DOI: https://doi.org/10.4314/njbmb.v39i2.7 NJBMB (2024) 39(2) 82-90

e-ISSN: 2659-0042

Nigerian Journal of Biochemistry and Molecular Biology

The Official Publication of the Nigerian Society of Biochemistry & Molecular Biology (NSBMB). Journal homepage: https://www.nsbmb.org.ng/journals/

Growth Performance and Toxicological Implications of Mixed Fungal Fermented Maize Cob Feed in Experimental Rats

Abbas Olagunju*[1], Elewechi Onyike[1], Danladi A. Ameh[1], Sunday E. Atawodi[2], Uche S. Ndidi[1*]

[1] *Department of Biochemistry, Ahmadu Bello University Zaria, Nigeria*
[2] *Department of Biochemistry, Federal University Lokoja, Nigeria*

OPEN ACCESS

***CORRESPONDENCE**
Olagunju, A.
nutribas@gmail.com,
aolagunju@abu.edu.ng
+2348038534580

ARTICLE HISTORY
Received: 19/01/2024
Reviewed: 06/04/2024
Revised: 15/04/2024
Accepted: 01/05/2024
Published: 30/06/2024

CITATION
Olagunju, A, Onyike, E., Ameh, D.A., Atawodi, S.E. and Ndidi, U.S. (2024). Growth Performance and Toxicological Implications of Mixed Fungal Fermented Maize Cob Feed in Experimental Rats. *Nigerian Journal of Biochemistry and Molecular Biology.* 39(2), 89-90
https://doi.org/10.4314/njbmb.v39i2.7

ABSTRACT

Mixed fungal fermentation was carried out to upgrade the nutritive value of the maize cobs for use as inclusion in developing a balanced feed for animal production. Grounded maize cobs were alkaline pretreated, and fermented with mixed and single lignocellulolytic fungi of *Lachnocladium flavidum* and *Aspergillus niger*. A feeding experiment assessed the growth performance and toxicological implications of graded levels (10%, 30%, and 50%) of fungal fermented maize cobs following their inclusion in the diet. Feed substituted with 10% *L. flavidum.* was found to have the most positive effect on animal weight and the growth rate, while for the feed consumption and the efficiency of feed conversion, statistical difference was not observed among the various substituted feeds. Toxicity studies reveal absolute and relative organ weights were stable with slight differences among substituted fermented-fed groups relative to the control. Liver and kidney weight appeared normal in various fermented substituted groups. Most markers of liver function analyzed appeared normal, glucose levels however increased significantly (P<0.05) in most of the fermented-fed groups. Serum electrolytes as markers of kidney function also showed a stable condition while urea and creatine levels increased significantly (P<0.05) but within the normal range. Hematological parameters did not show compromised values as all appeared within acceptable ranges. This research has shown that 10% *L. flavidum* and *A. niger* fermented maize cobs have been demonstrated to be efficacious and safe as a substitute for inclusion in animal feed production.

Keywords: *Mixed fermentation; Maize cobs; Growth; Liver function; Kidney function; Hematology*

INTRODUCTION

With the growing population, livestock feeding using grains-based diets is becoming increasingly challenging thus the need to source and re-focus attention on providing energy in feeding livestock and monogastric animals. Feed has been reported to contribute up to 80% of the total costs in livestock production (Kim *et al.,* 2021). The increasing demand for grains as raw materials in the production of high-value commodities is partly responsible for driving food and feed prices higher than ever recorded in history (Yafetto *et al.*, 2020). The major constraint to livestock production in Nigeria is the non-availability of sufficient feed throughout the year (Biaosheng *et al.,* 2020).

One of several alternatives considered in this direction, which has proved successful over the years, is the utilization of agro-industrial by-products in the production of monogastric feed (Atuahene *et al.*, 2000). The lignocellulosic residue is the cheapest and readily available form of carbohydrates for value addition (Bharathiraja *et al.*, 2017). However, the current level of use of agricultural agro-residues in animal feed is very low, and high utilization has been reported to produce poor performance (Yafetto *et al.*, 2020).

Maize is one of the most important cereal crops in sub-Saharan Africa and a major staple food crop in many parts of the world including Nigeria (USDA 2021). Nigeria is the twelfth-largest producer of maize in the world and the second-largest producer in Africa (USDA 2021). Maize production in Nigeria increased from 1.4 million tons in 1969 to 8 million tons in 2015 which further increased to 20 million in 2019, growing at an average annual rate of 6.72% (Oge, 2019). Maize cobs are a by-product of the maize crop, about 180-200 kg of cobs are obtained from each ton of maize shelled (Heuze *et al.*, 2017). The major components of corn cob include cellulose (45%), hemicellulose (35%) and lignin (15%) (Zhao *et al.*, 2022). However, the high amount of lignin content and level of crystallization of the residues limits optimal utilization as Lignin interferes by acting as a physical barrier that prevents the contact of cellulase with cellulose and other nutrients (Ravindra and Jaiswal, 2016).

The wider utilization of agricultural residues by livestock is affected by their compositions which necessitate different physical, chemical, and biological pretreatment processes (Xu *et al.*, 2022). Biological treatment using microorganisms has several advantages when compared with physical and chemical methods (Ma *et al.,* 2015). Several microorganisms such as *Aspergillus niger, Trichoderma reesei, Lachnocladium sp.*, and *Lenzites betulina* are good lignocellulolytic fungi (Andlar *et al.,* 2018). Solid-state fermentation (SSF) has been considered to be the most effective in producing value-added products from agricultural residues. However, optimization of the process parameters is necessary to achieve optimal results (Leite *et al.,* 2021).

With the increasing demand for livestock products in the world, the economy, and shrinking land area, the future hope of feeding the nations and safeguarding their food security will depend on better utilization of non-conventional feed resources, which are not used as food for humans or are not in high demand for human consumption (UNEP, 2021). There is a need to develop cheap, easy, and environmentally friendly methods of treating lignocellulosic agricultural residues like maize cob to improve nutritional quality for possible utilization in animal feed formulation (Zhao *et al.*, 2022). Mixed fungal fermentation using a consortium of degrading fungi was carried out to upgrade the nutritive value of the maize cobs for use as inclusion in developing a balanced feed for animal production and ascertain the efficacy of its usage.

MATERIALS AND METHODS

Animals feeding trials

Wistar albino rats, obtained from the laboratory animal units of the Faculty of Veterinary Medicine, Ahmadu Bello University, Zaria, Nigeria were used for the experiment. The animals were kept in a well-ventilated stainless-steel cage at room temperature and were acclimatized for 14 days, normal feed and clean drinking water were provided to them. Forty-two (42) well–fed fresh weaner's male rats of average age (5-6 weeks) and weight (80-90g) were used for this feeding trial. They were divided into seven groups of six rats each. Diets were formulated as isonitrogenous (appx. 19% crude protein) and isocaloric (appx. 4 kcal/g). Control diets were substituted with untreated maize cobs at 50% and treated maize cobs, at 10%, 30%, and 50%. Group 1 - *10% L. flavidum & A. niger,* Group 2 - *30% L. flavidum & A. niger,* Group 3 - *50% L. flavidum & A. niger,* Group 4 - *50% Lachnocladium flavidum,* Group 5 - *50% Aspergillus niger,* Group 7-control (commercial feed).

Spilt feed left over in the morning was collected, dried, and weighed. The level of feed intake or acceptability in g/day was determined by monitoring the decrease in the quantity of weighed feed served. Clean water was also provided in calibrated containers for four weeks. The rats were then weighed after the feeding trial to see changes in live weight before they were sacrificed and their blood collected for analysis. The feed consumption index and conversion ratio were calculated as shown below (Yi *et al* 2018);

$$\textit{Feed Consumption index} = \frac{\textit{feed consumed}}{\textit{average weight}} \times \textit{duration of feeding}$$

$$\textit{Feed Conversion ratio} = \frac{\textit{weight gain}}{\textit{feed consumed}}$$

Blood collection and sample preparation

At the end of 28 days of feeding trials for growth and performance, the animals were anesthetized by using chloroform after fasting for 12 hours. They were bled by cardiac puncture and a lateral section was cut through each. The kidney, liver, and heart were removed, blotted dry with filter paper, and weighed. Part of the whole blood was placed in test tubes containing EDTA and used for packed cell volume, hemoglobin (Hb), white blood cell (WBC) and red blood cell (RBC) counts determination. The remaining blood was allowed to clot and the serum was separated using a Pasteur pipette into cleaned labeled serum sample bottles. Serum was used for biochemical analysis.

Determination of hematological parameters in experimental rats

Red blood cell count (RBC), Packed Cell Volume (PCV), White blood cell count (WBC), and the hemoglobin (Hb) content of blood, were determined by methods as described by Dacie and Lewis, 1984.

Determination of biochemical parameters

Markers of liver function were determined using standard methods. Serum albumin was determined by the dye-binding method (Doumas e*t al.,* 1971), Serum protein concentration was estimated by the method of Plummer (1978), serum glucose was determined by the glucose oxidase method (Bauminger, 1974), serum total bilirubin, glutamine oxaloacetic transaminase (SGOT) and glutamine pyruvate transaminase (SGPT) were determined by the colorimetric methods of Reitmans and Frenkel (1957). Cholesterol was determined after enzymatic hydrolysis and oxidation (Abell *et al.*, 1952). Markers of kidney function were determined using standard methods. Urea was determined using the Diacetyl monoxamine method as described by Bhavadasan (1982), and creatinine was determined using the Jaffe method of Joris and Marijin (2011). Serum sodium and chloride electrolyte were determined using the colorimetric method (Tietz *et al.*, 1974). Bicarbonate (PEPC Method) by phosphoenolpyruvate carboxylase (Punzalan, 1990).

Statistical analysis

To address the biological variability of the samples, the experiment was repeated three times, and the results were expressed as mean ± standard deviation. Differences between the groups were analyzed by one-way analysis of variance (ANOVA) with the aid of the Statistical Package SPSS Version 26. $P<0.05$ were considered statistically significant for differences in mean using the least significant difference (LSD).

RESULTS

Effects of graded levels of fermented maize cobs on growth performance of experimental rats are shown in Table 1. The most significant effect on weight gain was observed in the group fed with 10% *L. flavidum/A. niger* included fermented cobs (29.00±2.00g). This was seen to decrease as the percentage of included fermented feed was increasing. The effects on growth rate followed similar pattern as the weight gain; group fed with 10% *L. flavidum /A. niger* included fermented cobs was seen to have the most significant effect on growth rate 1.77±0.50g/week when compared to the effect in rats fed with unfermented included feed (0.40±0.30g/week). The commercial feed/ control however gave a much higher weight gain and growth rate as compared to all fermented substituted fed groups. With the consumption index, statistical difference was not observed with 10%, 30% and 50% fermented cobs substituted groups. The unfermented substituted fed groups however showed a slightly positive increase in the consumption index as well as the groups fed commercial feed groups. In the feed conversion ratio, statistical difference (P>0.05) was not observed among all fermented substituted groups as well as the commercially fed groups.

Table 1. Effects of Feeding Graded Levels of Fermented Maize Cobs on the Growth of Experimental Rats

	Weight gain (g) (4wks)	Growth Rate (g/wk)	Consumption Index	Feed Conversion Ratio
50% Unfermented cobs	4.00 ± 1.00^{a}	0.40 ± 0.30^{a}	10.24 ± 1.00^{e}	8.51 ± 0.78^{a}
10% Lach. flavidum & A. niger	29.00 ± 2.00^{e}	1.77 ± 0.50^{d}	6.50 ± 0.40^{b}	12.96 ± 0.65^{c}
30% Lach. flavidum & A. niger	21.00 ± 2.00^{d}	1.47 ± 0.56^{d}	6.80 ± 0.35^{b}	11.71 ± 0.61^{bc}
50% Lach. flavidum & A. niger	10.00 ± 1.00^{bc}	0.73 ± 0.40^{c}	7.50 ± 0.50^{bc}	11.81 ± 0.95^{bc}
50% Lachnocladium flavidum	12.67 ± 2.31^{c}	0.73 ± 0.30^{c}	9.05 ± 0.57^{d}	11.23 ± 0.90^{b}
50% A. niger	11.51 ± 1.00^{bc}	0.71 ± 0.22^{c}	7.99 ± 0.45^{bc}	11.33 ± 0.43^{b}
Control (commercial feed)	32.67 ± 3.06^{e}	1.85 ± 0.70^{e}	$10.0.49\pm0.25^{a}$	11.50 ± 0.80^{bc}

Values are Mean ± SD, Values with different superscript letters down the column are significantly different at p <0.05

The pattern of the effects of graded levels of fermented maize cobs on weekly Feed Consumption Index of experimental rats shown in Figure 1, did not show statistically significant difference in the various groups on a weekly basis. The fourth week of consumption however showed slight positive increase in the consumption. With the feed conversion ratio (Figure 2), a progressive increase was observed in all fermented substituted groups as the weeks progressed.

Table 2 shows the effects of graded levels of fungal fermented maize cobs on absolute and relative organ weight of experimental rats. The percentage weight of the liver, kidney and heart were determined after the period of experimental trials. Statistical difference was not observed in the liver and kidney of experimental rats fed with fermented substituted maize cob feed. However statistically significant reduction (P<0.05) was observed in the group fed with unfermented substituted maize cobs. Significant reduction was also observed with the heart of rats fed with 30% *L. flavidum / A. niger* fermented maize cob substituted feed.

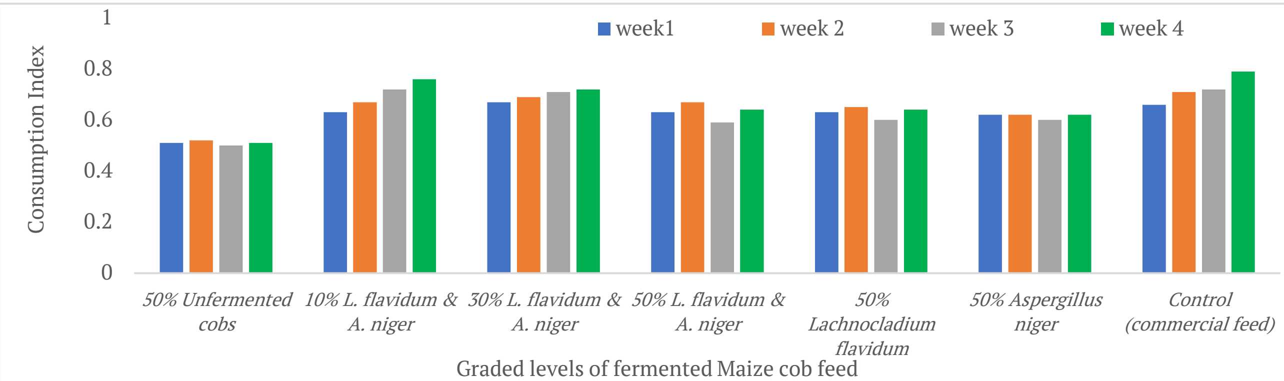

Figure 1. Effects of Graded Levels of Fermented Maize Cobs on Feed Consumption Index of Experimental Rats

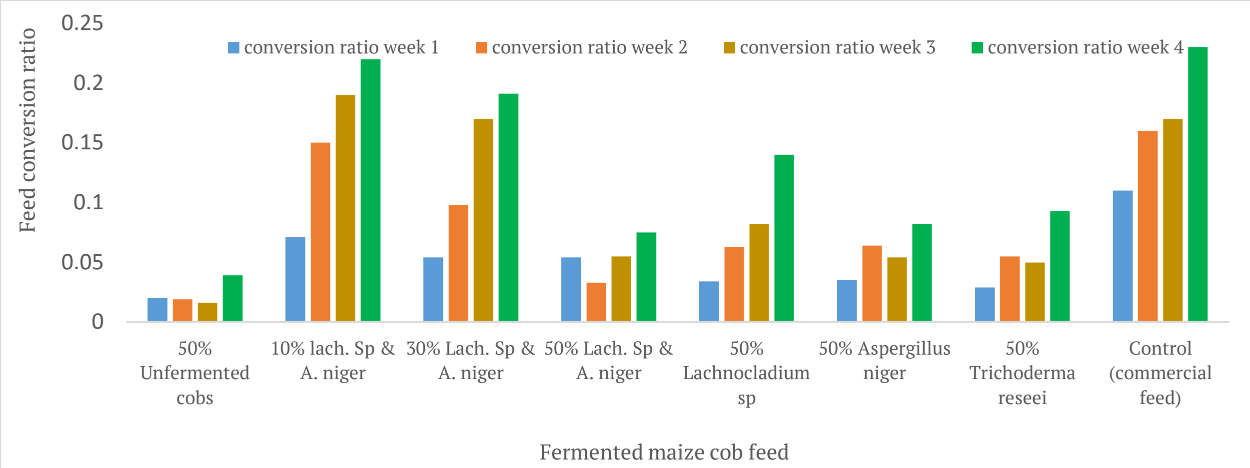

Figure 2. Effects of Graded Levels of Fermented Maize Cobs on Feed Conversion Ratio of Experimental Rats

Table 2. Effects of Graded Levels of Fungal Fermented Maize Cobs on Organ Weight of Experimental Rats

	Absolute organ weight				Relative organ weight		
	Body wt (g)	Liver (g)	Kidney (g)	Heart (g)	Liver (%)	Kidney (%)	Heart (%)
Control (commercial feed)	120.00 ± 5.00e	5.96 ± 0.80^{c}	0.86 ± 0.17^{a}	0.81 ± 0.12^{c}	4.97 ± 0.16^{a}	0.72 ± 0.06^{b}	0.68 ± 0.03^{b}
10% Lach. flavidum & A. niger	115.00 ± 4.50^{e}	5.11 ± 0.65^{b}	0.73 ± 0.20^{b}	0.80 ± 0.10^{c}	4.44 ± 0.1^{b}	0.63 ± 0.04^{ab}	0.70 ± 0.07b^{b}
30% Lach. flavidum & A. niger	100.00 ± 4.00^{c}	5.19 ± 0.45^{bc}	0.70 ± 0.30^{b}	0.78 ± 0.14^{cb}	5.19 ± 0.13^{a}	0.70 ± 0.10^{b}	0.78 ± 0.04^{b}
50% Lach. flavidum & A. niger	96.00 ± 3.00^{bc}	4.86 ± 0.40^{ab}	0.65 ± 0.20^{a}	0.70 ± 0.20^{b}	5.06 ± 0.08^{a}	0.68 ± 0.03^{b}	0.73 ± 0.03^{b}
50% Lachnocladium flavidum	105.00 ± 2.00^{c}	4.98 ± 0.50^{ab}	0.69 ± 0.10^{ab}	0.82 ± 0.06^{c}	4.74 ± 0.07^{b}	0.66 ± 0.06^{b}	0.78 ± 0.04^{b}
50% Aspergillus niger	97.00 ± 3.00^{bc}	4.39 ± 0.35^{ab}	0.61 ± 0.24^{a}	0.72 ± 0.10^{bc}	4.53 ± 0.04^{ab}	0.63 ± 0.04^{ab}	0.74 ± 0.04^{b}
50% Unfermented cobs	87.00 ± 1.00^{a}	4.44 ± 0.30^{ab}	0.51 ± 0.09^{a}	0.50 ± 0.05^{a}	5.10 ± 0.05^{a}	0.59 ± 0.03^{a}	0.59 ± 0.02^{a}

Values are Mean ± SD, Values with different superscript letters down the column are significantly different at p <0.0

Table 3 shows the effects of diets containing graded levels of fungal fermented maize cobs on some serum biochemical liver function parameters. Significant differences at P<0.05 observed among some parameters in the experimental groups. Significant increases were observed in total protein, AST, total bilirubin and glucose in most fed groups as compared to unfermented groups. The commercially fed group however had similar increment in these parameters, while reduction were seen to be significant (P<0.05) in cholesterol concentrations especially with 10% fed *Lach. flavidum*. Statistical differences where not observed in albumin levels (P>0.05). Table 4 shows the effects of diets containing graded levels of fungal fermented maize cobs on some serum Biochemical parameters of kidney function. Chloride, sodium, bicarbonate, urea and creatinine analyzed in the serum group of all experimental rats show significant difference (P<0.05) among the various substituted fermented groups in comparison with the control and unfermented groups. The differences observed where however within the normal acceptable ranges.

The effects of graded levels of fungal fermented maize cobs on hematological parameters in experimental rats is shown in Table 5. The packed cell volume (PCV), the red blood cells count (RBC), the hemoglobin (Hb), the Platelets and white blood cell (WBC) count were all found within the normal range. However statistical difference (P<0.05) in the WBC was observed among the groups fed with graded levels of fermented maize cobs as compared to the mono-unfermented groups. The differential count on the white blood cells shown in Table 6 also followed similar pattern as other hematological parameters, as no statistical difference was observed (P>0.05) in the lymphocytes, basophils, mesophils, eosinophils and neutrophils among all maize cob fermented, unfermented and control groups.

Table 3: Effects of Diets Containing Graded Levels of Fungal Fermented Maize Cobs on Some Serum Biochemical Indices of Liver Function

	Total protein (g/dl)	ALT(U/l)	AST (U/l)	T Bilirubin (mg/dl)	Glucose (mg/dl)	Albumin (g/dl)	Cholesterol
Control (commercial feed)	5.12±0.24^{ab}	14.00±2.00^{ab}	66.00±9.00^{ab}	0.43±0.09^{a}	107.51±12.50^{cd}	3.80±0.30^{ab}	(mg/dl)
10% Lach. flavidum & A. niger	6.33±0.75^{c}	13.40±0.70^{ab}	64.00±5.20^{ab}	0.49±0.11^{a}	105.34±7.80^{c}	4.50±0.50^{b}	45.06±8.10a
30% Lach. flavidum & A. niger	5.19±0.56^{ab}	14.90±0.74^{b}	67.30±4.90^{bc}	0.54±0.13^{ab}	115.58±6.20^{cd}	3.90±0.70^{ab}	42.00±5.00a
50% Lach. flavidum & A. niger	5.46±0.46^{ab}	16.20±0.50^{d}	77.50±5.00^{c}	0.60±0.10^{b}	120.22±7.30^{d}	3.85±0.55^{ab}	54.00±4.00b
50% Lach. flavidum	6.15±0.60^{b}	15.60±0.65^{c}	73.90±3.50^{bc}	0.49±0.19^{a}	89.55±5.50^{b}	4.43±0.31^{b}	59.00±6.00b
50% Aspergillus niger	4.98±0.40^{a}	14.30±0.60^{ab}	72.10±5.20^{bc}	0.53±0.08^{b}	74.67±4.60^{a}	4.11±0.61^{ab}	44.00±4.00a
50% Unfermented cobs	6.88±0.65^{d}	17.10±0.90^{e}	56.40±3.80^{a}	0.39±0.210^{a}	69.22±3.90^{a}	3.26±0.43^{a}	60.00±6.00c

Values are Mean ± SD, Values with different superscript letters down the column are significantly different at $p < 0.05$

Table 4. Effects of Diets Containing Graded Levels of Fungal Fermented Maize Cobs on Some Serum Biochemical Parameters (Kidney Function)

	Chloride (Cl^-) (mmol/L)	Sodium (Na^+) (mmol/L)	Bicarbonate (HCO_3^-) (mmol/L)	Urea (mg/dl)	Creatinine (mg/dl)
Control (commercial feed)	95.4±2.64^{ab}	132.5±2.60^{a}	16.80±0.30^{a}	14.00±1.20^{a}	0.49±0.02^{a}
10% Lach. flavidum & A. niger	94.3±1.22^{ab}	145.3±1.80^{b}	17.5±0.50^{ab}	16.00±1.80^{ab}	0.52±0.05^{ab}
30% Lach. flavidum & A. niger	98.5±0.56^{ab}	135.5±1.20^{a}	19.9±1.70^{b}	17.00±2.00^{ab}	0.64±0.20^{ab}
50% Lach. flavidum & A. niger	95.4±0.46^{ab}	140.2±2.30^{c}	16.5±0.55^{a}	15.00±1.50^{ab}	0.71±0.30^{ab}
50% Lach. flavidum	88.05±1.70^{bc}	136.5±1.50^{bc}	20.3±1.31^{b}	18.00±2.00^{b}	0.79±0.20^{ab}
50% Aspergillus niger	89.2±0.40^{cb}	141.7±0.90^{cb}	15.1±0.91^{a}	18.50±1.80^{c}	0.70±0.10^{ab}
50% Trichoderma reseei	90.4±1.50^{cb}	133.4±1.10^{a}	14.3±0.72^{a}	20.00±1.00^{d}	0.72±0.08^{ab}
50% Unfermented cobs	84.8±3.65^{d}	98.2±3.90^{d}	13.6±0.43^{a}	19.80±1.45^{d}	0.83±0.21^{b}

Values are Mean ± SD, Values with different superscript letters down the column are significantly different at $p < 0.05$

Table 5. Effects of Graded Levels of Fungal Fermented Maize Cobs on Hematological Parameters of Experimental Rats

	RBC ($x10^{12}$/L)	Hb (g/dl)	Plat ($x10^9$/L)	WBC ($x10^9$/l)
Control (commercial feed)	5.94±0.60^{b}	14.50±1.20^{a}	161.40±9.00^{a}	9.76±0.80^{b}
10% Lach. flavidum & A. niger	5.02±0.89^{ab}	13.70±2.30^{a}	157.20±10.00^{a}	10.30±2.70^{b}
30% Lach. flavidum & A. niger	4.98±0.80^{ab}	13.90±1.60^{a}	155.90±10.00^{a}	9.12±2.00^{b}
50% Lach. flavidum & A. niger	4.99±0.90^{ab}	14.40±1.60^{a}	149.40±15.00^{a}	8.92±2.40^{b}
50% Lachnocladium flavidum	4.53±0.70^{ab}	14.10±3.80^{a}	153.80±17.00^{a}	5.35±0.80^{a}
50% Aspergillus niger	4.62±0.90^{ab}	13.50±1.50^{a}	152.90±16.00^{a}	5.22±0.80^{a}
50% Trichoderma reseei	4.49±1.20^{ab}	13.90±2.30^{a}	154.70±12.40^{a}	5.99±0.60^{a}
50% Unfermented cobs	4.27±0.50^{a}	13.10±1.70^{a}	150.10±10.40^{a}	5.01±0.15^{a}

Values are Mean ± SD, Values with different superscript letters down the column are significantly different at $p < 0.05$

Table 6. Effects of Graded Levels of Fungal Fermented Maize Cobs on Differential Count of Experimental Rats

	Lymp (%)	Baso (%)	Meso (%)	Eosin (%)	Neut (%)
Control (commercial feed)	75.40±12.00^{a}	0.26±0.04^{a}	0.18±0.02^{a}	2.17±0.40^{a}	22.60±1.80^{ab}
10% Lach. flavidum & A. niger	74.50±14.50^{a}	0.31±0.08^{a}	0.16±0.09^{a}	2.11±0.90^{a}	20.50±4.50^{ab}
30% Lach. flavidum & A. niger	75.90±15.00^{a}	0.33±0.08^{a}	0.15±0.03^{a}	1.98±0.40^{a}	26.90±5.00^{b}
50% Lach. flavidum & A. niger	75.20±15.00^{a}	0.29±0.10^{a}	0.11±0.02^{a}	1.77±0.40^{a}	22.80±5.00^{ab}
50% Lachnocladium flavidum	74.90±15.00^{a}	0.30±0.04^{a}	0.15±0.01^{a}	1.65±0.40^{a}	23.80±5.00^{ab}
50% Trichoderma reseei	72.40±11.40^{a}	0.26±0.02^{a}	0.13±0.03^{a}	2.13±0.12^{a}	19.90±1.30^{ab}
50% Unfermented cobs	72.50±7.35^{a}	0.24±0.01^{a}	0.11±0.02^{a}	1.59±0.04^{a}	19.20±1.92^{a}

Values are Mean ± SD, Values with different superscript letters down the column are significantly different at p <0.05

DISCUSSION

Growth, diet, taste preference, and performance as key indicators of dietary adequacy and monitoring performance of experimental animals concerning expected patterns of weight gain and growth are important factors that may affect many types of experiments, especially those measuring food intake (Benli *et al.,* 2021). The results of preliminary feeding trials conducted for four weeks to study the effect of graded levels of fermented maize cobs on the growth and growth rate of experimental rats are in agreement with previous reports by Clement *et al.* (2020). The most significant effect on weight gain was observed in the group fed with 10% *L. flavidum /A. niger* included fermented cobs. This was seen to decrease as the percentage of included fermented feed was increasing. The average weight of the rats indicated that all test groups increased in weight throughout the feeding but showed a period of stunted growth towards the end of the trials. This could be due to a reduction in feed conversion with the increase in the age of the animals (Nasri et al., 2017). The 10% fermented fed group had a better weight gain compared to the 20%, 30%, and 50% fed groups probably due to a reduction in the feed efficiency ratio as the percentage of the less nutritional component of maize fermented cobs is increased. The decrease could also be due to poor utilization of a diet containing higher crude fiber (Sauvant *et al.,* 2004). This result agreed with that of Opara (1996) and Iyayi (2001) who observed that additional levels of fiber in the diets of animals depressed growth. Animals fed commercial feed and serving as the control however gave a much higher weight gain and growth rate as compared to all maize cob fermented substituted fed groups.

With the consumption index and feed consumption ratio, statistical difference was not observed with 10%, 30%, and 50% fermented corn cobs substituted groups. The unfermented substituted fed groups however showed a slightly positive increase in the consumption index as well as the groups fed commercial fed. The fourth week of consumption however showed a slight positive increase in the consumption. This could probably be due to improved adaptation to available feed as the feeding trials proceeded and lasted for 28 days. The fairly high feed intake observed could be due to the low levels of anti-nutritional factors caused by the effects of fermentation and also due to dietary enrichment through the development of diversity of flavor as reported by Steinkraus (2004), and Mcfeeters (2004). With the feed conversion ratio, a progressive increase was observed in all fermented substituted groups as the weeks progressed.

The percentage weight determined after the period of experimental trials did not show a statistical difference (P<0.05) in the liver and kidney of experimental rats fed with fermented substituted maize cob feed. However statistical difference (P<0.05) was observed in the group fed with unfermented substituted maize cobs. A slight difference was also observed with the hearts of rats fed with 30% *L. flavidum/A. niger* fermented maize cob substituted feed. It was observed that increasing the levels of the fungal-treated agro wastes in animal diets up to 50% had no significant effect P<0.05 on the liver, kidney, and heart relative weights when compared with the control. This could suggest that some key metabolic functions such as nutrients, transports, and excretion of metabolic products were not compromised (Elzubier and Jubarah, 1993). These findings were in agreement with that of Nasri *et al.* (2017) who reported Effect of age on feed efficiency and carcass yield characteristics of indigenous bull. Ogbonna *et al.* (1997) reported a decrease in gizzard, liver, and caecum weights of broilers fed increasing levels of cassava leaf meal. Deaton *et al.* (1979) also reported that gizzard and liver weights as a percentage of body weights were significantly influenced by dietary energy and fiber content of the diet. However, Elzubier and Juarah (1993), reported an increase in liver and viscera relative weights with an increase in the level of sorghum germ meal in broilers diet. The present study did not establish any relationship between the level of fungal-treated agro wastes and organ weights. This could be due to the biological modification of the agro wastes as a function of fungal treatment (Karunananda *et al.*, 1992). It can, however, be safely assumed that the fungal-treated agro wastes had no adverse effects on the liver and kidney of the animals.

Hematological parameters have commonly been used as indicators of physiological conditions and nutritional deficiency in animal dietary trials. The changes in hemoglobin concentration, erythrocyte count, hematocrit level, and differential leukocytes may indicate stress (Borges *et al* ., 2004), while the changes in erythrocyte, hemoglobin, and packed cell volume may reflect an alteration of energy status (Karadeniz *et al.,* 2008). Aside from the physiological and nutritional aspects, hematological variables can also be used as an indicator of health status and better disease resistance and immune response (Hrabčáková *et al.,* 2014). Indeed, several factors have been shown to influence the hematological variables including species, age, sex, environment, nutrition, infection, and physiological conditions (Borges *et al.,* 2004). In this present work, the effect of graded levels of fungal fermented maize cobs on hematological parameters in experimental rats shows the packed cell volume (PCV), the red blood cells (RBC), the hemoglobin (Hb), the platelets and white blood cell (WBC) count were all found within the normal range and no statistical difference was observed among the groups fed with graded levels of fermented maize cobs. No statistical difference was also seen when the fermented groups were compared with the control. The results of differential count on the white blood cells also followed a similar pattern as other hematological parameters, as no statistical difference was observed at P>0.05 on the lymphocytes, basophils, mesophiles, eosinophils, and neutrophils among all maize cob fermented, unfermented and control groups. The trend of results obtained indicates the non-toxic and unharmful nature of the fermented maize cobs to blood components.

Serum enzymes such as aspartate transaminase (AST), alanine transaminase (ALT), and alkaline phosphatase (ALP) are useful biomarkers of liver function (Diana, 2007). The levels of AST and ALT in serum are used in the diagnosis of the health status of the liver (Kasarala and Tillmann, 2016), while levels in the blood are directly related to the level of tissue damage (Botros and Sikaris, 2013). In this study, there was no correlation between fungal-treated agro wastes and the serum biomarkers alanine aminotransferase and aspartate aminotransferase. This normal level suggests that fermented residue inclusion in the diets had no noticeable adverse effects on the liver of the animals at the level of the inclusion studied.

Serum biochemical parameters of liver function; total protein, total bilirubin, glucose, albumin, and cholesterol were all assayed for following experimental feeding trial with a fermented, unfermented, and commercially prepared diet. A significant difference at P<0.05 was not observed among all experimental groups. However slight decreases were observed in the albumin, protein, and glucose with the levels of the fungal-treated residues. Increasing the levels of these residues could probably have resulted in reduced mobilization of glucose and amino acids from the diets, thereby leading to low serum levels of these biomolecules and reduced protein synthesis and growth (Cantalapiedra-Hijar *et al.*, 2014). High crude fiber has been reported to reduce the intestinal absorption of glucose, amino acids, and some mineral elements (McDonald *et al.*, 1994).

A healthy kidney removes creatinine and urea nitrogen from the blood, the higher the creatinine and urea value the less effective the kidney function (Rusul and Haider, 2014). Thus, the urea and creatinine concentration obtained in the samples of rats fed fermented diets implies no negative effect on kidney function. The chloride, sodium, and bicarbonate electrolytes analyzed in the serum of all groups of experimental rats followed a similar trend with no significant difference among the various substituted fermented groups as well as in comparison with the control.

CONCLUSION

Following a 28day feeding trials and toxicity studies, this research work established feed substituted with 10% *L. flavidum/ A. niger* mixed fermented residues among varying percentage of substituted feed was found to be safe and have an improved performance on growth rate.

AUTHORS' CONTRIBUTIONS

All authors (AO, EO, DAA, SEA & NUS) contributed to the study conception and design. The preparation, data collection and analysis were performed by author AO. The first draft of the manuscript was written by author AO and the design was done by author EO and DAA. All authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

FUNDING STATEMENT

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest

ACKNOWLEDGEMENT

The authors are very thankful to the laboratory technologists and academic staff of the Departments of Biochemistry, Microbiology, and Animal Science at Ahmadu Bello University Zaria.

REFERENCES

Abell, L.L., Levy, B.B., Brodie, F.E., and Kendall (1952). A simplified method for the estimation of total cholesterol in serum and demonstration of its specificity. *Journal of Biological Chemistry*, 195(1): 357-366.

Andlar, M., Rezić, T., Marđetko, N., Kracher, D., Ludwig, R., and Šantek, B. (2018). Lignocellulose degradation: An overview of fungi and fungal enzymes involved in lignocellulose degradation. *Engineering in Life Sciences*, 18(11): 225-231

Atuahene, C. C., Donkoh, A. and Ntim, I. (2000). Blend of oil palm slurry and rice bran as a feed ingredient for broiler chickens. *Animal Feed Science and Technology*, 83(3-4):185-193.

Bauminger, C. (1974). Determination of glucose using glucose oxidase. *Analytical Chemistry,* 36 (2): 726-729.

Benli, W, Long, H., Jing, C., Ye, Z., Xiajun, C., Cangcang, W., Xiaojie, D., Jing. G. and Jixiang, H. (2021). Effects of feeding frequency on growth performance, feed intake, metabolism, and expression of fgf21 in grass carp (*Ctenopharyngodon idellus*), *Aquaculture*. 545. pp 737196.

Bharathiraja, S., Suriya, J., Krishnan, M., Manivasagan, P., and Kim, S.K. (2017). Production of enzymes from agricultural wastes and their potential industrial applications. In *Advances in Food and Nutrition Research.* 80. pp 125-148.

Bhavadasan, M. K., Rajput, Y. S., Ganguli, N. C. (1982). A simple colorimetric method for the determination of urea in milk. *Indian Journal of Dairy Science*, 35(1): 263–266.

Biaosheng, L., Yan, J., Zhong, Z. and Zheng, X. (2020). A Study on the preparation of microbial and non-starch polysaccharide enzyme synergistic fermented maize cob feed and its feeding efficiency in finishing pigs. *BioMedical Research International*, 1(23): 1-11.

Borges, S., A., Fischer, da Silva, A. V., Majorka, A., Hooge, D. M., and Cummings, K. R. (2004). Physiological responses of broiler chickens to heat stress and dietary electrolyte balance (sodium plus potassium minus chloride, milliequivalents per kilogram). *Poultry Science*, 83(9): 1551-1558.

Botros, M., and Sikaris, K. A. (2013). The de rites ratio: the test of time. *Clinical Biochemistry Review*, 34(3): 117-130.

Cantalapiedra-Hijar, G., Sophie, L., Rodriguez-Lopez, J. M. and Isabelle, O. (2014). Diets rich in starch increase the posthepatic availability of amino acids in dairy cows fed diets at low and normal protein levels. *Journal of Dairy Science.* 97(8): 5151-5166

Clement, A., Dishi K., Altine, J. M., Yahaya, B., Isa J., Tiva, J., Joseph, U. I., and Asabe, I. (2020). Productive performance and cost benefits of feeding Wistar albino rats with processed tropical sicklepod (*Senna obtusifolia*) leaf meal-based diets. *Translational Animal Science*, 4(2):589–593.

Dacie, J. F., and Lewis, S.M. (1984). *Practical Haematology*. Churchhill living stone. Edingburgh., London, 6th Edition, pp22-27.

Deaton, J. N., Naughton, J. L., and Burdick, D. (1979). High-fiber sunflower meal as a replacement for soybean in layers diets. *Journal of British Poultry Science.* 20(2): 159-162.

Delanghe, J. R. and Speeckaert M. M. (2011). Creatinine determination according to Jaffe-what does it stand for? *NDT Plus*. 4(2):83-6.

Doumas, B. T., Watson, W. A., and Broggs, H. G. (1971). Albumin standard and measurement of serum albumin with bromocresol green (BCG). *Clinical Chemistry*, 31(1): 87- 90.

Elzubeir, E. A. and Jubarah, S. K. (1993). Nutritional evaluation of sorghum germ meal as a substitute for sorghum in broiler diets. *Animal Feed Science and Technology.* 44 (1-2):93-100

Hrabčáková P, Voslářová E, Bedáňová I, Pištěková V, Chloupek J, Večerek V. (2014). Hematological and biochemical parameters during the laying period in common pheasant hens housed in enhanced cages. *Scientific World Journal.* 2014(1): 364602.

Hueze V., Tran G., Eduard N., Lebs F., (2017). Maize green forage feedipedia e programme by INRA, CIRAD, AFZ and FAO. www.feedipedia.org/node 358

Iyayi, E. A. (2001). Cassava leaves supplementation for feeding weaner swine. *Tropical Animal Production Investigation* 4. pp 141 – 150.

Karadeniz, A., Simsek, N.and Cakir, S. (2008). Hematological effects of dietary L-carnitine supplementation in broiler chickens. Revue *Méd. Vét*, 159(8-9), 437-44.

Karunananda, K., Fales, S. L., Varga, G. A., and Royse, D. J. (1992). *Journal of the Science of Food and Agriculture,* 60(1): 105-112.

Kasarala, G. and Tillmann, H. L. (2016) Standard liver tests. *Clinical Liver Disease*. 8(1): 13-18.

Kim, O., Tim, M., Kim, S., Genet, M., Kebebe, E. G., Faith, O., Marcos C., Getahun L., and Karin W. (2021). Utilization of by-products and food waste in livestock production systems: a Canadian perspective. *Animal Frontiers*, 11(2): 55–63.

Leite, P., Belo, I., and Salgado, J. M. (2021). Co-management of agro-industrial wastes by solid-state fermentation for the production of bioactive compounds. *Industrial Crops and Products*, 172: 113990.

Ma, J., Zhao, QB., and Laurens, L.L.M. (2015). Mechanism, kinetics and microbiology of inhibition caused by long-chain fatty acids in anaerobic digestion of algal biomass. *Biotechnology and Biofuels* 8(1): 1-12.

Mcdonald, P., Edward, R. A. and Greenhall, J. F. D. (1994). Pretreatment of straw by ammonia and its effect on fiber digestibility. *In Animal Nutrition*. pp 423-429.

Mcfeeters, R. F. (2004). Fermentation microorganisms and flavor changes in fermented food. *Journal of Food Science.* 69(1):35–37.

Nasri, S., Huque, K. S., Rahman, N., and Gopal, D. (2017). Effect of age on feed efficiency and carcass yield characteristics of indigenous bull. *Bangladesh Journal of Animal Science*. 46(1):17-23

Ogbonna, C. I. C., and Popoola, A. R. (1997). Biodegradation of maize straw by fungi for use as ruminant feed. *Nigerian Journal of Biotechnology,* 8(1):46-56.

Oge U. (2019). Nigerian maize production increased more than twofold between 2015 and 2018 – Farmers Association, Retrieved fromhttps://www.premiumtimesg.com/news/more-news/323857-nigerianmaizeproduction-increasesmorethantwofoldbetween2015and2018farmers-association.html?tztc=1

Opara, C. C. (1996). Studies on the use of *Alchannia cordifor*m leaf meal as feed ingredients in poultry diets. M.Sc thesis Federal University of Technology, Owena Nigeria.

Plummer, T. D. (1978) *An introduction to practical biochemistry* 2nd ed. U K McGraw Hill Book Co. pp 144-146.

Punzalan, R. R., Johnson, G. F., Cunningham, B. A. and Feld, R. D. (1990). Kinetic measurement of bicarbonate in serum by thiocyanate inhibition of wheat germ phosphoenolpyruvate carboxylase. Clinical Chemistry, 36(12)1-2057–2062.

Ravindra, R., and Jaiswal, A. K. (2016). A comprehensive review on pre-treatment strategy for lignocellulosic food industry waste: Challenges and opportunities. *Bioresource Technology*. 199. pp92–10.

Reitman, S. and Frankel, S.A. (1957). A Colorimetric method for the determination of serum glutamic oxaloacetic and glutamic pyruvic transaminases. *American Journal of Clinical Pathology*, 28(1): 56-63.

Rusul, A., and Haider, S. (2014). A study of some biochemical changes in patients with chronic renal failure undergoing hemodialysis. *International Journal of Current Microbiology and Applied Sciences,* 3(5): 581–586.

Sauvant, D., Perez, J. M. and Tran, G. (2004). Tables of composition and nutritional values of feed Materials: pig, poultry, sheep, goats, rabbits, horses, fish. Wageningen Academic Publishers, Wageningen, Netherlands; INRA Editions, Paris, France. pp 43-51

Steinkraus, K. H., (2004). *Handbook of indigenous fermented foods*. 2nd ed. Marcel Dekker, New York, NY. pp 412–467.

Tietz, N.W., (1974). Fundamentals of Clinical Chemistry, W.B. Saunder Co., Phila, PA. pp 874.

United Nations Environment Programme (2021). Food Waste Index Report 2021. Nairobi. Retrieved from https://www.unep.org/resources/report/unep-food-waste-index-report-2021

United States Department of Agriculture, USDA. (2021). World agricultural production archives. Retrieved from https://fas.usda.gov/data/world-agricultural-production-03082024

Xu, Y., Fan, W., Huang, X., Liu, K., Xu, Y., Hu, B., and Chi, Z. (2022). Nutrition component adjustment of distilled dried grain with solubles via *Aspergillus niger* and its change about dynamic physiological metabolism. *Fermentation*. 8(6):264.

Yafetto, L. (2020). Application of solid-state fermentation by microbial biotechnology for bioprocessing of agro-industrial wastes from 1970 to 2020: A review and bibliometric analysis. *Heliyon*. 24:8(3).

Yi, Z., Li, X., Luo, W. *et al.* (2018) Feed conversion ratio, residual feed intake and cholecystokinin type A receptor gene polymorphisms are associated with feed intake and average daily gain in a Chinese local chicken population. *Journal of Animal Science and Biotechnology,* 9: 1-9.

Zhao, L., Sun, Z.F., Zhang, C.C., Nan, J., Ren, N.Q., Lee, D.J., and Chen, C. (2022). Advances in pretreatment of lignocellulosic biomass for bioenergy production: challenges and perspectives. *Bioresource Technology,* 343. pp 126-133.