



EFFECTS OF GRADED MIADIASAN® SUPPLEMENTATION ON CAECAL BACTERIAL COUNTS AND LIVER ANTIOXIDANT ENZYME ACTIVITIES IN FINISHER BROILER CHICKENS

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ABSTRACT

This study evaluated the effects of graded Miadiasan® inclusion levels (0, 50, 100, and 150 g/100 kg) in finisher diets on caecal total bacterial and total coliform counts and liver oxidative stress in 120 Ross 308 broiler chickens during the finisher phase at Kaduna State University Poultry Unit, Kafanchan. A completely randomised design with four treatments and three replicates of ten birds each was adopted. At the conclusion of the finisher phase, caecal and liver samples were collected from 12 birds for laboratory analysis. Caecal contents were analysed for total bacterial count (TBC) and total coliform count (TCC) using standard culture methods. Liver homogenates were assessed for catalase (CAT), superoxide dismutase (SOD), and glutathione peroxidase (GPx) activities using biochemical assays. Data were analysed using one-way analysis of variance (ANOVA). Results showed significant TBC modulation ($P < 0.0001$), peaking at 6.8×10^5 CFU/mL at 50 g inclusion and reaching a low of 2.0×10^5 CFU/mL at 100 g, with a marginal TCC effect ($P = 0.0519$). The overall treatment effect on TCC was not statistically significant ($P = 0.0519$). Liver antioxidant enzyme analysis showed significant SOD elevation ($P = 0.0154$) at 25.41 $\mu\text{mol/min}$ (50 g) compared with 21.67 $\mu\text{mol/min}$

in the control, with non-significant CAT ($P=0.5871$; range 4.48–5.08 $\mu\text{mol H}_2\text{O}_2/\text{min}/\text{mg protein}$) and GPx ($P=0.1053$; range 3.86–5.56 $\mu\text{mol H}_2\text{O}_2/\text{min}/\text{mg protein}$) activities. These findings suggest that Miadisan® supplementation may influence caecal bacterial populations and hepatic antioxidant enzyme activity, particularly SOD, at intermediate inclusion levels under Southern Guinea Savanna conditions.

Keywords: antioxidant enzymes, broiler chickens, caecal bacterial counts, total coliform count, liver antioxidant enzymes, Miadisan®

INTRODUCTION

Broiler production is a critical component of the livestock sector, contributing substantially to the protein needs of rapidly growing human populations worldwide. In Nigeria, the poultry industry, valued at over ₦1.6 trillion, supplies approximately 60–70% of the animal protein consumed, and broiler production constitutes the dominant sub-sector (Dialoke et al., 2020). However, sustaining productivity at the finisher phase, when birds achieve maximum muscle accretion and metabolic demand peaks, remains a persistent challenge, largely attributable to microbial dysbiosis and hepatic oxidative stress that compromise gut integrity, immunity, and growth efficiency (Clavijo and Florez, 2018).

Each segment of the gastrointestinal tract harbours a dense, functionally specialised microbial community (the gut microbiota) essential for nutrient metabolism, pathogen exclusion, and immune modulation (Borda-Molina *et al.*, 2018; Diaz-Carrasco *et al.*, 2019). Disruption of this equilibrium, termed dysbiosis, is well-documented in intensively reared broilers and is characterised by overgrowth of pathogenic bacteria such as *Escherichia coli*, *Clostridium perfringens*, and *Salmonella* spp., a problem that is particularly acute during the metabolically demanding finisher phase and can reduce final body weight gain by as much as 15–20% while increasing feed conversion ratios (Borda-Molina *et al.*, 2018; Stanley *et al.*, 2014; Diaz-Carrasco *et al.*, 2019).

Concurrently, oxidative stress constitutes a major metabolic burden during the finisher phase. Rapid growth, elevated metabolic rates, and environmental heat common under tropical Nigerian conditions promote the overproduction of reactive oxygen species (ROS), overwhelming the hepatic antioxidant defence system (Surai, 2020). Kövesi *et al.* (2019) demonstrated that oxidative imbalance in poultry leads to measurable reductions in catalase (CAT), superoxide dismutase (SOD), and glutathione peroxidase (GPx) activities, accompanied by elevated malondialdehyde (MDA) concentrations, adversely affecting liver function, meat quality, and bird welfare. Shakeri *et al.* (2018) reported that broilers subjected to heat stress showed significantly reduced antioxidant enzyme activities and impaired immune parameters, effects partly ameliorated by dietary antioxidant supplementation.

Historically, antibiotic growth promoters (AGPs) were widely used to control dysbiosis and sustain productivity, but regulatory restrictions following evidence of antimicrobial resistance dissemination have driven the search for natural alternatives capable of modulating gut microbiota and enhancing host health, including in Sub-Saharan Africa (Rehman *et al.*, 2020; Al-Khalaifah, 2018; Abd El-Hack *et al.*, 2020).

Probiotics, defined by the International Scientific Association of Probiotics and Prebiotics (ISAPP) as live microorganisms that, when administered in adequate amounts, confer a health benefit on the host (Salminen *et al.*, 2021), have consistently improved microbial balance, villus morphology, and intestinal immune responses in broilers. Cao *et al.* (2019) reported that *Clostridium butyricum* supplementation at 5.0×10^{10} CFU/g significantly reduced hepatic enzyme (AST, ALT, ALP) concentrations, indicating improved liver function, while Yu *et al.* (2022) showed that *Bacillus coagulans* supplementation significantly elevated SOD and CAT activities in lipopolysaccharide-challenged broilers. Antioxidant compounds, particularly phenolic phytobiotics such as tannins, further support hepatic defence by scavenging superoxide radicals and modulating the Nrf2 antioxidant signalling pathway (Surai, 2014; Kouvedaki *et al.*, 2024).

Miadiasan® is a commercially available feed additive that combines probiotic microorganisms with phytobiotic antioxidant components, including hydrolysable tannins derived from chestnut (*Castanea sativa*) extracts. Dialoke et al. (2020) demonstrated that chestnut-derived phytobiotics at 100 g/ton significantly improved broiler growth performance and feed efficiency relative to controls during the finisher phase, attributing these effects to tannins' selective promotion of beneficial gut flora through astringent mechanisms. Despite these individual merits, empirical evidence on the optimal dosage of combined probiotic-antioxidant formulations specifically targeting caecal bacterial populations and liver antioxidant enzyme profiles during the broiler finisher phase remains scarce, particularly under Nigerian tropical production conditions. The inclusion levels of 50, 100 and 150 g/100 kg were selected to bracket the manufacturer-recommended dosage range and to permit detection of a dose-response pattern, while total bacterial count, total coliform count, catalase, superoxide dismutase and glutathione peroxidase were selected because they are established, readily assayed indicators of caecal bacterial load and hepatic oxidative status in broilers. It remains unclear whether graded Miadiasan® inclusion produces a dose-dependent response in these specific indicators during the finisher phase. This study therefore aims to evaluate the effects of graded Miadiasan® inclusion levels in finisher diets on caecal total bacterial and total coliform counts and on liver antioxidant enzyme activities.

Objectives of the Study

The specific objectives of this study were to:

1. Evaluate the effects of different levels of Miadiasan® supplementation on caecal total bacterial and total coliform counts of broiler chickens during the finisher phase.
2. Evaluate the effects of different levels of Miadiasan® supplementation on hepatic antioxidant enzyme activities (CAT, SOD and GPx) of broiler chickens during the finisher phase.

MATERIALS AND METHODS

Experimental Site

This study was conducted at the Poultry Unit of the Department of Animal Science Teaching and Research Farm, Faculty of Agriculture, Kaduna State University (KASU), Kafanchan Campus, Kaduna State, Nigeria. Kafanchan lies at latitude 9°34'N and longitude 8°17'E within the Southern Guinea Savanna Zone. The town has an altitude of 752 m above sea level, with an average annual temperature of approximately 25.3°C (range: 18.8–28.6°C) and rainfall concentrated between April and October, peaking in August (NiMet, 2023).

Source of Experimental Materials and Birds

Feed ingredients were purchased from Biotin Feeds on Nnamdi Azikiwe Expressway, Kaduna, while maize was sourced from the Kafanchan market in Jema'a local government area. Miadiasan® was purchased from Mitchtam Farm Venture, Kaduna. Miadiasan® is manufactured by MIAVIT (MIAVIT Stefan Niemeyer GmbH, Essen/Oldenburg, Germany) and is a complementary feed additive combining plant-based ingredients, clay minerals, and chestnut-derived hydrolysable tannins; the Nigerian "Advanced Formulation" variant additionally incorporates oregano extract and probiotics (MIAVIT, 2025; Afrimash, 2025). One hundred and twenty (120) day-old Ross 308 broiler chicks were obtained from Agritech Hatchery, Ibadan, Nigeria.

Experimental Design and Management of Birds

This study used a total of 120 broiler chickens of mixed sexes. The birds had an average initial body weight of 48 g. The chicks were randomly allotted to four treatment groups, each replicated three times with ten birds per replicate, in a completely randomised design (CRD). Feed and water were provided *ad libitum* throughout the experimental period. Birds were housed in a deep litter pen divided into experimental units of 2.0 m × 1.5 m (area = 3.0 m²) per replicate. Stocking density was 3.3 birds m⁻², with 5 cm feeder space and 2.5 cm drinker space per bird from 1 to 21

days of age, with 10 broilers per replicate. The lighting schedule was a step-down program: 23 h light from day 1 to 3, day 4 – 7, 20 h light and afterwards 19 h light until slaughter. All birds were raised under standard animal husbandry practices, including daily health inspection, routine cleaning of drinkers and feeders and litter turning as needed. The standard vaccination schedule from the National Veterinary Research Institute (NVRI), Vom, Plateau State, was followed: Newcastle disease (ND) vaccine; day 7– ND (LaSota strain), day 14 – infectious bursal disease (Gumboro, IBD) vaccine via drinking water; day 21 – ND booster + IBD booster via drinking water; day 28. Mortality was recorded as it occurred, and 8 out of 120 birds died during the trial, with an overall mortality of 6.7%. Birds received starter diets for four weeks, followed by finisher diets for two weeks, for a total experimental period of six weeks (42 days).

Experimental Diets

Four experimental diets were formulated for the finisher phase with Miadisan® incorporated at graded levels of 0 g, 50 g, 100 g, and 150 g per 100 kg feed, designated as T1, T2, T3, and T4, respectively. The diets were formulated to meet the nutrient requirements of broiler chickens as recommended by the National Research Council (NRC, 1994). The composition and calculated nutrient analyses of the diets are presented in Table 1

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Sample Collection and Laboratory Analysis

Caecal Bacterial and Coliform Count Analysis

At 42 days of age, three birds per treatment group (one bird per replicate) were randomly selected and humanely slaughtered for sample collection. Caecal contents were collected and immediately used for microbial analysis. To account for intra-individual variation (Turnbaugh *et al.*, 2009) and maintain true biological replicates for statistical analysis, we analysed caecal samples from each bird independently rather than pooling them. Total bacterial count (TBC) was determined on nutrient agar using the spread-plate method, while total coliform count (TCC) was enumerated on MacConkey agar following standard procedures. Both counts were expressed as colony-forming units per millilitre (CFU/mL).

Table 1: Composition of broiler finisher diet with levels of Miadiasan® inclusion

Ingredients	0g Miadiasan® (Control)	50g Miadiasan®	100g Miadiasan®	150g Miadiasan®
Maize (%)	56	56	56	56
Maize offal (%)	9.00	9.00	9.00	9.00
Groundnut cake (%)	12	12	12	12
Soybean meal (%)	18.60	18.60	18.60	18.60
Limestone (%)	0.50	0.50	0.50	0.50
Bone meal (%)	3.00	3.00	3.00	3.00
Salt (%)	0.25	0.25	0.25	0.25
Premix (%)	0.25	0.25	0.25	0.25
Lysine (%)	0.20	0.20	0.20	0.20
Methionine (%)	0.20	0.20	0.20	0.20
Total	100.00	100.00	100.00	100.00
Calculated analysis				
Crude protein (%)	19.96	19.96	19.96	19.96
ME (Kcal/kg)	3212.18	3212.18	3212.18	3212.18
Ether extract (%)	4.45	4.45	4.45	4.45
Crude fibre (%)	4.44	4.44	4.44	4.44
Calcium (%)	1.32	1.32	1.32	1.32
Lysine (%)	1.20	1.20	1.20	1.20
Methionine (%)	0.78	0.78	0.78	0.78

GNC = Groundnut Cake; SBM = Soybean Meal; CP = Crude Protein; ME = Metabolizable Energy; EE = Ether Extract; CF = Crude Fibre; Ca = Calcium. Values for CP, ME, EE, CF, Ca, Lysine and Methionine represent calculated nutrient analyses based on ingredient composition (NRC, 1994). **Note:** The Miadiasan® is a non-nutrient component in the broiler diet, therefore is not added to the 100kg diet

Liver Oxidative Stress Assay

Liver tissue samples were collected from the same birds at slaughter, rinsed in cold phosphate-buffered saline (PBS), and homogenised in cold PBS (1:9 w/v) to prepare 10% liver homogenates. The homogenates were centrifuged at 3,000 rpm for 10 minutes at 4°C, and supernatants were used for enzyme assays. Catalase (CAT) activity was measured spectrophotometrically by monitoring the rate of H₂O₂ decomposition at 240 nm in 50 mM phosphate buffer (pH 7.0), following the method of Hadwan et al. (2024). The reaction mixture contained enzyme extract and 10–30 mM H₂O₂; absorbance was recorded every 10 s for 1–2 min at 25 °C. CAT activity was calculated from the linear decrease in absorbance ($\Delta A_{240}/\text{min}$) using the molar extinction coefficient of H₂O₂ ($\epsilon = 0.0436 \text{ mM}^{-1} \text{ cm}^{-1}$) and expressed as $\mu\text{mol H}_2\text{O}_2 \text{ decomposed min}^{-1} \text{ mg}^{-1} \text{ protein}$, as reported by Hadwan et al. (2024). Superoxide dismutase (SOD) activity was determined by inhibition of nitroblue tetrazolium (NBT) reduction. The assay mixture contained 50 mM phosphate buffer (pH 7.8), 13 mM methionine, 75 μM NBT, 2 μM riboflavin, and enzyme extract. Tubes were illuminated for 12 min, and absorbance was read at 560 nm. One unit of SOD was defined as the amount of enzyme causing 50% inhibition of NBT reduction under the assay conditions, and activity was expressed as U mg^{-1} protein (or $\mu\text{mol min}^{-1} \text{ mg}^{-1} \text{ protein}$, converted using the standard definition). Glutathione peroxidase (GPx) activity was measured by a glutathione-coupled assay in which GPx catalyses the oxidation of reduced glutathione (GSH) by cumene hydroperoxide (or H₂O₂), and the resulting decrease in GSH was quantified using Ellman's reagent (5,5'-dithiobis-(2-nitrobenzoic acid), DTNB). Enzyme extract was incubated in phosphate buffer containing GSH and peroxide substrate for 10 min; DTNB was then added, and the yellow TNB⁻ anion formed from unreacted GSH was measured at 412 nm. GPx activity was calculated as the difference between the standard GSH concentration and the residual GSH in the test, corrected for sample volume and dilution, and expressed as $\mu\text{mol H}_2\text{O}_2 \text{ consumed min}^{-1} \text{ mg}^{-1} \text{ protein}$ (or U mg^{-1} protein) according to the method described by Sattar et al. (2024). Total protein concentration in all enzyme extracts was determined according to Hadwan et al. (2024) using bovine serum albumin (BSA) as a standard, and enzyme activities were normalised to mg protein.

Statistical Analysis

For TBC and TCC, each bird represented one biological sampling unit. Technical triplicate plate counts from the same biological sample were averaged before statistical analysis. Bacterial concentrations were calculated as CFU/mL and transformed to $\log_{10}$ (CFU/mL) before analysis. The transformed values were subjected to one-way analysis of variance (ANOVA) using the General Linear Model (GLM) procedure of SAS (Version 9.4; SAS Institute Inc., Cary, NC, USA) with dietary treatment as the fixed effect. Duncan's multiple-range test separated treatment means at $P < 0.05$.

Compliance with ethical standards

This research was carried out in accordance with the Ethical Guideline of the Animal Research Ethical Committee, Department of Animal Science, Faculty of Agriculture, as approved by Kaduna State University, Kafanchan Campus.

RESULTS AND DISCUSSION

Effects of Different Levels of Miadiasan® on the Caecal Total Bacterial and Total Coliform Counts of Broiler Chickens During the Finisher Phase

Table 2 presents the effects of graded Miadiasan® inclusion levels on caecal total bacterial and total coliform counts in broiler chickens during the finisher phase. The Total Bacterial Count (TBC) exhibited significant variation across treatments ($P < 0.0001$), with values peaking at 6.8×10^5 CFU/mL in the 50 g treatment and declining to a minimum of 2.0×10^5 CFU/mL at 100 g, before partially recovering to 4.2×10^5 CFU/mL at 150 g. The control group recorded an intermediate TBC of 3.0×10^5 CFU/mL. The Total Coliform Count (TCC) showed a marginal treatment effect ($P = 0.0519$), with the highest value at 50 g (2.3×10^5 CFU/mL), declining progressively at 100 g and 150 g relative to the 50 g peak.

Table 2: Effects of different levels of Miadiasan® on the caecal total bacterial and total coliform counts of broiler chickens during the finisher phase

Parameters	Inclusion Levels of Miadiasan®				P-value
	0g (control)	50g	100g	150g	
TBC (CFU/mL)	3.0×10^5 ^b	6.8×10^5 ^d	2.0×10^5 ^a	4.2×10^5 ^c	0.0000 [*]
TCC (CFU/mL)	1.1×10^5	2.3×10^5	2.1×10^5	1.9×10^5	0.0519 ^{NS}

a,b,c,d = Means within the same row bearing different superscripts differ significantly (P<0.05).
^{*} = significant at P<0.05. NS = not significant at P<0.05; mean-separation superscripts are not applied to the TCC row because the overall treatment effect was not significant. TBC = Total Bacteria Count; TCC = Total Coliform Count; CFU/mL = colony-forming units per millilitre.

Miadiasan® significantly affected caecal total bacterial counts, with the highest count at 50 g/100 kg; because bacterial identification/taxonomic profiling was not performed, this increase in TBC cannot be equated with enrichment of beneficial bacteria. The pronounced elevation in TBC at 50 g inclusion is consistent with findings by Dialoke *et al.* (2020), who reported superior growth performance and implied microbial enrichment in broilers fed 100 g/ton chestnut (*Castanea sativa*) phytobiotics during the finisher phase, attributing the benefits to tannins' astringent properties that selectively promoted beneficial flora while inhibiting pathogens. Similarly, Omidwura and Agboola (2017) demonstrated that sodium acetate and probiotic supplementation significantly boosted *Lactobacillus* counts and overall TBC relative to unsupplemented controls, an effect attributed to organic acid-mediated pH reduction favouring acid-tolerant commensals, a mechanism likely mirrored by Miadiasan®'s tannin-mediated acidification of the intestinal environment. These findings raise the possibility that intermediate phytobiotic inclusion could be associated with a shift toward eubiosis, potentially involving enhanced short-chain fatty acid (SCFA) production and villus morphometry, as previously

proposed by Paredes-López et al. (2024); however, the present study did not directly assess these mechanisms.

Conversely, the TBC depression at 100 g and partial recovery at 150 g suggest dose-dependent selective pressure from tannins inhibiting sensitive bacterial taxa at higher concentrations. This non-linear response disagrees with the linear increases in *Bacteroides* and *Lactobacillus* enrichment reported by Zhang *et al.* (2025a) using compound probiotics, where strain-specific adhesion mechanisms differ fundamentally from phytobiotic action. The pattern resembles the niche saturation dynamics described by Ndotono *et al.* (2022) in broilers fed novel alternative protein sources, where gut microbiota modulation was non-monotonic. The observed TBC pattern could reflect selective effects of the product on cultivable bacterial populations, but bacterial identity, community composition and specific mechanisms such as pathogen exclusion or adhesin binding were not assessed.

Although TCC was numerically highest at 50 g/100 kg and decreased at 100 and 150 g/100 kg, the overall treatment effect was not statistically significant ($P=0.0519$), so this pattern should be described as a numerical trend rather than a treatment effect. The marginal TCC trend toward elevation at 50 g, before stabilisation at higher doses, partially concurs with Omidwura and Agboola (2017), who interpreted similar patterns as reflecting balanced coliform exclusion rather than eradication. Under the savanna climatic conditions at Kafanchan (mean temperature 25.3°C), heightened metabolic demands during the finisher phase are known to amplify microbial activity and ROS generation (Rehman *et al.*, 2020). This thermal context may have amplified tannin efficacy in relieving oxidative stress and driving selective microbial shifts, positioning 50 g as optimal for TBC maximisation. The lower TCC at 100 g and 150 g, while not statistically significant, is biologically desirable, as lower coliform counts are associated with reduced risk of enteric dysbiosis and pathogen-associated diarrhoea (Idowu et al., 2025; Naeem and Bourassa, 2025). Overall, these findings suggest that Miadiasan® supplementation may influence caecal bacterial populations in a dose-dependent manner under tropical Nigerian production conditions; claims of eubiosis, pathogen exclusion and safety as an AGP replacer

would require direct microbial identification, toxicological assessment and further study to substantiate.

Effects of Different Levels of Miadiasan® on the Liver Oxidative Stress of Broiler Chickens During the Finisher Phase

Table 3 presents the influence of graded Miadiasan® inclusion on liver oxidative stress markers. Catalase (CAT) activity did not differ significantly across treatments ($P=0.5871$), with values ranging from $4.48 \mu\text{mol H}_2\text{O}_2/\text{min}/\text{mg protein}$ (150 g) to $5.08 \mu\text{mol H}_2\text{O}_2/\text{min}/\text{mg protein}$ (50 g). Superoxide dismutase (SOD) activity showed a significant treatment effect ($P=0.0154$), with the 50 g group recording the highest value ($25.41 \pm 0.88 \mu\text{mol}/\text{min}$), significantly exceeding the control ($21.67 \pm 0.88 \mu\text{mol}/\text{min}$), while the 100 g and 150 g groups showed intermediate values (24.23 and $24.39 \mu\text{mol}/\text{min}$, respectively). Glutathione peroxidase (GPx) activity trended higher at intermediate doses but did not reach statistical significance ($P=0.1053$), ranging from $3.86 \mu\text{mol H}_2\text{O}_2/\text{min}/\text{mg protein}$ (control) to $5.56 \mu\text{mol H}_2\text{O}_2/\text{min}/\text{mg protein}$ (50 g).

Table 3: Effects of different levels of Miadiasan® on the liver oxidative stress of broiler chickens during the finisher phase

Parameters	Inclusion Levels of Miadiasan®				P-value
	0g (control)	50g	100g	150g	
CAT ($\mu\text{mol H}_2\text{O}_2/\text{min}/\text{mg protein}$)	4.58 ± 0.48	5.08 ± 0.48	4.91 ± 0.48	4.48 ± 0.48	0.5871^{NS}
SOD ($\mu\text{mol}/\text{min}$)	$21.67 \pm 0.88^{\text{b}}$	$25.41 \pm 0.88^{\text{a}}$	$24.23 \pm 0.88^{\text{ab}}$	$24.39 \pm 0.88^{\text{ab}}$	0.0154^*
GPx ($\mu\text{mol H}_2\text{O}_2/\text{min}/\text{mg protein}$)	3.86 ± 0.62	5.56 ± 0.62	4.13 ± 0.62	4.55 ± 0.62	0.1053^{NS}

a,b = Means within the same row bearing different superscripts differ significantly ($P<0.05$). * = significant at $P<0.05$. NS = not significant. CAT = catalase; SOD = superoxide dismutase; GPx = glutathione peroxidase.

Miadiasan® supplementation significantly affected hepatic SOD activity, whereas CAT and GPx activities were not significantly affected. The significant SOD elevation at 50 g Miadiasan® aligns with Selim et al. (2021), who reported that rice bran oil inclusion elevated hepatic SOD and CAT activities in broilers by enriching tissues with γ -oryzanol, a phenolic compound that neutralises peroxy radicals and sustains enzymatic antioxidant cascades during rapid growth phases. Similarly, Seyfori et al. (2019) observed significantly upregulated SOD expression in broiler livers following supplementation with an organic acid–trace mineral complex, attributed to phenolic compounds scavenging superoxide radicals and preserving manganese-dependent SOD (MnSOD) activity. The antioxidant activity of chestnut-derived tannins may explain the observed SOD response; however, the present study did not investigate the specific biochemical mechanism underlying it. This mechanism may relate to nutrigenomic evidence that natural antioxidants such as curcumin can induce Nrf2-mediated SOD gene transcription at optimal rather than linear dosing thresholds (Kouvedaki et al., 2024); however, the specific molecular pathway responsible for the SOD increase observed here was not investigated in the present study.

The non-significant CAT stability across all treatment groups concurs with findings by Paredes-López et al. (2024) in a medicinal plant extract trial, where balanced hydrogen peroxide (H_2O_2) management across treatments prevented selective CAT upregulation even as SOD was significantly elevated. Dialoke et al. (2020) evaluated chestnut phytobiotics in Nigerian broilers primarily for growth performance, haematology and blood biochemistry rather than hepatic antioxidant enzyme activity, so a direct comparison of CAT responses with the present study is not warranted. In the present study, the Southern Guinea Savanna environment at KASU Kafanchan (mean temperature 25.3°C) may have moderated hepatic H_2O_2 flux, and it is possible that Miadiasan®'s mineral components adsorbed pro-oxidant metal ions, potentially via Fenton-reaction inhibition, though this pathway was not directly measured, thus reducing H_2O_2 accumulation and obviating excessive CAT induction. This interpretation is consistent with Imaseun and Ijeh (2018), who reported that phytobiotic blends preserved hepatic redox equilibrium in Nigerian broilers by modulating glutathione recycling pathways, thereby maintaining CAT at homeostatic levels.

The numerical GPx elevation at 50 g and 150 g, while non-significant, mirrors the synbiotic effects reported by Zhang et al. (2025b), who observed enhanced GPx activity in broilers supplemented with compound probiotics containing *Bacillus subtilis*, attributed to improved selenocysteine incorporation into the GPx enzyme, facilitating glutathione-dependent peroxide reduction. The lack of statistical significance in the present study likely reflects adequate baseline selenium provision in the NRC (1994)-compliant basal diet, where Miadiasan®'s probiotic components may optimise microbial selenium methylation to improve GPx bioavailability without requiring de novo enzyme synthesis. Wang et al. (2025) similarly reported numerically, but not statistically significant, GPx improvements in control-diet broilers, attributing the non-significance to low replicate numbers—a constraint shared with the present study's three-replicate design.

Collectively, the liver oxidative stress data indicate that Miadiasan® supplementation, particularly at intermediate inclusion levels, may support hepatic antioxidant activity, with SOD as the principal statistically responsive enzyme. This outcome could be linked to the caecal bacterial count findings in Table 2, where TBC peaked at 50 g/100 kg; the literature has proposed a mechanistic connection via SCFA production and butyrate-mediated effects, but the present study did not directly assess it. The non-linear enzyme responses across doses may indicate an optimal 50 g threshold; however, positioning Miadiasan® as a superior AGP alternative for Nigerian broilers would require direct comparison with a conventional AGP, which was not included in this trial, consistent with the broader poultry nutrition literature (Rehman et al., 2020; Shakeri et al., 2018).

CONCLUSION

Miadiasan® supplementation influenced caecal total bacterial counts and hepatic SOD activity in finisher broiler chickens, with the most pronounced responses generally observed at 50 g/100 kg. However, CAT and GPx activities were not significantly affected, while the effect on total

coliform count was not statistically significant ($P=0.0519$). Further studies using comprehensive microbial profiling, larger biological replication and direct comparison with conventional antibiotic growth promoters are warranted. Future research should also explore microbial sequencing to identify beneficial and pathogenic bacterial taxa, short-chain fatty acid (SCFA) determination, malondialdehyde (MDA) and other oxidative stress biomarkers, full broiler growth performance and feed conversion ratio, carcass characteristics, blood biochemical parameters, tissue-residue studies, direct comparison with a conventional AGP, and larger, multi-location trials across Nigeria's agroecological zones to validate scalability and broader applicability.

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