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Original Article

Partial replacement of protein in soybean meal by moringa seed cake (*Moringa oleifera*) in bocourti's catfish (*Pangasius bocourti*)

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Abstract

The present study was undertaken in order to determine the effect of a dietary of moringa seed cake on digestibility, growth performance, blood chemistry and histopathologic of bocourti's catfish. Fish were fed with diets formulated by 0, 250, 500, 750, and 1000 g kg^{-1} of moringa seed cake to replace protein in soybean meal. Fish with mean wet weights of 21.50± 0.25 g per fish were fed experimental diets for 8 weeks. Significant differences ($p<0.05$) in weight gain, average daily gain and specific growth rate were detected between bocourti's catfish given the experimental diets. All fish grew normally and no significant difference was observed for survival rate and feed conversion ratio among fish fed tested diets. The highest FCR was generally observed that as moringa seed cake inclusion increased in the diets that were noted to exhibit slightly poor growth performance, feed utilization and pepsin digestibility tested. Blood chemistry and hepatosomatic index did not differ significantly for any of the diet treatments. No histopathological changes were found in distal intestines and liver. The study indicated that the dietary moringa seed cake contains ingredients that could be used for bocourti's catfish diets possibly not over up to for 500 g kg^{-1} soybean protein replacement without negative effect on growth, digestibility and histology.

Keywords: bocourti's catfish, moringa seed cake, growth, digestibility, histopathology

1. Introduction

Since farming aquatic animals in Thailand was broadly adopted and improved, it has caused a problem of high-priced feed as well as insufficient nutrition. A significant proportion of fish meal possesses a broad range of amino acids, and hence is high priced. It has been shown that fish meal constitutes the most suitable source of indispensable amino acids (IAA) for fish, given the high correlation between whole body IAA profile and the IAA requirement pattern (Mambrini and Kaushik, 1995). There has been an attempt to replace fish meal with soybean meal which possesses good quality of essential amino acids (EAA). As a result, soybean meal, both imported and locally made, is utilized with the hope of help decreasing the cost, but as it turns out, this is still somewhat expensive. Soybean meal is currently the most commonly used plant protein source in fish feeds and amounts to 500 g kg^{-1} of the diet of freshwater omnivorous fish species (Yue and Zhou, 2009), which indicates that commercial feed depends mostly on soybean meal as a fish meal replacer. However, the over-dependence will cause a hike in the price of soybean meal; therefore, utilization of other an inexpensive plant protein source would be beneficial in reducing the feed cost (Yue and Zhou, 2009). Herdsmen, no matter of undersized or oversized farms, are looking for a new cheaper raw material to decrease the cost, though it

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might not be as preferable as fish meal or soybean meal. This new material should be able to be produced locally, inexpensive and provide high nutrition. Certain plant materials offer the most promising alternative aqua feed ingredients and in fact locally produced materials have already been used in Thailand. Thailand is an agricultural country with a lot of plants in nature. The moringa (*Moringa oleifera*) is a fast-growing plant widely available in the tropics and subtropics with several economically important industrial and medicinal uses, and is a native food in Southeast Asia. *M. oleifera* represents a traditionally important food commodity as the leaves, flowers, fruits, and roots of this tree are locally used as vegetables (Siddhuraju and Becker, 2003). Its seeds have been extensively investigated as a source of oil. The seed protein contents are higher than those reported for important grain legumes and soybean varieties (Ferreira *et al*., 2008). Analyses of the proximate composition of *M. oleifera* seeds have showed high levels of protein with the dry seeds usually containing 332.50 to 383.00 g kg^{-1} of protein (Oliveira and Silveira, 1999; Abdulkarim *et al*., 2005). EAA composition in moringa seed cake has high essential amino acid, especially the sulfur amino acid such as methionine, cystine, tryptophan (Makkar and Becker, 1996) except for lysine (15.3 g kg^{-1} protein), threonine (30.8 g kg^{-1}) and valine (43.5 g kg^{-1}) (Oliveira and Silveira, 1999). These amino acids, however, are very low in soybean meal. It is found that methionine acid allows protein synthesis as well as being a reactant for homocysteine, cysteine, carnitine, creatine and choline. In general, there are low concentrations of antinutritional factors in the plant, although the seeds possess glucosinolates (65.5 μ mol g^{-1}) and phytates (41 g kg^{-1}) (Ferreira *et al*., 2008). To some degree, most plant proteins contain some antinutritional factors that vary with the processing, type and quality of the plant protein. Formulators should keep these concerns in mind so as to find the correct quality and type of plant protein for their purposes. Proteins of animal origin are generally more digestible than those of plant origin. Some technological treatments applied to plant proteins bring about a marked improvement in Apparent Digestibility Coefficient (ADC) by destroying antinutritional factors (Guillaume *et al*., 2001). However, there is no information regarding the utilization of moringa seed cake in fish feed. The relative lack of antinutritional components and the high protein and sulfur containing amino acid contents encourage the use of moringa seed cake as animal feed; it is an excellent source of proteins for animals. In addition, the waste from seed cake hold considerable potential for becoming animal and fish feed ingredients because of their high nutritional quality. This study offers an alternative of utilizing moringa seed cake as a protein source to replace soybean meal in bocourti's catfish diet and add value to raw materials. The aims of this study were to investigate protease activity and the *in vitro* digestibility, the effect of supplemented moringa seed cake in bocourti's catfish on growth performance, blood chemistry, and estimate the diets effect on histology of internal organs in bocourti's catfish.

2. Materials and Methods

2.1 Fish, diets and feeding protocol

Bocourti's catfish fingerlings from Phayao Inland Fisheries Research and development Center, located in Phayao Province, Thailand, were transferred to Khon Kaen University, Khon Kaen, Thailand and kept in 1000-L tank for acclimatization. They were fed with commercial diet with 35% protein for four weeks prior to feeding the experimental diets. After an acclimatization period of 30 days, 250 fish were randomly distributed into five groups with five replications; each replicate contained 10 fish (mean wet weight of 21.50± 0.25 g per fish) in an aquarium (100 L capacity) which 25 aquaria tanks

Moringa seed cake powder was obtained from Grenera Nutrients (P) LTD. (India). The diets were substituted by moringa seed cake, and protein in soybean was replaced as follows:

1. Control diet; without moringa seed cake,
2. Diet substituted by moringa seed cake with 250 g kg^{-1} replacement of soybean protein,
3. Diet substituted by moringa seed cake with 500 g kg^{-1} replacement of soybean protein,
4. Diet substituted by moringa seed cake with 750 g kg^{-1} replacement of soybean protein,
5. Diet substituted by moringa seed cake with 1000 g kg^{-1} replacement of soybean protein.

Five isonitrogenous and isoenergetic diets were formulated to contain approximately 350 g kg^{-1} protein and 14.30 kJ g^{-1} and to meet the known nutrient requirement of catfish. All diets were supplemented with L-Methionine, so the balance of this amino acid in the diets was similar in all cases.

The daily feeding was done by hand-fed method to apparent satiation twice a day (09.00 and 17.00) for eight weeks. Total feed was recorded weekly and fish from each tank were weighed to measure growth at the end of the experiment and growth performance calculated.

2.2 Chemical analysis

Proximate analysis of diets were analyzed as follows: dry matter after drying in an oven at 105°C until constant weight; ash content by incineration in a muffle furnace at 600°C for 6 h; crude protein (N x 6.25) by Kjeldahl method after acid digestion; lipid by petroleum ether extraction in a Soxlet apparatus by AOAC (1990) (Table 1). The phytic acid estimation was carried out by spectrophotometric methods (Talamond *et al*., 1998). Total tannin content was determined by the spectrophotometric methods described by Makkar *et al*. (1993). The amino acids of fish carcass and diets were analysed with an ultra fast liquid chromatography (UFLC), Shimadzu system (Shimadzu, Kyoto, Japan).

Table 1. Ingredients, proximate chemical composition, amino acid composition and antinutrient content of experimental diets (g kg^{-1} DM)

Ingredient (g Kg^{-1})	Protein replacement in soybean meal by moringa seed cake (g Kg^{-1})				
	0	250	500	750	1000
Fish meal	350.00	350.00	350.00	350.00	350.00
Soybean meal	250.00	190.00	125.00	65.00	00.00
Rice bran	173.20	163.10	163.00	127.90	142.80
Moringa seed cake	0.00	40.00	85.00	125.00	170.00
Corn	120.00	150.00	170.00	225.00	230.00
Fish oil	20.00	20.00	20.00	20.00	20.00
Soybean oil	20.00	20.00	20.00	20.00	20.00
α-starch	40.00	40.00	40.00	40.00	40.00
Dicalcuim phosphate	10.00	10.00	10.00	10.00	10.00
Premix	15.00	15.00	15.00	15.00	15.00
L-Methionine	1.80	1.90	2.00	2.10	2.20
Total	1000.00	1000.00	1000.00	1000.00	1000.00
Nutrient composition and antinutrient content by analysis (g Kg^{-1} dry weight on basis)					
Protein	356.3 ± 1.95	351.8 ± 0.03	354.6 ± 0.02	355.0 ± 0.07	351.2 ± 1.07
Fat	103.1 ± 0.01	102.5 ± 0.04	102.6 ± 0.06	107.7 ± 0.19	101.3 ± 0.14
Moisture	89.4 ± 0.23	86.1 ± 0.28	82.2 ± 0.35	89.1 ± 0.58	85.1 ± 0.25
Ash	101.9 ± 0.09	97.6 ± 0.05	94.0 ± 0.04	97.8 ± 0.11	99.3 ± 0.15
Phytic acid	34.7 ± 0.62	36.7 ± 0.30	36.8 ± 0.30	31.4 ± 0.16	34.6 ± 0.31
Total tannin	2.3 ± 0.03	2.4 ± 0.02	2.5 ± 0.17	2.7 ± 0.19	2.7 ± 0.02
Amino acid composition (g 100 g^{-1} dry weight on basis)					
Histidine	2.50 ± 0.01	2.58 ± 0.02	2.68 ± 0.02	20.4 ± 0.02	2.74 ± 0.01
Arginine	20.38 ± 0.01	18.50 ± 0.01	18.41 ± 0.03	18.87 ± 0.07	15.86 ± 0.10
Asparagine	3.28 ± 0.03	3.83 ± 0.02	4.07 ± 0.01	3.29 ± 0.01	5.88 ± 0.03
Glutamic acid	3.19 ± 0.01	3.44 ± 0.04	4.76 ± 0.02	3.94 ± 0.08	4.45 ± 0.04
Alanine	4.36 ± 0.09	4.08 ± 0.01	1.15 ± 0.07	1.52 ± 0.01	4.03 ± 0.01
Proline	2.52± 0.02	3.30 ± 0.04	3.78 ± 0.01	4.04 ± 0.01	3.87 ± 0.05
Methionine	1.27 ± 0.03	1.08 ± 0.01	1.47 ± 0.04	1.78 ± 0.03	1.79 ±0.01
Valine	3.33 ± 0.03	3.30 ± 0.03	3.47 ± 0.03	3.88 ± 0.01	3.52 ± 0.04
Trytophane	n.d.	n.d.	n.d.	n.d.	n.d.
Leucine	9.33 ± 0.06	9.30 ± 0.04	9.35 ± 0.02	9.64 ± 0.02	9.53 ± 0.04
Lysine	6.11 ± 0.02	9.08 ± 0.01	9.41 ± 0.04	10.18 ± 0.04	10.53 ± 0.02
Cysteine	10.06 ± 0.10	8.62 ± 0.03	9.76 ± 0.03	8.09 ± 0.03	8.86 ± 0.07

2.3 Crude enzyme preparations

The stomach and whole intestine were homogenized (1:2 w/v) with 50 mM Tris – HCl buffer at pH 7.5 (Fisher Scientific, Waltham, USA) in an ice water bath, using a tissue homogenizer. The preparation was centrifuged at 10,000 x g for 15 min at 4°C. The floating lipid fraction was removed and the aqueous supernatant was recovered and kept at -20°C until analysis completed (Gimenez *et al*., 1999).

2.4 Protease activity

Protease activity was monitored in triplicate by measuring the increase in cleavage of short chain poly-peptide (Bezerra *et al*., 2005). The total protease activity was determined by using 1 g L^{-1} (w/v) azocasein (Sigma- Aldrich, St. Louis, USA). The substrate (500 ml) was incubated with crude extract (20 ml) and buffer solution (200 ml) for 60 min at 30°C. Then, 500 ml of 200 g L^{-1} (w/v) trichloroacetic acid (Sigma-Aldrich, St. Louis, USA) was added to stop the reaction. After 15 min, centrifugation was carried out at 10,000 g for 10 min. The supernatant (1.0 ml) was added to 1 M NaOH (1.5 ml; Qrec, New Zealand) and the absorbance was measured at 440 nm against a blank similarly prepared but without the crude extract sample. The protease specific activity was expressed as unit of change in absorbance per min per mg protein of the enzyme extract (DAbs min^{-1} mg $protein^{-1}$).

2.5 Protein concentration

Protein concentration was determined by using bovine serum albumin (Sigma- Aldrich, St. Louis, USA) as a standard (Lowry *et al*., 1951).

2.6 Pepsin digestibility test

To assess the quality of experimental diets, digestible crude protein was determined by pepsin digestibility test (AOAC, 1990), using pepsin (obtained from porcine gastric mucosa; Sigma- Aldrich, St. Louis, USA).

2.7 Serum collecting

At the end of the growth trial, after final weighing, three fish per tank were anesthetized and blood was placed in non-heparinized tubes and left to clot at 4°C for 15 min. The sera were separated into aliquots for analysis of blood chemistry. Serum was analyzed for total protein, albumin, total bilirubin, alkaline phosphatase (ALP) alanine amino-transferase (ALT) and aspartate aminotransferase (AST) activity.

2.8 Hepatosomatic index and histological observation

At the termination of the feeding experiments, the fish were anesthetized and weighed individually. The fish were dissected; the liver was removed from four fish of each treatment and weighed for calculating the hepatosomatic index (HSI), and intestine was also taken. Buffered formalin-fixed samples of liver and distal intestine were dehydrated in ethanol, embedded in paraffin, sectioned, and stained with hematoxylin and eosin (H&E). The sections were examined under light microscopy, digital microscopy camera, and Motic Image Plus 2.1S software (Shimazu, Kyoto, Japan).

2.9 Statistical analysis

Mean value and standard deviation (S.D.) were calculated from the results. One way analysis of variance (ANOVA) was applied for comparison of the mean values; $p<0.05$ was established as significant.

3. Results and Discussion

3.1 Feed quality

Experimental diets were isonitrogenous and isocaloric, proximate chemical composition, amino acid composition and antinutrient content are presented in Table 1. The quantities of methionine fulfilled the needs of the channel catfish (NRC, 1993). The diets were found to contain phytate about 3.14-3.68 g kg^{-1} and contained of tannins was 0.23-0.27 g kg^{-1}. Thus, by increasing moringa seed cake content in feeds, phytate and tannins were increased.

3.2 Total protease activity

Digestive protease activity of digestive tract in fish after the end of experiment is displayed in Figure 1. Total protease activity in stomach and whole intestine were high ($p<0.05$) in the control group and fish fed with diet substituted by moringa seed cake replacing protein in soybean at 250 and 500 g $kg^{-1}$by 1.52 ± 0.29, 1.31 ± 0.36, and 1.11 ± 0.43 U mg $protein^{-1}min^{-1}$, respectively, which were higher activity of fish fed with diet substituted by moringa seed cake replacing protein in soybean at 750 and 1000 g kg^{-1}.

In the present study, total protease activity from stomach and whole intestine of bocourti's catfish was investigated, as presented in Figure 1. The activity of protease lower activity of fish fed with diet substituted by moringa seed cake replacing protein in soybean at 750 and 1000 g kg^{-1}. Protease activity decreased with increase moringa seed cake in bocourti's catfish diet. Similar results were observed by Kumar *et al*. (2011) and Santigosa *et al*. (2008). They found that protein digestibility (protease) activity decreased as plant protein inclusion increased in common carp and trout diet. The decrease in protease activity at higher inclusion level of moringa seed cake might be caused by the presence of phytic acid. Antinutritional factors such as phytic acid inhibit activities of some digestive enzymes such as pepsin, trysin and alpha-amylase (Alarcon *et al*., 1999). From the results, the activity of protease, which is essential for the utilization of protein from feed, contributes to a high growth rate in fish. Thus, characterization of digestive proteases is essential along with the quantitative estimations for a better understanding of digestive capability of the cultured species and for assessing protein ingredients in feed formulations (Moyano *et al*., 1996).

3.3 Pepsin digestibility study

The study of pepsin digestibility on experimental diets is presented in Figure 2. The results showed no difference ($p>0.05$) in pepsin digestibility study among the experimental diets.The percentage of protein digestibility ranged from 69.57 to 72.86. It was generally observed that as plant protein

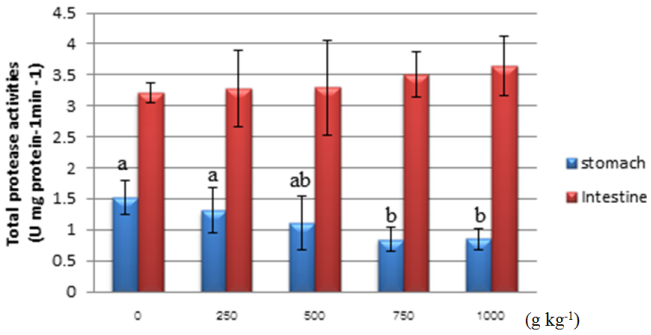

Figure 1. Total protease activities of digestive tract at terminal of experiment; values are means of five samples.

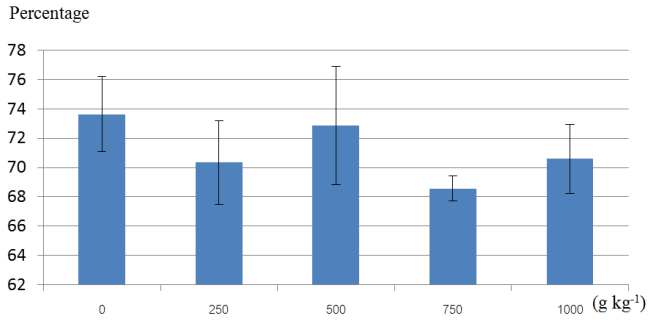

Figure 2. Pepsin digestibility study in experimental diets; values are means of three samples.

inclusion increased in the diets, protein digestibility decreased gradually.

Nowadays, plant sources have been used to replace the protein in fishmeal and soybean meal, either partially or totally. Practical fish feed has been an area of focus in aquaculture nutrition research recently (Gomes *et al*., 1995; Hossain *et al*., 2001; Ogunji, and Wirth, 2001). The use of plant protein sources in aqua feeds should be considered (SOFIA, 2007). Moringa has been widely studied as an alternative protein source in fish diet and seems to be a promising protein source. Moringa leaf can partially replace conventional diets without any depression in growth performance of Nile tilapia (*Oreochromis niloticus* L.) (Richter *et al*., 2003; Afuang *et al*., 2003).

None of the diets adversely affected the pepsin digestibility test compared to the control diet without moringa seed cake, but the diets supplemented with moringa seed cake above 500 g kg^{-1} of protein in soybean meal seemed to offer lower digestibility compared to the control diet. Generally, oil seed meal protein has percentage of digestibility of 80-95 for fish (Jauncey and Ross, 1982). The protein digestibility coefficient is a key factor in the evaluation of the quality of the diet for fish and the potential of the diet for the synthesis of new tissue. In the investigation, protein digestibility by pepsin digestibility tested to decrease with increased inclusion of moringa seed cake in the diet because the digestive enzymes act slowly on plant proteins, which are present in high amount in the kernel meal, which adversely affects the feed utilization (Kumar *et al*., 2011). Plant ingredients (bean meal, groundnut oilcake and sunflower oilcake) can efficiently substitute fishmeal at 250 g kg^{-1} in African catfish diets, and there were no significant differences in protein apparent digestibility coefficients (ADCs) (88-90 percent) with increased levels of dietary plant-based protein in diets (Nyina-Wamwiza *et al*., 2010). The ADCs in protein of plant leaf ingredients were determined in barnyard grass and dried maize leaves were found not only to offer poor digestibility but also to have a negative impact on the digestibility of the reference diet. On the contrary, fresh maize leaves were well digested for grass carp; with ADC 60.9, 70.5, and 84.7, respectively in protein compared to 94.1 in control diet. This indicated that dry plant materials seem to be poorly digestible and could even inhibit fish utilization of other nutrients contained in the diet (Dongmeza *et al*., 2010).

3.4 Growth performance and feed utilization

Growth parameters of bocourti's catfish are given in Table 2. Growth performance of fish revealed that final body weight and weight gain were significantly reduced by soybean meal replacement, resulting in reduced WG, ADG and SGR. Highest WG, ADG and SGR were observed for the fish fed with diets substituted protein by moringa seed cake with 250 and 500 g kg^{-1}, which were statically similar to those for the control group and significantly ($p<0.05$) higher than those for other groups. In terms of feed utilization, the data showed that there were also no significant differences in feed conversion ratio (FCR) and protein efficiency ratio (PER) among all groups. Nevertheless, the higher FCR was found in fish fed with the diets supplemented with moringa seed cake to replace protein in soybean at 750 and 1000 g kg^{-1}; lowest FCR was observed in diets substituting protein by moringa seed cake with 250 and 500 g kg^{-1} and the control group. Protein efficiency ratio (PER) ranged from 1.65 to 1.88,

Table 2. Growth performance and feed utilization of bocourti's catfish fed with experimental diets supplemented with moringa seed cake at terminal period (mean ± SD)

Growth performance and feed utilization	Protein replacement in soybean meal by moringa seed cake (g Kg^{-1})					p-value
	0	250	500	750	1000	
WG	13.23 ± 4.16^{ab}	15.50 ± 1.84^{a}	15.93 ± 1.26^{a}	10.93 ± 0.32^{b}	11.15 ± 1.45b	0.012
ADG	0.22 ± 0.07^{ab}	0.26 ± 0.03^{a}	0.27 ± 0.02^{a}	0.18 ± 0.01^{b}	0.19 ± 0.03^{b}	0.013
SGR	2.33 ± 0.44^{ab}	2.54 ± 0.18^{a}	2.59 ± 0.13^{a}	2.09 ± 0.05^{b}	2.12 ± 0.16^{b}	0.018
SR	100 ± 0.00	100 ± 0.00	100 ± 0.00	100 ± 0.00	100 ± 0.00	*
FCR	1.38 ± 0.31	1.30 ± 0.13	1.27 ± 0.08	1.50 ± 0.38	1.51 ± 0.46	0.725
PER	1.74 ± 0.35	1.84 ± 0.21	1.88 ± 0.12	1.65 ± 0.41	1.68 ± 0.52	0.831

Means with the different letters in the same row are significantly different at $p<0.05$.
Note: * is no variation

resulting in a reduction in PER by the high levels of moringa seed cake meal in the diet, similarly with Martínez-Llorens *et al*. (2009) and Nury *et al*., (2009). All fish grew normally, and no specific signs of disease were observed. No mortality occurred throughout the experiment.

In this study, the diets were progressively increasing replacement of the protein of soybean meal with moringa seed cake did not affect the survival of bocourti's catfish indicating that the experimental diets did not have any major negative effects on fish health. However, the dietary treatments significantly affected growth performance in the present study. As protein of moringa seed cake inclusion increased from 750 to 1000 g kg^{-1} there was a progressive reduction in growth. Growth performance and feed utilization were affected significantly by dietary treatment for all treatments diets. Plant protein based diets may reduce growth (Espe *et al*., 2006). This agrees with the study that growth reduction was mainly related to lower feed intake, because of an interaction effect of high plant protein (Olsvik *et al*., 2011). Slightly lower performance was found in carp fed a diet in which 750 g kg^{-1} of fish meal protein was replaced by *Jatropha curcas* kernel meal (Kumar *et al*., 2011). However, the study showed no effects of dietary supplement of methanol-extracted leaf meal containing 11, 220 and 330 g kg^{-1} found on the growth of Nile tilapia (*Oreochromis niloticus* L.) (SOFIA, 2007). A stydy of tilapia fed with raw moringa leaf meal revealed that 10% of replacement of fishmeal-based dietary protein did not cause any adverse effect on growth performance (Afuang *et al*, 2003). Most published research on the use of plant protein as a substitute of soy bean meal in fish feeds has focused on the inclusion of palm kernel meal (Ng and Chen, 2002), cotton seed meal (Yue and Zhou, 2009) and Faba beans (Azaza *et al*., 2009) with the goal of increasing inclusion of sustainable plant-based diet for fish and all results show that dietary protein source from plant origins did not affect in growth or survival of fish.

The depression of growth performance and growth parameters could likely be attributed to several factors, among which the presence of anti-nutrients will have been important (Francis *et al*., 2001). Anti-nutritional factors may limit the use of high levels of vegetable feedstuffs in fish feeds (Gatlin and Phillips, 1989). In fact, a decrease in nutrient utilization, mediated by soybean carbohydrates has been reported in salmonids (Arnesen *et al*., 1989). High dietary phytic acid (25.8 g kg^{-1}) dramatically depressed the growth rate in salmon fish (Richardson *et al*., 1985). In this present study, the level of phytic acid in the experimental diets ranged between 31.4 and 36.8 g kg^{-1} and tannin content 2.2 and 2.8 g kg^{-1}. These could be the mild factors causing growth retardation. Phytate contents of the moringa kernel samples were higher than those in the vegetative parts (Foidl *et al*., 2001). Phytic acid can reduce the protein digestibility by the formation of phytic acid–protein complexes and damage the pyloric caecum by depressing the absorption of nutrients (Thompson, 1993). Phytates present to an extent of 1 to 6 g kg^{-1} reduced mineral bioavailability in monogastric animals particularly, Zn^{2+} and Ca^{2+} (Spinelli *et al*., 1983). It has been reported that 50-60 g kg^{-1} diet can impair the growth of rainbow trout (Spinelli *et al*., 1983) and common carp (Hossain and Jauncey, 1993). With respect to total phenolics, Al-Owafeir (1999) showed that growth reduction in tilapia and has also been shown to significantly reduced the growth performance and feed utilisation in common carp (Siddhuraju and Becker, 2001). The tannin content appears to be directly related to protein digestibility (Richter *et al*., 2003). Giner-Chavez (1996) reported that levels from 5.0-20.0 g kg^{-1} can cause depression in growth and the levels of tannins above 50 g kg^{-1} of the diet, which are often lethal. However, plant protein ingredients are of low nutritional value; they have possible palatability problems and to lower feed intake (Tacon and Forster, 2000) but in the present study incorporating replacement protein in soybean meal with moringa seed cake in fish feed did not lead to mortality and slightly lower growth performance of fish fed more than 750 g kg^{-1} of soybean meal protein replacement. Thus, dietary moringa seed cake was readily consumed and safe for bocourti's catfish diet.

3.5 Amino acid composition in fish flesh

The amino acid composition of fish at the end of experiment is given in Table 3. With higher inclusion of moringa seed cake in the diets, the amino acid content remained constant in all experimental groups. None of the groups of test diets showed any statistical difference ($p > 0.05$) in muscle tissue amino acid content when compared to fish fed with the reference diet.

The amino acid compositions in all experimental diets of the present study were generally similar. Methionine content of the experimental diets supplemented with moringa seed cake ranged from 1.08 to 1.79 g kg^{-1}. The methionine contents in experimental diets were in agreement with the requirements cited by NRC (1993). In the current trial, the good EAA profile in experimental diets may have resulted in a normal growth and did not cause lowed growth or nutrient utilization. The high methionine contents in moringa seeds are close to those of human and cow milk and chicken eggs (WHO, 1985). This abundance of essential amino acids encourages the use of the seeds as an excellent food substitute for legumes or soybean, which is usually higher concentration of sulfur-containing amino acids. Methionine is generally limiting amino acid and methionine deficiency, frequently causing reduced growth (Jackson *et al*., 1982). Similar to a study report of low dietary levels of methionine, growth of juvenile hybrid striped bass and increased mortality has been shown (Keembiyehetty and Gatlin, 1993). However, EAA composition in moringa seed is sulfur amino acid such as methionine, cystine and tryptophan which should be used as supplementation (Goff and Gatlin, 2004).

Table 3. Amino acid composition (g $100g^{-1}$ dry weight on basis) in muscle of bocourti's catfish fed with experimental diets supplemented with moringa seed cake at terminal period

Amino acid	Protein replacement in soybean meal by moringa seed cake (g Kg^{-1})				
	0	200	500	750	1000
Histidine	8.82 ± 0.08	8.58 ± 0.08	9.27 ± 1.55	8.64 ± 0.02	8.52 ± 0.01
Arginine	2.79 ± 4.35	2.53 ± 5.71	2.61 ± 5.15	2.93± 0.07	2.12 ± 0.04
Asparagine	3.08 ± 0.59	2.72 ± 0.40	3.04 ± 0.25	2.44 ± 0.03	3.00 ± 0.04
Glutamic acid	2.81 ± 0.34	2.82 ± 0.58	3.17 ± 1.85	3.23 ± 0.04	2.41 ± 0.02
Alanine	5.28 ± 0.89	4.58 ± 0.06	4.43 ± 0.86	4.53 ± 0.03	4.62 ± 0.07
Proline	3.00 ± 0.53	2.71 ± 0.30	2.80 ± 0.51	2.50 ± 0.03	2.92 ± 0.04
Methionine	3.21 ± 1.81	2.84 ± 0.59	2.94 ± 0.22	2.42 ± 0.02	3.25 ± 0.01
Valine	2.97 ± 0.86	2.81 ± 0.35	2.12 ± 0.57	3.06 ± 0.01	2.56 ± 0.01
Trytophane	n.d.	n.d.	n.d.	n.d.	n.d.
Leucine	4.43 ± 1.94	3.85 ± 1.28	3.14 ± 0.61	4.75 ± 0.01	2.94 ± 0.01
Lysine	1.31 ± 0.31	0.97 ± 0.44	0.93 ± 0.35	0.28 ± 0.04	0.66 ± 0.04
Cysteine	2.69 ± 0.44	0.45 ± 0.32	0.99 ± 0.38	0.22 ± 0.01	0.67 ± 0.03

Table 4. Hepatosomatic index and serum biochemical parameters of bocourti's catfish at termination of the experiment

Serum biochemical parameters	Protein replacement in soybean meal by moringa seed cake (g Kg^{-1})					p-value
	0	250	500	750	1000	
Hepatosomatic index	2.61 ± 0.15	2.37 ± 0.34	2.57 ± 0.31	2.36 ± 0.35	2.56 ± 0.31	0.600
Haematocrit (%)	30.67 ± 3.06	28.33 ± 0.58	28.67 ± 0.58	29.67 ± 1.53	26.67 ± 2.52	0.203
ALP (U L^{-1})	117.33 ± 8.23	94.00 ± 9.66	102.67 ± 8.55	101.33 ± 4.38	93.00 ± 6.06	0.656
ALT (U L^{-1})	23.50 ± 2.12	25.50 ± 2.12	15.00 ± 2.64	25.00 ± 8.66	34.33 ± 20.26	0.405
AST (U L^{-1})	163.33 ± 3.08	170.00 ± 4.00	161.33 ± 2.08	153.67 ± 3.56	166.00 ± 7.24	0.984
Total protein (g L^{-1})	28.70 ± 0.23	30.30 ± 0.83	37.10 ± 3.66	34.30 ± 0.40	28.70 ± 0.92	0.494
Albumin (g L^{-1})	8.50 ± 0.07	8.50 ± 0.07	8.00 ± 0.17	7.50 ± 0.07	8.50 ± 0.35	0.969
Total bilirubin (μmol L^{-1})	31.81 ± 1.03	51.81 ± 0.83	63.44 ± 3.66	43.26 ± 1.37	80.71 ± 4.34	0.731

Means with the different letters in the same row are significantly different at $p<0.05$.

3.6 Serum biochemical values

Blood samples from fish at the termination of the experiment are shown in Table 4. Haematocrit determination and biochemical serum analyses were carried out on 3 fish in each of replications. Haematocrit determination and biochemical serum levels in blood were statistically similar ($p>0.05$). The ranges of haematocrit were 26.67 - 30.67 %. The ranges of enzyme activities in serum were: alkaline phosphatase (ALP) 93.00-117.33 U L^{-1}, aspartate aminotransferase (AST) 153.67-170.00 U L^{-1} and alanine aminotransferase (ALT) 15-34.33 U L^{-1}. The ranges of the other parameters analyzed in serum were: total protein 28.70-37.10 g L^{-1}, albumin 7.50-8.50 g L^{-1}, total bilirubin 31.81-80.71 μmol L^{-1}.

Haematocrit determination and biochemical serum levels were statistically similar. The haematocrit assay is normally used as a general indicator of fish health (NRC, 1993). Haematocrit level in all groups was within the normal range and did not differ significantly among the groups. In another study, Soltan *et al*., (2008) observed that FM protein replaced by mixture of plant proteins in Nile tilapia diets that lead to the lower haematocrit levels could be attributed to the binding of phytate to minerals (iron) and/or to be amine group of amino acids causing their low availabilities in the body and an increase in erythrocyte fragility. Fish have also been shown to exhibit stress reactions due to the presence of antinutrients like phorbol esters, trypsin inhibitors and saponins in the diet (Makkar and Becker, 1997; Martinez, 1976). Saponin can also increase O_2 consumption in common carp (Francis *et al*., 2001) and perch, *Anabas testudineus* (Martinez, 1976). Total blood protein concentration in all groups did not differ significantly. Among the blood protein, albumin and globulin are the major proteins, which play a significant role in the immune response. Total serum protein concentration is a measure of all of the different proteins in plasma with the exception of those consumed in clot forma-

tion such as fibrinogen and the clotting factors (Racicot *et al*., 1975). ALP, ALT and AST are released into blood during organ damage (Tietz, 1986). Thus, detection of high levels of ALP, ALT and AST in blood gives information on the damage of organs and in particular of liver cells. In this study, levels of ALP, ALT and AST were similar in all the diets, indicating normal organ function on feeding of moringa seed cake. Total bilirubin, an indicator of liver dysfunction (Kumar *et al*., 2011), was similar for all groups; the values did not differ significantly among the groups. These results show that moringa seed cake fed groups were normal and healthy. Similarly, replacement of 500 g kg^{-1} *Jatropha curcas* kernel meal by fish meal protein in rainbow trout diets did not cause lower growth or nutrient utilization and did not affect physiological and haematological parameters (Adamidous *et al*., 2011).

3.7 Histology

Histologic samples from the liver and distal intestine of sampled fish in each treatment group were examined and compared with fish maintained on control diet. Hepatosomatic index (HSI) in this study showed no statistical difference among the groups ($p>0.05$). In contrast, fish fed with moringa seed cake supplement replacing protein in soybean over 500 g kg^{-1} revealed apparent vacuolar and hemorrhage in liver (Figure 3). Histopathological examinations showed no pathological abnormalities of the distal intestines (Figure 4) among diet groups.

The liver histological change exhibited normality in fish fed with diet replaced protein of soybean meal with moringa seed cake. However, in this study mild histopathological changes in the liver were seen in fish fed more than 750 g kg^{-1} of soybean meal protein replacement; the liver was observed with slight vacuoles and hemorrhage. The results of the present study suggest that moringa seed cake can be included in the diet at a proportion of not over 500 g kg^{-1} by replacing protein in soybean meal without inducing morphological changes in the liver and distal intestine (DI) tract of bocourti's catfish. This conclusion is consistent with studies of gilthead sea bream fed with three legumes (field peas, chickpeas and faba beans) in diet up to 350 g kg^{-1} (Adamidous

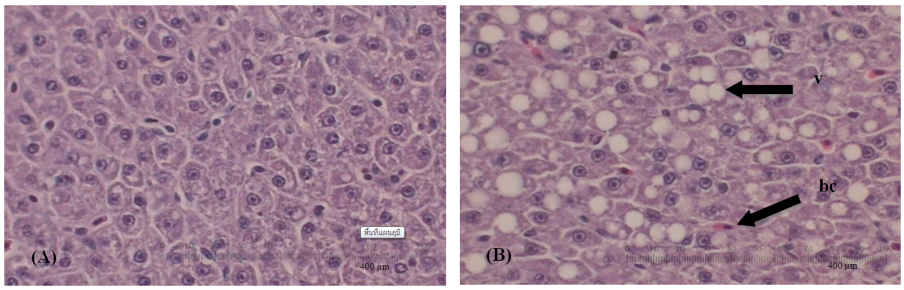

Figure 3. Representative histologic sections from bocourti's catfish fed with experimental diets, liver sample from fish with no noticeable difference (A) and mild histological changes (B); v, vacuolar; bc, blood cell; see Results section. Observed from three samples (400x).

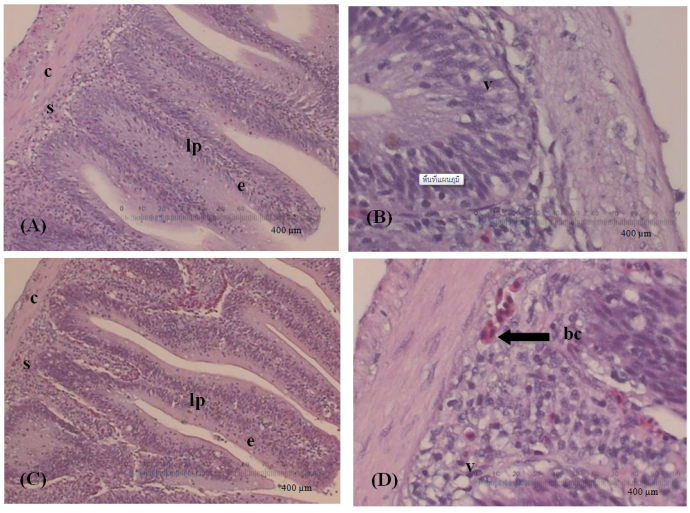

Figure 4. Distal intestine histology of fish with no noticeable difference (A, B) and mild histological changes (C, D). e, epithelium; lp, lamina propria; v, vacuole; bc, blood cell; C, circular muscular layer; S, submucosa; observed from three samples (400x).

et al., 2011) and juvenile cobias fed with defatted SBM in the diet with up to 285 g kg^{-1} (Romarheim *et al*., 2008) based on determination of histopathological investigations.

4. Conclusions

In conclusion, this study indicated that moringa seed cake can efficiency be used as a plant protein source. Moringa seed cake could replace at level of not over 500 g kg^{-1} for replacing protein in soybean meal in bocourti's catfish diet and could support the growth, adversely affected digestibility, haematological, serum biochemistry parameters and histopathological change in bocourti's catfish. Thus, moringa seed cake could be become an alternative plant protein source in fish diet to lower the production cost of fish diets and add value to a plant origin.

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