Increasing zinc levels in phytase-supplemented diets improves the performance and nutrient utilization of broiler chickens

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Abstract

Three hundred and thirty-six day-old Ross-308 male broiler chicks were used in a 35-day trial to investigate the effect of different concentrations of dietary Zn and phytase on broiler performance and energy utilization. Twelve day-old birds were used for the initial slaughter group to provide baseline body compositional data, while the remaining 324 birds were randomly distributed to six experimental diets. The treatments consisted of a 3 x 2 factorial arrangement with three levels of Zn (low, mid, and high; 30, 40, and 50 mg/kg, respectively) and two levels (0, 500 FTU/kg) of microbial phytase. Each dietary treatment was fed to 6 cages (9 birds/cage). Low Zn diet significantly decreased feed intake and body weight gain at days 1-24. Phytase supplementation improved body weight gain at d 24, irrespective of Zn level. The digestibility of P was improved in birds fed high-Zn diet with phytase supplementation, and the reverse was the case for Fe and Zn digestibility. High dietary Zn increased the Zn and Fe deposition in liver. The activity of AP, Ca-ATPase and Mg-ATPase in the jejunum was high in the phytase supplemented mid-level Zn diet. Phytase supplemented to the mid and high level Zn diets significantly improved most energy utilization parameters. This result indicate that the Zn concentrations used in this study were not inhibitory to phytase activity and broiler performance. Therefore, it can be concluded that dietary zinc level in phytase-supplemented diets could be increased up to 50 mg/kg without any negative effect on phytase-mediated broiler response.

Keywords: Bone quality, net energy, nutrient utilization

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Introduction

Zinc is an important trace mineral which plays a vital role in growth, feathering and disease resistance of chickens (Ao *et al*., 2011). A deficiency of Zn may cause retardation in growth, bone malformations, and suppression of disease resistance due to its essential role as a co-factor of many enzymes (Kfoury *et al.* 1968, Underwood & Suttle, 1999a). Although the NRC (1994) recommended 40 mg Zn/kg of diet, most commercial broiler diets are formulated to contain 100 - 120 mg Zn/kg. This excess of Zn in diets is ultimately excreted through the droppings and can pose a significant threat to the environment and crop production (Burrell *et al*., 2004). However, most Zn is bound with phytic acid. Despite a sufficient level of Zn in maize-soybean meal based-diets, the presence of fibre or phytate limits bioavailability in chickens (Underwood & Suttle, 1999).

Phytate forms complexes with different cations, such as Ca, P, Zn, Fe and Cu, and reduces their bioavailability in chickens (Maenz *et al*., 1999). Due to its high affinity for phytate, Zn forms a strong insoluble Zn-phytate complex (Reddy *et al*., 1982), which impairs the availability of Zn in chickens and can result in poor growth (O'Dell & Savage, 1960). Supplementation with microbial phytase has proved to be effective in dephosphorylating phytate and releasing the phytate-bound minerals (Kornegay, 2001). A previous *in vitro* study showed that incereased level of Zn (50 mg/kg) reduced phytate hydrolyis by phtyase (Akter *et al*., 2015).

Inclusion of phytase in maize–soybean-based diets containing low (5 ppm) Zn improved the body weight gain and feed intake of chickens (Yi *et al*., 1996b). Ao *et al.* (2007) found that supplementing phytase to diets with 12 mg Zn/kg resulted in increased feed intake and weight gain. In contrast, Mohanna and Nys (1999a) observed no significant difference in weight gain, feed intake and tibia ash content of birds fed diets

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containing 10 and 30 mg Zn/kg with or without phytase. Other researchers have reported that including phytase in chicken diets improves the availability of Zn (Thiel *et al*., 1993; Roberson & Edwards, 1994; Yi *et al*., 1996a), while the effect of a lack of phytase on Zn retention has also been reported in other studies (Roberson & Edwards, 1994; Sebastian *et al*., 1996). The lack of agreement between these studies may be the result of using different concentrations of Zn and phytase in diets, because, in addition to using varying levels of phytase, the concentrations of Zn in the experimental diets of these studies were either below the NRC (1994) recommendation for growth (40 mg/kg) or above the recommendation for maximum tissue deposition (85 mg/kg) (Mohanna & Nys, 1999b). Therefore, the present study was undertaken to evaluate the effect of different levels of Zn on phytase activity and impact on broiler growth performance, bone development, and nutrient utilization.

Materials and methods

The experiment was undertaken at the Centre for Animal Research and Teaching (CART) of the University of New England (UNE), Australia. All the procedures used in this study were approved by the Animal Ethics Committee of the same University (Approval No: AEC14-120).

The overall methodology that was adopted in this study has already been documented in Akter *et al.* (2017). However, a total of 336 day-old Ross 308 male broiler chicks (40.0 ± 0.7 g) were obtained from a local commercial hatchery (Baiada Poultry Pty. Ltd., Tamworth, Australia). On day one, 324 chicks were immediately randomly allocated to 6 treatments, with 54 chicks in each. Each treatment had six replicates with nine birds per replicate cage. The remaining 12 birds were used for the initial slaughter group, to provide baseline body compositional data.

Six experimental diets were formulated with low, mid, and high levels of Zn (30, 40, and 50 mg/kg, respectively) with 500 FTU/kg or without exogenous microbial phytase (Tables 1, 2 and 3). All diets were formulated either to meet or exceed the Aviagen (2009) nutrient recommendations and breed standards, with the exception of Zn. Diets were iso-energetic and iso-nitrogenous. In all grower diets, titanium dioxide (TiO_2), was added as an indigestible marker. The Ca, AvP and Na levels in the phytase-supplemented diets were calculated to include the mineral matrix (1.5 g, AvP, 1.65 g Ca, and 0.35 g Na per kg of diet) of the commercial *Escherichia coli*-derived phytase product used (Quantum Blue, AB Vista, Marlborough, UK). The activity of the phytase product was 5000 U/g where a unit (FTU) is defined as the quantity of enzyme that liberates one µmol of inorganic P per minute from sodium phytate at pH 5.5 and 37 °C. After mixing, the diets were pelleted at 65 °C temperature. The diets were fed to the birds as starter (0 - 10 d), grower (11 - 24 d), and finisher (25 - 35 d).

The collection, processing and chemical analysis of different samples (diets, ileal digesta, excreta, tibia bones, liver and part of jejunum) have been described previously (Akter *et al*., 2017).

On d 24, two birds from each replicate were killed and the whole intact carcases were frozen immediately and later processed. Both chicks from the same cage were pooled and processed together. After chopping and coarse-grinding individual chickens, they were thoroughly mixed and two subsamples (approximately 200 g each, wet weight) were finely ground and freeze-dried as described by Olukosi *et al*. (2008). The two subsamples were mixed together after drying, and ground again, from which a smaller sub-sample was taken for chemical analysis. The ground carcase samples were analysed for gross energy (GE), diethyl ether extractable fat (EE) and nitrogen (N).

The nitrogen content of the diets and meat samples was determined according to the Dumas combustion technique, as described by Sweeney (1989) using a LECO® FP-2000 automatic nitrogen analyser (Leco FP analyser model 602600; Leco Corp., St Joseph, Michigan, USA) with EDTA as a calibration standard. The crude protein (CP) equivalent of the ingredients was calculated as N (%) × 6.25. The EE was determined indirectly by the Soxhlet method for fat extraction. Around 6-8 g of finely ground sample was weighed into pre-weighed filter paper (No1 Whatman 185 mm) and extracted for 48 to 50 hours with chloroform, using a Soxhlet apparatus. After that, the samples were allowed to drain and dry at 80 °C for 72 hours. The EE was calculated as loss in weight and expressed as a proportion of dried sample weight.

To calculate the apparent metabolizable energy (AME), the following formulae were used:

$$AME\ (MJ/kg) = GEi - [GEo \times (Ti/To)]$$

where: GEi is gross energy (MJ/kg) in feed;
GEo is the gross energy (MJ/kg) in excreta,
Ti is the concentration of titanium dioxide in the diets; and
To is the concentration of titanium dioxide in the excreta.

Table 1 Ingredient and nutrient specifications of starter (0 - 10 days) diets

Ingredient composition (g/kg)	Without phytase			With phytase		
	Low Zn	Mid Zn	High Zn	Low Zn	Mid Zn	High Zn
Maize	570.1	569.8	569.5	586.7	586.9	586.6
Soybean meal	338.0	338.0	338.1	336.2	336.2	336.3
Meat meal	24.6	24.7	24.7	23.7	23.7	23.7
Canola oil	26.4	26.5	26.6	21.0	21.0	21.1
Limestone	11.2	11.2	11.2	11.5	11.5	11.5
Dicalcium phosphate	15.1	15.1	15.1	7.3	7.3	7.3
Salt	1.7	1.7	1.7	2.0	2.0	2.0
Sodium bicarbonate	2.0	2.0	2.0	0.4	0.4	0.4
Premix [A]	2.0	2.0	2.0	2.0	2.0	2.0
Choline Cl	0.9	0.9	0.9	1.1	0.9	0.9
L-lysine HCl	3.0	3.0	3.0	3.0	3.0	3.0
DL-methionine	4.1	4.1	4.1	4.1	4.1	4.1
L-threonine	1.9	1.9	1.9	1.9	1.9	1.9
Phytase	-	-	-	0.01	0.01	0.01
Calculated nutrient composition (g/kg)[2]						
Calcium	10.5	10.5	10.5	10.5	10.5	10.5
Available phosphorus	5.0	5.0	5.0	5.0	5.0	5.0
Total phosphorus	7.4	7.4	7.4	5.9	5.9	5.9
Sodium	1.6	1.6	1.6	1.6	1.6	1.6
Zinc (mg/kg)	30	40	50	30	40	50
Analysed nutrient composition (g/kg)						
Calcium	11.6	11.5	11.1	11.8	12.0	11.5
Total phosphorus	7.5	8.6	7.5	6.9	6.3	6.0
Sodium	1.6	1.8	1.7	1.5	1.6	1.4
Zinc (mg/kg)	36	45	58	35	43	55
Phytase FTU/kg	29	35	27	534	540	530

[1] Supplied per kg of diet (mg): 3.6 mg vitamin A (as all-trans retinol); 0.09 mg cholecalciferol; 44.7 mg vitamin E (as d-α-tocopherol); 2 mg vitamin K3; 2 mg thiamine; 6 mg riboflavin; 5 mg pyridoxine hydrochloride; 0.2 mg vitamin B12; 0.1 mg biotin; 50 mg niacin; 12 mg D-calcium pantothenate; 2 mg folic acid; 80 mg Mn; 60 mg Fe; 8 mg Cu; 1 mg I; 0.3 mg Co; 1 mg Mo.; [2] All diets were formulated to contain 12.7 MJ/kg metabolisable energy; 220 g/kg crude protein; 6.9 g/kg digestible methionine, 12.7 g/kg digestible lysine; 9.4 g/kg digestible methionine + cysteine; 8.3 g/kg digestible threonine, 13.7 g/kg digestible arginine.

Net energy of production (NEp) was calculated as follows:

Initial GE of carcase (kJ) = carcase GE (kJ/g) × body weight of bird (g) --- (1)

Final GE content of carcase (kJ) = carcase GE (kJ/g) × body weight of bird (g) --- (2)

NEp (kJ) = (2) - (1)

Table 2 Ingredient and nutrient specifications of grower (11 - 24 days) diets

Ingredient composition (g/kg)	Without phytase			With phytase		
	Low Zn	Mid Zn	High Zn	Low Zn	Mid Zn	High Zn
Maize	598.2	597.9	597.6	615.1	614.8	614.5
Soybean meal	282.0	282.1	282.1	280.2	280.2	280.3
Meat meal	50.0	50.0	50.0	49.0	49.0	49.0
Canola oil	38.7	38.8	38.9	33.2	33.3	33.4
Limestone	6.3	6.3	6.3	6.6	6.6	6.6
Dicalcium phosphate	7.9	7.9	7.9	0.0	0.0	0.0
Salt	1.7	1.7	1.7	1.6	1.6	1.6
Na bicarbonate	1.5	1.5	1.5	0.5	0.5	0.5
TiO_2	5.0	5.0	5.0	5.0	5.0	5.0
Premix[1]	2.0	2.0	2.0	2.0	2.0	2.0
Choline Cl	1.0	1.0	1.0	1.0	1.0	1.0
L-lysine HCl	1.9	1.9	1.9	2.0	2.0	2.0
DL-methionine	3.4	3.4	3.4	3.4	3.4	3.4
L-threonine	1.4	1.4	1.4	1.4	1.4	1.4
Phytase	-	-	-	0.01	0.01	0.01
Calculated nutrient composition (g/kg)[2]						
Calcium	9.0	9.0	9.0	9.0	9.0	9.0
Available phosphorus	4.5	4.5	4.5	4.5	4.5	4.5
Total phosphorus	5.9	5.9	5.9	5.3	5.3	5.3
Sodium	1.6	1.6	1.6	1.6	1.6	1.6
Zinc (mg/kg)	30	40	50	30	40	50
Analysed nutrient composition (g/kg)						
Calcium	10.0	9.5	9.6	9.0	9.5	9.4
Total phosphorus	7.6	7.3	7.1	6.0	6.1	5.9
Sodium	1.7	1.7	1.9	1.8	1.6	1.5
Zinc (mg/kg)	32	45	54	35	43	57
Phytase	40	35	40	545	535	540

[1]Composition as in Table1;
[2]All diets were formulated to contain 13.2 MJ/kg metabolisable energy; 210 g/kg crude protein; 6.1 g/kg digestible methionine, 11.0 g/kg digestible lysine;8.4 g/kg digestible methionine + cysteine; 7.3 g/kg digestible threonine, 12.6 g/kg digestible arginine.

Heat production (HP), which consists of the heat increment of feeding and fasting HP, was calculated as the difference between NEp and ME intake (MEI):

HP (kJ) = MEI - NEp

where, ME intake (MEI) was calculated using the following formula:

MEI (kJ) = ME (kJ/g) × feed intake (g)

Energy retained as fat (REf) and as protein (REp) was calculated as follows:

REf (kJ) = Carcase fat (g) × 38·2 kJ/g

REp (kJ) = Carcase crude protein content (g) × 23·6 kJ/g.

The values 38.2 and 23.6 kJ/g are energy values per gram of fat and protein, respectively, as derived by Larbier and Leclercq (1992):

Efficiency of ME use for energy retention (kRE) = NEp/MEI
Efficiency of ME use for lipid retention (kRE_f) = REf/MEI
Efficiency of ME use for protein retention (kRE_p) = REp/MEI.

The data were analysed using a factorial model of the GLM of Minitab software (Minitab, 2010). The statistical model inlcuded the Zn, phytase and their interacion effect. Differences within a significant effect were separated using Tukey's Honest Significance Difference test. Significant differences between diets were determined using Fisher's least significance difference test at $P \leq 0.05$.

Table 3 Ingredient and nutrient specifications of finisher (24 - 35 days) diets

Ingredient composition (g/kg)	Without phytase			With phytase		
	Low Zn	Mid Zn	High Zn	Low Zn	Mid Zn	High Zn
Maize	630.7	630.4	630.1	628	628.2	628.4
Soybean meal	257.8	257.8	257.9	282.8	282.1	281.4
Meat meal	50.0	50.0	50.0	31.9	32.4	32.8
Canola oil	39.7	39.8	39.9	39.5	39.5	39.4
Limestone	6.0	6.0	6.0	7.9	7.8	7.8
Dicalcium phosphate	6.4	6.4	6.4	1.8	1.7	1.6
Salt	2.0	2.0	2.0	2.0	2.0	2.0
Na bicarbonate	1.1	1.1	1.1	0.4	0.3	0.3
Premix[1]	2.0	2.0	2.0	2.0	2.0	2.0
Choline Cl	0.9	0.9	0.9	0.8	0.8	0.8
L-lysine HCl	1.0	1.0	1.0	0.6	0.6	0.6
DL-methionine	2.7	2.7	2.7	2.6	2.6	2.6
L-threonine	0.8	0.8	0.8	0.7	0.7	0.7
Phytase	-	-	-	0.01	0.01	0.01
Calculated nutrient composition (g/kg)[2]						
Calcium	8.5	8.5	8.5	8.5	8.5	8.5
Available phosphorus	4.2	4.2	4.2	4.2	4.2	4.2
Total phosphorus	6.4	6.4	6.4	5.0	5.0	5.0
Sodium	1.6	1.6	1.6	1.6	1.6	1.6
Zinc (mg/kg)	30	40	50	30	40	50
Analysed nutrient composition (g/kg)						
Calcium	9.1	9.2	9.0	8.6	8.8	9.1
Total phosphorus	6.5	6.5	6.5	5.0	5.0	5.0
Sodium	1.9	1.6	1.8	1.5	1.6	1.5
Zinc (mg/kg)	35	48	59	33	46	58
Phytase (U/kg)	28	36	30	540	550	545

[1] Composition as in Table 1;
[2] All diets were formulated to contain 13.4 MJ/kg metabolisable energy; 200 g/kg crude protein; 5.3 g/kg digestible methionine, 9.7 g/kg digestible lysine; 7.6 g/kg digestible methionine + cysteine; 6.5 g/kg digestible threonine, 12.3 g/kg digestible arginine.

Results

The gross response of birds fed different levels of Zn and phytase is shown in Table 4. There was no significant interaction between Zn and Phytase for FI and BWG for birds at any stage of rearing. At d 10, feed intake (FI) decreased (P <0.05) in birds on low-Zn diet. Phytase supplementation had no influence (P >0.05) on FI. Feeding diets with low Zn reduced the BWG of birds during 1 - 10 (P <0.001) and 1 - 24 (P <0.02) days of age. At d 24, the BWG was higher (P <0.02) in diets with phytase than in unsupplemented diets (1051 *vs* 1005 g/bird). The FCR of birds was not affected (P >0.05) by Zn, phytase or their interaction.

High Fe diet with phytase supplementation increased (Zn x phytase, P <0.01) the Fe deposition in the liver (Table 5). Birds fed high-Zn diet showed the highest (P <0.02) Zn content in liver (77.8 µg/g) than those on low or mid-Zn diet (70.8 and 72.7 µg/g, respectively). There was no phytase effect on the mineral contents of the liver. The Zn and phytase interaction was not significant for Cu and Mn content of liver.

Table 4 Effects of varying levels of dietary Zn with or without microbial phytase on feed intake (FI) and body weight gain (BWG), and feed conversion ratio (FCR) of broilers from day 0 to 35[1]

Treatment		FI (g/bird)			BWG (g/bird)			FCR		
Zn	**Phytase**	**1 - 10d**	**1 - 24d**	**1 - 35d**	**1 - 10d**	**1 - 24d**	**1 - 35d**	**1 - 10d**	**1 - 24d**	**1 - 35d**
Low	None	268	1383	4481	218	1000	2402	1.24	1.37	1.87
	Plus	255	1404	4382	212	1035	2498	1.20	1.36	1.75
Mid	None	272	1465	4260	223	965	2468	1.22	1.52	1.73
	Plus	277	1450	4408	228	1039	2417	1.21	1.40	1.84
High	None	281	1425	4501	239	1051	2386	1.18	1.40	1.89
	Plus	275	1456	4373	239	1078	2501	1.16	1.30	1.75
SEM		1.50	6.87	34.7	1.03	4.93	12.9	0.01	0.01	0.01
Source of variation										
Zn		<0.05	0.092	0.74	<0.001	<0.02	0.99	0.14	0.13	0.83
Phytase		0.376	0.610	0.825	0.989	<0.02	0.243	0.373	0.181	0.381
Zn x phytase		0.350	0.707	0.594	0.491	0.501	0.270	0.856	0.399	0.127

[1] Means were obtained from 6 replicate cages of 6-8 birds per cage;
SEM - Standard error of mean.

Table 5 Effects of varying levels of dietary Zn with or without phytase on liver mineral contents (µg/g) of broilers (24 d)[1]

Zn	Phytase	Fe	Zn	Cu	Mn
Low	None	384.3^{bc}	72.0	10.3	9.4
	Plus	321.1^{c}	69.6	9.0	10.3
Mid	None	421.3^{b}	73.5	10.2	10.2
	Plus	345.3^{bc}	71.8	10.6	8.5
High	None	422.4^{b}	79.3	10.6	11.1
	Plus	550.1^{a}	76.4	10.2	10.3
SEM		7.54	0.53	0.17	0.16
Source of variation					
Zn		<0.001	<0.02	0.531	0.192
Phytase		0.884	0.214	0.492	0.355
Zn x phytase		<0.01	0.963	0.583	0.191

[a-c] Means within a column without common superscript are significantly different at the level shown;
[1] Means were obtained from 6 replicate cages of 2 birds per cage; SEM - Standard error of mean.

The interaction between Zn and phytase significantly influenced the ileal digestibility of P, Mg, Fe and Zn (Table 6). Phytase supplementation to high-Zn diets improved the digestibility of P (P <0.001) but reduced the Mg (P <0.001), Fe (P <0.02), and Zn (P <0.01) digestibility. Birds fed phytase supplemented diets had poorer (P <0.01) Ca digestibility (0.41 *vs* 0.50) than those fed unsupplemented diets. There was no effect of Zn, phytase and their interaction on ileal digestibility of N.

The retention of Zn was significantly affected by the interaction between Zn and phytase (Table 7). Total tract retention of Zn was reduced (P <0.001) in birds fed high-Zn diet with phytase supplementation. There was a tendency to increase the retention of N (P =0.096) and Ca (P =0.085) in birds received phytase-supplemented high-Zn diets. Diets with phytase supplementation improved (P <0.01) the P retention better than enzyme unsupplemented diets (0.50 *vs* 0.41).

Table 6 Influence of different levels of dietary Zn with or without microbial phytase on the ileal digestibility of minerals at 24 d of age[1]

Zn	Phytase	N	Ca	P	Mg	Fe	Zn
Low	None	0.81	0.53	0.42^{c}	0.11^{c}	0.40^{c}	0.20^{bc}
	Plus	0.81	0.42	0.45^{bc}	0.24^{b}	0.52^{b}	0.24^{b}
Mid	None	0.83	0.50	0.51^{abc}	0.21^{bc}	0.47^{bc}	0.26^{b}
	Plus	0.82	0.44	0.52^{ab}	0.20^{bc}	0.46^{bc}	0.20^{bc}
High	None	0.83	0.53	0.55^{b}	0.34^{a}	0.56^{a}	0.35^{a}
	Plus	0.82	0.36	0.62^{a}	0.10^{c}	0.48^{bc}	0.16^{c}
SEM		0.003	0.01	0.01	0.01	0.01	0.01
Source of variation							
Zn		0.857	0.487	0.230	0.622	0.120	0.254
Phytase		0.130	<0.01	0.195	0.254	0.613	<0.02
Zn x phytase		0.192	0.527	<0.001	<0.001	<0.02	<0.01

[a-c] Means within a column without common superscript are significantly different at the level shown;
[1] Means were obtained from 6 replicate cages of 2 birds per cage; SEM - Standard error of mean.

Table 7 Effects of different levels of dietary Zn with or without microbial phytase on the total tract retention of minerals in broilers (22 - 24d)[1]

Zn	Phytase	N	Ca	P	Fe	Zn
Low	None	0.66	0.39	0.42	0.47	0.11^{cd}
	Plus	0.55	0.31	0.45	0.52	0.24^{ab}
Mid	None	0.58	0.23	0.35	0.47	0.22^{b}
	Plus	0.61	0.41	0.51	0.45	0.17^{bc}
High	None	0.69	0.38	0.46	0.56	0.32^{a}
	Plus	0.66	0.44	0.55	0.48	0.03^{d}
SEM		0.01	0.01	0.01	0.001	0.001
Source of variation						
Zn		<0.05	0.219	0.072	0.185	<0.001
Phytase		0.163	0.210	<0.01	0.595	<0.01
Zn x phytase		0.096	0.085	0.150	0.218	<0.001

[a-d] Means within a column without common superscript are significantly different at the level shown;
Means were obtained from 6 replicate cages of 6 birds per cage; SEM - Standard error of mean.

The length, width, breaking strength, ash and mineral content of tibia bone of birds were unaffected (*P* >0.05) by different levels of Zn and phytase (Table 8). The interaction between Zn and phytase was significant only for tibia Fe concentration. Birds fed high-Zn diet with phytase showed the highest (*P* <0.05) accumulation of Fe in tibia. Although not significant, Zn deposition in tibia tended to be highest (p = 0.082) in birds fed diets containing the mid-level of Zn. There was no significant effect of Zn, phytase or their interaction on different blood variables (data not shown).

There were significant interactions between Zn and phytase for protein and the activities of enzymes in the jejunum (Table 9). Phytase supplemented to high-Zn diets improved the protein content (*P* <0.01) and activities of AP and Ca-Mg-ATPase (*P* <0.001) in the jejunum of 24-d old birds. Birds fed mid-Zn diets with phytase supplementation showed the highest (*P* <0.001) activity of Ca-ATPase and Mg-ATPase in the jejunum mucosa.

Table 8 Effects of varying levels of dietary Zn with or without phytase on tibia morphometric parameters, breaking strength (BBS), ash, and mineral contents of broilers at 24 d of age[1]

Zn	Phytase	Length (mm)	Width (mm)	BBS (N)	Tibia ash%	Ca%	P%	Fe mg/kg	Zn mg/kg
Low	None	73.0	7.3	276.3	49.1	38.8	18.0	285.7^{ab}	349.1
	Plus	74.3	7.6	323.3	52.1	39.5	18.0	298.5^{ab}	369.5
Mid	None	74.2	7.3	284.9	47.9	39.1	18.0	310.0^{b}	398.9
	Plus	73.9	7.4	287.8	46.9	39.0	17.9	279.3^{ab}	407.8
High	None	72.7	7.2	277.6	47.8	39.5	18.0	275.4^{ab}	359.7
	Plus	74.7	7.6	277.0	48.9	39.1	18.0	315.2^{a}	361.5
SEM		0.22	0.04	3.27	0.62	0.12	0.06	3.20	5.06
Source of variation									
Zn		0.888	0.700	0.281	0.471	0.871	0.906	0.969	0.082
Phytase		0.208	0.092	0.157	0.636	0.811	0.839	0.515	0.559
Zn x phytase		0.438	0.656	0.179	0.748	0.545	0.858	<0.05	0.909

[a, b] Means within a column without common superscript are significantly different at the level shown;
[1] Means were obtained from 6 replicate cages of 2 birds per cage; SEM - Standard error of mean.

Phytase supplementation to diets containing mid to high-levels of Zn diets increased the ME (Zn x phytase, *P* <0.01) content of diets (Table 10). The Zn and phytase interaction was significant for MEI and NEp. The MEI (*P* <0.01) and NEp (*P* <0.01) were higher in birds that consumed diets containing mid or high levels of Zn with phytase supplementation. Heat production was not affected by Zn, phytase or their interaction. Increasing Zn levels in diets improved (*P* <0.001) the REp and protein deposition rate in the tissue. Phytase supplemented to low-Zn diet reduced the (Zn x phytase, *P* <0.01) the energy retention as fat (REf). Birds that consumed phytase-supplemented diets with mid or high levels of Zn deposited fat (Zn x phytase, *P* <0.01) and energy (Zn x phytase, *P* <0.01) at a faster rate than other groups of birds. The efficiency of ME utilization for energy (KRE), fat (KREf), and protein (KREp) deposition were 0.75 - 0.84, 0.16 - 0.18, and 0.41 - 0.46, respectively, and were not affected by Zn, phytase, or their interaction.

Discussion

In the current study, birds given the low-Zn diet showed reduced feed intake and body weight gain at 24 days of age. The poor body weight gain can be attributed to low feed intake, which is associated with a low dietary Zn level. Moreover, the weight gain of birds depends on effective utilisation of consumed feed and its subsequent use in cell proliferation; mainly in the muscle. Therefore, Zn deficiency may cause retarded growth and poor weight gain due to its role in DNA/RNA synthesis and carbohydrate, fat or protein metabolism (Wu and Wu, 1987). Previous studies (Bao *et al*., 2007; Ao *et al*., 2011) have reported the same trend in birds fed a low or deficient Zn diet. However, the exact mechanism by which dietary Zn influences feed intake is still unclear but it can be related to satiety regulation. It has been reported that Zn-deficient diets could increase the gene expression of mRNA for cholecystokinin (CCK) production in the intestine, which negatively affects the appetite of animals (Blanchard & Cousins, 2000; Mcdonald, 2011).

Table 9 Effects of varying levels of dietary Zn with or without phytase on jejunal protein and enzyme activity of broilers at 24 d of age[1]

Zn	Phytase	Protein	AP	Ca-Mg-ATPase	Ca-ATPase	Mg-ATPase
		(mg/g)	µm/mg protein/min	nmol/mg protein/min		
Low	None	73.0^{b}	1.23^{c}	173.0^{b}	72.6^{b}	148.9^{b}
	Plus	73.7^{b}	1.69^{b}	154.5^{c}	70.8^{b}	150.7^{b}
Mid	None	69.2^{bc}	1.48^{b}	148.9^{c}	72.6^{b}	129.1^{c}
	Plus	71.4^{b}	1.52^{b}	126.0^{d}	84.5^{a}	184.1^{a}
High	None	63.3^{c}	1.61^{b}	153.4^{c}	72.2^{b}	149.9^{b}
	Plus	83.5^{a}	1.97^{a}	200.1^{a}	73.1^{b}	152.1^{b}
SEM		0.52	0.17	1.21	0.26	11.57
Source of variation						
Zn		<0.001	<0.001	<0.001	<0.001	0.478
Phytase		0.850	0.424	0.679	<0.001	<0.001
Zn x phytase		<0.01	<0.001	<0.001	<0.001	<0.001

[a- d] Means within a column without common superscript are significantly different at the level shown;
[1] Means were obtained from 6 replicate cages of 2 birds per cage; SEM: Standard error of mean; AP: Alkaline phosphatase.

Table 10 Effects of varying levels of dietary Zn with or without phytase on net energy parameters[1]

Zn	Phytase	ME (MJ/kg)	MEI (KJ/bird/d)	NEp (KJ/d)	HP (KJ/d)	Energy retention		Rate of deposition			Efficiency of energy utilization		
						REp (KJ/d)	REf (KJ/d)	Protein (g/d)	Fat (g/d)	Energy (KJ/d)	K_{RE}	K_{REf}	K_{REp}
Low	None	14.9^{bc}	1045^{d}	865.6^{b}	199.7	436.4	178.8^{b}	18.0	4.7^{b}	874.7^{b}	0.84	0.17	0.42
	Plus	14.6^{c}	1035d	803.2^{c}	231.5	425.1	166.1^{c}	18.0	4.3^{c}	797.6^{c}	0.77	0.16	0.41
Mid	None	14.7^{c}	1063^{cd}	872.9^{b}	207.2	449.0	180.2^{b}	19.0	4.7^{b}	872.2^{b}	0.82	0.17	0.42
	Plus	15.7^{a}	1175^{a}	951.8^{a}	234.0	484.6	195.9^{a}	20.5	5.1^{a}	955.6^{a}	0.85	0.17	0.43
High	None	15.7^{a}	1129^{ab}	913.3$^{a}_{b}$	223.0	497.0	188.2^{ab}	21.5	4.9^{ab}	893.9^{ab}	0.74	0.16	0.43
	Plus	15.5^{ab}	1116^{bc}	945.2^{a}	211.2	506.4	195.0^{a}	21.5	5.1^{a}	945.3^{a}	0.85	0.18	0.46
SEM		0.05	4.45	5.04	6.72	3.60	1.02	0.13	0.03	5.04	0.01	0.001	0.004
Source of variation													
Zn		<0.002	<0.001	<0.001	0.984	<0.001	<0.001	<0.001	<0.001	<0.001	0.454	0.476	0.393
Phytase		0.391	0.064	0.362	0.508	0.374	0.363	0.306	0.363	0.280	0.414	0.542	0.594
Zn x phytase		<0.01	<0.01	<0.01	0.706	0.320	<0.01	0.310	<0.01	<0.01	0.058	0.337	0.696

[a-d] Means within a column without common superscript are significantly different at the level shown.
[1] Means were obtained from 6 replicate cages of 2 birds per cage;
NEp - Net energy for production; HP- Heat production;
REp - Energy retained as protein;
REf - Energy retained as fat;
K_{RE} – Efficiency of ME use for energy retention;
K_{REf} – Efficiency of ME use for fat retention;
K_{REp} – Efficiency of ME user for protein retention;
SEM - Standard error of mean.

In contrast, several studies (Salim *et al*., 2011; Salim *et al*., 2012; Štenclová *et al*., 2016) have reported that the overall performance of birds was not influenced by a varying concentration or source of Zn. The discrepancy may be due to the use of different amounts and sources of Zn in these experiments.

Different levels of Zn have no effect on FCR, a finding which is supported by previous study (Roy *et al*., 2014).

Phytase supplementation had no effect on FI but improved the BWG of birds, irrespective of Zn level. However, as a mineral nutrient matrix was applied with phytase supplementation and the dietary mineral content consequently reduced, an improvement in performance was not expected. This indicates that the diet was limiting in minerals such as P or Ca, or that phytase released other nutrients, possibly including energy and amino acids. This result also suggests that Zn levels used in the present study did not reduce the ability of the phytase to degrade phytate. As feed intake of birds was unaffected by phytase supplementation, the improved BWG of the same group of birds could be interpreted as being a consequence of the release of phytate-bound minerals and other nutrients by phytase. The beneficial effect of phytase on BWG has also been reported in previous studies (Dilger *et al*., 2004; Akter *et al*., 2017). The lack of response to phytase on FCR is in agreement with Roy *et al.* (2014).

An improvement in P utilization due to phytase supplementation was observed in diets containing Zn at a level of 40 - 50 mg/kg of diet. In contrast, supplementation of phytase in the high-Zn diet (50 mg/kg) reduced the digestibility of Fe and Zn, which is consistent with their low retention. The significant interaction between Zn and phytase in nutrient utilization (Fe and Zn) may indicate the possible formation of a Zn-phytate complex in the GIT of birds, which hindered phytate hydrolysis by phytase and limited the bioavailability of these minerals. Therefore, it can be assumed that phytase is less likely to release phytate-bound minerals at high dietary Zn concentration. However, body weight gain and tibia bone development have been considered to be the most sensitive criteria for assessing phytase benefit. As there was no effect of high-Zn diet with added phytase on bird performance and bone development, the observed significant negative interaction between high Zn and phytase on Fe and Zn utilization could be due to a unique absorption and reabsorption pattern of trace minerals in the GIT of broilers, leading to under- or over-estimation of the utilization of these minerals (Underwood & Shuttle, 1999b; Bao *et al.,* 2007).

The interaction between Zn and phytase significantly influenced the different variables of energy utilization. Phytase supplementation with mid or high Zn diet significantly improved ME, MEI, NEp, REp, REf and K_{RE}. The increase in MEI may be the result of higher feed intake, which is correlated with NEp. This is because, with a greater consumption of feed, there is a greater energy intake, which can be used for production rather than maintenance (Pirgozliev *et al*., 2011). Along with more feed consumption and the highest body weight gain, this group of birds showed increased utilization of different nutrients. It is possible that the release of phytate-bound nutrients by phytase increased feed intake due to high dietary Zn improving the energy utilization of birds. Moreover, the available extra energy is retained as fat or protein in the body, which contributes to improved weight gain. This relationship between improved BWG and an increased amount of energy deposited in tissues validates NEp as a more sensitive measure of energy utilization than ME.

It has been reported that fat retention increases in birds older than 35 days of age (Boekholt *et al*., 1994). In the present study birds deposited more energy as protein than as fat during the period of 0 - 24 d, which indicates better utilization of energy as protein and hence production of lean meat. Although the efficiency of ME retention as energy, fat and protein was unaffected by treatment differences, the K_{RE} value was higher in the present study than in the study of Olukosi *et al.* (2008). This may be due to differences in ME intake between the studies.

The accumulation of trace minerals in different tissues, for example the tibia, liver and plasma, is considered to be a good measure of the mineral status of birds (Salim *et al*., 2012). The concentration of Fe in tibia bone and liver was greater in birds that consumed the high-Zn diet with phytase supplementation. The liver is the main storage site of Fe, and it is possible that after Fe saturation of the liver, excess Fe moves to the tibia. This relocation of Fe is the result of the very limited amounts of Fe that are excreted through urine or sweat. Most of the excess Fe is usually excreted via sloughing of intestinal enterocytes (Cao *et al*., 1996). Therefore, the observed high concentration of Fe in the tibia and liver in the present study could be due to reduced excretion of Fe. Besides, the greater Zn concentration in the liver in the present study with mid to higher Zn diet partly supports the findings of Sunder *et al.* (2013). These authors reported that hepatic accumulation of Zn started to increase in birds fed diet with 160 mg Zn/kg and indicated that comparatively higher Zn supplementation is required to obtain a significant increase of this mineral in liver. There was no effect of phytase on accumulation of Zn in the liver and tibia, which is in accordance with other studies (Mohanna & Nys 1999a; Ao *et al*., 2007).

Tibia Zn concentration tended to increase in birds offered the diet with mid-level (40 mg/kg) Zn supplementation. This is partly in agreement with Mwangi *et al. (*2017) who reported that supplementing diet with 40 mg/kg Zn increased the tibia Zn concentration compared to a diet with 8 mg Zn/kg. According to Vieira *et al.* (2013) supplementation of 40 and 100 mg Zn/kg of diets had no statistically significant difference in tibia Zn deposition. Similarly, previous study (Ao *et al*., 2011) reported that tibia Zn concentration starts to

increase when dietary Zn ranges from 45 to 70 mg/kg. It has been reported that 40 mg of Zn/kg diet is adequate for optimizing BWG (Mohanna & Nys, 1999a), whereas increasing Zn level beyond this level causes an increased deposition in the tibia, but this plateaued at a level of 48 mg Zn/ kg (Bao *et al*., 2007).

In this study, the AP activity in the jejunal mucosa was reduced at low dietary Zn, which was counterbalanced by phytase supplementation. The release of Zn from phytate-mineral complex can be the possible cause of increased activity of AP in phytase-supplemented diets. According to Ghalehkandi *et al.* (2011) the activity of AP was higher in the intestine of male broilers fed diets containing 50 - 100 mg Zn /kg than in birds fed a diet without Zn supplementation. These authors found that the growth of intestinal coliform bacteria, responsible for damaging the intestinal mucosa and reduction of the absorption and digestion of nutrients, was inhibited by Zn. Further, Zn is required for maintaining the stability of AP, and deficiency of this mineral leads to poor activity of the enzyme (Reinhold *et al*., 1969; Cho *et al*., 2007). Phytase improved the activities of Ca-ATPase and Mg-ATPase in jejunal mucosa of birds on diets with a mid-level of Zn, which indicates increased absorption of Ca and therefore increased utilization (Bronner, 2003).

Conclusion

The results of the present study indicate that microbial phytase is effective in improving the performance and nutrient utilization of birds that consume diets with 40 - 50 mg Zn/kg. Improved growth performance and nutrient and energy utilization was mainly observed in birds given the highest Zn diets, irrespective of phytase supplementation. This suggests that using 50 mg Zn/kg of diet optimizes performance of birds and does not inhibit phytase activity. As most commercial poultry diets contain more than 100 mg Zn/kg of diet, further study is warranted to investigate whether this higher concentration of Zn has any effect on phytase activity or utilisation of other nutrients.

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Authors' Contribution

MA, PAI and HG were in charge of study design. MA carried out the animal experiment and did all laboratory and statistical analysis. MA wrote the first draft of manuscript. PAI and HG revised the manuscript.

Conflicts of Interest Declaration

The authors declare that they have no competing interests.

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