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 (2.83×10⁶/mm³), WBCs (7.76×10³/mm³), 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.

**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 viable feeds 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 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 the 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

| 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            | 15           | 15          | 15           |
| 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       | 100.0        |

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

\[
\text{ADC} = \frac{100 - \left( \frac{\% \text{ marker in diet}}{\% \text{ marker in feces} \times \% \text{ minerals in diet}} \right) \times \% \text{ minerals in feces}}{100}
\]

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% H₂SO₄ 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 WBCs 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:

\[\text{MCH} = \frac{\text{Hb}}{\text{RBC}} \times 10\]
\[\text{MCV} = \frac{\text{Hb}}{\text{PCV}} \times 10\]
\[\text{MCHC} = \frac{\text{Hb}}{\text{PCV}} \times 100\]

**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  | 0.010b  | 0.010b  | 0.013a  | 0.001  | 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-1 | 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                 | 58.37c  | 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.

**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...
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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       |

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 (%)  | 25.99c                 | 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 (Salih 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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