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EFFECT OF FERMENTED PEARL MILLET ON PERFORMANCE, PHYSIOLOGICAL RESPONSES, GUT MORPHOLOGY, AND CAECAL MICROBIOTAS IN BROILER CHICKENS

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ABSTRACT: This study evaluated the contribution of fermented pearl millet [*Pennisetum glaucum* (L) R. Br.,] on growth performance, physiological responses, gut morphology, and microbial composition in the caeca. One hundred and eighty day-old Arbor Acre broiler chicks were assigned into five groups and were fed starter (d 0 to 21) and finisher (d 22 to 42) diets. Test diets included a control composed of maize-soybean meal (0%); a similar diet with maize replaced with fermented pearl millet (FPM) at 25, 50, 75, and 100%. Results showed that there was no significant improvement in weight gain and feed conversion ratio although more feed was consumed (P = 0.035) as FPM increased in the diet. Carcass yield increased linearly (P = 0.05) at d 42. Bursa of Fabricius quadratically increased (P = 0.02) in weight particularly at 25% and 50% FPM levels at d 21. Concentrations of total protein (P = 0.026) and low-density lipoprotein (P = 0.037) increased linearly as FPM increased in the diets. Proventriculus weight, lymphocyte concentration in the blood, and size of gut segments linearly reduced (P < 0.05). Proventriculus and crop pH improved linearly (P = 0.05) while digesta pH in jejunum reduced linearly (P = 0.005) at d 21. Duodenal villus width increased quadratically (P = 0.008), and the highest width occurred in the 50% FPM group. Furthermore, dietary FPM did not influence caeca *Salmonella and Lactobacillus*. In conclusion, replacement of maize with FPM had no adverse effect on performance, physiological status, gut morphology and microbial composition of broiler chickens. Our results suggest that FPM represents a potential alternative in diets of broiler chickens without sacrificing the nutritional quality of the diet.

Keywords: Broiler chickens, Fermented pearl millet, Gut morphology, Gut microbiota, Nutritional quality.

INTRODUCTION

The poultry industry is challenged by scarcity and increasing cost of maize which has enthused the use of alternative source of energy (Owen et al., 2012; Ravindran and Blair, 2009). Cereal crops including wheat, sorghum, and barley have been investigated in partial or total replacement of maize (Silva et al., 2015; Viliene et al., 2022; Biesek et al., 2022). However, cereal grains contain considerable amount of anti-nutrients which inhibits nutrient utilization and growth of broiler chickens (Mathlouthi et al., 2002).

Grain fermentation, an important affordable processing method in poor tropical countries, has been reported to boost the nutritive value of cereals by enriching crude protein and fat content, and reduce anti-nutrient thereby improving nutrient digestibility. It also enhances micronutrient bioavailability and density accompanied by reduced crude fibre content (Feng et al., 2007; Wang et al., 2010; Kasprowicz-Potocka, 2015; Sugiharto et al., 2015; Zaworska et al., 2016). The fermentation process can be carried out naturally or with the use of starter culture. Natural fermentation process is a traditional method that is relatively simple, common, inexpensive, and effective method available to small scale poultry farmers in developing countries like Nigeria.

The use of fermented feed products in poultry production has been reported extensively. Fermented feed enhanced growth performance, antioxidant system, size of the immune organs, egg weight, and strength of the egg shell of laying hens (Engberg et al., 2009; Zhu et al., 2020). Fermented feed tends to produce beneficial bacteria which could improve the structure and function of the gut and stimulate establishment of beneficial bacteria population (Gao et al., 2009; Li et al., 2020). Drażbo et al. (2019) showed that inclusion of 15% fermented rapeseed cake improved body weight in turkeys without effect on carcass quality. Previous studies revealed that fermented feed modulated intestinal microflora, subsequently contributing to improved growth, intestine structure, and immunity when broiler chickens were fed fermented rice bran (Kang et al., 2015), and cottonseed meal (Ranjitkar et al., 2016; Jazi et al., 2017). Feeding broiler chickens *B. licheniformis* fermented products improved the body weight of coccidia infected broilers and regulated caeca microbial composition (Cheng et al., 2021). However, information is limited on the impact of fermented pearl millet in broiler chickens nutrition. Pearl millet (*Pennisetum glaucum,* PM) contains a high amount of nutrients comparable to maize, rich in antioxidants and fiber (Boncompagni et al., 2018; Punia et al., 2021). Pearl millet contains fewer, anti-nutrient compounds including phytate, tannins, polyphenols, and enzyme inhibitors which could influence nutrient availability and digestion (Osman, 2011; Boncompagni et al., 2018).

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To our knowledge, in literature, there is limited information on the effect of fermented pearl millet in broiler chickens. Therefore, this study aimed to evaluate whether fermented pearl millet affects growth performance, physiological responses, gut morphology, and microbial composition in the caeca of broiler chickens.

MATERIALS AND METHODS

Ethical approval

Experimental procedures adopted in this study were approved by the Bowen University research and ethical committee in conformation with AARIVE 2.0 guidelines (Du Sert et al., 2020).

Experimental units

The experiment was conducted at the Teaching and Research Farm of Bowen University. One hundred and eighty, 180, broiler chickens arrived at the facility, immediately weighed, and randomly distributed into 5 treatment groups. Each treatment was allotted to 3 replicates with 12 broiler chickens per replicate in a completely randomized design. Broiler chickens in each treatment group had ad libitum access to feed and water throughout the experimental period. The pens were maintained in identical environmental conditions. The experiment lasted for 42 days, starter (0 - 21 d) and finisher (22 - 42 d). The light regime was 1 h darkness (0 - 7 d) and 4 h darkness (8 - 42 d) respectively.

Experimental diets

The pearl millet grains used in this study were fermented naturally in water. The PM grains were fermented following modified procedure of Osman (2011). The PM grains were sterilized in brine solution for 30 min. After this time, the solution was drained, and the grains thoroughly rinsed. The grains were soaked and fermented in distilled water for 24 h in darkness at ± 30^{o}C. The solution was drained, grains thoroughly rinsed, dried for 2 days and stored. Table 1 presents the chemical composition of fermented PM according to previous procedure of AOAC (2005).

The present study formulated five treatment diets fed as mash. A maize-soybean meal was formulated at a standard 22.5% CP as the basal diet to meet or exceed NRC (1994) nutritional requirements for broiler chickens. The five treatments adopted include a control diet which contains 100% maize and four experimental diets with 25%, 50%, 75%, and 100% of maize replaced by fermented PM. The ingredients and nutrient composition of treatment diets were presented in Table 2. The broiler chickens were fed at two phase feeding. The starter diet was fed for the first 21 days and then finisher diets fed until the end of the experiment at d 42 of age.

Table 1 - Nutrient composition of fermented pearl millet.

Composition	Fermented pearl millet
Metabolizable energy (Kcal/kg)	3,441.60
Dry matter (%)	88.6
Protein (%)	10.9
Crude fibre (%)	1.9
Ash (%)	1.3
Crude fat (%)	6.79
Phosphorus, Available (%)	0.11
Phosphorus, Phytate (%)	0.19

Table 2 - Basic ingredients and composition of experimental diets.

Ingredients	FPM (d 0 to 21)					FPM (d 22 to 42)				
	0%	25%	50%	75%	100%	0%	25%	50%	75%	100%
Maize	52.09	39.28	27.09	15.74	-	59.70	45.77	31.35	16.69	-
Pearl millet	-	14.81	28.91	42.03	60.29	-	16.20	32.86	49.75	67.06
Soybean meal	40.90	39.52	38.23	37.02	35.31	34.10	32.50	30.98	29.45	28.54
Soybean oil	2.68	2.03	1.41	0.83	-	2.15	1.46	0.72	-	-
Salt	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.38
DCP	2.16	2.14	2.11	2.09	2.05	1.95	1.92	1.89	1.87	1.85
Limestone	1.49	1.52	1.54	1.56	1.6	1.39	1.42	1.45	1.47	1.51
Vit-Min Premix	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.38
Lysine	-	0.03	0.05	0.07	0.10	0.05	0.08	0.11	0.13	0.16
Methionine	0.18	0.17	0.16	0.16	0.15	0.16	0.15	0.14	0.14	0.12

Day 0 to 21 [crude protein: 22.5%; methionine: 0.50%; lysine: 1.21%; calcium: 1.10%; phosphorus: 0.50%; metabolizable energy: 2970 (kcal/kg)]; day 22 to 42 [crude protein: 20.00%; methionine: 0.45%; lysine: 1.10%; calcium: 1.00%; phosphorus: 0.45%; metabolizable energy: 3004 (kcal/kg)]; FPM = fermented pearl millet; DCP = dicalcium phosphate; vitamin-mineral premix supplied per kilogram of diet: vitamin A: 30,000 IU; vitamin D3: 6,250 IU; vitamin K: 5 mg; vitamin E: 75 mg; vitamin B1: 5.63 mg; vitamin B2: 15 mg; vitamin B6: 11.25 mg; vitamin B12; 0.0375 mcg; niacin; 100 mg; pantothenic acid: 37.5 mg; folic acid; 3.75 mg; choline chloride; 750 mg; manganese: 200mg; biotin: 0.125 mcg; zinc: 125 mg; iodine: 2.5 mg; copper: 12.5 mg; selenium; 0.5 mg; cobalt; 1.25 mg; iron; 50 mg; antioxidant; 312.5 mg.

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Performance, carcass and organ measurements

The body weight and feed intake were measured weekly. The FI, BWG and FCR were estimated for d 21 and d 42. Thirty broiler chickens (n = 30, 6 broiler chickens per treatment) were selected, fasted overnight, and weighed before slaughter. The resulting carcass were eviscerated, cut into different cuts and weighed. The carcass and internal organ weights were expressed as percentage live weight of each broiler chickens.

Hematological and biochemical determination

Blood samples were drawn from thirty broiler chickens (n = 30, 6 broiler chickens per treatment) at d 21 into vacutainer tubes for serum collection. Blood samples were centrifuged at 3000 rpm for 5 min at 4°C for serum collection. Serum samples were analyzed for cholesterol (CHL), low density lipoprotein (LDL), and high-density lipoprotein (HDL) using commercially available kits (AGAPPE Diagnostics Switzerland GmbH), albumin estimated by Bromocresol Green method (Wells et al., 1985), and total protein quantified by Biuret method (Kohn and Allen, 1995). The anti-coagulated blood samples were obtained for hematological analysis including white blood cell (WBC), lymphocytes (LYM), monocyte (Mono), packed cell volume (PCV), and red blood cell (RBC) were estimated using commercially available kits (RANDOX Laboratory Ltd, UK). Mean corpuscular hemoglobin concentration (MCHC) was determined using the equation described by Jain (1986).

Gut digesta pH

Broiler chickens (n = 30) were sampled at d 21 and d 42 to determine gut digesta pH. The entire intestine was separated into each segment. The pH in each segment from the crop to the caecum were measured by pH meter (Smart Spear pH tester, PH60S-Z Apera Instruments, USA). Stable readings were recorded for individual segments for each broiler chicken.

Gut morphology and caeca microbial composition

Gut morphology and caeca microbial composition of thirty broiler chickens (n = 30, 6 broiler chickens per treatment) were determined at d 21. The gut was removed and washed gently with Phosphate-buffered saline (PBS) solution. The gut segments including duodenum, jejunum, and ileum were separated, their individual length and weight subsequently measured and estimated as percentage of broiler chicken's live weight. For further morphological analysis, a 3-cm tissue sample of midpoint of each segment were placed in 10% formalin, dehydrated, cleared, and then embedded in paraffin (Suvarna et al., 2018). Cross sections of 5 µm for each sample were cut, mounted on glass slides, and hematoxylin-eosin stained. All morphometric images were taken and measured under a light microscope (VJ – 2005 DN Bio-microscope) and TX View CX Image® (Miotic Image 200, China). The contents of the caeca were collected, homogenized, and serially diluted 10-fold before plating. Samples were cultured on the Lactobacillus selective media, de Man, Rogosa and Sharpe agar (MERCK) and incubated for 48 h at 37°C before quantification. To enumerate Salmonella concentration, samples were incubated on Bismuth Sulfate agar for 24 h at 37°C. Bacterial colonies counted were reported as $\log_{10}$CFU for each gram of sample.

Statistical Analysis

A completely randomized design was adopted to analyse all data with analysis of variance (ANOVA) (Genstat Version 21.0, 2022). Bacteriological data was normalized using logarithmic transformation. Treatment means were separated at $P \leq 0.05$ by the Tukey least significant difference, post hoc test of ANOVA. Orthogonal polynomials were used to determine linear and quadratic effects of increasing levels of fermented pearl millet.

RESULTS

Growth performance of broiler chickens is shown in Table 3. During the experimental period, FPM did not affect BWG and FCR. On d 0 to 21 and d 0 to 42, there were no obvious effects of dietary FPM on FI. However, FI increased quadratically ($P = 0.035$) on d 22 to 42, with the highest FI obtained at the 25% and 100% FPM levels.

As presented in Table 4, on d 21, FPM in the diet had no significant effect on mean carcass yield. There was no impact of treatment diets on breast, thigh, and abdominal fat. However, drumstick at d 21 decreased quadratically ($P = 0.036$), with the lowest drumstick obtained at the 50% FPM level. Linear improvement in carcass yield was observed on d 42, although there was no significant effect on the breast, drumstick, thigh, and abdominal fat (Table 4).

The effects of FPM on organ weights of broiler chickens are shown in Table 5. By d 21, proventriculus weight reduced linearly ($P = 0.006$) as FPM increased in the diet while weight of bursa of Fabricius increased quadratically ($P = 0.02$), and highest weight was obtained in broiler chickens on 25% dietary FPM. Furthermore, FPM had no significant effect on weight of pancreas, gizzard, liver, and thymus. Similarly, by d 42, FPM did not influence organ weights.

Hematology and serum biochemistry of broiler chickens at d 21 are shown in Table 6. Increase in FPM up to 50% in the diets linearly decreased lymphocyte concentration in the blood ($P = 0.041$). Red blood cell concentration quadratically increased ($P = 0.048$) with the highest concentration obtained at 50% FPM level. The concentrations of Mono, WBC, PCV and MCHC were not altered. Similarly, FPM did not affect Albumin, CHL and HDL levels in the serum. Serum LDL

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concentration linearly increased {(P = 0.026) as FPM increased. Furthermore, TP level improved (P = 0.037) with up to 75% increase in FPM (Table 6).

The effects of FPM on both the length and weight of different segments of the intestine are shown in Table 7. On d 21, both ileum and cecum reduced in length linearly [(P = 0.003) and (P = 0.012), respectively] as FPM levels increased. In addition, both duodenum and jejunum lengths showed a decreasing trend. On d 42, complete replacement of maize with FPM reduced length of duodenum, jejunum and ileum at d 42. FPM also linearly reduced duodenum and ileum length [(P = 0.027) and (P = 0.015), respectively]. The least length was observed FPM had no effect on the length of the caecum (Table 7).

On d 21, FPM inclusion in the diets had no effect on empty weights of duodenum, jejunum, and caecum. On the contrary, empty weight of ileum (P = 0.034) decreased linearly as dietary FPM increased at d 21 while jejunum empty weight showed a similar decreasing trend at d 42 (Table 7).

The digesta pH in proventriculus and crop on d 21 increased linearly (P = 0.05) and jejunal pH decreased linearly (P = 0.005). However, FPM did not influence the pH of intestinal digesta from the gizzard, duodenum, ileum, and caecum. On d 42, FPM had no significant effect on the pH of the intestine. In contrast, crop digesta pH decreased quadratically (P = 0.048) with the addition of FPM to the diets (Table 8).

The dietary inclusion of FPM did not alter villus height and crypt depth of duodenum, however, duodenum villus height was quadratically increased (P = 0.008), and the highest observation was found in the 50% FPM group (Table 9). There was no effect of FPM on villus height, villus width and crypt depth in the jejunum (P > 0.05) at d 21 (Table 9). Furthermore, FPM did not influence (P > 0.05) *Lactobacillus* and *Salmonella* concentration in caeca of broiler chicken (Table 9).

Table 3 - Effect of fermented pearl millet on the performance parameters of broiler chickens during the rearing period

Parameters	FPM					SEM	P-value	
	0%	25%	50%	75%	100%		L	Q
BWG								
d 0 to 21	599	602	609	635	633	8.070	0.483	0.794
d 22 to 42	1154	1255	1116	1097	1164	25.200	0.411	0.226
d 0 to 42	1753	1858	1725	1732	1797	28.200	0.631	0.350
FI								
d 0 to 21	865	890	909	919	949	10.400	0.307	0.374
d 22 to 42	2039^{a}	2140^{ab}	2098^{ab}	2095^{ab}	2172^{b}	18.200	0.694	0.035
d 0 to 42	2904	3030	3007	3014	3121	26.900	0.488	0.076
FCR								
d 0 to 21	1.45	1.48	1.50	1.45	1.50	0.012	0.986	0.204
d 22 to 42	1.78	1.71	1.88	1.91	1.87	0.032	0.224	0.476
d 0 to 42	1.66	1.63	1.75	1.74	1.74	0.022	0.316	0.792

Different superscripts within rows indicate significance at P<0.05. Each mean represents 6 replicates. L = linear effects of increasing levels of fermented pearl millet in diets; Q = quadratic effects of increasing levels of fermented pearl millet; FPM = fermented pearl millet; BWG = body weight gain; FCR = feed conversion ratio; FI = feed intake.

Table 4 - Effect of fermented pearl millet on carcass traits of broiler chickens.

Parameters	FPM					SEM	P-value	
	0%	25%	50%	75%	100%		L	Q
d 21								
Carcass yield, %	52.10	50.40	50.40	52.70	52.80	0.550	0.717	0.148
Breast, %	19.40	18.30	18.70	20.50	20.00	0.480	0.754	0.276
Drumstick, %	9.09^{a}	8.45^{ab}	8.32^{b}	8.92^{ab}	9.05^{a}	0.140	0.106	0.036
Thigh, %	9.52^{ab}	8.93^{a}	9.78^{b}	9.80^{b}	10.08^{b}	0.170	0.718	0.301
AF, %	1.60^{ab}	2.24^{b}	0.89^{a}	0.95^{a}	0.95^{a}	0.180	0.122	0.230
d 42								
Carcass yield, %	58.50^{ab}	55.00^{a}	59.70^{ab}	58.60^{ab}	61.70^{b}	0.920	0.050	0.193
Breast, %	23.30	22.30	24.20	23.30	24.60	0.680	0.339	0.680
Drumstick, %	9.58	9.68	9.77	9.74	10.32	0.120	0.114	0.437
Thigh, %	11.10	11.70	11.30	11.10	12.20	0.160	0.114	0.211
AF, %	0.92	1.16	1.45	1.17	0.86	0.110	0.897	0.115

Different superscripts within rows indicate significance at P<0.05. Each mean represents 6 replicates. L = linear effects of increasing levels of fermented pearl millet in diets; Q = quadratic effects of increasing levels of fermented pearl millet; FPM = fermented pearl millet; AF = Abdominal fat.

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Table 5 - Effect of fermented pearl millet on digestive organ weights of broiler chickens.

Parameters		FPM					SEM	P-value	
		0%	25%	50%	75%	100%		L	Q
d 21	Pancreas, %	0.29	0.28	0.21	0.26	0.29	0.013	0.836	0.068
	Gizzard, %	1.82	2.00	1.91	1.89	1.90	0.039	0.853	0.462
	Proventriculus, %	0.58^{a}	0.55^{ab}	0.51^{ab}	0.49^{ab}	0.46^{b}	0.016	0.006	0.820
	Liver, %	3.03	3.17	2.68	2.80	2.67	0.097	0.135	0.934
	Bursa, %	0.13^{a}	0.21^{b}	0.19^{bc}	0.15^{ac}	0.16^{abc}	0.008	0.923	0.020
	Thymus, %	0.37	0.39	0.40	0.37	0.38	0.024	0.990	0.754
d 42	Pancreas, %	0.20	0.22	0.22	0.22	0.18	0.010	0.390	0.120
	Gizzard, %	1.53	1.54	1.54	1.78	1.51	0.050	0.560	0.460
	Proventriculus, %	0.43	0.31	0.33	0.31	0.30	0.020	0.090	0.380
	Liver, %	1.95	1.86	1.70	1.84	1.91	0.060	0.800	0.240
	Bursa, %	0.08	0.08	0.12	0.12	0.09	0.010	0.600	0.330
	Thymus, %	0.27	0.31	0.33	0.24	0.25	0.020	0.220	0.140

Different superscripts within rows indicate significance at P<0.05. Each mean represents 6 replicates. L = linear effects of increasing levels of fermented pearl millet in diets; Q = quadratic effects of increasing levels of fermented pearl millet; FPM = fermented pearl millet.

Table 6 - Effect of fermented pearl millet on haematology and serum biochemistry of broiler chickens fed fermented whole PM-based diet on day 21.

Parameters	FPM					SEM	P-value	
	0%	25%	50%	75%	100%		L	Q
LYM, %	66.70^{a}	63.60^{ab}	59.40^{b}	62.70^{ab}	66.20^{ab}	1.060	0.041	0.591
Mono, %	3.00	3.80	3.20	3.17	2.83	0.180	0.579	0.363
WBC, x 10^{3}/µL	18,242	18,300	20,270	18,067	18,617	415	0.616	0.365
PCV, %	29.50	31.20	32.80	29.70	30.30	0.540	0.410	0.079
MCHC, g/dl	33.10	33.40	33.10	33.50	33.90	0.260	0.870	0.977
RBC, x10^{6} /µL	3.34^{ab}	3.47^{ab}	3.61^{a}	3.32^{b}	3.46^{ab}	0.048	0.750	0.048
Albumin, g/dl	1.36	1.42	1.37	1.56	1.46	0.034	0.162	0.524
CHL, mg/dl	175	163	170	184	189	4.540	0.994	0.318
HDL, mg/dl	103	111	93	120	124	4.000	0.851	0.852
LDL, mg/dl	35.50^{a}	46.10^{ab}	46.60^{ab}	51.00^{b}	51.80^{b}	1.800	0.037	0.208
TP, g/dl	2.22^{a}	2.12^{ab}	2.70^{b}	2.62^{b}	2.30^{ab}	0.077	0.026	0.452

Different superscripts within rows indicate significance at P<0.05. Each mean represents 6 replicates. L = linear effects of increasing levels of fermented pearl millet in diets; Q = quadratic effects of increasing levels of fermented pearl millet; FPM = fermented pearl millet; LYM = lymphocytes; Mono = monocytes; MCHC = mean corpuscular hemoglobin concentration; PCV = packed cell volume; WBC = white blood cell; RBC = red blood cell; CHL = cholesterol; TP = Total protein; HDL = high-density lipoprotein; LDL = Low-density lipoprotein; µL = microlitre; mg = milligram; dl = decilitre; g = gram.

Table 7 - Effect of fermented pearl millet on the relative length and weight of intestine segments of broiler chickens.

Parameters	FPM					SEM	P-value	
	0%	25%	50%	75%	100%		L	Q
d 21 - Relative length (cm/g) of BW								
Duodenum	3.75	3.80	3.59	3.57	3.29	0.096	0.074	0.511
Jejunum	8.14	8.08	7.47	7.65	7.17	0.180	0.087	0.977
Ileum	8.58^{a}	8.20^{ab}	7.63^{b}	7.60^{b}	7.30^{b}	0.150	0.003	0.531
Caecum	1.88^{ab}	1.89^{a}	1.84^{ab}	1.69^{ab}	1.61^{b}	0.039	0.012	0.367
Relative weights (g/g) of BW								
Duodenum	0.42	0.49	0.40	0.44	0.34	0.023	0.118	0.195
Jejunum	0.70	0.72	0.66	0.66	0.62	0.023	0.190	0.761
Ileum	0.58^{ab}	0.64^{a}	0.52^{ab}	0.47^{b}	0.47^{b}	0.027	0.034	0.702
Caecum	0.25	0.32	0.25	0.25	0.21	0.016	0.160	0.207
d 42 - Relative length (cm/g) of BW								
Duodenum	2.13^{a}	1.98^{ab}	1.84^{ab}	2.04^{b}	1.64^{b}	0.066	0.027	0.690
Jejunum	3.96	3.83	3.70	4.23	3.29	0.120	0.135	0.193
Ileum	4.61^{a}	4.22^{a}	4.28^{a}	4.55^{a}	3.57^{b}	0.120	0.015	0.183
Caecum	1.04	1.00	1.03	1.11	0.96	0.022	0.803	0.369
Relative weights (g/g) of BW								
Duodenum	0.37	0.36	0.36	0.34	0.30	0.015	0.243	0.624
Jejunum	0.60	0.59	0.56	0.52	0.48	0.024	0.052	0.690
Ileum	0.49	0.55	0.56	0.46	0.47	0.027	0.327	0.206
Caecum	0.21	0.22	0.25	0.24	0.18	0.012	0.739	0.127

Different superscripts within rows indicate significance at P<0.05. Each mean represents 6 replicates. L = linear effects of increasing levels of fermented pearl millet in diets; Q = quadratic effects of increasing levels of fermented pearl millet; FPM = fermented pearl millet.

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Table 8 - Effect of fermented pearl millet on gut pH of broiler chickens.

Parameters	FPM					SEM	P-value	
	0%	25%	50%	75%	100%		L	Q
d 21								
Crop	4.60^{ab}	4.43^{a}	4.68^{ab}	4.63^{ab}	4.85^{b}	0.320	0.050	0.244
Proventriculus	1.83^{a}	2.60^{ab}	2.48^{ab}	2.27^{ab}	3.30^{b}	1.120	0.050	0.753
Gizzard	2.38	2.57	2.13	2.82	2.95	0.630	0.060	0.203
Duodenum	5.83	5.95	6.03	5.92	5.90	0.390	0.839	0.354
Jejunum	6.00^{a}	5.98^{a}	5.97^{ab}	5.88^{ab}	5.78^{b}	0.190	0.005	0.532
Ileum	6.42	6.33	6.33	6.68	6.60	0.710	0.495	0.774
Caecum	5.88	6.15	5.58	5.57	6.20	0.710	0.938	0.245
d 42								
Crop	4.87^{a}	4.62^{ab}	4.36^{b}	4.47^{ab}	4.62^{ab}	0.075	0.166	0.048
Proventriculus	3.52	3.83	2.15	3.12	3.02	0.058	0.298	0.351
Gizzard	2.53	2.50	2.79	2.46	2.77	0.095	0.595	0.951
Duodenum	5.69	5.72	5.98	5.50	5.90	0.100	0.809	0.989
Jejunum	5.95	6.19	6.19	6.00	6.13	0.089	0.573	0.275
Ileum	6.59	6.87	7.07	6.35	6.69	0.095	0.573	0.237
Caecum	6.40	6.62	6.06	6.45	6.10	0.120	0.450	0.888

Different superscripts within rows indicate significance at P<0.05. Each mean represents 6 replicates. L = linear effects of increasing levels of fermented pearl millet in diets; Q = quadratic effects of increasing levels of fermented pearl millet; FPM = fermented pearl millet.

Table 9 - Effect of fermented pearl millet on gut morphology and caecal microbial composition of broiler chickens on day 21.

Parameters	FPM					SEM	P-value	
	0%	25%	50%	75%	100%		L	Q
Duodenum, µm								
Villus height	786	740	645	646	764	45.000	0.706	0.340
Villus width	141^{a}	168^{ab}	229^{b}	191^{ab}	149^{a}	11.600	0.525	0.008
Crypt depth	86.50	83.60	81.20	71.20	75.60	2.710	0.122	0.774
Jejunum, µm								
Villus height	548	538	741	655	667	38.800	0.227	0.470
Villus width	176	150	174	217	174	12.200	0.524	0.883
Crypt depth	78.30	77.40	96.80	84.90	85.50	5.580	0.612	0.584
Lactobacillus, log 10 cfu/g	5.31	5.07	5.04	5.33	5.04	0.076	0.479	0.628
Salmonella, log 10 cfu/g	4.72	4.94	4.99	4.85	5.13	0.069	0.208	0.880

Different superscripts within rows indicate significance at P<0.05. Each mean represents 6 replicates. L = linear effects of increasing levels of fermented pearl millet in diets; Q = quadratic effects of increasing levels of fermented pearl millet; FPM = fermented pearl millet.

DISCUSSION

In the present study, dietary inclusion of FPM had no obvious effect on the performance of broiler chickens. Our results are consistent with Guo et al. (2020), who showed that fermented soybean meal had no influence on broiler chicken's growth performance. In contrast, other studies reported improved weight gain and FCR of broiler chickens feeding on fermented cotton seed meal of Sun et al. (2013) and Wang et al. (2017). The difference in findings observed by above researchers might be due to changes in the environment and nature of feed ingredients including size of and feed particles. The small size of pearl millet, (3-4 µm) when fed to broiler chickens ad libitum coupled with its volume and resident time of feed in the crop may have contributed to increased FI (Picard et al., 2000; Classen et al., 2016).

In the present study, FPM had no effect on carcass traits except for drumstick. This observation is similar to the report of Khempaka et al. (2018) that cassava pulp fermented with *Aspergilus oryzae* did not affect carcass composition of broiler chickens. On the other hand, Yeh et al. (2018) showed that improved drumstick and carcass yield of broilers may be attributed to higher digestible amino acids of fermented feed accompanied by enhanced growth performance. The proportion of abdominal fat has direct influence on carcass yield and economic value (Wen et al., 2018), since increased deposition of excess abdominal fat is usually considered a waste of dietary energy resulting in economic losses, decreasing feed efficiency, reduced carcass yield and carcass quality of broiler chicken (Fouad and El-Senousey, 2014).

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Other authors reported that feeding fermented mulberry leaf powder significantly improved muscle yield and reduced abdominal fat in broilers (Ding et al., 2021). In another study, the proportion of carcass yield and abdominal fat in broiler chickens were increased by feeding fermented cotton seed meal (Nie et al., 2015). In this study, however, dietary FPM had no effect on abdominal fat at the different feeding phases but increased carcass yield at the end of d 42 suggest that dietary FPM did not have negative effect on economic value of broiler chickens' carcass traits.

Changes in the weight of the immune organs including the bursa of Fabricius is an indicator of the condition of the immune system of chickens (Heckert et al., 2002; Tong et al., 2012), since they may partly redirect absorbed nutrients from growth towards development of the immune system. In our study, replacement of maize with FPM in diets resulted in increased weight of the bursa of Fabricius. Our study agrees with Sugiharto et al. (2020) and Ao et al. (2011), who found that fermented commercial feed improved immune responses including increase in weight of the immune organ in broilers. Furthermore, bursa of Fabricius increased in weight in broiler chickens fed fermented sour cherry kernel (Gungor and Erener, 2020).

Blood indices are important measures to determine the state of health in animals (Johnstone et al., 2017). Red blood cells function in transporting oxygen and supporting increased metabolism. In this study, the level of RBC was influenced by dietary FPM and was within the standard limit reported for chickens (Bounous and Stedman, 2000).

Lymphocytes are immune cells that generally increase in concentration in response to infection (Akhtar et al., 2015). In this study, lymphocyte concentration decreased in broiler chickens on FPM diets, comparable to the findings of Sugiharto et al. (2020), that fermented cassava pulp and *Moringa olifera* leaf meal reduced lymphocyte count in broiler chickens. Previous research reported that lactic acid bacteria present in fermented feed produce organic acids which reduces pH in the intestine, and hinder the establishment of pathogenic bacteria (Sugiharto and Ranjitkar, 2019). Furthermore, our results suggest that broiler chickens on the FPM diets were healthy, and their physiological state were not negatively affected. They had comparable nutrient utilization for blood production and other hematological indices, although LDL concentration increased. Fermentation reduced fiber content but increased protein contents in grains (Sugiharto et al., 2015), while serum cholesterol level may be lowered by increasing dietary crude fibre level in broiler diets (Delaney et al., 2003). Therefore, it could be stated that reduction in fiber content of PM through fermentation may have contributed to high LDL concentration.

The development of the intestine indicated by the relative weight and length reflects the physiological status and function of broiler chickens (Zhong et al., 2019). The size of broiler chicken's intestine including its length and weight have been used as indicators of digestive and absorptive capacities (Gao et al., 2010; Li et al, 2018). Furthermore, increased intestine length has been suggested to improve absorption of nutrients (Wang et al., 2015). In this study, intestinal relative length and weight decreased as levels of dietary FPM increased. This finding contrast with Naji et al. (2016), who showed that the length and weights of the digestive tract increased in broiler chickens fed fermented corn-soybean feeds. Furthermore, one factor that may have contributed to the small intestine length and weight is the fiber content (Jørgensen et al., 1996; Al-Marzooki et al., 2000). Recent studies have shown that high fibre content in diet of broiler chickens may promote the absorption of nutrients by the intestinal segments, which ultimately stimulate the intestine (Alyileili et al., 2020; Zhang et al., 2023). However, natural fermentation process reduces the crude fiber of grains (Akinola et al., 2017). In this study, the decreased intestinal length may be an adaptive response associated with the fiber content or small size of millet grain in the diet. However, reduction in length and weight of small intestinal segments as dietary FPM increased, did not negatively impact growth performance, carcass characteristics and organ weights, which suggest that nutrient absorption and availability were not impaired in this study.

Intestinal morphology greatly influences growth performance, nutrient digestion and utilization of broiler chickens (Ravindran and Abdollahi, 2021). In the present study, duodenum villus height, jejunum villus height, and jejunum crypt depth were not affected by dietary FPM. However, FPM significantly increased the duodenum villus width. Feng et al. (2007) showed that fermented commercial feeds improved the structure of intestinal morphology of broiler chickens. In addition, Naji et al. (2016) reported that villus height, crypt depth and their ratio increased for broiler chickens on fermented commercial feed of maize-soybean. Previous study reported that fermented cottonseed meal improved villi structure in duodenum and jejunum of broiler chickens (Jazi et al., 2017). The contrast in our study compared to other reports may be due to grain characteristics or the method of fermentation process.

Low intestinal pH is regarded as an important barrier to prevent significant colonization of pathogenic microorganisms in the intestine (Abouloifa et al., 2020). Reduction in digestive pH attributed to acidifying effect of fermented feeds provide favorable environment to increase colonization of lactic acid producing bacteria in broilers (Wyszyńska and Godlewska, 2021; Peng et al., 2022). Yaşar et al. (2016) showed that fermented feed encouraged establishment of barriers against pathogens through reduction of pH in the upper intestinal tract. Lin and Lee (2020) reported that wheat bran fermented with *Lactoporus sulphureus* reduced digesta pH in ileum and cecum of broiler chickens. In the present study, FPM in diets reduced digesta pH in the jejunum. The jejunum, the longest segment of the intestine is considered highly efficient in absorbing nutrients (Liu et al., 2021). Vicentini et al. (2021) showed that regulation of structure and function of the intestinal tract is controlled by the intestinal microbiota. FPM had no impact on concentration of caeca bacteria including *Lactobacillus and Salmonella*. Lv et al. (2022) similarly showed that the predominant bacterial species was not impacted in laying hens fed fermented diet. The activity of microbiota within the intestine played a critical role in growth performance and health of broilers through modulating the growth of pathogenic

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bacteria (Niba et al., 2009). Hence, the fact that FPM did not affect caeca microbial count may partly explain the lack of compromise in growth and FCR of broiler chickens.

CONCLUSION

In conclusion, BWG and FCR were not affected by replacement of maize with FPM although more feed was consumed. Weight of bursa of Fabricius and duodenum villus width improved on treatment diets. In addition, FPM reduced length and weight of small intestine but increased LDL without undermining growth performance, carcass traits, gut morphology, and beneficial microbiota. Based on the results on growth performance, physiological responses, and intestinal morphology, it can be recommended that, partial replacement of maize with FPM can be used as alternative to maize in the diets without compromising the overall performance of broiler chickens.

DECLARATIONS

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Consent to publish

Not applicable.

Author's contribution

O. Olasehinde. conceived, designed, conducted the experiment, analysed, and wrote the manuscript for publication. O. Olasehinde. and F. Aderemi reviewed the manuscript.

Competing interest

The authors declare that there is no competing interest to this research and publication.

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