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Effects of Phytase and Tannase Enzyme Supplementation on *in Vitro* Nutrient Digestibility and Tannin Degradation of Sorghum-Based Broiler Diets

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ABSTRACT: An *in vitro* study was conducted to assess the effects of phytase and tannase enzyme supplementation on nutrient digestibility and tannin degradability in sorghum-based broiler diets. A two-stage *in vitro* experiment involving simulation of gastric and ileal digestion *in vivo* was used. Data were analysed using Statistical Analysis System (SAS, 2011). The data were analysed for descriptive statistics and Chi-square tests were performed to check for possible associations among variables. Analysis of variance were performed to determine the effects of sorghum inclusion level as well as the effects of exogenous phytase and tannase supplementation on nutrients digestibility and tannin degradability using the GLM procedure. All tests were done at $p<0.05$ level of significance. The results of this study indicated that phytase and tannase supplementation significantly influenced calcium, phosphorus, fat and crude protein digestibility ($p< 0.01$). Sorghum inclusion level had no significant effect on calcium, phosphorus, fat and crude protein digestibility. Sorghum inclusion level significantly influenced tannin degradability ($p<0.01$). Chi-square test showed that there was an association between sorghum inclusion level and tannin hence increasing sorghum level increased tannin degradability. Both enzyme supplementation and sorghum inclusion level had significant effect on crude fibre and tannin degradability.

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KEYWORDS: Degradability, digestibility, nutrient, phytase, tannase, tannin.

INTRODUCTION

Sorghum has a good nutritional composition and is better adapted to semi-arid areas (Fracasso *et al*., 2016) compared to maize, the main energy source used in broiler ration formulation. However, its use is low because of anti-nutritional factors such as phytate and tannins (Legodimo and Madibela, 2013) which are poorly digested and bind about 60 – 90% of plant phosphorus rendering it unavailable for animal use. Additionally, phytate bind minerals such as calcium, zinc, iron and magnesium, amino acids and proteins forming strong insoluble complexes which are indigestible in the gastro-intestinal tract (Dersjant-Lia et al., 2018). Tannins on the other hand bind to proteins including digestive enzymes. Thus, these sorghum ANFs reduce the bioavailability of key minerals, amino acids and protein.

The use of microbial feed enzymes such as phytase is common in poultry diet formulation to facilitate the degradation of phytate. The efficacy of this enzyme is somewhat inconsistent between studies (Selle and Ravindran, 2007). Several factors are responsible for these inconsistencies including enzyme biochemistry and its microbial source, feed composition, substrate availability and gut physiology (Abd El Tawab and Khattab, 2018). However, the use of tannase in feed formulation remains unexplored. To date, studies emphasize the use of tannase in gallic acid production while its application in feed formulation is mainly presented as reviews with little attention paid to the practical research work. The current study explores the effects of use of phytase and tannase enzymes in sorghum-based broiler diets on *in vitro* nutrient digestibility and tannin degradation.

MATERIALS AND METHOD

Experimental diets

Maize, sorghum and soybean meal were milled and the dry matter (DM), gross energy (GE), CP, ash, fat, calcium and phosphorus composition analysed using the Association of Official Analytical Chemists standard procedure (AOAC, 2000). Condensed tannins content was quantified using the method of Folin-Denis (Pratik *et al*., 2016). Formulation of diets was done according to the recommendations of using maxi-packs by Capital Foods (Pvt ltd) and balancing of nutrients was done using the IDT Feed Formulation software® through substitution by weight of maize with sorghum at five levels (0, 25, 50, 75 and 100%). Four enzyme inclusion levels (none, 5000 FTU phytase, 25TU tannase as well as a combination of 5000FTU phytase and 25TU tannase) at each sorghum inclusion level were used. A total of 60 diets were formulated comprising of twenty starter, twenty grower and twenty finisher diets. Diets were formulated as per broiler nutrient recommendations (NRC, 1994). The formulated diets were analysed for DM, CP, GE, fat, calcium and phosphorus using the AOAC procedures (AOAC, 2000) and the analysed nutrients composition are as shown (Tables 1.1-1.3).

The in vitro experiment

A 2-step *in vitro* procedure involving simulation of stomach and ileal digestion (Boisen and Fernandez, 1995) was used followed in the current study.

Simulation of stomach digestion

For each of the experimental diets formulated, a total of 3.00g were accurately weighed using a Mettler scale, AE 100^{TM}. The weighed samples were transferred into 100ml conical flasks. To each flask, 25 ml phosphate buffer (0.1 mol/L, pH 6.0) was added and the mixture stirred for 1 min using a magnetic stirrer. The pH of the mixture was adjusted to 2.0 by adding hydrochloric acid (1 mol/L) and equilibrated at 37°C. A total of 1.5mg pepsin (pig pepsin, 3000 Sigma Units, Cat. No. 01152) and 0.5 ml chloramphenicol ethanol solution were added to the mixture. These were left to be digested in a water bath at 39 °C for 2 h. The mixture was slowly and continuously stirred during digestion to simulate feed digestion in the stomach.

Simulation of ileal digestion

After 2h of simulated stomach digestion, 10 ml of phosphate buffer (0.2 mol/L, pH 6.8) was added to the flasks. The pH was adjusted to 6.8 by adding sodium hydroxide (1 mol/L). A magnetic stirrer and 4mg of pancreatin (pig pancreatin, 3000 Sigma Units, Cat. No.1133B) were added to the mixture and allowed to digest for 6h in a water bath at 39 °C. The mixture was again slowly and constantly stirred to simulate feed digestion in the small intestine *in vivo*. After the 6h of incubation, 10% trichloroacetic acid was added to stop the reaction and the mixture were left for 2 hours. The digesta were filtered through 0.45μm filter membrane to allow for separate collection of supernatants and the residues. The residues were oven dried to a constant weight.

Calculations

The oven dried samples were accurately weighed and dry matter content of each sample determined using the formula: $\textit{Dry matter content} = 100 - [\frac{\textit{Residue weight} X 100}{\textit{Sample weight}}]$ *(Biagi et al., 2016)*

The digesta were analysed for the determination of protein, phosphorus, calcium, fat, energy and condensed tannins content according to the AOAC standard procedures (AOAC, 2000). Nutrients and tannins digestibility were calculated using the formula:

$$\%\ \textit{Nutrient digestibility} = \left(\frac{\textit{Nutrient in residue}}{\textit{Nutrient in feed sample}}\right) X\ 100$$

Table 1 Ingredient proportion and proximate composition of starter diet

Ingredient(g/kg)	0%				25%				50%				75%				100%			
Maize	560	560	560	560	429	429	429	429	289	286	286	282	144	144	144	142	0.00	0.00	0.00	0.00
Sorghum	0.00	0.00	0.00	0.00	149	144	144	144	289	286	286	282	429	429	429	426	585	585	585	580
SBM	400	395	395	390	382	382	382	377	382	383	383	386	387	382	382	382	375	370	370	370
LSF	12	12	12	12	12	12	12	12	12	12	12	12	12	12	12	12	12	12	12	12
Maxipack	28	28	28	28	28	28	28	28	28	28	28	28	28	28	28	28	28	28	28	28
Phytase	0.00	5.00	0.00	5.00	0.00	5.00	0.00	5.00	0.00	5.00	0.00	5.00	0.00	5.00	0.00	5.00	0.00	5.00	0.00	5.00
Tannase	0.00	0.00	5.00	5.00	0.00	0.00	5.00	5.00	0.00	0.00	5.00	5.00	0.00	0.00	5.00	5.00	0.00	0.00	5.00	5.00
Total	1000	1000	1000	1000	1000	1000	1000	1000	1000	1000	1000	1000	1000	1000	1000	1000	1000	1000	1000	1000
Proximate composition (%)																				
DM	92.9	92.9	92.9	92.9	93.1	93.1	93.1	93.1	93.0	93.0	93.0	93.0	92.4	92.4	92.4	92.4	91.8	91.8	91.8	91.8
CP	22.0	22.0	22.0	22.0	22.0	22.0	22.0	22.0	22.0	22.0	22.0	22.0	22.2	22.2	22.2	22.2	22.0	22.0	22.0	22.0
Fat (EE)	2.20	2.20	2.20	2.20	2.08	2.08	2.08	2.08	1.99	1.99	1.99	1.99	1.84	1.84	1.84	1.84	2.14	2.14	2.14	2.14
CF	2.73	2.73	2.73	2.73	2.80	2.80	2.80	2.80	3.88	3.88	3.88	3.88	3.98	3.98	3.98	3.98	4.21	4.21	4.21	4.21
Ca	0.88	0.88	0.88	0.88	0.88	0.88	0.88	0.88	0.90	0.90	0.90	0.90	0.90	0.90	0.90	0.90	0.90	0.90	0.90	0.90
P	0.46	0.46	0.46	0.46	0.46	0.46	0.46	0.46	0.46	0.46	0.46	0.46	0.46	0.46	0.46	0.46	0.46	0.46	0.46	0.46
CT	0.02	0.02	0.02	0.02	0.31	0.31	0.31	0.31	0.65	0.65	0.65	0.65	0.83	0.83	0.83	0.83	1.02	1.02	1.02	1.02
GE(MJ/Kg)	15.7	15.7	15.7	15.7	16.3	16.3	16.3	16.3	15.7	15.7	15.7	15.7	15.9	15.9	15.9	15.9	15.7	15.7	15.7	15.7

Table 2: Nutritional composition of ingredients and proximate composition of grower diets

Ingredient(g/kg)	**0%**				**25%**				**50%**				**75%**				**100%**			
Maize	644	644	644	640	485	483	483	480	324	322	322	320	164	161	161	160	0.00	0.00	0.00	0.00
Sorghum	0.00	0.00	0.00	0.00	164	161	161	160	324	322	322	320	485	483	483	480	644	644	644	640
SBM	316	311	311	310	311	311	311	310	312	311	311	310	311	311	311	310	316	311	311	310
LSF	16	16	16	16	16	16	16	16	16	16	16	16	16	16	16	16	16	16	16	16
Maxipack	24	24	24	24	24	24	24	24	24	24	24	24	24	24	24	24	24	24	24	24
Phytase	0.00	5.00	0.00	5.00	0.00	5.00	0.00	5.00	0.00	5.00	0.00	5.00	0.00	5.00	0.00	5.00	0.00	5.00	0.00	5.00
Tannase	0.00	0.00	5.00	5.00	0.00	0.00	5.00	5.00	0.00	0.00	5.00	5.00	0.00	0.00	5.00	5.00	0.00	0.00	5.00	5.00
Total	1000	1000	1000	1000	1000	1000	1000	1000	1000	1000	1000	1000	1000	1000	1000	1000	1000	1000	1000	1000
Proximate composition (%)																				
DM	93.0	93.0	93.0	93.0	94.3	94.3	94.3	94.3	92.5	92.5	92.5	92.5	93.6	93.6	93.6	93.6	92.9	92.9	92.9	92.9
CP	19.0	19.0	19.0	19.0	19.3	19.3	19.3	19.3	19.5	19.5	19.5	19.5	19.3	19.3	19.3	19.3	20.0	20.0	20.0	20.0
Fat (EE)	2.81	2.81	2.81	2.81	2.68	2.68	2.68	2.68	2.39	2.39	2.39	2.39	2.25	2.25	2.25	2.25	2.12	2.12	2.12	2.12
CF	3.61	3.61	3.61	3.61	3.68	3.68	3.68	3.68	4.04	4.04	4.04	4.04	4.12	4.12	4.12	4.12	4.39	4.39	4.39	4.39
Ca	0.85	0.85	0.85	0.85	0.85	0.85	0.85	0.85	0.85	0.85	0.85	0.85	0.84	0.84	0.84	0.84	0.84	0.84	0.84	0.84
P	0.42	0.42	0.42	0.42	0.42	0.42	0.42	0.42	0.42	0.42	0.42	0.42	0.41	0.41	0.41	0.41	0.41	0.41	0.41	0.41
CT	0.02	0.02	0.02	0.02	0.57	0.57	0.57	0.57	0.69	0.69	0.69	0.69	0.94	0.94	0.94	0.94	1.11	1.11	1.11	1.11
GE(MJ/Kg)	17.4	17.4	17.4	17.4	16.9	16.9	16.9	16.9	17.1	17.1	17.1	17.1	17.2	17.2	17.2	17.2	17.1	17.1	17.1	17.1

Table 3: Nutritional composition of ingredients and proximate composition of finisher diets

Ingredient(g/kg)	**0%**				**25%**				**50%**				**75%**				**100%**			
Maize	700	700	700	697	539	537	537	535	361	358	358	356	182	179	179	177	0.00	0.00	0.00	0.00
Sorghum	0.00	0.00	0.00	0.00	182	179	179	178	360	358	358	357	539	537	537	535	721	716	716	711
SBM	264	259	259	257	243	243	243	241	243	243	243	241	243	243	243	242	243	243	243	243
LSF	12	12	12	12	12	12	12	12	12	12	12	12	12	12	12	12	12	12	12	12
Maxipack	24	24	24	24	24	24	24	24	24	24	24	24	24	24	24	24	24	24	24	24
Phytase	0.00	5.00	0.00	5.00	0.00	5.00	0.00	5.00	0.00	5.00	0.00	5.00	0.00	5.00	0.00	5.00	0.00	5.00	0.00	5.00
Tannase	0.00	0.00	5.00	5.00	0.00	0.00	5.00	5.00	0.00	0.00	5.00	5.00	0.00	0.00	5.00	5.00	0.00	0.00	5.00	5.00
Total	1000	1000	1000	1000	1000	1000	1000	1000	1000	1000	1000	1000	1000	1000	1000	1000	1000	1000	1000	1000
Proximate composition (%)																				
DM	93.0	93.0	93.0	93.0	94.0	94.0	94.0	94.0	95.4	95.4	95.4	95.4	93.7	93.7	93.7	93.7	94.3	94.3	94.3	94.3
CP	17.17	17.17	17.17	17.17	17.5	17.5	17.5	17.5	17.0	17.0	17.0	17.0	17.3	17.3	17.3	17.3	17.8	17.8	17.8	17.8
Fat (EE)	2.32	2.32	2.32	2.32	2.40	2.40	2.40	2.40	2.42	2.42	2.42	2.42	2.25	2.25	2.25	2.25	2.13	2.13	2.13	2.13
CF	3.56	3.56	3.56	3.56	3.91	3.91	3.91	3.91	3.50	3.50	3.50	3.50	3.70	3.70	3.70	3.70	4.05	4.05	4.05	4.05
Ca	0.77	0.77	0.77	0.77	0.77	0.77	0.77	0.77	0.76	0.76	0.76	0.76	0.76	0.76	0.76	0.76	0.76	0.76	0.76	0.76
P	0.37	0.37	0.37	0.37	0.37	0.37	0.37	0.37	0.38	0.38	0.38	0.38	0.37	0.37	0.37	0.37	0.37	0.37	0.37	0.37
CT	0.02	0.02	0.02	0.02	0.59	0.59	0.59	0.59	0.73	0.73	0.73	0.73	1.27	1.27	1.27	1.27	1.38	1.38	1.38	1.38
GE(MJ/Kg)	17.77	17.77	17.77	17.77	17.6	17.6	17.6	17.6	17.8	17.8	17.8	17.8	17.4	17.4	17.4	17.4	17.6	17.6	17.6	17.6

Data analysis

Data were analysed using Statistical Analysis System (SAS, 2011). The data were analysed for descriptive statistics and Chi-square tests were performed to check for possible associations among variables. Analysis of variance were performed to determine the effects of sorghum inclusion level as well as the effects of exogenous phytase and tannase supplementation on nutrients digestibility and tannin degradability using the GLM procedure of SAS (2011). Least square means were calculated and in cases of significant differences being observed, post-hoc Tukey's test were performed to separate means. Analysis of variance were done at 5% level of significance ($p< 0.05$) and the following statistical model was used:

$Y_{ijkl} = \mu + E_i + S_j + IT_k + (ES)_{ij} + \varepsilon_{ijkl}$

Where:

Y_{ijkl} = response variable (% CP, Ca, P digestibility and tannins degradability)

μ = overall mean common to all observations

E_i = effect of the ith enzyme (none, phytase only, tannase only, phytase and tannase combination)

S_j = effect of the jth sorghum inclusion level (0, 25, 50, 75, 100% inclusion level)

IT_k = effect of the kth incubation time

$(ES)_{ij}$ = effect of interaction between ith enzyme and jth sorghum level

ε_{ijkl} = is the random error with mean 0 and variance σ^2

RESULTS

Phosphorus digestibility

Enzyme supplementation had a significant effect on phosphorus digestibility across all the 3 feed levels ($p < 0.05$). However, sorghum inclusion level had no significant effect on phosphorus digestibility ($p > 0.05$). The effect of enzyme supplementation, alone or in combination was also assessed. Phosphorus digestibility was significantly higher when phytase and tannase were used in combination for the grower diet only ($p < 0.05$) compared to when added as single enzymes. There was a significant association between sorghum inclusion level and phosphorus digestibility for the finisher feed level only ($\chi^2 = 196$ d f = 40 p = 0.05) such that increasing the level of sorghum in the finisher diet results in an increase in phosphorus digestibility. However, Chi-square test results indicated that there was no significant association between enzyme supplementation and phosphorus digestibility for the starter and grower feeds ($\chi^2 = 205$ d f = 51 p > 0.05).

Calcium digestibility

The results of this study indicated that enzyme supplementation had a significant effect on calcium digestibility across all the three feed levels (Table 1.4). Phytase and tannase enzyme combination showed higher calcium digestibility for the starter and grower diets compared to when used as single enzymes ($p < 0.05$). Sorghum inclusion level did not significantly affect calcium digestibility for the starter and finisher feed. However, there was a significant association between sorghum inclusion level and calcium digestibility for the grower diet ($\chi^2 = 40$ d f =35 p < 0.05). Chi-square test results showed that there was no significant association between enzyme supplementation and calcium digestibility ($\chi^2 = 52.3$ d f = 48 p > 0.05) across all the feeding levels.

Table 1.4*: The effect of phytase and tannase on *in vitro* calcium digestibility*

Feed type	Enzyme	Ca in feed	Ca in digesta	Digestibility
Starter	**No enzyme**	0.90 ± 0.011^a	0.89 ± 0.001^a	1.11 ± 11.453^a
	Phytase	0.90 ± 0.011^a	0.64 ± 0.132^b	28.17 ± 15.265^b
	Tannase	0.90 ± 0.011^a	0.88 ± 0.100^a	2.06 ± 11.43^a
	Phytase+Tannase	0.90 ± 0.011^a	0.55 ± 0.130^c	38.89 ± 15.186^c
Grower	**No enzyme**	0.84 ± 0.002^b	0.70 ± 0.136^a	0.71 ± 0.003^a
	Phytase	0.84 ± 0.002^b	0.61 ± 0.157^b	8.91 ± 0.003^b
	Tannase	0.84 ± 0.002^b	0.65 ± 0.118^b	8.80 ± 0.095^b
	Phytase+Tannase	0.84 ± 0.002^b	0.55 ± 0.125^c	19.60 ± 0.428^c
Finisher	**No enzyme**	0.76 ± 0.002^c	1.04 ± 0.119^a	-36.08 ± 15.987^a
	Phytase	0.76 ± 0.002^c	1.07 ± 0.099^b	-5.26 ± 12.643^b
	Tannase	0.76 ± 0.002^c	1.00 ± 0.030^a	-31.55 ± 3.600^a
	Phytase+Tannase	0.76 ± 0.002^c	0.95 ± 0.002^a	-25.00 ± 7.601^a

Ca means calcium, Dg means digestibility, least square means with different superscripts differ significantly ($P < 0.05$).

Crude protein (CP) digestibility

The effect of phytase and tannase enzyme supplementation as well as sorghum inclusion level on CP digestibility was evaluated. Phytase and tannase supplementation had a significant effect on crude protein digestibility ($p < 0.05$) as shown whereas sorghum inclusion level did not significantly affect crude protein digestibility across all the feed levels ($p > 0.05$).

Fat (Ether extract) digestibility

The mean fat content was 2.05 ± 0.289, 2.45 ±0.594 and 2.30 ± 0.0240 for the starter, grower and finisher diets respectively. Sorghum inclusion level had no significant effect on fat digestibility ($p > 0.05$). However, enzyme supplementation significantly affects fat digestibility ($P<0.0001$). Chi-square test results indicated that there was no significant association between enzyme supplementation and fat digestibility ($\chi^2 = 14$ d f =11 $p > 0.005$). Additionally, there was no significant association between sorghum inclusion level and fat digestibility ($\chi^2 =119$ d f = 4 $p > 0.05$).

Tannin degradability

The mean condensed tannin content of the diets was 0.571 ± 0.0830, 0.672 ± 0.0861 and 0.801 ± 0.113 for the starter, grower and finisher respectively. Enzyme supplementation had a significant effect on condensed tannins degradability ($p = 0.0001$) as shown (Table 1.5). Also, sorghum inclusion level had a significant effect on condensed tannin degradability ($p = 0.0001$). Chi-square test showed that there was a significant relationship between condensed tannin degradability and enzyme supplementation ($\chi^2 = 23$ d f = 4 $p < 0.05$). Additionally, there was a significant association between sorghum inclusion level and condensed tannins degradability ($\chi^2 = 23$ d ff= 4 $p = 0.001$).

Table 1.5: The effect of phytase and tannase (alone or in combination) on tannin digestibility

Feed type	Enzyme	CT in feed	CT in digesta	CT digestibility
Starter	**No enzyme**	0.57±0.180	0.51±0.002	31.73±0.499
	Phytase	0.57±0.180	0.50±0.001	32.93±0.736
	Tannase	0.57±0.180	0.37±0.001	50.53±0.326
	Phytase+Tannase	0.57±0.180	0.33±0.001	55.87±0.741
Grower	**No enzyme**	0.60±0.187	0.55±0.001	29.99±1.014
	Phytase	0.60±0.187	0.49±0.001	37.61±1.118
	Tannase	0.60±0.187	0.40±0.001	49.05±0.855
	Phytase+Tannase	0.60±0.187	0.37±0.001	52.85±0.955
Finisher	**No enzyme**	0.80±0.246	0.77±0.001	26.29±1.151
	Phytase	0.80±0.246	0.74±0.001	29.16±1.151
	Tannase	0.80±0.246	0.53±0.001	49.24±1.014
	Phytase+Tannase	0.80±0.246	0.50±0.001	52.10±1.111

DISCUSSION

The observation that supplementation of phytase in diets increased phosphorus digestibility is attributed to the fact that the enzyme catalyses the stepwise removal of phosphate from phytic acid and phytate yielding myo-inositol, phosphate, amino acids, minerals and other nutrients linked to phytate (Dersjant-Li et al., 2015). Phytase can hydrolyse phytic acid to increase accessibility of tannase to tannins (Woyengo and Nyachoti, 2011). The observation that phytase and tannase enzyme combination had higher phosphorus digestibility is explained by the fact that these enzymes could possibly act synergistically in improving phosphorus digestibility. However, the digestibility coefficients observed in the current study are lower than those obtained in a previous study (Dersjant-Li et al., 2021) who reported phosphorus digestibility of 72.3% at 1000FTU/kg phytase.

The calcium digestibility of maize-soyabean meal diets observed in the current study is comparable to previous observations for the starter diet only (Dersjant-Li et al., 2021). In their study, they observed calcium digestibility coefficients of 30.0, 34.5 and 38.8% for diets with no phytase, 500FTU/Kg phytase and 1000FTU/Kg phytase, respectively. However, current results are contrary to those cited by Allen et al (2014). In their study, the *in vitro* calcium digestibility of spinach, sweet potatoes and three Moringa leaves were 1.35 ± 0.21, 3.79 ± 0.23, 1.77 ± 1.04, 46.57 ± 8.34 and 37.69 ± 0.79% and these are relatively lower in contrast to the results of the current study. The differences are best explained by the fact in their study, Allen et al (2014) used leaves whereas grain samples were used for the purposes of the current study.

There are other factors which can possibly influence calcium digestibility. These include dietary calcium content, the particle size of calcium source and Ca: P ratio. These critically influence the efficacy of exogenous phytase in improving calcium digestibility (Angel et al., 2002). The addition of phytase results in reduced Ca-phytate complex formation by 75% and breaks phytate-phosphorus complexes hence improving calcium digestibility (Dersjant-Li et al., 2021). Particle size affects the hydrolysing action of phytase in that smaller calcium particles are more likely to form Ca-phytate complexes thus inhibiting the hydrolyzing actions of phytase on phytate (Manangi and Coon, 2007). In the current study, limestone flour was added to diets and chances are that such fine limestone is more soluble and may have hindered calcium digestibility.

The observation that addition of phytase in diets improve protein digestibility is consistent with previous results (Amerah et al., 2014). They observed an increase in amino acids digestibility in response to the addition of 1000 FTU/kg of Buttiauxella phytase. The *in vitro* protein digestibility determined in this study falls within the ranges of 40%–76% (Elkonin et al., 2013), 59–76 (Bhagyawant et al., 2018) and 74-79% (Bekele et al., 2021). However, the results are higher than those reported for Sudanese and Indian sorghum varieties of 6.82, 17.65 and 17.99% (Elnasikh et al., 2020). This could be a result of the differences in the pre-treatment procedures used. In their study, Elnasikh et al (2020) cooked the sorghum grains prior to the determination of crude protein digestibility compared to addition of exogenous phytase and tannase in the current study. The enzymes used in the current study could have degraded phytate-protein and tannin-protein complexes exposing more polypeptide bonds to proteolytic enzymes hence improving CP digestibility.

The effect of enzyme supplementation on ether extract digestibility in the current study is lower than those reported in previous studies. Abd El Tawab et al (2016) observed that supplementation of palm-fronts based diets with cellulase and tannase resulted in improvement from 72.2 ± 0.830 to 73.7 ± 0.325 ether extract digestibility. The previous observed digestibility is higher than those in the current study. This could be the effect of feed ingredient type which also influences level of substrate in the ingredients (Abd El Tawab and Khattab, 2018). In their study, Abd El Tawab et al (2016) used palm-fronts whereas maize/sorghum and soybean meal diets were used in the current study.

The observation that addition of enzymes had a significant effect on tannin content and nutrients digestibility is consistent with previous studies. The addition of tannase at 0.3% resulted in a reduction in tannin content by 1.8% in wheat straw diets (Raghuwanshi et al., 2014). Furthermore, Abd El Tawab et al (2016) suggested that adding tannase enzyme in dairy goat's diets resulted in a significant increase in nutrients digestibility. Increasing the level of sorghum in the diet tends to increase tannin digestibility such that the highest tannin degradability coefficient was observed when maize is completely replaced with sorghum. This is best explained by the effect of substrate on enzyme activity and rate of reaction. There is a linear relationship between substrate concentration and enzyme addition to a point where increasing the enzyme concentration does not improve digestibility because all the enzyme active sites are occupied (Vitolo, 2020).

The results of the current study showed that different sorghum inclusion levels had no significant effect on nutrients digestibility ($p > 0.05$). The results are contrary to previous observation. According to Farahat et al (2020), complete replacement of maize with sorghum led to a 5.25% decrease in crude protein digestibility coefficient *in vivo*. However, at 50% sorghum inclusion level, crude protein, dry matter and fat digestibility coefficients did not differ significantly (Farahat et al., 2020).

It was concluded that the inclusion of phytase and tannase; alone or in combination significantly improve phosphorus, calcium and crude protein digestibility as well as tannin degradability. Thus sorghum is a suitable energy source which can be used in broiler diets.

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