Indian J. Fish., 64(2): 33-39, 2017
DOI: 10.21077/ijf.2017.64.2.61610-06

Evaluation of mahua oil cake (*Bassia latifolia* Roxb.) as a non-conventional feed ingredient for *Labeo rohita* (Ham.) fingerlings

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ABSTRACT

Mahua (*Bassia latifolia* Roxb.) oil cake (MOC) is rich in protein (24%) and energy (19.0 KJ g^{-1}) with high levels of fatty acids comprising saturates (45%), monoenes (42%) and polyunsaturated fatty acids (PUFA, n-6) (7%). Saponin, phenol and flavonoids are the main metabolites. *Labeo rohita* (Hamilton, 1822) fingerlings (5.25±0.2 g) were fed with five iso-nitrogenous (28% CP) diets containing MOC at 0% (F1), 10% (F2), 20% (F3), 30% (F4) and 40% (F5) for 90 days in 300 l cement cisterns. Survival (%) of all groups were statistically similar ($p>0.05$). Weight gain (%) and specific growth rate (SGR) were significantly lower in F1, F2, F5 and higher in F3 and F4 groups ($p<0.05$). Feed conversion ratio (FCR) was significantly lower ($p<0.05$) in F4 as compared to the other groups. Among all the dietary treatments, significantly higher ($p<0.05$) protein efficiency ratio (PER) and net protein utilisation (NPU) were found in F4. Whole body protein and lipid was significantly higher ($p<0.05$) in F4 and F3, respectively. Hemoglobin, glucose, protein and cholesterol in blood were found to be at higher levels in F4 group. All these parameters declined, but serum glutamic oxaloacetic transaminase (SGOT) and serum glutamic pyruvic transaminase (SGPT) levels increased significantly ($p<0.05$) in F5. The findings of the study clearly indicated that MOC can be incorporated at 300 g kg^{-1} in the diet of *L. rohita* fingerlings without any adverse effect on growth, survival and nutrient utilisation.

Keywords: Fish feed, Mahua oil cake, Non-conventional ingredient

Introduction

Feed is the most crucial input that greatly influences the productivity and profitability in aquaculture. Reduction in feed cost and mechanisms that triggers nutrient efficiency would increase the net income in aquaculture. Competition for the conventional feed ingredients among livestock, poultry and aquaculture industries is becoming more severe and has tremendous impact on the market dynamics of aquaculture. Fish require more protein in their diet than the land animals and hence aquafeed demands more protein rich ingredients (Hasan *et al.,* 2007). Soybean meal and groundnut oil cake (GNOC) are the major plant proteins used in compounded aquafeeds, followed by other oil cakes (Barman and Karim, 2007; Manomaitis, 2009). Incorporation of soybean meal or cakes of edible oil seeds is becoming too competitive and costly, especially for carp culture. It is, therefore, necessary to search non-conventional plant resources for formulating cost effective carp feeds (FAO, 2010; Lenka *et al*., 2010; Rath *et al*., 2014; Daniel, 2016). *Bassia latifolia,* commonly known as mahua, belongs to Sapotaceae family, grows luxuriantly in the subtropical region of the Indian subcontinent (Jayasree *et al.,* 1998). As a forest product, mahua seed is collected in the unorganised livelihood sector, by tribals in India (Kulkarni *et al.,* 2013; Mishra and Pradhan, 2013; Verma *et al.,* 2014). The seed contains about 40% fat in the form of oil, mostly used in soap industry and the oil cake (expeller) is generally used as manure for horticultural crops (Ramadan *et al.,* 2016). According to Feedpedia (2016), the potential mahua oil cake (MOC) production in India is estimated at over 150 million t per year. Mahua oil cake is a good piscicide at 250 ppm due to presence of the metabolite saponin (mowrin) which is used to eradicate unwanted and predatory fishes during initial pond preparation in aquaculture. Saponin in the recommended concentration when absorbed through gill or mucus membrane causes hemolysis in fish (Dash *et al.,* 2013). Apart from its toxic principle, MOC is rich in macro and micro nutrients, which is not utilised rationally in the animal feed sector (Singh and Singh, 1991; Ramadan *et al.,* 2016). Very limited information is available on the use of MOC in the feed of livestock animals (Singh *et al*., 2011; Patil *et al.,* 2013; Jacob *et al.,* 2014; 2015). No published information is available so far on incorporation of MOC in fish feed. Therefore, the present investigation was carried out with the objective of evaluating MOC as a non-conventional ingredient in carp feed.

Materials and methods

Feed, fish and experimental set up

Mahua oil cake was collected from the local oil mill and analysed for its chemical composition in the National fish feed testing laboratory of ICAR-Central Institute of Freshwater Aquaculture (ICAR-CIFA) Bhubaneswar, India. MOC and other conventional ingredients such as GNOC and rice bran were milled to obtain powder. Five iso-nitrogenus (28% crude protein, CP) feeds were formulated incorporating raw MOC meal at 0% (F1), 10% (F2), 20% (F3), 30% (F4) and 40% (F5) along with GNOC, rice bran (RB) and vitamin and mineral premix (Supplevite-M, Jeco Vet Chem Pvt. Ltd, India) as co-ingredients (Table 2). Feed pellets of 2 mm were made with portable mechanical pelletiser and oven dried at 60°C. Pellets were crumbled to 0.5 mm size and stored in airtight plastic containers at room temperature.

The experiment was conducted at the wet laboratory facility of ICAR-CIFA, Bhubaneswar, India during September-December, 2014. Fifteen flow through cement tanks (300 l) with a flow rate of 0.5 l min^{-1} were used for rearing the fish. *L. rohita* fingerlings (5.25±0.2 g) were stocked in 15 tanks @ 12 no. per tank. Fish were fed *ad libitum*, twice daily at 09 00 and 16 00 hrs with the experimental diets (each diet in triplicate tanks), for 90 days with provision of continuous aeration. The unconsumed feed was siphoned out after 2 h of feeding, dried and weighed to calculate the daily feed intake. Fish were weighed at monthly intervals. Routine water quality parameters *viz*., temperature, transparency, total alkalinity, hardness, un-ionised ammonia, dissolved oxygen and pH were monitored twice a week as per APHA (1989). Proximate composition of ingredients as well as of the test feeds and initial and final carcass composition were determined. On termination of the experiment, blood was drawn from 10 fish from each dietary group by caudal vein puncture. A fraction of blood was collected in heparinised vial for whole blood hemoglobin assay and the rest was allowed to clot in normal vial for serum collection. The serum was separated by centrifugation (5000 rpm, 5 min). The whole blood hemoglobin was analysed immediately and serum was pooled treatment-wise and stored at -20°C for further analysis.

Proximate analysis

The proximate composition of MOC, experimental diets and whole body composition of the fish was determined according to AOAC (1990). Dry matter was estimated after drying the samples at 105°C for 24 h. Crude protein (CP) was determined by Kjeldahl method (Nx6.25) and ether extract (EE) by Soxhlet method using petroleum ether (boiling point 60-80^{0}C). Ash content was analysed following ignition of the sample at 550^{0}C in a muffle furnace for 3 h. Fiber content of the feed was determined using Fibertech and gross energy using Bomb caloriemeter (IKA Caloriemeter system, C5000 control).

Fatty acid analysis

Fatty acid composition of MOC was analysed. Lipids from the samples were extracted by adding (2:1 v/v) chloroform-methanol mixture containing 0.01% butylated hydroxytoluene (BHT) (Folch *et al.,* 1957). The weight of lipids was determined gravimetrically after evaporation of the solvent. Fatty acid methyl esters (FAME) were prepared by acid-catalysed transesterification of total lipids (Christie, 1982). Fatty acid methyl esters were separated by a gas chromatograph equipped with a flame-ionisation detector (Shimadzu GC-2010, Kyoto, Japan) on a DB- 25 capillary column (20 m×0.10 mm I.D., 0.10 µm J & W Scientific, Santa Clara, CA, USA). The fatty acids were identified using fatty acid methyl ester (FAME) standards. Percentage of the normalised area values of fatty acids were taken as weight percentage.

Screening for secondary metabolites

Aqueous and n-hexane extract of MOC, GNOC and rice bran was prepared and screened for major secondary metabolites *viz.,* tannin, phenol, saponin, alkaloids, glycosides and flavonoids as described by Evans (2000) and Harbone (1998).

Hematological analysis

Blood samples were analysed for hemoglobin, total protein, albumin, triglyceride, cholesterol, serum glutamic oxaloacetic transaminase (SGOT) and serum glutamic pyruvic transaminase (SGPT) using Greiner diagnostic kits (Bahlingen, Germany) as per the manufaturer's protocols. Globulin was calculated as: Globulin (g dl^{-1}) = total protein (g dl^{-1}) - albumin (g dl^{-1})

Growth, nutritional indices and survival rate

Fish were weighed at monthly intervals to ascertain the growth parameters during the experimental period of 90 days. The weight gain (%), specific growth rate (SGR), feed conversion ratio (FCR), protein efficiency ratio (PER), net protein utilisation (NPU) and survival rate (%) were calculated as mentioned below.

$$\text{Weight gain (\%)} = \frac{\text{Final weight - Initial weight}}{\text{Initial weight}} \times 100$$

$$\text{(SGR)} = \frac{\text{In final weight - In initial weight}}{\text{Days of experiment}} \times 100$$

$$\text{FCR} = \frac{\text{Feed consumed (dry weight)}}{\text{Live weight gain (wet weight)}}$$

$$\text{PER} = \frac{\text{Live weight gain}}{\text{Protein consumed}}$$

$$\text{NPU (\%)} = \frac{\text{Protein gain in carcass}}{\text{Protein intake in food}} \times 100$$

$$\text{Survival rate (\%)} = \frac{\text{Initial no. of larvae - Final no.of fry}}{\text{Initial no. of larvae}} \times 100$$

Statistical analysis

Data were analysed using one-way ANOVA (Snedecor and Cochran, 1967) and the difference between means was tested using Duncan's multiple range test (Duncan, 1955).

Results

The mean water quality parameters recorded in the experimental tanks during the feeding trial were: temperature 25-26°C; transparency 27-28 cm; total alkalinity ($CaCO_3$) 90-100 mg l^{-1}; hardness 80-92 mg l^{-1}; unionised ammonia <0.1 mg l^{-1}; dissolved oxygen 5-7 mg l^{-1} and pH 7.5-8.5. Chemical composition of all the ingredients is presented in Table 1 and that of the test feeds (F1-F5) is given in Table 2. The feeds were iso-nitrogenous with 280 g protein kg^{-1}. MOC was incorporated in the experimental feeds by replacing

Table 1. Chemical composition of mahua oil cake, groundnut oil cake and rice bran (g kg^{-1} dry matter basis)

Parameters	Mahua oil cake	Groundnut oil cake	Rice bran
Moisture	92.2±0.56	78.6±0.62	86 ± 0.48
Crude protein	234.9±0.62	421.6±0.84	122.6±0.66
Crude lipid	94.4±0.55	69.8±0.44	136.3±0.82
Crude fiber	86.0±0.11	54.2±0.46	94.4±0.88
Ash	62.3±0.25	63.6±0.68	68.8±0.44
Nitrogen free extract (NFE)	522.4±0.39	390.8±0.42	577.9±0.48
Phytochemicals			
Saponin	++++	-	+
Phenol	+++	+	++
Tannin	++	+	+
Flavonoid	+++	+	+
Alkaloid	+	-	-
Glycoside	++	-	+
Fatty acids (%)			
∑SFA	45.07±1.72	18.58±1.32	22.16±1.46
∑MUFA	42.39±0.23	45.22±1.88	37.39±1.68
∑PUFA n-6	6.72±0.11	32.41±0.86	33.18±1.82
∑PUFA n-3	0.63±0.08	0.58±0.04	2.64±0.64
Total	94.81±1.28	96.79±1.44	95.37±1.62

SFA: Saturated fatty acids, MUFA: Monounsaturated fatty acids, PUFA: Polyunsaturated fatty acids
+detected, ++ moderately detected, +++ adequately detected ++++ strongly detected, - not detected

Table 2. Composition of the mahua oil cake incorporated test diets used for feeding *L. rohita* fingerlings (g kg^{-1} dry matter basis)

Ingredients	Diets				
	F1 (0% MOC)	F2 (10% MOC)	F3 (20% MOC)	F4 (30% MOC)	F5(40% MOC)
Mahua oil cake	0	100	200	300	400
Groundnut oil cake	490	440	410	370	330
Rice bran	490	440	370	310	250
Vitamin-mineral pre-mix*	20	20	20	20	20
Chemical composition					
Crude protein	283.1±0.52^{a}	283.7±1.00^{a}	284.5±0.24^{a}	283.8±0.64^{a}	284.1±0.42^{a}
Ether extract	74.5±0.24^{a}	81.6±0.22^{b}	84.3±0.16^{c}	88.5±0.12^{d}	89.7±0.12^{e}
Crude fibre	71.1±4.48^{a}	81.9±0.24^{b}	85.8±0.14^{c}	88.1± 0.14^{d}	89.3±0.14^{d}
Ash	68.7±0.86^{a}	69.9±0.92^{a}	69.0±0.62^{a}	67.0±0.16^{a}	65.6±0.34^{a}
Nitrogen free extract (NFE)	502.6±4.52^{c}	482.9±1.94^{ab}	476.4±0.52^{ab}	472.5±0.44^{a}	471.3±0.62^{a}

*Supplevite-M (Jeco Vet Chem Pvt. Ltd, Mumbai, India). Each 1 kg of Supplevite-M contains: Vitamin A 200000 IU, Vitamin D_3 40000 IU, Vitamin B_2 0.8 g, Vitamin E 300 IU, Vitamin K 400 g, Calcium panthonate 1 g, Nicotinamide 4 g, Vitamin B_{12} 2.4 mg, Choline chloride 60 g, Calcium 300 g, Manganese 11 g, Iodine 0.4 g, Iron 3. g, Zinc 6 g, Copper 0.8 g, Cobalt 0.18 g
The data are mean±SE : Means bearing different superscripts in a row differ significantly ($p<0.05$)

GNOC at 50, 80, 120 and 160 g and rice bran at 50, 120, 180 and 240 g per kg of F2, F3, F4 and F5 feeds, respectively. Whole body composition and the growth indices along with survival rate of *L. rohita* fingerlings under different treatments are presented in Tables 3 and 4, respectively. At 0, 10, 20, 30 and 40% inclusion levels of MOC (F1-F5) survival rate did not differ significantly ($p>0.05$). Among all the dietary treatments, significantly higher ($p<0.05$) SGR (% day^{-1}) was found in F4 (30% MOC inclusion level), whereas it was similar ($p>0.05$) in F1 (10% MOC), F2 (20% MOC) and F5 (40% MOC). The FCR was significantly lower ($p<0.05$) in F4, than fish fed other diets. Similar to SGR, PER and NPU were also significantly higher ($p<0.05$) in F4 among all the dietary treatments (Table 4). The final whole body protein content was maximum in F4 and it was significantly ($p<0.05$) higher than the other MOC incorporated diet fed groups. Although there was an increase in whole body ether extract as compared to the initial value, the increase was not proportional to the increase in MOC level in the diets. The final whole body ash content differed significantly ($p<0.05$) and it showed an increasing trend from F1 to F5 (Table 3). Hemoglobin, total protein and globulin, glucose and cholesterol were significantly higher ($p<0.05$) in F4 group, and all these parameters declined in the F5 group (Table 5). No significant ($p> 0.05$) difference was observed in triglyceride levels among the different dietary treatments. There was no significant difference ($p>0.05$) in

Table 3. Whole body composition of *L. rohita* fingerling fed test feeds containing different levels of mahua oil cake for 90 days (g kg^{-1} dry matter basis)

Parameters	Initial	Diets				
		F1 (0% MOC)	F2 (10% MOC)	F3 (20% MOC)	F4 (30% MOC)	F5 (40% MOC)
Moisture	786.1±0.32	772.4± 0.24^{c}	761.5±0.33^{a}	761.7± 0.36^{a}	768.5±0.2^{b}	773.5±0.16^{d}
Crude protein	615.5±0.24	629.6±0.026^{a}	632.7±0.24^{c}	631.3±.0.82^{b}	644.2± 0.16^{d}	633.2±0.24^{c}
Ether extract	137.5±0.22	173.6±0.22^{b}	187.6±0.16^{d}	189.3± 0.25^{e}	180.6±0.24^{c}	166.2± 0.45^{a}
Ash	218.8±0.26	162.6±0.23^{a}	163.5±0.25^{b}	164.7±0.23^{c}	170.2±0.13^{d}	177.4±0.22^{e}

The data are mean±SE
Values bearing different superscripts in a row differ significantly ($p<0.05$)

Table 4. Survival, growth and nutritional indices of *L. rohita* fingerlings fed feeds containing different levels of mahua oil cake

Parameter	Diets				
	F1(0% MOC)	F2 (10% MOC)	F3 (20% MOC)	F4 (30% MOC)	F5 (40% MOC)
Survival (%)	97.2	100	100	97.2	97.2
Weight gain (g)	4.75±0.30^{a}	4.92±0.41^{a}	6.08±0.04^{b}	8.44±0.08^{c}	4.95±0.21^{a}
Weight gain (%)	90.59±5.90^{a}	93.83±7.90^{a}	114.12±2.3^{b}	160.75±1.61^{c}	94.34±4.01^{a}
SGR (% day^{-1})	0.72±0.034^{a}	0.73±0.046^{a}	0.85±0.039^{b}	1.06±0.069^{c}	0.73±0.022^{a}
FCR	2.91±0.25^{bc}	3.13±0.08^{b}	2.58±0.08^{b}	2.02±0.03^{a}	3.05±0.06^{c}
PER	1.23±0.09^{ab}	1.14±0.07^{a}	1.38±0.04^{b}	1.75±0.03^{c}	1.16±0.06^{a}
NPU (%)	18.53^{a}	17.71^{a}	19.72^{a}	26.39^{b}	16.66^{a}

The data are mean±SE. Values bearing different superscripts in a row differ significantly ($p<0.05$). SGR: Specific growth rate; FCR: Feed conversion ratio; PER: Protein efficiency ratio, NPU: Net protein utilisation. Average initial body weight (g) of the fingerlings was 5.25± 0.2

Table 5. Hemoglobin and biochemical constituents of the blood of *L. rohita* fingerlings fed feeds containing different level of mahua oil cake for 90 days

Attributes	F1 (0% MOC)	F2 (10% MOC)	F3 (20% MOC)	F4 (30% MOC)	F5 (40% MOC)
Hemoglobin (g dl^{-1})	7.85±0.23^{a}	7.91±0.33^{a}	7.77±0.27^{a}	9.23±0.03^{b}	7.69±0.05^{a}
Glucose (mg dl^{-1})	55.06±1.7^{d}	42.67±1.3^{b}	46.96±1.5^{c}	72.98±2.3^{e}	37.60±1.1^{a}
Protein (g dl^{-1})	3.84±0.02^{a}	3.96±0.05^{b}	4.08±0.03^{b}	5.12±0.06^{c}	3.75±0.01^{a}
Albumin (g dl^{-1})	2.39±0.03^{a}	2.35±0.05^{a}	2.40±0.03^{a}	2.34±0.05^{a}	2.32±0.03^{a}
Globulin (g dl^{-1})	1.45±0.02^{a}	1.61±0.02^{b}	1.68±0.03^{b}	2.78±0.03^{c}	1.62±0.01^{b}
Triglyceride (mg dl^{-1})	302.74±7.32	298.99±3.22	296.54±2.67	295.85±2.03	299.68±1.18
Cholesterol (mg dl^{-1})	186.41±1.73^{b}	175.93±1.32^{ab}	182.81±0.66^{b}	211.28±0.35^{c}	167.42±0.24^{a}
SGOT (u l^{-1})	148.63±2.17^{a}	152.74±1.03^{a}	156.77±0.53^{a}	159.23±0.52^{a}	219.16±2.34^{b}
SGPT (u l^{-1})	24.63±0.12^{a}	27.16±1.51^{a}	28.64±1.08^{a}	26.71±1.67^{a}	39.64±1.60^{b}

The data are mean ±SE. Values bearing different superscripts in a row differ significantly ($p<0.05$). SGOT: serum glutamic oxaloacetic transaminase; SGPT : serum glutamic pyruvic transaminase

SGOT and SGPT levels among groups F1 – F4 ($p>0.05$), but both SGOT and SGPT showed significantly higher value ($p<0.05$) in F5.

Discussion

The proximate composition of mahua oil cake and the toxic principle present in it have been reported by earlier workers (Singhaland Mudgal, 1984; Singh and Singh, 1991; Siddiqui *et al.,* 2004; and Chaudhary *et al.,* 2015). The crude protein content of MOC has been reported in the range of 19-30% and it is rich in many essential amino acids (Shanmugasundaram and Venkataraman, 1985; Ramadan *et al.,* 2016). Considering the fairly good amino acid profile of MOC, Singhal and Mudugal (1984) opined that MOC is comparable with GNOC except for cysteine. They also recorded higher levels of essential amino acids like lysine, valine, methionine, isoleucine and leucine in MOC compared to GNOC. The fatty acid profile of MOC has been studied in detail by several workers (Marikkar and Yanty, 2012; Kulkarni *et al.,* 2013; Munasinghe and Wansapala, 2015; Ramadan *et al.,* 2016). They reported that the seed of mahua in different climatic conditions contains fat in the range of 40-54% which is in the form of oil at ambient temperature. The total saturated and unsaturated fatty acid content varied from 40-47 and 50-53% respectively, dominated by palmitic, stearic, oleic and linoleic acids. In the present study, the proximate composition of MOC was as follows: crude protein 24%, crude lipids 9%, saturated fatty acids 45% and unsaturated fatty acids 49% which corroborates the observations of earlier workers. The anti-nutrient factors of MOC have been studied in detail in the recent past by Yadav *et al*. (2012), Mishra and Pradhan (2013), Verma *et al.* (2014) and Chaudhuri *et al.* (2015). They found saponin as the major factor with flavonoids, glycosides, tannin and alkaloids. In the present study, all these anti-nutrient factors were detected by qualitative test. Limited literature is available on the use of MOC in ruminant feed. Incorporation of raw MOC as one of the feed ingredients at 10-20% in cattle gives significantly higher growth performance (Jakhmola *et al.,* 1987; Tiwari *et al.,*1996; Singh *et al.,* 2011; Patil *et al.,* 2013; Jacob *et al.,* 2015). No literature is available on the use of MOC as feed ingredient for fish feed. Therefore, we are compelled to compare the findings of this experiment with the available literature on ruminants. In the present experiment, MOC was incorporated in the diets at 10, 20, 30 and 40% by partially replacing GNOC and rice bran. MOC incorporation at 20% (F3) and 30% (F4) resulted in better SGR (0.85 and 1.06), PER (1.38 and 1.75) and NPU (19.72 and 26.39). Similarly, the FCR was lowest (2.02) at 30% level of incorporation. Hence, incorporation of 30% MOC not only yielded significant growth performance and least FCR, but also replaced the conventional ingredients like GNOC and rice bran at 120 and 180 g kg^{-1} respectively. Ojha *et al.* (2013) and Jacob *et al.* (2015) reported better growth of crossbred calves with 10% of MOC incorporation. Tiwari *et al.* (1996) incorporated raw MOC at 20% level in the crossbred calves without affecting body weight gain. Considering the growth and economic gain, Khaing *et al.* (2015) opined that MOC could be incorporated in commercial concentrate for goat at 30% without any adverse effect on their performance. Although no literature is found on the incorporation of MOC in fish feed, Francis *et al.* (2005) reported that quillaja saponin acts as a growth promoter when incorporated in the fish feed. Recently, Serrano (2013) observed that by adding saponin to the feed, the average body weight of common carp increased significantly. Francis *et al.* (2002) opined that dietary saponin increases permeability of intestinal membrane to the digested dietary components, thereby increasing the feed utilisation efficiency. Serrano *et al.* (1998) expressed the view that dietary quillaja saponin could significantly increase the activity of amylase and trypsin in the gut as well as lactate dehydrogenease in the liver of carp. In the present study, the mahua saponin would have hastened the absorption of nutrient macromolecules across the gut and that might have optimised at 30% level of MOC incorporation. Inclusion of MOC at the next higher level (40%) reduced fish growth significantly. Chein *et al.* (2011) reported similar observation in Japanese flounder. In their experiment, enhanced growth was recorded in the feeding group where soybean saponin was incorporated at 0.8 g kg^{-1}feed, but growth was reduced at the next higher level of incorporation.

Fish receiving feed 4 (F4), which contained 30% MOC, showed significantly better hemoglobin (9.23 g dl^{-1}), globulin (2.78 g dl^{-1}) and cholesterol (211.28 mg dl^{-1}) levels in blood than the other dietary groups ($p<0.05$). These hematological parameters reduced significantly ($p<0.05$) when incorporation level of MOC was 40%. Increased SGOT and SGPT in serum is a sign of unhealthy liver. SGOT and SGPT in the present study were similar ($p>0.05$) in all the feeding groups up to 30% incorporation of MOC. These two parameters significantly increased (219.16 and 39.64 u l^{-1} respectively) when incorporation level was enhanced to 40%, which could be considered as another indication of incorporation limit. Francis *et al.* (2002) in their review discussed about hypoglycemic role of dietary saponin. They suggested that the hypoglycemic action may be due to the inhibition of glucose transport across the brush border of the small intestine. The drastic drop in blood glucose level with the diet containing 40% MOC in the present study may be due to higher dietary saponin. In India, use of MOC as

a piscicide in carp culture ponds is a common practice. The fish mortality occurs due to hemolytic effect of saponin, absorbed through gill. Das *et al.* (2013) reported the details of hematological alteration and morbidity in catla, rohu and mrigal with the application of MOC at 250 ppm in water. Rajput and Gaur (2015) recorded increased liver and muscle protein in *Clarias batrachus* when treated with lethal and sublethal doses of MOC, which they described as the sign of stress. In the present study, MOC when administered through feed in limited quantity did not manifest any toxic effect. Perhaps this is the first study which reveal that there is scope to use MOC in carp feed as a non-conventional ingredient not only to replace the conventional ingredient, but also as a growth promoter.

Acknowledgements

Authors are grateful to Dr. P. Jayasankar, former Director, ICAR-Central Institute of Freshwater Aquaculture (ICAR-CIFA), Kausalyaganga, Bhubaneswar, India for providing all required facilities to conduct the experiment.

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Date of Receipt : 17.09.2016
Date of Acceptance : 21.03.2017