

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

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25 ***Highlights***

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

32

33 **Abstract**

34

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

49 **1. Introduction**

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

63 Over the past twenty years there have been a suite of studies that have evaluated the
64 digestibility of specific raw materials (Merican and Shim, 1995; Brunsen et al., 1997; Glencross and
65 Smith, 1997; Smith et al., 2007, Cruz-Suarez et al., 2007; 2009; Yang et al., 2009; Carvalho et al.,
66 2016). Most of these studies have focused on specific ingredients. However, very few studies have
67 examined the digestibility of a comprehensive suite of raw materials, with those that do focused on
68 *Litopenaeus vannamei* (Lemos et al., 2009; Yang et al., 2009; Carvalho et al., 2016). In the study by
69 Lemos et al., 2009, the authors compared the digestibility of protein against the *in vitro* digestibility of
70 protein but did not report any of the other nutritional parameters (e.g. digestible dry matter, energy or
71 lipid). The study by Yang et al., 2009 assessed a range of plant and animal meals without assessing
72 their specific origin or the effects of post processing. Whereas the study by Carvalho et al., (2016) had
73 a focus on the use of various animal and vegetable meals but did also include an analysis of the effect
74 of inclusion level and reported variable effects of inclusion level across those raw materials studied.
75 Such databases on the digestible value of ingredients remain highly useful resources to underpin
76 future formulation of both practical and research diets and form the basis of understanding the key
77 raw material attributes that affect nutritional quality of raw materials.

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

85 **2. Materials and Methods**

86 *2.1 Raw material preparation*

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

96
97 *2.2 Diet manufacture*

98 A diet design strategy based on the diet-substitution ingredient digestibility method was used
99 as the basis for this study (as reviewed by Glencross et al., 2007). As the basis for this strategy a
100 reference diet was developed using a formulation specification of 42% protein and 7% lipid which
101 was a mimic of the commercial feeds typically used in the Australian shrimp farming industry and
102 which also acts as our industry equivalent performance benchmark (Glencross et al., 1999). A large
103 (100kg) batch of reference mash was prepared with a subsample pelleted to make the reference diet.
104 Test diets were made by blending a sample of the test ingredient with a subsample of the reference
105 mash in a 30:70 ratio on an as is basis (Table 3). Each diet was prepared by mixing samples of the test
106 raw material and reference mash in an upright planetary mixer (Hobart, Sydney, NSW, Australia).
107 Water was then added during the mixing to form a dough which was subsequently screw-pressed
108 (Dolly, La Monferrina, Castell'Alfero, Italy) through a 1.5mm die and cut to pellet lengths of about
109 6mm. The pellets were then steamed using a commercial steamer (Curtin & Son, Sydney, Australia)
110 at 100°C for 3 minutes before being oven dried at 60°C for 24h. Diets were kept at -20°C when not
111 being fed.

112
113 *2.3 Shrimp collection and trial management*

114 Several hundred individuals (~3.0 g/shrimp, subsample weight of n=40) of black tiger shrimp
115 were collected from two commercial farm grow-out ponds (Truloff's Prawn Farm, Alberton, Qld
116 4207 and Melivan Pty Lt, Kurrimine Beach, Qld 4871) by cast-netting and transferred to a holding
117 tank (10,000 L) where they were held pending allocation to trial tanks. During the holding period (~7
118 days) they were fed a standard commercial grower diet (Prawn Enhance™, Ridley Aquafeeds,
119 Narangba, QLD, Australia).

120 For the faecal collection part of the study, five shrimp were allocated to each of 60 x 100 L
121 circular (60cm D x 45cm H) tanks in an indoor laboratory system. Each of the tanks of shrimp were

122 maintained with flow-through seawater at a rate of 1 L/min. The temperature (assessed daily) across
123 all tanks was $28.9 \pm 1.0^{\circ}\text{C}$ and dissolved oxygen at 6.4 ± 0.14 mg/L over the experimental period.
124 Light was maintained on a 12 : 12 light : dark cycle for the duration of the study. All work undertaken
125 in the laboratory was done using red-light to ensure the shrimp were not disturbed. For each
126 treatment, each tank was used as the replicate unit ($n = 5$ per treatment). Three sub-experiments with
127 up to 12 treatments were conducted consecutively. In each of these sub-experiments the reference diet
128 and the SPC diet used were the same to provide two cross-trial references.

129 To acclimate the shrimp to their diets they were fed a fixed ration (1.0 g/tank/d) of their
130 respective treatment diet for one week prior to faecal collection commencing. During the faecal
131 collection period the shrimp were twice fed a ration (approx. 1.0g) 4 hours apart and allowed 30
132 minutes to consume the ration, before all uneaten food was siphoned to waste. Two hours after the
133 feed was first offered, all faeces were siphoned into a labelled bucket and allowed to settle briefly
134 before the faeces were then transferred to a 10 mL centrifuge tube. The seawater was then decanted
135 and replaced with deionised water and the volume made up to 10 mL before centrifuging at 5000 rpm
136 for 30 sec. All fluid was then decanted and the tube capped and frozen. The frozen pellet was then
137 transferred to a sample vial for pooling and sample preparation. This process was conducted over a
138 14-day period for each sub-experiment to collect adequate sample for analysis. Faeces were not
139 collected from any tanks with animals that had molted. No shrimp mortalities occurred during the
140 experiments. The methods used here were based on those reported previously (Glencross et al., 2002;
141 Smith and Tabrett, 2004; Smith et al., 2007; Glencross et al., 2013).

142

143 2.4 *Chemical and digestibility analysis*

144 All chemical analyses were carried out using methods consistent with AOAC (2005). Diet,
145 raw material and faecal samples were analysed for dry matter, yttrium, ash, nitrogen, total lipids, and
146 gross energy content. Only raw materials were analysed for amino acids. Dry matter was calculated
147 by gravimetric analysis following oven drying at 105°C for 24 h (Contherm Thermotec2000;
148 Thermofisher, Scoresby, VIC, Australia). Total yttrium concentrations were determined after mixed
149 acid digestion using an inductively coupled plasma atomic emission spectrophotometry (ICP-MS).
150 Protein levels were calculated from the determination of total nitrogen by CHNOS auto-analyser,
151 based on $\text{N} \times 6.25$ (Leco Corp., St. Joseph, MI, USA). Amino acid composition of samples were
152 determined by an acid hydrolysis prior to separation via HPLC (Shimadzu Nexera X2 series UHPLC,
153 Shimadzu Corporation, Kyoto, Japan; coupled with a Shimadzu 8030 Mass Spectrometer). The acid
154 hydrolysis destroyed tryptophan making it unable to be determined using this method. Total lipid
155 content of the samples was determined gravimetrically following extraction of the lipids using the
156 chloroform:methanol method. Gross ash content was determined gravimetrically following loss of
157 mass after combustion of a sample in a muffle furnace at 550°C for 12 h. Gross energy was
158 determined by adiabatic bomb calorimetry (Par Instrument Company, Moline, IL, USA).

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

$$163 \quad ADC_{diet} = 1 - \left(\frac{Y_{diet} \times Parameter_{faeces}}{Y_{faeces} \times Parameter_{diet}} \right)$$

164 where Y_{diet} and Y_{faeces} represent the yttrium content of the diet and faeces respectively, and
 165 $Parameter_{diet}$ and $Parameter_{faeces}$ represent the nutritional parameter of concern (organic matter, protein
 166 or energy) content of the diet and faeces respectively. The digestibility values for each of the test raw
 167 materials in the test diets examined in this study were calculated according to the formulae:

$$168 \quad Nutr .AD_{RM} = \frac{(AD_{test} \times Nutr_{test} - (AD_{basal} \times Nutr_{basal} \times 0.7))}{(0.3 \times Nutr_{RM})}$$

169 Where $Nutr .AD_{RM}$ is the digestibility of a given nutrient from the test raw material included in the test
 170 diet at 30%. AD_{test} is the apparent digestibility of the test diet. AD_{basal} is the apparent digestibility of
 171 the basal diet, which makes up 70% of the test diet. $Nutr_{RM}$, $Nutr_{test}$ and $Nutr_{basal}$ are the level of the
 172 nutrient of interest in the raw material, test diet and basal diet respectively. All raw material inclusion
 173 levels were also corrected for their respective dry matter contribution relative to the dry matter content
 174 of the basal mash (Bureau and Hua, 2006). Ingredients with less than 5% lipid or 10% carbohydrates
 175 (CHO) were not assessed for lipid or CHO digestibilities due to an unacceptable error rate being
 176 encountered below this level these nutrients in the raw materials.

177 Raw material digestibilities greater than 100% were not corrected because we consider they
 178 are potentially indicative of interactive effects between the diet and test raw material and should be
 179 stipulated as determined. However, for reasons of practicality, the total levels of digestible
 180 nutrients/energy were only calculated assuming a maximum digestibility of 100% or a minimum of
 181 0% when multiplied against the respective nutrient parameter of that raw material.

182 2.5 Statistical analysis

183 All values are means and standard error of the mean, unless otherwise specified. No ANOVA
 184 comparison of the digestibility values among all the raw materials was undertaken as this was
 185 considered largely pointless. For some specific comparisons an ANOVA was undertaken with a
 186 Tukey's HSD post hoc test applied. For some simple comparisons (e.g. extruded versus raw feed
 187 grains) a MANOVA analysis was undertaken with a Tukey's HSD post hoc test applied. To examine
 188 potential effects of composition on digestibility, correlation matrices between diet composition and
 189 diet digestibility, and again between raw material composition and raw material digestibility were
 190 undertaken using Microsoft Excel. Limits for all critical ranges were set at $P < 0.05$. Because of

196 nominal variance in the reference diet data across experiments, no standardisation of the inter-
197 experiment data was undertaken. Statistical analyses were conducted in the R-project statistical
198 environment, version 3.1.0 (R Core Team, 2014).
199

200 **3. Results**

201 *3.1 Raw material characterisation*

202 Across the 29 different raw materials examined in this study there was a substantial range in
203 the composition parameters observed (Tables 1 and 2). Concentrations of protein varied from 0.2%
204 DM in the pregelled starch to 93.4%DM in the blood meal. The concentrations of lipid were lowest in
205 the pregelled starch and wheat gluten (<1% DM), though were also low (<2%) in field peas, faba
206 beans and blood meals. By contrast the lipid concentrations were highest in the Camelina meal
207 (29.3% DM) and krill meal (21.1% DM). The concentrations of ash were lowest in the pregelled
208 starch and blood meal (~1.2% DM), though were also low (<2%) in wheat gluten, wheat flour and
209 corn gluten. By contrast the ash concentrations were highest in the meat and bone meals (24.6 and
210 27.7% DM) and the tuna by-product fish meal (21.9% DM). Carbohydrate (CHO) concentrations
211 were highest in the pregelled starch (98.6% DM), though were also high (>60%) in field peas, wheat
212 flour and faba beans. Several ingredients were devoid of any CHO (e.g. blood meal, Jack mackerel
213 meal, etc.). Energy densities were highest in the Camelina meal (26.3 MJ/kg DM) and lowest in the
214 faba beans and field peas (18.9 MJ/kg DM). Amino acid concentrations also varied substantially
215 among the different raw materials (Table 2). There was a strong relationship between the crude
216 protein and sum of amino acids across all raw materials ($R^2 = 0.973$).

217

218 *3.2 Diet nutrient and energy digestibilities*

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

230 Diet digestibility values for the 29 test diets ranged according to the different parameters
231 measured (Table 5). Dry matter digestibilities were on average 0.636 ± 0.106 (mean \pm SD), with a
232 CV% of 16.6%. ADC values for dry matter digestibilities ranged from 0.251 (camelina meal) to 0.783
233 (Vitamin-free casein). Protein digestibilities were on average 0.765 ± 0.095 (mean \pm SD), with a
234 CV% of 12.4%. ADC values for protein digestibilities ranged from 0.483 (hydrolysed feather meal) to
235 0.895 (wheat gluten). Lipid digestibilities were on average 0.790 ± 0.076 (mean \pm SD), with a CV%
236 of 9.6%. ADC values for lipid digestibilities ranged from 0.559 (Blood meal) to 0.870 (Anchovetta

237 fishmeal). Energy digestibilities were on average 0.704 ± 0.089 (mean \pm SD), with a CV% of 12.6%.
238 ADC values for energy digestibilities ranged from 0.438 (camelina meal) to 0.826 (wheat gluten).
239 Carbohydrate digestibilities were on average 0.722 ± 0.091 (mean \pm SD), with a CV% of 12.5%.
240 ADC values for carbohydrate digestibilities ranged from 0.423 (camelina meal) to 0.874 (blood meal).

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

247

248 3.2 *Raw material nutrient and energy digestibilities*

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

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

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

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

275 **4. Discussion**

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

288

289 *4.1 Raw material characterisation*

290 Although the focus of this study was to examine the effects of different raw materials on the
291 digestibilities of diets and subsequently, by calculation/inference, the raw materials being tested, the
292 large range of raw materials being assessed also offers the chance to examine the range in
293 composition of some key resources. This characterisation stage was extolled by Glencross et al.
294 (2007) as an often-missed point of many similar such studies and the results in the present study, we
295 believe exemplify why this is an important part of any raw material assessment study. It can be seen
296 by examination of the three fishmeals, three poultry offal meals, two canola meals and two soybean
297 meals, that substantial differences exist subject to factors such as genotype, origin and processing
298 variables. Simply describing a raw material as “soybean meal” or “fishmeal” without an
299 accompanying comprehensive chemical characterisation and identification of the products origin
300 substantially reduces the value of the data and limits the differentiation of good quality products from
301 inferior ones.

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

307

308 *4.2 Diet digestibilities*

309 The across experiment variability (coefficient of variation) in digestibility values observed of
310 the two common diets (the basal and SPC diets) used in each of the three experiments in this study,
311 while still less than 10%, was still substantially larger than that observed in other species that

312 examined digestibilities across separate experiments using the same diets (Glencross et al., 2015;
313 2017). In these other studies, where faeces were collected using stripping techniques from a
314 carnivorous fish (*Oncorhynchus mykiss*), the coefficients of variation across dry matter, protein and
315 energy digestibility ranged from only 1.2 % to 2.3%. We suspect that this lower level of variability
316 may be linked to the use of a settlement-type faecal collection method in the present study with
317 shrimp. An assessment of the methodology associated with shrimp faecal collection methods by
318 Tabrett and Smith (2004) identified that the duration the faeces spent in the water post-defaecation
319 had an appreciable impact on the digestibility determination with these species, but also noted that it
320 was virtually impossible to remove the post-defecation solubilisation effect that results in over-
321 estimation of ingredient digestibility.

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

332

333 4.3 *Raw material digestibilities*

334 The assessment of this suite of raw materials provided some clear indications on the nutritive
335 value of a range of raw materials currently used in commercial shrimp diets and some novel
336 prospective raw materials under consideration. Notable were the poor digestibilities associated with
337 camelina meal which despite being reported as a suitable raw material for salmonids (Hixson et al.,
338 2016), is clearly unsuitable for shrimp. Substantial variability in the digestibility could also be seen
339 among the three different fishmeals (jack mackerel, anchovetta and tuna by-product meal), and also
340 between the two soybean meals, with many of these differences significant. This later observation
341 contrasts that of Cruz-Suarez et al., (2009), who examined different processing effects on soybean and
342 found little impact on protein digestibility. However, our observations are consistent with that of Zhou
343 et al (2014) who reported substantial differences in performance and digestibility associated with the
344 use of a range of different soybean meals used in diets for *L. vannamei*. These differences further
345 support the importance of specific ingredient characterisation, as clearly not all fishmeals or soybean
346 meals are of equal nutritional value.

347 The use of different processing methods to produce meat and bone meals and poultry offal
348 meals had mixed results. The use of lower temperatures to render meat and bone meals had a minor

349 benefit to protein, lipid and energy digestibilities. The use of fresher starting material in the
350 production of poultry offal meal had minor benefits to protein and lipid digestibility, but ironically not
351 to energy digestibility. Based on the present digestibility data, the nutritive value of blood meal to
352 shrimp is questionable, as is that of hydrolysed feather meal. These findings are consistent with those
353 presented by others using growth studies with shrimp (Dominy and Ako, 1988; Ricque-Marie et al.,
354 1998; Cheng et al., 2002; Forster et al., 2003; Suresh et al., 2011).

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

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

374

375 4.4 Conclusions

376 The findings of this study demonstrate that there is a wide range in the nutritive values of
377 various raw materials when fed to shrimp. Importantly, a generalisation of the comparative
378 digestibility of animal protein sources against vegetable protein sources cannot be made, as there are
379 excellent and poor digestibilities in either class of raw materials. The collation of the digestibility
380 values in this study we consider to be an important step-forward for the shrimp aquaculture industry
381 as it continues to seek independence from fishery resources. Additionally, such datasets provide an
382 important resource for future meta-analyses and the development of robust *in vitro* and *in silico*
383 models to estimate raw material nutritional value (Lemos et al., 2004; 2007; 2009; Glencross et al.,
384 2015).

385

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391

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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, Sydney, 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
Pregelged 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 Mackerel)	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
Pregelged 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-ETM : BEC Feed Solutions, Carole Park, QLD, Australia. ^g Astaxanthin (10%) as Carophyll PinkTM and Stay CTM: 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; ^hYttrium 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
Pregelged 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 Mackerel)	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
Pregelld 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.