

Physiological and Adipokine Responses of Broiler Chickens Fed *Aspergillus niger*-Fermented Cassava Peel Diets as a Replacement for Maize

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Abstract

Cassava peels are abundant agro-industrial by-products, but their direct use is limited by high fibre and cyanogenic compounds. Microbial fermentation, particularly with *Aspergillus niger*, offers a potential approach to improving their nutritional and functional value. This study evaluated the physiological responses of broiler chickens fed *A. niger*-fermented cassava peels as partial replacements for maize. A total of 90 one-day-old Cobb 500 broilers were randomly allocated to three dietary treatments: a control diet (0% replacement) and diets with 25% and 50% maize replacement using *A. niger*-fermented cassava peels for 42 days. The results revealed that broilers fed the 25% replacement diet exhibited a significant reduction in serum ghrelin concentration ($p < 0.05$) compared with the control and 50% groups, whereas serum leptin levels showed no significant differences ($p > 0.05$) among dietary treatments. Inflammatory cytokine analysis revealed significant reductions in interleukin-6 and tumour necrosis factor- α concentrations in both replacement groups ($p < 0.05$), while interleukin-10 and interleukin-12 remained unchanged ($p > 0.05$). Antioxidant activity showed that fermented cassava peel diets significantly enhanced superoxide dismutase, glutathione S-transferase, and reduced glutathione activities in breast and thigh muscles ($p < 0.05$). *Aspergillus niger*-fermented cassava peel can effectively replace maize at moderate levels, supporting improved metabolic regulation, reduced systemic inflammation, and enhanced antioxidant defence in broiler chickens.

Categories: Functional foods, Poultry nutrition

Keywords: aspergillus niger, antioxidant enzymes, adipokines, inflammation, fermented feed

Introduction

The rising cost and increasing competition for maize among human food, livestock feed, and industrial uses have intensified the search for alternative feed ingredients in poultry nutrition, particularly in sub-Saharan Africa. Feed cost accounts for approximately 70%-80% of the total expenditure in broiler production, making the substitution of conventional ingredients with locally available agro-industrial by-products a viable option for sustainable poultry production [1,2].

Cassava (*Manihot esculenta* Crantz) is widely cultivated in tropical regions and produces large quantities of peels during processing, which are often underutilized and constitute environmental pollutants if improperly managed [3]. Although rich in carbohydrates, their direct utilization in monogastric diets is constrained by high fibre content, low crude protein, and the presence of cyanogenic glycosides [1]. To overcome these limitations, microbial fermentation techniques have

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been widely used to enhance the nutritional value and safety of cassava peel meal. Among the microorganisms used, *Aspergillus niger* has received considerable attention due to its strong enzymatic capacity for cellulose, hemicellulose, and starch degradation, as well as its effectiveness in cyanide detoxification [4,5]. Solid-state fermentation with *A. niger* has been shown to significantly increase crude protein content while reducing crude fibre and cyanide concentrations in cassava peels, making them more suitable as energy substitutes for maize in broiler diets [6,7].

Several recent studies have demonstrated that fermented cassava peel meal can partially replace maize in broiler diets without adverse effects on growth performance, nutrient digestibility, carcass traits, and economic returns when included at 20% to 40% replacement levels [6-8]. Meta-analytical evidence further suggests that wet- or microbially fermented cassava by-products may improve feed efficiency and weight gain when included at low levels, highlighting the importance of processing methods in determining nutritional outcomes [2].

Beyond growth performance, diet composition plays a crucial role in regulating physiological metabolism and lipid deposition in broiler chickens. Adipokines such as leptin and adiponectin have emerged as important metabolic regulators involved in energy homeostasis, lipid metabolism, and body fat accretion in poultry [9,10]. Adiponectin is particularly associated with fatty acid oxidation and reduced lipid accumulation, while leptin influences feed intake, energy balance, and adipose tissue signalling [9]. Recent studies have demonstrated that dietary manipulations, including the use of phytochemical additives such as turmeric, garlic, ginger, and cinnamon, can significantly modulate circulating adipokines and associated metabolic hormones in broilers [11].

Despite growing interest in fermented cassava by-products as maize substitutes, limited information is available on how *A. niger*-fermented cassava peel diets influence adipokine responses alongside classical physiological indicators in broiler chickens. Understanding these endocrine and metabolic responses is essential for evaluating the broader implications of alternative feed resources on broiler health, fat metabolism, and production performance.

The aim of this study was to investigate the physiological and adipokine responses of broiler chickens fed *A. niger*-fermented cassava peel diets as a maize replacement, addressing this gap in the hormonal and metabolic body of knowledge needed to support sustainable, non-conventional feed alternatives in poultry production.

Materials And Methods

Experimental sites

The study was assigned ethical approval number FUTA/ETH/23/121 and was conducted at the Poultry Unit of the Research and Teaching Farm, Federal University of Technology, Akure, Nigeria (FUTA), located at latitude 7°15' N and longitude 5°12' E within the humid tropical rainforest zone. The area is characterized by annual rainfall of approximately 1,300-1,650 mm and daily temperatures ranging from 27°C to 38°C [12].

Experimental materials

Pure fungal strains of *A. niger* were sourced from the Microbiology Laboratory of the Federal University of Technology, Akure. Cassava tubers were collected from within the university vicinity, washed with fresh water, peeled, and milled into pulp. The wastewater extracted from the pulp was reserved for microorganism inoculation. Cassava peels obtained during processing were dried and ground using a hammer mill at the university feed mill.

Sample preparation

The cassava pulp was inoculated with *A. niger* (strain ATCC 16404) inoculum at a concentration of 1×10^7 spores/mL, alongside a nutrient solution (750 mL containing 8.0 g urea, 1.3 g KH_2PO_4 , 1.1 g MgSO_4 , and 2.0 g citric acid). The moisture content was adjusted to 60-65%, and fermentation was carried out at 30°C under aerobic conditions for five days at pH 5.5. To prevent contamination, all equipment and fermenter trays were sterilized by autoclaving at 121°C for 15 min before use, and fermentation was conducted under aseptic conditions. Wastewater collected by pressing the fermented pulp was used for subsequent inoculation of cassava peels, while the pulp was further processed into flour and garri. Ground cassava peels were spread in sterilized fermenter trays to a depth of approximately 2 cm to ensure

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adequate aeration and inoculated with the collected pulp wastewater, nutrient solution, and *A. niger* inoculum at 1×10^7 spores/mL. Moisture content and pH were adjusted to 60% and 5.5, respectively, and maintained throughout the fermentation period [13]. Fermentation was conducted at 30°C for seven days before analysis, after which the cyanide content of the cassava peels was reduced from 0.04 to 0.02 mg g⁻¹. This was used to assess feed safety prior to use in poultry feed formulations.

Experimental dietary design

A total of 90 one-day-old Cobb 500 broilers were obtained from a hatchery and randomly distributed into three dietary treatments, with each treatment divided into three replicates (10 chicks per replicate) in a completely randomized design. The sample size of 10 birds per replicate (30 birds per treatment) was determined based on a power analysis using an estimated large effect size of 0.40 for adipokine and cytokine responses, with a significance level of $\alpha = 0.05$ and a desired statistical power of 80%, yielding a minimum required sample size of 22 birds per treatment group. Additionally, this replication structure is consistent with previous broiler studies validating the use of localized sample sizes for evaluating avian serum adipokine expression and systemic physiological parameters [14]. The three treatments corresponded to 0%, 25%, and 50% replacement of maize with *A. niger*-fermented cassava peels (MFCP). Starter diets (days 1-21) and finisher diets (days 22-42) were formulated for each treatment group (Table 1 and Table 2), and birds were fed ad libitum throughout the experimental period.

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Ingredients (%)	T1	T2 (ASP 25%)	T3 (ASP 50%)
MFCP	0.00	12.55	25.10
Maize	50.20	37.65	25.10
Soybean meal	35.60	35.60	35.60
Wheat offal	1.50	1.50	1.50
Fish meal	3.50	3.50	3.50
Soya oil	3.50	3.50	3.50
Limestone	1.10	1.10	1.10
Bone meal	1.60	1.60	1.60
Methionine	0.20	0.20	0.20
Lysine	0.10	0.10	0.10
Salt	0.20	0.20	0.20
Premix	0.50	0.50	0.50
Total	100.00	100.00	100.00
Crude protein (%)	23.01	22.98	23.11
ME (kcal/kg)	3,130.69	3,129.34	3,131.33

TABLE 1: Composition (%) of the broiler starter diet

T1 refers to Control Diet; T2 refers to *Aspergillus niger*-Fermented Cassava Peel 25% Diet; T3 refers to *Aspergillus niger*-Fermented Cassava Peel 50% Diet

ME, Metabolizable Energy; MFCP, Microbe-Fermented Cassava Peels

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Ingredients (%)	T1	T2 (ASP 25%)	T3 (ASP 50%)
MFCP	0.00	13.17	26.35
Maize	52.70	39.53	26.35
Soybean meal	28.90	28.90	28.90
Wheat offal	1.50	1.50	1.50
Fish meal	3.00	3.00	3.00
Soya oil	3.50	3.50	3.50
Limestone	1.00	1.00	1.00
Bone meal	1.40	1.40	1.40
Methionine	0.20	0.20	0.20
Lysine	0.10	0.10	0.10
Salt	0.20	0.20	0.20
Premix	0.50	0.50	0.50
Total	100.00	100.00	100.00
Crude protein (%)	20.00	19.96	20.07
ME (kcal/kg)	3,170.93	3,168.15	3,170.67

TABLE 2: Composition (%) of the broiler finisher diet

T1 refers to Control Diet; T2 refers to *Aspergillus niger*-Fermented Cassava Peel 25% Diet; T3 refers to *Aspergillus niger*-Fermented Cassava Peel 50% Diet

ME, Metabolizable Energy; MFCP, Microbe-Fermented Cassava Peels

Blood samples

At day 42, blood samples were collected from all birds through neck bleeding for further analyses. Serum was obtained by centrifugation of clotted blood at 3,000 × g for 10 min, and the resulting clear supernatant was used for the assessment of inflammatory markers, adipokines, and antioxidant enzymes [15].

Inflammation analysis

Serum concentrations of interleukin (IL)-6, IL-10, IL-12, and tumour necrosis factor (TNF) were quantified using species-specific sandwich enzyme-linked immunosorbent assay (ELISA) kits according to the manufacturer's instructions (Elabscience Biotechnology Co., Ltd., Wuhan, China). In brief, standards and serum samples were added to microplates

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pre-coated with cytokine-specific capture antibodies [16]. Following incubation and washing to remove unbound material, enzyme-linked detection antibodies were added and allowed to bind to captured cytokine complexes. A chromogenic substrate was then introduced, producing a colour change proportional to cytokine concentration, measured photometrically at the designated wavelength using a microplate reader. Cytokine concentrations were calculated by interpolation from a standard curve of known concentrations [17].

Adipokine analysis

Serum concentrations of adiponectin, leptin, and ghrelin were determined by ELISA using the following commercial kits: the Adiponectin ELISA Kit (DE/CA40/00809/18, Mediagnost), Leptin ELISA Kit (DE/CA40/00809/17, Mediagnost), and Ghrelin ELISA Kit (E309Ra, Bioassay Technology Laboratory, Shanghai, China), following the respective manufacturers' protocols [11].

Antioxidant activity

Determination of Reduced Glutathione

Plasma reduced glutathione (GSH) concentration was determined using a modified method described by Ellman [18]. Briefly, 250 μ L of plasma was combined with 125 μ L of Ellman's reagent (19.8 mg of 5,5'-dithiobisnitrobenzoic acid in 100 mL of 0.1% sodium citrate) and 750 μ L of 0.2 M phosphate buffer (pH 8.0), and absorbance was measured at 420 nm. Results were expressed as μ mol/mg protein.

Determination of Superoxide Dismutase Activity

Superoxide dismutase (SOD) activity was measured using a modified method described by Jewett and Rocklin [19]. The reaction mixture, consisting of 50 μ L tissue homogenate, 1,000 μ L of 50 mM sodium carbonate buffer (pH 10.2), and 17 μ L of adrenaline (0.6 mg/mL), was monitored spectrophotometrically at 480 nm at 15-sec intervals for 2 min. Activity was expressed as units/mg protein.

Determination of Catalase Activity

Catalase (CAT) activity was assessed following the method described by Sinha [20]. A 25 μ L sample was reacted with 100 μ L of 2 M H_2O_2 in 250 μ L of 0.01 M phosphate buffer (pH 7.0), and the reaction was terminated with 500 μ L of dichromate-acetic acid. Absorbance was measured at 620 nm at 30-sec intervals for 3 min, and activity was expressed as mmol H_2O_2 /min/mg protein.

Determination of Glutathione S-Transferase Activity

Glutathione S-transferase (GST) activity was measured using a modified method described by Habig et al. [21]. The reaction mixture consisted of 500 μ L of 0.1 M phosphate buffer (pH 6.5), 50 μ L of 30 mM 1-chloro-2,4-dinitrobenzene, and 350 μ L distilled water, pre-incubated at 37°C for 5 min, before initiating the reaction with 50 μ L each of plasma and 30 mM glutathione. Absorbance was measured at 340 nm at 1-min intervals for 3 min. Activity was expressed as nmol/min/mg protein.

Statistical analysis

Data were expressed as mean $\pm$ standard error of the mean (SEM), with replicate pens ($n = 3$ per treatment) as the experimental unit. Normality and homogeneity of variance were assessed using the Brown-Forsythe test and Bartlett's test, respectively, prior to analysis. Treatment effects were analysed by one-way analysis of variance (ANOVA) using GraphPad Prism (version 10; GraphPad Software, San Diego, CA, USA). A one-way ANOVA with Tukey's honestly significant difference (HSD) test was selected because the experimental design involved a single categorical factor (dietary treatment: 0%, 25%, and 50% maize replacement with *A. niger*-fermented cassava peel), and all three pairwise comparisons among treatments (control vs. 25%, control vs. 50%, and 25% vs. 50%) were of biological interest. Tukey's test controls the family-wise error rate across all possible pairwise comparisons while maintaining the type I error rate at $\alpha = 0.05$.

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Results And Discussion

Fermented feed has gained increasing attention due to its potential to enhance the nutritional quality of feed and boost animal growth performance [22]. Replacing maize with *A. niger*-fermented cassava peels (MFCP) triggers a coordinated endocrine, inflammatory, and antioxidant response, indicating an inclusion level-dependent effect on energy balance and physiological status in broilers. Fermentation, especially with microfungi such as *A. niger*, is known to hydrolyse structural carbohydrates, increase fermentative enzyme activities, and reduce anti-nutrients (e.g., cyanogenic glycosides), thereby improving the nutrient availability of cassava by-products [23]. These techniques likely support the results observed in this experiment. This finding also aligns with various reviews on cassava, which highlight the importance of processing and substitution levels, as performance and physiological responses may decline when cassava products exceed moderate dietary proportions unless processing methods or enzyme supplementation compensate for the lower energy density [24,25].

The effect of dietary replacement with *A. niger*-fermented cassava peels on serum adipokine profiles is presented in Table 3 and Figure 1. Dietary replacement significantly ($p < 0.05$) influenced ghrelin activity (Figure 1a), with broiler chickens fed the 25% replacement diet showing a marked reduction compared with the control, indicating improved energy utilization and reduced hunger signalling. Conversely, birds fed the 50% replacement diet showed ($p < 0.05$) higher ghrelin concentration than the 25% groups but similar and non-significant to the control group, suggesting enhanced appetite stimulation at higher inclusion levels.

Leptin concentration (Figure 1b) was slightly increased in broilers fed the 25% MFCP diet, though not statistically significant compared with both the control and 50% groups, reflecting improved lipid metabolism and energy sufficiency; however, leptin levels in birds fed diets across the groups did not differ significantly ($p > 0.05$).

In contrast, adiponectin activity (Figure 1c) was reduced in broilers fed the 50% replacement diet compared with the 25% group and showed no significant difference ($p > 0.05$) from the control, indicating that higher dietary inclusion of fermented cassava peels triggers a compensatory physiological shift that may diminish the beneficial adiponectin-mediated metabolic effects observed at moderate substitution levels.

Parameter	T1 (Control) Mean ± SEM	T2 (25% MFCP) Mean ± SEM	T3 (50% MFCP) Mean ± SEM	F (df)	p-value (T1 vs T2/T1 vs T3/T2 vs T3)	Sig.
Ghrelin (ng/mL)	35.30 ± 1.80	12.82 ± 1.21	29.08 ± 2.89	31.16 (2, 3)	0.0095/0.2340/0 .0236	a b a
Leptin (ng/mL)	0.56 ± 0.021	0.65 ± 0.032	0.58 ± 0.007	5.01 (2, 3)	0.1182/0.8986/0 .1695	No
Adiponectin (µg/mL)	24.29 ± 1.84	33.50 ± 1.02	22.87 ± 1.860	12.67 (2, 3)	0.0554/0.8201/0 .0381	ab a b

TABLE 3: Serum adipokine concentrations

Means within a row that do not share a common superscript letter (a–b) differ significantly according to one-way ANOVA with Tukey's HSD post-hoc test ($p < 0.05$). Values are presented as mean ± SEM ($n = 3$ replicates per treatment; 10 birds per replicate). ANOVA, Analysis of Variance; HSD, Honestly Significant Difference; MFCP, *Aspergillus niger*-Fermented Cassava Peels; SEM, Standard Error of the Mean

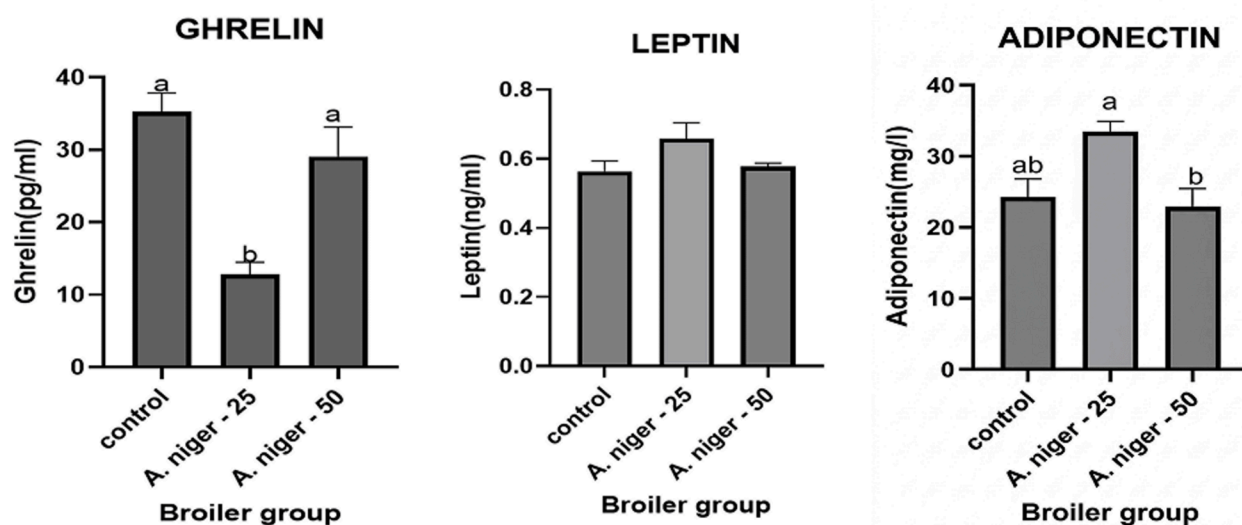


Figure 1a

Figure 1b

Figure 1c

FIGURE 1: Adipokine indices of broiler chickens fed *Aspergillus niger*-fermented cassava peel replacement diets: (a) ghrelin, (b) leptin, and (c) adiponectin

Data are presented as mean $\pm$ SEM (n = 3 replicates per treatment). Bars with different superscript letters differ significantly according to one-way ANOVA followed by Tukey's HSD post hoc test ($p < 0.05$).

ANOVA, Analysis of Variance; HSD, Honestly Significant Difference; SEM, Standard Error of the Mean

At 25% MFCP replacement, the observed reduction in serum ghrelin alongside increased leptin compared with the control is consistent with improved short-term satiety signalling and a state of energy sufficiency. In contrast, 50% MFCP elevated ghrelin without increasing leptin, suggesting a potential compensatory drive to eat and reflecting a relative energy shortfall at the higher replacement level. These findings are in line with the established roles of adipokines in poultry, where leptin and adiponectin integrate longer-term lipid metabolism and adiposity, whereas ghrelin frequently mirrors acute energy demand [26]. The directional shift at 50% MFCP corresponds with the meta-analytical report of Ogbuewu and Mbajiorgu [27], which noted that increasing cassava inclusion often reduces average daily gain unless the ingredient is optimally processed and used at moderate levels. Moreover, a review by Sugiharto et al. [28] demonstrated that diet composition can modulate adipokines in broilers (e.g., leptin and adiponectin changes with phytogetic supplementation), supporting the concept of endocrine adaptability in response to feed factors.

Adiponectin was maintained at 25% but declined at 50% MFCP; this may be due to a shift away from fatty-acid oxidation and insulin-sensitizing tone when energy density is constrained in chickens. Adiponectin and its receptors are expressed in adipose and multiple metabolic tissues and respond to nutritional status [29]. The adipokine profile suggests that 25% MFCP supports efficient energy handling, while 50% MFCP risks pushing birds toward hunger signalling.

The effect of dietary replacement with *A. niger*-fermented cassava peels on inflammatory markers is presented in Table 4 and Figure 2. Dietary replacement did not significantly ($p > 0.05$) alter IL-10 activity (Figure 2a). The relatively stable IL-10 profile across treatments suggests that dietary inclusion of fermented cassava peels did not adversely disrupt anti-inflammatory cytokine production. Meanwhile, IL-6 activity (Figure 2b) was significantly reduced in the treatment groups compared to the control ($p < 0.05$), suggesting a potential anti-inflammatory effect and improved immunity.

In contrast, Figure 2c shows no significant ($p > 0.05$) difference in the IL-12 activity. Nevertheless, the *A. niger*-fermented cassava peels replacement diet caused a significant ($p < 0.05$) decrease in TNF levels compared to the control group

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(Figure 2d), exerting anti-inflammatory effects of the diets in broiler chickens, with both replacement levels supporting reduced systemic inflammation compared with the control, thereby indicating potential immunomodulatory benefits of fermented cassava peels as a maize substitute.

Parameter	T1 (Control) Mean ± SEM	T2 (25% MFCP) Mean ± SEM	T3 (50% MFCP) Mean ± SEM	F (df)	p-value (T1 vs T2/T1 vs T3/T2 vs T3)	Sig.
Interleukin-10 (pg/mL)	102.3 ± 1.71	92.14 ± 3.6	92.14 ± 4.99	2.53 (2, 3)	0.2712/0.2711/ >0.9999	No
Interleukin-6 (pg/mL)	56.70 ± 2.06	44.59 ± 1.29	49.49 ± 1.03	15.95 (2, 3)	0.0227/0.0873/0 .2042	a b ab
Interleukin-12 (pg/mL)	44.98 ± 1.94	44.57 ± 2.39	43.70 ± 1.52	0.1092 (2, 3)	0.9881/0.8948/0 .9492	No
TNF-α (pg/mL)	40.46 ± 1.46	33.85 ± 0.38	32.54 ± 1.31	13.55 (2, 3)	0.0541/0.0336/0 .7281	a ab b

TABLE 4: Serum inflammatory cytokine concentrations

Means within a row that do not share a common superscript letter (a–b) differ significantly according to one-way ANOVA with Tukey's HSD post-hoc test ($p < 0.05$). Values are presented as mean ± SEM ($n = 3$ replicates per treatment; 10 birds per replicate). ANOVA, Analysis of Variance; HSD, Honestly Significant Difference; MFCP, *Aspergillus niger*-Fermented Cassava Peels; SEM, Standard Error of the Mean; TNF-α, Tumor Necrosis Factor Alpha

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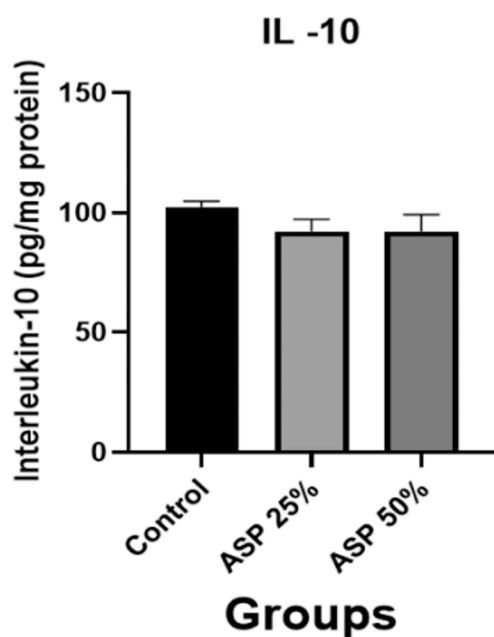


Figure 2a

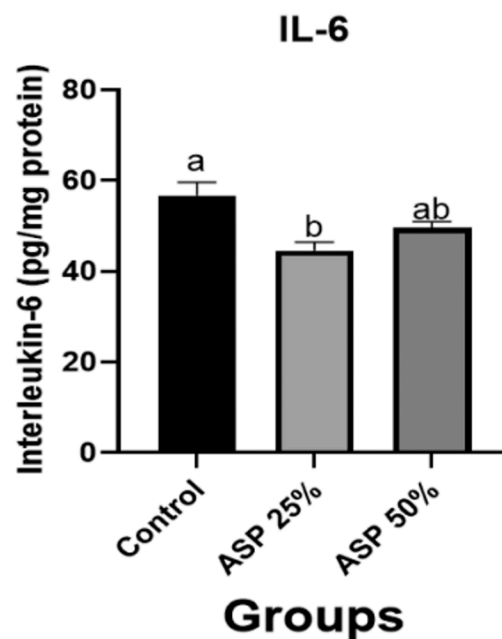


Figure 2b

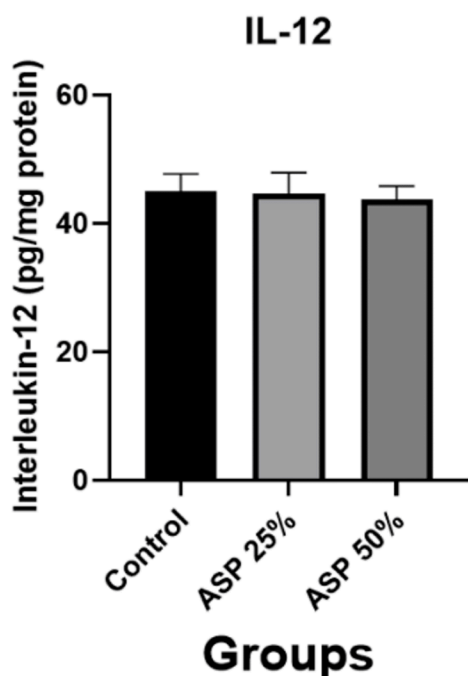


Figure 2c

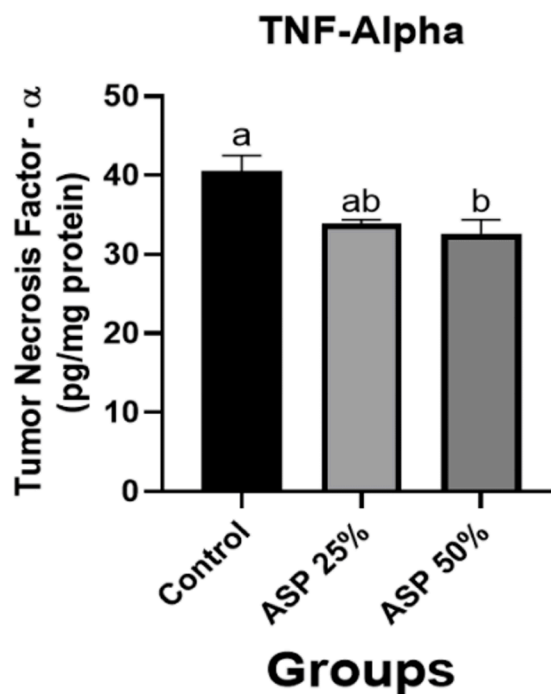


Figure 2d

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FIGURE 2: Inflammatory cytokine indices of broiler chickens fed *Aspergillus niger*-fermented cassava peel replacement diets: (a) interleukin-10, (b) interleukin-6, (c) interleukin-12, and (d) TNF- α

Data are presented as mean $\pm$ SEM (n = 3 replicates per treatment). Bars with different superscript letters differ significantly according to one-way ANOVA followed by Tukey's HSD post hoc test ($p < 0.05$).

ANOVA, Analysis of Variance; HSD, Honestly Significant Difference; SEM, Standard Error of the Mean; TNF- α , Tumor Necrosis Factor Alpha

Both levels of MFCP diets reduced IL-6 and TNF- α activity; the results for IL-6 and TNF- α are consistent with the findings of Zhu et al. [30], who recorded significantly lower values in laying birds fed fermented diets compared with birds fed unfermented diets. This aligns with existing research highlighting the benefits of dietary interventions in modulating inflammatory markers. Wang et al. [31] reported that individuals who underwent fibre, berry, whole wheat, and fatty acid dietary interventions showed decreased IL-6 levels compared with the control group, indicating that diets high in fibre can significantly lower IL-6. Katu et al. [32] also reviewed that fermented feeds can modulate cytokine production via improved gut microbiota composition and barrier function while reducing anti-nutritional burdens that otherwise provoke inflammation. Prebiotic and fermented feed studies further show enhanced total antioxidant capacity and cellular immune indices, supporting the claim that fermentation-derived metabolites can reduce systemic inflammation in broilers [29].

There was no significant difference in IL-10 and IL-12, indicating a shift toward a lower basal pro-inflammatory status without suppressing anti-inflammatory or Th1-related signalling. IL-12 plays a crucial role in immune responses, particularly in promoting the differentiation of T cells and enhancing the production of other cytokines. The lack of differences may imply that micro-fungi-fermented cassava peels do not adversely affect the immune response in broiler chickens, thereby maintaining a balanced inflammatory status [33].

The effect of *A. niger*-fermented cassava peels-replaced diets on activities of antioxidant indices such as GSH, CAT, SOD, and GST in the tissue analysis (breast and thigh) of broiler chickens following exposure to the diets for 6 weeks was studied and is shown in Table 5, Table 6, Figure 3, and Figure 4. Diets replaced with *A. niger*-fermented cassava peels exhibited significantly ($p < 0.05$) higher GST and SOD activities in the breast muscle of broiler chickens fed 50% MFCP diets (Figure 3c,d) compared with those of the control group, which were fed a normal diet.

Furthermore, the level of replacement (25% and 50%) affected the increase in GSH activity (Figure 3a and Figure 4a) in both muscles, especially in the 50% replacement diets. Tissue catalase activity in broiler chickens revealed that the replaced diets caused a decrease in breast tissue (Figure 3b) and an increase in thigh tissue (Figure 4b) catalase activity in birds fed the 25% replacement diets compared with those of the control and 50% groups.

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Parameter	T1 (Control) Mean ± SEM	T2 (25% MFCP) Mean ± SEM	T3 (50% MFCP) Mean ± SEM	F (df)	p-value (T2 vs T1/T3 vs T1/T2 vs T3)	Sig.
GSH (μmol/mg protein)	0.304 ± 0.007	0.241 ± 0.002	0.528 ± 0.015	231.5 (2, 3)	0.0399/0.0011/0 .0006	a b c
Catalase (mmol H ₂ O ₂ /min/mg protein)	0.84 ± 0.03	0.36 ± 0.02	0.66 ± 0.02	73.16 (2, 3)	0.0026/0.0474/0 .0094	a b c
SOD (units/mg protein)	5.43 ± 0.17	5.36 ± 0.09	6.81 ± 0.17	28.49 (2, 3)	0.9516/0.0159/0 .0139	b b a
GST (nmol/min/mg protein)	0.35 ± 0.04	0.54 ± 0.02	1.1 ± 0.03	126.7 (2, 3)	0.0601/0.0013/ 0.0030	b b a

TABLE 5: Antioxidant enzyme activities (breast muscle)

Means within a row that do not share a common superscript letter (a–c) differ significantly according to one-way ANOVA with Tukey's HSD post-hoc test ($p < 0.05$). Values are presented as mean ± SEM ($n = 3$ replicates per treatment; 10 birds per replicate). ANOVA, Analysis of Variance; HSD, Honestly Significant Difference; MFCP, *Aspergillus niger*-Fermented Cassava Peels; SEM, Standard Error of the Mean; GSH, Reduced Glutathione; SOD, Superoxide Dismutase; GST, Glutathione S-Transferase

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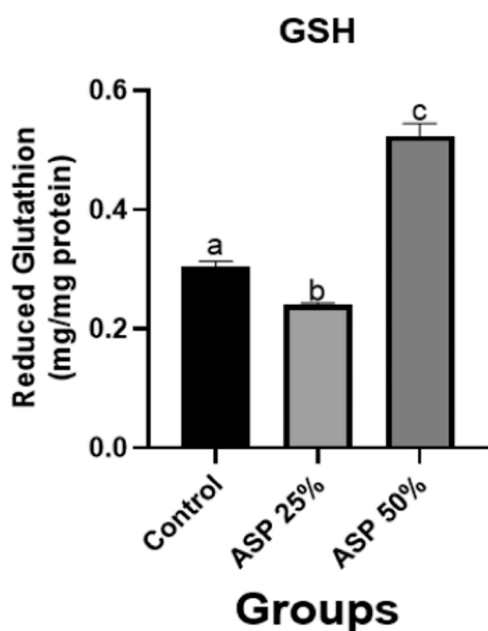


Figure 3a

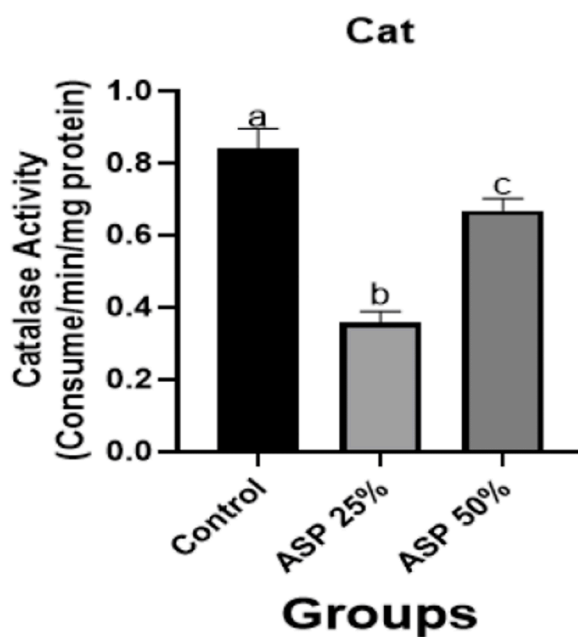


Figure 3b

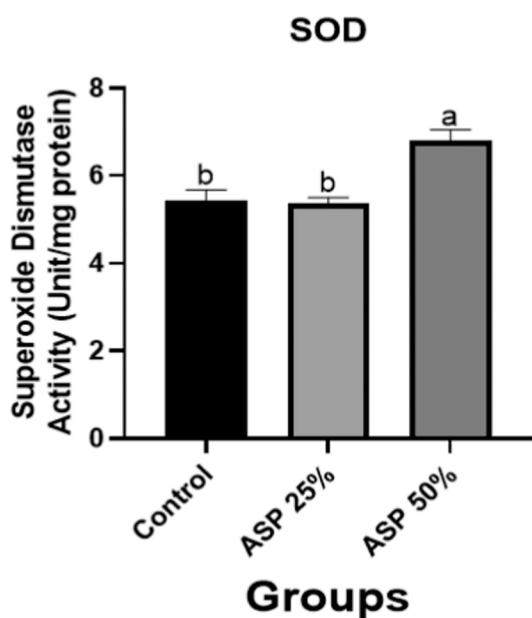


Figure 3c

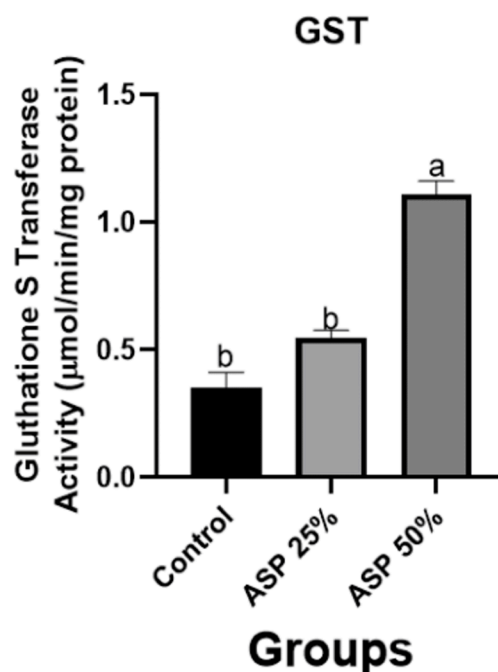


Figure 3d

How to cite this article:

FIGURE 3: Antioxidant status of broiler chicken breast muscle from chickens fed *Aspergillus niger*-fermented cassava peel replacement diets: (a) reduced glutathione (GSH), (b) catalase (CAT), (c) superoxide dismutase (SOD), and (d) glutathione S-transferase (GST)

Data are presented as mean $\pm$ SEM (n = 3 replicates per treatment). Bars with different superscript letters differ significantly according to one-way ANOVA followed by Tukey's HSD post hoc test (p < 0.05).

ANOVA, Analysis of Variance; HSD, Honestly Significant Difference; SEM, Standard Error of the Mean

Parameter	T1 (Control) Mean $\pm$ SEM	T2 (25% MFCP) Mean $\pm$ SEM	T3 (50% MFCP) Mean $\pm$ SEM	F (df)	p-value (T1 vs T2/T1 vs T3/T2 vs T3)	Sig.
GSH (μ mol/mg protein)	0.33 $\pm$ 0.01	0.52 $\pm$ 0.01	0.66 $\pm$ 0.01	121.5 (2, 3)	0.0064/0.0012/0 .0135	c b a
Catalase (mmol H ₂ O ₂ /min/mg protein)	1.17 $\pm$ 0.05	1.60 $\pm$ 0.01	1.10 $\pm$ 0.07	26.22 (2, 3)	0.0211/0.6433/0 .0137	b a b
SOD (units/mg protein)	32.59 $\pm$ 1.17	40.57 $\pm$ 2.41	15.31 $\pm$ 2.23	41.00 (2, 3)	0.1323/0.0183/0 .0062	a a b
GST (nmol/min/mg protein)	0.67 $\pm$ 0.01	0.53 $\pm$ 0.05	0.85 $\pm$ 0.03	19.47 (2, 3)	0.1420/0.0773/0 .0170	ab b a

TABLE 6: Antioxidant enzyme activities (thigh muscle)

Means within a row that do not share a common superscript letter (a–c) differ significantly according to one-way ANOVA with Tukey's HSD post-hoc test (p < 0.05). Values are presented as mean $\pm$ SEM (n = 3 replicates per treatment; 10 birds per replicate). ANOVA, Analysis of Variance; HSD, Honestly Significant Difference; MFCP, *Aspergillus niger*-Fermented Cassava Peels; SEM, Standard Error of the Mean; GSH, Reduced Glutathione; SOD, Superoxide Dismutase; GST, Glutathione S-Transferase

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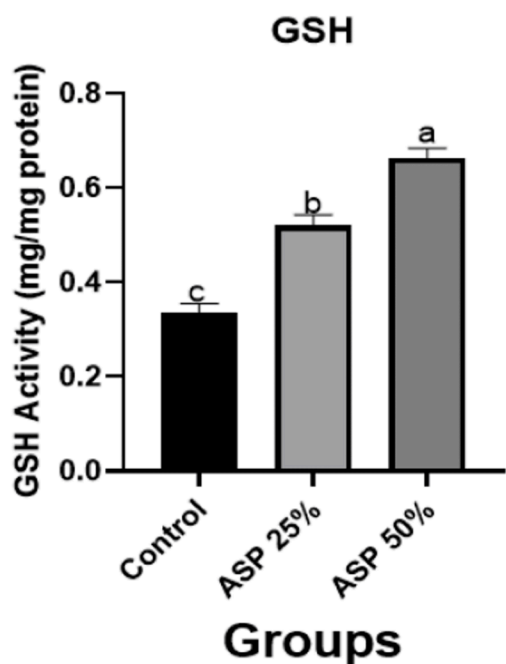


Figure 4a

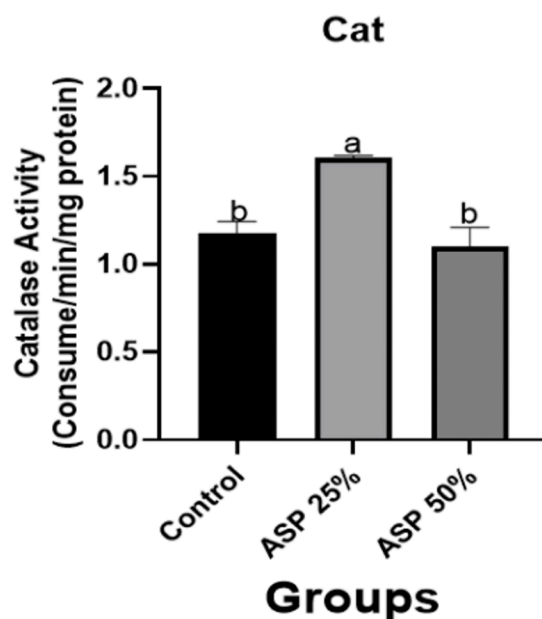


Figure 4b

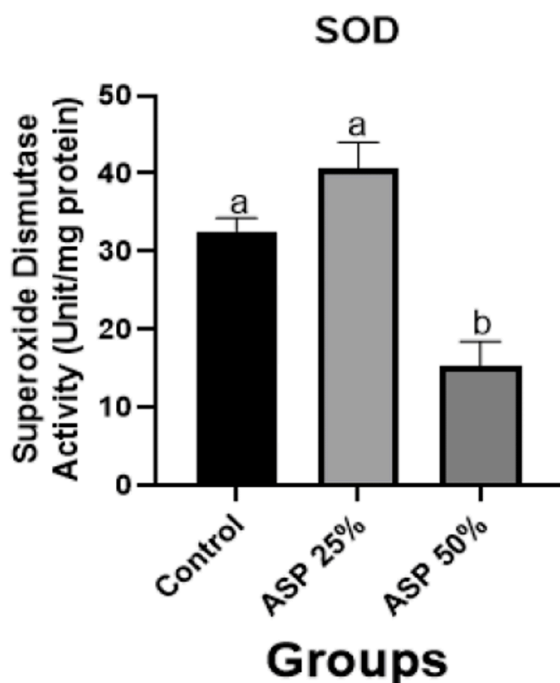


Figure 4c

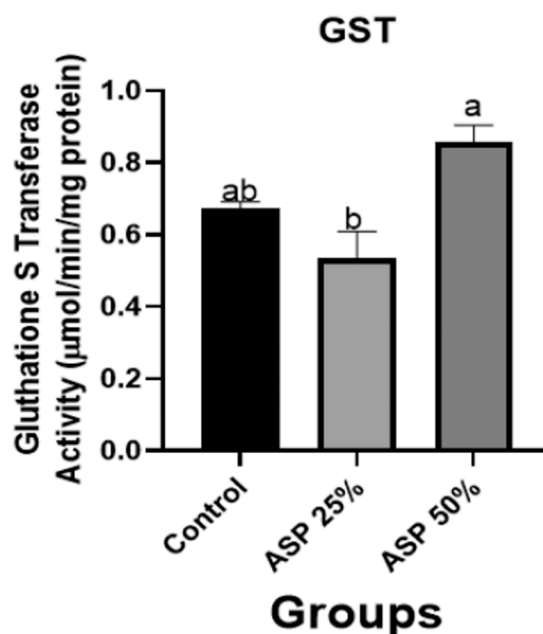


Figure 4d

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FIGURE 4: Antioxidant status of broiler chicken thigh muscle from chickens fed *Aspergillus niger*-fermented cassava peel replacement diets: (a) reduced glutathione (GSH), (b) catalase (CAT), (c) superoxide dismutase (SOD), and (d) glutathione S-transferase (GST)

Data are presented as mean $\pm$ SEM (n = 3 replicates per treatment). Bars with different superscript letters differ significantly according to one-way ANOVA followed by Tukey's HSD post hoc test (p < 0.05).

ANOVA, Analysis of Variance; HSD, Honestly Significant Difference; SEM, Standard Error of the Mean

Total antioxidant capacity can fully reflect the antioxidant potential of both enzymatic and non-enzymatic defence systems [34]. Antioxidant enzymes, including SOD and GSH-Px, eliminate superoxide anions and hydrogen peroxide in response to oxidative damage [34,35]. In the breast muscle, MFCP increased SOD and GST activities and elevated GSH (especially at 50%), while catalase declined at 25%. The increased GST activity observed in the breast and thigh tissues aligns with previous research highlighting the role of dietary antioxidants in enhancing detoxification pathways in poultry. Galal et al. [36] reported that phytochemicals from plants are known to induce GST activity, which plays a crucial role in detoxification and protection against oxidative stress, potentially contributing to cancer prevention.

In the thigh muscle, catalase increased at 25% MFCP, SOD peaked at 25% and then dropped at 50%, and GST rose at 50%, pointing to tissue-specific adjustments likely due to the differing oxidative demands of glycolytic (breast) and more oxidative (thigh) fibres, as well as inclusion-level-dependent redox pressure. The breast muscle of broilers is mainly composed of fast glycolytic fibres, which are more vulnerable to oxidative stress [37]. Similar results were reported by Zhu et al. [30], who observed higher antioxidant activity in laying hens fed fermented feed compared with those on unfermented diets. The findings are consistent with research by Salami et al. [38], which noted that an antioxidant-rich diet led to significant increases in SOD and catalase activities, thereby improving oxidative stress management in broilers.

Conclusions

Aspergillus niger-fermented cassava peels (MFCP) can replace a moderate inclusion of maize while supporting a satiety-lean endocrine profile, lower pro-inflammatory signalling, and enhanced antioxidant defences in muscle. These results suggest that a 25% maize replacement with *A. niger*-MFCP serves as a physiologically favourable option in standard broiler rations. However, the lack of companion data on growth performance, nutrient digestibility, carcass characteristics, and antinutritional factors (such as cyanide and mycotoxins) currently limits definitive practical recommendations. While higher substitution levels may be physiologically feasible if diets are carefully reformulated to equalize metabolizable energy and amino acid balance, full practical validation of MFCP as a maize replacement remains dependent on confirming these broader production, efficiency, and toxicological parameters.

Additional Information

Author Contributions

All authors have reviewed the final version to be published and agreed to be accountable for all aspects of the work.

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Disclosures

Plant subjects: All authors have confirmed that this study did not involve any herbarium specimen.

Human subjects: All authors have confirmed that this study did not involve human participants or tissue.

Animal subjects: The study involves the use of animal subjects, Research Ethics Committee, Centre for Research and Development, The Federal University of Technology, Akure ethics committee, protocol no FUTA/ETH/23/121.

Conflicts of interest: In compliance with [the ICMJE uniform disclosure form](#), all authors declare the following:

- **Payment/services info:** Payment was received from TETFUND-NRF, Nigeria.
- **Financial relationships:** All authors have declared that they have no financial relationships at present or within the previous three years with any organizations that might have an interest in the submitted work.
- **Other relationships:** All authors have declared that there are no other relationships or activities that could appear to have influenced the submitted work.

Data Availability Statements

The datasets (and/or code) supporting this study are available from the corresponding author upon reasonable request.

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