

Nutrient digestibility and intestinal morphology of broiler chickens fed graded levels of enzyme-supplemented sorghum as replacement for maize

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ABSTRACT

Aim/Background: This study evaluated the effects of enzyme-supplemented sorghum-diluted maize diets on nutrient digestibility and intestinal morphology of broiler chickens. Feed costs account for 60%–70% of total broiler production costs, and maize price volatility in Nigeria has driven interest in cost-effective alternatives. Sorghum is an alternative because of its similar energy profile and drought tolerance, but its anti-nutritional factors limit its direct use in poultry diets. Therefore, this study aimed to determine the optimal sorghum inclusion level when supplemented with a commercial enzyme blend.

Methods: The study was conducted in 2025 at the Poultry Unit of the Teaching and Research Farm, Federal University of Technology, Minna, Nigeria. Two-hundred-day-old Cobb 500 broiler chicks were randomly assigned to five dietary treatments in a completely randomized design with four replicates of ten birds each. The experimental diets consisted of 0%, 25%, 50%, 75%, and 100% sorghum replacing maize (T1–T5), formulated to meet nutrient requirements. All diets were supplemented with Birzyme Plus enzyme at 5 g per ton of feed. Nutrient digestibility was determined through total excreta collection during days 25 and 51. Two birds per replicate were housed in metabolic cages for 3 days of acclimatization, followed by 4 days of feeding, with 105 g of feed administered per bird daily. Excreta were collected, oven-dried, and analyzed for dry matter, crude protein, ether extract, crude fiber, and ash using AOAC methods. At day 56, two birds per replicate were sacrificed for gut morphology assessment. Data were analyzed using one-way Analysis of Variance with SPSS version 23.0, and treatment means were separated using Duncan Multiple Range Test at $p < 0.05$.

Results: T2 (25% sorghum) achieved the highest dry matter digestibility (17.38% and 17.18% for starter and finisher phases, respectively). Crude protein digestibility was highest in T4 and T3 (17.21% and 16.68%) during the starter phase. The 50% sorghum treatment recorded the highest small intestine weight (2.27%), large intestine weight (2.60%), and liver weight (1.19%). The 100% maize and 50% sorghum groups had the longest small intestines (153.00 cm). The 100% sorghum diet produced the longest absolute gastrointestinal tract (194.00 cm).

Conclusion: The study concluded that enzyme-supplemented sorghum at inclusion levels up to 50% optimizes nutrient digestibility and gut development in broiler chickens, providing a cost-effective alternative to maize in poultry production.

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Introduction

Poultry farming is crucial to global food security, with the poultry industry accounting for

approximately 40% of agricultural output in industrialized countries and 20% in developing nations like Nigeria [1,2]. Broiler chicken production

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dominates the poultry meat market, accounting for around 90% of total poultry production globally [3]. Despite its importance, the poultry industry faces rising challenges regarding feed procurement and costs, with feed expenses constituting approximately 60%–70% of total production costs [4].

Maize serves as a staple energy source in poultry diets, yet its price volatility and seasonal availability issues have negatively impacted feed costs. In 2016, Nigeria witnessed about a 100% increase in maize prices due to insurgency-related disruptions, flooding, and increased industrial demand [5]. This surge significantly heightened poultry feed costs, forcing farmers to ration feed, which detrimentally affected bird growth and production [5].

Given these challenges, sorghum has emerged as a promising substitute for maize due to its similar energy profile, lower cost, and greater resilience to drought conditions [6,7]. Sorghum is a drought-tolerant crop, particularly valuable in arid and semi-arid regions where water resources are limited [8]. However, sorghum utilization in poultry diets is hindered by antinutritional factors such as condensed tannins and phytates, which impair protein digestibility and mineral absorption, thereby compromising bird performance [9,10].

To counteract these limitations, enzyme supplementation targeting these antinutritional compounds has gained attention. Enzymes like phytase, xylanase, and proteases are used to boost nutrient availability from sorghum, improving feed conversion ratios and growth outcomes [11,12]. Phytase, for example, breaks down phytates, releasing phosphorus and other essential minerals, while xylanase improves the breakdown of non-starch polysaccharides, thus enhancing fiber digestibility [13,14].

Gut morphology is key to nutrient absorption, as the structural integrity of the intestine affects the surface area available for nutrient uptake. Previous studies have shown that enzyme supplementation can improve gut health by increasing villus height, crypt depth, and overall villus surface area, all of which contribute to better nutrient absorption [15]. However, there remains a key gap in research on the combined effects of multi-enzyme supplementation on both nutrient digestibility and intestinal morphology in broilers fed maize-sorghum diets. This study aimed to investigate the effects of enzyme-supplemented sorghum-based diets on nutrient digestibility and intestinal morphology of broiler chickens, contributing to the development of cost-effective and sustainable feeding strategies in the Nigerian poultry industry.

Materials and Methods

Experimental site

The experiment was conducted between April and July 2025 at the poultry unit of the Teaching and Research Farm, Department of Animal Production, School of Food and Agricultural Technology, Federal University of Technology, Minna, Nigeria. Minna is located at a latitude 9°37' North and a longitude 6°32' East, with an annual rainfall of approximately 1,312 mm. The temperature ranges between 20°C and 37°C, with relative humidity fluctuating from 21% to 73%. The area is characterized by Southern Guinea Savannah vegetation [16].

Experimental birds and management

Two hundred-day-old Cobb 500 broiler chicks were sourced from a reputable commercial hatchery and randomly assigned to five dietary treatment groups. The broilers were housed in a deep litter system with pen sizes measuring 1 square meter each. Wood shavings were used as litter material. Each pen was equipped with feeders and drinkers, providing ad libitum access to clean water and feed throughout the experimental period of 56 days. During the brooding phase, a heat source maintained optimal temperatures, starting at 32°C and gradually reducing according to age. Standard management and biosecurity protocols were strictly observed, including regular pen cleaning, use of footbaths at entry points, restricted access, and vaccination against common poultry diseases (Newcastle disease and Gumboro).

Experimental design

The experiment was arranged in a completely randomized design consisting of five dietary treatments, four replications, with 10 birds per replicate. The dietary treatments consisted of varying sorghum inclusion levels: Treatment 1 (T1), positive control group (100% maize); Treatment 2 (T2), 25% sorghum and 75% maize; Treatment 3 (T3), 50% sorghum and 50% maize; Treatment 4 (T4), 75% sorghum and 25% maize; Treatment 5 (T5), negative control group (100% sorghum). All diets were supplemented with enzymes. The sorghum used was a low-tannin variety and was decorticated to reduce tannin content.

Dietary treatment and feed formulation

Five experimental diets were formulated with varying maize-sorghum ratios as described above. All

diets were formulated to meet nutrient requirements according to NRC [17] recommendations. The experiment lasted for 56 days and was divided into two different growth phases: starter phase (1–28 days) and finisher phase (29–56 days). The ingredients and nutrient composition of experimental starter and finisher diets are presented in Tables 1 and 2.

Enzyme supplementation

Commercial enzyme preparation, Birzyme Plus, containing phytase, protease, cellulase, mannanase, alpha-amylase, and beta-glucanase, was used to improve nutrient utilization in the experimental diets. The enzyme blend was included at a recommended level of 5 g per ton of feed. Birzyme Plus was added to all treatments consistently during both the starter and finisher phases of the 56-day feeding trial.

Data collection

A nutrient digestibility trial was conducted during the starter phase (day 25) and finisher phase (day 51) of the study. A measured quantity of 105 g of feed was administered to each bird daily, and

excreta was collected per bird on a daily basis, and afterwards oven-dried for analysis purposes. Two birds were randomly selected per replicate and housed separately in metabolic cages fitted with feeders and drinkers. The birds were allowed to acclimatize for 3 days before commencing 4 days of feeding and excreta collection. A known weight of feed was administered to each bird, and excreta was collected per bird on a daily basis, and afterward oven-dried for analysis purposes. Proximate composition of the feed and dried excreta was analyzed for dry matter, crude protein, ether extract, crude fiber, and ash using standard methods described by AOAC [18]. Nutrient digestibility was calculated using the formula:

$$\text{Nutrient digestibility (\%)} = \frac{[(\text{Nutrient of feed} \times \text{feed intake}) - (\text{Nutrient of excreta} \times \text{excreta output})]}{(\text{Nutrient of feed} \times \text{feed intake})} \times 100.$$

At the end of the experiment (day 56), two birds per replicate were humanely sacrificed, and the gastrointestinal tract was collected. The weight and length of the crop, gizzard, proventriculus, small intestine, large intestine, heart, liver, lungs, spleen, and kidneys were recorded using a tape measure and digital scale to assess the effect of

Table 1. Ingredients and nutrient composition of experimental starter diets (1–28 days).

Ingredients (%)	T1 (0%)	T2 (25%)	T3 (50%)	T4 (75%)	T5 (100%)
Maize	52.50	39.38	26.25	13.12	0.00
Soybean meal	41.75	41.24	40.70	40.13	39.52
Sorghum	0.00	13.12	26.25	39.38	52.50
Soy oil	2.36	2.81	3.25	3.72	4.20
Di-calcium phosphate	1.80	1.90	2.06	2.19	2.41
Limestone	0.69	0.59	0.46	0.36	0.20
Vitamin-mineral premix	0.25	0.25	0.25	0.25	0.25
Lysine-HCl	0.24	0.28	0.33	0.37	0.42
DL Methionine	0.21	0.23	0.25	0.28	0.30
NaCl	0.20	0.20	0.20	0.20	0.20
Total	100.00	100.00	100.00	100.00	100.00
Calculated nutrients					
Energy (Kcal/Kg)	3,010.39	3,012.76	3,013.54	3,016.12	3,018.58
Crude protein (%)	23.04	23.09	23.13	23.16	23.16
Calcium (%)	1.00	1.00	1.01	1.02	1.04
Total Phosphorus (%)	0.73	0.74	0.76	0.77	0.80
Methionine (%)	0.57	0.57	0.57	0.58	0.59
Lysine (%)	1.45	1.45	1.46	1.46	1.47
Ether extract (%)	5.90	6.19	6.48	6.79	7.11
Fiber (%)	4.13	4.01	3.88	3.75	3.62

Table 2. Ingredients and nutrient composition of experimental finisher diets (29–56 days).

Ingredients (%)	T1 (0%)	T2 (25%)	T3 (50%)	T4 (75%)	T5 (100%)
Maize	60.17	45.13	30.09	15.04	0.00
Soybean meal	32.77	32.10	31.44	30.83	30.20
Sorghum	0.00	15.04	30.09	45.13	60.17
Soy oil	4.31	4.88	5.44	5.96	6.54
Di-calcium phosphate	1.50	1.67	1.83	1.96	2.05
Limestone	0.50	0.36	0.22	0.12	0.03
Vitamin-mineral premix	0.25	0.25	0.25	0.25	0.25
Lysine-HCl	0.15	0.20	0.25	0.29	0.33
DL Methionine	0.15	0.17	0.21	0.22	0.24
NaCl	0.20	0.20	0.20	0.20	0.20
Total	100.00	100.00	100.00	100.00	100.00
Calculated nutrients					
Energy (Kcal/Kg)	3,220.11	3,225.30	3,229.89	3,232.33	3,238.92
Crude protein (%)	19.77	19.79	19.82	19.86	19.91
Calcium (%)	0.81	0.82	0.82	0.83	0.83
Total Phosphorus (%)	0.64	0.65	0.68	0.69	0.70
Methionine (%)	0.47	0.47	0.48	0.48	0.48
Lysine (%)	1.16	1.16	1.16	1.16	1.16
Ether extract (%)	7.82	8.21	8.58	8.93	9.32
Fiber (%)	3.75	3.61	3.45	3.31	3.17

enzyme-supplemented sorghum-based diets on gut morphology. Relative organ weights were expressed as a percentage of live body weight, while relative organ lengths were expressed as cm per kg body weight.

Statistical analysis

Data collected were subjected to one-way analysis of variance in a completely randomized design using SPSS Version 23.0 software. Significant treatment means were separated using Duncan Multiple Range Test at the 0.05 level of significance.

Results

Nutrient digestibility at starter phase

Table 3 presents the nutrient digestibility values of broiler chickens fed enzyme-supplemented sorghum-diluted maize-soybean meal diets during the starter phase. There were significant differences among dietary treatments for all nutrient digestibility parameters measured. For dry matter digestibility, treatment T2 (25% sorghum) recorded the highest value of 17.38%, which was significantly different from all other treatments. This was

followed by T3 (50% sorghum) at 16.28%, T1 (control—100% maize) at 15.19%, T4 (75% sorghum) at 13.68%, and T5 (100% sorghum) with the lowest value of 12.88%.

Crude protein digestibility showed that treatments T4 and T3 recorded the highest values (17.21% and 16.68% respectively), which were not significantly different from each other. Treatment T2 recorded 12.78%, T5 had 12.10%, while the control treatment (T1) showed the lowest crude protein digestibility at 5.37%. This result indicates that enzyme supplementation particularly improved protein digestibility in diets with higher sorghum inclusion levels.

Nutrient digestibility at finisher phase

Table 4 shows the nutrient digestibility parameters during the finisher phase. The finisher phase results showed similar patterns to the starter phase, with significant differences among dietary treatments for all nutrient digestibility parameters. Dry matter digestibility showed a similar ranking, with T2 recording the highest value of 17.18%, followed by T3 (16.13%), T1 (15.08%), T4 (13.62%), and T5 with the lowest value of 12.68%. The consistency

in this pattern across both phases indicates that the combination of 25% sorghum with enzyme supplementation optimally balances grain composition with enzymatic activity for improved nutrient utilization.

Gut morphology

Table 5 presents the effects of enzyme-supplemented sorghum-diluted maize-soybean meal diets on the relative gut morphology of broiler chickens aged 56 days. The highest crop weight was recorded in the 100% sorghum diet (0.91%), significantly higher than the 100% maize diet (0.87%). The 25% sorghum group recorded the highest proventriculus weight (0.64%), significantly higher than the 100% sorghum group (0.49%). No significant differences were observed in gizzard weights across dietary treatments. The 50% sorghum group showed the highest small intestine weight (2.27%), significantly higher than the 75% sorghum group (1.55%). The 50% sorghum treatment also recorded the highest large intestine weight (2.60%), significantly higher than the 75% sorghum (1.28%) and 100% sorghum (1.41%) groups. The 50% sorghum group recorded the highest liver weight (1.19%), significantly higher than the 75% sorghum (0.67%) and 100% sorghum (0.73%) groups.

Table 6 shows the absolute and relative organ lengths of broiler chickens aged 56 days. The 100% sorghum diet resulted in the highest absolute GIT length (194.00 cm), significantly higher than the 75% sorghum (150.00 cm) and 25% sorghum (172.00 cm) groups. The 100% maize and 50% sorghum groups had the longest small intestines (153.00 cm), significantly longer than the 75% sorghum group (103.00 cm). The 50% sorghum group recorded the longest large intestine (67.00 cm), significantly longer than the 75% sorghum (47.00 cm) and 100% sorghum (59.00 cm) groups.

Discussion

Nutrient digestibility

Dry matter digestibility was highest in birds fed diets containing 25% sorghum and lowest in those on 100% sorghum. This demonstrates that moderate inclusion of sorghum improved feed utilization, likely due to the balanced nutrient composition achieved when maize and sorghum were combined. The decline observed at higher inclusion levels could be attributed to the increased presence of tannins and other anti-nutritional factors in sorghum, which reduce feed palatability and hinder nutrient breakdown [19]. These results are consistent with

Table 3. Nutrient digestibility of broiler chickens fed enzyme-supplemented sorghum diluted maize diets at starter phase.

Treatment	DM (%)	CP (%)	CF (%)	ASH (%)	FAT (%)
T1 (100% maize)	15.19 ± 0.14 ^c	5.37 ± 0.01 ^a	51.34 ± 1.07 ^d	21.40 ± 0.34 ^b	48.02 ± 0.87 ^c
T2 (25% sorghum)	17.38 ± 0.51 ^e	12.78 ± 0.68 ^b	52.93 ± 0.06 ^e	26.89 ± 0.40 ^d	48.13 ± 0.19 ^c
T3 (50% sorghum)	16.28 ± 0.21 ^d	16.68 ± 0.02 ^c	49.96 ± 0.16 ^c	13.12 ± 2.02 ^a	32.78 ± 3.50 ^b
T4 (75% sorghum)	13.68 ± 0.28 ^b	17.21 ± 0.02 ^c	47.04 ± 0.08 ^b	24.30 ± 0.92 ^c	37.36 ± 5.68 ^b
T5 (100% sorghum)	12.88 ± 0.46 ^a	12.10 ± 0.01 ^b	36.26 ± 0.06 ^a	21.54 ± 0.34 ^b	36.62 ± 2.17 ^b
<i>p</i> -value	<0.001	<0.001	<0.001	<0.001	<0.001

a–e Means within columns with different superscripts are significantly different ($p < 0.05$). DM = Dry Matter; CP = Crude Protein; CF = Crude Fiber; ASH = Ash content; FAT = Ether Extract.

Table 4. Nutrient digestibility of broiler chickens fed enzyme-supplemented sorghum diluted maize diets at finisher phase.

Treatment	DM (%)	CP (%)	CF (%)	ASH (%)	FAT (%)
T1 (100% maize)	15.08 ± 0.09 ^c	5.49 ± 0.21 ^a	51.07 ± 1.07 ^d	21.34 ± 0.34 ^b	48.02 ± 0.87 ^c
T2 (25% sorghum)	17.18 ± 0.33 ^e	10.59 ± 4.47 ^b	52.93 ± 0.06 ^e	26.89 ± 0.40 ^d	48.13 ± 0.19 ^c
T3 (50% sorghum)	16.13 ± 0.09 ^d	16.68 ± 0.02 ^c	49.96 ± 0.16 ^c	13.12 ± 2.02 ^a	32.78 ± 3.50 ^b
T4 (75% sorghum)	13.62 ± 0.14 ^b	17.21 ± 0.02 ^c	47.04 ± 0.08 ^b	24.30 ± 0.92 ^c	37.36 ± 5.68 ^b
T5 (100% sorghum)	12.68 ± 0.05 ^a	12.10 ± 0.01 ^b	36.26 ± 0.06 ^a	21.54 ± 0.34 ^b	36.62 ± 2.17 ^b
<i>p</i> -value	<0.001	<0.001	<0.001	<0.001	<0.001

a–e Means within columns bearing different superscripts are significantly different ($p < 0.05$). DM = Dry Matter; CP = Crude Protein; CF = Crude Fiber; ASH = Ash content; FAT = Ether Extract.

Table 5. Relative organ weights (% of live body weight) of broiler chickens fed enzyme-supplemented sorghum diluted maize diets at 56 days.

Parameters	T1 (0%)	T2 (25%)	T3 (50%)	T4 (75%)	T5 (100%)	SEM
Crop	0.87 ^b	0.98 ^a	0.85 ^b	0.89 ^b	0.91 ^b	0.015
Proventriculus	0.52 ^b	0.64 ^a	0.63 ^a	0.55 ^b	0.49 ^c	0.008
Gizzard	2.75 ^a	2.54 ^a	2.60 ^a	2.22 ^b	2.36 ^b	0.081
Small intestine	1.97 ^b	1.95 ^b	2.27 ^a	1.55 ^c	2.14 ^a	0.064
Large intestine	1.85 ^b	1.48 ^c	2.60 ^a	1.28 ^d	1.41 ^c	0.049
Heart	0.12 ^b	0.18 ^a	0.16 ^a	0.11 ^b	0.11 ^b	0.005
Liver	0.72 ^c	1.01 ^b	1.19 ^a	0.67 ^c	0.73 ^c	0.027
Kidney	0.18 ^b	0.24 ^a	0.16 ^b	0.17 ^b	0.17 ^b	0.006
Lung	0.18 ^a	0.18 ^a	0.16 ^a	0.11 ^b	0.17 ^a	0.005
Spleen	0.12	0.12	0.11	0.11	0.11	0.004

a-d Means within rows with different superscripts are significantly different ($p < 0.05$). SEM = Standard Error of Mean.

Table 6. Absolute and relative organ lengths of broiler chickens fed enzyme-supplemented sorghum diluted maize diets at 56 days.

Parameters	T1 (0%)	T2 (25%)	T3 (50%)	T4 (75%)	T5 (100%)	SEM
Absolute (cm)						
GIT	185.00 ^b	172.00 ^d	181.00 ^c	150.00 ^e	194.00 ^a	0.000
Small intestine	133.00 ^c	115.00 ^d	153.00 ^a	103.00 ^e	135.00 ^b	0.000
Large intestine	52.00 ^c	67.00 ^a	67.00 ^a	47.00 ^d	59.00 ^b	0.000
Relative (cm/kg BW)						
GIT	185.00 ^b	172.00 ^d	181.00 ^c	150.00 ^e	194.00 ^a	0.001
Small intestine	71.89 ^b	66.86 ^e	84.53 ^a	68.67 ^d	69.59 ^c	0.001
Large intestine	28.11 ^e	38.95 ^a	37.02 ^b	31.33 ^c	30.41 ^d	0.001

a–e Means within columns with different superscripts are significantly different ($p < 0.05$). GIT = Gastrointestinal Tract; BW = Body Weight; SEM = Standard Error of Mean.

a study, which reported that high-tannin sorghum reduces nutrient digestibility in broilers [20].

Crude protein digestibility improved with increasing sorghum inclusion up to 75%, after which it declined in the 100% sorghum treatment. The improvement at moderate inclusion levels suggests that enzymes facilitated better protein degradation and absorption. However, the decline at higher sorghum levels could be due to tannin-protein binding that reduces amino acid availability. This finding agrees with reports that observed reduced protein digestibility in broilers fed high-sorghum diets [21,22].

Crude fiber digestibility increased as sorghum inclusion rose to 25%, after which it declined. The improved fiber digestibility at moderate levels could be attributed to enhanced microbial and enzymatic breakdown of complex carbohydrates, while excessive fiber levels at 100% inclusion

likely limited fermentability and digestion. Fat digestibility was highest in birds fed 25% sorghum and lowest in those fed 50% sorghum. This indicates that moderate substitution improved lipid metabolism and energy availability. The decline at higher inclusion rates may be attributed to tannins interfering with lipid digestion and lipase enzyme activity [11].

Gut morphology

The significant increase in relative crop weight at 100% sorghum inclusion compared to 100% maize indicates adaptive enlargement, possibly due to higher fiber content or anti-nutritional factors in sorghum, even with enzyme supplementation. Similar trends in proventriculus and gizzard weights have been reported in sorghum-based diets, attributed to increased mechanical processing demands [23].

The increased relatively small and large intestine weights at 50% sorghum inclusion indicate enhanced intestinal development, potentially improving nutrient absorption surface area. This aligns with findings that moderate sorghum levels, when enzyme-supplemented, optimize gut hypertrophy without compromising efficiency [24]. Conversely, the reduced intestinal weights at 75% and 100% sorghum may reflect nutrient limitation or anti-nutritional effects overriding enzyme efficacy at high inclusion levels.

Liver and kidney weights were highest at intermediate sorghum levels, suggesting peak metabolic activity and detoxification demands. The consistency in crop, proventriculus, and gizzard lengths suggests these foregut segments are less responsive to dietary grain source when enzymes are included. The significantly longer small intestine in 100% maize and 50% sorghum groups supports optimal absorptive capacity under these regimens.

Enzyme supplementation significantly influenced gut morphology, likely through improved degradation of non-starch polysaccharides in sorghum. These ameliorated potential anti-nutritional effects, particularly at moderate inclusion levels (25%–50% sorghum), where intestinal development was enhanced. However, at higher sorghum levels ($\geq 75\%$), residual NSPs or tannins may have limited enzyme efficacy, leading to reduced organ weights and altered gut lengths. These morphological changes have implications for nutrient utilization, feed efficiency, and overall growth performance.

The study demonstrates that enzyme-supplemented sorghum can partially replace maize in broiler diets without compromising nutrient digestibility and gut morphology, with 25%–50% inclusion appearing most favorable. The optimal balance between enzyme efficacy and anti-nutritional factor concentration at these moderate inclusion levels resulted in superior nutrient utilization and gut development. The findings indicate that partial substitution of maize with enzyme-supplemented sorghum provides a cost-effective and sustainable alternative for broiler production, particularly in regions where maize availability is limited or expensive.

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