A compendium of raw material digestibilities for Barramundi, *Lates calcarifer*

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Abstract

A series of experiments were conducted to examine the nutrient and energy digestibility of a suite of diets and specific test raw materials when fed to juvenile (179g to 439g) barramundi, *Lates calcarifer*. Each of the diets was prepared using a twin-screw extruder to mimic modern aquafeed manufacturing processes. Each of the diets were fed to juvenile barramundi for a minimum of a week to allow acclimation to the diet before the faeces were collected using stripping methods. A broad range of digestible nutrient and energy values among the different raw materials were observed, with protein digestibilities ranging from 36% to 106%, and energy digestibilities ranging from 36% to 93%. This range in nutritional values of the different raw materials provides substantial utility in allowing the formulation of diets on a digestible nutrient and energy basis across the Asia Pacific region. These results also provide critical data to help underpin the replacement of both fishmeal and fishoil in barramundi diets.

Introduction

Aquaculture has long been perceived to be reliant on fishmeal as a protein source and fishoil as a lipid source (Tacon & Metian, 2008). However, over the recent decades there have been a multitude of studies examining a range of different raw materials that have potential application in reducing the reliance on these marine fishery resources as feed inputs for aquaculture (reviewed by Gatlin et al., 2007; reviewed by Glencross 2009). In order to reduce this reliance, it is critical to assess alternative raw materials. A series of key knowledge elements is recognised as being required to enable the effective utilisation of alternative raw materials by the feed production sector. Those being the characterisation of the raw material, the determination of its digestible nutrient and energy value, before assessing the palatability and utilisation value parameters (Glencross et al., 2007).

For barramundi (*Lates calcarifer*), there has been a significant volume of work examining elements of the raw material assessment process (Glencross et al., 2013; Blyth et al., 2015). Much of this work has focussed on either rendered animal meals (Williams et al., 2001; 2003a; 2003b; Glencross, 2011; Glencross et al., 2011) or feed grains (Glencross, 2011; Glencross et al., 2011; 2012). In both cases it has been demonstrated that either rendered animal meals or feed grains can replace substantial amounts of fishmeal in diets for this species. However, it has also been suggested that a critical threshold of around 15% fishmeal was pertinent to barramundi to induce adequate feed intake when fed a diet balanced for digestible protein, energy and amino acids using a plant protein concentrate as the alternative (Glencross et al., 2011; Glencross et al., 2016).

There has been somewhat less work on examining fishoil replacement in feeds for barramundi, though there has been much work done on other fish species (reviewed by Glencross, 2009). Despite this there have been some recent studies that have demonstrated that it has been possible to replace virtually all the fish oil in barramundi diets, so long as high inclusions of fishmeal were present and a minimum level of LC-PUFA maintained (Alhazzaa et al., 2011; Salini et al., 2015).

However, in order to progress the effective replacement of fishmeal and fish oils in feeds for barramundi it is essential that a compendium of raw material digestibilities for this species is assembled, similar that that has been done for Atlantic salmon (Aslaksen et al., 2007). Therefore, the present study was undertaken to determine the digestible value of suite of raw materials and also compile this data with other data from the literature into a single compendium.

Methods

Study design

The experiment design to determine the digestibilities of a series of test ingredients was based on the diet substitution approach (reviewed by Glencross et al., 2007), whereby a basal diet formulation to which each test ingredient was added at 30% by weight to a reciprocal 70% weight of the basal diet formulation. For each experiment a single batch of basal mash was formulated and prepared (Table 1).

To compare and contrast the data from this series of experiments a literature search was also undertaken of all public domain (peer-reviewed journal literature, reports, etc) barramundi digestibility data.

Raw material preparation

A range of raw materials were obtained for use in this study from various sources. The types and origins of each raw material are presented in Table 3. Each of the test ingredients was thoroughly ground using a RetschTM ZM200 rotor mill (Retsch Pty Ltd, North Ryde, NSW, Australia) such that they passed through a 750 μm square-holed screen. Following processing, and prior to diet preparation a sample of each ingredient was collected for chemical analysis.

Diet preparation

A laboratory-scale, twin-screw extruder (APV MFP19:25; APV-Baker, Peterborough, United Kingdom), with intermeshing, co-rotating screws was used to process all diets in this study. Each diet was extruded using the same processing parameters (Glencross et al., 2012). Water was peristaltically pumped (Watson-Marlow 504U, Falmouth, England) into the barrel at between 25 and 36 mL min^{-1}. Water addition was varied among diets based on maximising the expansion potential of each diet, with measurements taken during initial running phases with incremental variations in water addition and measurement of the expansion using vernier callipers (TradeToolsDirect, Ormeau, Australia). A 4 mm ∅ die was used for all diets and pellets were cut into 5 to 6 mm lengths using a four-bladed variable speed cutter and collected on large aluminium oven trays (650 x 450 x 25 mm, length x width x depth) before being dried at 65°C for 12 h. All other operational parameters and extrusion configurations were maintained constant for each of the diets. For some diets (the oil specific ones) the specified oil allocation was vacuum infused into the dried pellets using the methods reported in Glencross et al. (2012).

Barramundi handling and faecal collection

Juvenile barramundi were kept in an experimental tank array (24 x 300 L or 24 x 1000L) supplied with flow-through seawater (salinity =35 PSU; dissolved oxygen ~6.0 mg L^{-1}) of ~30°C at a rate of about 4 L min^{-1}. Each of the tanks were stocked with 20 fish. The specific fish sizes and environmental conditions used in each trial are presented in Table 2. Treatments were randomly assigned amongst the 24 tanks, with each treatment having four replicates, but the experiment being conducted over two block events (duplicates of each treatment were used within each of two blocked events) to achieve this level of replication. The same batch of fish was used for both blocks, but a complete randomised design was applied to each block to ensure experimental validity. The fish were allowed to acclimatise to their allocated dietary treatment for at least seven days before faecal collection commenced (Blyth et al., 2015).

For faecal collection the barramundi were hand fed their respective diets once daily to apparent satiation based on their response to an offering of 3 meals between 0800 and 0900h. Faeces were then collected the same afternoon (1500 – 1630) from each fish following anaesthesia using AQUI-S™ (0.02 mL L^{-1}) using stripping techniques based on those reported by Blyth et al. (2015). Fish were not stripped on consecutive days in order to minimise stress on the animal (as determined by loss of appetite and physical damage, of which none was observed) and to maximise feed intake prior to faecal collection. Faecal samples from different days were pooled within tank, and kept frozen at –20°C before being freeze-dried in preparation for analysis.

Chemical and digestibility analysis

The chemical analyses undertaken varied from experiment to experiment subject to the amount of faecal sample that was available. Dry matter content was calculated following oven drying at 105ºC for 24 h. Gross ash content was determined gravimetrically following loss of mass after combustion of a sample in a muffle furnace at 550°C for 12 h. Protein was determined based on measurement of total nitrogen by CHNOS auto-analyser, and then multiplied by 6.25. Total lipid content of the diets was determined gravimetrically following extraction of the lipids using chloroform:methanol (2:1). Gross energy was determined by ballistic bomb calorimetry. Total starch content was measured using enzymatic methods with the Megazyme Total Starch Kit, K-TSTA, following a modified AOAC Method 996.11. Total carbohydrates were calculated based on the dry matter content of a sample minus the protein, lipid and ash. Total non-starch polysaccharides were determined based on total carbohydrates minus total starch content.

Amino acid analysis involved the samples being hydrolysed at 110ºC for 24 h in 6 M HCl with 0.05 % Phenol. Cystine was derivatized during hydrolysis by the addition of 0.05 % 3-3-dithiodipropoinic acid. The acid hydrolysis destroyed tryptophan making it unable to be determined. Separation of the amino acids was performed by HPLC on a Hypersil AA-ODS 5µm column using an 1100 series Hewlett Packard HPLC system. Fatty acids were analysed as methyl ester derivatives. Lipids were esterified by the method of O'Fallon et al. (2007) and analysed by gas chromatography (GC) using flame ionisation detection. Specific fatty acid peaks were identified by comparing retention times relative to standards. Total yttrium and phosphorus concentrations were determined using inductively coupled plasma mass spectrometry (ICP-MS) after mixed acid digestion based on the method described by McQuaker et al., (1979).

The apparent digestibility (AD_{diet}) for each of the nutritional parameters examined in each diet was calculated based on the following formula (Maynard and Loosli, 1979):

$$AD_{diet} = \left(1 - \left(\frac{Y_{diet} \times Parameter_{faeces}}{Y_{faeces} \times Parameter_{diet}}\right)\right) \times 100$$

where Y_{diet} and Y_{faeces} represent the yttrium content of the diet and faeces respectively, and $Parameter_{diet}$ and $Parameter_{faeces}$ represent the nutritional parameter of concern (dry matter, protein [and amino acids in one study] or energy) content of the diet and faeces respectively. The digestibility values for each of the test ingredients in the test diets examined in this study were calculated according to the formulae:

$$Nutr.AD_{ingredient} = \frac{\left(AD_{test} \times Nutr_{test} - \left(AD_{basal} \times Nutr_{basal} \times 0.7\right)\right)}{\left(0.3 \times Nutr._{ingredient}\right)}$$

Where $Nutr.AD_{ingredient}$ is the digestibility of a given nutrient from the test ingredient included in the test diet at 30%. AD_{test} is the apparent digestibility of the test diet. AD_{basal} is the apparent digestibility of the basal diet, which makes up 70% of the test diet. $Nutr_{Ingredient}$, $Nutr_{test}$ and $Nutr_{basal}$ are the level of the nutrient of interest in the ingredient, test diet and basal diet respectively (as reviewed by Glencross et al., 2007).

Statistical analysis

All values are mean ± SE unless otherwise specified. Effects of raw material type were not statistically evaluated as it was perceived that such a statistical comparison was irrelevant to the

utility of this data in this study. Regression analysis was undertaken using the data analysis package of MSExcel.

Results

Raw material characterisation

As expected there was a substantial range in the composition parameters observed for the different raw materials assessed (Table 3). Protein concentrations in the raw materials varied from 271 g kg^{-1} DM in the Camelina meal to 965 g kg^{-1} DM in the Soy protein isolate. Other than the different oil raw materials, the lipid concentrations were lowest in each of the Blood meals (~1 g kg^{-1} DM) and highest in the Camelina meal (311 g kg^{-1} DM). Energy densities were highest in the oil raw materials (fish oil, ricebran oil and poultry oil) at 38.4 to 39.7 MJ kg^{-1}. The lowest energy densities were observed from one of the soy protein concentrates (17.5 MJ kg-1) though the Faba bean meal was also among the lower energy dense raw materials at 18.8 MJ kg^{-1}. Amino acid concentrations also varied substantially among the different raw materials (Table 3).

Raw material digestibility

There was a substantial range in the digestibilities of each of the parameters examined (dry matter, protein, lipid, energy, sum of amino acids and individual amino acids) across each of the three experiments presented. This largely reflected the different raw materials that were assessed, though there were some notable variations within specific raw material types. Raw material dry matter digestibilities ranged from 31% to 96% across the different raw materials, with an average of 59 ± 2.4%. Raw material protein digestibilities ranged from 36% to 106% across the twelve treatments, with an average of 81 ± 2.6%. Raw material lipid digestibilities ranged from 21% to 487% across the range of raw materials, with an average of 110 ± 14.6%. Raw material energy digestibilities ranged from 36% to 93%, with an average of 67 ± 2.3% (Table 4). Raw material sum of amino acid digestibilities ranged from 77% to 89% across the range of raw materials, with an average of 83 ± 1.2% (Table 5). Among the individual amino acids the mean digestibilities ranged from 72% for threonine to 120% for proline. Some amino acids, like lysine, were consistently highly digestible across the different raw materials (Table 5).

Between certain digestibility parameters there were clear relationships. Raw material energy digestibility was clearly linked (R=0.860, p=0.001) to protein digestibility (among those non-oil raw materials) (Table 4). However other expected relationships, like that between protein digestibility and of the sum of amino acids (sAA) digestibility (another way of examining protein digestibility) produced a poor regressions (R=-0.244, p=0.675) (Table 5).

Discussion

This study examined the nutritional value of a series of alternative raw materials to the use of both fishmeal and fish oil in diets for juvenile barramundi. The focus of this assessment was the examination of the digestible nutrient and energy value of each of these raw materials so as to provide data suitable for the formulation of diets on a digestible nutrient and energy basis (Glencross et al., 2007). Additional to this a literature survey was conducted to compile this new data, with other digestibility data available for this species, into a single compendium (McMeniman, 1998; Glencross 2011; Glencross et al., 2011; 2012; 2014; Tabrett et al.. 2012; Blyth et al., 2014; Diu et al., 2015).

Plant raw material digestibility

A wide range of plant derived raw materials was evaluated in the present digestibility study. Among those examined was a series of soybean products, including solvent extracted soybean meals, soy protein concentrates (SPC) and soy protein isolate (SPI) and several other feed grain varieties. Soy products have generally been favourably used in diets for barramundi without many issues, despite a lack of digestibility data (Boonyaratpailin et al., 1998; Tantikitti et al., 2005). Of the different feed grain varieties studied in the present work the protein digestibility was generally high at around 90%, though the dry matter and energy digestibilities were typically lower, reflecting the lower concentration of protein found in these raw materials (the exception being the highly processed products of SPC and SPI). Both McMeniman (1998) and Glencross (2011) previously examined the digestibility of solvent-extracted soybean meal in diets fed to barramundi and observed protein digestibilities of 85% and 103% respectively (Table 6). While it is often difficult to compare digestibility values across studies, without some form of reference, it can be seen that the range in protein digestibility values for such a common raw material can be quite expansive (65% to 103%) across all the combined studies. These differences could be due to a range of factors including the soybean genotype, growing environment, processing and not withstanding also the experimental methodologies used (Glencross et al., 2007).

However, substantial differences were also seen between the two SPC raw materials evaluated in the present studies and although there were subtle differences in the basal diets between the two experiments, otherwise experimental methodologies were kept uniform. Despite these consistencies in methodologies the protein digestibilities of the two SPC raw materials varied from 49% to 95% and the energy digestibilities observed for each raw material were consistent with there being such a substantial difference in protein digestibility between the two.

Such differences may be explained by processing methods used to produce either product (Gatlin et al., 2007). Interesting was the observation of the protein digestibility of SPC-1 in terms of both nitrogen and sum of amino acids, both used as proxies for determining protein digestibility, in that they were quite divergent. This observation perhaps suggests that there might have been a significant level of non-protein nitrogen associated with SPC-1 that was not absorbed by the animal. This observation also raises the question as to the more appropriate way to assess protein digestibility, from nitrogen or sum of amino acid data. While in other studies there has been a good regression between these two parameters in the present study this divergence casts some doubt on the validity of either method (Glencross et al., 2008). Logic suggests that the use of sum of amino acids provides a more valid assessment as it is less likely that there are non-protein amino acids in the raw materials than non-protein nitrogen sources (Krober & Gibbons, 1962). However, sum of amino acids does not account for tryptophan, albeit levels of this amino acid in most raw materials are very low and the comparison is still consistent across all samples.

There were also no other published reports on the digestible value of SPCs when fed to barramundi, but other data on protein concentrates from lupins were found (Glencross, 2011). Each of the lupin protein concentrates studied also had high protein and energy digestibilities. However, protein concentrates from other grain species such as canola/rapeseed, field peas or faba beans remain to be explored. Certainly studies on understanding the influence of different carbohydrate classes and non-starch polysaccharides on the digestibility of diets by this species provides a clear mechanism for understanding why some substantial differences are observed among some of the plant protein raw materials (Irvin et al., 2015).

Among the other plant protein raw materials examined in the present study a consistently high level of protein digestibility (90% to 95%) was observed. The exception to this was the Camelina meal which produced both protein and energy digestibility values of 36%. There was no other data in the literature on digestibility values of Camelina meal when fed to barramundi in which to compare, however some data was found on growth responses from work with Atlantic salmon (Hixson et al., 2014). That work found little impact from the inclusion of 100 g kg^{-1} of camelina meal, although the diet did contain high fishmeal levels (~318 g kg^{-1}). There were however several studies on the digestibility of lupin and canola meals (Glencross, 2011; Tabrett et al., 2012; Diu et al., 2015). The literature values found for lupin kernel meal protein digestibilities varied among the different lupins species evaluated, but ranged from 81% to 109%. For the *Lupinus angustifolius* species evaluated in the present study this range was substantially smaller (86% to 98%). Notably in some studies where the same sample of lupin kernel meal was used

across studies a highly conserved range of protein digestibility values were observed. Values of 96% to 97% were seen for the *L. angustifolius* cv. Myallie variety (Glencross, 2011; Tabrett et al., 2012) and 86% to 90% were seen for the *L. angustifolius* cv. Coromup variety (Diu et al., 2015; present study). This observation suggests that the between study variation is perhaps smaller than the between variety variation.

Animal raw material digestibility

A range of animal derived raw materials were also evaluated in the present digestibility study. Among those examined was a series of blood meal products and poultry offal meals. Each of the three blood meals examined had protein digestibilities that were observed to be similar to or better than that of the poultry offal meal or fish (tuna by-product) meal. Notably two of the blood meals had protein digestibilities above 100%, with one clearly lower at 83%. Reasons for why this difference existed among the blood meals are not clear as no information was provided from the supplier on the basis of the sample origin variability, other than it is suspected they were from different rendering plants. Clearly to follow this up further a more direct approach to rendering plants needs to be undertaken rather than obtaining samples from a feed producer. Other studies examining rendered mammalian meals with both barramundi and other species have also identified substantial variability in digestible values for these raw materials (McMeniman, 1998; Bureau et al., 1999).

Amino acid digestibilities of the poultry offal meal and tuna offal meal were quite similar, except for one or two amino acids. Those amino acids that were quite different between these two raw materials included cysteine and serine. Overall the sum of amino acid digestibilities were also quite different at 77% and 87% and these contrasted those of the nitrogen digestibilities 87% and 71% respectively for the same two samples. As with the plant protein raw materials there is a range of reasons why this difference may exist, however this cannot be reasonably explored based on the assessment of two raw material samples and clearly further work on this issue is warranted.

Lipid raw material digestibility

Each of the three lipid raw materials examined in the present study had lipid and/or energy digestibilities that were observed to be similar amongst each other with no clear better or inferior product. Although there have been a few studies examining lipid raw materials in barramundi, no data was found determining the digestibility of any lipid resources in this species (Alhazzaa et al.,

2011; Salini et al., 2015). It would be useful to not only follow this work up with assessment of additional lipid raw materials, but also to assess the discrete digestibilities of individual fatty acids from within the different lipid raw materials.

Conclusions

The findings from this compendium provide a useful resource to enable nutritionists to formulate diets for barramundi on a digestible nutrient and energy basis. To further reduce feed risk, additional raw materials need evaluation and dissemination of this data remains one of the highest priorities to provide enhanced flexibility for formulation options for use in barramundi feeds (Glencross et al., 2007). In addition to assessing the digestibility of additional raw materials, it was clear from this study that there is considerable variability in the nutritional value of raw materials, not only between types, but even within types. Therefore, to follow from this work further effort needs to be spent on defining those factors that affect the nutritional value within classes of raw materials. This can most notably be achieved by defining their digestible value relative to their chemical composition (Glencross, 2011; Glencross et al., 2007; 2011).

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References

Alhazzaa, R., Bridle, A.R., Nichols, P.D. & Carter, C.G., (2011) Replacing dietary fish oil with Echium oil enriched barramundi with C18 PUFA rather than long-chain PUFA. Aquaculture 312, 162–171.

Aslaksen, M.A., Kraugerud, O.F., Penn, M., Svihus, B., Denstadli, V., Jorgensen, H.Y., Hillestad, M., Krogdahl, A. & Storebakken, T., (2007) Screening of nutrient digestibilities and intestinal pathologies in Atlantic salmon, *Salmo salar*, fed diets with legumes, oilseeds, or cereals. Aquaculture 272, 541-555.

Blyth, D., Tabrett, S.J. & Glencross, B.D., (2015) A study of the effects of faecal collection method and acclimation time on the digestibility of diets and ingredients when fed to juvenile barramundi (*Lates calcarifer*). Aquaculture Nutrition 21, 248–255. DOI: 10.1111/anu.12159.

Boonyaratpalin, M., Suraneiranat, P. & Tunpibal, T., (1998) Replacement of fish meal with various types of soybean products in diets for the Asian sea bass, *Lates calcarifer*. Aquaculture 161, 67-78.

Bureau, D.P., Harris, A.M. & Cho, C.Y., (1999) Apparent digestibility of rendered animal protein ingredients for rainbow trout (*Oncorhynchus mykiss*). Aquaculture 180, 345-358.

Diu, N.T., Pirozzi, I. & Glencross, B.D., (2015). Digestibility of canola meals in barramundi (Asian seabass; *Lates calcarifer*). Aquaculture 435, 442–449.

Gatlin, D.M., Barrows, F.T., Brown, P., Dabrowski, K., Gaylord, T.G., Hardy, R.W., Herman, E., Hu, G., Krogdahl, A., Nelson, R., Overturf, K., Rust, M., Sealy, W., Skonberg, D., Souza, E.J., Stone, D., Wilson, R. & Wurtele, E. (2007) Expanding the utilisation of sustainable plant products in aquafeeds: a review. Aquaculture Research 38, 551-579.

Glencross, B.D., (2006) Nutritional management of barramundi, *Lates calcarifer* – A review. Aquaculture Nutrition 12, 291-309.

Glencross, B.D., (2009) Exploring the nutritional demand for essential fatty acids by aquaculture species. Reviews in Aquaculture 1, 71-124

Glencross, B.D., (2011) A comparison of the diet and raw material digestibilities between rainbow trout (*Oncorhynchus mykiss*) and barramundi (*Lates calcarifer*) – Implications for inferences of digestibility among species. Aquaculture Nutrition 17, e207-e215.

Glencross, B.D., Booth, M. & Allan, G.L., (2007) A feed is only as good as its ingredients – A review of ingredient evaluation for aquaculture feeds. Aquaculture Nutrition 13, 17 – 34.

Glencross, B.D., Hawkins, W.E., Evans, D., Rutherford, N., McCafferty, P., Dods, K. & Sipsas, S., (2008) Assessing the implications of variability in the digestible protein and energy value of lupin kernel meals when fed to rainbow trout, *Oncorhynchus mykiss*. Aquaculture 277, 251-262.

Glencross, B.D., Rutherford, N.R. & Jones, J.B., (2011) Fishmeal replacement options for juvenile barramundi (*Lates calcarifer*). Aquaculture Nutrition 17; e722–e732.

Glencross, B.D., Blyth D., Tabrett, S.J., Bourne, N., Irvin, S., Fox-Smith, T. & Smullen, R.P., (2012). An examination of digestibility and technical qualities of a range of cereal grains when fed to juvenile barramundi (*Lates calcarifer*) in extruded diets. Aquaculture Nutrition 18, 388-399.

Glencross, B., Wade, N. & Morton, K., (2013) Chapter 8. *Lates calcarifer* nutrition and feeding practices. In: Barramundi. (D.R. Jerry, F. Ayson. Eds.) CAB International, Wallingford UK. pp 178-228.

Glencross, B.D., Blyth, D., Bourne, N., Irvin, S. & Wade, N.P., (2014) An analysis of the effects of different dietary macronutrient energy sources on the growth and energy partitioning by juvenile barramundi, *Lates calcarifer*, reveal a preference for protein-derived energy. Aquaculture Nutrition 20, 583-594.

Glencross, B.D., Irvin, S., Blyth, D., Bourne, N., Campet, M., Boisot, P. & Wade, N., (2016) An evaluation of the complete replacement of both fishmeal and fish oil in diets for juvenile Asian seabass, *Lates calcarifer*. Aquaculture 451, 298-309.

Hixson, S.M., Parrish, C.C. & Anderson, D.M., (2014) Full substitution of fish oil with camelina (*Camelina sativa*) oil, with partial substitution of fish meal with camelina meal, in diets for farmed Atlantic salmon (*Salmo salar*) and its effect on tissue lipids an sensory quality. Food Chemistry 157, 51-61.

Irvin, S., Blyth, D., Bourne, N. & Glencross, B.D., (2015) Examining the discrete and interactive effect of different NSP non-starch polysaccharide (NSP) sources on feed digestibility by barramundi, *Lates calcarifer*. Aquaculture Nutrition DOI: 10.1111/anu.12321.

Krober, O.A. & Gibbons, S.J., (1962) Composition of Feedstuffs, Nonprotein Nitrogen in Soybeans. J. Agric. Food Chem. 10, 57-59.

Maynard, L.A. & Loosli, J.K. (1979) Animal Nutrition, 6th Edition. New York, NY: McGraw-Hill Book Co.

McMeniman, N., (1998) The apparent digestibility of feed ingredients based on stripping methods. In: Fishmeal Replacement in Aquaculture Feeds for Barramundi (K.C. Williams Ed.).

Project 93/120-04. Final Report to Fisheries R&D Corporation. Canberra, Australia. pp 46-70.

McQuaker, N.R., Brown, D.F. & Kluckner, P.D., (1979) Digestion of environmental materials for analysis by Inductively Coupled Plasma – Atomic Emission Spectrometry. Analytical Chemistry 51, 1082-1084.

O'Fallon, J.V., Busboom, J.R., Nelson, M.L. & Gaskins, C.T., (2007) A direct method for fatty acid methyl ester (FAME) synthesis: Application to wet meat, tissues, oils & feedstuffs. Journal of Animal Science 85,1511-1522.

Salini, M., Irvin, S., Bourne, N., Blyth, D., Cheers, S., Habilay, N. & Glencross, B.D., (2015) Marginal efficiencies of long chain-polyunsaturated fatty acid use by barramundi (Lates calcarifer) when fed diets with varying blends of fish oil and poultry fat. Aquaculture 449, 48-57. doi.org/10.1016/j.aquaculture

Tabrett, S.J., Blyth D. & Glencross, B.D., (2012) An examination of the variability in the digestibility of kernel meals of different cultivars of the white lupin, *Lupinus albus* fed to juvenile barramundi (*Lates calcarifer*). Aquaculture 364-365. 1-5.

Tacon, A.G.J. & Metian, M., (2008) Global overview on the use of fish meal and fish oil in industrially compounded aquafeeds: Trends and future prospects. Aquaculture 285, 146–158

Tantikitti, C., Sangpong, W. & Chiavareesajja, S., (2005) Effects of defatted soybean protein levels on growth performance and nitrogen and phosphorus excretion in Asian seabass (*Lates calcarifer*). Aquaculture 248, 41-50.

Williams, K.C., Barlow, C.G. & Rodgers, L., (2001) Efficacy of crystalline and protein-bound amino acids for amino acid enrichment of diets for barramundi/Asian seabass (*Lates calcarifer* Bloch). Aquaculture Research 32, 415-429.

Williams, K.C., Barlow, C.G., Rodgers, L., Hockings, I., Agcopra, C. & Ruscoe, I., (2003a) Asian seabass *Lates calcarifer* perform well when fed pellet diets high in protein and lipid. Aquaculture 225, 191-206.

Williams, K.C., Barlow, C.G., Rodgers, L. & Ruscoe, I., (2003b) Potential of meat meal to replace fish meal in extruded dry diets for barramundi *Lates calcarifer* (Bloch). I. Growth performance. Aquaculture Research 34, 23-32.

Table 1. Formulations and composition of each of the basal diets used in each experiment (all values are g kg^{-1} as used unless otherwise indicated).

Experiment	BAR-10-1	BAR-12-2	BAR-14-1
Raw material			
Fishmeal (Anchovetta)	640	764	750
Fish oil	100	50	20
Wheat flour	-	80	224
Wheat gluten	130	-	-
Cellulose	124	100	-
Vitamin and mineral premix*	5	5	5
Yttrium oxide	1	1	1
Diet composition			
Dry matter (g kg^{-1})	959	972	973
Protein	546	546	598
Lipid	129	136	97
Ash	106	143	160
Energy (MJ kg^{-1} DM)	22.1	20.7	21.5
sum Amino Acids	509	502	524
Alanine	38	34	32
Arginine	28	30	27
Asparagine	31	49	30
Cysteine	6	6	6
Glutamate	54	69	47
Glycine	36	34	23
Histidine	16	17	16
Isoleucine	39	23	41
Leucine	84	40	98
Lysine	39	42	38
Methionine	13	18	40
Phenylalanine	31	21	29
Proline	26	25	20
Serine	20	24	18
Taurine	4	5	3
Threonine	20	24	20
Tyrosine	20	17	16
Valine	20	25	20

* Vitamin and mineral premix includes (IU/kg or g/kg of premix): Vitamin A, 2.5MIU; Vitamin D3, 0.25 MIU; Vitamin E, 16.7 g; Vitamin K,3, 1.7 g; Vitamin B1, 2.5 g; Vitamin B2, 4.2 g; Vitamin B3, 25 g; Vitamin B5, 8.3; Vitamin B6, 2.0 g; Vitamin B9, 0.8; Vitamin B12, 0.005 g; Biotin, 0.17 g; Vitamin C, 75 g; Choline, 166.7 g; Inositol, 58.3 g; Ethoxyquin, 20.8 g; Copper, 2.5 g; Ferrous iron, 10.0 g; Magnesium, 16.6 g; Manganese, 15.0 g; Zinc, 25.0 g.

Table 2. Operational parameters of each experiment.

Experiment	Temperature ºC	DO mg L^{-1}	Tank Volume L	Fish Weight g $fish^{-1}$
BAR-10-1	28.8 ± 0.22	6.4 ± 0.15	250	398 ± 68.8
BAR-12-2	29.9 ± 0.12	5.5 ± 0.56	250	179 ± 73.0
BAR-14-1	30.3 ± 1.50	6.2 ± 0.1	1000	439 ± 97.2

Table 3. Composition of test ingredients

	Tuna Offal Meal	Poultry Offal Meal	Blood Meal 1	Blood Meal 2	Blood Meal 3	Soybean Meal 1	Soybean Meal 2	Camelina Meal	Lupin Kernel Meal	Faba Bean Meal	SPC 1 (Wilmar)	SPC 2 (Selecta)	SPI (ADM)	Fish oil	Ricebran Oil	Poultry Oil
Experiment	B-12-2	B-12-2	B-10-1	B-10-1	B-10-1	B-12-2	B-14-1	B-14-1	B-14-1	B-14-1	B-12-2	B-14-1	B-12-2	B-12-2	B-12-2	B-12-2
Dry matter (g kg^{-1})	920	974	935	937	872	877	897	933	916	905	871	923	896	1000	997	990
Protein (N x 6.25)	657	530	936	887	953	515	456	271	465	309	723	657	965	4	6	13
Lipid	85	179	1	1	1	27	94	311	87	22	14	29	57	993	912	939
CHO	7	138	46	94	26	386	390	372	417	641	197	237	0	3	80	59
Ash	243	149	17	18	20	68	60	46	31	28	66	77	33	0	0	0
Energy (MJ kg^{-1})	19.4	22.6	24.5	24.6	25.1	20.1	21.9	26.3	21.3	18.8	17.5	21.4	20.6	38.6	39.7	38.4
∑Amino Acids	590	619	989	983	939	478	413	246	390	248	644	590	855	-	-	-
Alanine	41	41	77	77	74	22	22	12	17	12	28	24	35	-	-	-
Arginine	38	45	43	43	51	36	29	20	44	24	50	45	68	-	-	-
Aspartic acid	59	52	101	100	89	57	34	15	31	23	77	45	103	-	-	-
Cysteine	9	13	16	16	18	9	7	6	5	3	10	9	13	-	-	-
Glutamic acid	78	83	91	90	99	89	34	26	34	20	123	47	172	-	-	-
Glycine	44	58	40	40	37	20	29	17	35	18	27	39	35	-	-	-
Histidine	17	12	58	57	48	14	12	7	12	6	17	20	22	-	-	-
Isoleucine	26	26	16	16	36	21	34	18	31	19	28	45	38	-	-	-
Leucine	48	48	119	118	102	38	60	30	50	45	52	67	68	-	-	-
Lysine	47	33	88	87	81	26	32	18	29	19	37	42	46	-	-	-
Methionine	19	15	16	17	15	8	8	11	4	6	9	25	12	-	-	-
Phenylalanine	26	28	70	70	60	26	34	15	23	15	35	58	47	-	-	-
Proline	29	46	44	44	43	24	20	13	16	11	31	29	43	-	-	-
Serine	28	39	50	50	44	28	20	11	18	11	38	28	50	-	-	-
Taurine	1	2	0	0	0	0	0	0	0	0	0	0	1	-	-	-
Threonine	30	27	53	51	50	21	16	10	14	8	28	24	34	-	-	-
Tyrosine	20	20	31	32	33	18	7	6	12	0	24	22	30	-	-	-
Valine	30	31	76	75	59	21	15	11	13	9	30	22	38	-	-	-
C14:0	3.0	1.2	n/a	n/a	n/a	0.0	n/a	n/a	n/a	n/a	0.6	n/a	0.0	8.2	0.5	1.1

C16:0	25.3	24.6	n/a	n/a	n/a	17.4	n/a	n/a	n/a	n/a	17.3	n/a	14.9	18.9	19.8	0.0
C18:0	9.6	9.1	n/a	n/a	n/a	4.9	n/a	n/a	n/a	n/a	4.7	n/a	5.2	3.7	2.2	0.0
C16:1	3.6	6.9	n/a	n/a	n/a	0.0	n/a	n/a	n/a	n/a	0.8	n/a	0.0	10.2	0.0	6.9
C18:1	17.7	43.4	n/a	n/a	n/a	15.6	n/a	n/a	n/a	n/a	26.3	n/a	23.2	13.6	41.8	66.1
C18:2n-6	2.8	11.3	n/a	n/a	n/a	53.4	n/a	n/a	n/a	n/a	41.7	n/a	49.8	1.9	32.4	20.6
C18:3n-3	0.0	1.5	n/a	n/a	n/a	8.2	n/a	n/a	n/a	n/a	4.6	n/a	6.9	0.7	1.2	2.5
C20:4n-6	2.6	1.3	n/a	n/a	n/a	0.0	n/a	n/a	n/a	n/a	0.0	n/a	0.0	1.1	0.0	0.0
C20:5n-3	4.6	0.0	n/a	n/a	n/a	0.0	n/a	n/a	n/a	n/a	0.4	n/a	0.0	17.6	0.0	0.0
C22:6n-3	23.5	0.8	n/a	n/a	n/a	0.0	n/a	n/a	n/a	n/a	0.0	n/a	0.0	13.9	0.0	0.0
∑SFA	40.8	34.9	n/a	n/a	n/a	22.8	n/a	n/a	n/a	n/a	24.6	n/a	20.1	33.1	23.7	2.1
∑MUFA	24.4	50.2	n/a	n/a	n/a	15.6	n/a	n/a	n/a	n/a	28.7	n/a	23.2	26.2	42.3	73.9
∑PUFA	2.8	12.8	n/a	n/a	n/a	61.6	n/a	n/a	n/a	n/a	46.2	n/a	56.6	5.9	33.6	23.5
∑LC-PUFA	32.0	2.1	n/a	n/a	n/a	0.0	n/a	n/a	n/a	n/a	0.4	n/a	0.0	34.9	0.5	0.4
∑n-3	29.3	2.3	n/a	n/a	n/a	8.2	n/a	n/a	n/a	n/a	5.0	n/a	6.9	37.4	1.6	3.2
∑n-6	5.5	12.6	n/a	n/a	n/a	53.4	n/a	n/a	n/a	n/a	41.7	n/a	49.8	3.0	32.4	20.8

Unless otherwise indicated all data is g/kg DM except fatty acid data which is % of total fatty acids. n/a: not analysed. ADM, Decatur, IL, USA.; Alfaone, Condell Park, NSW, Australia; Aus-Oils, Kojonup, WA, Australia; BEC Feed Solutions, Carole Park, QLD, Australia; CSIRO Plant Industries, Black Mountain, ACT, Australia; COGGO, Winthrop, WA, Australia; Coorow Seed Cleaners, Coorow, WA, Australia; Manildra, Auburn, NSW, Australia; Ridley Aquafeeds, Narangba, QLD, Australia; Selecta, Araguari, Brazil; Skretting Australia, Cambridge, TAS, Australia ; Wilmar, Singapore.

Table 4. Raw material digestibilities (%)

Experiment	Ingredient	Origin	Dry matter	Protein	Lipid	Energy
BAR-10-1	Blood Meal 1	Skretting Australia	96	83	-	73
BAR-10-1	Blood Meal 2	Skretting Australia	84	102	-	80
BAR-10-1	Blood Meal 3	Skretting Australia	83	106	-	76
BAR-12-2	Soy Protein Concentrate	Wilmar	53	49	85	49
BAR-12-2	Soybean Meal (Solvent-Extracted)	BEC Feed Solutions	31	68	57	35
BAR-12-2	Soy Protein Isolate	ADM	53	74	21	56
BAR-12-2	Poultry Offal Meal	BEC Feed Solutions	42	87	89	65
BAR-12-2	Fishmeal (Tuna Offal Meal)	BEC Feed Solutions	49	71	45	56
BAR-12-2	Ricebran oil	Alfaone	-	-	82	93
BAR-12-2	Fish oil (Peruvian anchovetta)	Ridley Aquafeeds	-	-	84	92
BAR-12-2	Poultry oil	Ridley Aquafeeds	-	-	80	83
BAR-14-1	Soy Protein Concentrate	Selecta	65	95	136	77
BAR-14-1	Camelina Meal	Aus-Oils	41	36	64	36
BAR-14-1	Lupin (*L. angustifolius* cv. Coromup) Kernel Meal	Coorow Seed Cleaners	67	90	100	61
BAR-14-1	Soybean Meal (Solvent-Extracted)	Ridley Aquafeeds	60	92	103	63
BAR-14-1	Faba Bean Meal	Ridley Aquafeeds	42	95	487	67

Table 5. Raw material amino acid digestibilities (%) derived from Experiment BAR-12-2.

Ingredient	SPC-1	SBM-1	SPI	POM	FISH	Pooled SEM
Protein*	49	68	74	87	71	2.8
sum Amino Acids	81	89	83	77	87	1.2
Alanine	89	93	78	83	88	2.1
Arginine	86	126	81	81	87	2.1
Asparagine	68	85	85	71	82	1.6
Cysteine	65	53	67	69	92	2.3
Glutamate	79	90	87	76	80	1.2
Glycine	82	79	97	87	91	2.8
Histidine	68	50	62	94	91	2.5
Isoleucine	105	140	86	90	104	2.6
Leucine	89	104	82	79	87	1.4
Lysine	97	97	101	115	109	3.0
Methionine	102	82	80	92	95	3.5
Phenylalanine	85	97	78	59	66	1.7
Proline	123	158	118	89	110	3.3
Serine	81	85	86	65	85	1.5
Taurine	0	0	0	104	90	8.5
Threonine	67	51	77	78	85	2.2
Tyrosine	97	135	97	86	103	2.2
Valine	94	112	77	81	97	1.8

SPC: Soybean protein concentrate. Soy: Soybean meal. SPI: Soybean protein isolate. POM: Poultry offal meal. FISH: Tuna offal (by-product) fishmeal. *Cross referenced against protein (Nitrogen) digestibility from Table 4.

Table 6. Literature raw material composition (g kg^{-1} DM) and digestibilities (%)

	Raw Material Composition				Raw Material Digestibility				
Raw material	Protein	Lipid	Ash	CHO	DM	Protein	Energy	Starch	Published as
Meat meal A	581	110	339	0	-	54	58	-	McMeniman, 1998
Meat meal B	608	146	242	4	-	64	66	-	"
Poultry offal meal	658	145	178	20	-	79	77	-	"
Fishmeal (Danish)	760	114	130	0	-	88	83	-	"
Fishmeal (Tuna meal)	567	111	303	18	-	92	69	-	"
Soybean meal (solvent-extracted)	448	184	53	315	-	85	76	-	"
Soybean meal (full-fat)	530	16	73	381	-	86	69	-	"
Peanut meal	321	480	27	172	-	92	69	-	"
Canola meal (solvent-extracted)	409	30	68	492	-	81	56	-	"
Lupin (*L. angustifolius*) kernel meal	440	88	27	445	-	98	61	-	"
Wheat gluten	841	24	16	119	-	102	99	-	"
Lupin (*L. luteus* cv. Wodjil) kernel meal	567	67	39	327	65	81	83	-	Glencross, 2011
Lupin (*L. angustifolius* cv. Myallie) kernel meal	412	64	35	489	37	96	73	-	"
Yellow lupin protein concentrate	819	112	29	40	92	99	113	-	"
Narrowleaf lupin protein concentrate	754	153	23	70	89	86	100	-	"
Soybean meal (solvent extracted)	500	17	86	397	57	103	65	-	"
Canola meal (expeller extracted)	388	133	53	559	21	63	60	-	"
Poultry offal meal	608	119	160	113	10	40	52	-	"
Hydrolysed feather meal	802	144	17	37	37	75	68	-	"
Barley (871)	269	81	28	622	50	63	56	96	Glencross et al., 2012
Barley (Waxiro)	184	41	19	756	59	94	63	57	"
Barley (Torrens)	252	36	24	687	85	79	77	41	"
Wheat	196	31	15	758	66	100	65	30	"

Oats	135	91	25	749	58	98	52	55	“
Barley	151	44	21	784	47	153	55	46	“
Sorghum	138	39	15	808	56	110	54	18	“
Tapioca	7	3	4	986	74	-	58	19	“
Triticale	205	26	20	749	64	111	57	37	“
Corn	52	26	18	905	81	150	43	18	“
Faba	380	63	36	521	65	104	62	41	“
Lupin (*L. albus* cv. Kiev mutant) kernel meal	502	82	37	379	58	102	68	-	Tabrett et al., 2012
Lupin (*L. albus* cv. Andromeda) kernel meal	482	86	37	395	75	109	79	-	“
Lupin (*L. albus* cv. WALAB2014) kernel meal	488	82	38	392	62	105	75	-	“
Lupin (*L. angustifolius* cv. Myallie) kernel meal	383	54	34	529	41	97	51	-	“
Fishmeal (Anchovetta)	721	85	158	36	92	93	95	-	Glencross et al., 2014
Pregelled Wheat Starch	10	1	3	986	84	-	86	-	“
Vitamin Free Casein	811	1	13	175	85	100	87	-	“
Wheat Gluten	710	46	8	236	90	100	98	-	“
Canola meal SE (Footscray)	370	57	67	506	55	82	66	-	Diu et al., 2015
Canola meal SE (Newcastle)	423	44	69	464	59	84	71	-	“
Canola meal SE (Nurmurkah)	381	56	78	485	58	84	68	-	“
Canola meal EX (Pinjarra)	348	92	70	490	56	80	68	-	“
Lupin (*L. angustifolius* cv. Coromup) kernel meal	408	64	31	497	58	86	71	-	“

ADM, Decatur, IL, USA.; Alfaone, Condell Park, NSW, Australia; BEC Feed Solutions, Carole Park, QLD, Australia; CSIRO Plant Industries, Black Mountain, ACT, Australia; COGGO, Winthrop, WA, Australia; Coorow Seed Cleaners, Coorow, WA, Australia; Manildra, Auburn, NSW, Australia; Ridley Aquafeeds, Narangba, QLD, Australia; Skretting Australia, Cambridge, TAS, Australia . SE: Solvent extracted. EX: Expeller extracted.