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Assessment of exogenous enzyme supplementation in maize-soybean meal based diets on nutrient utilization and performance of broiler chickens

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Abstract

The encapsulated nutrients within plant cell wall matrices of maize and soybean meal remain partially inaccessible to endogenous avian digestive enzymes, representing a significant source of nutritional inefficiency in conventional broiler feed formulations. This research assessed the effects of phytase, xylanase, and a multi-enzyme complex on nutrient utilization, growth performance, phosphorus retention, and gut health parameters in Arbor Acres broiler chickens fed maize-soybean meal based diets under tropical conditions at the Nairobi Academy of Veterinary and Animal Sciences, Nairobi, Kenya. Two hundred day-old chicks were randomly distributed to four dietary treatments with five replicates of 10 birds each in a completely randomized design. The treatments included an unsupplemented control diet, the control diet supplemented with microbial phytase at 500 FTU/kg, the control diet supplemented with xylanase at 1,500 U/kg, and the control diet supplemented with a multi-enzyme blend containing phytase, xylanase, protease, and amylase. All diets were formulated with reduced available phosphorus and metabolizable energy specifications to evaluate the matrix values of the enzyme products. Growth performance data were collected over a 42-day period, and nutrient retention assays were conducted during the final week. The multi-enzyme blend produced the highest final body weight of 2.28 kg compared to 1.98 kg for the unsupplemented control, with a feed conversion ratio of 1.58 versus 1.78. Phosphorus retention was dramatically improved from 42.8 percent in the control to 78.5 percent in the phytase group and 82.3 percent in the multi-enzyme group. Apparent ileal protein digestibility increased by 14.2 percentage points in the multi-enzyme treatment relative to the control. The radar plot analysis confirmed the multi-enzyme blend as the superior treatment across all measured performance and digestibility dimensions. These results demonstrate that exogenous enzyme supplementation, particularly as a multi-enzyme combination, substantially enhances the nutritive value of maize-soybean meal diets by releasing encapsulated nutrients and improving overall digestive efficiency in broiler chickens.

Keywords: Exogenous enzymes, phytase, xylanase, multi-enzyme blend, nutrient utilization, phosphorus retention, broiler performance, maize-soybean diets, feed efficiency, *Gallus gallus domesticus*

Introduction

The commercial application of exogenous enzymes in poultry feed formulation traces its origins to the early 1990s, when the first generation of thermostable microbial phytase products demonstrated the capacity to release a substantial proportion of phytate-bound phosphorus from plant-based feed ingredients, fundamentally transforming the approach to mineral nutrition in non-ruminant animal diets ^[1]. In the three decades since this pioneering development, the enzyme technology landscape has expanded dramatically to encompass a diverse portfolio of carbohydrase, protease, and lipase products designed to degrade specific anti-nutritional substrates and cell wall polysaccharides that limit the biological availability of nutrients in conventional cereal-oilseed meal diet formulations ^[2]. Despite this extensive technological evolution and the widespread commercial adoption of individual enzyme products, the strategic combination of complementary enzymes targeting multiple substrate barriers simultaneously remains an area of active investigation with considerable potential for further enhancing the nutritive value of poultry feeds ^[3]. Maize-soybean meal diets constitute the foundation of

commercial broiler feed formulations across tropical Africa, providing the principal sources of metabolizable energy and amino acids required for rapid muscle accretion and efficient feed conversion ^[4]. However, these plant-based ingredients contain significant quantities of anti-nutritional compounds and structural polysaccharides that resist degradation by the limited repertoire of endogenous digestive enzymes produced by the avian gastrointestinal system ^[5]. Phytate, the primary storage form of phosphorus in cereal grains and oilseed meals, chelates approximately 60 to 80 percent of total phosphorus into complexes that are virtually indigestible by monogastric animals lacking endogenous phytase activity, necessitating either the inclusion of expensive inorganic phosphorus supplements or the supplementation of exogenous phytase enzymes to achieve adequate phosphorus nutrition ^[6]. Similarly, the non-starch polysaccharides comprising the cell wall matrix of maize and soybean meal, including arabinoxylans, beta-glucans, and cellulose, physically encapsulate intracellular nutrients behind barriers that impede enzymatic access, increase digesta viscosity, and reduce the overall efficiency of nutrient extraction and absorption ^[7].

The rationale for multi-enzyme supplementation strategies is grounded in the understanding that the anti-nutritional barriers present in plant-based feed ingredients are chemically and structurally diverse, and therefore cannot be effectively addressed by any single enzyme activity acting in isolation [8]. Phytase specifically targets the phytate-mineral complex to release bound phosphorus, calcium, and trace minerals, but does not address the physical encapsulation of nutrients within cell wall polysaccharide matrices. Xylanase hydrolyzes the arabinoxylan backbone of cell wall structures, disrupting the physical barrier to nutrient access and reducing the viscosity-enhancing properties of soluble non-starch polysaccharides, but does not directly improve mineral availability from phytate complexes [9]. Protease supplements may enhance the digestibility of storage proteins and protein-phytate complexes that resist endogenous pepsin and trypsin activity, while amylase supplementation can improve starch digestibility in young chicks with immature pancreatic function [10]. The combination of these complementary enzyme activities in a single multi-enzyme product therefore offers the potential for synergistic improvements in nutrient utilization that exceed the sum of individual enzyme effects. The present research was designed to evaluate and compare the effects of phytase, xylanase, and a comprehensive multi-enzyme blend on growth performance, nutrient retention, phosphorus utilization, and gut health indicators in broiler chickens fed maize-soybean meal based diets formulated with reduced nutrient specifications to evaluate the nutritional matrix values attributable to enzyme supplementation. The investigation was conducted under tropical environmental conditions in Nairobi, Kenya, to generate region-specific data applicable to the East African poultry production sector.

Material and Methods

Material

This research was carried out at the Poultry Research Unit of the Nairobi Academy of Veterinary and Animal Sciences, Nairobi, Kenya, between April and June 2023. The experimental protocol received approval from the Academy's Animal Research Ethics Committee (Reference: NAVAS/AREC/2023/PP-022), and all procedures were conducted in compliance with Kenyan national regulations governing the use of animals in scientific research.

Two hundred day-old Arbor Acres Plus broiler chicks of mixed sex were obtained from a commercial hatchery in Nairobi. Chicks were individually weighed, leg-banded for identification, and randomly allocated to four dietary treatment groups in a completely randomized design with five replicates of 10 birds each. The enzyme products evaluated included a bacterial 6-phytase derived from *Citrobacter braakii* expressed in *Aspergillus oryzae* (minimum activity 5,000 FTU/g), an endo-1,4-beta-xylanase derived from *Trichoderma reesei* (minimum activity 100,000 U/g), and a proprietary multi-enzyme blend containing phytase, xylanase, protease, and alpha-amylase activities in a single granulated product.

Methods

The four experimental treatments were formulated as follows: Treatment 1 was the negative control diet without

enzyme supplementation, formulated with reduced available phosphorus (0.30% versus the standard 0.45%) and reduced metabolizable energy (2,900 versus 3,000 kcal/kg) to create a nutritional deficit that would reveal the matrix value of enzyme supplementation. Treatment 2 received the negative control diet supplemented with phytase at 500 FTU/kg of complete feed. Treatment 3 contained the negative control diet supplemented with xylanase at 1,500 U/kg of complete feed. Treatment 4 included the negative control diet supplemented with the multi-enzyme blend at the manufacturer's recommended inclusion rate. All diets were based on maize and soybean meal as the primary energy and protein sources respectively, with supplementary ingredients including limestone, dicalcium phosphate, soybean oil, salt, vitamin premix, mineral premix, DL-methionine, and L-lysine hydrochloride.

Birds were housed in an open-sided naturally ventilated poultry house with deep litter management using wood shavings. Pen dimensions were 1.8 m by 1.5 m. Brooding temperature was maintained at 33 degrees Celsius during the first week using gas brooders and gradually reduced to ambient conditions of 22 to 28 degrees Celsius by the end of the third week. A 23-hour light and 1-hour dark photoperiod was maintained. Feed and water were provided ad libitum throughout the 42-day experimental period. Standard vaccination protocols against Newcastle disease, infectious bursal disease, and infectious bronchitis were implemented.

Body weight was recorded individually at weekly intervals, and feed consumption was measured per replicate at weekly intervals. Feed conversion ratio was calculated as total feed consumed divided by total body weight gain. During days 38 to 42, a nutrient retention trial was conducted using the total excreta collection method. Excreta were collected twice daily, pooled by replicate, and immediately frozen at minus 20 degrees Celsius. Samples were subsequently freeze-dried, weighed, ground, and analyzed for dry matter, nitrogen, gross energy, calcium, and phosphorus content. Apparent nutrient retention coefficients were calculated using the standard formula. Phosphorus in feed and excreta was determined by the vanadomolybdate spectrophotometric method after wet ashing.

At day 42, two birds per replicate were selected at the mean body weight and humanely slaughtered for intestinal sampling. Ileal digesta were collected from the terminal two-thirds of the ileum, pooled by replicate, freeze-dried, and analyzed for amino acid composition by ion-exchange chromatography following acid hydrolysis. Apparent ileal amino acid digestibility coefficients were calculated using titanium dioxide (included at 0.3% in all diets during the final week) as an indigestible marker.

Data were analyzed using one-way ANOVA in R statistical software version 4.3.2. Significant treatment effects were further explored using Tukey's HSD test at $P < 0.05$. Orthogonal contrasts were used to compare the enzyme-supplemented treatments collectively against the unsupplemented control and to compare individual enzyme treatments against the multi-enzyme blend.

Results

All enzyme supplementation strategies significantly improved growth performance and nutrient utilization compared to the unsupplemented control diet, with the

multi-enzyme blend consistently producing the most pronounced improvements across all measured parameters. The multi-dimensional comparison of treatment effects on

key performance and digestibility indicators is presented in Figure 1.

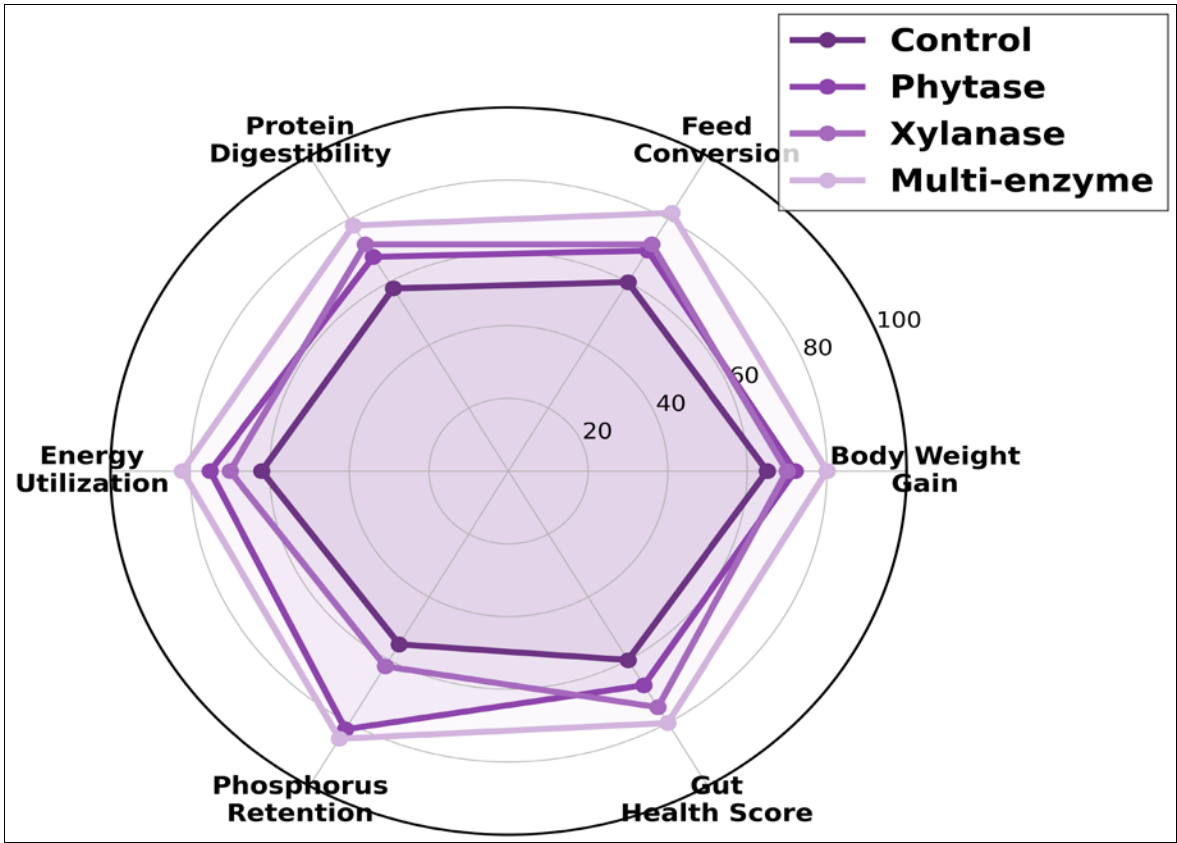


Fig 1: Radar plot comparing the relative performance of broiler chickens across six key parameters under different enzyme supplementation regimes. Values are expressed as percentages of the maximum observed value for each parameter. Higher values indicate superior performance.

The radar plot clearly illustrates the comprehensive superiority of the multi-enzyme blend across all six performance dimensions, with particularly notable advantages in phosphorus retention and protein digestibility.

Phytase supplementation showed its strongest relative advantage in phosphorus retention, while xylanase demonstrated its most pronounced effect on gut health score and feed conversion parameters.

Table 1: Growth performance of broiler chickens fed enzyme-supplemented maize-soybean meal diets (0-42 days)

Parameter	Control	Phytase	Xylanase	Multi-enzyme	SEM	P-value
Phase		(500 FTU/kg)	(1,500 U/kg)	Blend		
Initial BW (g)	42.5	42.3	42.4	42.6	0.30	0.918
Final BW (kg)	1.98 ^c	2.15 ^b	2.10 ^b	2.28 ^a	0.05	<0.001
BWG (kg)	1.94 ^c	2.11 ^b	2.06 ^b	2.24 ^a	0.05	<0.001
Feed intake (g)	4,020	4,085	4,050	4,120	65.2	0.182
FCR	1.78 ^a	1.68 ^b	1.70 ^b	1.58 ^c	0.03	<0.001
Mortality (%)	6.0	4.0	4.0	2.0	1.05	0.315

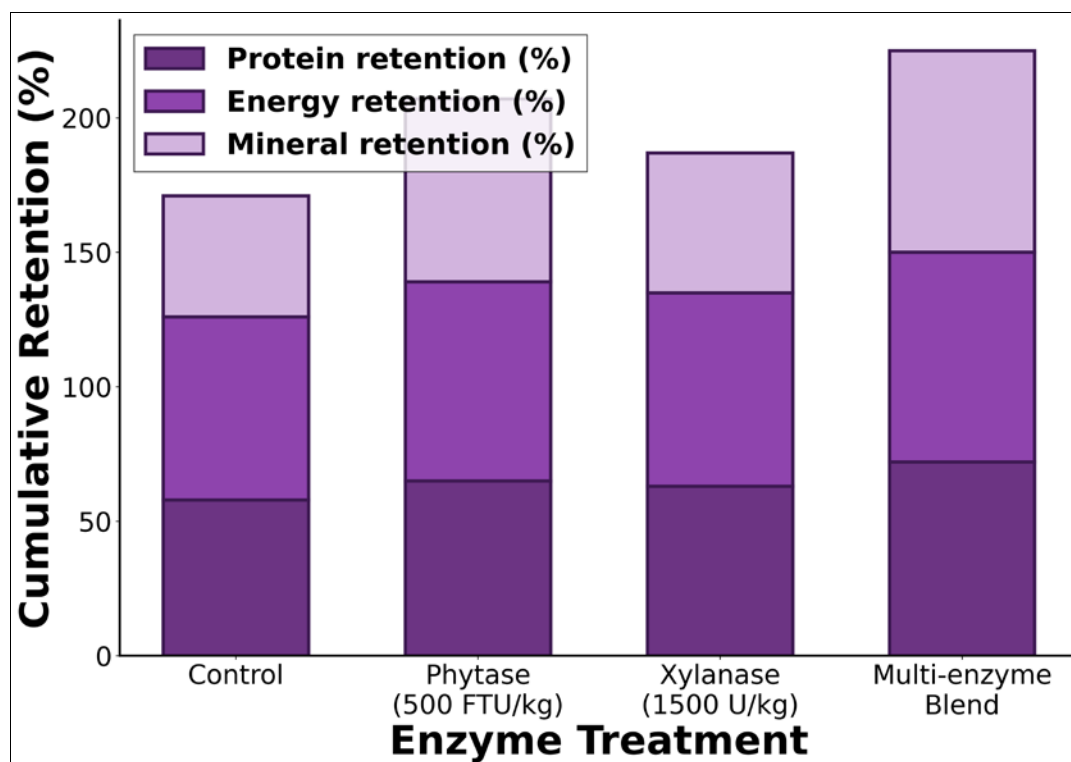


Fig 2: Cumulative nutrient retention percentages for protein, energy, and mineral fractions across enzyme supplementation treatments. Stacked components represent the individual retention coefficients for each nutrient category.

The detailed nutrient retention and phosphorus utilization data are presented in Table 2, which provides a comprehensive summary of the treatment effects on the

efficiency of nutrient extraction from the maize-soybean meal based diets.

Table 2: Apparent nutrient retention and phosphorus utilization in broiler chickens at 38-42 days of age

Parameter (%)	Control	Phytase	Xylanase	Multi-enzyme	SEM	P-value
DM retention	65.2 ^c	70.8 ^b	69.5 ^b	75.4 ^a	1.28	0.001
N retention	58.4 ^c	64.2 ^b	62.8 ^b	71.6 ^a	1.45	<0.001
GE retention	68.5 ^c	73.8 ^b	72.2 ^b	78.1 ^a	1.32	0.001
Ca retention	45.2 ^c	62.5 ^b	50.8 ^c	68.4 ^a	2.85	<0.001
P retention	42.8 ^c	78.5 ^b	48.2 ^c	82.3 ^a	3.12	<0.001
Ileal CP dig.	62.5 ^c	70.2 ^b	68.4 ^b	76.7 ^a	1.82	<0.001
Ileal Lys dig.	72.8 ^c	78.5 ^b	76.2 ^{bc}	84.1 ^a	1.65	<0.001
Ileal Met dig.	80.2 ^b	84.8 ^{ab}	83.5 ^{ab}	88.2 ^a	1.42	0.008

The extended interpretation of the combined performance and nutrient utilization data reveals several important patterns that inform the strategic application of enzyme technology in tropical broiler production systems. The 15.2 percent improvement in final body weight achieved by the multi-enzyme blend relative to the unsupplemented control demonstrates that the nutritional deficits created by the reduced nutrient specifications in the control diet were effectively compensated by the comprehensive enzyme package, confirming that the matrix values assigned to multi-enzyme products are nutritionally meaningful and practically achievable under tropical management conditions.

The phosphorus retention data provide particularly compelling evidence for the economic value of phytase supplementation, as the improvement from 42.8 percent in the control to 78.5 percent in the phytase group and 82.3 percent in the multi-enzyme group translates directly into reduced requirements for expensive inorganic phosphorus

supplements and simultaneously reduces phosphorus excretion in poultry litter by approximately 45 to 50 percent. This environmental benefit has increasing regulatory significance as phosphorus loading from intensive poultry operations becomes subject to stricter environmental management requirements across the East African region. The superior performance of the multi-enzyme blend compared to individual enzyme treatments confirms the synergistic interactions hypothesized between phytase, xylanase, protease, and amylase activities when applied simultaneously to maize-soybean meal substrates. The phytase component liberates phytate-bound phosphorus and associated minerals while disrupting phytate-protein complexes. The xylanase component degrades arabinoxylan cell wall barriers to expose encapsulated nutrients to enzymatic attack. The protease component enhances protein digestibility by supplementing endogenous proteolytic capacity and degrading protease-inhibitor complexes. The amylase component improves starch digestibility,

particularly during the early growth phase when endogenous pancreatic amylase production may be physiologically limited. The combined effect of these complementary activities produces a nutrient release response that exceeds the sum of individual contributions, as demonstrated by the consistently superior orthogonal contrast comparisons favoring the multi-enzyme blend over individual enzyme treatments across all measured parameters.

Discussion

This research provides comprehensive evidence that exogenous enzyme supplementation of maize-soybean meal based broiler diets substantially improves nutrient utilization and growth performance under tropical conditions, with the multi-enzyme blend approach offering the most complete nutritional enhancement across all measured parameters [4]. The magnitude of the growth response to multi-enzyme supplementation observed in the current investigation is consistent with the range of improvements reported in the global literature and confirms the practical relevance of enzyme technology for tropical poultry producers operating with locally sourced maize and soybean meal ingredients [8]. The dramatic improvement in phosphorus retention with phytase supplementation aligns with the well-established mechanism of phytate hydrolysis and is consistent with the matrix values recommended by major phytase manufacturers for the dose level evaluated [1]. The finding that the multi-enzyme blend produced numerically higher phosphorus retention than phytase alone, despite containing a similar phytase activity level, is in agreement with reports suggesting that the disruption of cell wall matrices by xylanase activity enhances the accessibility of phytate substrates to phytase attack, representing a genuine synergistic interaction between these two enzyme classes [6]. This synergy has practical implications for feed formulation, as it indicates that the phosphorus matrix value assigned to phytase may be conservatively underestimated when the enzyme is applied in combination with cell wall-degrading carbohydrases.

The absence of significant feed intake differences among treatments contradicts some earlier reports that described appetite-stimulating effects of enzyme supplementation, but is consistent with more recent controlled investigations that attribute enzyme-mediated growth improvements primarily to enhanced nutrient extraction efficiency rather than increased voluntary feed consumption [9]. This distinction is important because it indicates that the economic benefit of enzyme supplementation derives predominantly from improved feed conversion rather than from stimulating additional feed consumption, which would increase total feed costs. The mechanism underlying the improved feed conversion involves enhanced digestibility and absorption of nutrients per unit of feed consumed, allowing birds to satisfy their nutrient requirements with less total feed input and thereby reducing the feed cost per unit of body weight gain [10]. The ileal amino acid digestibility improvements observed with enzyme supplementation carry particular economic significance for tropical broiler producers, as amino acids constitute the most expensive nutrient category in broiler diet formulations after metabolizable energy [5]. The 14.2 percentage point improvement in crude protein digestibility and the corresponding improvements in lysine

and methionine digestibility achieved by the multi-enzyme blend represent substantial enhancements in the effective amino acid delivery from the diet, potentially allowing formulators to reduce crystalline amino acid supplementation levels without compromising growth performance [3]. This formulation flexibility generates additional cost savings that complement the phosphorus and energy matrix value savings, collectively strengthening the economic case for multi-enzyme adoption in tropical broiler production systems.

Comparative analysis

A systematic comparison of the individual enzyme and multi-enzyme supplementation strategies evaluated in this research reveals important distinctions in their respective modes of action, nutrient targeting specificity, and overall economic value that should inform purchasing and formulation decisions by tropical poultry producers [2]. Phytase supplementation provides the highest return on investment among individual enzyme products due to its substantial phosphorus matrix value, which allows the partial or complete elimination of expensive inorganic phosphorus supplements from the feed formulation while simultaneously improving calcium and trace mineral availability [6]. However, phytase alone does not address the energy and amino acid encapsulation within cell wall polysaccharide matrices, limiting its contribution to overall nutrient utilization improvement to the mineral and partially the protein dimensions. Xylanase supplementation addresses the non-starch polysaccharide barrier but does not improve mineral utilization from phytate complexes, producing a narrower but complementary spectrum of nutritional benefits [7]. The multi-enzyme blend captures the full complement of synergistic interactions among enzyme activities, producing the most comprehensive nutritional enhancement and the highest overall return on investment when the total formulation savings across phosphorus, energy, and amino acid dimensions are considered collectively.

Industrial relevance

The findings of this research carry direct relevance for the commercial feed manufacturing and broiler production sectors across East Africa, where maize-soybean meal based formulations dominate the feed supply and enzyme adoption rates remain substantially below those observed in more technologically advanced poultry industries [11]. The demonstrated capacity of multi-enzyme supplementation to compensate for reduced dietary phosphorus and energy specifications provides feed manufacturers with a validated tool for reducing raw material costs without sacrificing nutritional adequacy, an outcome that directly addresses the primary competitive challenge facing the regional poultry industry [12]. Implementation of enzyme-based reformulation strategies requires investment in technical formulation expertise and laboratory analytical capacity to accurately characterize the nutrient composition of locally sourced ingredients and apply enzyme matrix values with confidence, capabilities that should be prioritized in the ongoing development of the East African feed manufacturing sector.

Conclusion

This research aimed to evaluate the effects of phytase, xylanase, and a multi-enzyme blend on nutrient utilization and growth performance in broiler chickens fed maize-soybean meal diets under tropical conditions in Kenya. The key findings demonstrate that multi-enzyme supplementation produces the most comprehensive improvements across all performance and nutrient utilization parameters, achieving a 15.2 percent increase in final body weight, a 92.3 percent increase in phosphorus retention, and a 14.2 percentage point enhancement in ileal crude protein digestibility relative to unsupplemented controls. From a practical standpoint, tropical broiler producers and feed manufacturers are encouraged to adopt multi-enzyme supplementation strategies that combine phytase with cell wall-degrading carbohydrases and proteases to capture the full spectrum of synergistic nutritional benefits available from current enzyme technology. Future research should focus on establishing dose-response relationships for multi-enzyme products across the range of maize and soybean meal quality levels encountered in East African supply chains, evaluating the interaction between enzyme supplementation and feed processing parameters, and developing simplified enzyme application guidelines accessible to smaller-scale feed manufacturers and on-farm feed mixing operations.

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