


Combined Effects of Citric Acid and Phytase Supplementation on Growth Performance, Nutrient Digestibility and Body Composition of *Labeo rohita* Fingerlings

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Abstract

Aquaculturists have been concentrating their efforts to design aquafeeds using agro by-products to reduce feed costs. However, the presence of a wide variety of anti-nutritional factors (ANFs) in plant materials is one of the major obstacles. For this purpose, a 2x2 factorial experiment was established to evaluate the suitability of canola meal with citric acid (CA) and phytase (PHY) supplementation for growth performance, nutrient digestibility and body composition of *Labeo rohita* fingerlings. Five isocaloric and isonitrogenous diets; D1 (control; without CA and PHY), D2 (CA 15 g/kg+PHY 1000 FTU/kg), D3 (CA 30 g/kg+PHY 1000 FTU/kg), D4 (CA 15 g/kg+PHY 2000 FTU/kg) and D5 (CA 30 g/kg+PHY 2000 FTU/kg) were fed to fish (mean initial weight 7.61±0.31 g) in triplicates for 90-days. The growth performance was significantly increased while feed conversion ratio was decreased with D3 compared to control and other CA and PHY supplemented groups (P<0.05). Furthermore, higher digestibility (%) of nutrients (dry matter, crude protein and ash) and improved whole-body composition was also exhibited by fish fed with D3 (P<0.05). Hence, the above results elucidate that supplementation of 30 g/kg CA with 1000 FTU/kg PHY could be a useful approach for improved growth performance, nutrients digestibility and body composition of *L. rohita*.

Introduction

Sustainable aquaculture necessitates the development of nutritionally balanced aquafeeds comprising of essential fatty acids (EFAs), amino acids, vitamins and minerals (Zhou et al., 2004). Whereas to meet the increasing demands of the aquaculture industry, there is a need to explore cost-effective and readily available alternative feed ingredients (Lunger et al., 2007; Gatlin et al., 2007; Pham et al., 2008; NRC 2011; Bano and Afzal, 2017). In this regard, the inclusion of plant-based feed ingredients as a protein source is capturing the attention of researchers because of their

easier availability and least cost (Hussian et al., 2011). Recently, employed fish feed ingredients among plant sources are soybean meal (SBM), rapeseed meal (RSM), canola meal (CM), cottonseed meal (CSM), sunflower meal (SFM), peanut meal (PNM), palm kernel meal (PKM), sesame meal (SM), coconut meal (CM), linseed meal (LSM) and other meals based on aquatic plants such as *Azolla* etc. (Mohammadi et al., 2016). By far, a number of studies have been undertaken to study the effect of these feed ingredients on growth performance and health parameters of various commercially valuable fish species, such as hybrid grouper (*Epinephelus lanceolatus* x *E. fuscoguttatus*), turbot (*Scophthalmus*

maximus), Senegalese sole (*Solea senegalensis*), European catfish (*Silurus glanis*), marbled spinefoot (*Siganus rivulatus*), Nile tilapia (*Oreochromis niloticus*) and red sea bream (*Pagrus major*), etc. (Faudzi et al., 2017; Bai et al., 2017; Kumar et al., 2017; Monzer et al., 2017; Boonanuntanasarn et al., 2018).

Canola meal (CM) is a promising alternative to animal proteins due to its high protein content (38-40%) and low contents of anti-nutritional factors (ANF) such as erucic acid (< 2 %) and glucosinolates (< 30 $\mu\text{mol/g}$) (McDonald et al., 1999; Naseem et al., 2006). One of the major obstacles has been the presence of a wide variety of noxious ANFs such as phytate, fibre, tannin, etc. concerning the inclusion of plant-based ingredients in aquafeeds (Baruah et al., 2004; Hussian et al., 2011). Phytate or phytic acid (Myo-inositol 1, 2, 3, 4, 5, 6-hexaphosphate) is the main phosphorous storage component with a percentage of about 80% phosphorous in plants. It tends to build insoluble complexes with minerals, protein, carbohydrates, lipids and vitamins in the guts of fish, thereby making them unavailable to the fish (Papatryphon et al., 1999; Helland et al., 2006).

In this context, the provision of exogenous enzymes in aquafeeds has been successfully employed. For instance, phytase (PHY) hydrolyse phytate-complexes hence, making phosphorus more available to fish (Baruah et al., 2007a). The PHY shows its optimum activity at two pH levels of 2.5 and 5.0-5.5 (Simons et al., 1990). Furthermore, it is not equally active in the entire digestive tract and the highest phytate hydrolysis by phytase is observed in the stomach (Yi et al., 1996). It has also been reported that dietary intake of PHY enhanced the growth performance of channel catfish, *Ictalurus punctatus* (Jackson et al., 1996; Li and Robinson, 1997), rainbow trout, *Oncorhynchus mykiss* (Roehustscord and Pfeffer 1995; Vielma et al., 1998), Korean rockfish, *Sebastes schlegelii* (Yoo et al., 2005) and rohu *Labeo rohita* (Hussian et al., 2011; Bano and Afzal, 2017).

Additionally, agastric fishes such as carps lack an acid secreting-stomach; therefore, most of the nutrients and minerals remain unavailable to fish and excreted to the environment (Cao et al., 2007). The previous studies reported that the inclusion of organic acids in feed formulations reduces intestinal pH, leads to solubilization, absorption and utilization of nutrients in the intestine (Ravindran and Kornegay 1993; Khajepour et al., 2012; Liu et al., 2014). Among various organic acids dietary supplementation of citric acid (CA) in feeds showed desirable results; particularly in the case of poultry (Brenes et al., 2003; Kopecky et al., 2012) and pigs (Jongbloed et al., 2000; Gerritsen et al., 2010). In an *in vitro* study, dietary CA inclusion has resulted in hydrolyses of phytate complexes resulting in the release of phosphorous (Zyla et al., 1995). Moreover, dietary supplementation of CA and PHY resulted in improved growth performance, nutrient digestibility and mineral bioavailability in some important fish species such as

Indian carp (Baruah et al., 2007b), red sea bream (Hossian et al., 2007), common carp (Khajepour et al., 2012) and African catfish (Kemigabo et al., 2018).

Indian major carps (IMC) especially *Labeo rohita* commonly known as rohu, is largely consumed and cultured in the Asian region due to their good taste and high market attributes (Bano and Afzal, 2017). The present study has been designed to investigate the suitability of canola meal as a dietary protein source along with the combined effects of dietary CA and PHY supplementation on growth performance, nutrient digestibility and body composition of *L. rohita*.

Material and Methods

Fish Husbandry

All the experimental and analytical works were conducted according to the guidelines of the ethical committee of the University of Agriculture, Faisalabad (UAF), Pakistan. *Labeo rohita* fingerlings were transported from Government Fish Seed Hatchery to Fish Nutrition Laboratory Department of Zoology, UAF Pakistan. Fish were given a prophylactic dip (5 g NaCl/1000 mL distilled water) for 5 minutes to avoid infections (Rowland and Ingram, 1991). During acclimatization, fish were fed once daily on the control diet for two weeks (Allan and Rowland, 1992). A total of 225 fingerlings (mean initial weight 7.62 ± 0.16 g) were randomly stocked in V-shaped steel tanks (60 cm height \times 30 cm length \times 30 cm width) at the density of 15 fish/tank (120 L water holding capacity).

Experimental Diets and Design

The raw feed ingredients were acquired from the local feed market, ground and sieved (0.5 mm). Chromic oxide was used as an inert marker at 1% to estimate nutrient digestibility coefficients (%) of experimental diets. After weighing and mixing dry ingredients (Table 1), fish oil and distilled water (10-15%) were added to acquire a suitable texture of dough. The floating pellets (3 mm) were prepared by using an extruder (Jinan Saibainuo Machinery Co., Ltd., Model no. SYSLG30-IV Experimental Extruder) and excessive moisture of pellets was reduced by air drying. A 2x2 factorial experiment was established by supplementing two different doses (15 and 30 g/kg) of citric acid (Sigma-Aldrich® W230618) and two different concentrations (1000 and 2000 FTU/kg) of microbial phytase (Phyzyme® XP 10000 FTU/g; Danisco Animal Nutrition, Fin-65101 Vaasa, Finland). Phytase (PHY) solution was prepared by adding 1000 mL of distilled water in 2g of microbial phytase (powder form). The amount of phytase that generates 1 μmol inorganic phosphorus/minute at 37°C and pH 5.5 from the concentration 0.005 mmol/L of the substrate (sodium phytate) is defined as one unit phytase activity (Tayyab et al., 2017). Spraying method was used for supplementation of phytase and five

experimental diets were prepared; control diet was set as CA 0 g/kg+PHY 0 FTU/kg named as D1 whereas, D2, D3, D4 and D5 supplemented with 15 g/kg CA+1000 FTU/kg PHY, 30 g/kg CA+1000 FTU/kg PHY, 15 g/kg CA+2000 FTU/kg PHY and 30 g/kg CA+2000 FTU/kg PHY, respectively (Table 1). Each experimental diet was given to three replicated tanks. All the diets were stored at 4 °C in airtight bags until feeding.

Feeding Protocol and Faeces Collection

Fish were hand-fed with their respective diet in two feeding sessions (morning and afternoon) at 3% body weight for 90 days. The uneaten diet particles were collected after 2 hours of feeding, dried and stored for feed consumption analysis. The faecal material was collected through a faecal collection tube from each tank separately after 02 hours of washing (Figure 1 and 2). Thin faecal strings (4-5g) were carefully collected, dried at 60 °C for 12 hours and stored separately to study the nutrient digestibility of experimental diets. Water quality variables such as temperature (27.5 ± 0.4 °C), pH (7.8 ± 0.4) and dissolved oxygen (6.5±0.07 mg/L) were monitored on daily basis through digital meters (HANNA, model HI 9147 and AMPROBE pH and DO meter). Natural photoperiod (12 hours light and 12 hours dark) was provided and aeration was maintained by a capillary system in all tanks.

Calculations

The mean initial weight (g) of fish was recorded at the beginning of the feeding experiment and weight

gain (g) was evaluated on a bi-weekly basis by weighing each tank individually. Initial length (cm) was recorded by random selection of three ($n = 3$) fish from each tank and final length (cm) was measured at the termination of the trial to calculate the condition factor. Growth parameters and feed conversion ratio (FCR) was determined by applying the following equations;

$$AWG(g) = \text{Final weight}(g) - \text{Initial weight}(g)$$

AWG: Absolute Weight Gain

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

WG: Weight Gain

$$SGR (\%) = \frac{\text{Ln}(\text{Final weight}) - \text{Ln}(\text{Initial weight})}{\text{Initial weight (g)}} \times 100$$

SGR: Specific Growth Rate

$$\text{Fulton condition factor (K)} = \frac{\text{Fish weight (g)}}{\text{Fish length (cm)}} \times 100$$

$$\text{Feed conversion ratio} = \frac{\text{Dry feed intake (g)}}{\text{Wet weight gain (g)}}$$

The apparent digestibility coefficient (ADC) for the nutrients of CA and PHY supplemented canola meal-based diets was calculated by using formula given below;

Table 1. Ingredients and nutrient composition of experimental diets

Ingredients (g/kg)	D1	D2	D3	D4	D5
Fish meal	120	120	120	120	120
Canola meal	540	540	540	540	540
Wheat flour	100	100	100	100	100
Rice polish	120	120	120	120	120
Fish oil	90	90	90	90	90
Mineral mixture ¹	20	20	20	20	20
Vitamin premix ²	20	20	20	20	20
Ascorbic acid	10	10	10	10	10
Chromic oxide	10	10	10	10	10
Analyzed nutrient composition of diets (on dry matter basis)					
MC (g/kg)	9.4±0.2	9.5±0.2	9.5±0.4	9.7±0.0	9.8±0.1
CP (g/kg)	320.4±0.7	320.4±0.4	32.3±0.3	321.8±0.5	321.7±0.2
CL (g/kg)	110.1±0.2	111.8±0.5	110.4±0.2	110.4±0.8	110.7±0.1
Ash (g/kg)	88.8±0.3	90.6±0.5	89.9±0.2	88.9±0.3	90.5±0.5
GE (kcal/g)	19.0±0.3	18.9±0.4	19.5±0.2	19.9±0.3	19.6±0.5
CA (g/kg)	0	15	30	15	30
PHY (FTU/kg)	0	1000	1000	2000	2000

¹Each 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.

²Each kg of Vitamin premix contains; Vitamin A (Retinoic acid) 5.0 mg, Vitamin B1 (Thiamine) 0.5 mg, Vitamin B2 (Riboflavin) 3.0 mg, Vitamin B3 (Niacin) 5.0 mg, Vitamin B6 (Pyridoxine) 1.0 mg, Vitamin B7 (Biotin) 0.05 mg, Vitamin B9 (Folic acid) 0.18 mg, Vitamin B12 (Cobalamin) 0.002 mg, Vitamin C (Ascorbic acid) 5.0 mg, Vitamin D3 (Cholecalciferol) 0.002 mg, Cellulose 815.26 mg, Choline 100 mg. Abbreviations: MC = moisture content, CP = crude protein, CL = crude lipid, GE = gross energy, CA = citric acid, PHY = phytase.

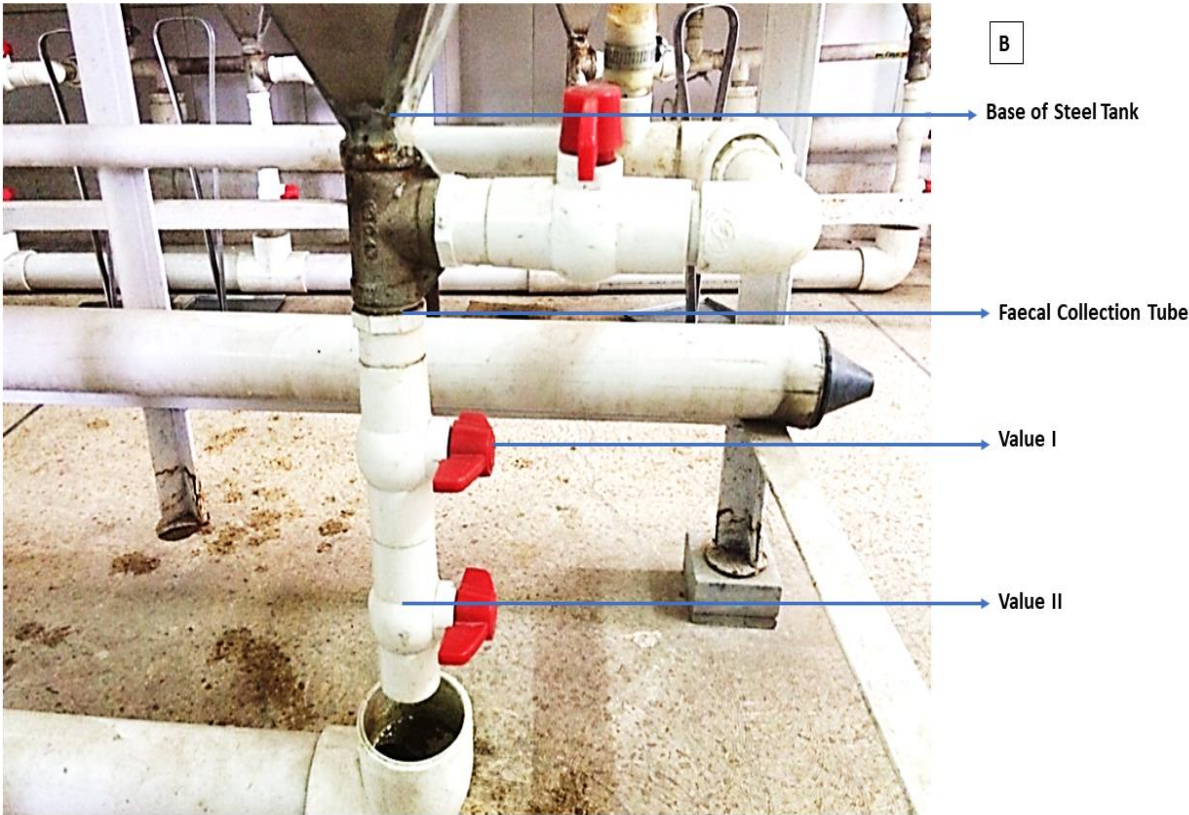
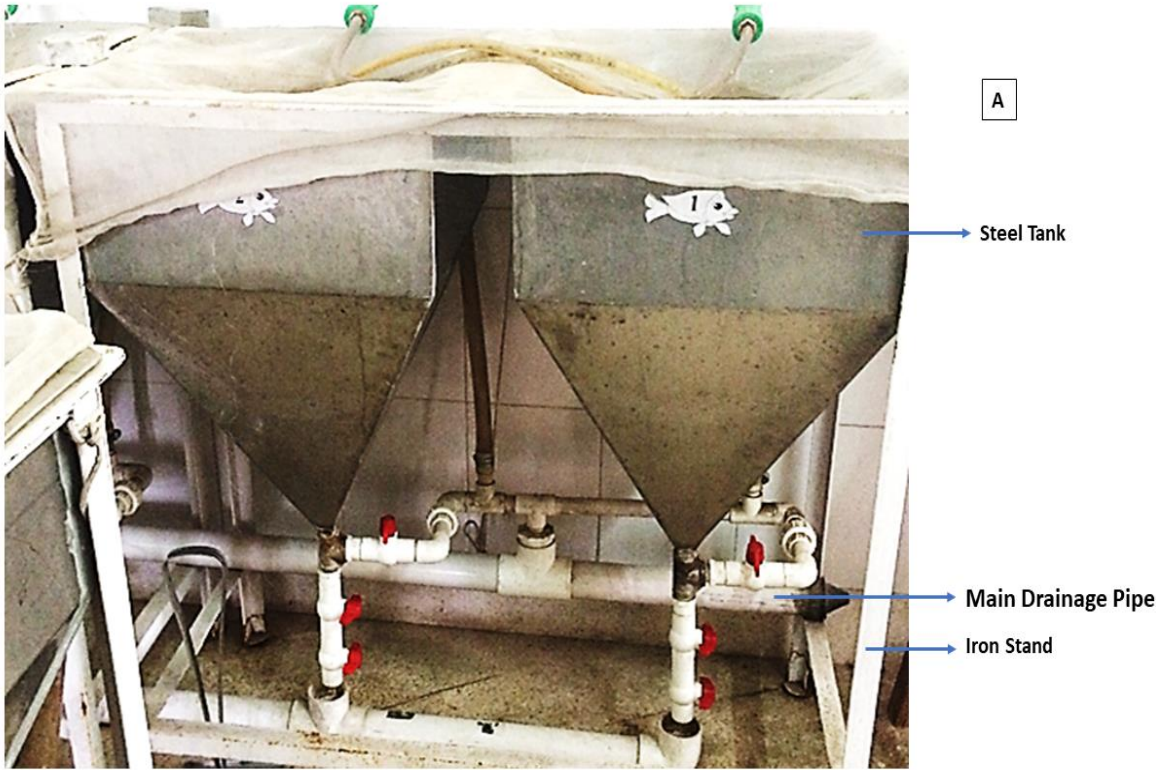


Figure 1. The outer view of (A) fish rearing steel tanks and (B) faeces collection tube

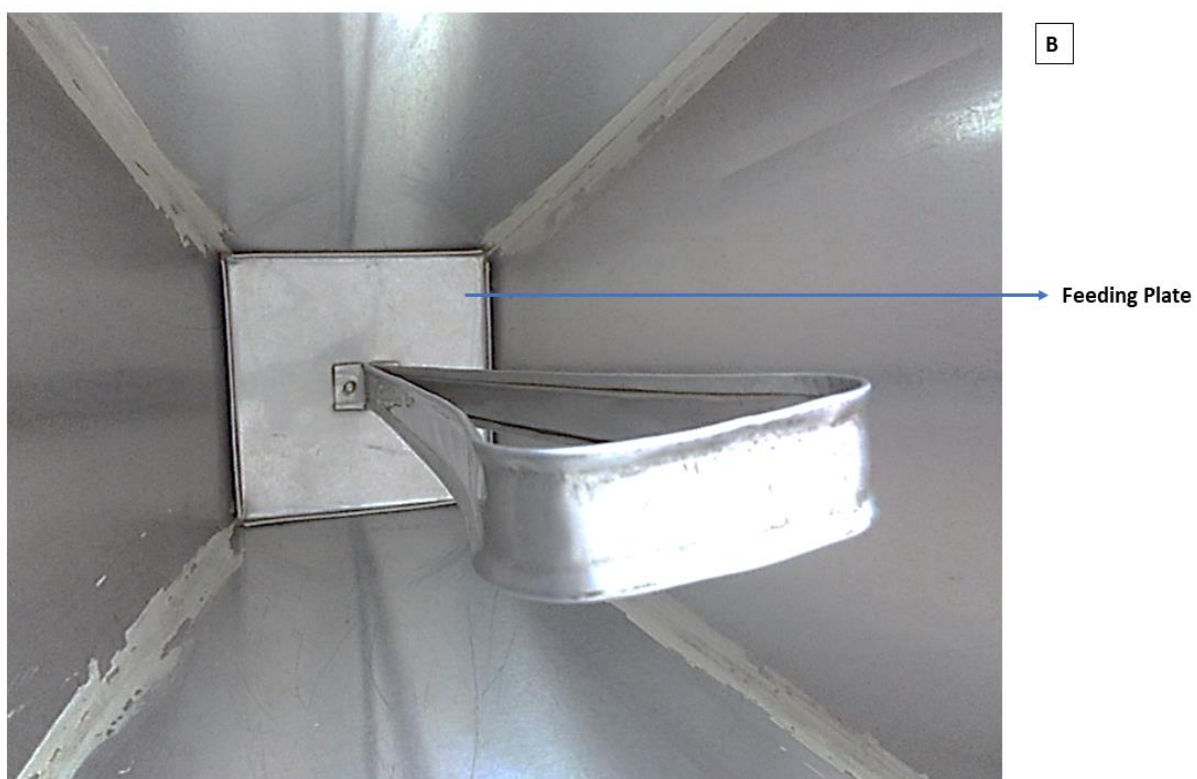
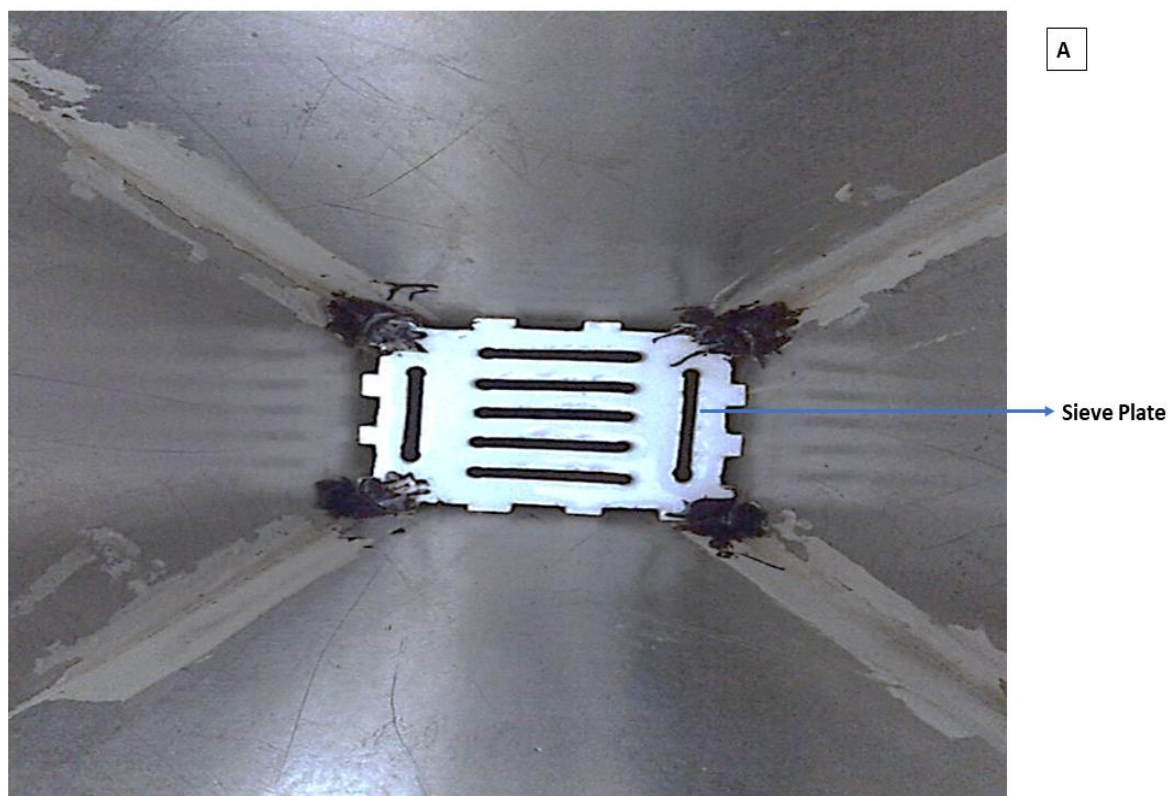


Figure 2. The inner view of fish rearing tanks with the (A) sieve plate and (B) feeding plate setup

$$\text{ADC of nutrient (\%)} = 100 - 100 \frac{\% \text{ of cod} \times \% \text{ of nf}}{\% \text{ of cof.} \times \% \text{ of nd}}$$

cod: chromic oxide in diet

nf: nutrient in feces

cof: chromic oxide in feces

nd: nutrient in diet

Proximate Composition of Diets, Faeces and Experimental Fish

The chemical composition of experimental diets, faeces and fish whole-body was analyzed (AOAC, 2006). At the termination of the growth experiment, five ($n=5$; total 15 fish/diet) fish were randomly selected from each tank and oven dried (Memmert UN30). Dry matter content was calculated by constant drying at 105°C for 6 hours. Crude protein content was evaluated by nitrogen percentage ($N_2 \times 6.25$) through the Kjeldhal method (Hanon K1100F micro- Kjeldhal auto analyzer). The ether extraction method was applied to determine crude lipid content by using the ether extraction method (Soxtec system HT2 1045) and an oxygen bomb calorimeter was used for gross energy calculation.

Analysis of Chromic Oxide

The chromic oxide contents in diets and faeces were estimated after oxidation with molybdate reagent using UV-VIS spectrophotometer (AE-S70-1U UK Co., Ltd.) at 440 nm (Divakaran et al., 2002).

Statistical Analysis

All data were collected as mean of three replicates ($n=3$) \pm standard error of mean (SE). Two-way analysis

of variance (ANOVA) was used to evaluate the effects of CA and PHY supplementation on growth performance, nutrient digestibility and whole-body composition of *L. rohita* fingerlings. Tukey's test was applied to determine the difference among the means of experimental groups and considered significant at $P<0.05$. SPSS software Version 22.0 IBM for Windows was used for all the statistical analysis of data.

Results

Growth Performance

The data of growth performance in terms of final weight (FW), absolute weight gain (AWG), weight gain percentage (WGP), specific growth rate (SGR) and Fulton's condition factor (K) of *L. rohita* fingerlings in response to citric acid (CA) and phytase (PHY) supplementation are summarized in Table 2. After the feeding period of 90-days, significant ($P<0.05$) interaction was observed for growth performance and FCR. FW, AWG, WGP, SGR and K was significantly ($P<0.05$) higher however, FCR was significantly ($P<0.05$) lower in fish fed with D3 (30 g/kg CA+1000 FTU/kg PHY) compared to D1; control (0 g/kg CA+0 FTU/kg PHY) and other CA and PHY supplemented groups (Table 2).

Apparent Nutrient Digestibility (%)

The supplementation of CA and PHY had a significant effect on apparent digestibility (%) of nutrients i. e. dry matter (DM), crude protein (CP), crude lipids (CL), and gross energy (GE) for canola meal-based diets fed to *L. rohita* fingerlings (Table 3). The apparent digestibility (%) of DM, CP, CL and GE was significantly

Table 2. Growth performance and survival (%) of *L. rohita* fingerlings fed with canola meal-based citric acid and phytase supplemented diets

Parameters	D1	D2	D3	D4	D5	CA	PYH	CA \times PHY
IW (g)	7.30 \pm 0.10	7.78 \pm 0.11	7.62 \pm 0.10	7.67 \pm 0.11	7.55 \pm 0.14			
FW (g)	12.38 \pm 0.31 ^d	16.31 \pm 0.39 ^c	23.49 \pm 0.72 ^a	15.02 \pm 0.19 ^c	18.94 \pm 0.71 ^b	0.000	0.000	0.000
AWG (g)	5.08 \pm 0.38 ^d	8.52 \pm 0.40 ^c	15.87 \pm 0.82 ^a	7.35 \pm 0.08 ^c	11.39 \pm 0.84 ^b	0.000	0.000	0.000
WG (%)	69.68 \pm 6.03 ^c	109.59 \pm 5.83 ^{bc}	208.60 \pm 13.87 ^a	95.83 \pm 0.28 ^c	151.22 \pm 13.79 ^b	0.000	0.000	0.000
SGR (% day ⁻¹)	1.79 \pm 0.08 ^d	2.37 \pm 0.05 ^c	3.06 \pm 0.05 ^a	2.21 \pm 0.01 ^c	2.69 \pm 0.08 ^b	0.000	0.000	0.000
K	1.21 \pm 0.03 ^d	1.60 \pm 0.03 ^c	2.30 \pm 0.07 ^a	1.47 \pm 0.01 ^c	1.86 \pm 0.07 ^b	0.000	0.000	0.000
FCR	2.40 \pm 0.23 ^a	1.60 \pm 0.03 ^{bc}	1.12 \pm 0.05 ^d	1.74 \pm 0.16 ^b	1.33 \pm 0.02 ^{cd}	0.000	0.008	0.000

The data are presented as mean ($n=3$) \pm standard error of mean (SE). Means within the same row having different superscript are significantly different ($P<0.05$). Abbreviations: IW=initial weight, FW=final weight, AWG=absolute weight gain, WG (%)=weight gain percentage, SGR=specific growth rate, K=Fulton condition factor, FCR=feed conversion ratio.

Table 3. The nutrient digestibility coefficient (%) of citric acid and phytase supplemented canola meal-based diets fed to *L. rohita* fingerlings

Digestibility (%)	D1	D2	D3	D4	D5	CA	PYH	CA \times PHY
DM	52.64 \pm 0.26 ^d	53.16 \pm 0.43 ^c	65.24 \pm 0.86 ^a	56.29 \pm 0.35 ^c	60.29 \pm 0.035 ^b	0.000	0.007	0.000
CP	64.74 \pm 0.55 ^d	67.64 \pm 0.93 ^c	76.86 \pm 0.58 ^a	65.48 \pm 0.47 ^{cd}	71.44 \pm 0.28 ^b	0.001	0.000	0.000
CL	61.83 \pm 0.37 ^d	64.58 \pm 0.30 ^c	72.63 \pm 0.31 ^a	64.34 \pm 0.24 ^c	69.21 \pm 0.17 ^b	0.000	0.000	0.000
GE	52.35 \pm 1.11 ^c	55.19 \pm 0.42 ^c	67.64 \pm 0.57 ^a	58.61 \pm 0.42 ^b	60.74 \pm 0.20 ^b	0.000	0.000	0.000

The data are presented as mean ($n=3$) \pm standard error of mean (SE). Means within the same row having different superscript are significantly different ($P<0.05$). Abbreviations: DM=dry matter, CP=crude protein, CL=crude lipids, GE=gross energy.

($P < 0.05$) increased in group fed with D3 (30 g/kg CA+1000 FTU/kg PHY), followed by group fed D5 (30 g/kg CA+2000 FTU/kg PHY). Whereas, significantly ($P < 0.05$) decreased nutrient i. e. DM, CP, CL and GE digestibility (%) was exhibited by control diet (0 g/kg CA+0 FTU/kg PHY) compared to other experimental groups (Table 3).

Proximate Composition of Whole-Body

The significant interaction of CA and PHY supplementation was observed for whole-body composition of *L. rohita* fingerlings, after the feeding period of 90-days. The significantly ($P < 0.05$) improved whole-body dry matter, crude protein and ash content was displayed by the group fed with D3 (30 g/kg CA+1000 FTU/kg PHY), followed by D5 (30 g/kg CA+1000 FTU/kg PHY) and significantly lowest DM, CP and ash content was observed in fish fed D1 (0 g/kg CA+0 FTU/kg PHY) (Figure 3 A, B, D). Inversely, significantly ($P < 0.05$) lowest whole-body crude lipid content was exhibited by groups fed D3 (30 g/kg CA+1000 FTU/kg PHY) however, highest crude lipid content was observed in fish fed control diet (0 g/kg CA+0 FTU/kg PHY) (Figure 3, C).

Discussion

In the present study, positive interaction was observed between CA and PHY which improved growth performance of *L. rohita*. Similar findings were reported by Khajepour et al. (2012) with common carp, *Cyprinus carpio* and Khajepour and Hosseini (2012) in beluga, *Huso huso*. The dietary acidification stimulates the activity of digestive enzymes in fish gut and optimizes the digestion and utilization of nutrients and minerals (Hussain et al., 2011a). Additionally, acidification provides favorable environmental conditions for phytase to diminish the level of phytate in digesta, thus basically inhibits the formation of phytate-protein-mineral complexes (Liebert and Portz, 2005). Citric acid has been evaluated to make stronger dephosphorylation of phytate in vitro (Sakata et al., 1995). Whereas, this increased growth with CA at 30 g/kg and PHY at 1000 FTU/kg clearly indicates that dietary supplementation of CA and PHY showed dose dependent response for growth performance in *L. rohita* in current study. Contrary, no significant interaction of PHY and CA supplementation on growth performance was reported by Zhu et al. (2015) which can be due to the suitability of stomach pH of yellow catfish. This contradictory interaction of PHY and CA on growth performance may be mainly due to different experimental fish species, which may have a stomach or not (Shah et al., 2015a).

Furthermore, significantly reduced FCR was measured with CA at 30 g/kg and PHY at 1000 FTU/kg supplementation compared to control and other CA and PHY supplemented groups in present study which is in agreement with the previous results with Indian major

carps, red sea bream, rainbow trout and yellowtail (Baruah et al., 2007a; Hussian et al., 2011a; Hussian et al., 2011b; Sarker et al., 2012). Evidences suggested that dietary intake of CA and PHY results in the availability of various nutrients such as, phosphorous and calcium which makes the diet nutrient efficient. Additionally, CA and PHY improves the epithelial proliferation and adsorption of nutrients by enhancing the pancreatic secretion in gastrointestinal tract (De Wet, 2005; Baruah et al., 2007b).

In the present study, improved digestibility of dry matter (DM) was observed with dietary supplementation of CA and PHY in *L. rohita* compared to control. However, highest digestibility of DM was observed with 30 g/kg CA and 1000 FTU/kg PHY which is supported by the findings of Phromkunthong et al. (2010) and Sari et al. (2012) in *C. carpio* and hens, respectively. CA has antimicrobial activities thus, reduces the pathogenic load in fish intestine consequently enhancing the absorption and utilization of nutrients which improved the digestibility of DM (Shah et al., 2015a). Additionally, CA improves gut health and facilitates digestion by lowering intestinal pH (Baruah et al., 2005). Moreover, phytase breakdowns the phytate-phosphorous complexes and convert tricalcium phosphate into the available form of phosphorus and other nutrients (Sarker et al., 2005; Liebert and Portz, 2005).

Similarly, increased digestibility of crude protein (CP) was observed due to the positive interaction of CA (30 g/kg) and PHY (1000 FTU/kg) in *L. rohita* which is similar to the reports of Bano and Afzal (2017) in *L. rohita* and Phromkunthong et al. (2010) in *Cyprinus carpio*. Some previous studies have suggested improved digestibility of CP with the inclusion of organic acids and exogenous enzymes (Baruah et al., 2007b; Khajepour and Hosseini, 2012). CA disrupts the cell wall of plant materials and stimulates the proteolytic enzymes activity such as pepsin and trypsin resulting in improved protein digestibility (Bano and Afzal, 2017; Hussain et al., 2017). Also, phytate present in plant material may chelate with amino acids in the stomach of fish thus, reduces the availability of amino acid. Whereas, the dietary supplementation of PHY improves the liberation of nutrients (especially amino acids) from plant materials in fish intestine (Usmani and Jafri, 2002). Though, the digestibility of nutrients is also (CA and PHY) dose dependent and species-specific function (Cao et al., 2007).

The digestibility of crude lipids (CL) was significantly increased with CA at 30 g/kg and PHY at 1000 FTU/kg compared to control and other CA and PHY supplemented groups in the present work which is in line with the findings of Ashraf and Goda (2007) and Portz and Liebert (2005) in Nile tilapia (*Oreochromis niloticus*). It has been reported that CA supplementation lowers the rate of gastric emptying by modifying gastric pH, hence improves the CL digestibility (Shah et al., 2015a). Phytase in the presence of CA improves the

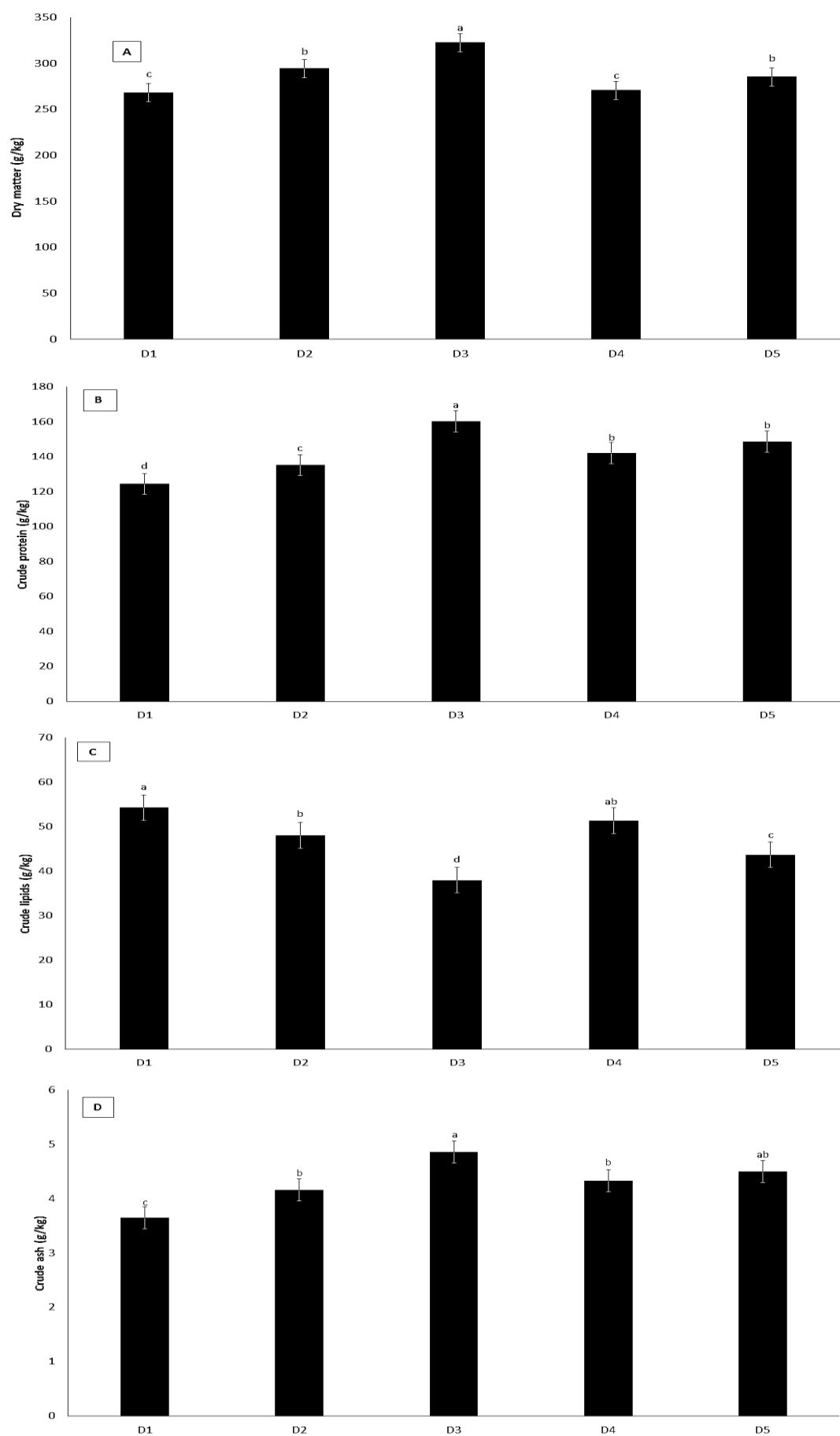


Figure 3. The whole-body dry matter, crude protein, crude lipids and crude ash (g/kg) content of *L. rohita* fingerlings fed with citric acid and phytase supplemented canola meal-based diets. Graph bars showing mean value of three replicates (n=3). The dietary treatments having different superscripts are significantly different at $P < 0.05$.

availability of phosphorous thus, it inhibits the β -oxidation of fatty acids resulting from the deficiency of phosphorous (Atapattu and Nelligaswatta, 2005; Shah et al., 2015b; Schafer et al., 1995).

Similarly, digestibility of gross energy (GE) was also significantly highest due to the positive interaction of CA at 30 g/kg and PHY at 1000 FTU/kg supplementation which is comparable to some previous reports (Boiling et al., 2000; Nourmohammadi et al., 2012; Hussain et al., 2015). CA regulates different vital energy yielding intermediary metabolic processes like ATP formation in TCA cycle, thus provides the energy to the cell (Diebold and Eidelsburger, 2006). Furthermore, metallic soap is formed by the accretion of lipids, calcium and phytate which diminishes the apparent digestibility of energy. While, hydrolyzation of phytate-nutrient complex by phytase, may not discharge the nutrients into faeces and become available to the fish thus, enhanced the digestibility of energy (Bano and Afzal 2017; Hussain et al., 2015; Gatlin III, 2000). Some studies suggested that 5% CA and 750 FTU/kg was sufficient to improve nutrient digestibility (Hussain et al., 2015). The plausible explanation of this contradiction in doses might be differences in feed manufacturing technique, plant material, enzyme sources and concentrations and difference in fish species (Mroz et al., 2000; Sarker et al., 2005; Cao et al., 2007).

The dietary supplementation of CA and PHY showed positive influence on the whole-body proximate composition of *L. rohita*, in present study. The dry matter (DM), crude protein (CP) and ash content was significantly higher however, crude lipid (CL) content was lower with 30 g/kg CA and 1000 FTU/kg PHY compared to control and other CA and PHY supplemented groups. This result is in line with the findings of Khajepour and Hosseini (2012) in *C. carpio*. CA along with the dietary supplementation of PHY is considered as a significant factor for improved uptake of dietary nutrients and minerals (calcium, phosphorous and zinc) for aquatic as well as for terrestrial cultured animals (Bano and Afzal, 2017). Thus, improved proximate composition of *L. rohita* in the present study might be result of synergistic effect of CA and PHY supplementation on fish body. Whereas, Hossain et al. (2007) found no significant effect of CA supplementation on whole-body composition of red sea bream which might be the result of difference in fish species used for study.

In conclusion, interaction of CA and PHY enhances the nutrient digestibility which leads to improved growth performance and body composition; additionally, this effect of CA was dose dependent. Hence, canola meal might be a suitable protein source for improved growth performance, nutrients digestibility and body composition of *L. rohita* fingerlings along with the dietary supplementation of CA at 30 g/kg and PHY at 1000 FTU/kg.

Data Availability Statement

The data that support the findings of the present study is already provided within the manuscript.

Ethical Statement

The experimental protocol was approved by the Ethics Committee of University of Agriculture, Faisalabad (UAF) Pakistan.

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Not applicable.

Author Contribution

Maryam Iqbal: Conceptualization, Methodology, Data Curation, Formal Analysis, Writing -review and editing; Muhammad Afzal: Supervision, Investigation, Visualization, review and editing; Atif Yaqub: Writing - review and editing; Khalid Mahmood Anjum: Resources and Analysis; Komal Tayyab: Experimentation and Methodology.

Conflict of Interest

The authors declare that they have no known competing financial or non-financial, professional, or personal conflict of interest.

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