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Growth, Muscle Proximate Composition and Whole-Body Nutrient Status of *Labeo rohita* Fed Acidified and Phytase Pre-Treated Sunflower Meal Based Diet

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MA and MF designed the experiment, supervised the study and wrote manuscript. AQ and MF conducted the experiment and performed post-trial analysis. SZHS helped in feeding trial execution, statistically analyzed the data and finalized the manuscript.

Key words
Body mineral status, Citric acid, Feed performance, Proximate composition, Phytase pretreatment.

ABSTRACT

Present study was conducted to evaluate the effect of citric acid (CA) and phytase (PHY) pre-treatment on growth, muscles proximate composition, whole body proximate composition and minerals status of *Labeo rohita* juveniles. Four experimental diets were prepared by supplementing citric acid (0 and 2%) and phytase (0 and 1000 FTU/kg) in 2×2 factorial arrangements. Total 180 juveniles (3.45±0.013) were distributed to 12 tanks having triplicate tank for each test diet. Weight gain of each replicate was recorded on weekly basis. Results showed that growth of *L. rohita* juveniles was significantly improved by the supplementation of CA and PHY. Improved (p<0.05) dry mater, crude protein, crude fat, and crude ash contents in the muscles and whole body of juveniles in response to CA and PHY supplementations were observed. Again, dietary acidification with CA also improved (p<0.05) the whole-body mineralization. Moreover, PHY pretreatment also resulted in higher (p<0.05) mineral deposition in the body as compared to control group. Both supplements interacted positively (p<0.05) to enhance most of body minerals. In short, CA as well as PHY efficiently enhanced growth performance, muscle proximate composition and whole-body nutrient status of *L. rohita* when fed on sunflower meal-based diet.

INTRODUCTION

The development of aquaculture industry mainly depends upon the formulation of balanced feed (Shaheen *et al*., 2000) because it costs about 45-50% of production expenditures (Craig and Helfrish, 2002). Fishmeal is considered as vital ingredient during feed formulation to provide fundamental nutrients including essential fatty acids, amino acids, vitamins and major as well as trace minerals (Zhou *et al.,* 2004). However, due to high price, increased requirement and unstable supply of fishmeal, it is compulsory to search for other alternative protein sources (Pham *et al.,* 2008). Agricultural by-products like sunflower meal are considered most proficient sources of energy and protein (Hardy, 2000). The feed formulations having plant protein sources are assumed to be environmental friendly due to less P contents and economical aqua feeds (Cheng and Hardy, 2002).

Sunflower meal (SFM) is nutritionally important feed ingredient containing up to 40% protein contents which depends on the dehulling techniques and oil mining methods (Mushtaq *et al.,* 2006). Its global production has been increased for the past few years which renders the need of a highly suitable alternate plant based protein source in fish diet for future accessibility. The one major problem related to use of plant protein is the occurrence of anti-nutritional factors. Anti-nutrients have been defined as substances which by themselves, or through their metabolic products arising in living systems, reduce feed utilization, imbalance the amino acid profile and affect the health and production of animals (Makkar, 1993).

Phytic acid or phytate (myo-inositol-1, 2, 3, 4, 5, 6-hexakisphosphates) is the most common anti nutrient present in plant seeds (Francis *et al.,* 2001) that contains about two-third of the total P in plants. However, fish is unable to utilize phytate-P (Usmani and Jafri, 2002) and this unused phytate bound P is excreted out and can contribute to the eutrophication of water body. Phytate forms the insoluble chelated complexes with essential minerals such as Zn, K, Mn, Ca, Cu, Na, Fe and Mg

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(Knuckles *et al.,* 1989). It also complexes with proteins and vitamins and reduces their absorption and utilization. Furthermore, it interferes with lipid and starch digestibility (Cosgrove, 1966). There are several ways to release these bound nutrients from phytate including, heat treatment, fermentation but more important are chelation with an organic acid and enzyme treatment. Agastric fishes lack the acid secretions as well as enzyme to hydrolyze phytate to release the bound nutrients (Storebakken *et al.,* 2000).

Organic acids are used in feed to physically degrade phytate complex and to release chelated nutrients including bound P (Zyla *et al.,* 1995). It has been reported that organic acids are efficiently used in aquaculture to improve the disease resistance, growth performance and nutrients utilization (Ng and Koh, 2011; Koh *et al.,* 2014) due to this phytate hydrolyzing capability. Citric acid (CA) may also work as chelating agents that bind with various cations in the intestine and make them easy to be absorbed (Ravindran and Kornegay, 1993). It was observed that CA addition in fish diet improved utilization of P and other minerals (Sugiura *et al.,* 2001).

Use of hydrolase enzymes is another technique widely used in fish nutrition to degrade phytate. Microbial phytase (PHY) is a hydrolase enzyme capable of hydrolysis of phytate results in the release of bound nutrients. It also have the potential to increase the absorption of minerals (Cheng and Hardy, 2003; Yoo *et al.,* 2005). Studies have shown the effectiveness of PHY in improving P availability from phytate leading to enhanced growth of several species including carps (Schafer *et al.,* 1995; Sardar *et al.,* 2007; Baruah *et al.,* 2007a; Phromkunthong *et al.,* 2010). Phytase supplementation also resulted in increased body protein level in *Oreochromis niloticus*, fed plant meal based diet (Olusola and Nwanna, 2014). Improved P and other mineral contents in the body in response to PHY supplementation was reported in Atlantic salmon (Carter and Sajjadi, 2011), bible carp *Carassius auratus gibelio* (Liu *et al.,* 2011), rainbow trout (Dalsgaard *et al.,* 2009) and olive flounder (Pham *et al.,* 2008).

Like all other enzymes PHY work efficiently under specific conditions, most importantly at pH ranges of 5.0-5.5 and 2.5. Citric acid not only solubilize phytate but also provides optimum pH for PHY action (Ravindran and Kornegay, 1993). An interaction between PHY and CA was reported for bone ash and mineral contents in *Labeo rohita* juveniles (Baruah *et al.,* 2005). The addition of 5% CA and PHY in the diet of rainbow trout, *Oncorhynchus mykiss* also significantly increased the apparent absorption of Mg and P (Sugiura *et al.,* 2001). Significant interaction between CA and PHY has been found on the specific growth rate, protein efficiency ratio and weight gain in *L. rohita* (Baruah *et al.,* 2007a). The nutritional value of diet was increased when it is supplemented with CA and PHY (Forster *et al.,* 1999). Both the supplements also enhanced the uptake and utilization of nutrients and Na, Mn, K, P, Cu, Mg and Zn in rainbow trout (*Oncorhynchus mykiss*) (Cheng and Hardy, 2003) and Korean rockfish *Sebastes schlegeli* (Yoo *et al.,* 2005). Phytase and CA collectively showed the potential to reduce phosphorus discharge in Atlantic salmon, *Salmo salar* (Sajjadi and Carter, 2004), rainbow trout *Oncorhynchus mykiss* (Vielma *et al.,* 2002) and *Cyprinus carpio* (Phromkunthong *et al.,* 2010).

Previous work conducted in our laboratory (Akram *et al.,* 2016; Shah *et al.,* 2015a, b, c, 2016; Afzal et al., 2019) reports the dietary benefits of CA and PHY post-treatment (top spraying after pellet formation). The present experiment was planned to study the effect of CA and PHY pre-treatment on growth, muscle proximate composition and body nutrient profile of *L. rohita* when fed sunflower meal-based diet.

MATERIALS AND METHODS

The present experiment was conducted in Fish Nutrition Laboratory, Department of Zoology, Wildlife and Fisheries, University of Agriculture, Faisalabad, Pakistan.

Experimental diets and design

A 60 days 2×2 factorial experiment was conducted under completely randomized design to examine the effects of CA and PHY pre-treatment on growth, muscles proximate composition and whole body status of *L. rohita* juveniles. Four SFM based experimental diets were formulated. All diets were supplemented with CA (%) and PHY (FTU/kg) at the level of 0,0; 2,0; 0,1000 and 2,1000, respectively. SFM1 was incubated without supplementation of CA and PHY, SFM2 was incubated with 2% CA supplementation, SFM3 was incubated with the supplementation of 1000 FTU/kg PHY, while, SFM4 was incubated with both supplements *i.e.* 2% CA and 1000 FTU/kg PHY. The process of pre-treatment of the diets was as follows: 1 kg of the finely ground (0.05 mm) ingredients, CA and PHY were mixed with 1.5 L of distilled water to form paste. The paste was first incubated at 40°C for 15.5 h and later oven dried at 60°C for 12.5 h. This dried paste of ingredients was again blended into powdery form (Nwanna *et al.,* 2008). Again, dough for pelleting was made by adding distilled water (15%) and pellets of 2 mm were prepared by hand pelletizer, blow dried and stored at -20°C throughout the experimental trial. Phytase solution was prepared by dissolving 2 g of powder microbial PHY in 1000 ml of distilled water (Robinson *et al.,* 2002). One FTU is the phytase activity unit that liberates 1 µmol of inorganic orthophosphate/min from 5.1 mmol/L substrate (sodium phosphate) at 5.5 pH

and 37°C temperature (Engelen *et al.,* 1994). Composition of experimental diets is mentioned in Table I.

Table I.- Ingredient and proximate composition (%) of sunflower meal based diet.

Ingredients	SFM1	SFM2	SFM3	SFM4
SFM	65	65	65	65
Wheat flour	14	12	13.95	11.95
Rice polish	10	10	10	10
Fish meal	5	5	5	5
Soybean oil	3	3	3	3
Vitamin premix[1]	1	1	1	1
Mineral mixture[2]	1	1	1	1
Ascorbic acid	1	1	1	1
Citric acid	0	2	0	2
Phytase	0	0	0.05	0.05
Phytase (FTU/kg)[3]	0	0	1000	1000
Total	100	100	100	100
Proximate composition				
Dry matter	97.59±0.04	97.51±0.05	97.94±0.20	97.67±0.10
Crude protein	33.93±0.73	34.62±0.84	34.06±0.52	34.44±1.0
Crude fat	10.81±0.41	11.17±0.06	11.11±0.25	11.26±0.33

[1]Each kg of vitamin premix contains Vitamin A (15 MIU) Vitamin D_3 (3 MIU) Vitamin E (6000 IU), Vitamin K (4000 mg), Vitamin B_1 (5000 mg), Vitamin B_2 (6000 mg) Vitamin B6 (4000 mg), B_9 (750 mg), Vitamin B_{12} (9000 ug), Calcium pantothenate (10000mg),Vitamin C (15000mg), Nicotinic acid (25000mg). [2]Each kg of mineral mixture contains; Ca (Calcium) 155 gm, P (Phosphorous) 135gm, Mg (Magnesium) 55gm, Na (Sodium) 45gm, Zn (Zinc) 3000 mg, Mn (Manganese) 2000 mg, Fe (Iron) 1000 mg, Cu (Copper) 600 mg, Co (Cobalt) 40 mg, I (Iodine) 40mg, Se (Selenium) 3mg. [3]The 0.05 g of PHY provides 1000 FTU, where, the FTU is one phytase activity unit that liberates 1 µmol of inorganic orthophosphate/min from 5.1mmol/L substrate (sodium phosphate) at 5.5 pH and 37 ○C temperature (Engelen *et al*., 1994).

Experimental conditions and fish feeding

Labeo rohita juveniles were obtained from Government Fish Seed Hatchery, Faisalabad. The juveniles were stocked in V-shaped tanks with proper aeration to acclimatize to the laboratory conditions. During acclamation period the fish were fed once daily on basal diet (Allan and Rowland, 1992). Before beginning of experiment, *L. rohita* juveniles were given the prophylactic dip in NaCl solution (5g/L) to avoid the fungal infection and to remove the ectoparasites (Rowland and Ingram, 1991). A group of 15 fish (initial weight, 3.45±0.013) were randomly stocked in each experimental tank and fed twice a day, 6 days a week. Triplicates tanks were allotted to each experimental treatment. Fish were initially fed a ration equal to 2% of their live wet weight which was adjusted according to apparent satiation of fish throughout the feeding trail. Fish were fed for three h and then remaining diet was recollected to determine feed conversion ratio (FCR). After that, fish were moved to clean water tanks, experimental tanks were washed and refilled with filtered fresh water. Aeration was provided to all the tanks round-the clock through capillary system. Important water quality variables were monitored constant *i.e.* dissolved oxygen in the range of 5.8-7.3 mg/L by using D.O. meter (Jenway, Model 970), pH in range of 7.4-8.6 and temperature in the limit of 24.9-28.7°C by using pH meter (Jenway, Model 3510).

Sample collection and chemical analysis

Fish of each experimental tank were bulk weighed at the initiation of feeding trial and weight gain was recorded weekly to determine growth performance. The chemical analyses of feed, muscles and whole body fish were performed according to the standard methods (AOAC, 2000). At the end of feeding trial fish were starved for 24 h,10 fish from each tank were collected, anesthetized by immersing in 3000 mg/L clove oil solution for 40-60s and sacrificed by sharp blow on head. Five fish were minced as such and used for whole body analysis while muscles of other 5 fish were separated and analyzed for muscle proximate composition. The proximate composition of diet, muscles and whole body was determined as follows: dry matter contents by oven drying at 105°C for 12 h; crude protein (N_2 ×6.25) was estimated by micro Kjeldahl method; crude fat contents were determined by petroleum ether extraction method through Soxtec HT2 1045 system; crude ash was measured by igniting the sample at 650°C for 12 h in electric furnace (Eyela-TMF 3100) to a constant weight. For mineral estimation the samples of whole body were digested in a boiling nitric acid and percholoric acid mixture (3:1). After appropriate dilution, Ca, Mg, Cu, Fe, Mn and Zn were measured by atomic absorption spectrophotometer (Hitachi Polarized Zeeman AAS, Z-8200, Japan). The P was analyzed colorimetrically by UV/VIS spectrophotometer (Shimadzu, UV 265 FW, Kyoto, Japan) at 750 nm wavelength. The Na and K were analyzed by using flamephotometer (Jenway PFP-7, UK).

Statistical analysis

The main and interaction effects of CA and PHY were calculated by applying two way analysis of variance on obtained data. The results were considered significant at $p<0.05$ (Snedecor and Cochran, 1991). As only two levels of each additive (0 and 2% for CA and 0 and 1000 FTUkg^{-1} for PHY) were used, the significant or non-significant response of these factors and their interaction for observed

responses can be clearly confirmed by the *p*-value of two way analysis of variance. Therefore, no post hoc test was applied. CoStat computer package (Version 6.303, PMB 320, Monterey, CA, 93940 USA) was used for statistical analyses.

RESULTS

The growth performance of *L. rohita* as a result of dietary CA and PHY supplementation is shown in Table II. Citric acid addition in feed improved weight gain percent (WG%), specific growth rate (SGR), and feed conversion ratio (FCR) by37.91%, 19.85%, and 9.091%, respectively. Similarly, supplementation of 1000 FTU/kg phytase also increased WG%, SGR, and FCR by, 38.23, 19.85, and 10.20% when compared to control diet. Citric acid and PHY interacted positively and resulted in increased (p<0.05) SGR by 19.85%, FCR was decreased by 9.09% as compare to control diet, while non-significant interaction among additives was observed for WG%.

Muscle proximate composition of *L. rohita* was significantly (p<0.05) improved by supplementation of CA in feed (Table III). Citric acid improved dry matter, crude protein, crude fat and crude ash contents of muscles upto 4.20, 9.14, 11.47 and 23.82%, respectively. Similar improvement in dry matter, crude protein, crude fat and crude ash upto 5.99%, 11.78%, 15.36% and 23.82% were observed as a result of phytase pre-treatment of feed. Although both supplements showed the positive interaction potential to further improve the nutrient profile of muscles but this interaction effect remained non-significant.

Effects of PHY, CA and their combination on whole body proximate composition are summarized in Table IV. Fish group fed CA supplemented diet showed significantly (p<0.05) improved percentage of whole body dry matter, crude protein, crude fat and crude ash contents by 7.10%,

Table II.- Growth performance of *L. rohita* fed citric acid and phytase pre-treated sunflower meal based diets.

PHY (FTU/kg)	CA (%)	Test diets	Initial weight (g)	Final weight (g)	Weight gain %[1]	Specific growth rate[2]	Feed conversion ratio[3]	Survival rate (%)
0	0	SFM1	3.46	11.25	224.57	1.31	1.08	100
	2	SFM2	3.43	14.07	309.71	1.57	0.99	100
1000	0	SFM3	3.45	14.16	310.43	1.57	0.98	100
	2	SFM4	3.45	17.27	399.72	1.57	0.99	100
PSE				0.01	0.90	0.00	0.00	0.00
				Analysis of variance				
p values	CA			<0.05	<0.05	<0.05	<0.05	
	PHY			<0.05	<0.05	<0.05	<0.05	
	PHY×CA			<0.05	0.051	<0.05	<0.05	

The data are means of two replicates; PSE, pooled; SE = √MSE/n (where MSE= mean-squared error). [1]Weight gain(%) = (Final weight – Initial weight) / Initial weight × 100. [2]SGR = ln (Final weight – Initial weight) / day × 100. [3]FCR = Total dry feed intake (g) / Wet weight gain (g).

Table III.- Muscle proximate composition of *L. rohita* fed citric acid and phytase pre-treated sunflower meal based diets.

PHY (FTU/kg)	CA (%)	Test diets	Dry matter (g/kg)	Crude protein (g/kg)	Crude fat (g/kg)	Crude ash (g/kg)
0	0	SFM1	254.25	127.55	55.92	3.56
	2	SFM2	262.82	136.37	48.87	4.05
1000	0	SFM3	265.21	138.54	48.42	4.11
	2	SFM4	274.18	147.3	40.67	4.63
PSE			0.84	0.70	0.43	0.01
			Analysis of variance			
p values	PHY		<0.05	<0.05	<0.05	<0.05
	CA		<0.05	<0.05	<0.05	<0.05
	PHY×CA		0.82	0.10	0.44	0.10

The data are means of two replicates; PSE, pooled; SE, √MSE/n (where MSE= mean-squared error).

6.83%, 12.02% and 11.16%, respectively as compared to control group. Similarly, addition of 1000 FTU/kg PHY significantly (p<0.05) enhanced the concentration of dry matter, crude protein, crude fat and crude ash by 8.41%, 8.51%, 14.88% and 12.69%, respectively in comparison of fish fed without PHY supplemented diet. A significant (p<0.05) interaction was observed between CA and PHY for dry matter, crude fat and crude ash which had resulted in 13.89%, 27.20% and 22.08% increased nutrient contents in the fish body, respectively in comparison of their independent effects. The crude protein of whole body was not significantly affected by interaction of both supplements.

Effects of CA and PHY pre-treatment on whole body mineral status are presented in Table V. Dietary acidification with CA had resulted in improved (p<0.05) whole body Ca, Mg, Cu, Zn, Mn, Fe, P, Na and K by 10.29%, 25.64%, 14.97%, 8.73%, 16.07%, 8.45%, 21.63%, 12.76% and 14.66%, respectively. Also, supplementation of phytase (1000 FTU/kg) significantly improved the concentration of Ca, Mg, Cu, Zn, Mn, Fe, P, Na and K in whole body of fingerlings by 12.01%, 29.75%, 15.39%, 9.479%, 17.80%, 8.69%, 22.67%, 12.76% and 15.45%, respectively. However, Interaction of both the supplements caused further deposition (p<0.05) of Ca (4.39%), Cu (31.14%), Fe (29.31%), P (46.01%), Na (27.34%) and K (23.37%), while no significant interaction effect was observed for Mg, Zn and Mn.

Table IV.- Whole body proximate composition of *L. rohita* fed citric acid and phytase pre-treated sunflower meal based diets.

PHY (FTU/kg)	CA (%)	Test diets	Dry matter (g/kg)	Crude protein (g/kg)	Crude fat (g/kg)	Crude ash (g/kg)
0	0	SFM1	251.90	126.92	47.89	3.94
	2	SFM2	254.99	128.91	42.75	4.38
1000	0	SFM3	254.99	128.91	41.68	4.44
	2	SFM4	267.86	136.55	37.65	4.81
PSE			0.42	0.38	0.67	0.01
			Analysis of variance			
p values	PHY		<0.05	<0.05	<0.05	<0.05
	CA		<0.05	<0.05	<0.05	<0.05
	PHY×CA		<0.05	0.55	<0.05	<0.05

The data are means of two replicates; PSE = pooled SE = √MSE/n (where MSE= mean-squared error).

Table V.- Whole body mineral composition of *L. rohita* fed citric acid and phytase pre-treated sunflower meal based diets.

PHY (FTU/kg)	CA (%)	Test diets	Ca (mg/g)	Mg (mg/g)	Cu (ug/g)	Zn (ug/g)	Mn (ug/g)	Fe (ug/g)	P (mg/g)	Na (mg/g)	K (mg/g)
0	0	SFM1	5.83	0.40	13.12	16.77	1.04	17.87	5.47	1.28	4.25
	2	SFM2	6.43	0.51	15.09	18.24	1.20	19.38	6.65	1.44	4.87
1000	0	SFM3	6.53	0.52	15.14	18.36	1.22	19.42	6.71	1.44	4.91
	2	SFM4	6.09	0.62	17.21	19.7	1.41	23.11	7.99	1.63	5.24
PSE			0.00	0.00	0.01	0.03	0.00	0.03	0.01	0.01	0.01
			Analysis of variance								
p values	PHY		<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05
	CA		<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05
	PHY×CA		<0.05	0.79	<0.05	0.07	0.25	<0.05	<0.05	<0.05	<0.05

The data are means of two replicates; PSE = pooled SE = √MSE/n (where MSE= mean-squared error).

DISCUSSION

Citric acid supplementation in SFM based diet improved growth and feed performance in the present study. This growth improvement may owe to more P availability and utilization by *L. rohita,* which was liberated as a result of phytateP solubility at lowered gut pH provided by CA present in the diet (Cross *et al.,* 1990). Phytate breakdown also results in release of other bound nutrients and minerals as well which are responsible for increase in growth rate. Growth improvement is also perhaps due to the physiological fact that during high intake of protein rich feed or during early age of animal, the hydrochloric acid level in stomach becomes insufficient for proper activation of pepsin and other pancreatic enzymes. This problem is resolved by addition of acidifiers in diet (Eidelsburger, 1997). Increased growth performance was also recorded for red sea bream (Sarker *et al.,* 2007) and rainbow trout (Pandey and Satoh, 2008), which is in close agreement with our results.

Growth performance, in the present experiment, was also increased by PHY pre-treatment of diet. Growth increment may attribute to phytate hydrolyzing capability of phytase (Cao *et al.,* 2007). Similar to our results, improved growth performance by PHY pre-treatment of plant based diet was also observed for rainbow trout by Wang *et al*. (2009). Similarly, phytase supplementation has resulted in improved growth performance of various fish species including common carp (Sardar *et al.,* 2007; Phromkunthong *et al.,* 2010), gibel carp (Liu *et al.*, 2011), catfish (Nwanna *et al.*, 2005; Kim and Hung, 2007; Hung *et al.,* 2014), tilpia (Cao *et al.,* 2008; Tahoun *et al.,* 2009) and rohu (Baruah *et al.,* 2007a; Hussain *et al.,* 2011).

In this study both supplements interacted positively (p<0.05) to enhance growth performance of juveniles. Citric acid supplementation might have favored the activity of phytase by lowering the gut pH of juveniles in the range of its optimum activity. In contrast to our results, a non-significant interaction was observed by Zhu *et al.* (2014) between these two supplements for growth performance of yellow catfish. This discrepancy of results depends on varying fish species, physiological conditions of fish, culture conditions, processing methods and feed ingredients used for experimentation (Liebert and Portz, 2005).

In the present study, improved (p<0.05) muscles and whole body dry mater was observed by feeding citric acid supplemented diet to rohu juveniles. Increased dry mater may attribute to the fact that citric acid efficiently hydrolyzed phytate and made chelated nutrients available (Koh *et al.,* 2014). Similar to our results, increased dry matter contents in whole body of *Seriola quinqueradiata* was observed in response to citric acid supplementation as compared to control group (Sarker *et al.,* 2012). Like dry matter elevated (p<0.05) contents of crude protein in the juvenile's muscles as well as whole body were recorded by CA supplementation. Higher crude protein contents were may be due to two related factors: (i) the effect of dietary acidification and (ii) solubilization of protein-phytate complexes (Khajepour and Hosseini, 2012; Sarker *et al.,* 2012a). However, in contrast to our results, Khajepour and Hosseini (2012) analyzed no significant differences in crude protein contents of common carp, *Cyprinus carpio* fed CA supplemented diets. Sarker *et al.* (2012) also observed that CA supplementation in different dietary groups showed no significant differences in whole body crude protein contents of yellowtail, *Sariola quinqueradiata*. A decrease in muscles and body crude fat contents were observed by dietary CA supplementation in the present study. In contrast to our results, *Pagrus major* which had 1% dietary CA supplementation showed no significant difference in crude fat contents compared to other supplementary organic acids and control group (Hossain *et al.,* 2007). On the other hand, Pandey and Satoh (2008) observed significantly enhanced lipid contents in rainbow trout when fed on CA (1%) supplemented fishmeal based diet. No or negative response to CA may likely be due to low level (1%) of CA used in these studies as compare to present experiment as well as differences in feed formulation and culture species.

The dry matter contents of *L. rohita* juvenile were found significantly (p<0.05) improved, in the present study, when fed phytase supplemented diet. This owe to the fact that PHY supplementation increased bioavailability of nutrients which intern reduced the moisture contents of tissues. Similar to our findings, Denstadli *et al.* (2007) also observed increased dry matter contents with PHY supplementation in *Salmo salar*. However, there was no significant improvement in dry matter contents with PHY supplementation in different studies on olive flounder (*Paralichthys olivaceus*) (Lee *et al.,* 2008) and Nile tilapia (Liebert and Portz, 2005).

Phytate can directly react with charged groups of protein mediated by mineral cations, and thus adversely affect the bioavailability of protein (Urbano *et al.,* 2000). In the present experiment, PHY supplementation had significantly (p<0.05) improved the crude protein contents in the muscles and whole body of *L. rohita* juveniles. This might be due to the reduction of phytate-protein complexes by PHY action. Vielma *et al.* (2004) also reported positive effects of PHY supplementation on protein utilization in rainbow trout. Dephytinization of dietary phytate-protein complexes was also observed in Atlantic salmon (Sugiura *et al.,* 1998) and common carp (Schafer *et al.,* 1995) fed

PHY supplemented diets. Again, *L. rohita* muscles and whole body crude fat contents, in the present study were decreased when fed on diet supplemented with PHY. Similar decreased in whole body crude fat in response to dietary PHY supplementation has been reported for grass carp (Liu *et al.,* 2013) and Nile tilapia (Cao *et al.,* 2008). Enhanced P concentration by PHY supplemented diets is probably the main cause of decreased crude fat of whole body, as, P causes ß-oxidation of fatty acids. That's why the fish feeding on control diet (least P availability) showed maximum whole body crude fat contents (Schafer *et al.,* 1995).

In the present study, a non-significant interaction was observed for muscles proximate composition of *L. rohita*. Both supplements (CA and PHY) interacted significantly to enhance the whole body dry matter and to lower the crude fat contents while interaction for crude protein was non-significant. Dietary microbial PHY and CA may act synergistically to enhance the availability of these nutrients by hydrolyzing the phytate moiety present in plant ingredients, and as a result improving the body composition of juveniles (Baruah *et al*., 2005, 2007a, b). Similarly, Baruah *et al.* (2007a) observed an interaction between CA and PHY to improve whole body proximate composition in *L. rohita* juveniles.

Citric acid, in the present study, had enhanced (p<0.05) the minerals contents in the whole body of *L. rohita* juveniles. Dietary acidification might had reduced the pH of the experimental diet which led to gut acidification. The phytate-mineral complexes are likely to be solubilized at lower pH, resulting in the release of bound cations. Subsequent chelation of these released cations by supplemented CA might had also played important role in improving the mineralization (Shah *et al.,* 2015c). Similar findings were reported by Khajepour and Hosseini (2012) in beluga while feeding it with CA acidified diet. Improved whole body mineralization was recorded in response to dietary acidification in rainbow trout (Vielma *et al.* 1999).

Results from present study indicated elevated (p<0.05) levels of Mn, Mg, Na, Ca, Cu, Fe, P and Zn in the whole body of *L. rohita* juveniles having PHY pretreated diet. During pre-treatment PHY might had hydrolyzed the phytate-mineral complexes present in plant ingredients hence released bound P as well as other chelated minerals leading to improved whole body mineralization. Laining *et al.* (2011) also found improved minerals deposition in juvenile tiger puffer, *Takifugu rubripes* in response to PHY supplementation. Improved whole body P, Ca and Mn contents were also reported in African catfish fed diet added with PHY (Nwanna *et al.,* 2005). Similarly, in common carp, an agastric fish, PHY supplementation had resulted in increased whole body P concentration (Nwanna and Schwarz, 2007).

The present study showed synergism between supplements to improve mineral status of fish whole body. Acidification of diet with CA decreased the pH of gut and thus enhanced the effectiveness of microbial PHY (Baruah *et al.,* 2007b) leading to enhanced minerals deposition. Baruah *et al.* (2005), in *L. rohita* juveniles, also found significant interaction between CA and PHY to improve whole body mineralization. From the present study, it can be concluded that pre-treatment of CA and PHY efficiently improved growth and nutrient status of *L. rohita* by acting individually as well as in combination.

CONCLUSION

The present study concluded that pre-treatment of sunflower meal with CA and PHY improved growth performance and chemical composition of muscles and whole body. Additionally, both supplements interacted significantly positively to improve growth, whole body proximate and some mineral parameters.

Statement of conflict of interest

The authors declare no conflict of interest.

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