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

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Abstract

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

1. Introduction

Canola (rapeseed) meals (*Brassica spp*.) (CM) have considerable potential for fish meal replacement in fish diets as they contain a relatively high protein content, varying from 32% to 45% dry matter (Burel *et al.*, 2000b) with a good amino acid profile, notably higher in lysine and sulphur containing amino acids (methionine and cysteine) compared to soybean meal, and are also a source of some minerals and vitamins (reference). Canola protein has been shown to be well digested by a number of species (Cho & Slinger, 1979; Hilton & Slinger, 1986; Anderson *et al.*, 1992; Hajen *et al.*, 1993; Higgs *et al.*, 1995; Higgs *et al.*, 1996; Mwachireya *et al.*, 1999; Allan *et al.*, 2000; Burel *et al.*, 2000b; Glencross *et al.*, 2004a). Indeed, among aquaculture species, many species have been shown to have good growth and feed utilisation efficiency when fed diets containing canola meal. These include rainbow trout (Yurkowski *et al.*, 1978; Hilton & Slinger, 1986; McCurdy & March, 1992; Gomes *et al.*, 1993), juvenile Chinook salmon (Higgs *et al.*, 1982), gilthead seabream (Kissil *et al.*, 2000), red seabream (Glencross *et al.*, 2004b), channel catfish (Webster *et al.*, 1997), Japanese seabass (Cheng *et al.*, 2010), and cobia (Luo *et al.*, 2012). However, growth performance is restricted in some species when fed diets with canola meal over 20% to 30% due to deleterious effects attributed to anti-nutritional factors present in canola meal such as fibre, breakdown products of glucosinolates, tannins, phytic acid, sinapine, oligosaccharides and other anti-nutritional factors (Higgs *et al.*, 1982; Leatherland *et al.*, 1987; Teskeredžić *et al.*, 1995; Burel *et al.*, 2000b; Burel *et al.*, 2001)

Like other tropical species, there has been relatively little effort carried out for barramundi in seeking a replacement of fish meal for this species. The limited studies on replacement of fish meal by plant protein sources such as soybean meal and lupin meal suggested that different raw materials can be effectively used with as little as 15% fish meal remaining in the diet (Glencross *et al*. 2011). The few available studies on canola meal use in the diet for barramundi indicate that the introduction of canola meal into diets for barramundi have been acceptable (Glencross, 2011; Glencross *et al.*, 2011b). However, there is limited information on the nutritional value of canola meal for barramundi. Therefore a comprehensive study is suggested to provide clear data and guidelines for the use of this ingredient in diets for barramundi.

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

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

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

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

2. Materials and Methods

2.1 Ingredient preparation and characterisation

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

2.2 Diet and experiment design

The experiment design was based on a strategy that allowed for the diet-substitution digestibility method to be used (Glencross et al., 2007). For this method, a basal diet was formulated and prepared with the composition of approximately 530 g/kg DM protein, 100 g/kg DM fat and an inert marker (yttrium oxide at 1 g/kg) (Table 2). Initially a basal mash was prepared and thoroughly mixed, forming the basis for all diets used in this study. Each canola meal was supplemented at a ratio of 30%: 70% to the basal mash to prepare each of the test diets; the reference diet was made from 100% of basal mash, without addition of any other ingredients.

After the various diets were prepared, each mash was mixed by using a 60L upright Hobart mixer (HL 600, Hobart, Pinkenba, QLD, Australia). The mash was then made into pellets using a laboratory-scale, twin-screw extruder with intermeshing, co-rotating screws (MPF24:25, Baker Perkins, Peterborough, United Kingdom). All diets were extruded operational through a 4mm Ø die at the same parameters for consistency. Pellets were cut into 6 to 8mm lengths using two-bladed variable speed cutter and collected on an aluminium tray and dried at 65^0C for 12h in a fan-forced drying oven. The pellets were then stored frozen for later use. The formulation and composition of the test and basal diets are presented in Table 2.

2.3 Fish handling and faecal collection

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

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

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

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

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

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

2.5 Digestibility analysis

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

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

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

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

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

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

2.6 Statistical analysis

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

3. Results

3.1 Variation in raw materials

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

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

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

3.2 Dietary digestibility

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

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

3.3 Ingredient digestibility

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

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

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

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

4. Discussion

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

4.1 Variation in raw materials

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

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

4.2 Variation in ingredient digestibility

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

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

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

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

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

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

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

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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)

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

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

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

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

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

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

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)

641

642 **Table 3** Diet apparent digestibility coefficients (%)
643

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
Argin**ine**	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

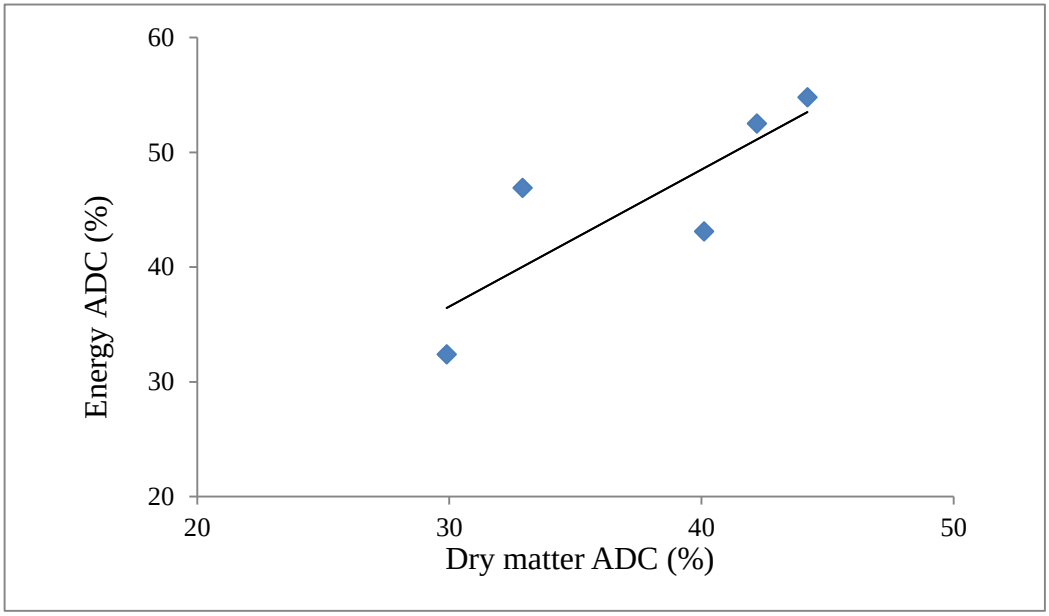

Figure 1 Correlation between dry matter ADC and energy ADC values across all test ingredients ($y = 1.1927x + 0.786$, $R^2 = 0.6889$)