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Carob seed germ meal as a partial soybean meal replacement in the diets of red hybrid tilapia

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ABSTRACT

The feasibility of carob seed germ meal (CSGM) as a soybean meal (SBM) replacement in the diet of red tilapia hybrid was evaluated in an 8-week feeding trial. Five isonitrogenous and isocaloric diets with increasing CSGM (0, 10, 20, 30 and 40%), at the expense of SBM, were fed to triplicated groups of tilapia fingerlings. Their growth, feeding efficiency, whole body proximate composition, selected plasma biochemical parameters, and liver and gut histopathology were assessed. The survival and growth of red tilapia were unaffected by dietary CSGM inclusion up to 30%. Growth and feeding efficiencies were significantly reduced at 40% CSGM inclusion. Hematocrit and body crude lipid were significantly lower ($p < .05$) in fish fed 30–40% CSGM while plasma ALT and protein were significantly higher compared to the control (0% CSGM). In addition, some instances of lipofuscin and cellular degradation were shown in liver while morphological changes were observed in fish fed 30 and 40% CSGM. Some of these included a 60% and 34% reduction in goblet cell prevelance and villi length, respectively, as well as a thickening of the intestinal mucosal and submucosa layers of 51 and 27%, respectively as dietary CSGM increased from 0 to 40%, likely due to increased anti-nutritional factors. In conclusion, a dietary inclusion only up to 20% untreated CSGM is recommended for red hybrid tilapia.

Introduction

The increasing cost and decreasing availability of fishmeal (FM) have led to the utilization of soybean meal (SBM) as the main protein source in most commercial tilapia diets (Sharma et al., 2014). Unfortunately, however, SBM is also becoming more expensive with prices increasing from USD 300 ton^{-1} in 2004 to USD 566 ton^{-1} in 2014 (FAO, 2014). Thus, for improved sustainability and cost-effectiveness, numerous alternatives to SBM are increasingly being investigated (Ng and Romano, 2013). The majority of these are from plant sources that are often produced at a much lower scale compared to SBM, however, they can be more locally available and therefore can offer greater flexibility to farmers and feed millers to improve cost-effectiveness. Among these alternatives, carob seed (*Ceratonia siliqua*), also known as locust bean appears to have some potential as an alternative to SBM (Alexis et al., 1985; Martínez-Llorens et al., 2012; Couto et al., 2016). Carob seed is widely produced throughout the Mediterranean and Middle East countries and consists mainly of an outer gum (endosperm) layer and a smaller inner germ (Battle and Tous, 1997). The gum is commercially extracted and used as a thickening agent in many food products and, while the germ can be used as a chocolate substitute, this is often considered as a discarded by-product. The germ, however, has a relatively high protein content that could be utilised as a protein source in fish diets (Alexis et al., 1985; Martínez-Llorens et al., 2012; Couto et al., 2016). Alexis et al. (1985) found that diets containing CSGM lowered the growth rate of rainbow trout (*Salmo gairdneri*) compared to either SBM or FM-based diets. In a subsequent study, it was observed that increasing CSGM (at the expense of SBM) decreased the growth of rainbow trout fingerlings (Alexis, 1990). Based on the increase in total plasma protein and decrease in body protein, it was suggested that the lower protein digestibility of CGSM was due to the presence of anti-nutritional factors (ANF), particularly tannins, in the meal.

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Carob seed has indeed been shown to contain relatively high amounts of tannins (Avallone et al., 1997) and these are well known to create protein aggregates that makes the proteins unavailable to fish (Francis et al., 2001). Tannins have also been implicated as a contributor for suppressing growth, decreasing protein and energy digestibility as well as causing intestinal histopathological changes in sea bream (*Sparus aurata*) fed a diet containing 52% CSGM (Martínez-Llorens et al., 2012). In addition, Martínez-Llorens et al. (2012) suggested that low dietary methionine, a high fibre content and other ANFs also contributed to these symptoms. Recently, Couto et al. (2016) found that meagre (*Argyrosomus regius*) juveniles can accept up to 22.5% CSGM in their diet without growth or gut histopathology being affected, but there was decreased digestive enzyme activity and nitrogen retention. Beyond these levels, however, dietary CSGM compromised fish growth but suggested that tannins may not be solely responsible for the reduction in nutrient utilization (Couto et al., 2016).

The aim of this study was to investigate the effect of increasing dietary inclusions of CSGM, at the expense of SBM, on the survival, growth, whole-body proximate composition, plasma parameters and the liver and intestine histopathology of red tilapia hybrid (*Oreochromis* sp.). Nowadays, red tilapia is the main tilapia variety produced in Malaysia and accounts for about 85% of total tilapia production, with the annual production of red tilapia is 31,227.08 tonnes (DOF, 2015). To the best of our knowledge, the use of dietary CSGM has not yet been reported in tilapia and such findings would likely benefit the tilapia aquaculture industry.

Table 1
Chemical composition of fishmeal (FM), carob seed germ meal (CSGM) and soybean meal (SBM) used in this study (% dry weight basis).

Component	FM	CSGM	SBM
Dry matter	91.00	93.39	89.91
Crude protein	56.64	34.80	45.97
Crude lipid	1.55	2.33	1.42
Ash	23.40	6.78	6.10
Crude fibre	–	12.47	3.36
Acid detergent fibre	–	20.65	–
Neutral detergent fibre	–	33.61	–
Essential amino acids			
Arginine	3.86	4.90	2.88
Histidine	1.73	1.22	2.35
Isoleucine	2.49	1.35	2.11
Leucine	4.26	2.46	3.86
Lysine	4.32	2.26	2.53
Methionine	1.83	0.49	0.56
Phenylalanine	2.10	1.31	2.25
Threonine	2.78	1.44	1.64
Valine	1.99	1.50	1.86
Antinutritional factors			
Total phenols[a]		3.93 ± 0.10	
Tannins[a]		2.73 ± 0.13	
Condensed tannins[b]		1.92 ± 0.03	
Hydrolysable tannins[c]		0.88 ± 0.01	
Phytic acid		0.98 ± 0.03	
Saponins[d]		0.33 ± 0.01	
Trypsin inhibitor (mg g^{-1})		6.45 ± 0.61	

[a] As tannic acid equivalent, [b]As leucocyanidin equivalent, [c]As by different, [d]As diosgenin equivalent.

Materials and methods

Source and biochemical analysis of carob seed germ meal, soybean meal and fishmeal

The carob seeds were obtained from Turkey (Dutpinar Gida Tic. San. Ltd. Sti) and brought to the Universiti Putra Malaysia lab. Both the fishmeal (FM, of mixed fish species) and soybean meal (SBM) were locally obtained. The carob seed were milled to separate the gum and germ, and then using a hammer mill, the germ was then ground into a fine powder. The SBM was also hammer milled to a fine powder while the FM was sieved. After preparing the protein sources, the proximate composition was measured according to standard AOAC (1997) methods. The essential amino acid composition was measured using HPLC according to Strydom and Cohen (1994), using a precolumn AQC reagent (6-aminoquinolyl—N-hydroxysuccinimdyl carbamate, Waters, USA). The acid detergent fibre and neutral detergent fibre of CSGM were measured according to Van Soest et al. (1991). All analyses were performed in triplicate. Table 1 shows the proximate and essential amino acid composition of the FM, SBM and carob seed germ meal (CSGM). The proximate composition among the diets were generally the same, except that crude fibre, acid detergent fibre and neutral detergent fibre increased with increasing CSGM inclusion levels. Meanwhile, among the essential amino acids, methionine, phenylalanine, and isoleucine continually decreased with increasing CSGM.

Experimental diets

Five isonitrogenous (32%) and isolipidic (7%) diets were formulated with increasing levels of CSGM (0, 10, 20, 30 and 40%) at the expense of SBM (Table 2). The dry ingredients were mixed together for 15 min, and then the lipid was added slowly and further mixed for another 15 min. Next, distilled water was added at 20% of the total ingredient weight. This resulting mash was then extruded through a single-screw extruder (Brabender KE19; Brabender GmbH, Germany) into 1.0 mm diameter pellets. The barrel temperatures were maintained at 60, 100 and 120 °C while the die head temperature was 160 °C. These pellets were dried at 55 °C in an oven overnight and then stored in zip lock plastic bags at −20 °C until feeding. Various ANFs that included the total phenol, total tannin, and condensed tannins of CSGM and diets were measured according to Makkar et al. (1993). The amount of total phenols were quantified by using a Folin–Ciocalteu reagent while the amount of tannins were estimated as the difference in phenolics before and after the removal of tannins from the extract using insoluble polyvinylpyrolidone. Condensed tannins were determined by using a butanol–HCl–Fe^{3+} reagent according to Porter et al. (1985). The total phenols as well as total tannins were expressed as tannic acid equivalents while condensed tannins were expressed as leucocyanidin equivalents. Hydrolysable tannins were determined by subtracting condensed tannins from the total tannins. Phytic acid was measured in CSGM and diets using slightly modified methods according to Roohani et al. (2012) in which cerium replaced iron in the precipitation step while microwaving was applied to completely dissolve malachite green in ethanol. Trypsin inhibitor activities of CSGM and diets were determined according to Smith et al. (1980) using trypsin (bovine pancreas, T-4665, Sigma) and BAPNA (B-4875, Sigma) and results were expressed as mg trypsin inhibition g^{-1} of sample. All the measured ANF continually increased with increasing CSGM inclusion levels, and the greatest increase were trypsin inhibitors of over 20 times higher from the control to the 40% CSGM diet (Table 2).

Experimental set up

Red hybrid tilapia fingerlings were purchased from a local tilapia farm and placed in a 1000 L fiberglass tank. After two days, 300 fish were randomly and equally stocked into glass aquaria for a two-week acclimation period and fed with a commercial diet designed for tilapia (Star Feed 32% protein). All fish in the aquaria

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