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Partial and complete displacement of inorganic feed phosphates by microbial phytase decreases phosphorus excretion and maintains tibia integrity in broiler chickens fed maize-soybean meal or wheat-soybean meal based diets

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Abstract

Supplementation of conventional poultry diets with inorganic phosphates is common practice owing to the poor bioavailability of phosphorus from vegetable feedstuffs. However these inorganic feed phosphates may pose risks of contamination to underground and surface water bodies, hence controlled reduction or elimination of inorganic feed phosphates by phytases is being considered a safer alternative. The effect of a partial or complete displacement of inorganic feed phosphates by a microbial phytase on performance, phosphorus excretion, tibial integrity and serum mineral bioavailability in chickens fed either maize or wheat-soybean meal based diets was investigated in a 49d trial. One hundred and eighty unsexed day-old broiler chicks were allocated into six dietary treatments with three replicates in each treatment containing 10 birds per replicate. The experiment was arranged in a completely randomized design. Control diets based on either of maize or wheat were formulated, with dicalcium phosphate (DCP) included as the inorganic feed phosphate i.e. IFP only diets. Similar test diets were also formulated with 50% and 100% less DCP and supplemented with a microbial phytase included at 300g/tonne of feed, i.e. 50% IFP + phytase and phytase only diets. At 21- 49d, excreta was collected for estimation of phosphorus excretion, blood was collected to determine serum mineral availability and birds were slaughtered, tibia harvested for determination of tibia ash and morphology. Daily feed intake of chickens on the low non-phytate phosphorus (NPP) diets with phytase supplementation compared favourably with the IFP only treatments at 1-21d, 22-49d and 2-49d periods. Complete displacement of IFP reduced DLWG of chickens at 1-21d when compared to partial displacement in both maize (50%IFP + phytase vs phytase only, $p < 0.001$) and wheat-soybean meal-based diets (50%IFP + phytase vs phytase only, $p = 0.02$), despite phytase supplementation. A significant increase in FCR was observed between chickens on the IFP only diets and the phytase only diets in both cereal-based diet groups and between both low NPP maize-soybean meal-based diets supplemented with phytase (50% IFP + phytase vs phytase only, $p < 0.001$) at 0-21d. Chickens on the control diets showed comparable DLWG with chickens on the 50% IFP diet supplemented with phytase at 1-21d and 22-49d in both maize- and wheat-soybean meal-based groups. There was no significant influence of the dietary treatments on serum calcium and phosphorus concentration as well as tibia ash percentage and tibia morphology. Partial and complete displacement of inorganic phosphate by microbial phytase further showed a decrease in phosphorus excretion to the environment. In conclusion, phytase supplementation of a partially displaced IFP maize or wheat-soybean meal-based diet improved performance of broiler chickens with no negative influence on serum mineral concentration or tibia integrity, while complete displacement of IFP in broiler diets further reduced phosphorus excretion.

Keywords: Dicalcium phosphate, non-phytate phosphorus, performance, cereals, serum phosphorus

Le déplacement partiel et complet des phosphates alimentaires inorganiques par la phytase microbienne diminue l'excrétion de phosphore et maintient l'intégrité du tibia chez les poulets à griller nourris avec des régimes à base de maïs et de tourteau de soja ou de blé et de tourteau de soja

Résumé

La supplémentation des régimes alimentaires conventionnels de la volaille avec des phosphates inorganiques est une pratique courante en raison de la faible biodisponibilité du phosphore provenant des aliments végétaux. Cependant, ces phosphates alimentaires inorganiques peuvent présenter des risques de contamination des masses d'eau souterraines et de surface, c'est pourquoi la réduction ou l'élimination contrôlée des phosphates alimentaires inorganiques par les phytases est considérée comme une alternative plus sûre. L'effet d'un déplacement partiel ou complet des phosphates alimentaires inorganiques par une phytase microbienne sur les performances, l'excrétion de phosphore, l'intégrité tibiale et la biodisponibilité des minéraux sériques chez des poulets nourris avec des régimes à base de maïs ou de farine de blé-soja a été étudié dans un essai de 49 jours. Cent quatre-vingt poussins de chair d'un jour non sexés ont été répartis en six traitements diététiques avec trois répétitions dans chaque traitement contenant 10 oiseaux par répétition. L'expérience a été arrangée dans une conception complètement randomisée. Des régimes témoins à base de maïs ou de blé ont été formulés, avec du phosphate dicalcique (DCP) inclus comme phosphate alimentaire inorganique, c'est-à-dire des régimes IFP uniquement. Des régimes de test similaires ont également été formulés avec 50 % et 100 % de moins de DCP et complétés par une phytase microbienne incluse à 300 g/tonne d'aliment, c'est-à-dire 50 % d'IFP + phytase et régimes à base de phytase uniquement. À 21-49 jours, les excréments ont été collectés pour estimer l'excrétion de phosphore et le sang a été collecté pour déterminer la disponibilité des minéraux sériques et les oiseaux ont été abattus, le tibia récolté pour la détermination de la cendre et de la morphologie du tibia. L'apport alimentaire quotidien des poulets sur les régimes à faible teneur en NPP avec une supplémentation en phytase se comparait favorablement avec les traitements IFP uniquement aux périodes de 1-21 jours, 22-49 jours et 2-49 jours. Le déplacement complet de l'IFP a réduit le DLWG des poulets à 1-21 jours par rapport au déplacement partiel dans les régimes à base de maïs (50 % IFP + phytase vs phytase uniquement, $p < 0{,}001$) et de farine de blé-soja (50 % IFP + phytase vs phytase seulement, $p = 0{,}02$), malgré la supplémentation en phytase. Une augmentation significative du FCR a été observée entre les poulets sur les régimes IFP uniquement et les régimes uniquement à base de phytase dans les deux groupes de régime à base de céréales et entre les deux régimes à base de farine de maïs et de soja à faible NPP complétés par de la phytase (50 % IFP + phytase vs phytase uniquement , $p < 0{,}001$) à 0-21 j. Les poulets des régimes témoins ont montré un DLWG comparable à celui des poulets du régime IFP à 50 % supplémenté en phytase à 1-21 jours et à 22-49 jours dans les groupes à base de farine de maïs et de blé-soja. Il n'y avait pas d'influence significative des traitements diététiques sur la concentration sérique de calcium et de phosphore ainsi que sur le pourcentage de cendres du tibia et la morphologie du tibia. Le déplacement partiel et complet du phosphate inorganique par la phytase microbienne a en outre montré une diminution de l'excrétion de phosphore dans l'environnement. En conclusion, la supplémentation en phytase d'un régime à base de maïs ou de farine de soja partiellement déplacé IFP a amélioré les performances des poulets de chair sans influence négative sur la concentration minérale sérique ou l'intégrité du tibia, tandis que le déplacement complet de l'IFP dans les régimes pour poulets de chair a encore réduit l'excrétion de phosphore.

Mots clés : Phosphate dicalcique, phosphore non phytate, performance, céréales, phosphore sérique

Introduction

Nutrition is the singular most important contributor to skeletal integrity besides genetics. Phosphorus supplied via the diet is essential for the formation of bones and teeth, and other physiological processes such as protein synthesis, nervous function, transfer of genetic information, acid balance, plus repairs of cells and tissues. Plant phosphorus is primarily stored in cereals, legumes, nuts and oilseeds as a mineral complex – phytate (myo-inositol hexakisphosphate (IP6)) which is indigestible and insoluble under physiological conditions in monogastric animals. Dietary phytate also poses a challenge to the bioavailability of other essential nutrients through its strong chelation with proteins, starch, and mono- and divalent cations such as calcium (Ca), magnesium (Mg), iron (Fe), copper (Cu), and zinc (Zn) (Cosgrove, 1980; Kumar and Sinha, 2018). About 86% and 50% of the total phosphorus in corn and wheat grains respectively, is bound in phytate complexes and subsequently excreted as waste (Jacela *et al.*, 2010). Inorganic sources of phosphorus are offered as a secondary supply of phosphorus in the diets of monogastric animals owing to the unavailability of plant phosphorus bound in phytate. Calcined bone meal (bone ash, $Ca_5(OH)(PO_4)_3$) and dicalcium phosphate (DCP) are the inorganic feed phosphates of choice of the Nigeria poultry production industry. However, the use of inorganic feed phosphates may increase soluble phosphorus in litter with the potential to contaminate underground and surface water bodies (Junqueira *et al.*, 2011). Commercially available exogenous phytases (enzymes that exhibit substrate-specificity for phytates) derived from either fungi or bacteria, such as *Aspergillus niger* and *Escherichia coli* have found use in monogastric nutrition to improve the availability of phosphorus in plant-based ingredients hitherto bound in phytate complexes as well as to mitigate phosphorus excretion into the environment. Phytases have also been instrumental to improving performance and nutrient utilization in monogastric species due to its ability to hydrolyze the inositol-phosphate linkages of the phytate complex in a stepwise manner, releasing bound amino acids and minerals in the process (De Cuyper *et al.*, 2020; Kies *et al.*, 2001; Syed *et al.*, 2017).Phytase requirement in monogastric animals varies in response to type of plant-based feed ingredients as ingredients differ in endogenous phytase content, as well as the degree of heat treatment to which they plant-based ingredients are subjected (Nielsen and Wenzel, 2007).Against this backdrop, this study was designed to appraise a partial and total displacement of an inorganic feed phosphate by a commercial 6-phytase in performance, mineralization, tibial integrity and phosphorus excretion of 49d-old broiler chickens fed either a corn-soybean or wheat-soybean-based diet.

Materials and methods

Birds, housing and diets

This research was conducted at the poultry unit of the Teaching and Research Farm, University of Ibadan, Ibadan, Nigeria. One hundred and eighty 1-d-old unsexed broiler chickens (Arbor acres) were obtained from a commercial hatchery, weighed, wing tagged and randomly allocated to 18 deep litter floor pens (0.8m × 0.8m) based on weight equalization using the Experimental Animal Allotment Program (Kim and Lindemann, 2007). The ambient temperature and illumination regimen in the animal house were 32°C and 24L:0D, respectively for the first seven days and subsequently 24-29°C and 14L:10D (natural illumination) for the rest of the study period. A 2-phase rearing system was adopted: starter; 1-21d and grower; 22-49d, and the IFP used in this study was feed grade dicalcium phosphate (DCP). The experimental design was a completely randomized design with six treatments per phase; control diets based on either of maize or wheat with DCP included as the inorganic feed phosphate plus similar diets formulated with 50% and 100% less DCP and supplemented with a microbial phytase included at 300g/tonne of feed (Tables 1 and 2). The commercial microbial phytase (6-phytase) was obtained from *Aspergillus niger* and contained 5000 FYT/g. Diets were formulated to meet the requirements of broiler chicken (NRC, 1994). Each experimental diet was offered to three replicates as a mash and with water *ad libitum*.

Table 1: Gross composition and layout of experimental diets (starter; 1-21d)

Cereal	Maize			Wheat		
Inorganic Feed Phosphate	+	+	-	+	+	-
Microbial phytase[1], U/kg feed	0	1500		0	1500	
Ingredients	g/kg					
Maize	504.0	509.5	512.0			
Wheat				550.0	554.5	557.0
Maize starch	44.0	44.0	44.0	69.0	69.0	69.0
Soybean meal	340.0	340.0	340.0	280.0	280.0	280.0
Fish meal, 70% CP	50.0	50.0	50.0	40.0	40.0	40.0
Soy oil	30.0	30.0	30.0	30.0	30.0	30.0
Limestone	6.0	11.0	19.0	6.0	11.0	19.0
Dicalcium phosphate	21.0	10.5		19.0	9.5	
Vitamin-mineral premix[2]	2.0	2.0	2.0	2.0	2.0	2.0
DL-Methionine	1.0	1.0	1.0	2.0	2.0	2.0
Salt	2.0	2.0	2.0	2.0	2.0	2.0
Titanium dioxide[3]	+	+	+	+	+	+
Calculated analysis[4]						
Metabolizable energy, kcal/kg	3017.23	3035.08	3043.19	2979.07	2992.78	2997.40
Crude Protein, %	23.14	23.18	23.19	21.89	21.94	21.95
Methionine, %	0.49	0.49	0.49	0.55	0.55	0.55
Digestible methionine, %	0.45	0.45	0.45	0.51	0.51	0.51
Methionine + cysteine, %	0.82	0.82	0.82	0.88	0.88	0.88
Digestible methionine + cysteine, %	0.71	0.71	0.71	0.78	0.78	0.78
Lysine, %	1.27	1.27	1.27	1.12	1.12	1.12
Digestible lysine, %	1.10	1.10	1.10	0.99	0.99	0.99
Ether extract, %	6.12	6.14	6.15	5.01	5.01	5.01
Fibre, %	3.32	3.33	3.33	3.27	3.28	3.28
Calcium, %	0.86	0.83	0.90	0.78	0.78	0.87
Total phosphorus, %	0.76	0.57	0.38	0.73	0.56	0.39
Non-phytate phosphorus, %	0.52	0.38	0.23	0.50	0.37	0.24

[1]Ronozyme P 5000 CT, Koninklijke DSM N.V, Netherlands, contains 5000FYT/g, included at 300g/tonne of feed

[2] Optimix premix, Animal Care, Nigeria. Provided the following per kg/diet: vitamin A, 20,000IU; vitamin D3, 4,000 IU; vitamin E, 20 mg; vitamin K3, 4 mg, vitamin B1, 3 mg; vitamin B2, 10 mg; niacin 16mg; calpan 16 mg; vitamin B6, 6 mg; vitamin B12, 0.03 mg; choline chloride, 200 mg; folic acid, 2 mg; biotin,0.1 mg; manganese, 120 mg; iron, 40 mg; zinc, 100 mg, copper, 10 mg; iodine, 2 mg; cobalt, 0.4 mg; selenium,0.4 mg; antioxidant, 250 mg

[3] Titanium dioxide included at 0.5% of feed as an inert tracer

[4] Calculated analysis based on nutritional values of feed ingredients (NRC,1994; Feedipedia)

Table 2: Gross composition and layout of experimental diets (finisher; 22-49d)

Cereal	Maize			Wheat		
Inorganic Feed Phosphate	+	+	-	+	+	-
Microbial phytase[1], U/kg feed	0	1500		0	1500	
Ingredients	g/kg					
Maize	571.0	573.8	576.5			
Wheat				586.5	588.3	590.8
Maize starch	40.0	40.0	40.0	75.0	75.0	75.0
Soybean meal	340.0	340.0	340.0	270.0	270.0	270.0
Fish meal, 72% CP	10.0	10.0	10.0	30.0	30.0	30.0
Soy oil	10.0	10.0	10.0	10.0	10.0	10.0
Limestone	9.0	14.0	19.0	8.0	13.0	17.2
Dicalcium phosphate	15.5	7.70		13.5	6.70	
Vitamin-mineral premix[2]	2.0	2.0	2.0	2.0	2.0	2.0
Lysine-HCl				1.8	1.8	1.8
DL-Methionine	0.5	0.5	0.5	1.2	1.2	1.2
Salt	2.0	2.0	2.0	2.0	2.0	2.0
Titanium dioxide[3]	+	+	+	+	+	+
Calculated analysis[4]						
Metabolizable energy, kcal/kg	2901.70	2910.79	2919.55	2878.50	2883.98	2891.60
Crude Protein, %	20.83	20.85	20.87	21.26	21.28	21.31
Methionine, %	0.37	0.37	0.37	0.45	0.45	0.45
Digestible methionine, %	0.34	0.34	0.34	0.42	0.42	0.42
Methionine + cysteine, %	0.69	0.69	0.69	0.78	0.78	0.78
Digestible methionine + cysteine, %	0.58	0.58	0.58	0.68	0.68	0.68
Lysine, %	1.08	1.08	1.08	1.20	1.20	1.20
Digestible lysine, %	0.92	0.92	0.92	1.07	1.07	1.07
Ether extract, %	3.96	3.97	3.98	2.95	2.95	2.95
Fibre, %	3.40	3.42	3.42	3.29	3.30	3.30
Calcium, %	0.74	0.76	0.79	0.72	0.76	0.78
Total phosphorus, %	0.60	0.46	0.32	0.62	0.50	0.38
Non-phytate phosphorus, %	0.37	0.26	0.15	0.40	0.31	0.22

[1]Ronozyme P 5000 CT, Koninklijke DSM N.V, Netherlands, contains 5000 FYT/g, included at 300g/tonne of feed

[2] Optimix premix, Animal Care, Nigeria. Provided the following per kg/diet: vitamin A, 20,000IU; vitamin D3, 4,000 IU; vitamin E, 20 mg; vitamin K3, 4 mg, vitamin B1, 3 mg; vitamin B2, 10 mg; niacin 16mg; calpan 16 mg; vitamin B6, 6 mg; vitamin B12, 0.03 mg; choline chloride, 200 mg; folic acid, 2 mg; biotin,0.1 mg; manganese, 120 mg; iron, 40 mg; zinc, 100 mg, copper, 10 mg; iodine, 2 mg; cobalt, 0.4 mg; selenium,0.4 mg; antioxidant, 250 mg

[3] Titanium dioxide included at 0.5% of feed as an inert tracer

[4] Calculated analysis based on nutritional values of feed ingredients (NRC,1994; Feedipedia)

Sampling, chemical analyses and calculations

Body weight and feed intake were recorded weekly. Daily live weight gain (DLWG) was estimated as the linear slope of the response of live body weight of the birds to age, feed conversion ratio (FCR) calculated as the ratio of feed consumed to body weight gained per time and flock uniformity expressed as the percentage of bird weights occurring within 10% of the average group weight. Age at market weight (pegged at 2.45kg liveweight)
was predicted using the logistic non-linear growth model [Equation 1] (Robertson, 1923, Aggrey, 2002).

$$W_t = {W_A}/{[1 + exp - k(t - t_i)]} \qquad --- \; Equation\ 1$$

On day 21, two birds per replicate were moved into metabolic cages with dimensions: 120cm × 69cm × 85cm and equipped with feeding troughs, nipple drinkers and excreta collection tray. After a five day acclimatization period, excreta was collected 12 hourly for three days. Excreta was oven dried at 55°C till constant weight was attained and milled, and sub-samples of excreta were assayed for phosphorus (Francesch *et al.*, 2005). On day 42 of the study, two birds per replicate were bled, blood samples centrifuged at 3000 rpm for 15 mins and serum harvested. Serum calcium and phosphorus were assayed by a spectrophotometric method using the relevant biochemical assay kits (Quimica Clinica Aplicada, S.A). On day 49, four birds per replicate were weighed, euthanized and tibia harvested for analyses. Two right tibia per replicate were dried to constant weight in a forced air oven at 105ºC, defatted and calcined in a muffle furnace at 550ºC to determine tibia ash. Another two right tibia were subjected to tibia morphology assessment.

Statistical analysis

Data were subjected to analysis of variance (ANOVA) using JASP statistical package (version 0.14.1) (JASP, 2020), and the effect of partial or total displacement of inorganic feed phosphates by a microbial phytase within each cereal-based diet group were tested by a set of contrasts. Significance was based on a 5% probability level.

Results

No visual indicators of skeletal degeneration or deformity were observed in the birds throughout the study period. The effect of partial or complete displacement of inorganic feed phosphates by a microbial phytase on growth performance; daily feed intake (g), Daily live weight gain, DLWG (g) and feed conversion ratio, FCR, are summarized in Figures 1-3. Phytase supplementation of the completely displaced IFP diet resulted in poorer daily weight gain when compared to the IFP only 24.83 ± 3.48g *vs* 20.49 ± 4.34g, $p < 0.001$) and partially displaced IFP (24.82± 3.34g *vs* 20.49 ± 4.34g, $p < 0.001$) maize-soybean based diets in the starter phase. Similarly, phytase supplementation of the completely displaced IFP wheat-soybean meal-based diet resulted in poorer daily weight gain when compared to the IFP only 24.83 ± 3.48g *vs* 20.49 ± 4.34g, $p < 0.001$) and partially displaced IFP (24.82 ± 3.34g *vs* 20.49 ± 4.34g, $p < 0.001$) maize-soybean meal-based diets in the starter phase. In the finisher phase of growth, a decline in DLWG was observed in response to total displacement of IFP in phytase-supplemented maize (71.61 ± 11.46g *vs* 63.78 ± 10.21g, p=0.02) and wheat (70.32 ± 7.98g *vs* 63.78 ± 10.21g, p=0.02) diets. In both grower and finisher phases, no effect of phytase supplementation was observed on DLWG of chickens on the partially displaced IFP diets (both maize-and wheat-soybean meal based diets) compared to the IFP diets. Overall, i.e. from 1-49d, phytase supplementation of a partially displaced IFP corn- or wheat-soybean meal-based diet produced birds with DLWG comparable with those on the IFP only diet.

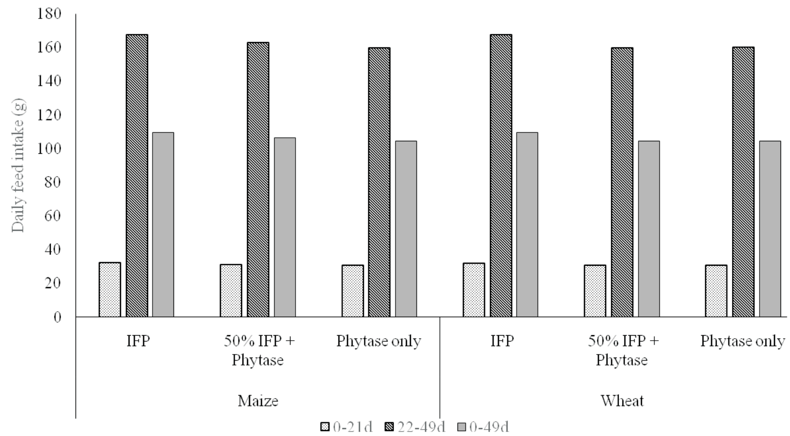

Figure 1: Effect of a partial or total displacement of inorganic feed phosphates by a microbial phytase on daily feed intake of broiler chickens fed a maize-soybean or wheat-soybean-based diet (IFP – Inorganic feed phosphate (dicalcium phosphate))

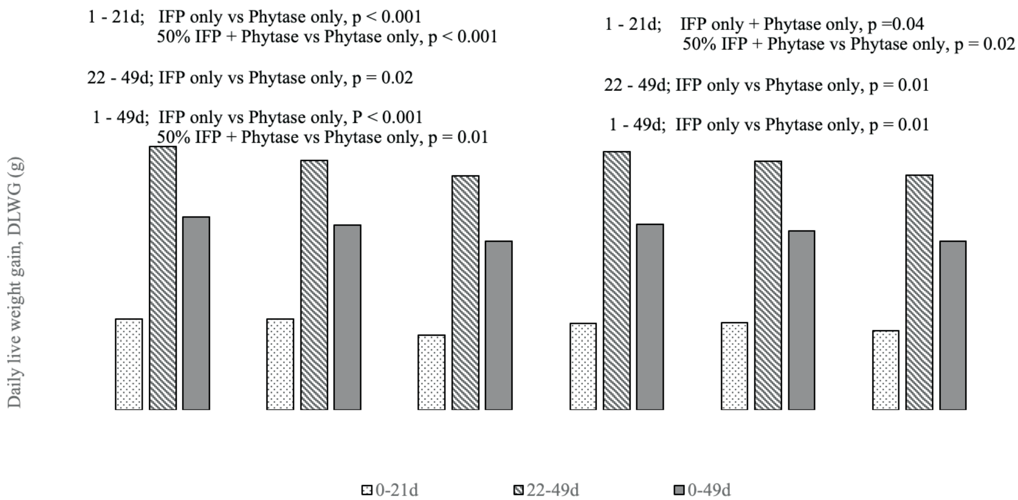

Figure 2: Effect of a partial or total displacement of inorganic feed phosphates by a microbial phytase on daily live weight gain, DLWG of broiler chickens fed a maize-soybean or wheat-soybean-based diet (IFP–Inorganic feed phosphate (dicalcium phosphate))

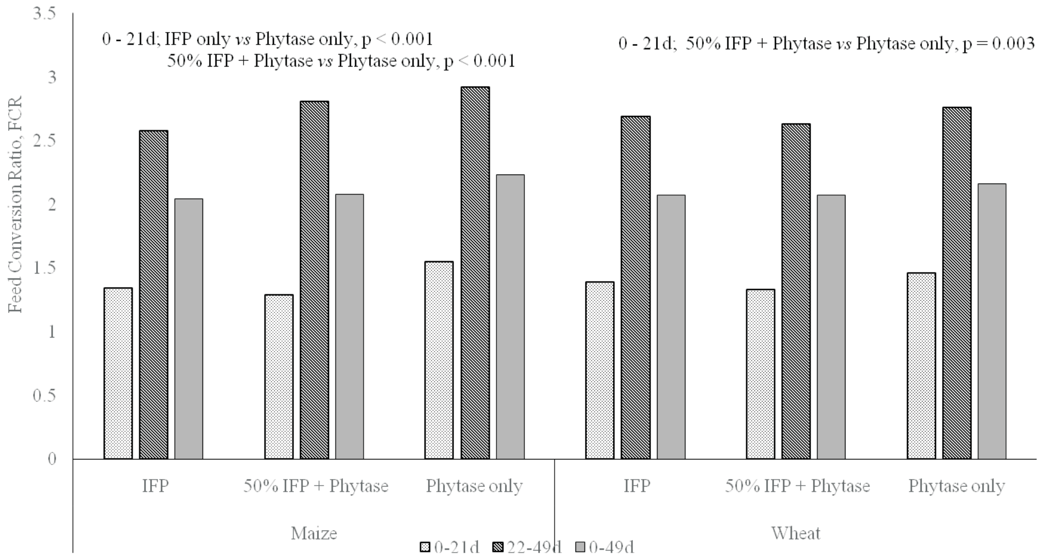

Figure 3: Effect of a partial or total displacement of inorganic feed phosphates by a microbial phytase on feed conversion ratio, FCR of broiler chickens fed a maize-soybean or wheat-soybean-based diet (IFP – Inorganic feed phosphate (dicalcium phosphate))

However, chickens on the phytase only diet had a lower DLWG which differed significantly from those on the IFP only maize- (45.90 ± 7.091g *vs* 52.48 ± 7.37g, $p<0.001$) wheat- (45.97 ± 4.35g vs 50.60 ± 5.57g, $p=0.008$) soybean meal-based diets, as well as from chickens on the partially displaced IFP maize-soybean meal-based diet supplemented with phytase (45.90 ± 7.09g *vs* 50.38 ± 4.94g, $p=0.02$). Feed conversion ratio of the birds on the IFP only maize-soybean-based diets compared favourably with the birds on the partially displaced IFP maize diet with enzyme supplementation during the starter phase, while the chickens on the phytase only maize-soybean-based diet showed a poorer FCR in the starter phase compared to the partially displaced IFP with phytase supplemented maize-soybean-based diet (1.55 ± 0.51 *vs* 1.29 ± 0.04, $p< 0.001$) and the IFP only maize-soybean-based diet (1.55 ± 0.51 *vs* 1.34 ± 0.06, $p< 0.001$). Also, a poorer FCR was observed in the starter phase when chickens on the phytase only wheat-soybean meal-based diet were compared with chickens on the partially displaced IFP with phytase supplemented wheat-soybean-based diet (1.46 ± 0.04*vs* 1.33 ± 0.03, $p = 0.003$) No effect of phytase supplementation on partial or complete displacement of IFP in maize- or wheat-soybean meal-based diets was observed in the finisher phase or in the overall assessment of the study. The effect of partial or complete displacement of inorganic feed phosphates by a microbial phytase on final weight (g), flock uniformity, % and age at 2.45kg (days) are summarized in Table 3. While flock uniformity was not influenced by partial or complete displacement of inorganic feed phosphates by phytase, it ranged from ~ 54-83% for all chickens in the study. Final weight of chickens was similar for chickens on the IFP only and partial IFP displacement with enzyme supplementation treatments for both maize- and wheat-soybean meal-based groups. However, complete displacement of IFP with phytase supplementation was observed to lower weight of birds at 49d compared to chickens on the IFP only maize- (2242.58 ± 350.4g *vs* 2545.48 ± 349.97g, $p < 0.001$) and wheat (2297.50 ± 207.37g *vs* 2494.17 ± 266.05g, $p = 0.02$) soybean meal-based diets. Chicken on the partial displacement of IFP maize-soybean meal-based diet with phytase supplementation also fared better than chickens on the phytase only maize-soybean meal-based diet (2455.83 ± 221.46g *vs* 2242.58 ± 350.4g, $p = 0.01$). The age at 2.45kg also seemed uninfluenced by partial or complete displacement of inorganic feed phosphates by phytase, except between IFP only *vs* phytase only birds on the maize-soybean meal-based diets (49.71 ± 5.74d *vs* 52.45 ± 5.91d, $p = 0.02$).The effect of partial or complete displacement of inorganic feed phosphates by a microbial phytase on phosphorus excretion, tibia ash, and serum concentration of phosphorus and calcium are summarized in Table 4. Partial and complete displacement of IFP in the maize-soybean meal-based diets reflected in a significant decline in excreta phosphorus concentration when compared with the IFP only diets (Phytase only < 50% IFP + phytase < IFP only treatments). Also, complete and partial displacement of IFP with phytase supplementation significantly lowered phosphorus excretion compared to the IFP only treatment (1.27 ± 0.36 *vs* 2.06 ± 0.54 and 1.10 ± 0.20 *vs* 2.06 ± 0.54 g/100g DM, respectively) in the wheat-soybean meal-based treatments, however, no significant difference in excreta phosphorus content was observed between chickens on the 50% IFP + phytase *vs* chickens on the phytase only wheat-soybean meal-based diets. Partial or complete displacement of inorganic feed phosphates by a microbial phytase did not significantly impact on tibia mineralization or serum mineral concentration in chickens on both the maize- and wheat-soybean meal-based treatments. Evaluation of the effect of partial or complete displacement of inorganic feed phosphates by a microbial phytase on tibia morphology of broiler chickens (Table 5A and 5B) showed no effect on indices except for a decline in the tibia length of chickens on the 50% IFP (98.91 ± 2.09 mm) *vs* 0% IFP + phytase (92.11 ± 5.67 mm), and reduction in the thickness of medial wall of chickens on the 100% IFP (2.36 ± 0.70 mm) *vs* 50% IFP + phytase (1.99 ± 0.48 mm) and chickens on the 100% IFP (2.36 ± 0.70 mm) *vs* 0% IFP + phytase (0.88 ± 0.12 mm) maize-soybean meal-based treatments.

Table 3: Effect of a partial or total displacement of inorganic feed phosphates by phytase on final weight, flock uniformity, and age at market, 2.45kg (days) of broiler chickens fed a maize-soybean or wheat-soybean-based diet

Dietary treatments			Performance Indices			
Cereal	IFP inclusion, %	Phytase (U/kg feed)	Initial weight (g)	Final weight (g)	Flock uniformity, %	Age at market, 2.45kg (days)
Maize	100	0	44.10 ± 2.95	2545.48 ± 349.97	61.33 ± 10.60	49.71 ± 5.74
	50	1500	44.17 ± 2.90	2455.83 ± 221.46	83.00 ± 7.00	50.13 ± 3.92
	0	1500	44.07 ± 2.98	2242.58 ± 350.4	58.33 ± 28.87	52.45 ± 5.91
Wheat	100	0	44.10 ± 3.00	2494.17 ± 266.05	65.00 ± 9.17	49.20 ± 2.99
	50	1500	44.10 ± 2.92	2419.17 ± 267.31	54.33 ± 18.88	49.82 ± 3.03
	0	1500	44.13 ± 2.96	2297.50 ± 207.37	79.33 ± 35.80	51.15 ± 2.26
Contrast p-value						
Maize: 100% IFP *vs* 50% IFP + Phytase				NS	NS	NS
100% IFP *vs* 0% IFP				< 0.001	NS	0.02
50% IFP vs 0% IFP + Phytase				0.01	NS	NS
Wheat: 100% IFP *vs* 50% IFP + Phytase				NS	NS	NS
100% IFP *vs* 0% IFP				0.02	NS	NS
50% IFP vs 0% IFP + Phytase				NS	NS	NS

IFP – Inorganic feed phosphate; NS – not significant at $p > 0.05$

Table 4: Effect of a partial or total displacement of inorganic feed phosphates by phytase on phosphorus excretion and tibia mineralization of broiler chickens fed a maize-soybean or wheat-soybean-based diet

Dietary treatments			Excreta phosphorus content (g/100gDM)	Tibia ash (g/100g DM)	Serum minerals	
Cereal	IFP inclusion, %	Phytase (U/kg feed)			P (mg/dL)	Ca (mg/dL)
Maize	100	0	2.14 ± 0.58	41.58 ± 2.58	6.30 ± 1.18	3.61 ± 1.49
	50	1500	1.61 ± 0.43	41.32 ± 2.46	6.06 ± 1.54	5.25 ± 3.07
	0	1500	0.81 ± 0.09	38.50 ± 3.91	5.65 ± 1.45	5.74 ± 3.11
Wheat	100	0	2.06 ± 0.54	41.00 ± 2.49	5.25 ± 1.24	8.14 ± 2.81
	50	1500	1.10 ± 0.20	41.63 ± 2.71	6.10 ± 1.18	8.60 ± 3.59
	0	1500	1.27 ± 0.36	40.69 ± 2.76	4.43 ± 1.11	6.57 ± 0.60
Contrast p-value						
Maize: 100% IFP *vs* 50% IFP + Phytase			[illegible]	NS	NS	NS
100% IFP *vs* 0% IFP			< 0.001 (↓62%)	NS	NS	NS
50% IFP + Phytase vs 0% IFP + Phytase			< 0.001 (↓50%)	NS	NS	NS
Wheat: 100% IFP *vs* 50% IFP + Phytase			< 0.001 (↓47%)	NS	NS	NS
100% IFP *vs* 0% IFP			< 0.001 (↓38%)	NS	NS	NS
50% IFP + Phytase *vs* 0% IFP + Phytase			NS	NS	NS	NS

"IFP - Inorganic feed phosphate; NS - not significant at $p > 0.05$

Table 5a: Effect of a partial or total displacement of inorganic feed phosphates by phytase on the tibia morphology of broiler chickens fed a maize-soybean or wheat-soybean-based diet : mean ± standard deviation values

	Dietary Treatments					
Cereal	Maize			Wheat		
IFP inclusion, %	100	50	0	100	50	0
Phytase (U/kg feed)	0	1500	1500	0	1500	1500
Tibia morphology characteristics						
Tibia weight (g)	13.00 ± 1.66	12.68 ± 2.38	10.96 ± 1.80	13.08 ± 1.63	11.97 ± 2.28	12.12 ± 1.70
Tibia length (mm)	96.36 ± 5.68	98.91 ± 2.09	92.11 ± 5.67	98.82 ± 2.05	98.26 ± 4.35	96.62 ± 3.07
Tibia weight/length ratio	0.14 ± 0.02	0.13 ± 0.02	0.12 ± 0.02	0.13 ± 0.01	0.12 ± 0.02	0.13 ± 0.02
Thickness of lateral wall (mm)	2.36 ± 0.70	1.99 ± 0.48	2.01 ± 0.60	2.07 ± 0.36	1.68 ± 0.24	1.76 ± 0.44
Thickness of medial wall (mm)	1.27 ± 0.41	0.94 ± 0.17	0.88 ± 0.12	1.10 ± 0.16	1.03 ± 0.20	0.97 ± 0.13
Diaphyseal diameter (mm)	9.03 ± 0.75	8.66 ± 0.69	8.57 ± 0.91	8.99 ± 0.42	8.44 ± 0.69	8.41 ± 0.83
Medullary diameter (mm)	5.40 ± 1.00	5.74 ± 0.33	5.68 ± 0.51	5.82 ± 0.25	5.73 ± 0.52	5.68 ± 0.61
Robusticity index	41.13 ± 3.31	42.66 ± 1.83	41.62 ± 2.22	42.03 ± 0.97	43.14 ± 1.06	42.20 ± 2.11
Tibiotarsal index	40.08 ± 9.93	33.56 ± 4.36	33.39 ± 5.32	35.15 ± 4.15	32.11 ± 2.62	32.37 ± 4.04
Crossectional cortical area (mm^2)	60.55 ± 10.81	56.18 ± 14.15	52.53 ± 22.95	64.17 ± 12.09	47.44 ± 12.91	53.98 ± 8.21
Crossectional medullary area (mm^2)	44.86 ± 9.38	44.32 ± 8.93	47.06 ± 8.44	41.98 ± 5.91	41.32 ± 8.94	43.95 ± 8.79

"IFP - Inorganic feed phosphate; NS - not significant at $p > 0.05$

Table 5b: Effect of a partial or total displacement of inorganic feed phosphates by phytase on the tibia morphology of broiler chic kens fed a maize-soybean or wheat-soybean-based diet : contrast p -values

	Dietary Treatments					
Cereal	Maize			Wheat		
Contrasts	100% IFP *vs* 50% IFP + Phytase	100% IFP *vs* 0% IFP	50% IFP vs 0% IFP + Phytase	100% IFP *vs* 50% IFP + Phytase	100% IFP *vs* 0% IFP	50% IFP vs 0% IFP + Phytase
Contrast p-values						
Tibia weight (g)	NS	NS	NS	NS	NS	NS
Tibia length (mm)	NS	NS	0.01	NS	NS	NS
Tibia weight/length ratio	NS	NS	NS	NS	NS	NS
Thickness of lateral wall (mm)	NS	NS	NS	NS	NS	NS
Thickness of medial wall (mm)	0.01	0.02	NS	NS	NS	NS
Diaphyseal diameter (mm)	NS	NS	NS	NS	NS	NS
Medullary diameter (mm)	NS	NS	NS	NS	NS	NS
Robusticity index	NS	NS	NS	NS	NS	NS
Tibiotarsal index	NS	NS	NS	NS	NS	NS
Crossectional cortical area (mm^2)	NS	NS	NS	NS	NS	NS
Crossectional medullary area (mm^2)	NS	NS	NS	NS	NS	NS

Discussion

The possibility of replacement of inorganic feed phosphate by a commercial 6-phytase preparation for broiler chickens was investigated in maize- and wheat-soybean meal-based diets. Maize and wheat are staple cereal grains used as energy sources in monogastric feed formulation with the former preferred in the US, Brazil and most Asian countries, and the latter predominantly used in Europe, Canada, Australia, New Zealand and the Russian Federation (Poultry Hub Australia, 2021). Higher endogenous phytase content in wheat compared to maize is believed to result in a higher phosphorus digestibility (50% *vs* 15%) in monogastric animals (Mavromichalis, 2013). However, other studies have reported 98.9 ± 1.2% and 99.7 ± 1.2% residual inositol hexaphosphate ($InsP_6$) + inositol pentaphosphate ($InsP_5$) for wheat and maize respectively, after incubation with wheat bran phytase (Brejnholt *et al.*, 2011), reflecting negligible digestion by endogenous phytases. In the current study, the maize, wheat and soybean meal in the diets are the target substrates for phytase activity, and the inorganic phosphorus in the diets supplied as dicalcium phosphate. In similar studies investigating supplementation of a low phosphorus (up to 50% of IFP in the positive control diet) maize-soybean diet by a microbial phytase at 500-300 FTU/kg diet, similar compensatory responses in feed intake, feed conversion ratio, body weight and body weight gain in comparison to the positive control group were reported (Gautier *et al.*, 2018; Mondal *et al.*, 2007; Walters *et al.*, 2019). The amount of phosphates released by phytase supplementation (1500phytase U/kg diet) compensates for the 0.9-0.14% non-phytate phosphorus reduction in the 50% IFP maize- and wheat-soybean meal-based diets. However, phosphates released by the same level of phytase supplementation in the light of further displacement of inorganic phosphorus in the diets up to 0.18-0.27% NPP in the diets failed to sufficiently compensate for IFP displacement in the diets. Higher doses of phytase is postulated to enable improved performance of chickens on lower phosphorus diets (Scholey *et al.*, 2018; Walters *et al.*, 2019). On the other hand, partial or complete displacement of IFP showed a steady decline in phosphorus excretion despite the singular supplementation level of phytase. Inclusion of phytase in the diets of broiler chickens decreased phosphorus excretion in broiler chickens up to 40% (Abdel-Megeed and Tahir, 2015; Almeida *et al.*, 2013), while phytase supplementation of layer chicken diets at 1000U/kg decrease phosphorus excretion by 47% (Casartelli *et al.*, 2005). In agreement with our current research, compensatory phosphate release by phytase was able to mask possible variability in serum calcium (Attia *et al.*, 2021; Taheri and Abbasi, 2020) and phosphorus concentration (Attia *et al.*, 2021) of chickens on low NPP diets. Yet, higher supplementation of phytase >500U/kg diet have been reported to either significantly increase serum phosphorus concentration in chickens (Bilal *et al.*, 2015; Taheri and Abbasi, 2020) or reduce serum P levels when compared with the control group (Viveros *et al.*, 2002). The effect of displacing IFP in broiler chicken diets on tibia ash percentage was also ameliorated by supplemental phytase in a study by Viveros *et al.,* (2005) where the low NPP diet + 500U Natuphos/kg diet compared favourably with the positive control group. Higher dosing of phytase up to 3000 FTU phytase/kg diet still showed similar responses (Taheri and Abbasi, 2020). Consequent on these observations, the presence of only marginal effects of displacement of inorganic feed phosphates by phytase on the tibia morphology; tibia length and thickness of medial wall - of broiler chickens was as expected.

Conclusion

The study showed that partial displacement of inorganic feed phosphates in broiler chicken diets supplemented with 1500U phytase/kg diet compared favourably in performance; daily feed intake, daily live weight gain, feed conversion ratio, flock uniformity and age at market weight, with the 100% IFP diet without phytase supplementation. Partial and complete displacement of IFP in broiler chicken diets supplemented with 1500U phytase/kg diet maintained tibia ash, tibia morphology, or serum calcium and phosphorus concentration of broiler chickens, and, partial and complete displacement of IFP in broiler chicken diets supplemented with 1500U phytase/kg diet significantly reduced phosphorus excretion of broiler chickens by 25-62%.

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