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Critical variability exists in the digestible value of raw materials fed to black tiger shrimp, *Penaeus monodon*: The characterisation and digestibility assessment of a series of research and commercial raw materials

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Highlights

- The digestibilities of each of 29 raw materials were determined for *Penaeus monodon*.
- Significant variability was observed in both diet and subsequent ingredient digestibilities.
- The combined variation in composition and digestibility was shown to magnify differences in quality between raw materials.
- This data provides an improved basis from which to formulate shrimp diets on a digestible nutrient basis.

Abstract

The digestibility of a suite of raw materials was determined when fed to black tiger shrimp (*Penaeus monodon*) in a series of three experiments. A total of 29 commercial and research raw materials were evaluated using the diet replacement digestibility method. Each of the reference and test diets were fed to tanks of shrimp for one-week prior to commencing faecal collection. The collected faecal samples were kept separate from any feed residue through using a discrete feeding period, after which uneaten feed was removed before a separate faecal collecting period. The same reference diet and soy protein concentrate diet were used across each of the three experiments and demonstrated consistent digestibility using this method. Most raw materials demonstrated some utility for use in diets for shrimp, with digestible protein or energy values greater than 0.800. However, there were some raw materials (e.g. Camelina meal) that provided very little nutritive value for shrimp. This study presents data on the digestibility and digestible nutrient content of a wide variety of raw materials, providing a clear basis for progressing to formulating shrimp diets on a digestible protein and energy basis, thereby optimising dietary formulation, maximising ingredient utilisation and reducing impacts of uneaten feed.

1. Introduction

Progress in the use of raw materials, other than fishmeal and fish oils, in diets for shrimp has resulted in significant advancements in the ability to utilize a range of different terrestrial derived grain and animal resources (Davis and Arnold, 2000; Davis et al., 2002; Alvarez et al., 2007; Cruz-Suarez et al., 2001; 2007; Smith et al., 2007; Luo et al., 2012; Carvalho et al., 2016). However, the capacity to effectively utilize raw materials in diets for any aquaculture species, including shrimp, relies on an ability to formulate diets to consistent digestible nutrient and digestible energy specifications (Glencross et al., 2007). Failure to formulate on an equivalent digestible nutrient and energy basis can result in a misleading interpretation of the value of a raw material through a failure of diet specifications, not a failure in the raw material per se. However, in many cases, the assessment of alternative raw materials has occurred with excess nutrient supply masking any potential deficiencies through the formulation of diets to crude nutrient and gross energy specifications only and as such the variability in the nutritional value of those alternatives is missed because of that over supply of nutrients (Glencross et al., 2008).

Over the past twenty years there have been a suite of studies that have evaluated the digestibility of specific raw materials (Merican and Shim, 1995; Brunsen et al., 1997; Glencross and Smith, 1997; Smith et al., 2007, Cruz-Suarez et al., 2007; 2009; Yang et al., 2009; Carvalho et al., 2016). Most of these studies have focused on specific ingredients. However, very few studies have examined the digestibility of a comprehensive suite of raw materials, with those that do focused on *Litopenaeus vannamei* (Lemos et al., 2009; Yang et al., 2009; Carvalho et al., 2016). In the study by Lemos et al., 2009, the authors compared the digestibility of protein against the *in vitro* digestibility of protein but did not report any of the other nutritional parameters (e.g. digestible dry matter, energy or lipid). The study by Yang et al., 2009 assessed a range of plant and animal meals without assessing their specific origin or the effects of post processing. Whereas the study by Carvalho et al., (2016) had a focus on the use of various animal and vegetable meals but did also include an analysis of the effect of inclusion level and reported variable effects of inclusion level across those raw materials studied. Such databases on the digestible value of ingredients remain highly useful resources to underpin future formulation of both practical and research diets and form the basis of understanding the key raw material attributes that affect nutritional quality of raw materials.

In the present study, a series of digestibility experiments were undertaken with black tiger shrimp (*Penaeus monodon*) to define the digestible nutrient and energy values of a suite of raw materials for use in shrimp diets. It was postulated that shrimp would exhibit different capacities to digest this range of different raw materials. We considered that the generation of this data is an essential step to improve the basis by which shrimp diets are formulated. The variation in chemical and digestible composition of the different raw materials is discussed, as are some of the key observational determinants of variability in digestibility values encountered in this study.

2. Materials and Methods

2.1 Raw material preparation

A suite of raw materials with potential for or currently being used in the shrimp feed sector were sourced from a commercial feed company (Ridley Aquafeeds, Narangba, QLD, Australia) and raw material producers throughout Australia. A mixture of plant protein and rendered animal by-products were obtained. Some additional raw materials for use in research diets were also evaluated (e.g. vitamin-free casein). Each of the raw materials was milled using a Retsch mill (ZM200 Centrifugal Mill; MEP Instruments, Brendale, QLD, Australia) with a 750 µm screen to create a consistent flour from each product. After milling, all raw materials were held at -20ºC pending diet manufacture. Details and composition of all raw materials used in this study are presented in Tables 1 and 2.

2.2 Diet manufacture

A diet design strategy based on the diet-substitution ingredient digestibility method was used as the basis for this study (as reviewed by Glencross et al., 2007). As the basis for this strategy a reference diet was developed using a formulation specification of 42% protein and 7% lipid which was a mimic of the commercial feeds typically used in the Australian shrimp farming industry and which also acts as our industry equivalent performance benchmark (Glencross et al., 1999). A large (100kg) batch of reference mash was prepared with a subsample pelleted to make the reference diet. Test diets were made by blending a sample of the test ingredient with a subsample of the reference mash in a 30:70 ratio on an as is basis (Table 3). Each diet was prepared by mixing samples of the test raw material and reference mash in an upright planetary mixer (Hobart, Sydney, NSW, Australia). Water was then added during the mixing to form a dough which was subsequently screw-pressed (Dolly, La Monferrina, Castell'Alfero, Italy) through a 1.5mm die and cut to pellet lengths of about 6mm. The pellets were then steamed using a commercial steamer (Curtin & Son, Sydney, Australia) at 100ºC for 3 minutes before being oven dried at 60ºC for 24h. Diets were kept at -20ºC when not being fed.

2.3 Shrimp collection and trial management

Several hundred individuals (~3.0 g/shrimp, subsample weight of n=40) of black tiger shrimp were collected from two commercial farm grow-out ponds (Truloff's Prawn Farm, Alberton, Qld 4207 and Melivan Pty Lt, Kurrimine Beach, Qld 4871) by cast-netting and transferred to a holding tank (10,000 L) where they were held pending allocation to trial tanks. During the holding period (~7 days) they were fed a standard commercial grower diet (Prawn Enhance™, Ridley Aquafeeds, Narangba, QLD, Australia).

For the faecal collection part of the study, five shrimp were allocated to each of 60 x 100 L circular (60cm D x 45cm H) tanks in an indoor laboratory system. Each of the tanks of shrimp were maintained with flow-through seawater at a rate of 1 L/min. The temperature (assessed daily) across all tanks was 28.9 ± 1.0ºC and dissolved oxygen at 6.4 ± 0.14 mg/L over the experimental period. Light was maintained on a 12 : 12 light : dark cycle for the duration of the study. All work undertaken in the laboratory was done using red-light to ensure the shrimp were not disturbed. For each treatment, each tank was used as the replicate unit (n = 5 per treatment). Three sub-experiments with up to 12 treatments were conducted consecutively. In each of these sub-experiments the reference diet and the SPC diet used were the same to provide two cross-trial references.

To acclimate the shrimp to their diets they were fed a fixed ration (1.0 g/tank/d) of their respective treatment diet for one week prior to faecal collection commencing. During the faecal collection period the shrimp were twice fed a ration (approx. 1.0g) 4 hours apart and allowed 30 minutes to consume the ration, before all uneaten food was siphoned to waste. Two hours after the feed was first offered, all faeces were siphoned into a labelled bucket and allowed to settle briefly before the faeces were then transferred to a 10 mL centrifuge tube. The seawater was then decanted and replaced with deionised water and the volume made up to 10 mL before centrifuging at 5000 rpm for 30 sec. All fluid was then decanted and the tube capped and frozen. The frozen pellet was then transferred to a sample vial for pooling and sample preparation. This process was conducted over a 14-day period for each sub-experiment to collected adequate sample for analysis. Faeces were not collected from any tanks with animals that had molted. No shrimp mortalities occurred during the experiments. The methods used here were based on those reported previously (Glencross et al., 2002; Smith and Tabrett, 2004; Smith et al., 2007; Glencross et al., 2013).

2.4 Chemical and digestibility analysis

All chemical analyses were carried out using methods consistent with AOAC (2005). Diet, raw material and faecal samples were analysed for dry matter, yttrium, ash, nitrogen, total lipids, and gross energy content. Only raw materials were analysed for amino acids. Dry matter was calculated by gravimetric analysis following oven drying at 105ºC for 24 h (Contherm Thermotec2000; Thermofisher, Scoresby, VIC, Australia). Total yttrium concentrations were determined after mixed acid digestion using an inductively coupled plasma atomic emission spectrophotometry (ICP-MS). Protein levels were calculated from the determination of total nitrogen by CHNOS auto-analyser, based on N x 6.25 (Leco Corp., St. Joseph, MI, USA). Amino acid composition of samples were determined by an acid hydrolysis prior to separation via HPLC (Shimadzu Nexera X2 series UHPLC, Shimadzu Corporation, Kyoto, Japan; coupled with a Shimadzu 8030 Mass Spectrometer). The acid hydrolysis destroyed tryptophan making it unable to be determined using this method. Total lipid content of the samples was determined gravimetrically following extraction of the lipids using the chloroform:methanol method. 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. Gross energy was determined by adiabatic bomb calorimetry (Par Instrument Company, Moline, IL, USA).

Differences in the ratios of the parameters of dry matter, protein, lipids, carbohydrates or gross energy to yttrium, in the feed and faeces in each treatment were calculated to determine the apparent digestibility coefficient (ADC_{diet}) for each of the nutritional parameters examined in each diet based on the following formula:

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

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 (organic matter, protein or energy) content of the diet and faeces respectively. The digestibility values for each of the test raw materials in the test diets examined in this study were calculated according to the formulae:

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

Where $Nutr.AD_{RM}$ is the digestibility of a given nutrient from the test raw material 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. $Nutr_{RM}$, $Nutr_{test}$ and $Nutr_{basal}$ are the level of the nutrient of interest in the raw material, test diet and basal diet respectively. All raw material inclusion levels were also corrected for their respective dry matter contribution relative to the dry matter content of the basal mash (Bureau and Hua, 2006). Ingredients with less than 5% lipid or 10% carbohydrates (CHO) were not assessed for lipid or CHO digestibilities due to an unacceptable error rate being encountered below this level these nutrients in the raw materials.

Raw material digestibilities greater than 100% were not corrected because we consider they are potentially indicative of interactive effects between the diet and test raw material and should be stipulated as determined. However, for reasons of practicality, the total levels of digestible nutrients/energy were only calculated assuming a maximum digestibility of 100% or a minimum of 0% when multiplied against the respective nutrient parameter of that raw material.

2.5 Statistical analysis

All values are means and standard error of the mean, unless otherwise specified. No ANOVA comparison of the digestibility values among all the raw materials was undertaken as this was considered largely pointless. For some specific comparisons an ANOVA was undertaken with a Tukey's HSD post hoc test applied. For some simple comparisons (e.g. extruded versus raw feed grains) a MANOVA analysis was undertaken with a Tukey's HSD post hoc test applied. To examine potential effects of composition on digestibility, correlation matrices between diet composition and diet digestibility, and again between raw material composition and raw material digestibility were undertaken using Microsoft Excel. Limits for all critical ranges were set at $P < 0.05$. Because of nominal variance in the reference diet data across experiments, no standardisation of the inter–experiment data was undertaken. Statistical analyses were conducted in the R-project statistical environment, version 3.1.0 (R Core Team, 2014).

3. Results

3.1 Raw material characterisation

Across the 29 different raw materials examined in this study there was a substantial range in the composition parameters observed (Tables 1 and 2). Concentrations of protein varied from 0.2% DM in the pregelled starch to 93.4%DM in the blood meal. The concentrations of lipid were lowest in the pregelled starch and wheat gluten (<1% DM), though were also low (<2%) in field peas, faba beans and blood meals. By contrast the lipid concentrations were highest in the Camelina meal (29.3% DM) and krill meal (21.1% DM). The concentrations of ash were lowest in the pregelled starch and blood meal (~1.2% DM), though were also low (<2%) in wheat gluten, wheat flour and corn gluten. By contrast the ash concentrations were highest in the meat and bone meals (24.6 and 27.7% DM) and the tuna by-product fish meal (21.9% DM). Carbohydrate (CHO) concentrations were highest in the pregelled starch (98.6% DM), though were also high (>60%) in field peas, wheat flour and faba beans. Several ingredients were devoid of any CHO (e.g. blood meal, Jack mackerel meal, etc.). Energy densities were highest in the Camelina meal (26.3 MJ/kg DM) and lowest in the faba beans and field peas (18.9 MJ/kg DM). Amino acid concentrations also varied substantially among the different raw materials (Table 2). There was a strong relationship between the crude protein and sum of amino acids across all raw materials (R^2= 0.973).

3.2 Diet nutrient and energy digestibilities

Across the three experiments there was a low level of variability (CV% < 10%) among the various digestibility values of the two common reference diets (the basal and SPC diets) (Table 4). However, the extent of this variation was significant among the different experiments on the basal diet for the CHO digestibility, and on the SPC diet for most parameters except lipid digestibility. The coefficients of variation (CV%) in the digestibility of these diets ranged from 0.8% for dry matter digestibility of the basal diet to 9.2% for dry matter digestibility of the SPC diet across the three experiments. Variation in the digestibility values for protein, lipid and energy of these two diets were otherwise between these two values observed for the dry matter digestibilities. Variation in the ingredient digestibility values across the three experiments was somewhat larger with coefficients of variation ranging from 9.8% for protein digestibility to 29.9% for energy digestibility. No significant effects of experiment were observed for any of the other parameters.

Diet digestibility values for the 29 test diets ranged according to the different parameters measured (Table 5). Dry matter digestibilities were on average 0.636 ± 0.106 (mean ± SD), with a CV% of 16.6%. ADC values for dry matter digestibilities ranged from 0.251 (camelina meal) to 0.783 (Vitamin-free casein). Protein digestibilities were on average 0.765 ± 0.095 (mean ± SD), with a CV% of 12.4%. ADC values for protein digestibilities ranged from 0.483 (hydrolysed feather meal) to 0.895 (wheat gluten). Lipid digestibilities were on average 0.790 ± 0.076 (mean ± SD), with a CV% of 9.6%. ADC values for lipid digestibilities ranged from 0.559 (Blood meal) to 0.870 (Anchovetta fishmeal). Energy digestibilities were on average 0.704 ± 0.089 (mean ± SD), with a CV% of 12.6%. ADC values for energy digestibilities ranged from 0.438 (camelina meal) to 0.826 (wheat gluten). Carbohydrate digestibilities were on average 0.722 ± 0.091 (mean ± SD), with a CV% of 12.5%. ADC values for carbohydrate digestibilities ranged from 0.423 (camelina meal) to 0.874 (blood meal).

Across the 30 different diets (including the basal diet) a correlation matrix examining 35 combinations was created to examine potential relationships between diet composition (dry matter, ash, protein, lipid, carbohydrate, protein+lipid and organic matter) and diet digestibilities for dry matter, protein, lipid, energy and carbohydrates. Several significant relationships were observed; Diet ADC-CHO vs. Diet Lipid (R =-0.487, P=0.006), Diet ADC-CHO vs. Diet DM (R =-0.430, P=0.018) and Diet ADC-Lipid vs. Diet Ash (R =0.397, P=0.030).

3.2 Raw material nutrient and energy digestibilities

Consistent with the observations from the diet digestibilities there was also a substantial range in the digestibilities of each of the parameters examined (dry matter, protein, lipid and energy) across each of the raw materials studied (Table 5). Raw material dry matter digestibilities ranged from -0.818 (camelina meal) to 0.929 (dried fish solubles) across the different raw materials. Raw material protein digestibilities ranged from -0.247 (camelina meal) to 1.347 (wheat gluten). Lipid digestibilities ranged from -0.028 (raw field peas) to 1.693 (wheat flour) across the range of raw materials. CHO digestibilities ranged from -0.527 (camelina) to 1.002 (extruded field peas) across the range of raw materials. Raw material energy digestibilities ranged from -0.109 (camelina meal) to 0.953 (vitamin-free casein).

The factorial arrangement of field pea/faba bean x extrusion/raw demonstrated some notable effects. A significant effect of both grain type (P<0.000) and processing (P=0.001) on the digestibility of energy was observed, with improvements in energy digestibility occurring with the use of pre-extruded grains and peas being more digestible than faba beans. However, there was no interaction effect (P=0.307). There was also a significant effect (P=0.003) of extrusion on the dry matter digestibility of faba beans, but not field peas and a significant difference between the two grain types (P<0.000). No interaction effect was observed (P=0.152). There were no significant effects of grain type, processing or interaction on protein digestibility. There was no effect of grain type on lipid digestibility (P=0.678), or processing (P=0.244), but there was a significant interaction effect (P=0.025). There was also an effect of grain type on carbohydrate digestibility (P<0.000), but not processing (P=0.240) or interaction (P=0.351).

Across the 29 different raw materials a correlation matrix examining 32 combinations was created to examine potential relationships between raw material composition (dry matter, ash, protein, lipid, energy, carbohydrate, protein+lipid and organic matter) and raw material digestibilities for dry matter, protein, lipid and energy. No significant relationships were observed.

The digestible nutrient and energy contents of each of the tested ingredients is presented in Table 6.

4. Discussion

To reduce reliance on fishmeal in shrimp diets, it has long been recognised that assessment of alternative raw materials is one of the critical steps underpinning the optimal use of alternative raw materials (Gatlin et al., 2007). One of the foundational assessment strategies in evaluating raw materials for any animal species is to measure the digestibility of nutrients and energy from the specific raw materials of interest (Glencross et al., 2007). In this regard, the present study was undertaken to measure the digestible nutrient and energy values of a suite of raw materials for use in shrimp diets. It was anticipated that there would be substantial differences among the various test raw materials on diet digestibility, which was observed in several instances. The generation of this data is an essential step to improve the future basis to formulate shrimp diets. Not only does this data broaden the range of raw materials available for use in shrimp diets by providing a better understanding of their nutritional limitations, it also provides a basis from which to formulate diets on a digestible nutrient basis and so better design diets to meet the needs of shrimp.

4.1 Raw material characterisation

Although the focus of this study was to examine the effects of different raw materials on the digestibilities of diets and subsequently, by calculation/inference, the raw materials being tested, the large range of raw materials being assessed also offers the chance to examine the range in composition of some key resources. This characterisation stage was extolled by Glencross et al. (2007) as an often-missed point of many similar such studies and the results in the present study, we believe exemplify why this is an important part of any raw material assessment study. It can be seen by examination of the three fishmeals, three poultry offal meals, two canola meals and two soybean meals, that substantial differences exist subject to factors such as genotype, origin and processing variables. Simply describing a raw material as “soybean meal” or “fishmeal” without an accompanying comprehensive chemical characterisation and identification of the products origin substantially reduces the value of the data and limits the differentiation of good quality products from inferior ones.

As anticipated, the extrusion of faba beans and field peas had no significant effect on their proximate chemical composition, supporting that any nutritional impacts are due to secondary changes in the composition of these raw materials. Another observation in this study was the strong relationship (R^2= 0.973) between crude protein of sum of amino acids, supporting that sAA is an excellent proxy for protein.

4.2 Diet digestibilities

The across experiment variability (coefficient of variation) in digestibility values observed of the two common diets (the basal and SPC diets) used in each of the three experiments in this study, while still less than 10%, was still substantially larger than that observed in other species that examined digestibilities across separate experiments using the same diets (Glencross et al., 2015; 2017). In these other studies, where faeces were collected using stripping techniques from a carnivorous fish (*Oncorhynchus mykiss*), the coefficients of variation across dry matter, protein and energy digestibility ranged from only 1.2 % to 2.3%. We suspect that this lower level of variability may be linked to the use of a settlement-type faecal collection method in the present study with shrimp. An assessment of the methodology associated with shrimp faecal collection methods by Tabrett and Smith (2004) identified that the duration the faeces spent in the water post-defaecation had an appreciable impact on the digestibility determination with these species, but also noted that it was virtually impossible to remove the post-defecation solubilisation effect that results in over-estimation of ingredient digestibility.

The large data set of diet digestibilities was also used to explore for diet compositional factors that may influence diet digestibility. Although three significant correlations were found across the 35 different diet compositional and digestibility combinations, only the one associated with the diet carbohydrate digestibility and diet lipid content appears plausible. Earlier studies have shown that higher lipid levels can negatively impact lipid digestibility in shrimp, so this link may extend to impacting other nutrients (Glencross et al., 2002). However, it was noted in the present study that there was no significant correlation between diet lipid level and lipid digestibility, so this weakens this hypothesis. The general absence of clear correlations between diet proximate compositional parameters and diet digestibility parameters infers that diet digestibility is largely affected by factors other than those ones examined.

4.3 Raw material digestibilities

The assessment of this suite of raw materials provided some clear indications on the nutritive value of a range of raw materials currently used in commercial shrimp diets and some novel prospective raw materials under consideration. Notable were the poor digestibilities associated with camelina meal which despite being reported as a suitable raw material for salmonids (Hixson et al., 2016), is clearly unsuitable for shrimp. Substantial variability in the digestibility could also be seen among the three different fishmeals (jack mackerel, anchovetta and tuna by-product meal), and also between the two soybean meals, with many of these differences significant. This later observation contrasts that of Cruz-Suarez et al., (2009), who examined different processing effects on soybean and found little impact on protein digestibility. However, our observations are consistent with that of Zhou et al (2014) who reported substantial differences in performance and digestibility associated with the use of a range of different soybean meals used in diets for *L. vannamei*. These differences further support the importance of specific ingredient characterisation, as clearly not all fishmeals or soybean meals are of equal nutritional value.

The use of different processing methods to produce meat and bone meals and poultry offal meals had mixed results. The use of lower temperatures to render meat and bone meals had a minor benefit to protein, lipid and energy digestibilities. The use of fresher starting material in the production of poultry offal meal had minor benefits to protein and lipid digestibility, but ironically not to energy digestibility. Based on the present digestibility data, the nutritive value of blood meal to shrimp is questionable, as is that of hydrolysed feather meal. These findings are consistent with those presented by others using growth studies with shrimp (Dominy and Ako, 1988; Ricque-Marie et al., 1998; Cheng et al., 2002; Forster et al., 2003; Suresh et al., 2011).

The examination of the effects of pre-extrusion on the nutritional value of both field peas and faba beans demonstrated some important findings. There was no significant effect of extrusion on the protein digestibility of either faba beans or field peas. However, some significant effects on the energy and dry matter digestibilities were observed supporting the notion that with both faba beans and field peas the main benefit of extrusion is from improving the nutritive value of the starch content of the grain. We suspect that this is related to an improvement in the starch digestibility which can be inferred from effects on both the dry matter and energy digestibilities. Similar effects have also been seen with several fish species (Booth et al., 2002; Davies and Gouviea, 2008). Inclusion of un-extruded field peas in diets for shrimp has been reported before, along with diet digestibility values that indicate that when peas are used to replace soybean that there is a significant improvement in both dry matter and protein digestibility (Bautista et al., 2003).

Across all the raw materials, a correlation matrix examining 32 combinations failed to find significant relationships between any of the raw material composition and raw material digestibility parameters. This suggests that there are underlying factors driving the variation in digestibility, either at a chemical classification level finer than the proximate analyses used in the present study, or as the result of a combination of factors. One successful study using a similar approach to define the factors affecting the digestibility of lupins used a greater number of samples (n=75) and had a greater degree of compositional characterisation and further relied on multivariate statistics to define those factors responsible (Glencross et al., 2008b).

4.4 Conclusions

The findings of this study demonstrate that there is a wide range in the nutritive values of various raw materials when fed to shrimp. Importantly, a generalisation of the comparative digestibility of animal protein sources against vegetable protein sources cannot be made, as there are excellent and poor digestibilities in either class of raw materials. The collation of the digestibility values in this study we consider to be an important step-forward for the shrimp aquaculture industry as it continues to seek independence from fishery resources. Additionally, such datasets provide an important resource for future meta-analyses and the development of robust *in vitro* and *in silico* models to estimate raw material nutritional value (Lemos et al., 2004; 2007; 2009; Glencross et al., 2015).

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References

AOAC (Association of Official Analytical Chemists). 2005. Official Methods of Analysis of the Association of Official Analytical Chemists. 15th edition. Association of Official Analytical Chemists. Washington, DC, USA.

Alvarez, J. S., Hernández-Llamas, A., Galindo, J., Fraga, I., García, T., Villareal, H., 2007. Substitution of fishmeal with soybean meal in practical diets for juvenile white shrimp *Litopenaeus schmitti*. Aquaculture Research 38, 689–695.

Brunson, J.F., Romaire, R.P., Reigh, R.C. (1997) Apparent digestibility of selected ingredients in diets for white shrimp *Penaeus setiferus* L. Aquaculture Nutrition 3, 9-16.

Bautista, M.N., Eusebio, P.S., Welsh, T.P. (2003) Utilization of feed pea, *Pisum sativum*, meal as a protein source in practical diets for juvenile tiger shrimp, *Penaeus monodon*. Aquaculture 225, 121-131.

Booth, M.A., Allan, G.L., Evans, A.J., Gleeson, V.P., (2002) Effects of steam pelleting or extrusion on digestibility and performance of silver perch *Bidyanus bidyanus*. Aquaculture Research, 33, 1163-1173.

Bureau, D.P., Hua, K., (2006) Letter to the Editor of Aquaculture. Aquaculture 252,103-105.

Carvalho, R.A., Ota, R.H., Kadry, V.O., Tacon, A.G., Lemos, D., (2016) Apparent digestibility of protein, energy and amino acids of six protein sources included at three levels in diets for juvenile white shrimp *Litopenaeus vannamei* reared in high performance conditions. Aquaculture 465, 223-234.

Cheng, Z.J., Behnke, K.C., Dominy, W.G. (2002b) Effect of feather meal on growth and body composition of the juvenile Pacific white shrimp, *Litopenaeus vannamei*. Journal of Applied Aquaculture 12, 57-69.

Cruz-Suarez, L.E., Ricque-Marie, D., Tapia-Salazar, M., McCallum, I.M., Hickling, D., 2001. Assessment of differently processed feed pea (*Pisum sativum*) meals and canola meal (*Brassica* sp.) in diets for blue shrimp (*Litopenaeus stylirostris*). Aquaculture 196, 87–101.

Cruz-Suárez, L.E., Nieto-López, M., Guajardo-Barbosa, C., Tapia-Salazar, M., Scholz, U., Ricque-Marie, D., 2007. Replacement of fish meal with poultry by-product meal in practical diets for *Litopenaeus vannamei*, and digestibility of the tested ingredients and diets. Aquaculture 272, 466-476.

Cruz-Suárez, L.E., Tapia-Salazar, M., Villarreal-Cavazos, D., Beltran-Rocha, J., Nieto-López, M.G., Lemme, A., Ricque-Marie, D. (2009) Apparent dry matter, energy, protein and amino acid digestibility of four soybean ingredients in white shrimp *Litopenaeus vannamei* juveniles. Aquaculture 292, 87-94.

Davies, S.J., Gouveia, A., 2010. Response of common carp fry fed diets containing a pea seed meal (*Pisum sativum*) subjected to different thermal processing methods. Aquaculture, 305, 117-123.

Davis, D.A., Arnold, C.R., 2000. Replacement of fish meal in practical diets for the Pacific white shrimp, *Litopenaeus vannamei.* Aquaculture 185, 291–298.

Davis, D.A., Arnold, C.R., McCallum, I., 2002. Nutritional value of feed peas (*Pisum sativum*) in practical diet formulations for *Litopenaeus vannamei*. Aquac. Nutr. 8, 87–94.

Dominy, W.G., Ako, H. (1988) The utilization of blood meal as a protein ingredient in the diet of the marine shrimp *Penaeus vannamei*. Aquaculture 70, 289-299.

Francis, G., Makkar, H.P.S. and Becker, K. (2001) Anti-nutritional factors present in plant-derived alternate fish feed ingredients and their effect in fish. Aquaculture 199, 197-227.

Forster, I.P., Dominy, W.G., Obaldo, L.G., Tacon, A.G.J. (2003) Rendered meat and bone meals as ingredients of diets for shrimp *Litopenaeus vannamei* (Boone, 1931). Aquaculture 219, 655-670.

Gatlin, D.M., Barrows, F.T., Brown, P., Dabrowski, K., Gaylord, T.G., Hardy, R.W., Herman, E., Hu, G., Krogdahl, Å., Nelson, R. and Overturf, K., (2007) Expanding the utilization of sustainable plant products in aquafeeds: A review. Aquaculture Research 38, 551-579.

Glencross, B.D. and Smith, D.M., (1997) A comparison of the utilisation of triacylglycerols, esterified and free fatty acids by the prawn, *Penaeus monodon*. Aquaculture 159, 67-86.

Glencross, B. and Smith, D.M., (2010) Raw material evaluation strategies for shrimp diets - Optimizing raw material sustainability. In: The Shrimp Book (V. Alday and D.A. Davis, Eds.). Nottingham University Press. pp 938.

Glencross, B.D., Smith, D.M., Tonks, M.L., Tabrett, S.M., Williams, K.C., 1999. A reference diet for nutritional studies of the prawn, *Penaeus monodon*. Aquaculture Nutrition 5, 33-40.

Glencross, B.D., Smith, D.M., Thomas, M.R., Williams, K.C., 2002. The effects of dietary lipid amount and fatty acid composition on the digestibility of lipids by the prawn, *Penaeus monodon*. Aquaculture 205, 157-169.

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., and Sipsas, S. (2008a) 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., Hawkins, W.E., Evans, D., Rutherford, N., McCafferty, P., Dods, K., Karopoulos M., Veitch, C., Sipsas, S., Buirchell, B. 2008b. Variability in the composition of lupin (*Lupinus angustifolius*) meals influences their digestible nutrient and energy value when fed to rainbow trout (*Oncorhynchus mykiss*). Aquaculture 277, 220-230.

Glencross, B.D., Tabrett, S.J., Irvin, S., Wade, N., Smith, D.M., Coman, G.E., Preston, N.P., 2013. An examination of the genotype by diet interaction effects in the Black Tiger shrimp, *Penaeus monodon*. – why do selected shrimp grow faster? Aquaculture Nutrition 19, 128-138.

Glencross, B.D., Bourne, N., Rutherford, N.R., Hawkins, W.E., Burridge, P., Dods, K., Sipsas, S., Sweetingham, M., 2015. Using NIRS to develop calibrations to predict the digestible protein and energy value of a feed grain – the lupin case study. Aquaculture Nutrition 21, 54-62.

Glencross, B.D., Bourne, N., Irvin, S., Blyth, D., 2017. Using near-infrared reflectance spectroscopy to predict the digestible protein and digestible energy values of diets when fed to barramundi, *Lates calcarifer*. Aquaculture Nutrition 23, 397-405.

Hixson, S.M., Parrish, C.C., Wells, J.S., Winkowski, E.M., Anderson, D.M. and Bullerwell, C.N., 2016. Inclusion of camelina meal as a protein source in diets for farmed salmonids. Aquaculture Nutrition 22, 615-630.

Lemos, D., Nunes, A.J.P. (2007) Prediction of culture performance of juvenile *Litopenaeus vannamei* by in vitro (pH-stat) degree of feed protein hydrolysis with species-specific enzymes. Aquaculture Nutrition 13, 1-11.

Lemos, D., Ezquerra, J.M., Garcia-Carreño, F.M. (2000) Protein digestion in penaeid shrimp: digestive proteinases, proteinase inhibitors and feed digestibility. Aquaculture 186, 89-105.

Lemos, D., Navarrete del Toro, A., Córdova-Murueta, J.H., Garcia-Carreño, F. (2004) Testing feeds and feed ingredients for juvenile pink shrimp *Farfantepenaeus paulensis*: in vitro determination of protein digestibility and proteinase inhibition. Aquaculture 239, 307-321.

Lemos, D., Lawrence, A.L., Siccardi, A.J. (2009) Prediction of apparent protein digestibility of ingredients and diets by in vitro pH-stat degree of protein hydrolysis with species-specific enzymes for juvenile Pacific white shrimp *Litopenaeus vannamei*. Aquaculture 295, 89-98.

Luo, L., Wang, J., Pan, Q., Xue, M., Wang, Y., Wu, X., Li, P., (2012) Apparent digestibility coefficient of poultry by-product meal (PBM) in diets of *Penaeus monodon* (Fabricius) and *Litopenaeus vannamei* (Boone), and replacement of fishmeal with PBM in diets of *P. monodon*. Aquaculture Research 43. 1223-1231.

Merican, Z.O. and Shim, K.F. (1995) Apparent digestibility of lipid and fatty acids in residual lipids of meals by adult *Penaeus monodon*. Aquaculture 133, 275-286.

R Core Team, 2014. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL http://www.R-project.org/.

Ricque-Marie, D., Abdo-de La Parra, M.I., Cruz-Suarez, L.E., Cuzon, G., Cousin, M., Aquacop, Pike, I.H. (1998) Raw material freshness, a quality criterion for fish meal fed to shrimp. Aquaculture 165, 95-109.

Smith, D.M. and Tabrett, S.J. (2004) Accurate measurement of in vivo digestibility of shrimp feeds. Aquaculture 232, 563-580.

Smith, D.M., Tabrett, S.J., Glencross, B.D., Irvin, S.J., Barclay, MC., 2007. Digestibility of lupin kernel meals in feeds for the black tiger shrimp, *Penaeus monodon*. Aquaculture 264, 353-362.

Suresh, V.A., Kumaraguru Vasagam, K.P., Nates, S. (2011) Attractability and palatability of protein ingredients of aquatic and terrestrial animal origin, and their practical value for blue shrimp, *Litopenaeus stylirostris* fed diets formulated with high levels of poultry by-product meal. Aquaculture 319, 132-140.

Yang, Q., Zhou, X., Zhou, Q., Tan, B., Chi, S., Dong, X. (2009) Apparent digestibility of selected feed ingredients for white shrimp *Litopenaeus vannamei*, Boone. Aquaculture Research 41, 78-86

Zhou, Y.G., Davis, D.A. and Buentello, A., 2015. Use of new soybean varieties in practical diets for the Pacific white shrimp, *Litopenaeus vannamei*. Aquaculture Nutrition 21, 635-643.

Table 1. Composition and origin of the experimental raw materials. Indicated also is which of the three sub-experiments each ingredient was evaluated in.

Ingredient	Source	Experiment	Dry Matter	Protein	Lipid	Ash	CHO	Energy
Blood meal	AJ Bush, Beaudesert, QLD, Australia	1	93.2	93.4	1.6	1.2	-	23.4
Dried Fish Solubles	Aquativ, Elven, France	2	93.5	71.8	13.9	14.2	0.1	22.4
Fishmeal (Anchoveta)	Ridley, Narangba, QLD, Australia	1	90.9	70.5	12.5	16.4	0.6	22.3
Fishmeal (Jack Mackerel)	Ridley, Narangba, QLD, Australia	3	92.7	74.3	11.4	15.5	-	21.6
Fishmeal (Tuna By-Product)	Ridley, Narangba, QLD, Australia	3	96.4	67.1	10.5	21.9	-	20.3
Krill meal	Akerbiomarine, Lysaker, Norway	3	94.9	64.4	21.1	11.8	-	24.5
Meat and bone meal 1 (Low temp)	CSF, Laverton, VIC, Australia	2	93.6	51.3	12.3	27.7	8.7	19.2
Meat and bone meal 2 (High temp)	CSF, Laverton, VIC, Australia	2	96.0	53.2	13.5	24.6	8.6	20.0
Hydrolysed feather meal	Camilleri, Maroota, NSW, Australia	1	94.8	82.3	7.3	5.3	-	22.6
Poultry offal meal (FAQ)	Camilleri, Maroota, NSW, Australia	3	94.7	69.7	16.6	15.1	-	23.3
Poultry offal meal (HQ)	CSF, Laverton, VIC, Australia	1	95.7	72.2	13.7	13.5	0.6	22.2
Poultry offal meal (LQ)	CSF, Laverton, VIC, Australia	1	96.5	65.9	15.0	14.6	4.5	22.6
Vitamin free casein	Sigma-Aldrich, Syndey, NSW, Australia	1	94.7	82.2	0.8	8.0	9.0	22.4
Camelina meal	Aus-Oils, Kojonup, WA, Australia	1	92.1	27.2	29.3	5.2	38.3	26.2
Canola meal - Expeller	Riverland Oilseeds, Pinjarra, WA, Australia	1	94.8	36.2	9.6	7.3	47.0	21.2
Canola meal – Solvent Extracted	Riverland Oilseeds, Footscray, VIC, Australia	1	89.6	37.5	6.6	8.4	47.5	20.9
Corn gluten	Arrow Commodities, Surrey Hills, NSW, Australia	2	92.3	65.1	6.0	1.6	27.3	23.7
Faba bean - extruded	Ridley, Narangba, QLD, Australia	2	96.3	29.9	1.5	3.3	65.3	18.9
Faba bean - raw	Ridley, Narangba, QLD, Australia	2	90.5	30.3	1.8	3.6	64.3	19.0
Field peas - extruded	Ridley, Narangba, QLD, Australia	2	96.0	25.2	1.4	3.1	70.3	18.9
Field peas - raw	Ridley, Narangba, QLD, Australia	2	90.6	24.9	2.1	3.3	69.7	19.0
Lupin kernel meal (cv. Coromup)	Coorow Seeds, Coorow, WA, Australia	3	91.8	46.0	8.2	4.1	33.6	21.0
Pregelled starch	Manildra, Auburn, NSW, Australia	3	85.6	0.2	0.0	1.2	98.6	20.5
Soybean meal (Hifeed)	Ridley, Narangba, QLD, Australia	3	92.5	48.5	11.8	8.2	31.5	23.4
Soybean meal (Trifecta)	Ridley, Narangba, QLD, Australia	3	92.1	69.3	2.6	4.3	23.8	21.7
Soy Protein Concentrate	Selecta, Araguari, Brazil	1, 2, 3	90.2	69.8	2.4	7.3	20.5	21.9
Soy Protein Isolate	ADM, Decatur, IL, United States	1	93.7	89.7	5.3	5.0	-	23.3
Wheat flour (Plain)	Manildra, Auburn, NSW, Australia	3	87.5	15.3	1.9	1.7	81.2	21.5
Wheat gluten	Manildra, Auburn, NSW, Australia	3	92.1	86.5	0.7	1.5	3.4	24.1

All values are percent dry basis. Except for Dry matter, which is on a percent as received basis and for Energy which is on a MJ/kg dry basis.

Table 2. Amino acid compositions of the experimental raw materials

Ingredient	sAA	ALA	ARG	ASP	CYS	GLU	GLY	HIS	ISO	LEU	LYS	MET	PHE	PRO	SER	TAU	THR	TYR	VAL
Blood meal	850	68	41	54	10	76	49	35	51	140	70	12	69	41	32	6	36	23	38
Dried Fish Solubles	640	49	41	59	6	92	68	12	26	46	47	17	24	36	30	10	28	18	31
Fishmeal (Anchoveta)	703	29	40	43	9	73	33	27	49	100	50	44	46	41	26	8	30	26	28
Fishmeal (Jack Mackeral)	685	43	40	42	9	71	33	25	46	107	46	48	41	30	24	7	29	17	26
Fishmeal (Tuna By-Product)	661	26	38	45	6	70	34	21	50	126	46	46	39	24	23	3	26	12	25
Hydrolysed Feather Meal	822	39	56	54	44	89	65	6	38	67	18	5	40	82	94	2	38	25	60
Krill meal	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
Meat and bone meal (Low temp)	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
Meat and bone meal (High temp)	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
Poultry offal meal (FAQ)	619	41	45	52	13	83	58	12	26	48	33	15	28	46	39	2	27	20	31
Poultry offal meal (HQ)	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
Poultry offal meal (LQ)	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
Vitamin free casein	814	1	31	41	4	29	67	27	67	133	71	79	73	38	42	0	35	35	41
Camelina meal	246	12	20	15	6	26	17	7	18	30	18	11	15	13	11	0	10	6	11
Canola meal – Expeller	312	16	21	25	9	60	16	9	14	25	12	7	15	23	16	0	16	11	18
Canola meal – Solvent Extracted	323	16	22	25	10	62	16	9	14	26	16	7	15	23	16	0	16	12	18
Corn gluten	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
Faba bean - extruded	256	13	26	21	3	23	17	7	18	46	20	7	16	11	10	0	7	1	10
Faba bean - raw	248	12	24	23	3	20	18	6	19	45	19	6	15	11	11	0	8	0	9
Field peas - extruded	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
Field peas - raw	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
Lupin kernel meal (cv. Coromup)	390	17	44	31	5	34	35	12	31	50	29	4	23	16	18	0	14	12	13
Pregelled starch	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
Soybean meal (Hifeed)	478	22	36	57	9	89	20	14	21	38	26	8	26	24	28	0	21	18	21
Soybean meal (Trifecta)	535	26	42	63	10	92	22	16	23	42	30	11	28	28	32	0	25	20	25
Soy Protein Concentrate	590	24	45	45	9	47	39	20	45	67	42	25	58	29	28	0	24	22	22
Soy Protein Isolate	855	35	68	103	13	172	35	22	38	68	46	12	47	43	50	1	34	30	38
Wheat flour (Plain)	104	4	4	4	3	8	15	3	8	8	8	5	8	12	5	0	3	2	4
Wheat gluten	800	21	26	26	19	297	27	15	27	55	12	14	41	99	42	0	22	26	30

sAA : Sum of all amino acids. n/a : not assessed.

Table 3. Formulations for the experimental diets

Ingredient	Reference	Test
Fishmeal (Anchoveta)	500.0	350.0
Wheat gluten	70.0	49.0
Wheat flour	399.3	279.5
Lecithin	10.0	7.0
Fish oil	15.0	10.5
Yttrium oxide	1.0	0.7
Astaxanthin	0.5	0.4
BanoxE	0.2	0.1
Cholesterol	1.0	0.7
Vitamin C	1.0	0.7
Vitamin and Mineral Premix	2.0	1.4
Test ingredient	-	300.0
TOTAL	1000.0	1000.0

[e] Cholesterol : MP Bio, Aurora, OH, USA. [f] Banox-E™ : BEC Feed Solutions, Carole Park, QLD, Australia. [g] Astaxanthin (10%) as Carophyll Pink™ and Stay C™: DSM, Wagga Wagga, NSW, Australia. [h] Vitamin and mineral premix : Rabar, Beaudesert, QLD, Australia; includes (IU/kg or g/kg of premix): Vitamin A, 2.5MIU; Vitamin D3, 1.25 MIU; Vitamin E, 100 g; Vitamin K3, 10 g; Vitamin B1, 25 g; Vitamin B2, 20 g; Vitamin B3, 100 g; Vitamin B5, 100; Vitamin B6, 30 g; Vitamin B9, 5; Vitamin B12, 0.05 g; Biotin, 1 g; Vitamin C, 250 g; Banox-E, 13 g; [h]Yttrium oxide: Stanford Materials, Aliso Viejo, CA, USA.

Table 4. Cross experiment statistics

	Diet Digestibilities					Raw Material Digestibilities			
	Dry Matter	Protein	Lipid	Energy	CHO	Dry Matter	Protein	Lipid	Energy
	Basal Diet								
Exp-1	0.696	0.774	0.842	0.778	0.914				
Exp-2	0.690	0.779	0.758	0.731	0.823				
Exp-3	0.701	0.810	0.788	0.749	0.794				
mean	0.696	0.788	0.796	0.753	0.844				
SEM	0.0005	0.0005	0.0033	0.0012	0.0017				
CV	0.8%	2.5%	5.3%	3.2%	7.4%				
ANOVA	p=0.740	p=0.055	p=0.104	p=0.146	p=0.001				
	Soy Protein Concentrate								
Exp-1	0.612	0.750	0.861	0.684	0.583	0.506	0.784	0.869	0.430
Exp-2	0.584	0.742	0.780	0.643	0.641	0.464	0.728	1.014	0.588
Exp-3	0.695	0.838	0.795	0.761	0.736	0.717	0.882	0.761	0.789
mean	0.630	0.777	0.812	0.696	0.654	0.562	0.798	0.881	0.602
SEM	0.0040	0.0018	0.0044	0.0028	0.0029	0.0270	0.0093	0.3968	0.0274
CV	9.2%	6.9%	5.3%	8.6%	11.8%	24.2%	9.8%	14.4%	29.9%
ANOVA	p=0.044	p=0.006	p=0.163	p=0.014	p=0.003	p=0.145	p=0.139	p=0.788	p=0.134

SEM = standard error of the mean; CV=coefficient of variation (standard deviation / mean *100).

Table 5 Diet and raw material digestibility coefficients.

Diet	Diet Digestibility Coefficients					Raw Material Digestibility Coefficients				
	Dry Matter	Protein	Lipid	Energy	CHO	Dry Matter	Protein	Lipid	Energy	CHO
Basal	0.696	0.788	0.796	0.753	0.844					
Blood meal	0.547	0.541	0.559	0.569	0.874	0.387	0.452	-	0.389	-
Dried Fish Solubles	0.774	0.846	0.777	0.812	0.852	0.929	0.795	0.225	0.953	-
Fishmeal (Anchoveta)	0.659	0.789	0.734	0.709	0.749	0.587 ^{x}	0.837 ^{x}	0.673 ^{x}	0.651 ^{x}	-
Fishmeal (Jack Mackerel)	0.586	0.765	0.870	0.730	0.664	0.486 ^{y}	0.815 ^{x}	1.114 ^{z}	0.530 ^{y}	-
Fishmeal (Tuna By-Product)	0.556	0.745	0.843	0.685	0.639	0.355 ^{z}	0.735 ^{y}	0.952 ^{y}	0.521 ^{y}	-
Hydrolysed Feather Meal	0.485	0.483	0.690	0.517	0.734	-0.005	0.071	0.568	0.061	-
Krill meal	0.704	0.815	0.859	0.766	0.700	0.789	0.951	1.045	0.717	-
Meat and bone meal (Low temp)	0.704	0.807	0.796	0.756	0.761	0.719 ^{j}	0.758 ^{j}	1.318 ^{j}	0.767 ^{j}	-
Meat and bone meal (High temp)	0.706	0.823	0.814	0.731	0.749	0.717 ^{j}	0.715 ^{j}	0.919 ^{k}	0.710 ^{k}	-
Poultry offal meal (FAQ)	0.625	0.756	0.867	0.726	0.734	0.578 ^{m}	0.724 ^{m}	0.961 ^{m}	0.666 ^{m}	-
Poultry offal meal (HQ)	0.627	0.749	0.800	0.685	0.730	0.453 ^{n}	0.684 ^{m}	0.820 ^{n}	0.554 ^{n}	-
Poultry offal meal (LQ)	0.628	0.714	0.783	0.680	0.786	0.473 ^{n}	0.583 ^{n}	0.791 ^{n}	0.552 ^{n}	-
Vitamin free casein	0.783	0.873	0.810	0.818	0.811	0.940	0.906	-	0.977	-
Camelina meal	0.251	0.577	0.633	0.438	0.423	-0.818	-0.247	0.540	-0.109	-0.527
Canola meal – Expeller	0.555	0.752	0.722	0.620	0.596	0.394 ^{o}	0.738 ^{o}	0.616 ^{o}	0.545 ^{o}	0.296 ^{o}
Canola meal – Solvent Extracted	0.555	0.758	0.706	0.575	0.592	0.345 ^{o}	0.750 ^{o}	0.716 ^{p}	0.265 ^{p}	0.236 ^{o}
Corn gluten	0.747	0.853	0.838	0.783	0.742	0.798	0.816	0.810	0.798	0.687
Faba bean - extruded	0.732	0.835	0.807	0.754	0.813	0.843 ^{a}	0.736	0.635 ^{a}	0.783 ^{a}	0.747 ^{a}
Faba bean - raw	0.709	0.813	0.794	0.717	0.734	0.758 ^{b}	0.575	0.878 ^{ab}	0.688 ^{b}	0.648 ^{b}
Field peas - extruded	0.718	0.828	0.842	0.748	0.774	0.709 ^{b}	0.742	1.162 ^{b}	0.696 ^{b}	1.002 ^{c}
Field peas - raw	0.646	0.838	0.747	0.655	0.713	0.491 ^{c}	0.795	-0.028 ^{c}	0.406 ^{c}	0.990 ^{c}
Lupin kernel meal (cv. Coromup)	0.566	0.748	0.862	0.699	0.628	0.322	0.770	0.953	0.556	0.083
Pregelled starch	0.575	0.677	0.826	0.698	0.783	0.379	-	-	0.464	0.767
Soybean meal (Hifeed)	0.674	0.811	0.859	0.759	0.709	0.784 ^{r}	0.983 ^{r}	0.609 ^{r}	0.731 ^{r}	0.410 ^{r}
Soybean meal (Trifecta)	0.637	0.718	0.836	0.751	0.811	0.680 ^{s}	0.647 ^{s}	0.351 ^{s}	0.706 ^{r}	0.566 ^{s}
Soy Protein Concentrate	0.630	0.777	0.812	0.696	0.654	0.562	0.798	0.881	0.602	0.663
Soy Protein Isolate	0.695	0.838	0.710	0.755	0.745	0.774	0.877	0.551	0.892	-
Wheat flour (Plain)	0.639	0.772	0.863	0.750	0.771	0.633	0.629	1.693	0.682	0.688
Wheat gluten	0.733	0.895	0.849	0.826	0.683	0.830	1.347	0.730	0.883	-
Pooled SEM	0.01	0.01	0.01	0.01	0.01	0.03	0.02	0.02	0.03	0.03

Each data point is the mean (n=5). For the faba beans and field pea raw material digestibilities, the indicated different superscripts (a, b, c) imply a significant difference at $P<0.05$ from a MANOVA analysis. For the other superscripts comparisons are only made within the fishmeals (x, y, z), meat and bone meals (j, k), poultry meals (m, n), canola meals (o, p) or soybean meals (r, s), but not among the different meals.

Table 6 Raw material digestible nutrient values (% as received). Based on raw material digestibility* x composition.

	Dry Matter	Protein	Lipid	CHO	Energy
Blood meal	36.1	39.3	-	-	8.5
Dried Fish Solubles	86.9	50.0	2.7	-	18.6
Fishmeal (Anchoveta)	53.4	48.8	6.9	-	12.0
Fishmeal (Jack Mackeral)	45.0	56.1	10.6	-	10.6
Fishmeal (Tuna By-Product)	34.2	47.5	9.7	-	10.2
Hydrolysed Feather Meal	0.0	5.5	3.9	-	1.3
Krill meal	74.8	58.1	20.0	-	16.7
Meat and bone meal (Low temp)	67.3	34.1	10.8	-	12.9
Meat and bone meal (High temp)	68.9	35.1	11.5	-	13.1
Poultry offal meal (FAQ)	54.7	45.3	14.3	-	13.9
Poultry offal meal (HQ)	43.4	45.3	10.3	-	11.3
Poultry offal meal (LQ)	45.6	35.8	11.1	-	11.6
Vitamin free casein	89.0	66.8	-	-	19.6
Camelina meal	0.0	0.0	13.4	0.0	0.0
Canola meal – Expeller	30.9	22.6	3.8	9.2	4.4
Canola meal – Solvent Extracted	37.3	24.0	5.3	12.6	10.4
Corn gluten	73.6	45.3	4.1	16.0	16.1
Faba bean - extruded	81.2	20.4	0.9	45.3	13.7
Faba bean - raw	68.6	14.3	1.3	34.3	10.7
Field peas - extruded	68.1	17.2	1.3	65.0	12.2
Field peas - raw	44.5	16.3	0.0	56.9	6.3
Lupin kernel meal (cv. Coromup)	29.6	32.5	7.2	3.2	10.7
Pregelled starch	32.5	0.0	0.0	64.8	7.0
Soybean meal (Hifeed)	72.5	40.7	6.1	13.9	14.6
Soybean meal (Trifecta)	62.6	38.1	0.8	15.5	13.0
Soy Protein Concentrate	51.5	46.5	1.9	12.2	11.1
Soy Protein Isolate	72.5	69.1	2.6	-	18.2
Wheat flour (Plain)	55.4	7.3	1.5	50.3	11.2
Wheat gluten	76.4	79.7	4.5	-	19.6

*where values were >100% they were rounded to 100. Where values <0 they were rounded to 0.