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

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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<sup>0</sup>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  $390 \pm 85$  g (mean  $\pm$  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  $\pm$  SD of water temperature, pH,  $\text{NO}_2$ ,  
138  $\text{NH}_3$  were  $29.8 \pm 0.3^\circ\text{C}$ ,  $7.3 \pm 0.1$  units,  $0.5 \pm 0.3$  mg  $\text{L}^{-1}$  and  $0.3 \pm 0.2$  mg  $\text{L}^{-1}$  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 anaesthetised 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  $-20^\circ\text{C}$ . 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  $-20^\circ\text{C}$  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 105°C 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 °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™ 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):

$$206 \quad \text{ADC (\%)} = \left[ 1 - \frac{Y_2O_3 \text{ diet} \times \text{Nutr}_{\text{faeces}}}{Y_2O_3 \text{ faeces} \times \text{Nutr}_{\text{diet}}} \right] \times 100$$

209 where  $Y_2O_3$ <sub>diet</sub> and  $Y_2O_3$ <sub>faeces</sub> are the yttrium content of the diet and faeces respectively, and  
210  $\text{Nutr}_{\text{diet}}$  and  $\text{Nutr}_{\text{faeces}}$  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

$$214 \quad \text{ADC}_{\text{ing}} (\%) = \frac{\text{ADC}_{\text{test}} \times \text{Nutr}_{\text{test}} - \text{ADC}_{\text{basal}} \times \text{Nutr}_{\text{basal}} \times 0.7}{0.3 \times \text{Nutr}_{\text{ing}}}$$

217 where  $\text{ADC}_{\text{test}}$  and  $\text{ADC}_{\text{basal}}$  are apparent digestibility of test diet and basal (reference) diet  
218 respectively;  $\text{Nutr}_{\text{test}}$ ,  $\text{Nutr}_{\text{basal}}$  and  $\text{Nutr}_{\text{ing}}$  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  $\pm$  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 to 19.9 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  $\mu\text{mol/g DM}$ ). 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<sup>0</sup>C and 150<sup>0</sup>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<sup>0</sup>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 than that 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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626 **Table 1** Chemical composition of raw materials (values are g/kg DM unless otherwise  
627 indicated)

	FM <sup>a</sup>	LM <sup>b</sup>	CM				Mean +SD	CV (%)
			SE-CM Foo <sup>c</sup>	SE-CM New <sup>d</sup>	SE-CM Num <sup>e</sup>	EX-CM Pin <sup>f</sup>		
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
<i>Amino acids</i>								
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)

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- 628 <sup>a</sup> Peruvian fish meal, supplied by Ridley Aquafeeds, Narangba, QLD, Australia
- 629 <sup>b</sup> Lupin kernel meal, supplied by Coorow Seed Cleaners Pty Ltd, Coorow, WA, Australia
- 630 <sup>c</sup> Solvent extracted canola meal, supplied by Cargill, Footscray, Victoria, Australia
- 631 <sup>d</sup> Solvent extracted canola meal, supplied by Cargill, Newcastle, New South Wales, Australia
- 632 <sup>e</sup> Solvent extracted canola meal, supplied by Riverland Oilseeds, Numurkah, Victoria, Australia
- 633 <sup>f</sup> Expeller extracted canola meal, supplied by Riverland Oilseeds, Pinjarra, WA, Australia
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	FM	LM	SE-CM Foo	SE-CM New	SE-CM Num	EX-CM Pin
<i>Ingredient (g/kg)</i>						
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 <sup>a</sup>	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
<i>Diet composition as analysed (all values are g/kg DM unless otherwise indicated)</i>						
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 <sup>b</sup>	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 <sup>a</sup> 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<sub>3</sub>, 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 <sup>b</sup> Determined as DM – (ash + protein + lipid)

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

Nutrient	Reference	LM	SE-CM Foo	SE-CM New	SE-CM Num	EX-CM Pin	Pooled SEM
Dry matter	66.1 <sup>b</sup>	58.5 <sup>a</sup>	54.8 <sup>a</sup>	58.9 <sup>a</sup>	57.7 <sup>a</sup>	55.7 <sup>a</sup>	0.99
Protein	85 .7 <sup>c</sup> d	8 6. 3 <sup>d</sup>	8 2. 0 <sup>b</sup>	83 .8 bc	83 .8 <sup>b</sup> c	79 .7 <sup>a</sup>	0 .5 3
Energy	78 .3 <sup>c</sup>	7 1. 2 <sup>b</sup>	6 6. 4 <sup>a</sup>	70 .6 b	68 .0 <sup>a</sup> b	67 .6 <sup>a</sup> b	0 .9 2
<i>Amino acids</i>							
Aspartic acid	82.5 <sup>b</sup>	83.3 <sup>b</sup>	79.7 <sup>b</sup>	81.7 <sup>b</sup>	80.9 <sup>b</sup>	76.2 <sup>a</sup>	0.64
Glutamic acid	93.0 <sup>c</sup>	92.9 <sup>c</sup>	90.6 <sup>b</sup>	91.8 <sup>bc</sup>	91.5 <sup>bc</sup>	88.6 <sup>a</sup>	0.37
Serine	88.1 <sup>c</sup>	87.6 <sup>c</sup>	83.2 <sup>ab</sup>	85.0 <sup>bc</sup>	84.8 <sup>bc</sup>	80.4 <sup>a</sup>	0.68
Histidine	89.5 <sup>c</sup>	88.5 <sup>bc</sup>	81.3 <sup>a</sup>	86.8 <sup>b*</sup>	86.1 <sup>b</sup>	79.6 <sup>a</sup>	1.07
Glycine	84.2	83.6	80.6	82.5	83.0	77.1	0.65
Threonine	90.7 <sup>d</sup>	89.6 <sup>cd</sup>	86.0 <sup>b</sup>	87.9 <sup>bc</sup>	87.3 <sup>bc</sup>	83.4 <sup>a</sup>	0.58
Cysteine-X	73.8 <sup>c</sup>	69.4 <sup>bc</sup>	64.7 <sup>b</sup>	74.8 <sup>c</sup>	67.7 <sup>bc</sup>	56.6 <sup>a</sup>	1.51
<b>Arginine</b>	93.1 <sup>cd</sup>	94.4 <sup>d</sup>	90.8 <sup>ab</sup>	92.1 <sup>bc</sup>	92.0 <sup>bc</sup>	90.1 <sup>a</sup>	0.36
Alanine	92.3 <sup>c</sup>	91.6 <sup>c</sup>	89.6 <sup>ab</sup>	90.7 <sup>bc</sup>	90.5 <sup>bc</sup>	88.1 <sup>a</sup>	0.35
Taurine	79.6 <sup>b</sup>	72.3 <sup>ab</sup>	63.8 <sup>a</sup>	69.6 <sup>ab</sup>	70.5 <sup>ab</sup>	69.3 <sup>ab</sup>	1.59
Tyrosine	91.4 <sup>c</sup>	91.1 <sup>c</sup>	86.5 <sup>ab</sup>	88.2 <sup>b</sup>	87.8 <sup>ab</sup>	85.4 <sup>a</sup>	0.56
Valine	91.8 <sup>c</sup>	91.0 <sup>c</sup>	88.1 <sup>ab</sup>	89.3 <sup>bc</sup>	88.2 <sup>ab</sup>	85.7 <sup>a</sup>	0.52
Methionine	91.5 <sup>c</sup>	89.9 <sup>bc</sup>	89.0 <sup>ab</sup>	90.7 <sup>bc</sup>	90.3 <sup>bc</sup>	87.6 <sup>a</sup>	0.36
Phenylalanine	92.2 <sup>b</sup>	92.1 <sup>b</sup>	90.7 <sup>ab</sup>	91.1 <sup>ab</sup>	89.6 <sup>a</sup>	89.2 <sup>a</sup>	0.32
Isoleucine	92.7 <sup>d</sup>	91.8 <sup>cd</sup>	89.0 <sup>ab</sup>	90.0 <sup>bc</sup>	89.4 <sup>ab</sup>	87.3 <sup>a</sup>	0.46
Leucine	94.1 <sup>d</sup>	93.6 <sup>cd</sup>	91.5 <sup>ab</sup>	92.3 <sup>bc</sup>	92.2 <sup>ab</sup>	90.3 <sup>a</sup>	0.33
Lysine	92.4 <sup>d</sup>	91.0 <sup>cd</sup>	87.2 <sup>ab</sup>	89.3 <sup>bc</sup>	90.1 <sup>cd</sup>	86.2 <sup>a</sup>	0.52
Proline	81.8 <sup>a</sup>	82.3 <sup>a</sup>	87.0 <sup>bc</sup>	88.8 <sup>c*</sup>	85.7 <sup>b</sup>	81.4 <sup>a*</sup>	0.64

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

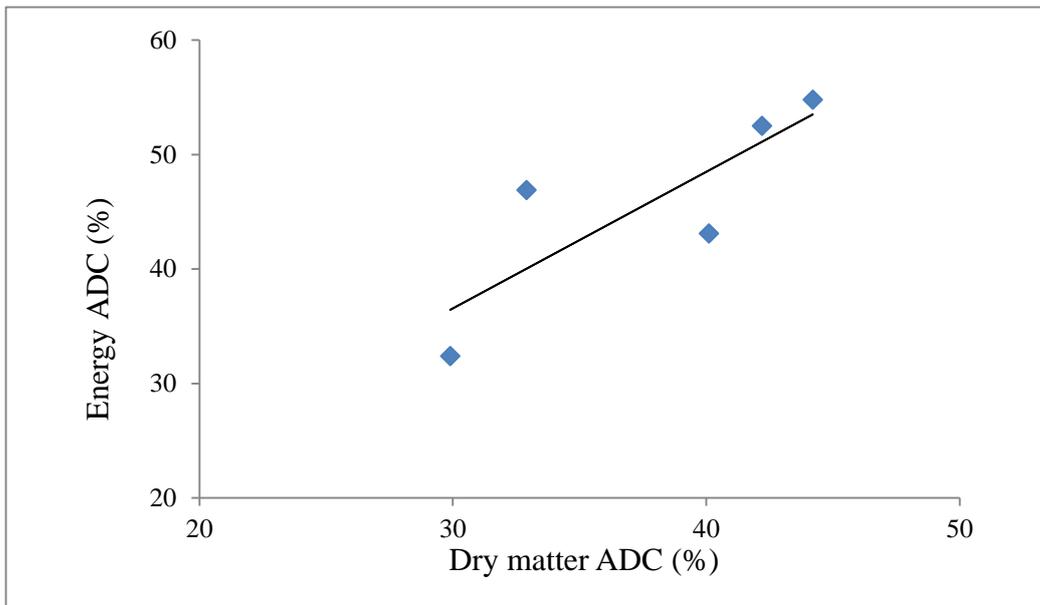
Nutrient	LM	SE-CM Foo	SE-CM New	SE-CM Num	EX-CM Pin	Pooled S.E.M
Dry matter	44.2	29.9	42.2	40.1	32.9	2.98
Protein	92.7 <sup>c</sup>	74.5 <sup>b</sup>	86.6 <sup>c</sup>	84.1 <sup>bc</sup>	63.1 <sup>a</sup>	2.78
Energy	54.8 <sup>b</sup>	32.4 <sup>a</sup>	52.5 <sup>b</sup>	43.1 <sup>ab</sup>	46.9 <sup>b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

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

653 (\*) 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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