Original Research

Journal of Advanced Veterinary Research
(2024) Volume 14, Issue 6, 1033-1041

Effectiveness of phytase and nonstarch polysaccharides-degrading enzymes on performance, bone mineralization, litter, and gene expression in broiler chickens fed nutritionally reduced diets

Basma M. Bawish[1*], Elshaimaa Ismael[1], Samar H. Abdelfatah[2], Shaimaa Kamel[3], Khaled N.E. Fahmy[2]

[1]*Department of Veterinary Hygiene and Management, Faculty of Veterinary Medicine, Cairo University, Giza, 12211 Egypt.*
[2]*Department of Nutrition and Clinical Nutrition, Faculty of Veterinary Medicine, Cairo University, Giza, 12211, Egypt.*
[3]*Department of Biochemistry and Molecular Biology, Faculty of Veterinary Medicine, Cairo University, Giza, 12211, Egypt.*

ARTICLE INFO

Recieved: 08 April 2024

Accepted: 15 June 2024

***Correspondence:**

Corresponding author: Basma M. Bawish
E-mail address: basmabawish@cu.edu.eg

Keywords:

Exogenous enzymes, Energy reduced diet, Growth performance, Bone minerals, Litter quality, IGF-1/ TLR4 and CPT1A

ABSTRACT

Effects of dietary non-starch polysaccharides-degrading enzymes (NSPase) and phytase complex on performance, carcass, bone minerals, litter, and gene expression (IGF, IL-1β, IL-10, TLR-4, CPT1A) were determined in broilers fed corn-soybean nutrient-reduced diets. Totally, 1200 Ross-308 one-day-old broiler chicks were randomly assigned into 4 treatments, with 6 replicates of 50 birds each; (G1) Control group received nutrient-adequate diet without enzymes supplementation; (G2) received energy-reduced diet (-100 kcal/kg) with NSPase (100 g/ton Econase®) + phytase enzymes (100 g/ton Quantum Blue®; 5,000 FTU/g); (G3) received energy-reduced diet (-80 kcal/kg) with NSPase (250 g/ton Enziver®) + phytase enzymes (100 g/ton Phytonex®; 5,000 FTU/g); (G4) received as G3 diet with a 0.5% decrease in crude protein (CP). For all energy-reduced diets, the nutritional matrix of phytase with reductions of phosphorus (P) (0.15%), and calcium (Ca) (0.165%) was considered. Dietary NSPase and phytase supplementation to a low-energy diet significantly ($P<0.05$) enhanced body weights, weight gain, feed conversion ratio, and litter quality (lowered nitrogen, phosphorous, and calcium excretion in broiler manure), with constant bone mineralization. No significant effects ($P>0.05$) on carcass or blood biochemistry. Energy and CP-reduced diet showed better feed intake, immune organ weights, and mineral bioavailability by decreasing Alkaline phosphatase activity. Moreover, upregulated gene expression of IGF-1 in muscles, inflammatory cytokines (IL-1B and IL-10), immune-related genes (TLR-4) in the liver, and (CPT1A) responsible for energy production. Conclusively, dietary NSPase with phytase enzymes compensated for up to 0.5% CP, 100 kcal ME/kg, and 0.15% and 0.165% units of Av.P and total Ca, with improving broiler performance and environmental impacts.

Introduction

Nowadays, nutritional approaches to support the production of high-quality, affordable, and safe animal products are necessary, and the relationship between nutrition, health, animal welfare, and the environment must be considered. In recent years, broiler performance has been improved by increasing the metabolizability of corn and soybean meal-based diets using highly specialized exogenous enzymes (Chaves *et al*., 2020). In many parts of the world, corn and soybean meal are the main sources of energy and protein for poultry and livestock (Poernama *et al*., 2021). However, they are also abundant in antinutritional components, such as non-starch polysaccharides (NSP), which contain approximately 10-12% of total NSP (Knudsen, 2001; Morgan *et al*., 2021). Broilers are extremely sensitive to dietary NSP quantity and composition because NSP directly affects digestion passage rate, intestinal health, and microbiota composition (Morgan *et al*., 2022a), therefore influencing nutrient utilization and productive performance (Mateos *et al*., 2012; Kheravii *et al*., 2018; Mahmood & Guo, 2020).

Water-soluble NSP raises digesta viscosity, which, at high concentrations, decreases nutritional digestibility by limiting enzyme accessibility to substrates and physically impeding absorption through the intestinal wall (Choct, 2015). Moreover, the insoluble NSP acts as a nutrient diluent and physical barrier to digestive enzymes, which reduces nutrient utilization (Hetland *et al*., 2004). The commercial use of NSP-degrading enzymes is ubiquitous in poultry diets. The NSP-degrading enzyme cocktails generally contain xylanase as the main activity, in combination with other enzymes (Aftab, 2012; O'Neill *et al*., 2014). NSP-degrading enzyme such as xylanase counteracts the antinutritive effects of NSP by breaking down soluble arabinoxylans, which lowers digesta viscosity and increases nutrient digestibility (Bedford, 2002; Choct, 2004). Using NSP-degrading enzyme may enhance disruption of cell walls and solubilization of NSP, resulting in increased nutrient release and utilization, producing a more diverse range of prebiotic oligosaccharides (Morgan *et al*., 2022b).

Phytate is one of the anti-nutritional components that have a detrimental effect on broiler performance (Selle & Ravindran, 2007). Approximately two-thirds of the phosphate (P) in vegetable feedstuffs are poorly digested by chickens due to its binding to phytate which is poorly hydrolyzed by chickens (Woyengo & Nyachoti, 2011). Phytate may precipitate proteins under acid-to-neutral pH, lowering their solubility and digestibility and accelerating the loss of endogenous proteins (Cowieson & Bedford, 2009). Furthermore, Phytate may combine with other divalent cations to produce complexes that decrease the availability of calcium (Ca), manganese (Mn), iron (Fe), and zinc (Zn) (Newman, 1991). The adverse effects of phytate on nutrient utilization can be partly ameliorated by exogenous phytase enzyme, which degrades phytate (Attia *et al*., 2019). Phytase is known to hydrolyze phytate P in plants, boosting the available P (Av. P) content of the diet, enabling the reductions of added inorganic P and the feed cost (Walk & Poernama, 2019). Phytase enzyme can reduce the antinutritional effect of phytate by catalyzing the stepwise hydrolysis of inositol phosphate esters (InsPs) and myo-inositol (González-Ortiz *et al*., 2020). As a result, increases the digestibility of phosphorous (P), calcium (Ca), amino acids, and energy while decreasing the excretion of inorganic P into the environment (Humer *et al*., 2015). Although the efficacy and utility of phytase and the NSP enzyme (NSPase) have been proven independently over the past two decades, their combined values are not well defined (Gehring *et al*., 2013; Schramm *et al*., 2017).

Putatively, NSPase may increase the effectiveness of phytase by facilitating access to phytate, which would otherwise be encapsulated within the cell walls, thus improving the absorption of minerals, amino acids, and energy sources, and subsequently, increasing the productive perfor-

ISSN: 2090-6277/2090-6269/

mance (Poernama *et al*., 2021). It is essential to give precise and unambiguous instructions for using a variety of enzyme products simultaneously, as nutritionists place a growing emphasis on precision in formulation systems. So, the objective of the present study was to investigate the effect of phytase and nonstarch polysaccharides-degrading enzymes on growth performance, carcass yield, bone mineralization, litter quality, and gene expression in broiler fed a nutritionally reduced diet.

Materials and methods

Birds and Housing

The trial was carried out at the Animal and Poultry Research Center of Cairo University's Faculty of Veterinary Medicine in Giza, Egypt. All the animal experiment procedures in the current studies were approved by the Institutional Animal Care and Use Committee, Cairo University, Egypt (Vet CU 03162023685).

A total of 1200 Ross-308 one-day-old broiler chicks were weighed and allocated in a completely randomized design with four dietary treatments and six replicates of 50 broilers per experimental unit. Upon arrival, birds were placed immediately in 24-floor pens with clean wood shavings in a naturally ventilated open house for 35 days. Each pen for broilers was (2.9×1.7 m) in size. All pens were equipped with a round feeder and a bell drinker. Experimental diets and water were provided ad libitum throughout the trials. The pens were preheated to 33°C 12 hours before the beginning of the experiment and kept at 33°C for the first 3 days, then pen's temperature was gradually reduced to 2.8°C each week until it reached 24°C on day 21 and was kept until the end of the trial. Throughout the whole trial, the relative humidity was maintained between 55-60% and the lighting schedule was 24L for the first three days, then subsequently kept under 23L:1D for the remainder of the study.

Experimental diets

Dietary treatments consisted of a basal diet (G1) (control group) fully meeting the nutritional requirements without NSPase or phytase enzyme; the 2nd group (G2) was fed a basal diet with a reduction of 100 kcal/kg AMEn with NSPase "Econase XT 25P" at a rate of 100 g/ton of feed(derived from *Trichoderma reesei*; AB Vista, Marlborough, UK) + phytase "Quantum Blue 5G" 100 g/ton of feed (5000 phytase units (FTU), derived from *E. coli*; AB Vista, Marlborough, UK); the 3rd group (G3) was fed a basal diet with AMEn reduction of 80 kcal/kg, with NSPase "Enziver" 250 g/ton of feed (each gram contains 1000 phytase units (FTU), 7500 IU amylase, 5500 IU protease, 10.000 IU cellulase, 5000 IU xylanase, 800 IU b-glucanase and 900 IU pectinase; derived from *Aspergillus*, *Trichoderma*, *Bacillus*, and a pathogenic *E. coli*; Zoetis, New Jersey, USA), and phytase "Phytonex" 100g/ton of feed (5,000 FTU/g, derived from *E. coli* and *Aspergillus niger*; Zoetis, New Jersey, USA); the 4th group (G4) was based on the G3 diet with a reduction in crude protein (CP) by 0.5%. The nutritional matrix of phytase, with reductions of 0.15% P and 0.165% Ca, was considered for all energy-reduced diets (Shelton *et al*., 2004).

The birds in the control group were fed a corn-soya bean meal-based diet that was formulated to satisfy the necessary nutrient requirements for broiler chickens (NRC, 1994). Diet formulation and nutrient values of each dietary treatment are presented in Tables 1. Three-phase feeding programs, including starter (0 to 14 days) provided in crumble form, while grower (15 to 28 days) and finisher (29 to 35 days) were provided in pellet form.

Growth Performance

The birds and feeds were weighed at the beginning of the experiment and weekly to determine average live body weight (BW), body weight gain (BWG), and feed intake (FI), and to calculate the feed conversion ratio (FCR) (Bawish *et al*., 2023). Daily dead or culled birds were recorded. FI and subsequently FCR were adjusted according to the mortality rate. The European Production Efficiency Factor (EPEF) was calculated (Huff *et al*., 2013) as follows:

EPEF = (livability × live weight (kg) / (age in days × FCR) × 100.

Carcass traits

Six birds from each group close to the average live BW, were chosen at the end of the trials, weighed, and slaughtered by neck cutting after fasting for 12 hours to allow the gut to completely evacuate. Following the removal of the head, neck, and legs, each bird was scalded, de-feathered, and eviscerated. The carcass yield was calculated by weighing the carcass without giblets and expressing it as a percentage of its live weight. Breast (Pectoralis major and Pectoralis minor) and thigh and drumstick muscles were removed and weighed relative to the live weight. The weight of the liver, gizzard, heart, spleen, and bursa of Fabricius for each bird was recorded, and then the relative indices were calculated (Biswas *et al*., 2019; Zarghi *et al*., 2020; Hussien *et al*., 2022).

Physical and Chemical examinations of litter

A total of 6 litter samples were collected from each group. From each replicate, 5 litter subsamples were collected from different spots within the pen, and then well mixed. For estimating litter moisture, 10 g of each litter sample was dried at 100.0±5.0°C in the hot air oven (Dumas *et al*., 2011). After 24 – 48 h, dry litter weights were subtracted from the initial litter weights to obtain Moisture %. Additionally, litter samples were analyzed for total N using total Kjeldahl N, and Ca (Jackson, 1973), and total P using ascorbic acid (Houba *et al*., 1995) by a spectrophotometer at 880 nm (Spectronic 21D).

Biochemical analysis

At the end of the experiment, blood samples were collected and centrifuged at 4000 rpm for 15 min for separation of serum (Hussien *et al*., 2023). Serum samples were used for the determination of protein profile (total protein and albumin), lipid profile (total cholesterol and triglycerides), liver function (Alanine transaminase (ALT) and Aspartate transaminase (AST)), kidney function (uric acid), and bone-related parameters (Ca, P, and Alkaline Phosphatase (ALP)). All these parameters were determined using Spectrum diagnostics kits.

Gene expression analysis

Different tissue samples were collected at the end of the experiment. Breast and thigh muscles for determination of the level of expression of the IGF gene. Liver samples for determination of the level of expression of IL-1β, IL-10, TLR-4, and CPT1A genes. Total RNA isolation from the different samples was done by ABT Total RNA Mini Extraction Kit, Applied Biotechnology. RNA quantity and quality were determined by Nanodrop Technology (Ali *et al*., 2022). The cDNA synthesis was done using ABT H-minus cDNA synthesis kit, Applied Biotechnology. qRT-PCR was performed by ABT 2X SYBR GREEN Master Mix, Applied Biotechnology. The β-actin gene was used as a reference gene (Elleithy *et al*., 2023). The Primers used in qRT-PCR were designed using a primer designing tool (https://www.ncbi.nlm.nih.gov/tools/primer-blast/). The qRT-PCR primers were shown in Table (2). Each qRT-PCR was done three times (Hassanen *et al*., 2023). The obtained data were analyzed using the equations of ΔCT, ΔΔCT, and $2^{-\Delta\Delta CT}$ (Kasas *et al*., 2022).

Bone mineral profile

At the end of the trial, six birds from each group were randomly se-

Table 1. Physical and chemical compositions of the basal and experimental diets for each growing period.

Items	G1 diet			G2 diet			G3 diet			G4 diet		
	Starter (0 to 14 days)	Grower (15 to 28 days)	Finisher (29 to 35 days)	Starter (0 to 14 days)	Grower (15 to 28 days)	Finisher (29 to 35 days)	Starter (0 to 14 days)	Grower (15 to 28 days)	Finisher (29 to 35 days)	Starter (0 to 14 days)	Grower (15 to 28 days)	Finisher (29 to 35 days
Ingredients%												
Yellow corn	54.25	59.4	65	55.8	61	66	55.6	61	66.22	57.04	62.3	67.55
Soybean meal 46%	29	13	6	35.5	26.6	17.7	32.5	21.17	10.5	33	24	13.75
High fat SBM	12	23	24.45	4.8	8.7	12.7	8	14	19.5	6	10	15
Monocalcium phosphate	1.6	1.5	1.5	0.9	0.8	0.7	0.9	0.8	0.8	0.9	0.75	0.75
Limestone	1.8	1.7	1.6	1.7	1.55	1.5	1.65	1.6	1.5	1.7	1.57	1.5
NaCl	0.28	0.28	0.28	0.28	0.28	0.38	0.28	0.28	0.28	0.28	0.28	0.28
Sod bicarbonate	0.22	0.2	0.2	0.22	0.22	0.22	0.22	0.2	0.2	0.22	0.22	0.22
L-Lysine	0.15	0.25	0.3	0.1	0.15	0.2	0.12	0.25	0.3	0.15	0.2	0.27
DL-Methionine	0.25	0.22	0.22	0.23	0.23	0.23	0.25	0.22	0.22	0.23	0.2	0.2
Toxin binder	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Lincomix	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
Anticoccidial	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02
Biostrong	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02
Econase XT 25P	0	0	0	0.01	0.01	0.01	0	0	0	0	0	0
Quantum Blue 5G	0	0	0	0.01	0.01	0.01	0	0	0	0	0	0
Enziver	0	0	0	0	0	0	0.03	0.03	0.03	0.03	0.03	0.03
Phytonex	0	0	0	0	0	0	0.01	0.01	0.01	0.01	0.01	0.01
Broiler premix[1]	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3
Chemical analysis:												
ME (Kcal/kg)	2935.11	3082.78	3161.45	3007.94	3125.07	3206.1	3016.66	3118.73	3226.4	3010.48	3101.83	3200.62
Crude Protein (%)	23.06	21.17	19.08	23.01	21.07	19.17	23.1	21.1	19.2	23.02	21.07	19.02
Crude Fat (%)	3.14	3.9	4.12	2.77	3.12	3.48	2.95	3.43	3.87	2.87	3.23	3.65
Calcium (%)	1.01	0.94	0.89	1.03	0.95	0.9	1.01	0.96	0.91	1.03	0.94	0.9
P. Available (%)	0.49	0.45	0.44	0.49	0.46	0.43	0.49	0.45	0.44	0.49	0.45	0.43

[1]Vitamin and mineral mixture contained: 13000000 IU vitamin A; 6000000 IU vitamin D3; 80000 mg vitamin E; 4000 mg vitamin K; 5000 mg vitamin B1; 9000 mg vitamin B2; 5000 mg vitamin B6; 35 mg vitamin B12; 20000 mg pantothenic acid; 70000 mg Nicotinic acid; 2000 mg Folic acid; 250 mg Biotin; 400000 mg choline chloride; 120000 mg Manganese oxide; 100000 mg Zinc oxide; 15000 mg Copper sulphate; 1000 calcium Iodide; 50000 mg ferrous sulphate; 350 mg Selenium selenite.

Table 2. Sequences of primers used in qRT-PCR.

Gene symbol	Gene description	Accession number	Primer Sequence
CPT1A	Carnitinepalmitoyltransferase 1A	NM_001012898.1	F:5′-TGGTGGATTTGGACCTGTGG -3′ R:5′-TGCTTCGTGGCAATAACCCA -3′
IGF1	Insulin Like Growth Factor 1	NM_001004384.2	F:5′-ACTGTGTGGTGCTGAGCTGGTT-3′ R:5′-AGCGTGCAGATTTAGGTGGCTT-3′
IL-1β	Interleukin-1beta	NM_204524.2	F:5′-GCCTGCAGAAGAAGCCTCG-3′ R:5′-GACGGGCTCAAAAACCTCCT-3′
IL-10	Interleukin-10	NM_001004414.4	F:5′-ATGGCAGCTTAACGTTCGGT-3′ R:5′-ATGGCAAATGCAGAGCCAGA-3′
TLR-4	Toll-like receptor 4	NM_001030693.1	F:5′-ATGTCCTCTTGCCATCCCAA-3′ R:5′-TCTCCCCTTTCTGCAGAGTG-3′
β-actin	Beta-actin	L08165.1	F:5′-CCCACACCCCTGTGATGAAA-3′ R:5′-TAGAACTTTGGGGGCGTTCG-3′

lected for determination of bone mineral profile. The right tibia was separated, and adhering tissue was removed manually. The weight of the tibia was recorded and expressed as a percentage (%) relative to the live body weight (tibia index). Tibia length was measured from the proximal end to the distal end. Bone was pooled and frozen until analyzed. Tibia fat was extracted via Soxhlet extraction using 100% ethyl ether according to the modified methods of Watson *et al.* (2006). Tibia bones were dried for 24 h at 100°C in a hot air oven and then were ashed overnight at 600°C using a muffle furnace. Tibias were analyzed for total ash, P, and Ca using AOAC (2007) protocol.

Statistical analysis

Data analysis was applied using PASW Statistics, Version 18.0 software (SPSS Inc., Chicago, IL, USA). Findings were summarized as means and pooled standard error of means (SEM). Statistical inference was assessed by applying one-way analysis of variance (ANOVA) test, and Tukey post-hoc test for multiple comparisons. Significance was determined at P≤0.05. Box plots and bar plots were executed with "ggpubr" package (Kassambara, 2022) using R for Statistical Computing (https://www.r-project.org/).

Results

Performance parameters

Tables 3, 4 illustrate the performance of broiler chickens subjected to the four dietary treatments. From 14 to 28 d of age, broilers in G2 had the highest live BW (+37.5, 85.9, 163.8 g/bird; +9.6, 11.0, 7.6 %) (P=0.0001) and BWG (+30.6, 48.3, 72.9 g/bird; +18.5, 12.4, 13.7 %) (P<0.05) compared to the control group. On day 35, the highest values of BW relative to chickens fed the nutrient-adequate basal diet (G1) were recorded in G2 (+163.8 g/bird; +7.6%), followed by G3 and G4 (+95.5, +82.2 g/bird; +4.4, 3.8 %), respectively (P=0.0001) (Table 3). Moreover, the overall BWG was improved in G2 (+163.8 g/bird; 7.7%) (P=0.0001) followed by G3 and G4 (+95.5 g, 82,2 g/bird; 4.5, 3.9 %) than those fed basal diet (G1) (Table 4).

Feed intake was significantly (P<0.05) affected by energy and nutrient reductions from 21 to 35 days of age and for the overall experimental period. FI was lower in birds fed energy and CP-reduced diet (G4) from 21-28 d (-73.4 g/bird; -9.3 %) and for the entire period (-194.4 g/bird; -6.2 %) (P=0.038, 0.006) than those fed nutrient-adequate basal diet. Moreover, at 35 d the lowest FI was observed in G2, G3, and G4 (-85.3, -93.5, -82.7 g/bird; - 6.1, 6.7, 5.9 %) respectively, compared to the control group (P<0.05). On average, better FCR was recorded from 28 to 35 d and for the entire period with improved EPEF in the nutrient-reduced dietary groups with reducing its matrix value (G2, G3, and G4) compared with those fed basal diet (P<0.05) (Table 3,4). Overall, mortality was 4.3% lower in birds fed energy and CP-reduced diet (G4) than those in G3 (Table 4).

Carcass traits and immune organs' relative weights

The effects of dietary treatment on carcass traits are presented in Table 5. No significant effect was found between different dietary treatments for carcass traits (dressing yield, breast, thigh, drum, liver, gizzard, and heart relative weights) (P > 0.05). However, the immune organs' relative weight (spleen and bursa of Fabricius) was significantly improved by 0.08% (P=0.0001, P=0.006) respectively in G4 when compared to the control group. Spleen's relative weight also recorded 0.03% increases in G3 versus the control group.

Physical and Chemical examinations of litter

Litter moisture % and total N, P, and Ca contents of bird groups were displayed in (Figs. 1 and 2). The litter Ca was lowered in G3 by 18.75% and

Table 3. Effect of dietary supplementation of phytase and non-starch polysaccharides-degrading enzymes on performance parameters of broiler chickens fed a nutritionally reduced diet.

Groups	Body weight (g)					Weight gain (g)					Feed intake (g)					FCR (g/g)				
	D 7	D 14	D 21	D 28	D 35	D 7	D 14	D 21	D 28	D 35	D 7	D 14	D 21	D 28	D 35	D 7	D 14	D 21	D 28	D 35
G1	164	391.0^{b}	781.1^{b}	1322.3^{b}	2156.2^{c}	124.8	226.2^{c}	390.1^{b}	541.3^{b}	833.8	141.3	289.7	506.0^{ab}	792.6^{a}	1396.5^{a}	1.14	1.28	1.3	1.47^{a}	1.68^{a}
G2	171.8	428.5^{a}	867.0^{a}	1481.1^{a}	2320.0^{a}	131.8	256.8^{a}	438.4^{a}	614.1^{a}	838.9	141.8	294.4	529.1^{a}	762.9^{b}	1311.2^{b}	1.08	1.15	1.21	1.26^{b}	1.57^{ab}
G3	165.4	408.1^{b}	784.5^{b}	1374.4^{b}	2251.7^{b}	125.4	242.8^{ab}	376.3^{b}	590.0^{ab}	877.3	137.5	295.8	472.3^{b}	755.7^{ab}	1303.0^{b}	1.1	1.22	1.26	1.28^{b}	1.49^{b}
G4	162.6	392.8^{b}	773.1^{b}	1358.7^{b}	2238.3^{b}	122.6	230.3^{bc}	380.3^{b}	585.6^{ab}	879.7	139	289.1	469.6^{b}	719.2^{b}	1313.8^{b}	1.14	1.26	1.23	1.23^{b}	1.50^{b}
SEM[1]	1.58	3.76	8.45	14.86	22.28	1.58	3.11	5.64	9.25	8.8	1.7	3.33	8.84	8.04	13.59	0.02	0.02	0.02	0.03	0.02
P-value	NS[2]	0.00	0.00	0.00	0.00	NS	0.00	0.00	0.03	NS	NS	NS	0.04	0.01	0.04	NS	NS	NS	0.00	0.01

[a,b,c] Mean values with different superscripts in the same column indicate significant difference (Tukey's test; P≤0.05).
G1, Control group (basal diet); G2, basal diet (-100 Kcal, -0.15% Av.P., -0.165% Ca) + 100 g Econase + 100 g Quantum blue/ton of feed; G3, basal diet (-80 Kcal, -0.15% Av.P., -0.165% Ca)+ 250 g Enziver + 100g Phytonex/ton of feed; G4, basal diet (-80 Kcal & -0.5% CP, -0.15% Av.P., -0.165% Ca) + 250 g Enziver + 100g Phytonex/ton of feed.
FCR, Feed Conversion Ratio (g of feed / g of weight gain).
[1]SEM: Pooled standard error of the mean.
[2] NS: Not significant.

15.38% on days 24 and 35, respectively, compared to the control group. While in G4, litter Ca was reduced by 31.25% on day 24. The litter N was lowered in G2 on day 24 by 4%, while in G3 by 2.3% and 19.8% on days 24 and 35, respectively. The litter P was decreased in G2 by 28.4% and 12.2% on days 24 and 35, respectively. The litter P was reduced in G3 by 14.9% on day 24, while in G4 was declined by 21.3% and 14.4% on days 24 and 35, respectively. On day 24, G4 exhibited lower litter moisture compared to G2 (-5.5%) and G3 (-8.2%). However, insignificant differences were observed in litter moisture between the different groups on days 24 and 35 (P>0.05).

Blood biochemical analysis

Supplementation of NSP enzymes and phytases in the diet of broiler chickens didn't affect the total protein, albumin, total cholesterol, and triglycerides concentrations. Moreover, the activity of liver enzymes (ALT and AST) weren't affected (P>0.05). Concerning the bone-related parameters, Ca and P concentrations didn't differ in all groups. While uric acid concentration and ALP activity were significantly (P<0.05) decreased in all groups than the control group. The highest decrease in uric acid concentration was in G3, while ALP was in G4 (Table 6).

Gene expression analysis

The expression level of IGF gene in both thigh and breast muscles was upregulated in all groups (P=0.008) by dietary NSP enzymes and phytases supplementation. The highest upregulation was in G4 in the thigh muscles (Fig. 3). The expression level of IL-1β was upregulated in G4 (P=0.0001) (Fig. 4). The expression level of IL-10 was upregulated significantly (P=0.009) in G3 and G4 and non-significantly (P>0.05) in G2

Table 4. Effect of dietary supplementation of phytase and non-starch polysaccharides-degrading enzymes on cumulative growth performance of broiler chickens fed a nutritionally reduced diet (days 1-35).

Groups	Body weight (g)	Weight gain (g)	Feed intake (g)	FCR (g/g)	EPEF	Mortality (%)
G1	2156.16^{c}	2116.17^{c}	3125.27^{a}	1.48^{a}	390.96^{b}	6.33^{ab}
G2	2320.00^{a}	2280.00^{a}	3039.43^{ab}	1.33^{b}	462.97^{a}	7.00^{ab}
G3	2251.67^{b}	2211.67^{b}	2964.72^{ab}	1.34^{b}	438.68^{a}	8.67^{a}
G4	2238.33^{b}	2198.33^{b}	2930.92^{b}	1.33^{b}	459.57^{a}	4.33^{b}
SEM[1]	22.28	13.94	25.04	0.02	7.24	0.53
P- value	0.00	0.00	0.02	0.00	0.00	0.02

^{a,b,c} Mean values with different superscripts in the same column indicate significant difference (Tukey's test; P≤0.05).
G1, Control group (basal diet); G2, basal diet (-100 Kcal, -0.15% Av.P., -0.165% Ca) + 100 g Econase + 100 g Quantum blue/ton of feed; G3, basal diet (-80 Kcal, -0.15% Av.P., -0.165% Ca)+ 250 g Enziver + 100g Phytonex/ton of feed; G4, basal diet (-80 Kcal & -0.5% CP, -0.15% Av.P., -0.165% Ca) + 250 g Enziver + 100g Phytonex/ton of feed
FCR, Feed Conversion Ratio, and EPEF, European production efficiency factors.
[1]SEM: Pooled standard error of the mean.

Table 5. Effect of dietary supplementation of phytase and non-starch polysaccharides-degrading enzymes on carcass traits of broiler chickens fed a nutritionally reduced diet.

Groups	Dressing (%)	Breast (%)	Thigh (%)	Drum (%)	Liver (%)	Gizzard (%)	Heart (%)	Spleen (%)	Bursa (%)
G1	75.18	23.88	19.41	9.73	2.43	1.3	0.5	0.11^{c}	0.19^{b}
G2	74.55	26.07	19.77	9.97	2.27	1.15	0.52	0.13^{bc}	0.16^{b}
G3	73.95	22.87	19.22	9.69	2.59	1.27	0.57	0.14^{b}	0.18^{b}
G4	73.3	23.68	19.28	9.31	2.47	1.34	0.56	0.19^{a}	0.27^{a}
SEM[1]	0.43	0.36	0.21	0.12	0.04	0.05	0.01	0.01	0.01
P- value	NS[2]	NS	NS	NS	NS	NS	NS	0.00	0.01

^{a,b,c} Mean values with different superscripts in the same column indicate significant differences (Tukey's test; P≤0.05).
G1, Control group (basal diet); G2, basal diet (-100 Kcal, -0.15% Av.P., -0.165% Ca) + 100 g Econase + 100 g Quantum blue/ton of feed; G3, basal diet (-80 Kcal, -0.15% Av.P., -0.165% Ca)+ 250 g Enziver + 100g Phytonex/ton of feed; G4, basal diet (-80 Kcal & -0.5% CP, -0.15% Av.P., -0.165% Ca) + 250 g Enziver + 100g Phytonex/ton of feed
[1]SEM: Pooled standard error of the mean.
[2]NS: Not significant.

Table 6. Effect of dietary supplementation of phytase and non-starch polysaccharides-degrading enzymes on some blood biochemical parameters of broiler chickens fed a nutritionally reduced diet.

Groups	Total protein (g\dl)	Albumin (g/dl)	TC (mgdl)	TAG (mg/dl)	ALT (U/L)	AST (U/L)	Uric acid (mg/dl)	ALP (U/L)	Ca (mg/dl)	Ph (mg/dl)
G1	2	1.3	114.8	63.2	16.2	266.7	4.60^{a}	1913.90^{a}	7.7	7.1
G2	3.1	1.05	118	52.35	13.7	256.7	3.80^{ab}	1112.60^{b}	7.06	7.1
G3	2.2	1.7	119.1	52.5	15.9	248.8	3.50^{b}	829.40^{bc}	6.3	7.3
G4	3	1.6	102.9	56.4	11.6	228.5	3.80^{ab}	771.60^{c}	6.7	6.9
SEM	0.17	0.06	2.7	2	0.7	5.9	0.14	110.7	0.2	0.15
P- value	NS[2]	NS	NS	NS	NS	NS	0.02	0.00	NS	NS

^{a,b,c} Mean values with different superscripts in the same column indicate significant differences (Tukey's test; P≤0.05).
G1, Control group (basal diet); G2, basal diet (-100 Kcal, -0.15% Av.P., -0.165% Ca) + 100 g Econase + 100 g Quantum blue/ton of feed; G3, basal diet (-80 Kcal, -0.15% Av.P., -0.165% Ca)+ 250 g Enziver + 100g Phytonex/ton of feed; G4, basal diet (-80 Kcal & -0.5% CP, -0.15% Av.P., -0.165% Ca) + 250 g Enziver + 100g Phytonex/ton of feed
[1]SEM: Pooled standard error of the mean.
[2]NS: Not significant.

Table 7. Effect of dietary supplementation of phytase and non-starch polysaccharides-degrading enzymes on bone mineralization of broiler chickens (days 24 and 35) fed a nutritionally reduced diet.

Groups	Bone weight (%)	Bone length	Total ash (%)	Ca (%)	P (%)
G1	0.23	6.72	29.63	23	16.25
G2	0.23	6.82	29.62	20.73	14.77
G3	0.22	6.8	29.5	20.5	14.47
G4	0.22	6.82	30.19	20.52	14.82
SEM[1]	0.00	0.07	0.45	0.41	0.38
P- value	NS[2]	NS	NS	NS	NS

G1, Control group (basal diet); G2, basal diet (-100 Kcal, -0.15% Av.P., -0.165% Ca) + 100 g Econase + 100 g Quantum blue/ton of feed; G3, basal diet (-80 Kcal, -0.15% Av.P., -0.165% Ca)+ 250 g Enziver + 100g Phytonex/ton of feed; G4, basal diet (-80 Kcal & -0.5% CP, -0.15% Av.P., -0.165% Ca) + 250 g Enziver + 100g Phytonex/ton of feed
[1]SEM Pooled standard error of the mean.
[2]NS: Not significant.

(Fig. 5). TLR-4 expression level was significantly (P=0.0001) upregulated in G3 and G4 (Fig. 6). CPT1A m-RNA was overexpressed (P=0.0001) in all groups rather than the control one (Fig. 7).

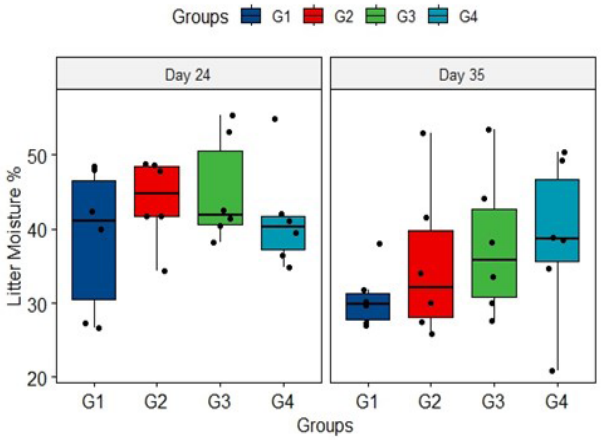

Fig. 1. Effect of dietary supplementation of phytase and non-starch polysaccharides-degrading enzymes on litter moisture % of broiler chickens (days 24 and 35) fed a nutritionally reduced diet. G1, Control group (basal diet); G2, basal diet (-100 Kcal, -0.15% Av.P., -0.165% Ca) + 100 g Econase + 100 g Quantum blue/ton of feed; G3, basal diet (-80 Kcal, -0.15% Av.P., -0.165% Ca)+ 250 g Enziver + 100g Phytonex/ton of feed; G4, basal diet (-80 Kcal & -0.5% CP, -0.15% Av.P., -0.165% Ca) + 250 g Enziver + 100g Phytonex/ton of feed.

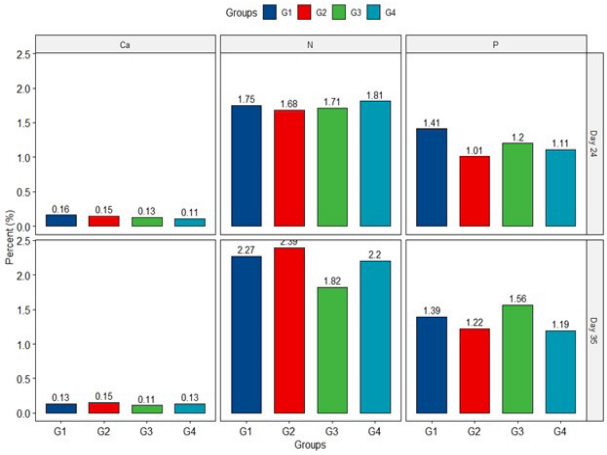

Fig. 2. Effect of dietary supplementation of phytase and non-starch polysaccharides-degrading enzymes on litter nutrients % (Ca, N, and P) of broiler chickens (days 24 and 35) fed a nutritionally reduced diet. G1, Control group (basal diet); G2, basal diet (-100 Kcal, -0.15% Av.P., -0.165% Ca) + 100 g Econase + 100 g Quantum blue/ton of feed; G3, basal diet (-80 Kcal, -0.15% Av.P., -0.165% Ca)+ 250 g Enziver + 100g Phytonex/ton of feed; G4, basal diet (-80 Kcal & -0.5% CP, -0.15% Av.P., -0.165% Ca) + 250 g Enziver + 100g Phytonex/ton of feed.

Bone mineral profile

The effects of dietary treatment on bone mineral profile are presented in Table 7. No significant effect was found between different dietary treatments (nutrient-adequate and reduced diets) for the bone mineral profile (tibia bone weight %, tibia length, tibia ash %, Ca %, and P %) (P > 0.05).

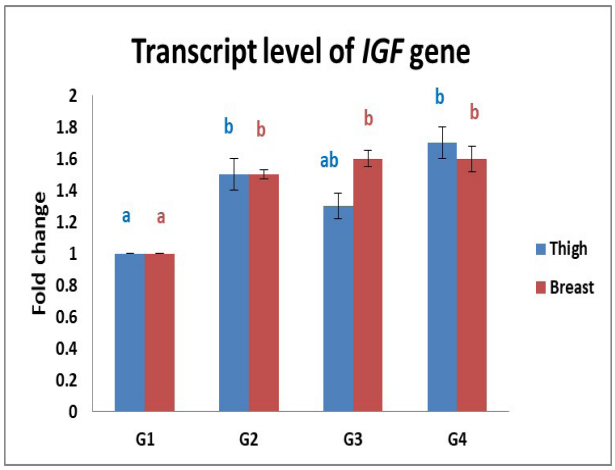

Fig. 3. Effect of dietary supplementation of phytase and non-starch polysaccharides-degrading enzymes on the expression of IGF gene in both thigh and breast muscles of broiler chickens fed a nutritionally reduced diet. G1, Control group (basal diet); G2, basal diet (-100 Kcal, -0.15% Av.P., -0.165% Ca) + 100 g Econase + 100 g Quantum blue/ton of feed; G3, basal diet (-80 Kcal, -0.15% Av.P., -0.165% Ca)+ 250 g Enziver + 100g Phytonex/ton of feed; G4, basal diet (-80 Kcal & -0.5% CP, -0.15% Av.P., -0.165% Ca) + 250 g Enziver + 100g Phytonex/ton of feed. Data are represented as mean ± SEM. Groups having different letters are significantly different from each other at P<0.05. Groups having similar letters are not-significantly different from each other at P<0.05.

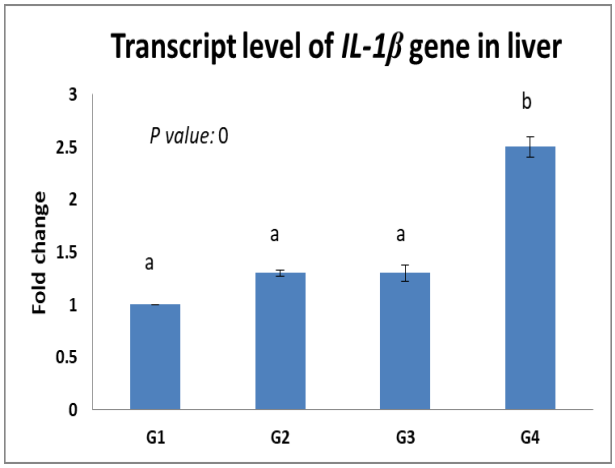

Fig. 4. Effect of dietary supplementation of phytase and non-starch polysaccharides-degrading enzymes on the expression of IL-1β gene in the liver of broiler chickens fed a nutritionally reduced diet. G1, Control group (basal diet); G2, basal diet (-100 Kcal, -0.15% Av.P., -0.165% Ca) + 100 g Econase + 100 g Quantum blue/ton of feed; G3, basal diet (-80 Kcal, -0.15% Av.P., -0.165% Ca)+ 250 g Enziver + 100g Phytonex/ton of feed; G4, basal diet (-80 Kcal & -0.5% CP, -0.15% Av.P., -0.165% Ca) + 250 g Enziver + 100g Phytonex/ton of feed. Data are represented as mean ± SEM. Groups having different letters are significantly different from each other at P<0.05. Groups having similar letters are non-significantly different from each other at P<0.05.

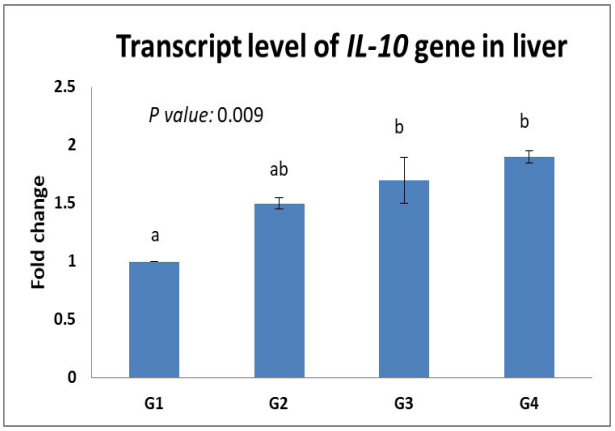

Fig. 5. Effect of dietary supplementation of phytase and non-starch polysaccharides-degrading enzymes on the expression of IL-10 gene in the liver of broiler chickens fed a nutritionally reduced diet. G1, Control group (basal diet); G2, basal diet (-100 Kcal, -0.15% Av.P., -0.165% Ca) + 100 g Econase + 100 g Quantum blue/ton of feed; G3, basal diet (-80 Kcal, -0.15% Av.P., -0.165% Ca)+ 250 g Enziver + 100g Phytonex/ton of feed; G4, basal diet (-80 Kcal & -0.5% CP, -0.15% Av.P., -0.165% Ca) + 250 g Enziver + 100g Phytonex/ton of feed. Data are represented as mean ± SEM. Groups having different letters are significantly different from each other at P<0.05. Groups having similar letters are non-significantly different from each other at P<0.05.

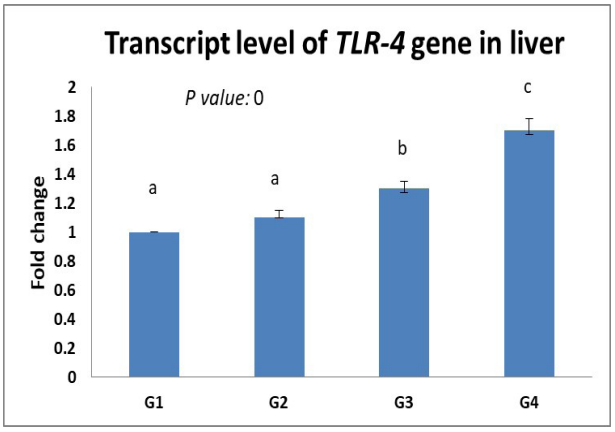

Fig. 6. Effect of dietary supplementation of phytase and non-starch polysaccharides-degrading enzymes on the expression of TLR-4genein the liver of broiler chickens fed a nutritionally reduced diet. G1, Control group (basal diet); G2, basal diet (-100 Kcal, -0.15% Av.P., -0.165% Ca) + 100 g Econase + 100 g Quantum blue/ton of feed; G3, basal diet (-80 Kcal, -0.15% Av.P., -0.165% Ca)+ 250 g Enziver + 100g Phytonex/ton of feed; G4, basal diet (-80 Kcal & -0.5% CP, -0.15% Av.P., -0.165% Ca) + 250 g Enziver + 100g Phytonex/ton of feed. Data are represented as mean ± SEM. Groups having different letters are significantly different from each other at P<0.05. Groups having similar letters are non-significantly different from each other at P<0.05.

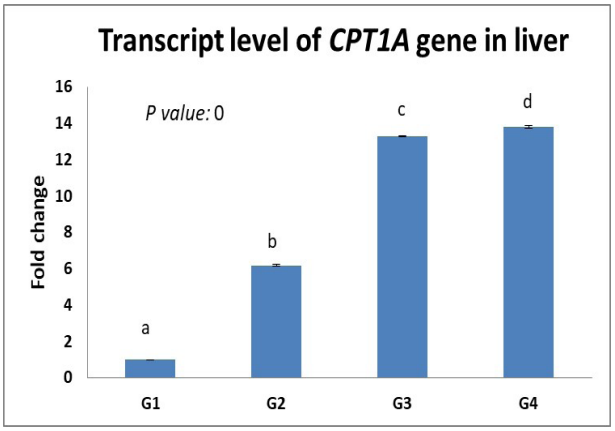

Fig. 7. Effect of dietary supplementation of phytase and non-starch polysaccharides-degrading enzymes on the expression of TLR-4genein the liver of broiler chickens fed a nutritionally reduced diet. G1, Control group (basal diet); G2, basal diet (-100 Kcal, -0.15% Av.P., -0.165% Ca) + 100 g Econase + 100 g Quantum blue/ton of feed; G3, basal diet (-80 Kcal, -0.15% Av.P., -0.165% Ca)+ 250 g Enziver + 100g Phytonex/ton of feed; G4, basal diet (-80 Kcal & -0.5% CP, -0.15% Av.P., -0.165% Ca) + 250 g Enziver + 100g Phytonex/ton of feed. Data are represented as mean ± SEM. Groups having different letters are significantly different from each other at P<0.05. Groups having similar letters are non-significantly different from each other at P<0.05.

Discussion

The objective of this study was to determine the nutritional potential of NSPase and phytase enzymes via performance, carcass traits, bone mineralization, litter quality, and gene expression in broilers fed diets reduced in energy, Av. P, Ca, and CP. Our results revealed that the inclusion of both NSPase and phytase enzymes in combination had a positive impact on the growth performance of birds fed nutrient-reduced diets. The live BW and BWG from 14 to 28 days and the overall values were optimized at G2 followed by G3 and G4, respectively. Additionally, the overall FCR and EPEF were improved in birds fed the nutrient-reduced diets compared with those fed an adequate-nutrient diet. Phytase was expected to increase the feed efficiency and growth performance in broiler chickens because: In its ingredients, about 2/3 of the P included in its substrate, phytate (Kornegay, 2001). Phytate, in its natural state is combined with macronutrients including proteins and lipids inside the intestinal lumen, potentially reducing nutritional solubility and intestinal absorption (Cowieson *et al*., 2014). Moreover, phytate is negatively charged in the GIT at all pH levels, so it can bind to positively charged nutrients and some endogenous enzymes, reducing nutrient utilization (Adeola and Sands, 2003). Recent studies regarding phytase supplementation into poultry diets have reported consistent beneficial effects on performance, and nutrient digestibility (Wu *et al*., 2015; Pieniazek *et al*., 2017; Walters *et al*., 2019).

Possibly, NSPase may increase phytase activity by facilitating access to phytate, which would otherwise be trapped within cell walls (Poernama *et al*., 2021), leading to an improvement in the absorption of minerals, amino acids, and energy-producing sources (Cozannet *et al*., 2017; Schramm *et al*., 2017). A previous study by Lee *et al*. (2010) showed the combined effects of phytase and NSPase, on the intestinal viscosity, BWG, and FCR of broilers which subsequently improved performance. Interestingly, it was reported that the overall FI as significantly affected by energy and nutrient reductions as it was more efficient in birds fed energy and CP-reduced diet with simultaneous improvement in FCR and decrease in mortality rate than birds fed adequate-nutrient diet. The observed decrease in FI in the current experiment may be an indication of optimum nutrient use which may be cost-effective and allow the possibility to reduce energy, P, Ca, and CP in poultry diets. Francesch and Geraert, (2009); Attia *et al*., (2020) reported that it is possible to minimize dietary AME and amino acids while maintaining cost-effective feed formulation and optimum poultry performance by employing NSPase and phytase enzyme complexes. Moreover, the reduced mortality rate may be related to the enhanced immune status in birds fed energy and CP-reduced diet in our findings.

For the carcass traits, NSPase and phytase combination showed no significant impact on the carcass traits, and similar findings were reported by Poernama *et al*. (2021); Abdel-Hafeez *et al*. (2018). Dietary supplementation with NSPase and phytase significantly improved immune organs (spleen and bursa Fabricius) relative weights in birds fed energy and CP-reduced diet as reported by Attia *et al*. (2020). Immune organs are the foundation for achieving the immune function and an increase in immune organ weight is associated with a better immune response in healthy broilers (Lan *et al*., 2020). However, other studies encountered no effect of enzyme supplementation on the relative weight of immune organs (Khaksar *et al*., 2012).

During the rearing period, the litter conditions change primarily due to the addition and buildup of bird excreta and moisture (Brink *et al*., 2022). The examined litter samples of NSPase and phytase-supplemented birds showed lower nutrients and element levels (N, P, and Ca). In poultry litter, microbes utilize urea and uric acid, resulting in emitting 80% of litter N as ammonia (NH3) (Kelleher *et al*., 2002; Ritz *et al*. 2004). On the other hand, dietary P is an expensive ingredient in broiler diets, and the application of litter containing excessive P for soil fertilization leads to the runoff and pollution of surface and groundwater with P (Dankowiakowska *et al*. 2013). Ismael *et al*., (2022) reported that fortification of a low-energy broiler diet with NSPase (xylanase) improved N and P absorption and lowered their excretion in broiler dropping and litter, which would reduce environmental pollution with poultry waste (deep-litter). NSPase could augment phytase activity and improves the assimilation and absorption of nutrients, minerals, amino acids, and energy sources (Poernama *et al*., 2021). The obtained results agreed with Woyengo *et al*. (2019), who stated that adding NSPase to phytase-supplemented diets enhanced the digestibility of phosphorous for the broiler. Similar findings were described by Yang *et al*. (2020), that supplementing a swine corn-based diet with both NSPase and phytase improved the digestibility of P and Ca. Litter moisture showed a relatively lower level in G4 on day 24. This finding agreed with Alfonso-Avila *et al*. (2022), who indicated that low-protein feed lowers litter moisture due to reducing the water intake and uric acid, which are excreted through the kidneys.

Blood biochemical parameters (TP, Albumin, TC, TAG, UA, ALT, AST, Ca, and P) were measured to evaluate the body performance of the bird.

The changes in serum uric acid levels between the control and treated groups showed significance. This finding agreed with Alfonso-Avila *et al*. (2022), who indicated that each 1% decrease in CP fed to broiler chicken's diet, induced a 9.4% decrease in plasma uric acid levels. On the other hand, the changes in TP, Albumin, TC, TAG, ALT, AST, Ca, and P levels between the different groups were non-significant. This indicated that the reduction in the calories, crude protein, Ca, and P in the diet formulation didn't affect their serum concentrations in broiler chickens. This may return to the effects of NSPase and phytase that may improve nutrient utilization in broiler chickens by breaking down anti-nutritional factors and releasing nutrients that are otherwise unavailable. For example, phytase can break down phytate, which is a form of P that broiler chickens poorly absorb. Also, phytic acid can bind to minerals like Ca and make them less available to the animal. By breaking down phytic acid, phytase can increase the bioavailability of Ca and other minerals in the feed (Zanu *et al*., 2020). Likewise, NSPase breaks down NSPs which bind to Ca and other minerals (Costa *et al*., 2013). This can lead to improved growth performance and reduced feed costs.

The use of phytase and NSPase in broiler chicken diets can provide feed formulation flexibility by allowing for the use of alternative feed ingredients that may be lower in nutrient density. This can reduce feed costs and increase the sustainability of broiler chicken production (Zanu *et al*., 2020; Costa *et al*., 2013). ALP activity was significantly decreased in all treated groups, and G4 showed the lowest ALP activity. ALP is an enzyme that is commonly used as a biomarker for bone metabolism in animals. The activity of ALP in the blood can indicate changes in bone formation or resorption (Elleithy *et al*., 2023). Phytase and NSPase can enhance mineral bioavailability in animals. This, in turn, can improve bone formation and mineralization, which may lead to changes in ALP activity. These agreed with our results, which showed a beneficial effect on growth performance and tibia mineralization by phytase and NSPase addition. Ciurescu *et al*. (2020) showed that phytase supplementation for the broiler turkey diet, significantly increased plasma total cholesterol, total protein, Ca, P, and Fe contents, with a higher AST activity and tended to increase ALT activity.

Studies showed that adding phytase and NSPase to broilers' chicken diets can improve growth performance (Anwar *et al*., 2023). Our study reported that the addition of phytase and NSPase upregulated the IGF-1 expression in both thigh and breast muscles. The best result was shown in G4. IGF-1 is produced in the liver under the control of growth hormone. It stimulates the body's growth and provides anabolic effects on different body cells (Yakar *et al*., 2002). The results of Muszyński *et al*. (2018) showed an increase in the weight gain and serum IGF-1 concentration. The addition of phytase and NSPase in broiler chicken diets can affect the immune response of broiler chickens. For example, some studies have reported that the addition of phytase and NSPase to the diet can modulate the expression of cytokines such as IL-1B and IL-10, which are involved in the immune response. This modulation may have implications for the disease resistance and overall health of broiler chickens. Phytase can activate the cellular and humoral immune response. It affects the immune system of broiler chickens by altering the gut microbiota and/or stimulating the production of cytokines, which are signaling molecules that play a key role in the immune response (Jarosz *et al*., 2017). Our study reported the upregulation of the expression levels of inflammatory cytokines (IL-1B and IL-10) and immune response-related genes (TLR-4) in the liver of broiler chickens. Studies have investigated the effect of phytase and NSPase on various metabolic pathways, including lipid metabolism, which may indirectly affect the expression of CPT1A. The CPT1A gene encodes for the CPT1A enzyme, which is found in the liver and responsible for fatty acid oxidation which takes place within mitochondria. Long-chain fatty acids cannot enter mitochondria unless they are attached to carnitine. Carnitine palmitoyl transferase 1A connects carnitine to long-chain fatty acids so they can cross the inner mitochondrial membrane for fatty acid oxidation and energy production (Bonnefont *et al*., 2004). Our results reported the upregulation of CPT1A expression levels. Liu *et al*., (2010) reported that phytase can affect lipase activity and lipid metabolism of broiler chickens.

The bone mineral profile data showed that the addition of phytase enzyme and NSPase (xylanase) to reducing diet in AME, Av. P, Ca, and protein % can conserve the bone mineralization constant without any adverse effect on the total ash, Ca, and P% of bone. Our findings agreed with Poernama *et al*. (2021); Francesch and Geraert (2009); Avila *et al*. (2012) who reported that the fortification of phytase and NSPase enzyme can restore the bone mineralization profile by reducing diet to the group fed on basal diet. This finding may be related to the effects of NSPase and phytase which improve nutrient utilization by breaking down anti-nutritional factors and phytic acid. Also, phytic acid can bind to minerals like Ca and make them less available to the animal (Zanu *et al*., 2020). Likewise, NSPase breaks down NSPs which bind to Ca and other minerals (Costa *et al*., 2013). Also, NSPase, able to break down the cell wall NSP matrix, can facilitate the access of phytase to phytate molecules (Olukosi *et al*., 2007), supporting the hypothesis that the use of a combination of NSPase and phytase can fully synergist their effects. Ravindran *et al*. (2006) reported that a combination of xylanase, amylase, and protease was able to increase the availability of energy by 3%, and the apparent N retention by 11.7%. Moreover, phytase alone might improve dietary AME, apparent amino acid digestibility, apparent CP digestibility, and mineral absorption of reduced nutrient C-SBM diets, according to Santos *et al*. (2008). Similar responses to those obtained in the present experiment were recorded by Cowieson *et al*. (2006). So, we can use the Econase or Enziver (NSPase) and Quantum blue or Phytonex (Phytase) in reducing diet at AME, Av. P (- 0.15%), and Ca (-0.165%) without any adverse effect on bone mineralization profile. Also, Enziver® (NSPase) and Phytonex® (phytase) can be added to the reduced nutrient diet as mentioned above plus the diet reduced in CP (-0.5%) without any effect on growth performance or bone mineralization profile.

Conclusion

Broiler chicken fedenergy-reduced diet supplemented with NSPase and phytase combination could improve the overall birds' performance, constant bone mineralization, with improved litter quality from an environmental perspective. Furthermore, energy and CP-reduced diet enhanced overall feed intake and mineral bioavailability, decreased mortality rate, improved immune status via increased immune organ weights, and upregulation of immune-related genes. Therefore, the use of exogenous enzymes containing NSPase and phytase in reduced-nutrient diets based on the matrix value of both NSPase and phytase is a potential approach to decrease the cost of feed formulation, with improvement in the performance that a diet with adequate nutritional levels would provide.

Conflict of interest

The authors declare that they have no competing interests.

References

Abdel-Hafeez, H.M., Saleh, E.S.E., Tawfeek, S.S., Youssef, I.M.I., Abdel-Daim, A.S.A., 2018. Utilization of potato peels and sugar beet pulp with and without enzyme supplementation in broiler chicken diets: effects on performance, serum biochemical indices and carcass traits. Journal of Animal Physiology and Animal Nutrition 102, 56-66.

Adeola, O., Sands, J.S., 2003. Does supplemental dietary microbial phytase improve amino acid utilization? A perspective that it does not. Journal of Animal Science 81, E78-E85.

Aftab, U., 2012. Exogenous carbohydrase in corn-soy diets for broilers. World's Poultry Science Journal 68, 447-464.

Alfonso-Avila, A.R., Cirot, O., Lambert, W., Létourneau-Montminy, M.P., 2022. Effect of low-protein corn and soybean meal-based diets on nitrogen utilization, litter quality, and water consumption in broiler chicken production: insight from meta-analysis. Animal 16, 100458.

Ali, W.A., Moselhy, W.A., Ibrahim, M.A., Amin, M.M., Kamel, S., Eldomany, E.B., 2022. Protective effect of rutin and β-cyclodextrin against hepatotoxicity and nephrotoxicity induced by lambda-cyhalothrin in Wistar rats: biochemical, pathological indices and molecular analysis. Biomarkers 27, 625-636.

Anwar, U., Riaz, M., Farooq Khalid, M., Mustafa, R., Farooq, U., Ashraf, M., Munir, H., Auon, M., Hussain, M., Hussain, M., Ayaz Chisti, M.F., 2023. Impact of exogenous xylanase and phytase, individually or in combination, on performance, digesta viscosity and carcass characteristics in broiler birds fed wheat-based diets. Animals 13, 278. https://doi.org/10.3390/ani13020278

AOAC International. 2007. Official Methods of Analysis of AOAC International. 18th ed. Rev. 2. AOAC Int., Gaithersburg, MD..

Attia, Y., El-Kelawy, M., Al-Harthi, M., El-Shafey, A., 2020. Impact of multienzymes dose supplemented continuously or intermittently in drinking water on growth performance, nutrient digestibility, and blood constituents of broiler chickens. Animals 10, 375.

Attia, Y.A., Addeo, N.F., Abd Al-Hamid, A.A.H.E., Bovera, F., 2019. Effects of phytase supplementation to diets with or without zinc addition on growth performance and zinc utilization of white pekin ducks. Animals 9, 280.

Avila, E., Arce, J., Soto, C., Rosas, F., Ceccantini, M., McIntyre, D.R., 2012. Evaluation of an enzyme complex containing nonstarch polysaccharide enzymes and phytase on the performance of broilers fed a sorghum and soybean meal diet. Journal of Applied Poultry Research 21, 279-286.

Bawish, B.M., Zahran, M.F.S., Ismael, E., Kamel, S., Ahmed, Y.H., Hamza, D., Attia, T., Fahmy, K.N.E., 2023. Impact of buffered sodium butyrate as a partial or total dietary alternative to lincomycin on performance, IGF-1 and TLR 4 genes expression, serum indices, intestinal histomorphometry, Clostridia, and litter hygiene of broiler chickens. Acta Veterinaria Scandinavica 65, 44.

Bedford, M., 2002. The role of carbohydrases in feedstuff digestion. Poultry feedstuffs: supply, composition and nutritive value. J. M. McNab and K. N. Boorman, eds. CABI, Roslin, Midlothian, UK, 319-336.

Biswas, A., Mohan, N., Raza, M., Mir, N.A., Mandal, A., 2019. Production performance, immune response and blood biochemical parameters in broiler chickens fed diet incorporated with prebiotics. Journal of animal physiology and animal nutrition 103, 493-500.

Bonnefont, J.P., Djouadi, F., Prip-Buus, C., Gobin, S., Munnich, A., Bastin, J., 2004. Carnitine palmitoyltransferases 1 and 2: biochemical, molecular and medical aspects. Molecular aspects of medicine 25, 495-520.

Brink, M., Janssens, G.P., Delezie, E., 2022. How do moisture content, friability, and crust development of litter influence ammonia concentrations in broiler production?. Livestock Science 265, 105-109.

Chaves, N.R., Nascimento, K.M., Kiefer, C., Rosa, M.S., Feitas, H.B., Paiva, L.L., Silva, T.R., Silva, L.A., Macie, V.A., Leal, C.R., Souza, A.I., 2020. Phytase and xylanase in diets with nutritional adjustments and their effects on serum biochemistry, morphometry and intestinal health of broilers. Anais da Academia Brasileira de Ciências 92, 1-16.

Choct, M., Kocher, A., Waters, D.L.E., Pettersson, D., Ross, G., 2004. A comparison of three xylanases on the nutritive value of two wheats for broiler chickens. British Journal of Nutrition 92, 53-61. https:// doi.org/10.1079/BJN20041166.

Choct, M., 2015. Feed non-starch polysaccharides for monogastric animals: classification and function. Animal Production Science 55, 1360-1366.

Ciurescu, G., Vasilachi, A., Grosu, H., 2020. Efficacy of microbial phytase on growth performance, carcass traits, bone mineralization, and blood biochemistry parameters in broiler turkeys fed raw chickpea (*Cicer arietinum* L., cv. Burnas) diets. Journal of applied poultry research 29, 71-184. https://doi.org/10.1016/j.japr.2019.10.004

Costa, F.G.P., Morais, S.A.N., Lima, M.R., Souza, R.B., Santos, T.T., Gomes, G.A., Pinheiro, S.G., 2013. Use of enzymes in diets with different percentages of added fat for broilers. Arquivo Brasileiro de Medicina Veterinária e Zootecnia 65, 894-901.

Cowieson, A.J., Aureli, R., Guggenbuhl, P., Fru-Nji, F., 2014. Possible involvement of myo-inositol in the physiological response of broilers to high doses of microbial phytase. Animal Production Science 55, 710-719.

Cowieson, A.J., Bedford, M.R., 2009. The effect of phytase and carbohydrase on ileal amino acid digestibility in monogastric diets: complimentary mode of action?. World's Poultry Science Journal 65, 609-624.

Cowieson, A.J., Singh, D.N., Adeola, O., 2006. Prediction of ingredient quality and the effect of a combination of xylanase, amylase, protease and phytase in the diets of broiler chicks. 1. Growth performance and digestible nutrient intake. British Poultry Science 47, 477-489.

Cozannet, P., Kidd, M.T., Neto, R.M., Geraert, P.A., 2017. Next-generation non-starch polysaccharide-degrading, multi-carbohydrase complex rich in xylanase and arabinofuranosidase to enhance broiler feed digestibility. Poultry Science 96, 2743-2750.

Dankowiakowska, A., Kozłowska, I., Bednarczyk, M., 2013. Probiotics, prebiotics and snybiotics in Poultry–mode of action, limitation, and achievements. Journal of Central European Agriculture 14, 467-478. https://doi.org/10.5513/ JCEA01/14.1.1222

Dumas, M.D., Polson, S.W., Ritter, D., Ravel, J., Gelb Jr, J., Morgan, R., Wommack, K.E., 2011. Impacts of poultry house environment on poultry litter bacterial community composition. PLoS One 6, e24785.

Elleithy, E.M., Bawish, B.M., Kamel, S., Ismael, E., Bashir, D.W., Hamza, D., Fahmy, K.N.E.D., 2023. Influence of dietary *Bacillus coagulans* and/or *Bacillus licheniformis*-based probiotics on performance, gut health, gene expression, and litter quality of broiler chickens. Tropical Animal Health and Production 55, 38.

Francesch, M., Geraert, P.A., 2009. Enzyme complex containing carbohydrases and phytase improves growth performance and bone mineralization of broilers fed reduced nutrient corn-soybean-based diets. Poultry Science 88, 1915-1924.

Gehring, C.K., Bedford, M.R., Dozier III, W.A., 2013. Extra-phosphoric effects of phytase with and without xylanase in corn-soybean meal-based diets fed to broilers. Poultry Science 92, 979-991.

González-Ortiz, G., Olukosi, O.A., Jurgens, G., Apajalahti, J., Bedford, M.R., 2020. Short-chain fatty acids and ceca microbiota profiles in broilers and turkeys in response to diets supplemented with phytase at varying concentrations, with or without xylanase. Poultry science 99, 2068-2077.

Hassanen, E.I., Kamel, S., Mohamed, W.A., Mansour, H.A., Mahmoud, M.A., 2023. The potential mechanism of histamine-inducing cardiopulmonary inflammation and apoptosis in a novel oral model of rat intoxication. Toxicology,484, 53410. doi:10.1016/j.tox.2022.153410.

Hetland, H., Choct, M., Svihus, B., 2004. Role of insoluble non-starch polysaccharides in poultry nutrition. World's Poultry Science Journal 60, 415-422.

Houba, V.J.G., Novozamsky, I., Van der Lee, J.J., 1995. Influence of storage of plant samples on their chemical composition. Science of the Total Environment 176, 73-79.

Huff, G.R., Huff, W.E., Jalukar, S., Oppy, J., Rath, N.C., Packialakshmi, B., 2013. The effects of yeast feed supplementation on turkey performance and pathogen colonization in a transport stress/Escherichia coli challenge. Poultry Science 92, 655-662.

Humer, E., Schwarz, C., Schedle, K., 2015. Phytate in pig and poultry nutrition. Journal of Animal Physiology and Animal Nutrition 99, 605-625.

Hussien, A., Ismael, E., Bawish, B.M., Kamel, S., Ismail, E.Y., El Bendari, E.K., Fahmy, K.N.E.D., 2022. Response of Broiler Chickens to the Dietary Fortification of Bile Acid. Journal of Advanced Veterinary Research 12, 82-587.

Hussien, A., Ismael, E., Elleithy, E.M., Kamel, S., Hamza, D.A., Ismail, E.Y., Fahmy, K.N.E.D., 2023. Influence of *Bacillus* subtilis and *Bacillus* licheniformis probiotic supplementation via the drinking water on performance and gut health of broiler chickens. Journal of Advanced Veterinary Research 13, 94-102.

Ismael, E., Ismail, E.M., Khalefa, H.S., Elleithy, E.M., Elmosalamy, S.H., Marouf, S. and Fahmy, K.N.E.D., 2022. Evaluation of Saccharomyces cerevisiae yeast fermentate and xylanase in reduced energy diet fed to broiler chicken. International Journal of Veterinary Science 11, 141-150.

Jackson, M.L., 1973. Soil chemical analysis, pentice hall of India Pvt. Ltd., New Delhi, India 498, 151-154.

Jarosz, Ł., Marek, A., Grądzki, Z., Kwiecień, M., Kalinowski, M., 2017. The effect of feed supplementation with zinc chelate and zinc sulphate on selected humoral and cell-mediated immune parameters and cytokine concentration in broiler chickens. Research in Veterinary Science 112, 59-65. doi:10.1016/j.rvsc.2016.09.007.

kasas, A.E., Farag, I.M., Darwish, H.R., Soliman, Y.A., Nagar, E.E., Ibrahim, M.A., Kamel, S., Warda, M., 2022. Molecular characterization of alpha subunit 1 of sodium pump (ATP1A1) gene in Camelus dromedarius: its differential tissue expression potentially interprets the role in osmoregulation. Molecular Biology Reports 49, 3849-3861.

Kassambara, A., 2022. ggpubr R Package: 'ggplot2' Based Publication Ready Plots. Version 0.5.0.. https://rpkgs.datanovia.com/ggpubr/

Kelleher, B.P., Leahy, J.J., Henihan, A.M., O'dwyer, T.F., Sutton, D., Leahy, M.J., 2002. Advances in poultry litter disposal technology–a review. Bioresource technology 83, 27-36.

Khaksar, V., Golian, A., Kermanshahi, H., 2012. Immune response and ileal microflora in broilers fed wheat-based diet with or without enzyme Endofeed W and supplementation of thyme essential oil or probiotic PrimaLac?. African Journal of Biotechnology 11, 14716.

Kheravii, S.K., Morgan, N.K., Swick, R.A., Choct, M., Wu, S.B., 2018. Roles of dietary fibre and ingredient particle size in broiler nutrition. World's Poultry Science Journal 74, 301-316. https://doi. org/10.1017/s0043933918000259

Knudsen, K.B., 2001. The nutritional significance of "dietary fibre" analysis. Animal Feed Science and Technology 90, 3-20. https://doi. org/10.1016/S0377-8401(01)00193-6

Kornegay, E.T., 2001. Digestion of phosphorus and other nutrients: the role of phytases and factors influencing their activity. Enzymes in farm animal nutrition. 237-271.

Lan, R., Li, S., Chang, Q., An, L., Zhao, Z., 2020. Sodium butyrate enhances growth performance and intestinal development in broilers. Czech Journal of Animal Science 65, 1-2.

Lee, S.Y., Kim, J.S., Kim, J.M., An, B.K.., Kang, C.W., 2010. Effects of multiple enzyme (Rovabio® Max) containing carbohydrolases and phytase on growth performance and intestinal viscosity in broiler chicks fed corn-wheat-soybean meal based diets. Asian-Australasian Journal of Animal Sciences 23, 1198-1204.

Liu, N., Ru, Y., Wang, J., Xu, T., 2010. Effect of dietary sodium phytate and microbial phytase on the lipase activity and lipid metabolism of broiler chickens. British Journal of Nutrition 103, 862-868.

Mahmood, T., Guo, Y., 2020. Dietary fiber and chicken microbiome interaction: Where will it lead to?. Animal Nutrition 6, 1-8. https:// doi.org/10.1016/j.aninu.2019.11.004

Mateos, G.G., Jiménez-Moreno, E., Serrano, M.P., Lázaro, R.P., 2012. Poultry response to high levels of dietary fiber sources varying in physical and chemical characteristics. Journal of Applied Poultry Research 21, 156-174. https://doi.org/10.3382/japr.2011-00477

Morgan, N., Bhuiyan, M.M., Hopcroft, R., 2022a. Non-starch polysaccharide degradation in the gastrointestinal tract of broiler chickens fed commercial-type diets supplemented with either a single dose of xylanase, a double dose of xylanase, or a cocktail of non-starch polysaccharide-degrading enzymes. Poultry Science 101, 101846.

Morgan, N., Bhuiyan, M.M., Nguyen, T.N.A., Middlebrook, T., Hopcroft, R., 2021. Dietary soluble non-starch polysaccharide level and composition influences grower and finisher phase performance, excreta moisture content and total tract nutrient digestibility in broilers. British Poultry Science 62, 759-770. https://doi.org/10.1 080/00071668.2021.1919995

Morgan, N., Bhuiyan, M.M., Wallace, A., Hopcroft, R., 2022b. Comparing a single dose of xylanase to a double dose or cocktail of non-starch polysaccharide-degrading enzymes in broiler chicken diets. Journal of Applied Animal Nutrition 10, 91-102.

Muszyński, S., Tomaszewska, E., Kwiecień, M., Dobrowolski, P., Tomczyk, A., 2018. Effect of dietary phytase supplementation on bone and hyaline cartilage development of broilers fed with organically complexed copper in a Cu-deficient diet. Biological Trace Element Research 182, 339-353.

Newman, K., 1991. Phytase: the enzyme, its origin and characteristics: impact and potential for increasing phosphorus availability. Biotechnology in the Feed Industry, Proc. Alltech's 7th Ann. Symp.(T.P. Lyons, Ed.). Alltech Technical Publications, Nicholasville (Kentucky) 7, 169-177.

NRC, 1994. Nutrient Requirements of Poultry. National Research Council, 9th ed. National Academy of Sciences, Washington.

O'Neill, H.M., Smith, J.A., Bedford, M.R., 2014. Multicarbohydrase enzymes for non-ruminants. Asian-Australasian journal of Animal Sciences 27, 290. https://doi.org/10.5713/ajas.2013.13261

Olukosi, O.A., Cowieson, A.J., Adeola, O., 2007. Age-related influence of a cocktail of xylanase, amylase, and protease or phytase individually or in combination in broilers. Poultry Science 86, 77-86.

Pieniazek, J., Smith, K.A., Williams, M.P., Manangi, M.K., Vazquez-Anon, M., Solbak, A., Miller, M., Lee, J.T., 2017. Evaluation of increasing levels of a microbial phytase in phosphorus deficient broiler diets via live broiler performance, tibia bone ash, apparent metabolizable energy, and amino acid digestibility. Poultry Science 96, 370-382.

Poernama, F., Wibowo, T.A., Liu, Y.G., 2021. The effect of feeding phytase alone or in combination with nonstarch polysaccharides-degrading enzymes on broiler performance, bone mineralization, and carcass traits. Journal of Applied Poultry Research 30, 100134.

Ravindran, V., Morel, P.C., Partridge, G.G., Hruby, M., Sands, J.S., 2006. Influence of an Escherichia coli-derived phytase on nutrient utilization in broiler starters fed diets containing varying concentrations of phytic acid. Poultry Science 85, 82-89.

Ritz, C.W., Fairchild, B.D., Lacy, M.P., 2004. Implications of ammonia production and emissions from commercial poultry facilities: A review. Journal of Applied Poultry Research 13, 684-692. https://doi.org/10.1093/japr/13.4.684

Santos, F.R., Hruby, M., Pierson, E.E.M., Remus, J.C., Sakomura, N.K., 2008. Effect of phytase supplementation in diets on nutrient digestibility and performance in broiler chicks. Journal of Applied Poultry Research 17, 191-201.

Schramm, V.G., Durau, J.F., Barrilli, L.N.E., Sorbara, J.O.B., Cowieson, A.J., Félix, A.P., Maiorka, A., 2017. Interaction between xylanase and phytase on the digestibility of corn and a corn/soy diet for broiler chickens. Poultry Science 96, 1204-1211.

Selle, P.H., Ravindran, V., 2007. Microbial phytase in poultry nutrition. Animal Feed Science and Technology 135, 1-41.

Shelton, J.L., Southern, L.L., Gaston, L.A., Foster, A., 2004. Evaluation of the nutrient matrix values for phytase in broilers. Journal of Applied Poultry Research 13, 213-221.

Walk, C.L., Poernama, F., 2019. Evaluation of phytase, xylanase, and protease in reduced nutrient diets fed to broilers. Journal of Applied Poultry Research, 28, 85-93.

Walters, H.G., Coelho, M., Coufal, C.D., Lee, J.T., 2019. Effects of increasing phytase inclusion levels on broiler performance, nutrient digestibility, and bone mineralization in low-phosphorus diets. Journal of Applied Poultry Research 28, 1210-1225.

Watson, B.C., Matthews, J.O., Southern, L.L., Shelton, J.L., 2006. The effects of phytase on growth performance and intestinal transit time of broilers fed nutritionally adequate diets and diets deficient in calcium and phosphorus. Poultry science 85, 493-497.

Woyengo, T.A., Bogota, K.J., Noll, S.L., Wilson, J., 2019. Enhancing nutrient utilization of broiler chickens through supplemental enzymes. Poultry science 98, 1302-1309.

Woyengo, T.A., Nyachoti, C.M., 2011. Supplementation of phytase and carbohydrases to diets for poultry. Canadian Journal of Animal Science 91, 177-192.

Wu, D., Wu, S.B., Choct, M., Swick, R.A., 2015. Comparison of 3 phytases on energy utilization of a nutritionally marginal wheat-soybean meal broiler diet. Poultry science 94, 2670-2676.

Yakar, S., Rosen, C.J., Beamer, W.G., Ackert-Bicknell, C.L., Wu, Y., Liu, J.L., Ooi, G.T., Setser, J., Frystyk, J., Boisclair, Y.R., LeRoith, D., 2002. Circulating levels of IGF-1 directly regulate bone growth and density. The Journal of Clinical Investigation, 110, 771-781.

Yang, J.C., Wang, L., Huang, Y.K., Zhang, L., Ma, R., Gao, S., Hu, C.M., Maamer, J., Pierre, C., Preynat, A., Lei, X.G., 2020. Effect of a multi-carbohydrase and phytase complex on the ileal and total tract digestibility of nutrients in cannulated growing pigs. Animals 10, 1434.

Zanu, H.K., Kheravii, S.K., Morgan, N.K., Bedford, M.R., Swick, R.A., 2020. Interactive effect of dietary calcium and phytase on broilers challenged with subclinical necrotic enteritis: part 2. Gut permeability, phytate ester concentrations, jejunal gene expression, and intestinal morphology. Poultry Science 99, 4914-4928.

Zarghi, H., Golian, A., Tabatabaei Yazdi, F., 2020. Effect of dietary sulphur amino acid levels and guanidinoacetic acid supplementation on performance, carcase yield and energetic molecular metabolites in broiler chickens fed wheat-soy diets. Italian Journal of Animal Science 19, 951-959.