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Effects of canola meal on growth, feed utilisation, plasma biochemistry, histology of digestive organs and hepatic gene expression of barramundi (Asian seabass; *Lates calcarifer*)

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ABSTRACT

The serial replacement of fish meal (anchovetta) by canola meal (CM) (100, 200, 300 g kg^{-1} as either solvent extracted (SE) CM or expeller extracted (EX) CM was undertaken to investigate the effects of increasing dietary CM levels on feed intake, growth, protein and energy retention, plasma biochemistry and the expression of a suite of hepatic genes in barramundi (Asian seabass; *Lates calcarifer*) over an eight week feeding trial. An additional diet using lupin kernel meal (LM) to replace the fish meal was also included as a comparative reference. Eight iso-digestible nitrogenous (423 ± 29 g kg^{-1}) and iso-digestible energetic (14.6 ± 8 MJ kg^{-1} DM) diets were formulated. Each diet was randomly allocated to triplicate groups of fish in seawater tanks (600 L), and each tank was stocked with 15 fish (53.4 ± 7.0 g). Fish were fed once daily (9:00–10:00) to apparent satiation, and uneaten feed was collected to determine feed consumption. The results showed that the survival, feed intake, growth, FCR, energy and protein retention of fish fed the diet containing SE CM were similar or even higher to those of fish fed the fish meal reference diet (FM) and the LM diet. Fish fed with the diet containing 300 g kg^{-1} SE CM did not show any changes in biochemistry and gene expression in a suite of detoxification genes. However, the diet with 300 g kg^{-1} EX CM depressed feed intake, growth performance and increased feed conversion ratio (FCR). Transcription of genes involving in fatty acid synthesis and the TCA cycle were not changed by different diets. The down regulation of gene expression in certain detoxification genes (*Lc CYP1A1*, *Lc CYP3A*, *Lc CYP2N* and *Lc GST*) was observed in fish fed with the diet containing 300 g kg^{-1} EX CM compared to the FM control diet and other experimental diets. In general, the SE CM can be used up to 300 g kg^{-1} diet without negative performance effects or signs of clinical plasma biochemistry. By contrast the maximum acceptable level of the EX CM for barramundi was only 200 g kg^{-1}. Higher inclusion level of the EX CM induced negative effects on growth performance, feed utilisation, plasma biochemistry and gene expression in relation to detoxification.

Statement of relevance: Previous research has demonstrated that canola meal is a potential plant protein source for fish meal replacement in diets for many fish species. The present work is designed to be the first to assess both two types of Australian canola meal in regard to different processing methods (solvent extraction and expeller extraction) in use for barramundi (Asian seabass) diets. The research not only assesses effects of canola meal on growth performance and feed utilisation but also investigate effects of canola meal use on fish health status using broad approach including plasma chemistry, histology and gene expression analysis. The present findings provide practical information for barramundi's diet formulation using canola meal as an additional plant ingredient. These also provide implications of potential health effects relating to using canola meal for barramundi.

1. Introduction

Canola meal (CM) is considered a potentially useful plant protein source for fish meal replacement in diets for aquaculture species (Burel and Kaushik, 2008). Canola is the second biggest oilseed product with production around 59 million tons in 2010, in which the production of CM was 32 million tons (Enami, 2011). It has high nutrition value with protein content varying between 320 and 450 g kg^{-1} of dry matter (Burel et al., 2000a) and favorable amino acid compared to other available plant proteins (Friedman, 1996), and it is also the source of mineral, vitamin and other microelement. Many fish species have been shown to have good growth performance when fed with diets containing CMs. These include rainbow trout (Gomes et al., 1993; Hardy and Sullivan, 1983; Leatherland et al., 1987; Mccurdy and March, 1992; Yurkowski et al., 1978), juvenile Chinook salmon (Higgs

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et al., 1982), gilthead seabream (Kissil et al., 2000), red seabream (Glencross et al., 2004b), channel catfish (Lim et al., 1998; Webster et al., 1997), and tilapia (Zhou and Yue, 2010). However, CM also contains many anti-nutritional factors (ANFs) which limit its utilisation. A decrease in growth performance has been reported when fish were fed with high levels of CM in their diets (Burel et al., 2000c; Cheng et al., 2010; Luo et al., 2012; Satoh, 1998; Webster et al., 1997).

Using plant ingredients has raised considerations of the effects of ANFs on the growth performance and health status of fish (Francis et al., 2001). As with other plant ingredients, CM contains many ANFs including fibre, oligosaccharides, phenolic compounds, tannins, phytic acid, glucosinolates (GSLs) and their derivatives (Bell, 1993; Higgs et al., 1995). Rapeseed meal/CM and ANFs caused goitrogenicity and internal organ abnormalities in animals (Mawson et al., 1994). In fish, although the GSL content in most of commercial CMs is considerably reduced compared to earlier varieties of rapeseed, there are still concerns about the effect of these compounds on thyroid function, such as thyroid hypertrophy or a reduction in the plasma thyroid hormone levels triiodothyronine (T3) and thyroxine (T4) (Burel et al., 2000c, 2001; Hilton and Slinger, 1986; Yurkowski et al., 1978). In addition, the activities of some protein metabolism enzymes in liver (e.g. asparate aminotransferase (AST), alanine aminotransferase (ALAT)) have been reduced with increasing dietary CM levels (Cheng et al., 2010; Luo et al., 2012).

Understanding the molecular pathways that regulate the utilisation of dietary nutrients and energy are additional elements to understanding the feeding and growth response in fish when fed with a particular diet. It is generally assumed that the replacement of fish meal by plant materials is likely to change the biological values of diets thereby also likely affecting molecular metabolism in certain pathways (Panserat et al., 2008, 2009). Detoxification plays an important role in the protection of the body against the damage of toxic compounds from endo- and exogenous sources (Xu et al., 2005). The detoxifying mechanisms in the liver rely on the involvement of phase 1 and 2 biotransformation enzymes. Phase 1 (cytochrome P450-CYP450) involves in oxidation, reduction and hydrolysis reactions to produce polar metabolites and if they are sufficiently polar they may be readily excreted at this point (Parkinson, 2001). However most phase 1 products are not eliminated rapidly and undergo subsequent reactions. Phase 2 (such as glutathione group - GSH) comprises conjugation reactions with phase 1 metabolites to produce more polar metabolites that are readily excreted (Parkinson, 2001). Various studies have shown the presence of CYP genes and phase 2 enzymes in fish, and investigated function of these genes in detoxification mechanism in fish (George, 1994; Uno et al., 2012). In mammals, ingestion of GSLs or their breakdown products which are primarily presented in cruciferous vegetables, is generally related to the inhibition of metabolic phase 1 and induction of the phase 2 enzymes (Mahéo et al., 1997; Nho and Jeffery, 2001; Wang et al., 1997; Zhang and Talalay, 1994). Nevertheless, the effect of GSLs and their derivatives are still unclear and controversial (Paolini and Legator, 1992). An induction of CYP450 enzymes has been reported as a consequence of the presence of brassica GSL breakdown products in the diet (Kumar et al., 2004; Paolini et al., 2004; Perocco et al., 2006). A study on fish has indicated that CYP450 (CYP1A1) activity increased by the broccoli and sulforaphane enriched diet (Villa-Cruz et al., 2009).

Barramundi (or Asian Seabass; *Lates calcarifer*) is a commercially important species in Australia and Southeast Asia (Tucker et al., 2002). Barramundi are a fast growing species, with a growth rate of approximately 1 kg/year and can reach a marketable size (350 g–5 kg) in 6–24 months (Boonyaratpalin, 1997; Rajaguru, 2002; Yue et al., 2009). Like other marine carnivorous species, barramundi require a relatively high dietary protein intake. The few studies on fish meal replacement with barramundi using plant protein sources suggest that different raw materials can be effectively used with as little as 15% fish meal remaining in diet (Glencross et al., 2011b). The few available studies on CM use in the diet for juvenile barramundi indicate that the introduction of CM into diets for barramundi have been acceptable (Glencross et al., 2011b). However, in that study only one type at a single inclusion level of expeller extracted CM was evaluated. Therefore, this study used a serial inclusion experiment to study nutrient utilisation and the inclusion level limitations of two canola meals from solvent (SE) and expeller (EX) extraction. The utility of these ingredients was based on examining the growth and feed utilisation parameters such as weight gain, daily growth coefficient, feed intake, feed conversion ratio (FCR), protein and energy retention. The alternations of plasma biochemistry, histology and hepatic gene expression in relation to fatty acid synthesis, energy production and detoxification were also studied.

2. Materials and methods

2.1. Experimental diets

The experiment included eight diets. Six diets were used to generate a serial inclusion level design (100, 200 and 300 g kg^{-1}) of each of SE CM and EX CM. These diets were compared to two reference diets (a fish meal (FM) based diet and a lupin kernel meal (LM) diet with 300 g kg^{-1} of LM). Diets were formulated to iso-digestible nitrogenous (423 ± 29 g kg^{-1}) and iso-digestible energetic (14.6 ± 8 MJ kg^{-1} DM) specifications, based on previous digestibility data (Blyth et al., 2015; Ngo et al., 2015). The two CMs selected to use in the growth experiment were SE CM (Numurkah, Vic, Australia) and EX CM (Pinjarra, WA, Australia). Chemical composition of each ingredient is described in Table 1.

After the various diets were prepared, each mash was mixed by using a 60 L upright Hobart mixer (HL600, 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 through a 4 mm die at the same parameters for consistency. Pellets were cut into 6 mm to 8 mm lengths using two-bladed

Table 1
Chemical composition of ingredients (values are g kg^{-1} DM unless otherwise indicated).

	FM[a]	LM[b]	SE CM[c]	EX CM[d]	Wheat gluten[e]	Pregelled starch	Fish oil[f]
Dry matter	929	906	903	974	900	950	990
Protein	642	408	381	348	848	1	0
Lipid	117	64	56	92	9	1	985
Carbohydrate	4	497	485	490	120	993	0
Ash	237	31	78	70	23	5	5
Gross energy (MJ kg^{-1})	20.4	21.1	20.3	20.6	22.9	17.9	38.4
Essential amino acids							
Lysine	49	15	18	12	15		
Threonine	25	14	18	16	20		
Methionine	17	3	8	7	10		
Isoleucine	28	17	15	14	27		
Leucine	46	29	28	25	49		
Tryptophan	8	3	3	3	2		
Valine	32	17	20	19	30		
Phenylalanine	24	17	17	15	34		
Histidine	15	10	10	10	1		
Arginine	46	25	25	21	27		

[a] 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 Riverland Oilseeds, Numurkah, Victoria, Australia.
[d] Expeller extracted canola meal, supplied by Riverland Oilseeds, Pinjarra, WA, Australia.
[e] Supplied by Manildra, Auburn, NSW, Australia.
[f] Supplied by Ridley Aquafeeds, Narangba, QLD, Australia.