Agriculture and Natural Resources 51 (2017) 286–291

Contents lists available at ScienceDirect

Agriculture and Natural Resources

journal homepage: http://www.journals.elsevier.com/agriculture-and-natural-resources/

Original Article

Effect of moringa (*Moringa oleifera*) leaf supplementation on growth performance and feed utilization of Bocourti's catfish (*Pangasius bocourti*)

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ARTICLE INFO

Article history:
Received 6 May 2015
Accepted 30 December 2016
Available online 7 November 2017

Keywords:
Moringa
Bocourti's catfish
Growth
Digestibility

ABSTRACT

The optimal level of dietary moringa leaf was determined on the growth performance, feed utilization, digestibility and serum biochemistry of Bocourti's catfish with mean wet weights of 3.72 ± 0.06 g per fish. Fish were fed with diets containing isonitrogenouse (350 g/kg crude protein) and isoenergetic (3000 kcal/kg) supplemented with dietary moringa leaf at 0 g/kg fish, 100 g/kg fish, 150 g/kg fish and 200 g/kg fish for 60 d. Fish were hand fed to apparent satiation twice a day. At the end of the experiment, a significant ($p < 0.05$) reduction in the growth performance was found as the moringa leaf inclusion increased in the diets, along with slightly poorer growth performance and feed utilization. Fish fed the diet containing moringa leaf at 100 g/kg demonstrated better growth than at the other inclusion levels, but it was not significantly different from the control group. The digestibility coefficient and protein digestibility were lower in fish fed with a higher inclusion of moringa leaf in the diets ($p < 0.05$). Pepsin digestibility and serum biochemical parameters were not different among all treatments ($p > 0.05$). The study indicated that dietary moringa leaf could be included in the Bocourti's catfish diet at possibly not over 100 g/kg fish without a negative effect on the growth, feed utilization, digestibility and serum biochemistry.

Introduction

Commercial aquaculture production of Pangasius in Southeast Asia, specifically Basa (*Pangasius bocurti*) and Tra (*Pangasianodon hypophthalmus*), has increased rapidly in recent years (Globefish, 2009). Pangasius catfish is a leading aquaculture commodity in Thailand and in the early 1990s imports of these species into the USA grew steadily until anti-dumping laws were enforced in 2003 and imports were cut by half (Seafood Watch, 2007). Basa and Tra are both native to the Mekong River and Delta, and to the Chao Praya River in Thailand (Tyson, 1991). It is clear that fish meal supplies from these finite fisheries are strictly limited and if aquaculture continues to expand worldwide, the requirements for fish meal will soon exceed global supplies (Food and Agricultural Organization, 2006). Because fish meal is a limited primary source and plants are widely available and reasonably priced, the use of plant protein sources in aquafeeds should be considered (State of World Fisheries and Aquaculture, 2007). Soybean meal is one of the most nutritious of all plant protein sources because of its high protein content, high digestibility, and relatively well-balanced amino acid profile (Lovell, 1988). Owing to its reasonable price and steady supply, soybean meal is widely used as a cost-effective feed ingredient for many aquaculture species (Storebakken et al., 2000). It is currently the most commonly used plant protein source in fish feeds (Hardy, 2010). However, soybean meal has to compete with human food use, and hence there is a need to identify other protein-rich plant resources that could be used in fish diets. Another potential alternative plant protein source for fish feeds is moringa (*Moringa oleifera*), as this plant is receiving much attention because its leaves, flowers and seeds can all be used as food (Makkar and Becker, 1997). Moringa leaf contains crude protein (CP) with about 260 g/kg of leaf, of which about 87% is true protein (Makkar and Becker, 1996). Essential amino acids found in moringa leaf are methionine, cysteine, typtophan and lysine (Makkar and Becker, 1996). A comparison between the amino acid

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https://doi.org/10.1016/j.anres.2017.10.001

composition of raw moringa leaf and that of soybean revealed an almost identical pattern of all the essential amino acids (Foidl et al., 2001). There is an abundant total amount of these essential amino acids is plenty in the leaf that can be used as animal feed (Afuang et al., 2003). Therefore, the objective of the present experiment was to investigate the effect of different levels of dietary moringa leaf in the diet of Bocourti's catfish on the growth performance, nutrient digestibility, serum biochemical and liver histopathology.

Materials and methods

Fish, diets and feeding protocol

Bocourti's catfish fingerlings were kept in a 1000 L tank for acclimatization on a commercial diet with 35% protein for 4 wk prior to feeding the experimental diets. After acclimatization, fish were randomly distributed into four groups with five replications; each replicate contained 10 fish (mean wet weight of 3.72 ± 0.06 g) in an aquarium (50 L capacity).

Four isonitrogenouse and isoenergetic diets were formulated to contain approximately 350 g/kg protein and 3000 kcal/kg with moringa leaf powder at 0 g/kg, 100 g/kg, 150 g/kg and 200 g/kg. Experimental diets were isonitrogenous and isocaloric (the proximate chemical composition is presented in Table 1). The diets' chemical compositions of protein, moisture, fat, fiber and ash were analyzed according to the methods of Association of Official Analytical Chemists (1990). The fish were hand fed hand to apparent satiation twice a day (0900 h and 1700 h) for 60 d. The total feed was recorded weekly and each fish from each tank was weighed to measure growth at the end of the experiment and growth performance was calculated.

Phytochemicals in moringa leaf and experimental diets

Moringa leaf and test diets were determined for their phytochemical content, that is, the total tannin content (Association of Official Analytical Chemists, 1990) and phytic acid (Talamond et al., 1998).

Protein digestibility

The digestibility coefficients of the protein in the test diets were calculated according to Austreng (1978). To assess the quality of experimental diets, digestible crude protein was determined using the pepsin digestibility test (Association of Official Analytical Chemists, 1990).

Serum biochemistry and liver function

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

Hepatosomatic index and histological observation

At the termination of the feeding experiments, the fish were anesthetized and weighed individually. They were dissected; the livers were removed from four fish of each treatment and weighed for calculating the hepatosomatic index (HSI). Buffered formalin-fixed samples of liver were dehydrated in ethanol, embedded in paraffin, sectioned and stained with hematoxylin and eosin. The sections were examined using light microscopy, a digital microscopy camera, and the Motic Image Plus 2.1S software (Shimadzu; Kyoto, Japan).

Statistical analysis

Mean values and SD were calculated from the results. One way analysis of variance was applied for comparison of the mean values; $p < 0.05$ was established as significant.

Results and discussion

Feed quality

The quantified phytochemical content of the moringa leaf and test diets is shown in Table 2. Similar results for the total tannin content and phytic acid content were observed by Gupta et al. (1989). Thus, by increasing moringa leaf in the diets, the amounts of tannins and phytate were increased.

Table 1
Ingredients and chemical composition of experimental diets.

Ingredient (g/kg)	Moringa leaf at different levels in experimental diets (g/kg fish)			
	0	100	150	200
Fish meal	330	330	330	330
Soybean meal	250	200	180	156
Corn meal	140	110	110	90
Rice bran	130	120	110	90
Moringa leaf	0	100	150	200
Soya oil	35	35	30	32
Fish oil	35	35	35	32
Alpha-starch	50	50	35	50
Methyl cellulose	10	–	–	–
Dicalcium phosphate	10	10	10	10
Premix[a]	10	10	10	10
Chemical composition by proximate analysis (g/kg dry weight basis; mean ± SD)				
Protein	350.13 ± 2.00	349.40 ± 1.15	350.37 ± 0.58	350.16 ± 0.56
Fat	120.83 ± 0.55	118.23 ± 0.32	116.70 ± 0.21	115.21 ± 0.10
Fiber	15.30 ± 0.15	15.13 ± 0.36	15.27 ± 0.32	15.57 ± 0.66
Ash	127.70 ± 0.50	132.93 ± 0.38	136.67 ± 0.32	138.74 ± 0.13
Moisture	58.16 ± 0.20	57.03 ± 0.25	56.30 ± 0.15	57.89 ± 0.60

[a] Vitamin and mineral mix/kg: vitamin A 36,000 international units (IU), vitamin D3 9000 IU, vitamin E 187 mg, vitamin K3 19 mg, vitamin B1 52 mg, vitamin B2 97 mg, vitamin B6 46 mg, vitamin C (coated) 68,800 mg activity., vitamin B12 60 mg, panthothenic acid 93 mg, niacin 130 mg, folic acid 10 mg, inositol 225 mg, biotin 450 mg, manganese (Mn) 105 mg, copper (Cu) 9 mg, iron (Fe) 90 mg, Zinc (Zn) 90 mg, iodine (KI) 1.8 mg, cobalt (Co) 450 mg, magnesium (Mg) 1900 mg, selenium (Se) 150 mg, sodium (Na) 117 mg, potassium (K) 150 mg, calcium (Ca) 219 mg.

Table 2
Phytochemical content of moringa leaf and experimental diets (mean ± SD).

Phytochemical (%)	Moringa leaf	Moringa leaf of different levels in experimental diets (g/kg fish)				
		0	100	150	200	*p*-value
Total tannin	2.85 ± 0.39	0.91 ± 0.04^{d*}	2.07 ± 0.25^{b}	2.39 ± 0.14^{a,b}	2.61 ± 0.26^{a}	0.0001
Phytic acid	0.49 ± 0.12	0.49 ± 0.03^{d}	1.12 ± 0.14^{b}	1.29 ± 0.07^{a,b}	1.41 ± 0.14^{a}	0.0001

*Means within the same row with different, lowercase, superscript letters are significantly different ($p < 0.05$).

Table 3
Protein digestibility and pepsin digestibility in Bocourti's catfish fed with experimental diets (mean ± SD).

Digestibility	Moringa leaf of different levels in experimental diets (g/kg fish)				
	0	100	150	200	*p*-value
Digestibility coefficient (%)	81.95 ± 1.69^{a*}	73.39 ± 0.46^{b}	75.21 ± 1.54^{b}	69.43 ± 1.88^{c}	0.0001
Protein digestibility (%)	91.87 ± 0.39^{a}	89.96 ± 0.25^{b}	89.56 ± 0.44^{b}	87.37 ± 0.38^{c}	0.0001
Pepsin digestibility (%)	62.84 ± 4.37	55.47 ± 3.06	55.00 ± 4.88	52.71 ± 1.75	0.0502

*Means within the same row with different, lowercase, superscript letters are significantly different ($p < 0.05$).

In the present study, the tannin content in the experimental diets ranged between 0.91 ± 0.04% and 2.61 ± 0.26% and the phytic acid content ranged between 0.49 ± 0.12% and 1.41 ± 0.14%. Giner-Chavez (1996) reported that levels of tannin from 0.5% to 2.0% can cause depression in growth and levels of tannins above 5.0% in the diet are often lethal. High dietary phytic acid (2.5%) dramatically depressed the growth rate in salmon fish (Richardson et al., 1985) and it has been reported that 0.5%–0.6% can impair the growth of rainbow trout (Spinelli et al., 1983) and common carp (Hossain and Jauncey, 1993). Therefore, the use of plant materials in animal feed should take into account the toxic chemicals contained in raw materials for animal feed (Siddhuraju et al., 2000).

Protein digestibility

The study of protein digestibility and pepsin digestibility on experimental diets is presented in Table 3. The digestibility coefficient and protein digestibility were significantly reduced by supplementation with moringa leaf. The results showed no significant difference in the pepsin digestibility of all experimental diets; the percentage of protein digestibility ranged from 52.27 ± 1.75% to 62.84 ± 4.37%. It was generally observed that as the amount of moringa leaf increased in the diets, the protein digestibility decreased gradually.

The use of plant protein sources in aqua feeds should be considered (State of World Fisheries and Aquaculture, 2007). Moringa has been widely studied as an alternative protein source in fish diets and seems to be a promising protein source. Moringa leaf can partially replace conventional diets of Nile tilapia (*Oreochromis niloticus* L.) without any depression in growth performance (Richter et al., 2003; Afuang et al., 2003). In the present study, none of the diets adversely affected pepsin digestibility compared to the control diet without moringa leaf, but the *in vivo* digestibility was affected by the diets supplemented with moringa leaf above 200 g/kg compared to the control diet. Generally, the digestibility percentage of protein is within the range 31–95% for fish (Halver and Hardy, 2002). Plant ingredients (bean meal, groundnut oilcake and sunflower oilcake) can efficiently substitute fishmeal at 250 g/kg in African catfish diets, and there were no significant differences in protein antibody-drug conjugates (ADCs; 88–90) with increased levels of dietary plant-based protein in diets (Nyina-Wamwiza et al., 2010). The ADC in the protein of plant leaf ingredients, determined in barnyard grass and dried maize leaf, were found to be not only poorly digestible but also to have a negative impact on the digestibility of the reference diet while fresh maize leaves were well digested (Dongmeza et al., 2010). The results of this study indicated that fresh maize leaves have good potential to be used as a supplement in diets for grass carp. This indicated that dry plant materials seem to result in low digestibility and could even inhibit fish utilization of other nutrients contained in the diet (Dongmeza et al., 2010).

Growth performance and feed utilization

The growth parameters of Bocourti's catfish are given in Table 4. The results from studying the growth performance of the fish revealed that the final body weight and weight gain were significantly reduced by moringa leaf supplement, resulting in reduced average daily gain (ADG) and specific growth rate (SGR). The highest ADG and SGR were observed in the fish fed with moringa leaf supplemented diets at 100 g/kg, which were statistically similar to the control group and significantly higher than other groups. In terms of feed utilization, data showed that there were also significant differences in the feed conversion ratio (FCR) among all groups. The lowest FCR was observed in diets supplemented with moringa leaf at 100 g/kg and 150 g/kg in the control

Table 4
Growth performance and feed utilization of Bocourti's catfish fed with experimental diets for 60 days (mean ± SD).

Growth performance and feed efficiency	Moringa leaf of different levels in experimental diets (g/kg fish)				
	0	100	150	200	*p*-value
Average daily gain (g/fish/d)	1.93 ± 0.03^{a*}	1.70 ± 0.19^{ab}	1.56 ± 0.15^{b}	1.46 ± 0.15^{b}	0.0221
Specific growth rate (%/d)	15.05 ± 0.09^{a}	14.24 ± 0.61^{ab}	13.76 ± 0.50^{b}	13.34 ± 0.45^{b}	0.0166
Feed conversion ratio	0.98 ± 0.03^{c}	1.02 ± 0.01^{bc}	1.06 ± 0.01^{ab}	1.09 ± 0.03^{a}	0.0018
Protein efficiency ratio	0.63 ± 0.01	0.63 ± 0.03	0.62 ± 0.02	0.59 ± 0.01	0.0521
Survival rate (%)	100	100	100	100	–

*Means within the same row with different, lowercase, superscript letters are significantly different ($p < 0.05$).

Table 5
Serum biochemical parameters of Bocourti's catfish fed with experimental diets for 60 d (mean ± SD).

Serum biochemical parameter[a]	Moringa leaf of different levels in experimental diets (g/kg fish)				
	0	100	150	200	*p*-value
Hct %	29.00 ± 2.65	31.66 ± 3.05	31.00 ± 1.73	30.00 ± 1.00	0.2795
TP g/L	0.35 ± 0.05	0.32 ± 0.20	0.34 ± 0.28	0.34 ± 0.23	0.2484
ALB μmol/L	1.49 ± 0.05	1.54 ± 0.05	1.54 ± 0.05	1.64 ± 0.05	0.1610
BT μmol/L	122.78 ± 0.98	115.25 ± 0.97	110.98 ± 0.70	97.30 ± 1.83	0.3213
ALP IU/L	300.33 ± 14.64	261.66 ± 19.50	263.00 ± 13.48	163.33 ± 13.70	0.5157
AST IU/L	172.66 ± 12.36	114.33 ± 13.50	146.33 ± 17.78	163.00 ± 14.42	0.2537
ALT IU/L	26.00 ± 7.93a	15.66 ± 20.08	22.66 ± 9.07	19.00 ± 7.93	0.5555
his	2.64 ± 0.30	2.68 ± 0.04	2.52 ± 0.74	2.92 ± 0.52	0.8116

[a] Hct = haematocrit, ALP = alkaline phosphatase, AST = aspartate aminotransferase, ALT = alanine amiotransferase, BT = total bilirubin, TP = total protein, ALB = albumin, HSI = hepatosomatic index.

group. Protein efficiency (PER) was not significantly different, ranging from 0.59 ± 0.01 to 0.63 ± 0.03. All fish grew normally, and no specific signs of disease were observed. No mortality occurred throughout the experiment.

In this study, growth performance and feed utilization were affected significantly by all treatments diets. Plant protein based diets may reduce growth (Espe et al., 2006). This agreed with the study by Olsvik et al. (2011) that reported growth reduction was mainly related to a lower feed intake, because of an interaction effect with high plant protein. However, a study performed on the growth of Nile tilapia (*Oreochromis niloticus* L.) showed no effects of dietary supplement using methanol-extracted leaf meal containing 11 g/kg, 220 g/kg and 330 g/kg (Makkar and Becker, 1996). Tilapia fed with a diet containing moringa leaf meal at 100 g/kg, 130 g/kg and 150 g/kg replacement with fishmeal-based dietary protein did not cause any adverse effect on growth performance (Richter et al., 2003; Afuang et al., 2003).

Serum biochemistry

The results of the serum biochemistry from fish sampled at the termination of the experiment are shown in Table 5. Haematocrit

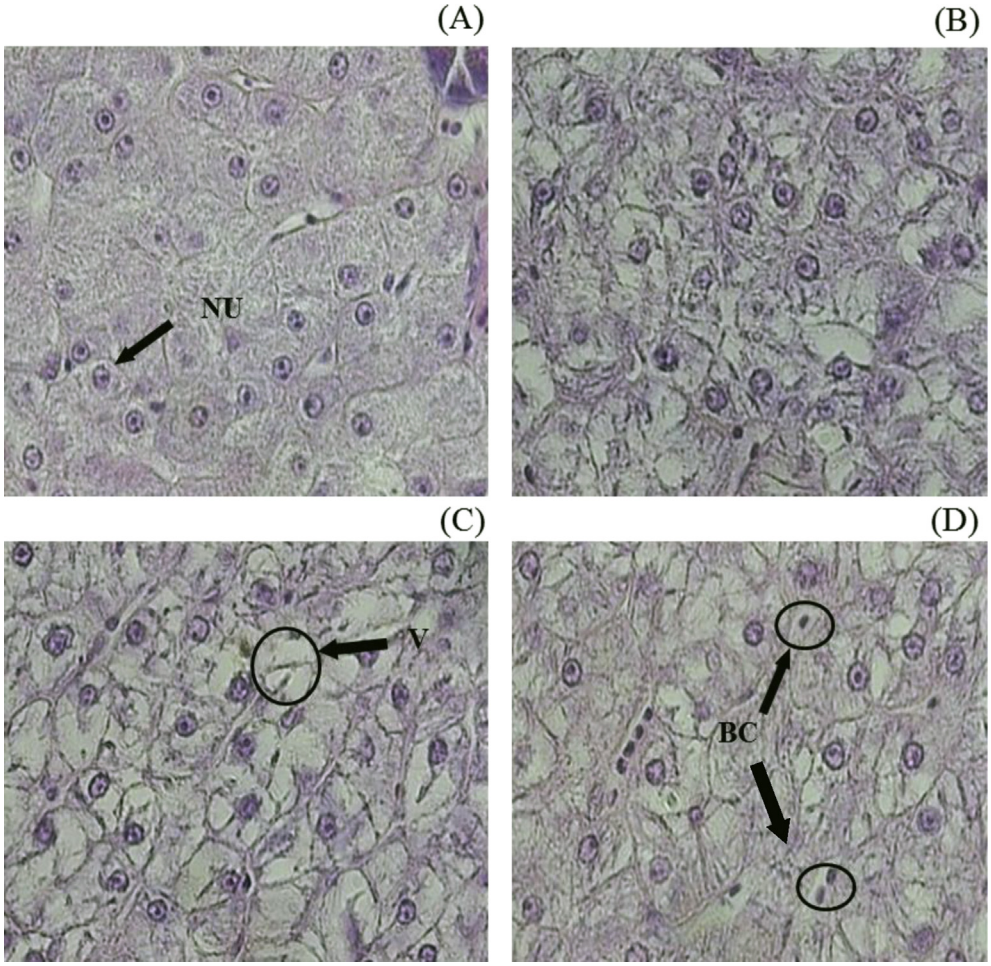

Fig. 1. Representative liver histological sections stained with hematoxylin and eosin at × 400 magnification from Bocourti's catfish fed for 60 d with dietary moringa leaf at: (A) 0 g/kg fish; (B) 100 g/kg fish; (C) 150 g/kg fish; (D) 200 g/kg fish (NU = nucleus, V = vacuolar, BC = blood cell).

determination and biochemical serum analyses were carried out on three fish in each of the replications. Haematocrit determination and biochemical serum levels in blood were not statistically different. The range in levels of haematocrit was 29.00 ± 2.65% to 31.66 ± 3.05%. The ranges of the sample means of hepatic enzyme activity in the serum samples were: alkaline phosphatase (ALP) 163.33 ± 13.70 international units (IU)/L to 300.33 ± 14.64 IU/L, aspartate aminotransferase (AST) 114.33 ± 13.50 U/L to 172.66 ± 12.36 IU/L and alanine aminotransferase (ALT) 15.66 ± 20.08 IU/L to 26.00 ± 7.93 IU/L. The ranges of the other parameters analyzed in the serum samples were: total protein 0.32 ± 0.20 g/L to 0.35 ± 0.05 g/L, albumin 1.49 ± 0.05 μmol/L to 1.64 ± 0.05 μmol/L, total bilirubin 97.30 ± 1.83 μmol/L to 122.78 ± 0.98 μmol/L. The hepato somatic index (HSI) in this study showed no statistical differences among all groups.

Haematocrit determination and the biochemical serum levels were statistically similar. The haematocrit assay is normally used as a general indicator of fish health (National Research Council, 1983). The 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 fish meal as animal protein replaced by a mixture of plant proteins in Nile tilapia diets led to lower haematocrit levels and could be attributed to the binding of phytate to minerals (iron) and/or amine the group of amino acids causing their low availabilities in the body and an increase in erythrocyte fragility. At the trial levels used (100–200 g/kg fish), substitution of moringa leaf for soybean meal is considered to be in the normal range in healthy fish compared with the control group. The total blood protein content is taken as an index of nutritional status (Gad, 2007). The total blood protein concentration in all groups did not differ significantly. Among the blood proteins, albumin and globulin are the major proteins, which play a significant role in the immune response (Kaneko et al., 1997).

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 provides information on the damage of organs and in particular of liver cells. In the present study, the levels of ALP, ALT and AST were similar in all the diets, indicating normal organ function resulting from feeding the fish with dietary moringa leaf. Total bilirubin, an indicator of liver dysfunction (Kumar et al., 2011), was similar for all groups and did not differ significantly among the groups. These results showed that fish fed with dietary moringa leaf were normal and displayed no effects in terms of serum biochemical parameters.

Histological study of fish liver

The histological study on liver samples of Bocourti's catfish fed with diets containing moringa leaves at 0 g/kg fish, 100 g/kg fish, 150 g/kg fish and 200 g/kg fish for 60 d showed cytoplasm cytosine and round nuclei in the central cells in the liver tissue of fish sampled in all experiments (Fig. 1). In some cells, the cell nucleus was pushed to the edge of the cell, creating a larger gap in the cytoplasm. Small amounts of bleeding with slight hemorrhaging were observed in fish fed with moringa leaves mixed at 200 g/kg.

The histological study of the liver samples of the fish fed with dietary moringa leaves supplementation at 100 g/kg fish, 150 g/kg fish and 200 g/kg fish for 60 d compared with the histological appearance of the liver in control group. The results indicated that hepatocytes nuclei were round and dispersed in the center of cells in all experimental groups. In some cells, the cell nucleus was pushed to the edge of the cell causing a larger gap in the cytoplasm cytosine or vacuolation. The observed gap in the cytosine cytoplasm due to glycogen and fat accumulation in the liver cells was diminished until a gap formed in the liver (Roberts, 1989). Liver cells of fish fed with moringa leaf diet supplementation at 100 g/kg, 150 g/kg and 200 g/kg showed a cloudy swelling which indicated cell degradation observed by small particles inside cytoplasm. Bocourti's catfish fed with feed mixed with moringa leaves at 150 g/kg and 200 g/kg had small amounts of bleeding, an indication of slight hemorrhaging. However, the deterioration of cells or small amounts of bleeding and hemorrhaging do not necessarily cause severe debilitation; the fish body can recondition itself and resume a normal condition (Kaneko et al., 1997). The histological analysis revealed that inclusion of moringa leaf supplement had some effect on fish liver cells but did not affect the function of the liver.

In conclusion, this study indicated that moringa leaf can be efficiently used as a plant protein source. Moringa leaf could be used as a supplement at no more than 100 g/kg fish to support growth with no adverse affect on the digestibility and serum biochemistry parameters in Bocourti's catfish. Moreover, moringa leaf had some effect on liver cells, but the liver function was normal in all feeding groups. Thus, moringa leaf could be used as an alternative plant protein source in the diet of Bocourti's catfish to lower the production cost of feed and to add value to the plant's use.

Conflict of interest

There is no conflict of interest.

References

Afuang, W., Siddhuraju, P., Becker, K., 2003. Comparative nutritional evaluation of raw, methanol extracted residues and methanol extracts of moringa (*Moringa oleiera* lam.) leaf on growth performance and feed utilisation in Nile tilapia (*Oreochromis niloticus* L.). Aquac. Res. 34, 1147–1159.

Association of Official Analytical Chemists, 1990. Official Methods of Analysis, Fifteenth ed. Association of Official Analytical Chemists, Washington, DC, USA.

Austreng, E., 1978. Digestibility determination in fish using chromic oxide marking and analysis of contents from different segments of the gastro-intestinal tract. Aquaculture 13, 265–272.

Dongmeza, E.B., Francis, G., Steinbronn, S., Focken, U., Becker, K., 2010. Investigations on the digestibility and metabolizability of the major nutrients and energy of maize leaves and barnyard grass in grass carp (*Ctenopharyngodon idella*). Aquacult. Nutr. 16, 313–326.

Espe, M., Lemme, A., Petri, A., El-Mowafi, A., 2006. Can Atlantic salmon (*Salmo salar*) grow on diets devoid of fish meal? Aquaculture 255, 255–262.

Foidl, N., Makkar, H.P.S., Becker, K., 2001. The potential of Moringa oleifera for agricultural and industrial uses. In: Fuglie, L.J. (Ed.), The Miracle Tree. CTA and CWS, Dakar, Senegal, pp. 45–77.

Food and Agricultural Organization, 2006. State of World Aquaculture 2006. FAO Fisheries Technical Paper No. 500. FAO, Rome.

Gad, S.C., 2007. Animal Models in Toxicology. CRC Press, Boca Raton, FL, USA.

Giner-Chavez, B.I., 1996. Condensed tannins in tropical forages. Ph. D. Thesis. Cornell University, Ithaca, NY, USA.

Globefish, 2009. Pangasisus Market Report. July 2009. Food and Agriculture Organization website. http://www.fao.org/in-action/globefish/market-reports/resource-detail/en/c/336932/. (Accessed 19 July 2017).

Gupta, K., Barat, G.K., Wagle, D.S., Chawla, H.K.L., 1989. Nutrient contents and antinutritional factors in conventional and non-conventional leafy vegetables. Food Chem. 31, 105–116.

Halver, J.E., Hardy, R.W., 2002. Fish Nutrition, third ed. Academic Press, New York, NY, USA.

Hardy, R.W., 2010. Utilization of plant proteins in fish diets: effects of global demand and supplies of fish meal. Aquac. Res. 41, 770–776.

Hossain, M.A., Jauncey, K., 1993. The effect of varying dietary phytic acid, calcium and magnesium levels on the nutrition of common carp, Cyprinus carpio. In: Kaushik, S.J., Luquent, P. (Eds.), Fish Nutrition in Practice. Proceedings of International Conference, Biarritz, France, June 24–27, 1991, pp. 705–715.

Kaneko, J.J., John, W.H., Michael, L.B., 1997. Clinical Biochemistry of Domestic Animals. Academic Press, San Diego, CA, USA.

Kumar, V., Makkar, H.P.S., Becker, K., 2011. Nutritional, physiological and haematological responses in rainbow trout (*Oncorhynchus mykiss*) juveniles fed with detoxified *Jatropha curcas* kernel meal. Aquacult. Nutr. 17, 451–467.

Lovell, R.T., 1988. Use of soybean products in diets for aquaculture species. J. Aqua. Prod. 2, 27–52.

Makkar, H.P.S., Becker, K., 1996. Nutritional value and antinutritional components of whole and ethanol extracted *Moringa oleifera* leaf. Anim. Feed Sci. Tech. 63, 211–228.

Makkar, H.P.S., Becker, K., 1997. Nutrient and antiquality factors in different morphological parts of *Moringa oleifera* tree. J. Agr. Sci. 128, 311–322.

National Research Council, 1983. Nutrient Requirement of Warmwater Fishes and Shell Fish. National Academy Press, Washington DC, USA.
Nyina-Wamwiza, L., Wathelet, B., Richir, J., Rollin, X., Kestemont, P., 2010. Partial or total replacement of fish meal by local agricultural by-products in diets of juvenile African catfish (*Clarias gariepinus*): growth performance, feed efficiency and digestibility. Aquacult. Nutr. 16, 237–247.
Olsvik, P.A., Tostensen, B.E., Hemre, G.I., Sanden, M., Waagbo, R., 2011. Hepatic oxidative stress in Atlantic salmon (*Salmo salar* L.) transferred from a diet based on marine feed ingredients to a diet based on plant ingredients. Aquacult. Nutr. 17, e424–e436.
Richardson, N.L., Higgs, D.A., Beames, R.M., McBride, J.R., 1985. Influence of dietary calcium, phosphorus, zinc and sodium phytate level on cataract incidence, growth and histopathology in juvenile chinook salmon (*Oncorhynchus tshawytscha*). J. Nutr. 115, 553–567.
Richter, N., Siddhuraju, P., Becker, K., 2003. Evaluation of the quality of (*Moringa oleifera* Lam.) leaf as an alternative protein source for Nile tilapia (*Oreochromis niloliticus* L.). Aquaculture 217, 599–611.
Roberts, R.J., 1989. Fish Pathology. Bailliere Tindall, London, UK.
Seafood Watch, 2007. Farmed Pangasius. Monterey Bay Aquarium, Monterey, CA, USA.
State of World Fisheries and Aquaculture, 2007. The State of World Fisheries and Aquaculture 2006, Pp. 1–180. Italy, FAO Fisheries and Aquaculture Department. Rome.
Siddhuraju, P., Becker, K., Makkar, H.P.S., 2000. Studies on the nutritional composition and antinutritional factors of three different germplasm seed materials of an under-utilised tropical legume, *Mucuna pruriens* var. *utilis*. J. Agric. Food Chem. 48, 6048–6060.
Soltan, M.A., Hanafy, M.A., Wafa, M.I.A., 2008. Effect of replacing fish meal by a mixture of different plant protein sources in Nile tilapia (*Oreochromis niloticus* L.) diets. Glob. Veterinaria 2, 157–164.
Storebakken, T., Refstie, S., Ruyter, B., 2000. Soy products as fat and protein sources in fish feeds for intensive aquaculture. In: Drackley, J.K. (Ed.), Soy in Animal Nutrition. Federation Animal Science Societies. Savoy, IL, USA, pp. 127–170.
Spinelli, J., Houle, C.R., Wekell, J.C., 1983. The effect of phytates on the growth of rainbow trout (*Salmo gairdneri*) fed with purified diets containing varying quantities of calcium and magnesium. Aquaculture 30, 71–83.
Talamond, P., Gallon, G., Guyot, J.P., Lape, I.M., Treche, S., 1998. Comparison of high-performance ion chromatography and absorptiometric methods for the determination of phytic acid in food samples. Analusis 26, 396–400.
Tietz, N.W., 1986. Textbook of Clinical Chemistry. W. B. Saunders, Philadelphia, PA, USA.
Tyson, R.R., 1991. Systematic revision of the Asian catfish family Pangasiidae, with biological observation and description of three new species. Proc. Acad. Nat. Sci. U. S. A 143, 97–144.