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1 **Digestibility of canola meals in barramundi (Asian seabass; *Lates calcarifer*)**

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3 Diu Thi Ngo[1,2*], Igor Pirozzi[2], Brett Glencross[1#]

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5 1. CSIRO Aquaculture, 41 Boggo Rd, Dutton Park, QLD 4102, Australia

6 2. Centre for Sustainable Tropical Fisheries and Aquaculture & College of Marine and
7 Tropical Biology, James Cook University, Townsville, QLD 4811, Australia.

8

9 [*]Present details

10 (p) +61-416-570-068

11 (e) ngodiuait7@gmail.com

12 [#]Corresponding author

13 (p) 61-7-3833-5926

14 (e) brett.glencross@sciro.au

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16

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18 **Abstract**

19 The influence of two different oil processing methods and four different meal origins
20 on the digestibility of canola meals when fed to barramundi (*Lates calcarifer*) were
21 examined in this study. The apparent digestibility coefficients were determined using the
22 diet-substitution method with faeces collected from fish using stripping techniques. The
23 protein content of the solvent extracted (SE) canola meals (370-423 g/kg DM) was higher
24 than that of the expeller extracted (EX) canola meal (348 g/kg DM), but the lipid content was
25 lower than that of the expeller extracted canola meal. Amongst the SE canola meals, the
26 protein digestibility of the canola meals from Numurkah and Newcastle was similar (84.1%
27 and 86.6% respectively), but significantly higher than that of the canola meal from Footscray
28 (74.5%). The protein digestibility was the lowest (63.1%) for the EX canola meal. The
29 energy digestibility of the canola meals (43.1-52.5%) was similar to that of the lupin (54.8%)
30 except for the lower of SE canola from Footscray (32.4%). The SE canola meals provide
31 276-366 g/kg DM of protein while that of the EX is only 220g/kg DM. The digestible energy
32 content of the SE canola meal Footscray (6.5 MJ/kg) was lower than the other canola meals
33 (8.7-10.6 MJ/kg DM).

34

35 **1. Introduction**

36

37 Canola (rapeseed) meals (*Brassica spp.*) (CM) have considerable potential for fish
38 meal replacement in fish diets as they contain a relatively high protein content, varying from
39 32% to 45% dry matter (Burel *et al.*, 2000b) with a good amino acid profile, notably higher
40 in lysine and sulphur containing amino acids (methionine and cysteine) compared to soybean
41 meal, and are also a source of some minerals and vitamins (reference). Canola protein has
42 been shown to be well digested by a number of species (Cho & Slinger, 1979; Hilton &
43 Slinger, 1986; Anderson *et al.*, 1992; Hajen *et al.*, 1993; Higgs *et al.*, 1995; Higgs *et al.*,
44 1996; Mwachireya *et al.*, 1999; Allan *et al.*, 2000; Burel *et al.*, 2000b; Glencross *et al.*,
45 2004a). Indeed, among aquaculture species, many species have been shown to have good
46 growth and feed utilisation efficiency when fed diets containing canola meal. These include
47 rainbow trout (Yurkowski *et al.*, 1978; Hilton & Slinger, 1986; McCurdy & March, 1992;
48 Gomes *et al.*, 1993), juvenile Chinook salmon (Higgs *et al.*, 1982), gilthead seabream (Kissil
49 *et al.*, 2000), red seabream (Glencross *et al.*, 2004b), channel catfish (Webster *et al.*, 1997),
50 Japanese seabass (Cheng *et al.*, 2010), and cobia (Luo *et al.*, 2012). However, growth
51 performance is restricted in some species when fed diets with canola meal over 20% to 30%
52 due to deleterious effects attributed to anti-nutritional factors present in canola meal such as
53 fibre, breakdown products of glucosinolates, tannins, phytic acid, sinapine, oligosaccharides
54 and other anti-nutritional factors (Higgs *et al.*, 1982; Leatherland *et al.*, 1987; Teskeredžić *et*
55 *al.*, 1995; Burel *et al.*, 2000b; Burel *et al.*, 2001)
56 Like other tropical species, there has been relatively little effort carried out for
57 barramundi in seeking a replacement of fish meal for this species. The limited studies on
58 replacement of fish meal by plant protein sources such as soybean meal and lupin meal
59 suggested that different raw materials can be effectively used with as little as 15% fish meal
60 remaining in the diet (Glencross *et al*. 2011). The few available studies on canola meal use in
61 the diet for barramundi indicate that the introduction of canola meal into diets for barramundi
62 have been acceptable (Glencross, 2011; Glencross *et al.*, 2011b). However, there is limited
63 information on the nutritional value of canola meal for barramundi. Therefore a
64 comprehensive study is suggested to provide clear data and guidelines for the use of this
65 ingredient in diets for barramundi.

66 The nutritional value of canola meal varies according to the amount of residual oil
67 content, which is a direct consequence of the oil extraction technique used. Solvent
68 extraction and expeller pressing are the two main canola oil extraction methods used which
69 produce different qualities of canola meals (Glencross *et al.*, 2004b). Other aspects, such as

70 different growing conditions (e.g. weather and soil type), are also able to influence the
71 nutrient composition of canola meal. Moreover, crushing plants may have effects on quality
72 of CM products by adding some of the gums or soapstocks into the meal (Bell, 1993;
73 Hickling, 2001). Therefore, a comprehensive assessment of this ingredient should include an
74 examination of the variation in nutritional value of canola meal based on different processing
75 methods and origin.

76 There are several key steps to effectively assess a raw material for aquafeed. Initially,
77 the raw material needs to be comprehensively characterised, so the composition and history
78 of raw material are documented in order to allow a meaningful comparison with other raw
79 materials. Secondly, the digestible values of the ingredient needs to be measured so as to
80 allow for an understanding of the nutritional values of the ingredient via digestible values for
81 a species rather than crude values; then the formulation of diets based on digestible values
82 will be more nutritionally appropriate and economical. Once these fundamental assessments
83 have been made then the acceptable levels of inclusion of the ingredient in the fish diets can
84 be investigated by conducting feeding trials through the assessment of feed palatability,
85 intake, growth performance and effects of replaced diets on fish health or any biochemical,
86 physical changes as well (Glencross *et al.*, 2007).

87 This study therefore aims to assess the variation of the nutritive composition of the
88 four canola meals (from four crushing factories in four different regions in Australia -
89 Newcastle, Footscray, Pinjarra and Numurkah, which are produced from the two different oil
90 extraction techniques (solvent and expeller). Further to this the apparent digestibility of dry
91 matter, protein, amino acids and energy of each of the four canola meals were determined
92 when fed to barramundi (*Lates calcarifer*).

93

94 2. **Materials and Methods**

95 *2.1 Ingredient preparation and characterisation*

96 Four samples of canola meal produced from mixed genotypes were used in this
97 experiment (including three solvent-extracted (SE) CMs and one expeller (EX) CM) were
98 obtained from four different crushing plants (Newcastle, New South Wales; Footscray,
99 Victoria; Pinjarra, Western Australia; Numurkah, Victoria), and a Lupin kernel meal
100 (*Lupinus anguitifolius* cv. Coromup) used as a plant reference ingredient. These ingredients
101 were ground to pass through a 750 µm screen prior to being included in a series of
102 experimental diets. The chemical composition of four canola meals and reference ingredients
103 are described in Table 1.

104

105 *2.2 Diet and experiment design*

106 The experiment design was based on a strategy that allowed for the diet-substitution
107 digestibility method to be used (Glencross et al., 2007). For this method, a basal diet was
108 formulated and prepared with the composition of approximately 530 g/kg DM protein, 100
109 g/kg DM fat and an inert marker (yttrium oxide at 1 g/kg) (Table 2). Initially a basal mash
110 was prepared and thoroughly mixed, forming the basis for all diets used in this study. Each
111 canola meal was supplemented at a ratio of 30%: 70% to the basal mash to prepare each of
112 the test diets; the reference diet was made from 100% of basal mash, without addition of any
113 other ingredients.
114 After the various diets were prepared, each mash was mixed by using a 60L upright
115 Hobart mixer (HL 600, Hobart, Pinkenba, QLD, Australia). The mash was then made into
116 pellets using a laboratory-scale, twin-screw extruder with intermeshing, co-rotating screws
117 (MPF24:25, Baker Perkins, Peterborough, United Kingdom). All diets were extruded
118 operational through a 4mm Ø die at the same parameters for consistency. Pellets were cut
119 into 6 to 8mm lengths using two-bladed variable speed cutter and collected on an aluminium
120 tray and dried at $65^0$C for 12h in a fan-forced drying oven. The pellets were then stored
121 frozen for later use. The formulation and composition of the test and basal diets are presented
122 in Table 2.

123

124 *2.3 Fish handling and faecal collection*

125 Hatchery produced barramundi (Gladstone, Queensland) were reared in a stock
126 holding tank on a commercial pellet (Ridley Aquafeeds, Narangba, Australia) before being

127 used in this experiment. Fish were acclimatised to their dietary treatment for one week prior
128 to faecal collection which has been shown to be adequate for establishing an equilibrium in
129 digestibility values (Blyth *et al.*, 2012).

130 The experiment included 6 treatments, with each treatment having 4 replicates. Each
131 of the 24 cages was stocked with 5 fish of 390± 85 g (mean ± SD, n = 120). Treatments were
132 randomly allocated and replicates evenly distributed across 6 x 2500 L tanks each with four
133 HDPE mesh cages (300 L) per tank. No replicate cage of the same treatment occurred more
134 than once per tank. Cages were rotated once per week across tanks after stripping events.
135 This removed potential confounding effects due to tank effects. Tanks were supplied with
136 aeration and temperature controlled recirculated freshwater. Water quality data was
137 monitored on a daily basis during the experiment. Mean± SD of water temperature, pH, $NO_2$,
138 $NH_3$ were 29.8±0.3℃, 7.3±0.1 units**,** 0.5±0.3 mg $L^{-1}$ and 0.3±0.2 mg $L^{-1}$ respectively over
139 the 30 day experiment duration.

140 Barramundi were manually fed once daily to apparent satiety, as determined over
141 three separate feeding events between 1600 and 1700 each day. The experiment was
142 designed with two blocks over time, with 12 cages for each block. The fish within the same
143 block had their faeces collected on the same day. Faeces were collected in the following
144 morning (0800 – 0900) from each fish within each tank using stripping techniques based on
145 those reported by Glencross *et al.* (2011a). Fish were anesthetised using AQUI-S (20 ppm) in
146 a small oxygenated tank (120 L). Once loss of equilibrium was observed, close attention was
147 paid to the relaxation of the ventral abdominal muscles of the fish to ensure the fish were
148 removed from the water before they defecated in the anaesthetic tank. The faeces were then
149 expelled from the distal intestine using gentle abdominal pressure. Faecal samples were
150 expelled into small plastic jars (70 mL) and stored in a freezer at -20°C. To ensure accuracy
151 for determination of digestion values, faecal collection was carefully handled to avoid
152 contaminating the faeces with mucus and urine. No fish were stripped on consecutive days in
153 order to minimise stress on the animal and maximise feed intake prior to faecal collection.
154 Faeces were collected until sufficient sample for chemical analysis (over a twenty-day period
155 of faeces collection for this experiment), with each fish being stripped six times, once every
156 second day. Faecal samples from different stripping days from each tank were pooled within
157 replicate, and kept frozen at –20°C before being freeze-dried in preparation for analysis.

158

159 *2.4 Chemical analyses*

160 Diets, ingredients and faecal samples were analysed for dry matter, yttrium, ash, total
161 lipid, nitrogen, amino acids and gross energy content. Canola meals were also analysed for
162 neutral detergent fibre (NDF), acid detergent fibre (ADF), lignin, phytic acid, tannins,
163 polyphenolic compounds and glucosinolates.

164 Dry matter was calculated by gravimetric analysis following oven drying at 105ºC for
165 24 h. Total yttrium concentration was determined after mixed acid digestion using
166 inductively coupled plasma mass spectrometry (ICP-MS: ELAN DRC II, Perkin Elmer)
167 based on the method described by (McQuaker *et al.*, 1979). Protein levels were calculated
168 from the determination of total nitrogen by organic elemental analyser (Flash 2000, Thermo
169 Fishery Scientific), based on N x 6.25. Amino acid composition of samples, except for
170 tryptophan, was determined by an acid hydrolysis (HCl) at 110 $^{0}$C for 24 h prior to
171 separation via HPLC. Total lipid content of the diets and ingredients was determined
172 gravimetrically following extraction of the lipids using chloroform: methanol (2:1), based on
173 method of Folch *et al.* (1957). Gross ash content was determined gravimetrically following
174 loss of mass after combustion of a sample in a muffle furnace at 550°C for 12 h. Gross
175 energy was determined using a ballistic bomb calorimeter (PARR 6200, USA).

176 Total glucosinolates content in four canola meals were determined according to
177 method AOF4-1.22 of AOF (2007). On the basis of this method, CMs were heated to
178 destroy the natural myrosinase enzyme in these meals. Glucosinolates were then extracted by
179 water onto a solid phase extraction column. Myrosinase was then added and the samples
180 were incubated to allow the myrosinase enzyme to cleave the glucose molecules from the
181 glucosinolate moleculars. The glucose molecules were washed off the solid phase extraction
182 and the concentration determined by calorimetric reaction. A calculation was then used to
183 determine glucosinolate concentration.

184 Total poly phenolics and total tannins were assayed based on the method of Makkar
185 *et al.* (1993). Briefly, phenolic compounds from canola meals and lupin were extracted in
186 ethanol solution with the Folin Ciocalteu reagent and sodium carbonate added. The
187 supernatant containing phenols was measured at 725 nm using Merck standard tannic acid
188 solution for calibration. Then tannins from phenol containing extract were precipitated using
189 insoluble polyvinyl pyrrolidone (polyvinyl polypyrrolidone, PVPP), and the second
190 supernatant containing simple phenols was measured as above method. Total tannins were
191 determined by difference between the total phenolic content and the single phenolic content.

192 Phytic acid in samples were separated and concentrated by ion-exchange
193 chromatography. The phytic acid concentrate is then quantitatively determined as
194 phosphorus by inductively coupled plasma atomic emission spectrometry (ICP – AES).

195 NDF content was determined by using FibreCap$^{TM}$ 2021/2023 following to the
196 method described in the standard of EN ISO 16472. This method is based on the principle
197 that a neutral detergent solution, with a heat-stable alpha amylase, is used to dissolve the
198 easily-digested proteins, lipids, sugars, starches and pectins in samples, leaving fibrous
199 residue (aNDF). ADF and Lignin were determined following the standard of EN ISO 13906:
200 2008.

201

202 *2.5 Digestibility analysis*

203 Apparent digestibility coefficients (ADCs) of dry matter, protein, amino acids and
204 gross energy for reference and test diets were calculated by following formula (Maynard *et*
205 *al.*, 1979):

206–208

$$ADC\ (\%) = \left[ 1 - \frac{Y_2O_{3\ diet} \times Nutr_{faeces}}{Y_2O_{3\ faeces} \times Nutr_{diet}} \right] \times 100$$

209 where $Y_2O_{3diet}$ and $Y_2O_{3faeces}$ are the yttrium content of the diet and faeces respectively, and
210 $Nutrt_{diet}$ and $Nutr_{faeces}$ are the nutritional parameters (dry matter, protein, amino acid and
211 energy) of the diets and faeces respectively. Then, the ADCs of ingredients were determined
212 according to the formula:

213

214–216

$$ADC_{ing}\ (\%) = \frac{ADC_{test} \times Nutr_{test} - ADC_{basal} \times Nutr_{basal} \times 0.7}{0.3 \times Nutr_{ing}}$$

217 where $ADC_{test}$ and $ADC_{basal}$ are apparent digestibility of test diet and basal (reference) diet
218 respectively; $Nutr_{test}$, $Nutr_{basal}$ and $Nutr_{ing}$ represent the nutritional parameters (dry matter,
219 protein, amino acids and energy) of test diet, basal diet and ingredient respectively. All raw
220 material inclusion levels were corrected on dry matter basis and an actual ratio of basal diet
221 to test ingredient was used for digestibility calculation of test ingredient (Bureau & Hua,
222 2006).

223 Digestibility values calculated exceeding 100% were not corrected because they
224 indicate potential effects of interaction between diet and test ingredient and are reported as
225 determined. However, for practical reasons, only digestibility values in a range of 0% to
226 100% were used for calculation of digestible nutrients and energy as per recommendations
227 from Glencross *et al*. (2007).

228

229 *2.6 Statistical analysis*

230 All figures are mean ± SEM. Data were analysed for homogeneity of variation by
231 Levene's test before being analysed with a one-way analysis of variance (ANOVA) using
232 SPSS 11.0 for Windows. Differences among the means were tested by Duncan's multiple
233 range tests with the level of significance $P < 0.05$. Three outliers of homogeneity of
234 variances were identified and removed from data set with degrees of freedom adjusted
235 accordingly for subsequent statistical analyses (Table 3 and Table 4). These outliers were
236 dietary ADCs of proline in the SE-CM New and EX-CM Pin diets and one ingredient ADC
237 of histidine for SE CM Newcastle.

238

239 **3. Results**

240 *3.1 Variation in raw materials*

241 The chemical composition of the ingredients is presented in Table 1. The difference
242 in nutrient composition of canola meals was mainly observed in protein and lipid content.
243 The crude protein content of solvent-extracted (SE) CMs varied from 370 to 423 g/kg DM,
244 and was higher compared to that of the expeller CM (348 g/kg DM). However, lipid content
245 of the SE CMs was lower (44 g to 56g /kg DM) compared to that of expeller extracted (EX)
246 CM 92g/kg DM). There was also a variation in the chemical composition among the SE
247 CMs. The CM from Newcastle had higher protein content than the CM from Footscray and
248 Numurkah. Energy values were relatively consistent among the different CMs, range of from
249 20.1 to 20.6 MJ/ kg DM. The lupin kernel meal had a relatively similar composition to SE
250 CMs (Table 1) but was lower in ash content (31g/kg DM) compared to canola meals (67-
251 70g/ kg DM).

252 Similar to protein, amino acid content was fairly consistent among solvent CMs,
253 while lower content of almost all amino acids of EX compared to SE were observed. Lysine
254 content was significantly lower in EX. In general, although some lower amino acid content
255 was recorded for CMs, sulfur containing amino acids and lysine were higher in the CMs than
256 in the lupin meal (Table 1).

257 In addition to the nutritive values, anti-nutritional factors were also characterised in
258 this study. These include phenolic compounds (14.3 to19.9 g/kg DM), tannins (3.3 to 6.6
259 g/kg DM), phytic acid (26.6 to 45.2 g/kg DM) and glucosinolates (3.1 to 6.6 µmol/g DM). In
260 comparison with the lupin meal, all antinutritional compounds presented in the CMs were
261 consistently higher (Table 1). Fibre (reported as NDF, ADF and lignin) content was higher in
262 the expeller CM than in the solvent meals (NDF 310 vs 240 to 250 g/kg DM respectively).

263

264 *3.2 Dietary digestibility*

265 Dietary ADCs of protein were virtually identical (82.0% to 83.8%) among the
266 different SE CM diets and were higher than that of EX CM diet (79.7%). Overall, the dietary
267 protein digestibility of SE CM diets was relatively similar to the reference diet (85.7%) but
268 less than that of the lupin diet (86.3%). The same trend was seen for amino acid
269 digestibilities (Table 4). Lower dietary amino acid digestibilities were recorded for the EX
270 CM than for the SE CMs. The amino acid ADCs of the SE CMs were similar to those of the
271 lupin meal except for those of the SE CM from Footscray.

272 , The digestibility values of the test diets were consistent for both dry matter and
273 energy (except for lower values of SE-CM Footscray diet), and were lower than those of the
274 reference diet (detailed in Table 3).

275

276 *3.3 Ingredient digestibility*

277 The findings from the present study indicate that there is an influence of oil extraction
278 methods on the ingredient protein digestibility of CMs. Protein digestibility of EX CM was
279 significantly lower than that of SE CMs (63.1% vs a range of 74.5-84.1%). Furthermore,
280 there was also a difference in protein digestibility amongst SE CMs. Protein digestibility of
281 CM Footscray was lower than those of CM Newcastle and Numurkah. There were no
282 significant differences amongst protein digestibility values of CM Footscray, CM Numurkah
283 and lupin meal; however a higher value was still recorded for the lupin meal (92.7 %).

284 There was no significant difference in the ADCs of dry matter among the different
285 CMs, although the lower value was still seen for SE CM Footscray (29.9%). The results
286 showed that dry matter digestibility did not exceed 50% for any of the CMs or the lupin
287 meal.

288 There was a correlation between DM digestibility and energy digestibility (Fig. 1),
289 therefore low DM digestibility reflected poor energy digestibility of CMs and lupin, except
290 for EX (poor DM digestibility but high energy digestibility). Energy digestibility of the SE
291 CMs and EX CM was similar and equivalent to that of lupin, excluding a significant lower
292 value (32.4%) recorded for solvent CM Footscray.

293 In general, amino acid availability reflected protein digestibility (Table 3). Indeed,
294 many amino acid digestibility values were recorded exceeding 70% for canola meals which
295 were similar to protein values; however, for some amino acids, very low digestibility values
296 were observed (some below 50%), such as for histidine, cysteine, methionine and lysine in
297 expeller meal. There was substantial variation in amino acid digestibility among ingredients,
298 and a significant decrease in digestibility of almost all amino acids was reported for EX CM
299 compared to other ingredients. In some cases digestibility values over 100% were recorded,
300 such as for proline in all ingredients, and some other amino acids in the SE CM Newcastle.

301

302 **4. Discussion**

303

304 The findings of this study provide a comprehensive assessment of the influence of oil
305 extraction methods on the bioavailability of nutrients from various Australian canola meals
306 when fed to barramundi. These ingredient digestibility values were compared to a lupin
307 kernel meal which have previously been shown to have good acceptability as a plant protein
308 ingredient for use in barramundi (Glencross *et al.*, 2011b).

309

310 *4.1 Variation in raw materials*

311 Results of the present study showed that the processing method applied in canola oil
312 extraction process affects the nutritional composition of the canola meals and their
313 subsequent digestibility by barramundi. Indeed, a 61-109% higher level of oil, accompanied
314 with a reduction of 6-22% of protein content, was observed in the expeller meal compared
315 with the solvent-extracted meals. In terms of "protein quality", the loss of lysine content in
316 expeller canola meal was probably due to heat damage in canola processing (Carpenter,
317 1973).

318 The variation in composition of the four canola meals from different regions suggests
319 that growing conditions (e.g. weather, soil quality) may also affect quality of canola meal.
320 Furthermore, canola meal crushers probably also influence the quality of produced canola
321 meal by adjusting quality parameters in processing (Clandinin *et al.*, 1959; Bell, 1993;
322 Hickling, 2001). Moreover, different cultivars which were not identified in this study may be
323 a reason for dissimilarity in the qualities of the canola meals. In general, the Australian SE
324 CMs characterised in our study had protein (370- 423g/kg DM) equivalent to European
325 meals and Canadian meals, but were higher in lipid content (40 - 57g/kg DM) compared to
326 European meal (French Feed Database, 2005) and the Australian meal in the study of
327 Glencross *et al.* (2004a). For the EX meal, the protein content reported in this study was
328 consistent with European and Canadian expeller meals' but the lipid content was lower
329 (French Feed Database, 2005). For amino acids, the greatest differences were seen for lysine.
330 The lysine content of the EX CM in this study (12.3g/kg DM) was lower than that of other
331 EX Australian meals (17.7-21.1 g/kg wet basis) in report of (Spragg & Mailer, 2007), that of
332 Australian EX meal (20g/kg DM) (Glencross *et al.*, 2004a) that of European (39g/kg DM)
333 (French Feed Database, 2005), despite having similar protein levels.

334 *4.2 Variation in ingredient digestibility*

335 The findings of the current study indicate that the processes applied in oil extraction
336 to canola seed have affected not only their composition but also the digestibility of the meals
337 when fed to barramundi. Indeed, protein digestibility of the EX meal was lower than that of
338 SE meals (63.1% vs. 74.5-86.6%). The results of our study were dissimilar to the results of
339 Glencross *et al.* (2004b) where protein digestibility of Australian canola meals was
340 determined for red seabream. In that work, there were no significant differences in protein
341 digestibility between expeller and solvent meals but a higher value was still seen for expeller
342 (93.6% for expeller meal vs 83.2% for solvent meal. However, heat treatment of this EX CM
343 at $130^0$C and $150^0$C substantially depressed its digestible protein to 51.3% and 23.1%
344 respectively. In the present study, although operation temperature in oil processing of the
345 CMs was not described, substantial depletion of protein digestibility of the EX CM suggests
346 that high temperature was probably applied in the processing which might have caused
347 Maillard reactions leading to a modification of protein quality due to cross-linkages of amino
348 acids (Carpenter, 1973). Spragg and Mailer (2007) described that in some canola oil
349 extraction plants the temperature can be increased up to $135^0$C to increase oil production.
350 However, there are also other reasons which can explain a decrease of 10% in protein ADC
351 of EX meal. The higher phytic acid content together with higher fibre (expressed as ADF and
352 NDF content) presented in the EX CM than in the SE meals could adversely affect protein
353 digestion of barramundi. Mwachireya *et al.* (1999) reported that high levels of fibre either
354 alone or together with phytate adversely impacted the digestibility of CM for rainbow trout.
355 In terms of fibre (reported as NSP), a certain decrease in protein digestibility was observed
356 when fish fed increased dietary NSP classes (Glencross, 2009; Glencross *et al.*, 2012b). The
357 effect of fibre on nutrient digestibility is thought to interfere with the transport of nutrients
358 along the gastrointestinal tract and consequently the efficiency of nutrient absorption is
359 limited. In that study, the glucosinolate content was reported to be higher in the expeller
360 meal, but might not compromise its protein digestibility. In the present study, glucosinolate
361 content in the EX was similar or lower compared to those in the SE CMs; however, protein
362 digestibility of the EX CM was still much lower. This suggests that in our study with
363 barramundi, glucosinolates were not a factor depressing protein digestibility of the CMs.

364 The current results of digestibility from the two SE CM samples (Newcastle and
365 Numurkah) were consistent with the digestibility results reported for solvent-extracted canola
366 meal fed to Chinook salmon (Hajen *et al.*, 1993), Atlantic salmon (Higgs *et al.*, 1996)
367 rainbow trout (Mwachireya *et al.*, 1999), turbot (Burel *et al.*, 2000b), silver perch (Allan *et*
368 *al.*, 2000), red seabream (Glencross *et al.*, 2004b). Compared to results of Burel *et al.*

369 (2000a), the protein digestibility of Australian CM for barramundi (74.5% to 86.6%) was
370 lower than that of European solvent-extracted rapeseed meal for trout (89-91%); however, in
371 that study, the canola meal was dehulled to reduce fibre content of the ingredient. In the
372 present study, the protein digestibility of the SE CM Footscray was lower compared to that
373 of SE CM Newcastle, which indicated that there was a certain variation in digestibility of the
374 CMs from different growing regions and different plants. These comparisons suggest that the
375 different canola meals significantly affect the digestible values determined for each species.
376 In regards to the expeller meal, the protein digestibility determined for barramundi in this
377 study was much lower than that reported for both for silver perch (Allan *et al.*, 2000) and red
378 seabream (Glencross *et al.*, 2004b).

379 While amino acid digestibility generally reflects protein digestibility, in some cases,
380 there were some major differences in amino acid digestibility (Table 4). In terms of different
381 types of processing, amino acid ADCs of the EX CM was significantly lower than those of
382 the SE CMs. In case of the EX CM, many amino acid ADCs were below 50% which were far
383 lower than those of the SE CMs in this study for barramundi and those of different solvent
384 meals for other species (Hilton & Slinger, 1986; Anderson *et al.*, 1992; Allan *et al.*, 2000).
385 Maillard reactions could also occur during the expeller processing resulting in cross-linkages
386 of amino acids, typically with lysine, leading to its limited digestibility value (34.8% for the
387 EX meal compared to >80.6% for the SE meals). Newkirk *et al.* (2003) also showed that
388 high temperature decreased digestible amino acids of canola meal in broiler chickens. In our
389 results, several digestibility values of amino acids were calculated exceeding 100% (Table
390 4). In several previous studies, unusual observations for digestibility parameters were also
391 reported (Allan *et al.*, 2000; Glencross *et al.*, 2004c; Glencross *et al.*, 2012a). These could be
392 explained through errors relating to measurement or interactions among ingredients.
393 Glencross *et al.* (2007) recommended that these values should be reported but values
394 rounded 0% to 100% used to formulate diets on digestible nutrient basis.

395 In general, carnivorous species tend to ineffectively utilise dry matter and energy
396 from plant ingredients (Cho *et al.*, 1982; Sullivan & Reigh, 1995). In the present study, the
397 low DM digestibility was determined for both the EX and SE meals (29.9% to 40.1%), and
398 they were much lower than that of European meals (46% to 71%) (Burel *et al.*, 2000b) and
399 still less that than of Canadian meals (38% to 60%) (Cho & Slinger, 1979; Hajen *et al.*, 1993;
400 Higgs *et al.*, 1996; Mwachireya *et al.*, 1999; Allan *et al.*, 2000). As with to DM digestibility,
401 the energy ADCs of the Australian canola meals were also lower for barramundi (32.4% to
402 52.5%) than those of other canola meals for other fish species such as chinook salmon (51%
403 – 71%) , Atlantic salmon (62% to 73%), turbot (69% – 81%), gilthead seabream (79%) silver

404 perch (58%), red seabream (62%) (reviewed of Burel and Kaushik (2008)) and snakehead
405 (57.2%) (Yu *et al.*, 2013). Low ADC values of dry matter and energy suggests that
406 carbohydrates in canola meals are poorly digestible. This is consistent with a previous report
407 regarding the composition of carbohydrates, which indicated that carbohydrates in canola
408 appeared to be predominantly non-starch polysaccharides (NSPs) (Van Barneveld, 1998). A
409 number of studies have reported effects of NSPs or their classes on digestible values and in
410 most cases NSPs have negative effects on DM and energy digestibility of ingredients or diets
411 (Hansen & Storebakken, 2007; Glencross, 2009; Glencross *et al.*, 2012b). The low digestible
412 energy of canola meals may limit their inclusion in diets as the critical specification of a diet
413 is to meet the energy requirement for an animal. Further work is suggested to focus on the
414 reduction of fibre and anti-nutritional compounds to maximise digestible nutrients and
415 energy of Australian canola meals for barramundi.

416 In conclusion, although low protein and amino acid digestibility of EX CM were
417 observed for barramundi, other SE CMs were fairly well digested, and similar to that seen for
418 lupin meal. The digestibility profiles of nutrients and energy in this study may provide useful
419 information for the formulation of nutritionally balanced diets for barramundi. Additional
420 research should be considered to assess palatability and utilisation of canola meals when fed
421 to this fish species.

422

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437 ## 5. References

438 Allan, G. L., Parkinson, S., Booth, M. A., Stone, D. A., Rowland, S. J., Frances, J., Warner-
439 Smith, R. (2000). Replacement of fish meal in diets for Australian silver perch,
440 *Bidyanus bidyanus*: I. Digestibility of alternative ingredients. *Aquaculture, 186*(3),
441 293-310.

442 Anderson, J. S., Lall, S. P., Anderson, D. M., Chandrasoma, J. (1992). Apparent and true
443 availability of amino acids from common feed ingredients for Atlantic salmon (*Salmo*
444 *salar*) reared in sea water. *Aquaculture, 108*(1-2), 111-124.

445 AOF. (2007). AOF 4-1.22, *Australian Oilseed Federation standards manual.*: Australian
446 Oilseeds Federation, Sydney.

447 Bell, J. M. (1993). Factors affecting the nutritional value of canola meal: A review.
448 *Canadian Journal of Animal Science, 73*(4), 689-697. doi: 10.4141/cjas93-075

449 Blyth, D., Irvin, S., Bourne, N., Glencross, B. D. (2012). *Comparison of feacal collection*
450 *methods, and diet acclimation times for the measurement of digestibility coefficients*
451 *in barramundi (Lates calcarifer)*. Paper presented at the International Society for Fish
452 Nutrition and Feeding Meeting, Molde, Norway.

453 Bureau, D., Hua, K. (2006). Letter to the Editor of Aquaculture. *Aquaculture, 252*(2), 103-
454 105.

455 Burel, C., Boujard, T., Escaffre, A. M., Kaushik, S. J., Boeuf, G., Mol, K. A., . . . KuÈhn, E.
456 R. (2000a). Dietary low-glucosinolate rapeseed meal affects thyroid status and
457 nutrient utilization in rainbow trout (*Oncorhynchus mykiss*). *British Journal of*
458 *Nutrition, 83*(06), 653-664.

459 Burel, C., Boujard, T., Kaushik, S., Boeuf, G., Mol, K., Van der Geyten, S., . . . Querat, B.
460 (2001). Effects of rapeseed meal-glucosinolates on thyroid metabolism and feed
461 utilization in rainbow trout. *General and comparative endocrinology, 124*(3), 343-
462 358.

463 Burel, C., Boujard, T., Tulli, F., Kaushik, S. J. (2000b). Digestibility of extruded peas,
464 extruded lupin, and rapeseed meal in rainbow trout (*Oncorhynchus mykiss*) and turbot
465 (*Psetta maxima*). *Aquaculture, 188*(3), 285-298.

466 Burel, C., Kaushik, S. J. (2008). Use of rapeseed/canola meal in diets of aquaculture species.
467 In C. Lim, C. D. Webster & C. S. Lee (Eds.), Alternative protein sources in

468 aquaculture diets (pp. 343-408): The Haworth PressTaylor and Francis Group (New
469 York and London). doi: 10.1300/5892_13

470 Carpenter, K. (1973). *Damage to lysine in food processing, its measurement and its*
471 *significance, KJ Carpenter, VH Booth.* Paper presented at the Nutrition abstracts and
472 reviews.

473 Cheng, Z., Ai, Q., Mai, K., Xu, W., Ma, H., Li, Y., Zhang, J. (2010). Effects of dietary
474 canola meal on growth performance, digestion and metabolism of Japanese
475 seabass,(*Lateolabrax japonicus*). *Aquaculture, 305*(1), 102-108.

476 Cho, C., Slinger, S. (1979). *Apparent digestibility measurement in feedstuffs for rainbow*
477 *trout.* Paper presented at the Proceedings of the world symposium on finfish nutrition
478 and fishfeed technology, Hamburg.

479 Cho, C., Slinger, S., Bayley, H. (1982). Bioenergetics of salmonid fishes: energy intake,
480 expenditure and productivity. *Comparative Biochemistry and Physiology Part B:*
481 *Comparative Biochemistry, 73*(1), 25-41.

482 Clandinin, D., Renner, R., Robblee, A. (1959). Rapeseed Oil Meal Studies 1. Effects of
483 Variety of Rapeseed, Growing Environment and Processing Temperatures on the
484 Nutritive Value and Chemical Composition of Rapeseed Oil Meal. *Poultry Science,*
485 *38*(6), 1367-1372.

486 Folch, J., Lees, M., Sloane-Stanley, G. (1957). A simple method for the isolation and
487 purification of total lipids from animal tissues. *J. biol. Chem, 226*(1), 497-509.

488 French Feed Database. (2005). Technical data. from The French Association of Animal
489 production (AFZ) http://www.feedbase.com

490 Glencross, B. (2009). The influence of soluble and insoluble lupin non-starch
491 polysaccharides on the digestibility of diets fed to rainbow trout (*Oncorhynchus*
492 *mykiss*). *Aquaculture, 294*(3), 256-261.

493 Glencross, B. (2011). A comparison of the digestibility of diets and ingredients fed to
494 rainbow trout (*Oncorhynchus mykiss*) or barramundi (*Lates calcarifer*)–the potential
495 for inference of digestibility values among species. *Aquaculture Nutrition, 17*(2),
496 e207-e215.

497 Glencross, B., Blyth, D., Tabrett, S., Bourne, N., Irvin, S., Anderson, M., . Smullen, R.
498 (2012a). An assessment of cereal grains and other starch sources in diets for
499 barramundi (*Lates calcarifer*) - implications for nutritional and functional qualities of
500 extruded feeds. *Aquaculture Nutrition, 18*(4), 388-399.

501 Glencross, B., Hawkins, W., Curnow, J. (2004a). Nutritional assessment of Australian canola
502 meals. I. Evaluation of canola oil extraction method and meal processing conditions
503 on the digestible value of canola meals fed to the red seabream (*Pagrus auratus,*
504 *Paulin*). *Aquaculture Research, 35*(1), 15-24.

505 Glencross, B., Hawkins, W., Curnow, J. (2004b). Nutritional assessment of Australian canola
506 meals. II. Evaluation of the influence of the canola oil extraction method on the
507 protein value of canola meals fed to the red seabream (*Pagrus auratus, Paulin*).
508 *Aquaculture Research, 35*(1), 25-34.

509 Glencross, B., Hawkins, W., Evans, D., Rutherford, N., McCafferty, P., Dods, K., Hauler, R.
510 (2011a). A comparison of the effect of diet extrusion or screw-press pelleting on the
511 digestibility of grain protein products when fed to rainbow trout (*Oncorhynchus*
512 *mykiss*). *Aquaculture, 312*(1-4), 154-161.

513 Glencross, B., Rutherford, N., Bourne, N. (2012b). The influence of various starch and non-
514 starch polysaccharides on the digestibility of diets fed to rainbow trout (
515 *Oncorhynchus mykiss*). *Aquaculture, 356*, 141-146.

516 Glencross, B., Rutherford, N., Jones, B. (2011b). Evaluating options for fish meal
517 replacement in diets for juvenile barramundi (*Lates calcarifer*). *Aquaculture*
518 *Nutrition, 17*(3), 722-732.

519 Glencross, B. D., Booth, M., Allan, G. L. (2007). A feed is only as good as its ingredients - A
520 review of ingredient evaluation strategies for aquaculture feeds. *Aquaculture*
521 *Nutrition, 13*(1), 17-34.

522 Glencross, B. D., Carter, C. G., Duijster, N., Evans, D. R., Dods, K., McCafferty, P., . . .
523 Sipsas, S. (2004c). A comparison of the digestibility of a range of lupin and soybean
524 protein products when fed to either Atlantic salmon (*Salmo salar*) or rainbow trout
525 (*Oncorhynchus mykiss*). *Aquaculture, 237*(1), 333-346.

526 Gomes, E. F., Corraze, G., Kaushik, S. (1993). Effects of dietary incorporation of a co-
527 extruded plant protein (rapeseed and peas) on growth, nutrient utilization and muscle
528 fatty acid composition of rainbow trout (*Oncorhynchus mykiss*). *Aquaculture, 113*(4),
529 339-353.

530 Hajen, W., Beames, R., Higgs, D., Dosanjh, B. (1993). Digestibility of various feedstuffs by
531 post-juvenile chinook salmon (*Oncorhynchus tshawytscha*) in sea water. 1.
532 Validation of technique. *Aquaculture, 112*(4), 321-332.

533 Hansen, J. Ø., Storebakken, T. (2007). Effects of dietary cellulose level on pellet quality and
534 nutrient digestibilities in rainbow trout (*Oncorhynchus mykiss*). *Aquaculture, 272*(1),
535 458-465.

536 Hickling, D. (2001). Canola Meal: Feed Industry Guide (pp. 39): Canadian International
537 Grains Institute, Winnipeg, Manitoba, Canada.

538 Higgs, D., Dosanjh, B., Beames, R., Prendergast, A., Mwachireya, S., Deacon, G. (1996).
539 *Nutritive value of rapeseed/canola protein products for salmonids.* Paper presented at
540 the Eastern Nutrition Conference.

541 Higgs, D., Dosanjh, B., Prendergast, A., Beames, R., Hardy, R., Riley, W., Deacon, G.
542 (1995). Use of rapeseed/canola protein products in finfish diets. *Nutrition and*
543 *Utilization Technology in Aquaculture*, 130-156.

544 Higgs, D. A., McBride, J. R., Markert, J. R., Dosanjh, B. S., Plotnikoff, M. D., Clarke, W. C.
545 (1982). Evaluation of Tower and Candle rapeseed (canola) meal and Bronowski
546 rapeseed protein concentrate as protein supplements in practical dry diets for juvenile
547 chinook salmon (*Oncorhynchus tshawytscha*). *Aquaculture, 29*(1), 1-31.

548 Hilton, J., Slinger, S. (1986). Digestibility and utilization of canola meal in practical-type
549 diets for rainbow trout (*Salmo gairdneri*). *Canadian Journal of Fisheries and Aquatic*
550 *Sciences, 43*(6), 1149-1155.

551 Kissil, G. W., Lupatsch, I., Higgs, D., & Hardy, R. (2000). Dietary substitution of soy and
552 rapeseed protein concentrates for fish meal, and their effects on growth and nutrient
553 utilization in gilthead seabream *Sparus aurata* L. *Aquaculture Research, 31*(7), 595-
554 601.

555 Leatherland, J., Hilton, J., & Slinger, S. (1987). Effects of thyroid hormone supplementation
556 of canola meal-based diets on growth, and interrenal and thyroid gland physiology of
557 rainbow trout (*Salmo gairdneri*). *Fish Physiology and Biochemistry, 3*(2), 73-82.

558 Luo, Y., Ai, Q., Mai, K., Zhang, W., Xu, W., & Zhang, Y. (2012). Effects of dietary
559 rapeseed meal on growth performance, digestion and protein metabolism in relation
560 to gene expression of juvenile cobia (*Rachycentron canadum*). *Aquaculture*.

561 Makkar, H. P., Blümmel, M., Borowy, N. K., & Becker, K. (1993). Gravimetric
562 determination of tannins and their correlations with chemical and protein
563 precipitation methods. *Journal of the Science of Food and Agriculture, 61*(2), 161-
564 165.

565 Maynard, L. A., Loosli, J. K., Hintz, H. F., & Warner, R. G. (1979). *ANIMAL NUTRITION*
566 *7TH EDITION*.

567 McCurdy, S., & March, B. (1992). Processing of canola meal for incorporation in trout and
568 salmon diets. *Journal of the American Oil Chemists' Society, 69*(3), 213-220.

569 McQuaker, N. R., Brown, D. F., & Kluckner, P. D. (1979). Digestion of environmental
570 materials for analysis by inductively coupled plasma-atomic emission spectrometry.
571 *Analytical Chemistry, 51*(7), 1082-1084.

572 Mwachireya, S., Beames, R., Higgs, D., & Dosanjh, B. (1999). Digestibility of canola
573 protein products derived from the physical, enzymatic and chemical processing of
574 commercial canola meal in rainbow trout *Oncorhynchus mykiss* (Walbaum) held in
575 fresh water. *Aquaculture Nutrition, 5*(2), 73-82.

576 Newkirk, R., Classen, H., Scott, T., & Edney, M. (2003). The digestibility and content of
577 amino acids in toasted and non-toasted canola meals. *Canadian Journal of Animal*
578 *Science, 83*(1), 131-139.

579 Spragg, J., & Mailer, R. (2007). Canola meal value chain quality improvement. *Canola meal*
580 *value chain quality improvement*.

581 Sullivan, J. A., & Reigh, R. C. (1995). Apparent digestibility of selected feedstuffs in diets
582 for hybrid striped bass (*Morone saxatilis*♀ X *Morone chrysops*♂). *Aquaculture,*
583 *138*(1), 313-322.

584 Teskeredžić, Z., Higgs, D., Dosanjh, B., McBride, J., Hardy, R., Beames, R., . . . Bridges, R.
585 (1995). Assessment of undephytinized and dephytinized rapeseed protein concentrate
586 as sources of dietary protein for juvenile rainbow trout (*Oncorhynchus mykiss*).
587 *Aquaculture, 131*(3), 261-277.

588 Van Barneveld, R. J. (1998). Infuence of oil extraction method on the nutritional value of
589 canola meal for growing pigs (pp. 44). Pig Research and Development
590 Corporation,Canberra, Australia.

591 Webster, C. D., Tiu, L. G., Tidwell, J. H., & Grizzle, J. M. (1997). Growth and body
592 composition of channel catfish (*Ictalurus punctatus*) fed diets containing various
593 percentages of canola meal. *Aquaculture, 150*(1), 103-112.

594 Yu, H. R., Zhang, Q., Cao, H., Wang, X. Z., Huang, G. Q., Zhang, B. R., . . . Cui, Y. (2013).
595 Apparent digestibility coefficients of selected feed ingredients for juvenile snakehead,
596 Ophiocephalus argus. *Aquaculture Nutrition*.

597 Yurkowski, M., Bailey, J., Evans, R., Tabachek, J. A. L., Ayles, G. B., & Eales, J. (1978).
598 Acceptability of rapeseed proteins in diets of rainbow trout (*Salmo gairdneri*).
599 *Journal of the Fisheries Board of Canada, 35*(7), 951-962.
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625 **Tables and figures**

626 **Table 1** Chemical composition of raw materials (values are g/kg DM unless otherwise
627 indicated)

| | FM [a] | LM [b] | CM | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | **SE-CM Foo** [c] | **SE-CM New** [d] | **SE-CM Num** [e] | **EX-CM Pin** [f] | **Mean ±SD** | **CV (%)** |
| Dry matter (g/kg) | 925 | 906 | 900 | 908 | 903 | 974 | 921±35.3 | 3.8 |
| Crude protein | 721 | 408 | 370 | 423 | 381 | 348 | 381±31.5 | 8.3 |
| Total lipid | 91 | 64 | 57 | 44 | 56 | 92 | 62±20.7 | 33.2 |
| Total ash | 175 | 31 | 67 | 69 | 78 | 70 | 71±4.8 | 6.8 |
| Gross energy (MJ/kg DM) | 20.6 | 21.1 | 20.1 | 20.2 | 20.3 | 20.6 | 20±0.2 | 1.1 |
| NDF | n/a | n/a | 250 | 240 | 249 | 310 | 262±32.1 | 12.3 |
| ADF | n/a | n/a | 191 | 182 | 196 | 216 | 196±14.4 | 7.3 |
| Lignin | n/a | n/a | 94 | 95 | 111 | 134 | 109±18.7 | 17.2 |
| Total poly-phenolics | n/a | 3.3 | 15.6 | 14.3 | 19.9 | 16.4 | 16.6±2.4 | 14.6 |
| Total tannins | n/a | <1.1 | 4.4 | 3.3 | 6.6 | 4.1 | 4.6±1.4 | 30.9 |
| Phytic acid | n/a | 9.9 | 44.4 | 35.2 | 26.6 | 45.2 | 37.9±8.8 | 23.2 |
| Glucosinolates (µmol/g DM) | n/a | | <3.3 | 3.3 | 6.6 | 3.1 | 4.3±2.0 | 45.4 |
| *Amino acids* | | | | | | | | |
| Aspartic acid | n/a | 41.8 (102.5) | 29.8 (80.5) | 29.8 (70.4) | 28.1 (73.8) | 25.7 (73.9) | 28±1.9 | 6.8 |
| Glutamic acid | n/a | 87.5 (214.5) | 72.1 (194.9) | 77.0 (182.0) | 68.5 (179.8) | 61.8 (177.6) | 70±6.4 | 9.2 |
| Serine | n/a | 21.3 (52.2) | 18.6 (50.3) | 19.1 (45.2) | 17.9 (47.0) | 16.2 (46.6) | 18±1.3 | 7.1 |
| Histidine | n/a | 10.0 (24.5) | 11.2 (30.3) | 11.6 (27.4) | 10.0 (26.2) | 9.5 (27.3) | 11±1.0 | 9.3 |
| Glycine | n/a | 14.9 (36.5) | 18.1 (48.9) | 18.6 (44.0) | 17.8 (46.7) | 16.1 (46.3) | 18±1.1 | 6.1 |
| Threonine | n/a | 14.3 (35.0) | 18.1 (48.9) | 18.3 (43.3) | 17.8 (46.7) | 16.1 (46.3) | 18±1.0 | 5.7 |
| Cysteine-X | n/a | 5.5 (13.5) | 10.7 (28.9) | 11.3 (26.7) | 10.8 (28.3) | 9.2 (26.4) | 11±0.9 | 8.6 |
| Arginine | n/a | 45.7 (112.0) | 24.7 (66.8) | 25.6 (60.5) | 24.8 (65.1) | 21.3 (61.2) | 24±1.9 | 7.9 |
| Alanine | n/a | 13.9 (34.1) | 18.1 (48.9) | 18.8 (44.4) | 17.6 (46.2) | 16.1 (46.3) | 18±1.1 | 6.5 |
| Tyrosine | n/a | 16.6 (40.7) | 13.1 (35.4) | 13.0 (30.7) | 12.9 (33.9) | 11.7 (33.6) | 13±0.7 | 5.2 |
| Valine | n/a | 16.5 (40.4) | 21.0 (56.8) | 20.8 (49.2) | 20.0 (52.5) | 18.8 (54.0) | 20±1.0 | 5.0 |
| Methionine | n/a | 2.6 (6.4) | 7.5 (20.3) | 8.5 (20.1) | 7.7 (20.2) | 6.8 (19.5) | 8±0.7 | 9.2 |
| Phenylalanine | n/a | 17.1 (41.9) | 16.7 (45.1) | 17.4 (41.1) | 16.8 (44.1) | 14.9 (42.8) | 16±1.1 | 6.6 |
| Isoleucine | n/a | 16.5 (40.4) | 15.7 (42.4) | 16.0 (37.8) | 15.2 (39.9) | 14.1 (40.5) | 15±0.8 | 5.5 |
| Leucine | n/a | 28.6 (70.1) | 29.0 (78.4) | 30.1 (71.2) | 28.3 (74.3) | 25.7 (73.9) | 28±1.9 | 6.6 |
| Lysine | n/a | 14.6 (35.8) | 17.3 (46.8) | 17.4 (41.1) | 17.7 (46.5) | 12.3 (35.3) | 16±2.6 | 16.0 |
| Proline | n/a | 18.0 | 20.1 | 30.8 | 25.7 | 23.6 | 25±4.5 | 17.9 |

| (44.1) | (54.3) | (72.8) | (67.5) | (67.8) |
|---|---|---|---|---|

628 [a] Peruvian fish meal, supplied by Ridley Aquafeeds, Narangba, QLD, Australia

629 [b] Lupin kernel meal, supplied by Coorow Seed Cleaners Pty Ltd, Coorow, WA, Australia

630 [c] Solvent extracted canola meal, supplied by Cargill, Footscray, Victoria, Australia

631 [d] Solvent extracted canola meal, supplied by Cargill, Newcastle, New South Wales, Australia

632 [e] Solvent extracted canola meal, supplied by Riverland Oilseeds, Numurkah, Victoria, Australia

633 [f] Expeller extracted canola meal, supplied by Riverland Oilseeds, Pinjarra, WA, Australia

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635 **Table 2** Diet formulation and chemical composition

| | FM | LM | SE-CM Foo | SE-CM New | SE-CM Num | EX-CM Pin |
|---|---|---|---|---|---|---|
| *Ingredient (g/kg)* | | | | | | |
| Fish meal | 740 | 518 | 518 | 518 | 518 | 518 |
| Fish oil | 20 | 14 | 14 | 14 | 14 | 14 |
| Wheat flour | 133.0 | 93.1 | 93.1 | 93.1 | 93.1 | 93.1 |
| SE CM Newcastle | - | - | 300 | - | - | - |
| SE CM Footscray | - | - | - | 300 | - | - |
| SE CM Numurkah | - | - | - | - | 300 | - |
| EX CM Pinjarra | - | - | - | - | - | 300 |
| Lupin kernel meal | - | 300 | - | - | - | - |
| Cellulose | 101.0 | 70.7 | 70.7 | 70.7 | 70.7 | 70.7 |
| Vitamin and mineral premix[a] | 5.0 | 3.5 | 3.5 | 3.5 | 3.5 | 3.5 |
| Yttrium oxide | 1.0 | 0.7 | 0.7 | 0.7 | 0.7 | 0.7 |
| *Diet composition as analysed (all values are g/kg DM unless otherwise indicated)* | | | | | | |
| Dry matter | 968 | 976 | 975 | 960 | 971 | 975 |
| Protein | 536 | 505 | 496 | 516 | 500 | 486 |
| Total lipid | 92 | 89 | 81 | 79 | 74 | 98 |
| Ash | 138 | 106 | 118 | 113 | 119 | 113 |
| Carbohydrate[b] | 203 | 275 | 280 | 253 | 277 | 278 |
| Energy (MJ/kg DM) | 20.4 | 20.7 | 20.0 | 20.5 | 20.5 | 20.8 |
| Aspartic acid | 47.41 | 46.35 | 43.09 | 44.98 | 41.52 | 40.47 |
| Glutamic acid | 71.03 | 76.23 | 71.23 | 78.03 | 69.20 | 67.70 |
| Serine | 21.83 | 21.94 | 21.02 | 22.58 | 20.52 | 19.99 |
| Histidine | 15.98 | 14.75 | 13.74 | 15.34 | 14.35 | 13.30 |
| Glycine | 29.33 | 25.54 | 26.76 | 28.09 | 25.73 | 25.03 |
| Threonine | 22.17 | 20.22 | 21.50 | 22.76 | 20.75 | 20.27 |
| Cysteine-X | 6.10 | 5.74 | 7.21 | 9.08 | 6.97 | 6.75 |
| Arginine | 29.70 | 34.72 | 28.75 | 30.66 | 28.51 | 27.12 |
| Alanine | 32.45 | 27.51 | 28.73 | 30.34 | 27.97 | 27.55 |
| Taurine | 5.27 | 3.97 | 3.83 | 4.13 | 3.76 | 3.82 |
| Tyrosine | 16.87 | 16.97 | 15.95 | 16.75 | 15.52 | 15.27 |
| Valine | 26.52 | 23.99 | 25.61 | 26.71 | 24.36 | 23.87 |
| Methionine | 15.44 | 11.76 | 13.08 | 14.22 | 12.66 | 12.40 |
| Phenylalanine | 22.09 | 21.14 | 21.10 | 22.21 | 19.85 | 19.38 |
| Isoleucine | 21.62 | 20.49 | 20.34 | 21.20 | 19.48 | 19.01 |
| Leucine | 37.99 | 35.94 | 35.90 | 37.89 | 35.21 | 34.44 |
| Lysine | 31.52 | 27.50 | 28.16 | 29.60 | 27.79 | 25.13 |
| Proline | 19.51 | 23.76 | 26.62 | 28.69 | 25.41 | 24.66 |

636 [a] Vitamin and mineral premix includes (IU/kg or g/kg of premix): Vitamin A, 2.5MIU; Vitamin D3, 0.25 MIU; Vitamin E,
637 16.7 g; Vitamin K,3, 1.7 g; Vitamin B1, 2.5 g; Vitamin B2, 4.2 g; Vitamin B3, 25 g; Vitamin B5, 8.3; Vitamin B6, 2.0 g;
638 Vitamin B9, 0.8; Vitamin B12, 0.005 g; Biotin, 0.17 g; Vitamin C, 75 g; Choline, 166.7 g; Inositol, 58.3 g; Ethoxyquin, 20.8
639 g; Copper, 2.5 g; Ferrous iron, 10.0 g; Magnesium, 16.6 g; Manganese, 15.0 g; Zinc, 25.0 g.

640 [b] Determined as DM – (ash + protein + lipid)

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642 **Table 3** Diet apparent digestibility coefficients (%)

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| Nutrient | Reference | LM | SE-CM Foo | SE-CM New | SE-CM Num | EX-CM Pin | Pooled SEM |
|---|---|---|---|---|---|---|---|
| Dry matter | 66.1$^{b}$ | 58.5$^{a}$ | 54.8$^{a}$ | 58.9$^{a}$ | 57.7$^{a}$ | 55.7$^{a}$ | 0.99 |
| Protein | 85.7$^{cd}$ | 86.3$^{d}$ | 82.0$^{b}$ | 83.8$^{bc}$ | 83.8$^{bc}$ | 79.7$^{a}$ | 0.53 |
| Energy | 78.3$^{c}$ | 71.2$^{b}$ | 66.4$^{a}$ | 70.6$^{b}$ | 68.0$^{ab}$ | 67.6$^{ab}$ | 0.92 |
| *Amino acids* | | | | | | | |
| Aspartic acid | 82.5$^{b}$ | 83.3$^{b}$ | 79.7$^{b}$ | 81.7$^{b}$ | 80.9$^{b}$ | 76.2$^{a}$ | 0.64 |
| Glutamic acid | 93.0$^{c}$ | 92.9$^{c}$ | 90.6$^{b}$ | 91.8$^{bc}$ | 91.5$^{bc}$ | 88.6$^{a}$ | 0.37 |
| Serine | 88.1$^{c}$ | 87.6$^{c}$ | 83.2$^{ab}$ | 85.0$^{bc}$ | 84.8$^{bc}$ | 80.4$^{a}$ | 0.68 |
| Histidine | 89.5$^{c}$ | 88.5$^{bc}$ | 81.3$^{a}$ | 86.8$^{b*}$ | 86.1$^{b}$ | 79.6$^{a}$ | 1.07 |
| Glycine | 84.2 | 83.6 | 80.6 | 82.5 | 83.0 | 77.1 | 0.65 |
| Threonine | 90.7$^{d}$ | 89.6$^{cd}$ | 86.0$^{b}$ | 87.9$^{bc}$ | 87.3$^{bc}$ | 83.4$^{a}$ | 0.58 |
| Cysteine-X | 73.8$^{c}$ | 69.4$^{bc}$ | 64.7$^{b}$ | 74.8$^{c}$ | 67.7$^{bc}$ | 56.6$^{a}$ | 1.51 |
| Arginine | 93.1$^{cd}$ | 94.4$^{d}$ | 90.8$^{ab}$ | 92.1$^{bc}$ | 92.0$^{bc}$ | 90.1$^{a}$ | 0.36 |
| Alanine | 92.3$^{c}$ | 91.6$^{c}$ | 89.6$^{ab}$ | 90.7$^{bc}$ | 90.5$^{bc}$ | 88.1$^{a}$ | 0.35 |
| Taurine | 79.6$^{b}$ | 72.3$^{ab}$ | 63.8$^{a}$ | 69.6$^{ab}$ | 70.5$^{ab}$ | 69.3$^{ab}$ | 1.59 |
| Tyrosine | 91.4$^{c}$ | 91.1$^{c}$ | 86.5$^{ab}$ | 88.2$^{b}$ | 87.8$^{ab}$ | 85.4$^{a}$ | 0.56 |
| Valine | 91.8$^{c}$ | 91.0$^{c}$ | 88.1$^{ab}$ | 89.3$^{bc}$ | 88.2$^{ab}$ | 85.7$^{a}$ | 0.52 |
| Methionine | 91.5$^{c}$ | 89.9$^{bc}$ | 89.0$^{ab}$ | 90.7$^{bc}$ | 90.3$^{bc}$ | 87.6$^{a}$ | 0.36 |
| Phenylalanine | 92.2$^{b}$ | 92.1$^{b}$ | 90.7$^{ab}$ | 91.1$^{ab}$ | 89.6$^{a}$ | 89.2$^{a}$ | 0.32 |
| Isoleucine | 92.7$^{d}$ | 91.8$^{cd}$ | 89.0$^{ab}$ | 90.0$^{bc}$ | 89.4$^{ab}$ | 87.3$^{a}$ | 0.46 |
| Leucine | 94.1$^{d}$ | 93.6$^{cd}$ | 91.5$^{ab}$ | 92.3$^{bc}$ | 92.2$^{ab}$ | 90.3$^{a}$ | 0.33 |
| Lysine | 92.4$^{d}$ | 91.0$^{cd}$ | 87.2$^{ab}$ | 89.3$^{bc}$ | 90.1$^{cd}$ | 86.2$^{a}$ | 0.52 |
| Proline | 81.8$^{a}$ | 82.3$^{a}$ | 87.0$^{bc}$ | 88.8$^{c*}$ | 85.7$^{b}$ | 81.4$^{a*}$ | 0.64 |

644 Different superscripts within rows indicate significant differences between means among ingredients, but not between
645 parameters ($P < 0.05$).
646 (*) mean for three replicates after removal of extreme outlier

**Table 4** Ingredient apparent digestibility coefficients and digestible nutrient and energy values of test ingredients

| Nutrient | LM | SE-CM Foo | SE-CM New | SE-CM Num | EX-CM Pin | Pooled S.E.M |
|---|---|---|---|---|---|---|
| Dry matter | 44.2 | 29.9 | 42.2 | 40.1 | 32.9 | 2.98 |
| Protein | 92.7[c] | 74.5[b] | 86.6[c] | 84.1[bc] | 63.1[a] | 2.78 |
| Energy | 54.8[b] | 32.4[a] | 52.5[b] | 43.1[ab] | 46.9[b] | 2.42 |
| *Amino acids* | | | | | | |
| Aspartic acid | 89.3[bc] | 78.0[b] | 104.6[c] | 73.3[b] | 44.8[a] | 5.28 |
| Glutamic acid | 93.7[bc] | 84.8[b] | 110.0[c] | 83.3[b] | 74.3[a] | 2.92 |
| Serine | 89.6[c] | 71.7[b] | 99.8[c] | 73.3[b] | 53.5[a] | 4.18 |
| Histidine | 101.0[c] | 34.5[a] | 93.5[c]* | 77.9[b] | 24.0[a] | 7.92 |
| Glycine | 90.8[bc] | 79.2[b] | 105.6[d] | 76.3[b] | 42.0[a] | 5.62 |
| Threonine | 94.2[c] | 81.3[b] | 108.1[d] | 75.4[b] | 58.7[a] | 4.18 |
| Cysteine-X | 50.4[b] | 47.1[b] | 107.4[c] | 48.6[b] | 24.0[a] | 6.86 |
| Arginine | 97.9[b] | 90.9[b] | 115.7[d] | 92.7[b] | 79.5[a] | 2.92 |
| Alanine | 101.6[c] | 88.2[b] | 116.5[d] | 82.5[b] | 68.7[a] | 4.08 |
| Taurine | - | - | - | - | - | - |
| Tyrosine | 94.2[b] | 76.6[a] | 102.1[b] | 73.3[a] | 63.9[a] | 3.67 |
| Valine | 97.0[cd] | 87.9[d] | 109.0[c] | 73.9[b] | 60.3[a] | 4.26 |
| Methionine | 88.5[c] | 77.9[bc] | 118.2[d] | 66.7[ab] | 48.1[a] | 5.90 |
| Phenylalanine | 101.6[b] | 97.5[b] | 114.9[c] | 70.2[a] | 67.8[a] | 4.39 |
| Isoleucine | 96.4[cd] | 86.5[c] | 105.8[d] | 74.3[b] | 60.5[a] | 3.95 |
| Leucine | 100.3[c] | 90.1[b] | 110.4[d] | 87.7[b] | 78.9[a] | 2.73 |
| Lysine | 106.5[c] | 80.6[b] | 115.9[c] | 87.6[b] | 34.8[a] | 6.67 |
| Proline | 155.7[c] | 198.5[d] | 154.3[c]* | 137.5[b] | 127.0[a]* | 6.83 |
| *Digestible nutrients* | | | | | | |
| DM (g/kg) | 401 | 269 | 383 | 362 | 320 | |
| Protein (g/kg DM) | 378 | 276 | 366 | 320 | 220 | |
| Energy (MJ/kg DM) | 11.5 | 6.5 | 10.6 | 8.7 | 9.7 | |

Different superscripts within rows indicate significant differences between means among ingredients, but not between parameters (*$P < 0.05$*).

(*) mean for three replicates after removal of extreme outlier

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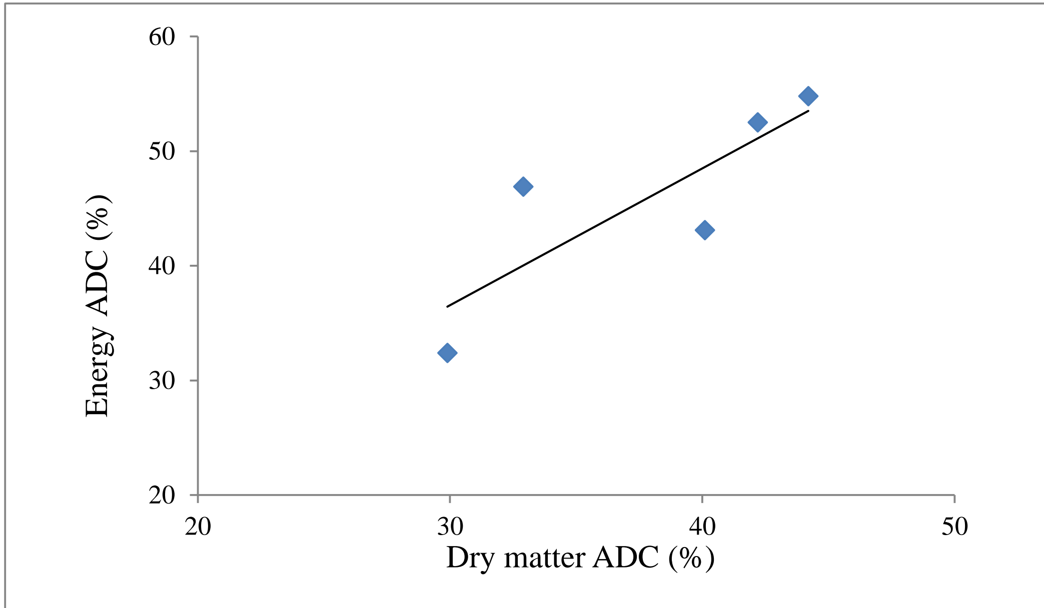


665 **Figure 1** Correlation between dry matter ADC and energy ADC values across all test
666 ingredients (y = 1.1927x + 0.786, $R^2 = 0.6889$)

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