Comparative evaluation of commercially available supplementary sources of inorganic phosphorus in broiler feed

Khattak, FM; Shahzad, MA; Pasha, TN; Saleem, G

Published in:
The Journal of Animal and Plant Sciences

First published: 01/12/2017

Document Version
Peer reviewed version

The Journal of Animal & Plant Sciences, 26(6): 2016, Page: 1576-1581
ISSN: 1018-7081

COMPARATIVE EVALUATION OF COMMERCIALLY AVAILABLE SUPPLEMENTARY SOURCES OF INORGANIC PHOSPHOROUS IN BROILER FEED

F. Khattak[*], M. A. Shahzad[1], T. N. Pasha[1] and G. Saleem[1]

[*]MSRC, SRUC, West Mains Road, Edinburgh, EH9 3JG, Scotland
[1]University of Veterinary & Animal Sciences, outfall Road Lahore, Pakistan
Corresponding Author Email: farina.khattak@sruc.ac.uk

ABSTRACT

The study was designed to compare the growth performance, digestibility and concentration of phosphorous (P) and calcium (Ca) in blood plasma, tibia bone and excreta in juvenile birds fed different commercially available sources of inorganic P. Day-old Hubbard broiler chickens (180) were randomly assigned to one of 4 dietary treatments (A-D), replicated three times with 15 birds per cage. All diets were corn soyabean based and the only difference was the source of inorganic P. The diets were supplemented with 1.20% MCP imported, 1.65% DCP local, 1.65% DCP imported and 1.65% bone ash and were designated as treatment A, B, C and D respectively. The diets were fed for the period of 21 days. On day 21, 3 birds per cage were randomly selected and euthanized. Blood plasma, tibia bone and total excreta were collected to calculate total Ca and P concentration. Feed intake and total excreta collected over the last 3 days (day 19 to 21) were used to calculate dry matter digestibilities (DMD). The results showed that growth performance of broilers up to 21 days of age was not affected ($P>0.05$) by the different sources of inorganic P, provided that the level of available P in diet remains 0.4%. However, diets containing DCP local outperformed compared to all P sources in terms of digestibility. The concentration of total Ca and P values showed no differences ($P>0.05$) in bone and excreta samples but the values were higher ($P<0.05$) in blood plasma from birds fed diets containing MCP and DCP diets compared to those on bone ash. The study suggests that indigenous source of DCP used in this study could replace the imported source and thus have potential to improve P digestibility.

Key words: MCP, DCP**,** inorganic phosphorus, plasma, tibia, digestibility.

INTRODUCTION

The importance of P and Ca in poultry nutrition is widely established in relation to optimum bone growth and remolding, it is now imperative to formulate poultry diets to fulfill the requirement of P. The poor availability of P from different feed ingredients has resulted in supplementation of inorganic P in poultry diet to fulfill the growth and metabolic requirements of fast growing broilers (Viljoen 2001, Viljoen 2003). Commercially available dicalcium Phosphate (DCP), monocalcium phosphate (MCP), deflourinated rock phosphate and bone ash are added in poultry diet to ensure that adequate phosphorus is available to meet P and Ca requirement of birds. Other in-feed P sources include meat meal, fish meal, bone meal, bone ash, whereas Ca sources include limestone (Ca carbonate), Ca sulfate, Ca fluoride, chips and marble. However among different inorganic P sources, supplementation of poultry diets with MCP and DCP is a common practice all over the world. The biological availability of P has been shown to vary depending upon the source, molecular formula, age and species of poultry utilized in the bioassay (Ammerman *et al*., 1995; Axe, 1998). Excessive Ca can increase the pH in gut resulting in decreased absorption of phosphorus from the intestines as well as that of magnesium, manganese and zinc (Ensminger, 1992; Van der Klis *et al*., 1996). Williams *et al*., (2000 and 2004) postulated that rapid bone modelling and remodeling in modern birds with inadequate supply of mineral (primarily Ca and phosphorus) Ca and P deprivation leads to increased risk of fractures, poor meat and rachitic bones, osteoporosis and osteomalacia (William *et al.*, 2004).

The P utilization from all commercially available inorganic feed phosphates is not equal and therefore can lead to over or under formulation of P that as a consequence can either depress growth performance and/or lead to excessive P being excreted to the environment (Lima *et al*., 1997; IFP, 2006). Although extensive research has been conducted to evaluate feed phosphates available to poultry industry (Ravindran *et. al*., 1995; Fernandes *et al*., 1999; Leske and Coon *et al*., 2002), however, studies regarding the comparison of different available sources of inorganic P used in poultry feed are limited. Therefore, the current study was thus designed to compare the growth performance, digestibility and concentration of P and Ca in blood plasma, tibia bone and excreta in response to different local and imported sources of inorganic P commercially available and routinely used in poultry industry in Pakistan.

MATERIALS AND METHODS

Experiment was conducted at the poultry research farm of University of Veterinary and Animal Sciences (UVAS) Lahore, Pakistan and all the analytical work was done at the Department of Animal Nutrition, UVAS.

Birds and husbandry: One hundred and eighty day-old Hubbard broiler chickens were obtained from a commercial hatchery. Birds were randomly assigned to one of four dietary treatments (A-D), replicated three times with 15 birds per cage. Three experimental cages were randomly assigned to each treatment. Chicks were given *ad libitum* access to feed and water and 24 h light was provided throughout the experimental period. The trial was conducted in an environmentally controlled poultry house where ventilation, temperature and other requirements were managed as per standard good husbandry practices. Birds were vaccinated against New Castle Disease (ND) during 1st week and Infectious Bursal Disease during the 2nd and ND again during 3rd week as per normal commercial practice.

Experimental diets: A corn soyabean based basal ration was used for all 4 diets (diet A to D). MCP imported, DCP local, DCP imported & bone ash were added in diet A, B, C and D respectively. Both DCP and MCP used were feed grade imported DCP and MCP were supplied by TIMAB, Dinars, France whereas, local DCP and MCP were from Liner Pak, Lahore and Sitara Chemicals, Faisalabad, Pakistan, respectively. Bone ash was obtained from local suppliers from Lahore, Pakistan. Feed formulation and calculated nutrient composition of experimental diets is presented in Table 1. The level of supplemental sources of P was 1.20, 1.65, 1.65 and 1.65% for diet A, B, C and D respectively, as recommended by the manufacturer for use in broiler diet. The level of available P in diets A to C was 0.45% and 0.39% for diet D. All four experimental diets were iso-caloric and iso-nitrogenous. The experimental diets were fed for a period of 21 days.

Sampling schedule and procedure: All birds were bulk weighed per cage at the start of the experiment (day 0) and then at 7, 14 and 21 days of age. Feed and weigh backs were recorded per cage at day 0, 7, 14 and 21. Mortality was recorded as and when occurred. Average body weight gains and feed intake data was calculated for days 0 to 7, 7 to 14, 14 to 21 and 0 to 21. Mortality adjusted FCR (MFCR) was calculated as:

MFCR = Total feed intake of period per pen / (total live weight of pen + total weight of dead birds in pen) – total live weight of pen in previous period

At the end of the study (day 21), three birds were randomly selected from each replicate cage and were euthanised with an overdose of sodium pentabarbitone following blood collection in heparin tubes through wing vein. The plasma was separated by centrifugation of blood for 15 minutes at 3000 revolutions per minute (rpm) in centrifuge machine. The plasma was refrigerated at -4°C. Plasma was treated with 10% trichloroacetic acid (TCA). After dilution samples were analyzed on atomic absorption spectrophotometer and spectrophotometer for Ca and P respectively (AOAC, 1990).

After blood collection, same birds were dissected and left tibia was removed. Each tibia was individually packed in self sealed polythene bags and stored at -20 °C until analysed. Following thawing, tibia was placed in boiling water for 10 min in a glass beaker. Adhering muscle and connective tissue were removed manually. Tibia bone was rinsed with distill water, weighed (in a pre-weighed crucibles).and placed in an oven at 105°C for 48 h to determine dry matter. After 48 hours, the tibia's were removed from oven and placed carefully in desiccator to enable cooling. The dry matter (DM) was determined by dividing the dry tibia weight by fresh tibia weight. The dried tibia were than placed in labeled crucibles and weighed before placing them in an electric furnace to be ashed at 600 °C for 16 hours and than at 800°C for 4 hours. The crucibles were weighed and the differences between the dried and ashed tibia weights were used to determine ash content. Tibia ash (0.025g) was taken in a 50ml flask & 0.2ml 5N HCl was added in this flask to dissolve ash. The flask was shaken for 5 minutes. The deionized water was added to make up the volume to 50ml and quantified by atomic-absorption spectrophotometer & spectrophotometer for Ca and P concentrations respectively (Hunt, 1969).

The total excreta per cage were collected from day 19 to 21 by using trays underneath each cage. The excreta samples were weighed & dried in an oven at 105 °C for 48hrs. The DM content of excreta was calculated in the same way as mentioned above for calculating tibia DM. The dry matter of excreta was determined. The apparent total tract nutrient dry matter digestibility (DMD) was calculated using the following formula:

$$\text{Percent DMD} = [(DM_{in} - DM_{out})/DM_{in}] \times 100$$

Where DM_{in} and DM_{out} = Dry matter content in the diet and excreta respectively.

For Ca and P estimation, 5g of dried excreta was ashed at 650°C for 4 hours in crucibles in an electric furnace. 0.5g of ashed sample was taken in a flask and 10 ml of nitric acid was added and the flask was kept in hot water bath at 65°C for 15 minutes. 5 ml of perchloric acid was added to the flask and was kept again in hot water bath at 75°C for 15 minutes. The sample was than dried on hot plate till 0.5 ml of the fluid was left. After filtration the sample was raised to 50ml solution by repeated washing (Elaroussi *et al.*, 1994). The solution was prepared for Ca and P estimation by using atomic

absorption spectrophotometer (Perkin Elmer AA 400) and spectrophotometer, respectively.

Statistical Analysis: Statistical analysis was performed using a randomized complete block design. Each replicate pen was an experimental unit. The data obtained was analysed using Generalized ANOVA (Genstat 14 for Windows, IACR Rothamstead, England). The model included blocks and diets to test for the effect of diets on the response criteria.Values were considered significant if P<0.05. In case of significant differences, the Duncan's multiple range tests was used to compare differences among treatment means.

RESULTS AND DISCUSSION

Growth Performance: Feed intake, weight gain and feed conversion ratio were not affected by (P>0.05) by different sources of inorganic phosphorus used in the present study (Table 2). DCP is most widely used phosphorus supplement and often utilized as a standard reference product in studies of phosphorus availability. Phosphorus in DCP is assumed by the nutritionist to be 100% available. Overall, bird performance was not different (P>0.05) among the commercial phosphate sources tested. Most of previous studies on P, used phosphorus deficient basal diets, resulting in loss of appetite along with reduction in weight gain as a consequence of impaired feed efficiency (Anselme, 2003., IFP 2004), however in the present study, all of the experimental diets were having available phosphorus within the range of 0.39 to 0.45% which falls within the acceptable level for broilers. Having no effect of inorganic P source on growth parameters is consistent with other studies where diets were supplemented with different sources of DCP and growth depression was only observed when birds were fed diets deficient in inorganic P source (Lima *et al*., 1997; Leskeand Coon, 2002). However, Tahir *et al*., (2011) reported that substituting 25% of indigenous source of rock phosphate (Hazara rock phosphate; HRP) for DCP in the diet increased (*P*< 0.05) average body weight gain whereas 100% HRP substitution decreased (*P*< 0.05) body weight gain. The authors suggested that decrease in weight gain was due to higher levels of flourine (562 mg/kg) in HRP.

Similar to current study earlier researcher (Payne, 2005) demonstrated that different P sources did not have any effect on growth performance of birds. However in later experiments he found decreased performance of birds receiving different P sources compared to commercial broiler starter. He attributed this difference to high Ca content in that diet resulting in reduced performance and depressed growth. The reason why no growth depression was observed in the current study could be possibility due to the fact that none of the experimental diets were deficient in P and level of Ca was similar across the diets.

Available Calcium and Phosphorus in plasma, bone and excreta: Ca and P concentration in blood plasma of broiler chickens fed different sources of inorganic P showed significant (p<0.05) differences (Table 3). Birds fed diets containing MCP and DCP (diets A to C) had higher (P<0.05) Ca levels in blood plasma compared to those fed on diets containing bone ash (diet D). Whereas, in case of P, birds fed imported DCP had lowest level (P<0.05) of plasma P compared to all other diets. Birds fed diets containing bone ash had intermediate level of plasma P compared to those fed diets containing MCP imported and DCP local (diet A and B) where highest levels of plasma P were observed.

Several factors may affect Ca and P metabolism; these could include dietary, physiological, or animal factors. Different plant sources of feed ingredients have different levels of Ca and P. In addition different manufacturing conditions employed in the production of raw materials may lead to subtle changes in their chemical and physical composition and result in altered plasma P levels in animals (Adeola and Cowieson, 2011, Adedokun and Adeola (2013). Since P is present in different chemical form in different sources that could result in variation of plasma level in different sources of P (Lima *et al*., 1995 and. Lima *et al*., 1997) reported plasma P values ranged between 6.3 -7.7mg/100mL of eight phosphorus sources. The authors concluded that plasma phosphorus level was increased by level of phosphorus supplementation regardless of the source utilized.

Bone and excreta from broiler chickens fed different sources of inorganic P showed no differences (P>0.05) in Ca and P values is presented (Table 3).

Previous studies suggested inclusion of P in test diet at sub optimal level through extremely low P levels in basal diet to get best results. However severely deficient diets have been reported to result in considerable mortality (Ammerman *et al*., 1995, Van der Klis *et al*., 1996). In the present study inclusion of different commercially available inorganic P products to experimental diets was according to manufacturer recommendations and this could probably explain why no differences (P>0.05) were observed in bone and excreta samples.

Dry matter digestibility (DMD): The DMD values showed differences between different inorganic sources of P used in this study (Table 4). The digestibility was highest (P<0.05) in birds fed diets containing DCP local (diet B) compared to all other experimental diet (diet A, C and D). The results suggested that the DCP local was more biologically available when fed to juvenile broiler birds up to 21 days of age. Inorganic P exits in the divalent form (HPO_4^{-2}) and the monovalent form ($H_2PO_4^-$

) in the lumen of the gastrointestinal tract (Quamme, 1985; Gropper *et al*., 2008; Hill *et al*., 2008). Therefore, dietary P in the organic form must be first hydrolysed by enzymes (phytases, phospholipase C and alkaline phosphatase to release P from bound form (Gopper *et al*., 2008). Thus any P not absorbed by poultry is simply excreted (Leytem *et al.*, 2007). Dietary factors (vitamin D3 and other minerals mainly Ca, magnesium, zinc, manganese etc), environment, age, breed and sex are all identified as factors affecting the P digestibility which may influence the phytase and alkaline phosphatse activity in chicken (Mutucumarana *et al*., 2014).

Table 1. Calculated ingredient and nutrient composition of experimental diets.

Ingredients (%)	Experimental diets			
	A	B	C	D
Corn	36.67	36.67	36.67	36.67
Rice	15.00	15.00	15.00	15.00
Rice Polishing	14.81	14.58	14.58	15.29
Soybean Meal	15.41	15.41	15.41	15.41
Canola Meal (36%)	12.52	12.52	12.52	12.52
Sunflower Meal (26%)	1.00	1.00	1.00	1.00
Chips	1.63	1.41	1.41	1.41
Vi t-Min-premix	1.00	1.00	1.00	1.00
L Lysine-HCL	0.31	0.31	0.31	0.31
MHA	0.22	0.22	0.22	0.22
Sodium Chloride	0.23	0.23	0.23	0.23
Monocalcium Phosphate (I)*	1.20	-	-	-
Dicalcium phosphate (L)*	-	1.65	-	-
Dicalcium phosphate (I)*	-	-	1.65	-
Bone Ash	-	-	-	1.65
Total	100	100	100	100
Calculated nutrient composition (%)				
ME (Kcal/Kg)	2750	2750	2750	2750
CP%	19	19	19	19
CF%	5.89	5.89	5.89	5.89
EE%	3.88	3.88	3.88	3.88
Ca%	1.00	0.87	0.87	0.87
Ava. P%	0.45	0.45	0.45	0.39
Total P%	0.94	0.89	0.89	0.89
Linoleic acid	1.13	1.13	1.13	1.13
Methionine%	0.53	0.53	0.53	0.53
Lysine%	1.17	1.17	1.17	1.17

Diet A=MCP imported; Diet B= DCP local; Diet C= DCP imported; Diet D=Bone ash; I* = imported and L* = local

Table 2. Growth performance of broilers fed diets containing different commercially available supplementary sources of phosphorus (days 0-21 post hatch).

Treatment	Growth Performance		
	WG (g/bird)	F1 (g/bird)	MFCR
A	531	924	1.75
B	574	957	1.66
C	537	953	1.78
D	564	955	1.69
SEM	27.8	50.1	0.13
P-value	0.584	0.360	0.920

A=MCP imported; B= DCP local; C= DCP imported; D=Bone ash; WG= weight gain; FI= feed intake; MFCR=Mortality adjusted FCR; SEM=Standard error of means; n=15

Table 3. Concentration of total phosphorus (P) and calcium (Ca) in blood plasma (mg/100ml), tibia bone and excreta (mg/100gm) of broilers fed diets containing different commercially available supplementary sources of phosphorus (days 0-21 post hatch)

Treatment	Plasma (mg/100ml)		Bone (mg/100gm)		Excreta (mg/100g)	
	Ca	P	Ca	P	Ca	P
A	12.1[b]	6.5[c]	7.52	196.4	2.4	184
B	12.2[b]	6.9[c]	7.98	257.9	2.4	166
C	11.8[b]	4.2[a]	7.15	170.7	2.4	176
D	10.4[a]	5.9[b]	7.53	197.1	2.2	200
SEM	0.41	0.19	0.27	22.06	0.19	7.2
P-value	0.024	<0.001	0.188	0.138	0.256	0.071

A=MCP imported; B= DCP local; C= DCP imported; D=Bone ash; SEM=Standard error of means; [abc]Means within a column with different superscript differ significantly (P<0.05); n= 15

Table 4. Effect of experimental diets on mean dry matter digestibility (DMD) measured by total excreta collection method

Treatments	DMD (%)
A	75.8[a]
B	79.2[b]
C	73.4[a]
D	75.4[a]
SEM	2.10
P-value	<0.001

A=MCP imported; B= DCP local; C= DCP imported; D=Bone ash; SEM=Standard error of means; [ab]Means within a column with different superscript differ significantly (P<0.05; n=15.

Conclusion: In conclusion, the present study revealed that when different inorganic P sources were compared in young broilers, local DCP out performed in terms of digestibility. However, higher digestibility was not translated in improved growth performance but this could be due to the reason that the level of available P was optimum (0.4%) in all experimental diets and thus leaving little room for further improvement. The study does suggest that indigenous sources of DCP could replace the imported source and thus have potential to improve P digestibility.

Acknowledgements: The research work was funded by Higher Education Commission (HEC), Pakistan.

REFERENCES

Adedokun, S. A. and O. Adeola (2013). Calcium and phosphorus digestibility: Metabolic limits**.** J. Appl. Poult. Res. 22 (3): 600-608

Adeola O. and A. J. Cowieson (2011). Board-Invited Review: Opportunities and challenges in using exogenous enzymes to improve nonruminant animal production. J. Anim. Sci. 89:3189–3218.

Ammerman, C. B., D. H. Baker and A. J. Lewis. (1995). Bioavailability of Nutrients for Animals: Amino Acids, Minerals, and Vitamins. Academic Press, San Diego, CA

Anselme, P. (2003). Phosphorous in poultry nutrition. Key elements for performance of animal health. Feed Magazine. 01 March:1-4.

AOAC. (1990). Official methods of analysis of the Association of Official Analytical Chemists. 15th edition. Washington, DC.

Axe, D. E. (1998). Phosphorus value in ingredient sources examined. Feedstuffs 70:22. June 1:17–28

Elaroussi, M. A., L. R. Forte, S. L. Eber and H. V. Biellier (1994). Calcium homeostasis in the laying hen. 1. Age and dietary calcium effects. Poult. Sci. 73:1581-1589.

Ensminger, M.E. (1992). Poultry Science. Interstate Publishing, INC., Danville, Illinois, USA.

Gropper, S.G., J. L. Smith and J. L. Groff. (2008). Advance Nutrition and Human Metabolism. 5th ed. Wadsworth Publishing Company, Belmont, C.A.

Hill, S., K. Knowlton, E. Kebreab, J. France, M. Hanigan. (2008). A model of phosphorous digestion and metabolism in the lactating dairy cow. J. Dairy Sci. 91: 2021-2032.

Hunt, B.J. (1969). The estimation of magnesium in plasma, muscle and bone, by atomic absorption spectrophotometry. Clinical chemistry. 15 (10). 979-996.

IFP. (2004). Inorganic Feed Phosphates. http://www.feedphosphates.org

IFP. (2006). Optimising phosphorus utilisation in pig and poultry diets.Cefic, Brussels.

Leske, K. and C. Coon.. (2002). The development of feedstuff retainable phosphorus values for broilers. Poult. Sci., 81(11), 1681-1693.

Leytem, A. B., P. A. Thacker and B. L. Turner (2007). Phosphorous characteristics in feces from broiler chicks fed low-phytate barley diets. J. Sci. Food and Agr. 87: 1495-1501.

Lima, F., C. Mendonça, J. Alvarez, G. Ratti, S. Lenharo, H. Kahn and J. Garzillo (1995). Chemical and physical evaluations of commercial dicalcium phosphates as sources of phosphorus in animal nutrition. Poult. Sci. *74*(10), 1659-1670.

Lima, F.R., C. X. Mendonca, J. C. Alvarez, J. M. Garzillo, E. Ghion and P. M. Leal (1997). Biological evaluations of commercial dicalcium phosphates as sources of available phosphorus for broiler chicks. Poult. Sci. *76*:1707–1713.

Mutucumarana, R. K., V. Ravindran, G. Ravindran and A. J. Cowieson (2014). Measurement of true ileal digestibility and total tract retention of phosphorus in corn and canola meal for broiler chickens. Poult. Sci. 93 (2):412-419.

Payne, S. G. (2005). Phosphorus availability of feed phosphates in broilers. Thesis (MscAgric (Animal Sciences))--University of Stellenbosch.

Ravindran, V., E. Kornegay, L. Potter, B. Ogunabameru, M. Welten, J. Wilson and M. Potchanakorn (1995). An evaluation of various response criteria in assessing biological availability of phosphorus for broilers. Poult. Sci. 74(11), 1820-1830.

Tahir, M., A. B. Lughmani and G. M. Pesti (2011). Evaluation of an indigenous source of rock phosphate as a supplement for broiler chickens. Poult. Sci. 90 (9): 1983-1991.

Van der Klis, J., H. Versteegh, P. Garnsworthy, J. Wiseman and W. Haresign (1996). Phosphorus nutrition of poultry. Paper presented at the Recent advances in animal nutrition 1996.

Viljoen, J. (2001). Utilisation of feed phosphates: Fact or confusion. Afma matrix, 24-27.

Viljoen, J. (2003). Quality of feed phosphate supplements for animal nutrition. South African Society of Ani. Sci.: http://www. sasas. co. za/Popular/ Popular. html.

Williams, B., S. Solomon, D. Waddington, B. Thorp and C. Farquharson (2000). Skeletal development in the meat-type chicken. Brit.Poult. Sci. *41*(2), 141-149.

Williams, B., D. Waddington, D. Murray and C. Farquharson (2004). Bone strength during growth: influence of growth rate on cortical porosity and mineralization. Calcified tissue Int., *74*(3), 236-245.