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Effect of Phytase, Protease, and their Combination on the Nutrients Bioavailability and Performance Indices of Broilers Fed a Sorghum-Based Diet Under Local Climatic Conditions of Khyber Pakhtunkhwa Pakistan

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ABSTRACT

The present study evaluated the effect of protease and phytase in a sorghum-based diet on nutrient bioavailability and performance indices of broilers. A total of 160-day-old broiler chicks were purchased from the local hatchery and assigned to four treatment groups replicated four times with 10 birds per replicate in a completely randomized design. There were four dietary treatments designated as control contained 100% maize-based diets without sorghum and enzyme, HDV-100+protease (T1) had 100% sorghum without any maize and supplemented with protease enzyme; HDV-100+phytase (T2) had 100% sorghum without maize and supplemented with phytase enzyme; HDV-100+protease+phytase (T3) had 100% sorghum without maize and supplemented with the combination of protease and phytase enzyme. The result showed improved body weight gain and feed conversion ratio during the overall experimental period in T1, T2, and T3 diet groups than in the control group. During the entire experimental period feed intake and livability remind unchanged. On days, 21 and 28 of the experimental period, the litter moisture content was not effected but at day 35 of the experimental period, the litter moisture content was calculated significantly higher in the T2 and T3 diet groups than in the control and T1 diet groups. Relative organ weight was not effected throughout the experimental period. The crude protein and apparent ileal metabolizable energy were calculated significantly higher in the T3 diet groups as compared to the remaining groups. Overall, the digestibility of essential amino and non-essential amino acid were recorded ($P<0.05$) higher in the T1, T2, and T3 diet group as compared to the control group. In conclusion, the replacement of maize with sorghum in combination with exogenous enzyme protease and phytase can effectively improve the growth performance and nutrient digestibility in the broiler.

INTRODUCTION

The poultry industry for feed production mostly relies on highly digestible feed for energy and protein, such as soybean meal and maize (Zhu *et al*., 2014). In tropical countries, maize is mostly used for bioethanol production and human consumption (Morgan and Choct, 2016). Due to higher metabolizable energy in compression to other cereal grains, it is commonly used in poultry feed as an energy source (Bhuiyan *et al*., 2013). Maize is used to feeding poultry in Pakistan, but it is also a principal food for humans. Maize production in some parts of Asia is hampered by low yields caused by drought (Wang *et al*., 2018), making it an unusual and expensive product, mainly for poultry production. The inadequate quantity and high cost of maize have posed a risk to the poultry industry because maize prices are high and unpredictable, using substitute feedstuffs may be the best approach in formulating feeds for profitable poultry production. Sorghum has long been used as a substitute feed component for maize in broiler diets due to its lower production cost. However, most poultry studies have focused on the effects

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of maize or sorghum-based diets and have not considered the incomplete replacement of the dietary cereal grain (Tancharoenrat *et al*., 2014). The response of exogenous enzyme supplementation has different results due to the composition of the cereal grains. Moreover, the phytate arabinoxylan and crude protein may affect the efficacy of exogenous enzymes like phytase and protease of the sorghum and maize grains-based diet in broilers (Choct, 2015). Although sorghums have higher protein content, the digestibility of amino acids is lower as compared to maize (Rostagno *et al*., 2011). The main protein in sorghum is kafirin, which has a low digestibility (Li *et al*., 2018). Kafirin, content in sorghum contains tannin and phytate, both of which are anti-nutrients that can impair energy and nutrient utilization (Pasquali *et al*., 2017). Tannin may bind with other nutrients rendering them inaccessible, while phytate binds to minerals and reduced its availability (Woyengo and Nyachoti, 2013). Exogenous enzymes such as phytase and protease have been widely studied in the broiler to reduce the negative effects of these anti-nutritional factors. As a result, the first goal of this study was designed to evaluate the effect of exogenous enzymes in broiler fed with sorghum-based diets.

MATERIALS AND METHODS

Bird's husbandry and experimental diets

A total of 160 days old broiler chicks were purchased from the local hatchery and the birds were assigned to four treatment groups replicated four times having 10 birds per replicate in a completely randomized design. Before the experiment, the experimental house and all other equipment were washed and disinfected. Chicks were reared in a small brooding area where the temperature was kept at 34^{o}C for the first week and gradually decreased, as the birds grew older, and continued at 22^{o}C for the rest of the experimental period. All commercial and experimental diets were provided ad libitum, and birds had 24-h access to water and feed. Individually installed feeders and drinkers in each pen were used. Every day, the feeders and drinkers were cleaned. The birds in all groups were vaccinated according to the standard schedule used in the area. Four experimental diets were formulated fulfilling all the nutritional requirements of the broiler. In experimental diets, the sorghum replacement level was 0%, and 100% with diets formulated to be iso-nutritive and isoenergetic. There were four dietary treatments designated as control contained 100% maize-based diets without sorghum and enzyme, HDV-100+protease (T1) had only 100% sorghum without any maize and supplemented with protease enzyme; HDV-100+phytase (T2) had only 100% sorghum with without any maize and supplemented with phytase enzyme; HDV-100 (T3) had only 100% sorghum with without any maize and supplemented with the combination of protease and phytase enzyme. Protease, phytase, and their combination were added at the rate of 0.01% in the final diet after mixing. The dietary composition and calculated analysis of both the starter and finisher experimental diets are shown in Table I.

Table I. Dietary composition and calculated nutrient content of the experimental diets used in the study.

Ingredients (g)	Control	Diets		
		T1	T2	T3
Maize corn	566	0.00	0.00	0.00
Sorghum grain	0.00	566	566	566
Soybean meal	346	346	346	346
Poultry oil	50.2	50.2	50.2	50.2
Di-calcium phosphate	17.5	17.0	17.0	17.0
Limestone	9.06	8.16	8.16	8.16
Salt	4.46	4.13	4.13	4.13
Lysine-HCL	2.18	2.20	2.20	2.20
Threonine	1.26	1.24	1.24	1.24
DL- Methionine	2.64	2.80	2.80	2.80
Choline and Coban 90	2.25	2.22	2.22	2.22
Vitamin premix*	0.25	0.25	0.25	0.25

*Vitamin mineral premix (each kg contained): retinol, 200,000 IU; tocopherol, 1072 IU; ascorbic acid, 26000 IU; cholecalciferol, 80,000 IU; thiamine, 11666 IU; menadione, 11,333 IU; pyridoxine, 33333 IU; niacin, 5,36,000 IU; folic acid, 13600 IU; riboflavin, 54,000 IU; methylcobalamin, 223 IU; biotin, 1340 IU; Ca, 195 g; Mg, 6 g; Fe, 2,000 mg; Na, 18 g; Zn, 1,200 mg; K, 70 g; Mn, 1,200 mg; Cu, 400 mg; I, 40 mg, Co, 20 mg and Se, 8 mg.

Growth performance parameters

Body weight gain and feed intake were calculated on weekly basis. Total body weight gain was calculated by subtracting initial weight from final body weight. The refused feed was subtracted from the offered feed to calculate feed intake. Data obtained from total body weight gain and feed intake were used to calculate the feed conversion ratio (FCR) (Shuaib *et al*., 2022). At 21 and 35 d of the experimental period, two birds of average body weight were randomly selected from each experimental unit and sacrificed after fasting for 2 h. The relative weight of the organs was determined concerning the body weight. On the days 21 and 35 of the experimental period litter samples were collected from four different places in each cage from surface to bottom from the area near drinkers and feeders. The collected litter samples were stored were mixed and stored in plastic bags. From each group, 70 grams were taken and weighed into trays. The samples were dried in a forced air oven at 65 °C for 24 h. The moisture content in the litter was determined according to the methods described by (AOAC, 2000).

Hematological analysis and nutrient digestibility

On day 35 of the experimental period, blood samples were collected after fasting the birds for 12 h before sampling. From representative birds, blood samples were collected in tubes containing EDTA as an anticoagulant. Samples were transported in ice packs to the laboratory to prevent deterioration. Blood samples were analyzed as described by Ritchie *et al*. (1994). On day 36 of the experimental period, five birds of uniform body weight and size were randomly selected and transferred to metabolic cages for the digestibility assy. The birds in the experimental groups were fed with experimental diets from day 37 to day 42 of age. Diets were provided in a mesh form and birds have free excess to water and feed all the time. During the last four days, excreta were collected and dried in a forced air oven and stored until further analysis. Feed and digesta proximate analysis was performed as outlined in the AOAC (2005) procedure. For the determination of indigestible contents, a UV absorption spectrophotometer was used (Williams *et al*., 1962). An adiabatic bomb calorimeter was used to determine the gross energy of feed and fecal samples. For the determination of apparent metabolizable energy (AME), the digestive marker method was used. The final AME values were calculated by using the formula described by (Adejumo *et al*., 2021). For ileal amino acid digestibility, the test ingredient and ileal digesta samples were oxidized with a hydrogen peroxide-formic acid-phenol solution, and the excess oxidation reagent was decomposed with sodium disulfite. After oxidation, samples were hydrolyzed for 24 hs in 6M HCl. The pH of the hydrolysate was adjusted to 2.20 before it was centrifuged, filtered, and then injected into an amino acid analyzer for the determination of the amino acid profile and then the apparent ileal amino acid digestibility was determined as described by (Ullah *et al*., 2016).

Statistical analysis

The data was analyzed by completely randomized design through SAS software employing one-way ANOVA. The mean difference was compared using the Least Significant Difference (Steel, 1997) test.

RESULTS

The results regarding the effect of the complete replacement of maize with sorghum and supplementation of protease and phytase enzymes individually and in combination on feed intake, weight gain, FCR, livability, and litter moisture are presented in Table II. The supplementation of enzymes alone or in combination did not effect the growth performance parameters like BWG, feed intake, and FCR during the starter phase from day 1-21. However from day 22-35 and day 1-35 higher ($p<0.05$) body weight gain and best FCR was recorded for the T3 diet group as compared to the T1, T2, and control groups. During the entire experimental period feed intake and livability remind unchanged. On days, 21 and 28 of the experimental period, the litter moisture content was not effected but on day 35 of the experimental period, the litter moisture content was calculated significantly higher in the T2 and T3 diet groups than in the control and T1 diet groups. Relative organ weight was not effected throughout the experimental period. The result of the hematological parameters and nutrient digestibility are presented in Table III. All the dietary treatments had no significant ($p>0.05$) effect on all the blood parameters. It is evident from the data that enzyme supplementation did not affect the dry matter and crude fat digestibility compared with a maize-based diet without enzyme supplementation. However, crude protein and apparent ileal metabolizable energy were calculated significantly ($P<0.05$) higher T3 diet groups as compared to the remaining group. Overall, the digestibility of essential amino and non-essential amino acid were recorded ($P < 0.05$) higher in the T1, T2, and T3 diet group as compared to the control group.

DISCUSSION

In the present study, the use of an exogenous enzyme in poultry improved the production performance parameters which is attributed to the enzyme's ability to cause non-starch polysaccharide (NSP) degradation in the cell wall matrix of feed ingredients. This resulted in the release of encapsulated nutrients, as well as lower viscosity of digesta caused by soluble NSP and an improved rate of diffusion between enzyme and digestion end products (Selle *et al*., 2010; Gidado *et al*., 2020). Exogenous enzymes, by stimulating intestinal motility in broiler chicken, expose the locked nutrient in the intestine to endogenous enzymes for proper unlocking of the nutrients. The activity of endogenous enzymes like amylase, trypsin, and lipase increase with age (Sakomura *et al*., 2004). The exogenous protease perfected the action of endogenous proteolytic enzymes in the GIT thus improving the growth performance by improving nutrient digestibility (Pasquali *et al*., 2017). The relative weight of the organs remained unaffected in the present study. Similarly, Thomas and Ravindran (2008) found that sorghum-based diets did not affect the relative weight of the different organs. Gizzard and small intestine

Table II. Effect of enzyme supplementation on broiler production performance, relative organ weight and liter moisture content fed with sorghum-based diet.

Parameters		Control	Treatments			P-value
			T1	T2	T3	
Starter phase (day 1-21)	BWG (g)	633.9±0.08	646.4±0.090	629.2±0.03	733.4±0.96	0.061
	FI (g)	1140±0.021	1110±0.021	1105±0.031	1120±0.022	0.097
	FCR	1.79±0.023	1.71±0.03	1.75±0.04	1.52±0.05	0.102
	Livability (%)	96.23±0.49	95.60±0.56	96.82±0.44	96.20±0.52	0.234
Finisher phase (day 22-35)	BWG (g)	1198.8±0.97^{b}	1196.8±0.61^{b}	1189.7±0.18^{b}	1220.4±0.24^{a}	0.045
	FI (g)	2160±0.92	2159.7±0.94	2155.2±0.73	2151±0.45	0.098
	FCR	1.80^{a}±0.03	1.80^{a}±0.04	1.81^{a}±0.03	1.76^{b}±0.03	0.041
	Livability (%)	96.31±0.39	95.60±0.46	96.93±0.37	96.0±0.61	0.122
Overall period (day 1-35)	BWG (g)	1832.7±0.21^{b}	1843.2±0.75^{b}	1818.9±0.50^{b}	1953.8±0.16^{a}	0.000
	FI (g)	3300.2±0.36	3259.2±0.74	3260.2±0.94	3270±0.53	0.378
	FCR	1.80±0.03^{a}	1.76±0.04^{a}	1.79±0.04^{a}	1.67±0.02^{b}	0.000
	Livability (%)	96.31±0.12	95.71±0.53	96.82±0.74	96.02±0.75	0.000
Relative organ weight (%)	Gizzard	1.18±0.04	1.19± 0.05	1.20 ± 0.06	1.25±0.05	0.152
	Pancreas	0.24±0.02	0.26±0.03	0.25±0.02	0.24±0.03	0.228
	Liver	1.75±0.03	1.74±0.02	1.78±0.04	1.76±0.03	0.457
	Cecum	0.54±0.05	0.58±0.02	0.55±0.01	0.59±0.09	0.256
Intestinal segment weight (%)	Duodenum	1.47±0.18	1.48±0.30	1.45±0.70	1.44±0.51	0.674
	Jejunum	1.76±0.11	1.76±0.18	1.76±0.28	1.74±0.01	0.951
	Ileum	2.47±0.01	2.46±0.50	2.47±0.01	2.46±0.03	0.070
Intestinal segment length (cm)	Duodenum	30.00±0.36	30.53±0.56	30.65±0.42	30.34±0.49	0.344
	Jejunum	80.35±0.42	81.03±0.54	79.93±0.44	77.68±0.56	0.342
	Ileum	87.16±0.70	88.50±0.76	89.16±0.61	85.66±0.42	0.895
Moisture content (%)	Day 21	21.10±0.25	21.50±0.34	21.83±0.30	21.50±0.56	0.518
	Day 28	24.62±0.41	24.52±0.41	24.32±0.53	24.31±0.21	0.078
	Day 35	27.36±0.21^{b}	27.00±0.57^{b}	28.26±0.42^{a}	28.16±0.30^{a}	0.048

Means in rows having different superscripts are significantly different. BWG, body weight gain; FI, feed intake; FCR, feed conversion ratio.

weight were decreased numerically. It may be due to the presence of NSP in diets that harm access of digestive enzymes to their substrates, thus, adapting the structure and functions of digestive organs. The supplementation of exogenous enzymes reduced the reactions in organs of the digestive thus, reducing the size of the gizzard and small intestine (Zhu *et al*., 2014). Litter moisture content was increased by the supplementation of exogenous enzymes into the broiler diet at 35 of the experimental period which is in agreement with the findings of Pos *et al*. (2003), and Farahat *et al*. (2013) who observed an increase in litter moisture content in turkeys and broiler, respectively. This effect could also be attributed to an increase in digesta osmolarity in broilers fed with phytase-containing diets, which is due to the release of more cations than anions in the early stages of phytate degradation, because 6-phytase acts cyclically, gradually releasing cations and anions in unequal proportions (Letourneau *et al*., 2011). There was significant interaction among the supplementation of different exogenous enzymes individually in broilers when maize was replaced by sorghum in the diet. Enzyme supplementation significantly increased the crude protein digestibility and AME of the sorghum-based diet compared with the maize-based diet. These results of the present study are in agreement with the findings of Gidado *et al*. (2020), and Pasquali *et al*. (2017) who observed a significant effect of exogenous enzymes on the crude digestibility sorghum-based diet. The exogenous enzymes can form a chelate with minerals and thus increase the crude protein digestibility. Moreover, phytate binds with protein through complexes

Table III. Effect of enzyme supplementation on the hematology and nutrient digestibility of broiler fed with sorghum-based diet.

Parameters	Control	Treatments			P-value
		T1	T2	T3	
Hematological parameters					
PCV (%)	27.12±0.04	27.25±0.05	27.27±0.06	27.46±0.07	0.484
Hb (mg/dl)	9.58±0.20	9.82±0.13	9.48±0.25	9.52±0.29	0.787
RBC (cells x10^6/mm^3)	2.63±0.02	2.54±0.04	2.61±0.03	2.60±0.03	0.981
WBC (cells x10^3/mm^3)	10.67±0.03	10.38±0.04	10.47±0.06	10.47±0.01	0.898
Neutrophils (%)	11.47±0.04	11.54±0.01	10.35±0.01	12.36±0.06	0.186
Heterophils/Lymphocyte (%)	88.45±0.03	90.46±0.09	91.47±0.02	91.42±0.03	0.073
Nutrient composition					
Dry matter (%)	77.30±0.02	78.26±0.01	79.90±0.02	81.90±0.01	0.070
Crude protein (%)	79.73±0.50^{c}	79.78±0.31^{c}	80.61±0.20^{b}	82.70±0.33^{a}	0.001
Crude fat (%)	82.20±0.33	83.60±0.33	84.32±0.23	85.45±0.23	0.078
AME (Kcal kg^{-1})	2721.7±0.39^{b}	2799.2±0.18^{b}	2796.2±0.40^{b}	2888.2±0.41^{a}	0.000
Essential amino acid (%)					
Arginine	82±0.24	84±0.11	84±0.13	85±0.17	0.066
Histidine	77±0.37	77±0.31	77±0.32	78±0.15	0.001
Leucine	85±0.24^{b}	86±0.14^{b}	88±0.21^{a}	89±0.23^{a}	0.000
Lysine	81±0.24^{c}	85±0.14^{a}	84±0.27^{b}	86±0.21^{a}	0.016
Methionine	81 ±0.17^{c}	83±0.21^{b}	84 ±0.15^{b}	85±0.23^{a}	0.014
Phenylalanine	84±0.24^{c}	86±0.13^{b}	87±0.17^{a}	88±0.37^{a}	0.012
Threonine	70±0.12^{b}	74±0.13^{b}	74±0.11^{b}	76±0.27^{a}	0.013
Valine	80±0.12^{c}	83±0.14^{b}	83±0.34^{b}	85±0.21^{a}	0.011
Non-essential amino acid (%)					
Alanine	85±0.32^{c}	87±0.12^{b}	88±0.37^{b}	89±0.15^{a}	0.012
Aspartic acid	80 ±0.33^{c}	84±0.31^{b}	83±0.15^{b}	85±0.13^{a}	0.010
Glutamine	84±0.37^{c}	88±0.15^{b}	87±0.24^{b}	89±0.13^{a}	0.011
Glycine	73±0.31^{c}	77±0.37^{b}	77±0.24^{b}	79 ±0.39^{a}	0.011
Serine	78±0.36^{c}	81±0.21^{b}	81±0.25^{b}	83±0.35^{a}	0.013
Tyrosine	76±0.32^{b}	80±0.11^{a}	81±0.36^{a}	82±0.27^{a}	0.014

Means in rows having different superscripts are significantly different. PCV, packed cell volume; Hb, hemoglobin; RBC, red blood cells; WBC, white blood cells.

and binds with starch directly or indirectly through starch granule-associated protein thus improving the protein digestibility and apparent metabolizable energy. The AME values were significantly higher in the sorghum-based diet compared to the maize-based diet when supplemented with enzymes and this effect can be attributed to longer feed retention time, which is evidenced by a longer transit time when sorghum-based diets are used (Mateos *et al*., 1982). In addition, sorghum may have lower starch digestion coefficients than maize, and feedstuffs with low starch digestion tend to stay longer in the small intestine of birds (Weurding *et al*., 2001). Ileal amino acid digestibility was improved by the supplementation of exogenous enzymes, phytase, protease, and their combination. The digestibility of some essential amino acids like leucine, lysine, methionine, phenylalanine, threonine, and valine was significantly improved by the supplementation of the exogenous enzyme. The maximum increase in the digestibility of these amino acids was observed in the group supplemented with a combination of protease and phytase enzymes compare with the individual use and control group. Complete replacement of sorghum may compromise the bird's performance, nutrient utilization, and amino acid digestibility. However, enzymes could be the capability to restore the compromised bird performance and increase the availability of nutrients (Wu *et al*., 2017). The exogenous enzymes such as phytase and protease when added in combination can further enhance the nutrient availability of birds.

CONCLUSION

The current study found that adding dietary enzymes could improve the nutrient digestibility of a sorghum-based diet in the broiler. Furthermore, supplementation of exogenous enzymes in a sorghum-based diet has a synergistic effect on nutrient digestion when used in combination.

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IRB approval

The experimental work was approved by the Board of Studies meeting (No. 458/PS/UAP dated 04/01/2021), The University of Agriculture Peshawar, KP, Pakistan.

Ethical statement

Before the start of the experiment, approval was taken from the Ethical Review Committee of the Faculty of Animal Husbandry and Veterinary Sciences, The University of Agriculture, Peshawar, Pakistan, and all the measures and tools were considered to minimize the pain and discomfort of birds during the conduction of this experiment.

Statement of conflict of interest

The authors have declared no conflict of interest.

REFERENCES

Adejumo, I.O., Bryson, B., Olojede, O.C., Bedford, M.R. and Adedokun, S.A., 2021. Effect of sodium sources and exogenous phytase supplementation on growth performance, nutrient digestibility, and digesta pH of 21-day-old broilers. *Poult. Sci*., **100**: 101467. https://doi.org/10.1016/j.psj.2021.101467

AOAC, 2000. *Methods of analysis of AOAC*. International. AOAC International, Maryland, USA (2003).

AOAC, Official Method, 2005. Official methods of analysis of AOAC International, 2.

Bhuiyan, M.M., Islam, A.F. and Iji, P.A., 2013. High levels of maize in broiler diets with or without microbial enzyme supplementation. *S. Afr. J. Anim. Sci*., **43**: 44-55. https://doi.org/10.4314/sajas.v43i1.5

Choct, M., 2015. Feed non-starch polysaccharides for monogastric animals: Classification and function. *Anim. Prod. Sci*., **55**: 1360–1366. https://doi.org/10.1071/AN15276

Farahat, M.H., Abdel-Razik, W.M., Hassanein, E.I. and Noll, S.L., 2013. Effect of phytase supplementation to diets varying in chloride level on performance, litter moisture, footpad score, and gait score of growing turkeys. *Poult. Sci*., **92**: 1837–1847. https://doi.org/10.3382/ps.2012-02869

Gidado, A.S., Oguntoye, M.A. and Akintunde, A.R., 2020. Growth performance, nutrient digestibility and serum biochemical indices of finisher broiler chickens fed varying levels of sorghum in replacement of maize with the supplementation of exogenous enzymes. *Niger. J. Anim. Sci*., **22**: 81-89.

Letourneau, M.P., Narcy, A., Lescoat, P., Magnin, M., Bernier, J.F., Sauvant, D., Jondreville, C. and Pomar, C., 2011. Modeling the fate of dietary phosphorus in the digestive tract of growing pigs. *J. Anim. Sci*., **89**: 3596–3611. https://doi.org/10.2527/jas.2010-3397

Li, A., Jia, S., Yobi, A., Ge, Z., Sato, S.J., Zhang, C. and Holding, D.R., 2018. Editing of an alpha-kafirin gene family increases, digestibility and protein quality in sorghum. *Pl. Physiol*., **177**: 1425-1438. https://doi.org/10.1104/pp.18.00200

Mateos, G.G., Sell, J.L. and Eastwood, J.A., 1982. Rate of food passage (transit time) as influenced by level of supplemental fat. *Poult. Sci*., **61**: 94–100. https://doi.org/10.3382/ps.0610094

Morgan, N.K. and Choct, M., 2016. Cassava: Nutrient composition and nutritive value in poultry diets. *Anim. Nutr*., **2**: 253-261. https://doi.org/10.1016/j.aninu.2016.08.010

Pasquali, G.A.M., Fascina, V.B., Silva, A.L., Aoyagi, M.M., Muro, E.M., Serpa, P.G., Berto, D.A. and Sartori, J.R., 2017. Maize replacement with sorghum and a combination of protease, xylanase, and phytase on performance, nutrient utilization, litter moisture, and digestive organ size in broiler chicken. *Can. J. Anim. Sci*., **97**: 328–337. https://

doi.org/10.1139/CJAS-2016-0133

Pos, J., Enting, H. and Veldman, A., 2003. *Effect of phytase and dietary calcium level on litter quality and broiler performance*. In: Proceedings of 14th European symposium of poultry nutrition. pp. 10-14.

Ritchie, B.W., Harrison, G.J. and Harrison, L.R., 1994. *Avian medicine principles and application*. Wingers Publishing Incorporation, Lake Worth, Florida, U.S.A. pp. 176-198.

Rostagno, H.S., Albino, L.F.T., Donzele, D.L., Gomes, P.C., Oliveira, R.F., Lopes, D.C., Ferreira, A.S., Barreto S.L.T. and Euclides, R.F., 2011. *Brazilian tables for poultry and swine: Feed composition and nutritional requirements*. 3rd ed. Universidade Federal de Viçosa, Viçosa, MG, Brazil. pp. 119.

Sakomura, N.K., Bianchi, M.D., Pizauro, J.M., Café, M.B. and Freitas, 2004. Effect of age on enzyme activity and nutrients digestibility for broilers fed soybean meal and full fat soybean. *Braz. J. Anim. Sci*., **33**: 924–935. https://doi.org/10.1590/S1516-35982004000400013

Selle, P.H., Cadagan, D.J., Li, X. and Bryden, W.L., 2010. Implications of sorghum in broiler chicken nutrition. *Anim. Feed Sci. Technol*., **156**: 57-74. https://doi.org/10.1016/j.anifeedsci.2010.01.004

Shuaib, M., Hafeez, A., Chand, N. and Tahir, M., 2022. Effect of dietary inclusion of soybean hull on production performance and nutrient digestibility during peak egg production period with different phases in laying hens. *Pakistan J. Zool*., **55**: 397-405. https://doi.org/10.17582/journal.pjz/20211105091115

Steel, R.G.D., 1997. Analysis of variance II: multiway classifications. In: *Principles and procedures of statistics: A biometrical approach*. pp. 204-252.

Tancharoenrat, P., Ravindran, V. and Ravindran, G., 2014. Influence of cereal type and fat source on the performance and fat utilization of broiler starters. *Anim. Prod. Sci*., **55**: 74–79. https://doi.org/10.1071/AN13375

Thomas, D.V. and Ravindran, V., 2008. Effect of cereal type on the performance, gastrointestinal tract development and intestinal morphology of the newly hatched broiler chick. *J. Poult. Sci*., **45**: 46–50. https://doi.org/10.2141/jpsa.45.46

Ullah, Z., Ahmed, M.G., Nisa, U. and Sarwar, M., 2016. Standardized ileal amino acid digestibility of commonly used feed ingredients in growing broilers. *Asian Australas. J. Anim. Sci*., **29**: 1322-1330. https://doi.org/10.5713/ajas.15.0703

Wang, Z., Li, J., Lai, C., Wang, R.Y., Chen, X. and Lian, Y., 2018. Drying tendency dominating the global grain production area. *Glob. Fd. Secur*., **16**: 138-149. https://doi.org/10.1016/j.gfs.2018.02.001

Weurding, R.E., Veldman, A., Veen, W.A., Van der Aar, P.J. and Verstegen, M.W., 2001. Starch digestion rate in the small intestine of broiler chickens differs among feedstuffs. *J. Nutr*., **131**: 2329–2335. https://doi.org/10.1093/jn/131.9.2329

Williams, C.H., David, D.J. and Iismaa, O., 1962. The determination of chromic oxide in faeces samples by atomic absorption spectrophotometry. *J. agric. Sci*., **59**: 381-385. https://doi.org/10.1017/S002185960001546X

Woyengo, T.A. and Nyachoti, C.M., 2013. Review: Anti-nutritional effects of phytic acid in diets for pigs and poultry current knowledge and directions for future research. *Can. J. Anim. Sci*., **93**: 9-21. https://doi.org/10.4141/cjas2012-017

Wu, D., Wu, S.B., Choct, M. and Swick, R.A., 2017. Performance, intestinal microflora, and amino acid digestibility altered by exogenous enzymes in broilers fed wheat- or sorghum-based diets. *J. Anim. Sci*., **95**: 740–751. https://doi.org/10.2527/jas.2016.0411

Zhu, H.L., Hu, L.L., Hou, Y.Q., Zhang, J. and Ding, B.Y., 2014. The effects of enzyme supplementation on performance and digestive parameters of broilers fed corn–soybean diets. *Poult. Sci*., **93**: 1704–1712. https://doi.org/10.3382/ps.2013-03626