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Original Article

Influence of Phytase with Or without Organic Acid (Sodium Di-Formate) Supplementation on Growth Performance, Carcass Response, Protein and Mineral Digestibility in Starter Phase of Broilers

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Phytase, organic acid, growth, digestibility.

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ABSTRACT

This experiment was planned to evaluate the effects of phytase supplementation with or without organic acid (OA) on performance, protein, calcium (Ca), phosphorus (P) and sodium (Na) digestibility and carcass parameters in broiler chickens fed low Ca and low P diets in comparison to a high Ca and high P diet with standard specifications. For this purpose, two iso-nitrogenous and iso-caloric diets were prepared in such a way that one diet had high Ca (9.80) and high P (4.50) and second diet had low Ca (8.50) and low P (2.40), respectively. Low Ca and low P diet was further supplemented with enzyme phytase @ 500 FTU/kg, and phytase @ 500 FTU/kg + organic acid (1 kg/ton). Rations were offered to seven replicates of ten birds each, from day 1 to 21. Results revealed that the experimental treatments had no effect on feed intake and growth performance of birds ($p>0.05$). Carcass parameter results showed highest thigh meat yield % and liver weight % in the birds fed diet with low Ca & P and supplemented with enzymes phytase ($p<0.05$). Fecal P and Na digestiblities were high ($p<0.05$) in experimental broilers fed low Ca low P with enzyme phytase supplementation as compared to the diet without phytase supplementation. It is concluded that phytase and OA supplementation in the starter diet with low Ca and low P level did not influence intake, growth and feed conversion of the broilers. However, thigh meat yield percent and mineral digestiblities (P and Na) increased in the broilers that received the diet with low Ca and low P level and supplemented with enzyme phytase.

INTRODUCTION

In poultry nutrition, plant base ingredients form the major part of ration and more than 60% of the total phosphorus (P) present in plant base ingredients are in the form of phytate (Gautier *et al.*, 2017). Poultry birds do not have the ability to use this phytate P (Waldroup *et al.*, 2000). This P in the form of phytate is present in aleurone layers and outer bran of grains (Steiner *et al.*, 2007). It has been reported that phytate is the storage form of P and inositol. It forms complexes with proteins, lipids and with many other micro-minerals in the gastro intestinal tract, thus reduces the solubility and absorption of nutrients (Cowieson *et al.*, 2011; Alshamiri *et al.*, 2021). It has also been reported that phytate also forms complexes with other minerals and nutrient like sodium (Na), calcium (Ca), iron and protein resulting in their deprived availability to the poultry birds. It also inhibits the efficiency of protein digesting enzymes like pepsin and trypsin and hence reduces digestibility of major nutrient proteins (Adeola & Cowieson, 2011), that are considered crucial for the growth of broilers.

The supplementation of exogenous enzymes phytase @ 500 FTU/kg has been beneficial to reduce the negative impact for birds due to low P availability, results in the release of phytate P, increase in nutrient digestibility, increase in ash content in bones and finally have good effect on the growth performance of birds (Onyango *et al.*, 2005). Phytase reduces the endogenous secretions which lead to high energy consumption (Pirgozliev *et al.*, 2008). Dibner & Buttin (2002) reported that different organic acids (OA) such as acetic acid, formic acid, propionic acid, butyric acids and other carboxylic acids such as lactic, tartaric, citric, malic and fumaric are being used in the poultry diet, and known for their antimicrobial activity and improved bird performance (Adil *et al.*, 2010; Ashraf *et al.*, 2020; Shawky *et al.*, 2020). Organic acids lower the pH of digesta (Dibner & Buttin 2002; Masood *et al.*, 2020) especially in GIT and increase the activity of pepsinogens and zymogens (Jongbloed *et al.*, 2000; Tammam *et al.*, 2020). A previous study also demonstrated that the pH of the crop in birds also affects the activity of phytase enzymes (Murai *et al.*, 2001). As described earlier, organic acids lower the pH of the gut in broilers and thus supplementation of organic acids in the diet may improve efficiency of supplemental phytase. It has also been reported that supplementation of chicken diet with organic acid reduces the production of toxins produced by bacteria or by the pathogens residing in the GIT thus lower the damage of the epithelial wall of the GIT (Langhout, 2000; Khan *et al.*, 2019; Khan *et al.*, 2020). OAs increase the digestibility of protein, Ca, magnesium, P and zinc by lowering the pH of the GIT and thus making the phytase more effective at lowering the intestinal pH and increase the growth performance of the birds (Kirchgessner & Roth, 2012).

Thus, the purpose of the study reported was to evaluate the effect of phytase and OA supplementation on feed intake, growth performance, carcass characteristics, protein and mineral digestibility in broiler chickens fed low Ca and low P diets in comparison to a high Ca and high P diet with standard specifications in starter phase of broiler chicken.

MATERIALS AND METHODS

The experimental procedure of the current study was approved by the Director Graduate Studies, University of Agriculture, Faisalabad, Pakistan. Institute of Animal and the Dairy Sciences, University of Agriculture Faisalabad also approved the current study. Experimental feeds were formulated to be isocaloric and isonitrogenous for starter phase (d zero to 21) (Table 1). The experimental feeds were offered to experimental birds from 1 to 21 days. The specifications of the ingredients used in the experimental diet preparation were taken from Brazilian Tables for Poultry and Swine (Rostagno *et al.*, 2011). The experimental diets fed to the birds were in the form of pellets from day 1 to 21 of the experimental trial. For this experiment, two iso-nitrogenous and iso-caloric diets were prepared in such a way that one diet had high Ca (9.80) and high P (4.50) and second diet had low Ca (8.50) and low P (2.40). Low Ca and low P diet were further supplemented with phytase enzyme@ 500 FTU/kg, phytase enzyme@500 FTU/kg + OA (1 kg/ton).

Table 1 – Ingredients composition and nutrient profile of experimental diets.

Ingredients	Experimental diets	
	Starter Ration	
	(High Ca and P)	(Low Ca and P)
Corn	590.69	570.32
Rice polishing	50.00	90.00
Soybean meal	300.32	290.55
Vegetable oil	0.680	-
Limestone	10.45	10.48
Mono-calcium phosphate	10.62	8.760
Sodium chloride	2.60	2.20
Sodium-bicarbonate	2.90	2.40
Lysine sulphate	4.10	4.20
DL-Methionine	3.00	3.00
L-Threonine	1.00	1.00
Premix3	5.00	5.00
Total	1000	1000
Calculated nutrients composition	g/kg	
Crude protein	20	20
Metabolizeable energy (MJ/kg)	12.13	12.13
Calcium	9.80	8.50
Available P	4.50	3.00
Sodium	2.00	1.70
Potassium	8.10	8.50
Chloride	2.30	2.00
DEB4	2310	2340
Dig. Lys	11.5	11.5
Met+Cys	8.50	8.50
Dig. Thr	7.70	7.70
Dig. Tryp	2.20	2.18

[1]PC=positive control (CP:20%, ME:2900 kcal/kg, Ca: 0.98%, P: 0.45%).

[2]NC=negative control (CP:20%, ME:2900 kcal/kg, Ca: 0.85%, P: 0.30%).

[3]Phytase was added @ 500FTU/kg while organic acid was added @ 1kg/ton in premix. Each kg of premix will provide per kg of diet: 10,000 IU Vitamin A, 11.0 IU Vitamin E, 1.1 mg Vitamin K, 1100 IU Vitamin D3, 5 mg Riboflavin, 12 mg Ca Pantothenate, 12.1 µg Vitamin B12, 2.2 mg Vitamin B6. 2.2 mg thiamin, 44 mg nicotinic acid, 250 mg choline chloride, 1.55 mg Folic acid, 0.11 mg d-biotin, 60 mg Mn, 50 mg Zn, 0.3 mg I, 0.1 mg Co, 30 mg Fe, 5 mg Cu and 1 mg Se.

[4]Dietary electrolyte balance.

Animal Husbandry and Experimental Procedure

The experiment was carried out at the Research Center of Animal Nutrition, Faculty of Animal Husbandry, University of Agriculture, Faisalabad. Two hundred and eighty Cobb 500 (day old) male broiler chicks were purchased from a local hatchery. The number of birds and samples were ruled by the available resources and ethical considerations. Procured broiler chicks (Cobb 500) were randomly distributed into 28 replicates in that manner that each replicate had10 birds. Each replicate was housed in a pen having dimension of 1.2 x 0.9 x 0.8 m. Litter was spread in each pen. Gas brooder was used during the brooding period. Sugar solution (1kg sugar/5L water) was used for flushing on the very first day of experiment. For the comfort of the experimental birds, house temperature was set at 95 °F before the arrival of the chicks, and this temperature was maintained for the first week of the experiment to serve the purpose of brooding. After that brooding temperature was reduced by 5° F every week until it reached 75 °F. In the current experiment, the chicks had free access to feed and water round the clock. Shed temperature and ventilation was maintained with the help of electric water cooler, and ceiling fans. Standard management conditions were ensured in the shed throughout the experimental trial. The birds were free from fear of thrust and hunger, and welfare conditions were ensured as described in recent studies of animal's research (Aziz ur Rahman *et al.*, 2017; Aziz ur Rahman *et al.*, 2019).

Data Collection

Feed intake was calculated by subtracting the feed refused from the total feed offered during the week. The weight gain (WG) data was obtained on a weekly basis. The weekly average WG was determined by simply subtracting the average body weight (BW) of the previous week from the average BW at the end of the next week. The data collected for intake and WG were adjusted for mortality and feed conversion ratio (FCR) was calculated by dividing intake on total WG.

Digestibility Assay

Protein, P, Ca and Na digestibility was determined on the 21st day by using an external marker that was acid insoluble ash (AIA). For this objective, acid insoluble ash source, Celite® was included in the experimental feeds @ 1% of feed (Sales & Janssens, 2003) three days before digestibility trial. Feed samples were analyzed for dry matter, ether extract, crude protein (CP), ash and crude fiber and ash. On the 21stday fecal digesta from 3 birds per replicate was collected. These samples were oven dried at 65°C and then they were grinded for further analysis.

Chemical Analysis

Dry matter, crude protein, crude fiber, ether extract and crude were determined according to the guideline of AOAC, 1990. Sodium concentration of excreta samples were quantified by using flame absorption spectrophotometry. Phosphorus was determined by using molybdo-vanadate method (method 965.17, AOAC, 2000). Calcium concentration was determined by titration method (AOAC, 2000). All chemical analysis were done carefully, considering the protocol of previous studies (Muhammad *et al.*, 2016; Wang *et al.*, 2016; Niu *et al.*, 2017; He *et al.*, 2018; Hussain *et al.*, 2018; Sharif *et al.*, 2018; Xia *et al.*, 2018; Xia *et al.*, 2018; Xia *et al.*, 2018; Hussain *et al.*, 2020).

Calculation and Statistical Analysis

The apparent digestibility coefficient of the nutrient was calculated by the method described by Ravindran *et al.* (1999).

$$\text{Nutrient Digestibility (\%)} = \frac{\left(\frac{N}{AIA}\right)\text{diet} \times \left(\frac{N}{AIA}\right)\text{digest}}{\left(\frac{N}{AIA}\right)\text{diet}}$$

N: Nutrient

AIA: Acid insoluble ash

General Linear Model of Minitab Statistical Software 17 (Minitab Inc. 2010) under CRD was used to analyze collected data. Tukey's test was used to compare the means.

RESULTS

Growth Performance

Data of growth performance parameter is present in Table 2. Result revealed that the experimental treatments had no effect on feed intake during starter phase (*p>0.05*). Similarly, there was no difference in the WG of the birds by giving different dietary treatments during starter phase. Feed conversion ratio was also not changed by the experimental treatments in starter phase (*p>0.05*). However, numerically better FCR was observed in the broilers offered the experimental diet low in Ca & P and supplemented with phytase (*p>0.05*) for1-21 days lifespan.

Table 2 – Influence of phytase with or without organic acid (sodium di-formate) supplementation on growth performance of broiler chicken in starter phase.

Parameters	Dietary treatments				
	1HCHP	2LCLP	3LCLPPH	4LCLPPHOA	*p*-Values
Feed intake (g)	645±34	632±53	656±23	664±33	0.41
Weight gain (g)	768±63	792±53	809±36	788±45	0.537
Feed conversion ratio	1.55±0.07	1.50±0.18	1.49±0.16	1.54±0.17	0.551

[1]HCHP=High calcium high phosphorus (CP:20%, ME:2900 kcal/kg, Ca:0.98%, P: 0.45%).

[2]LCLP=Low calcium low phosphorus (CP:20%, ME:2900 kcal/kg, Ca: 0.85% and P: 0.30%).

[3]LCLPPH=Low calcium low phosphorus+ Phytase.

[4]LCLPPHOA=Low calcium low phosphorus+ Phytase+ Organic acid.

Means with different superscripts in rows differ significantly ($p<0.05$).

Carcass Characteristics

Results of carcass characteristics are presented in Table 3. Findings of carcass characteristics explored that live weight %, and dressing percentage were similar for all groups ($p>0.05$). However, dietary treatments influenced the thigh meat yield %, chest meat yield %, heart weight, liver weight %, gizzard weight % and spleen weight % ($p<0.05$). In the current experiment, the highest breast meat yield % was observed in the birds fed high Ca & P diet ($p<0.05$). While, the highest thigh meat yield % and liver weight % were observed in the birds fed the diet that had low Ca & P and was supplemented with phytase ($p<0.05$). However, the highest heart weight %, gizzard weight % and spleen weight % was observed in the birds on high Ca & P supplemented diet with phytase and OA ($p<0.05$).

Table 3 – Influence of phytase with or without organic acid (sodium di-formate) supplementation on carcass characteristics of broiler chicken in starter phase.

Carcass characteristics	Dietary Treatments				
	1HCHP	2LCLP	3LCLPPH	4LCLPPHOA	*p*-Values
Dressing%	56±0.16	54±0.42	57±1.01	59±0.01	0.408
Breast meat yield%	24a±0.24	23ab±0.38	22b±0.36	22b±0.25	0.009
Thigh meat yield%	10b±0.09	10ab±0.09	11a±0.17	11ab±0.16	0.004
Liver weight%	2.1b±0.02	2.2ab±0.05	2.4a±0.05	2.3ab±0.05	0.040
Heart weight%	0.43c±0.01	0.50bc±0.00	0.54ab±0.01	0.56a±0.01	0.003
Gizzard weight%	1.21ab±0.02	1.14b±0.01	1.20ab±0.01	1.28a±0.02	0.011
Spleen weight%	0.08b±0.00	0.11ab±0.00	0.12ab±0.00	0.13a±0.00	0.039

[1]HCHP=High calcium high phosphorus (CP:20%, ME:2900 kcal/kg, Ca:0.98%, P: 0.45%).

[2]LCLP=Low calcium low phosphorus (CP:20%, ME:2900 kcal/kg, Ca: 0.85% and P: 0.30%).

[3]LCLPPH=Low calcium low phosphorus+ Phytase.

[4]LCLPPHOA=Low calcium low phosphorus+ Phytase+ Organic acid.

Means with different superscripts in rows differ significantly ($p<0.05$).

Serum Phosphorus Level

Results of blood serum P level are presented in Figure 1. On day 21, better serum P level was noted in the birds fed High Ca, high P (HCHP) and Low Ca, low P+ Phytase+ OA (LCLPPHOA) diet (p<0.05). Poor serum P was observed in the birds fed Low Ca, low P (LCLP) and Low Ca, low P+ Phytase (LCLPPH) (p<0.05).

Crude Protein and Mineral Digestibility

Results of CP and mineral digestibility are exhibited in Table 4. Better CP digestibility on day 21 was noted in the birds fed HCHP diet ($p<0.05$) as compared to other experimental treatments. While, the lowest CP digestibility on day 21 was noted in the birds offered the LCLP experimental diet ($p<0.05$). On day 21, the results of Ca digestibility revealed better Ca digestibility in the birds offered HCHP experimental diet ($p<0.05$). Poor Ca digestibility on day 21 was noted in the birds offered the LCLP experimental feed ($p<0.05$). Calcium digestibility in treatment LCLPPH was better than in treatment LCLP and poorer than in HCHP ($p<0.05$). Phosphorus digestibility was changed due to dietary treatments on day 21 ($p>0.05$). Phosphorus digestibility results on day 21 presented that the birds fed HCHP diet had better P digestibility ($p<0.05$). Poor P digestibility on day 21 was observed in the birds fed LCLP diet ($p<0.05$). Sodium digestibility on day 21 showed that the birds fed HCHP diet had better Na digestibility ($p<0.05$).

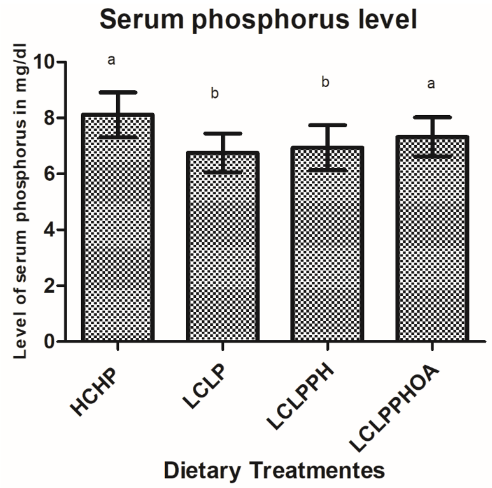

Figure 1 – Influence of phytase with or without organic acid (sodium di-formate) supplementation on serum phosphorus level of broiler chicken in starter phase.

DISCUSSION

In the present experiment, feed intake was not changed by phytase in diets with LCLP compared to HCHP diet which agrees with the results reported by previous researchers (Namkung & Leeson, 1999; Lan *et al.*, 2002; Tizziani *et al.*, 2016). Namkung & Leeson (1999), reported that feed intake (629.38) from day 1-14 of broiler chicks offered feed that contained exogenous enzyme phytase with the low Ca (0.79%) and available P (0.35%) were similar to the feed intake (639.54g) of the control group chicks fed the diet that contained normal levels of available Ca (0.90%) and available P (0.45%). In contrary to the current findings, Lan *et al.* (2002) reported that the supplementation of different levels of phytase-producing bacterial culture (equivalent to 250, 500, 750, and 1,000 U of phytase/ kg of feed) to the low available P diet (0.24%) increased the feed intake of broiler chickens to a level similar to broiler chicks offered feed with high Ca and P level (0.45%). The difference in results with Lan *et al.*´s (2002) study could be explained by the age difference of broilers. In the current study, we just observed the intake in starter phase while in Lan *et al.* (2002) study reported the result of the overall study period of broiler growth. Similarly, OA and phytase supplementation in LCLP and HCHP starter diet of broiler didn't affect the intake. Interesting results of WG was observed in the current study. In the current study WG was similar among all treatments, which is contradictory to the previous research of Gehring *et al.*, (2013). Gehring *et al.*, (2013) examined the impact of the supplementation of phytase enzymes with variable levels of available P in sorghum soybean meal corn gluten diets from day one to day 21 of broiler. They reported that WG was increased in diets supplemented with phytase when compared to the LCLP diets. They conducted another experiment with different levels of available P and with and without phytase supplementation, WG was increased at the level of P which was high and available to the bird for its proper functioning of the body. The level of P, which was high without supplementation of phytase, has lower WG when compared to the phytase supplementation. The current study´s findings, which based the diets in corn-soya diets, disagree with Gehring *et al.* (2013) study, which based their diets in sorghum soybean meal corn gluten. Moreover, no difference of the experimental diets on FCR in starter phase of broiler was observed in the current study, which indicates that all dietary treatments have the same dietary nutrient absorption efficiency in starter phase. Our results agree with the study of Rutherfurd *et al.* (2012), who stated that the FCR was the same for the birds fed phytase enzymes supplemented feed as compared with the diets with low level of P and with high Ca and P diet. Interestingly, HCHP, LCLP diets, with the supplementation of OA, behave the same in terms of growth and FCR compared to other treatments in the current experiment.

Table 4 – Influence of phytase with or without organic acid (sodium di-formate) supplementation on crude protein, calcium, phosphorus and sodium digestibility of broiler chicken in starter phase.

Digestibility	Dietary Treatments				
	1HCHP	2LCLP	3LCLPPH	4LCLPPHOA	*p*-Values
Crude protein	70a±3	51b± 4	57ab±4	65ab±3	0.024
Ca	80a±2	53c±2	67b±2	74ab ±2	0.000
p	82a±1	52c±3	75ab ±1	71b ±1	0.000
Na	76a±1	63b±3	70ab±2	64b±3	0.018

[1]HCHP=High calcium high phosphorus (CP:20%, ME:2900 kcal/kg, Ca:0.98%, P: 0.45%).

[2]LCLP=Low calcium low phosphorus (CP:20%, ME:2900 kcal/kg, Ca: 0.85% and P: 0.30%).

[3]LCLPPH=Low calcium low phosphorus+ Phytase.

[4]LCLPPHOA=Low calcium low phosphorus+ Phytase+ Organic acid.

Means with different superscripts in rows differ significantly (p<0.05).

Although, a lot of work has been done on phytase supplementation and its impact on carcass parameters of pigs the impact of phytase on carcass characteristics in broilers is also important, but information regarding characteristics of carcass is limited. Research of phytase supplementation on P deficient diets had been studied in pigs to evaluate its effect on carcass characteristics and it has been documented that results on carcass characteristics were variable with phytase supplementation (Liu *et al.*, 1998; Lysenko *et al.*, 2021). For example, Walz & Pallauf (2003) conducted a trial on barrows and stated that phytase enhanced digestibility of minerals but had no effect on carcass and meat characteristics. Similarly, research on drakes reported by Attia (2003) demonstrated that supplementation of phytase had no effect on carcass yield and meat quality. However, Shelton *et al.* (2004) reported phytase inclusion in the mineral deficient diet reversed the negative effects of mineral deficient diets on carcass characteristics. In the current study, thigh meat yield was higher in diets with low level of P supplemented with phytase compared to diets with low level of P without phytase supplementation. So, it could be hypothesized that the inclusion of the phytase in broiler diets with deficient P, not only prevented negative effects on thigh meat yield in birds, but also enhance its yield. Our finding is in agreement with Scheideler & Ferket (2000) who also reported that thigh yield was higher in broilers fed LCLP diets with phytase supplementation. Our result is in line with Pillai *et al.*, (2006) who stated that the addition of phytase in P deficient feed of broiler not only reduce the negative effect on thigh yield but also on breast yield. Preston *et al.* (2000) found interesting results of phytase enzymes addition in P deficient feed in broiler and claimed that the addition of enzyme phytase in P deficient feed of broiler can increased the yield of important parts of carcass; however, Angel *et al.* (2006) opposed these findings. Similarly, in the current study, breast yield results revealed that phytase supplementation failed to reduce the negative effect of low P diet on breast meat yield. Similarly, OA and phytase supplementation with LCLP diet failed to overcome the negative effect of breast meat yield in broiler fed low P diet.

The observed increase in internal organ´s weights, e.g. heart and liver in the broilers fed in low available P without phytase supplementation diets, is perhaps due to lower supply of P. Temprano *et al.* (2004) reported that insufficient supply of P resulted in heart hypertrophy due to hypophosphatemia. Hypertrophy along with hypophosphatemia resulted in reduced contraction of the heart due to reduced myosin ATPase activity and lower cyclic adenosine monophosphate. This may cause heart failure, increasing diameter of myocardial fiber and decrease in the systolic pressure as reported by Martinez *et al.*, (2004). The increase in liver weight could be attributed to low ATP creation which stimulates the formation of liver enzymes. The findings of the current study are supported with the findings of de Oliveira *et al.* (2009) who reported decreased weight of internal organs including heart and liver when broilers were fed low available P. However, the increase in internal organs including heart and liver weight in diets with low available P plus phytase could be attributed to higher mineral digestibility and higher growth with this treatment.

Previous researcher reported that CP digestibility is not changed by the supplementation of phytase enzyme in low P feed compared to diets with normal P level (Woyengo *et al.*, 2010; Rutherfurd *et al.*, 2012; Lalpanmawia *et al.*, 2014). However, in the current study, on day 21 fecal CP digestibility was improved with the supplementation of phytase in LCLP diet which is in line with the results of the previous studies (Chung *et al.*, 2013; Cowieson *et al.*, 2006; Selle *et al.*, 2006). Ravindran *et al.* (2006) examined the impact of phytase enzyme supplementation feed having low P on broiler digestibility and reported that digestibility of CP was increased with phytase enzyme supplementation compared to feed having LCLP. Similarly, Cowieson *et al.*, (2006) also stated that phytase enzymes supplementation in the feed of broiler fed low P diet enhance apparent CP protein digestibility compared to control feed. Pirgozliev *et al.*, (2010) stated that superdose of phytase (12 500 FTU) in the diet of broiler deficient in P further enhance total tract amino acid digestibility. These findings are further confirmed by Rutherfurd *et al.* (2012a) and Chung *et al.* (2013) who stated that the apparent amino acids digestibility increased with the supplementation of phytase in low P feed of broilers. Furthermore, other researchers also have similar findings (Mansoori & Acamovic, 1998; Cowieson *et al.*, 2004). Furthermore, in the current experiment, phytase supplementation in diets with low available P improved Ca and P fecal digestibility on day 21. It has been reported in many studies that P and Ca digestibility were improved by phytase supplementation (Ravindran *et al.*, 2006; Lalpanmawia *et al.*, 2014). Several scientists reported similar findings and it is well documented that P digestibility increased with the supplementation of phytase in low P diets (Wu *et al.*, 2004; Olukosi *et al.*, 2007; Ravindran *et al.*, 2008; Rutherfurd *et al.*, 2012; Walk *et al.*, 2013). The increase in P digestibility could be explained by the mechanism of action of phytase enzyme on phytate which probably release or remove minerals from the phytate bond as well as stoppage of bounding mineral with phytate. The release of P from phytate is well recognized, however, impact of phytase on release and bioavailability of other minerals is not clear as documented by Chung *et al.* (2013). However, current study findings reported that phytase have the ability to increase the digestibility of Ca and Na. Formic acid addition is known to improve apparent digestibility of nutrients in broilers (Hernandez *et al.*, 2006; Garcia *et al.*, 2007). Emami *et al.* (2013) noted that supplementing diets with LCLPPHOA improve the digestibility of P compared to LCLP. Our results of P digestibility of low available P diet with phytase and OA agree with the study of Emami *et al.*, 2013. Furthermore, current study findings explored that Ca and Na digestibility of LCLP diet with phytase and OA is increased compare to LCLP diet without phytase supplementation.

In case of serum P level, some studies indicated that phytase supplementation can increase significantly serum P (Scheideler & Ferket 2000; Ghasemi *et al.*, 2006). Shirley & Edwards Jr (2003) observed that phytase supplementation in LCLP diet enhance blood P level because phytase in low Ca and P diet enhance more P removal/released from phytate. However, our findings showed that phytase in LCLP diet did not alter the blood P level in starter phase. Findings are consistent with other studies that reported that phytase supplementation into feeds for broiler chickens did not increase the P concentration in blood serum of birds (Bozkurt *et al.*, 2006; Plumstead *et al.*, 2008; Kliment & Angelovičová 2012; Amerah *et al.*, 2014).

CONCLUSION

Based on the results, it is concluded that phytase and organic acid supplementation in the starter diet with low Ca and low P level did not influence intake, growth and feed conversion of the broilers. However, thigh meat yield percentage and mineral digestibility (P and Na) increased in the broilers that received the diet with low Ca and low P level and supplemented with phytase in the starter phase of broiler.

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