Pakistan J. Zool., vol. 54(4), pp 1737-1745, 2022 DOI: https://dx.doi.org/10.17582/journal.pjz/20190531050527

Effect of Citric Acid Acidified *Moringa oleifera* Seed Meal based Diet on Minerals Absorption, Carcass Composition and Hematological Indices of *Cirrhinus mrigala* Fingerlings

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ABSTRACT

This study was conducted to evaluate the effect of citric acid (CA) treated *Moringa oleifera* seed meal (MOSM) based diet on mineral absorption, carcass composition and hematological indices in *Cirrhinus mrigala* fingerlings. Basal diet was supplemented with 0%, 1%, 2%, 3%, 4% and 5% CA resulting in the formulation of six experimental diets. Ten fingerlings were stocked in tanks in triplicate for each treatment. Feed was given at 5% live wet body weight of fingerlings for 90 days. Results showed that diet acidification with CA significantly ($p< 0.05$) improved the mineral absorption, carcass composition and hematological indices of *C. mrigala* fingerlings compared to control diet. Data shows that mineral absorption was higher ($p<0.05$) at medium levels of CA supplementation (2%, 3% or 4% levels) compared to extreme levels (1% and 5%). Maximum body crude protein and crude fat contents were observed in *C. mrigala* fed 2 % and 3% CA supplemented diets, respectively. Moreover, fingerlings fed CA acidified diets showed significant improvement ($p< 0.05$) in hematological parameters compared to control diet. Comparison of treatments showed maximum values of RBCs ($2.83\times10^{6}mm^{-3}$), WBCs ($7.76\times10^{3}mm^{-3}$), PLT (65.96), Hb (8.47 g/100ml), PCV (24.51 %), and MCV (187.11 fl) in fingerlings fed 3% CA supplemented diet. In conclusion, 3% CA acidified MOSM based diet performed better regarding mineral absorption, carcass composition and hematological indices *C. mrigala* fingerlings.

Article Information
Received 31 May 2019
Revised 30 June 2020
Accepted 01 April 2021
Available online 04 August 2021 (early access)
Published 20 April 2022

Authors' Contribution
MH conducted the trial and did analysis. SMH planned, supervised, provided research materials and prepared manuscript. RI co-supervised the research. MMS helped in statistical analysis. RI and MMS preparation of manuscript. SZHS helped in compiling the results. SH helped in chemical analysis. NA helped in collection of results and analysis. MZHA formulated fish feed and helped in data collection.

Key words
Citric acid, Minerals absorption, Carcass, Hematological indices, Fingerlings.

INTRODUCTION

Cirrhinus mrigala is one of major Indian carps commonly consumed throughout Pakistan because of its good meat quality and taste. It has wide distribution in freshwater reservoirs of Pakistan of substantial economic importance and market value (Rauf, 2015; Hussain *et al*., 2017). Supplementary feed constitutes more than 50% expenditure in carp. To meet the future requirements of food production through aquaculture, economically viablefeeds of good quality are necessary (FAO, 2012). Fishmeal is being used as a major component in the formulation of domestic livestock and aquaculture diets and also serves as a taste attractant for herbivorous and omnivorous fish species (Davis and Arnold, 2000; FAO, 2007). In the previous three decades fish meal prices have increased in real terms and are expected to be increased further with continuous growth in demand (FAO, 2016). Due to the limited supply and increased cost of fishmeal, aquaculture feed industry and research institutions have conducted a large number of studies to reduce the dependency of the aquaculture industry on fishmeal (Rana *et al*., 2009; Tacon *et al*., 2006). In order to obtain economically sustainable, environment friendly and viable production researchers are evaluating unconventional protein sources predominantly from plant products such as leaves, seeds and other agricultural byproducts due to their high protein contents (Richter *et al*., 2003; Abo-State *et al*., 2014).

Moringa oleifera plant is one of the potential plant protein sources for inclusion in aquaculture diets (Chuks *et*

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0030-9923/2022/0004-1737 $ 9.00/0

al., 2013). The leaves and pods of plant contain high profile minerals like magnesium (Mg), zinc (Zn), phosphorus (P), manganese (Mn), calcium (Ca) in trace amount, and are a good source of vitamins, amino acids, protein, beta-carotene and various phenolics (Majhi, 2013). Moringa kernel and the fat free kernel meals have 36.7% and 61.4% of crude protein, respectively.

These plant ingredients contain anti-nutritional compounds which are very bitter in taste and result in their poor acceptability to fish (Francis *et al*., 2001). Phytic acid chelates with minerals in plant seeds (Jorquera *et al*., 2008) which practically become non-available for agastric and monogastric fishes (Baruah *et al*., 2007). Phytate forms mineral-phytate complexes leading to reduced mineral bioavailability from the digestive tract and an adverse impact on carcass composition and retention of nutrients (Greiner and Konietzny, 2006).

The problem can be solved by supplementing organic acids in plant-based diets (Reda *et al*., 2016; Hussain *et al*., 2017). Citric acid (CA) is one of the organic acids with high buffering capacity and unique flavor, which has been widely used in diets of fish (Hossain *et al*., 2007). It also increases the efficacy of exogenous as well as endogenous phytases by providing an optimum gut pH. Besides, it acts as an antimicrobial agent and stimulates feeding in fish (Shah *et al*., 2015a). Significantly higher mineral absorption has been revealed in broiler chicks (Boling-Frankenbach *et al*., 2011), *L. rohita* fingerlings (Baruah *et al*., 2007) and *C. mrigala* fingerlings (Hussain *et al*., 2018) fed CA acidified diets. The present study was designed to study the impact of CA supplementation in *Moringa oleifera* seed meal (MOSM) based diets on minerals absorption, carcass composition and hematological indices of *C. mrigala* fingerlings.

MATERIALS AND METHODS

Procurement of fish and experimental conditions

C. mrigala fingerlings were procured from Government Fish Seed Hatchery, Faisalabad. Before the start of experiment, fingerlings were bathed in NaCl (5g/L) solution for specific time period to disinfect them. V-shape like water tanks were designed especially for the collection of fish fecal material. Fingerlings were acclimatized in these tanks for two weeks during which they were fed basal diet once in a day to apparent satiation (Allan and Rowland, 1992). Water quality parameters like pH, dissolved oxygen and temperature were recorded on daily basis. Tap water was used throughout the experiment.

Processing of feed ingredients and experimental diets

Feed ingredients were purchased from local commercial feed market. Before the formulation of the experimental diets standard methods (AOAC, 1995) were used to analyze ingredient chemical composition (Table I). After fine grinding, feed ingredients were passed through (0.5mm) mesh size, mixed in a food-mixer for 5 min and fish oil was added gradually. One control diet (0% CA) and five test diets with 1%, 2%, 3%, 4% and 5% CA were prepared, respectively, using MOSM as main test ingredient. Diets were blended with water in food-mixer to form suitable dough and subsequently pellets (Lovell, 1989).

Plan of feeding and sample collection

Ten fingerlings of *C. mrigala* stocked in each tank were fed first at 8:00 am and then at 2:00 pm daily on their prescribed diet as of 5 % live wet body weight. The whole experiment was triplicated. After the completion of two hours feeding session, the unutilized diet was collected through the valves from each tank. The tanks were washed thoroughly to remove remaining diet particles and then water was refilled in each tank. Three hours after tanks washing, fecal material was collected through valves of fecal collection pipes. Total of 5 g fecal material was collected from each tank until the completion of 90 days feeding period. Breakdown of fecal strings was minimized by extreme care during fecal collection to avoid nutrient

Table I.- Ingredients composition (%) of test diets.

Ingredients	Test diet-I (control)	Test diet-II	Test diet-III	Test diet-IV	Test diet-V	Test diet-VI
MOSM	35	35	35	35	35	35
Fish meal	15	15	15	15	15	15
Soybean meal	15	15	15	15	15	15
Wheat flour	17	16	15	14	13	12
Rice polish	8	8	8	8	8	8
Fish oil	6	6	6	6	6	6
Vitamin premix	1.0	1.0	1.0	1.0	1.0	1.0
Mineral premix	1.0	1.0	1.0	1.0	1.0	1.0
Ascorbic acid	1.0	1.0	1.0	1.0	1.0	1.0
Chromic oxide	1.0	1.0	1.0	1.0	1.0	1.0
Citric acid level	0 %	1 %	2 %	3 %	4 %	5 %
Total	100.0	100.0	100.0	100.0	100.0	100

MOSM, *Moringa oleifera* seed meal; Test diet-I, with 0% CA; Test diet-II-VI, with 1%, 2%, 3%, 4% and 5% CA.

Table II.- Analyzed minerals composition in MOSM based diets.

Diets/ Minerals	Citric acid levels						PSE	P
	Test diet-I (control)	Test diet-II	Test diet-III	Test diet-IV	Test diet-V	Test diet-VI		
Ca	0.87	0.88	0.89	0.87	0.88	0.88	0.0384	0.9994
Na	0.0085	0.0085	0.0084	0.0085	0.0086	0.0084	0.0367	0.9952
K	1.4	1.39	1.4	1.39	1.4	1.39	0.0267	0.9976
P	2.02	2.03	2.01	2.02	2.01	2.02	0.0274	0.9981
Fe	0.046	0.047	0.047	0.048	0.047	0.048	0.0018	0.9735
Cu	0.0054	0.0056	0.0055	0.0055	0.0055	0.0056	0.0002	0.9954
Zn	0.042	0.041	0.042	0.042	0.043	0.043	0.0022	0.9912
Mn	0.024	0.024	0.025	0.025	0.024	0.025	0.0025	0.9964
Mg	0.0093	0.0092	0.0093	0.0094	0.0093	0.0094	0.0003	0.9973
Cr	0.028	0.028	0.027	0.028	0.027	0.027	0.0021	0.9929
Al	0.00064	0.00063	0.00062	0.00063	0.00063	0.00062	2.24024	0.9857

For diet treatment, see Table I.

leaching. For chemical analysis, fecal material was oven dried at 60°C, grinded completely and stored in laboratory.

Chemical analysis of feed and feces

Analyzed minerals composition in MOSM based diets is presented in Table II. Feed ingredients, experimental diets and feces samples were homogenized separately by motor and pestle and standard procedures (AOAC, 1995) were applied for analysis. For minerals estimation, diets and feces samples were digested separately in a perchloric acid and boiling nitric acid (1:2) mixture (AOAC, 1995). Distilled water used for appropriate dilution of samples and minerals contents were estimated by atomic absorption (Hitachi Polarized Atomic Absorption Spectrometer, Z-8200). Calibrated standards were prepared using commercially available standards (AppliChem® Gmbh Ottoweg4, DE-64291 Darmstadt, Germany) to estimate mineral contents. Phosphorus content was estimated calorimetrically (UV/VIS spectrophotometer) at 350nm. Sodium and potassium were estimated using Flame photometer (Jenway PFP-7, UK). Analyzed minerals composition in MOSM based diets is presented in Table II.

Estimation of chromic oxide

Chromic oxide in test diets was added as an inert marker to determine minerals absorption. After experimental diets and feces ash samples oxidation with perchloric reagent, acid digestion method (Divakaran *et al*., 2002) through UV-VIS 2001 spectrophotometer at 350 nm was used to estimate chromic oxide content.

Digestibility calculation

Standard formula (NRC, 1993) was used to determine ADC% (apparent minerals digestibility coefficients) for test diets.

$$ADC\ (\%) = 100 - 100 \times \frac{\%\ \text{marker in diet} \times \%\ \text{minerals in feces}}{\%\ \text{marker in feces} \times \%\ \text{minerals in diet}}$$

Carcass composition

Three fishes were randomly selected from each tank after the completion of experiment for proximate composition of whole fish body. Fish samples were thoroughly homogenized by mortar and pestle and analyzed by standard methods (AOAC, 1995). Samples were oven-dried at 105°C for 12 h to determine moisture contents. Crude protein (N × 6.25) was determined by micro kjeldahl apparatus whereas crude fat was determined by petroleum ether extraction method (Soxtec HT2 1045 system). Crude fiber was determined as loss on ignition of dried lipid-free residues after digestion with 1.25% H_2SO_4 and 1.25% NaOH, whereas ash was determined by sample ignition at 650°C for 12 h in electric furnace (Eyela-TMF 3100). Total carbohydrates were determined by using following formula: Total carbohydrate % = 100 - (crude protein % + crude fat % + crude fiber % + ash % + moisture %).

Hematological analysis

After the completion of 90 days experimental period fishes were tranquilized with 150 mg/1 tricane methanesulfonate solution (Wagner *et al*., 1997) for the collection of blood samples. The samples were sent to Molcare Lab, Biochemistry Department, University of

Agriculture, Faisalabad for hematological analysis. Blood RBCs and WBCc were determined with a haemocytometer with improved Neubauer counting chamber (Blaxhall and Daisley, 1973) while Hb concentrations were estimated following Wedemeyer and Yastuke (1977). Haematocrit (PCV) was estimated by the Wintrobe and Westergreen method using micro haematocrit centrifuge (Blaxhall and Daisley, 1973) and heparinized capillary tubes of 25 mm. The MCH, MCV and MCHC were also calculated by following formulae:

$$MCH = \frac{Hb}{RBC} \times 10$$

$$MCV = \frac{PCV}{RBC} \times 10$$

$$MCHC = \frac{Hb}{PCV} \times 100$$

Statistical analysis

At the end, one-way analysis of variance (Steel *et al*., 1996) was applied to analyze minerals absorption, carcass composition and hematological parameters. Differences among treatments were compared using Tukey's Honesty Significant Difference Test and considered significant at $p<0.05$ (Snedecor and Cochran, 1991). Statistical analysis was completed by Co-Stat Computer Package (version 6.303, PMB 320, Monterey, CA, 93940 USA).

RESULTS

The composition (%) of minerals in feces of *C. mrigala* fingerlings fed MOSM based diets is presented in Table III. The data shows that significantly ($p<0.05$) higher concentrations of mineral were excreted through feces by fingerlings fed the control diet. Mineral absorption (%) by *C. mrigala* fingerlings fed MOSM based diets is presented in Table IV. Significant improvement in mineral absorption was observed by the addition of CA in MOSM based diets. The data shows that various minerals were absorbed

Table III.- Analyzed composition (%) of minerals in feces of *C. mrigala* fingerlings fed citric acid acidified MOSM based diets.

Diets	Citric acid levels (%)						PSE	P
	Test diet-I (control)	Test diet-II	Test diet-III	Test diet-IV	Test diet-V	Test diet-VI		
Ca	0.48a	0.46a	0.31b	0.31b	0.35b	0.46a	0.016	0
Na	0.0048a	0.0050a	0.0034b	0.0030b	0.0034b	0.0049a	0.00016	0
K	0.75a	0.72a	0.54b	0.48b	0.53b	0.71a	0.016	0
P	1.11a	0.91b	0.70c	0.68c	0.70c	0.93b	0.017	0
Fe	0.023ab	0.025a	0.018bc	0.019bc	0.018c	0.024a	0.001	0.0003
Cu	0.0027a	0.0025ab	0.0022ab	0.0020b	0.0020b	0.0023ab	0.00012	0.0098
Zn	0.022a	0.023a	0.020b	0.020b	0.019b	0.023a	0.001	0.0423
Mn	0.013a	0.012a	0.009b	0.009b	0.010b	0.013a	0.0011	0.0624
Mg	0.0044a	0.0044a	0.0036b	0.0035b	0.0041ab	0.0046a	0.00013	0.0002
Cr	0.016a	0.015a	0.014a	0.015a	0.014a	0.014a	1.34E-	0.001
Al	0.00041a	0.00034b	0.00030c	0.00029c	0.00029c	0.00033b	0.00116	0.0005

For diet treatment, see Table I.

Table IV.- Minerals absorption (%) by *C. mrigala* fingerlings fed citric acid acidified MOSM based diets.

Diets	Citric acid levels (%)						PSE	P
	Test diet-I (control)	Test diet-II	Test diet-III	Test diet-IV	Test diet-V	Test diet-VI		
Ca	49.44d	52.34c	67.24a	66.92a	61.46b	51.72cd	0.4995	0
Na	47.37c	45.84c	62.26b	67.45a	62.30b	46.53c	0.5257	0
K	50.44d	52.43cd	64.33b	67.62a	63.73b	53.42c	0.533	0
P	49.41d	58.79c	67.51b	68.52a	66.78b	57.66c	0.4706	0
Fe	54.64b	51.43c	64.03a	63.30a	63.95a	53.11bc	0.5544	0
Cu	53.54e	58.47d	62.52bc	65.24a	64.50ab	61.78c	0.456	0
Zn	51.69c	49.36d	56.41b	55.93b	57.61a	50.31cd	0.4414	0
Mn	50.34d	53.43c	67.57a	66.85a	60.68b	54.12c	0.5137	0
Mg	55.71c	56.38bc	64.42a	65.27a	58.41b	55.07c	0.48067	0
Cr	49.45cd	48.74d	52.23ab	51.46abc	53.14a	50.43bcd	0.4725	0
Al	41.13c	50.69b	54.31a	56.51a	55.52a	51.50b	0.5447	0

For diet treatment, see Table I.

Table V.- Carcass proximate composition (%) of *C. mrigala* fingerlings fed citric acid acidified MOSM based diets.

Diets	Citric acid levels (%)						PSE	P
	Test diet-I (control)	Test diet-II	Test diet-III	Test diet-IV	Test diet-V	Test diet-VI		
Crude protein	54.75d	56.00c	60.54a	58.70b	58.34b	56.62c	0.15952	0
Crude fat	9.23d	11.47c	13.01b	13.52a	12.82b	11.73c	0.17325	0
Ash	9.34a	9.63a	8.26bc	7.61c	8.42b	9.44a	0.15539	0
Moisture	7.10a	6.42b	6.14b	5.17c	5.46c	6.40b	0.12885	0
Crude fiber	1.26a	1.18b	1.06c	1.02c	1.19b	1.24a	0.06726	0.0032
Carbohydrate	18.32a	15.31b	10.98c	13.99b	13.77b	14.57b	0.3409	0

For diet treatment, see Table I.

Table VI.- Hematological indices of *C. mrigala* fingerlings fed citric acid acidified MOSM based test diets.

Diets	Citric acid levels (%)						PSE	P
	Test diet-I (control)	Test diet-II	Test diet-III	Test diet-IV	Test diet-V	Test diet-VI		
RBC ($10^6 mm^{-3}$)	1.25d	1.57c	2.65ab	2.83a	2.54b	1.63c	0.04831	0
WBC ($10^3 mm^{-3}$)	6.73d	7.12c	7.71a	7.76a	7.49ab	7.55b	0.06075	0
PLT	54.36d	60.76c	63.76b	65.96a	63.52b	60.34c	0.09712	0
Hb (g/100ml)	6.35d	6.38d	7.23c	8.47a	8.13b	7.32c	0.06269	0
PCV (%)	21.44d	22.24c	23.60b	24.51a	23.45b	22.66c	0.16374	0
MCHC (%)	25.99e	27.70d	32.28c	33.81b	34.93a	32.12c	0.17407	0
MCH (pg)	38.64d	38.86d	42.01c	49.97b	56.84a	50.02b	0.10383	0
MCV (fl)	92.26f	103.99e	184.87b	187.11a	183.01c	173.69d	0.16843	0

Data are means of three replicates. PSE =pooled SE = √MSE/n (where MSE=mean-squared error). WBC, white blood cell; RBC, red blood cell; PCV, packed cell volume; Hb, hemoglobin concentration; PLT, platelet; MCV, mean corpuscular volume; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration.
For diet treatment, see Table I.

significantly ($p<0.05$) better at 2%, 3% or 4% CA levels. However, significantly lower mineral absorption was observed at 1% and 5% CA levels compared to other CA levels. Hence the results indicated that by increasing CA % in MOSM based diets, absorption of minerals by *C. mrigala* fingerlings also increased. Maximum minerals were absorbed at 2%, 3% or 4% CA levels while absorption of minerals decreased with further increase in CA %.

Carcass proximate composition (%) of *C. mrigala* fingerlings fed MOSM based diets is presented in Table V. Inclusion of CA in MOSM based diets caused significant variation in body proximate composition of *C. mrigala* among different treatments. Maximum body crude protein (60.54%) and crude fat (13.52%) contents were observed in *C. mrigala* fed 2% and 3% CA supplemented diets, respectively. Whereas, *C. mrigala* fed a 3% CA supplemented diet showed minimum ash, moisture and crude fiber in their body proximate composition. The results revealed maximum retention of crude protein and crude fat by *C. mrigala* at 2% and 3% CA levels, respectively in MOSM based diets.

Fingerlings of *C. mrigala* fed CA acidified MOSM based diets showed significant improvement ($p<0.05$) in hematological parameters compared to the control diet (Table VI). Comparison of means showed that maximum values of RBCs (2.83×10^6 mm^{-3}), WBCs (7.76×10^3 mm^{-3}), PLT (65.96), Hb (8.47 g/100ml), PCV (24.51%), and MCV (187.11 fl) were observed in fingerlings fed 3% CA acidified MOSM based diets. However maximum values of MCHC (34.93%), MCH (56.84 pg) were observed in fingerlings fed a 4% CA acidified MOSM based diet. Minimum values of above said hematological parameters were observed in fingerlings fed the control diet.

DISCUSSION

Phytate present in plant feed ingredient chelates with minerals and makes them unavailable to fish by reducing their availability and absorption (Hussain *et al*., 2011a). Dietary CA inclusion in plant based diets enhances the activities of intestinal digestive enzymes (Shah *et al*.,

2015b) which helps in releasing minerals from the phytic acid complex (Baruah *et al*., 2007) and thus enhances dietary minerals absorption by fish (Sarker *et al*., 2005). The results of present study also revealed that *C. mrigala* fingerlings fed MOSM based diets supplemented with CA excreted significantly lower minerals through feces compared to control diets. Supplementation of CA in MOSM based diets significantly enhanced Ca, Na, K, P, Fe, Cu, Zn, Mn, Mg, Al and Cr absorption by *C. mrigala* fingerlings compared to control diets. These results coincide with the findings of Rabia *et al*. (2017) who reported improved absorption of P, Na, K, Ca, Mg, Cu, Zn, Mn and Fe in the body of fish fed CA supplemented diets as compared to control diet. Highest absorption of Ca (67.24 %), Fe (64.03 %) and Mn (67.57 %) was observed at 2% CA level. Whereas significantly higher ($p<0.05$) absorption of Na, K, P, Cu, Mg and Al was observed at 3% CA level. In agreement to our results Baruah *et al*. (2005) also reported significantly better mineral absorption by *L. rohita* fingerlings fed 3% CA acidified diet. Baruah *et al*. (2007) reported that dietary CA supplementation at 3 % significantly enhanced the absorption of Na, K, P, Fe, Mg, Mn, Ca, N and Cu. Khajepour and Hosseini (2010, 2011 and 2012) reported significant increase in Ca and P content of muscle and serum when fed 2% or 3% CA supplemented diets. Hisano *et al*. (2017) also reported relative improvement in Ca and P in Pacu juveniles fed 3% CA acidified diet compared to control diet. In contrary to our results Sarker *et al*. (2007) reported that supplementation of 1% CA was adequate in retention of nutrient and keeping aquatic loading levels low. Moreover, Zhu *et al*. (2015) reported no significant impact of CA on minerals absorption by juvenile yellow cat fish. However, absorption of particular nutrient may be species specific and also depend on the feed ingredients used. This area needs further research (Baruah *et al*., 2007).

Fish flesh is considered a better protein source than eggs, milk, cereals and other animal proteins because of balanced fatty acid and amino acid profiles along with essential minerals (Hussain *et al*., 2011b). Through proximate analysis scientist monitor the health and physiological condition of fish (Saliu *et al*., 2007; Aberoumad and Pourshafi, 2010). Results of present study revealed significant improvement in body proximate composition of *C. mrigala* fingerlings fed CA supplemented MOSM based diets compared to control diet. By the addition of CA, crude protein and crude fat contents increased while moisture, ash, crude fiber and carbohydrate contents decreased. In agreement to present study Reda *et al*. (2016) also reported significant improvement in carcass composition of Nile tilapia fed acidified diet. Nuez-Ortin (2011) reported that Nile tilapia retained significantly higher protein and fat when fed acidified diets. In contrary to our results, Sarker *et al*. (2007) and Zhu *et al*. (2015) reported no significant effect of CA on body proximate composition of red sea bream *Pagrus major* and yellow catfish *Pelteobagrus fulvidraco*, respectively. This contradiction in results of various scientists may be due to the different feed composition, method of feed formulation, ecological variables and species difference and therefore needs further exploration.

Hematological studies are least studied in fish, necessary to access fish health and to check the quality of formulated diets (Schutt *et al*., 1997; Shahzad *et al*., 2016). The results of present study revealed no adverse effect of CA acidified MOSM based diets on hematological indices of *C. mrigala* fingerlings. Acidification of MOSM based diets with CA improved growth and nutrients availability to fish from diet which in turn also improved hematological indices. The results showed the safe use of CA in the diet of *C. mrigala* fingerlings to improve body status. The improvement in hematological indices may be attributed to the liberation of, Fe P, Ca and Cu from MOSM based diets by acid supplementation (Khajepour and Hosseini, 2010, 2012). The results are in agreement with Kubena (1996) and Baruah *et al*. (2009) who also reported positive impact of nutrients availability on fish hematology and immune system although no effect of CA on RBCs count was observed (Baruah *et al*., 2009). Whereas, positive effect of dietary acidification on fish blood WBCs, RBCs, platelets, Hb, MCV and MCH counts is also reported by Reda *et al*. (2016). Most of the hematological indices of *C. mrigala* fingerlings showed significant improvement at 3% CA level. In agreement to our study Baruah *et al*. (2009) and Khajepour *et al*. (2011) also reported significantly ($P<0.001$) improved blood Hct and Hb in fish fed 3% CA added diet.

CONCLUSION

In conclusion CA supplementation in MOSM based diet caused significant improvement in overall minerals absorption, carcass proximate composition and hematological indices of *C. mrigala* fingerlings. These parameters showed significantly better improvement at 3% CA level. Hence, 3% CA acidified MOSM based diet is recommended for better minerals absorption and carcass proximate composition of *C. mrigala* fingerlings without any negative impact on fish hematological indices.

Statement of conflict of interest

The authors have declared no conflict of interests.

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