



Growth Performance and Lipid Profile of Broiler Chickens Fed Fermented Millet Stalks

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Authors' contributions

This work was carried out in collaboration among all authors. Author YAI designed the study, wrote the protocol, and wrote the first draft of the manuscript. Authors HMI, LIA, AA, MF and KM managed the analyses of the study. Authors MKI, SZA, KIH, SAA and NA managed the literature searches. Authors YAI and HMI performed the statistical analysis. All authors read and approved the final manuscript.

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ABSTRACT

High cost of feed production has been a persistent thorn in the flesh on the Nigerian poultry production sector. The price of maize a key source of carbohydrate in feed formulation is above board. The study revealed the potential of using *Aspergillus niger* fermented millet stalk (FMS) as

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maize replacement in broiler feed formulation. Millet stalk sample was fermented using *Aspergillus niger* at pH 7 and temperature of 35°C for 5 days to enhance its nutritional composition for inclusion in broiler feed formulation. Five diets were prepared with five different levels of FMS as maize replacement, namely 0% FMS (D1) 25% FMS (D2), 50% FMS (D3), 75% FMS (D4), and 100% (D5). The experimental Broilers were given the formulated diet for eight consecutive weeks. The Broilers were subjected to growth performance and lipid profile assessment. The mean weight, average daily weight gain and average weight gain of the studied chicken were highest in group D3 (2055g, 48.93g, 1630g) and least in group D4 (1741g, 41.45g, 1343g), the gain to feed ratio was highest in group D3 (2.78g) and least in group D5 (1.30g). The study findings demonstrated that FMS had a significant ($p < 0.05$) effect on average body weight gain, feed conversion ratio and growth rate. Significant influences were not observed for all the lipid profile parameters. The findings underscore the viability of fermented millet stalk as a sustainable and cost-effective substitute for maize in poultry diets.

Keywords: Broilers; millet; fermentation; feed; poultry; maize; food security.

1. INTRODUCTION

The poultry industry is a significant contributor to the global food supply, providing a valuable source of protein-rich meat and egg (Okike *et al.*, 2017). The goal of poultry production research is to reduce production costs while preserving poultry comfort and enhancing performance and product quality (Egbune and Tonukari, 2023). The Nigerian poultry production has been limited by the exorbitant cost of feed production which gulps up to 80% of total production cost, an ugly scenario affecting the level of chicken survival and the profitability of poultry farming (Yafetto, 2018, Azizi *et al.*, 2021, Ndego *et al.*, 2022). Katsina State in northern Nigeria the study area of this study has seen a rise in the prevalence of banditry and kidnapping that has led to a decrease in agricultural production. This has led to a continuous increase in the cost of food stuffs including the raw materials specifically maize for poultry feed formulation (Yaradua *et al.*, 2022). In feed production, maize is the carbohydrate source of choice. But its availability and affordability is usually affected by the supply chain. Leading to its demand usually exceeding its supply with a resultant increase of above 2000% over the last 2 decades (Ndego *et al.*, 2022). This gloomy condition has resulted to the high cost of poultry feeds and a sharp rise in poultry production cost. As a boomerang effect feed producers are left with no option but to scout for alternatives that will substitute for the conventional feed raw materials but at the same time maintaining quality and standards. Several initiatives have been implemented, such as finding cheaper and locally available materials as partially substitutable energy source instead of maize in poultry feed formulations (Ojo *et al.*, 2022).

The world generates a large volume of agro-waste byproducts that might be beneficial alternative feedstuffs (Egbune and Tonukari, 2023). Millet stalk, a by-product of millet production, is a potential alternative source of carbohydrates for poultry feed. Millet is a cereal crop that is widely grown in many parts of the world, and its stalk is rich in carbohydrates, proteins, and fiber (Adebayo-Oyetoro *et al.*, 2019). However, the use of millet stalk as a feed ingredient is limited due to its low digestibility and high fiber content (Pourreza *et al.*, 2017). Therefore the use of millet stalks as feed requires an appropriate technologic approach to increase its nutritional value. One strategy that can be done is to use it as fermented feed (Yanti *et al.*, 2020). Many previous studies have shown that feeding fermented feed had beneficial effects on growth performance in chickens (Lv *et al.*, 2020, Hamza and Gunyar, 2022, Zhu *et al.*, 2023).

Fermentation, one of the most ancient and economical methods of food preparation in the world, is defined as a technology in which microorganisms' growth and metabolic activities are used to preserve foods. It is an inexpensive process that requires comparatively little energy, and therefore it is the main strategy for food production in some cultures (Sanlier *et al.*, 2019). It is a process that enhances the nutritional value of food by enhancing the quality of proteins, improving the absorption of fiber, and increasing the synthesis of essential amino acids, vitamins, and proteins. It also facilitates micronutrient availability while reducing anti-nutritional compound levels (Egbune and Tonukari, 2023, Ndego *et al.*, 2022). The use of fermented feed products in poultry production has been reported extensively. Fermented feed enhanced

growth performance, antioxidant system and size of the immune organs (Zhu *et al.*, 2023, Olasehinde and Aderemi, 2023, Vandenberghe *et al.*, 2021).

There are two fermentation techniques: Solid state and submerged fermentation. Solid-state fermentation (SSF) is a process with a porous solid substrate or support for the growth of microorganisms with a continuous gas phase. It is arguably the most natural condition for the growth of microorganisms whose natural habitats are solid materials, such as plant and rock surfaces, soils and decomposing organic matter such as leaves, bark and wood (Vandenberghe *et al.*, 2021). To initiate solid state-fermentation (SSF), bacterial and fungal species that are generally recognized as safe (GRAS) organism are utilized (Egbune and Tonukari, 2023). During fermentation, microorganisms break down fermentable carbohydrates into end products such as organic acid, carbon dioxide, and alcohol, as well as anti-microbial metabolites such as bacteriocins that increase food safety by killing or inhibiting food-borne pathogens (Sanlier *et al.*, 2019).

In published works, there is paucity of results on the effect of fermented millet stalk inclusion in broiler chickens diet as carbohydrate source replacement for maize. As such, this work aimed to evaluate whether fermented millet stalk affects growth performance and lipid profile of broiler chickens.

The results obtained in the study will be potentially useful in the poultry production sector, by provision of a cheaper and viable maize replacement in broiler feed formulation with a focus on its effects on growth performance, nutritional value, and economic viability.

2. MATERIALS AND METHODS

2.1 Sampling Area

The study was carried out in Katsina State, Nigeria in the year 2024. The State is located between latitude 12°15'N and longitude of 7°30'E in the North West Zone of Nigeria, with an area of 24,192km² (9,341 sq meters). Katsina State has two distinct seasons: rainy and dry. The rainy season begins in April and ends in October, while the dry season starts in November and ends in March. The average annual rainfall, temperature, and relative humidity of Katsina State are 1,312 mm, 27.3°C and 50.2%, respectively (Yaradua *et al.*, 2019).

2.2 Sample Preparation

Millet stalks were obtained from farms located within Umaru Musa Yar'adua University Katsina, Katsina State, Nigeria. The millet stalks were sorted cleaned to remove any contaminants or foreign materials, they were sun dried for two weeks to reduce moisture content, which helps prevent mold growth during storage. The dried millet stalks were chopped into smaller pieces using a cutlass and then grinded into smaller particles using a grinding machine which increases the surface area. The grinded sample was treated using concentrated sulphuric acid (H₂SO₄) to reduce contamination and thus was used as the substrate.

2.3 Preparation of Fermentation Medium

Exactly 60 g each, of the sample were measured and placed into ten different fermentation vessels. 140mls of solution containing 28 g of peptone dissolved in 1000mls of distilled water, 0.28 g of Disodium Hydrogen Phosphate, 1.4 g of Sodium Chloride, was measured and placed into each fermentation vessel, this mixture was shaken vigorously and was then autoclaved at 121°C for 30 minutes.

2.4 Inoculum Preparation

A pure culture of *Aspergillus Niger* was obtained from a previously isolated strain at the laboratory in the department of Microbiology of Umaru Musa Yaradua University, Katsina State. The microorganism was subcultured in a Potato Dextrose Agar at 4°C, a sterilized loop was used to inoculate the microbe (3% v/v) into the fermentation medium containing the fermentation substrate, inoculum concentration was kept constant throughout the vessel. The fermentation was carried out at a pH of 7 and a temperature of 35°C for 5 days.

2.4.1 Experimental design for feed formulations and feeding trial

Two hundred and fifty broiler chicks were purchased from certified sale outlets in Katsina state. The broilers were raised from a day old on a starter feed (premix and maize). The recommended medications and vaccines was administered to ensure good health status of the experimental birds. Brooding was done for two weeks, after which the birds were weighed and divided into five groups in a completely randomized design and fed with the formulated

broiler feed. The broiler finisher feed was formulated with fermented millet stalk to replace maize in the premix /maize formulation; Diets 1 to 5 were produced using the following maize to fermented millet stalk as ratios: 4:0 (control), 3:1, 1:1, 1:3, and 0:4. Each diet was fed to each group for six weeks. Throughout the experiment, feed and water were provided *ad libitum*.

2.4.2 Growth performance and survival of experimental broilers

Each broiler was weighed daily and weight was recorded. The broiler samples were assessed after six weeks for growth performance analysis using the formulae recommended by Kari *et al.* (2021):

Survival rate (%) = (Total number of survived broilers / Total number of experimental broilers at the beginning of the experiment) × 100%

Weight gain (%) = (Final weight – initial weight) × 100% / initial weight

Specific growth rate (%) = (log Final weight – log initial weight) × 100% / Experiment duration

Feed conversion rate (FCR) = Total feed consumption / broiler weight gain

2.4.3 Blood sampling and biochemical analysis

At the end of the feeding trial, venous blood was taken with a sterile syringe and needle from pronounced veins in the experimental broilers' wings and transferred to a test tube. After allowing the blood to coagulate for a while, it was dislodged and centrifuged at 2000 g for 10 minutes to get the serum as supernatant. The supernatants were utilized for lipid profile investigations. Serum TC concentration was measured by the end point colorimetric method (Allain *et al.*, 1974). Friedewald *et al.* (1972) method was used to evaluate the serum HDL-Cholesterol. Serum LDL-Cholesterol concentration was measured using the method of Wiecland and Siedel, (1983). Tietz (1990) method was used to evaluate the serum Triglyceride concentration.

2.5 Statistical Analysis

The statistical analysis of this research was performed using the Statistical Package for the

Social Sciences (SPSS) version 26.0. The results were presented as mean ± SD in normally distributed data, median and interquartile range for data that is not normally distributed. The Broiler growth performances and lipid profile were evaluated using a one-way analysis of variance (ANOVA) and Kruskal Wallis test. A p-value < 0.05 was considered statistically significant.

3. RESULTS AND DISCUSSION

The current study evaluated the effects of fermented millet stalks at different inclusion levels on broiler chickens growth performance and lipid profile. The results of this study show significant variations in growth performance across the different experimental broiler chicken groups. The mean/median weight, average daily weight gain and average weight gain of the studied chicken were highest in group D3 and least in group D4, the gain to feed ratio was highest in group D3 and least in group D5 (Table 1). Likewise, from Table 1 the growth rate was highest in group D3 and least in groups D2 and D5. The feed conversion ratio was highest in group D3 and least in group D4 (Table 1). The study findings demonstrated that that FMS had a significant ($p < 0.05$) effect on average body weight gain, feed conversion ratio and growth rate. (Table 2). These observations are in agreement with the earlier work of Kumar *et al.* (2017) that reported a significant increase in body weight of broiler chickens fed with fermented feed, but differ from the report of Olasehinde and Aderemi, (2023) that reported no effect on growth on their experimental broilers fed with fermented pearl millet. Adebayo-Oyetero *et al.* (2019) reported a significant improvement in feed conversion ratio of broiler chickens fed with fermented feed, which is in agreement with the observation made in the current study. No mortality among the experimental broiler chickens was recorded.

Risk of diseases linked to fat metabolism in broiler chickens and consumers can best be understood by performing lipid profile test in broiler chickens. Table 3 exhibits the blood lipid profile of experimental broilers fed with the different formulated feeds. The group that was fed with the D5 feed demonstrated the highest total-cholesterol, followed by the D2 feed group. Conversely, the experimental broilers fed with D4 feed demonstrated the lowest total-cholesterol. Meanwhile, the D3 group recorded the highest mean triglyceride concentration.

Table 1. Growth performance of experimental broilers

Group	W (g)	ADWG (g)	ABWG (g)	FCR	GR	GFR	MR (%)
D1 _(n=50)	1898 ± 53.79	45.19	1461	0.55	3.35	1.83	0
D2 _(n=50)	1855 ± 177.	45.19	1413	0.70	3.20	1.47	0
D3 _(n=50)	2055 ± 220	48.93	1630	0.86	4.20	2.78	0
D4 _(n=50)	1741 ± 156	41.45	1343	0.36	4.00	2.76	0
D5 _(n=50)	1894 ± 206	46.14	1558	0.78	3.20	1.30	0

Key = W=Weight; ADWG =Average daily weight gain; ABWG =Average body weight gain; FCR =Feed conversion ratio; GR =Growth rate; GFR =Gain to feed ratio; MR =Mortality rate

Table 2. Comparison of growth performance of experimental broilers after feeding trial among groups

Parameter	Test statistics (A)	P- value
Mean Weight	182	0.046*
Average daily weight gain	0.861	0.91
Average body weight gain	119	0.037*
Growth rate	6.23	0.025*
Feed conversion ratio	0.012	0.041*
Gain to feed ratio	1.03	0.06

Key: A= ANOVA, *= statistically significant

Table 3. Lipid profile among groups of experimental broilers

Group	TC (mmol/l)	TG (mmol/l)	HDL-C (mmol/l)	LDL-C(mmol/l)
D1 _(n=25)	2.9 ± 0.7	3.1 ± 1.2	0.9 ± 0.1	2.7 ± 0.9
D2 _(n=25)	4.0 ± 0.6	2.0 ± 0.5	1.2 ± 0.2	2.6 ± 0.8
D3 _(n=25)	3.7 ± 1.0	3.4 ± 1.4	0.8 ± 0.1	2.8 ± 2.2
D4 _(n=25)	2.8 ± 0.5	2.3 ± 0.3	0.8 ± 0.1	2.8 ± 0.7
D5 _(n=25)	4.4 ± 0.9	2.5 ± 1.4	0.7 ± 0.1	3.7 ± 1.0

Values are presented as in mean ± Standard deviation; Key: TC = Total Cholesterol, LDL-C – Low density lipoprotein Cholesterol, HDL –C = High density lipoprotein Cholesterol, TG = Triglyceride

Table 4. Comparison of lipid profile among groups of experimental broilers

Parameter	Test statistic (F)	P-value
TC	1.15	0.29
HDL-C	0.124	0.78
LDL-C	0.818	0.69
TG	0.540	0.73

Key: F= Analysis of variance

On the other hand, the D2 feed fed group has the highest. All groups observed no significant difference in their lipid profile mean concentrations (Table 4). There was an observed increase in LDL-C level with increase in the inclusion ratio of the FMS, but the increase is not significant ($p=0.69$). High LDL is a risk factor of sudden deaths syndrome in broilers (Shariat *et al.*, 2013). There was also an observed reduction of the “good cholesterol” HDL-C. These findings differ from the reports of Shariat *et al.* (2013) and Johnson *et al.* (2023) that reported a reduction of

LDL-C and an increase in HDL-C concentrations in their broiler samples.

4. CONCLUSION

The current study has demonstrated that fermented millet stalk is a promising maize alternative that could improve the growth performance of broiler chickens. At 50% inclusion in the broiler formulated feed, fermented millet stalk enhanced the broiler growth performance. The study findings also

revealed a decline in growth performance when the fermented millet stalk level exceeded 50%. In addition, this study will provide the baseline information for researchers to discover the full potential of fermented millet stalk as a possible maize replacement in broiler chicken feed formulation.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models and text-to-image generators have been used during writing or editing of this manuscript.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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