Apparent Digestibility Coefficients of Feed Raw Materials for Barramundi *Lates calcarifer* (Bloch).

by

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Submitted in fulfilment of the requirements for the degree of Master of Applied Science.
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October 1996.

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6 MARCH 1997.

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Acknowledgment

I would like to thank Mr. Manuel Pando for his assistance in the determination of analytical values for this study.

I would also acknowledge the assistance of my two original supervisors of this program of study Dr. K. L. Wee and the late Dr. N. C. Gillespie.

I also thank my current supervisor, Dr. C. Carter for his invaluable assistance.

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ABSTRACT

Apparent Digestibility Coefficients of Feed Raw Materials for Barramundi *Lates calcarifer* (Bloch)

This study was undertaken to define the availability of different nutrients within feed raw materials for the tropical species barramundi *Lates calcarifer* (Bloch). Availability was measured as the apparent digestibility coefficient (A.D.C.) measured as the difference in composition between feed and faeces. Feed materials investigated include blood meal, fish meals, meat and bone meal, soy meal, soy protein meals, yeast meal, squid meal, prawn shell meal and wheat.

Initial trials were conducted to determine the best method for faecal collection, including continual filtration and stripping. Individual aquariums holding ten fish were connected to purpose built filtration units for the collection of faeces. Experiments one, two and four used the method of continual filtration of faecal material to determine the apparent digestibility coefficient of raw materials, using fish with a mean weight of 396.65+/- 65g. Considerable variation was found in protein and lipid A.D.C.s for the ingredients tested. Fish meals tested had significantly different absorption efficiencies.

Experiment two assessed the effect of temperature on faecal collection chambers. No differences were found on the nutritional parameters of crude protein and crude lipid.

Experiment three, carried out with experiment four was a practical evaluation of A.D.C.s for crude protein and crude lipid of a reference diet with faeces collected by continual filtration and stripping.

Experiment four assessed production method for diet manufacture found a significant difference between the methods of cold extrusion and steam pelleting. Different processing techniques of raw materials and of finished diets and their effect of apparent digestibility coefficient are discussed.

Results from this study were used to formulate a diet for barramundi using commercial production techniques, for trialing with existing commercial barramundi feeds. Results from this six week trial indicated that through the use of protein and lipid A.D.C.s the reformulation of diets for barramundi can see significant savings to the barramundi farming industry. Growth rates and food conversion rates were comparable to existing commercial diets but at a greatly reduced cost.

In all experiments faecal material was collected daily, analysed and used to calculate apparent digestibility coefficients. Results are also compared with existing information available on cold water species to elucidate any major differences.

Introduction

This program of study was instigated with a major aim of investigating the nutrition of barramundi *Lates calcarifer* (Bloch), through the use of apparent digestibility coefficients (A.D.C.s) so as to reduce the cost of existing commercial compounded feeds. The nutrition and farming of the barramundi has been investigated to an extent, mainly in Asian countries, Chou (1984); Avila (1986); New (1986); Cheong & Yeng (1986); Gosh et al. (1987) ; Wong & Chou (1989); Pechmanee (1989); Tubongbanua-Marasigan (1989); Boonyaratpalin, (1990); Boonyaratpalin et al. (1990); Boonyaratpalin (1991); Cuzon et al. (1990); Dhert et al. (1990); Wanakowat et al. (1990); Parazo et al. (1991); Tacon et al. (1991); Wanakowat et al. (1991); Phromkunthong et al. (1994). However, only limited investigation have been carried out in Australia, MacKinnon (1987); Krogh (1994); Rimmer et al. (1994); Barlow et al. (1993);Williams et al. (1996), when compared to other well researched fish groups, such as the salmonids.

Barramundi or sea bass is an important coastal, estuarine and freshwater fish of the indo-pacific region. The species is widely distributed from the Arabian Gulf to China and Taiwan, and to Papua New Guinea and northern Australia. The fish is a popular and highly valued food fish through out the region which commands a high market price when available. It is also fast growing and euryhaline. The latter two attributes are important factors for pond and cage culture in the monsoonal regions in which it is cultured. Techniques for the culture of barramundi were developed in Thailand in the early 1970's (Grey 1987). The nutritional requirements of barramundi have advanced from this point of feeding trash fish, which however a majority of Asian producers still rely on. The requirements for protein have been described by Boonyaratpalin (1990); Aquacop et al. (1993), lipid requirements by Cuzon et al. (1990); Wanakowat et al. (1993), vitamin and mineral requirements by Boonyaratpalin (1990); Wanakowat et al. (1990); Boonyaratpalin et al. (1990); Phromkunthong et al. (1994); Boonyaratpalin and Wanakowat (1993).

Krogh (1994), identified the need for increasing the knowledge of the nutrition of this species particularly in relation to the manufacture of commercially compounded feeds using commonly available ingredients. Feeds for the barramundi were originally developed using a salmonid model for diet design. This model essentially relied on the use of large proportions of high quality fish meal and oils, with little if any substitution of other ingredients. Growth, survival and food conversion ratios were considered acceptable for the industry , (Tucker et al. 1988). However, the cost of production could not be sustained for an extended period if the industry was to grow. The need for a reduction in the cost of feeds became increasingly evident.

The use of digestibility coefficients for the formulation of commercial diets for intensively cultured animals has been common practice within the feed industry for many years. However adequate knowledge of this type of data has been lacking for a number of fish species. Cho et al. (1982) reported A.D.C.s of protein, lipid and energy for rainbow trout using chromic oxide as a marker and a gravity settling technique for faecal collection. The methods for the determination of digestibility include the direct and indirect methods. The direct method requires measurement of all feed consumed and faecal material collected, whereas the indirect method, through the use of markers eliminates the need to quantitatively collect all faecal material. Smith (1971, 1976) and Smith et al. (1980) (in NRC 1993) used modified metabolism chambers to hold rainbow trout for using the direct method of digestibility assessment. The fish were force fed a measured amount of diet and excrements were collected from the chambers posterior and anterior sections. This method allowed for the determination of carbon and nitrogen budgets as well as digestible and metabolizable energies. However, this method has been criticised as the fish are immobilised and force fed which leads to a level of stress that could compromise the utilisation of the feed and subsequent determinations of absorption of nutrients.

The indirect method of assessment of digestibility relies on the use of markers either internal or external to the food source.

The validity of this method relies on the assumption that the amount of marker in the feed and the faeces remains constant through out the experimental period, should be transported through the gut at the same rate as the food components, that the marker is indigestible and will not affect the faeces, have no effect on the digestive metabolism of the animal and that all of the marker will appear in the faeces. The use of chromic oxide (Cr_2O_3) as a digestibility marker has been common practice originally adapted from digestibility research on terrestrial animals (Stevenson and De Langen, 1960). De Silva and Perera (1983), however report on doubts raised on the use of Cr_2O_3 due to differential passage in the digestive tract, the authors reported on the use of internal markers in the determination of digestibility of the diet of fish rather than the use of external markers such as Cr_2O_3 that overcome the potential problem of differential passage. Internal markers used in digestibility studies have included silica, cellulose, hydrolysis restante ash and hydrolysis resistant organic matter and acid resistant ash (Hickling, 1966; Buddington, 1979; Buddington, 1980; Bowen 1981 In De Silva and Perera 1983: Perera et al. 1995 *a,b*)

The measurement of feed consumption rates of individual fish in nutritional trials is important because effects on feed consumption as a result of dietary manipulation can be separated from the effects of quantity of diet consumed, Perera et al. (1995*a,b*). This has, however only been possible due to recent advances in radiography. The factor of consumption rates has not been a feature of nutritional trials, with the exception of the direct metabolism chamber methods where fish were force fed a known amount of food. The use of radiography does however allow for the calculation of nutritional budgets without the potential effects of stressed and immobilised fish.

The reduction in cost of ingredients through reformulation of practical commercial diets was a major aim of this study, through the measurement of A.D.C.s for protein and lipid for this species. Reformulated and cheaper feeds contribute significantly to the reduction of costs to the Australian barramundi industry.

Experiment one addressed this aim as well as comparing the growth of test animals through regression analysis to examine whether A.D.C.s could be used as a rapid method to predict performance of an ingredient in relation to the digestibility of the macronutrients in that ingredient.

Growth rates of fish were not severely compromised due to tank effects as fish fed with the reference diet had similar growth rates as those reported by Cash (1994), on the production of barramundi in recirculating tank systems for commercial production of flesh.

The use of the "Guelph system" a gravity settling principle for faecal collection was chosen for this experiment. The system although, originally developed for use with cold water salmonids showed characteristics suitable for use with tropical species, although with some modifications. Recognising the fact that the "Guelph system" was initially designed for salmonids that were held in cooler waters than those of species from tropical regions, experiment two evaluated the effect of increased temperature on A.D.C.s. The potential for microbial action increases with temperature and with the potential for effect on A.D.C.s by action on collected faeces. This experiment evaluated the potential for this effect by assessing any difference in A.D.C. for protein of the reference diet determined from faeces collected at two different temperatures.

Faecal collection chambers were either encased with a jacket of ambient water (25^{o}C +/- 2^{o}C) or with ice. The jacketing material was contained in an insulated container to reduce temperature transfer, especially with the ice treatment.

Experiments three and four were run concurrently, experiment three was a practical investigation into the effect of fish size on protein and lipid A.D.C.s. Experiment four however was evaluating the effect of processing technique on the protein and lipid A.D.C.s. With the exception of experiment four all feeds were produced on a scaled down commercial feed mill to duplicate conditions in commercially produced compounded feeds.

Feeds used in experiment four were produced with either the scaled down commercial mill or with traditional laboratory methods using food mincers and a drying oven. The major difference between the two methods is the heat generated in the commercial mill that provides some gelatinisation of starches as well as heat effects on protein and lipid ingredients.

The type of processing required for a list of ingredients in the form of a commercial diet is of considerable importance to commercial feed manufacturers. Processing systems for compounded feeds are expensive and complex, as well as being persistent in regards to usable life. Therefore the decision to install a particular option is very important, as the effect of the processing technique on the digestibility and potentially the ultimate growth of particular fish species, will in part decide the type of processing technique utilised to produce the feeds for that species.

Validation of data collected from this study, particularly the protein and lipid A.D.C.s, obtained from experiment one was performed by the formulation of a diet that could be produced under full scale commercial conditions and trialed against existing commercial formulations for barramundi. Four diets were evaluated in a 4x2 factorial experiment with 3 replications. Two temperature regimes were used to simulate the different seasonal water temperatures experienced between summer and winter in the major commercial growout area for barramundi in Australia.

MATERIALS AND METHODS

Fish and Experimental Organisation

Fish

Five hundred juvenile barramundi *Lates calcarifer* (Bloch), of 30g mean weight were obtained from a commercial hatchery (Barramundi Waters) in North Queensland, and flown to the research facility in Brisbane. Stock fish were held in together in a 2000 L capacity circular tank. Water was recirculated (15 L per minute) through a combined particulate and biological filter, temperature was maintained at 25 C+/- 2 C using electric immersion heaters. Water was pumped from the filter system and entered the tank via a tangential spray bar. Stock fish were measured for total length and weight and randomly assigned to faecal collection tanks. Ten fish were assigned to each tank.

Length of Experiments and Feeding Regime

Fish were acclimatised to diets for a period of one week before collection of faeces. Faeces was then collected over an eight week period. This was done to ensure that sufficient quantities of faeces was available for analysis. Fish were fed to satiation twice per day. First feeding was in the mornings after removal of faeces from collectors and in the afternoon prior to cleaning in preparation for the evenings collection. Care was taken on the timing between feeding and cleaning so as to reduce stress. A minimum of one hour between feeding and commencement of cleaning was allowed in the afternoons. As no direct contact was made with fish in the mornings during removal of faeces from collectors, fish were fed immediately after faeces was removed.

Faecal Sampling

All tanks and faeces collectors were thoroughly cleaned at the end of each day after final feeding for that day at approximately 1700 hrs. At approximately 0700 hrs the next morning faeces was removed from collectors. Faeces from tanks of the same test ingredient were pooled on that day. Wet faeces whether from filtering or stripping was immediately removed from the aquarium area into the laboratory and excess water was removed by

filtering through ashless filter paper and stored at -18°C until required for chemical analysis. Daily samples were identified by date and feed type.

Calculation of apparent digestibility coefficients

The determination of apparent digestibility coefficients (A.D.C.) in either the reference of test diets is calculated from the ratio of nutrient to indicator (chromicIII oxide) using the following expressions.

Formulae 1:

$$\left(\frac{\%\text{ nutrient in diet}}{\%\text{ indicator in diet}} - \frac{\%\text{ nutrient in faeces}}{\%\text{ indicator in faeces}}\right) \times 100 = \text{A.D.C.}(\%)$$

$$\frac{\%\text{ nutrient in diet}}{\%\text{ indicator in diet}}$$

(Cho et al.,1982.).The digestibility coefficient for a nutrient in the ingredients can then be calculated from the digestibility coefficients for the reference and test diets on the basis of a 30% substitution of the test ingredient for the reference diet using the following expression.

Formulae 2:

$$\frac{100}{30\ \text{A.D.C.t}} - \frac{70}{100\ \text{A.D.C.r}} = \text{A.D.C. ingredient.}$$

(Cho et al.,1982.)

Where: A.D.C.t = Apparent Digestibility Coefficient ; test diet

A.D.C.r = Apparent Digestibility Coefficient ; reference diet

Analytical Methods

The analytical methods used were carried out on both feeds and faeces. A.O.A.C., 1984, methods were used throughout this study with minor modifications made due to operating procedures in the laboratory . Crude protein was determined as macro kjeldahl nitrogen x 6.25 (A.O.A.C., 1984). This factor was used throughout the study as a standard rather than use different factors for different ingredients (ie: Soya products). Crude lipid was determined by standard soxhlet extraction (A.O.A.C., 1984). Petroleum Spirit (Boiling Range 40-60°C) was used as the solvent to replace diethyl ether. Moisture was determined, by drying at a temperature of 115°C in laboratory convection oven (AOAC,1984). Crude ash was determined by ignition in a muffle furnace (600° C) for a period of 6 hours (AOAC,1985). ChromicIII Oxide determination was by the colourmetric method described by Stevenson & De Langen, (1960).

Formulation of Diets

All diets were formulated using the computer based feed formulation package FORMAT®, Single mix and parametrics®, Format International Inc. U.K., Table 1 describes the ingredients used to formulate diets. Table 2 summarise the formulations used. All formulations were based on the original test (Guelph C201) and trial diets proposed by Cho et al. (1982). Table 3 shows the calculated analysis from the formulae. A number of experiments have evaluated the desirable protein and lipid levels for barramundi (Tucker et al. 1988; Wong & Chou, 1989; Cuzon et al., 1990; Catacutan & Coloso, 1995). It was following these findings that crude lipid was set from the original 14% in the Guelph C201 to 10%. Crude protein was targeted at 43.0 % for formulation.

Most commercial feeds for this species are formulated with this crude lipid level and have supported adequate growth therefore this study used this lipid level as a guide. As a indication of accuracy of formulation and to provide a basis for calculation of A.D.C.s each diet was analysed for proximate composition (% as is) (Table 3).

Table 1.

Proximate composition (*% as is* +/- SEM) of ingredients used in reference and test diets

Ingredient	Crude Protein n=3	Crude Lipid n=3	Moisture n=3
Defatted Soybean Meal (Sol.)	45.4(±0.08)	1.4(±0.01)	12.4 (±0.01)
Fish Meal (Red Tobis, Denmark)	71.2(±0.36)	10.4(±0.0)	10.3 (±0.01)
Fish Meal (Tuna Offal)	52.5(±0.91)	10.4(±0.01)	9.5 (±0.08)
Modified Soy Protein	70.3(±0.00)	1.5(±0.00)	8.4 (±0.05)
Whole Wheat	10.6(±0.03)	2.4(±0.00)	11.1 (±0.02)
Full Fat Soy Meal (Ext)	38.3(±0.06)	18.5(±0.00)	11.0 (±0.02)
Torula Yeast	60.2(±0.06)	6.2(±0.01)	8.2 (±0.03)
Fish Meal (Mackerel, Chile)	69.2(±0.02)	9.3(±0.02)	10.6 (±0.00)
Whole Squid Meal	68.4(±0.14)	5.4(±0.00)	11.8 (±0.01)
Blood Meal (Spray)	86.3(±0.03)	3.3(±0.01)	11.2 (±0.03)
Meat & Bone Meal	51.5(±0.01)	11.9(±0.00)	11.8 (±0.02)
Fish Meal (Mullet Offal)	55.1(+/-0.70)	14.4(±0.61)	10.4 (±0.02)
Prawn Head & Shell Meal	40.4(±0.06)	1.7(±0.00)	10.3 (±0.00)

Table 2.

Ingredients used in reference and test diets

Ingredients

(%)

Diet type	Wheat	Fish Meal Danish	Soy Bean Meal (DF)	Fish Oil	Vit /Min Premix	Filler	Test Ing.
Reference	30	53	10	3.2	0.2	3.1	0
Soya (DF)	21	37.2	37	2.2	0.2	1.9	0
Wheat	51	37.2	7	2.2	0.2	1.9	0
Fish Meal (Danish)	21	67.2	7	2.2	0.2	1.9	0
Blood Meal	21	37.1	7	3.4	0.2	0.8	30
Fish Meal (Chile)	21	37.1	7	2.7	0.2	1.5	30
Meat & Bone	21	37.1	7	1.6	0.2	2.6	30
Fish Meal (Mullet)	21	37.1	7	3.2	0.2	1	30
Prawn Shell Meal	21	37.1	7	4.2	0.2	0	30
Soya (FF)	21	37.1	7	0	0.2	4.2	30
Squid Meal	21	37.1	7	3.6	0.2	0.6	30
Fish Meal (Tuna)	21	37.1	7	3.6	0.2	0.6	30
Yeast (Torula)	21	37.1	7	4.2	0.2	0	30

Table 3.

Measured composition (% as is) of reference and test diets

Type	Crude Protein n=3	Crude Lipid n=3	Crude Fibre n=3	Crude Ash n=3	Moisture n=3	N.F.E. (1) n=3
Reference	46.3	10.2	1.5	7.9	9.8	24.3
Soya (DF)	46.5	8.6	2.8	7.7	8	26.4
Wheat	36.7	7.9	1.8	6.1	9.1	38.4
Fish Meal (Danish)	54.2	10.4	1.2	9.3	7.8	17.1
Blood Meal	58.5	10.1	1.3	7.5	5.7	16.9
Fish Meal (Chile)	53.6	10.1	1	9.7	8.6	17
Meat & Bone	48.7	10.3	1.9	13.9	8	17.2
Fish Meal (Mullet)	48.8	10.1	1	12.8	10.4	16.9
Prawn Shell Meal	43.9	9.9	4.2	11.4	9.9	20.7
Soya (FF)	45.3	10.7	2.6	7.1	7.2	27.1
Squid Meal	56.8	10.1	1	8.6	6.6	16.9
Fish Meal (Tuna)	56.8	10.1	1	8.6	6.6	16.9
Yeast (Torulla)	45.1	9.9	1.4	7.3	9.7	26.6

1: N.F.E. = Nitrogen Free Extract, calculated by difference

Source of Ingredients

Materials for the production of diets were sourced either through a commercial aquaculture feed manufacturer, Ridley Agriproducts, Narangba, Qld. Australia or directly through other suppliers listed below. Ingredients when received were checked visually for uniformity and for any insect infestation. Ingredients were stored in sealed plastic drums in an air conditioned laboratory until required for analysis. Where indicated in diet, diatomaceous earth (sourced from swimming pool supply store) is used as an inert filler.

Table 4

Source of ingredients for current study

Ingredient	Supplier
Defatted Soya Bean Meal (Solvent).	Ridley Agriproducts, Narangba, Qld. Australia.
Fish Meal (Red Tobis, Denmark).	Esbjerg Fiskeindustri,Esbjerg, Denmark.
Fish Meal, (Tuna Offal).	Solomon Is. Fishing Corp., Solomon Is.
Modified Soya Protein.	Oppenheimer P/L, Sydney, NSW, Australia.
Wheat (Whole Grain).	Ridley Agriproducts, Narangba, Qld. Australia.
Full Fat Soya Meal (Extruded).	Ridley Agriproducts, Narangba, Qld. Australia.
Torula Yeast	Ridley Agriproducts, Narangba, Qld. Australia.
Fish Meal (Mackerel, Chile).	Compania Pesquera Camanchaca S.A., Santiago, Chile.
Squid Meal.	Tawin Grand Asia Co. Ltd., Taipei, Taiwan, ROC.
Blood Meal.	A.J. Bush & Sons P/L, Brisbane, Qld, Australia.
Meat & Bone Meal.	A.J. Bush & Sons P/L, Brisbane, Qld, Australia.
Fish Meal (Mullet spp. Offal).	A.J. Bush & Sons P/L, Brisbane, Qld, Australia.
Prawn Head & Shell Meal.	Tawin Grand Asia Co. Ltd., Taipei, Taiwan, ROC.

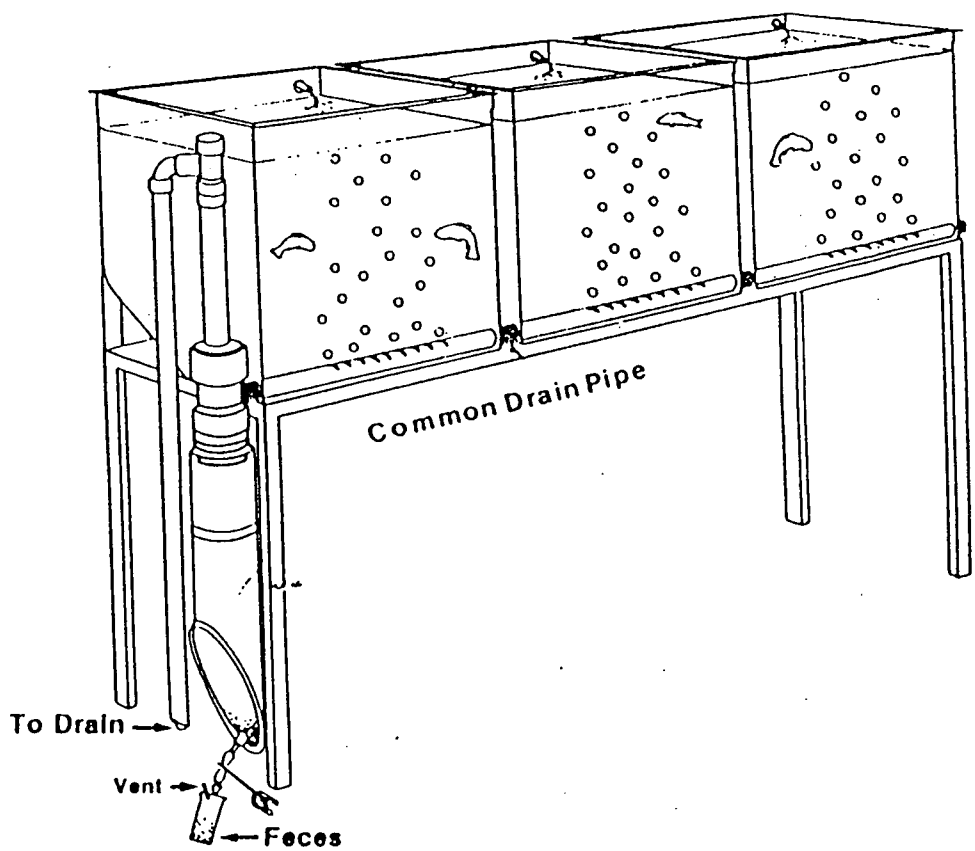
Experiment 1.

Determination of Apparent Digestibility Coefficients using Continual Filtration Units Tank Systems

The faecal collection systems used in this part of the project was a modification of the Guelph System, Cho et al. (1982). The major differences, was that the Guelph System used a common collection pipe for faeces from three tanks (Fig.1), and the tanks used in the present experiment used individual collection chambers (Fig.2). Other minor differences included the materials of construction and design of the collection chamber.

Figure 1

CYAQ-2 Digestibility system (Guelph System) consisting of three fish tanks with common drains to faeces settling column . (after Cho et al. 1982)



All tanks were of the same design containing 70 L. of water, consisting of a floor sloped at 35 degrees from the back of the tanks, falling to the front of the tank. A collection pipe was located along the bottom of the front wall of the tank and joined via a 'T' piece through the centre of the front wall of the tank.

Tank systems were constructed of 19mm waterproof building plywood coated with several layers of two part epoxy sealant. The system consisted of nine individual tanks linked to a common water supply system(Fig. 2). The entire system was located in a temperature controlled room (25°C±2°C) with a photo-period of 12 Hours light 12 Hours dark.

Water supply for the system was of a recirculating design. Water exiting from the bottom of the tanks via 25 mm diameter pipes entered the tank's faecal collector. The faecal collector was constructed of 100mm diameter pipe with an outlet to a common drain (Fig. 3). Water from the common drain passed first through a particulate filter and then through a mixed media biological filter via gravity. Water was then drawn from the biological filter via the pump to return the water to individual tanks. Water entered individual tanks at the rear and top.

Each individual tank was also equipped with an overflow system separate from the faeces collection system. This overflow connected direct to the common drain and entered the filter system. Each overflow was of an open design so as to negate the potential for self siphoning (Fig 2). Flow rates into each faeces collector was controlled by gate valves.

Water transfer through each tank was calculated to be three complete exchanges per hour. Freshwater, sourced from town water supply was used throughout the experiment. Water from the entire system was replaced at least three times per week.

Experimental organisation followed the plan that of the nine available tanks in the recirculating system, three were randomly assigned the reference diet and the remaining six tanks were randomly allocated to two test diets. This procedure was repeated until all test diets had been evaluated. Fish from each testing cycle were removed from the test tanks and replaced with fish from the holding tank. Fish from the most recent testing cycle were not replaced into the holding tank before the removal of new test fish to overcome the possibility of those individual fish going through two consecutive test cycles.

All fish were weighed before and after placement into continual filtration tanks for assessment of correlation between growth and A.D.C.. Specific growth rate (SGR) was also calculated from this data for each diet type, as total weight increment divided by number of days feed diet and expressed as g g^{-1} day^{-1}. Length of experiment was 8 weeks.

Tables 5 and 6 show the A.D.C.s for protein and lipid for the test diets only. These were calculated using formulae 1 (page 7). After analysis of variance identified that significant differences existed , a Tukey-Kramer analysis was carried out to identify which means were similar. Test diets are identified as not being significantly different by similar letter subscript.

By the use of formulae 2 (page 9) the A.D.C. for protein and lipid for individual ingredients is presented in tables 7 and 8 on pages 28 and 29 respectively. The same statistical analysis was carried out on ingredient data as was on diet data.

Figure 2.

Tank design used in current study

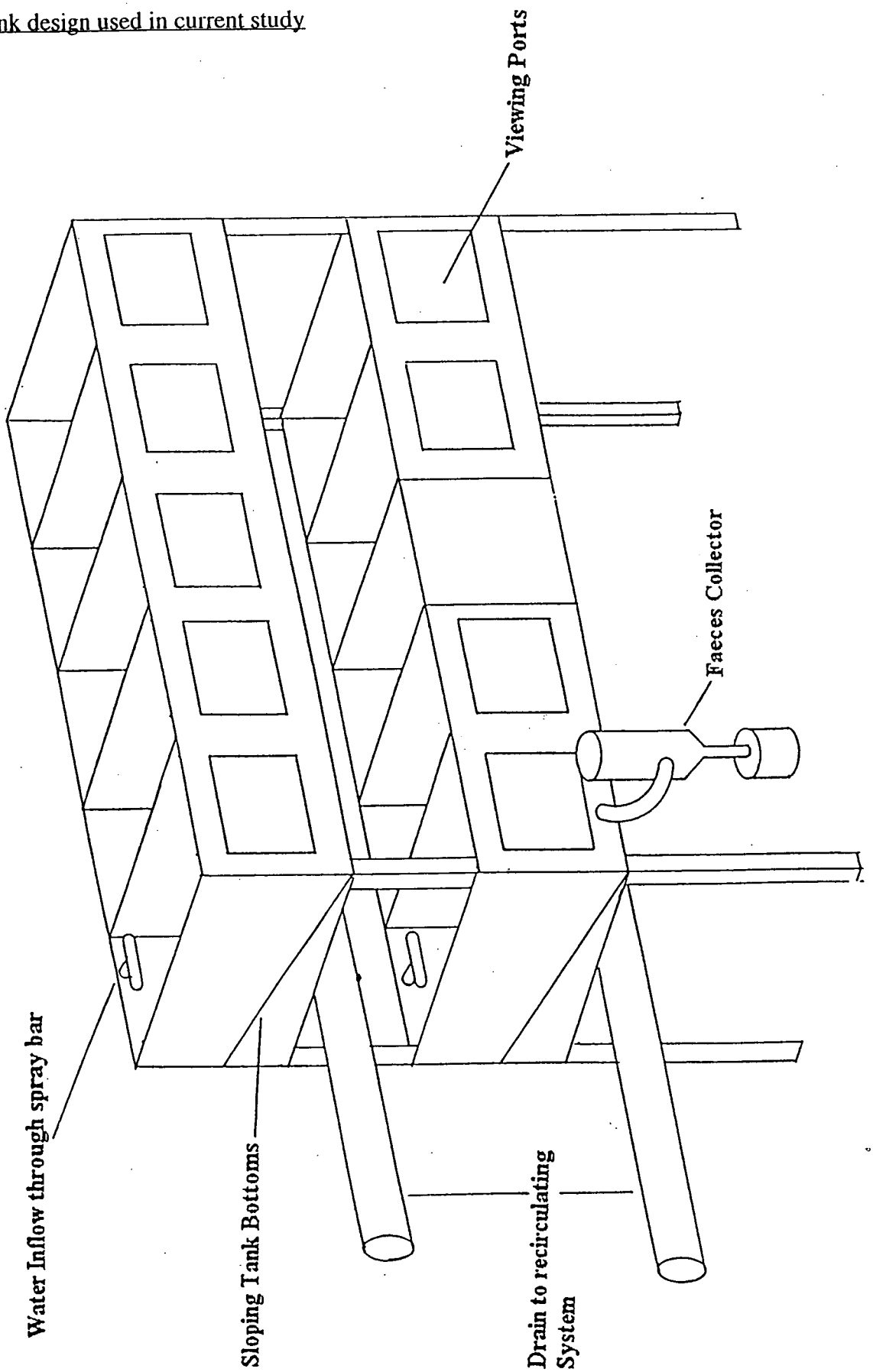
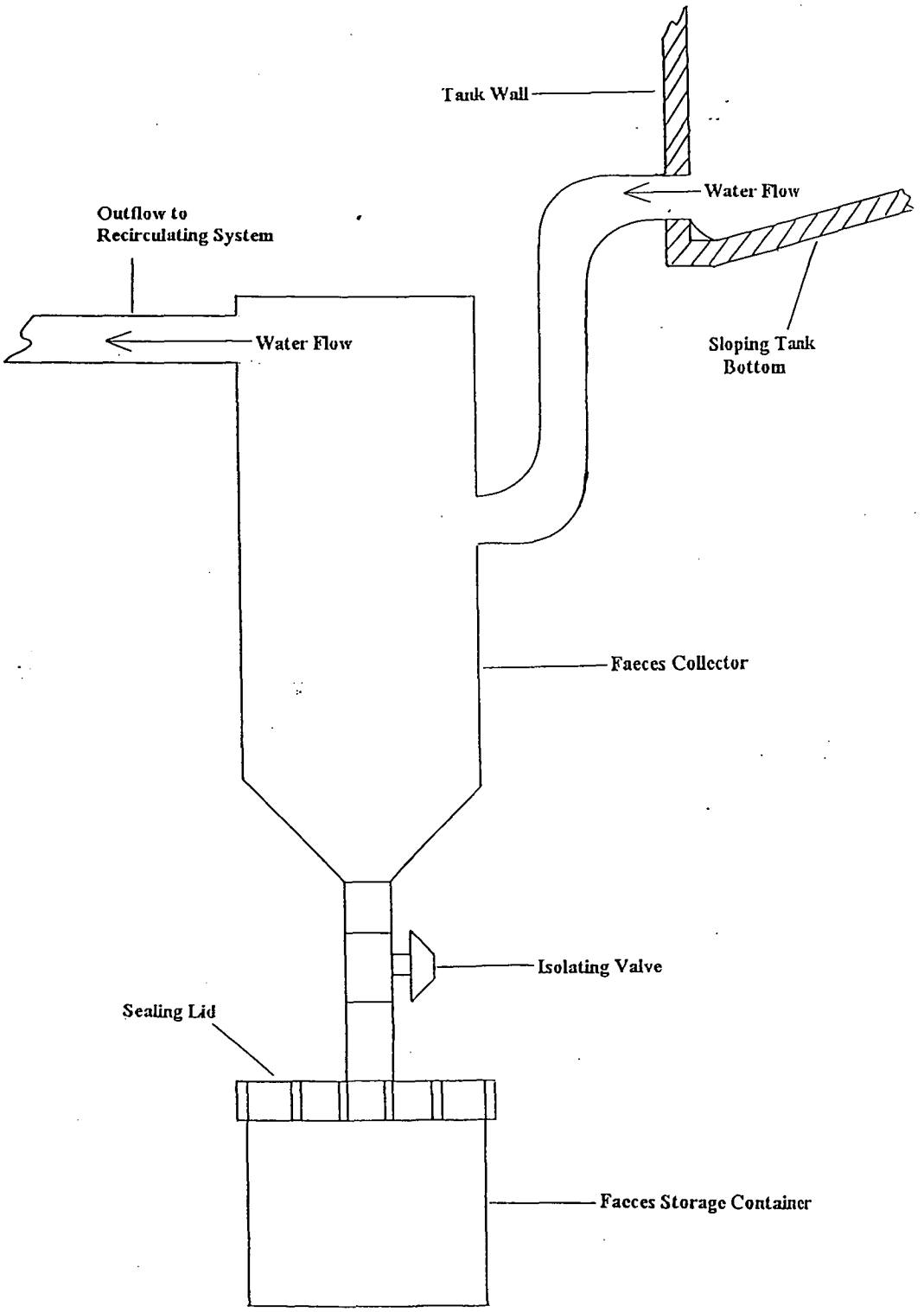


Figure 3.

Detail of faeces collector



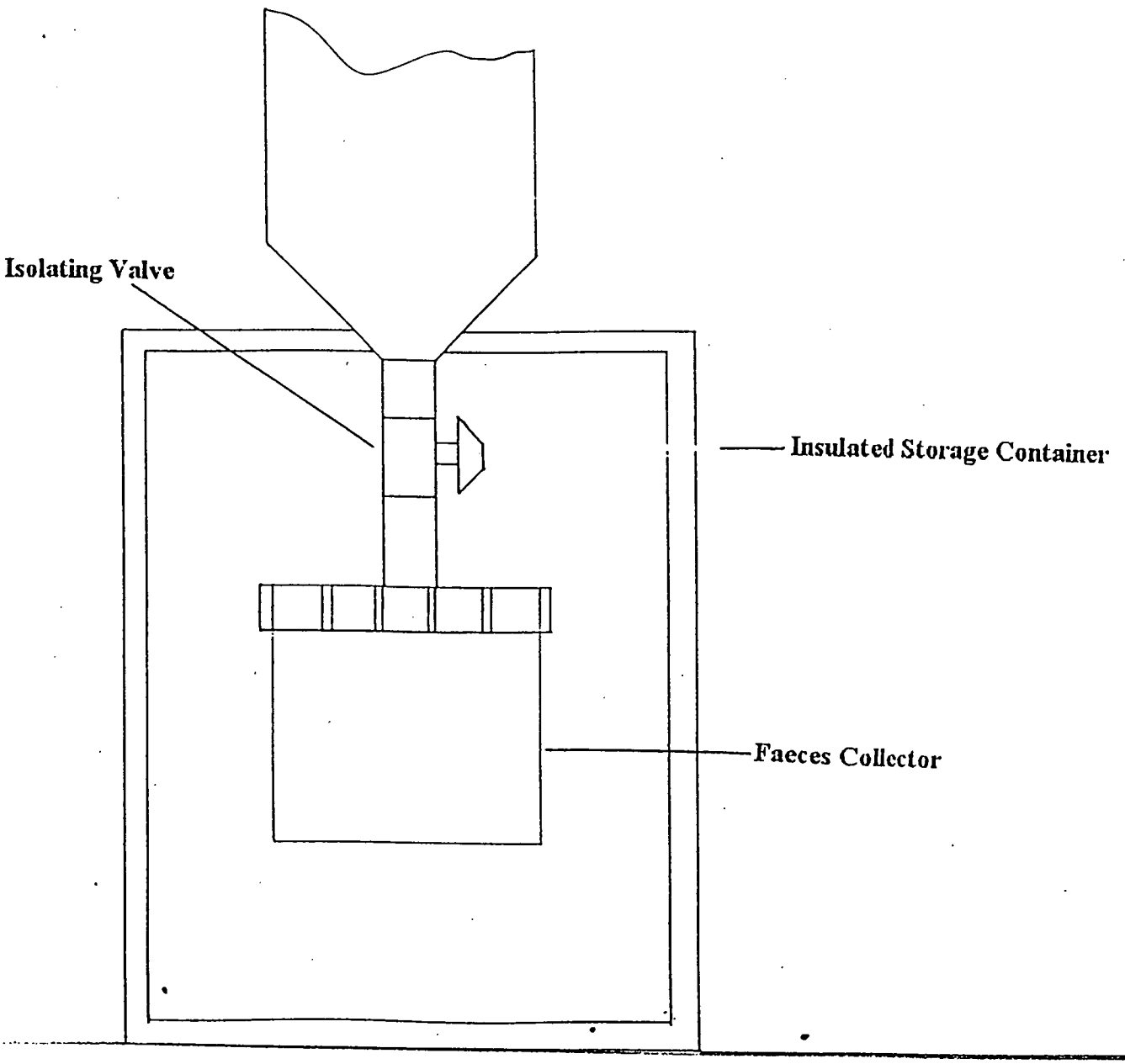
Experiment 2

Reduced Temperature Storage

Faecal collection chambers were housed in 20 L plastic containers filled with either ice (initially -18°C ± 1°C) or water at ambient temperature. Containers were wrapped in expanded foam as insulation with a lid of polystyrene foam (Fig 4). These faecal collection chambers were housed in ice or ambient water overnight for a period of 12 hours. Samples were retrieved and treated the same as all other collected samples.

Figure. 4.

Detail of Faecal Collection Chamber with Insulated Container.



Experiment 3

As a practical comparison of techniques fish of a larger size were tested to see if body size/collection technique had any effect on the values of protein apparent digestibility coefficient. As facilities did not allow for large fish, mean weight 2,400 g ± 160g, to be housed in continual filtration units, stripping was used to collect faeces from large fish. Faeces from small fish 396.65g ± 98.65 was collected using the continual filtration method as size of fish precluded them from stripping. The cold extruded reference diet was fed to all fish. It is acknowledged that as two variables were being compared in this evaluation the data provides useful information only. No definitive answers may be drawn to the effect of size of fish or to the method of diet production due to the combining of these two variables into one procedure.

Stripping Techniques.

The tank system for this section of the project was identical to the holding tank for smaller fish. Large fish were anaesthetised with Benzocaine, 50ppm. At a point just prior to complete anaesthesia they were removed from the tank and stripped for faeces. Fish were held firmly cradling the anterior end of the body on the right forearm with the dorsal side facing towards the body. The left hand supporting the posterior end of the body. The thumb and the two first fingers of the right hand are placed on either side of the fish on the ventral side and moved along and down broadly applying gentle pressure following the large intestine so that faecal material was expelled into a large plastic tray (modified version Austreng 1978. - S. Percival personal communication). Faeces samples were treated as described above.

Experiment 4

Effect of Production Method on Apparent Digestibility Coefficients.

Methods of Manufacture

Feed (Reference diet) was produced in two forms. Cold extruded pellets were made using a kitchen mincer apparatus and extruding the mix onto aluminium baking trays where the pellets were dried in a laboratory air convection oven at 50° C for a period of 12 Hours. Moisture content was lowered to approximately 10% for the cold extruded reference diet. All other diets were produced, using a laboratory pellet mill, California Pellet Mill model CL-2 (appendix 1). This machine is a vertical, ring type, die mill. This type of machinery was chosen to simulate commercial milling practices. Ingredients for diets were weighed individually and batched to produce a 5 Kg batch together into a bucket. This was then thoroughly mixed using a mixing attachment on an electric drill. All ingredients were ground through a laboratory hammer mill to a particle size of <500 microns. The batch was then remixed. Ingredients were then placed in the feed hopper of the pelleting mill. Pellets were produced of standard length, 4mm diameter and 8 mm in length.

Pellets were cooled using a air bypass pellet cooler until they were equal to ambient temperature. Pellets were placed in sealed plastic drums and stored in an air-conditioned laboratory. Diets were not stored for a period longer than two months.

Statistical Analysis

Experiment 1

Data from continual filtration experiment was analysed using one way analysis of variance after a arcsin transformation of percentage data. A Tukey-Kramer multiple comparison analysis was then carried out to identify means that were significantly different at $P < 0.05$. Statistical analysis was carried out using JMP version 2.02.

Regression analysis performed on protein A.D.C. and S.G.R. using Lotus 1.2.3. spreadsheet for windows, Version 5. (Anon. 1994)

Experiments 2, 3 & 4

Data from these experiments was analysed using Student's t. test to ascertain if means were significantly different at $P < 0.05$. (Hoel, 1976)

Results

Reference and trial diets showed considerable variation in mean protein and lipids A.D.C., calculated using formulae one, (tables 5 and 6) even between fish meal based diets tested. Protein A.D.C. of Danish, Chillian and tuna fish meal diets were not significantly different, the mullet based fish meal diet was significantly different to all other fish meal based diets. However, in regard to lipid A.D.C. mullet and tuna fish meal diets were not significantly different but were different to Danish and Chillian fish meal test diets.

Other marine protein meal based test diets; squid; prawn head and shell meal, were similar in respect to protein and lipid A.D.C.. Of the terrestrial ingredient test diets the blood meal test diet was significantly different to all other diets with the exception of the reference and defatted soybean test diets. The yeast test diet was similar to mullet fish meal diet only in respect of protein A.D.C. but lipid A.D.C. was also similar to soya protein, wheat, meat and bone meal, and prawn head and shell meal diets

Table 5.

Apparent Digestibility Coefficients of Crude Protein for reference and test diets measured using the Continual Filtration Method
(Fish Mean Wt. 396.65 ± 98.65 g)

Test Diet	Crude Protein Apparent Digestibility Coefficient, +/- SEM % n=3	Identifiers denoting no significant difference
Reference Diet	95.1 +/- 0.37	a,b,c,d
Defatted Soybean Meal (Sol) Diet	93.9 +/- 0.33	a,b,c,d,e,f,g
Fish Meal(Red Tobis, Denmark) Diet	92.2 +/- 0.26	c,d,e,f,g,h,i
Fish Meal (Tuna Offal) Diet	89.7 +/- 0.26	g,h,i,j,k,l
Modified Soy Protein Diet	88.9 +/- 0.09	i,j,k,l,m
Whole Wheat Diet	89.9 +/- 0.92	f,g,h,i,j,k,l
Full Fat Soy Meal (Ext) Diet	93.7 +/- 0.01	b,c,d,e,f,g
Torula Yeast Diet	86.1 +/- 0.51	m,n
Fish Meal (Mackerel, Chile) Diet	91.9 +/- 0.24	c,d,e,f,g,h,i,j
Whole Squid Meal Diet	92.4 +/- 0.44	c,d,e,f,g,h
Blood Meal (Spray) Diet	95.8 +/- 0.10	a
Meat & Bone Meal Diet	88.4 +/- 0.28	h,i,j,k,l,m,n
Fish Meal (Mullet Offal) Diet	83.6 +/- 1.21	m,n
Prawn Head & Shell Meal Diet	90.6 +/- 0.31	e,f,g,h,i,j,k,l

Means with same letter not significantly different at 5% level. Data analysis after arcsine transformation of % data

Table 6.

Apparent Digestibility Coefficients of Crude Lipid for reference and test diets measured using the Continual Filtration Method
(Fish Mean Wt. 396.65 ± 98.65 g)

Test Diet	Crude Lipid Apparent Digestability Coefficient, +/- SEM % n=3	Identifiers denoting no significant difference
Reference Diet	98.8 +/- 0.02	a,b
Defatted Soybean Meal (Sol) Diet	93.8 +/- 0.24	a,b,c,d,e,f
Fish Meal(Red Tobis, Denmark) Diet	88.5 +/- 0.04	b,c,d,e,f,g,h, i
Fish Meal (Tuna Offal) Diet	90.1 +/- 0.68	l,m
Modified Soy Protein Diet	98.6 +/- 0.11	g,h,i,j,k
Whole Wheat Diet	98.1 +/- 0.08	d,e,f,g,h,i,j,k
Full Fat Soy Meal (Ext) Diet	95.9 +/- 0.29	a,b,c,d,e,f
Torula Yeast Diet	95.5 +/- 0.28	k,l,m
Fish Meal (Mackerel, Chile) Diet	94.5 +/- 0.08	b,c,d,e,f,g,h, i
Whole Squid Meal Diet	90.1 +/- 0.20	b,c,d,e,f,g,h
Blood Meal (Spray) Diet	89.7 +/- 0.83	a
Meat & Bone Meal Diet	93.2 +/- 0.80	h,i,j,k,l
Fish Meal (Mullet Offal) Diet	89.9 +/- 0.25	l,m
Prawn Head & Shell Meal Diet	96.3 +/- 0.32	d,e,f,g,h,i,j,k

Means with same letter not significantly different at 5% level. Data analysis after arcsine transformation of % data.

The mean A.D.C. data for the test ingredients as discrete components of the test diets differed from the results of the test diets. Through the use of formulae 2 the individual test ingredient A.D.C.s for protein and lipid could be separated from the whole diet data.

Where as the data for protein A.D.C. of whole diet found no differences between Danish, Chillian and tuna fish meal as integral components of a diet, when assessed as individual ingredients tuna fish meal differed significantly from Danish and Chillian fish meals and was grouped with mullet fish meal. The probability of interactions or synergistic effects could account for this difference between whole diet data and discrete ingredient A.D.C.

Much greater differences were also noted with lipid A.D.C. between whole test diets and discrete ingredients. Chillian fish meal as a discrete ingredient was significantly different to all other marine meals with the exception of prawn head and shell meal.

The lipid A.D.C. for defatted and full fat soybean meal were not different however modified soya protein was different to both of these ingredients.

Of interest is the difference noted in lipid A.D.C. between blood meal and meat and bone meal. As both products are sourced from the same animal, beef cattle and therefore lipid types would be similar, however processing conditions vary greatly between production of the two meal types which explains the difference in lipid A.D.C..

Table 7.

Apparent Digestibility Coefficients of Crude Protein for test diet ingredients measured using the Continual Filtration Method
(Fish Mean Wt. 396.65 ± 98.65 g)

Test Diet	Crude Protein Apparent Digestibility Coefficient, +/- SEM % n=3	Identifiers denoting no significant difference
Defatted Soybean Meal (Sol)	89.5 +/- 1.10	b,c,d,e,f,g
Fish Meal(Red Tobis, Denmark)	83.8 +/- 0.85	c,d,e,f,g,h,i
Fish Meal (Tuna Offal)	75.4 +/- 2.14	m
Modified Soy Protein	72.9 +/- 0.29	h,i,j,k,l
Whole Wheat	76.2 +/- 3.07	f,g,h,i,k,l
Full Fat Soy Meal (Ext)	88.7 +/- 0.04	b,c,d,e,f,g
Torula Yeast	63.5 +/- 0.50	m
Fish Meal (Mackerel, Chile)	82.6 +/- 0.78	c,d,e,f,g,h,i, j
Whole Squid Meal	84.4 +/- 1.47	c,d,e,f,g,h
Blood Meal (Spray)	95.8 +/- 0.35	a,b
Meat & Bone Meal	71.0 +/- 0.93	h,i,j,k,l,m
Fish Meal (Mullet Offal)	55.1 +/- 4.03	m
Prawn Head & Shell Meal	78.7 +/- 1.02	e,f,g,h,i,j,k, l

Means with same letter not significantly different at 5% level. Data analysis after arcsine transformation of % data.

Table 8.

Apparent Digestibility Coefficients of Crude Lipid for test diet ingredients measured using the Continual Filtration Method
(Fish Mean Wt. 396.65 ± 98.65 g)

Test Diet	Crude Lipid Apparent Digestability Coefficient, +/- SEM % n=3	Identifiers denoting no significant difference
Defatted Soybean Meal (Sol) Diet	81.9 +/- 0.79	d,e,f,g
Fish Meal(Red Tobis, Denmark) Diet	77.2 +/- 0.13	h
Fish Meal (Tuna Offal) Diet	73.8 +/- 2.13	h
Modified Soy Protein Diet	98.0 +/- 0.37	a
Whole Wheat Diet	96.3 +/- 0.26	a
Full Fat Soy Meal (Ext) Diet	88.9 +/- 0.98	d,e,f,g
Torula Yeast Diet	87.5 +/- 0.95	c,d,e,f
Fish Meal (Mackerel, Chile) Diet	84.4 +/- 0.28	c,d,e,f,g
Whole Squid Meal Diet	69.7 +/- 0.67	h
Blood Meal (Spray) Diet	68.2 +/- 2.75	h
Meat & Bone Meal Diet	80.0 +/- 2.68	e,f,g
Fish Meal (Mullet Offal) Diet	68.7 +/- 0.83	h
Prawn Head & Shell Meal Diet	90.4 +/- 1.05	b,c,d

Means with same letter not significantly different at 5% level. Data analysis after arcsine transformation of % data.

Experiment 2

Results of Reduced Temperature Storage Experiment

Experiment two was conducted to ascertain the effect of cold storage of faecal material once inside the faecal collection chamber but prior to collection and processing. No significant difference was found between the two treatments of cold storage and ambient storage (Student's t. test $P > 0.05$) (Table 9).

Table 9.

Effect of Temperature Storage: Crude Protein Apparent Digestibility Coefficients.

Diet	Crude Protein Digestibility Coefficient %
Ice Storage	95.51 (+/- 0.07)
No Ice Storage	94.31 (+/- 0.42)

t. test, No difference between means ($P>0.05$)

Experiment 3

Practical evaluation of effect of fish size on digestibility coefficients

This experiment was carried out in tandem with experiment 4 as a practical comparison of techniques for the collection of faeces. Analysis of data (Student's t test $P > 0.05$) found that no significant difference existed between the two treatments, small and large fish. However the difference between stripped fish varied considerably as indicated by the large standard error of the mean. Although no significant difference existed between the two treatments a large difference was noted and the non signifigance is attributed partly to the large SEM of the data from the large fish treatment.

Table 10.

Effect of fish size: Crude Protein (Cold Extruded Reference Diet)

Diet	Crude Protein Digestibility Coefficient %
Small Fish	86.2 (+/- 1.49)
Large Fish	72.31 (+/- 13.49)

t test, No difference between means ($P>0.05$)

Experiment 4

Practical evaluation of effect of production method on digestibility coefficients

This experiment was carried out in tandem with experiment 3 as a practical comparison of techniques for the production of diets. Analysis of data (Student's t test $P > 0.05$) found that a significant difference existed between the two treatments.

Table 11.

Effect of production method: Crude Protein (reference diet)

Diet	Crude Protein Digestibility Coefficient	Crude Lipid Digestibility Coefficient
Steam Pelleted	95.1 (+/- 0.27)	98.8 (+/- 0.0)
Cold Extruded	86.2 (+/- 1.49)	86.7 (+/- 1.39)

t test, Significant difference between means ($P<0.05$) down columns.

Weight gain of fish fed reference and test diets

Fish were weighed at the beginning and end of each diet trial period. This was done to ascertain if growth was compromised by the test diets. Specific growth rate for each diet type was calculated as total weight increment divided by number of days feed diet and expressed as g g^{-1} day^{-1}. All fish gained weight over the trial period (Table 12)

Table 12.
Weight Gain of fish held in continual filtration units fed reference and test diets.

Test Diet	Mean Initial Weight, g (SEM)	Mean Final Weight,g (SEM)	Weight Change, g	SGR (g g -1 day-1)
Reference Diet	301.6+/- 3.9	384.2+/- 3.2	82.6	1.47
Defatted Soybean Meal (Sol) Diet	304.4+/- 4.6	407.4+/- 6.9	103.0	1.83
Fish Meal(Red Tobis, Denmark) Diet	314.8+/- 2.5	531.7+/- 3.6	216.9	3.85
Fish Meal (Tuna Offal) Diet	324.7+/- 1.9	453.2+/- 4.5	128.5	2.29
Modified Soy Protein Diet	307.8+/- 4.1	406.7+/- 6.8	98.9	1.76
Whole Wheat Diet	325.8+/- 3.9	400.8+/- 6.9	75.0	1.33
Full Fat Soy Meal (Ext) Diet	300.8+/- 4.0	396.1+/- 4.2	95.3	1.7
Torulla Yeast Diet	320.8+/- 3.0	390.6+/- 3.7	69.8	1.24
Fish Meal (Mackerel, Chile) Diet	453.6+/- 2.9	520.5+/- 3.4	66.9	1.19
Whole Squid Meal Diet	443.1+/- 4.4	511.1+/- 4.4	68.0	1.21
Blood Meal (Spray) Diet	440.6+/- 5.4	549.9+/- 3.5	109.3	1.95
Meat & Bone Meal Diet	443.0+/- 4.8	539.4+/- 2.5	96.4	1.72
Fish Meal (Mullet Offal) Diet	524.5+/- 2.8	653.5+/- 1.5	129.0	2.3
Prawn Head & Shell Meal Diet	608.7+/- 3.1	661.9+/- 2.6	53.2	0.95
Cold Extruded Reference	541.2+/- 2.3	613.9+/- 2.68	72.7	1.29

Regression Analysis of Apparent Digestibility Coefficients verses Specific Growth Rate

Regression analysis (Anon. 1994) was carried out on data derived from experiment one. Protein A.D.C. of diets and test ingredients was analysed with specific daily growth rates to ascertain what correlations existed between the digestibility of individual ingredients and the growth rate displayed by groups of test fish. Results of the regression analysis of data for protein A.D.C. of diets returned a formulae of $Y = 148.2$ (+/- 25.7) - 1.5 (+/-2) X, where ($n = 14$; $R^2 = 0.04$; $P > 0.05$), which indicated no relationship.

A similar result was shown by the data of protein A.D.C. for ingredients which returned a formulae of $Y = 45.3$ (+/- 26.8) - 0.4 (+/- 0.6) X, where ($n = 14$; $R^2 = 0.03$; $P > 0.05$)

Discussion

Introduction

This study has been focused on the determination of Apparent Digestibility Coefficients of common ingredients currently available in Australia for use in aquaculture feeds by the commercial feed milling industry. New, (1986), documented the feeds for barramundi in the Malaysian industry and found that the most common source of feed was trash fish. However, the Australian barramundi industry could not be supported on such a source of nutrition as the amount of trash fish available in Australia is in relatively small supply when compared with Asian countries culturing the same species due to high consumption rates of wet material. Also the ecological impact of large quantities of trash fish is not sustainable in the Australian environment as most farms are situated within or close to particularly sensitive coastal and marine environments that have a low ecological resilience to nutrient inputs. The Australian feed milling industry responded in the late 1980's with the supply of feeds that were derivations on salmonid diets, (MacKinnon et al., 1987). These diets did produce reasonable growth rates but the cost was high due to the inherent basis of the formulation of the diets being based on salmonid requirements with little substitution for marine protein and lipid sources.

This study has provided information on A.D.C.s of common ingredients of fish diets which have been used to formulate more productive commercial feeds without increasing costs to farmers. Ingredients chosen reflected the requirement of feed mills for a range of fish meal sources, potential marine sourced alternatives to fish meals and the substitution of marine proteins and lipids by terrestrial ingredients, either from rendered animal by-products or processed vegetable sources.

Methodology of Collection

A range of methods for the determination of the A.D.C. of ingredients have been evaluated by a number of researchers, including intestinal dissection, anal suction, siphoning, stripping and continual filtration (Smith et al., 1980; Windell et al., 1978; Cho et al., 1982; Choubert et al., 1982) . Cho & Slinger 1979; Spyridakis et al., 1989, found that the methods of faecal material affects the value of the apparent A.D.C. for crude protein and lipid. Spyridakis et al., 1989 found that protein A.D.C. varied from 82.5% for stripping to 94.2% continual filtration. This study utilised only two methods, the continual filtration method as described by Cho et al., 1982 and a modified stripping technique as described by Austreng, 1978.

Cho et al., (1982), compared the protein and lipid A.D.C. of herring meal using the continual filtration and stripping methods. Stripping techniques returned 77.5% ± 1.0 and 62.2% ± 5.1 for protein and lipid A.D.C.s respectively. The continual filtration method however, returned the much higher figures of 91.0% ± 0.8 and 97.3% ± 1.0 for protein and lipid A.D.C..

This study only investigated protein A.D.C.. The continual filtration method returned a A.D.C. of 86.2% ± 1.49 and stripping protein A.D.C. of 72.3% ± 13.49 (Table 14). These figures concur with those reported by Cho et al., 1982, showing the difference between the methods. The large SEM associated with the stripping technique is an indication of the difficulty experienced using this technique. Dilution from urine, mucus and water can effect the sample by varying amounts of dilution as well as the addition of extra nitrogen from the urine source. This variable addition of extraneous fluids is the probable cause of the large SEM associated with this technique. However, the compounding effect of differences in size is recognised (see above).

This experiment also attempted to provide a practical comparison of the influence of fish size and the effect on protein A.D.C.. Fish irrespective of size were fed the cold extruded, diet small fish (396.65g ± 98.65) returned a protein A.D.C. of 86.2% ± 1.49 and larger fish (2400g ± 160), returned a protein A.D.C. of 72.31% ± 13.49. Although not statistically significantly different due to the large SEM of the large fish data could have been due to the varied inclusion of endogenous addition from mucus and urine. Further investigation is warranted in this area with improvement in technique for stripping or larger continual filtration tanks to hold large fish as well as standardisation of techniques between size of fish. This would allow the hypothesis, such as as whether smaller fish digest selected proteins at a greater rate than large fish to be tested.

Spyridakis et al., (1989) found that A.D.C. tended to decrease over time due to the effect of bacterial action on faeces collected using the continual filtration method. A significant difference existed for protein A.D.C. for European sea bass *Dicentrarchus labrax* when microbial action was allowed to occur over a period of 20 hours. The protein A.D.C.. for the first hour after collection was significantly different from measurements taken at 4, 12 and 20 hours. However no significant difference was found between the longer collection times.

Williams et al. (1996), also reported a difference in protein and lipid A.D.C. when faecal water contact time was increased from 3 to 16 hours. Protein and lipid A.D.C.s ($P< 0.05$) increased.

The design of the "Guelph" continual filtration system requires the maintenance of a temperature regime that is required for the test animals. This temperature regime may not however be conducive for the short term storage of faecal material in the collection chambers (Fig. 4., materials & methods section). This is particularly so when the test animals require a tropical temperature regime as is the case with the barramundi. Microbial action could possibly alter the faecal material to such an extent that A.D.C. were effected.

An experiment was carried out to ascertain the effect of temperature of the faecal collection chambers and the subsequent effect on the A.D.C. for a selected diet. As the study was investigating crude nutritional parameters of protein and lipid A.D.C. these were selected to be trialed with reduced temperature storage. Faecal collection chambers were encased in a plastic container to which either ice or ambient water was added. The collection chambers were treated the same as all other collections. This study however found no significant difference between the two treatments measuring crude nutritional parameters.

For some period of time the principle of gravity settling of faeces for collection has been questioned, much of the criticism arises from the issue of leaching of nutrients. Satoh et al. (1992), however refute the criticism of the method. The authors found no significant difference between measured A.D.C.s between 3 and 15 hours settling period (Table 13).

Table 13.

Apparent Digestibility Coefficients over time

	3 hour ADC %	6 hour ADC %	9 hour ADC %	.12 hour ADC %	15 hour ADC %
Apparent Digestibility Coefficient: Protein	91.1 c	91.4 a,b,c	91.4 a,b,c	91.7 b,c	90.7 a,b,c
Apparent Digestibility Coefficient: Lipid	95.4 b	94.7 a,b	94.8 a,b	94.8 a,b	93.7 a,b

(after Satoh, et al. 1992.)

Faeces is described as the residue of a digestion process in the gastrointestinal system of fish and little, if any, soluble material (except some of endogenous material) would remain in the faecal residue of dietary origin in the rectum. Therefore, little material would be lost in neutral water over the 8-16 hour settling period. Any losses are mainly the result of handling and physical damage. Feeding and defecating periods must be separated and the complete system must be thoroughly cleaned prior to the defecating period.

Williams (pers comm.), described the faecal pellets that were expelled from barramundi were not a solid mass and this was attributed to the reference diet utilised in this study, the comments raised by Satoh et al. (1992), may well explain the differences found in protein and lipid A.D.C. across time in the Williams et al. (1996) study.

If however the analysis was to be carried out on more discrete nutritional components either amino acids or the fatty acids it is possible that microbial action could alter the A.D.C. for these components. This does raise the issue of the effectiveness of the method for tropical species. The design of the system would possibly require a modification to account for the effect of increased microbial action caused by elevated temperatures. Spyridakis et al. 1989 proposed the use of an antibacterial agent (.2% formalin solution) in the collection chambers. Choubert et al., 1979; 1982, described an immediate freezing system to overcome the potential effect of bacterial alteration of A.D.C. measurements.

The use of the "Guelph system" for crude nutritional parameters of feed ingredients for tropical species is however a sound principle. Of great importance though is the quality of the reference diet used so that faecal material is solid in nature

Perera et al. (1995*a*) evaluated the nutritive value of Bacterial Single Cell Protein,(BSCP), but instead of using a single test diet replacing 30% of a reference diet, four diets with incremental levels of BSCP were tested. Not only did this approach define a single value for absorption efficiency of a test ingredient it provided an insight into other effects

attributable to the test ingredient that would not become evident in a single evaluation using a 70/30 combination of reference and test ingredient. This study also utilised methodology for the evaluation of consumption of feed by individual fish by radiography that elucidated the nitrogen budgets associated with this test ingredient (Perera,et al. 1995). Gudmundsson et al. (1994) also evaluated the use of radiographic methods for elucidation of consumption data with salmonids and found the method suitable, however commented on the variability of the method making small differences in the feed consumption difficult to detect. Perera et al. (1995) however did not report the same difficulties.

The use of graded levels of a test diet rather than the use of a single test diet with a fixed level of substitution, reveals more information about a test ingredient than the approach of using a 70/30 substitution of a reference diet. Effect of inclusion level on consumption rates of feed can be assessed and in combination with radiographic methods individual consumption rates and therefore nutrient budgets evaluated. For the evaluation of large numbers of discrete ingredients this method would however be very time consuming. The use of a single reference/test diet combination for assessment of absorption efficiency has the advantage of a large number of ingredients can be evaluated relatively rapidly. However the information is limited, no quantitative evaluation of effect on consumption rates may be made only subjective evaluation. However the use of graded inclusion of a test ingredient reveals far greater detail of the nutritional value and effects of levels of inclusion of an ingredient.

The provision of a single digestibility coefficient to a formulator of commercial diets is helpful but without the knowledge of maximum inclusion levels and the reasons for these levels the importance of the digestibility coefficient is negated as diets can not confidently formulated as the effects of varying inclusion levels is unknown.

Therefore the use of a 70/30 substitution of an ingredient has merit when large numbers of ingredients need to be evaluated. This method could perform a screening function, however full elucidation of the nutritive value of an ingredient could only be achieved by the use of graded levels of an ingredient combined with an assessment of consumption of feed and the evaluation of nutrient budgets.

The effect of processing techniques used to manufacture finished feeds is also of great interest to the milling industry. The answer to the question of what processing technique will give the same groups of ingredients an advantage over alternative processing forms is keenly sought by most manufacturers.

The results of this study were used to develop a commercial formulation which was trialed against existing commercial barramundi formulations. The results of this trial will be fully discussed at the end of this section. Appendix one contains a copy of the commercial company's internal report, reprinted with permission.

Crude Protein

Protein in fish feeds is incorporated to meet the minimum amino acid requirements and growth potential of the species. Investigations of this study have focused on the digestibility of the macronutrient protein rather than the amino acid requirements of the barramundi as to the knowledge of the author, amino acid requirements have not been fully described for this species.

Protein requirement is little affected by increasing temperatures, Brett (1979, in NRC 1993) described a parallel increase in growth and feeding functions with temperature increase but attributed increased growth to improved feed conversion efficiency and higher intakes, however no investigation of variations to absorption efficiency related to temperature were described. As is common with most digestibility studies my work was carried out at a constant temperature.

However the effect of temperature on digestibility is worthy of future investigation to elucidate if an increase in growth and food utilisation is a function of changed digestibilities of ingredients due to temperature or increases in metabolic functions of the fish. Methodology of collection of faeces and the effect on absorption efficiencies has been examined recently by a number of authors ,Perera et al. (1995*b*); reported significant differences between the markers used and Williams et al. (1996) reported significant differences between collection methods of faeces.

The protein A.D.C. for various fish meals has been investigated by Hajen et al.*a,b*, (1993) using post juvenile chinook salmon *Oncorhynchus tshawytscha* as a test animal, table fourteen summarises the fish meal components of the study. The "Guelph" continual filtration method was employed in the derivation of protein A.D.C.s. The processing of some fish meals is described by and using the criteria proposed by Pike et al., 1990, the quality of the herring meals and Menhaden meal described, would be of a high nutritional quality . Given these criteria of low processing temperatures the protein A.D.C.s would be expected to be high for these meals. No description was given though for fish meal supplied from Anchovy (Chillian in origin).

This study utilised four fish meals, two processed from fish captured specifically for the production of industrial fish meal (Danish & Chilean Fish Meals) and two produced from offal after fish have been processed for human consumption (Tuna & Mullet Fish Meals). All of the fish meals used in the study carried out by Hajen, et al.*a,b*, (1993) could by classified as industrial fish meals. There exists some similarity in the results of this study and that carried out by Hajen, et al.*a,b*, (1993).

As can be seen from table 14, the industrial fish meals with high quality have high protein A.D.C.s. Menhaden meal which processing is not described shows a much lower protein A.D.C. similar to that displayed by both Danish and Chillian Meals from this study. Both of the industrial meals used in this study although described as suitable for use in

aquaculture diets have not been produced by the " Low Temperature" method as described by Pike et al., (1990). Offal meals used in this study (Tuna & Mullet) returned lower protein A.D.C.s than the industrial fish meals used in this study and much lower than those reported by Hajen et al.*a,b*, (1993). This would support the study carried out by Andorsdottir et al., (1989 In Pike et al. 1990) on the significance of fish meal quality and its effect on protein A.D.C.. Foster (1992) also reviewed the quality of fish meals, particularly those available to the Australian aquaculture feed industry and found wide variation across suppliers and even within batches from suppliers. Basing an assessment of quality of freshness of raw product through total volatile nitrogen (TVN), processing temperature of meal, levels of biogenic amines and pepsin digestibility concluded that Danish sourced fish meals were of high quality but were expensive, relative to fish meals sourced from South America.

Williams et al. (1996), reports a protein A.D.C. of barramundi for Danish and Tuna fish meals of 86.8% ± 1.24 and 83.2% ± 1.24 respectively which concurs with the results generated from this study. The study by Williams et al., (1996) also addressed the issue of leaching of nutrients from the faecal material in contact with water over time, and concluded that leaching of soluble nutrients tended to over-estimate protein A.D.C.s. Titanium dioxide was used in the Williams et al., (1996) research but was not validated against other methods or markers such as Cr_2O_3.

Cho et al. (1982) found a protein A.D.C. for defatted soybean meal of 96% in rainbow trout, However, Hajen et al.*a,b*,(1993) reported for the same ingredient a protein A.D.C. of 77.0 % ± 4.25 for chinook salmon. Ogino & Chen (1973), reported a protein A.D.C. of soybean using common carp (*Cyprino carpio* L.) as the test species as 86.15 and Eid & Matty (1989), also using common carp reported a protein A.D.C. of defatted soybean meal of 83.2 ± 1.2. This current study found a protein A.D.C. of 89.5 ± 1.1. From these results the protein A.D.C. of defatted soybean meal would fall within the mid range of reported values for this ingredient in Barramundi.

The protein A.D.C. of Extruded Full Fat Soybean Meal found in this study was 88.7% ± 0.04. Cho et al., 1982 however reported a protein A.D.C. in rainbow trout of 96% . Pongmaneerat & Watanabe, (1993*a*), used defatted soybean meal as the sole protein source in experiments with rainbow trout and found protein A.D.C. was high at 92% however importantly growth and protein efficiency was low which was attributable to an inadequate amino acid profile of the soybean meal.

The effect of soybean proteinase inhibitors in fish have been investigated by a number of researchers, Krogdahl et al., (1994) and Kaushik et al. (1995), found that increasing the levels of soybean proteinase inhibitors in diets containing high levels of fishmeal reduced the protein A.D.C. from 93% to 70% in rainbow trout. Lipid A.D.C. was however not affected. However Olli et al., (1994) found, in similar experiments with Atlantic salmon, that both protein and lipid A.D.C.s were affected. It is apparent that the effect of soybean proteinase inhibitors is common across fish species, however an additional effect on lipid A.D.C. has been noted and would seem to be species specific. However the effect of soybean proteinase inhibitors would in a practical application have potentially less effect in extruded feeds than pelleted feeds if the processing of the feeds in the extrusion system was such that they were deactivated due to sufficient heat and time exposure. But extrusion of soybean products within a compounded feed does not often fully deactivate proteinase inhibitors possibly due to the lower temperatures that compounded feeds are extruded at compared to soybeans destined for meal production, and the potential for masking of discrete particles of soybean by other lipids in a compounded feed that acts as a protectant to heat deactivation of proteinase inhibitors. This is however very dependent on the type of extrusion system and the processing parameters used with that system.

Rainbow trout used in the study by Cho et al. 1982 reported a protein A.D.C. for Soybean Protein Isolate of 97 % however Chinook Salmon returned a lower protein A.D.C. for this ingredient 86.3 ± 1.08 in a study carried out by Hajen et al.(1993). This study found a much lower protein A.D.C. for this ingredient of 72.9 ± 0.29.

Sources of ingredients were not described in the literature for the ingredient so it is difficult to assume that a direct comparison can be made. The removal of soluble carbohydrates by chemical washing will increase the proportion of the protein components including the anti-nutritional factors (eg., trypsin inhibitors) and concentrations will be higher in this type of ingredient than other heat treated soy products (eg., defatted and extruded full fat meals) (Carter 1994).

The protein A.D.C. of soya protein isolate is significantly different from the other two soya products investigated in this study. Soya protein isolates have higher levels of anti-nutritional factors which would manifest their effect on growth but possibly not as an effect on protein A.D.C. (Carter 1995). Why this lowered level has been found is unclear. It is suggested that some effect may be imparted by the processing of soya beans into the protein isolate that is altering the protein A.D.C..

Ingredients sourced from terrestrial animals have been indicated as potential's for the replacement of fish meals as a protein source for fish diets, (Manzi, 1989; New, 1991; Allan, 1994). meat & bone meal and blood meals have been investigated by other researchers in respect to their protein A.D.C. for various species. Cho et al. (1982) found protein A.D.C. of 85 %, 91% and 55% for meat & bone meal, spray dried blood meal and flame dried blood meal respectively, for rainbow trout. Hajen et al., (1993) only determined the protein A.D.C. of batch dried blood meal, similar in manufacturing process to flame dried blood meal, for chinook salmon and reported a level of 29.4 % ± 1.20. The protein A.D.C. of meat & bone meal and spray dried blood meal found for this study was 71.0% ± 0.93 and 95.8% ± 0.35 respectively.

It is predicted that in common with other fish species, significant differences will exist in protein A.D.C. of blood meals in barramundi, that are produced using different manufacturing methodologies.

The yeast chosen for this study was done due to the cost which is a feature of interest to the feed milling industry. Other yeasts and single cell proteins (SCP) that have been studied in relation to fish nutrition have been expensive and they have been not utilised by commercial feed millers because of this fact. Rumsey et al. (1991) found that the disruption of the cell wall of brewer's yeast increased the absorption of nutrients and the complete removal of the cell wall further increased absorption. The cell wall is resistant to enzymatic action which does reduce the absorption of nutrients. Perera et al. (1995) postulated the same effect with bacterial single cell protein (BSCP) but also found that the urea nitrogen increased but the NH_3 nitrogen did not change. The authors suggested that nucleic acid intake and catabolism increased with increasing levels of BSCP due to the high levels of nucleic acid found in this ingredient. The pathways for the reduction of nucleic acids favoured the formation of urea rather than the formation of tissue, thus explaining the lowered SGR for fish fed elevated levels of BSCP.

As the yeasts are characterised by high levels of high nucleic acids like the BSCP the lowered digestibility could also be a function of this. It is probable that the relatively low A.D.C. for this ingredient is a function of the effects of the cell wall and the levels of nucleic acids found.

Nitrogen excretion was not measured in this work but further investigation would be warranted to ascertain the effect of graded levels of yeast on the total nitrogen excretion. Also the effect of disruption yeast cell walls as a factor affecting the absorption of nutrients would be justified.

The protein A.D.C. of sweet torula yeast found by this study was 63.5% ± 0.50. This ingredient has not been reported in the literature in respect to fish A.D.C.s. However Cho et al. (1982) reported a protein A.D.C. for brewers yeast of 91% (no SEM stated). Shcherbina et al. (1987) investigated the protein A.D.C. of species of *Candida* yeasts fed to common carp and reported a protein A.D.C. of 85%.

Differences in the two studies could be explained by different types of yeast used as well as a species response difference. Variations from the literature and results from this study indicate variations of protein A.D.C. for yeast species.

As there has been no direct comparison of a particular yeast species across different fish species these reported variations could be attributable to different fish species ability to digest and assimilate yeast protein, variation within the proteins of different species of yeast that alter their digestion and assimilation or a combination of both of these rationale.

The use of prawn head and shell meal is not a common ingredient in fish diets, its use is more common in shrimp and prawn diets, (Evans,1992 ; Devresse, 1995). Robertson, (1988) found that barramundi were the single most important predator of juvenile *Penaeus merguiensis* prawns in the north of Australia. These prawns made up to 22% of the diet of barramundi in the area. Sabapathy and Teo (1993), subsequently investigated the digestive enzymes of barramundi and found that of the carbohydrases, the chitinase activity was significant in the intestine and pyloric caeca. With chiton forming a major part of the composition of prawn shell its use as a practical ingredient has thought to be limited and possibly deleterious to both the protein and lipid A.D.C.s. Although the assessment of chiton digestibility was outside the scope of this investigation the protein A.D.C. did not appear to be affected by the high levels of chiton in the test diets. Prawn head and shell meal protein A.D.C. was not significantly different to other protein sources that would have insignificant amounts of chiton such as meat & bone meal and modified soya protein.

Although not often used as a direct nutritional ingredient for practical or experimental diets, wheat and wheat by-products do however comprise significant proportions of aquaculture feeds. More often their use is as a binder or filler . Cho et al., (1982) reported a protein A.D.C. of 92 % for wheat middlings in rainbow trout. Hajen et al.*a,b*,(1993) reported a protein A.D.C. for the same ingredient in chinook salmon of 85.7% ± 1.37 and 84.3% ± 0.95 for extruded wheat was reported.

This study found a protein A.D.C. of 76.2 ± 3.07. The addition of wheat to a diet can affect the protein or lipid A.D.C.s of certain species. Arnensen & Krogdahl, (1993) found that increasing the levels of wheat in diets fed to Atlantic salmon decreased growth but had no effect on protein or lipid A.D.C.s. The same effect has been noted for rainbow trout (Bergot, 1991). However, Hemre et al. (1995) found that increasing levels of undigested starch from wheat, although not effecting protein digestibility did lower lipid A.D.C. in Atlantic salmon. A species ability to digest starch would seem to play a significant effect on any deleterious effects of increasing starch levels in the diet on the A.D.C. of protein and lipids. Herold et al., (1995) found that in the white sturgeon *Acipenser transmontanus* both protein and lipid A.D.C. were affected by increasing starch levels in the diet. The protein A.D.C. of wheat reported in this study although lower than reported for other species could possibly have been reduced due to high levels of carbohydrate in the diet for a carnivorous species. However a separate trial would have had to be conducted to evaluate this proposed effect, with incremental levels of starch which was outside the scope of this study.

As the significant protein fraction of wheat is in the form of gluten the addition of this ingredient *de novo* shows potential as a nutritional ingredient. The inclusion of wheat gluten has mostly been as a form of binder for fish foods. This study however confirms the nutritional contribution of this ingredient to barramundi.

However it is noted that the faecal pellets were very different to all other ingredients tested. Where all other faecal pellets were well defined those collected for the wheat test diet were very plastic in appearance and form. Also growth rate of the fish fed this diet was the lowest recorded. Similar differences were noted by Williams (personal comment) with increasing levels of this ingredient and barramundi.

Lipids

Fish have a requirement for lipids not only as an energy source but as a supply of essential fatty acids. The majority of fish, have a requirement for w-3 fatty acids and there may be a small but significant requirement for the w-6 fatty acids. The commercial feeding of barramundi has relied on the supply of feeds based broadly on salmonid diets with the total added lipids being of marine origin (MacKinnon et al., 1987). However , the use of alternative sources of lipids other than those of marine origin, to supply dietary requirements for farmed fish is an area of active research. Reinitz and Yu (1981), found with rainbow trout fed dietary lipids consisting of 5% marine lipid or 9% lipids comprised of terrestrial animal lipids or soya bean oil showed no difference in growth.

This program of study although not measuring growth as a parameter investigated if any differences could be found between the Apparent Digestibility Coefficient (A.D.C.) of lipids derived from ingredients of both terrestrial and marine origin.

Borolongan and Parazo (1991), conducted experiments where soybean oil, cod liver oil and coconut oil were tested either singly or in combination as a source of dietary lipid for barramundi. The highest growth rates were obtained with a 1:1 mix of cod liver oil and soybean oil. Buranapanidgit (1989 in NRC 1993) described a requirement for barramundi of 1% of 20:5(n-3) and 22:6(n-3) . Wanakowat et al. (1991) refined these findings by examination of total n-3 requirements of barramundi and found that the total n-3 requirements for the species was 1%.

Given that the essential n-3 requirements of barramundi are similar to other species documented in the literature (NRC 1993), the lipid types that are able to be incorporated as practical ingredients is relatively broad when compared to such species. This information from the literature has enabled the investigation of lipid sources from both terrestrial and marine origins.

It has been proposed that lipids derived from marine sources generally show a higher A.D.C. than lipids derived from terrestrial sources, as the fatty acid chain length in length from C18 to C22, the digestibility increases and the digestibility of unsaturated fatty acids is higher than that of saturated fatty acids of the same chain length (Austreng et al.,1979; Cho et al 1982; Hajen et al.*a,b* (1993)). As the terrestrial sources of lipid have a tendency to have greater proportions of saturated fatty acids than marine lipids. However this study found that differences exist between the type of marine lipid and the A.D.C. that was found. Six marine lipid sources were investigated and of the lipids sources from fish meals, that derived from mullet fish meal showed the lowest A.D.C.. Chillian fish meal showed the highest lipid A.D.C. of all fish meals tested. Cho et al. (1982), reported a lipid A.D.C. for herring fish meal of 97%, which is higher than all lipid A.D.C.s for this study. The quality of lipid component of fish meal has an important effect on the feeding value of the fish meal when feed to fish, any oxidation is detrimental to intake and potentially digestibility of those lipids, Pike et al. (1990). Fish and fish products including fish meals are particularly prone to detrimental oxidative lipid damage due to the high degree of polyunsaturation of their lipids. The effect of oxidative rancidity can extend even to final products obtained from cultured fish (Baker and Davies 1996). This may explain the differences in lipid A.D.C.s found. The quality of the type of meal reported by Cho et al. 1982 was however, not discussed. However if the quality was high as is to be expected for herring meal then this would account for the high lipid A.D.C..

This study found a lipid A.D.C. for Danish fish meal(standard A grade) of 83.8% ± 0.85, Williams et al., (1996) also using barramundi as a test animal reported a lipid A.D.C. for the same ingredient of 83.2% ± 1.24.

Ackman, et. al. (1987 in Pike,1990), discusses the large differences found in the basic fatty acid composition of lipids of marine origin. It is proposed that the lower A.D.C. found in lipids derived from tuna fish meal (mean 73.8 % ± 2.13) could be attributable to the predominance of longer chain fatty acids in this lipid when compared to other sources of marine lipids (Cho pers comm).Williams et al. (1996) also reported a lipid A.D.C. of 64.65% ± 1.20 for the same ingredient. This later study of Williams et al. (1996) supports the results generated from my study.

The low lipid A.D.C. for squid meal (69.7 % ± 0.67) is unusual for a marine lipid source. However the quality of processing this meal may explain the lower than expected A.D.C. for a marine source. The supplier quotes a processing time of 120 minutes above 100° C. Andorsdottir et al. 1989, (In Pike et al. 1990), reported that salmon fed two different fish meals showed significant differences in lipid A.D.C.. A severe effect was noticed on lipid A.D.C. for fish fed fish meal that had been processed at 140° C as compared with fish fed with fish meal produced at the lower temperature of 60° C. The processing techniques of this meal correlate with the upper extreme in the study carried out by Andorsdottir et al. 1989 (In Pike et al. 1990), and the results of this study would concur with this earlier investigation.

The different types of soya products chosen for this study, reflected their commercial importance. All three are either recognised or proposed as major sources of protein and lipid in the formulation of aquatic diets (Akiyama, 1989*a*; Akiyama, 1989*b*; Lim & Dominy, 1990; Lovell 1991). However the type and level of inclusion of soya products may well vary with species and the age and size of the species Lim & Akiyama, (1991).
The production of these 3 types of products and their significance to commercial feed production are fully explained by Carter,(1995).

Significant differences exist between the lipid A.D.C. for the different types of soya derived ingredients used in this study. Carter (1995) points out that the precise conditions of processing of the soya products is rarely available and therefore the effects on A.D.C. of different sources could be confused by this factor. Hajen et al. (1993) found that the solvent extraction process of canola meal as described by Naczk et al. (1986), which is similar to the process used in the hexane extraction of lipids from soya products, lowered the lipid A.D.C. in salmonid species when compared to un-extracted ingredient sources. The reduction in A.D.C. was attributed to possible losses in readily soluble carbohydrates and proteins, however the physical or chemical effect on lipid sources were not discussed. It was proposed that the extraction process as a result of loss of these elements of the ingredient lead to the increase in the proportion of more indigestible components of the ingredient. Carter (1995), reported the same effect for soya products when fed to pigs. From a practical perspective the variation that is evident in soybean products poses great difficulties to manufactures of commercial aquaculture feeds. The performance of feeds may be compromised from the variability that is caused by nutrient absorption between different products.

Diet Production Techniques

It has been proposed by a number of researchers that the method of manufacture for the diet that is to be fed to test animals will affect the A.D.C., (Cruz, 1975; Wee, 1992;Wilson & Poe, 1985) the most significant effects noted were due to extrusion as compared to pressed feeds. An experiment was incorporated into this present study to elucidate the effect of different production techniques on the same diet and the subsequent effect on lipid and protein A.D.C.. As the production of feed in suitably small quantities by the steam extrusion method was not possible it was decided to compare feed produced with moderate amounts of treatment by steam pressing and with the same formulae produced with no heat at all, via cold extrusion.

The reference diet used through out this study was produced from the same mixed batch of ingredients and was either produced using a laboratory pellet mill (CPM CL-2) or extruded through a kitchen mincer and dried in a laboratory drying oven (convection). For both protein and lipid A.D.C. a significant difference (student's t test p= 0.05) was noted between the two treatments. Steam pelleting of the reference diet had the effect of increasing protein A.D.C. from 86.2% ± 1.49 to 95.1% ± 0.27. Steam pelleting uses heat, moisture and pressure to produce pellets that are water stable. The process also partially or fully gelatinises, dependent on processing conditions, the starch found in the ingredients. As protein digestibility tends to be depressed as the concentration of carbohydrates increase (Inaba et al. 1963; Kitamikado et al. 1964 *a,b*; Nose 1967; Page and Andrews 1973; Smith and Lovell 1973; Austreng et al. 1977; Rychly and Spannhof 1979 In NRC 1993) which in part is due to replacement of protein by the increasing levels of starch. However, conversely as the levels of starch become more nutritionally available through the process of gelatinisation the protein digestibility could rise. Borlongan and Parazo (1991) when evaluating barramundi nutrition found that the fish did not favour carbohydrate as an energy source, however the gelatinisation of the starch molecules in diets and the subsequent potential increase in availability of nutrients from carbohydrates may in turn affect the absorption of other nutrients such as protein.

Lovell, 1984, found that an increase in protein A.D.C. in channel catfish when fed extruded as compared to non extruded feeds. However the same change was not noted for lipid A.D.C. in the same experiment. This study however found a significant difference in lipid A.D.C. for steam pelleted feeds of 98.8% ± 0.0 and 86.7% ± 1.39 for cold extruded pellets(Table 11).

Hajen et al.(1993)*a,b*, found a significant difference in lipid A.D.C. when two similar wheat products were compared for lipid A.D.C. in chinook salmon, although not identical, the ingredients, wheat middlings and wheat are comparable. The wheat component was extruded but the wheat middlings was presented to test animals in an uncooked form.

Extruded wheat returned a lipid A.D.C. of 71.3% ± 2.82 but the uncooked wheat middlings only reported a lipid A.D.C. of 45.3% ± 1.95.

Growth and Performance

The results of the regression analysis of protein A.D.C. for diets and ingredients verses the specific growth rate showed a poor correlation for both tests. The analysis of diets and S.G.R. returned a R squared value of only 0.04 and the ingredients verses S.G.R. reported a R squared of 0.03.

This study investigated the digestion and absorption of nutrients in ingredients but not the assimilation of these nutrients. However, these results suggest that although discrete nutrients are digested and absorbed no single ingredient provided all the nutrient requirements for growth, measured as weight gain over time. The amino acid profile and levels is one possible area where individual ingredients may not meet the requirements for growth of the fish. Mohsen and Lovell (1990, in N.R.C., 1993) describe that some individual protein sources have been found to have beneficial effects in catfish diets not fully explained simply on the basis of meeting the amino acid requirements of the fish. The results of fish growth rates (SGR) and weight change (Table12) show that some combinations of test ingredients (defatted soya meal, Danish fish meal, tuna fish meal, soya protein, full fat soya meal, blood meal and mullet fish meal) and reference diet gave greater growth rates and SGR than the reference diet alone. The original concept of the reference diet as proposed by Cho et al. (1982) was of a diet that was similar to a commercial diet and was able to sustain growth not maximum growth. The contribution of test ingredients therefore potentially can enhance the nutritional value to the tested species by direct addition of nutritional components or even through a synergistic effect with components of the reference diet. If a synergistic effect is occurring however the difference between the true and the apparent digestibility will become greater and the nutritional value of the

ingredient could be overestimated. This situation would concur with the results of the regression analysis.

The use of graded inclusion of ingredients for the determination of digestibility coefficients would give greater detail of the interaction between different ingredients and their level of incorporation on the digestibility coefficients, effects on consumption rates and the potential affect of any antinutrients or adventitious toxins that effects, could be possibly masked by the use of single levels of inclusion in a test diet. A further extension of this investigation should involve the elucidation of pathways of discrete nutrients within the test fish. Perera et al. (1995*a,b*) used a graded inclusion of bacterial single cell protein in the investigation of absorption and nutrient budgeting. The use of graded inclusion of the test ingredient found significant differences in the absorption of amino acids (AA) between different levels of inclusion in the diet and importantly suggested that the AA absorption efficiency is a more useful measurement of nutritional quality of an ingredient than the dietary AA composition. The same proposal is useful for the description of the macronutrient of protein absorption rather than the level in the ingredient.

This situation does however, indicate the limitations of using A.D.C.s for the preparation of practical fish diets. Determining the digestibility of individual components of ingredients does not indicate the assimilation of these nutrients or the relative importance of discrete nutrients within ingredients.

Comparison of commercial and experimental diets for grow-out barramundi

Summary

A commercial feed company, Ridley Agriproducts, were interested in a practical evaluation of the commercial viability of data generated from this study. The company had already been suppling the Australian barramundi industry with pelleted feeds for a number of years but saw the opportunity for cost reduction of their diets through utilisation of some of the findings from this study.

A six week growth experiment was conducted in cooperation with and at the Queensland Department of Primary Industries, Walkamin Freshwater Fisheries and Aquaculture Research Centre, North Queensland, Australia, to evaluate 4 diets under two different temperature regimes to simulate summer and winter growth periods (full report, appendix one). An existing commercial diet (R15MJ)was used as the control and will be described as having a cost of raw materials as factor of 1.00 to retain confidentiality for the commercial feed company involved. The diet that resulted from this study is assigned the code of (R14MJ), and had a cost of raw materials of 0.86 relative to the existing commercial diet. Two other experimental diets identified as (Wlk-S) and (Wlk-W) were also trialed with respective ingredient costs factors of 0.92 and 0.76.

At a water temperature of 23°C (Mean winter temperature), there was no significant difference between diets measured as weight gain of fish although the diets R15MJ and Wlk-W tended to perform at a lower level. However at a higher water temperature of 28°C (Mean summer temperature) the diet Wlk-W was markedly inferior to all other diets trialed.

Efficiency of food conversion was found to best for the diet Wlk-S and superior to the Commercial diet R15MJ which was inturn better than R14MJ and Wlk-W.

Irrespective of water temperature the most cost effective diet was R14MJ(measured as Kg. live weight gain/$ Unit.) at 121.6 units which is the diet developed from results of this study. The next best performing diet was Wlk-S diet with a result of 111.1units equalled by the Wlk-W diet with 111.1 units followed by the existing commercial R15MJ with 97.5 units.

These results concur with the original concept that the use of salmonid specifications was incorrect for this species. The salmonid model is fairly inflexible in regard to the substitution of alternative ingredients to fish meal and oils which can present supply problems to commercial feed manufacturers. Also with this constraint on substitution of ingredients the cost of diets is relatively high due to the high levels of fish meals and oils. The substitution of other ingredients once their individual apparent digestibility coefficients for major nutritional components was known, would enable the formulation of diets that would be able to maintain growth and food conversion efficiencies acceptable to commercial producers of this species at a reasonable cost.

Table 14.

Apparent Digestibility Coefficients from different studies.

Ingredient	ADCp % (+/- SEM)	Identifier	ADCl (+/-SEM)	Identifier
Herring Meal (Canada)	90.5 +/- 0.51	1	97.0 (no SEM)	4
Anchovy Meal (Chile)	91.7+/- 1.50	1		
Menhaden Meal (Unknown Origin)	83.1+/- 1.45	1		
Low Temperature Herring/Caplin Meal (Norway)	93.6+/- 0.81	1		
Danish Fish Meal (Denmark)	83.8+/- 0.85 86.8+/- 1.24	2 3	83.8+/- 0.85 83.2+/- 1.24	2 3
Mackerel Meal (Chile)	82.6+/- 0.78	2	84.4+/- 0.28	2
Tuna Meal (Solomon Is.)	75.4+/- 2.14 83.2+/-1.24	2 3	73.8+/- 2.13 b 64.6+/- 1.20 c	2 3
Mullet Meal (Australia)	55.1+/- 4.03	2	68.7+/- 0.83	2

Where:

A.D.C.p = Apparent Digestibility Coefficient, Protein.

A.D.C. l = Apparent Digestibility Coefficient, Lipid.

1; Hajen et al. (1993) (chinook salmon)

2; Current Study (barramundi)

3; Williams et al. 1996. (barramundi)

4; Cho et al. 1982. (rainbow trout)

Summary Conclusions

- Processing techniques do have an effect on the apparent digestibility coefficient of ingredients when applied to the barramundi. The indication is that with more processing the greater the digestibility for proteins and for some lipids.

- The quality of proteins and lipids of marine origin does have a profound effect on the digestibility in the test animal. Ingredients with suspected poor processing during manufacture did not perform as those with specific manufacturing controls that are directed towards aquaculture ingredients.

- The procedure of stripping for the collection of faeces for tropical species may well have merit once procedures are in place to obviate the influence of extraneous body fluids.

- The literature reports that the production of diets using extrusion technology has little or no effect on protein A.D.C. however lipid A.D.C. is often effected as is energy and carbohydrate A.D.C.s. This study found a significant difference between protein A.D.C. for steam pelleted and cold extruded methods of production.

- The proportion of effect of soybean ingredients on protein and lipid A.D.C.s is species specific. The extent to which the effect occurs in barramundi was outside of the scope of this research but further investigation utilising graded inclusion of soybean ingredients would ascertain the effect on protein and lipid A.D.C.s.

- The effect of increasing levels of starch on protein and lipid A.D.C.s in barramundi is also an area that warrants further investigation.

- The use of a salmonid model with little substitution of fish meals and marine oils for the nutrition of the barramundi, *Lates calcarifer* Bloch does not allow the flexibility in formulation of diets attributal to the barramundi's ability to digest a diverse range of ingredients that are not of marine origin. The continued use of this model is therefore not recommended.

- The principle of gravity settling for the collection of faeces is appropriate for use in tropical species. The methodology is however dependent on the use of reference diets that produce faecal pellets that are solid or semi-solid.

- The use of the continual filtration method although developed for use with cold water species has application in the determination of Apparent Digestibility Coefficients for crude nutritional parameters. However if a more detailed investigation of A.D.C. were to be carried out on amino acid or fatty acid components of the diet it is suggested that modifications would be required to the procedure to reduce the risk of results affected, due to microbial action.

- The carrying out of more detailed investigation using the concept of graded inclusion of ingredients to determine the effect of inclusion level on A.D.C. for protein and lipid as well as the effect on palatability and consumption is a logical next step in the evaluation of the nutrition of the barramundi. Concepts and methods employed by Perera et al. (1995*a,b*) are appropriate for this continued evaluation.

- The use of digestibility coefficients does not elucidate the assimilation or metabolism of nutrients from ingredients. This information would however give more detail of the importance of particular ingredients and is a recommended for further evaluation following measurement of A.D.C..

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Appendix 1

Comparison of Commercial and Experimental Diets for Growout Barramundi: Walkamin 1995.

Ridley Corporation Internal Report

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Introduction

Research at QDPI's Walkamin and Bribie Island Aquaculture Centres has clarified the nutrient requirements of grow-out barramundi and evaluated the suitability of a number of terrestrial protein sources as substitutes for fishmeal. Barramundi are able to digest the protein and fat of a wide range of terrestrial animal and vegetable food sources but have a reduced capacity to metabolise diets high in digestible carbohydrate. Abattoir by-product meals such as meat and bone appear to be very suitable as alternatives to fishmeal provided that due care is taken to ensure dietary essential amino acid profiles are maintained. Importantly, the research has also shown that water temperature has a profound effect on appetite and hence on the growth capacity of the fish. In cool water (<23-24°C), fish have a poor appetite and consequently, their requirement for dietary protein (essential amino acids) is lower than is the case in warm water (>26 - <31°C) where appetite and growth are high. Similarly, requirements for poly unsaturated fatty acids (PUFA's) such as docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA) are lower in cool water than in warm water.

In practical terms, these findings imply that it may be more cost effective to provide fish in winter with a low-protein 'holding' diet formulated with high inclusions of terrestrial protein meals, while in summer the diet should be of a higher protein content (containing substantial inclusions of terrestrial protein sources) and providing adequate amounts of essential PUFA's. These findings need further substantiation before being recommended for use in commercial diets.

This Report describes the results of an experiment at QDPI's Walkamin Fisheries Centre undertaken to evaluate four diets for grow-out barramundi. The experiment compared the two currently available commercial diets (referred to as Ridley 15MJ and Ridley 14MJ) with two experimental diets which were formulated to contain high inclusions of terrestrial protein meals and to satisfy the fish's nutrient requirements for growth at either summer (Wlk-S) or winter (Wlk-W) water temperatures, respectively.

Methodology

Design 4x2 factorial with 3 replications to compare four diets (Table 1) and two water temperatures (23 vs 28°C). Replicates were positioned within the experimental facility as three (3) blocks with fish being selected on the basis of weight uniformity. Two independent water recirculation systems were used for each of the two water temperatures. The experimental unit was the individual tank (n = 24) which had a capacity of 1 000 L.

Fish Prior to allocation to experimental tanks, fish were held in water of 26°C and fed a common diet. Fish were sorted by weight into an experimental group of 600 fish each of approximately 200 g with these being randomly and equally distributed to the 24 tanks (25 fish/tank). Fish were acclimatised to the experimental conditions for three weeks prior to the start of the experiment. In this acclimatisation period, all fish received a salinity change (12 ppt salinity for 2 hr) for preventative ectoparasite management.

Table 1 Formulation and estimated nutrient composition of the diets

Ingredients	R15MJ	R14MJ	Wlk-S	Wlk-W
			(%)	
Maize		9.5		
Wheat	27.1	9.5	18.2	29.6
Fish meal 65%	54.2	14.8	10.0	5.0
Blood meal	6.5	14.7		
Meat meal 52%		20.0	45.0	35.0
Soybean meal FF			16.0	20.0
Soybean meal DF	6.5	13.0		
Lupin meal		12.6		
Gluten			5.0	5.0
Lysine HCl	0.66	0.55	0.6	0.3
Methionine		0.4	0.3	0.15
Salt			0.25	0.25
Fish oil	4.22	4.3	4.0	4.0
Ca Proprionate	0.28	0.25	0.25	0.25
Choline Cl 60%	0.28	0.125	0.1	0.1
V+M premix	0.28	0.2	0.2	0.2
Vit C - coated	0.06	0.125	0.125	0.125
TOTAL	100	100	100	100
			Estimated nutrient content[2]	
DE kJ/g	15.3	14.8	15.0	15.2
CP%	47.5	44.0	43.0	37.1
Fat%	9.3	9.0	12.7	12.2
Lys%	4.19	3.25	2.62	1.96
M+C%	1.72	1.55	1.41	1.18
Thr%	2.10	1.73	1.43	1.21
Try%	0.56	0.50	0.38	0.33
Arg%	2.67	2.53	2.67	2.27
C20:5%	0.80	0.52	0.46	0.42
C22:6%	1.40	0.78	0.67	0.59
n-3:n-6	5.76	1.58	0.89	0.75

1 Stated prices of raw ingredients in October 1995 at Ridley's Narangba plant.
2 Nutrient content estimates based on Author's feed's database.

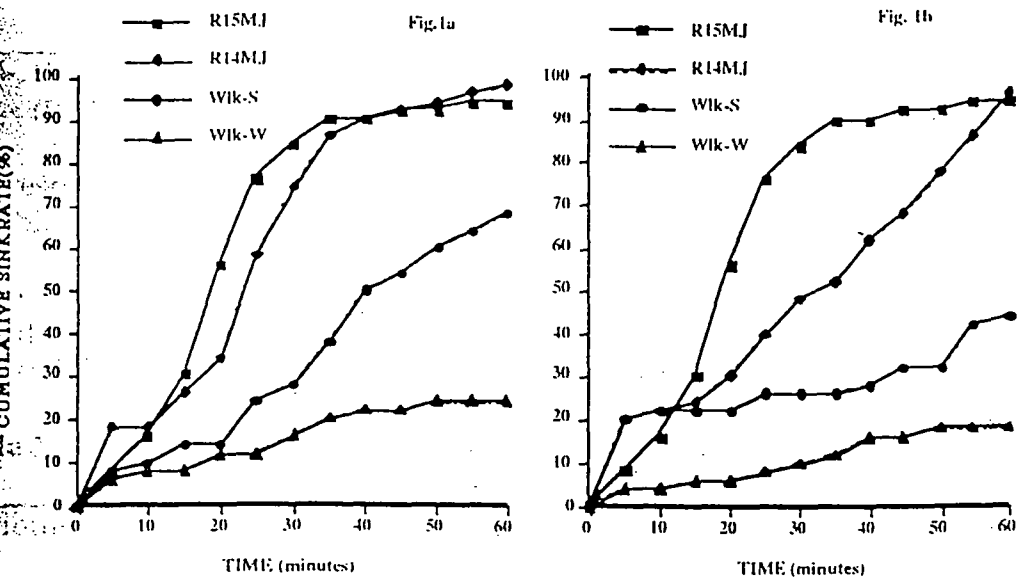
Management The experiment commenced on 24/25 October 1995 and continued for six weeks until 5/6 December 1995. Fish were fed to satiety twice daily except on the day of the fortnightly weighing. After 45 minutes from food allocation, uneaten pellets were counted and removed from the tank. Actual food consumption was determined by reconciling the amount of food given and the weight of uneaten pellets (using relationships between dry pellet number and weight derived for each diet). All fish were salt bathed (12 ppt for 2 hr) on day of weighing. Water temperature measurements were monitored daily and water quality determinations (dissolved oxygen, pH, nitrite and free ammonia) were made twice weekly.

Results and discussion

Diets

The 6 mm extruded diets contained a minimum of fines and pellets were of uniform size within each diet type. However, marked differences were observed in the density of the various diets. Both of the commercial diets sank faster than either of the experimental diets with the Wlk-W diet being the most buoyant with fewer than 20% of the pellets sinking after 60 minutes (Figure 1). The high buoyancy of the Wlk diets undoubtably was due to the small size of the mix used in their preparation rather than to any peculiarity of the dietary ingredients. Fish were initially reluctant to feed at the surface, particularly those in 23°C water but this was overcome by lowering the light intensity in the laboratory.

Figure 1 Cumulative sink rate of the test diets in water at 23°C (Fig. 1a) or 28°C (Fig, 1b)



Fish health

The health of the fish was good throughout the experiment with only 2 of the 600 fish being removed because of disease (peritonitis). However, a number of fish had a distended swim bladder necessitating the removal of nine fish because of disoriented swimming. Veterinary examination (Annex A) showed the absence of any infectious agent. Affected fish were equally distributed amongst all tanks (Tables 3 and 4) and were unlikely to have influenced treatment responses. The condition is thought to have been a genetic abnormality.

Response to diets

There was a significant ($P<0.05$) interaction between water temperature and fish growth rate. Differences between diets were minimal (and not significant) at 23°C whereas at 28 °C the low protein winter formulation diet (Wlk-W) was inferior to all other diets (Table 2). The ingredient cost to convert feed into gain was similarly less differentiated at 23 than at 28°C.

Table 2. Interaction effects between water temperature and diet

Water temp (°C)	Diets				
	R15MJ	R14MJ	Wlk-S	Wlk-W	±sem
			Average daily gain (g/day)		
23	1.12^{C}	1.25^{C}	1.32^{C}	1.14^{C}	0.099
28	3.53^{A}	3.73^{A}	3.56^{A}	2.87^{B}	
			Specific growth rate (%/day)		
23	0.52^{C}	0.58^{C}	0.58^{C}	0.54^{C}	0.305
28	1.25^{A}	1.28^{A}	1.23^{A}	1.23^{A}	

A,B,C Within response attributes, means with a similar letter do not differ (P>0.05).

The responses of fish to the main effects of water temperature and diet are shown in Tables 3 and 4 respectively.

Water temperature had the expected marked effect on all growth attributes. The ingredient cost to convert feed into fish gain was significantly less (P<0.05) at 28 than at 23 °C.

Table 3. Effect of water temperature on fish response

Attribute	Effect of water temperature (°C)		
	23	28	±sem
Weights (g)			
Start	193.8^{X}	219.7^{Y}	2.15
End	244.6^{X}	363.5^{Y}	3.56
Daily gain (g/d)	1.21^{X}	3.42^{Y}	0.050
Specific growth rate (%/d)	0.55^{X}	1.20^{Y}	0.015
Food conversion (g:g)	1.36^{X}	1.21^{Y}	0.018
Food intake (g/d)	1.63^{X}	4.10^{Y}	0.049
Survival (%)	100.0^{X}	95.7^{Y}	0.61
Survival (degrees)[1]	90.0^{X}	76.4^{Y}	1.94
Swim bladder defects	3.2^{X}	3.3^{X}	0.40
Swim bladder (degrees)[1]	7.3^{X}	7.7^{X}	0.92
Ingredient cost ($/kg gain)	0.87^{X}	0.76^{Y}	0.012

1 Arsine transformed response to ensure normality of percentage data.
X,Y Within response attributes, means with a similar letter do not differ (P>0.05).

Food intake was increased and food conversion was worse for the R14MJ diet compared to all other diets (P<0.05). Apart from the already mentioned interaction effects between diet and water temperature for fish growth, fish on

the R14MJ diet consumed more food but converted it into growth less efficiently than those on all other diets. Importantly, the ingredient cost to convert food into fish growth differed significantly between all diets with the two Ridley diets being the most expensive. The summer Walkamin formulation (Wlk-S) was the least expensive and 25% cheaper than the high specification Ridley diet (R15MJ).

Table 4 - Effect of diet on fish response

Attribute	Effect of diet				
	R15MJ	R14MJ	Wlk-S	Wlk-W	±sem
Weights (g)					
Start	203.9	205.0	211.1	207.1	3.05
End	301.4^{AB}	309.6^{A}	313.4^{A}	291.6^{B}	5.03
Daily gain (g/d)	2.32^{A}	2.49^{A}	2.44^{A}	2.01^{B}	0.070
Specific growth rate (%/d)	0.88^{A}	0.93^{A}	0.90^{A}	0.78^{B}	0.022
Food conversion (g:g)	1.26^{B}	1.34^{C}	1.17^{A}	1.37^{C}	0.026
Food intake (g/d)	2.76^{B}	3.20^{A}	2.79^{B}	2.72^{B}	0.069
Survival (%)	98.0^{A}	95.3^{B}	99.3^{A}	98.7^{A}	0.86
Survival (degrees)[1]	83.4^{A}	77.6^{A}	87.3^{A}	84.6^{A}	2.74
Swim bladder defects	3.2^{A}	3.5^{A}	3.0^{A}	3.3^{A}	0.56
Swim bladder (degrees)[1]	7.3^{A}	8.0^{A}	6.9^{A}	7.7^{A}	1.30

[1] Arsine transformed response to ensure normality of percentage data.

A,B,C,D Within response attributes, means with a similar letter do not differ ($P>0.05$).

Appendix 2

Record of Data Collected and

Statistical Analysis Performed

Explanation Notes Appendix 2

P. 81

PROTEIN FEED = Protein apparent digestibility coefficient for test diets

PROTEIN ING.= Protein apparent digestibility coefficient for test ingredients within test diets.

MEAN SEM = Mean of values and standard error of mean.

LIPID FEED = Lipid apparent digestibility coefficient for test diets.

LIPID ING. = Lipid apparent digestibility coefficient for test ingredients within test diets.

P 82- 84 Crude nutritional data collected for experiments.

P 85 - 88 Calculation of apparent digestibility coefficients from experiments.

P 89 - 92 Crude nutritional data of ingredients.

24/5/96

Diet		PROTEIN FEED	MEAN SEM	PROTEIN ING.	MEAN SEM	LIPID FEED	SEM	LIPID ING.	SEM
Reference		95.8				98.8			
Reference	R	95.2	95.1			98.8	98.8		
Reference		94.5	0.37			98.9	0.02		
SoyMeal Defat.		94.5		91.5		93.4		80.5	
SoyMeal Defat.	S	93.8	93.9	89.2	89.5	94.2	93.8	83.2	81.9
SoyMeal Defat.		93.4	0.33	87.6	1.1	93.8	0.24	82	0.79
Fish Meal (Danish)		91.7		82.2		88.5		77.3	
Fish Meal (Danish)	FM	92.3	92.2	84.3	83.8	88.4	88.5	76.9	77.2
Fish Meal (Danish)		92.5	0.26	85	0.85	88.5	0.04	77.3	0.13
Fish Meal (Tuna)		89.8		75.8		90.5		70.9	
Fish Meal (Tuna)	FMT	90.7	89.7	78.9	75.4	90.9	90.1	72.4	73.8
Fish Meal (Tuna)		88.5	0.26	71.5	2.14	88.7	0.68	77.9	2.13
Mod. Soy Protein		89.1		73.2		98.6		97.8	
Mod. Soy Protein	SP	88.7	88.9	72.3	72.9	98.8	98.6	98.7	98
Mod. Soy Protein		89	0.09	73.2	0.29	98.4	0.11	97.4	0.37
Whole Wheat		89.6		75.2		98.3		96.8	
Whole Wheat	W	91.6	89.9	81.9	76.2	97.9	98.1	95.9	96.3
Whole Wheat		88.5	0.92	71.4	3.07	98.1	0.08	96.4	0.26
Soy Meal Full Fat		93.7		88.7		96.4		90.7	
Soy Meal Full Fat	FF	93.6	93.7	88.6	88.7	95.8	95.9	88.7	88.9
Soy Meal Full Fat		93.6	0.01	88.7	0.04	95.4	0.29	87.3	0.98
Yeast (Torulla)		86.2		63.9		95.8		88.5	
Yeast (Torulla)	Y	86.2	86.1	64.1	63.5	94.9	95.5	85.6	87.5
Yeast (Torulla)		85.1	0.15	62.6	0.5	95.8	0.28	88.5	0.95
Fish Meal (Chile)		91.9		82.8		94.7		84.9	
Fish Meal (Chile)	FMC	91.5	91.9	81.4	82.6	94.4	94.5	84.1	84.4
Fish Meal (Chile)		92.3	0.24	84.1	0.78	94.4	0.08	84.1	0.28
Whole Squid Meal		92.3		84.1		90.5		70.9	
Whole Squid Meal	WSM	91.6	92.4	82	84.4	90.1	90.1	69.4	69.7
Whole Squid Meal		93.2	0.44	87.1	1.47	89.8	0.2	68.6	0.67
Blood Meal (spray)		95.9		96.2		88.1		63.1	
Blood Meal (spray)	BM	95.9	95.8	96.2	95.8	90.9	89.7	72.4	68.2
Blood Meal (spray)		95.6	0.1	95.1	0.35	89.5	0.83	69.1	2.75
Meat & Bone Meal		88.8		72.6		94.6		84.6	
Meat & Bone Meal	MB	88.4	88.4	71	71	91.8	93.2	75.3	80
Meat & Bone Meal		87.9	0.28	69.4	0.93	93.3	0.8	80.1	2.68
Fish Meal (Mullet)		85.9		62.9		89.3		67.1	
Fish Meal (Mullet)	FMM	82.8	83.6	52.7	55.1	89.9	89.9	69.1	68.7
Fish Meal (Mullet)		81.9	1.21	49.6	4.03	90.1	0.25	69.9	0.83
Prawn Shell Meal		90.5		78.3		96.9		92.4	
Prawn Shell Meal	PSM	90.2	90.6	77.2	78.7	95.8	96.3	88.8	90.4
Prawn Shell Meal		91.2	0.31	80.7	1.02	96.2	0.32	90.1	1.05

↑
Red Label

done O

Crude Nutritional Data Collected for Test & Reference Diets;(Feces)

Diet	Crude Protein	variance	s. dev		Crude Lipid	variance	s. dev
Reference	2.8				0.29		
Reference	2.5				0.25		
Reference	2.9	0.03	0.17		0.28	0.000	0.02
SoyMeal Defat.	2.29				0.45		
SoyMeal Defat.	2.6				0.4		
SoyMeal Defat.	2.8	0.04			0.42	0.000	
Fish Meal (Danish)	3.9				0.89		
Fish Meal (Danish)	3.6				0.8		
Fish Meal (Danish)	3.5	0.03			0.81	0.002	
Fish Meal (Tuna)	3.3				0.81		
Fish Meal (Tuna)	3				0.87		
Fish Meal (Tuna)	3.7	0.08			0.89	0.001	
Mod. Soy Protein	3.7				0.24		
Mod. Soy Protein	3.5				0.2		
Mod. Soy Protein	3.9	0.03			0.26	0.001	
Whole Wheat	2.6				0.23		
Whole Wheat	2.1				0.27		
Whole Wheat	2.9	0.11			0.25	0.000	
Soy Meal Full Fat	1.9				0.17		
Soy Meal Full Fat	2.3				0.2		
Soy Meal Full Fat	2.1	0.03			0.22	0.000	
Yeast (Torulla)	3.1				0.09		
Yeast (Torulla)	3.4				0.12		
Yeast (Torulla)	2.7	0.08			0.1	0.000	
Fish Meal (Chile)	3.9				0.85		
Fish Meal (Chile)	4.1				0.89		
Fish Meal (Chile)	3.7	0.03			0.9	0.000	
Whole Squid Meal	3.5				0.84		
Whole Squid Meal	3.8				0.88		
Whole Squid Meal	3.1	0.08			0.9	0.001	
Blood Meal (spray)	11.4				1.3		
Blood Meal (spray)	11.1				1		
Blood Meal (spray)	11.9	0.11			1.1	0.016	
Meat & Bone Meal	4.4				0.4		
Meat & Bone Meal	4.6				0.6		
Meat & Bone Meal	4.8	0.03			0.5	0.007	
Fish Meal (Mullet)	3.2				0.8		
Fish Meal (Mullet)	3.9				0.84		
Fish Meal (Mullet)	4.1	0.15			0.81	0.000	
Prawn Shell Meal	2.7				0.2		
Prawn Shell Meal	2.8				0.27		
Prawn Shell Meal	2.5	0.02			0.25	0.001	
Ref Cold ext	4.2				4		
Ref Cold ext	3.7				3.8		
Ref Cold ext	3.9	0.04	0.21		4.2	0.027	0.16
Ref Large Fish	3.5				3.8		
Ref Large Fish	4.8				4.1		

Crude Nutritional Data Collected for Test & Reference Diets

Diet	Ash	variance	Moisture	variance
Reference			83.9	
Reference			81.9	82.6
Reference			82	0.846667
SoyMeal Defat.			89.5	
SoyMeal Defat.			88.8	
SoyMeal Defat.			88.7	0.126667
Fish Meal (Danish)			83.8	
Fish Meal (Danish)			84.4	
Fish Meal (Danish)			83.7	0.095556
Fish Meal (Tuna)			83.7	
Fish Meal (Tuna)			84.3	
Fish Meal (Tuna)			83.7	0.08
Mod. Soy Protein			86	
Mod. Soy Protein			87.1	
Mod. Soy Protein			86.5	0.202222
Whole Wheat			83.4	
Whole Wheat			83.6	
Whole Wheat			84.2	0.115556
Soy Meal Full Fat			86.7	
Soy Meal Full Fat			86.4	
Soy Meal Full Fat			87.1	0.082222
Yeast (Torulla)			85.9	
Yeast (Torulla)			86.5	
Yeast (Torulla)			87.3	0.328889
Fish Meal (Chile)			83.3	
Fish Meal (Chile)			82.2	
Fish Meal (Chile)			83.4	0.295556
Whole Squid Meal			82.5	
Whole Squid Meal			83.5	
Whole Squid Meal			82.9	0.168889
Blood Meal (spray)			75.4	
Blood Meal (spray)			76.5	
Blood Meal (spray)			76.4	0.246667
Meat & Bone Meal			79.7	
Meat & Bone Meal			79.8	
Meat & Bone Meal			79.9	0.006667
Fish Meal (Mullet)			78.3	
Fish Meal (Mullet)			78.6	
Fish Meal (Mullet)			79	0.082222
Prawn Shell Meal			89.2	
Prawn Shell Meal			89.6	
Prawn Shell Meal			88.5	0.206667
Bef Cold ext			78.3	
Bef Cold ext			78.1	
Bef Cold ext			77.5	0.115556
Bef Large Fish			85.9	
Bef Large Fish			87.5	

Ref Large Fish	86.7	0.426667

Production Method Trial
Small Fish

Calculation of Crude Protein ADC

	Ref 1	Ref 1	Ref1	Ref 2	Ref 2	Ref2
Nut. to Dry Basis						
Feed Nutrient.	46.30	45.50	45.10	46.40	45.10	45.70
Feed Moisture.	10.10	10.20	10.20	10.40	10.40	10.10
Fecal Nutrient level.	2.80	2.90	3.10	4.50	3.70	3.90
Fecal Moisture Level.	90.10	90.30	91.40	78.30	78.10	77.50
Feed Nutrient, Dry Matter Level.	51.50	50.67	50.22	51.79	50.33	50.83
Feces Nutrient, Dry Matter Level	28.28	29.90	36.05	20.74	16.89	17.33
Dry Cr2 O3 Feces	6.50	6.20	6.40	1.30	1.20	1.40
Dry Cr2 O3 Feed	0.50	0.51	0.49	0.50	0.49	0.51
ADC in Feed	95.78	95.15	94.50	84.60	86.29	87.58
ADC in Ingred.						

Ref 1= Steam pelleting: Continual Filtration
Ref 2 = Cold Extruded Small Fish: Continual Filtration

Ref1 Mean	95.1
Ref1Standard Deviation	0.52
Ref 1 Variance	0.27
Ref 2 Mean	86.2
Ref 2 Standard Deviation	1.22
Ref 2 VAriance	1.49

Production Method Trial
Small fish

Calculation of Crude Lipid ADC

	Ref 1	Ref 1	Ref1	Ref 2	Ref 2	Ref2
Nut. to Dry Basis						
Feed Nutrient.	9.60	9.70	9.50	9.70	9.50	9.60
Feed Moisture.	10.10	10.20	10.20	10.10	10.50	10.40
Fecal Nutrient level.	0.29	0.25	0.28	2.50	2.60	2.70
Fecal Moisture Level.	82.00	83.90	81.90	83.30	85.80	84.30
Feed Nutrient, Dry Matter Level.	10.68	10.80	10.58	10.79	10.61	10.71
Feces Nutrient, Dry Matter Level	1.61	1.55	1.55	14.97	18.31	17.20
Dry Cr2 O3 Feces	6.50	6.20	6.40	5.50	5.90	4.90
Dry Cr2 O3 Feed	0.50	0.51	0.49	0.47	0.45	0.45
ADC in Feed	98.84	98.82	98.88	88.14	86.84	85.26
ADC in Ingred.						

Ref 1= Steam pelleting: Continual Filtration
Ref 2 = Cold Extruded Small Fish: Continual Filtration

Ref1 Mean	98.8
Ref1Standard Deviation	0.03
Ref 1 Variance	0.00
Ref 2 Mean	86.7
Ref 2 Standard Deviation	1.18
Ref 2 VAriance	1.39

Fish Size Experiment: 2 Fish Sizes each Fed Reference Diet Cold Extruded & Stripped
Calculation of Crude Protein ADC

	Ref 1	Ref 1	Ref1	Ref 2	Ref 2	Ref2
Nut. to Dry Basis						
Feed Nutrient.	46.40	45.10	45.70	45.20	45.80	45.10
Feed Moisture.	10.40	10.40	10.10	10.40	10.00	10.10
Fecal Nutrient level.	4.50	3.70	3.90	3.40	2.70	3.40
Fecal Moisture Level.	78.30	78.10	77.50	91.20	92.20	90.20
Feed Nutrient, Dry Matter Level.	51.79	50.33	50.83	50.45	50.89	50.17
Feces Nutrient, Dry Matter Level	20.74	16.89	17.33	38.64	34.62	34.69
Dry Cr2 O3 Feces	1.30	1.20	1.40	1.18	1.23	1.50
Dry Cr2 O3 Feed	0.50	0.49	0.51	0.50	0.49	0.51
ADC In Feed	84.60	86.29	87.58	67.55	72.90	76.49

ADC in Ingred.

Ref 1= Steam pelleting: Continual Filtration
Ref 2 = Cold Extruded, Large Fish :Stripped

Ref1 Mean	86.16
Ref1Standard Deviation	1.22
Ref 1 Variance	1.49
Ref 2 Mean	72.31
Ref 2 Standard Deviation	3.67
Ref 2 VAriance	13.49

Ice Storage Experiment; Small Fish
Calculation of Crude Protein ADC

	Ref 1	Ref 1	Ref1	Ref 2	Ref 2	Ref2
Nut. to Dry Basis						
Feed Nutrient.	46.30	45.50	45.10	46.40	45.20	45.30
Feed Moisture.	10.10	10.20	10.20	10.40	10.00	10.10
Fecal Nutrient level.	2.80	2.90	2.79	2.68	2.70	2.90
Fecal Moisture Level.	90.10	90.30	90.30	91.20	92.20	90.20
Feed Nutrient, Dry Matter Level.	51.50	50.67	50.22	51.79	50.22	50.39
Feces Nutrient, Dry Matter Level	28.28	29.90	28.76	30.45	34.62	29.59
Dry Cr2 O3 Feces	6.50	6.20	6.40	6.00	5.20	5.30
Dry Cr2 O3 Feed	0.50	0.51	0.49	0.50	0.49	0.51
ADC in Feed	95.78	95.15	95.62	95.10	93.51	94.35
ADC in Ingred.						

Ref 1= Ice Stored
Ref 2 = No Ice Storage

Ref1 Mean	95.51
Ref1Standard Deviation	0.27
Ref 1 Variance	0.07
Ref 2 Mean	94.32
Ref 2 Standard Deviation	0.65
Ref 2 VAriance	0.42

Crude Nutritional Data Collected for Ingredients.

Diet	Crude Protein	variance	Crude Lipid	
SoyMeal Defat.	45.40		1.3	
SoyMeal Defat.	45.70	45.37	1.5	1.37
SoyMeal Defat.	45.00	0.08	1.3	0.01
Fish Meal (Danish)	71.50		10.4	
Fish Meal (Danish)	70.40	71.23	10.3	10.37
Fish Meal (Danish)	71.80	0.36	10.4	0.00
Fish Meal (Tuna)	51.50		10.5	
Fish Meal (Tuna)	52.30	52.53	10.4	10.40
Fish Meal (Tuna)	53.80	0.91	10.3	0.01
Mod. Soy Protein	70.30		1.5	
Mod. Soy Protein	70.20	70.27	1.5	1.53
Mod. Soy Protein	70.30	0.00	1.6	0.00
Whole Wheat	10.40		2.3	
Whole Wheat	10.60	10.60	2.4	2.37
Whole Wheat	10.80	0.03	2.4	0.00
Soy Meal Full Fat	38.00		18.5	
Soy Meal Full Fat	38.50	38.33	18.5	18.47
Soy Meal Full Fat	38.50	0.06	18.4	0.00
Yeast (Torulla)	59.90		6.3	
Yeast (Torulla)	60.20	60.20	6.3	6.23
Yeast (Torulla)	60.50	0.06	6.1	0.01
Fish Meal (Chile)	69.10		9.1	
Fish Meal (Chile)	69.40	69.23	9.4	9.27
Fish Meal (Chile)	69.20	0.02	9.3	0.02
Whole Squid Meal	68.30		5.3	
Whole Squid Meal	68.90	68.40	5.4	5.37
Whole Squid Meal	68.00	0.14	5.4	0.00
Blood Meal (spray)	86.50		3.3	
Blood Meal (spray)	86.10	86.30	3.4	3.30
Blood Meal (spray)	86.30	0.03	3.2	0.01
Meat & Bone Meal	51.40		11.9	
Meat & Bone Meal	51.50	51.50	11.8	11.87
Meat & Bone Meal	51.60	0.01	11.9	0.00
Fish Meal (Mullet)	56.00		13.5	
Fish Meal (Mullet)	54.00	55.13	15.4	14.40
Fish Meal (Mullet)	55.40	0.70	14.3	0.61
Prawn Shell Meal	40.50		1.7	
Prawn Shell Meal	40.70	40.43	1.6	1.67
Prawn Shell Meal	40.10	0.06	1.7	0.00

Crude Nutritional Data Collected for Ingredients.

Diet	Ash		variance	Moisture	variance
SoyMeal Defat.	ND			12.3	
SoyMeal Defat.	ND			12.4	~~11.77~~ 12.4
SoyMeal Defat.	ND			12.5	0.01
Fish Meal (Danish)		12.4		10.4	
Fish Meal (Danish)		12.7	12.53	10.3	10.33
Fish Meal (Danish)		12.5	0.02	10.5	0.01
Fish Meal (Tuna)		19.6		10.2	
Fish Meal (Tuna)		20	19.67	10.9	9.50
Fish Meal (Tuna)		19.4	0.06	10.6	0.08
Mod. Soy Protein		2.1		7	
Mod. Soy Protein		2.5	2.30	7.5	8.43
Mod. Soy Protein		2.3	0.03	7.4	0.05
Whole Wheat	ND			10.4	
Whole Wheat	ND			10.3	11.10
Whole Wheat	ND			10.6	0.02
Soy Meal Full Fat		5.4		12.4	
Soy Meal Full Fat		5.5	5.37	12.5	11.00
Soy Meal Full Fat		5.2	0.02	12.7	0.02
Yeast (Torulla)	ND			7.8	
Yeast (Torulla)	ND			7.5	8.17
Yeast (Torulla)	ND			7.4	0.03
Fish Meal (Chile)	ND			9.6	
Fish Meal (Chile)	ND			9.5	10.57
Fish Meal (Chile)	ND			9.6	0.00
Whole Squid Meal	ND			12.6	
Whole Squid Meal	ND			12.5	11.80
Whole Squid Meal	ND			12.4	0.01
Blood Meal (spray)	ND			10.5	
Blood Meal (spray)	ND			10.4	11.23
Blood Meal (spray)	ND			10.8	0.03
Meat & Bone Meal	ND			12.5	
Meat & Bone Meal	ND			12.3	11.80
Meat & Bone Meal	ND			12.6	0.02
Fish Meal (Mullet)	ND			10.5	
Fish Meal (Mullet)	ND			10.4	10.43
Fish Meal (Mullet)	ND			10.7	0.02
Prawn Shell Meal	ND			10.2	
Prawn Shell Meal	ND			10.2	10.25
Prawn Shell Meal	ND			10.3	0.00

ND= NOT DETERMINED

Calculation of Crude Ash ADC

	REF	DFM	TFM	MSP	FFSO
Nut. to Dry Basis					
Feed Nutrient.	12.46	16.04	15.88	9.08	9.10
Feed Moisture.	10.10	10.20	10.20	10.40	10.40
Fecal Nutrient level.	1.09	1.41	0.86	2.50	2.23
Fecal Moisture Level.	90.10	90.30	91.40	78.30	78.10
Feed Nutrient, Dry Matter Level.	13.86	17.86	17.68	10.13	10.16
Feces Nutrient, Dry Matter Level	11.01	14.54	10.00	11.52	10.18
Dry Cr2 O3 Feces	3.58	4.00	3.58	3.58	2.26
Dry Cr2 O3 Feed	0.50	0.55	0.51	0.50	0.49
ADC in Feed	88.91	88.81	91.94	84.12	78.26
ADC in Ingred.		89.12	99.03	72.95	53.42

REF = Reference Diet, Steam Pelleted
DFM = De fatted Soy Meal, Expellor.
TFM = Tuna Fish Meal
MSP = Modified Soy Protein.
FFSO = Full Fat Soy Meal, Extruded.

Calculation of Crude Protein ADC

	Ref 1	DFMP	YSTF	YSTP	DFMF
Nut. to Dry Basis					
Feed Nutrient.	46.30	50.30	10.50	50.30	9.80
Feed Moisture.	10.10	10.70	10.20	10.50	9.00
Fecal Nutrient level.	2.80	3.93	0.10	0.30	0.80
Fecal Moisture Level.	90.10	90.50	90.30	90.50	90.50
Feed Nutrient, Dry Matter Level.	51.50	56.33	11.69	56.20	10.77
Feces Nutrient, Dry Matter Level	28.28	41.37	1.03	3.16	8.42
Dry Cr2 O3 Feces	6.50	6.50	0.32	0.32	4.00
Dry Cr2 O3 Feed	0.50	0.50	0.51	0.49	0.50
ADC in Feed	95.78	94.35	85.95	91.40	90.23
ADC in Ingred.		91.02	63.01	81.17	77.27

Ref 1= Reference, steam pelleted
DFMP= DANISH FISH MEAL, PROTEIN
DFMF = DANISH FISH MEAL LIPID
YSTP = YEAST, SWEET TORULA, PROTEIN
YSTF = YEAST, SWEET TORULA, LIPID