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Preliminary evaluation of meatmeal in aquaculture diets for silver perch M.561

1994

Prepared by:
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ISBN: 1 74036 908 4
Published: August 1994

MEAT & LIVESTOCK
AUSTRALIA

Preliminary evaluation of meatmeal in aquaculture diets for silver perch (*Bidyanus bidyanus*) - Final report to MRC

Contents

1 Summary

The replacement of fishmeal in aquaculture diets is recognised as a major international research priority. Most aquaculture diets are based primarily on fishmeal, although this protein source is expensive, decreasing in availability and mostly imported into Australia. Meatmeal and meat products are produced in abundance in Australia (over 470 000 t/yr) and are a relatively inexpensive source of protein. They have potential for use in Australian aquaculture feeds, and for export for use in the enormous global aquaculture feed industry. The market for aquaculture feeds in Asia is estimated to be approximately 2.6 million tonnes per year.

The first task in evaluating alternative protein sources is to assess their digestibility to target species. The aim of the present study was to assess digestibility of meat products for silver perch (*Bidyanus bidyanus*), a native Australian freshwater fish with great potential for aquaculture. As total protein for 'normal' meatmeal is relatively low compared with fishmeal, two modified, high protein meat products were also evaluated. The four products evaluated were beef meal, lamb meal, a high protein meal from mixed species and Provine®, a high protein meal based on selected ingredients.

Silver perch readily accepted diets with up to 30% meatmeal. Digestibility coefficients for dry matter, energy, protein and amino acids were determined which will assist with formulating diets to meet assumed requirements for this species. Dry matter digestibility and digestible energy for meat products was similar or superior to that for low quality fishmeal, oilseed meals and grain legumes. Protein digestibility was lower for meat products than for vegetable protein sources.

Digestibility coefficients for dry matter, energy and protein all increased with increasing protein content in the meat products. However, digestibility of most essential amino acids was lower in higher protein meals than in beef meal or lamb meal. The reverse was true for non-essential proline and alanine. All meat products were deficient in lysine, with phenylalanine, isoleucine and histidine low in some products. An increase in total protein content, through removal of bone, improved the value of meatmeal in silver perch diets.

Consistency is one of the major problems when using meatmeal in animal feeds and in this study three separate batches of lamb meal and Provine® were analysed. Composition of these meals over the period was consistent, but longer term studies are needed and establishment of industry standards, especially for high protein specialised meals are recommended.

A research strategy to determine the maximum amount of meat products which can be used in silver perch diets is described. This strategy is based on two experiments; one in large tanks which can be used as model ponds and the other in commercial size production ponds. Additional research to evaluate new meat products should start with digestibility studies.

Meatmeals with reduced ash (through removal of bone) and/or reduced fat contents will be more suitable for use in aquaculture diets than common meat and bone meals (for example the beef meal and lamb meal evaluated in this study).

2 Background

Meatmeal and meat products may have potential use in aquaculture feeds. In Australia, 479 973 tonnes of meatmeal was produced in 1991/92 and production is increasing (Australasian Agribusiness Services, 1993). On a cost per unit protein basis, meatmeal is an attractive protein source and, for aquaculture species, absence of significant quantities of carbohydrate, especially fibre, is a significant advantage over vegetable protein sources.

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

There are, however, some major problems with fishmeal. Fishmeal and fish oil production is declining (Barlow, 1989). The aquaculture feed industry currently uses more than 3 million tonnes of the global fisheries catch (New and Wijkstrom, 1990) excluding `trash fish' fed directly to aquaculture species. As aquaculture production increases, demand for fishmeal will also increase, inevitably forcing prices to rise. As higher quality fishmeal is generally required for aquaculture feeds, species of fish currently used for human consumption will increasingly be targeted by fishmeal manufacturers. In Malaysia, much of the cheap fish which was used to produce salted fish for human consumption is now used as aquaculture feed instead (New, 1991).

Apart from a relatively small quantity of fishmeal produced in Tasmania during a limited period each year, very little fishmeal is produced in Australia (Foster, 1992) and most required for aquaculture feeds is imported (ABARE, 1991). Imported fishmeal varies in quality and prices in Australia have risen to about AUS $1 100/tonne for high quality Danish fishmeal. Improved growth and food conversion efficiency have been recorded for salmonids when low-temperature fishmeals have been used. These special `aquaculture grade' fishmeals are more expensive than ordinary fishmeal, some by as much as 35% (Foster, 1992).

If Australian aquaculture is to develop, suitable alternatives to fishmeal must be found.

The need to replace fishmeal in aquaculture diets is recognised as a major international research priority (Manzi, 1989; New, 1991) and was recognised as one of the major challenges facing aquaculture nutrition researchers at the Aquaculture Nutrition Workshop held in 1991 (Allan and Dall, 1992).

Australian agriculture has much to gain from developing new products for use in aquaculture feeds and from selling existing products in this market. Forecasts of the world's aquaculture feed production for the year 2000 range from a projected 3.5 million tonnes (New, 1991) to 6.6 million tonnes (Akiyama, 1991). By far the largest consumer is the Asian region, with a market estimated at 2.6 million tonnes in 1990 (Akiyama, 1991). New et al's., (1993) estimate is more conservative, predicting an Asian market of 2.6 million tonnes by 2000. This market is expanding, and will continue to expand rapidly.

The push throughout Asia to increase aquaculture production is leading to a much greater demand for formulated feeds. This is evident in the much greater increase in the production of aquaculture diets than in production of fish and crustaceans from aquaculture. Between 1986 and 1990, production of aquaculture feeds increased more than four fold (Akiyama, 1991). The aquaculture feed market could offer an outlet for tens or even hundreds of thousands of tonnes of Australian products if these are shown to be well utilised by fish and crustaceans and are competitively priced.

Australian feed manufacturers also have the opportunity to enter the rapidly expanding aquaculture feeds market. Although Asian fish and crustacean feed manufacturing technology is currently at the forefront of international feed development, Australian companies could access this market if low cost ingredients could be produced from Australian agricultural products. This would require appropriate formulations and rigorous evaluation of diets. The development of new technology to improve the digestibility of Australian agricultural products to fish and the manufacture of new protein or amino acid supplements could give Australian feed manufacturing companies significant advantages over rival overseas companies. Value adding to our agricultural products by combining them into high value exportable aquaculture diets could greatly increase export earnings.

A large number of studies using different species and ingredients have already been conducted. The majority have investigated the potential of soybean meals or soybean products to replace fishmeal (eg Dabrowski et al., 1989; Shiau et al., 1989; Smith et al., 1988; Mohsen and Lovell, 1990; Balogun and Ologhobo, 1989; Lim and Dominy, 1990) because of the excellent amino acid profile of soybeans.

Limited studies with meat products have been undertaken, although in general results from those that have been completed for catfish have been positive (Lovell, 1992). Other studies have investigated a range of different products including

rapeseed meal (Davies et al., 1990; Smith et al., 1988), cottonseed meal (El-Sayed, 1990; Robinson and Brent, 1989), mustard oil cake, linseed and sesame meals (Hosain and Jauncey, 1989a, 1989b) and other less common vegetable proteins (Martinez-Palacios et al., 1988; Olvera-Novoa et al., 1990). Unfortunately, many of these studies have been conducted on an *ad-hoc* basis and, with the exception of channel catfish, very little systematic research has been conducted for warmwater species. Although the first task in evaluating the potential use of a feed ingredient is to assess its digestibility (Cho et al., 1982), digestibility of alternative protein sources to fishmeal has not been determined for many warmwater species apart from catfish (NRC, 1993; Halver, 1989). The measurement of digestibility involves measuring the amount of energy, or a specific nutrient such as protein or fat, which is ingested, and subtracting the amount remaining in the faeces. For highly digestible ingredients like fishmeal, very little energy or specific nutrients remain in the faeces. In terms of digestibility to fish, fishmeal is generally superior to terrestrial protein sources, which are in turn superior to vegetable protein sources (Lovell, 1989). Fish do not have well developed mechanisms to digest the large amounts of carbohydrate or fibre often present in vegetable protein ingredients (New, 1987), although omnivorous or herbivorous species are more capable of utilising carbohydrates than carnivorous species.

If digestibility of ingredients is not considered when diets to compare different ingredients are formulated, the different diets may vary considerably in the digestible energy levels and in the amounts of specific nutrients (eg protein) actually available to the fish.

3 Objectives

1 Determine the apparent digestibility coefficients for energy, protein and essential amino acids for four different meatmeals (to be determined in conjunction with MRC) in diets for silver perch (*Bidyanus bidyanus*).

2 Analyse the composition of the same meatmeals on three different occasions to measure consistency in nutritional composition over time.

3 Recommend the most appropriate research strategy to:

- a determine the maximum content of meatmeal(s) which can be included in practical diets for aquaculture species
- b evaluate the effect of including large amounts of meatmeal in fish diets on composition of fish, particularly fatty acid profiles
- c investigate whether meatmeal content in aquaculture diets can be increased by improving the palatability of 'meatmeal diets', or increasing the utilisation of meatmeal through the addition of enzymes, amino acids or other supplements and
- d assess the effects of processing diets containing meatmeal on the utilisation of meatmeal by aquaculture species.

4 Methods

4.1 Meat products and experimental diets

Four meat products (Table 4.1) were evaluated during this study. These included:

1 Beef meal (with bone) (M)

2 Lamb meal (with bone) (L)

3 Mixed species meal (with reduced bone content to give elevated protein) (W)

4 High protein derived meatmeal (Provine®) (P)

TABLE 4.1

Suppliers and prices for meat products

Ingredient	Supplier	Address	Tel/Fax	Price $/t	Contact
Beef meal (M)	Beef City	PO Box 886 Toowoomba 4350	076 915188 076 915205(F)	445	Roger Jeffcote
Lamb meal (L)	Fletcher International	PO Box 764 Dubbo 2830	068 845833 067 842965 (F)	453	Peter Breen
Mixed (W)	Midcoast Meat Company	PO Box 40 Macksville 2447	065 607200 065 607255 (F)	500	Kevin Whita
Provine® (P)	Aspen Technology	2 Cope Street Preston 3072	03 4806200 03 4804542 (F)	775	Martin Flavin

The other components of experimental diets (Table 4.2) were the silver perch experimental diet (at 70 or 85% inclusion) (SP35; see Appendix 1) and chromic oxide, used as an inert marker. SP35 was also the control diet. The silver perch experimental diet and all meat products were ground or sieved to ensure all particles passed through a 710 μm screen. Dry ingredients (SP35, meat products plus chromic oxide) were thoroughly mixed in a Hobart mixer (Troy Pty Ltd, Ohio 45374, USA) then combined with approximately 400 ml water/kg dry mix before being extruded through a meat mincer (Barnco Australia Pty Ltd, Leichhardt, NSW, 2040) with a 2 mm diameter die. Pellets were dried at <35°C in a convection dryer for about 6 hours until the moisture content was between 20 and 30%.

TABLE 4.2

Composition of experimental diets (100 g dry basis)

	Experimental Diet								
Ingredient	1	2	3	4	5	6	7	8	9
SP35[1]	99	84.2	69.3	84.2	69.3	84.2	69.3	84.2	69.3
Beef meal (M)	-	14.8	29.7	-	-	-	-	-	-
Lamb meal (L)	-	-	-	14.8	29.7	-	-	-	-
Mixed meat meal (W)	-	-	-	-	-	14.8	29.7	-	-
Provine (P)	-	-	-	-	-	-	-	14.8	29.7
Chromic oxide	1	1	1	1	1	1	1	1	1

[1]SP35 = Silver perch experimental diet (see Appendix 1 for composition)

4.2 Experimental fish

Eight juvenile silver perch (mean initial starting weight 6.1 g) were stocked into each tank. Fish were bred at NSW Fisheries Grafton Research Centre by Dr Stuart Rowland. Fish were anaesthetised using a bath of 25 mg/l ethyl p-aminobenzoate for 5 minutes, then groups of 2 or 3 fish were caught at random, weighed and distributed among 27 tanks by systematic interspersion.

4.3 Experimental facilities and procedures

Experimental tanks were 160 l cylindro-conical tanks (conical base sloped at 35°) fitted with a 65 mm diameter, 250 mm long settlement chamber which tapered into a 12 mm diameter, 150 mm long length of silicon tubing. Continuously-flowing, preheated water (mean 25.0°C; range 24.7-25.9°C) was filtered through a sand filter and a diatomaceous earth filter, then passed through a UV sterilizer before being supplied to experimental tanks at a flow-rate of 600 ml/min. Effluent water from each tank flowed out the side of the cylindro-conical tanks into a 25 mm diameter pipe. 20-25% of this flowed to waste and the rest was collected and recirculated through a $2m^3$ biological filter, a diatomaceous earth filter, a UV sterilizer and was then reused.

Each tank was aerated using two air-stone diffusers. The inside of each tank was black.

Fish were stocked seven days prior to the start of the faecal collection period to allow for acclimation to experimental conditions. During this period, fish were fed the silver perch experimental diet (Appendix 1). Three days prior to the start of the faecal collection period the test diets were introduced. Fish were fed to excess using automatic conveyor belt-type feeders for three hours each day from 0900-1200. One hour after feeding ceased, all uneaten food was drained from the tanks and the walls of the tank and the settlement chamber were thoroughly cleaned to remove any faeces, uneaten food or bacterial slime. The faeces were collected over 16 h. The silicon tubing into which the faeces settled was packed in ice and kept at ≤4°C throughout this period. Faeces were collected over 15 days and, for each tank, faeces were pooled over this period. There were three replicate tanks for each diet. During experiments dissolved oxygen was always above 6.3 mg/l, pH was between 7.7 and 8.3, and nitrite and ammonia were less than 0.1 mg NO_2-N/l and 0.1 mg total ammonia - N/l respectively.

4.4 Biochemical analyses

Proximate and chromium analyses were done at NSW Agriculture's Wollongbar Agricultural Institute using methods described in Allan and Frances (1994). Nitrogen was determined using the macro or semi-micro kjeldahl methods. Amino acids were analysed following acid hydrolysis using high pressure liquid chromotography and Waters Pico-Tag (Waters Pty Ltd, Lane Cove, NSW, 2066, Australia). Sulphur amino acids were determined separately following performic acid digestion, and tryptophan, which is lost during acid hydrolysis, was not determined.

4.5 Digestibility determinations

The measurement of digestibility is the first task in evaluating the potential of any feed ingredient for inclusion in a diet. This involves measuring the amount of a specific nutrient ingested and subtracting what remains in the faeces. The indirect method of Cho and Kaushik (1990) was used here, with chromic oxide as the inert indicator. Faeces were collected by settlement. The apparent digestibility coefficients (ADC's) for energy, protein (Nx6.25) and essential amino acids in experimental diets were calculated as follows:

$$100 - \frac{\%\ Cr_2O_3 \text{ in diet}}{\%\ Cr_2O_3 \text{ in faeces}} \times \frac{\%\ \text{nutrient in faeces}}{\%\ \text{nutrient in diet}}$$

As very few ingredients can be used as the sole diet, the meat products were evaluated by preparing diets comprising SP35 (Appendix 1) and the meat products. The ADC's for the meat products were calculated as follows:

(ADC of experimental diet - ADC SP35 diet x proportion of SP35 in experimental diet)/proportion of test ingredient in experimental diet.

It was planned to analyse for calcium and phosphorus as well as proximates and amino acids. However, phosphorus analysis in faeces produced very anomolous results; possibly bone fragments affected phosphorus digestibility. This is being further investigated. Calcium analysis was not possible, but calcium digestibility should not affect potential use of meatmeals in aquaculture diets.

4.6 Statistical analysis

The experiment was designed for analysis using two-factor Analysis of Variance with meat product (M, L, W or P) as the first factor and inclusion level (15 or 30%) as the second factor. Homogeneity of variance for each of the 8 treatments was assessed using Cochrans Test (Winer, 1971) and comparison between means were made using Student Newman-Keuls multiple range test.

5 Results

Objective 1

Analytical results for ingredients, diets and faeces (Appendix 2) were used to calculate digestibility coefficients for dry matter, energy and protein (Table 5.1) and essential amino acids (Table 5.2). For dry matter, digestibility coefficients for the reference diet (SP35) were slightly higher than for beef or lamb meal but lower than for the high protein mixed meal or the Provine®. Digestibility coefficients for energy tended to be higher for diets containing meat products than for the reference diet. Digestibility coefficients for protein were higher for the high protein mixed meal and Provine® compared with the beef and lamb meals. For essential amino acids, digestibility coefficients were always higher for the reference diet (SP35) than for any of the diets containing meat products. This difference was significant ($P<0.05$) for arginine, histidine, lysine, threonine and valine.

Digestibility coefficients for ingredients were calculated using the values for the reference diet and the proportion of the ingredient used (Tables 5.3 and 5.4). Two-way ANOVA revealed significant differences between ingredients, but that neither the inclusion level or the interaction between inclusion level and ingredient type were significant ($P>0.05$). For dry matter, energy and protein, digestibility coefficients increased with protein content; Provine® was the highest, followed by the mixed meal, lamb meal then beef meal. Significant differences are indicated in Tables 5.3 and 5.4. Digestibility coefficients for isoleucine, leucine, lysine, phenylalanine, tryosine, threonine and value were all significantly less for Provine® than for other meat products. Values for histidine for Provine® were slightly but not significantly lower, while for arginine digestibility coefficients for Provine® were higher than for beef or lamb meal but lower than for the mixed meal. Interestingly, digestibility coefficients for non-essential amino acids, especially proline and

alanine, were similar or higher for Provine® compared with other products.

Objective 2

Results from sampling three different batches of lamb meal and Provine® are presented in Table 5.5. For energy, protein and fat, differences between batches of lamb meal were less than 10%, while for Provine® the third batch was 3-4 MJ/kg lower in energy, 1-2% lower in protein and 1.4-2.6% higher in fat than the first batches. These differences are still relatively minor. For both ingredients, differences in essential amino acids between the three batches were rarely more than 20%, indicating that in general composition of these two ingredients was relatively consistent. Analyses of different batches over a longer period than was possible in this study (3 months) is necessary before final conclusions about consistency can be made.

Composition of fish fed experimental diets was analysed for protein, energy and fat. There were no significant differences ($P>0.05$) between body composition of fish fed different diets. Similarly, growth of fish during the experiment was similar for all treatments ($P>0.05$) (mean 7.9g/fish weight gain, range 4.1-9.9g/fish).

TABLE 5.1

Dry matter, energy and protein digestibility coefficients for diets

Diet[1]	Digestibility coefficient[2,3] (%)		
	Dry matter	Energy	Protein
Reference (SP35)	64.9±0.9^{bc}	75.5±0.7^{ab}	88.2±0.3^{e}
Beef meal 15%	60.7±1.0^{a}	74.6±2.6^{a}	84.6±0.3^{b}
Beef meal 30%	59.8±2.9^{a}	75.8±1.2^{abc}	82.3±1.9^{a}
Lamb meal 15%	63.6±0.6^{b}	76.4±0.9^{abc}	85.3±0.8^{b}
Lamb meal 30%	61.4±0.5^{a}	77.3±0.7^{bc}	82.8±0.2^{a}
Mixed 15%	66.4±2.6^{c}	77.3±1.9^{bc}	87.5±1.0^{de}
Mixed 30%	68.3±1.0^{d}	77.7±0.8^{c}	86.2±0.8^{c}
Provine® 15%	68.6±1.5^{d}	79.2±1.6^{d}	87.5±0.6^{de}
Provine® 30%	71.7±1.0^{e}	79.9±1.0^{d}	86.9±0.3^{cd}

1 The percentage indicates inclusion level.

2 Digestibility coefficients for diets were calculated using the equation 100 (1- nutrient in faeces/nutrient in diet * chromium in diet/chromium in faeces).

3 Values are means ± SE. Means with similar letters in the superscript are not significantly different (P>0.05). Data for protein digestibility coefficients were transferred (arcsine $x^{0.5}$) prior to statistical analysis.

TABLE 5.2

Digestibility coefficients for essential amino acids for diets

Diet[1]	Digestibility coefficients[2,3]								
	Arg	Hist	Iso	Leuc	Lys	Phenyl	Meth[4]	Threo	Val
Reference (SP35)	91.3±0.4^{e}	92.2±0.1^{c}	91.6±0.2^{e}	92.5±0.4^{e}	92.6±0.3^{d}	91.2±0.7^{d}	89.0±1.9^{a}	90.6±0.2^{d}	91.1±0.4^{d}
Beef meal 15%	86.9±0.6^{c}	90.4±0.8^{b}	90.3±0.3^{de}	91.3±0.4^{de}	90.2±0.3^{c}	89.7±0.5^{cd}	86.8±4.4^{a}	89.0±0.9^{c}	89.0±0.2^{c}
Beef meal 30%	81.8±0.9^{a}	88.9±0.3^{ab}	88.1±0.6^{cd}	89.3±0.4^{bc}	87.5±0.9^{b}	87.4±0.8^{b}	85.3±3.9^{ab}	86.6±0.6^{b}	86.3±0.5^{b}
Lamb meal 15%	86.3±0.6^{bc}	90.7±1.5^{b}	90.1±1.4^{de}	91.0±1.1^{de}	90.1±1.0^{c}	89.5±1.2^{cd}	86.2±2.0^{a}	88.8±1.8^{bc}	89.0±1.2^{c}
Lamb meal 30%	82.4±1.8^{a}	90.8±0.3^{b}	89.5±0.9^{d}	90.2±0.8^{cd}	88.4±1.1^{b}	88.6±0.9^{bc}	86.5±1.3^{a}	87.2±0.5^{bc}	87.7±1.3^{bc}
Mixed 15%	89.0±0.7^{d}	90.3±1.3^{b}	89.0±1.4^{d}	90.5±1.2^{cd}	90.1±1.5^{c}	89.3±1.4^{c}	84.2±4.9^{ab}	88.4±1.5^{bc}	88.1±1.5^{bc}
Mixed 30%	88.0±1.8^{cd}	89.1±1.5^{ab}	86.9±1.6^{bc}	88.5±1.5^{b}	88.3±1.2^{b}	87.4±1.8^{b}	84.0±2.3^{ab}	86.8±1.7^{bc}	86.0±1.8^{b}
Provine® 15%	87.4±2.2^{cd}	89.6±1.4^{b}	86.1±2.0^{b}	88.6±1.9^{b}	88.9±1.3^{bc}	87.2±2.0^{b}	83.9±3.4^{ab}	86.9±2.3^{bc}	86.2±2.0^{b}
Provine® 30%	85.1±2.2^{b}	87.9±2.1^{a}	82.9±3.0^{a}	85.8±2.1^{a}	86.4±1.8^{a}	84.4±2.3^{a}	78.4±11.6^{b}	84.2±2.1^{a}	83.4±2.9^{a}

1 The percentage indicates the inclusion level
2 Digestibility coefficient = 100 (1-nutrient in faeces/nutrient in diet * chromium in diet/chromium in faeces)
3 Values are means ± SE. Means with similar letters in the superscript are not significantly different (P>0.05)
4 Data for methionine were transformed (arcsine $x^{0.5}$) prior to analyses

TABLE 5.3

Dry matter, energy and protein digestibility coefficients for ingredients

Diet[1]	Digestibility coefficient[2,3] (%)		
	Dry matter	Energy	Protein
Beef meal 15%	37.2±6.9	69.4±17.5	64.2±2.0
Beef meal 30%	48.1±9.8 }a	76.4±4.1 }a	68.7±6.4 }a
Lamb meal 15%	56.4±4.1	81.6±5.9	68.7±5.4
Lamb meal 30%	53.3±1.5 }b	81.4±2.2 }b	70.1±0.8 }a
Mixed 15%	75.0±17.3	87.5±12.5	83.9±6.9
Mixed 30%	76.4±3.4 }c	82.8±2.7 }b	81.5±2.7 }b
Provine® 15%	90.1±9.8	100.2±10.5	83.5±3.9
Provine® 30%	87.7±3.2 }d	90.3±3.4 }c	83.7±0.8 }b

1 The percentage indicates inclusion level

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

3 Values are means ± SE. A similar letter in the superscript after the parentheses indicates differences between ingredients are not significant ($P>0.05$). Digestibility coefficients were not affected by inclusion level ($P>0.05$) and there was no interaction ($P>0.05$). Data for protein digestibility coefficients were transformed (arcsine $x^{0.5}$) prior to statistical analysis.

TABLE 5.4

Digestibility coefficients for essential amino acids for ingredients

Diet[1]	Digestibility coefficients[2,3]								
	Arg	Hist	Iso	Leuc	Lys	Phenyl	Meth	Threo	Val
Beef meal 15%	61.8±3.7	80.3±5.0	82.6±2.1	84.6±2.4	76.2±2.0	81.3±3.3	74.3±29.1^{a}	79.6±6.1	77.1±1.2
Beef meal 30%	60.0±3.1^{a}	81.2±0.9^{ab}	80.0±2.1^{c}	81.7±1.3^{b}	75.6±3.1^{b}	78.4±2.7^{b}	76.7±13.1^{a}	77.2±2.1^{b}	75.1±1.5^{b}
Lamb meal 15%	58.1±4.2	82.1±9.8	81.3±9.6	82.5±7.5	75.7±6.8	80.1±8.1	70.4±13.1^{a}	78.3±12.3	77.3±7.7
Lamb meal 30%	61.4±6.0^{a}	87.4±0.9^{b}	84.4±3.0^{c}	84.8±2.7^{b}	78.5±3.6^{b}	82.6±2.9^{b}	80.8±4.4^{a}	79.1±1.6^{b}	79.9±4.3^{b}
Mixed 15%	76.1±4.7	79.7±8.5	74.1±9.5	79.2±7.9	75.4±9.8	78.5±9.0	57.0±33.0^{a}	76.1±10.2	71.0±10.1
Mixed 30%	80.1±5.9^{c}	81.7±5.1^{ab}	76.0±5.2^{b}	79.2±4.9^{b}	78.1±3.9^{b}	78.5±6.1^{b}	72.4±7.8^{a}	77.8±5.5^{b}	74.3±6.1^{b}
Provine® 15%	65.1±14.5	74.9±9.5	54.7±13.2	66.5±12.6	67.9±8.7	64.4±13.6	55.0±22.4^{a}	66.0±15.2	58.5±13.0
Provine® 30%	70.7±7.3^{b}	77.9±7.0^{a}	62.7±10.0^{a}	70.1±6.9^{a}	71.7±6.0^{a}	68.5±7.7^{a}	53.5±38.7^{a}	69.1±7.1^{a}	65.6±9.6^{a}

1 The percentage indicates the inclusion level

2 Digestibility coefficient = (digestibility coefficient of experimental diet - digestibility coefficient of SP35 * proportion of SP35 in experimental diet)/proportion of test ingredient in experimental diet

3 Values are means ± SE. A similar letter in the superscript after the parentheses indicates differences between ingredients are not significant (P>0.05). Digestibility coefficients were not affected by inclusion level (P>0.05) and there was no interaction (P>0.05).

TABLE 5.5

Comparative analyses of different batches of lamb meal and Provine® (dry basis)

Ingredient	Batch	Nutrient											
					Amino acid (g/100 g)								
		Energy (MJ/kg)	Protein (g/100g)	Fat (g/100g)	Arg	Hist	Iso	Leuc	Lys	Phenyl	Meth	Threo	Val
Lamb meal	1	16.2	54.3	7.2	4.3	1.2	1.8	3.5	3.5	1.9	1.1	2.1	2.4
	2	15.6	53.4	7.4	4.0	1.0	1.6	3.2	3.1	1.7	0.8	2.1	2.2
	3	15.2	52.6	7.6	4.7	1.2	2.0	3.9	3.7	2.1	0.8	2.4	2.7
Provine®	1	25.7	81.0	10.4	6.8	1.6	3.7	6.2	4.9	3.4	1.7	3.6	4.3
	2	26.1	80.0	11.6	5.6	1.4	3.2	5.5	4.5	3.1	1.6	3.4	3.7
	3	22.6	79.0	13.0	5.4	1.3	3.2	5.4	4.3	3.0	1.5	3.2	3.8

6 Discussion

Based on digestibility coefficients for dry matter, (a good indication of the total amount of an ingredient digested), meat products were improved when bone was removed. This is evident in the increase in dry matter digestibility from between 37-56% for beef and lamb meal (with bones) to 75-90% for high protein mixed meal and Provine®. Much of the bone was removed from these latter two products as indicated by the analyses for ash (Appendix 2) which was 36.0, 34.5, 12.1 and 3.0% for beef meal, lamb meal, mixed meal and Provine® respectively. Cost-effective methods to remove ash from meatmeals will improve their value in diets for silver perch.

Food conversion efficiency is influenced by dry matter digestibility. Dry matter digestibility coefficients for the mixed meal and Provine® compared favourably with coefficients for lower quality Peruvian fishmeal, oilseed meals and grain legumes (see Appendix 2).

Published dry matter digestibility coefficients for meat products used in fish diets are scarce. For rainbow trout, dry matter digestibility coefficients of 43.2 and 38.8% were determined for fat extracted meat and bone meals (60.2% protein, 2.5% fat, 27.2% ash and 63.1% protein, 3.5% fat and 24.4% ash respectively) (Alexis et al., 1988).

Digestibility coefficients for energy for Provine® were similar to those for Peruvian fishmeal, although energy digestibility for other meat products was lower. Digestible energy from all meat products compared favourably with those from oilseed meals and grain legumes.

For silver perch in this study the digestible energy values for beef meal (49.2% protein, 9.2% fat and 36.0% ash) and lamb meal (54.3% protein, 7.2% fat and 34.5% ash) were 12.29 MJ/kg and 13.27 MJ/kg respectively. These are comparable with digestible energy values determined for meat and bone meal with 54.1% protein, 10.3% fat, 31.1% ash for channel catfish (12.26 MJ.kg) and rainbow trout (13.33 MJ/kg) (NRC, 1993).

Digestibility coefficients for protein in the present study were lower than those previously recorded with silver perch for fish meals, oilseed meals and cereals but similar to those recorded for grain legumes (Table 5.3; Appendix 3). Digestibility of crude protein was better for the higher protein mixed meal and Provine® than for beef meal or lamb meal. However, except for arginine, digestibility coefficients for essential amino acids tended to be lower for the higher protein meals than for beef meal or lamb meal. This apparent anomoly was caused by much higher digestibility coefficients for the non-essential amino acids proline and alanine for the mixed meal and Provine® compared with those for beef meal and lamb meal. (Digestibility coefficients for proline for the mixed meal, Provine®, beef meal and lamb meal included at 30% in the test diet were 75.5, 79.0, 30.7 and 33.4% respectively. Digestibility coefficients for alanine for the mixed meal, Provine®, beef meal and

lamb meal included at 30% in the test diet were 76.2, 73.4, 50.5 and 55.2% respectively.)

For pigs, digestibility coefficients for lysine for three low protein meatmeals (43-43.5% protein) ranged from 87-92%, while coefficients for seven high protein meatmeals (49.4-59.1% protein) were more variable, ranging from 68-98% (personal communication, Ted Batterham, 1993). This increase in variability was attributed to a greater chance of processing damage with low bone content meals, mainly due to Maillard reaction. In the present study, lysine digestibility was similar for beef meal, lamb meal and the mixed meal despite a removal of bone in the mixed meal. Lysine digestibility in Provine® was lower but this may reflect the quite different production process for this material compared with the other meals.

Protein digestibility values for silver perch fed meat products compared favourably with published values for rainbow trout. Asgard (1988) calculated a protein digestibility value for meat and bone meal with 51.3% protein of 59%, while Alexis et al. (1988) determined protein digestibility values of 60.9 and 59.7% for defatted meat and bone meals (60.2% protein, 2.5% fat, 27.2% ash and 63.1% protein, 3.5% fat, 24.4% ash respectively).

Digestible energy and digestible nutrients in the meat products used in the present study were calculated using analysed composition and digestibility coefficients (for diets with meat products included at 30%). These are compared with values for fishmeal and with requirements for channel catfish (Table 6.1). Nutritional requirements for silver perch are not well known (Appendix 4) but indications are that a diet with 35% crude protein containing sufficient concentrations of essential amino acids to meet requirements for channel catfish (on a percent of dietary protein basis) is suitable. On this basis, total crude protein content would restrict use of beef meal and lamb meal. All meat products investigated were deficient in lysine, lamb meal and mixed meal were also deficient in phenylalanine and the beef meal was also deficient in histidine, isoleucine and phenylalanine.

The cost of digestible protein for meat products used in the present study were calculated (on a $/kg protein basis) and compared with fishmeals and vegetable proteins (Table 6.2). For digestible protein, meat products ranked in the following order: mixed meal, Provine®, lamb meal and beef meal. All meat products were more cost-effective as a supply of digestible protein than Danish fishmeal, although Peruvian fishmeal was superior to beef meal. Meat products were inferior, on the basis of the cost of digestible protein, to oilseed meals and grain legumes. The trend for improved cost-effectiveness with an increase in protein (through a reduction in bone) was evident in the difference between beef meal, lamb meal and the mixed meal.

Maximum inclusion levels of ingredients in formulated diets will depend not only upon composition and digestibility but also upon the presence of anti-nutritional factors. Although meatmeal has fewer anti-nutritional factors than plant protein sources, it can contain high contents of bone fragments which can be deleterious.

TABLE 6.1

Digestible energy and digestible nutrients for low temperature Danish fish meal and meat products compared with requirements for channal catfish (NRC, 1993)

Nutrient	Ingredient					
	Danish fishmeal	Beef meal	Lamb meal	Mixed meal	Provine®	Requirements
Digestible dry matter (%)	91.4	48.1	53.3	76.4	87.7	
Digestible energy (MJ/kg)	21.5	12.3	13.2	19.4	23.2	12.6
Digestible protein (%)	72.2	33.8	38.1	49.4	67.8	28.0
Amino acids (g/16 g nitrogen)						
Digestible arginine	8.1	4.7	4.9	6.0	5.9	4.3
Digestible histidine	2.6	1.3	2.0	1.6	1.6	1.5
Digestible isoleucine	4.6	2.2	2.8	2.7	2.9	2.6
Digestible leucine	7.7	4.6	5.5	5.7	5.4	3.5
Digestible lysine	8.5	3.9	5.0	4.7	4.4	5.1
Digestible methionine[1]	3.0	*	*	*	*	2.3
Digestible phenylalanine[2]	7.2	2.9	3.9	4.6	5.9	5.0
Digestible threonine	5.0	2.5	3.1	3.3	3.0	2.0
Digestible valine	5.2	3.0	3.6	3.6	3.5	3.0

1 Including cystine. * Values for meat products are being recalculated using new analyses.

2 Including tryosine

TABLE 6.2

Cost of digestible protein for meat products, fishmeal and selected vegetable protein sources

Ingredient	Digestible protein	Digestible lysine	Price		Price
	(%)	(%)	($/t)	$/kg digestible protein	$/kg digestible lysine
Beef meal (M)	33.8	1.91	445	1.32	23.3
Lamb meal (L)	38.1	2.74	453	1.19	16.5
Mixed (W)	49.4	2.85	500	1.01	17.5
Provine® (P)	67.8	3.53	775	1.14	22.0
Danish fishmeal	68.1	5.23	1200	1.76	22.9
Danish fishmeal (low temp)	72.2	6.19	1500	2.08	24.2
Peruvian fishmeal	62.0	5.23	750	1.21	14.3
Soybean meal	47.6	2.82	440	0.92	15.6
Canola meal	40.3	2.25	330	0.82	14.7
Peanut meal	39.4	1.56	380	0.96	24.4
Cottonseed meal	41.6	1.49	370	0.89	24.8
Lupins (*Lupinus argustifilis*) hulls on	30.84	1.58	298	0.97	18.9

Excessive heat during the rendering process can damage proteins, especially lysine, and may contribute to lower protein digestibility. Consistent temperature throughout rendering facilities is important (Carpenter and Booth, 1973).

Excessive amounts of hair or wool also make processing difficult as can high contents of fat. In general, provided essential fatty acid requirements are met, saturated animal fats have no adverse effects on fish (Reinitz, 1980) and they are a good, cheap source of energy. However, fish fed diets with high concentrations of saturated fat tend to have a body composition lower in unsaturated fatty acids which may become a marketing disadvantage. Reduction of fat content, through mechanical or chemical extraction, will result in meals with a higher protein content which is an advantage for aquaculture diet formulation. Contamination of meatmeal products with pesticides or bacteria, particularly salmonella, are a genuine concern and industry specifications on these contaminants is needed (Australasian Agribusiness Services, 1993). Concern with exotic diseases like bovine spongiform encephalopathy (or Mad Cow Disease) has reduced use of meat products overseas (Australasian Agribusiness Services, 1993).

One of the major factors which has prevented the use of meatmeals in animal feeds has been inconsistent composition. This was recognised in the review commissioned by the Meat Research Corporation into the meatmeal and tallow industry and markets (Australasian Agribusiness Services, 1993). The inconsistency is especially notable when compared with vegetable protein sources such as soybean meal. The variability is a result of a number of factors, including the differing nature of raw materials, especially where mixed species are rendered. The practice of rendering processers to 'take what's left' contributes to this variability. In the present study, we assessed composition of three batches of two materials, lamb meal and Provine®. Consistency of these products was good, although the three batches were produced over a relatively short period of three months. Industry standards are desirable, especially for 'high grade meatmeals'.

One of the objectives of this study was to recommend research strategies to further quantify the potential to use meat products in aquaculture diets. Results of the digestibility study show meat products are well digested and that their value is improved by reducing the content of bone and increasing total protein content. Armed with data on digestibility, further research is needed to determine the maximum amount of products that can be included in diets. The following experiment was designed to address this question.

Future meatmeal research with silver perch

Experiment 1

Aims

1 To determine the potential to partially or totally replace fishmeal with meat products in diets for silver perch.

2 To determine the effects on fish growth, food conversion efficiency and fish body composition of feeding silver perch diets containing varying amounts of meat products.

3 To determine if deficiencies in lysine, methionine and threonine restrict inclusion of meat products and if synthetic amino acids can overcome this deficiency.

Methods

Examples of experimental diets which might address the above aims are listed in Table 6.3. The rationale for these diets is as follows:

1 Diet 1 is SP35 which gives known fish performance.

2 Diets 2-5 were designed to give similar contents (within 5%) of digestible protein, digestible energy, digestible phosphorus, fat and fibre to Diet 1.

3 Diets 2-4 were designed to give similar contents (within 5%) of digestible essential amino acids, lysine, methionine plus cystine, isoleucince, leucine, arginine, histidine, phenylalanine plus tryosine, valine and threonine, through manipulation of intact protein sources and if necessary addition of synthetic amino acids.

4 Diet 5 has no synthetic amino acids.

To determine effects of meat diets on growth and food conversion efficiency, fish should be on-grown for as long as possible in facilities conducive to rapid growth rate (equivalent to those recorded in commercial facilities if possible). Fish should be fed twice daily (to mimic commercial practice) to satiation to allow fish on inferior diets to consume more if necessary.

At the conclusion of the experiment, fish should be examined for total protein content, total fat content, fatty acid profile and for any signs of nutritional deficiency.

This experiment will address Objectives 3a and 3b. Objective 3c was to investigate whether meatmeal contents in aquaculture diets can be increased by improving palatability of meatmeal diets or increasing the utilisation of meatmeal through addition of enzymes, amino acids or other supplements. The treatment without amino acids was included to address this objective. Food consumption data and food conversion efficiency data will indicate whether meatmeal diets have problems with palatability.

Research is being conducted at the University of Tasmania to investigate the efficacy of enzymes in improving digestibility of feed ingredients to silver perch. Results for this project (part of the Fisheries Research and Development Corporation, Replacement of Fishmeal in Aquaculture Diets Sub-Program) should be

reviewed before enzyme studies are with meat products are undertaken.

The next phase of the research is to grow silver perch to market size in commercial size ponds using the 'best' meat-based diets. These diets need to be formulated following the results from the experiment described above. Ideally, two diets would be compared; the SP35 reference diet which is known to give good fish performance and a meat-based diet. Fish would need to be stocked into at least six ponds, three for each diet.

To carry results into the commercial arena of aquaculture feed manufacture, the effects of processing on diets containing meat also need to be quantified. Most dry aquaculture diets are produced using a pellet press or an extruder. Pre-conditioning using steam is often incorporated into both types of process.

To determine the effects of processing on meatmeal, the first step should be a digestibility study using at least two meat products; a high protein and a low protein meal and several processing treatments. This research should not delay large scale, commercially orientated trials with untreated meat products evaluated in this present study.

The value of increasing protein content through the removal of bone from meat meals has been discussed. Other methods to increase protein content include the removal of fat through improved mechanical means or by chemical (solvent) extraction. The first task in evaluating high protein meatmeals for use in aquaculture is to conduct further digestibility studies. These results would then provide the basis for practical formulations which meet nutritional requirements for target species.

TABLE 6.3

Experimental diets to evaluate meatmeal use for silver perch

Ingredient	Diet				
	1	2	3	4	5
Fishmeal (Danish)	27.0	13.0	6.0	0	0
Soybean meal	20.0	20.0	20.0	20.0	20.0
Blood meal	2.0	3.4	3.0	3.9	3.9
Lamb meal	-	6.3	7.8	8.9	8.9
Provine®	-	9.1	14.7	18.1	18.9
Corn gluten meal	4.0	6.0	6.0	6.0	6.0
DL-methionine	0.15	0.26	0.38	0.48	-
L-Lysine	-	0.06	0.23	0.33	-
L-Threonine	-	-	0.68	0.12	-
Wheat	26.9	22.1	21.9	22.0	22.2
Sorghum	11.0	11.0	11.0	11.0	11.0
Millrun	2.0	2.0	2.0	2.0	2.0
Fish oil	1.0	1.1	1.1	1.1	1.1
Di-calcium phosphate	2.0	1.7	1.9	2.0	2.0
Vitamin/mineral premix	4.0	4.0	4.0	4.0	4.0
Composition (calculated)					
Digestible protein (%)	32.1	34.0	34.1	34.1	34.0
Digestible energy (MJ/kg)	13.0	13.4	13.3	13.2	13.3
Ash	47.0	7.5	7.5	7.5	7.5
ADF (%)	3.1	3.3	3.3	3.3	3.4
Fat (%)	6.4	6.2	6.0	5.7	5.8
Linolenic series fatty acids (%)	0.28	0.30	0.30	0.3	0.3
Digestible lysine (%)	2.05	2.00	2.00	2.0	1.8
" meth + cys (%)	1.44	1.40	1.40	1.40	0.94
" isoleucine (%)	1.42	1.37	1.31	1.25	1.26
" leucine (%)	2.94	3.11	3.00	2.96	3.00
" arginine (%)	1.99	2.08	2.08	2.06	2.10
" histidine (%)	0.82	0.85	0.80	0.80	0.81
" pheny + try (%)	2.66	2.79	2.71	2.68	2.71
" valine (%)	1.69	1.72	1.64	1.61	1.64
" threonine (%)	1.42	1.40	1.40	1.40	1.30

7 Conclusions and Recommendations

7.1 Dry matter digestibility of meat products was higher (better) for products with higher protein contents and reduced ash content. Beef and lamb meal, with 49.2 and 54.3% protein and of 36.0 and 34.5% ash respectively, had dry matter digestibility coefficients of between 37 and 56%. In comparison, the high protein mixed meal and Provine® with 60.6 and 81.0% protein and 12.1 and 3.0% ash respectively had dry matter digestibility coefficients of between 75 and 90% respectively.

7.2 Dry matter digestibility coefficients for the high protein mixed meal and Provine® compared favourably with lower quality Peruvian fishmeal, oilseed meals and grain legumes.

7.3 Digestibility coefficients for energy for Provine® were similar to those for Peruvian fishmeal, although those for other meal products were lower. Digestibility coefficients for energy for all meat products compared favourably with those for oilseeds and grain legumes and were similar to those reported for rainbow trout and channel catfish for meat and bone meal.

7.4 Digestibility coefficients for protein for meat products were lower than for fishmeals, oilseed meals and cereals and similar to those for grain legumes. Digestibility of essential amino acids tended to be lower for high protein meat products than beef meal and lamb meal, although digestibility coefficients for non-essential proline and alanine were much higher. Overall, crude protein digestibility was higher for higher protein meat products.

7.5 Previous problems with poor digestibility of lysine for pigs fed meatmeals with reduced bone contents were not evident in this study but should be carefully assessed in future evaluation of reduced ash (and elevated protein) meatmeals.

7.6 For aquaculture diets, total protein content of beef meal and lamb meal (and products with similar protein contents) will limit inclusion.

7.7 All meat products were deficient in lysine; lamb meal and the high protein mixed meal were also deficient in phenylalanine, and beef meal was deficient in histidine, isoleucine and phenylalanine.

7.8 On the basis of cost of digestible protein, the meat products ranked as follows: mixed meal, Provine®, lamb meal and beef meal.

7.9 Lamb meal and Provine® from three separate batches were analysed and found to be consistent in proximate and amino acid composition. Consistency over a longer period should be assessed and the introduction of industry standards for product quality will improve the marketability of meat products for use in aquaculture diets.

7.10 Future research should be conducted to further evaluate meat products in aquaculture diets. An experiment to compare the effects of feeding silver perch diets with different amounts of meat products on growth, food conversion efficiency and fish body composition is described. This experiment would also assess the potential to overcome amino acid deficiencies in meat products with synthetic amino acids.

8 Acknowledgements

I would like to thank the following for their technical assistance: Mr Scott Parkinson, Ms Jane Frances, Mr David Stone, Ms Rebecca Warner-Smith (Port Stephens Research Centre), Mr Ken O'Brien and Ms Wendy Peasley (Wollongbar Agricultural Institute). Ms Jane Frances also assisted with preparing the report and Ms Jo Pickles expertly typed the manuscript.

In addition to the funding support from the Meat Research Corporation, the Fisheries Research and Development Corporation have provided major support for aquaculture nutrition research, particularly on the use of alternative protein sources to fishmeal, through the Replacement of Fishmeal in Aquaculture Feeds Sub-Program. In particular I would like to thank Mr Peter Dundas-Smith (FRDC Executive Director) and Mr Simon Prattley (FRDC Program Manager) for their assistance. Funding for complimentary research has also been provided by the Australian Centre for International Agricultural Research; Mr Barney Smith's assistance is also gratefully acknowledged.

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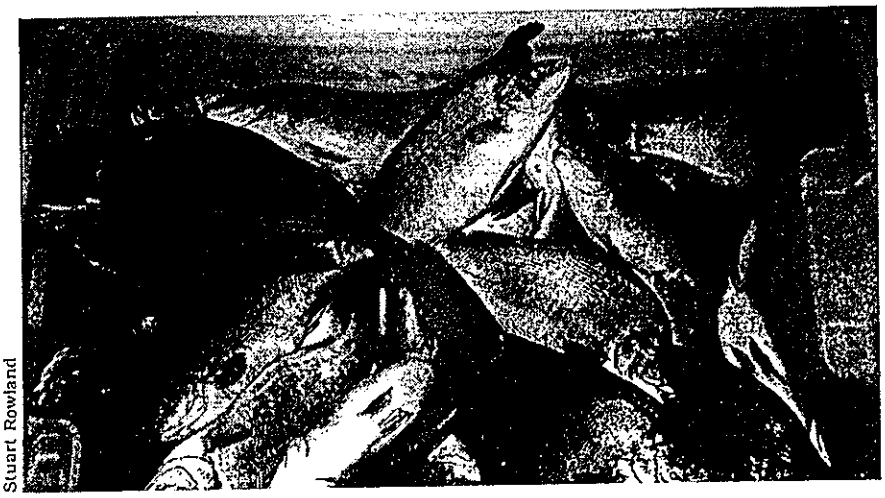
Development of an experimental diet for silver perch (*Bidyanus bidyanus*)



Stuart Rowland

NSW Fisheries is conducting a research project aimed at developing technology for growing silver perch in earthen ponds. This article details a diet that has been formulated for the initial experiments and which has produced encouraging results.

By Geoff Allan[1] & Stuart Rowland[2]

Freshwater finfish is the major component of world aquaculture production. In 1987, approximately 6.8 million t of finfish were produced, of which 88.4% was farmed in freshwater (Nash and Kensler, 1990). Although there are many indigenous freshwater fish in Australia that are highly regarded for their edible qualities, many of these species are no longer abundant. Hatchery techniques have been developed for some species (Rowland, 1989); however, with the exception of barramundi (*Lates calcarifer*) there has been no research into the grow-out of native finfish.

Currently there is only a small industry (1613 t in 1989-90) based on the freshwater production of the exotic rainbow trout, *Oncorhynchus mykiss* (O'Sullivan, 1992).

Rowland and Barlow (1991) suggested that the native freshwater fish silver perch (*Bidyanus bidyanus*) has high potential for aquaculture because hatchery techniques are established and the species is hardy, grows rapidly in farm dams, is omnivorous and readily accepts pellets.

A major research project to determine the feasibility and develop techniques for the intensive culture of silver perch commenced at NSW Fisheries', Eastern Freshwater Fish Research Hatchery (EFFRH), Grafton, in 1990. A component of the project, the evaluation of feeds, is being partly funded by the Fisheries Research and Development Corporation. Formulated feed represents one of the major costs in finfish aquaculture, accounting for up to 60% of total operating costs (Manzi, 1989). The development of nutritionally adequate, cost-effective diets is therefore one of the major factors limiting the establishment of an economically successfully aquaculture industry. One of the research priorities for the silver perch project at EFFRH is to determine protein requirements. Requirements for other omnivorous freshwater species, such as channel catfish (*Ictalurus punctatus*) are in the range 25-36% protein (Robinson, 1989) while requirements for carnivorous freshwater species such as rainbow trout (*Oncorhynchus mykiss*) are higher (40-45%; Halver, 1989).

The initial nutrition experiment was conducted with fry (0.6 g) stocked in 1,000 litre aerated tanks and fed isoenergetic diets with protein contents of 21, 36 and 49%. The fastest growth was recorded with the 36% protein diet; however, differences in growth between fish on this diet and the 49% protein diet were not significant (Allan and Rowland, 1991). The results indicated that the dietary protein requirement for juvenile silver perch would exceed 21% and would probably be closer to those required by other omnivorous freshwater species than to those required by carnivorous freshwater species such as rainbow trout.

These results provided the basis for the formulation of a diet to be used in pond trials. The diet, SP35 (Table 1) was also formulated to satisfy or exceed the published requirements for channel catfish of essential amino acids, digestible energy to protein ratio and available phosphorus (NRC, 1983; Lovell, 1989; Robinson, 1989). Published results for nutrient digestibility and phosphorus availability for catfish (NRC, 1983; Robinson, 1989) were also used. Even though requirements for essential fatty acids are likely to be lower for silver perch than for carnivorous marine species (Anderson and Arthington, 1992), fish oil was added to the diet to ensure that essential fatty acid deficiencies did not depress growth in in silver perch.

The diet was manufactured in the form of crumbles (2 mm; 3 mm) for fry and fingerlings, and pellets (3 x 12 mm; 6 x 12 mm) for larger fish. All experimental diets were manufactured by Janos Hoey Pty Ltd, Forbes, NSW, and stored at 15°C until used.

SP35 was first used in a fingerling production experiment. Silver perch fry (0.6 g) were stocked into six, aerated 0.1 ha earthen ponds and fed 2 mm and 3 mm crumbles at rates up to 3% body weight per day. Within two weeks, fry were readily feeding on the crumbles. Fingerlings (16 g) were harvested after 12 weeks; survival rates ranged from 97 to 100% and the food conversion ratios ranged from 1.0 to 1.3 (S. Rowland,

[1]Brackish Water Fish Culture Research Station, Salamander Bay, NSW 2301
[2]Eastern Freshwater Fish Research Hatchery, Grafton, NSW 2460

unpublished data, 1992).

A grow-out phase experiment is currently underway in the earthen ponds. Fingerlings were stocked in May and fed SP35 at rates up to 3% body weight daily. Fish fed throughout winter, and growth has been rapid since late spring. Mean weights of silver perch in six ponds at the end of February, 1992, ranged from 436 to 581 g and assuming high survival, estimated standing crops in some ponds may exceed 8 t/ha (S.Rowland, unpublished data, 1992).

Harvesting fingerlings from EFFRH ponds at Grafton, NSW

The results of the nutrition and production research to date, suggest that the experimental diet, SP35, is suitable for the pond production of fingerling and market size (400-500 g) silver perch.

The formulation of this diet will probably be modified after further nutrition experiments. Experiments to define the optimum protein requirements and to determine the digestibility of a number of protein sources are underway at EFFRH and Brackish Water Fish Culture Research Station. Subsequent research will concentrate on ways to reduce the cost of silver perch diets by defining optimum protein to energy ratios, and formulating practical diets with reduced fishmeal and increased soybean (or other plant protein) content.

TABLE 1: Formulation and biochemical composition of the experimental diet, SP35, for silver perch.

Ingredients	%
Fish meal	27.0
Soybean meal	20.0
Blood meal	2.0
Corn gluten meal	4.0
Wheat	28.4
Sorghum	11.0
Millrun	2.0
Cod liver oil	1.0
Di-calcium phosphate	2.0
Vitamin/mineral premix[1]	2.5
L-methionine	0.15
Proximate composition (as fed basis)	%
Crude protein (N x 6.25)[2]	35.6
Crude fat (ether extract)[2]	5.5
Linolenic series (n-3) fatty acids[3]	1.1
Fibre (acid detergent)[2]	4.4
Carbohydrate (difference)[2]	52.1
	(g/kg)
total methionine[4]	7.4
Total lysine[4]	22.6

[1] Included the following (per kg diet) Retinol 2.4 mg; Cholecalciferol 25 µg; ∞-Tocopherol acetate 125 mg; Menadione sodium bisulfite 16.5 kg; Thiamin. HCl 10 mg; Riboflavin 25.5 mg; Nicotinamide 200 mg; Ca-pantothenate 54.5 mg; Pyridoxine. HCl 15 mg; Cyanobalamin 20 µg; folic acid 4 mg; Biotin 1 mg; Ascorbic acid 450 mg; Myo-inositol 600 mg; Choline chloride 1500 mg; $CaCO_3$ 7.5 g; $MnSO_4$ 0.3 g; $ZnSO_4$ $7H_2O$ 0.7 g; $FeSO_4$ $7H_2O$ 0.5 g; $CuSO_4$ 60 mg; NaCl 7.5 g; KIO_3 2 mg.

[2] Methods described by Faichney and White (1983).

[3] Neutral and polar lipid fractions were separated by chromatoghraphy and lipid classes were separated by thin layer chromatography.

[4] Amino acid profiles analysed using high pressure liquid chromatography and Waters (Lane Cove, NSW) Pico-Tag.

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DIGESTIBILITY EXPT - JULY 1994 (D794)
MEATMEALS-PROXIMATE ANALYSIS
db=DRY BASIS

DIET	TYPE	REP	DM%	dbCr mg/g	dbGEMJ/kg	dbN%	dbPROT%	dbFAT%	dbASH%
M	I		96.98		16.09	7.87	49.17	9.19	36.03
W	I		97.7		23.49	9.70	60.64	17.19	12.07
L	I		97.15		16.24	8.69	54.30	7.20	34.45
L batch 2	I		97.56		15.58	8.55	53.43	7.39	36.62
L batch 3	I		97.77		15.17	8.42	52.61	7.62	35.13
P	I		94.34		25.70	12.95	80.96	10.42	3.04
P batch 2	I		90.75		26.13	12.80	80.03	11.64	2.89
P batch 3	I		90.81		22.63	12.64	79.01	12.97	2.79
R	I		94.44		18.12	6.25	39.05	2.75	15.97
M15	D		96.1	10.19	17.51	6.40	40.00	2.47	19.35
M30	D		96.6	9.42	17.39	6.64	41.47	4.04	22.35
W15	D		95.86	9.49	18.82	6.76	42.25	3.81	15.61
W30	D		96.34	9.59	19.57	7.12	44.50	6.49	14.88
L15	D		97.06	9.56	17.54	6.52	40.76	1.98	18.55
L30	D		97	9.42	17.41	6.85	42.78	2.99	21.73
P15	D		96.71	9.55	18.93	7.13	44.59	3.20	14.31
P30	D		96.46	9.70	20.16	8.07	50.41	4.74	12.67
R	D		97.4	9.63	17.83	6.16	38.50	0.73	16.05
R	F	1	97.2	27.75	12.37	2.06	12.86		35.35
R	F	2	97	26.97	12.42	2.06	12.89		35.71
R	F	3	97.8	27.49	12.49	2.10	13.10		34.92
M15	F	1	96.4	25.53	11.87	2.50	15.63		40.73
M15	F	2	97.6	26.32	10.88	2.52	15.75		44.30
M15	F	3	98.21	25.93	11.20	2.52	15.72		41.31
M30	F	1	98.8	22.37	10.29	2.97	18.53		46.62
M30	F	2	96.81	23.87	10.68	2.94	18.40		46.85
M30	F	3	97.4	24.18	10.49	2.83	17.71		44.91
L15	F	1	96.41	26.13	11.53	2.63	16.47		41.79
L15	F	2	97.21	26.55	11.22	2.58	16.14		42.25
L15	F	3	95.81	26.09	11.31	2.70	16.90		41.97
W30	F	1	97.8	29.98	13.88	3.05	19.04		35.47
W30	F	2	98.6	30.92	13.75	3.08	19.27		32.65
W30	F	3	97.99	29.92	13.72	3.18	19.90		35.14
W15	F	1	98.8	27.98	12.87	2.60	16.26		33.33
W15	F	2	99.19	27.16	12.61	2.41	15.06		33.81
W15	F	3	97.41	29.66	12.62	2.50	15.66		36.39
L30	F	1	98.6	24.44	10.11	3.06	19.14		47.22
L30	F	2	99.4	24.56	10.28	3.10	19.37		46.67
L30	F	3	99.4	24.23	10.35	3.01	18.80		46.97
P15	F	1	99.4	30.38	12.59	2.92	18.23		34.61
P15	F	2	98.2	31.35	12.32	2.85	17.82		34.29
P15	F	3	98.4	29.71	12.73	2.78	17.40		34.56
P30	F	1	98.79	34.47	14.28	3.81	23.79		30.98
P30	F	2	98.2	33.58	14.45	3.68	22.98		32.08
P30	F	3	98.19	34.87	14.17	3.77	23.55		33.18

M=MEATMEAL, W=WHITE, L=LAMBMEAL, P=PROVINE, R=REFERENCE, 15=15% INCLUSION, 30=30% INCLUSION
I=INGREDIENT; D=DIET; F=FAECES; DM=DRY MATTER; GE=GROSS ENERGY; Cr=CHROMIC OXIDE.

DIGESTIBILITY EXPT - JULY 1994 (D794)
MEATMEALS-AMINO ACID ANALYSIS
db=DRY BASIS

DIET	TYPE	REP	dbASP%	dbGLUT A%	dbSER%	dbHIST%	dbARG%	dbTHREO%	dbALA%	dbPRO%	dbTYRO%	dbVAL%	dbISO%	dbLEUC%	dbPHENYL%	dbLYS%	dbCYS%	dbMETH%
M	I		3.31	5.71	2.14	0.79	3.87	1 60	3 88	4 98	1.14	1.97	1.34	2.74	1.52	2.52	0.55	0.75
W	I		4.92	7.85	3.01	1 18	4 53	2 55	4 32	4 52	1.85	2.96	2.12	4.34	2.34	3.65	0.82	1.11
L	I		3.77	6.96	2.35	1.21	4.29	2 14	4 01	4.58	1.52	2.44	1.82	3.53	1.89	3.49	0.65	1.12
L batch 2	I		3.19	6.24	2.22	1.02	4 01	2.05	3 92	4 25	1.28	2.22	1.63	3.15	1.72	3.13	0.78	0.80
L batch 3	I		4.16	7.60	2.73	1.15	4.65	2.37	4.49	4.76	1.53	2.68	1.96	3.85	2.06	3.74	0.74	0.79
P	I		6.93	9.88	3.56	1.62	6 81	3 55	5 27	5 24	2.97	4.34	3.72	6 20	3.37	4.93	1.78	1.69
P batch 2	I		5.96	8.76	3.31	1.44	5.64	3 37	4 53	4 27	2.62	3.74	3.21	5.48	3.10	4.50	1.42	1.55
P batch 3	I		5.88	8.62	3.10	1.33	5 38	3 20	4 44	4 19	2.54	3.77	3.24	5.38	3.02	4.29	1.34	1.47
R	I		3.56	6.40	1.88	1.01	2.55	1 64	2 37	2.41	1.51	2.14	1.75	3.37	1.85	2.66	0.61	1.12
M15	D		3.61	6.30	1.86	0.87	2.76	1 72	2.66	3.15	1.40	2.12	3.42	1.63	1.82	2.64	0.57	1.03
M30	D		3.55	6.23	1.92	0.93	2.97	1 64	2 78	3.23	1.35	2.06	1.61	3.13	1.74	2.61	0.55	0.98
W15	D		4.30	7.43	2.25	1.14	3.21	1 98	2 99	3 20	1.72	2.47	2.00	3.90	2.14	3.04	0.76	1.13
W30	D		3.93	6.73	2.11	0.99	3.01	1 84	2.82	3.11	1.58	2.27	1.81	3.50	1.91	2.78	0.84	1.11
L15	D		3.87	6.73	2.08	0.97	2.62	1 79	2 75	3.04	1.50	2.08	1.73	3.51	1.89	2.80	0.65	1.05
L30	D		3.58	6.42	2.02	1.02	2.93	1 76	2.79	3 27	1.37	2.04	1.65	3.26	1.79	2.72	0.56	1.02
P15	D		4.04	6.86	2.11	1.07	3.10	1 89	2.80	2.87	1.69	2.41	2.01	3.74	2.07	3.03	0.58	1.00
P30	D		4.55	7.35	2.35	1.12	3.69	2 17	3 20	3.28	1.91	2.74	2.31	4.23	2.32	3.52	0.76	1.30
R	D		3.38	6.32	2.01	1.05	2.58	1.69	2.39	2.23	1.39	2.07	1.74	3.39	1.82	2.55	0.63	0.98
R	F	1	0.74	1.14	0.52	0.24	0.63	0.46	0 71	0.77	0.33	0.53	0.42	0.72	0.45	0.53	0.44	0.28
R	F	2	0.74	1.13	0.51	0.23	0.63	0.44	0.70	0.76	0.30	0.51	0.40	0.70	0.43	0.52	0.41	0.33
R	F	3	0.75	1.23	0.54	0.24	0.65	0.45	0 72	0.77	0.33	0.54	0.42	0.76	0.48	0.55	0.45	0.31
M15	F	1	0.83	1.41	0.65	0.28	0.90	0.52	1.03	1.20	0.33	0.61	0.45	0.80	0.51	0.67	0.53	0.39
M15	F	2	0.91	1.49	0.65	0.27	0.94	0 51	1 05	1.25	0.33	0.63	0.47	0.81	0.51	0.69	0.40	0.28
M15	F	3	0.76	1.34	0.61	0.26	0.89	0 48	1.00	1.17	0.33	0.61	0.45	0.77	0.49	0.66	0.47	0.37
M30	F	1	0.94	1.66	0.68	0.27	1.11	0.53	1.27	1.55	0.33	0.67	0.48	0.85	0.54	0.75	0.59	0.37
M30	F	2	1.07	1.82	0.73	0.29	1.18	0 58	1 35	1.66	0.37	0.71	0.53	0.92	0.59	0.82	0.62	0.37
M30	F	3	1.09	1.77	0.73	0.29	1.14	0 55	1.25	1.55	0.36	0.70	0.50	0.89	0.55	0.76	0.47	0.30
L15	F	1	0.79	1.43	0.64	0.25	0.95	0 47	1.04	1.26	0.36	0.63	0.48	0.83	0.51	0.66	0.98	0.39
L15	F	2	0.84	1.46	0.66	0.26	0.95	0.52	1 02	1.20	0.35	0.59	0.43	0.78	0.49	0.67	0.62	0.34
L15	F	3	0.99	1.70	0.72	0.29	0.98	0.56	1.08	1.26	0.39	0.65	0.50	0.89	0.55	0.73	0.85	0.37
W30	F	1	1.24	1.89	0.79	0.37	0.99	0.73	1 06	1.09	0.50	0.90	0.71	1.23	0.73	0.93	0.92	0.53
W30	F	2	1.19	1.82	0.74	0.33	0.90	0.66	1.03	1 04	0.47	0.86	0.68	1.16	0.67	0.90	0.73	0.48
W30	F	3	1.14	1.98	0.86	0.38	1.03	0.72	1.15	1.18	0.51	0.97	0.76	1.30	0.77	0.98	0.78	0.46
W15	F	1	1.00	1.58	0.67	0.31	0.85	0.60	0.94	0.98	0.41	0.77	0.59	0.99	0.60	0.78	0.69	0.45
W15	F	2	0.81	1.33	0.56	0.26	0.77	0.50	0.82	0.86	0.36	0.65	0.49	0.84	0.50	0.65	0.97	0.51
W15	F	3	1.06	1.67	0.73	0.32	0.87	0.63	0.96	1.04	0.45	0.77	0.61	1.02	0.62	0.81	0.62	0.39
L30	F	1	0.86	1.62	0.71	0.24	1.13	0.56	1.23	1.60	0.34	0.62	0.45	0.82	0.51	0.75	0.46	0.32
L30	F	2	0.89	1.68	0.73	0.25	1.24	0.56	1 36	1.72	0.36	0.69	0.49	0.90	0.55	0.79	0.51	0.32
L30	F	3	0.80	1.52	0.65	0.24	1.10	0.53	1.19	1.50	0.35	0.62	0.45	0.81	0.51	0.70	0.61	0.35
P15	F	1	1.09	1.61	0.69	0.32	0.97	0.65	0 98	0.96	0.51	0.88	0.74	1.19	0.71	0.88	0.61	0.55
P15	F	2	1.19	1.62	0.73	0.35	1.00	0.68	1.01	1.02	0.52	0.88	0.73	1.16	0.70	0.87	0.74	0.45
P15	F	3	1.29	1.91	0.84	0.37	1.12	0.76	1.12	1.14	0.59	0.97	0.81	1.32	0.79	0.93	0.73	0.49
P30	F	1	1.82	2.45	1.06	0.47	1.44	0 99	1.36	1.22	0.80	1.30	1.11	1.79	1.05	1.28	0.79	0.60
P30	F	2	1.65	2.35	1.05	0.48	1.41	0.99	1.35	1.22	0.78	1.27	1.10	1.78	1.05	1.28	1.23	1.00
P30	F	3	1.56	2.13	0.98	0.41	1.25	0.89	1.19	1 07	0.70	1.10	0.95	1.58	0.93	1.15	0.75	0.65

M=MEATMEAL, W=WHITE, L=LAMBMEAL, P=PROVINE, R=REFERENCE, 15=15% INCLUSION, 30=30% INCLUSION
I=INGREDIENT; D=DIET; F=FAECES.

2 FISHMEAL REPLACEMENT IN AQUACULTURE FEEDS FOR SILVER PERCH

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Project Objectives

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

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

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

4 To identify areas where inadequate knowledge of nutritional requirements may restrict fishmeal substitution and determine these requirements for silver perch

5 To formulate and evaluate diets with reduced contents of fishmeal for silver perch

Milestones - Year 1

1 Validation of analytical and experimental techniques

   a comparison of *in vitro* and *in vivo* methods for digestibility

   b comparison of carcass composition and stable isotope analyses to evaluate availability of nutrients in ingredients

2 Determination of digestibility coefficients for 10-15 new ingredients

PROGRESS REPORT

Introduction

This section summarises progress with the silver perch research during Year 1 at NSW Fisheries Port Stephens Research Centre and Grafton Research Centre. Results from collaborative studies by Alex Anderson (QUT) and Nigel Preston (CSIRO) are presented separately. (See Chapters 5 and 6).

This section includes:

1 *In vivo* digestibility work; including four separate validation experiments and results from a further five experiments using different ingredients or cultivars sometimes at different inclusion levels, sometimes subjected to dehulling

2 Preliminary results of a pond trial comparing a low-cost diet with a reference diet

3 Results from a protein and energy experiment

4 An outline of proposed research for Year II

In vivo digestibility experiments

Methodology

For all digestibility experiments the following methods were used:

1 170 l tanks supplied with continuously-flowing freshwater at approximately 600 ml/min.

2 Water was recirculated through a $2m^3$ biological filter, UV steriliser and a diatomaceous earth filter before being supplied to tanks. 75% of water was recirculated daily, 25% was fresh. Water was heated to 26°C.

3 Chromic oxide was used as a marker at 1.0% in all diets.

4 All experiments included a control diet (the 35% protein silver perch reference diet) and the experimental diets which comprised the test ingredient (usually at 30%) and the control diet (usually at 70%).

5 Fish were acclimated to experimental tanks for at least one week before the experiment commenced.

6 Fish were fed experimental diets for between 3-10 days before faeces were collected.

7 Fish were fed using continuous feeders for three hours each day.

8 Tanks were thoroughly cleaned 1 hour after the end of feeding.

9 Faeces were chilled when the period before collection exceeded 2 hours.

10 Fish were weighed at the start and end of each experiment.

11 Digestibility coefficients were calculated on described by Cho and Kaushik (1990).

Validation experiments

Determination of digestibility *in vivo* relies upon the collection of faeces. A series of experiments was designed to determine the best method of collecting faeces for reliable digestibility determination.

Experiment 1

Aim

To compare digestibility coefficients determined from faeces collected using different methods.

Design

7 different methods/periods of collecting faeces (treatments) including:

1	faeces collected by settlement after	1-2 h
2	" " " " "	6 h
3	" " " " "	12 h
4	" " " " "	18 h
5	" " " " "	18 h (with centrifugation)
6	faeces collected by stripping	

7 faeces collected by dissection (with faeces from the anterior half of the intestine separated from the posterior half)

Three replicates for each treatment except for 4) which had 6 replicates. Faeces from 6) and 7) were also collected by settlement after 18 h.

Results

1 Faeces could not be collected by stripping; fish were too small to obtain sufficient faeces and regirgutation of uneaten food contaminated the samples.

2 Results for digestibility coefficients for dry matter, energy and nitrogen are presented in Figure 1. Insufficient faeces from dissection were obtained to calculate digestibility coefficients for energy and nitrogen.

3 The results from 13 combinations of 3 tanks where faeces were collected after settlement over 18 h were used to predict the ability to detect differences in digestibility coefficients with an $\propto$ of 0.001 and a β of 0.1. With 3 replicate tanks, differences of 3.0, 3.0 and 4.0% for digestibility coefficients for dry matter, nitrogen and energy respectively were significant.

Comments

1 Stripping is not a practical method for collecting faeces from small (<10 g) silver perch.

2 Digestibility coefficients determined using faeces collected by dissection probably underestimate digestibility; differences in faeces from the anterior and posterior portions indicate digestion occurs along the length of the intestine.

3 For faeces collected by settlement, digestibility coefficients for dry matter and energy increase with time of collection. This may indicate some loss of dry matter with time, possibly by leaching. Differences for nitrogen were, however, minor.

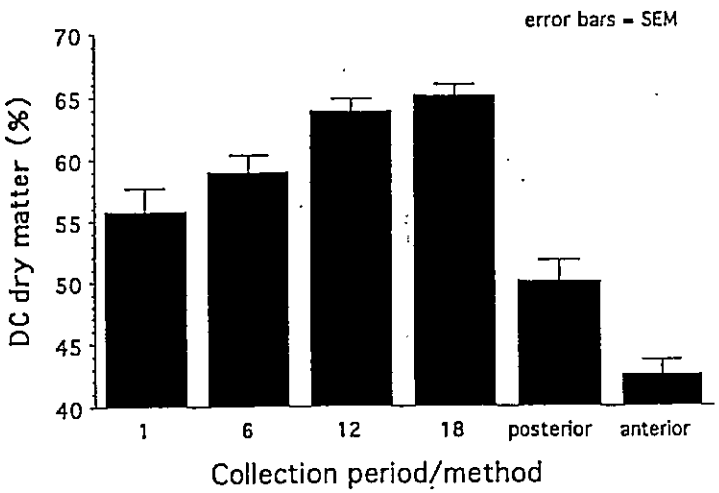
Experiment 2

Aim

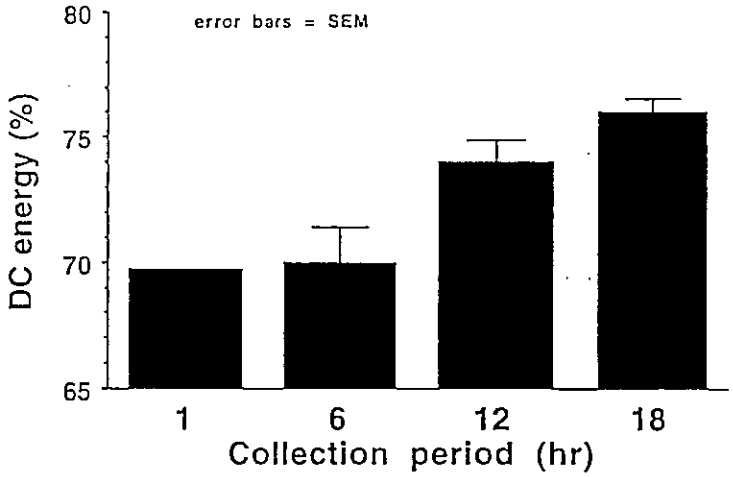
To compare the effects of two periods of collecting faeces by settlement on digestibility coefficients for different ingredients.

Figure 1

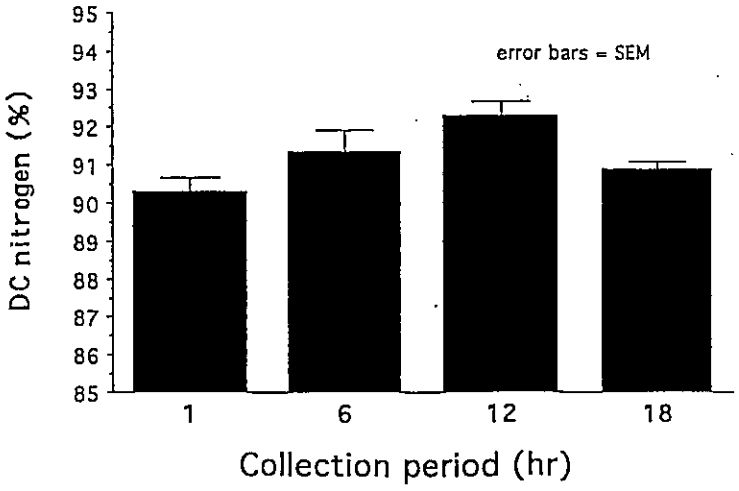
Digestibility coefficients for dry matter



Digestibility coefficients for energy



Digestibility coefficients for nitrogen



Design

This experiment was designed following the results of the previous experiment which indicated that leaching over time may lead to losses in faecal material. The design was a two factor ANOVA; factor one was ingredient (7 levels, Australian fishmeal, Danish fishmeal, wheat gluten meal, wheat variety 1, wheat variety 2, corn starch, lard) and factor two was period of collection (2 or 18 h). Three replicate tanks were used for each ingredient and faeces were collected after 2 h and then again from the same tanks after 18 h.

Results

Digestibility coefficients are presented in Table 1.

TABLE 1

Dry matter digestibility coefficients for different ingredients calculated using faeces collected after 2 or 18 h. Values are means ± standard error of the mean for n=3 replicate tanks.

Ingredient	Period of collection	Digestibility coefficient for dry matter (%)
Danish fishmeal	2	75.5±8.7
	18	91.4±1.2
Australian fishmeal	2	87.5±3.0
	18	80.7±2.2
Wheat gluten meal	2	81.4±9.8
	18	97.4±3.2
Wheat (Var 1)	2	4.2±2.2
	18	39.8±2.1
Wheat (Var 2)	2	5.7±3.5
	18	33.9±4.5
Corn starch	2	5.8±0.8
	18	21.9±5.2
Lard	2	54.7±7.3
	18	53.9±6.1

Comments

1 Ingredient, period of collection and their interaction were all significant ($P<0.05$).

2 The large variability in digestibility coefficients for ingredients after 2 h may have been due to the small amount of faeces collected and difficulties with analyses.

3 Period was not significant for the more digestible nutrients; including fishmeals, wheat gluten meal and lard, but was highly significant for corn starch and wheat.

4 Period of time food is retained in digestive tract may affect digestibility coefficient.

Experiment 3

Aim

To further investigate the effects of period of collection and time of collection on digestibility coefficients calculated from faeces collected by settlement.

Design

Treatments

1	Faeces collected after			0-2 h
2	"	"	"	2-4
3	"	"	"	4-6
4	"	"	"	6-8
5	"	"	"	8-10
6	"	"	"	10-12
7	"	"	"	12-14
8	"	"	"	14-16
9	"	"	"	16-18
10	Faeces collected after			6 h
11	"	"	"	12 h
12	"	"	"	18 h

For treatments 1-9, faeces from 6 tanks were combined for each of 3 replicate groups.

For Treatments 10-12 there were 3 replicate tanks/treatment.

Results

Digestibility coefficients for dry matter, energy and nitrogen and presented in Figure 2.

Comments

a Period of time food was in the digestive system affected the composition of faeces and the calculated digestibility coefficients.

b For representative estimation of digestibility of ingredients, faeces should be collected over an extended period.

c Digestibility coefficients for energy and dry matter calculated from faeces collected over one 18 h period were similar to digestibility coefficients averaged from faeces collected every 2 hours over an 18 h period. This indicates that leaching from 2 h - 18 h was not a major pathway for loss.

Experiment 4

Aim

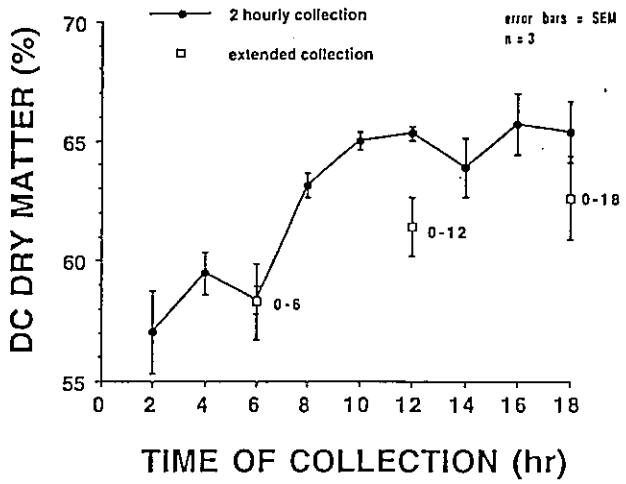
To validate the assumption that digestibility coefficients for different ingredients are additive.

Design

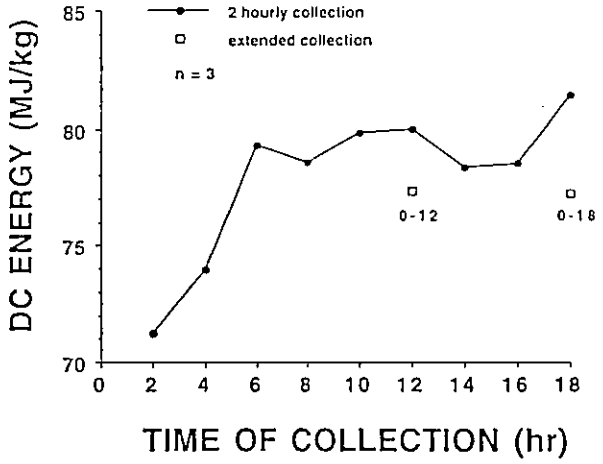
This experiment included 9 treatments (diets) with 3 replicate tanks each. The diets were the control (Table 1) and eight others which comprised 70% control diet plus 30% of one of the ingredients in the control diet (except for the fish oil diet, which comprised 90% of the control diet plus 10% fish oil).

Figure 2

Digestibility coefficients for dry matter



Digestibility coefficients for energy



Digestibility coefficients for nitrogen

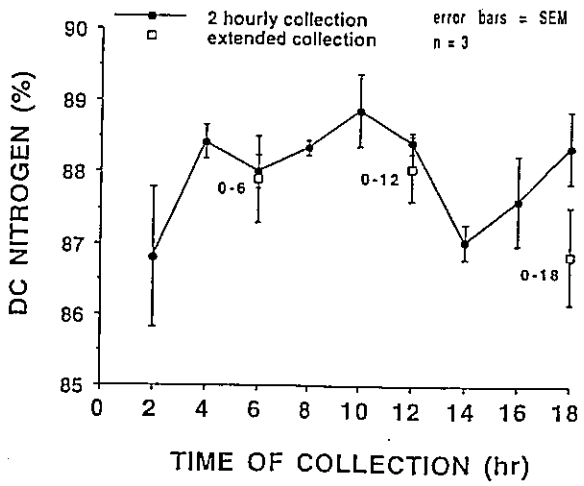


TABLE 2

Ingredients in 35% protein silver perch reference diet[1]

Ingredient	g/100 g
Danish fishmeal	27.0
Soybean meal	20.0
Blood meal	2.0
Corn gluten meal	4.0
Wheat	26.9
Sorghum	11.0
Millrun	2.0
Cod-liver oil	1.0
Di-calc phosphate	2.5
Vit/min premix	4.0
DL-methionine	0.15

[1] Allan and Rowland (1992)

Results

The digestibility coefficients for the ingredients in the control diet were calculated, multiplied by their proportion in the control diet and compared with coefficients determined for the control diet (Table 3). The digestibility coefficients for dry matter, energy, protein and phosphorus derived from separate ingredients agreed to within 5.7% of those calculated directly from the control diet.

Comments

This experiment demonstrated that the assumption that digestibility coefficients for ingredients are additive is valid for ingredients in the silver perch diet. Differences between the determined digestibility coefficients and those derived from the sum of coefficients for individual ingredients did not differ by more than about 5%. If the contribution from the vitamin/mineral premix, di-calc phosphate and DL-methionine were included, the differences would have been even less.

TABLE 3

Determined digestibility coefficients (DC's) for the control diet compared with DC's derived from the sum of DC's from component ingredients

Ingredient	Digestibility Coefficients (%) multiplied by inclusion level			
	Dry matter	Energy	N	P
Danish fishmeal	24.73	27.34	25.44	13.93
Soybean meal	16.09	16.65	19.08	8.42
Blood meal	1.97	2.09	1.85	1.31
Corn gluten meal	3.93	3.86	3.91	1.42
Wheat	11.99	14.25	23.40	13.89
Sorghum	4.85	5.74	9.59	4.19
Millrun	1.11	1.12	1.72	0.90
Cod-liver oil	1.07	1.22	-	-
Di-calc phosphate	nd	nd	nd	nd
Vit/min premix	nd	nd	nd	nd
DL-methionine	nd	nd	nd	nd
Sum	**65.74**	**72.27**	**84.99**	**44.06**
Calculated[1]	**68.61**	**76.63**	**90.30**	**46.77**

nd = not determined

[1] Calculated directly from control diet treatment during same experiment. Digestibility coefficients derived from the sum of ingredients do not include vit/min mix, Di-calc, phosphate or DL-methionine.

Experiments 5-10

Aim

To determine digestibility coefficients for ingredients with potential to partially or completely replace fishmeal.

Design

All experiments included a control and experimental diets comprising 30% of test ingredients and 70% of the control diet except for the following:

- Lard - 10% test ingredient + 90% control diet
- Artemia - 8% test ingredient + 92% control diet

Results

Results are presented below in Table 4. Standard error for all values and individual amino acids for all ingredients are available on request.

TABLE 4

Digestibility coefficients for dry matter, energy and nitrogen for different ingredients (values are means, n=3)

Ingredient	Digestibility Coefficient (%)		
	Dry matter	Energy	Nitrogen
Danish fishmeal	86.9	97.0	91.2
Danish fishmeal (LT)*	91.4	100	98.9
Aust fishmeal	80.7	97.2	97.4
Peruvian fishmeal	75.0	89.5	88.8
Bloodmeal	92.7	92.8	82.5
Meatmeal (beef)	40.1	76.4	68.7
Meatmeal (lamb)	53.3	81.4	70.1
Meatmeal (high P)	76.4	82.8	81.5
Provine	87.7	90.3	83.7
Soybean meal (defatted, hexane)	76.5	81.6	94.3
Canola meal	66.6	72.6	92.4
Peanut meal	74.8	80.1	95.8
Cottonseed meal	54.3	53.9	86.6

* LT = Low temperature

TABLE 4 (cont)

Digestibility coefficients

Ingredient	Digestibility Coefficient (%)		
	Dry matter	Energy	Nitrogen
Wheat gluten meal	97.4	100	100
Corn gluten meal	95.8	97.4	94.1
Lupins (*L. albus*; hulls on)[1]	64.7	72.7	96.1
(*L. albus*; dehulled)[1]	77.8	85.2	100
(*L. angustifolius*; hulls on)[1]	50.3	59.4	96.6
(*L. angustifolius*; dehulled)[1]	67.2	74.0	100
(*L. angustifolius*; hulls on)[2]	48.6	45.6	100
Field peas	51.0	52.0	86.5
Chick peas	30.8	48.7	82.9
Cow peas	43.4	45.8	82.6
Wheat (low P)	39.8	47.7	92.1
Wheat (high P)	33.9	36.4	100
Sorghum	44.7	44.6	89.4
Millrun	50.5	54.5	87.7
Artemia	90.2		100
Corn starch	21.9	26.5	
Lard	53.9	57.2	

[1] Determined in 1994
[2] Determined in 1993

Preliminary results for pond trial comparing control diet with a 'least-cost' vegetable protein diet

Background

Experimental data on digestibility coefficients were available for a number of oilseed and grain legume meals. The four meals with the highest digestibility coefficients for protein and energy were further evaluated in a 'dose response' experiment. In this dose response experiment, 3-4 diets with increasing amounts of each meal (used to

replace fishmeal) were formulated to give equal digestible protein and digestible energy contents. Regression analysis was used to model the effect of inclusion level on growth and to predict quantities of each meal which would give 5 and 10% reductions in growth. The amounts which were predicted to give 5% reduction in growth were used as upper restrictions and a linear least cost computer program used to formulate a diet with a similar nutritional profile to the control diet but including the four evaluated meals.

Design

The two diets were as described in Table 5.

TABLE 5

Control and 'least-cost' diet for silver perch trial in ponds

Ingredient	Diets	
	Control	Least-cost
Fishmeal	27	10
Soybean meal	20	8
Blood meal	2	5
Corn gluten meal	4	
Peanut meal		20
Canola meal		14
Lupin meal		17
Wheat	27	19
Sorghum	11	
Millrun	2	
d-calc phosphate	2	2
Fish oil	1	2
Vit/min	4	4
DL-methionine	0.2	0.3
L-lysine		0.2

Fish were stocked into six 0.1 ha ponds at the Grafton Research Centre. Initial mean size at stocking was 11.5 g/fish and stocking density of 0.75 fish/m^2 was used.

Results

Initially fish performance on the two diets was similar. Average initial weight was 11.5 g/fish (16 April 1994) and at the last weight check (5 December 1994) fish were 187.8±5.5 and 160.2±5.3 g/fish for the control and 'least-cost' diets respectively.

Comments

Over the winter months growth on both diets was slow but similar. As growth increased with higher temperatures differences in growth on diets became apparent. Observations of feeding behaviour suggests that the least cost diet has a lower acceptance, possibly due to factors such as a high fibre content, poor palatability or synergistic effects of anti-nutritional factors in plant ingredients.

Effects of diets with different protein and energy contents

Background

Preliminary research with silver perch indicated that diets with 35% protein give performance as good as diets with higher protein contents. As silver perch deposit large amounts of fat in the muscle tissue and in the visceral cavity, diets with low energy to protein ratios has been assumed to be preferable. Neither optimum protein or energy contents nor protein:energy ratios have been established for this species.

Design

Fifteen diets containing five protein (25, 30, 35, 40 and 45%) and three digestible energy (DE) (10.5, 12.6 and 14.6 MJ/kg) concentrations were prepared using Danish fishmeal as the sole protein source, cod-liver oil to balance omega-3 fatty acid contents, soybean oil to provide omega-6 fatty acids and a mixture of lard and corn starch (at a ratio of 1:3) to provide required DE contents (Table 6).

Published DE values for other species of fish of 16.7, 36.9, 37.4, 35.6 and 10.5 MJ/kg for fishmeal, fish oil, soy oil, lard and corn starch respectively were used. Cellulose was used as a filler and a vitamin and mineral premix was added. Three extra diets were also provided; a practical diet with 35% protein which has been used previously in large scale rearing trials, and two diets with 35% protein and 12.6 MJ/kg DE but with different ratios of lard and corn starch. Silver perch (mean initial weight 1.2 g, range 1.0-1.4 g) were placed in aerated 70 l aquaria (four replicates per diet) with continuously-flowing recirculated and freshwater (3:1) at a flow rate of 250 ml/minute. Temperature was 26°±1°C. Fish were acclimated to experimental conditions for seven days and then fed experimental diets twice daily to satiation for a further 36 days.

TABLE 6

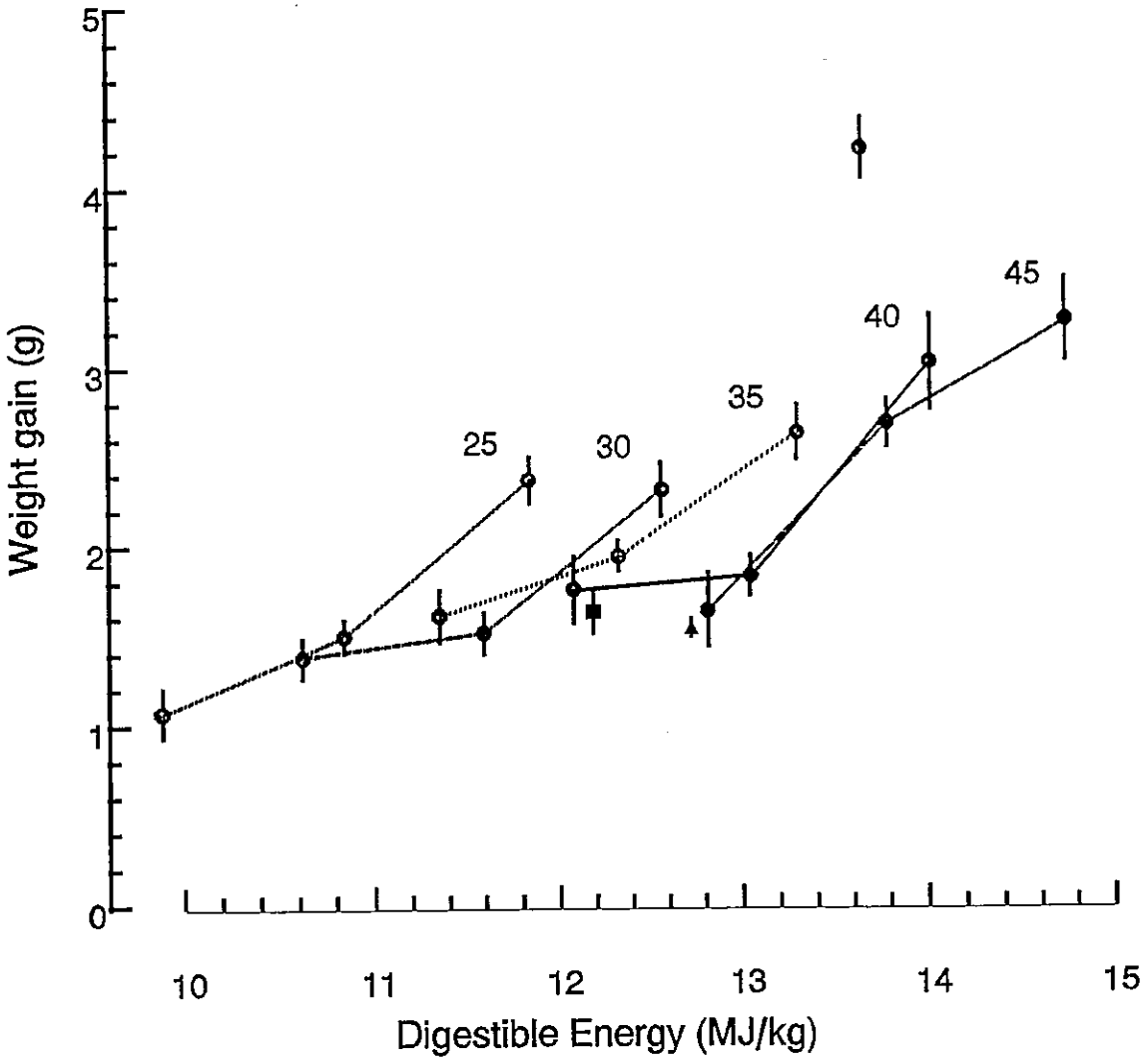
Experimental diets for protein and energy experiment

Protein (%)	DE (MJ/kg)	Analysed DE (MJ/kg)	Fish meal (%)	Fish oil (%)	Lard (%)	Corn starch (%)	Cellulose (%)
25	10	10.0	32	3	3	25	32
25	13	10.9	32	3	5	40	16
25	15	11.9	32	3	6	54	0
30	10	10.7	38	2	2	20	32
30	13	11.7	38	2	4	34	17
30	15	12.7	38	2	6	48	1
35	10	11.4	44	1	2	15	33
35	13	12.4	44	1	3	29	17
35	15	13.4	44	1	8	43	1
40	13	12.2	51	1	1	9	33
40	13	13.1	51	1	3	23	17
40	15	14.1	51	1	5	38	2
45	10	12.9	57	0	0	4	34
45	13	13.9	57	0	2	18	18
45	15	14.8	57	0	4	32	2
35HF	13	12.8	44	1	8	12	29
35LF	13	12.3	44	1	0	40	9

Results

Results are presented in Figure 3. Growth increased with protein and energy ($P<0.001$), although there was a significant interaction between these factors ($P<0.05$). For fish fed the lowest and highest DE diets, growth was not significantly increased by increasing protein from 40 to 45%. Conversely, for diets with 12.6 MJ/kg DE, growth was increased with an increase in protein from 40 to 45%. For all protein contents, growth of fish fed diets with 14.6 MJ/kg DE was significantly ($P<0.05$) greater than for fish fed diets with lower DE contents. Protein efficiency

Figure 3 Weight gain of silver perch fed diets with one of five digestible protein contents (25, 30, 35, 40 or 45) and different digestible energy contents



ratios (PER) were highest for fish fed diets with DE contents of 14.6 MJ/kg, and tended to decrease with increasing protien content. Food conversion ratios (FCR) (dry weight feed/wet weight gain fish) were lowest (best) for fish fed diets with higher protein and energy contents. Growth, PER and FCR were similar ($P>0.05$) for fish fed a diet with 40% protein and 14.6 MJ/kg, and a diet with 45% protein and 12.6 MJ/kg DE.

Comments

Optimum requirements for protein and energy were not established, although the faster growth of fish fed lower protein and higher DE diets, compared with fish fed higher protein and lower DE diets, indicates the potential to replace protein in silver perch diets with other sources of energy. In collaborative research with Professor Roberts (Newcastle University), reducing the protein to energy ratio was found to result in significantly higher amounts of fat in fish tissue (Hunter et al., 1994).

Growth of silver perch on experimental diets (range of mean weights 1.1-3.2 g/fish) was less than on the control diet (4.2 g/fish). Possible reasons for this include differences in digestible energy of test ingredients between published values and actual values for silver perch; differences in the palatability and consumption between test diets and the control diet; and growth inhibiting factors in one or more of the ingredients in the test diets.

Published digestible energy values for channal catfish for fishmeal were lower and those for corn starch and lard higher than for silver perch. Consequently energy levels increased with protein.

Outline of proposed research for Year II

Digestibility

Experiments to determine digestibility of new ingredients will include different varieties and types of grain legumes, and oilseed meals extracted using different processes. Other ingredients with potential will be evaluated if time permits.

The focus for digestibility studies will be to determine the effects of processing on digestibility. Experiments will be designed to investigate the effects of: grinding, dehulling, extrusion and other types of cooking, and the addition of enzymes.

Growth studies

Growth studies will be designed as dose-response experiments to determine maximum inclusion levels for different ingredients and combinations of ingredients.

Nutritional requirements

Experiments will be designed to determine optimum content of the first limiting amino acid - lysine. Experimental designs used very successfully for pigs and poultry will be adopted and tried with fish. If these are successful, further experiments will be conducted to determine effects of different energy contents on performance to determine optimum energy to lysine ratios.

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Acknowledgements

I would like to thank Mr Scott Parkinson, Ms Jane Frances, Mr David Stone and Ms Rebecca Warner-Smith for their excellent technical assistance with this research. Complimentary funding for aspects of this research was received from the Australian Centre for International Agricultural Research.

Development of Artificial Diets for Silver Perch

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ABSTRACT

To successfully develop artificial diets for silver perch information is needed on nutritional requirements, the chemical composition, availability, price and value to the fish of potential feed ingredients, and the best way to present and feed diets in culture facilities. In the wild, silver perch is an omnivore. Small fish feed preferentially on crustaceans and zooplankton and the proportion of algae and other plant material in their diet increases as fish grow. Very little research on nutritional requirements has been done with silver perch. However, like all fish, silver perch require amino acids (protein), lipid, vitamins and minerals. Sufficient energy from these nutrients and from carbohydrates must be available. In this paper, the role of amino acids, lipid, carbohydrate, vitamins and minerals, energy and other dietary additives in fish nutrition is reviewed and, where available, requirements for silver perch are presented. Like other commercially farmed omnivores, silver perch require diets with approximately 35% protein. They are able to chain elongate and desaturate fatty acids, indicating they do not require high contents of long-chain, highly unsaturated fatty acids (HUFA's) in their diets. The preferred protein source for fish diets is fishmeal, yet this ingredient is expensive, can be difficult to obtain, is of variable composition and global production of it is declining. Fortunately, silver perch can effectively utilise some plant proteins and tolerate quite high contents of some oilseed meals, eg soybean meal and peanut meal, and grain legumes, eg lupins. Methods of evaluating different ingredients are reviewed and results for silver perch presented. Different methods of processing ingredients are discussed, as these can greatly affect the value of artificial diets. The differences between extruded and steam-pelleted diets are defined.

Farmers can often achieve considerable savings by improving feeding strategies. Successful feeding rates and frequencies used by NSW Fisheries to grow silver perch are presented. Feeding rates range from 1-10% of fish weight per day and feeding frequencies from 1-5 times per day depending upon fish size and water temperature. Finally, the priorities for research to accelerate the commercial development of low-cost, efficient, artificial diets for silver perch are discussed.

INTRODUCTION

Information on aquaculture nutrition has increased rapidly over the past decade, reflecting the enormous increases in aquaculture production. Since 1986, aquaculture production has increased by more than 40% (Anon., 1990, 1994) to 20.8 million tonnes per year (including aquatic plants). One reason for this increase has been the trend towards more intensive culture practices necessitating a greater reliance on formulated feeds. In the same period, production of aquaculture feeds has risen even faster, and recent estimates predict the Asian aquaculture feed market alone will be about 2.6 million tonnes per annum by the year 2000 (New and Csavas, 1993). As feed and feeding costs can contribute up to 70% of the total operating costs for fish farming (Wee, 1992) the development of nutritionally adequate, economical diets is of crucial importance.

The development of aquaculture diets began with farmers feeding fish available foods such as fresh animal meat, kitchen wastes and fishery by-products (Lall, 1991; Jantrarotai, 1991). Subsequently, information on the composition of natural food items for wild fish was used as a nutritional basis for formulated diets. Silver perch is usually described as an omnivore following feeding studies of wild fish (Burchmore and Battaglene, unpublished data, 1988) and fish in farm dams (Barlow et al., 1987). Burchmore and Battaglene (unpublished data, 1988) examined stomach contents of 917 silver perch and found the diet consisted of 32% algae and that the proportion of algae and other plant material in the diet increased as fish grew.

To further the development of cost-effective aquaculture diets and feeding strategies, more research is needed on:

- requirements for essential nutrients and energy,
- composition, digestibility and availability of potential feed ingredients,
- effects and benefits of processing ingredients and diets, and
- strategies for effectively and economically feeding fish.

Although a large number of fish species are cultured throughout the world, only the nutritional requirements of rainbow trout (*Oncorhynchus mykiss*) and channel catfish (*Ictalurus punctatus*) have been extensively studied (Lall, 1991). Very little nutritional research has been done with silver perch (*Bidyanus bidyanus*). This paper will describe some of the principles of fish nutrition and specific information for silver perch will be presented wherever possible. Although nutritional requirements for different life stages (ie larvae, juveniles, adults and broodstock) can be very different, this paper will focus on the development of grower diets (for juveniles and rapidly growing adult fish) as these are by far the most important economically.

NUTRITIONAL REQUIREMENTS

Fish require amino acids, fatty acids, vitamins, minerals and energy from protein, lipid and carbohydrate. To investigate nutritional requirements, most researchers use measures of fish performance to assess response to various manipulated diets. Such measures include survival, growth, food consumption and conversion efficiency, nutrient deposition and gross or histological appearance. Maximum performance is usually considered optimal although this is not always the case. Most rapid growth, for example, does not always correlate with absence of disease or with longevity, and diets which promote the most rapid growth are often not the most economical (Lall, 1991).

Protein

Protein is comprised of various amino acids; ten of which are essential (NRC, 1993; Lovell, 1989). Essential amino acids are those which cannot be synthesised by the animal or cannot be synthesised in sufficient quantity to support maximum growth (Lovell, 1989). These are: argine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan and valine. Insufficient protein in the diet results in a reduction in growth or a loss of weight as fish withdraw protein from some tissues to support functions of more vital ones (NRC, 1993). Excess protein will be metabolised for energy. Protein requirements are influenced by a range of variables, including fish size, culture conditions (including stocking density and availability of natural food items), water temperature, feeding strategy (whether fish are fed to satiation or on a restricted regime), composition of the diet (particularly the energy concentration) and the quality of the protein. Gross requirements decrease as fish grow and increase as water temperature increases.

Estimated protein and amino acid requirements for juvenile fish are presented in Tables 1 and 2.

TABLE 1

Estimated protein requirements for juvenile fish[1]

Species	Protein source(s)	Estimated requirement (%)
Channel catfish (*Ictalurus punctatus*)	Whole egg protein	32-36
Common carp (*Cyprinus carpio*)	Casein	31-38
Grass carp (*Ctenopharyngodon idella*)	Casein	41-43
Japanese eel (*Anguilla japonica*)	Casein and arginine plus cystine	44.5
Estuary grouper (*Epinephelus striatus*)	Tuna muscle meal	40-50
Milkfish (fry) (*Chanos chanos*)	Casein	40
Snapper (*Pagrus auratus*)	Casein	55
Smallmouth bass (*Micropterus dolomieui*)	Casein and fish protein concentrate	45
Largemouth bass (*Micropterus salmoides*)	Casein and fish protein concentrate	40
Tilapia		
Tilapia aurea (fry)	Casein and egg albumin	56
Tilapia aurea	Casein and egg albumin	34
Oreochromis mossambica	White fishmeal	40
Tilapia zillii	Casein	35
Snakehead (*Channa micropeltes*)	Fishmeal	52
Chinook salmon (*Oncorhynchus tshawytscha*)	Casein, gelatin and amino acids	40
Coho salmon (*Oncorhynchus kisutch*)	Casein	40
Rainbow trout (*Oncorhynchus mykiss*)	Fishmeal	40
	Casein and gelatin	40
	Casein, gelatin and amino acids	45
Sockeye salmon (*Oncorhynchus nerka*)	Casein, gelatin and amino acids	45
Yellowtail (*Seriola quinqueradiata*)	Sand eel and fishmeal	55

[1] Based on NRC (1993) and Wilson (1989)

TABLE 2

Amino acid requirements for juvenile fish[1] (% of protein)

Amino acid	Common carp	Channel catfish	Chinook salmon	*Tilapia nilotica*
Arginine	4.2	4.3	6.0	4.2
Histidine	2.1	1.5	1.8	1.7
Isoleucine	2.3	2.6	2.2	3.1
Leucine	3.4	3.5	3.9	3.4
Lysine	5.7	5.1	5.0	5.1
Methionine[2]	3.1	2.3	4.0	3.2
Phenylalanine[3]	6.5	5.0	5.1	5.7
Threonine	3.9	2.0	2.2	3.6
Tryptophan	0.8	0.5	0.5	1.0
Valine	3.6	3.0	3.2	2.8

1 Based on Lovell (1989)
2 Plus cystine
3 Plus tyrosine

Preliminary studies with silver perch using diets based on fishmeal and soymeal indicate protein contents of around 35% should be sufficient (Allan and Rowland, 1991).

Lipid

Lipid is a term often used synonymously with fat or oil and covers fats, sterols, waxes, phospholipids and sphingomyelins (New, 1987). Fats are the storage esters of glycerol and are the major vehicle many animals use to store energy. Sterols are components or precursors of hormones, waxes are energy storage compounds, phospholipids are components of cellular membranes and sphingomyelins are found in brain and nerve tissue compounds (New, 1987).

Lipids are a concentrated energy source for fish and are important in the palatability of feeds (New, 1987). They are comprised of fatty acids, some of which are essential (New, 1987; Lovell, 1989). The nomenclature describing fatty acids can be confusing. Besides having a common name, fatty acids are also given a numerical designation such as 14:0, 18:3 n-3, 20:5 n3 or 22:6 n-3. This designation describes the number of carbon atoms present, the number of double bonds and the position of the first double bond (New, 1987). For eicosapentaenoic acid (EPA) or 20:5 n-3, there are 20 carbon atoms and five double bonds, the first of which occurs on the third carbon atom, numbering from the terminal methyl end. Saturated, monounsaturated and polyunsaturated fatty acids (or PUFA's) are those which have 0, 1 or more than 1 double bond respectively. The term HUFA's is used for those PUFA's with four or more double bonds (New, 1987).

Aquatic animals have a greater requirement for n3 (or omega [ω]3) series fatty acids than terrestrial animals, which have a greater requirement for n6 fatty acids (New, 1987; Hepher, 1988). Among fish, cold water species have a greater requirement for n3 series fatty acids than warmwater species. One hypothesis to explain this is that the n3 structure permits a greater degree of unsaturation which is necessary in membrane phospholipids to maintain flexibility and permeability at low temperatures (NRC, 1993). Animals can further

desaturate and elongate chains of unsaturated fatty acids to form PUFA's, although different speices have different capacities to do so (NRC, 1993; Hepher, 1988). The ability to desaturate and chain elongate fatty acids allows reduction in the dietary content of aquatic animal oils, which can be expensive, and permits substitution with less expensive oils. Anderson and Arthington (1992) found silver perch were capable of rapid desaturation and chain elongation, comparable with rainbow trout.

NRC (1993) summarised published information on essential fatty acid requirements for warmwater fish and crustaceans. Most fish needing n3 or n6 fatty acids require 0.5 - 2.0 % of these fatty acids (NRC, 1993; Lall, 1991). In commercial rations, lipid contents range from around 5-6% for diets for channel catfish (Robinson, 1989) to 20% for some salmon and trout diets (Lovell, 1989). Elevated lipid contents are used to spare protein. Commercial rations for snapper commonly have lipid contents of 15-16% (Foscarini, 1988) and for tilapias, 5-12% (El-Sayed and Teshima, 1991; Luquet, 1991). Experimental diets for silver perch (designed for commercial culture conditions) contain between 5-10% lipid (Allan and Rowland, 1992; unpublished data, 1993).

Carbohydrate

Carbohydrate includes starches, sugars, cellulose (and other cell wall material) and gums and is usually the cheapest source of energy in fish diets (New, 1987). Fish do not have a specific requirement for carbohydrate (NRC 1993) and some studies indicate that, like diabetics, fish are incapable of maximum carbohydrate utilisation (Robinson, 1989). Although enzymes necessary for carbohydrate digestion have been detected in fish, some species are clearly better able to digest carbohydrates than others (NRC, 1993). High carbohydrate diets appeared to stimulate lipogenic enzyme activity in channel catfish, indicating this species may be able to convert energy from carbohydrate into lipid, and therefore utilise carbohydrate more efficiently than species lacking this ability (Robinson, 1989). The digestibility of carbohydrates is influenced by the digestive system of fish and herbivorous and omnivorous fish are better equipped to digest carbohydrates than carnivores. Carbohydrate digestibility is also influenced by processing, eg cooking or steam treatment, and by the structural complexity of the carbohydrate (NRC, 1993; Robinson, 1989).

In addition to an energy source and to spare protein for growth, carbohydrates may act as precursors for metabolic intermediates necessary for growth, and play a vital role in pellet formulation and binding of commercial fish diets.

Complex carbohydrates, including plant cell wall material, such as cellulose, hemicellulose, lignin and pentosans offer little nutritional benefit and ultimately become a pollutant in the culture environment. Fish feed formulations aim to keep the fibre content in diets as low as possible.

Vitamins

Vitamins are organic compounds which are only required in small quantities for growth, health and function (Lovell, 1989). Table 3 lists minimum requirements for channel catfish, carp, and red sea bream. Different species have different essential vitamins and deficiency signs of these essential vitamins range from poor appetite to severe tissue deformity and death. Deficiency signs for vitamins for a range of species are presented in Table 4. The vitamin contents of the experimental diet used by NSW Fisheries for growth trials are listed in Table 5.

Minerals

Minerals are inorganic compounds some of which are constituents of bone, fins, scales, tissue and blood. Some minerals function as components or activators of hormones and enzymes (eg zinc) (Lovell, 1989). One of the major differences in mineral requirements between fish and other animals is the role minerals play in osmoregulation in fish. The mineral contents of NSW Fisheries' silver perch reference diet (SP35) are given in Table 5 and the mineral requirements of several species are listed in Table 6.

Fish can absorb minerals from the water through the gills or digestive tract (Lovell, 1989). For this reason requirements for saltwater and freshwater fish differ. The availability of phosphorus depends largely upon the source. Phytate phosphorus from grains is poorly available to fish, phosphorus of fishmeal is about 40-70% available, and inorganic phosphorus, from sodium or monocalcium phosphate, is highly available to all fish (Lovell, 1989).

TABLE 3

Minimum requirements (mg/kg) of vitamins to prevent signs of deficiency[1,2]

Vitamin	Channel catfish *Ictalurus punctatus*	Common carp *Cyprinus carpio*	Red sea bream *Pagurs major*
Thiamin	1.0		R
Riboflavin	9.0	7.0	R
Pyridoxine	3.0	5-6	5-6
Pantothenic acid	10-20	30-50	R
Nicotinic acid	14	28	R
Biotin	R	1	N
Folic acid	N	N	N
Vitamin B_{12}	R	N	R
Choline	R	4 000	R
Inositol	N	440	550-900
Ascorbic acid	60	NT	R
Vitamin A	1 000 - 2 000	10 000 IU	NT
Vitamin D	500 - 1 000	N	NT
Vitamin E	30	200-300	NT
Vitamin K	R	N	NT

1 Based on NRC (1993)
2 Minimum requirements not allowing for storage or processing losses
R Required
N No dietary requirement demonstrated
NT Not tested
IU International units

TABLE 4

Deficiency signs for vitamins[1]

Vitamin	Deficiency signs for salmon, catfish and other species
Thiamin[2]	Poor appetite, muscle atrophy, convulsions, instability and loss of equilibrium, oedema, poor growth
Riboflavin[2]	Corneal vascularisation, cloudy lens, haemorrhagic eyes, photophobia, dim vision, incoordination, abnormal pigmentation of iris, striated constrictions of abdominal wall, dark colouration, poor appetite, anaemia, poor growth
Pyridoxine[2]	Nervous disorders, epileptiform fits, hyperirritability, ataxia, anaemia, loss of appetite, oedema of peritoneal cavity, colourless serous fluid, rapid postmortem rigor mortis, rapid and gasping breathing, flexing of opercles
Pantothenic acid[2]	Clubbed gills, prostration, loss of appetite, necrosis and scarring, cellular atrophy, gill exudate, sluggishness, poor growth
Inositol[2,3]	Poor growth, distended stomach, increased gastric emptying time, skin lesions
Biotin[2]	Loss of appetite, lesions in colon, discolouration, muscle atrophy, spastic convulsions, fragmentation of erythrocytes, skin lesions, poor growth
Folic acid[2]	Poor growth, lethargy, fragility of caudal fin, dark colouration, macrocytic anaemia
Choline[2]	Poor growth, poor food conversion, haemorrhagic kidney and intestine
Niacin[2]	Loss of appetite, lesions in colon, jerky or difficult motion, weakness, oedema of stomach and colon, muscle spasms while resting, poor growth
Vitamin B_{12}[2]	Poor appetite, low haemoglobin, fragmentation of erythrocytes, macrocytic anaemia
C[2]	Scoliosis, lordosis, impaired collagen formation, altered cartilage, eye lesions, haemorrhagic skin, liver, kidney, intestine, and muscle
A[4]	Impaired growth, exophthalmos, eye lends displacement, oedema, ascites, depigmentation, corneal thinning and expansion, degeneration of retina
D[4]	Poor growth, tetany of white skeletal muscle, impaired calcium homoeostasis
E[4]	Reduced survival, poor growth, anaemia, ascites, immature erythrocytes, variable-sized erythrocytes, erythrocyte fragility and fragmentation, nutritional muscular dystrophy, elevated body water
K[4]	Prolonged blood clotting, anaemia, lipid peroxidation, reduced hematocrit

1 Based on Halver (1989)
2 Water soluble vitamins
3 No deficiency signs found when channel catfish fed diets without inositol
4 Fat soluble vitamins

TABLE 5

Vitamin and mineral contents of SP35[1] (NSW Fisheries experimental grower diet for silver perch)

Thiamin HCl	10 mg
Riboflavin	25.5 mg
Pyridoxine HCl	15 mg
Ca-Pantothenate	54.5 mg
Nicotinamide	200 mg
Biotin	1 mg
Folic acid	4 mg
Cyanobalamin (Vitamin B_{12})	20 μg
Choline chloride	1.5 g
Myo-inositol	600 mg
Ascorbic acid	450 mg
Retinol (Vitamin A)	2.4 mg
Cholecalciferol (Vitamin D_3)	25 μg
α-Tocopherol acetate (Vitamin E)	125 mg
Menadione sodium bisulphate (Vitamin K_3)	16.5 mg
Calcium carbonate ($CaCO_3$)	7.5 g
Manganese sulphate ($MnSO_4$)	0.3 g
Zinc sulphate ($ZnSO_4.7\ H_2O$)	0.7 g
Iron sulphate ($FeSO_4.7\ H_2O$)	0.5 g
Copper sulphate ($CuSO_4$)	60 mg
Sodium chloride (NaCl)	7.5 g
Potassium iodate ($KI0_3$)	2 mg

[1] Allan and Rowland (1992)

TABLE 6

Mineral requirements of some freshwater fish[1]

Mineral	Rainbow trout	Channel catfish	Common carp
Calcium (%)	<0.1	<0.1	<0.1
Phosphorus (%)	0.7	0.4	0.7
Magnesium (%)	0.05	0.04	0.05
Iron (mg/kg)	R	30	-
Copper (mg/kg)	3	5	3
Manganese (mg/kg)	13	2.4	13
Zinc (mg/kg)	15-30	20	15-30
Iodine (μg/kg)	R	-	-
Selenium (mg/kg)	0.15-0.38	0.25	R

[1] Based on Lall (1991)
R Required

Energy

Energy is not a nutrient but is required by all animals to sustain life (Smith, 1989). One of the most notable differences between fish nutrition and nutrition of homoeothermic land animals is that fish require less energy. This is because:

1 they do not have to maintain a constant body temperature,
2 they use less energy to maintain position and to move about in water than animals do on land, and
3 they lose less energy in protein catabolism and excretion than land animals (Lovell, 1989; Smith, 1989).

One of the manifestations of this lower energy requirement is the much higher crude protein content (and protein to energy ratio) in fish diets than in diets for homoeothermic land animals.

Both an excess and deficiency of energy can reduce growth. Energy needs for maintenance and movement must be satisfied first and if insufficient energy is available in the food, essential nutrients, eg protein, will be used for energy rather than growth. Conversely, if excess energy is supplied, food consumption will be reduced before enough essential nutrients for maximum growth have been consumed. Excess energy:protein ratios can also lead to the deposition of large amounts of body fat which can be undesirable (Lovell, 1989). Protein and digestible energy requirements for different size channel catfish for maximum protein synthesis are given in Table 7.

TABLE 7

Protein and digestible energy (DE) requirements for different size channel catfish[1]

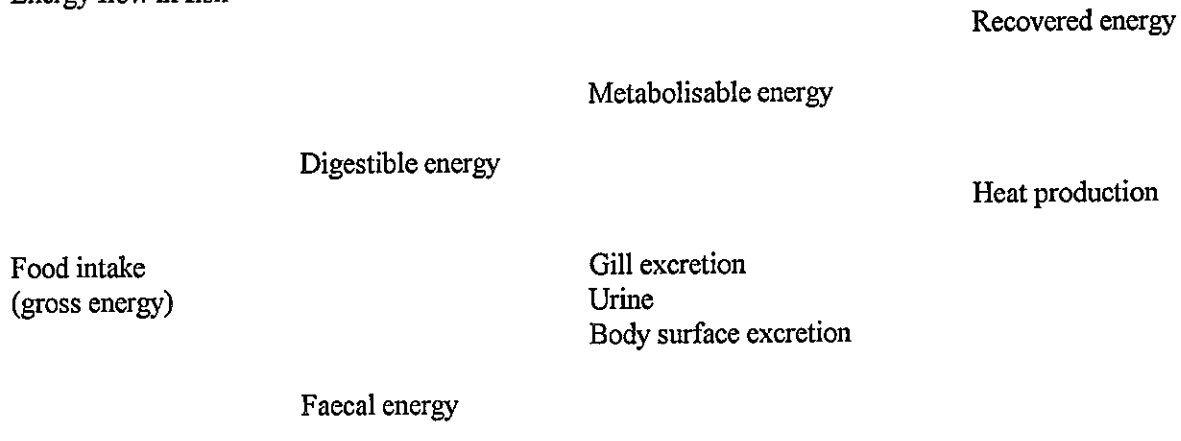
Fish size (g)	Protein (g/100 g fish/day)	Energy (MJ/100 g fish/day)	DE/Protein ratio (MJ/kg)
10	1.1	47.7	42.7
56	0.8	37.7	47.7
266	0.4	20.9	48.5

[1] Based on Mangalik (1986); cited in Lovell (1989)

Gross energy is defined as the heat released when the compound is completely oxidised to water, carbon dioxide and other gases. Digestible energy is the difference between gross energy and the energy lost in faeces, and metabolisable energy is the difference between digestible energy and the energy lost through urine and skin and gill excretions (Lovell, 1989); Cho and Kaushik, 1990). Recovered (or available) energy differs widely between ingredients and is very difficult to measure. In practical terms, digestion accounts for most of the difference between ingredients and diets for fish (Lovell, 1989a). When evaluating feed ingredients or diets it is important to measure or estimate the amounts of energy (and essential nutrients) which will be available to the fish. Bioenergetics is the study of energy intake and utilisation. The energy flow is illustrated in Figure 1.

FIGURE 1

Energy flow in fish[1]



[1] Based on Lovell (1989)

FEED INGREDIENTS

Once a fish nutritionist has some understanding of the nutritional requirements of the species to be cultured, they can then combine different feed ingredients to make a formulated diet. Selecting the best (and most cost-effective) ingredients is a major challenge for feed formulators. Feed ingredients are selected on the basis of nutrient composition, nutrient availability, presence of anti-nutritional factors or toxins, price and availability. Extensive information is available on analysed nutrient composition for a large number of feed ingredients, although for fish nutritionists the most commonly used information is the United States - Canadian tables of feed composition (NRC, 1993). In general, nutrient compositions in these tables reflect averages, and different batches of ingredients from different regions can vary substantially.

Satisfying protein requirements is usually the most expensive task. The protein source of choice for aquaculture diets is fishmeal, and diets can contain as much as 70% (Wee, 1992). Fishmeal is excellent because it has a high total protein content, has a very well balanced amino acid profile and has a high proportion of desirable unsaturated fatty acids. It is also low in carbohydrate and fibre, is very palatable and, when processed well, is highly digestible with few anti-nutritional factors. Unfortunately, the price and availability of fishmeal will restrict future aquaculture development unless suitable alternatives can be found. In 1991, 31% of the total world fish and shellfish catch (or 26 269 000 t) was reduced into 6 367 000 t of fishmeal (Tacon, 1993). Approximately 14% was used in aquafeeds, and (if current trends continue) this proportion is likely to increase to around 25% by the year 2000. Unfortunately, while world aquaculture production is increasing rapidly, especially in Asia (New and Csavas, 1993), production of fishmeal is expected to remain stable or decline by about 5% by the year 2000 (Barlow, 1989). In Australia, we produce very little high quality fishmeal (<7 000 t), leading to the importation of $17 million worth (x tons) in 1993. Evaluating suitable alternative protein sources to fishmeal and ways of improving the value of alternative protein sources is an international research priority (Manzi, 1989; New, 1991).

We are fortunate in Australia in having a large number of protein sources which have potential for use in aquafeeds. These include vegetable protein, such as oilseed meals like soybean meal, canola meal, peanut meal and cotton seed; grain legumes like lupins, chick peas and field peas, and terrestrial animal meals like meat and blood meal and poultry offal meals. Although these ingredients are generally inferior to fishmeal, they are cheaper and more readily available. Having a large number of ingredients to choose from when formulating diets gives nutritionists a greater chance of balancing nutrient requirements, more flexibility when some ingredients are scarce or expensive, and allows the use of linear computer programs to formulate least-cost, effective diets.

The first step in evaluating a new ingredient is to determine its' digestibility. This involves measuring the energy and nutrient content of the ingredient and subtracting what is voided in the faeces.

Cho et al (1982) and Cho and Kaushik (1990) have reviewed methods involved in determing digestibility coefficients in feed ingredients for fish. Using this approach, Allan and Rowland (in press) determined apparent digestibility coefficients from energy and protein for a number of oilseeds and from legumes for silver perch. Results are presented in Table 8. These data indicate that vegetable proteins are readily digested but that energy was less digestibile in the vegetable meals tested compared with fishmeal, and further that energy in the grain legumes was less well digested than in the oilseed meals. When ingredients contain anti-nutritional substances such as trypsin inhibitors, gossypol, tannins, glucosinolates, aflatoxins etc, data on maximum inclusion contents are needed in addition to information on digestibility. For the ingredients with the highest protein digestibility coefficients in Table 8, Allan et al. (1993) formulated diets with different inclusion levels, but similar digestible energy and digestible protein contents. Growth responses of silver perch on these diets were modelled and the maximum inclusion levels predicted to give 5 and 10% reductions in growth determined (Table 9).

TABLE 8

Apparent digestibility coefficients (ADC), digestible protein (DP) and digestible energy (DE) of Australian oilseeds and grain legumes and two fishmeals fed to silver perch (*Bidyanus bidyanus*)

	ADC(%)[1,2]			
	CP[3]	GE[4]	DP[5](%)	DE[6](MJ/kg)
Soybean meal	94.5±0.4	77.9±3.6	45.8±0.02	15.4±0.7
Canola meal	92.4±0.03	72.6±0.2	40.3±0.02	14.5±0.03
Cottonseed meal	86.6±1.7	53.9±5.2	41.6±0.9	10.7±1.0
Peanut meal	95.8±1.4	80.1±6.1	39.5±0.5	15.8±1.2
Lupins	100.0±1.2	45.6±5.2	30.8±0.2	9.0±1.0
Field peas	86.5±1.0	52.0±9.0	23.9±0.2	9.7±1.7
Cow peas	83.2±1.7	45.8±10.2	21.0±0.5	8.6±1.9
Chick peas	82.9±1.2	48.7±4.3	18.9±0.3	9.2±0.9
Danish fishmeal	91.2±1.4	97.3±3.3	68.0±1.0	21.5±0.7
Peruvian fishmeal	88.8±3.5	89.5±1.0	61.9±2.4	18.5±0.2

1 Apparent digestibility coefficient
2 Values are means ± SD for 3 replicate tanks
3 Crude protein
4 Gross energy
5 Digestible protein = CP x ADC CP/100
6 Digestible energy = GE x ADC GE/100

TABLE 9

Relationship between fishmeal content and weight gain and the amount of fishmeal which can be replaced to result in a 5 and 10% reduction in weight gain[1]

	Correlation coefficient	Amount of fishmeal (%)	
	r	A[2]	B[3]
Soybean meal	0.69	27	46
Canola meal	0.91	16	28
Peanut meal	0.66	30	48
Lupin	0.52	20	41

1 Data from Allan et al. (unpublished data, 1993)
2 A = 5% reduction in weight gain
3 B = 10% reduction in weight gain

Canola meal, which contains glucosinolates and erucic acid as well as other anti-nutritional factors, reduced growth when added at a lower amount than ingredients with fewer anti-nutritional substances such as lupins. As a protein source, vegetable meals are generally inferior to terrestrial animal meals which in turn are inferior to fishmeal.

Some of the reasons for these differences and possible solutions are listed in Table 10.

TABLE 10

Reasons why vegetable and terrestrial animal protein sources are inferior to fishmeal and possible solutions

Problems	Possible solutions	Comments
Amino acid deficiency	Addition of synthetic amino acids or polypeptides	Not all amino acids are synthesised. Crystalline amino acids are subject to leaching and rapid absorption but poor assimilation. Polypeptides are not available commercially
Fatty acid deficiencies	Supplement with fish oil	Fish oil is expensive and supplies are not increasing
Poor digestibility	Processing, eg by cooking, extrusion or micronisation, can improve digestibility of some nutrients in some ingredients	Can also reduce availability of some nutrients in some ingredients, can be expensive
	Addition of enzymes	Expensive, needs to be shown to be beneficial for fish
Non-palatability or reduces attractability of diet	Addition of attractants such as fish silage extracts, amino or fatty acids	Can be expensive

OTHER DIETARY COMPONENTS

In addition to feed ingredients, vitamins and minerals, diets may contain other materials that can influence fish growth. These include binders, antioxidants, mould inhibitors, pigments, hormones, antibiotics, feeding stimulants and attractants. Common binders include sodium and calcium bentonites, lignosulfonates, hemicellulose, carboxymethylcellulose, alginate, guar gum, gelatinised starch from cereals, wheat gluten, whey and molasses (Lall, 1991). Most binders are usually added at about 0.5 - 4.0% of the diet, except for gelatinised starches, which may be added at up to 20% (Lovell, 1989). Commonly used antioxidants and mould inhibitors are listed in Table 11. For a thorough review of pigments, hormones, antibiotics, feeding stimulants and attractants please refer to Lovell (1989), Tacon (1990) and NRC (1993).

TABLE 11

Commonly used antioxidants and preservatives[1]

Antioxidants

Octyl gallate
Dodecyl gallate
N-propyl gallate
BHA (Mixture of 3- and 2-*tert* butyl 4-hydroxyanisole)[2,3]
BHT (2, 6-di (*tert* butyl) -4-methylphenol)[2,3]
Ethoxyquin (6-ethoxy-1, 2-dihydro-2, 2, 4-trimethyl-quinoline)[2,4]

Preservatives

Propionic acid or Ca, Na or K salt[5]
Sorbic acid or Ca, Na or K salt[5]
Benzoic acid or Na salt
Acetic acid
Formic acid
Citric acid
Ascorbic acid or Ca or Na salt
Gentian violet
Potassium and sodium bisulphite
Potassium and sodium metabisulphite
Propylene glycol
Salt

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1 Adapted from Tacon (1990)
2 Major synthetic antioxidants
3 Maximum level permitted in the USA is 0.2% of the total fat content
4 Maximum level permitted in the USA is 150 mg/kg feed
5 Most common. Inclusion level about 0.2 - 1.0% of diet

PROCESSING

Processing includes grinding, classification, sieving, mixing, heating, drying, crumbing, pelleting and extruding. It can affect the digestibility and availability of energy and nutrients in ingredients and the physical and water stability, buoyance, texture, hardness and price of the diet. Some diets are fed as moist or semi-moist feeds but most aquaculture feeds are dry feeds and contain about 8-10% moisture.

Silver perch feeds are available as pellets produced either through a pelleting press (with or without starch conditioning) or through an extruder. For the same ingredients, feeds processed through a pelleting press should be cheaper as this is a simpler operation requiring less expensive equipment. It relies on the use of moisture, heat and pressure to combine the ingredients into a mash which is then forced through dies of varying size openings and cut to varying lengths (Lall, 1991). Where a steam conditioner is used, steam is generally added to increase the moisture content to approximately 5-6% and elevate the temperature to 70-90°C (NRC, 1993). This partially gelatinises starch which helps bind the diet and affects digestibility.

Extrusion uses more sophisticated equipment. Here the finely ground feed with a moisture content of around 25% is heated in a conditioning chamber to 104-148°C with dry steam under pressure. The sudden reduction in pressure as the material is forced through the die holes at the end of the barrel allows the water vapour to expand and air is trapped in the feed matrix (Lall, 1991; NRC, 1993). By modifying this process, buoyancy of the feed can be controlled. The feed then passes through a drying tunnel to reduce moisture content. Extrusion allows almost complete gelatinisation and feeds are more firmly ground resulting in better water stability and less dust than for pelleted feeds (Lovell, 1989). Because some vitamins are destroyed by heat, and for feeds where high contents of lipid are required (eg for some salmonids), feeds are sometimes coated with vitamin mixtures or fat after processing. Cooling prior to bagging and shipment is important for both pelleted and extruded feeds to reduce condensation and restrict the growth of mould.

FEEDING RATES AND FREQUENCIES

Feeding rates and frequencies are as important as the nutritional characteristics of the diet in determining growth rates, feed conversion efficiencies and costs of feeding. The feeding strategies will depend upon a number of factors including fish size, water temperature, culture facilities and the type of diets used (pelleted or extruded; floating or sinking). Research to optimise these important variables for silver perch culture has not begun, although the feeding rates and frequencies used successfully by Dr Stuart Rowland during NSW Fisheries' growout trials are presented in Table 12.

TABLE 12

Suggested feeding rates and frequencies[1] for silver perch

Fish size (g)	Pellet size[2]	Water temp (°C)	Feed rate (% body wt)	Feed frequency (times/day)
0.5 - 4	2C	18-23	5	3
		24-30	10	5
5 - 50	3C	10-14	1	1
		15-18	2	2
		19-23	3	3
		24-30	5	3
51 - 250	3P	10-18	1	1
		19-23	2	2
		24-30	3	2
251 - 1 000	6P	10-18	1	1
		19-23	2	2
		24-30	3	2

1 Developed at NSW Fisheries, Grafton Research Centre, using SP35 (35% protein diet) (Appendix 1)

2 Diameter (mm) Crumble (C) or Pellet (P)

RESEARCH PRIORITIES

Development of artificial diets for silver perch has just commenced. Based on research described in this paper we have formulated and trialled a successful, relatively low protein diet, SP35. The composition of this diet is listed in Appendix 1. To reduce the cost of this diet and improve its efficiency and ability to meet many of the yet unknown nutritional requirements of silver perch, considerable nutritional research still needs to be done. Priorities for NSW Fisheries are to:

1 continue searching for potential ingredients,
2 thoroughly evaluate promising ingredients in terms of their digestibility and assimilation,
3 develop and evaluate methods of improving the value of ingredients through processing (eg extrusion) and the use of enzymes and supplements,
4 identify the nutrients which are most expensive to supply, and which may restrict the development of cheap, efficient diets, and determine the requirements for these nutrients, and
5 develop and evaluate low-cost, efficient diets for use in commercial silver perch farming.

FINAL NOTES

The development of cost-effective diets and sound feeding strategies will go a long way towards reducing operating costs for silver perch farming. To ensure that diets and feeding strategies are appropriate and to evaluate the cost of feeding, farmers need to keep records on feeding. Records on the following are essential: total size and number of fish fed, total quantity of feed used, amount of fines in the feed, feeding rates and frequencies used, periods when fish were not fed and fish feeding behaviour. Feeding should be reduced or stopped when water quality deteriorates (particularly if dissolved oxygen decreases or ammonia increases). Changes to feeding strategies, eg changing brands of feed or increasing feeding rates or frequencies, should be made gradually.

ACKNOWLEDGEMENTS

I would like to acknowledge the contribution to nutritional research described in this paper by Dr Stuart Rowland and technical staff from the NSW Fisheries Grafton Research Centre and Jane Frances, Scott Parkinson, Rebecca Warner-Smith and David Stone from the NSW Fisheries Port Stephens Research Centre. Grant funds for the research were provided by the Fisheries Research and Development Corporation, the Grains Research and Development Corporation and the Australian Centre for International Agricultural Research. Mr Wayne O'Connor made many useful comments on a draft manuscript. Jo Pickles expertly typed the manuscript.

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