efficient at digesting protein from wheat and lupin (Allan *et al.* 2000a). As the protein digestibility of diets which contained wheat or dehulled lupin was similar, the difference in energy digestibility may be attributed to the higher levels of digestible carbohydrate present in the wheat. When compared to lupin, wheat has higher levels of starch (wheat 57%; dehulled lupin <1%) and lower levels of NSP (wheat ~24%; dehulled lupin ~50%). Values for starch digestibility of the wheat diets were high in this study (>90%), while NSP digestibility for lupin has been reported to be negligible for silver perch (Allan & Rowland 1998).

The role of the Natugrain-blend® is to negate the anti-nutritional effects of NSPs by reducing their solubility. The enzymes present in Natugrain-blend® may also degrade the intact NSP cell walls of plant ingredients and release bound nutrients, making them available for digestion (Chesson 1993.). The enzymes present in the Natugrain-blend® are classed as endo-enzymes (Table 1). Endo-enzymes are relatively inactive against oligosaccharides and their activity is to randomly hydrolyse polysaccharides into shorter chained oligosaccharides (Chesson 1993).

For poultry and pigs, the application of endo-NSPases, β-glucanases and β-xylanases, to diets containing NSP has been reported to lead to a reduction in chain length and solubility of NSPs. The reduction in NSP solubility has also been reported to reduce the viscosity of the digesta and enhance the digestion of other macromolecules (Dudley-Cash 1997; Campbell & Bedford 1992; Chesson 1993). There was no evidence of enhanced nutrient digestibility for silver perch due to the application of the Natugrain-blend®.

The digestion of NSP requires total de-polymerisation to monomers (Dudley-Cash 1997). A combination of exo-enzymes, specific to NSP, which sequentially cleave and liberate the terminal sugar units from the polysaccharide chains, and β–glycosidases, which hydrolyse oligosaccharides to their respective monosaccharide monomers, are required for complete de-polymerisation. However, even if monomers of NSPs were made available for digestion, previous research has demonstrated that silver perch are not able to tolerate high levels of galactose and xylose in the blood (Stone, Allan & Anderson, in press c). Galactosemia, prolonged elevation of blood galactose concentrations, has been reported to have negative effects on fish performance. Shikata, Iwanaga & Shimeno (1994) reported a remarkable reduction in growth, reduced feeding activity, low body and serum fat levels and reduced hepatopancreatic enzyme activity in carp fed diets containing 30% galactose. Inefficient utilisation of galactose has also been reported for Chinook salmon (*Oncorhynchus tshawytscha*), brook trout (*Salvelinus fontinalis*) and chum salmon fry (*Oncorhynchus keta*) (Buhler & Halver 1961; McCartney 1971; Akiyama, Murai & Nose 1982).

Commercial silver perch diets may contain up to 30% starch (Allan *et al.* 2000c). The pre-digestion of the starch component using Natustarch® would be beneficial as dextrin and gelatinised wheat starch have been found to be more digestible than raw wheat starch by silver perch (reducing sugar increase indicates increasing degree of dextrinisation) (Stone *et al.* in press a). Silver perch also utilise dextrin and gelatinised wheat starch more efficiently than raw wheat starch for growth (Stone, Allan & Anderson, in press d). However, for commercial diets manufactured using either cooking-extrusion or steam pelleting technology that result in gelatinisation and dextrinisation of the starch component of the diet, it appears that Natustarch® will not further improve digestibility for silver perch. Enhanced energy digestibility of diets containing ingredients high in NSP was not observed in this study.

Acknowledgments

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Table 1.   Enzyme complement and activity levels of Natustarch® and Natugrain-blend®.

Enzyme supplement[1]	Enzyme type and activity		
	α-amylase[2]	endo-1, 3(4)- β-glucanase[3]	endo-1,4-β-xylanase[4]
Natustarch®	45.000 RAU^{-1}g	-	-
Natugrain-blend®	-	960 BGU^{-1}ml	44 000 EXU^{-1}ml

1 Products and enzyme activities were supplied by BASF, Sydney, NSW, Australia.
2 RAU = reference amylase unit = the quantity of enzyme that will convert under standardised conditions (pH 6.6 at 30°C with reaction time of between 15 and 25 min) 1 mg of soluble starch per minute in a product, having equal absorption to reference colour at 620 nm after reaction with iodine.
3 BGU = β-glucanase unit = the amount of enzyme which liberates 0.258 micromole of reducing sugars, measured as glucose equivalents, out of β-glucan per minute at 40°C and pH 3.5.
4 EXU = endo-xylanase unit = the amount of enzyme which liberates 4.53 micromole of reducing sugars, measured as xylose equivalents, from xylan per minute at 40°C and pH 3.5.

Table 2.   Composition of test ingredients used in this study (dry basis).

Ingredient	Energy (MJ/kg)	Protein (%)	Lipid (%)	Starch (%)	Ash (%)	Total CHO[1] (%)
Raw wheat starch[2]	17.2	<0.1	<0.1	98.0	<0.1	98.0
Gelatinised wheat starch[2,3]	18.3	<0.1	<0.1	98.0	<0.1	98.0
Wheat[4]	16.4	14.8	1.7	57.0	2.7	80.8
Lupin (*Lupinus angustifolius*)[5]	18.8	39.6	6.4	<0.5	4.0	50.0

1 Carbohydrate calculated by difference; CHO (including fibre) (%) = 100-(protein % + lipid % + ash %).
2 Supplied by Starch Australasia, Summer Hill, Sydney, NSW, Australia.
3 The degree of gelatinisation for gelatinised wheat starch was 100%.
4 Wheat supplied by Janos Hoey Pty. Ltd., Forbes, NSW, Australia.
5 Lupin supplied by: M.C. Croker Pty Ltd, Wagga Wagga, NSW, Australia.

Table 3.   Basal diet formulation (dry basis).

Ingredient	g kg^{-1}
Chilean fish meal	801.5
Corn gluten	113.5
Cod liver oil	70.0
Vitamins[1]	7.5
Minerals[2]	7.5

[1] Vitamin premix, (active ingredients in 1 kg feed): Vitamin A, 8000 iu; vitamin D3 1000 iu; vitamin E 50, 125 mg; vitamin K, 16.5 mg; thiamine HCl (89.3%), 10 mg; riboflavin (80%), 25.5 mg; pyridoxine HCl (81%), 15 mg; cal DL pantothenate (45%), 55 mg; biotin (2%), 1 mg; niacin, 200 mg; folic acid, 4 mg; vitamin B12 (1%), 0.02 mg; vitamin C stab (96%), 1000 mg; choline Cl (50%), 1500 mg; inositol, 600 mg.
[2] Mineral premix, (active ingredients in 1 kg feed): Ca (as calcium iodate, 62% I), 0.465 mg; Cu (as copper sulphate, 25% Cu) 3 mg; Fe (as ferrous sulphate, 30% Fe), 30 mg; Mg (as magnesium sulphate; Epson salt, 10%), 500 mg; Mn (as manganese sulphate, 31% Mn)10 mg; Se (as sodium selenite, 44% Se), 0.33 mg; Zn (as zinc sulphate, 34% Zn), 100 mg; filler (lime), 2.1 mg.

Table 4. Formulation and measured composition of diets containing either 30% raw or gelatinised wheat starch and graded levels of Natustarch® for Experiment 1.

Diet[1]	Basal	Raw starch	Raw starch	Raw starch	Raw starch	Gel[3] starch	Gel starch	Gel starch	Gel starch
Natustarch® level (mg kg⁻¹ diet)	0	0	50	100[2]	150	0	50	100[2]	150
Basal diet (g kg⁻¹ diet)	1000	700	700	700	700	700	700	700	700
Raw wheat starch (g kg⁻¹ diet)	-	300	300	300	300	-	-	-	-
Gel wheat starch (g kg⁻¹ diet)	-	-	-	-	-	300	300	300	300
Composition (Dry basis)									
Protein (%)	70.3	49.2	49.2	49.2	49.2	49.2	49.2	49.2	49.2
Energy (MJ kg⁻¹)	22.6	20.9	20.9	20.9	20.9	21.3	21.3	21.3	21.3
Lipid (%)	15.8	11.1	11.1	11.1	11.1	11.1	11.1	11.1	11.1
Starch (%)	<0.3	29.4	29.4	29.4	29.4	29.4		29.4	29.4
Ash (%)	12.1	8.5	8.5	8.5	8.5	8.5	8.5	8.5	8.5

[1] Ytterbium chloride was used at 0.1g kg⁻¹ as the inert indicator.
[2] Recommended application rate of Natustarch® (BASF, Australia).
[3] Gel = gelatinised.

Table 5. Formulation and measured composition of diets containing either 30% wheat or lupin and graded levels of Natugrain-blend[®] for Experiment 2.

Diet[1]	Basal	Wheat	Wheat	Wheat	Wheat	Lupin	Lupin	Lupin	Lupin
Natugrain-blend[®] level (μL kg^{-1} diet)	0	0	75	150[2]	300	0	75	150[2]	300
Basal diet (g kg^{-1} diet)	1000	700	700	700	700	700	700	700	700
Wheat (g kg^{-1} diet)	-	300	300	300	300	-	-	-	-
Lupin (g kg^{-1} diet)	-	-	-	-	-	300	300	300	300
Composition (Dry basis)									
Protein (%)	70.5	53.8	53.8	53.8	53.8	61.2	61.2	61.2	61.2
Energy (MJ kg^{-1})	22.8	20.9	20.9	20.9	20.9	21.6	21.6	21.6	21.6
Lipid (%)	15.1	11.1	11.1	11.1	11.1	12.5	12.5	12.5	12.5
Total CHO[3] (%)	2.2	25.7	25.7	25.7	25.7	15.6	15.6	15.6	15.6
Starch (%)	<0.3	17.0	17.0	17.0	17.0	<0.3	<0.3	<0.3	<0.3
Ash (%)	12.2	9.4	9.4	9.4	9.4	9.7	9.7	9.7	9.7

[1] Ytterbium chloride was used at 0.1g kg^{-1} as the inert indicator.
[2] Recommended application rate of Natugrain-blend[®] (BASF, Australia).
[3] Total carbohydrate calculated by difference; CHO (including fibre) (%) = 100-(protein % + lipid % + ash %).

Table 6. Apparent digestibility coefficients (%) of diets and ingredients by silver perch for Experiment 1[1].

Index	Ingredient type (A)		se	A (P)	Natustarch® level (mg kg^{-1} diet) (B)				se	B (P)	Interaction A X B (P)
	Raw	Gel			0	50	100	150			
Dry matter ADC (Diet)	80.1	84.7*	0.39	<0.01	80.9^a	83.1^b	83.4^b	82.1^{ab}	0.55	0.02	0.42 ns
Starch ADC[2]	88.5	99.5*	0.52	<0.01	92.2^a	95.0^b	93.4^{ab}	95.6^b	1.00	0.02	0.02
Energy ADC (Diet)	88.6	92.7*	0.31	<0.01	90.0^a	91.0^a	91.2^a	90.5^a	0.44	0.24	0.86 ns
Energy ADC (Ingredient)	76.3	92.9*	1.22	<0.01	81.9^a	86.0^a	86.6^a	83.7^a	1.72	0.23	0.87 ns
Protein ADC (Diet)	91.9	91.7	0.25	0.70	90.6^a	92.4^b	92.4^b	91.8^b	0.35	0.01	0.39 ns

[1] Values are means. For ingredient type * denotes significant difference (n = 12 pooled replicate tanks for each starch type). For Natustarch® level means in the same row with the same letter in the superscript are not significantly different (n = 6 pooled replicate tanks) (two-factor ANOVA; P > 0.05; SNK).
[2] Denotes a significant interaction between ingredient type and Natustarch® level (P < 0.05).
ns = not significant.

Table 7.   Total reducing sugar content of diets following the addition of Natustarch®.

Diet and Natustarch® level (mg kg⁻¹ diet)	Total reducing sugar (mg g⁻¹ diet as fed)[1]	Increase in total reducing sugar (%)[2]
30% raw wheat starch (0)	5.5^{ab}	-
30% raw wheat starch (50)	8.6^{c}	60
30% raw wheat starch (100)	7.3^{bc}	35
30% raw wheat starch (150)	10.9^{d}	99
30% gelatinised wheat starch (0)	3.1^{a}	-
30% gelatinised wheat starch (50)	11.9^{de}	289
30% gelatinised wheat starch (100)	14.1^{e}	366
30% gelatinised wheat starch (150)	14.0^{e}	363
Pooled se	0.68	

[1] Means ($n = 2$) in the same column which share the same superscript are not significantly different (one-factor ANOVA; SNK; $P > 0.05$).

[2] The percent increases of total reducing sugar for diets from each series (raw or gelatinised wheat starch) which had Natustarch® added, when compared to the diet within each series which contained no Natustarch® were determined as follows = (reducing sugar in test diet-reducing sugar in control diet)/reducing sugar in control diet) x 100.

Table 8. Apparent digestibility coefficients (%) of ingredients and diets for silver perch following the addition of Natugrain-blend® for Experiment 2.

ADC%	Ingredient type (A)		se	A	Natugrain blend® level (µL kg-1 diet) (B)				se	B	Interaction
	Wheat	Lupin		(P)	0	75	150	300		(P)	A X B (P)
DM (Diet)	74.0	66.4*	0.56	<0.01	72.1[a]	69.3[a]	70.0[a]	69.4[a]	0.80	0.08	0.20 ns
Energy (Diet)	83.3	77.8*	0.44	<0.01	81.6[a]	80.5[a]	80.4[a]	79.7[a]	0.62	0.22	0.14 ns
Energy (Ingredient)	60.0	41.6*	1.76	<0.01	54.9[a]	50.5[a]	50.1[a]	47.7[a]	2.48	0.26	0.16 ns
Protein (Diet)	88.2	88.6	0.26	0.24	88.8[a]	88.0[a]	88.6[a]	88.2[a]	0.36	0.35	0.40 ns

[1] Values are means. For ingredient type * denotes significant difference ($n = 12$ pooled replicate tanks for each starch type). For Natugrain blend® level means in the same row with the same letter in the superscript are not significantly different ($n = 6$ pooled replicate tanks) (two-factor ANOVA; $P > 0.05$; SNK).
ns = not significant

4.8.   Effect of the addition of two enzymes to two commercial diets for silver perch

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Introduction

The addition of enzymes to diets has been shown to improve digestibility of diets for pigs and poultry. Their effectiveness has also been evaluated for fish, with varying results. The aim of this study was to investigate the effect on digestibility of adding Natuphos® to two commercial diets currently being used for silver perch. The diets differed in their ingredient compositions but had similar nutrient specifications.

Natuphos® contains phytase, specific for phytic acid and has been reported to improve the availability of phosphorous and also increase protein utilisation.

Materials and Methods

The addition of Natuphos®, a feed additive containing phytase, to two diet formulations, SP35 and 95LC2 (Allan & Rowland 1998), was evaluated during this study. Natuphos®, in a liquid form, was supplied by BASF Australia. Prior to the experiment the phytase activity of the Natuphos® additive was determined (5700 FTU/g). Based on this activity level the Natuphos® was added to each of the diets at 3 nominal levels (Table 2). An additional diet (SP35 autoclaved at 100°C for 5 minutes prior to the application of 500 FTU/kg Natuphos®) was also included in the study to estimate effects of steam conditioning on enzyme effectiveness. The Natuphos® was added to distilled water and sprayed on to the diets after they were pelleted. Three separate 20 g samples of each diet were analysed to evaluate phytase activity and homogeneity of enzyme application (Table 3).

Eight silver perch (mean individual weight, 11.6g) were stocked into each of twenty seven 170l cylindroconical tanks (n = 3 tanks /diet) and fed their respective diets at 8% bw /day (Table 2). Faeces were collected by settlement and apparent digestibility coefficients (ADC's) for dry matter, phosphorous and nitrogen were calculated using ytterbium chloride (0.01%) as the inert indicator.

The digestibility coefficients were analysed using two-factor ANOVA, with diet as the first factor and Natuphos® level as the second factor. The SP35 diets (raw and steamed) containing 500 FTU/kg of Natuphos® were compared using one factor ANOVA.

Table 2.   Natuphos® inclusion experimental diets.

Natuphos® inclusion level (FTU/kg)[1]	Diet		
	SP35 Cold pelleted	95LC2 Steam pelleted	SP35 Steamed[2]
0	x	x	
500	x	x	x
1000	x	x	
2000	x	x	

[1] Natuphos® phytase activity 5700 FTU/g.
[2] Autoclaved at 100°C for 5 minutes to mimic the effect of a steam pelleting process.

Table 3.   Nominal and measured Phytase levels in experimental diets.

Nominal phytase inclusion level (FTU/kg)[1]	Measured phytase level (FTU/kg)[2]		
	SP35 Cold pelleted	95LC2 Steam pelleted	SP35 Steamed
0	360	70	290
500	660±10	335±5	455±15
1000	785±13	713±9	-
2000	1617±46	1520±17	-

[1] Natuphos® phytase activity 5700 FTU/g.
[2] Except for diets containing no additional phytase (mean, $n = 2$) all values are mean±se, $n = 3$.

Results

The two-factor ANOVA indicated that there was a significant difference between the DM, P and N digestibility of SP35 and 95LC2 ($P < 0.05$), however, there was no significant effect of phytase on DM, P and N digestibility for any of the diets ($P > 0.05$) (Table 4). There was no interaction between diet and phytase level ($P > 0.05$).

Autoclaving had no significant effect on the phosphorous digestibility of SP35 containing 500 FTU/kg Natuphos® by silver perch.

Table 4.   Dry matter, phosphorous and nitrogen apparent digestibility coefficients for diets[1].

Diet	Apparent digestibility coefficient (%)		
	Dry matter	Phosphorous	Nitrogen
SP35	68.54±0.64	41.81±2.57	90.66±0.19
SP35 Natuphos® 500 FTU/kg	71.18±0.42	43.09±3.01	91.08±0.40
SP35 Natuphos® 1000 FTU/kg	69.60±0.49	42.60±1.58	91.15±0.28
SP35 Natuphos® 2000 FTU/kg	69.74±1.00	50.27±2.04	90.94±0.84
95LC2	57.16±1.03	15.84±0.76	80.09±0.44
95LC2 Natuphos® 500 FTU/kg	56.06±1.68	14.60±6.04	79.60±1.02
95LC2 Natuphos® 1000 FTU/kg	57.86±0.89	19.71±2.29	81.26±0.67
95LC2 Natuphos® 2000 FTU/kg	56.44±0.84	15.26±1.59	79.53±0.88
Autoclaved SP35 Natuphos® 500 FTU/kg	68.84±0.40	42.89±1.48	90.90±0.18

[1] Mean±se, *n* = 3.

Conclusions

The addition of Natuphos® to the two commercial diet formulations fed to silver perch did not lead to an improvement in phosphorous digestibility. Dry matter and nitrogen digestibility were also unaffected. There are several possible explanations for the poor performance of the enzyme additive. Leaching of the Natuphos® from the feed pellets may have contributed to the loss of some of the phytase from the pellet. However, as the pellet is available for the fish to eat for a maximum of 16 seconds this is unlikely. The recommended level of Natuphos® for pig and poultry diets is approximately 500 FTU/kg of diet. Even with the high levels of Natuphos® (2000 FTU/kg) used in this study, there was no evidence of enhanced phosphorous digestibility for silver perch.

Another factor influencing the performance of Natuphos® on the digestibility of phosphorous is pH. The activity of Natuphos® is highly dependent on pH, and maximum activity is achieved at a pH of approximately 5.0. The pH of the digestive tract of silver perch is approximately 7.0 (Anderson, 1998), and at this pH, the activity of Natuphos® is practically nil (Hoppe, 1992).

To aid in the effectiveness of this enzyme for the digestibility of phosphorous in silver perch diets, further work should investigate the leaching of Natuphos® into the water column. Additionally the development of a phytase enzyme additive with an optimum pH more suited to that in the digestive tract of silver perch may also be beneficial.

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4.9.   **Metabolic studies on carbohydrate utilisation by barramundi and tilapia**

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Abstract

Barramundi, unlike tilapia, are slow to clear a dose of glucose injected into the peritoneal cavity. They also appear to be unable to metabolise galactose and xylose at any appreciable rate. From studies of blood glucose changes after meals, it appears that their capacity to digest starches, dextrins and maltose is limited, but that they absorb free glucose rapidly. Unlike tilapia, the absorbed glucose is only slowly removed from the blood, resulting in an extended hyperglycaemia after feeding on diets containing free glucose. From studies on the accumulation of glycogen and lipid in the muscle and liver, it seems that there are differences between barramundi and tilapia in terms of their metabolism of energy-containing nutrients such as lipid and carbohydrate.

Introduction

Increasing effort is being made to use more plant ingredients, such as wheat, lupins and field peas as protein sources, or to spare energy in aquaculture diets, in order to obtain increased economic efficiencies (Tacon, 1994; Wilson, 1994; Allan, 1998; Allan *et al.* 2000a). One of the key limitations to the increased use of plant ingredients is the ability of fish to utilise some or perhaps all of the carbohydrates normally present in plants (Allan *et al.* 2000a).

Plant carbohydrates are generally considered to be in two categories, reserve polysaccharides and structural polysaccharides. The polysaccharide, starch, is an energy reserve in most plants and seeds and one of the predominant carbohydrates in wheat (~80%) and field peas (~40%). When totally digested, glucose is the major component of starch. However, the ability of many fish to digest starch appears to be quite limited, thus there is interest in inclusion into feeds of starch derivatives processed in ways to improve digestibility. Processes of interest in this area include gelatinisation and dextrinisation. Non-starch polysaccharides (NSP) have a structural role in plants and seeds, and are predominantly composed of glucans and pentosans. When NSPs are digested they yield a high proportion of monosaccharides such as galactose and xylose. Lupins contain 30 - 40% NSP, of which galactose and xylose comprises 40-70% and 5-10% respectively.

Glucose tolerance tests have been conducted on a range of fish species. (Palmer and Ryman, 1972; Furuichi and Yone, 1982; Shimeno, 1982; Furuichi *et al.* 1986; Wilson, 1994; Lin *et al.* 1995; Shiau and Chuang, 1995). In general, fish of a lower trophic level (herbivorous, omnivorous) tend to be more efficient at both the uptake and clearance of glucose compared to carnivorous species (Furuichi and Yone, 1981; Garcia-Riera and Hemre, 1996; Peres *et al.* 1999). However, few studies have been made on sugars other than glucose, particularly those which are present in the NSP fraction of plants. Investigation of the ability of fish to utilise the monosaccharide galactose has been reported (Hung, 1991; Shikata *et al.* 1994). Hung (1991) reported that following an oral administration of galactose sturgeon (*Acipenser transmontanus*) actively transported galactose and convert galactose into glucose *in situ*. Shikata *et al.* (1994) reported that after an oral administration of glucose or galactose, carp exhibited similar uptake rates for both sugars, but the clearance rate of

glucose from the blood stream was greater than galactose. A thorough search of the literature has indicated that there is a dearth of data reported on xylose tolerance in fish.

This work was carried out as a comparative study of two species, the carnivorous barramundi (*Lates calcarifer*) and the omnivorous/detritivorous tilapia (*Oreochromis mossambicus*). The aim was to investigate the tolerance of two species to glucose, galactose and xylose, and to examine the metabolic effects of dietary starch, processed starch and starch derivatives on blood glucose levels and on accumulation of fat and glycogen in the tissues.

Materials and Methods

Barramundi juveniles (about 25 mm total length) were obtained from a commercial hatchery and grown to the required size on a commercial pelleted diet. Tilapia from a captive population obtained from the North Pine Dam were bred, and the juveniles grown to the required size, again using a commercial pelleted barramundi feed. Fish were housed in groups in 60 x 60 x 30 cm glass tanks with a recirculating water supply.

For the experiments on carbohydrate tolerance, fish were selected randomly from the available stock to give group mean weights of about 25 g, with a coefficient of variation of 25%, and placed in groups of 5 fish per treatment. Fish were lightly anaesthetised with MS-222 (Sigma Chemical Co., Castle Hill, NSW) and injected intraperitoneally (i.p.) with the glucose solution at a dose rate of 1, 2 or 4 g/kg, or with galactose or xylose at a rate of 1 g/kg. They were then returned to the tank and blood samples taken from 5 fish at each time interval, under anaesthesia using a 26-gauge needle and collecting from the caudal vein. The blood was immediately deproteinated using the barium hydroxide-zinc sulphate precipitant and the supernatant stored frozen until analysed for the appropriate carbohydrate. Glucose was measured using the glucose oxidase method with a test kit form Sigma (Castle Hill, NSW), while galactose was measured as total reducing sugar (Dische, 1962a) corrected for the presence of glucose. Xylose was measured colorimetrically using the orcinol reaction (Dische, 1962b).

For the experiments on the effect of diet on circulating glucose levels, a similar procedure was used except that the fish were fed a ration of 2% of body weight of the appropriate diet instead of being injected.

For the 6 week feeding trials, groups of fish were fed with the appropriate diet twice a day to satiation for 6 weeks. The feeding rate was estimated to be approximately 4% body weight per day. After 6 weeks the fish were sacrificed by over-anaesthetisation in MS-222, following which the liver and a sample of muscle were taken and frozen for glycogen and triacylglycerol measurement. . Liver and muscle glycogen concentrations were analysed by the amyloglucosidase digestion method of Murat & Serfaty (1974). Liver and muscle triacylglycerol concentrations were analysed using the Hantzsch reaction following extraction and saponification (Teitz 1987).

Nine different diets were prepared as indicated in Table 1, using raw and gelled wheat starch, 3 dextrins (Fieldoses 9, 17 and 30 from Goodman Fielder), pea starch, glucose and maltose as carbohydrate sources. All ingredients used in the basal diet and test ingredients were ground using a hammer mill (C-E Raymond Inc., IL, USA) or sieved to ensure all particles passed through a 710 μm screen. Dry ingredients were thoroughly mixed in a Hobart mixer (Troy Pty. Ltd., OH, USA). Ytterbium chloride, an inert indicator, was then sprayed onto each batch of diet mash at 0.1g kg^{-1} dry basis. The mash was then combined with approximately 400 mL distilled water kg^{-1} dry mix before being cold pelleted through a meat mincer (Barnco Australia Pty. Ltd., Leichhardt, NSW, Australia) with a 4 mm die. The diet was then dried in a convection oven at 35C until the moisture content was approximately 20%. The diets were then reground in a hammer mill to ensure

homogenous mixing of the ytterbium chloride throughout the mash. The appropriate amount of oil was added to each batch of mash and then each diet was re-pelleted with a 2 mm die. Pellets were dried at 35C in a convection drier for approximately 6 h until the moisture content was between 10 to 15%, to produce a dry, sinking pellet.

Results were analysed by ANOVA, and comparison of means performed by Tukey's test.

Results

Initial experiments with non-injected fish showed that the handling procedure did not cause any increase in blood glucose levels. When the fish were injected with glucose, a marked difference between the two species in glucose tolerance was found (Figure 1). Although the blood glucose level reached similar levels with the 1 and 2 g/kg doses, the time at which the maximum was reached was different. In tilapia, the maximum blood glucose level was obtained 1 hour after injection with a dose rate of 1 g/kg, and after 2 hours with a dose of 2 g/kg. In contrast, barramundi took 8 hours to reach the maximum with both the 1 and 2 g/kg doses, and with the 2 g/kg dose the blood glucose level was still significantly elevated after 16 hours. A dose of 4 g/kg resulted in blood glucose levels remaining close to 300 mg/100 mL for over 24 hours.

There were major differences between barramundi and tilapia in their tolerance of galactose and xylose (Figure 2). At a dose of 1 g/kg there was a prolonged elevation, lasting over 24 hours, of reducing sugar in the blood of barramundi, but in tilapia only a small elevation lasting less than 4 hours was observed. Xylose administered at a dose of 1 g/kg caused an extended elevation of blood levels in both species, but the level in tilapia dropped at a faster rate than in barramundi (Figure 2).

When the blood glucose levels were followed after feeding the 9 diets (basal and with 8 with different carbohydrates) only very small differences were seen, except when glucose was incorporated into the diet (Figure 3). There was a small rise in blood glucose when the basal diet was fed to both species, but the increase from 60 up to 80 (mg/100 ml) was smaller in tilapia than in barramundi, where the increase was from 60 to 120. Incorporating raw or pre-gelled starch into the diet of tilapia caused no noticeable change to this pattern (Figure 3). The same result was obtained when dextrins, maltose or pea starch were included in the diets (data not shown). In barramundi raw wheat starch caused an increased blood glucose level after 1 hour, and pre-gelled wheat starch caused a higher level still, and extended to 2 hours. As in tilapia, the inclusion of dextrins, pea starch or maltose gave blood glucose profiles indistinguishable from that given by the basal diet.

The inclusion of glucose had a marked effect on blood glucose levels in both species. In tilapia a significantly elevated level was observed only at 4 hours after feeding, while in barramundi the level was significantly elevated after 1 hour and remained so for between 8 and 16 hours (Figure 3).

Feeding on diets containing different carbohydrates showed substantial differences between diets and between species in the accumulation of lipid and glycogen (Figures 4 to 7 and Table 2). Between species, barramundi liver (mean of all diets 151 mg/g) and muscle (20 mg/g) always contained more lipid than tilapia liver (57 mg/g) and muscle (6 mg/g). Also, where barramundi liver had a relatively constant level of glycogen, tilapia liver had levels that appeared to vary more with diets. Muscle glycogen levels were generally low and varied much more between individual animals. In tilapia, the diet containing pre-gelled wheat starch was associated with a higher level of liver lipid than the raw wheat starch diet, but the opposite association with liver glycogen levels was found. Between diets there were a number of significant differences found with both liver and muscle lipid levels.

Discussion

It was apparent that barramundi are far less tolerant of all three monosaccharides than are tilapia. In tilapia, glucose appeared rapidly in the blood after i.p. injection, peaking after 1 or 2 hours depending on the dose (1 or 2 g/kg) and returning to normal within 8 hours. In contrast, the same doses in barramundi caused blood levels to peak 8 hours after administration, and returning to normal in 16 hours after a dose of 1 g/kg, but taking over 16 hours to return to normal after a dose of 2 g/kg. This is taken to indicate that the processes which transfer glucose from the peritoneum to the blood operate much more quickly in tilapia, and that the uptake from the blood to the tissues is also more rapid in tilapia. This is consistent with tilapia possessing glucose metabolic processes that are more active and efficient than those of barramundi. Similarly, barramundi take much longer to clear galactose and xylose from the blood following i.p. injection of these sugars than do tilapia. However, it is not known if tilapia actually metabolise xylose, or whether it is simply slowly excreted. With galactose, only a small elevation in blood reducing sugar level was observed, which indicates that these fish can metabolise galactose as rapidly as it appears in the blood, or that galactose is very inefficiently transferred from the peritoneum to the blood. The conclusion is that attempts to improve DM and energy digestibility by pre-digestion of NSP will not be successful in barramundi, as they have a very limited, if any, capacity to metabolise the products of NSP digestion.

Tilapia are known to tolerate a much higher dietary starch level than do barramundi (Wilson, 1994), and are also known to digest starch efficiently. In contrast, barramundi do not appear to show high digestibility coefficients towards starch (McMeniman, unpublished results). A series of diets were set up containing the products of partial starch digestion – dextrins, maltose and glucose – with the aim of detecting whether these products would be contribute more glucose than the carbohydrate-free basal diet or raw starch itself. In tilapia, none of these diets, except the one containing glucose, caused any elevation in blood glucose concentration over that caused by the basal diet. The elevation caused by the basal diet is presumed to be associated with the increased metabolic activity associated with digestion and absorption. At a feeding rate of 2% body weight, a diet containing the equivalent of 30% carbohydrate as glucose would result in an oral loading of 6 g/kg. An i.p. dose of 2 g/kg raised glucose levels to over 250 mg/100 mL, however, the increase in blood glucose level resulting from the oral dose of approximately 6 g/kg was only 122 mg/100 mL and peaked 4 hours after feeding. It is concluded that the process of uptake from the blood into the tissues is as rapid as the processes of digestion and absorption in these fish.

Only slight effects were seen in the starch-containing diets when fed to barramundi. With raw starch, there was an increased level of blood glucose 1 hour after feeding when compared to the basal diet, and with pre-gelled starch the level was significantly elevated at 1 and 2 hours after feeding. However, the diets containing the dextrins and maltose did not show any effect on blood glucose levels, but the diet containing glucose gave a large and sustained increase in blood glucose levels. This is taken to indicate that (a) pre-gelled starch is more quickly digested and absorbed than raw starch, (b) the digestion of dextrins and maltose does not proceed at a more rapid rate than with starch itself, and (c) the absorption of glucose from the intestine is very rapid compared to the rate of digestion and the rate of uptake from the blood. Consequently, inclusion of pre-digested starch products in barramundi diets will probably not result in better utilisation of dietary carbohydrate by barramundi.

Lipid (triacylglycerol) and glycogen were present, as expected, in both the liver and the muscle of fish which had been fed the diets for 6 weeks. Barramundi liver contained higher levels of lipid, between 95 and 190 mg/g, or approximately 10 to 20% by weight. Higher levels were associated with the control diet, starch and low dextrin, while lower levels were associated with the medium and high dextrins, maltose and glucose. In tilapia, only pre-gelled starch was associated with high liver lipid levels, while the control diet was associated with low levels. However, raw starch was

associated with higher glycogen levels than the basal diet or pre-gelled starch. Muscle lipid and glycogen levels showed some differences between diets, but were considerably more variable. The control diet contained no carbohydrate and 70 g/kg fish oil, while the carbohydrate diets contained 300 g/kg of the different carbohydrates and 4.9 g/kg oil. The conclusion is that there are substantial differences in energy metabolism between the species in terms of energy storage and lipogenesis from dietary nutrients, which would need considerable further investigation to elucidate.

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Table 1.   Diet formulation (dry basis).

Ingredient	g kg-1
Chilean fish meal	801.5
Corn gluten	113.5
Fish oil	70.0
Vitamins1	7.5
Minerals2	7.5

[1] Vitamin premix, (active ingredients in 1 kg feed): Vitamin A, 8000iu; vitamin D3 1000 iu; vitamin E 50, 125 mg; vitamin K, 16.5 mg; thiamine HCl 89.3%, 10 mg; riboflavin S/D 80%, 25.5 mg; pyridoxine HCl 81%, 15 mg; cal DL Pant 45%, 55 mg; biotin 2%, 1 mg; niacin, 200 mg; folic acid, 4 mg; vitamin B12 1%, 0.02 mg; vitamin C stab 96%, 1000 mg; choline Cl 50%, 1500 mg; inisitol, 600 mg.

[2] Mineral premix, (active ingredients in 1 kg feed): Calcium iodate 62% I, 0.465 mg; copper sulphate 25% Cu, 3 mg; ferrous sulphate 30% Fe, 30 mg; magnesium sulphate (Epson salt 10% mg), 500 mg; manganese sulphate 31% Mn, 10 mg; sodium selenite 44% Se, 0.33 mg; zinc sulphate 34% Zn, 100 mg; filler (lime), 2.1 mg.

Test diets

Diet number	Basal mash (g kg^{-1})	Test ingredient (g kg^{-1})
1. Basal diet	1000	-
2. Raw wheat starch	700	300
3. Gelatinised wheat starch	700	300
4. Dextrin, Fieldose 9	700	300
5. Dextrin, Fieldose 17	700	300
6. Dextrin, Fieldose 30	700	300
7. Maltose	700	300
8. Glucose	700	300
9. Raw pea starch	700	300

[1] Ytterbium chloride was used at 0.1g kg^{-1} as the inert indicator.

Table 2.   Significance of differences in tissue lipid and glycogen levels between diets as
                    determined by Tukey's test.

Barramundi liver lipid

Diet	2	3	4	5	6	7	8	9
1	n.s.							
2	n.s.	n.s.						
3	n.s.	n.s.	n.s.					
4	p<0.01	p<0.01	p<0.01	p<0.01				
5	p<0.01	p<0.01	p<0.01	p<0.01	n.s.			
6	p<0.01	p<0.05	n.s.	p<0.05	n.s.	n.s.		
7	p<0.01	p<0.01	p<0.01	p<0.01	n.s.	n.s.	n.s.	
8	n.s.	n.s.	n.s.	n.s.	n.s.	p<0.01	n.s.	n.s.

Tilapia liver lipid

Diet	2	3	4	5	6	7	8	9
1	p<0.01							
2	p<0.01	p<0.01						
3	n.s.	n.s.	p<0.01					
4	n.s.	n.s.	p<0.01	n.s.				
5	n.s.	p<0.05	p<0.01	n.s.	n.s.			
6	n.s.	p<0.01	p<0.01	n.s.	n.s.	n.s.		
7	n.s.	n.s.	p<0.01	n.s.	n.s.	n.s.	n.s.	
8	n.s.	n.s.	p<0.01	n.s.	n.s.	n.s.	n.s.	n.s.

Barramundi liver glycogen

Diet	2	3	4	5	6	7	8	9
1	p<0.01							
2	p<0.01	n.s.						
3	p<0.01	n.s.	n.s.					
4	n.s.	p<0.01	p<0.01	n.s.				
5	p<0.01	p<0.05	p<0.05	n.s.	n.s.			
6	p<0.01	p<0.05	p<0.05	n.s.	n.s.	n.s.		
7	n.s.	p<0.01	p<0.01	n.s.	n.s.	n.s.	n.s.	
8	n.s.	p<0.01	p<0.01	n.s.	n.s.	n.s.	n.s.	n.s.

Tilapia liver glycogen

Diet	2	3	4	5	6	7	8	9
1	p<0.01							
2	n.s.	p<0.01						
3	p<0.05	n.s.	n.s.					
4	p<0.05	n.s.	n.s.	n.s.				
5	n.s.	p<0.05	n.s.	n.s.	n.s.			
6	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.		
7	n.s.	p<0.01	n.s.	n.s.	n.s.	n.s.	n.s.	
8	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.

Table 2.   Continued

Barramundi muscle lipid

Diet	2	3	4	5	6	7	8	9
1	n.s.							
2	n.s.	n.s.						
3	n.s.	n.s.	n.s.					
4	p<0.05	n.s.	p<0.05	n.s.				
5	p<0.05	n.s.	p<0.05	n.s.	n.s.			
6	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.		
7	p<0.01	n.s.	p<0.01	n.s.	n.s.	n.s.	n.s.	
8	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.

Tilapia muscle lipid

Diet	2	3	4	5	6	7	8	9
1	n.s.							
2	n.s.	n.s.						
3	n.s.	n.s.	n.s.					
4	n.s.	n.s.	n.s.	n.s.				
5	n.s.	n.s.	p<0.05	n.s.	p<0.05			
6	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.		
7	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	
8	n.s.	n.s.	n.s.	n.s.	n.s.	p<0.05	n.s.	n.s.

Barramundi muscle glycogen

Diet	2	3	4	5	6	7	8	9
1	n.s.							
2	p<0.01	p<0.01						
3	n.s.	n.s.	p<0.05					
4	n.s.	n.s.	p<0.01	n.s.				
5	n.s.	n.s.	p<0.01	n.s.	n.s.			
6	n.s.	n.s.	p<0.01	n.s.	n.s.	n.s.		
7	n.s.	n.s.	n.s.	n.s.	n.s.	p<0.05	n.s.	
8	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.

Tilapia muscle glycogen

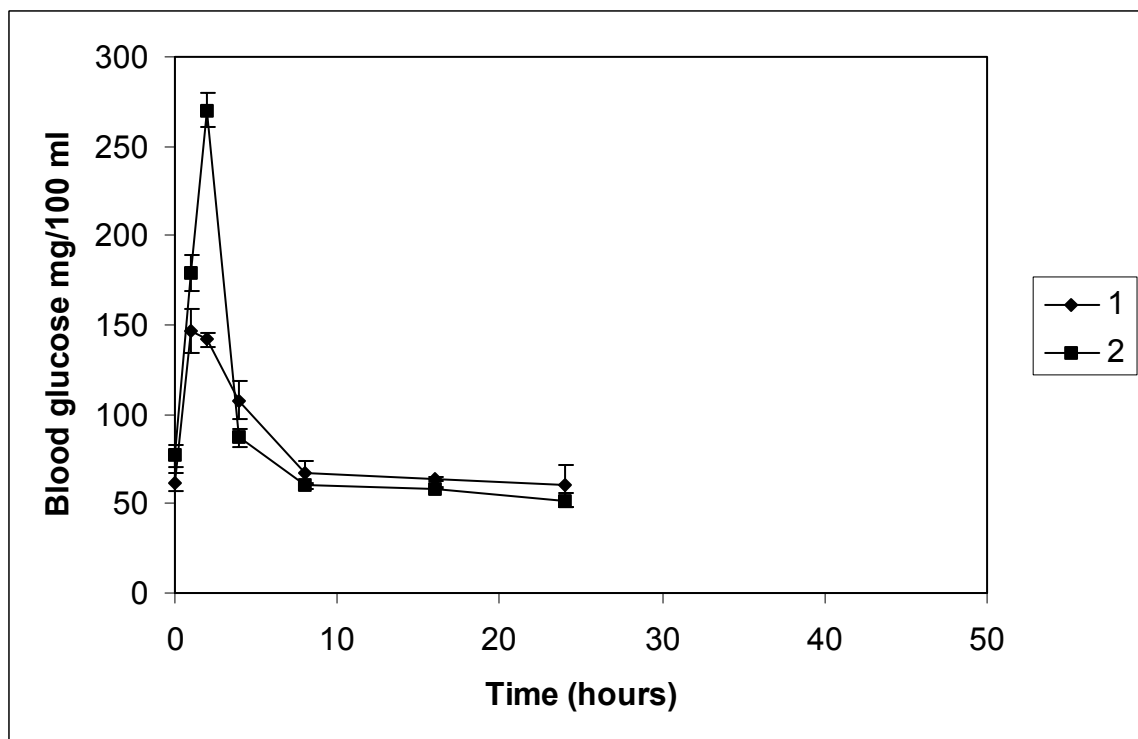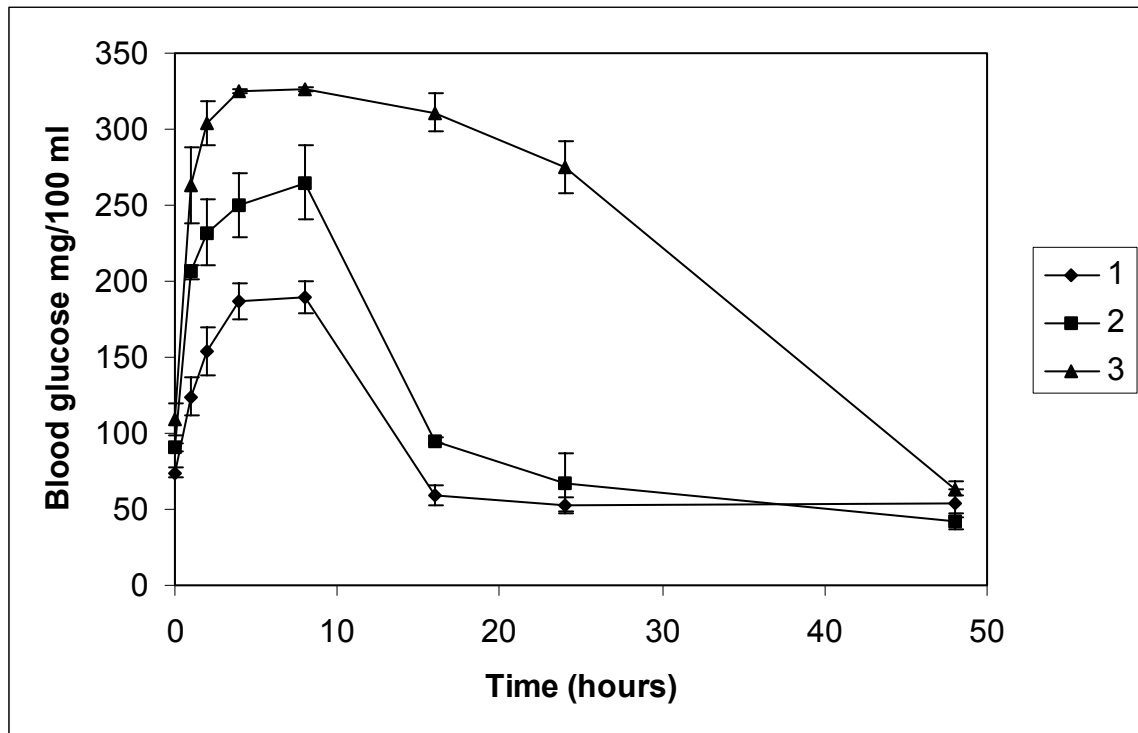| Diet	2	3	4	5	6	7	8	9
1	n.s.							
2	n.s.	n.s.						
3	n.s.	n.s.	n.s.					
4	n.s.	n.s.	n.s.	n.s.				
5	n.s.	n.s.	n.s.	n.s.	n.s.			
6	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.		
7	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	
8	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.

Figure 1. Blood glucose levels (means ± s.e.m.) in barramundi (upper panel) and tilapia (lower panel) following i.p. injection with a dose of 1 g/kg (line 1), 2 g/kg (line 2) or 4 g/kg (line 3).
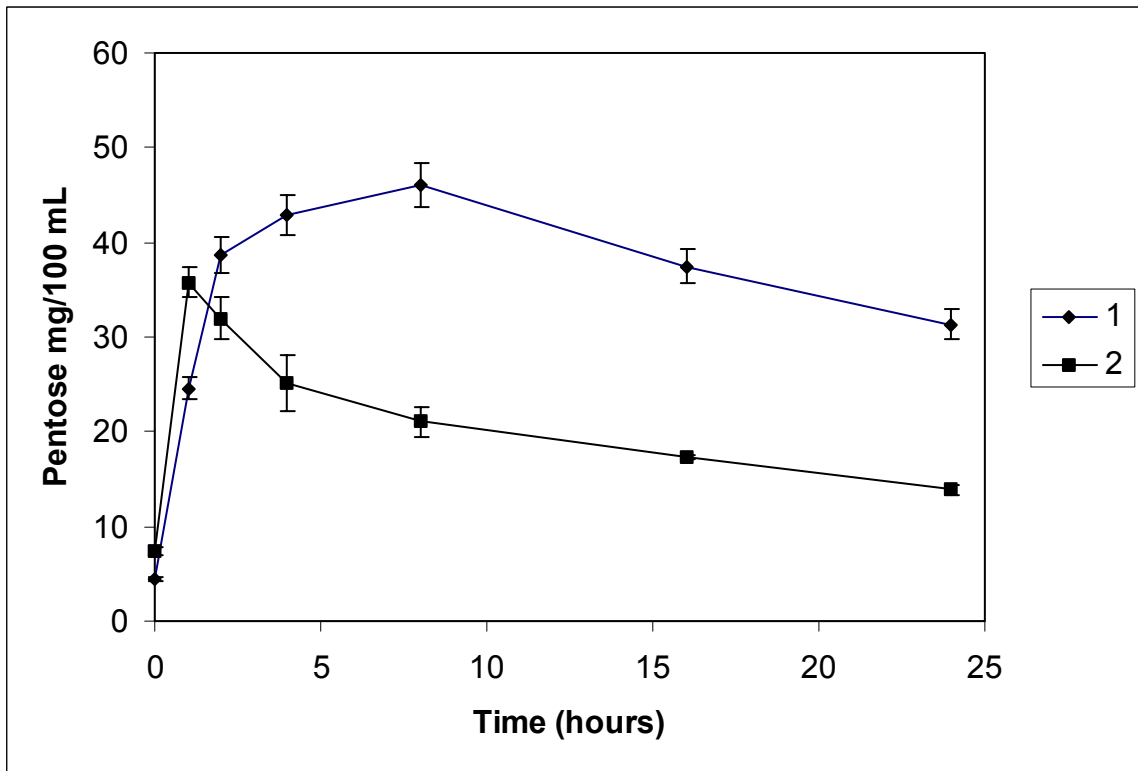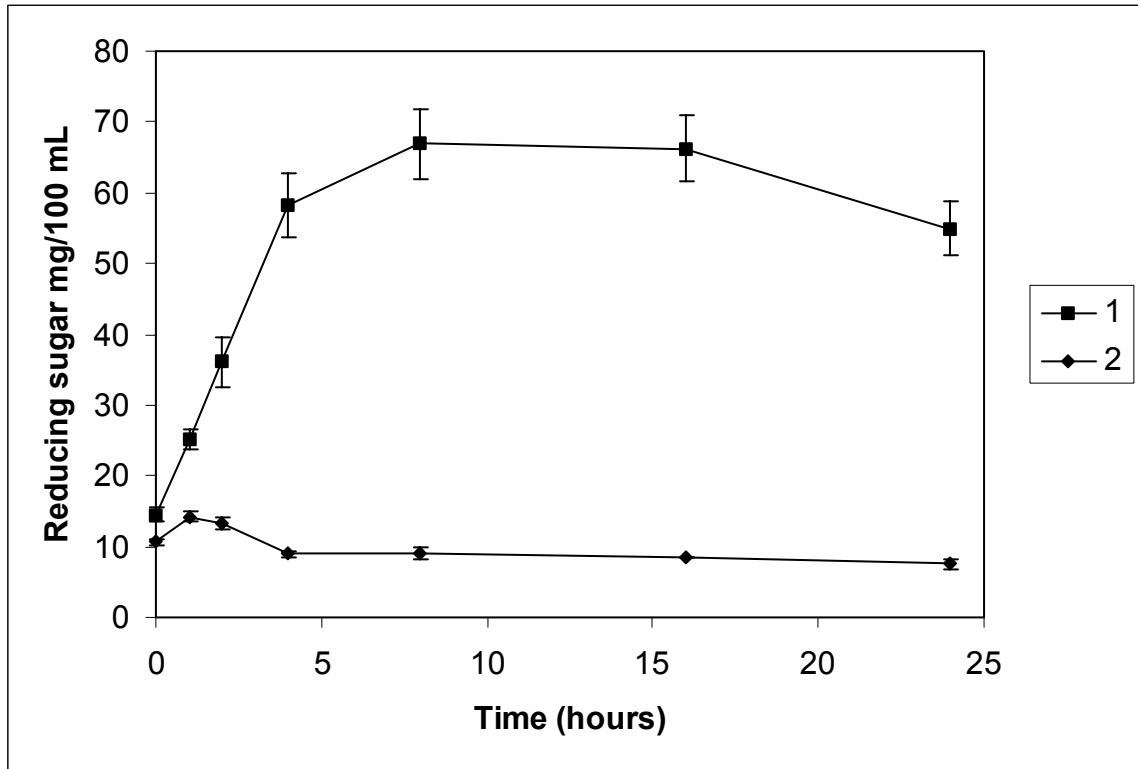
Figure 2. Upper panel: blood reducing sugar level (corrected for glucose) following i.p. injection of galactose at a dose of 1 g/kg in Lower panel: blood pentose level following i.p. injection of xylose at a dose of 1 g/kg in barramundi (1) and tilapia (2).
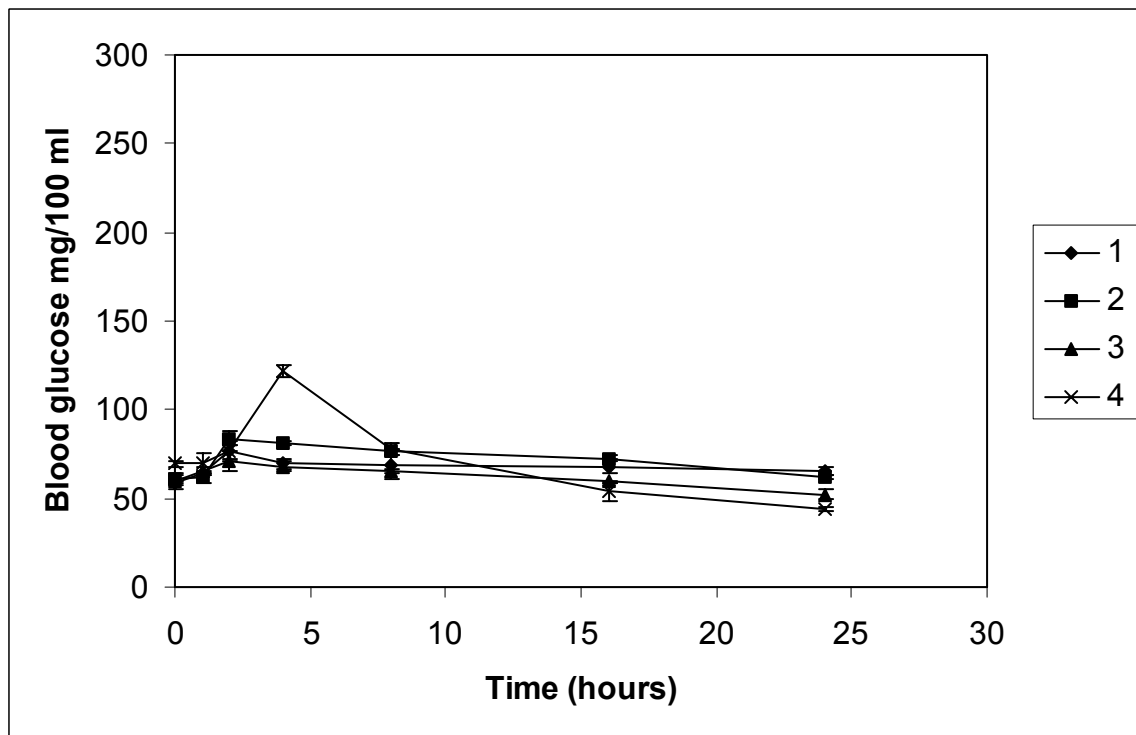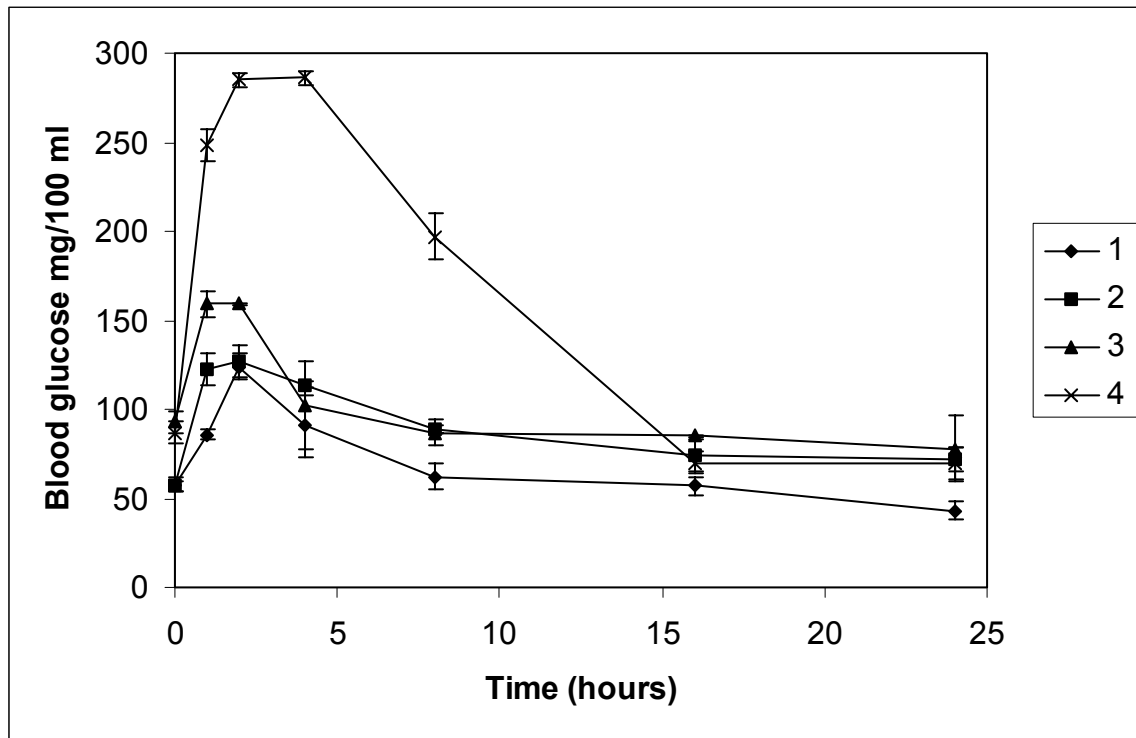
Figure 3.   Blood glucose levels (means ± s.e.m.) in barramundi (upper panel) and tilapia (lower panel) at times after feeding different diets. Line 1: basal (carbohydrate free) diet; line 2: diet containing raw starch; line 3: diet containing pre-gelled starch; line 4: diet containing glucose.

Figure 4. Triacylglycerol levels (means ± s.e.m.) in the livers of barramundi (upper panel) and tilapia (lower panel) after feeding for 6 weeks on diets containing different carbohydrates.

Figure 5.   Glycogen levels (means ± s.e.m.) in the livers of barramundi (upper panel) and tilapia
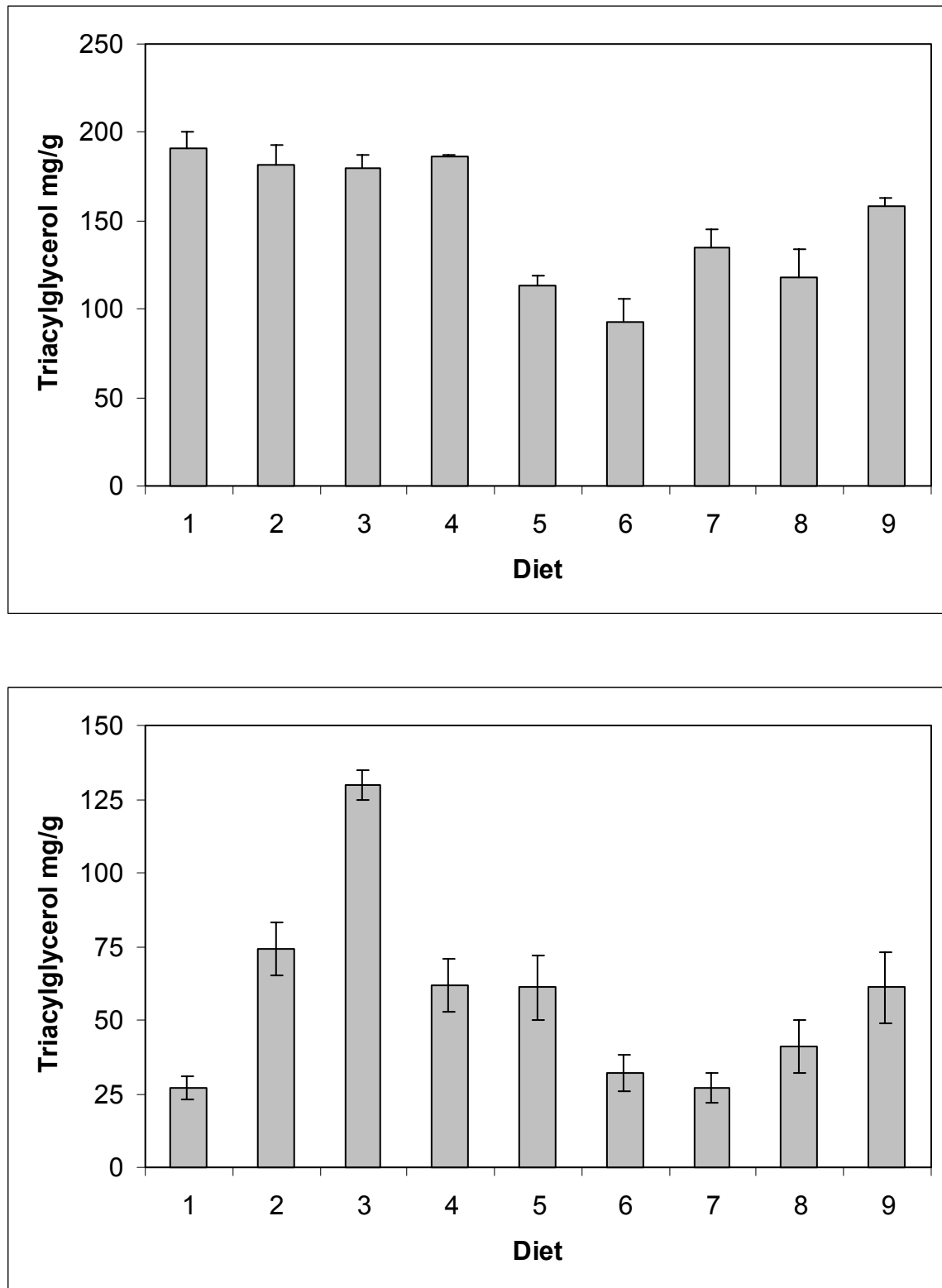(lower panel) after feeding for 6 weeks on diets containing different carbohydrates.

Figure 6. Triacylglycerol levels (means ± s.e.m.) in the muscle of barramundi (upper panel) and tilapia (lower panel) after feeding for 6 weeks on diets containing different carbohydrates.

Figure 7. Glycogen levels (means ± s.e.m.) in the muscle of barramundi (upper panel) and tilapia (lower panel) after feeding for 6 weeks on diets containing different carbohydrates.
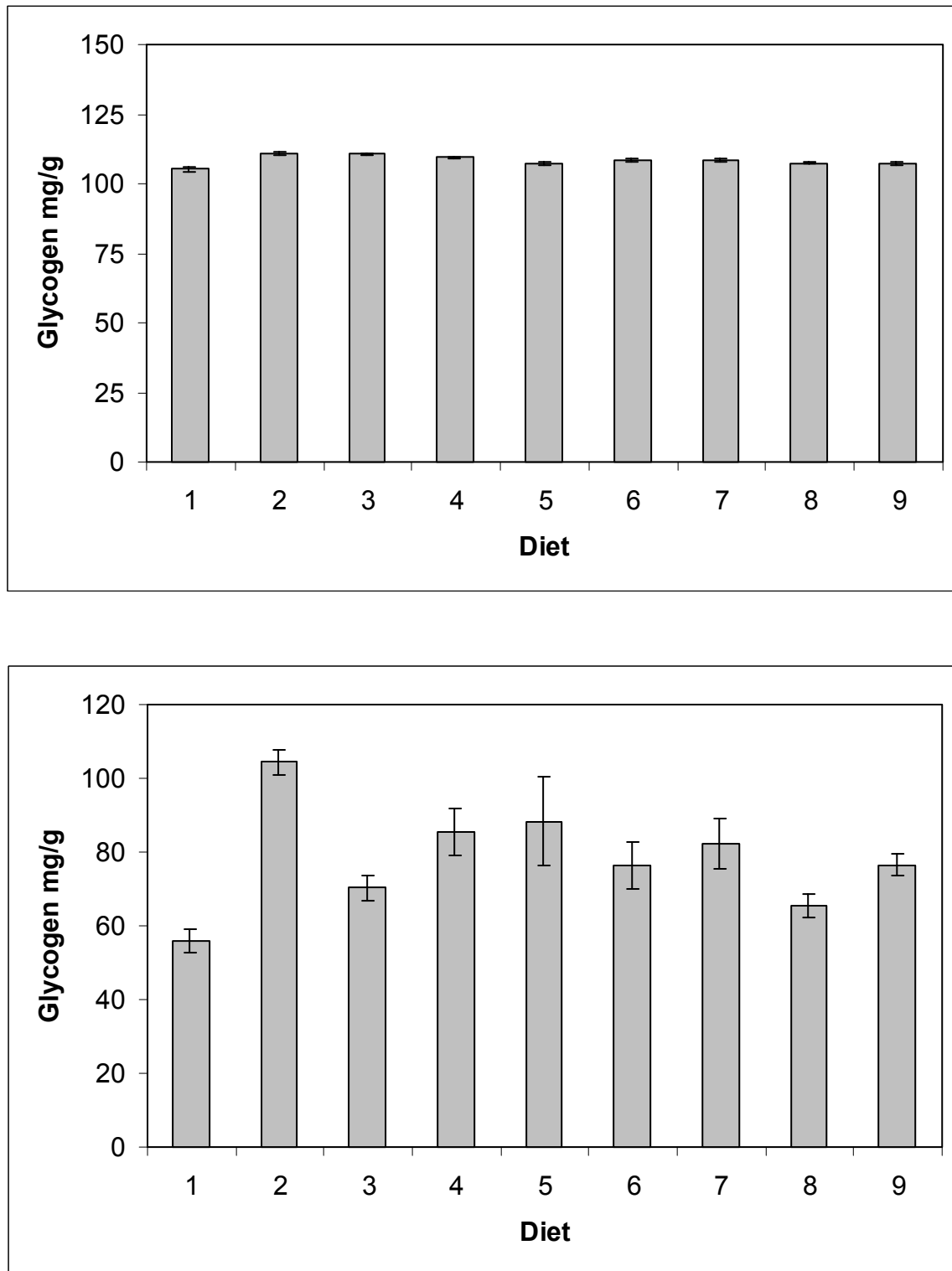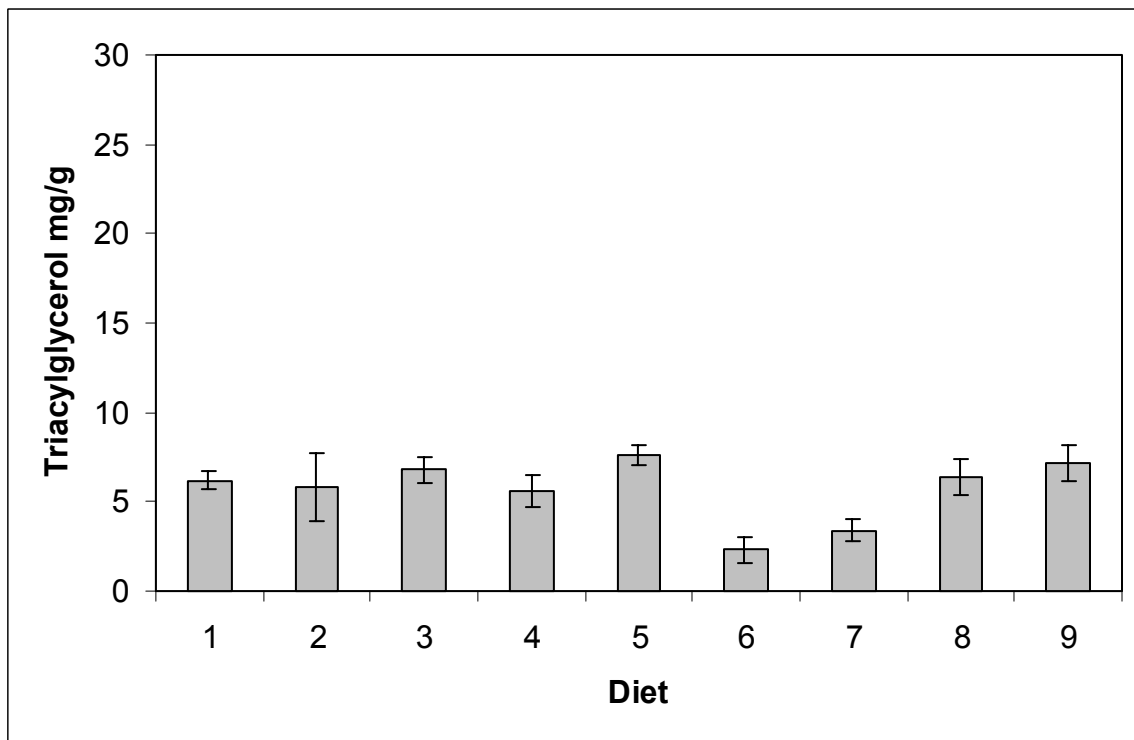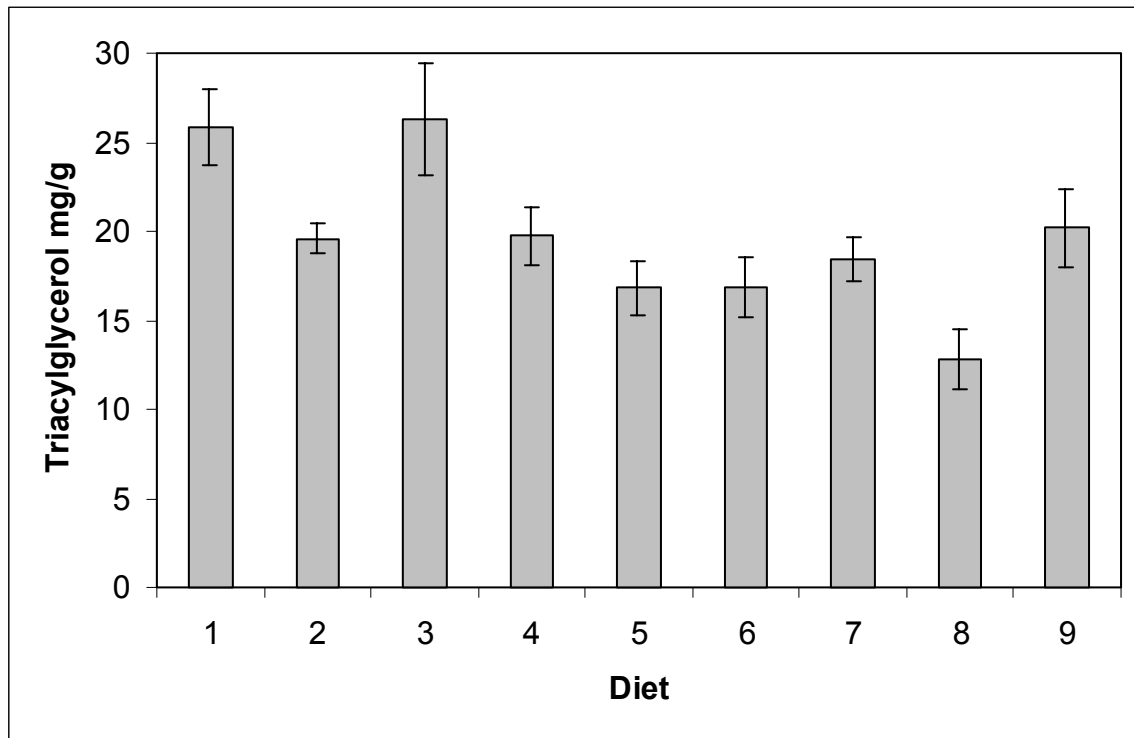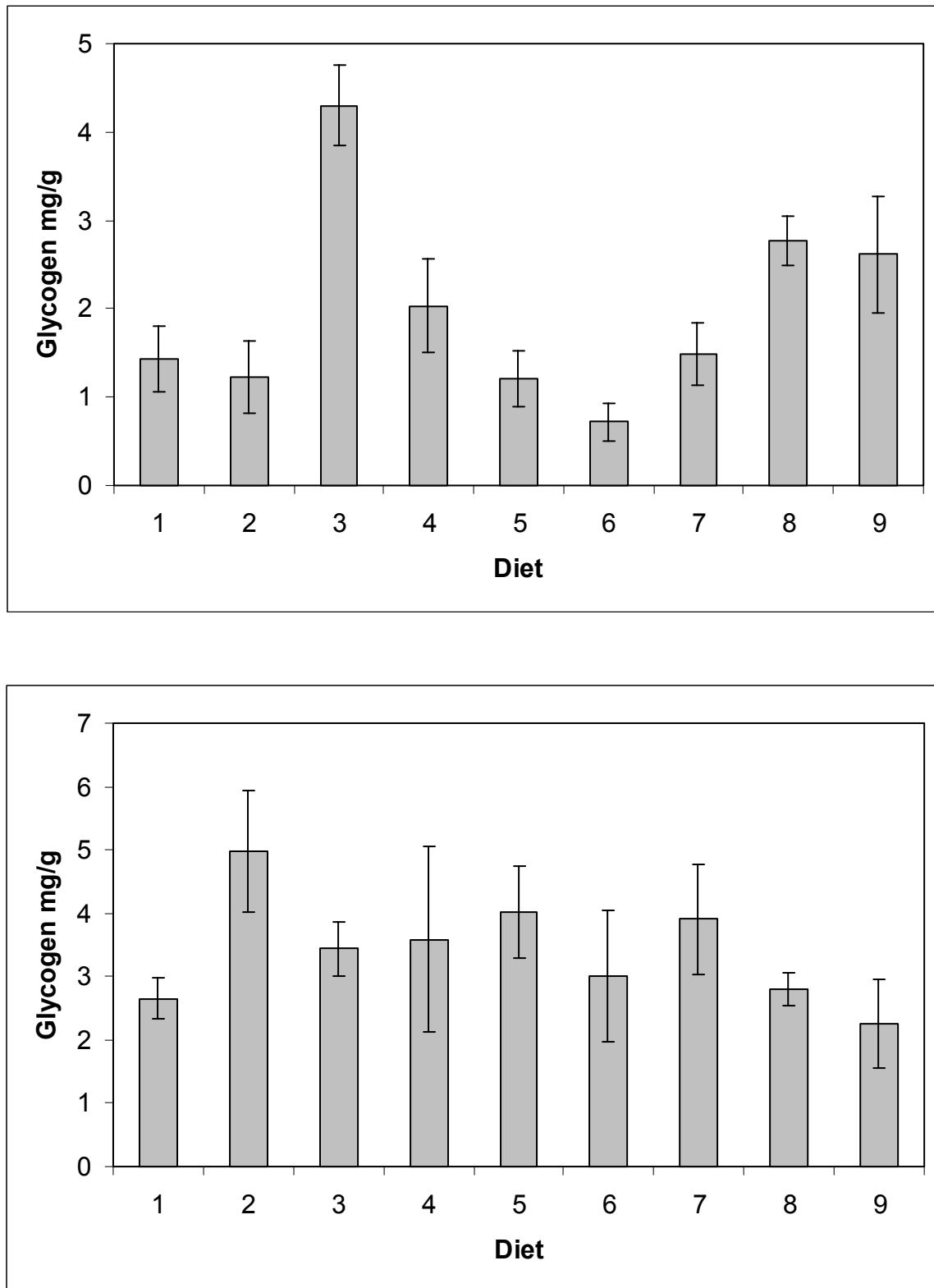
4.10. Digestibility and utilisation of starch by barramundi

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This paper describes a series of experiments to determine the extent to which barramundi digest (four experiments) and utilise (two experiments) starch. The experiments are not reported in the order in which they were conducted because results of the earlier experiments suggested that more fundamental questions be asked.

Materials and Methods

Fish used in the experiments were kept in tanks that were housed in an air-conditioned room. The temperature of the water in the tanks was kept at 27 ^{o}C with, when necessary, submersed heaters. The oxygen tension and ammonia levels in the water were measured daily. A recirculating system delivered water to the 200 l tanks. Approximately 20% of the water in the system was replaced with conditioned tap water every day.

Digestibility experiments

In all experiments starch, glucose or maltose replaced portion of the control diet. Digestibility of the control and test diets was calculated by reference to the faecal concentration of the inert marker ytterbium. Ytterbium chloride was mixed into each diet to give a ytterbium concentration in the diets of approximately 60 mg/kg DM. Digestibility of the starch dry matter and energy was calculated by difference assuming no associative effects between the starch and the control diet. The composition (g/kg) of the control diet was: fish meal, 801.5; gluten, 113.5; vitamin mix, 7.5; mineral mix, 7.5; fish oil, 70. The composition (mg/kg) of the vitamin mix was: vitamin A, 6; vitamin B1, 15; vitamin B2, 20; vitamin B6, 15; vitamin B12, 0.06; biotin, 0.6; vitamin C, 2000; vitamin D3, 1.275; vitamin E, 225; vitamin K3, 10; calcium pantothenate, 50; folic acid, 4; niacin, 75; inositol, 250; choline chloride, 1150; para-aminobenzoic acid, 50; ethoxyquin, 125; citric acid 6000. The composition (mg/kg) of the mineral mix was : aluminium, 0.5; chromium, 0.5; cobalt, 0.5; iodine, 4; selenium, 0.1; copper, 5; magnesium, 297; manganese, 25; zinc, 100. Both mixes were supplied by Rhone Poulenc Animal Nutrition Pty., Ltd.

The diets fed in the four digestibility experiments were:

Experiment 1. The control diet and test diets containing 15 and 30% wheat starch each fed to four fish (average liveweight 800 g) in four tanks.
Experiment 2. The control diet and test diets containing wheat starch with 0, 25, 50 and 80% degrees of gelatinisation which replaced 30 and 60% of the control diet. Each diet was fed to eight fish (average liveweight 200 g) in four tanks.
Experiment 3. The control diet and test diets containing 10, 15 and 20% starch treated and untreated with amylase. Each diet was fed to eight fish (average liveweight 350 g) in three tanks.
Experiment 4. The control diet and test diets containing 27.9% wheat starch, pregelled wheat starch, starch with low, medium and high dextrin content, pea starch, maltose and glucose. Each diet was fed to eight fish (average liveweight 200 g) in four tanks.

Diets were thoroughly mixed in a mechanical mixer and after addition of water (approximately 10%) were pelleted with a laboratory pelleter. The pellets were the dried in an oven at 40 ^{o}C and

stored frozen before use. The fish were fed once a day (0800 h) at a rate of 80% of satiety with satiety being calculated from the expression 0.32 x fish liveweight[0.42] (Williams, pers.com.).

Faeces samples were obtained from the fish by manual stripping. The faeces from fish in any one tank were bulked and stored frozen before being freeze dried and then finely ground with a mortar and pestle. For some experiments each fish had to be stripped on more than one occasion to obtain a large enough sample for analysis.

Growth experiments

The diets fed in the two growth trials were:
Experiment 1. Control diet and test diets containing 15 and 30% starch or the inert filler diatomaceous earth. Each diet was fed to six fish in four tanks.
Experiment 2. Control diet and test diets containing 15 and 30% starch or diatomaceous earth. In two further treatments the control diet was fed at 85 and 70% of the control rate so that fish in these treatments were consuming similar amounts of the control diet to fish fed the diets containing 15 and 30% diatomaceous earth, respectively. Each diet was fed to 10 fish in three tanks.

The diets were fed daily (0900 h) at 80% of satiety with satiety being calculated as detailed previously. Fish were weighed every two weeks and the amounts of feed offered were adjusted accordingly. Any uneaten food was collected from the bottom of the tanks at the end of each daily feeding period and subsequently dried and weighed. Each experiment was of six weeks duration.

At the end of each experiment the fish were euthanased by immersing them in a solution containing 200 mg/l 2-phenoxyethanol and weighed. Homogenous samples of whole fish were then obtained using autoclave and high speed blending procedures (Williams *et al.* 1995). Fish samples were frozen, freeze dried and finely ground prior to analysis.

Finely ground samples of feed, faeces and fish were mainly analysed by standard laboratory procedures (AOAC, 1990). Dry matter (DM) was determined by drying to constant weight at 105 ^{o}C, organic matter (OM) by ashing at 500 ^{o}C for 5hours, nitrogen (N) with a Leco combustion autoanalyser and fat by soxhlet extraction with petroleum ether. Gross energy (GE) was determined with a DDS bomb calorimeter. Ytterbium in faeces was measured by inductively coupled plasma mass spectroscopy after microwave assisted nitric acid:hydroflouric acid (2:1 v/v) digestion.

Data from each experiment were subjected to an analysis of variance with differences between treatment effects being examined with "t" tests only where the "F" test of the ANOVA was significant (P < at least 0.05).

Results

The results for the digestibility experiments are shown in Tables 1 to 6.

Table 1. Digestibility experiment 1. The digestibility (%) of dry matter, nitrogen and energy for the control diet and diets containing 15 and 30% starch and the derived digestibility coefficients for starch.

	Diets				Derived coefficients		
	Control (C)	C + 15% starch	C + 30% starch	sem	Starch (in 15% diet)	Starch (in 30% diet)	sem
Dry matter	82^a	75^b	63^c	0.9	35	18	6.9
Nitrogen	91	91	89	0.7			
Energy	92^a	85b	74^c	0.8	29	19	5.5

Values on the same line with different superscripts are significantly different, P at least < 0.05.
sem = standard error of the mean.

Table 2. Digestibility experiment 2. The digestibility (%) of dry matter, nitrogen and energy for the control diet and for diets containing 30 and 60% gelatinised and ungelatinised starch.

		Digestibility		
Starch in diet, %	% gelatinised	Dry matter	Nitrogen	Energy
Control	-	79^a	89^a	90^a
30	0	62^b	87^{ab}	74^{bc}
30	25	66^b	87^{ab}	77^b
30	50	63^b	86^{ab}	74^{bc}
30	80	61^b	84^{cd}	72^c
60	0	40^c	83^d	50^d
60	25	39^c	84^{cd}	49^d
60	50	34^c	78^e	44^e
60	80	34^c	79^e	44^e
sem		1.5	0.8	1.2

Values in the same column with different superscripts are significantly different, P < at least 0.05.
sem = standard error of the mean.

Table 3. Digestibility experiment 2. Effect of level of inclusion of starch and degree of gelatinisation of starch on digestibility (%) of dry matter, nitrogen and energy in the diets and the calculated digestibilities of dry matter and energy in the starch.

	Starch inclusion %			Gelatinisation %				
	30	60	sem	0	25	50	80	sem
Dry matter	64^a	38^b	1.2	55	52	50	48	1.7
Nitrogen	87^a	81^b	0.5	85^a	86^a	82^b	82^b	0.6
Energy	76^a	48^b	1.2	66^a	63^{ab}	60^{bc}	59^{bc}	1.6
Derived coefficients for starch:								
Dry matter	27^a	8^b	2.2	19	24	16	12	3.1
Energy	27^a	8^b	2.1	20^b	25^{ab}	14^{bc}	11^c	3.0

Values on the same line with different superscripts are significantly different, P < at least 0.05.
sem = standard error of the mean.

Table 4.   Digestibility experiment 3. Digestibility (%) of dry matter, nitrogen and energy in the control (C) diet and diets containing 10, 15 and 20% starch treated (A) and untreated with amylase. The calculated digestibilities of dry matter and energy in starch are also shown.

	Diets							sem
	Control	C + 10A	C + 15A	C + 20A	C + 10	C + 15	C + 20	
Dry matter	82[a]	72[b]	70[c]	67[d]	72[b]	69[cd]	65[e]	0.7
Nitrogen	90[a]	86[b]	85[b]	85[b]	86[b]	85[b]	85[b]	0.5
Energy	91[a]	83[b]	80[c]	77[d]	82[b]	80[c]	76[d]	0.5
Derived coefficients for starch:								
Dry matter		19[b]	0.9[a]	9[a]	-20[b]	-3[a]	-3[a]	4.8
Energy		-12	-2	8	-15	-1	-4	3.4

Values on the same line with different superscripts are significantly different, P < at least 0.05.
sem = standard error of the mean.

Table 5.   Digestibility experiment 3. Main effects of amylase (A) treatment of diets and level of inclusion of starch on calculated digestibility (%) of dry matter and energy in the starch.

	Amylase			% starch in diet			
	Yes	No	sem	10	15	20	sem
Dry matter	-3	-9	3.902.0	-20[b]	-1[a]	3[a]	3.9
Energy	-7	-2		-14[b]	-1[a]	2[a]	2.4

Values on the same line with different superscripts are significantly different, P < at least 0.05.
sem = standard error of the mean.

Table 6.   Digestibility experiment 4. The digestibility (%) of dry matter (DM), nitrogen and energy in diets containing glucose, maltose and different starch sources. The calculated digestibility of the dry matter and energy in the glucose, maltose and starch sources are also shown.

Diet	Digestibilities of components of diets			Derived digestibilities	
	DM	Nitrogen	Energy	DM	Energy
Control diet	82[a]	88[ab]	90[a]		
Wheat starch	61[d]	84[bc]	72[cd]	10[d]	8[d]
Pregelled wheat starch	57[d]	78[c]	69[d]	-2[d]	-7[c]
Low dextrin	76[ab]	89[a]	82[bc]	86[a]	78[a]
Medium dextrin	77[ab]	90[a]	83[ab]	65[b]	56[b]
High dextrin	77[ab]	89[a]	84[ab]	66[b]	62[b]
Maltose	76[ab]	88[a]	83[ab]	62[b]	59[b]
Glucose	61[cd]	76[c]	73[cd]	28[c]	40[c]
Pea starch	69[bc]	87[ab]	78[bc]	39	40[c]
sem	3.0	1.6	2.3	4.7	5.6

Values on the same column with different superscripts are significantly different, P < at least 0.05.
sem = standard error of the mean.

The results of the growth experiments are shown in Tables 7 and 8.

Table 7. Growth experiment 1. The growth and feed conversion efficiencies (FCR) of fish fed the control diet and the control diet diluted with 15 and 30% starch and diatomaceous earth (diat).

	Diets					
	Control	15% starch	30% starch	15% diat	30% diat	sem
Mean fish weight at the start of the experiment, g	470	466	452	444	437	15.3
Mean fish weight at the end of the experiment, g	641[a]	584[b]	603[ab]	485[c]	515[c]	17.6
%LWG[A]	0.36[a]	0.33[b]	0.03[c]	0.18[d]	0.09[c]	0.007
FCR, g fish/g food[B]	1.09[a]	1.00[b]	0.78[c]	0.52[d]	0.27[c]	0.018
FCR, g dry fish/g dry food	0.43[a]	0.40[a]	0.38[a]	0.21[b]	0.12[c]	0.020
FCR, g fish N/g food N	0.31[a]	0.31[a]	0.33[a]	0.18[b]	0.12[c]	0.009
FCR, g fish E/g food E[C]	0.49[a]	0.50[a]	0.49[a]	0.31[b]	0.22[b]	0.034
G fat deposited/MJ E consumed	7.32[a]	8.00[b]	9.07[a]	4.81[b]	3.81[c]	0.931

[A] %LWG, increase in weight as a % of start weight
[B] g wet fish/g food DM consumed
[C] E = energy

Table 8. Growth experiment 2. The growth and feed conversion efficiencies of fish fed the control diet and the control diet diluted with 15 and 30% starch and diatomaceous earth (diatom).

Treatment	g fish/g DMI	g fish/g DDMI	g fish DM/g DMI	g fish DM/g DDMI	g fish N/g food N	MJ fish/MJ food	MJ fish/MJ DE	g fat/MJ food	g fat/MJ DE
Control (C)	1.44[a]	1.75[abc]	0.48[a]	0.59[a]	0.39[b]	0.52[a]	0.57[a]	7.25[a]	7.90[a]
C + 15% starch	1.27[b]	1.70[bc]	0.41[b]	0.55[bc]	0.39[b]	0.45[c]	0.53[b]	5.85[b]	6.91[b]
C + 30% starch	1.08[d]	1.72[abc]	0.35[d]	0.55[bc]	0.39[b]	0.40[d]	0.53[b]	5.18[c]	6.99[b]
C + 15% diatom	1.16[c]	1.67[c]	0.37[c]	0.53[c]	0.37[b]	0.46[bc]	0.59[a]	5.72[bc]	7.34[ab]
C + 30% diatom	0.62[d]	1.08[d]	0.17[e]	0.30[d]	0.21[c]	0.25[e]	0.38[d]	3.00[e]	4.66[c]
85% C	1.46[a]	1.79[ab]	0.47[a]	0.57[ab]	0.39[b]	0.48[b]	0.53[b]	6.08[b]	6.63[b]
70% C	1.47[a]	1.80[a]	0.47[a]	0.57[ab]	0.41[a]	0.47[bc]	0.51[c]	4.26[d]	5.04[c]
sem	0.023	0.023	0.006	0.010	0.008	0.010	0.012	0.199	0.26

Values in the same column with different superscripts are significantly different. *P* < at least 5%.
DMI = dry matter intake, DDMI = digestible DMI, MJ = mega joule, DE = digestible energy.

Conclusion

The conclusions from these experiments are that:
1. Wheat starch is poorly digested by barramundi;
2. The more wheat starch there is in the diet the lower the digestibility of the starch;
3. The efficiency of food conversion is reduced by including wheat starch in barramundi diets, and
4. Diatomaceous earth is not a suitable inert filler for barramundi experimental diets when included in the diet at 15% or greater.

5.   BENEFITS

1. Aquaculturists will gain from better, cheaper diets for silver perch and barramundi which will reduce costs of growing this species and lead to increases in production. Silver perch diets, although still too expensive for the liking of silver perch farmers, are the cheapest pellets and diets for any species of fish or prawn found in Australia. Production of silver perch is based almost entirely on diets developed during this Subprogram (or its precursor the Replacement of Fishmeal in Aquaculture Diets Subprogram) and has increased from less than 50 t in 1995/96 to over 310 t in 2000/01. (Predictions for 2002/03 are over 400 t) (Stuart Rowland, NSW Fisheries, Grafton Aquaculture Centre, September 2002, personal communication). Diets for barramundi, although more expensive are more cost effective. Production of barramundi has increased from 555 t in 1996/97 to 898 t in 2000/01 (ABARE, Fisheries Statistics 2000; 2001). Prawns have increased in production from 1565 t in 1995/96 to 2819 t in 2000/01 (ABARE, Fisheries Statistics, 1997; 2001). It is difficult to attribute production increases to any single factor but improvements in diets and feeding have played a significant part, especially for silver perch and barramundi.

2. The Australian public will benefit from increased production of fish which will reduce some of the more than 80 000 t of fish and fish products imported annually (including 45 800 t of live, fresh or frozen fish or fillets). Unfortunately, despite increases in aquaculture production, imports of edible fisheries products have increased from 112 706 t in 1995/96 to 144 407 t in 2000/01 (ABARE, Fisheries Statistics, 1997; 2001).

3. Agriculture producers will benefit from increased marketing opportunities for Australian agriculture products. This includes the rapidly increasing domestic aquaculture feed market which has nearly doubled in the last two years (1991/92 – 1993/94) and the rapidly increasing Asian aquaculture feed market predicted to reach 2.6 million tonnes in 2000. Statistics for use of agriculture ingredients for aquaculture feeds produced domestically or exported for feeds produced overseas are difficult to obtain. However, thousands of tonnes of Australian ingredients, particularly lupins and meat meals are now being sold offshore, largely as a result of research conducted under this subprogram and its precursor.

4. Feed manufacturers will benefit from having more information to use in silver perch diet formulation including many more ingredients which have been evaluated and new information on nutritional requirements. Demonstrated performance of Australian produced aquaculture feeds in Australia will also lead to marketing opportunities for these diets overseas. Two major manufacturers dominate aquafeed production in Australia – Ridley's Aquafeeds and Skrettings, Australia. Both have expanded their production considerably since the inception of this subprogram. Silver perch diets are also made by Select Nutrition, a small feed manufacturer located at Windsor, NSW. This company sells over 500 t of silver perch feed annually. It did not produce any aquafeed prior to the inception of this subprogram.

6. FURTHER DEVELOPMENT

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

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

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

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

Nutrition and Aquatic Animal Health
- ***Interaction between nutrition and aquatic animal health***
- *Nutritional prevention of disease and maintenance of animal health*

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

Nutrition and Product Quality
- *Influence of manufactured aquatic feeds on aquaculture product quality and food safety hazards*

7.   CONCLUSION

The major findings of this study were:

1.   Dehulling greatly improved digestibility of lupins but was of little benefit for field peas fed to silver perch.
2.   Extrusion greatly improved digestibility (dry matter and energy) of starch rich peas, slightly improved soybean, had no effect on lupins (very little starch) and actually reduced digestibility of canola fed to silver perch.
3.   Steam conditioning and extrusion of silver perch diets both improved fish performance compared with cold pelleted diets. Extrusion improved food conversion efficiency (i.e. utilization) but reduced voluntary intake. Overall fish performance was best on steam pelleted diets.
4.   Utilization of nitrogen and energy from four key ingredients in silver perch diets was measured. Utilization was not affected for diets with less than 45% peanut meal or 75% field peas or canola. Utilization of meat meal was not affected regardless of the content.
5.   Juvenile silver perch are efficient at digesting and utilizing carbohydrates.
6.   Barramundi are inefficient at digesting and utilizing carbohydrates as an energy source.
7.   Silver perch and barramundi lack the ability to digest and utilize non-starch polysaccharide (NSP).
8.   The botanical origin of starch, starch inclusion content and processing of starch all had significant effects on starch digestibility for silver perch.
9.   Silver perch digested wheat starch more efficiently than maize starch, pea starch or potato starch.
10. Silver perch digest the energy from wheat more efficiently than the energy from dehulled lupins.
11. When injected with 1 g carbohydrate kg BW^{-1} silver perch were glucose tolerant, but galactose and xylose intolerant. However, when the dose rate of glucose was increased from 1 to 2 to 4 g kg BW^{-1}, glucose tolerance was compromised.
12. When injected with 1 g carbohydrate kg BW^{-1} barramundi were much less tolerant of glucose than silver perch. They were galactose and xylose intolerant.
13. Silver perch were more efficient at utilizing processed wheat starch (either in the gelatinised or dextrinised form) for growth than wheat meal or raw wheat starch.
14. The optimum utilization of dietary carbohydrate for protein sparing and growth was observed when either gelatinised starch or dextrin was included in the diet at 30%.
15. Adding α-amalse (an enzyme designed to increase starch digestibility) to silver perch diets improved digestibility of raw wheat starch, but not to anywhere near the same extent as gelatinisation. It did not give further improvement to gelatinised starch.
16. Addition of a blend of β-glucanase and β-xylanase (designed to improve digestibility of non-starch polysaccharides) had no effect on digestibility of lupins fed to silver perch.
17. Addition of phytase (to improve digestibility of phosphorus and utilization of protein) did not deliver clear benefits for silver perch.

8.   STAFF

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9.   PUBLICATIONS

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

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Allan G.L., Jantrarotai W. & Kosutark P. (1999) Extension of aquaculture diet development research.

Booth M.A., Allan G.L. & Stone D.A.J. (1999) Utilisation of four agricultural ingredients by silver perch.

Stone D.A.J., Tostin N., Allan G.L. & Booth M.A. (1999) Benzocaine, Aqui-S™ and clove oil as anaesthetics for silver perch.

Abstracts of papers presented at the AQUA 2000 (World Aquaculture Society Conference) 2-6 May, 2000, Nice, France:

Allan G.L., Booth M.A. & Stone D.A.J. (2000) Effects of extrusion on digestibility of field peas, lupins, soybean meal and canola meal fed to silver perch.

Booth M.A., Allan G.L. & Stone, D.A.J. (2000) Maintenance requirements of juvenile silverperch.

10. APPENDICES

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Nutrient Requirements and Feeding of Finfish for Aquaculture

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This book is dedicated to the memory of

Dr Tippawan Paripatananont,

a colleague and good friend, whose efforts and
dedication to complete the chapter on snakehead
and *Pangasius* catfish inspired us all to accomplish the task.

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Silver Perch, Bidyanus bidyanus

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Introduction

Silver perch, Bidyanus bidyanus (Mitchell) (Teraponidae) is a freshwater, temperate fish, endemic to the Murray–Darling river system west of the Great Dividing Range in south-eastern Australia. It is a schooling, omnivorous fish that feeds naturally on zooplankton, insects, crustaceans, molluscs, small fish and some aquatic plants, in particular filamentous algae. Silver perch is an attractive, small-scaled, laterally compressed fish with premium-quality white flesh, a mild, delicate flavour, a fine texture, few bones and about 40% meat recovery. A recent study of over 200 Australian seafood products found that farmed silver perch had the third highest levels of fatty acids beneficial to human health (Nichols et al., 1998).

Hatchery techniques, based on hormone-induced spawning and extensive pond rearing of larvae, were developed in the early 1980s (Rowland et al., 1983; Rowland, 1984) and up to 5 million fingerlings are produced annually for aquaculture, stock enhancement and conservation purposes. However, it was not until the early 1990s that research into the grow-out of silver perch commenced at the New South Wales (NSW) Fisheries' Grafton Aquaculture Centre (GAC), Australia (29° 41' S; 152° 56' E). The research demonstrated that silver perch is an excellent species for semi-intensive or intensive culture in earthen ponds. High survival rates (> 90%) and fast growth rates from fingerlings (2–5 g fish⁻¹ day⁻¹) to market-size (~500 g), at high stocking densities (20,000 fish ha⁻¹), have routinely produced high production rates of around 10 t ha⁻¹ year⁻¹ in static, aerated, 0.1 ha earthen ponds (Rowland, 1995a: Rowland et al., 1995).

Most silver perch cultured commercially are grown in static, aerated, earthen ponds that range in surface area from 0.1 to 0.5 ha. Recent research suggests that cages may also be suitable for culture. A three-phase production strategy is recommended for this species: I, hatchery; II, fingerling; III, grow-out (Rowland, 1995b). This strategy is usually combined with a single-batch system, where

each pond only has fish of the same age or batch, which are totally harvested before the next batch is stocked. Fry (30 mm, 0.5 g) become available from hatcheries in mid- to late summer (January to February), and are stocked at densities up to 150,000 fish ha⁻¹ in fingerling ponds for 3 or 4 months. The fingerlings are then harvested, graded and stocked at a density of 10,000–30,000 ha⁻¹ for grow-out. The grow-out phase takes 10–18 months, depending on the temperature regime, to reach market size (400–800 g). Fish and ponds are closely managed because of the intensity of production. Major water-quality variables (temperature, dissolved oxygen, pH and ammonia) are monitored every 2–3 days. Fish are sampled monthly to estimate the mean weight and biomass, and the daily ration is adjusted accordingly. Sampled fish are checked for disease; there are no major disease problems in the industry at present. Fish are harvested using seine nets on most farms, and are placed live in clean water for 7 days to purge off-flavours and to ensure a uniform, high-quality product. Silver perch are sold live, principally into Asian communities, or whole chilled with prices ranging from A$7 to A$15 kg⁻¹. The industry is expected to have a large processing component in the future.

Although the silver perch farming industry is currently in its infancy, it has great potential for growth. Australia has limited wild fisheries, most of which are fully or overexploited, and approximately 70% of the white-fleshed finfish consumed is imported. There are abundant sites with high-quality water available for aquaculture and freshwater effluent is easily managed, making possible environmentally sound practices with no release of effluent to natural waterways. Considerable opportunities exist for the integration of silver-perch culture with established agricultural industries, especially where irrigation is used.

Silver perch are farmed predominantly in the states of NSW and Queensland, with small quantities also produced in Victoria, South Australia and Western Australia. Only 2.6 t were farmed in NSW in 1992/93, but production is now increasing rapidly as more farms are constructed and farming practices improve. The established culture techniques, the availability of sites and the premium quality of the product provide a basis for a dramatic increase in production over the next 5–10 years, which is predicted to exceed 1000 t by 2005. There is also much interest in the culture of silver perch in other countries – especially in the People's Republic of China, Taiwan and Israel. Fingerlings and broodfish have been exported to these countries from some hatcheries in Australia.

Nutrient Requirements

Protein and energy

Early research indicated that protein requirements of silver perch were similar to those reported for other omnivores, such as channel catfish, and lower than those reported for carnivores (Allan and Rowland, 1991; Allan et al., 1994). Protein efficiency ratios increased with increasing digestible energy (DE) (in the range

12–15 MJ kg⁻¹) and tended to decrease with increasing digestible protein (in the range 25–45%) (Allan *et al.*, 1994). Harpaz *et al.* (1999) reported increased growth of 3 g silver perch fingerlings when fed 41% protein diets compared with the growth of fish fed 23% protein diets. Although gross energy was similar for Harpaz *et al.*'s diets, the DE content of the 23% protein diet was much lower than that of the 45% protein diet. Unfortunately, such differences in the DE content of experimental diets have confounded interpretation of this and other experiments that have tried to estimate the protein requirements of silver perch and other fish species.

Protein and energy need to be kept in balance (NRC, 1993). Research with pigs and poultry has led to the development of the concept of two phases in protein deposition: a protein-dependent phase and an energy-dependent phase. At a constant energy content, protein deposition will increase with increasing protein intake until a plateau is reached, after which there will be no response to increasing protein intake. At a higher energy intake, protein deposition will increase with a higher protein intake (Bikker, 1994).

A study was conducted at our laboratory to estimate requirements of silver perch for digestible protein using isocaloric diets (14 MJ of DE kg⁻¹) made using practical ingredients (fish-meal, maize gluten, peanut meal, wheat, wheat starch and fish-oil) with varying protein contents (11–40% digestible protein). Fish were fed to satiation. The minimum dietary protein content before growth was retarded was 28% (Fig. 25.1; Allan *et al.*, 2001).

The optimum protein requirements for juvenile silver perch at three different DE contents were also evaluated. Using similar methods to those described above, but with three series of diets, each with a different DE content, and with fish fed at about 90% of satiation level, protein retention efficiency was plotted against digestible dietary protein for each series of diets. Results indicate that optimum
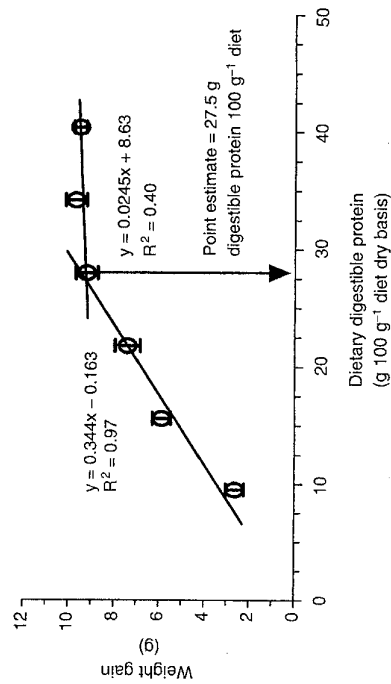

Fig. 25.1. Effect of dietary digestible protein content and a digestible energy content of 14 MJ kg⁻¹ on individual weight gain of juvenile silver perch. Error bars indicate standard error of mean (SEM).

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digestible protein requirements were 25% for diets with 12–14 MJ of DE kg⁻¹, 26% for diets with 14–16 MJ of DE kg⁻¹ and 29% for diets with 16–17 MJ of DE kg⁻¹ (G.L. Allan, unpublished data).

Requirements of protein and energy for maintenance were estimated by feeding juvenile silver perch restricted rations (approximately 90% of satiation) of a nutritionally adequate reference diet or diets with different substitution levels of an inert filler (diatomaceous earth). Using this approach, Booth *et al.* (2000a) estimated requirements of protein and energy for maintenance as approximately 2.1 g digestible protein kg⁻¹ body weight day⁻¹ and 116 kJ of DE kg⁻¹ body weight day⁻¹. These amounts were slightly higher than those estimated for some other species (Lupatsch *et al.*, 1998), possibly because in the study by Booth *et al.* (2000a) fish were still expending energy for feeding activity, whereas in the other studies maintenance requirements were estimated by progressively reducing food intake, thereby also reducing feeding activity.

Protein is made up of 20 α-amino acids linked into chains by peptide bonds (NRC, 1993). The protein supplied to fish must contain sufficient quantities of the amino acids that fish cannot synthesize (there are ten of these essential amino acids) to provide for tissue protein synthesis.

The most common method for estimating amino acid requirements involves making a series of diets using an intact protein (e.g. casein plus gelatin) plus a mixture of crystalline amino acids. Graded amounts of the amino acid under investigation are supplied in the crystalline amino acid mix to give a series of diets. The total protein content of the crystalline amino acid mix is balanced in the series of diets using non-essential amino acids. Ngamsnae *et al.* (1999) used this method to estimate requirements of silver perch for arginine and phenylalanine and then estimated the requirements for each of the other indispensable amino acids by multiplying the requirement for arginine by the content of each amino acid (as a proportion of total amino acids) (see Table 25.1).

Growth rates of fish fed diets composed of purified sources of protein plus mixtures of crystalline amino acids are usually inferior to those of fish fed diets based on intact, practical protein sources, probably due to a relatively poor utilization of crystalline amino acids and purified protein sources (Nose and Murai, 1990; NRC, 1993; Cowey, 1994; Ngamsnae *et al.*, 1999). Allan *et al.* (2001) reported that, for diets with 14 MJ of DE kg⁻¹, requirements for essential amino acids could not be as high as those reported by Ngamsnae *et al.* (1999) because the growth of fish did not increase when additional amino acids were added past the contents in the 28% protein diet (see Fig. 25.1). They suggested that the amino acid content of the 28% protein diet could be considered as 'recommended values' for diets at 14 MJ of DE kg⁻¹ (Table 25.1).

Lipids and fatty acids

Dietary lipids are an important source of energy and essential fatty acids and help with the absorption of fat-soluble vitamins (NRC, 1993). The DE content of

Table 25.1. Requirements and 'recommended' values of essential amino acids (% of diet) for juvenile silver perch.

Amino acid	Estimated requirements*	Recommended values[†]
Arginine	2.7	1.9
Histidine	1.0	0.7
Isoleucine	1.8	1.1
Leucine	3.6	2.0
Lysine	3.7	1.5
Methionine	1.5	0.6
Phenylalanine	2.2	1.2
Threonine	2.8	1.0
Valine	2.3	1.3
Tryptophan[‡]		

* Ngamsnae et al. (1999) estimated requirements of juvenile silver perch after fish were fed diets based on casein and gelatin plus a mixture of crystalline amino acids with graded contents of crystalline arginine or phenylalanine.

[†] Allan et al. (2001) fed juvenile silver perch diets with 14 MJ of DE kg^{-1} but graded digestible protein contents, all from intact protein sources rather than crystalline amino acids. The amino acid contents of the lowest-protein diet where growth or protein deposition was not retarded (28% protein diet – see Fig. 25.1) have been listed as the 'recommended' amino acid content for practical diets (digestible protein level 28% – see Fig. 25.1).

[‡] Tryptophan was not measured in either study.

fish-oil and lard fed to silver perch was determined as described by Allan et al. (1999) (with revised calculations for ingredient digestibility recommended by Sugiura et al. (1998)). Digestibility coefficients and DE contents were 92% and 36 MJ of DE kg^{-1} for cod-liver oil and 52% and 21 MJ of DE kg^{-1} for lard. Practical diets for silver perch typically contain 6–10% crude lipid.

Anderson and Arthington (1989) measured the deposition of fatty acids in silver perch fed a soybean meal-based diet. They found that fatty acids accumulated in both the depot lipids and the phospholipids of the fish. When fish were transferred on to a fat-free diet, the preferential incorporation of both arachidonic (20:4n-6) and docosahexaenoic (22:6n-3) acids into the phospholipids during lipid turnover led the authors to conclude that silver perch require both n-3 and n-6 series fatty acids.

Silver perch were able to chain elongate and desaturate dietary linoleic acid (LA) (18:2n-6) and linolenic acid (LNA) (18:3n-3) to their longer-chain metabolites (Anderson and Arthington, 1992). This is in agreement with Hunter (2000), who found that silver perch require both LA and LNA and can chain-elongate and desaturate LA and LNA into their longer-chain polyunsaturated fatty acids. However, as growth was enhanced by the addition of longer-chain n-3 fatty acids to the diet, the conversion process alone is inadequate to optimize growth performance.

Dietary lipid has a major impact on fish lipid content (Buckley and Groves, 1979; Shearer, 1994). Measurement of lipid and fatty acid composition of whole silver perch and fillets confirms this and indicates that the fatty acid profile of farmed fish is strongly influenced by dietary lipid source (Hunter et al., 1994, 2000). The whole-body lipid content of cultured silver perch is relatively high and increases with size. For silver perch grown in different experiments but all on the same diet (SP35–35% crude protein, 14–15 MJ of DE kg^{-1}), carcass contents were 24.6% lipid (dry basis) and 75.1% moisture for 2 g fish; 33.9% and 68.0% for 6 g fish; 41.9% and 59.7% for 72 g fish; and 50.9% and 58.8% for 395 g fish (Allan et al., 2000b; Hunter, 2000; Stone et al., 2000). Indicative fatty acid contents of farmed silver perch are listed in Table 25.2.

Hunter et al. (1994) reported that, for juvenile silver perch fed diets with protein contents ranging from 25 to 45% and 10.6 to 14.6 MJ of DE kg^{-1}, there was an inverse linear relationship between dietary protein : energy content and fish-fillet lipid content.

Carbohydrates

Although fish have no requirement for carbohydrate, provision of some carbohydrate is important in formulated diets to facilitate pellet binding, and to provide an alternative, lower-cost energy source to spare protein and/or lipid (Lovell, 1989; NRC, 1993). One of the most noticeable differences between diets for different warm-water fish species is that omnivores (e.g. channel catfish, common carp and tilapia) tend to be fed less protein in their diets than strictly carnivorous species (e.g. Asian sea bass = barramundi, gilt-head sea bream and red drum) (Wilson, 1991). However, as the overall protein composition of different fish species (especially on a lipid-free basis) does not vary greatly and net protein retention rates are similar (NRC, 1993), it is likely that this difference reflects the ability of different species to digest and utilize carbohydrate for energy.

One approach to investigating whether a species can tolerate different types or amounts of carbohydrate is to measure the uptake and clearance rates of carbohydrates (e.g. glucose) following intraperitoneal injection. Figure 25.2 shows the uptake and clearance rates of glucose for silver perch and barramundi, demonstrating that silver perch are more efficient at initial uptake and clearance of glucose than barramundi (A.J. Anderson, Z.S. Lipovsek and D.A.J. Stone, unpublished data; D.A.J. Stone, G.L. Allen and A.J. Anderson, unpublished data). Silver perch were also better able to utilize galactose than barramundi but both species were xylose-intolerant (A.J. Anderson, Z.S. Lipovsek and D.A.J. Stone, unpublished data; D.A.J. Stone, G.L. Allen and A.J. Anderson, unpublished data).

Unpublished data from our laboratory indicate that silver perch can efficiently digest diets containing 30% wheat starch, regardless of the degree of gelatinization, without adverse effects on liver enlargement, as indicated by the hepatosomatic index (HIS). However, when starch content was increased to 60%,

Fig. 25.2. Plasma glucose levels of silver perch and barramundi following an intraperitoneal injection of glucose (dose rate = 0.1% body weight).

Table 25.3. Apparent digestibility coefficients (%) for different carbohydrate sources for silver perch and barramundi (all ingredients were included at 30% with a basal diet of fish-meal 80.2%, wheat gluten 10%, fish oil 7%, vitamins and minerals 27%) (D.A.J. Stone, G.L. Allan and A.J. Anderson, unpublished data).

Starch/ingredient	Silver perch	Barramundi
Pea starch	75.1	39.6
Wheat starch	82.7	7.6
Pregelatinized wheat starch	95.2	−6.5
Dextrin (9%)	97.6	78.4
Dextrin (17%)	95.5	56.2
Dextrin (30%)	97.7	62.4
Maltose	98.4	59.0
Glucose	99.5	40.0

(Allan et al., 1999), while those of barramundi were collected by stripping. Although this difference may have led to an overestimation of digestibility for silver perch and/or an underestimation for barramundi, previous results for protein-rich ingredients have been in much closer agreement (Williams et al., 1998), indicating that the differences reflect differences between the species.

Vitamins and minerals

No research has been conducted on vitamin or mineral requirements for silver perch. The vitamin and mineral mixes used in experimental and commercial diets

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Table 25.2. Fatty acid composition of various sizes of silver perch (% of total fatty acids).

Fatty acid	Juvenile fish			Adult fish
	2 g*	6 g*	72 g†	(~ 500 g)‡
C12:0	0.1	0.1	Nm	Nm
C14:0	3.0	3.6	2.9	3.7
C15:0	0.2	0.3	0.2	Nm
C16:0	24.7	28.3	28.2	24.6
C17:0	0.3	0.2	Tr	Nm
C18:0	4.3	4.6	3.9	3.6
C20:0	0.2	0.1	Nd	Nm
C22:0	0.1	Tr	Nm	Nm
C14:1	0.1	0.1	Nm	Nm
C16:1	8.9	8.3	8.2	12.3
C17:1	0.2	Tr	Nd	Nm
C18:1n-9	27.5	32.0	34.6	31.5
C18:1n-7	2.2	2.2	1.9	2.5
C20:1n-11	0.1	0.6	0.2	Nm
C20:1n-9	0.8	2.7	1.4	1.1
C20:1n-12	Nm	Nm	Nm	0.7
C22:1n-11	0.2	1.6	1.0	Nm
C22:1n-9	0.1	0.3	Nm	Nm
C24:1n-9	0.4	0.4	Nm	Nm
C18:2n-6	9.3	7.3	8.2	7.7
C18:2n-9	Nm	Nm	Nm	0.8
C18:3n-6	0.9	0.4	0.5	Nm
C20:2n-6	0.3	0.2	Tr	Nm
C20:3n-6	0.7	0.3	0.2	Nm
C20:4n-6	0.6	0.2	Tr	Nm
C18:3n-3	1.2	0.7	0.9	1.0
C18:4n-3	0.6	0.5	0.5	Nm
C20:4n-3	0.4	0.3	Nm	Nm
C20:5n-3	2.1	0.8	1.5	1.8
C22:3n-3	0.2	Tr	Nm	Nm
C22:5n-3	1.8	0.6	1.2	1.8
C22:6n-3	5.8	2.3	4.6	6.3

* Ground whole fish (results are means for $n = 4$ fish) (Hunter, 2000).
† Ground, head-off, gutted fish (results are means for $n = 4$ fish) (Hunter et al., 2000).
‡ Skinless fillets (results are means for $n = 4$ fillets) (B.J. Hunter, personal communication).
Tr, trace; Nd, not detected; Nm, not measured.

digestibility was positively correlated with the degree of gelatinization and HIS was elevated. Digestibility coefficients of various carbohydrate sources by silver perch and barramundi are presented in Table 25.3. The experimental diets were the same for both species but faeces of silver perch were collected by settlement
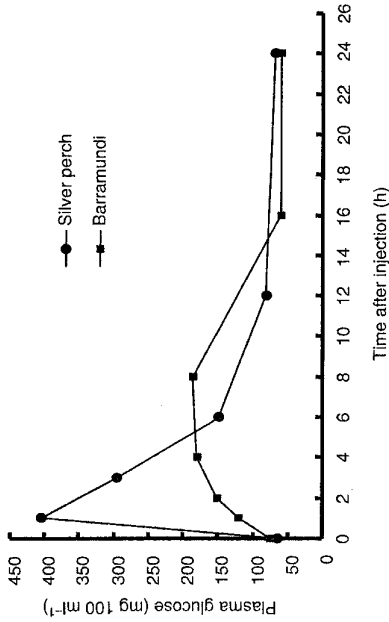
Table 25.4. Recommended vitamin and mineral mixes in experimental and some commercial silver-perch diets.

Vitamin*	IU kg⁻¹ diet*	mg kg⁻¹ diet	Mineral*	mg kg⁻¹ diet
Retinol (A)	8000		Calcium iodate (62% I)	0.47
Cholecalciferol (D$_3$)	1000		Manganese sulphate monohydrate (31% Mn)	10.0
D,L-α-Tocopherol acetate (E)		125.0	Zinc sulphate monohydrate	100.0
Menadione sodium bisulphite (K$_3$)		16.5	Copper sulphate pentahydrate	3.0
Thiamine hydrochloride (B$_1$)		10.0	Ferrous sulphate heptahydrate	29.9
Pyridoxine hydrochloride (B$_6$)		15.0	Sodium chloride	0.33
Folic acid		3.8	Magnesium sulphate (10% Mg)	500.0
Ascorbic acid (C)		1000.0	Lime	2.0
Calcium D-pantothenate		49.6		
Myoinositol		600.0		
Choline chloride		1500.0		
Niacin		200.3		
Cyanocobalamin (B$_{12}$)		0.02		
Ethoxyquin (antioxidant)		150.0		
Calcium propionate (mould inhibitor)		250.0		

* Amount of active ingredient.

of silver perch are given in Table 25.4. Diets supplemented with these premixes have been used successfully for silver perch grown in static, earthen ponds. Premixes containing similar vitamin and mineral levels have been used in the commercial feeds for silver perch for around 5 years and deficiency symptoms have not been reported to date.

Practical Diets

The proximate composition and digestibility coefficients for dry matter, energy and protein of various ingredients for silver perch were determined by Allan et al. (2000a) and are presented in Table 25.5. Booth et al. (2000b) showed that, for silver perch > 17 g, grinding ingredients to ensure that 80% of particles were < 500 μm did not improve digestibility compared with when 80% of particles were between 710 and 1000 μm. They also demonstrated that steam conditioning or extrusion processing significantly improved digestibility, weight gain and feed conversion ratio (FCR) compared with when diets were pelleted without steam.

In Australia, several diets are commercially available for silver perch. Initially, formulations (e.g. SP35) were based on published requirements for other commonly cultured omnivorous species and on high-quality feed ingredients not readily available in Australia (Allan and Rowland, 1992). Following research on nutritional requirements and ingredient digestibility, diets with relatively low protein (35%–28% digestible protein) and energy (< approximately 15 MJ of DE kg⁻¹) were formulated (Allan and Rowland, 1999; Allan et al., 2000b;

Table 25.5. Dry-basis proximate composition and apparent digestibility coefficients of ingredients for silver perch (Allan et al., 2000a).

Test ingredient	Protein* (%)	Fat (%)	Ash (%)	GE (MJ kg⁻¹)	Apparent digestibility coefficient (%) Protein	Energy
Fish meals						
Australian fish-meal†	73.2	9.9	14.2	21.3	92.3	89.6
Danish fish-meal	72.9	11.4	13.0	21.5	94.2	98.0
Peruvian fish-meal	70.2	11.3	17.6	20.9	89.0	88.6
Animal meals						
Blood meal (spray dried)	94.9	–	3.1	23.9	90.2	99.9
Meat-and-bone meal (beef)	49.2	9.2	36.0	16.1	71.5	75.2
Meat-and-bone meal (lamb)	54.3	7.2	34.5	16.2	73.9	80.8
Poultry meal	60.3	18.2	15.0	22.7	85.4	93.7
Feather meal (hydrolysed, ring-dried)	84.3	11.2	3.0	24.9	92.8	101.0
Oilseeds						
Soybean meal (solvent)	47.8	3.7	8.0	17.0	94.8	78.0
Soybean meal (whole, expeller)	47.5	6.4	6.3	20.9	95.7	84.1
Soybean meal (dehulled, full-fat)	35.8	19.5	5.5	23.3	92.3	79.8
Canola meal (solvent)	36.6	2.6	7.4	19.9	83	58.1
Canola meal (whole, expeller)	31.8	12.5	6.6	21.8	79.6	58.6
Groundnut meal	41.2	1.3	5.2	19.7	98.2	7.0
Cottonseed meal (dehulled)	48.1	4.6	8.3	19.9	83.0	53.1
Linola (linseed meal)	29.8	11.3	6.1	21.2	77.7	44.3
Legumes (whole)						
Lupins, Lupinus angustifolius (cv. Gungurru)	34.1	5.7	2.8	17.9	97.1	51.2
Lupins, Lupinus albus	37.6	6.2	3.7	20.9	95.9	70.1
Field pea, Pisum sativum (cv. Dunn)	25.5	1.1	3.4	17.0	81.0	51.0
Faba bean, Vicia faba (cv. Fjord)	27.7	1.3	3.6	17.3	90.5	59.2
Chick-pea, Cicer arietinum (cv. Desi)	20.8	4.7	3.4	19.4	82.2	54.8
Vetch, Vicia sativa (cv. Blanch-fleur)	30.9	0.9	3.3	17.9	71.2	52.8
Cow-pea, Vigna unguiculata	25.2	2.3	3.7	18.8	96.5	44.7
Cereals						
Wheat gluten	76.9	–	–	23.1	99.8	94.0
Maize gluten meal	62.0	–	1.1	24.1	95.4	104.5
Wheat 1 (Aust. Std. Wheat)	12.2	–	–	18.3	99.5	53.2
Wheat 2 (high protein)	15.2	–	–	18.5	106.1	31.0
Mill-run	22.3	–	4.3	19.6	86.2	55.2
Sorghum	14.5	–	2.3	18.8	77.8	37.8

* N × 6.25.
† Sea Fish Pty Ltd, Triabunna, Tasmania, Australia.

Table 25.6). Lower-energy diets were used in an attempt to limit the excess carcass lipid deposition that some silver-perch farmers had reported to be a problem. Performances of silver perch on these diets were compared during a series of large-scale farming trials, with the fish being grown to over 400 g in replicate 0.1 ha earthen ponds at GAC, using very similar methods to those used by commercial farmers. A summary of the results of two experiments is given in Table 25.7. Sensory profiles of fish fed different diets were compared by accredited

taste panels. The most successful diets contained fish-oil but much less fish-meal (0 to 10%) and produced fish that tasted as good as or better than fish fed a fish-meal/soybean-meal-based diet (SP35). They were also much more cost-effective (Allan and Rowland. 1999; Allan et al., 2000b) and several (95LC2, GRC3) have been adopted or are being considered for adoption by commercial feed-manufacturing companies. The replacement of expensive imported fish-meal and other protein sources with high-quality Australian agricultural protein sources has reduced ingredient costs for silver-perch diets by approximately 60% to around A$0.74 kg^{-1} without compromising performance. Silver-perch diets are currently the cheapest diets for any fish cultured in Australia.

Feeding Practices

Feed currently constitutes approximately 20% of production costs in silver-perch culture and efficient delivery of the diet is necessary for economic viability. Poor feeding practices increase the cost of production; overfeeding wastes feed and adversely affects water quality, while underfeeding results in reduced growth. Practices vary across the industry, from feeding to satiation to feeding a restricted amount based on a proportion of body weight. Satiation can be difficult to determine in the characteristically turbid silver-perch ponds where not all fish feed at the surface. Until recently, a feeding strategy specifically for silver perch had not been developed, and recommendations to industry were based on regimes used in other warm-water finfish industries, such as the channel catfish industry in the USA (Allan, 1995).

Russell et al. (1996) conducted a 4-week study in tanks and suggested that feeding between 5% and 10% body weight day^{-1} twice daily at water temperatures around 25°C would produce optimum growth and FCR (food conversion ratio) in small (1.3 g) fingerlings. A series of experiments was recently conducted at GAC to identify appropriate feeding rates and frequencies for fingerling and market-size silver perch at different ambient water temperatures. In each experiment, fish were stocked in floating cages in a 0.32 ha aerated pond and fed the commercial least-cost diet 95LC2 (35% crude protein, 5% fish-meal; extruded, slow-sinking) that had been specifically developed for silver perch (Allan et al., 2000b). Silver perch performed well in these experiments, with very high survival (> 98%) and growth rates, similar to those reported in open pond culture (Rowland et al., 1995). The new recommended feeding regimes for fingerling and market-size silver perch are given in Table 25.8. It is also recommended that the application of feed does not exceed 150 kg ha^{-1} day^{-1} in individual ponds.

Silver-perch broodfish are fed 2% body weight daily, commencing in early spring in the lead up to the breeding season when the water temperature reaches about 18°C; this rate is maintained during the summer. At lower water temperatures, rates of 0.5–1.0% are adequate for growth, health and gonadal development. First-feeding larvae (4.5 mm) are stocked in ponds, where they feed

Table 25.6. Composition of practical diets SP35,* 95LC1,* 95LC2,*† GRC2*† and GRC3† used in two separate experiments, each using nine 0.1 ha static, aerated earthen ponds.

Ingredient*	Per cent in diet (dry matter basis)				
	SP35	95LC1	95LC2	GRC2	GRC3
Fish-meal (Danish)	27.0	10.0	5.0	0	0
Meat meal (lamb meal)	–	21.7	36.9	37.5	29.4
Blood meal (ring-dried)	2.0	2.1	–	1.7	0
Maize-gluten meal	4.0	3.8	5.2	0	0
Soybean meal (solvent-extracted)	20.0	–	–	–	–
Canola	–	–	5.0	5.0	0
Groundnut meal	–	–	5.0	0	0
Field peas (Pisum sativum)	–	14.9	10.4	0	0
Lupins (cv. Gungurru) dehulled	–	25.5	7.4	0	0
Wheat	26.9	–	–	10.6	25.9
Sorghum	11.0	4.7	–	0	0
Mill-run	2.0	10.0	17.7	20.0	20.0
Fish-oil (cod-liver oil)	1.0	2.9	3.2	3.2	7.6
D,L-Methionine	0.2	0.4	0.3	0.1	0
Vit./min. premix	4.0	4.0	4.0	1.5	1.5
Dicalcium phosphate	2.0	–	–	0	0

* Allan et al., 2000b.
† 95LC2 was manufactured separately for both experiments. For the second experiment, minor changes to the vitamin and mineral premixes were made and an additional 2.5% mill-run was added (Allan and Rowland, 1999).

Table 25.7. Final weight, growth rate, food conversion ratio and production rate for silver perch fed different diets during two separate experiments in 0.1 ha earthen ponds.*

Experiment†	Diet	Final weight (g)	Growth rate (g fish^{-1} day^{-1})	FCR	Production (t ha^{-1})
1	SP35	395.4 ± 11.9[a]	2.2 ± 0.07[a]	2.2	5.8 ± 0.13[a]
1	95LC1	433.8 ± 10.8[b]	2.5 ± 0.09[b]	2.0	6.3 ± 0.12[b]
1	95LC2	441.7 ± 7.2[b]	2.5 ± 0.03[b]	1.9	6.5 ± 0.08[b]
2	95LC2	461 ± 9.7[a]	2.4 ± 0.05[a]	1.6	6.5 ± 0.14[a]
2	GRC2	453 ± 6.1[a]	2.4 ± 0.03[a]	1.7	6.5 ± 0.10[a]
2	GRC3	433 ± 6.9[a]	2.3 ± 0.05[a]	1.7	6.2 ± 0.11[a]

* Values are means ± SEM for three replicate ponds. For each experiment means in columns which share the same superscript were not significantly different (P < 0.05).
† Experiment 1 fish were stocked at 15,000 fish ha^{-1} at a mean weight of 81 g and cultured for 143 days (Allan et al., 2000b). Experiment 2 fish with a mean weight of 58 g were stocked at 15,000 fish ha^{-1} and cultured for 187 days (Allan and Rowland, 1999).

Table 25.8. Suggested feeding rates and frequencies for fingerling and large silver perch at different water temperatures.

Water temperature(°C)	Fingerlings (2–50 g)		Large fish (> 50 g)	
	Feeding rate (% BW day⁻¹)	Feeding frequency (no. of times day⁻¹)	Feeding rate (% BW day⁻¹)	Feeding frequency (no. of times day⁻¹)
10–12	0.5	1	0.5	Alternate days
12–15	1	1	0.5	1
15–20	2.5	1	2	1
20–25	5	2	3	2 (50–500 g)
			1.5	2 (> 500 g)
25–30	7.5	2	2	2 (50–500 g)
			1	1 (> 500 g)

BW, body weight.

on zooplankton for several weeks before being weaned on to a commercially available diet containing 50% protein. Post-larvae and fry (up to 2 g) are fed this diet to satiation, four times daily.

A range of feeding techniques are used in the silver-perch industry. At the GAC and smaller farms fish are fed by hand, while on larger farms (> 10 ha of ponds) vehicle-mounted blowers are used to deliver the feed. Several farmers have recently started using automatic feeders.

Initially cold-pressed pellets were used (Allan and Rowland, 1992), but since the formulation and evaluation of 95LC2 (Allan et al., 2000b) a majority of silver-perch farmers use extruded, slow-sinking or floating pellets. Although some silver perch feed aggressively at and near the surface, particularly in the warmer months, many fish feed mid-water. Thus, slow-sinking pellets are recommended for this species to ensure that all fish receive their daily ration.

Future Nutrition Research Priorities

Relatively low-cost production of silver perch is possible because the species performs well on low-protein diets composed of agricultural ingredients. An examination of the relative cost–benefit of using different proportions of protein, lipid and carbohydrates as energy sources could also lead to more cost-effective diets. Further cost savings may be possible if reduction in the content of expensive vitamins can be achieved without compromising performance or immune response. Research to clarify amino acid requirements and the ability of silver perch to utilize crystalline amino acids is warranted. Recent reports of apparently stress-related 'winter diseases' indicate that research on immunostimulants may be of value. Further research to determine requirements for long-chain n-3 fatty acids to optimize performance is also needed. In all nutrition research with silver perch, it is necessary to investigate the impacts of diet on carcass composition and sensory characteristics.

Conclusions

1. Silver perch is a new species for aquaculture, is relatively easy to culture and performs well on low-protein (e.g. 28% digestible protein) and low-energy (e.g. 15 MJ of DE kg⁻¹) diets because of the efficient digestion and utilization of dietary carbohydrates, especially starch.

2. Maintenance requirements for protein and energy were estimated as 2.1 g digestible protein kg⁻¹ body weight day⁻¹ and 116 kJ of DE kg⁻¹ body weight day⁻¹.

3. Recommended amino acid contents were estimated as those contents sufficient to ensure that growth was not retarded for diets made from intact protein sources with 15 MJ of DE kg⁻¹.

4. Silver perch are 'fatty' and, although they are able to chain-elongate and desaturate dietary linoleic and linolenic acid, growth is enhanced by the addition of longer-chain n-3 fatty acids in the diet. There is an inverse linear relationship between dietary protein : energy content and fish lipid content.

5. Australian agricultural ingredients, such as meat and poultry meals, lupins, peas and wheat, can be used in low-cost rations to replace most or all fish-meal without compromising performance or taste.

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