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Ingredient apparent digestibility coefficients for the Australian short-finned eel (*Anguilla australis australis*, Richardson)

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Abstract

*Fish meal is the most widely used protein source in commercial eel foods and information on the nutritive value of more economical protein sources is needed in order to improve cost-effectiveness of diets. This investigation was conducted to determine apparent digestibility coefficients of available plant and animal meals for juvenile Australian short-finned eel (*Anguilla australis australis*, Richardson). The suitability of a modified Guelph-type settlement faecal collector in eel digestibility studies was assessed. Animal by-product (spray-dried meat solubles; blood meal; high fat poultry meal) and plant protein (soya bean; canola; maize gluten; lupin; field pea) meals were mixed with a reference diet and marker (0·3 : 0·69 : 0·01) and the resultant test diets given to the juvenile eels (3·15 (s.e. 0·42) g) at 0·05 live weight per day. The reference diet and all the test diets were well accepted by the fish. Apparent digestibility coefficients for crude protein (ADC_{CP}) for maize gluten meal (MGM), lupin meal (LM) and blood meal (BM) were found to be 0·97, 0·96 and 0·96 respectively and they were significantly ($P < 0·001$) higher than that for the other ingredients. However, apparent digestibilities for dry matter (ADC_{DM}) and energy (ADC_{kJ}) were significantly ($P < 0·0001$) higher for animal by-products than for plant proteins except for maize gluten meal. This was explained by the higher content of nitrogen free extractives in the former plant proteins. There was a strong positive correlation between ADC_{DM} and ADC_{kJ} for all ingredients ($P < 0·01$). Weaker positive correlations were also found between ADC_{CP} and ADC_{DM} and between ADC_{CP} and ADC_{kJ}. Similar results obtained for warm water species using similar faecal collection techniques and over limited eel digestibility data support the suitability of the modified Guelph-type settlement collector system in digestibility studies with juvenile eels.*

Keywords: Anguilla australis australis, *animal protein, digestibility, fish culture, plant protein.*

Introduction

It is well documented that the continuing expansion of aquaculture production necessitates the identification of alternative protein sources to fish meal since fish meal is a major and expensive component of commercial fish foods (Robaina *et al.*, 1995; Hardy, 1996; Carter and Hauler, 2000). However, the quality and suitability of a protein source is mainly dependent on its digestible protein content and amino acid profile (Kaushik and Cowey, 1991; Watanabe *et al.*, 1996; García-Gallego *et al.*, 1998; Gomes *et al.*, 1998). Apart from unbalanced amino acid profiles and endogenous antinutritional factors, the quantity and chemical composition of carbohydrates prevent the use of high levels of plant proteins in fish foods (Wilson, 1994; Robaina *et al.*, 1995; Refstie *et al.*, 1998; Mwachireya *et al.*, 1999). Nutrients that are not retained by the fish are excreted into the water and an effective management of aquaculture waste begins with the understanding of nutrient digestibility and utilization of food ingredients in aquaculture foods (Sugiura *et al.*, 1998). Several investigations showed that non-protein components (mainly starch and the fibre fractions) of many plant proteins could not be efficiently utilized by fish, resulting in much lower digestibility coefficients for dry matter and energy than fish-meal-based diets (Hilton and Slinger, 1986;

Morales *et al.,* 1994; McGoogan and Reigh, 1996; Degani *et al.,* 1997; da Silva and Oliva-Teles, 1998; Sugiura *et al.,* 1998; de Silva *et al.,* 2000). This is perhaps due to the digestible energy value of proteins being greater than that of carbohydrates; the relative proportions of these two components largely determine the available gross energy content in diets for fish (Bell, 1993). Animal by-products may also greatly differ in terms of protein quality resulting from the manufacturing practices used (Johnson *et al.,* 1998). As many of these factors may influence digestibility, it is important to determine apparent digestibility coefficients using accurate measurement techniques in order to limit the confounding error of a measurement technique on digestibility coefficients (Storebakken *et al.,* 1998).

Various techniques have been used to collect faecal material from fish including settlement type collectors, mechanical collectors, stripping or dissecting the anterior or posterior sections of the intestinal tract or siphoning faeces from the tanks (Choubert *et al.,* 1982; Cho *et al.,* 1982; Storebakken *et al.,* 1998; Percival *et al.,* 2001). Guelph-type settlement faecal collectors are widely used in digestibility studies since they allow the use of smaller sizes of fish and create very little disturbance to faeces or fish during collection compared with siphoning (Hajen *et al.,* 1993; da Silva and Oliva-Teles, 1998). However, overestimation of digestibility coefficients may occur due to nutrient leaching from the faeces in settlement type collectors as opposed to underestimation of digestibility with stripping or dissecting the intestinal tract. Several studies have reported digestibility coefficients of balanced diets in the European eel measured with modified Guelph-type settlement collectors (García-Gallego *et al.,* 1995 and 1998). However only two studies on eels investigated the apparent digestibility coefficients of different ingredients (Schmitz *et al.,* 1984; de Silva *et al.,* 2000) and they used a specifically designed metabolic chamber and siphoned faeces, respectively.

Australia has a relatively large variety of cheap non-fish meal protein sources and the nutritional quality of these sources requires investigation prior to the development of balanced diets for feasible farming of the Australian short-finned eel, *Anguilla australis australis* (Richardson) : considered as one of the prime candidates for inland aquaculture in Australia (Brown *et al.,* 1997; de Silva *et al.,* 2000). Traditionally, commercial eel foods contain high levels of high quality fish meal and research on the possibility of using plant proteins and animal by-products as alternative protein sources to fish meal is limited. The aim of this experiment was to measure the apparent digestibility levels of available Australian plant protein (soya bean, canola, maize gluten, lupin and field pea) and animal by-product (blood meal, spray-dried meat solubles, high fat poultry meal) meals.

Material and methods

Fish and maintenance

Elvers were supplied by Inland Fisheries Commission, Tasmania. Prior to experimentation, elvers were kept in 380-l stock tanks and weaned on to a commercial eel diet (made of 500 to 550 g/kg of crude protein, 100 to 150 g/kg of crude lipid and 200 to 250 g/kg of gelatinized potato starch, originally a powdered diet but prepared as 1-mm dry pellets using a laboratory pellet mill, manufactured by Chinda Corporation, Taiwan). Complete weaning of elvers to this commercial diet took 3 weeks. The digestibility trial was conducted in three modified 19·8-l carboys incorporated into a recirculation system (Engin and Carter, 2001). Three weeks before beginning the experiment, all elvers were transferred to a 15-carboy recirculation system and given the commercial eel diet. Seventy elvers (3·15 (s.e. 0·42) g) were randomly selected from these tanks, weighed and allocated to each carboy of the digestibility system. Elvers were anaesthetized during the allocation (80 mg/l, Benzocaine).

Because the experimental system of three tanks did not allow the eight experimental diets and a reference diet to be offered simultaneously in triplicate, the digestibility trial was conducted in 13-day cycles. After a 6-day acclimation period to each diet, faeces were collected for the following 7-day period. Dietary treatments were randomly allocated over time so that each of the eight test diets and a reference diet was given in triplicate (once per tank). Elvers were offered food at 0·05 live weight in two equal meals from 09:00 to 10:00 h and from 17:00 to 18:00 h each day.

Faeces from each tank were collected using a modified Guelph-type settlement collector (Cho *et al.,* 1982) attached to each carboy. A 5-mm plastic mesh was firmly attached to the effluent pipe at the bottom of tank in order to prevent elvers swimming into the collectors. Mesh size was selected to be large enough to allow the passage of faeces into the faecal collectors. Two 32-mm PVC pipes prepared as parallel units were used to prevent elvers aggregating on the mesh. Before feeding, 40-mm PVC pipes were plugged in to each digestibility tank to prevent pellets going through the mesh. Flow rate into each tank was adjusted to 1·1 l/min and turned off during feeding. After feeding, the pipes were removed and all the uneaten food flushed out using the valves beneath the tanks. For each replicate, food

Table 1 *Formulation and chemical composition of the reference diet (REF3)*

Ingredients (g/ kg diet)		
Fish meal	600·0	
Fish oil	170·0	
Dextrin	210·0	
CMC	10·0	
Minerals†	5·0	
Vitamins‡	5·0	
Chemical composition	Mean	s.e.
Moisture (g/kg diet)	116·7	1·2
Crude protein (g/kg DM)	411·9	1·8
Crude fat (g/kg DM)	256·8	7·9
Ash (g/kg DM)	96·5	1·6
Gross energy (MJ/kg)	21·0	0·04

†Mineral mixture (g/kg food) according to de la Higuera *et al*. (1989): CaH_2PO_4 1·37; $CaCO_3$ 1·306; KH_2PO_4 0·954, KCl 0·096; NaCl 0·577, $MnSO_4.H_2O$ 0·036, $FeSO_4.7H_2O$ 0·144, $MgSO_4$ 0·48, KI 0·0018, $CuSO_4.5H_2O$ 0·0048, $ZnSO_4.7H_2O$ 0·024, $CoSO_4$ 0·0028, Na_2MoO_4 0·0008, Na_2SeO_3 0·002, $Al_2(SO_4)_3.18H_20$ 0·0016.

‡ Vitamin mixture (g/kg food) according to de la Higuera *et al*. (1989): calcium pantothenate 0·13, thiamine 0·044, riboflavin 0·109, pyridoxine 0·033, inositol 0·874, biotin 0·001, folic acid 0·011, choline chloride 2·623, nicotinic acid 0·219, cyanocobalamin 0·002, ascorbic acid 0·874, retinol 0·044, menadione 0·022, ∞-tocopherol 0·007, cholecalciferol 0·009. Individual ingredients were supplied by Sigma-Aldrich Pty Ltd and ICN Biochemicals Pty Ltd, Australia.

consumption was measured on the 2nd and 5th day of the faecal collection period. Approximately two thirds of the water volume in each tank was replaced with clean freshwater from a 1000-l reservoir tank. Elvers were prevented from escaping using mesh cloth under the lids. Over the experiment the mean water quality parameters were : temperature, 26·1 (s.e. 0·3)ºC; dissolved oxygen, 6·5 (s.e. 0·3) mg/l; pH, 6·9 (s.e. 0·4); total ammonia nitrogen, 0·12 (s.e. 0·03) mg/l. Photoperiod was 11 h:13 h light : dark.

Diet formulation and preparation

Eight test diets were formulated to contain 0·69 of a reference diet (REF3, Table 1), 0·30 of the test ingredient and 0·01 chromium III oxide as an inert marker (Cho *et al.,* 1982). The ingredients tested for digestibility were : soya-bean meal (SBM; solvent extracted soya bean, Pivot Aquaculture, Tasmania, Australia); canola meal (CM; solvent extracted, Pivot Aquaculture, Tasmania, Australia); maize gluten meal (MGM; Pivot Aquaculture, Tasmania, Australia); lupin meal (LM; whole Australian sweet lupin, *Lupinus angustifolius*, autoclaved at 105ºC for 10 min and ground, Milne Feeds Pty Ltd, Western Australia); field pea meal (FPM; whole field pea, *Pisum sativum*, autoclaved at 105ºC for 10 min and ground, Milne Feeds Pty Ltd, Western Australia); meat meal (MM; wet pressed and spray-dried meat

Table 2 *Chemical composition and energy content of test diets used in the digestibility experiment*

	Diets													
	SBM		CM		MGM		LM		FPM		MM		BM		PM	
	Mean	s.e.	Mean	s.e.	Mean	s.e.	Mean	s.e.	Mean	s.e.	Mean	s.e.	Mean	s.e.	Mean	s.e.
Dry matter (DM, g/kg diet)	873·9	1·2	878·2	2·1	881·6	2·7	882·6	2·6	872·1	2·1	866·2	2·5	887·1	3·9	879·1	1·3
Crude protein (g/kg DM)	434·0	0·0	399·0	2·8	471·0	2·8	396·5	4·9	352·5	19·1	484·5	9·2	562·5	0·7	450·5	28·9
Crude fat (g/kg DM)	199·0	0·3	191·4	9·9	215·6	13·0	210·8	0·6	197·6	0·6	210·2	0·3	185·8	5·9	318·2	16·7
Ash (g/kg DM)	88·3	1·7	86·6	0·2	78·9	2·1	77·4	1·7	76·2	0·6	91·3	1·6	75·2	0·8	91·9	0·2
NFE + crude fibre† (g/kg DM)	278·7	0·9	323·0	4·9	234·5	5·1	315·3	2·2	373·7	10·6	214·0	4·8	176·5	3·0	139·4	14·4
Gross energy (MJ/kg DM)	20·4	0·1	20·0	0·0	21·1	0·2	20·3	0·007	19·7	0·05	20·6	0·04	21·4	0·06	22·2	0·007

† NFE = nitrogen-free extractives. Calculated as the remainder of the diet (as fed) after crude protein + crude fat + ash accounted for (Carter and Hauler, 2000).

Table 3 *Chemical composition, gross energy and the essential amino acid content of the ingredients used in the digestibility experiment*

	Ingredient															
	SBM		CM		MGM		LM		FPM		MM		BM		PM	
	Mean	s.e.	Mean	s.e.	Mean	s.e.	Mean	s.e.	Mean	s.e.	Mean	s.e.	Mean	s.e.	Mean	s.e.
Dry matter (g/kg diet)	880·3	9·1	918·1	10·8	915·8	5·7	921·4	2·9	891·8	9·1	825·5	6·6	962·4	7·8	916·9	6·2
Crude protein (g/kg DM)	515·9	3·1	423·8	4·5	679·7	7·5	298·5	5·7	247·6	19·4	744·7	5·8	958·2	13·2	452·5	5·2
Crude fat (g/kg DM)	40·3	0·4	49·6	0·7	86·0	1·1	78·3	0·2	26·1	0·7	140·3	0·5	28·8	0·4	454·9	11·9
Ash (g/kg DM)	89·1	17·4	108·8	9·5	96·9	31·1	119·8	0·8	63·9	6·6	154·4	14·2	53·2	2·7	99·2	26·4
GE (MJ/kg DM)	18·6	0·3	17·5	0·3	21·9	0·7	18·2	0·4	17·2	0·1	20·6	0·1	23·2	0·0	26·6	0·1
Essential amino acids† (g/kgDM)																
Arginine	37·6	0·9	24·7	0·2	22·8	0·2	43·4	3·8	11·8		54·2	1·3	43·9	0·6	41·3	0·1
Histidine	12·5	0·8	8·9	0·1	13·4	0·1	9·0	0·9	26·2		11·2	1·5	55·0	0·4	9·3	0·5
Isoleucine	23·5	0·6	16·1	0·1	28·5	0·1	14·9	1·3	6·3		18·5	1·3	15·1	0·2	22·6	0·1
Leucine	38·3	1·0	27·7	0·1	112·4	0·6	23·4	1·8	11·2		39·3	1·7	113·3	0·6	40·3	0·3
Lysine	30·5	0·1	18·8	0·1	9·2	0·7	15·4	1·4	18·8		30·9	1·4	81·0	2·8	28·5	1·2
Methionine	5·1	0·3	7·6	0·1	16·3	0·1	2·3	0·2	17·0‡		8·4	0·1	14·6	0·1	9·0	0·6
Phenylalanine	25·2	0·6	15·9	0·1	43·3	0·1	14·2	1·0	3·6§		19·1	1·2	64·9	0·1	23·5	0·3
Threonine	21·0	0·6	18·1	0·2	24·1	0·1	12·4	1·0	NA		21·6	1·2	49·2	0·3	23·7	0·3
Tryptophan	4·8		2·7		1·5		2·6		NA		4·5		6·0		4·3	
Valine	24·4	0·6	20·6	0·1	30·9	0·1	14·0	1·0	21·8		26·8	0·5	79·0	0·1	29·9	0·1

† According to C. G. Carter, unpublished. NA not analysed.
‡ Includes cysteine.
§ Includes tyrosine.

soluble, Daka, A. M. B. A, Denmark); blood meal (BM; co-agulated, dried and ground, Peerless Holdings Pty Ltd, Victoria, Australia) and high fat poultry meal (PM; Edgell, Tasmania, Australia). The chemical composition of the test diets and ingredients is shown in Tables 2 and 3. Fish meal and fish oil were from jack mackerel, *Trachurus picturatus* (Pivot Aquaculture, Tasmania, Australia). Vitamin and mineral mixtures were prepared according to de la Higuera *et al.* (1989) (Table 1).

Dry ingredients of the reference diet were mixed with a Hobart mixer for 30 min. Fish oil and the vitamin and mineral mixtures were then added and the mixture was mixed for a further 20 min. The reference diet was stored at -20ºC until used. Whole ingredients and those containing large particles (≥ 0·5 mm) were ground (M20, IKA Labortechnik, Germany) before being mixed with the reference diet. After the combination of the reference diet, test ingredients, chromium III oxide (10 g/kg diet) and water (50 g/kg diet), each test diet was mixed for 30 min. Diets were manufactured as pellets (1-mm die) using a laboratory pellet mill (model CL-2, California Pellet Mill Co., USA). All the diets were dried overnight at 37ºC in a fan forced oven. Dried diets were individually bagged and stored at -20ºC until used.

Sampling and calculation of apparent digestibility coefficients

Because the system was kept in a warm temperature-controlled room, faecal samples were collected in 75 ml sample jars held in crushed-ice-filled foam boxes in order to prevent the bacterial degradation of the faeces. Faecal samples were collected from the settlement collector between 18:00 and 09:00 h on each day during each 7-day faecal collection period. During collection, sample jars were carefully unscrewed from the collectors and frozen immediately at -20ºC without draining the excess water in jars. All the frozen sample jars were freeze dried. Following freeze drying, the faecal samples from each replicate tank of each treatment throughout the 7-day collection period were ground, pooled (by equal weight) and stored at -20ºC until analysis. Freeze dried samples were used in the analysis of chromium III oxide and nutrients (see below). The apparent digestibility coefficients (ADC) for the reference diet and test diets were calculated using the standard formula:

$$ADC\ (\%) = 100\text{-}[100(\%I_{diet}/\%I_{faeces})\times(\%N_{faeces}/\%N_{diet})]$$

(Maynard and Loosli, 1969) where I is the inert marker and N the nutrient. The ADC for dry matter (ADC_{DM}), crude protein (ADC_{CP}) and energy (ADC_{kJ}) and for each ingredient was calculated as:

$$ADCi\ (\%) = ADC_{test} + ((0{\cdot}7\times N_{REF3})/(0{\cdot}3\times Ni))\times(ADC_{test}\text{-}ADC_{REF3})$$

(Sugiura *et al.*, 1998) where ADCi is the apparent digestibility coefficient for each ingredient; ADC_{test} is the apparent digestibility coefficient of the test diet; N_{REF3} the nutrient content of the reference diet; Ni the nutrient content of each test ingredient; ADC_{REF3} the apparent digestibility of the reference diet.

Chemical analysis

Diets, ingredients and faeces were analysed for crude protein (Kjeldahl, selenium catalyst; %N × 6·25), gross energy (bomb calorimeter; Gallenkamp Autobomb, calibrated with benzoic acid). Diets and ingredients were analysed for crude fat (Bligh and Dyer, 1959), dry matter (g per kg DM) and ash (AOAC, 1995). Chromic oxide was determined according to Furukawa and Tsukahara (1966).

Statistical analysis

Data are reported as mean±s.e. throughout the text. The apparent digestibility coefficients for dry matter, crude protein and energy calculated for each of the test ingredients were arcsin-transformed prior to analysis and normality and homogeneity of variance were confirmed for each parameter (JMP version 3·2·1). Means were compared by one-way ANOVA. When a significant treatment effect was observed a Tukey-Kramer HSD test was used to compare means. Correlations between apparent digestibility coefficients of ingredients for dry matter, energy and crude protein were conducted by Pearson correlation test. Significance was accepted at probabilities of 0·05 or less.

Results

There was no mortality during the experiment. All the test diets and the reference diet were well accepted by the elvers. Apparent digestibility coefficients for dry matter (ADC_{DM}) of test ingredients ranged between 0·37 and 0·93 (Table 4). FPM had a significantly lower ADC_{DM} than the other meals whereas MGM had a significantly higher ADC_{DM} than all the other meals except BM. There was no significant difference between ADC_{DM} values for SBM and CM and these values did not differ significantly from the ADC_{DM} value for PM. The ADC_{DM} of LM was the second lowest among the plant proteins and significantly different from both that of the plant proteins and animal by-products tested. The ADC_{DM} values for animal by-products (MM, PM and BM) ranged from 0·74 to 0·90 and they were higher than plant proteins except MGM and CM (Table 4). The highest ADC_{DM} amongst them was obtained on BM and it was significantly different from PM. However, there was no significant

difference between the ADC_{DM} values of BM and MM. PM had the lowest ADC_{DM} between animal by-products and it was not significantly different than the ADC_{DM} of MM.

Apparent digestibility coefficients for crude protein (ADC_{CP}) of all the test ingredients varied between 0·85 and 0·97 (Table 4). It appeared that the range of ADC_{CP} values for plant proteins and animal by-products was similar (Table 4). The lowest ADC_{CP} was obtained on FPM and it was significantly lower than that of the other plant proteins tested except SBM. Although ADC_{CP} of FPM was significantly lower than that of the other plant proteins, the scale of the difference was not as large as for ADC_{DM} or ADC_{kJ} among plant proteins (e. g. 0·12 units difference between the ADC_{CP} of FPM and MGM *v.* 0·56 units difference between ADC_{DM} in the same ingredients) (Table 4). There was no significant difference between ADC_{CP} of MGM, SBM, CM, LM, BM and MM. ADC_{CP} of MGM was significantly higher than that of PM and FPM (Table 4).

Apparent digestibility coefficients for energy (ADC_{kJ}) followed a similar trend to ADC_{DM} both in plant proteins and animal by-products. ADC_{kJ} of plant proteins except MGM were significantly lower than those of animal by-products (Table 4). The range of ADC_{kJ} among plant proteins was greater than the range of ADC_{kJ} among animal by-products (Table 4). The highest ADC_{kJ} was obtained for MGM and BM and they were significantly higher than the other ingredients. FPM and LM had the lowest and the second lowest ADC_{kJ} digestibilities of all ingredients, respectively.

A positive strong correlation was found between ADC_{DM} and ADC_{kJ} of all ingredients ($r = 0{\cdot}98$; no. = 24; $P < 0{\cdot}01$). Similarly, there were significant positive correlations between ADC_{CP} and both ADC_{DM} ($r = 0{\cdot}62$; no. = 24; $P < 0{\cdot}01$) and ADC_{kJ} ($r = 0{\cdot}55$; no. = 24; $P < 0{\cdot}01$).

Discussion

The stripping and dissection of the anterior or posterior sections of the intestinal tract were demonstrated to be an ineffective faecal collection method with smaller sizes of fish (Cho *et al.*, 1982; Allan *et al.*, 1999). Settlement allows digestibility to be measured with smaller fish and causes minimal disturbance to faeces during collection. To minimize the breakage and leaching of nutrients from faeces that may result in a significant overestimation of digestibility coefficients, modified Guelph-type of settlement collectors held in crushed ice were used in the present study. Because similar digestibility coefficients for eels (Schmitz *et al.*, 1984) and other

Table 4 *Apparent digestibility coefficients for dry matter (ADC_{DM}), crude protein (ADC_{CP}) and energy (ADC_{kJ}) for the Australian short-finned eel given a variety of plant and animal proteins (mean of three replicates)*

	Ingredient																	
	SBM		CM		MGM		LM		FPM		MM		BM		PM			
	Mean	s.e.	Mean	s.e.	Mean	s.e.	Mean	s.e.	Mean	s.e.	Mean	s.e.	Mean	s.e.	Mean	s.e.	Significance
ADC_{DM}	0·70^{c}	0·02	0·77^{cd}	0·01	0·93^{f}	0·01	0·57^{b}	0·03	0·37^{a}	0·07	0·82de	0·04	0·90^{ef}	0·01	0·74^{cd}	0·06	***
ADC_{CP}	0·91^{ab}	0·02	0·94^{b}	0·00	0·97^{b}	0·01	0·96^{b}	0·04	0·85^{a}	0·04	0·92^{ab}	0·00	0·96^{b}	0·03	0·87^{n}	0·04	**
ADC_{kJ}	0·76^{c}	0·03	0·77^{c}	0·01	0·97^{e}	0·01	0·61^{b}	0·01	0·46^{a}	0·07	0·89^{d}	0·04	0·97^{e}	0·01^{e}	0·82^{cd}	0·06	***

^{a,b,c,d,e,f} Means with different superscripts in the same row are significantly different (Tukey-Kramer HSD multiple comparison).

fish species like the Australian silver perch (Allan *et al.,* 1999) and trout (Yamamoto *et al.,* 1998) were reported for the same type of ingredient using almost similar or exactly the same type of collection technique to the present study, the validity of the technique could be supported by being comparable to other studies. For example, previous studies have also used the same time-frame for the collection of faeces in digestibility studies with eels (de Silva *et al.,* 2000; Tibbetts *et al.,* 2000). In contrast to findings by Watanabe *et al.* (1996), Allan *et al.* (1999) demonstrated that prolonged collection (18 h following previous meal) of faeces in collectors when held in ice caused negligible leaching of dry matter and protein.

The Australian short-finned eel digested the dry matter and energy in animal by-products significantly better than most of the plant proteins. Although not significantly different, the apparent digestibility coefficients of MM for dry matter, crude protein and energy were higher than those of PM but lower than those of BM. The only animal by-product previously tested for digestibility in the short-finned eel has been the meat meal (de Silva *et al.,* 2000). Their result was in contrast to that in the present study (0·82 and 0·89 dry matter and energy digestibility coefficients of meat meal, respectively) and a relatively high ash content (0·29 DM) of meat meal may have caused lower dry matter and energy digestibilities in their study. Similar observations were made with salmonids leading to the conclusion that a large amount of poorly digested ash in the meat or meat and bone meal results in markedly lower dry matter digestibility (Cho *et al.,* 1982; Bureau *et al.,* 1999). Protein digestibility of meat meals appears not to be related to the amount of ash in the products but a slight increase in protein digestibility of meat meal products was observed with air classification process as a reduction in collagen content in these products (Bureau *et al.,* 1999).

Although there are no available eel digestibility results for poultry and blood meal in the literature to compare with, our findings are in line with what was reported with salmonid digestibility values for these products (Hajen *et al.,* 1993; Pfeffer *et al.,* 1995; Suguira *et al.,* 1998; Bureau *et al.,* 1999). It appears that a significant improvement in the crude protein digestibility has occurred over the years through better manufacturing practices (Miller, 1996). Spray-dried blood meal gave high digestibility values in this study and this is in line with findings with other studies (Cho *et al.,* 1982). Compared with spray-drying, other processing techniques available to produce blood meal like rotoplate, steam-tube and ring-drying have been shown to give a significantly lower digestibility coefficients in fish due to excessive heat damage to proteins (Cho *et al.,* 1982).

Apparent protein digestibility coefficients for plant proteins ranged between 0·85 and 0·97 in the present study. This is in agreement with findings from previous studies which consistently report high levels of digestive utilization of plant proteins by carnivorous and omnivorous fish, (Cho and Cowey, 1991; McGoogan and Reigh, 1996). However, plant proteins contain high levels of complex carbohydrates and several anti-nutritional factors like trypsin inhibitor which may be detrimental for fish growth (Wilson and Poe, 1985). It is well documented that the ability to utilize plant carbohydrates as energy sources varies among species and it is rather limited in many carnivorous fish (Cho *et al.,* 1982; Cowey and Walton, 1989; Kaushik *et al.,* 1989; Morales *et al.,* 1994; Wilson, 1994; García-Gallego *et al.,* 1995). The significantly lower energy and dry matter digestibilities of lupin and field pea meals than that of other plant proteins, found in the present study, seem to be associated with the quantity and the chemical composition of the carbohydrates they contain (McGoogan and Reigh, 1996). The fundamental structure of the plant cell wall is formed by cellulose which is a very stable and most abundant polysaccharide in nature (de Silva and Anderson, 1995). Although cellulose can be hydrolysed by strong acid treatment, with the exception of micro-organisms, few non-ruminant animals have the necessary endogenous enzymes (i.e. cellulases) capable of hydrolysing and digesting cellulose (Wee and Tacon, 1989). Cellulase enzyme activity (acquired from intestinal microflora) has been found in channel catfish (Stickney and Shumway, 1974). However, the amount of cellulose digested during passage of food along the gut can be considered negligible (Stickney and Shumway, 1974). Previously in eels, soya-bean meals have been shown to have the same ADC_{CP} as fish meal but had lower ADC_{DM} (Schmitz *et al.,* 1984). Adult European eels (weighing between 170-230 g) had ADC_{DM} of 0·68 from soya-bean meal compared with the 0·87 from fish meal (Schmitz *et al.,* 1984). In contrast to 0·70 and 0·76 ADC_{DM} and ADC_{kJ} for a soya-bean meal reported in the present study, de Silva *et al.* (2000) demonstrated that ADC_{DM} and ADC_{kJ} of soya-bean meal were 0·82 and 0·56, respectively in the Australian short-finned eel weighing about 40 g. It is likely that faeces collected with disturbance (siphoning) and held in tanks for a period without cooling had an impact and resulted in higher ADC_{DM} and ADC_{kJ} of soya-bean meal in their study. Cooling the faeces after settlement in collectors has been shown to prevent bacterial decomposition of faeces

hence decreasing the chance of overestimating digestibility coefficients (Spyridakis *et al.*, 1989). However, the almost 0·20 digestibility units lower ADC_{kJ} of soya-bean meal reported by de Silva *et al.* (2000) must also be related to the quality of the protein source. In fact, previous studies support the fact that carbohydrates of soya-bean meals are largely in the form of undigestible higher polysaccharides (Arneson *et al.*, 1989; Pongmaneerat and Watanabe, 1993). Therefore, digestibility coefficients reported for the same type of meals in different studies are currently hard to compare since values are affected by the techniques used to measure digestibility, the quality of the ingredients, dietary composition, fish size, ration level and the water temperature employed in each experiment (Wilson and Poe, 1985; Anderson *et al.*, 1993; Watanabe *et al.*, 1996; Yamamoto *et al.*, 1997; da Silva and Oliva-Teles, 1998; Bureau *et al.*, 1999).

Apparent digestibility coefficients for dry matter, crude protein and energy in maize gluten were over 0·90 in the present study. Maize gluten is a major co-product of maize wet milling and contains high protein and low fibre (Park *et al.*, 1997). Although there are no published apparent crude protein digestibility values of maize gluten for eels, generally high values were reported with other carnivorous species like salmonids (Cho and Slinger, 1979; Morales *et al.*, 1994; Yamamoto *et al.*, 1997 and 1998; Suguira *et al.*, 1998) and red sea bream (Yamamoto *et al.*, 1998). Yamamoto *et al.* (1997) reported that protein digestibility was 0·96 for maize gluten meal in fingerling rainbow trout at 15ºC and demonstrated that the availabilities of amino acids from maize gluten meal almost approximated to the apparent protein digestibility value. A similar assumption was made in order to calculate the digestible essential amino acid content of each protein source in Table 5. Although there are not many apparent dry matter and energy digestibilities reported for maize gluten in different species, the 0·93 and 0·97 dry matter and energy digestibility values found in the present study were comparable with the results obtained with the Australian silver perch (Allan *et al.*, 1999) (Table 6). However lower maize gluten lipid and carbohydrate digestibilities were shown in rainbow trout (Morales, ,994). This may not be surprising since the European eel has been shown to have a comparatively greater ability to utilize high levels (over 30% of diets) of maize starch in balanced diets than the rainbow trout (García-Gallego *et al.*, 1995). Warm water fish species are able to tolerate much higher levels of dietary carbohydrate than cold water or marine fish due possibly to higher amylase activity present in the digestive system of these fishes (Wilson, 1994; de Silva and Anderson, 1995).

Table 5 *Digestible nutrient contents for ingredients consumed by the Australian short-finned eel*

	Ingredient															
	SBM		CM		MGM		LM		FPM		MM		BM		PM	
	Mean	s.e.	Mean	s.e.	Mean	s.e.	Mean	s.e.	Mean	s.e.	Mean	s.e.	Mean	s.e.	Mean	s.e.
Dry matter (DM, g/kg)	614·4	6·4	705·1	8·3	849·9	5·2	525·2	1·6	330·8	3·4	680·3	5·6	863·3	7·0	676·6	4·5
Crude protein (g/kg DM)	467·0	2·8	399·2	4·2	659·4	7·3	287·1	5·5	210·2	16·5	685·9	5·3	920·8	12·7	422·0	4·7
Gross energy (MJ/kg DM)	14·1	0·2	13·5	0·3	21·2	0·7	11·1	0·2	8·0	0·1	18·3	0·1	22·4	0·1	21·9	0·1
Essential amino acids† (g/kg DM)																
Arginine	34·0	0·8	23·2	0·2	22·1	0·2	41·8	3·6	10·0		49·9	1·2	42·2	0·6	35·9	0·1
Histidine	11·3	0·7	8·4	0·1	13·0	0·1	8·6	0·8	22·2		10·3	1·3	52·9	0·4	8·0	0·4
Isoleucine	21·2	0·6	15·2	0·1	27·6	0·1	14·4	1·2	5·3		17·1	1·2	14·5	0·2	19·7	0·1
Leucine	34·7	1·0	26·1	0·1	109·0	0·6	22·6	1·8	9·5		36·2	1·6	108·9	0·6	35·0	0·30
Lysine	27·6	0·1	17·7	0·1	9·0	0·6	14·9	1·3	16·0		28·5	1·3	77·8	2·7	24·8	1·1
Methionine	4·6	0·3	7·2	0·1	15·8	0·1	2·2	0·2	14·4		7·7	0·1	14·0	0·1	7·9	0·5
Phenylalanine	22·8	0·6	15·0	0·1	42·0	0·1	13·6	1·0	3·1		17·6	1·1	62·4	0·1	20·4	0·3
Threonine	19·0	0·6	17·0	0·1	23·4	0·1	12·0	0·9	NA		19·9	1·1	47·3	0·3	20·6	0·3
Tryptophan	4·3		2·5		1·5		2·5		NA		4·1		5·8		3·7	
Valine	22·0	0·6	19·4	0·1	29·9	0·1	13·5	0·9	18·5		24·7	0·5	75·9	0·1	26·0	0·1

† Values calculated assuming approximation of the availability of amino acids to crude protein digestibility coefficients in each ingredient (Yamamoto *et al.*, 1997).
NA = not analysed.

Table 6 *Apparent digestibility coefficients calculated for two Australian warm-water species (silver perch,* Bidyanus bidyanus *and the short-finned eel,* A. australis australis*) given the same type of plant and animal proteins in different studies*

	Ingredients								
	SBM		MGM		BM		MM		
Parameter	Mean	s.e.	Mean	s. e.	Mean	s.e.	Mean	s.e.	Reference
Silver perch									
ADC_{DM}	0·81	0·02	0·98	0·02	0·99	0·02	NA		Allan *et al*. (1999)†
ADC_{CP}	0·95	0·01	0·98	0·00	0·92	0·03	NA		Allan *et al*. (1999)†
ADC_{kJ}	0·83	0·02	0·96	0·00	1·04	0·08	NA		Allan *et al*. (1999)†
Short-finned eel									
ADC_{DM}	0·82	0·02	NA		NA		0·33	0·03	de Silva *et al*. (2000)‡
	0·70	0·02	0·93	0·01	0·90	0·01	0·82	0·04	The present study
ADC_{CP}	0·92	0·01	NA		NA		0·53	0·04	de Silva *et al*. (2000)‡
	0·91	0·02	0·97	0·01	0·96	0·00	0·92	0·00	The present study
ADC_{kJ}	0·56	0·06	NA		NA		0·64	0·05	de Silva *et al*. (2000)‡
	0·76	0·03	0·97	0·01	0·97	0·01	0·89	0·04	The present study

† Faeces from juvenile silver perch (9·8 to 11·2 g) were collected by settlement over 18 h on each day of a 12-day faecal collection period.
‡ Faeces from medium size short-finned eel (average 40 g) were collected by siphoning between 18:30 and 08:30 h on each day of faecal collection period. NA not analysed.

However, diets with high levels of crude or raw starch have been shown to inhibit the digestibility of these diets by the European eel (Spannhof and Kühne, 1977) not because of a decrease in amylase secretion rate but an increased chance of adsorption of the amylase to the crude or raw starch, thus inhibiting starch hydrolysis (Spannhof and Plantikow, 1983). Better digestive utilization of dietary ingredients would promote greater efficiency in the utilization of dietary protein and energy and result in lower waste production (Kaushik and Médale, 1994; Morales *et al.*, 1994; Robaina *et al.*, 1995). However, a recent investigation by Bureau *et al.* (1998) showed that energy from digestible carbohydrate is poorly retained by rainbow trout. Therefore, more research is needed in order to understand the dietary energy efficiency of practical diets containing alternative plant protein meals for the Australian short-finned eel.

According to Cho and Kaushik (1990) the ADC of energy of food ingredients closely correlates with those of dry matter which are lower in all cases than energy digestibility coefficients. A strong positive correlation ($r = 0{\cdot}98$; no. = 24; $P < 0{\cdot}01$) between ADC_{DM} and ADC_{kJ} of ingredients found in the present study is in agreement with the findings for rainbow trout (Cho *et al.*, 1982) and the sea bass (da Silva and Oliva-Teles, 1998).

The digestibility coefficients for separate ingredients are assumed to be additive and can be used in least cost diet formulations for fish species (Cho *et al.*, 1982; Allan *et al.*, 1999). This assumption was put to the test for some of the ingredients (SBM, MGM, LM and MM) by using digestibility values of the reference diet and individual ingredients and then comparing the sum of these (on a proportional basis) with direct measurement of test diets (Cho *et al.*, 1982) (Table 7). There was a similarity between the digestibilities of test diets determined and calculated confirming the previous comparisons obtained for rainbow trout (Cho *et al.*, 1982; Watanabe *et al.*, 1996), channel catfish (Wilson and Poe, 1985), carp, tilapia and ayu (Watanabe *et al.*, 1996), sea bass (da Silva and Oliva-Teles, 1998) and the Australian silver perch (Allan *et al.*, 1999).

In conclusion, the present study demonstrated that the juvenile Australian short-finned eel digested the dry matter and energy in animal by-products better than in plant proteins with the exception of maize gluten. The effectiveness of diets formulated upon the basis of digestibilities of the nutrients and energy in individual ingredients can be evaluated by observation of weight gain, food efficiency and body composition of fish receiving the diets under particular culture regimes (Cho *et al.*, 1982). It is a necessity to conduct growth trials with potential alternative protein ingredients (decided upon their digestibility values, price or the availability of amino acids to a particular fish species) for the success of food development studies. This research successfully identified the highly digestible Australian plant and

Table 7 *Apparent digestibility coefficients (ADC) for test diets (SBM, MGM, LM and MM) determined and calculated with the Australian short-finned eel*

Ingredient/diet	Inclusion level (g/100g dry basis)	Dry matter (g/100g)		Energy (MJ/kg)		Crude protein (g/100g)	
		ADC†	proportional ADC‡	ADC†	proportional ADC‡	ADC†	proportional ADC‡
Reference diet (REF3)	69	85·6(1·4)	59·1(0·9)	94·1(1·6)	64·9(1·1)	93·9(1·6)	64·8(1·1)
Cr_2O_3	1	-	-	-	-	-	-
SBM	30	69·8(1·7)	20·9(0·5)	75·6(3·2)	22·7(0·9)	90·5(1·6)	27·2(0·5)
Test diets (determined)§	100	81·1(0·8)	-	89·2(1·0)	-	92·6(0·5)	-
Test diets (calculated)¶	100	80·0(1·4)	-	87·6(2·0)	-	92·0(1·6)	-
Difference ‖		1·4	1·8	0·6			
Reference diet (REF3)	69	85·6(1·4)	59·1(0·9)	94·1(1·6)	64·9(1·1)	93·9(1·6)	64·8(1·1)
Cr_2O_3	1						
MGM	30	92·8(1·0)	27·8(0·3)	96·8(1·1)	29·0(0·3)	97·0(0·5)	29·1(0·2)
Test diets (determined)§	100	88·2(0·5)	-	94·9(0·3)	-	95·1(0·4)	-
Test diets (calculated)¶	100	86·9(1·2)	-	3·9(1·4)	-	93·9(1·1)	-
Difference ‖		1·5	1·1	1·3			
Reference diet (REF3)	69	85·6(1·4)	59·1(0·9)	94·1(1·6)	64·9(1·1)	93·9(1·6)	64·8(1·1)
Cr_2O_3	1						
LM	30	57·1(2·6)	17·1(0·8)	60·8(1·4)	18·3(0·4)	96·2(4·0)	28·9(0·9)
Test diets (determined)§	100	76·8(1·2)	-	85·1(0·9)	-	94·4(0·7)	-
Test diets (calculated)¶	100	76·2(1·7)	-	83·2(1·5)	-	93·7(2·0)	-
Difference ‖		0·8	2·2	0·7			
Reference diet (REF3)	69	85·6(1·4)	59·1(0·9)	94·1(1·6)	64·9(1·1)	93·9(1·6)	64·8(1·1)
Cr_2O_3	1						
MM	30	82·4(3·9)	24·7(1·2)	88·5(4·2)	25·7(1·5)	92·1(0·4)	27·6(0·2)
Test diets (determined)§	100	84·6(1·1)	-	92·4(1·2)	-	93·1(0·2)	-
Test diets (calculated) ¶	100	83·8(2·1)	-	90·6(2·6)	-	92·4(1·3)	-
Difference ‖	0·9	1·9	0·8				

† Values are means for three replicate tanks. All means have s.e. indicated in parentheses.
‡ ADC × inclusion level/100 (Allan *et al.*, 1999).
§ The determined ADC is based on the average of the test diets analysed from digestibility runs.
¶ Sum of proportional ADCs.
‖ Percent difference = (test diet [determined]-test diet [calculated])/test diet [determined] × 100.

animal proteins that may replace fish meal protein in balanced diets for juvenile Australian eel and more research is needed to further it.

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