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Ileal Villi Morphological Characteristics of Cobb 500 Broilers Fed Phytase and Tannase Treated Sorghum Based Diets

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Abstract

The effects of phytase and tannase enzyme treatment of sorghum-based broiler diets on ileal villi characteristics were evaluated in Cobb 500 broilers. A total of three sorghum levels, 0, 50, and 100%, with 4 enzyme levels; 0, 5% phytase, 5% tannase and 5% phytase+tannase combination were used to develop 12 different dietary treatments. Three hundred and sixty broilers were randomly allocated to the 12 dietary treatments in a completely randomized design experiment. On day 42, two birds from each replicate were randomly selected and slaughtered for ileal villi morphometry analyses. A 2cm tissue sample of the ileum was cut and prepared for histological analyses. Villus height and width, muscularis externa thickness, and crypt depth were measured on a light microscope using a calibrated eyepiece graticule. The total villi surface area was calculated, which indicates the digestive and absorptive capacity of the ileum. The General Linear Models (GLM) procedure of the Statistical Analysis System ver 9.4 (SAS Institute Inc., 2011) was used to analyse the data. All tests were performed at $p < 0.05$ significance. Villus height, width, and muscularis externa thickness significantly increased with increasing levels of sorghum in the diet ($p<0.001$). Birds fed complete sorghum diets supplemented with phytase enzyme had the longest villi ($p<0.001$). The 0% and 100% sorghum levels exhibited comparable crypt depth. Treatment significantly affected the apparent villi surface area ($p<0.0001$). The apparent villi surface area increased with increasing sorghum inclusion. Birds fed a complete sorghum diet supplemented with phytase had the highest villi surface area (15.48±0.241 mm). It can be concluded that phytase and tannase can be added to complete sorghum broiler diets without compromising ileal villi integrity. Hence, we recommend the addition of phytase and tannase in sorghum-based broiler diets to counteract the effects of sorghum antinutrients.

Introduction

Chicken meat has become the world's most consumed meat type (OECD, 2021). This is attributed to the fact that there are no negative cultural or religious perceptions associated with chicken consumption (Barbut and Leishman, 2022) and it has remarkable nutritional benefits (Farrell, 2022). However, the high cost of feed because of the unavailability of traditional feed ingredients limits poultry production (Ntuli and Oladele, 2013; Amponsah et al., 2015). Of major concern is the unavailability of maize, a key energy source used in poultry diets. Overall, 61% of global maize is used as livestock feed; however, its production is reduced by increasing trends of heat stress, severe droughts, and changing agroecological conditions (Challinor et al., 2014). Maize deficit because of climate change elicited the need to evaluate other potential energy sources.

Sorghum is drought tolerant and has a low risk of failure under drought conditions compared with maize (Amelework et al., 2017; Widiyono et al., 2021; Ali et al., 2023) and can thus potentially substitute maize in poultry diets to rump up broiler feed production. The use of sorghum in broiler feeds remains low due to the presence of tannins and phytates, which reduce nutrient digestibility, absorption, and bioavailability (Rahman and Osman, 2011).

The secondary negative effects associated with phytic acid involve altering the digestion process and this may negatively affect the intestinal health and intestinal microbiota of broilers (Ptak et al., 2015; Moita et al., 2021), resulting in poor performance.

The benefits of phytase supplementation in reducing the effects of phytate are well documented (Amerah et al., 2014; Moita et al., 2021; Selle et al., 2023). Exogenous phytases liberate phytate-bound phosphorus and reduce the direct antinutritive properties of phytate or myo-inositol hexaphosphate (IP6) (Selle et al., 2023). The improved bioavailability of phosphorus and calcium may express different effects on intestinal health, morphology, and microbiota diversity of broiler chickens (Moita et al., 2021). The use of tannase to curb the effects of tannins in sorghum-based broiler feeds remains unexplored.Additionally, the effects of phytase and tannase enzyme combination on gastrointestinal tract organs and ileal villi morphology are poorly understood.

Morphology of the small intestine mucosa is an important indicator of intestinal health and integrity (Paiva et al., 2014) and hence has a significant effect on feed digestion, nutrients absorption, immune status (animal health), and animal performance at large (Kogut and Arsenault, 2016). The aim of the current study was to evaluate the effects of phytase and tannase supplementation alone or in combination on the morphology of the ileal villi of Cobb 500 broilers fed sorghum-based diets.

Materials and Methods

Animal ethics

All procedures followed in this study comply with national guidelines for the good care and management of research animals. The Zimbabwe's National Animal Research Ethics Committee approved the procedures outlined in this study and granted the animal ethics (Reference Number: 013/22).

Study site

The study was conducted at Henderson Research Station, Poultry Section in Mazowe district, Zimbabwe. It is located in the agroecological region IIb characterized by an annual rainfall range of 750-1000 mm (Mavhura et al., 2021). The latitude and longitude for the area are 17.35^{o}E and 300.58^{o}S, respectively, while the altitude is 1300m. The temperature range for this area is between 15 and 29^{o}C. The area is suitable for all farming systems, including dairy, piggery, horticulture, poultry, beef, and crop production (Mavhura et al., 2021).

Experimental design and diets

All the ingredients were milled and analysed for dry matter, gross energy, crude protein, ash, fat, calcium, and phosphorus composition according to the Association of Official Analytical Chemists standards (AOAC, 1995). Condensed tannins in sorghum were quantified using the method of Folin-Denis (Pratik et al., 2016). Formulation was done using the IDT Feed Formulation software® through substitution by weight of maize with sorghum at three levels (0, 50 and 100%). There were four enzyme inclusion levels (none, 5% Phytase, 5% Tannase as well as a combination of 5% phytase and 5% tannase) at each sorghum inclusion level. A total of 12 diets were developed comprising a starter, grower, and finishing diets.

Diets were developed to meet the recommended nutrient levels for broilers (NRC, 1994). The diets were analysed for dry matter, crude protein, gross energy, fat, calcium, and phosphorus using the AOAC procedures (AOAC, 1995) and the analysed nutrients composition are shown in Tables 1-3. Diets 1 to 4 are complete maize-based diets with different enzyme treatments (1 has no enzyme, 2 has 5% phytase, 3 has 5% tannase and 4 has 5% of both phase and tannase). Additionally, diets 5 to 8 are described by 50% substitution of maize with sorghum on weight bases and have different enzyme levels (5 has no enzyme, 6 has 5% phytase, 7 has 5% tannase and 8 has 5% of both phase and tannase). Diets 9 to 12 are complete sorghum-based diets with variations in enzyme levels; thus, 9 has no enzyme, 10 has 5% phytase, 11 has 5% tannase, and 12 has 5% of both phase and tannase.

A total of 360 unsexed Cobb 500-day-old chicks were randomly allocated to thirty-six 1 m × 2 m pens. The experiment followed a completely randomized design experiment with 12 dietary treatments replicated three times. A total of ten birds were placed in each pen. A 10 cm thick layer of dry grass was placed on the floor as bedding in the pens. Heat and lighting were provided using 75 W infra-red lamps. The starter, grower, and finisher diets were fed from day 1 to 14, day 15 to 28, and day 29 to 42, respectively. Feed and water were offered *ad libitum* throughout the feeding trial. Chicks were offered vitamins C and E as well as biotin (Stress pac®, Irvine's Zimbabwe) in drinking water on arrival to combat the stress experienced during transportation. A foot bath drenched with disinfectant (Virukill®, Veterinary distributors, Pvt Ltd, Zimbabwe) was placed at the entrance to the brooding house. Mortality was recorded during the entire experimental period.

Data collection

On day 42, seventy-two birds from each dietary treatment comprise 2 birds/replicate were randomly selected for ileal villi morphology analyses. Birds were fasted overnight to limit intestinal throughput. The birds were humanely slaughtered using the neck dislocation method, carcasses were scalded in hot water at about 60°C for approximately 63 s, and the feathers were plucked manually.

Table 1. Ingredients and chemical composition of starter diets

Ingredient (gkg^{-1})	The percentage inclusion level of sorghum											
	0%				50%				100%			
Diet	1	2	3	4	5	6	7	8	9	10	11	12
Maize meal	560	560	560	555	289	286	286	282	0	0	0	0
Sorghum meal	0	0	0	0	289	286	286	282	585	585	585	580
Soya meal	400	395	395	395	382	383	383	386	375	370	370	370
Limestone flour	12	12	12	12	12	12	12	12	12	12	12	12
Broiler maxipack	28	28	28	28	28	28	28	28	28	28	28	28
Phytase	0	5	0	5	0	5	0	5	0	5	0	5
Tannase	0	0	5	5	0	0	5	5	0	0	5	5
Total	1000	1000	1000	1000	1000	1000	1000	1000	1000	1000	1000	1000
	Proximate Composition (%)											
DM	92.9	92.9	92.9	92.9	93.0	93.0	93.0	93.0	91.8	91.8	91.8	91.8
CP	22.0	21.9	22.0	22.0	22.0	22.0	22.0	22.0	22.0	22.0	22.0	22.0
Fat (EE)	2.20	2.20	2.20	2.20	1.99	1.99	1.99	1.99	2.14	2.14	2.14	2.14
CF	2.73	2.73	2.73	2.73	3.88	3.88	3.88	3.88	4.21	4.21	4.21	4.21
Ca	0.88	0.88	0.88	0.88	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
CT	0.02	0.02	0.02	0.02	0.65	0.65	0.65	0.65	1.02	1.02	1.02	1.02
GE(MJ/Kg)	15.7	15.7	15.7	15.7	15.7	15.7	15.7	15.7	15.7	15.7	15.7	15.7

Table 2. Total mesophile aerobic microbial population of poultry feeds

Ingredient (gkg^{-1})	The percentage inclusion level of sorghum											
	0%				50%				100%			
Diet	1	2	3	4	5	6	7	8	9	10	11	12
Maize meal	644	644	644	640	324	322	322	320	0	0	0	0
Sorghum meal	0	0	0	0	324	322	322	320	644	644	644	640
Soya meal	316	311	311	310	312	311	311	310	316	311	311	310
Limestone flour	16	16	16	16	16	16	16	16	16	16	16	16
Broiler maxipack	24	24	24	24	24	24	24	24	24	24	24	24
Phytase	0	5	0	5	0	5	0	5	0	5	0	5
Tannase	0	0	5	5	0	0	5	5	0	0	5	5
Total	1000	1000	1000	1000	1000	1000	1000	1000	1000	1000	1000	1000
	Proximate Composition (%)											
DM	93.0	93.0	93.0	93.0	92.5	92.5	92.5	92.5	92.9	92.9	92.9	92.9
CP	19.0	19.0	19.0	19.0	19.5	19.5	19.5	19.5	20.0	20.0	20.0	20.0
Fat (EE)	2.81	2.81	2.81	2.81	2.39	2.39	2.39	2.39	2.12	2.12	2.12	2.12
CF	3.61	3.61	3.61	3.61	4.04	4.04	4.04	4.04	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	0.84	0.84	0.84	0.84
P	0.42	0.42	0.42	0.42	0.42	0.42	0.42	0.42	0.41	0.41	0.41	0.41
CT	0.02	0.02	0.02	0.02	0.69	0.69	0.69	0.69	1.11	1.11	1.11	1.11
GE(MJ/Kg)	17.4	17.4	17.4	17.4	17.1	17.1	17.1	17.1	17.1	17.1	17.1	17.1

Table 3. Ingredients and chemical composition of finisher diets

	The percentage inclusion level of sorghum											
	0%				50%				100%			
Diet	1	2	3	4	5	6	7	8	9	10	11	12
Maize meal	700	700	700	697	361	358	358	356	0	0	0	0
Sorghum meal	0	0	0	0	360	358	358	357	721	716	716	711
Soya meal	264	259	259	257	243	243	243	241	243	243	243	243
Limestone flour	12	12	12	12	12	12	12	12	12	12	12	12
Broiler maxipack	24	24	24	24	24	24	24	24	24	24	24	24
Phytase	0	5	0	5	0	5	0	5	0	5	0	5
Tannase	0	0	5	5	0	0	5	5	0	0	5	5
Total	1000	1000	1000	1000	1000	1000	1000	1000	1000	1000	1000	1000
	Proximate composition %											
DM	93.0	93.0	93.0	93.0	95.4	95.4	95.4	95.4	94.3	94.3	94.3	94.3
CP	17.17	17.17	17.17	17.17	17.0	17.0	17.0	17.0	17.8	17.8	17.8	17.8
Fat (EE)	2.32	2.32	2.32	2.32	2.42	2.42	2.42	2.42	2.13	2.13	2.13	2.13
CF	3.56	3.56	3.56	3.56	3.50	3.50	3.50	3.50	4.05	4.05	4.05	4.05
Ca	0.77	0.77	0.77	0.77	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	0.38	0.38	0.38	0.38	0.37	0.37	0.37	0.37
CT	0.02	0.02	0.02	0.02	0.73	0.73	0.73	0.73	1.38	1.38	1.38	1.38
GE(MJ/Kg)	17.77	17.77	17.77	17.77	17.8	17.8	17.8	17.8	17.6	17.6	17.6	17.6

	The percentage inclusion level of sorghum											
	0%				50%				100%			
Diet	1	2	3	4	5	6	7	8	9	10	11	12
maize meal	700	700	700	697	361	358	358	356	0	0	0	0
Sorghum meal	0	0	0	0	360	358	358	357	721	716	716	711
Soya meal	264	259	259	257	243	243	243	241	243	243	243	243
Limestone flour	12	12	12	12	12	12	12	12	12	12	12	12
Broiler maxipack	24	24	24	24	24	24	24	24	24	24	24	24
Phytase	0	5	0	5	0	5	0	5	0	5	0	5
Tannase	0	0	5	5	0	0	5	5	0	0	5	5
Total	1000	1000	1000	1000	1000	1000	1000	1000	1000	1000	1000	1000
	Proximate composition %											
DM	93.0	93.0	93.0	93.0	95.4	95.4	95.4	95.4	94.3	94.3	94.3	94.3
CP	17.17	17.17	17.17	17.17	17.0	17.0	17.0	17.0	17.8	17.8	17.8	17.8
Fat (EE)	2.32	2.32	2.32	2.32	2.42	2.42	2.42	2.42	2.13	2.13	2.13	2.13
CF	3.56	3.56	3.56	3.56	3.50	3.50	3.50	3.50	4.05	4.05	4.05	4.05
Ca	0.77	0.77	0.77	0.77	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	0.38	0.38	0.38	0.38	0.37	0.37	0.37	0.37
CT	0.02	0.02	0.02	0.02	0.73	0.73	0.73	0.73	1.38	1.38	1.38	1.38
GE(MJ/Kg)	17.77	17.77	17.77	17.77	17.8	17.8	17.8	17.8	17.6	17.6	17.6	17.6

Note: DM=Dry Matter, CP= Crude Protein, EE= Ether Extract, CF= Crude Fibre, Ca= Calcium, P= Phosphorus, CT= Condensed Tannins, GE= Gross Energy

The ileum was separated from the rest of the segments at the Meckel's diverticulum to ileocecal-colonic junction (Incharoen et al., 2010). A 2-cm - long ileal (1m proximal to the ileocecal junction) segment sample was collected, placed in a sterile plastic container, and was cleaned twice with saline solution (1% NaCL) to remove intestinal digesta. The samples were fixed in 10% formalin, dehydrated using ethyl alcohol, embedded in paraffin wax, and cut into 4-m thick sections using a microtome (Brudnicki et al., 2017). The tissue samples were stained in eosin and hematoxylin on a glass slide and examined under a Trinocular Research Microscope Model B-5127 of 2015, India at × 40 magnifications. Digital images were captured using IS capture model S300 that was on computer fitted to the microscope. Villi height and width, crypt depth, and muscularis externa thickness was measured according to the specifications of Nain et al. (2012). Villi absorptive surface area was calculated as follows: (Nain et al., 2012).

Total villi surface area= Average villus width × villus height

Statistical analyses

The data were tested for normality using the Shapiro-Wilk test and log10-transformed wherever necessary. The General Linear Models (GLM) procedure of the Statistical Analysis System ver 9.4 (SAS Institute Inc., 2011) was used to analyse the data. The following model was used:

$$Y_{ijkl} = \mu + T_i + + \varepsilon_{ijk}$$

Where: Y_{ijkl} = response variable (villi height, villi width, crypt depth, muscularis externa thickness and apparent villi surface area)

μ = general mean common to all observations

T_i = effect of the ith dietary treatment (0, 50 and 100% sorghum level with no enzyme or phytase only or tannase only or phytase plus tannase enzyme combination)

ε_{ijk} = random error term

Comparison of means was done using Tukey's test. All tests were performed at p< 0.05 significance.

Results

The significance of treatment on the villi parameters studied are shown in Table 4. Treatment had a significant effect (p<0.0001) on villi height. Villus height significantly increased (p<0.001) with increasing levels of sorghum in the diet (Table 5). Birds fed 100% sorghum supplemented with phytase had the tallest villi, which was not significantly different from the 100% diet supplemented with phytase and tannase enzyme combination (Table 5). The 0% sorghum diet supplemented with phytase only recorded the shortest villi, and this was not significantly different (P > 0.05) from the 0% sorghum diet supplemented with tannase and the control diet (Figure 1). Birds fed a 0% sorghum diet supplemented with a phytase and tannase enzyme combination resulted in significantly taller villi compared with those fed 0% sorghum with (without) enzyme supplementation (Table 5).

The 50% sorghum inclusion diets exhibited moderately taller villi, shorter than those from the 100% sorghum diets, and taller than the 0% sorghum diets. There were no significant differences in villi height for the 50% sorghum diets with or without enzyme supplementation (Table 5).

Apical and basal villi widths significantly increased with increasing sorghum inclusion in the diet (p<0.0001). The 100% sorghum without enzyme diet resulted in the widest apical villi (Table 6). There were no significant differences in apical villi width for 100% sorghum supplemented with either phytase or tannase as single enzymes or used in combination (Table 6). The narrowest villi were recorded in birds fed 0% sorghum diet supplemented with a phytase and tannase enzyme combination. Basal villi width was also highest in birds fed the 100% sorghum diet, and no significant differences emanated from enzyme supplementation (Table 6). Again, the 0% sorghum diet supplemented with a phytase and tannase enzyme combination resulted in the narrowest villi width.

Treatment significantly affected the apparent villi surface area (p<0.0001). The inclusion of sorghum increased the apparent villi surface area. Broilers fed complete sorghum-based diets showed higher villi surface area. Birds fed a complete sorghum diet supplemented with phytase had the highest villi surface area (15.48 ± 0.241 mm). The birds fed 0% sorghum diets had lower villi surface area compared to the 50 and 100% sorghum diets (Figure 2). The least clear villi surface area was recorded in broilers fed 0% sorghum supplemented with a phytase and tannase enzyme combinations (5.63 ±0.241mm). Data were tested for normality using the Shapiro - Wilk test and all the collected data were normal (S>0.90 for villus height, apical villi width, basal villi width, crypt depth, muscularis externa, and clear villi surface area). The crypt depth was highest in birds fed the 50% sorghum level with no significant differences resulting from enzyme supplementation (Table 5). The 0% and 100% sorghum levels exhibited comparable crypt depth. However, the 100% sorghum diet supplemented with a phytase and tannase enzyme combination had the least crypt depth measurement.

Dietary treatment significantly influenced live body weight at slaughter and carcass weights (p< 0.05). Generally, the highest live body and carcass weights were observed in birds fed diet at 50% sorghum inclusion level with no enzyme supplementation and this was not significantly different from the weights recorded in broilers fed 50% sorghum diets with

Table 4. Effects of phytase and tannase inclusions in sorghum-based broiler diets on villi parameters

Parameter	Effect
	Treatment
Villus height	**
Villi surface area	*
Apical villi widths	**
Basal villi widths	**
Muscularis externas thickness	**

*P < 0.001; **P < 0.0001. All tests were performed at p < 0. 05 level of significance.

Table 5. Effect of sorghum level and phytase and tannase supplementation on ileal villi parameters

The ileal villus	Dietary treatment											
Parameter (mm)	1*	2*	3*	4*	5*	6*	7*	8*	9*	10*	11*	12*
VH	5.35^{ghi}	5.30^{ghi}	5.40^{ghi}	4.41^{f}	5.73^{e}	5.54^{eg}	5.74^{e}	5.76^{e}	6.43^{bcd}	6.71^{abd}	6.40^{bcd}	6.60^{abcd}
aVW	1.49^{h}	1.57^{h}	1.40^{h}	1.11^{k}	2.09^{bde}	2.00^{bdefg}	1.89^{efg}	1.86^{efg}	2.47^{a}	2.15^{bde}	2.15^{bde}	2.09^{bde}
bVW	1.47^{ef}	1.64^{ef}	1.64^{ef}	1.45^{f}	1.63^{ef}	2.15^{bcd}	2.07^{cd}	2.02^{cd}	2.30^{abc}	2.47^{ab}	2.34^{ab}	2.28^{bcd}
CD	1.80^{defg}	1.85^{defg}	1.70^{efg}	1.78^{defg}	2.12^{abcfg}	2.15^{abcf}	2.13^{abc}	2.12^{abc}	2.06^{bcd}	1.89^{cdef}	1.70^{efgh}	1.52^{h}
ME	1.17^{ghi}	1.22^{ghi}	1.26^{gh}	1.11^{hi}	1.13^{ghi}	1.80^{f}	1.97^{e}	2.13^{cd}	2.16^{cd}	2.47^{a}	2.30^{bc}	2.24^{bcd}
VSA**	7.92^{f}	8.48^{f}	8.20^{f}	5.63^{g}	10.67^{de}	11.48^{cd}	11.36^{cd}	11.15^{cde}	15.3^{a}	15.48^{a}	14.37^{b}	14.44^{b}

Note: Means in the same row with different superscripts differ significantly (P < 0.05). VH=villi height, Avw =apical villi width, b VW=basal villi width, CD=crypt depth, ME= muscularis externa thickness, VSA**= villi surface area in mm2
Description of treatments: 1 =100% maize with no enzyme, 2* =100% maize with 5% phytase, 3*= 100% maize with 5% tannase, 4*= 100% with 5% phytase and tannase combination, 5*=50% maize and 50% sorghum with no enzyme, 6*= 50% maize and 50% sorghum with 5% phytase, 7*= 50% maize and 50% sorghum with 5% tannase, 8*= 50% maize and 50% sorghum with 5% phytase and tannase combination, 9* =100% sorghum with no enzyme, 10* =100% sorghum with 5% phytase, 11*= 100% sorghum with 5% tannase, 12*= 100 sorghum with 5% phytase and tannase combination

Table 6. Effect of dietary treatment on live body and carcass weights on day 42

Dietary treatment	Live body weight	SEM	Carcass weight	SEM
1	2100.5^{abc}	63.50	761.5^{abcde}	6.43
2	1926.5^{cdef}	63.50	747.1^{cde}	6.43
3	1952.3^{cdef}	63.50	754.5^{abcde}	6.43
4	2096.8^{abcde}	63.50	757.0^{abcde}	6.43
5	2159.7^{abc}	63.50	767.9^{abcd}	6.43
6	2004.2^{abcdef}	63.50	758.4^{abcd}	6.43
7	1967.5^{bcdef}	63.50	756.4^{abcde}	6.43
8	2102.3^{abcd}	63.50	764.1^{abcde}	6.43
9	1950.5^{cdef}	63.50	747.1^{abcd}	6.43
10	1912.8^{cdef}	63.50	752.9^{bcde}	6.43
11	1931.2^{cdef}	63.50	743.6^{de}	6.43
12	1920.2^{abcdef}	63.50	742.6^{de}	6.43

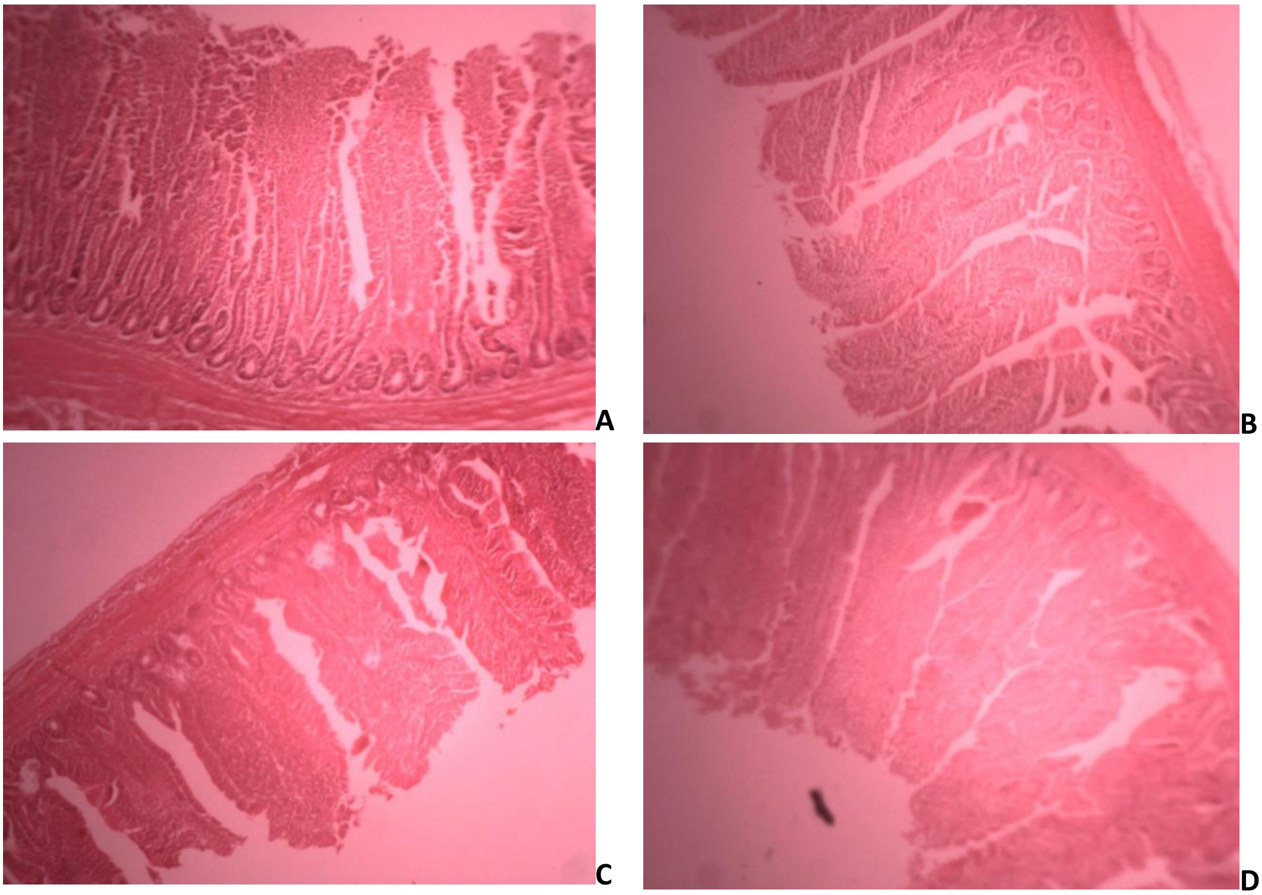

Figure 1. Ileal villi characteristics of Cobb 500 broilers fed: (A) 100% sorghum with phytase (B) 50% sorghum with phytase (C) 0% sorghum with phytase (D) 100% sorghum with phytase plus tannase enzyme combination at ×40 magnification

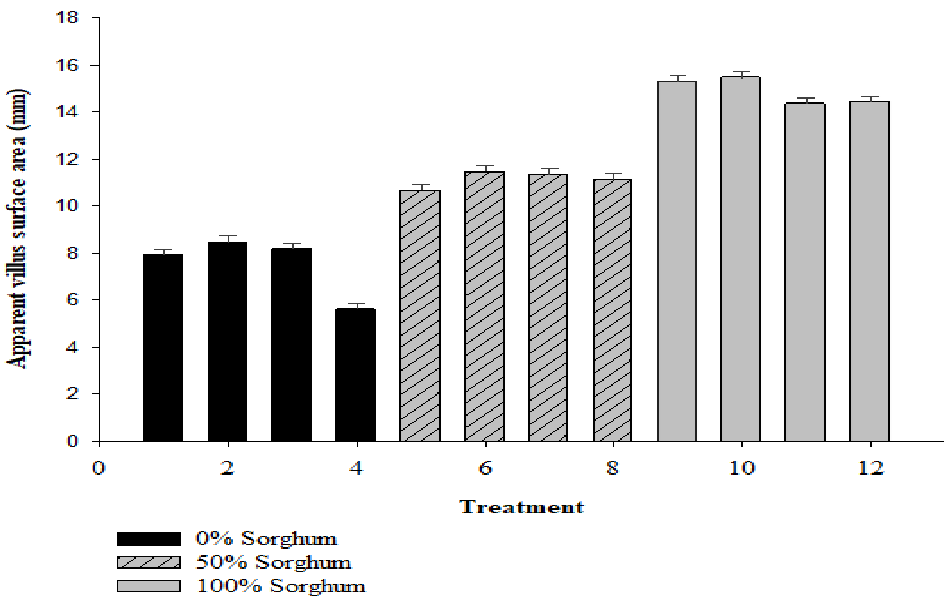

Figure 2. Effect of sorghum inclusion level and enzyme supplementation on apparent villus surface area

phytase only and with phytase and tannase enzyme combination as well as birds fed complete maize diets without enzyme, with phytase only and with phytase and tannase enzyme combination as shown in Table 6. The least live body and carcass weights were observed in broilers fed complete sorghum diets with a phytase and tannase enzyme combination.

Discussion

Generally, an increase in villi height is related to enhanced nutrient absorption, while deeper crypts are associated with higher tissue turnover rates (Choct, 2009; de Verdal et al., 2011). The results of the current study on villi height contradict previous results by Nyamambi et al. (2007). In their study, they observed that duodenal villus height and crypt depth were lowered with increasing tannin levels. Nyamambi et al. (2007) used Chirimaugute, Brown Tsweta, DC, and SV2 sorghum varieties with tannin contents of 1.68, 0.20, 2.48, and/g DM, respectively. They stipulated that day 7 and day 14 villi height of chicks fed 100% DC, Chirimaugute, and Brown Tsweta sorghum diets were lower than the villi height of chicks raised on total maize and 50% DC diets. Additionally, sorghum inclusion levels had no significant effect on villus height, width, and crypt depth of broilers at day 42 (Silva et al., 2015; Many*elo* et al., 2019). This is because the digesta arriving at the ileum had already been subjected to maximum enzyme activity and maximum absorption in the previous intestinal segments irrespective of the composition of feed consumed (Silva et al., 2015). Elongation of the villi with increasing sorghum level could be explained by the increase in tannins and their metabolites, which increase mitosis in the villi, implying increased proliferation (Jamroz et al., 2009; Brus et al., 2018). Tannins act as gut microbiota modulators (Tosi et al., 2013; Choi and Kim, 2020;) and their anti-oxidative traits enhance villus length (Buyse et al., 2022). The villi height obtained in this study was lower than that reported by Mutibvu (2016) in indigenous chickens. This can be attributed to the vitamin A supplemented in the diets, which enhances gut morphology (Kunisawa and Kiyono, 2013) as well as the indigenous chicken breed they used. The observation that broilers fed either complete maize or complete sorghum diets showed shallowest crypts is surprising given the fact that complete sorghum diets had higher levels of tannins and phytate which had negative effects on the intestinal epithelium. A shallower crypt depth indicates a decrease in the metabolic cost of intestinal epithelial renewal in the villi (Xu et al., 2022). On the other hand, deeper crypts promote rapid cell turnover, faster metabolism of tissue, and allow renewal of intestinal villi (Hamedi et al., 2011), resulting in rapid replacement of sloughed or inflamed villi (Jayaraman et al., 2013). The observation that completes substitution of maize by sorghum results in higher ileal villi surface area is contrary to previous findings (Silva et al., 2015).

They highlighted that sorghum level had no significant effect on the absorptive surface area in the jejunum; thus, the feed offered did not interfere with the absorption mechanisms. In their study, however, Silva et al. (2015) used a sorghum variety that contained 42.7 mg/kg equivalent to 0.00427% tannin and this is classified as a tannin free variety. It is possible that the impact of tannins in this variety on villi surface area could be negligible.

In a separate study, sorghum level had no effect on villus height and crypt depth in the small intestinal mucosa segments except for duodenal villus height, which was smaller in 7-d-old broilers fed the low-sorghum diet than in broilers fed the high-sorghum or control diets (Torres et al., 2013). Such a response is explained by the fact that during the initial stages of life, intestinal growth and differentiation occurs in the absence of exogenous feed nutrients and luminal and hormonal factors, thus the process is influenced by intrinsic factors (Drozdowski et al., 2010). However, Incharoen et al. (2010) highlighted that dietary nutrient composition influences intestinal development and mucosal architecture which subsequently influences digestive absorption and assimilation of digested nutrients. Shorter intestinal villi are related to a smaller number of absorptive cells, presence of toxic substances, and a larger number of secretory cells (Iwashita et al., 2003), whereas longer villi promote healthy digestion and high nutrients absorption efficiency (Itza-Ortiz et al., 2019). The current results showed villi widths higher than those reported by Ncube et al. (2017). This disparity could be a consequence of the protein source *Acacia angustissima*, which could have limited villi development in their study.

The observation that phytase and tannase enzyme supplementation had a significant effect on villi morphology is consistent with previous results. The addition of phytase maize-soyabean diet resulted in higher jejunal villi height (Pekel et al., 2017; Karami et al., 2020). The effects of phytase supplementation on villi width and crypt depth are inconsistent. The inclusion of phytase in broiler diets increased villi width (Karami et al., 2020; Moita et al., 2021). The effects of phytase or tannase enzyme combination in sorghum-based diets on intestinal villi morphology are still limited for comparison.

Although the total replacement of maize by sorghum-increased villi height and hence the absorptive capacity of the villi, complete sorghum diets negatively affected live body and carcass weights. The observation is consistent with previous results by Avila et al. (2009) reported that there was no positive association between villi height and nutrient absorption. However, this is surprising because longer villi provide higher absorptive area, better digestive enzyme activity and fast transport of nutrients (Wijtten et al., 2012) which translate to high live body weight.

Conclusion

It can be concluded that the broiler diet containing 50% sorghum with 5% phytase plus tannase enzyme combination can be fed to broilers without compromising ileal absorptive area, live body weight and carcass weight.

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