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

Diu Thi Ngo [a,b,*], Igor Pirozzi [b], Brett Glencross [a]

[a] CSIRO Aquaculture, 41 Boggo Rd, Dutton Park, QLD 4102, Australia
[b] Centre for Sustainable Tropical Fisheries and Aquaculture & College of Marine and Environmental Science, James Cook University, Townsville, QLD 4811, Australia

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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*) was 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. Among 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 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 220 g/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). This study shows that there can be significant variability in the digestibility of canola meals subject to potential processing and sourcing variables.

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. Canola protein has been shown to be well digested by a number of species (Allan et al., 2000; Anderson et al., 1992; Burel et al., 2000b; Cho and Slinger, 1979; Glencross et al., 2004a; Hajen et al., 1993; Higgs et al., 1995, 1996; Hilton and Slinger, 1986; Mwachireya et al., 1999). 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 (Gomes et al., 1993; Hilton and Slinger, 1986; McCurdy and March, 1992; Yurkowski et al., 1978), 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 (Burel et al., 2000b, 2001; Higgs et al., 1982; Leatherland et al., 1987; Teskeredžić et al., 1995)

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, 2011a; Glencross et al., 2011). 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 (Hickling,

* Corresponding author at: Centre for Sustainable Tropical Fisheries and Aquaculture & College of Marine and Environmental Science, James Cook University, Townsville, QLD 4811, Australia. Tel.: +61 416 570 068.
E-mail address: ngodiuait7@gmail.com (D.T. Ngo).

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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 need 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, and 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) and were obtained from four different crushing plants (Newcastle, New South Wales; Footscray, Victoria; Pinjarra, Western Australia; Numurkah, Victoria), and a lupin kernel meal (*Lupinus angustifolius* 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 is 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% basal mash, without addition of any other ingredients.

After the various diets were prepared, each mash was mixed by using a 60 L 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 operationally through a 4 mm Ø die at the same parameters for consistency. Pellets were cut into 6 to 8 mm lengths using two-bladed variable speed cutter and collected on an aluminium tray and dried at 65 °C for 12 h 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., 2014).

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 × 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 ± 0.3 °C, 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 (2011b) and Blyth et al. (2014). 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.

2.4. Chemical analyses

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

Dry matter was calculated by gravimetric analysis following oven drying at 105 °C for 24 h. Total yttrium concentration was determined after mixed acid digestion using inductively coupled plasma mass spectrometry (ICP-MS: ELAN DRC II, Perkin Elmer) based on the method described by McQuaker et al. (1979). Protein levels were calculated from the determination of total nitrogen by organic elemental analyser (Flash 2000, Thermo Fishery Scientific), based on N × 6.25. Amino acid composition of samples, except for tryptophan, was determined by an acid hydrolysis (HCl) at 110 °C for 24 h prior to separation via HPLC. Total lipid content of the diets and ingredients was determined gravimetrically following extraction of the lipids using chloroform: methanol (2:1), based on the method of Folch et al. (1957). Gross ash content was determined gravimetrically following loss of mass after